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Synthesis and biological evaluation of aeruginosin based compounds and self-assembly of glucosamine based compounds

Navneet goyal
ngoyal@uno.edu

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A Dissertation

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By

Navneet Goyal

B.S. Chemistry (Hons) Panjab University, India, 2002
M.S. Chemistry (Hons) Panjab University, India, 2004
M.S. University of New Orleans, New Orleans, 2010

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To my family

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ABBREVIATIONS

Ac	Acetyl
AIBN	Azobisisobutyronitrile
Bn	Benzyl
Bz	Benzoyl
DMF	*N*,*N*-Dimethylforamide
DMSO	Dimethylsulfoxide
DCM	Dichloromethane
DIEA	Diisopropyl ethyl amine
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
HOBt	1-hydroxy benzotriazole
m-CPBA	*m*-Chloroperoxybenzoic acid
Ms	Mesylate
PTSA	p-toluenesulfonic acid
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
PTSA	*p*-Toluenesulfonic acid

ABSTRACT

Aeruginosins are a family of marine natural products containing mostly non-proteogenic amino acids. These compounds contain a common 2-carboxy-6-hydroxy-octaindole (Choi) rigid bicyclic structure. Many aeruginosins are inhibitors for enzymes involved in the blood coagulation cascade, such as thrombin and Factor VIIa. In order to understand the structure activity relationship (SAR) of the aeruginosins and to discover novel anticoagulants with potentially improved inhibitory and pharmacokinetic properties, in the first part of my thesis I have discussed, synthesis of a series of novel analogs of aeruginosin 298-A, in which the Choi will be replaced with L-proline and oxygenated Choi analogs, and the Argol is replaced with various other functionalities. The preparation of oxygenated Choi analogs starting from glucose using a new method has been discussed.

In the second part of my dissertation, I have discussed the design, synthesis and self–assembly of glucosamine based hydro and organogelators**.** Carbohydrate-based low molecular weight gelators are an interesting class of molecules with many potential applications. A series of amides and ureas were prepared from the protected D-glucosamine from the corresponding acid chloride and isocyanates. The self-assembling properties of these compounds were studied in several solvents, including water and aqueous solutions. Most of these compounds were found to be efficient low molecular weight hydrogelators (LMHGs) for aqueous solutions. The preparation and characterization of these compounds will be elaborated.

Chapter 1

Introduction

1.1. Overview:

In this chapter, the background information related to aeruginosins and thrombin inhibitors will be discussed in the first part, this is adapted from paper: *Cardiovasc. Hematol. Agents Med. Chem.* **2009**, *7*, 147-165 by Wang, G. and Goyal, N. The second part composes a brief introduction of self-assembling of glucosamine derivatives will be introduced in the second part.

Part I. Aeruginosin analogs and other rigid bicyclic compounds as antithrombotic

1.2 Introduction to Blood Coagulation

Blood coagulation is a complex process of ordered events which involves both cellular (i.e., blood platelets and leukocytes) and proteinaceous components (i.e., the coagulation factors, serine proteases and cofactors).[1] (coagulation pathway). The several important enzymes involved in blood coagulation cascades include thrombin (factor IIa), factor Xa and factor VIIa. Most of these coagulation factors are trypsin like serine proteases and they catalyze the coagulation cascade at different stages.

The basic events in blood coagulation involve a primary hemostasis i.e. platelet aggregation to form a primary platelet plug, followed by secondary hemostasis which is activation of plasma

coagulation factors generating fibrin which intertwines and reinforces the aggregated platelets resulting in a strong fibrin clot.[2-5] The two pathways responsible for the biochemical coagulation cascade leading to the generation of fibrin are (i) an intrinsic contact activation system and (ii) an extrinsic tissue factor system (Figure 1.1). Both pathways involve a stepwise activation of proteases, and they converge upon generation of active thrombin which is responsible for the final cleavage of fibrinogen to fibrin. The positive-feedback mechanisms along both pathways accelerate the coagulation process.

However in recent years, the extrinsic system triggered by tissue factor has been considered as the major pathway to thrombinogenesis.[5]

1.3 Problems associated with blood coagulation

Under normal physiological conditions the coagulation system is balanced by an anticoagulation system and a fibrinolysis system.[6,7] The imbalance between these mechanisms results in blood clotting or bleeding depending on which system dominates. A pathogenic imbalance in favor of the coagulation system may lead to thrombosis. In this process, a clot forms in the blood vessels and affects the circulation of blood. Thrombosis can become a serious condition if the clot migrates to the heart or to the lungs which can trigger life threatening diseases like myocardial infarction, pulmonary embolism (PE), deep vein thrombosis (DVT), and thromboembolic stroke. Thrombosis and related complications are major causes of fatal cardiovascular and cerebrovascular disease throughout the world.[8] The risk of thrombosis related disorders increases with age, obesity, surgery etc. and these are the leading causes of death in developed countries and are now having increasing incidences in developing countries as well.[9]

Even though men and women can have clotting disorders, these conditions pose added difficulties for women because of their relationship to reproductive issues. Women with these disorders can develop serious complications during pregnancy leading to miscarriage. Pregnancy, oral contraceptives and post-menopausal hormone replacement therapy are all triggering events for DVT in women with thrombosis. Thrombosis may manifest itself as the formation or presence of a blood clot in a blood vessel or one of the cavities in the heart. In fact, emboli (clots or plugs brought by the blood from another blood vessel and forced into a smaller vessel so as to obstruct the circulation) from deep vein clots are a leading cause of death in hospitalized patients. Annually, 200,000 to 300,000 patients develop this form of clot for the first time during a hospitalization. Nearly 40% of these patients suffer from a complication known as pulmonary embolism (a clot that travels to the lung and obstructs a significant amount of blood flow to the organ). This complication is fatal in 30% of the cases.

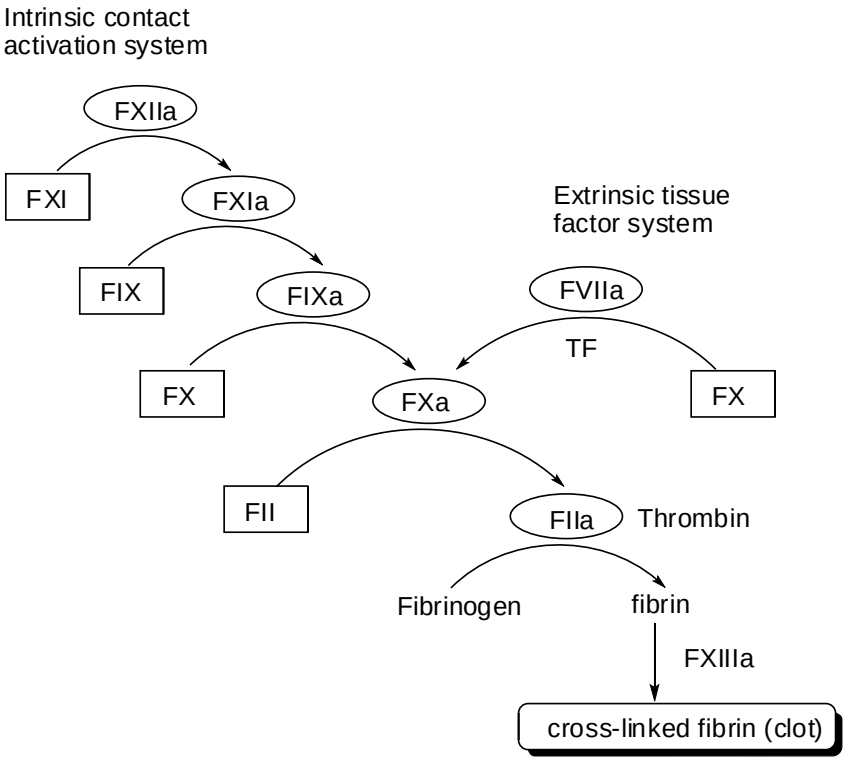


Figure 1.1. Schematic overview of blood coagulation cascade[7]

Recent research shows that these disorders contribute significantly to morbidity and mortality in the United States. Each year, more than 600,000 Americans die from abnormal blood clots. Genetic thrombosis is an inherited abnormality that leads to an increased risk of thrombosis throughout a person's life. These may be protein C deficiency, protein S deficiency, anti-thrombin deficiency and prothrombin mutations. Acquired thrombosis refers to a group of disorders that an individual is not born with, but may develop throughout his or her life due to another circumstance such as illness. An example of acquired thrombophilia is the development of a lupus anticoagulant or antiphospholipid antibody syndrome.

1.4. Factors in the coagulation pathway and their inhibitors

Thrombin (factor IIa) is a key trypsin-like multifunctional serine protease[10] of the blood coagulation cascade and is also the most potent activator of platelet aggregation. The important blood coagulation factors like thrombin, factor Xa and factor VIIa all belong to the trypsin family of serine proteases and they have active sites similar to trypsin. Thrombin cleaves fibrinogen to fibrin which further forms insoluble fibrin polymer clots. Factor Xa is the first member of coagulation factors in the final common coagulation pathway. It acts by cleaving prothrombin (factor II) into thrombin (factor IIa), and requires factor Va as a cofactor.

Thrombosis disorders can be prevented or controlled by the proper use of an anticoagulant. The research for developing effective anticoagulants has intensified during the past few decades, since there are not many drugs available in treating or preventing thrombotic diseases.

The two major drugs against thrombosis are heparin and warfarin which were introduced more than 50 years ago. Heparin or low molecular weight heparins are useful anticoagulants in acute conditions. However, the requirement of intravenous injection limits their application. The widespread use of heparin resulted in the occurrence of Heparin Induced Thrombocytopenia (HIT), a severe immune response caused by heparin-dependent antibodies. Warfarin (coumadin) is used as an oral drug in the chronic setting. However, the delayed onset of action, potential drug-drug interactions and the need for constant monitoring make this drug problematic. Despite these problems, they are still the major drugs for treating thrombosis disorders. The development of a new effective and safe drug to treat thrombosis disorder is very challenging but very important. Several of these anticoagulants compounds are in different stages of clinical trials.

Factor VIIa and tissue factor (TF) complex activates factor IX and factor X upon blood vessel damage, initiating the extrinsic pathway leading to activiated factor Xa. Therefore factor VIIa/TF has been considered as a promising target for anticogulants. Many efforts have been devoted to finding effective factor Xa inhibitors as anticoagulants.[11] Some reports also suggest that factor VIIa/TF inhibitors prevent thrombosis with a lower bleeding risks than other types of inhibitors.[12]

Currently only a few Direct Thrombin Inhibitors (DTIs) are approved for clinical use or are in the advanced stage of clinical trials.[13] These include the small molecule thrombin inhibitors, Argatroban, Ximelagatran, Dabigatran, and several peptide thrombin inhibitors, lepirudin, desirudin and bivalirudin. Lepirudin and desirudin are the recombinant analogs of hirudin (65 amino acids), a naturally occurring polypeptide isolated from medicinal leeches, *Hirudo medicinalis*.[14]

Hirudin, binds to thrombin in a bivalent fashion through interactions at both the active site and the remote "fibrinogen-binding exosite".[15] Bivalirudin is a synthetic polypeptide (20 amino acids) analog of hirudin.[16] Recombinant hirudins and the shorter peptide analog bivalirudin are approved by FDA for the treatment of thrombosis associated with HIT, and they are under investigations for other applications in treating thrombosis disorders.[17] These hirudin analogs are highly efficient direct bivalent thrombin inhibitors, i.e. they bind to both the active site (catalytic site) and the exosite (fibrinogen binding site) of thrombin. Argatroban **1** (Figure 1.2) is a synthetic small molecule thrombin inhibitor and has been recently approved for use as an anticoagulant for treating prophylaxis, HIT and associated thrombotic complications.[18] These

thrombin inhibitors are developed in response to the urgent need for an alternative anticoagulants other than heparin. However, they also need to be administered through IV injection. Exanta **3** (ximelagatran) which is the prodrug of melagatran **2**, the only orally available drug for treating thrombosis disorders, was approved for limited uses in Europe, but the liver toxicity found from recent clinical trial led to the overall withdrawal of this compound from the market.[19]

It has been a challenge to achieve selectivity among these enzymes in searching for new anticoagulants. Recently, it is hypothesized that dual inhibitors of these enzymes may be more effective antithrombotic agents since they inhibit two targets in the blood coagulation cascade.[20] Accordingly, there is a trend in developing dual inhibitors for factor Xa and thrombin, factor Xa and factor VIIa, thrombin and factor VIIa. Of the currently oral anticoagulants, the two agents that are in the most advanced stage are Dabigatran etexilate **4** (BIBR 1048)[21] and Rivaroxaban **5** (BAY59-7939).[22]

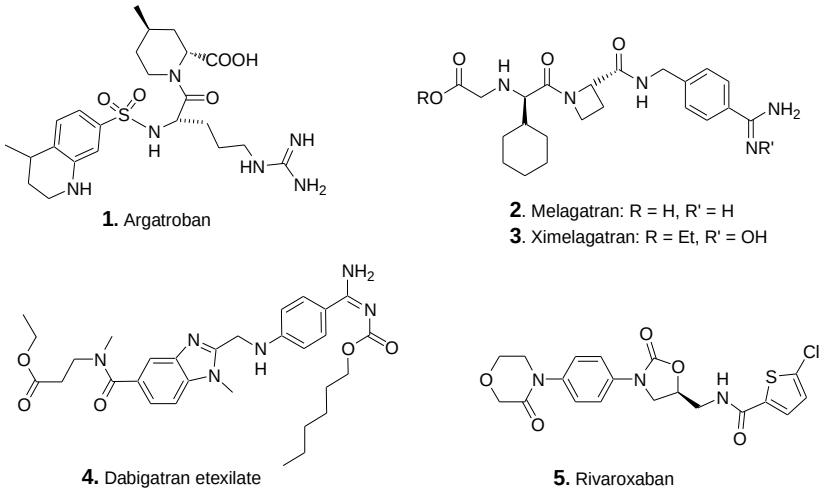


Figure 1.2. Structures of several anticoagulants.

Dabigatran etexilate **4** (BIBR 1048), the prodrug of dabigatran, is an orally available directly reversible nonpeptidic small molecule thrombin inhibitor. It is currently in phase III clinical trial for the treatment and prevention of secondary venous thromboembolism and other conditions.[17] Rivaroxaban **5** (BAY 59-7939) is an oral anticoagulant currently undergoing phase IIb clinical trials for treating DVT and PE and other thromboembolic complications. It is a factor Xa inhibitor, and it may replace warfarin for a number of indications.[23]

1.5. Aeruginosins: A class of naturally occurring serine protease inhibitors

In recent years, a new class of marine natural products, the aeroginosins were isolated from cyanobacterial blooms (**6-13**) which have long been a source of biologically interesting secondary metabolites y the isolation of aeruginosin 298A, a class of serine protease inhibitors (**2**) from cyanobacterium *Microcystis aeruginosa*.[26] 20 more compounds structurally and pharmacologically related to aeruginosin 298A have been isolated and identified so far and many of them are potent thrombin inhibitors.[27] Few of them are shown in Figure 1.3. These mark the introduction of a new class of peptidic serine protease inhibitors to the chemistry community.

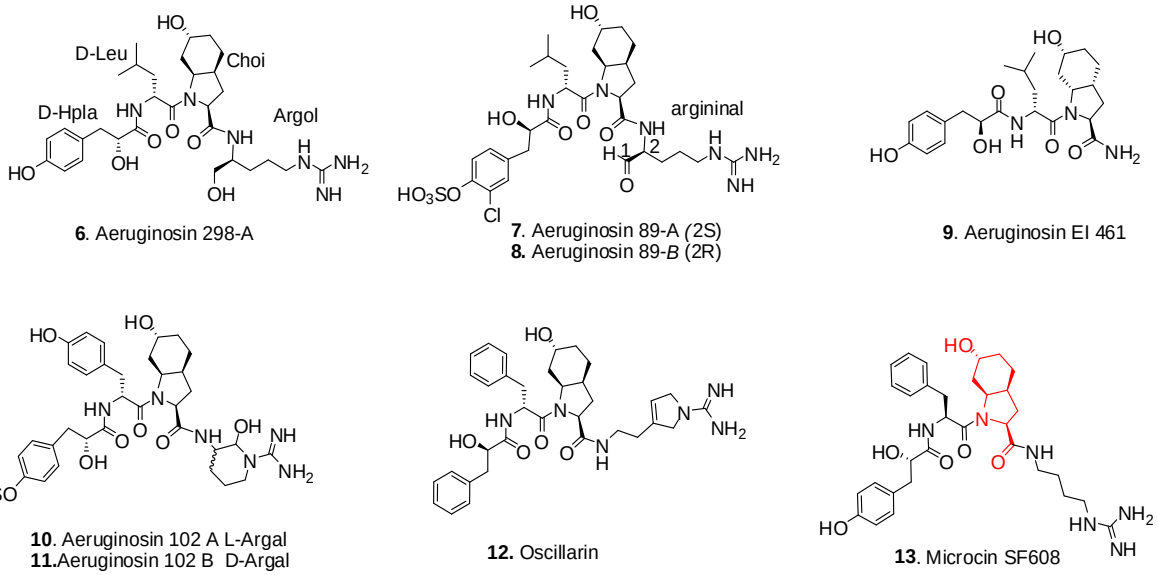


Figure 1.3. Structures of some aeruginosins (**6-13**) and 3 different Choi core structures (**28-30**).

Some of these have been gathered from geographically distinct locations and from sources having an unclear relationship to the Microcystis waterbloom and are small molecules/ small linear peptides containing natural or unnatural amino acids or their derivatives found to be serine protease inhibitors. Approximately 16 compounds in this family share a common new bicyclic amino acid core structure 2-carboxy-6-hydroxyoctahydroindole (Choi). Most of the known aeruginosins have been evaluated for inhibitory activity against thrombin by X-ray crystal structures of thrombin complexed with aeruginosin 298A (1A2C).[28]

1.6. Pharmacophore and Clinical application of direct thrombin inhibitors

The central role of thrombin in the blood coagulation cascade has made it an attractive target for the development of antithrombotic drugs. Several X-ray crystal structures of the enzyme in

complex with different inhibitors have been solved, providing important information about enzyme–inhibitor interactions. Currently, many efforts have been devoted to searching for better anticoagulant therapies. Special attention has been focused on small molecules that can function as direct thrombin inhibitors and exploit their potential of regulating hemostasis and thrombosis.[29-31]

Despite much research devoted to finding thrombosis inhibitors that could treat or prevent blood clotting disorders, an orally active, safe and effective anticoagulant is still elusive. Thrombin consists of two polypeptide chains: A(36 residues) and B (259 residues) linked by disulfide bridges.[10] The compounds belonging to the aeruginosin family inhibit the S1, S2, and the d-S3 subsites (Figure 1.4) of the thrombin active site. The S1 subsite, also known as the specificity pocket, is characterized by an aspartic acid residue (Asp189) in the bottom of the pocket. This Asp189 is able to recognize and engage in ionic interactions with inhibitors containing basic P1 side chains. An alanine residue (Ala190) of the S1 pocket of thrombin distinguishes it from trypsin, which has a serine in this position.[32]

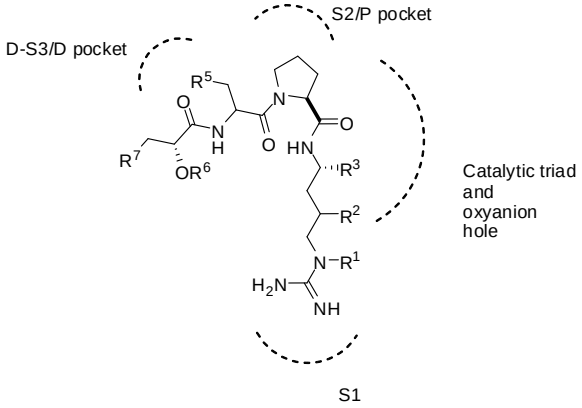


Figure 1.4.(a). Correlation of active site thrombin with ppack

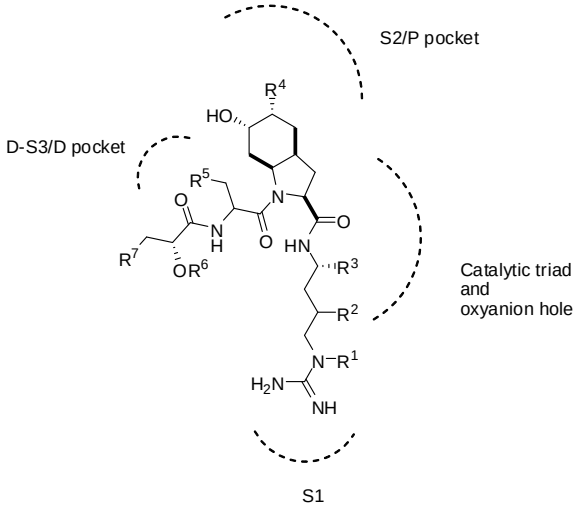


Figure 1.4.(b). Correlation of active site thrombin with aeruginosin

The S1 subsite is also in close proximity to the Ser195-His57-Asp102 catalytic triad and the oxyanion hole i.e. the backbone amide NH groups of Ser195 and Gly193. The S2 subsite, or the proximal P pocket, is a hydrophobic pocket primarily created in the Tyr60A-Pro60B-Pro60C-

Trp60D thrombin insertion loop by Tyr60A and Trp60D.[33,34] The S4 pocket or the D-pocket has been redefined as the distal S3 (d-S3) subsite based on the binding of d-phenylalanine in the X-ray crystal structure of the prototypical synthetic thrombin inhibitor d-Phe-Pro-Arg chloromethyl ketone (PPACK, Figure 1.4).[35]

In general, the design of synthetic thrombin inhibitors has been based on the D-Phe-Pro-Arg sequence of PPACK, originally developed based on the cleavage sites of thrombin's natural substrates such as fibrinogen.[36] The binding mode of aeruginosin 298-A resembles closely to that of D-Phe-Pro-Arg chloromethyl ketone **4a** and other serine protease inhibitors. Initially, the structure of aeruginosin 298A (**6a**) was elucidated through 2D NMR studies and the peptide was found to contain four subunits: hydroxyphenyllactic acid (Hpla), leucine, a Choi core, and a reduced arginine (Argol). Close inspection of NOE correlations from the Choi amide of aeruginosin EI461 revealed an axial hydrogen atom (H-6) at C6 and a cis-fused perhydroindole.

The fact that H-6 showed an NOE correlation to the ring-junction hydrogens (H-3a and H-7a) suggested a Choi configuration unique among the aeruginosins. In their initial report, Murakami and co-workers **[26]** assigned an l-configuration to the leucine amide subunit in aeruginosin 298A based on degradation and derivatization of the acid hydrolyzate. Later, in 1998 Tulinsky and co-workers**[42]** published an X-ray crystallographic structure of a ternary complex of 1 bound to a hirugen–thrombin complex at 2.1 Å resolution providing an absolute stereochemical configuration. The Hpla subunit was proposed to display a d-configuration and the leucine an l configuration. The stereogenic centers in the substituted Choi[26] amino acid were designated as 2S, 3aS, 6R, 7aS, and the absolute stereo-chemistry as l-Choi, while the Argol subunit was found

to also have an l-configuration.

The crystal structure of the ternary complex revealed some unexpected interactions between 1 and the binding pocket of thrombin. l-Leu was found to occupy the hydro-phobic d-enantiomorphic S3 subsite, despite its proposed l-stereochemical configuration. In addition, the Hpla residue was found to interact with the S3 subsite, which was expected to accommodate the l-leucine residue. These key observations cast some doubt on the stereochemical assignments initially made for aeruginosin 298A. The issue would not be resolved until the proposed structure (**6a**) was revised through total synthesis by the groups of Bonjoch[42,43] and Wipf[44] independently in 2000. This sequence is closely mimicked by the general structure of the Aeruginosins (Figure 1.5): a hydrophobic d-amino acid, a constrained bicyclic l-proline (Choi and OHChoi), and a basic arginine-mimetic subunit. The d-S3 subsite formed by the side chains of Leu99, Ile174, and Trp215 is also hydrophobic but larger than S2.

In addition to theaforementioned regions, hydrogen bonding to at least one of the Ser214-Trp215-Gly216 residues opposite the d-S3 subsite has been found to be important for inhibitory activity. Aeruginosin 298-A (**6**) inhibits thrombin (IC_{50} = 0.5 μM) and trypsin (IC_{50} = 1.7 μM), (but does not significantly affect papain, chymotrypsin and plasmin[37] and contains sequence D-Hpla-D-Leu-L-Choi-L-Argol.

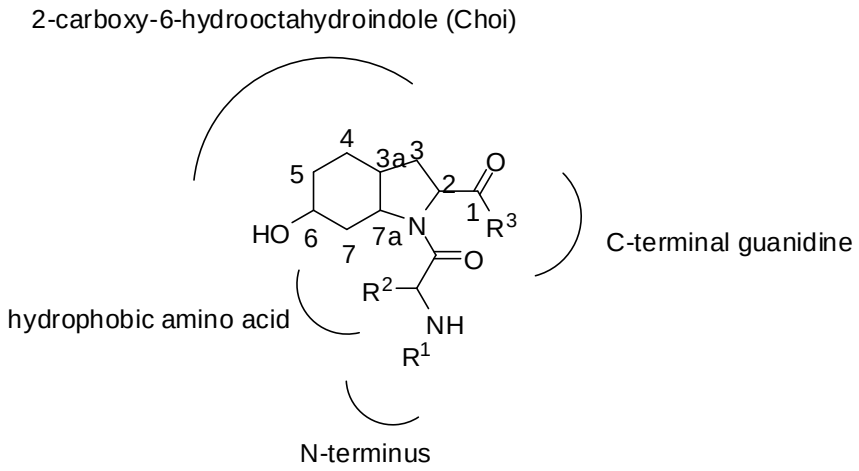


Figure 1.5. Generalized structure of the aeruginosins.

Aeruginosin 298-A binds to the active site of thrombin in a non-covalent fashion forming an antiparallel strand with thrombin. The P1 residue argininol fits into the binding site of thrombin. The guanidine of argininol forms a double hydrogen bond with Asp 189 of thrombin. The D-Leu and D-Hpla bind to the S_3 subsite. The 5-membered ring of the Choi residue occupies the hydrophobic S_2 binding site, while its 6-membered ring projects out and loosely interacts with Try60 and Tyr60 from thrombin. The X-ray crystal structure of thrombin complex with aeruginosin 298-A is shown in Figure 1.6a (PDB code 1A2C).[28]

Various aeruginosins exhibit varying degrees of inhibitory activity against serine proteases, and their activity profile can be explained by a high degree of pharmacophoric and structural homology within the family. Nearly all of the aeruginosins are composed of four subunits: an N-terminal hydroxy or acidic group, a bulky hydrophobic amino acid, a 2-carboxyperhydroindole core, and a C-terminal guanidine-containing group (Figure 1.6). This array of structural and

functional features is responsible for their affinity to the catalytic binding pocket of trypsin, thrombin, and other serine proteases involved in the blood coagulation cascade.

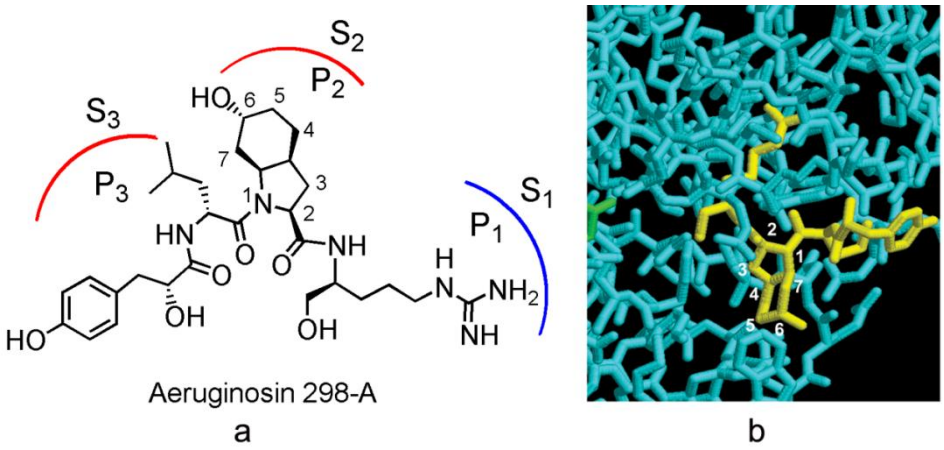


Figure 1.6. (a) The structure of aeruginosin 298-A **(b)** The conformation of Aeruginosin-298A in complex with thrombin.[28]

1.7. Role of Aeruginosins as DTI

As a result of their structural novelty and biological activity, the aeruginosins have also garnered considerable attention from synthetic organic chemists. Derivatives of the 2-carboxyperhydroindole core structure have received the most attention with respect to conceptually diverse synthetic approaches. To date, the total syntheses of seven aeruginosins have been completed; four of which involve revisions to the originally proposed structures. Initially the aeruginosins were classified as natural products produced by the cyanobacterium Microcystis aeruginosa incorporated with Choi, however in the years following the isolation of the thrombin and trypsin inhibitor aeruginosin 298A (**6a**),[26] linear peptides containing the core

bicyclic subunit having similar biological activities and structural features were isolated from the fresh-water cyanobacteria of the family Oscillatoria and from marine sponges of the family Dysideidae containing the distinctive cis-fused 2-carboxy-perhydroindole core structure, and many of the exhibited in vitro inhibitory activity against serine proteases.[38-41]

From the results of the enzyme assays listed in Table 1.1 it is evident that depending on the target enzyme different structural features are important. A basic P1 end group appears to be a prerequisite for achieving high potency against the trypsin-like serine proteases. One explanation for the lack in activity against chymotrypsin and elastase might be the substrate preferences of these enzymes, which favor peptides with bulky hydrophobic or small neutral P1 side chains, respectively. Consequently, a replacement of the basic P1 subunit in the aeruginosins by a hydrophobic group might result in a shift in inhibitory activity towards other enzymes. The main role of the octahydroindole core subunit seems to be to direct different parts of the tetrapeptide into specific regions in space conferring the bioactive conformation. Fine-tuning of the octahydroindole substituents has been further suggested to attain selectivity between different enzymes.[45,46]

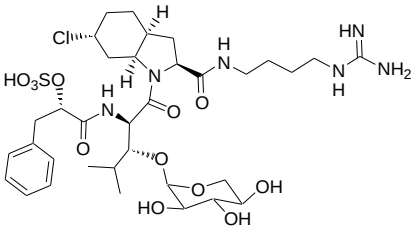
Small changes in the nature of the P3 substituent on the amino acid residue appear to have a strong influence on the affinity for the coagulation factors. A striking example is the remarkable effect of the chlorine atom in chlorodysinosin A compared to the hydrogen-substituted dysinosin A (Table 1). As mentioned above, it was reported that the presence of a sugar residue on the octahydroindole core [46] as in dysinosin B had an influence on the selectivity between FVIIa and thrombin. This sugar moiety also resulted in a slight increase in affinity for FVIIa. Moreover,

they found that an N-terminal P4 sulfate group seems to contribute to both thrombin and FVIIa binding (cf. dysinosin D with dysinosins A–C, Table 1.1). In accordance with this observation, oscillarin, which lacks a P4 sulfate, exhibits a weak activity against FVIIa (IC_{50} = 3.9 mm). Since oscillarin still shows a high activity against thrombin, a P4 sulfate group might be more important for inhibition of FVIIa. Chlorodysinosin A exhibits the highest inhibitory activity against FVIIa among all the aeruginosins (IC_{50} = 0.039 mm, Table 1.1).[47]

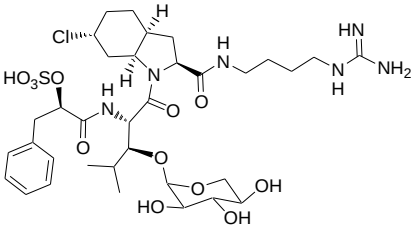
The large difference in activity between chlorodysinosin A and dysinosin A indicates a positive effect of the P3 chlorine atom upon binding to FVIIa, similar to that observed upon the binding to thrombin. The precise importance of the N-terminal sulfate group for different biological activities needs to be further elucidated. Beside the search for better activities and selectivities against target serine proteases[48], other structural and pharmacological aspects should be addressed to render this intriguing class of natural products more "druggable".[49] The prospects of achieving such a daunting task are nevertheless promising, especially since the natural products and the totally synthetic thrombin inhibitors share the same binding sites.[50,29a,30,34,51,52,53] As such, they should be amenable to a segment-coupling protocol in a structure-based organic synthesis paradigm[27a] to produce potent hybrid analogues with favorable pharmacological properties. Attention has been directed towards other coagulation cascade factors besides thrombin (FIIa). Although no X-ray crystal structure of the aeruginosins in complex with factor VIIa is available, the high homology of the active site with that found in thrombin suggests a similar binding mode.[54,55]

For aeruginosin 205 structure assignment, there are some dispute of the originally proposed

structures.[37] Bonjoch et al argued that the structures should be revised to place the chlorine as a substituent on the D-Leucine residue not on the octahydroindole core structure.[56-57] These are shown in Figure 1.7. This revision is supported by some later work by Hanessian based on observed biological activities of synthetic analogs containing D-chloro leucine unit.[58]

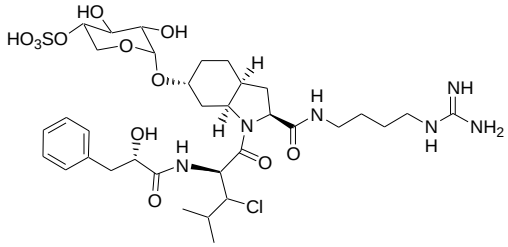


14 Aeruginosin 205A

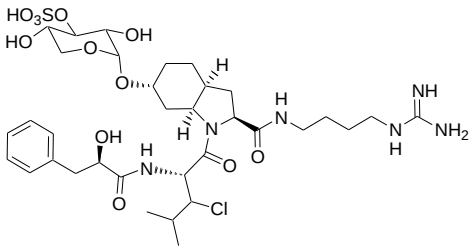


15 Aeruginosin 205B

These are proposed structures from JOC 1997, 1810



16 Propose revision of Aeruginosin 205A



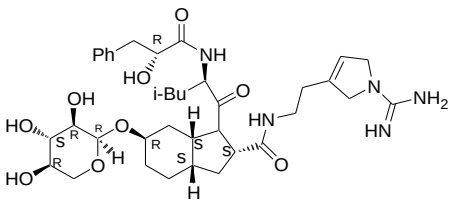
17 Propose revision of Aeruginosin 205B

Figure 1.7. The proposed and revised tentative structures of aeruginosin 205.

However, the correct structure assignments of aeruginosin 205 still remain to be further confirmed by total synthesis. Two other glycosylated compounds, termed aeruginoside 126A and 126B with a sugar moiety attached to the octahydroindole core have been identified recently.[59]

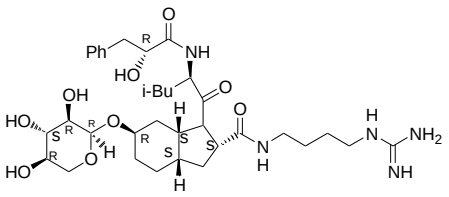
Other aeruginosins contain a different amino acid residue at the Argol position, aeruginosin 89 contains a *N*-4-amino-5-oxo-pentyl-guanidine (argininal or Argal) at the P1 position. The

aldehyde functional group of the Argal is important for the biological activity for aeruginosin 89 (**7, 8**).[19]



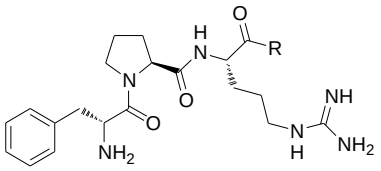
18

Aeruginoside 126A



19

Aeruginoside 126B



1. R = H

2. R = CH_2Cl

20

The Argal group exists as an equilibrium of the open chain form and a 6-membered ring cyclic hemiaminal as shown in the structures of aeruginosin 102 (**9-10**)**.** Aeruginosin 89-B **8** is very selective towards thrombin (IC_{50} = 0.05 μg/mL) but it's IC_{50} to trypsin is 6.6 μg/mL and it also doesn't inhibit papain, chymotrypsin and elastase.

The stereoisomer 89-A **7** is a very potent thrombin inhibitor with 0.03 μg/mL activity, however it's not very selective towards thrombin. It also inhibits trypsin at 0.4 μg/mL and plasmin at 0.02 μg/mL. Aeruginosin 102-A and B (**9, 10**) have the sequence of D-Hpla sulfate, D-Tyr, Choi, and Argal. Aeruginosin 102-A inhibits thrombin and trypsin at 0.04 μg/mL and 0.2 μg/mL respectively whereas aeruginosin102-B inhibits thrombin and trypsin at 0.1 μg/mL and 1.1 μg/mL respectively. The aeruginosins without an arginine like basic unit, such as 298B and

EI461 donot inhibit either thrombin or trypsin. Recently several new peptides banyasin A, banyaside A and banyaside B were isolated and their structures have been reported.[60]

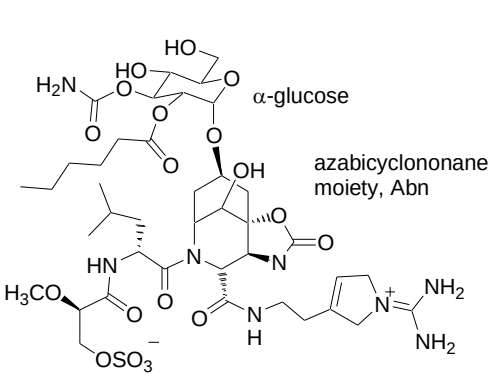
Although the unique new structure in banyaside represent perhaps a new class of serine protease inhibitors, their structure similarity with dysininosin is evident here. Banyaside A inhibit trypsin at 1.48ug/mL, and thrombin at 0.39ug/mL, IC_{50}. However, it seems a little challenging to synthesize the unusual azabicyclonanonane moiety (Abn).

1.8. Inhibition of Trypsin

Trypsin is a digestive enzyme frequently employed as a marker for inhibition of trypsin-like serine proteases. Inhibition of trypsin might also by itself be of interest for the treatment of pancreatic disorders, such as pancreatitis.[61] In general, the binding of aeruginosin 98B to trypsin is similar to that shown for the aeruginosins in complex with thrombin and closely resembles that of a d-Phe-Pro-Arg tripeptide.[62] The most striking feature of the structure of aeruginosin 98B cocrystallized in complex with trypsin[45] was the lack of interactions between aeruginosin 98B and the trypsin catalytic triad (Ser195-His57-Asp102), indicating a new possible mode of serine protease inhibition. The basic P1 agamatine side chain is buried in the S1 specificity pocket, forming two strong hydrogen bonds to the side-chain carboxylate of Asp189. The Choi moiety is situated in the S2 subsite with the 6-O-sulfate group reaching out into solution. It was speculated that the Choi sulfate group might be the primary determinant for selectivity for trypsin over thrombin.[74] The P3 d-alloIle side chain is located in the Trp215 aryl-binding site and makes van der Waals interactions with Trp215 and Leu99. The phenol hydroxy group utilizes a water

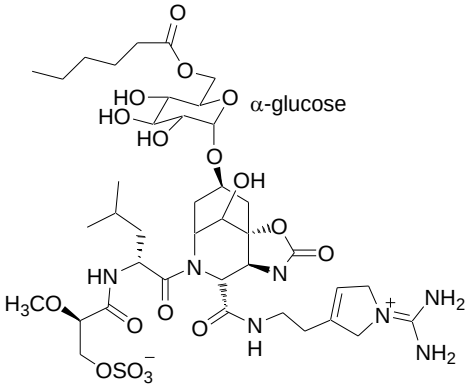
molecule in the P4 position, also observed in crystal structures of uncomplexed trypsin[63] as a bridge to interact with Cys220 and Ser146. Most of the aeruginosins have been assessed for trypsin inhibitory activity (Table 1.1). Not surprisingly, the structure-activity relationships (SARs) for trypsin are similar to those for the trypsin-like serine protease thrombin. To date, the most potent natural aeruginosins are chlorodysinosin A and oscillarin, both demonstrating IC_{50} values against trypsin of 0.037 mm (0.025 and 0.024 $mgmL^{-1}$, respectively, Table 1.1).

In accordance with the substrate preference of trypsin, a basic P1 end group appears to be critical for inhibition. Aeruginosin 298B, which lacks a basic P1 group, is inactive against trypsin (IC_{50} > 100 $mgmL^{-1}$, Table 1.1).[64] Moreover, the bulky P1 ethyl hemiaminal of aeruginosin103A to be responsible for its low trypsin inhibitory potency (IC50=51.0 $mgmL^{-1}$, Table 1.1).[65] Fukuta and co-workers[66] suggested that the difference in trypsin potency between aeruginosin 298A and 102A might be due to the P4 sulfate group found in the more potent 102A (IC_{50} = 1.0 and 0.2 $mgmL^{-1}$, respectively, Table 1.1).



Banyaside A

26



Banyaside B

27

The summary of the inhibition data against several enzymes is shown in Table 1.1.

Table 1.1. Summary of aeruginosins and their inhibition activities against thrombin and other enzymes. IC_{50} in μg/mL unless otherwise noted.

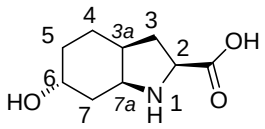
IC_{50} (μg/mL)	**Trypsin**	**Thrombin**	**Factor VIIa**
Aeruginosin 98-A	0.6	7.0	
Aeruginosin 98-B	0.6	10.0	
Aeruginosin 98-C	3.9	3.3	
Aeruginosin 101	3.0	3.2	
Aeruginosin 298-A	1.0	0.3	
Aeruginosin 298-B	>100	>100	
Aeruginosin 89-A	0.4	0.03	
Aeruginosin 89-B	6.6	0.05	
Aeruginosin 102-A	0.2	0.04	
Aeruginosin 102-B	1.0	0.1	
Aeruginosin 205-A	0.07	1.5	
Aeruginosin 205-B	0.07	0.17	
Microcon SF 608	0.5 ug/mL		
Oscillarin		28nM	3.9uM
Dysinosin A		0.452μM K_i	0.108μM K_i
		46 nM	326 nM
Dysinosin B		0.170	0.090μM K_i
Dysinosin C		0.550	0.124μM Ki
Dysinosin D		>5.1μM K_i	1.320μM K_i
Chlorodysinosin	37nM	5.7 nM	39 nM

Although aeruginosins and related compounds exhibit promising biological activity, they haven't been used as lead compounds and developed into useful therapeutic agents. One obstacle for aeruginosins to be used as thrombin inhibitors is their lack of selectivity among the trypsin like enzymes. The selectivity for thrombin over trypsin is not very high for most of the members. Another obstacle is the complexity of their structures. There are also confusions about the absolute stereochemistry assignment in the literature. The various natural products don't seem to have a clear trend of structure activity correlations.

1.9. Syntheses of Aeruginosins 298A and 298B (The P2 Choi core structures and the preparations.)

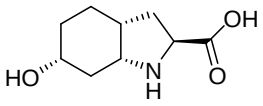
Several core structures for the P2 group from the structures of the natural products aeruginosins, are shown in Figure 1.8, the most common core being structure **23**. The Choi in EI461 **24** has the different stereochemistry and dysinosins contain the dihydroxylated octahydroindole **25**. The Choi core structure is crucial for their anti-thrombotic activity. Several total syntheses towards the natural products have been reported because of the interesting structure and biologically activities of these compounds and several methods have been developed for the synthesis of Choi or its analogs.[27, 42-44, 47,68-78]

The majority of strategies involve the use of amino acids as starting materials. The absolute configuration of Aeroginosins obtained from the crystal structure served as the basis for two total syntheses reported. An efficient syntheses of the (2S,3aS,6R,7aS)-6-hydroxyoctahydroindole-2-carboxylic acid (l-Choi) motif starting from l-Tyr(OMe)-OH (**26**) was found in many of the aeruginosins[**76**] and were featured in the first total synthesis of aeruginosins 298A[42] and 298B.[43]



2-carboxy-6-hydroxyotahydroinhole Choi

23



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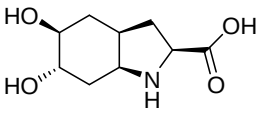

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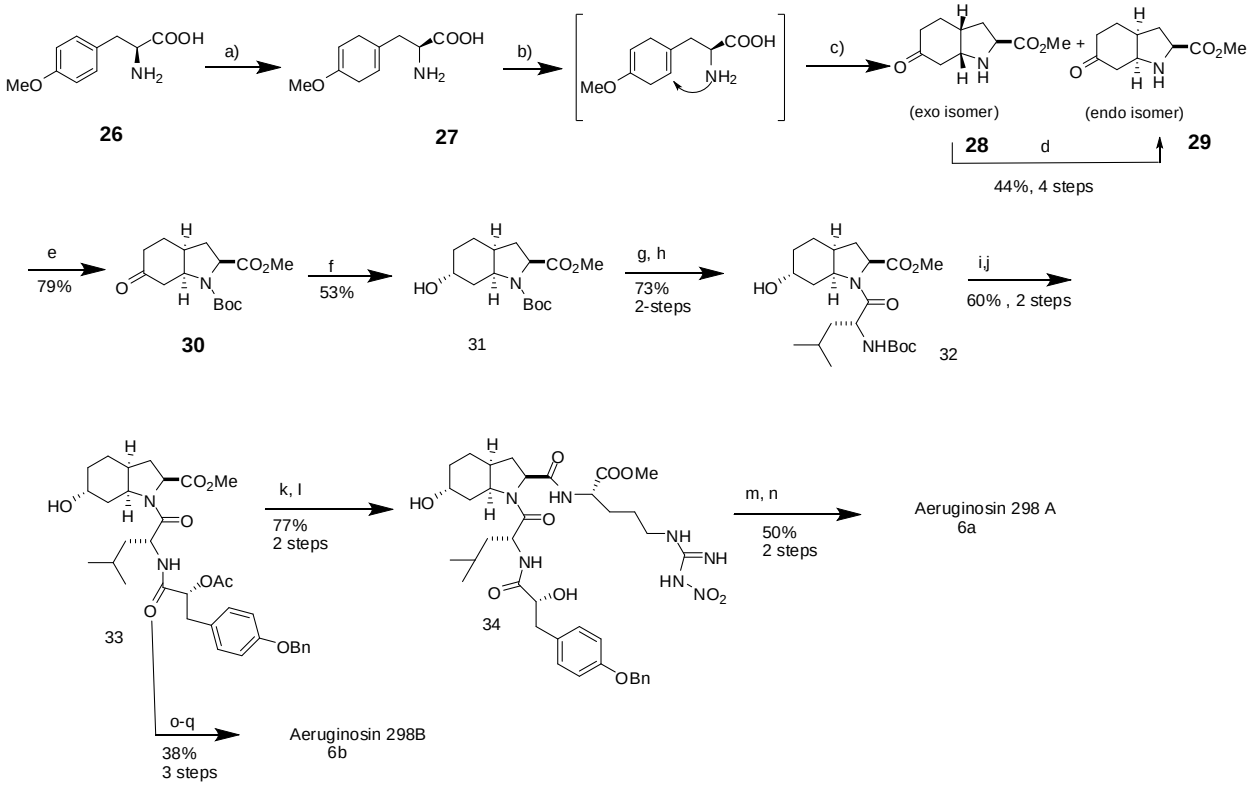
Figure1.8. The structures of the Choi P2 units present in aeruginosins

The azabicyclic core subunit was obtained in two steps by Birch reduction of O-methyl-L-tyrosine **26** followed by acidic cleavage of the enol ether and Michael-type addition of the pendant amine nucleophile (Scheme 1.1) by Bojoch *et al*.[75]

The resulting secondary amine was treated directly with BnBr to afford a mixture of isomers **28** and **29**. Thermodynamic equilibration of this mixture in the presence of MeOH/aq. 8 N HCl provided the more stable isomer **29** in 44% overall yield from **26**. For the purpose of completing the synthesis of aeruginosins 298A and 298B, intermediate **29** was converted into **31**. A variety of ketone-reduction conditions were examined in an effort to secure the desired 6R stereochemistry of the alcohol. Optimal selectivity in favor of the 6R isomer (8:1) was achieved by reduction with LS-Selectride.

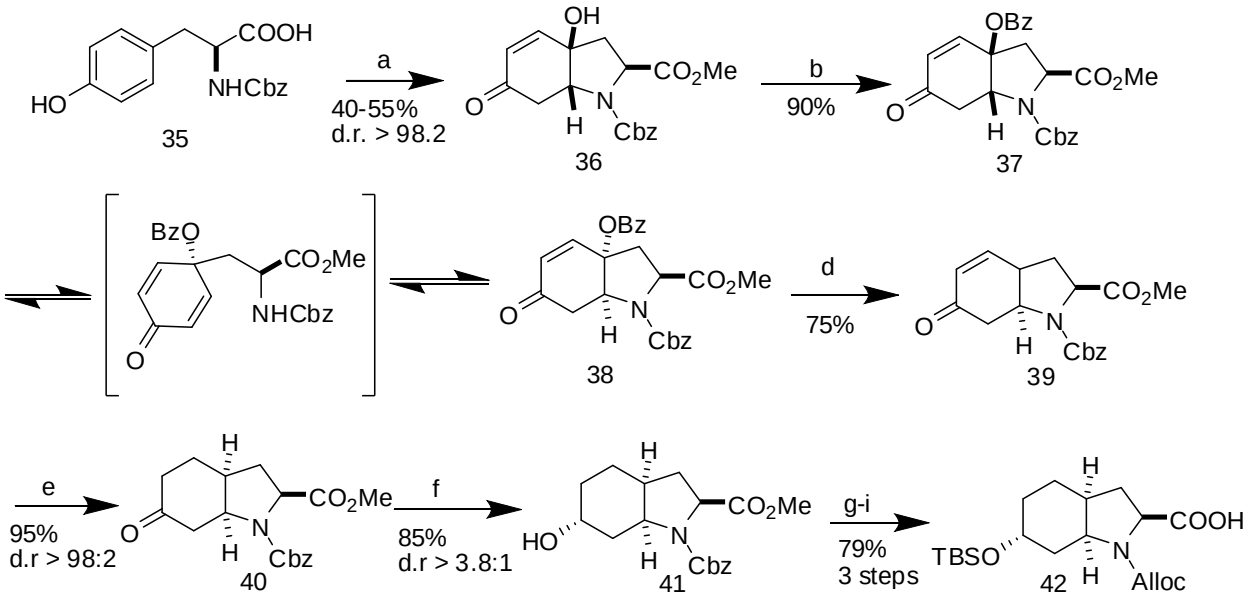


Scheme 1.1. Synthesis of aeruginosin 298A by Bonjoch *et al*. **(a)** Li, NH_3, THF/tBuOH, -78 °C; **(b)** MeOH, 3N HCl, 35 °C; **(c)** BnBr, $NaHCO_3$, EtOH, 70 °C; **(d)** MeOH, 8N HCl, 65 °C; **(e)** H_2, $Pd(OH)_2$, Boc_2O, EtOAc; **(f)** LS-Selectride, THF, -78°C; **(g)** TFA, CH_2Cl_2, 0 °C; **(h)** Boc-d-Leu, BOP, NMM, CH_2Cl_2 ; **(i)** TFA, CH_2Cl_2, 0 °C; **(j)** (O-Bn,O-Ac)-d-Hpla, BOP, NMM, CH_2Cl_2 ; **(k)** 0.1N LiOH/THF; **(l)** l-Arg(NO_2)-OMe, BOP, NMM, DMF; **(m)** $LiBH_4$, THF; **(n)** H_2, Pd/C, EtOAc/ MeOH, 6N HCl, 1 atm; **(o)** 0.1N LiOH/THF; **(p)** NH_4OH, PyBOP; **(q)** H_2, Pd/C.

With the protected l-Choi derivative **31** in hand, aeruginosin 298A was assembled using standard peptide-coupling techniques. Thus, Boc removal was followed by BOP-mediated coupling with Boc-d-Leu-OH to give dipeptide **32** in 73% yield.

A tyrosine-oxidation rearrangement route toward the key Choi bicyclic hydroindole, which is present in the aeruginosins as well as other alkaloidal natural products (Scheme 1.2) was developed by Wipf *et al*.[44] Treatment of *N*-Cbz-l-Tyr-OH (**35**) with $PhI(OAc)_2$ followed by

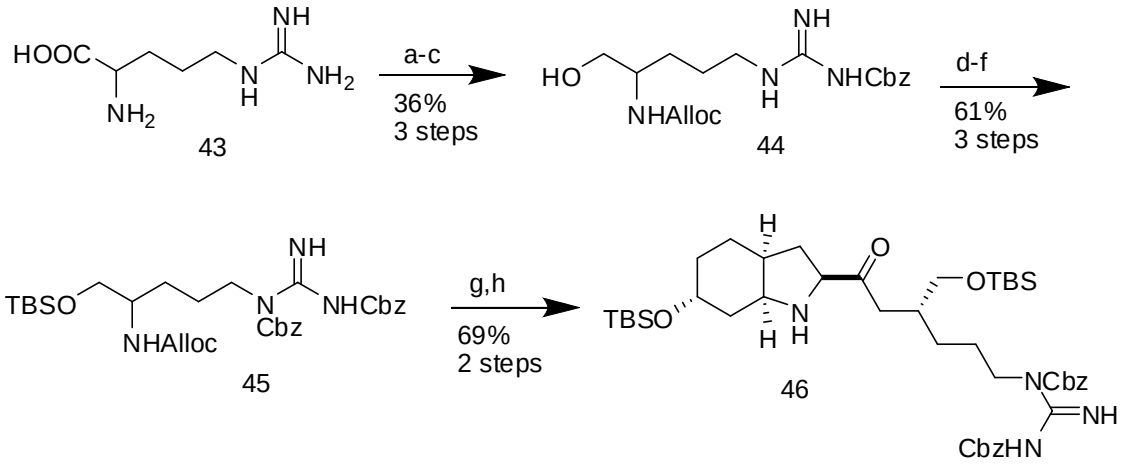
exposure to methanol/ $NaHCO_3$ afforded cyclic product **36** with a diastereoselectivity of d.r. > 98:2. Access to the desired stereochemistry at the ring-junction carbons was possible by the thermodynamic equilibration of benzoylated derivative **37** to give the more stable isomer **38**; this proceeded by ring-opening to the cyclohexadienone followed by Michael-type closure.



Scheme 1.2. Synthesis of l- Choi derivative **42** for synthesis of aeruginosin 298A by Wipf *et al.* a) $PhI(OAc)_2$, $NaHCO_3$, MeOH; b) Bz_2O, DMAP,pyridine, CH_2Cl_2 ; c) $NaHCO_3$, DMSO, 90 °C; d) Zn dust, AcOH/THF, 65°C; e) H_2, 5% PtO_2, 10% AcOH/EtOH, 0°C; f) L-Selectride, THF, -78 °C; g) TBSOTf, ImH, CH_2Cl_2; h) H_2, Pd/C, EtOH, then AllocCl, pyridine; i) LiOH, THF/H_2O, 40 °C.

After recycling of recovered **37**, the resulting 1.6:1 mixture of products was then separated to give **38** in 78% yield. Using activated Zn dust or SmI_2, reductive displacement of the benzoate ester gave the β,γ-unsaturated ketone **39**, which was hydrogenated to give cis-fused azabicyclic ketone **40**. Reduction of the ketone function with L-Selectride afforded a 3.8:1 mixture of products having exo and endo hydroxy groups. Choi derivative **42** was afforded by protection of

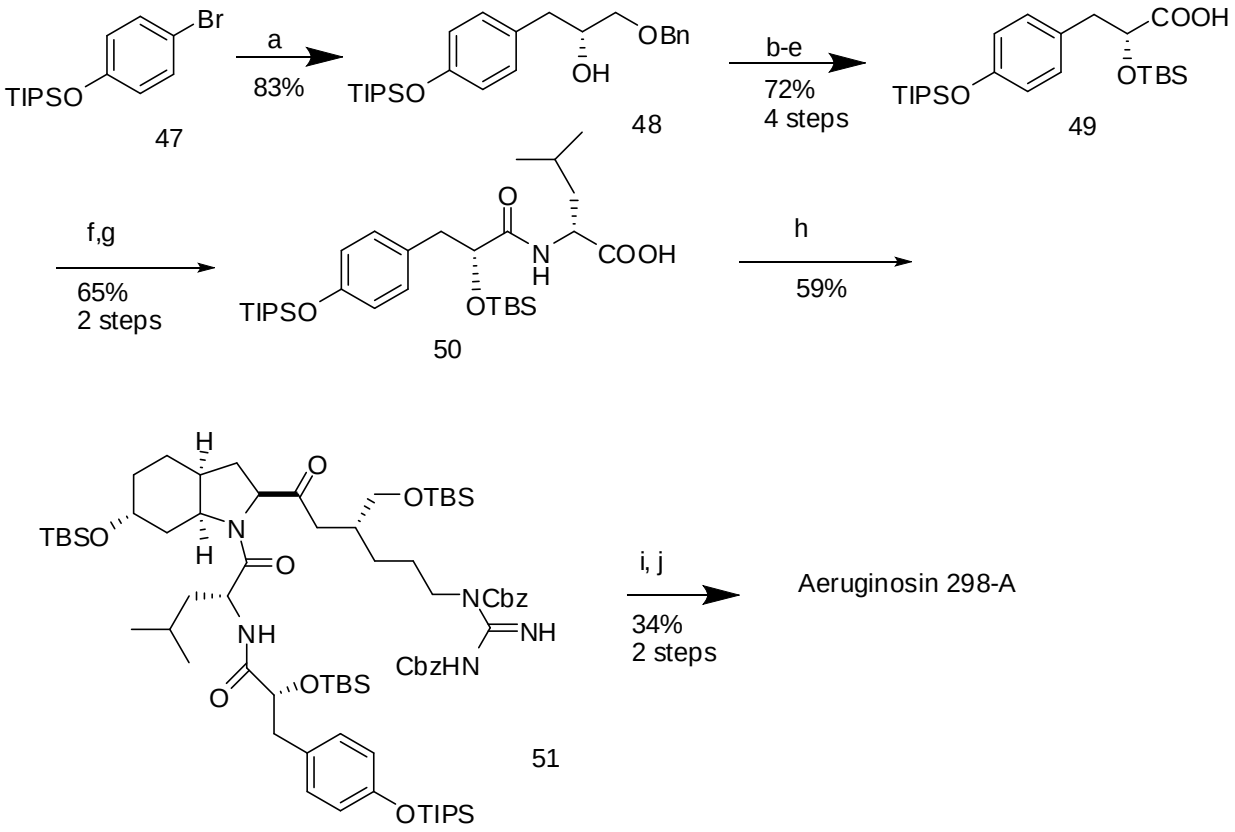
the major product **41** as TBS ether, followed by exchange of the Cbz protecting group for Alloc, and cleavage of the methyl ester.



Scheme 1.3. Synthesis of aeruginosin 298A by Wipf *et al.* synthesis of amide **46**. a) AllocCl, aq. NaOH; b) CbzCl, aq. NaOH, THF; c) IBCF, NMM, DMF, -20 °C, then $NaBH_4$; d) TBSOTf, ImH, CH_2Cl_2 ; e) CbzCl, DMAP, K_2CO_3, DMF; f) Bu3SnH, [$Pd(Ph_3)_4$], AcOH, THF; g) **42**, FDPP, DIEA, CH_2Cl_2; h) Bu_3SnH, [$Pd(PPh_3)_4$], AcOH, THF.

Following this, the argol fragment was prepared from l-Arg-OH **43** by sequential protection of the α-amine and guanidine groups and then reduction to the primary alcohol **44** (Scheme 1.3). Protecting-group manipulation gave **45** in 22% overall yield from arginine. The Choi-Argol fragment was obtained by pentafluorophenyl ester-mediated coupling of amine **45** to acid **42** in 69% yield. Removal of the Alloc group gave subunit **46**.

In Scheme 1.4, the synthesis of the appropriately protected Hpla-Leu fragment and final assembly of aeruginosin 298A is depicted.

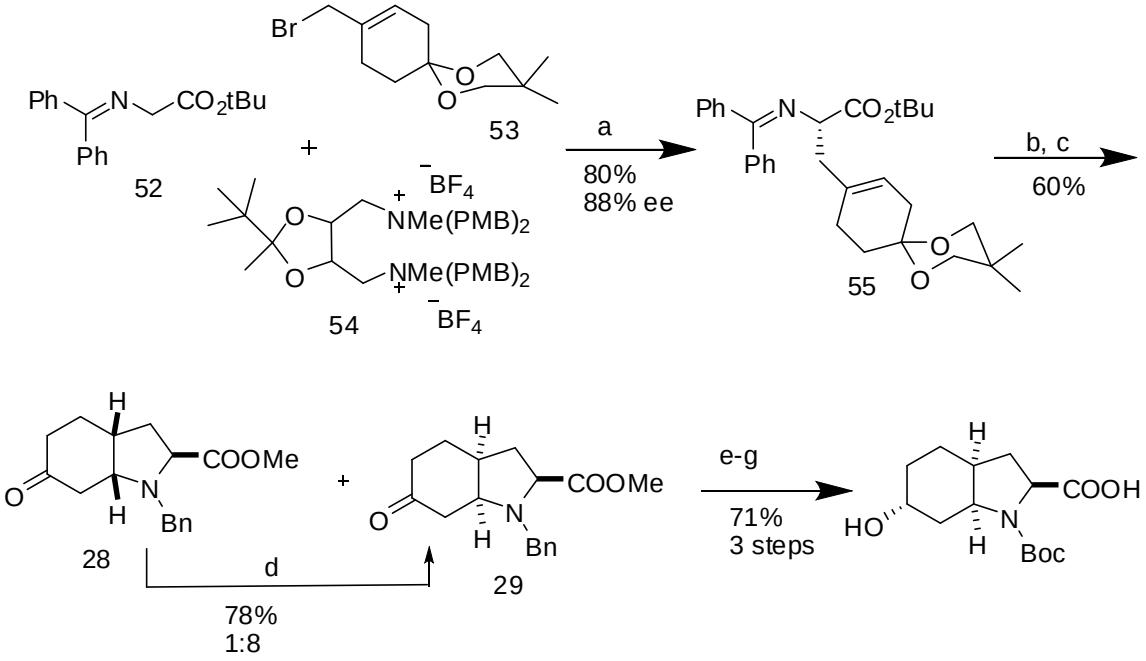


Scheme 1.4. Synthesis of aeruginosin 298A by Wipf *et al*. completion of the synthesis. a) ^{t}BuLi, CuBr·SMe_2, THF, -78 °C to -45 °C then (R)-benzylglycidol, BF_3·Et_2O, -45 °C to -20 °C; b) TBSOTf, ImH, CH_2Cl_2 ; c) H_2, Pd/C, EtOAc; d) DMP, CH_2Cl_2 ; e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, ^{t}BuOH/H_2O; f) l-Leu-OBn, DEPC, DIEA, CH_2Cl_2 ; g) H_2, Pd/C, EtOH; h) **46**, DEPBT, DIEA, CH_2Cl_2 ; i) aq. HF; j) H_2, Pd/C, EtOH.

The Hpla subunit was prepared by reaction of (R)-benzylglycidol with an aryl cuprate. Protection of the alcohol as the TBS ether was followed by hydrogenolysis of the benzyl ether and oxidation of the resulting alcohol to carboxylic acid **49**. Coupling to d-Leu-OBn, and ester cleavage by hydrogenolysis gave **50**. Then, fragments **50** and **46** were joined in the presence of DEPBT[44,77-78] to give intermediate **51** in 59% yield. Removal of the silyl and Cbz protecting groups afforded aeruginosin 298A **6a**.

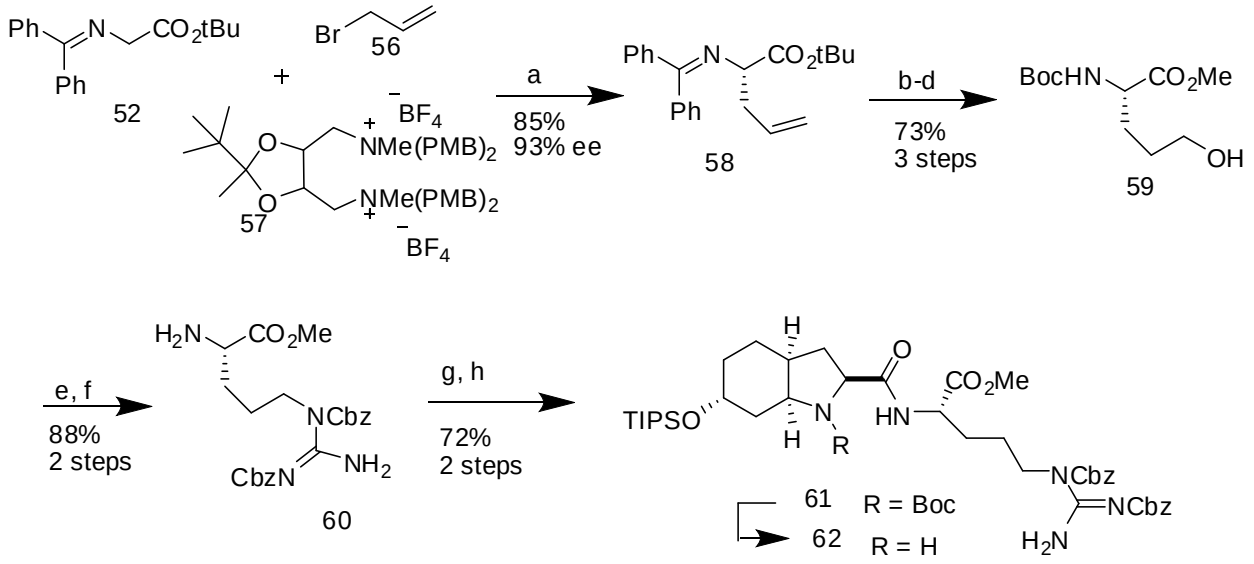
Application of catalytic asymmetric phase-transfer alkylation reactions was recently devised for

the synthesis of the l-Choi subunit by (Scheme 1.5)[66,72a] by Shibasaki *et al*.



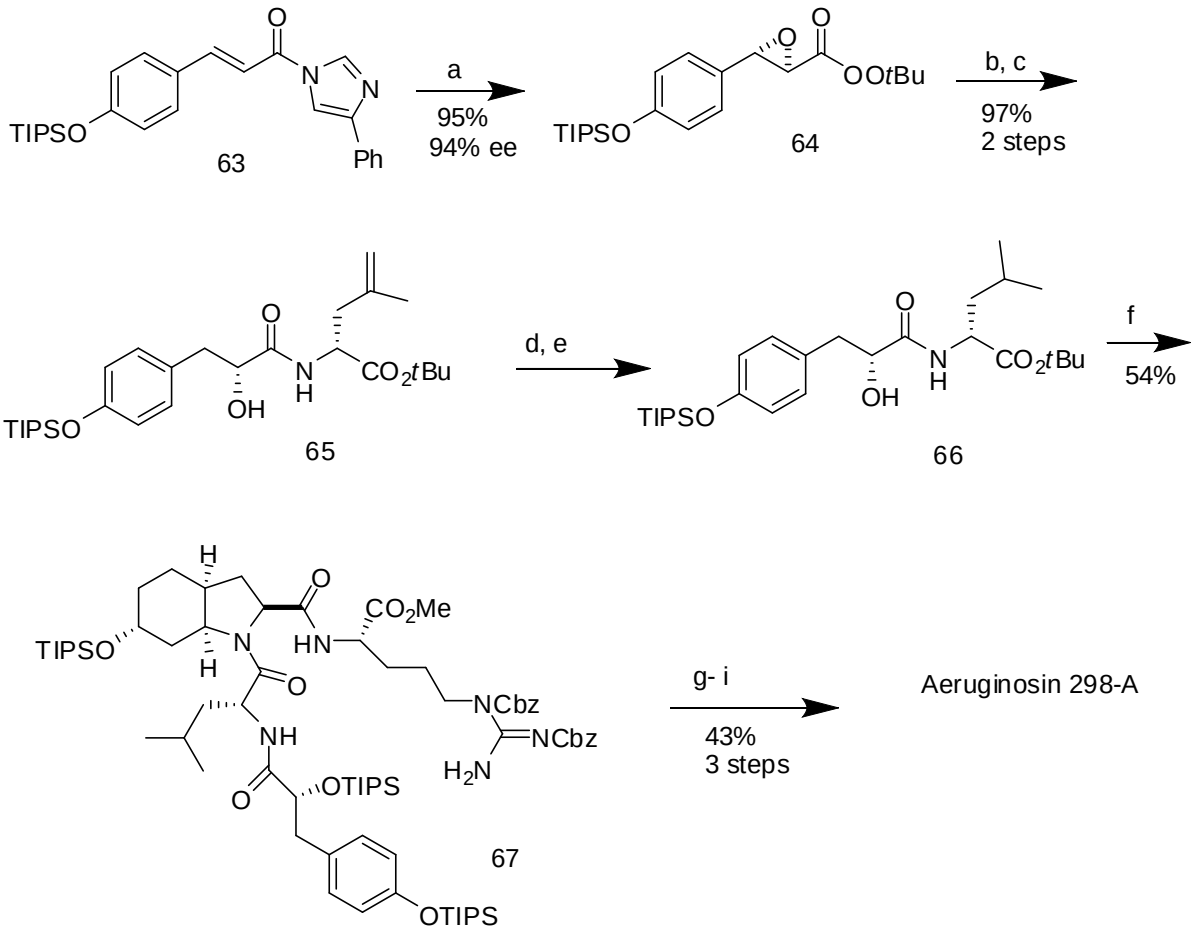
Scheme 1.5. Synthesis of l-Choi derivative **56** for synthesis of aeruginosin 298A by Shibasaki *et al*. a) $CsOH \cdot H_2O$, toluene/CH_2Cl_2 (7:3), -70 °C; b) 4N HCl, MeOH; c) BnBr, $NaHCO_3$, EtOH; d) conc. HCl; e) H_2, Pd/ C, Boc_2O; f) LS-Selectride, THF, -78 °C; g) aq. LiOH/MeOH.

For the synthesis of the Choi core of aeruginosin 298A, the enolate of **52** was alkylated with bromide **53** in the presence of 10 mol% **54** as a catalyst to give **55** in 80% yield and 88% ee. A point to consider is the fact that the enolate of glycine derivative **52** was previously shown to react with a variety of electrophiles to give α-amino acids of high enantiomeric purity.[81] Ketal deprotection and subsequent intramolecular Michael-type addition then afforded bicyclic ketones **28** and **29**, which were converted to **56** following the method previously described by Bonjoch and co-workers.[43]



Scheme 1.6. Synthesis of fragment **62** for synthesis of aeruginosin 298A by Shibasaki *et al.* a) $CsOH \cdot H_2O$, toluene/CH_2Cl_2 (7:3); b) HCl; c) Boc_2O, TEA; d) 9-BBN, then H_2O_2 ; e) DEAD, PPh_3, di-Cbz-guanidine; f) p-TsOH; g) **56**, EDCI, HOBt; h) TIPSOTf, DIEA; i) $ZnBr_2$.

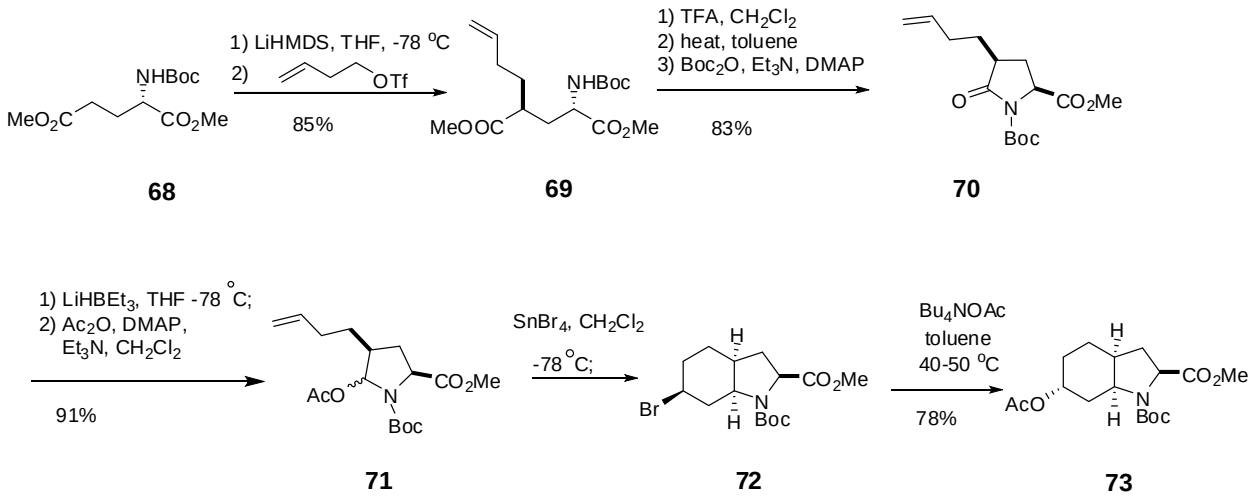
In Scheme 1.6, a synthesis of the l-Argol subunit utilizing asymmetric phase-transfer alkylation methodology is shown. Thus the allylglycine precursor **58** was obtained in excellent yield and enantioselectivity. Functional-group manipulation led to alcohol **59**, which was further transformed into guanidine intermediate **60**. Peptide coupling with **56** gave protected l-Choi-Argal fragment **61** in 72% yield. Boc removal then afforded compound **62**.



Scheme 1.7. Completion of the synthesis of aeruginosin 298A by Shibasaki *et al.* a) La-(S)-BINOL-PPh_3=O (10 mol%), TBHP, MS 4Å, THF; b) d-4,5-dehydroleucine-O^{t}Bu, THF; c) H_2, Pd/C; d) TIPSOTf, DIEA; e) TMSOTf; f) 62, HATU, DIEA, CH_2Cl_2 ; g) $LiBH_4$, THF; h) HF·py; i) H_2, Pd/C.

The Hpla subunit was prepared by asymmetric epoxidation of imidazolide **63** (Scheme 1.7).[79] Acid hydrolysis of **60** in methanol promoted deprotection of the imine and acetal, transesterification. Migration of the C-C double bond to the corresponding enone, which underwent Michael addition and benzylation to the bicyclic ketones **62, 63** in 2:1 ratio, led to the desired product **63.** The undesired isomer can be isomerized to **63** by treating it with HCl in

methanol. Exchange of protecting group on nitrogen and stereoselective reduction lead to the protected Choi **64**.



68 **69** **70**

71 **72** **73**

Scheme 1.8. Synthesis of Choi starting from L-glutamic acid

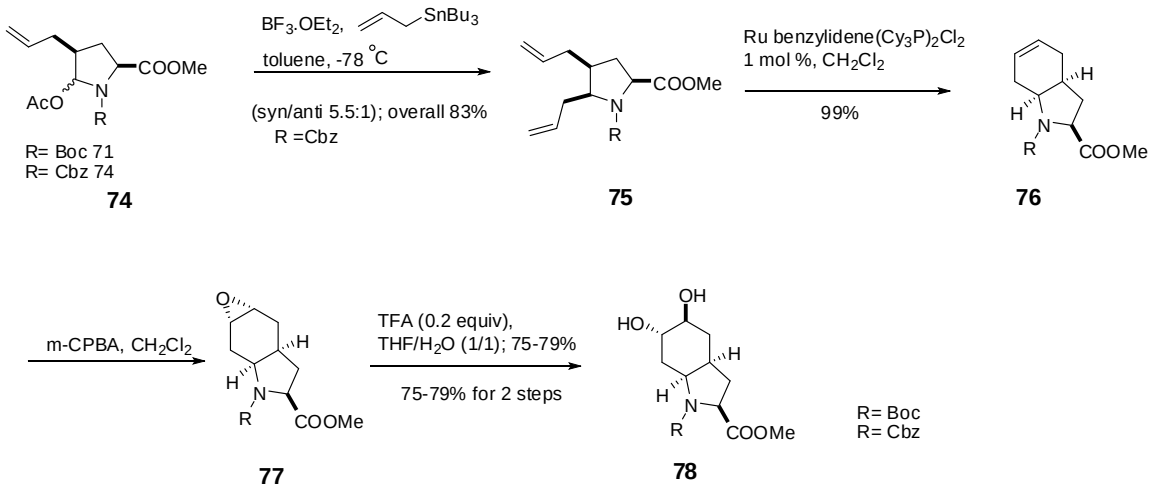
The resulting peroxyester **64** could then be coupled by simple mixing with d-4,5-dehydroleucine-OtBu to give amide **65**. Reduction of the double bond by catalytic hydrogenation, protection of the alcohol, and cleavage of the t-butyl ester then gave the Hpla-Leu fragment **66**. After extensive optimization, the final condensation reaction was performed in the presence of HATU to give **67** in 54% yields with minimal racemization of the Leu amide subunit (< 5%). Finally, removal of the methyl ester and further deprotection afforded aeruginosin 298A. Although the Argol and Leu subunits of the natural product are easily accessible from readily available chiral starting materials, Shibasaki's phase-transfer alkylation methodology proved particularly useful for the synthesis of analogues of aeruginosin 298A featuring unnatural amino acid residues.[66]

Another method for the formation of the Choi is using L-glutamic acid derivative as the starting

material. Hanessian et al have synthesized the Choi in aeruginosin 298-A (and dysinosin) by using L-glutamic acid as the starting material. As shown in Scheme 1.8, the protected glutamic acid **68** was alkylated with 3-butenol triflate to form the intermediate **69**. The removal of Boc protecting group followed by cyclization and protection of the amide again with Boc lead to the 5-membered ring lactam **70.**

Partial reduction of **70** followed by protection afforded the N-Boc carbinolamine acetate **71**, which then with $SnBr_4$ underwent cyclization reaction to afford the 6-bromo-ocathydroindole analog **72**, displacing the bromo with acetate group leading to the protected octahydroindole **73**. To prepare the dihydroxyl perhydroindole, intermediate **74** was used which was prepared by a similar method as shown in Scheme 1.6.

Allylation of **74** via N-acyl imiinium ion chemistry afforded the product **75** in a 5.5/1 syn/anti ratio and the syn isomer can be converted to the desired final product via olefin metathesis and epoxidation of the alkene followed by acidic hydrolysis. (Scheme 1.9)



Scheme 1.9. Synthesis of Choi

1.10. Aeruginosin analogs

To date, limited efforts have been made to prepare analogues of the aeruginosin natural products.[58,66, 80-84] Selected examples are depicted in Figure 1.9. Mainly aeruginosins 298A and 98B have been explored as starting points for the synthesis of new synthetic aeruginosin-like serine protease inhibitors. In one of these studies, a trypsin activator rather than inhibitor was surprisingly identified. Radau and co-workers[80-82] used l-proline as a core structure instead of the synthetically more demanding Choi. More recently, analogues possessing selective, albeit weak inhibition against thrombin versus trypsin (**79** and **80**, Scheme 1.10) and the screening of aeruginosin 298A analogues against trypsin[58,84] have been reported. Both of these studies supported the importance of the chemical structure of the basic P1 subunit for inhibitory activity. Manipulations in the P3 and P4 regions had a lower impact on the trypsin inhibition.

Analogue **84** prepared by Shibasaki and co-workers [66] showed anti-trypsin activity comparable to that of the lead compound aeruginosin 298A. Furthermore, Takahashi and co-workers[102] found the potent inhibitor **82**, which is 300 times more active than aeruginosin 298A. Hanessian and co-workers[58] recently reported on truncated analogues of the aeruginosins with nonnatural benzamidine in the P1 position and including sulfamides, exemplified by compound **83** Figure 1.9. However, only modest activities against trypsin, thrombin, and other coagulation factors were obtained. A more thorough investigation of the SARs against thrombin was subsequently carried out focusing on the "chlorine effect" in chlorodysinosin A and probing the importance of all four aeruginosin subunits. The nonnatural benzamidine was found most favorable in the P1 position, while the size and shape of the P2 Choi core was proven less important. The beneficial "chlorine effect" in the P3 position was confirmed, and similar effects were obtained with other β-branched P3 side chains, such as the β-cyclohexyl **85** and isoleucine **86** analogues, which showed inhibitory activities against thrombin at IC_{50} = 0.002 $mgmL^{-1}$. An N-terminal d-HPla was superior to simpler motifs, and the terminal sulfate group of chloriodysinosin A was shown to have only a small impact on the thrombin inhibition. To the best of our knowledge analogue 84, lacking the 6-hydroxy group but with a benzamidine P1 unit, is the most active in vitro thrombin inhibitor reported to date (IC_{50} = 0.0010 $mgmL^{-1}$, Figure 1.9).

Our group has also developed novel synthesis to synthesize bicyclic core structure starting from sugar and then incorporated them to synthesize aeruginosin 298-A.

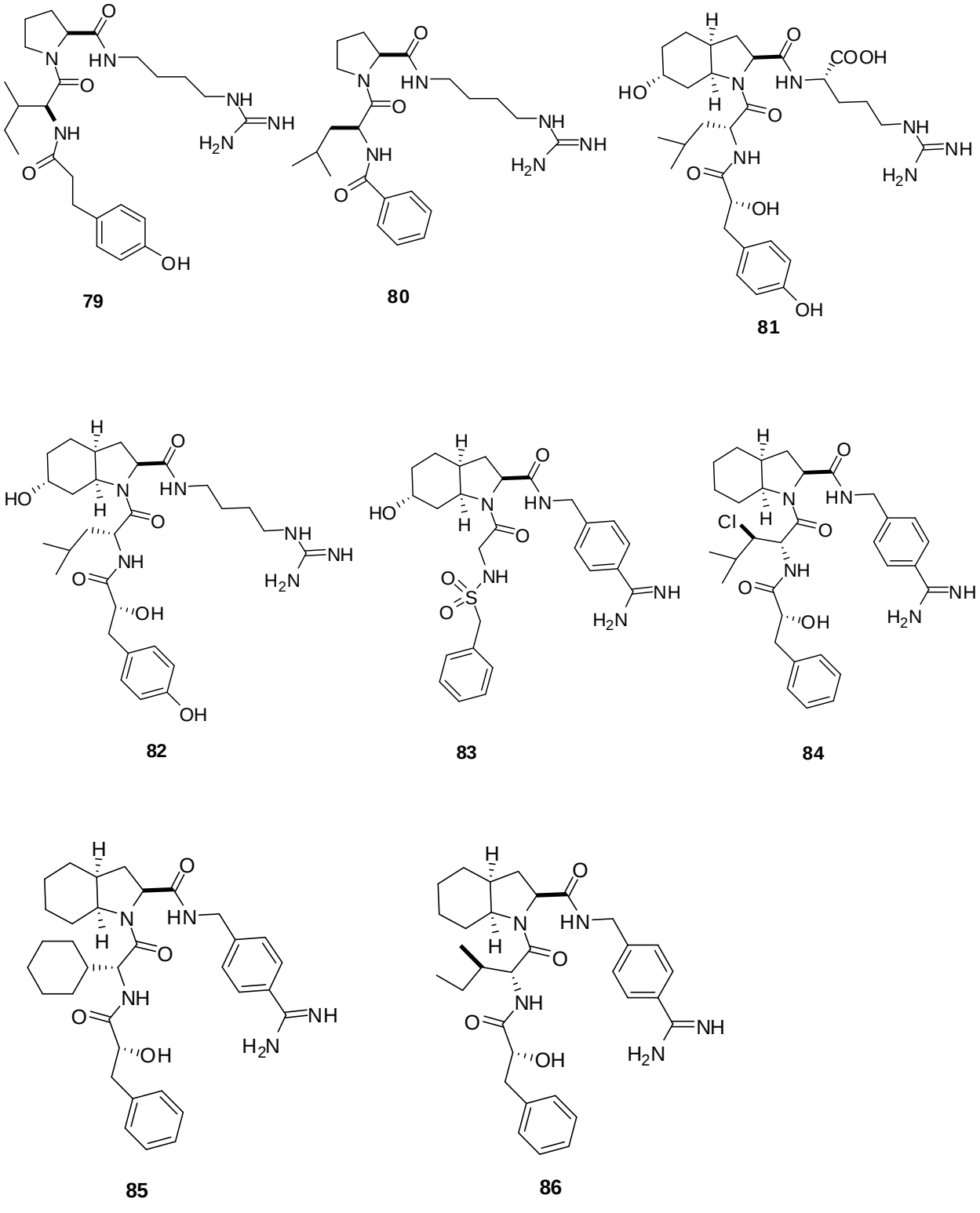
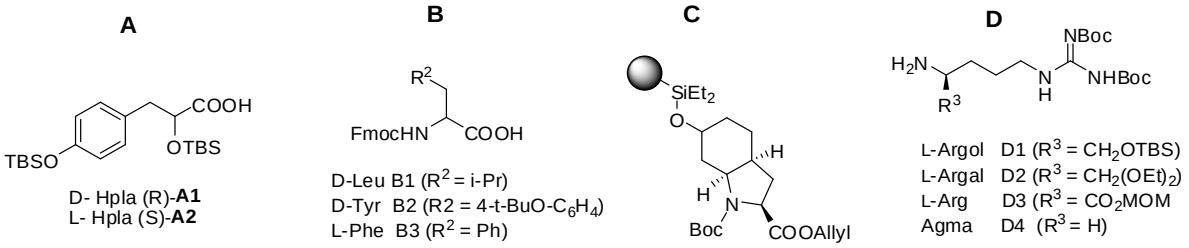


Figure 1.9. Examples of aeruginosin analogues.

Recently a combinatorial library of the compounds with varying amino acids on P1 and P3-P4 positions, keeping P2 the same as in aeruginosin 298-A[83,85] have prepared and the evaluated their activities against trypsin.



The structures **87a-87x** are synthesized by solid phase method and trypsin inhibition activities are shown in Table 1.2. The study showed that compound D-Hpla-D-Leu-L-Choi-Agma **87g**, is the most active one against trypsin and it inhibits trypsin at 0.043μg/mL. This is in the same range as the sulfated Agma containing Aeruginosins 205 A and B.

Table 1.2. The structures of compounds synthesized and their inhibition activities against trypsin.

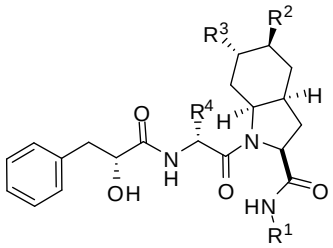
Compound No.	Compound Structure	Components	purity	IC_{50} (μg/mL)
87a	D-Hpla-D-Leu-L-Choi-L-Argol	A1, B1, C, D1	92	14 (1)c , (6)d
87b	L- Hpla -D-Leu-L-Choi-L-Argol	A2, B1, C, D1	91	7.9
87c	D- Hpla -D-Leu-L-Choi-L-Argal	A1, B1, C, D2	92	3.4
87d	L- Hpla -D-Leu-L-Choi-L-Argal	A2, B1, C, D2	90	3.4
87e	D- Hpla -D-Leu-L-Choi-L-Arg-OH	A1, B1, C, D3	84	0.48
87f	L- Hpla -D-Leu-L-Choi-L-Arg-OH	A2, B1, C, D3	81	3
87g	D- Hpla -D-Leu-L-Choi-Agma	A1, B1, C, D4	93	0.043

Compound No.	Compound Structure	Components	purity	IC_{50} (μg/mL)
87h	L-Hpla-D-Leu-L-Choi-Agma	A2, B1, C, D4	90	0.09
87i	D- Hpla -D-Tyr-L-Choi-L-Argol	A1, B2, C, D1	89	6.8 (28)d
87j	L- Hpla -D-Tyr-L-Choi-L-Argol	A2, B2, C, D1	89	100
87k	D- Hpla -D-Tyr -L-Choi-L-Argal	A1, B2, C, D2	89	4.8
87l	L- Hpla -D- Tyr -L-Choi-L-Argal	A2, B2, C, D2	91	4.6
87m	D- Hpla -D- Tyr –L-Choi-L-Arg-OH	A1, B2, C, D3	87	0.78 (8.7)d
87n	L- Hpla -D- Tyr –L-Choi-L-Arg-OH	A2, B2, C, D3	87	6.5
87o	D- Hpla -D- Tyr -L-Choi-Agma	A1, B2, C, D4	88	0.11
87p	L- Hpla -D- Tyr -L-Choi-L- Agma	A2, B2, C, D4	90	0.3
87q	D- Hpla -L-Phe-L-Choi-L-Argol	A1, B3, C, D1	94	>150
87r	L- Hpla -L-Phe-L-Choi-L-Argol	A2, B3, C, D1	93	1.1
87s	D- Hpla -L-Phe-L-Choi-L-Argal	A1, B3, C, D2	85	4
87t	L- Hpla -L-Phe-L-Choi-L-Argal	A2, B3, C, D2	95	4.6
87u	D- Hpla -L-Phe-L-Choi-L-Arg-OH	A1, B3, C, D3	82	>150
87v	L- Hpla -L-Phe-L-Choi-L-Arg-OH	A2, B2, C, D3	74	>150
87w	D -Hpla -L-Phe-L-Choi-Agma	A1, B3, C, D4	95	8.7
87x	L- Hpla -L-Phe-L-Choi-Agma	A2, B3, C, D4	96	11 (0.5)f

Hanessian *et al* also prepared quite a few analogs of the aeruginosins with varying P1, P2 and P3 units and evaluated their inhibitory activities against thrombin. The general structure for their

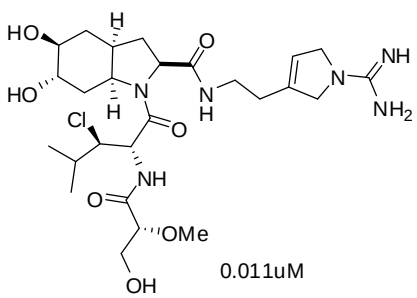
library compounds is **83**. Several sites can be modified as indicated, R^1, R^2, R^3, R^4. The results are shown in Table 1.3.

Again, the P1 unit is important for potent inhibition activities. Among the three P1 groups tested here, the 4-amidinobenzyl group (compounds **88g**) gave the best activity. The 1-amidino-δ3-pyrroline **88h, 88i** also gave reasonably good activity, while agamatine variants **88d, 88e** are the least potent among the groups of compounds tested.



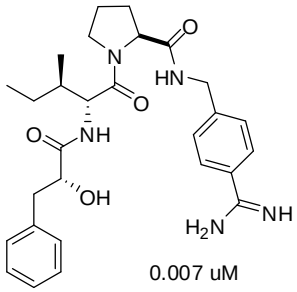
88

The bicyclic P_2 subunit generally is considered to maintain the molecule in a favorable conformation in binding to the active site. The authors reported that the 6-hydroxy group doesn't influence the inhibition activity against thrombin. The compound without the 6-hydroxyl group gave slightly improved activities comparing to the ones with 6-hydroxy group.



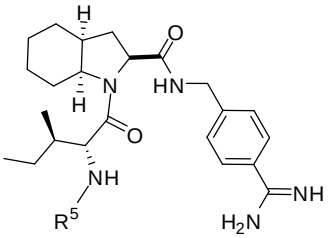
0.011uM

89



0.007 uM

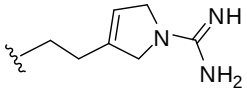
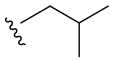
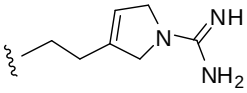
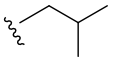
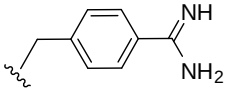
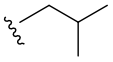
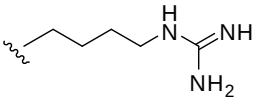
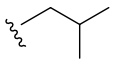
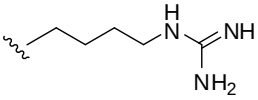
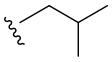
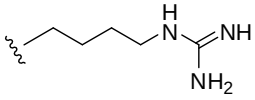
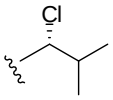
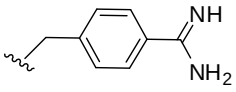
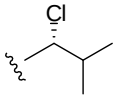
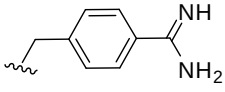
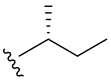
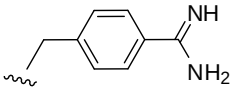
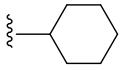
90



91

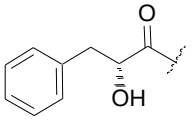
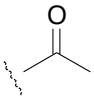
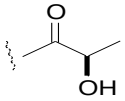
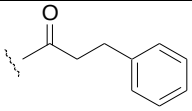
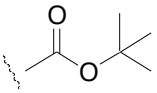
Proline has been used often as a P_2 subunit in many thrombin inhibitors, here the proline variant at P_2 also showed considerable inhibition. Compound **89** inhibits thrombin at 11nM while compound **90** in which the Choi is replaced by a L-pro inhibits thrombin at 7nM. To study the effect of the P3 position, compounds with different R^5 substituent were synthesized and the results are shown in Table 1.3. From this Table, we can see that the P_3 position seems to tolerate quite a few different functionalities. The 3-R chloro substituent showed improved activities. Isoleucine and cyclohexyl group also give good activities. The observed "chlorine effect" was considered being routed from a better fit in the active binding site.

Table 1.3. The inhibition activities of library compounds with general structure **88** (R^2 = H) towards thrombin and trypsin.

Compound	R^1	R^3	R^4	IC_{50} (μ M) Thrombin	IC_{50} (μ M) Trypsin
88a		OH		0.22	0.36
88b		H		0.12	0.37
88c		H		0.097	0.09
88d		H		1.5	0.12
88e		OH		5.6	
88f		OH		0.31	
88g		H		0.0016	0.0063
88h		H		0.0033	
88i		H		0.0034	

The authors also studied several different substituents at the P_4 position. Phenyllactic acid as appeared in oscillarin was chosen to be the main group and it also gave the best inhibition compared to other tested groups as shown in Table 1.4.

Table 1.4. The inhibition activities towards thrombin for compounds with general structure **91**.

Compound	**91a**	**91b**	**91c**	**91d**	**91e**	**91f**
R^5		H				
IC_{50} μM	0.0033	0.101	0.786	0.053	0.334	0.171

While these aeruginosin analogs have shown better inhibitory activity comparing to the natural product, they are also quite potent inhibitors towards trypsin. Recently several analogs have been obtained by varying the different attachments. From these studies and other known systems, perhaps one can find better selective thrombin inhibitors by modifying the P1 groups. The further studies in this area will help elucidating these compounds and lead to more advanced drug leads as useful anticoagulants.

1.11. Other compounds with rigid P2 units

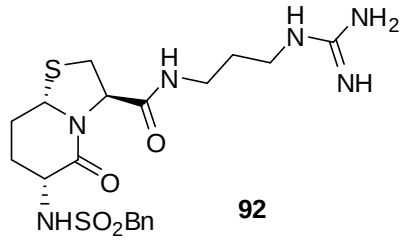
There are several other thrombin inhibitors containing a rigid P2 unit. The function of these rigid amino acids is to maintain the conformation of the overall molecule in a favorable form. Several of these compounds have appeared in recent reviews[5], including those containing bicyclic thioazalactams such as in **92-95**. These compounds contain a 7-thiaindolizidinone as the core structure, where the 6,5-fused bicyclic core occupies the S2 pocket[86].

Compound **92** inhibits thrombin and trypsin with K_i values of 0.145 μM and 5.10 μM, respectively. Compound **93** has stereochemistry opposite to **92** at the P3 binding site, but it inhibits thrombin and trypsin with K_i of 0.111 μM and 7.90 μM, respectively. The stereochemistry at the C-6 seems to have no influence on inhibitor potency[87].

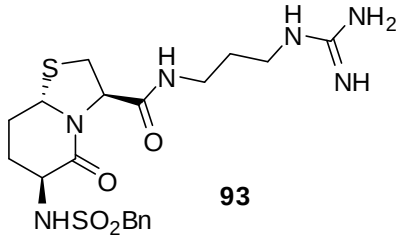
Compound **94** contains a different P1 group than **82**. It inhibits thrombin with an IC_{50} of 16.4 nM, and trypsin withan IC_{50} of 11.6 nM.[88] It seems that the additional formyl group reduced selectivity towards trypsin. From a series of compounds containing different P3 and P1 substituent, it was found that the optimal compound is **95**, with a phenyl propyl group at P3 position and a thiazolyl substituent at the P1 side chain inhibiting thrombin with K_i = 2 nM. Other bicyclic P3-P2 peptide compounds **96-98** showed promising activities against thrombin with K_i values in the nanomolar range, **96** (K_i = 7 nM), **97** (K_i = 10 nM) and **98** (K_i = 1.2nM) [89-91]. Some of them are found to be promising intravenous antithrombotic agents, but not orally available. Compound **96** contains a fused piperazinone system which was designed to include the P2-P3 unit together. This compound has also showed high *in vivo* efficacy. Compound **99** was found to be very potent towards thrombin with K_i = 0.11 nM; however, **95** and **99** are not very selective towards thrombin[92-93]. Selectivity can be improved by introducing more sterically demanding cyclic amino acids in the P1 position.

For example, inhibitors **100** and **101** retained activities towards thrombin in the low nanomolar range but their K_i values towards trypsin are more than 10 μM.[94-95] Other similar bicyclic

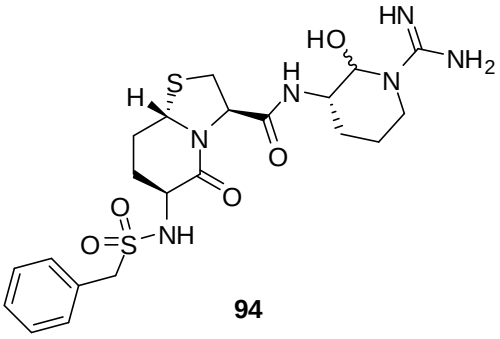
systems used in thrombin inhibitor design are shown in compounds **102-104**, which contain an indolizidinone motif.



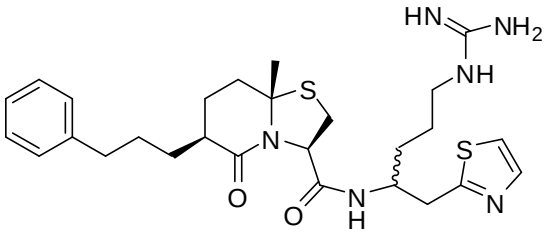
92



93



94



95

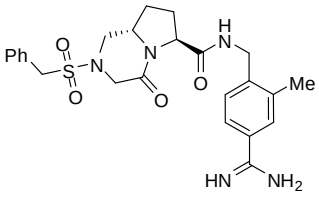
Compound **102** inhibits thrombin with K_i = 9nM and is about 20 fold selective over trypsin.[86] Compounds **103** and **104** are highly potent thrombin inhibitors, (**103**, K_i = 0.65 nM; **104**, K_i = 0.85 nM)[96]. They also showed some oral bioavailability, but further development of these compounds was stopped because of their high trypsin inhibitions with K_i values which were less than 1 nM. After incorporating a bulky cyclic group at the P1 position, selectivity can be improved. Compounds **105-107** contain a bicyclic β-strand mimetic.

The triazolopyridazine unit was synthesized by a hetero Diels-Alder reaction between a diene and the 1,2,4 triazilinedione.[96-98] These compounds are potent thrombin inhibitors, with K_i values of 23 pM for **105**, 11 nM for **106**, and 16 pM for **107**. They also showed some selectivity towards trypsin with K_i >40nM. The conformational constrained bicyclic pyridine **108** (K_i = 0.24 nM to thrombin)[99] had shown improved pharmacokinetics comparing to those of piperazinone **107**. The pyridinone systems **109** and **110** are synthesized to provide rigid conformational stability to the molecules; however, no thrombin inhibition data were reported for these two compounds.[100-101]

Other compounds with rigid fused ring systems include the carbohydrate derivatives **111-113**. The dianhydrohexitol- based benzamidines **111-113** inhibit factor VIIa and factor Xa. In these compounds, the amidine substituent position is important to the binding affinities. Compound **113** inhibits factor Xa with IC_{50} at 150 nM and factor VIIa at 100 nM. In contrast, the IC_{50} values of isomer **112** against Xa and VIIa are 3 μM and 6 μM, respectively. [102-103]

Several other compounds containing rigid structures were also prepared and had been shown to be active against blood coagulation factors, especially factor Xa. These include tetracyclic tetrahydroquinoline[104] **114**, compounds with bicyclic pyrazinones and pyridinones[105] **116a-b**, and compounds with a bicyclic dihydropyrazolopyridinone scaffold **116a-b.** Compound **116a** is a potent factor Xa inhibitor with K_i = 0.03nM.[106] The analog **116b** inhibits factor Xa with K_i = 0.18 nM, and it showed good enzyme selectivity and bioavailability.[106]

Based on the natural product **117**, isolated from extracts of *Lantana camara*, which inhibits thrombin with IC_{50} of 4 nM, a fused indane lactone containing compound **118** was synthesized and found to be a potent thrombin inhibitor [107].



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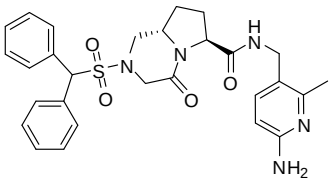

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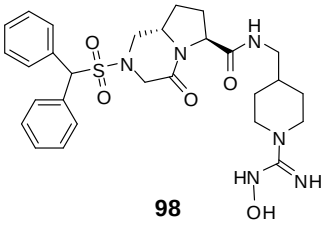

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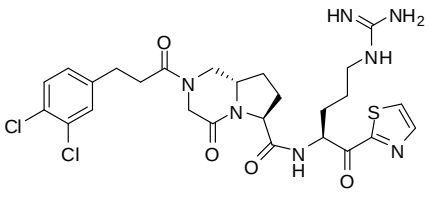
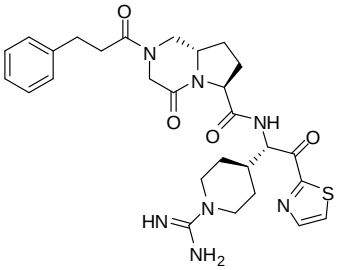


99



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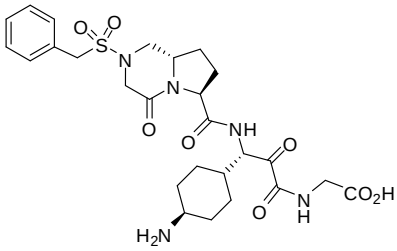

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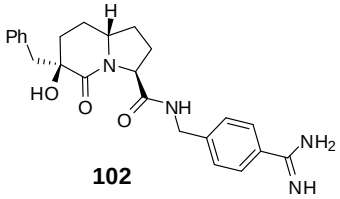

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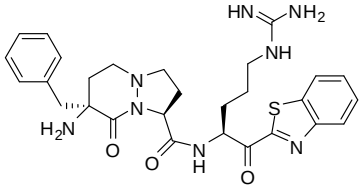
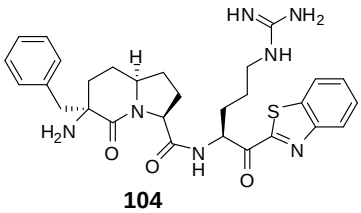
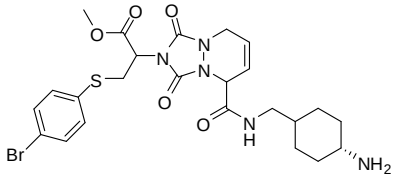


103



104

However, the lactone showed limited plasma stability. Thus structure optimization led to the *trans*-fused indane lactam **107** and **108**, which inhibited thrombin with IC_{50} =0.05 µM and 0.016 µM, respectively. Compound **108** showed improved stability compared to the lactone analog, but it was not developed further due to insufficient potency in APTT assays.[108]



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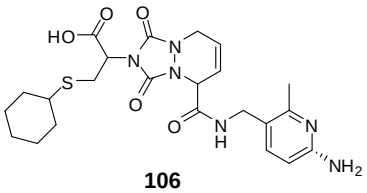

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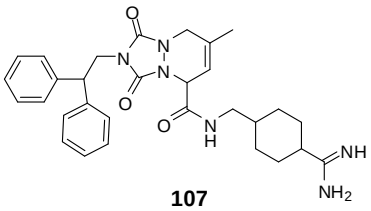

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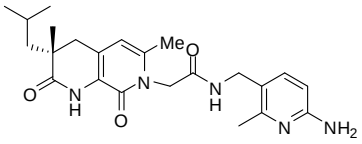
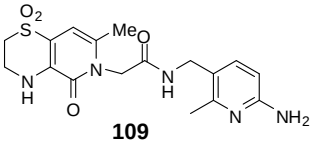
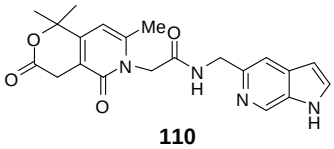


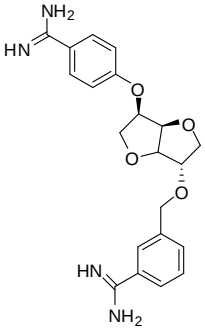
108



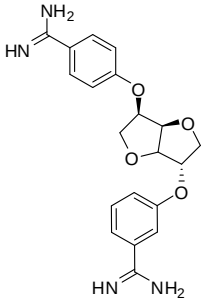
109



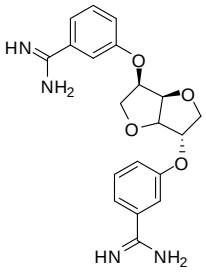
110



111



112



113

The X-ray crystal structure of **107** covalently bound to thrombin indicated that the amidine side chain occupies the S1 binding pocket, while the diethylcarboxamide substituent on the pendant phenyl ring occupies the lipopholic S3 pocket in the acylated enzyme complex. The indane core does not interact with the S2 pocket, but there is a hydrogen-bonding interaction between the carboxamide carbonyl oxygen and the hydroxyl of tyrosine 60A. The hydrogen bonding compensated for the fact that the indane core has poor interaction with the S2 site [108].

1.12. Conclusions

Despite the intense efforts in search of effective anticoagulants to replace the existing therapies using heparins or warfarins, a small molecule drug that is effective, safe and orally available is still elusive. Direct inhibitors of blood coagulation factors such as thrombin or factor Xa have occurring serine protease inhibitors. These compounds share a common rigid bicyclic amino acid at the P2 pocket. A few compounds in the aeruginosin family, such as oscillarin and chlorodysinosin, exhibited high potency against thrombin and factor VIIa. Several synthetic aeruginosin analogs have also been prepared and evaluated, and many of them showed promising activities against thrombin.

Despite the progress with these novel systems, further structure optimizations are necessary in order to achieve the desired selectivity and potency among the serine proteases. Other compounds containing conformationally rigid P_2 core structures have also been studied. Although several of them have shown great promises as thrombin inhibitors, much more effort is needed to understand the SAR and to further test these compounds *in vivo*, in order to discover new structure motifs as potential anticoagulants. Rigid molecular structures play an important role in maintaining molecular conformation. Aeruginosins and other compounds with a rigid, fused bicycle in their structures are potentially useful lead compounds for further SAR studies in order to find effective antithrombotic agents.

1.13 Part II: Introduction to self-assembly of D-glucosamine derivatives

The word “gel” comes from Latin word “gelatus”, which means to immobilize or to freeze.[109] While this definition is very broad, a more accurate definition given by the esteemed Nobel laureate P. J. Flory defined a gel as “a colloidal dispersion with a continuous structure over macroscopic dimensions, which is permanent on the analytical time scale, and which is solidlike in its rheological behavior”.[110] The colloidal dispersion causes an increase in the viscosity in the liquid medium by forming a matrix which entraps the liquid. In doing so, the system attains a semisolid consistency, lying somewhere between the liquid and solid state.

In recent years, low molecular weight gelators (LMWGs) have gained great attention because of their potential applications as advanced soft materials. LMWGs are small molecules that can self-assemble and form 3-dimensional network which allows the immobilization of solvents.[111-114] Based on the solvent system they are divided into two categories. Low molecular weight hydro gelator (LMHGs) and low molecular weight organo gelator (LMOGs). The resulting gels are usually called supramolecular gels or physical gels to differentiate from polymer gels.

The self assembly process depends on a variety of different forces and on the solvents being used. For aprotic, organic solvents, hydrogen bonding is often the most important force for guiding the self assembly process, though π-stacking interactions of aromatic rings, metal

coordination, and electrostatic interactions can also play a significant role.[113] For water, and to a lesser extent, polar protic organic solvents, hydrogen bonding and electrostatic interactions are not as significant, due to the ability of the solvent to participate in these types of interactions; hydrophobic forces (and other Van der Waals forces) and π-π stacking are the major contributors to self assembly of LMWGs in water.[114] Regardless of the nature of the solvent, a delicate balance between hydrophobicity and hydrophilicity of the gelator molecules must be maintained for gelation to occur; if the gelator molecule is too soluble, complete solvation will occur instead of self-assembly, while extreme solvophobicity can lead to exclusion of the solvent, causing precipitation or recrystallization.

The preparation of organogels or hydrogels using LMOGs is pretty straightforward. In general, a certain amount of a LMOG is placed in a vial containing the testing solvent. Then it is heated above the sol-gel temperature (Tgel) to dissolve the compound. Gels are formed when the solution is cooled below the Tgel.[115]

The resulting solidification or increase of the viscosity of the solvent(s) is caused by the formation of a fibrous network, which is created by the noncovalent inter and intramolecular forces. The minimum gelation concentration (MGC) is obtained through serial dilution and heating until a stable gel is no longer formed.

LMWGs have gained considerable amount of interest due to their implications in supramolecular chemistry, and their potential applications as advanced soft materials in biomedical and materials research. For instance, organogelators have been more and more explored as optical electronic

devices and found applications in semiconductors and photovoltaic cells. They have also been explored as sequester agent for oil spill and chemical spill cleanup. As for supramolecular hydrogels, they are being explored more and more for biomedical applications, such as matrix for cell growth, enzyme assays, drug delivery, and tissue engineering. The supramolecular gels are reversible and can be engineered to be stimuli-responsive. These provide the basic steps towards their use in biomedical research.[116-118]

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Chapter 2

Preparation of L-proline based aeruginosin 298-A analogs: Optimization of the P1-moiety

2.1. Abstract

Aeruginosin are a family of naturally occurring oligopeptides that share a common bicyclic amino acid core structure. Many compounds in the family are inhibitors of serine proteases, such as thrombin and trypsin. Thrombin is an important enzyme in the blood coagulation cascade, and is a promising target for anticoagulant drug development. In order to understand the structure–activity relationship (SAR) and to find selective thrombin inhibitors, we synthesized a series of aeruginosin 298-A analogs, in which the P2 bicyclic amino acid was replaced by a L-proline residue. The structure optimization was focused on modification of the P1 position. In choosing the P1 group, an effort was made to avoid using the highly basic guanidine groups present in nearly all naturally occurring aeruginosins. The synthesis and enzyme assays of these aeruginosin analogs against thrombin and trypsin are reported. We found that several compounds with neutral P1 groups exhibit excellent selectivity over trypsin and good potency against thrombin. The SAR data of the P1 groups obtained here can be used in preparing other thrombin inhibitors with better selectivity against trypsin.

This chapter is adapted from paper: Wang, G.; Goyal, N.; Hopkinson, B. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3798-3803.

2.2. Introduction

Blood coagulation disorders can lead to serious diseases, including deep vein thrombosis and pulmonary embolism. The two main drugs currently used to treat such disorders are heparin and warfarin, however both suffer certain limitations like narrow therapeutic windows, severe side effects, and/or the need for parenteral administration. Intensive efforts have been made to develop new anticoagulants relying on direct inhibition of coagulation enzymes. There have been intense efforts over the past few decades to develop safer and more effective anticoagulants.[1–5] Since thrombin is an important enzyme in the blood coagulation cascade, it has become an attractive target in the development of new antithrombotic drugs. Direct inhibition of thrombin has led to the development of several effective anticoagulants, such as argatroban and hirudin.[6,7] However, the discovery of new, orally available, safe drugs to treat thromboembolism is still very challenging.[8,9]

In the early 1990s Murakami and co-workers reported the isolation and characterization of the novel protease inhibitory peptides microginin and micropeptins A and B from Microcystis aeruginosa. This was soon followed by the isolation of Aeruginosin 298A from the same cyanobacterial source. The 20 member of natural product aeruginosin family have shown a lot of biological activity against serine protease inhibitor.[10-18]

Thus, the knowledge that several of the aeruginosins exhibit high inhibitory potency against blood coagulation factors have made them attractive small-molecule targets in the search for new anticoagulants. Some structures (**1-8**) related to aeruginosins are shown in Figure 2.1. Aeruginosins typically contain tetrapeptid *p*-hydroxylphenyllactic acid (Hpla), Leucine, 2-

carboxy-6-hydroxyotahydroinhole (Choi), argininol (Argol) or arigninal (Argal) or agamatine (Agma) through peptidic linkages.

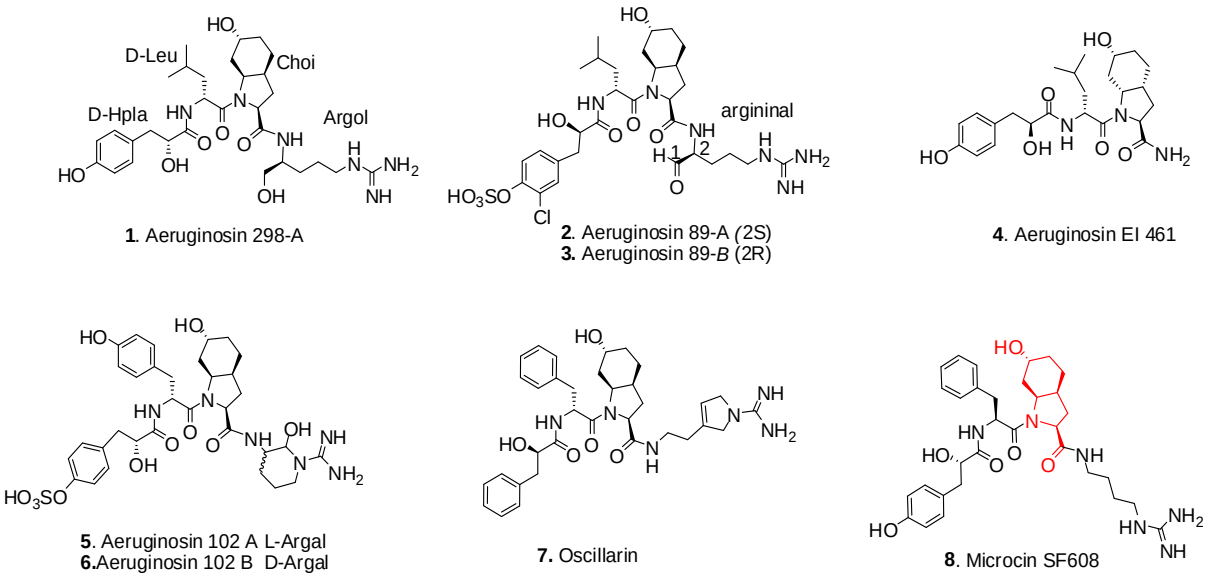


Figure 2.1. Structures of Aeruginosin class compounds.

These molecules are divided into three parts. P1 (S1), P2 (S2) and P3 (S3) subunits which are corresponds to the hydroxylphenyllactic acid (Hpla), Leucine, (P1), 2-carboxy-6-hydroxyotahydroinhole (Choi) (P2), and arigninal (Argal) at P3. The most important regions of the thrombin active site for inhibition by compounds belonging to the aeruginosin family are the S1, S2, and the d-S3 subsites. The S1 subsite, also known as the specificity pocket, is characterized by an aspartic acid residue (Asp189) in the bottom of the pocket, which is able to recognize and engage in ionic interactions with inhibitors containing basic P1 side chains. An alanine residue in position 190 of the S1 pocket of thrombin distinguishes it from trypsin, which has a serine in this position. The S1 subsite is also in close proximity to the oxyanion hole (the backbone amide NH groups of Ser195 and Gly193) and the Ser195-His57- Asp102 catalytic

triad. The S2 subsite, or the proximal P pocket, is a hydrophobic pocket primarily created by Tyr60A and Trp60D in the Tyr60A-Pro60B-Pro60C-Trp60D thrombin insertion loop.

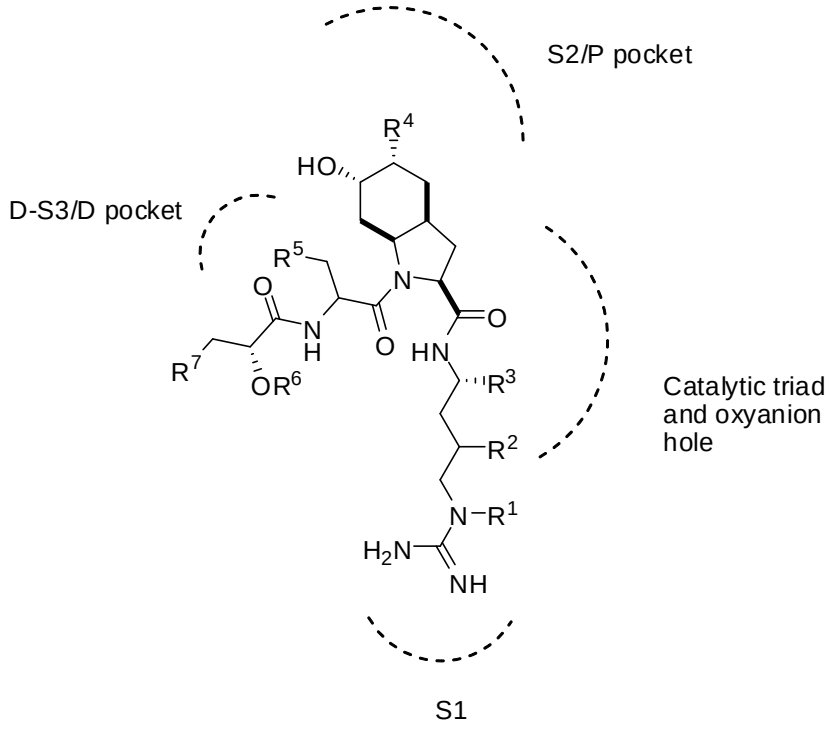


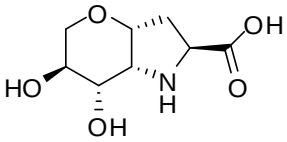
Figure 2.2 Schematic illustration of the thrombin active site bound to the Aeruginosins.

Although many aeruginosins exhibit promising biological activities, their structures need to be optimized to obtain useful therapeutic agents. One challenge is to obtain selectivity among the different serine proteases; other problems may include their peptide like structures and structural complexity. In recent years, there has been growing interest in synthesizing analogs of aeruginosins. [10, 11, 19-23]

L-Proline has been a commonly used P2 group in thrombin inhibitor designs including orally active thrombin inhibitors [24-26] and aeruginosin analogs.[27] Along with the seminal works reported

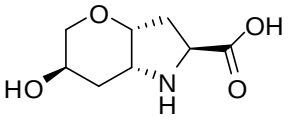
by Hanessian's group and several other research teams, a significant amount of knowledge has been obtained regarding the SAR of the aeruginosin families. Among the various modified aeruginosin analogs, many showed improved potency compared to their original natural products; however, many of these compounds are also potent inhibitors for trypsin. The blood coagulation factors, such as thrombin and factors Xa and VIIa, are all trypsin-like enzymes which share similar active sites and can thus bind similar compounds. Therefore, it is important to find molecules that can exhibit selectivity for thrombin and/or other blood coagulation factors over trypsin in order to avoid possible side effects. In the recent years many groups have made an attempt to synthesize a better antithrombin using small molecules. Few research groups have also incorporated proline for peptide synthesis. Taking that into account we designed our molecules based on aeruginosin and Proline and then performed our synthesis. The P1 moiety has also been changed by different polar and non-polar moiety.

Keeping the crystal structure binding in mind and to study more structure activity relationship (SAR) our group has synthesized various aeruginosin analogs, where the Choi core structure has been replaced with a different bicyclic oxygenated core structure derived from different sugars.



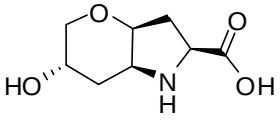
H-D-O-Choi

9



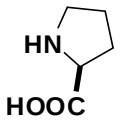
D-O-Choi

10



L-O-Choi

11



L-proline

12

Figure 2.3. Bicyclic oxygenated core structures.

Many different synthetic routes were followed to synthesize these bicyclic unnatural amino acids. And then there biological studies were performed for few molecules and rest of the compound biological studies is underway.

2.3. Results and Discussion

We first designed and synthesized Aeruginosin analogs by replacing P2 moiety by Proline and with other functionalities at P1. Figure 2.4 depicts the retro synthetic approach we applied for the synthesis of tetrapeptide to HPLA, dipeptide and the Argal group**.**

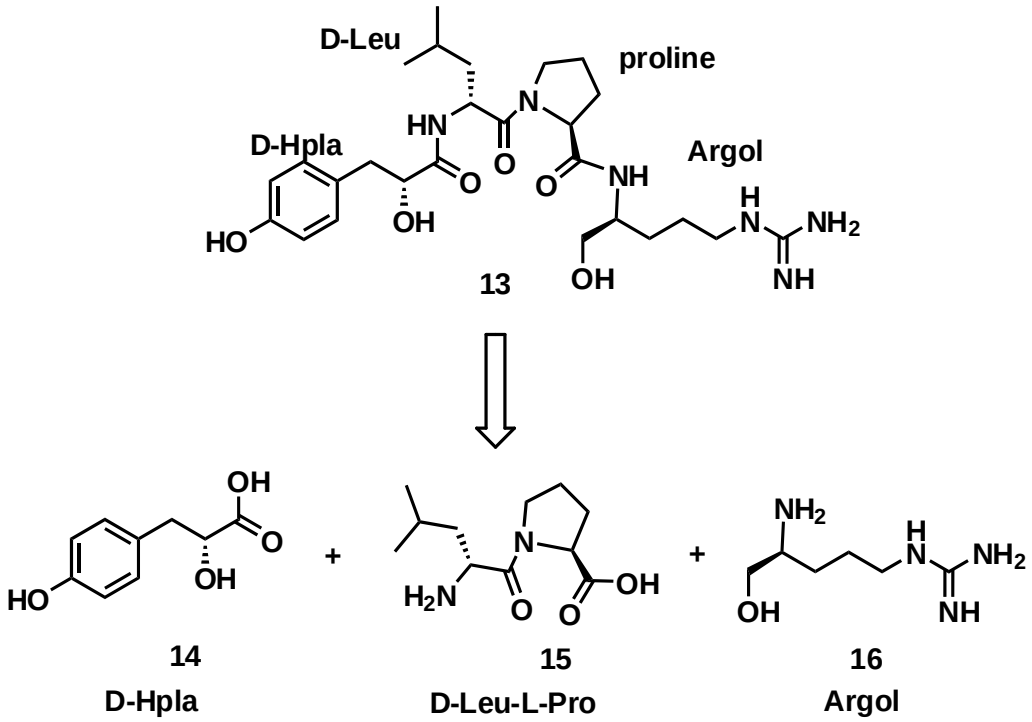
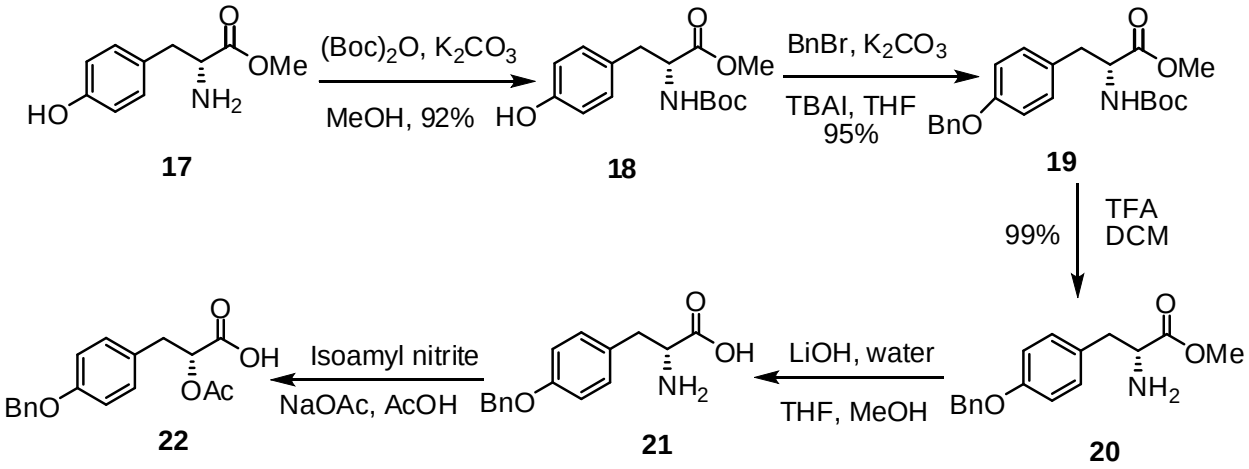


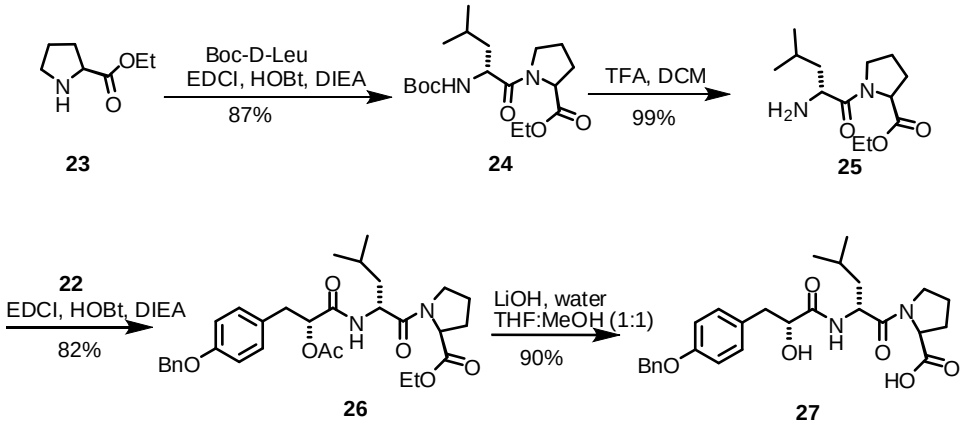
Figure 2.4. Reterosynthetic approach for synthesis of Aeruginosin

The aeruginosins have also garnered considerable attention from synthetic organic chemists as a result of their structural novelty and biological activity. Although these compounds are composed of similar subunits, isolation of new members has often been attended by the discovery of previously unknown amino acids and arginine-mimetic subunits as constituents. Derivatives of the 2-carboxyperhydroindole core structure have received the most attention with respect to conceptually diverse synthetic approaches.



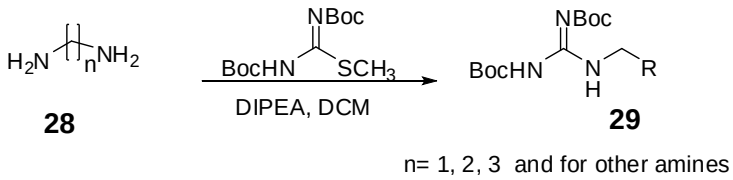
Scheme 2.1. Synthesis of D-HPLA

As shown in the Scheme 2.1, for the Preparation of D-HPLA the amino group of Tyrosine ester **17** was protected with Boc and the hydroxyl group was protected using benzyl. Then Boc group was removed using TFA in DCM and ester was hydrolysed using NaOH in THF: Water: MeOH. And then amino group was replaced by –OAc using isoamyl nitrite in NaOAc and AcOH.



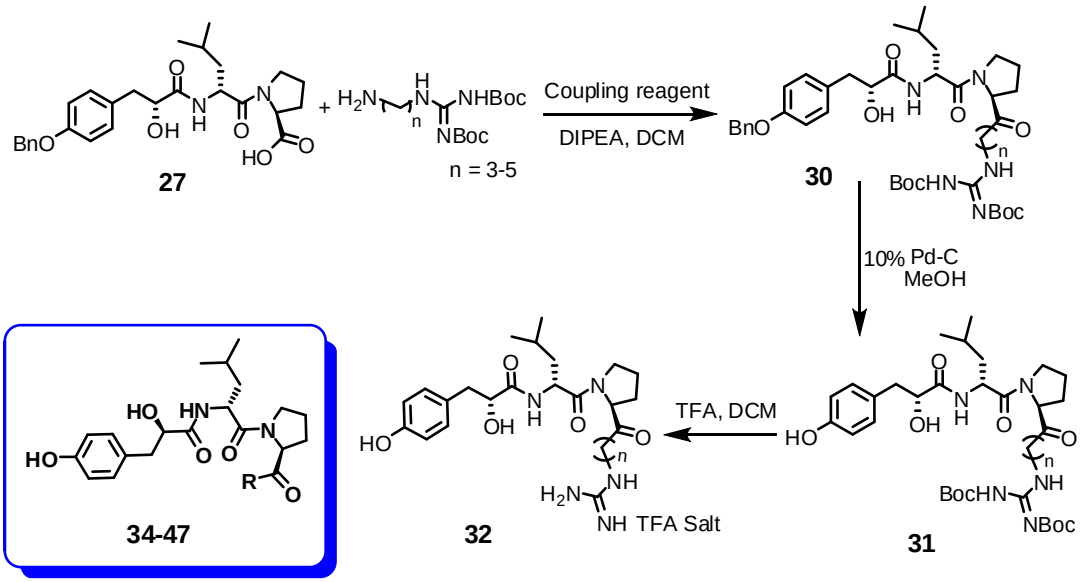
Scheme 2.2. Synthesis of dipeptide and tripeptide

The L-proline was converted to the ester **23**, and it was coupled with N-Boc-D-Leucine to give the dipeptide **24**. The Boc was removed to give the amine **25**, which was coupled with the protected form of D-Hpla **22** to afford the tripeptide **26**. The ester groups were hydrolyzed to form the free acid **27**, which is ready to couple with various P1 groups. Scheme 2.3 depicts one example of the coupling reaction to prepare the teterapeptide. The diamine **28** was treated with N-Boc protected thiomethyl pseudourea to give the intermediate **29**.



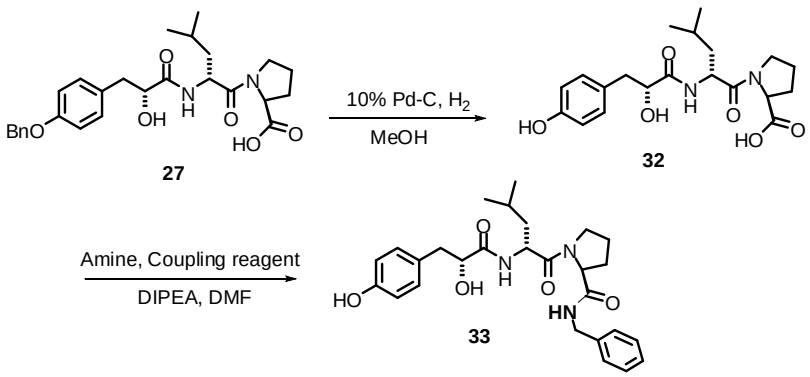
Scheme 2.3. Synthesis of P1 group

Compound **29** was coupled with the tripeptide **27** using typical coupling reagents such as EDCI, HOBt, HATU and PyBOP depending on the amine used. Compound **31** was then converted to the - 70 -ertapeptide **32** after removal of benzyl and Boc protecting groups.



Scheme 2.4. Synthesis of Tetrapeptide

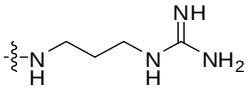
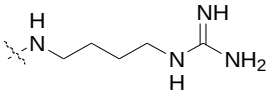
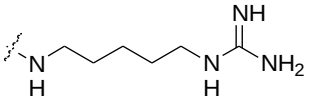
In case of the compounds where we wanted a benzyl group at P1 position we first removed the benzyl group of HPLA at P3 position. Followed by this, the tripeptide was coupled with the benzylated amine group to synthesize tetrapeptides.

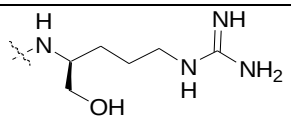
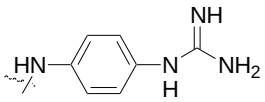
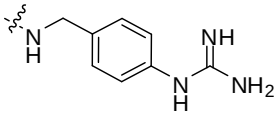
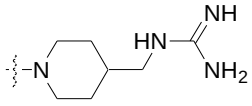
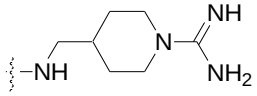
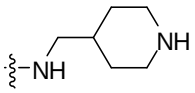
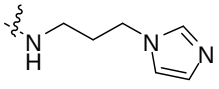


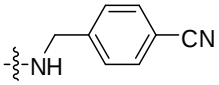
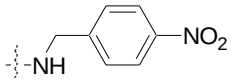
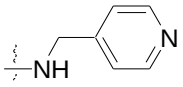
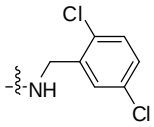
Scheme 2.5. Synthesis of Tetrapeptide with benzyl moieties at P1 position

To synthesize all these tetrapeptides, different coupling reagents were used. Here is the table for all the coupling reagents used and the respective percentage yields for the compounds.

Table 2.1: Structure of various P1 units to form final tertapeptide and the yields for the coupling reaction at the last step

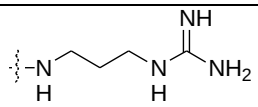
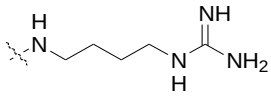
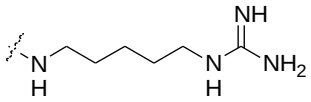
Compound Number	**P1 group R =**	**Coupling-reagent used**	**(%) yield**
34		EDCI	77
35		EDCI	72
36		EDCI	78

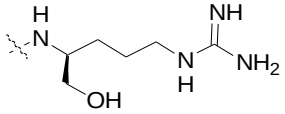
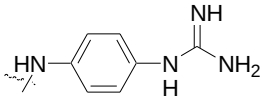
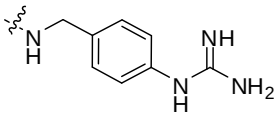
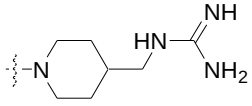
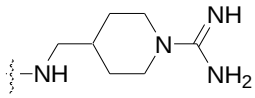
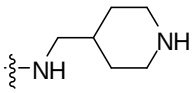
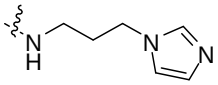
Compound Number	P1 group R =	Coupling-reagent used	(%) yield
37		PyBOP	80
38		HATU	68
39		EDCI	74
40		HATU	58
41		EDCI	74
42		EDCI	82
43		EDCI	79

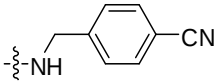
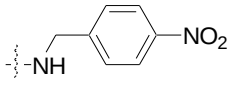
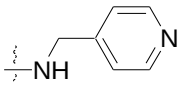
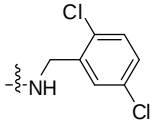
Compound Number	P1 group R =	Coupling-reagent used	(%) yield
44		EDCI	85
45		EDCI	75
46		EDCI	82
47		EDCI	89

Several compounds with the general structure of **32** are synthesized. The structures of P1 groups used are shown in Table 2.1.

Table 2.2: Structure of various P1 units and the inhibitory values against Thrombin and Trypsin[27]

Compound Number	P1 group R =	IC_{50} (μg/mL) Thrombin	IC_{50} (μg/mL) Trypsin
34		0.092	12.5
35		1.5	6.1
36		0.62	103

Compound Number	P1 group R =	IC_{50} (μg/mL) Thrombin	IC_{50} (μg/mL) Trypsin
37		3.0	14
38		0.50	No inhibition
39		0.43	150
40		0.57	No inhibition
41		3.5	55
42		0.65	No inhibition
43		0.084	No inhibition

Compound Number	P1 group R =	IC_{50} (μg/mL) Thrombin	IC_{50} (μg/mL) Trypsin
44		0.20	210
45		0.28	210
46		0.43	140
47		0.41	14.2

In Table 2.2 we also carried out the enzyme assays of these compounds. The 50% inhibition concentrations are shown in Table 2.2 as well. Commercial thrombin inhibitor PPACK and trypsin inhibitor purchased from Calibiochem and Sigma were used as the standards.

From the enzyme assay results, we can see that the P1 specificity group is important to the thrombin inhibition. For the compounds with guanidine groups (**34**–**41**), reasonable inhibition activity toward thrombin was typically exhibited. Compound 34 is the most potent among the three simplest compounds **34**– **36**, which have similar structures but different chain lengths of the P1 group. Compound **36**, with a 5-methylene linker, showed the best selectivity to thrombin over trypsin. Compound **37** has the same structure as aeruginosin 298-A, except that the Choi is replaced with L-proline; it seems to have maintained similar activities toward both enzymes.

Compounds **38-39** contain the phenyl guanidine group, which does not seem to inhibit trypsin well, but has good activity toward thrombin. Compounds **42–44** have a piperidinyl group; **42**, with the secondary amine, showed good selectivity to thrombin. Without the amidine group from **44**, compound **45** showed good activity to thrombin and no inhibition to trypsin. Several compounds without highly basic groups at the P1 positions gave good inhibition activity against thrombin while also maintaining good selectivity over trypsin. For example, compounds with C-4-piperidinyl-methyl (**42**), propyl imidazole (**43**), cyanobenzyl (**44**), nitrobenzyl (**45**), and C-4-pyridinyl-methyl (**46**) do not contain the highly basic guanidine group. These compounds exhibited reasonably good thrombin inhibition activities, and they did not inhibit trypsin at concentrations below 100 μg/mL. Compound **46** showed about 1000-fold selectivity for thrombin over trypsin. Several others exhibited even better selectivity toward thrombin. Compound **47** has a neutral 2,5-dichlorobenzyl P1 group, and it showed good activity against thrombin but is not as selective toward trypsin, with only about 35-fold selectivity. Several of the compounds showed diminished potency to thrombin binding, while compounds **36** and **43–47** showed better or comparable potencies to that of aeruginosin 298-A.

2.4. Conclusion

In conclusion, we have synthesized a series of aeruginosin 298- A analogs in which the Choi is replaced with L-proline and the P1 Argol is replaced with various functionalities. We did not change P3 moiety and used D-HPLA and D-Leucine for this position. Among these tetrapeptides, several of them exhibit excellent selectivity for thrombin inhibition over trypsin inhibition. These include cyanobenzyl, nitrobenzyl, propyl imidazole, cyanobenzyl, C-4-

piperidinylmethyl, and C-4-pyridinyl-methyl groups at the P1 positions. These P1 replacements are less basic or neutral, and generally have simpler or smaller groups than Argol or other guanidine containing units. The SAR data shown here is useful in finding thrombin inhibitors that are easy to synthesize and have superior selectivity toward thrombin. Further structure modifications can potentially lead to the discovery of potent and selective thrombin inhibitors that are also orally available.

2.5. Experimental Procedure

Synthesis of (R)-2-acetoxy-3-(4-(benzyloxy) phenyl) propanoic acid 22:

Isoamyl nitrite (1.50 g, 12.88 mmol) was added slowly to a mixture of (R)-2-amino-3-(4-(benzyloxy) phenyl) propanoic acid (1.0 g, 3.68 mmol) and NaOAc (1.05 g, 12.88 mmol) in glacial acetic acid. The reaction mixture was stirred for 24 hrs at room temperature. The mixture was concentrated to remove acetic acid. Residue was acidified using HCl and dissolved in EtOAc. The organic phase was extracted and washed with water and brine and dried over Na_2SO_4. The crude product was purified by flash chromatography on silica gel using a solvent system of AcOH: EtOAc: Hexane (1:9:1). The product was obtained as white powder, yield 75%, $[\alpha]_D$ = +7.2 (c = 0.70, $CHCl_3$); ee = 98%, reported[26] $[\alpha]_D$ = +7.4 (c = 0.70, $CHCl_3$); ee > 98%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.10 (s, 3H), 3.07 (dd, 1H, *J* = 14.2, 8.7 Hz), 3.17 (dd, 1H, *J* = 14.2, 3.6 Hz), 5.04 (s, 2H), 5.22-519 (m, 1H), 6.93 (d, 2H, *J* = 8.0 Hz), 7.17 (d, 2H, *J* = 8.4 Hz), 7.44-7.30 (m, 5H), 8.53 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.9, 22.7, 24.7, 36.6, 41.6, 50.2, 52.3, 69.9, 74.3, 114.7, 127.5, 128.0, 128.6, 130.7, 136.9, 157.8, 169.3, 173.1.

Synthesis of (S)-ethyl pyrrolidine-2-carboxylatester 23:

Thionyl chloride (1.02 g, 8.6 mmoles) was added dropwise to a mixture of L-proline (1.0 g, 8.6 mmoles) in ethanol (15 mL) as a solvent under cooling. Stirr the reaction mixture at RT for 30 minutes and then reflux it for 3 hrs. The mixture was concentrated on vacuum and then it was crystallized using ether to get compound **23** as a white solid. Yield 99%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (t, 3H, *J* = 7.1 Hz), 1.57 (m, 2H), 1.9 (m, 2H), 2.81 (m, 2H), 3.91 (m, 1H).

Synthesis of (S)-ethyl 1-((R)-2-(tert-butoxycarbonylamino)-4-methylpentanoyl)pyrrolidine-2-carboxylate 24:

Boc-D-Leucine (375 mg, 2.60 mmol) was dissolved in dichloromethane, EDCI (747mg, 3.9 mmol), HOBt (527 mg, 3.9 mmol) and DIPEA (1.006 ml, 7.8 mmol) were added to the reaction mixture. To this mixture, the ester-proline (500 mg, 2.17 mmol) in dichloromethane was added drop wise. The reaction was left stirring for 4 hrs at room temperature. After this, it was diluted with 100 mL dichloromethane and 25 mL water. The dichloromethane layer was separated and washed with 1N HCl, water, then saturated sodium bicarbonate and finally with brine. The compound was dried over on sodium sulfate and was concentrated using vaccum. The crude product was purified by flash chromatography on silica gel. The product was obtained as a light yellow solid. Yield 87%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.84 (m, 6H), 1.26 (t, 3H, *J* = 7.3 Hz), 1.42 (s, 9H), 1.59 (m, 2H), 1.94 (m, 2H), 2.75 (m, 2H), 3.84 (m, 1H), 4.12 (q, 2H, *J* = 6.9 Hz), 4.45 (m, 1H), 5.20 (d, 1H, *J* = 6.95 Hz).

Synthesis of (S)-ethyl 1-((R)-2-amino-4-methylpentanoyl)pyrrolidine-2-carboxylate 25: Compound **24** (500 mg, 1.40 mmoles) was dissolved in dichloromethane and then TFA (0.2 ml)

solution in dichloromethane (0.1 ml) was added to the reaction mixture. The reaction mixture was stirred for 2 hours. For work up dichloromethane was removed under reduced pressure and was crystallized using ether. Yield 99%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.84 (m, 6H), 1.27 (t, 3H, *J* = 7.3 Hz), 1.58 (m, 2H), 1.97 (m, 2H), 2.75 (m, 2H), 3.84 (m, 1H), 4.12 (q, 2H, *J* = 6.9 Hz), 4.45 (m, 1H).

1. Preparation of tripeptide **26**:

Synthesis of (R)-2-Acetoxy-3-(4 (benzyloxy) phenyl) propanoic acid 26:

22 (300 mg, 1.16 mmol) was dissolved in dichloromethane, EDCI (244 mg, 1.27 mmol), HOBt (171 mg, 1.27mmol) and DIPEA (0.64 mL, 4.99 mmol) were added to the reaction mixture. To this mixture, the dipeptide 16 (356 mg, 1.39 mmol) in dichloromethane was added drop wise. The reaction was left stirring for 5 hrs at room temperature. After this, it was diluted with 100mL dichloromethane and 50 mL water. The dichloromethane layer was separated and washed with 1N HCl, water, then saturated sodium bicarbonate and finally with brine. The compound was dried over on sodium sulfate and was concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel. The product was obtained as a white powder. Yield 80%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H) 1.21 (t, 2H, *J* = 9.5 Hz), 1.25 (t, 3H, *J* = 7.3 Hz), 2.01 (m, 2H), 2.04 (s, 3H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6Hz), 3.47 (t, 2H, *J* = 7.6Hz), 4.12 (q, 2H, *J* = 7.32), 4.35 (m, 1H), 4.29 (m, 1H), 4.74 (m, 1H), 4.98 (s, 2H), 6.93 (d, 2H, *J* = 8.0 Hz), 7.17 (d, 2H, *J* = 8.4 Hz), 7.44-7.30 (m, 5H), 8.53 (s, 1H).

Synthesis of (S)-1-((R)-2-((R)-2-acetoxy-3-(4-(benzyloxy) phenyl) propanamido)-4-methylpentanoyl) pyrrolidine-2-carboxylic acid 27:

To a stirred solution of compound **26** (200 mg, 0.361 mmol) in THF (5 mL) was added 0.2 N LiOH (5 mL) at 0° C. The reaction mixture was warmed at room temperature and stirred for 6 hours before the reaction mixture was acidified to pH 2-3, and extracted with ethyl acetate (100 mL) and then washed with brine. After removing the solvent on a rotavap, the product was obtained as a white powder. Yield 89%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.25 (t, 3H, *J* = 7.3 Hz), 2.01 (m, 2H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.35 (m, 1H), 4.29 (m, 1H), 4.74 (m, 1H), 4.98 (s, 2H), 6.93 (d, 2H, *J* = 8.0 Hz), 7.17 (d, 2H, *J* = 8.4 Hz), 7.44-7.30 (m, 5H), 8.53 (s, 1H).

General procedure for the synthesis of tetrapeptides 30:

Tripeptide (1 eq) was dissolved in dichloromethane and then EDCI (1.5 eq), HOBt (1.1 eq) and DIPEA (3 eq) were added to the reaction mixture. Various amines (1.2 eq) were dissolved in dichloromethane and then added drop wise to the reaction mixture. After stirring at room temperature for 5-7 hrs, the reaction mixture was diluted with dichloromethane and water. The dichloromethane layer was separated and washed with 1N HCl, water, saturated sodium bicarbonate and finally with brine. The compound was dried over sodium sulfate and was concentrated on a rotavap. The crude product was purified by flash chromatography on silica gel.

General procedure for debenzylation to 31 by catalytic hydrogenation:

10% Pd/C (20% by weight) was added to a solution of benzyl protected tetrapeptide (1 eq) in MeOH and the mixture was stirred under atmospheric hydrogen pressure (hydrogen balloon) for

20 h. The catalyst was removed by filtration through a short pad of celite, this was washed with MeOH. The filtrate and washing methanol were combined and concentrated to give the desired product.

General Procedure for Boc-deprotection to the target tetrapeptides 32:

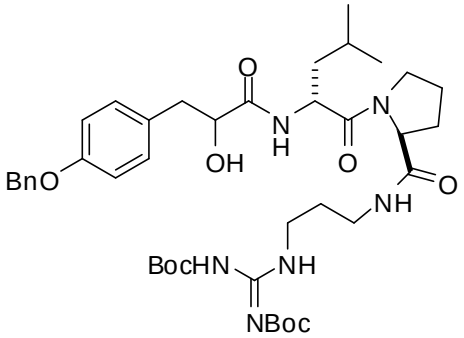
Trifluoroacetic acid (2 eq) was added to a solution of Boc protected tetrapeptide in small amount of dichloromethane. The reaction mixture was stirred at 0 °C for 2 hrs. Dichloromethane was removed under reduced pressure and the residue was treated with ether to produce the product as a precipitate. The precipitate was filtered and dried under reduced pressure using a vacuum pump.

2. Preparation of tetrapeptide

Preparation of Tetrapeptide 34

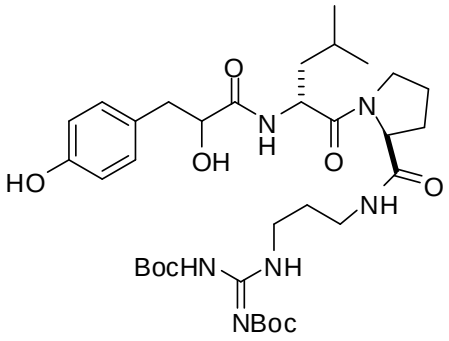
The intermediates for the preparation of compound **34**:

34a:



Yield 77%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.45 (s, 18H), 1.65 (m, 1H), 1.81 (m, 2H), 2.01 (q, 2H, *J* = 7.6 Hz), 2.25 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.11 (m, 2H), 3.25 (m, 2H), 3.42 (m, 2H), 4.44 (m, 1H,) 4.49 (m, 1H), 4.51 (m, 1H), 5.01 (s, 2H), 6.88 (d, 2H, *J* = 8.4 Hz), 7.16 (d, 2H, *J* = 8.7 Hz), 7.37 (m, 5H), 8.34 (s, 1H), 11.42 (s, 1H).

34b:



Yield 85%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.45 (s, 18H), 1.65 (m, 1H), 1.81 (m, 2H), 2.01 (q, 2H, *J* = 7.6 Hz), 2.25 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.11 (m, 2H), 3.25 (m, 2H), 3.42 (m, 2H), 4.44 (m, 1H,) 4.49 (m, 1H), 4.51 (m, 1H), 5.01 (s, 2H), 6.72 (d, 2H, *J* = 8.0 Hz), 7.07 (d, 2H, *J* = 8.0 Hz), 7.48 (s, 1H), 11.25 (s, 1H).

Compound 34:

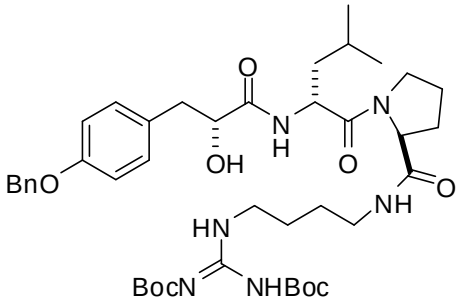
Gummy white material. Yield 99%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.45 (s, 18H), 1.65 (m, 1H), 1.81 (m, 2H), 2.01 (q, 2H, *J* = 7.6 Hz), 2.25 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.11 (m, 2H), 3.25 (m, 2H), 3.42 (m, 2H), 4.44 (m, 1H,)

4.49 (m, 1H), 4.51 (m, 1H), 5.01 (s, 2H), 6.72 (d, 2H, *J* = 8.0 Hz), 7.07 (d, 2H, *J* = 8.0 Hz), 9.0 (s, 1H), 11.25 (s, 1H); HRMS calcd for $C_{24}H_{38}N_6O_5$+H 491.2982, found 491.2977.

Preparation of teterapeptide 35

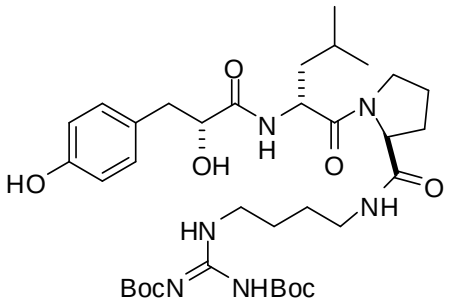
The intermediates for the preparation of compound **35**:

35a:



Yield 72 %, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.45 (s, 18H), 1.97 (m, 4H), 2.17 (m, 2H), 2.19 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6Hz), 3.20 (m, 4H,), 4.35 (m, 1H), 4.29 (m, 1H), 4.74 (m, 1H), 5.01 (s, 2H), 6.88 (d, 2H, *J* = 8.7 Hz), 7.16 (d, 2H, *J* = 8.4 Hz), 7.35 (m, 5H), 8.28 (s, 1H), 11.42 (s, 1H).

35b:



Yield 83%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.45 (s, 18H), 1.97 (m, 4H), 2.17 (m, 2H), 2.19 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.20 (m, 4H,), 4.35 (m, 1H), 4.29 (m, 1H), 4.74 (m, 1H), 5.01 (s, 2H), 6.88 (d, 2H, *J* = 8.7 Hz), 7.18 (d, 2H, *J* = 8.4 Hz), 9.51 (s, 1H), 11.21 (s, 1H).

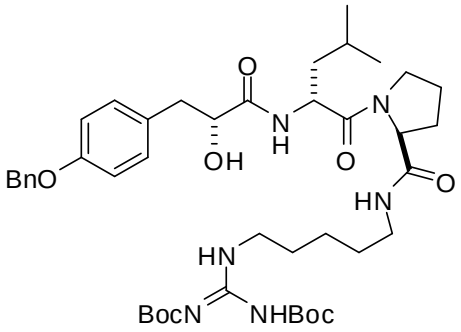
Compound 35:

Light yellow powder. Yield 91%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H),1.97 (m, 4H), 2.17 (m, 2H), 2.19 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.20 (m, 4H,), 4.35 (m, 1H), 4.29 (m, 1H), 4.74 (m, 1H), 5.01 (s, 2H), 6.88 (d, 2H, *J* = 8.7 Hz), 7.18 (d, 2H, *J* = 8.4 Hz), 9.51 (s, 1H), 11.21 (s, 1H); HRMS calcd for $C_{25}H_{40}N_6O_5$+H 505.3138, found 505.3140.

Preparation of teterapeptide 36

The intermediates for the preparation of compound **36**:

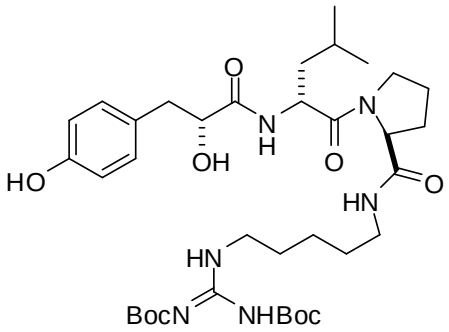
36a:



Yield 78%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (m, 6H), 1.30 (2H, m), 1.41 (s, 18H), 1.51 (m, 4H), 2.20 (m, 4H), 2.21 (m, 2H), 2.82 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.01 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.20 (m, 4H), 3.35 (2H, m), 4.02 (m, 2H), 4.33 (m, 1H,) 4.41 (m, 1H), 4.52 (m, 1H), 5.01

(s, 2H), 6.88 (d, 2H, *J* = 8.7 Hz), 7.16 (d, 2H, *J* = 8.4 Hz), 7.35 (m, 5H), 8.28 (s, 1H), 11.48 (s, 1H).

36b:



Yield 84%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.30 (2H, m), 1.41 (s, 18H), 1.51 (m, 4H), 2.20 (m, 4H), 2.21 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.20 (m, 4H), 3.35 (2H, m), 4.02 (m, 2H), 4.33 (m, 1H,) 4.41 (m, 1H), 4.52 (m, 1H), 5.01 (s, 2H), 6.73 (d, 2H, *J* = 8.0 Hz), 7.04 (d, 2H, *J* = 8.0 Hz), 9.52 (s, 1H), 11.13 (s, 1H).

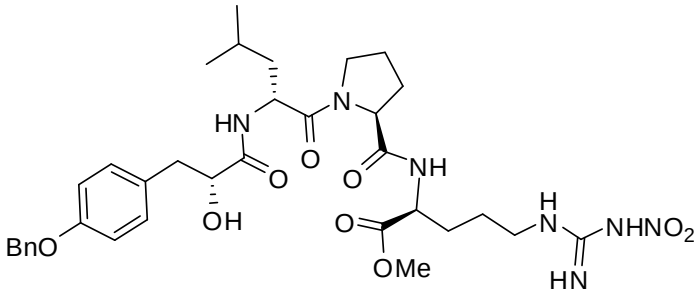
Compound 36:

White powder. Yield = 98%, ^{1}H NMR (CD_3OD, 400 MHz) δ 0.87 (m, 6H), 1.30 (2H, m), 1.51 (m, 4H), 2.20 (m, 4H), 2.21 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.20 (m, 4H), 3.35 (2H, m), 4.02 (m, 2H), 4.33 (m, 1H,) 4.41 (m, 1H), 4.52 (m, 1H), 5.01 (s, 2H), 6.67 (d, 2H, *J* = 8.4 Hz), 7.06 (d, 2H, *J* = 8.4 Hz), 7.77 (s, 1H), 7.78 (s, 1H); HRMS calcd for $C_{26}H_{42}N_6O_5$+H 519.3925, found 519.3928.

Preparation of teterapeptide 37

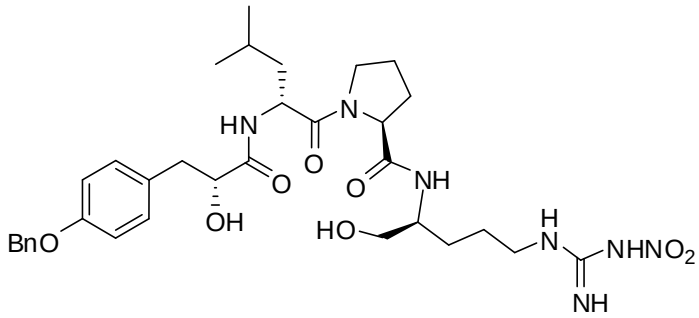
The intermediates for the preparation of compound **37**:

37a:



Yield 82%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.12 (m, 1H), 1.25 (t, 3H, *J* = 7.3 Hz), 1.90 (m, 2H), 2.01 (m, 2H), 2.10 (m, 2H), 2.51 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 3.72 (s, 3H), 4.45 (m, 1H), 4.68 (m, 1H), 4.74 (m, 1H), 5.01 (s, 2H), 6.93 (d, 2H, *J* = 8.0 Hz), 7.17 (d, 2H, *J* = 8.4 Hz), 7.44-7.30 (m, 5H), 8.43 (s, 1H).

37b:



Yield 79%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.12 (m, 1H), 1.25 (t, 3H, *J* = 7.3Hz), 1.90 (m, 2H), 2.01 (m, 2H), 2.10 (m, 2H), 2.51 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.25 (m, 2H), 3.47 (t, 2H, *J* = 7.6 Hz), 4.45 (m, 1H), 4.68 (m, 1H),

4.74 (m, 1H), 5.01 (s, 2H), 7.12 (d, 2H, *J* = 8.2 Hz), 7.17 (d, 2H, *J* = 8.3 Hz), 7.44-7.30 (m, 5H), 8.53 (s, 1H).

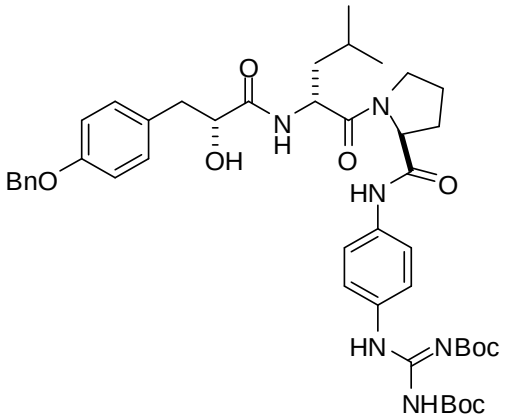
Compound 37:

White solid. Yield 82%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.12 (m, 1H), 1.25 (t, 3H, *J* = 7.3 Hz), 1.90 (m, 2H), 2.01 (m, 2H), 2.10 (m, 2H), 2.51 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.25 (m, 2H), 3.47 (t, 2H, *J* = 7.6 Hz), 4.45 (m, 1H), 4.68 (m, 1H), 4.74 (m, 1H), 5.01 (s, 2H), 7.12 (d, 2H, *J* = 8.2 Hz), 7.17 (d, 2H, *J* = 8.3 Hz). HRMS Calcd for $C_{26}H_{42}N_6O_6$+H is 535.3244, found 534.3234.

Preparation of teterapeptide 38

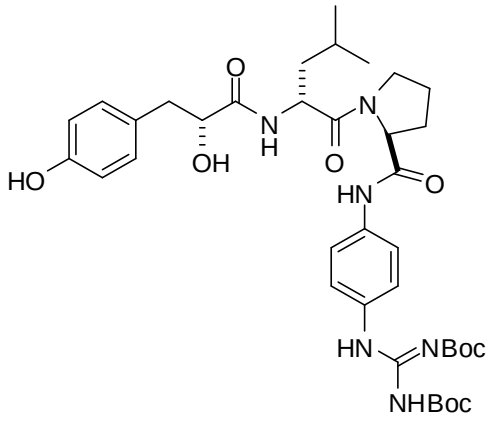
The intermediates for the preparation of compound **38**:

38a:



Yield 68%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.42 (s, 18H), 2.01 (m, 2H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.35 (m, 1H), 4.29 (m, 1H), 4.84 (m, 1H), 4.98 (s, 2H), 6.85 (d, 2H, *J* = 8.4 Hz), 7.15 (d, 2H, *J* = 8.4 Hz), 7.44-7.30 (m, 5H), 7.46 (d, 2H, *J* = 9.1 Hz) 7.65 (d, 2H, *J* = 9.1 Hz), 11.60 (s, 1H), 10.22 (s, 1H).

38b:



Yield 89 %, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.42 (s, 18H), 2.01 (m, 2H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.35 (m, 1H), 4.29 (m, 1H), 4.84 (m, 1H), 4.98 (s, 2H), 6.85 (d, 2H, *J* = 8.4 Hz), 7.15 (d, 2H, *J* = 8.4 Hz), 7.46 (d, 2H, *J* = 9.1 Hz) 7.65 (d, 2H, *J* = 9.1 Hz), 10.22 (s, 1H), 11.88 (s, 1H).

Compound 38:

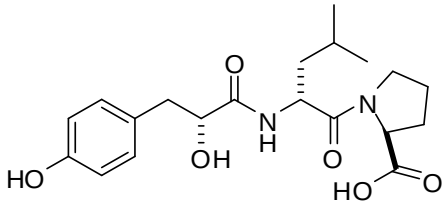
Yellow powder. Yield 99%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.25 (t, 3H, *J* = 7.3 Hz), 2.01 (m, 2H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.35 (m, 1H), 4.29 (m, 1H), 4.84 (m, 1H), 4.98 (s, 2H),

6.85 (d, 2H, *J* = 8.4 Hz), 7.15 (d, 2H, *J* = 8.4 Hz), 7.46 (d, 2H, *J* = 9.2 Hz), 7.65 (d, 2H, *J* = 9.1 Hz), 10.22 (s, 1H), 11. (s, 1H); HRMS calcd for $C_{27}H_{36}N_6O_5$+H 525.2825, found 525.2833.

Preparation of teterapeptide 39

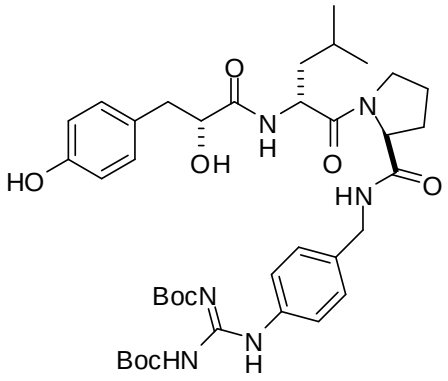
The intermediates for the preparation of compound **39**:

39a:



Yield 95%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.25 (t, 3H, *J* = 7.3 Hz), 2.01 (m, 2H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.02 (s, 2H), 4.35 (m, 1H), 4.29 (m, 1H), 4.74 (m, 1H), 6.93 (d, 2H, *J* = 8.0 Hz), 7.17 (d, 2H, *J* = 8.4 Hz).

39b:



Yield 75%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.42 (s, 18H), 2.01 (m, 2H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.35 (m, 1H), 4.29 (m, 1H), 4.84 (m, 1H), 4.98 (s, 2H), 6.85 (d, 2H, *J* = 8.4 Hz), 7.15 (d, 2H, *J* = 8.4 Hz), 7.46 (d, 2H, *J* = 9.1 Hz) 7.65 (d, 2H, *J* = 9.1 Hz), 11.60 (s, 1H), 10.22 (s, 1H).

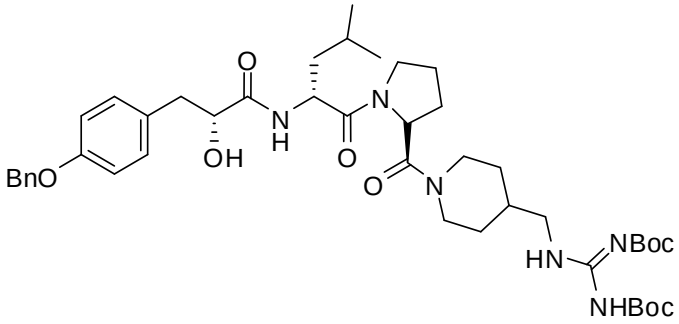
Compound 39:

Light yellow gummy solid. Yield 95%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.25 (t, 3H, *J* = 7.3 Hz), 2.01 (m, 2H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.35 (m, 1H), 4.29 (m, 1H), 4.84 (m, 1H), 4.98 (s, 2H), 6.85 (d, 2H, *J* = 8.4 Hz), 7.15 (d, 2H, *J* = 8.4 Hz), 7.46 (d, 2H, *J* = 8.7 Hz) 7.65 (d, 2H, *J* = 8.9 Hz), 10.22 (s, 1H), 11.00 (s, 1H); MS calcd for $C_{28}H_{38}N_6O_5$ 538.29, found 538.40.

Preparation of teterapeptide 40

The intermediates for the preparation of compound **40**

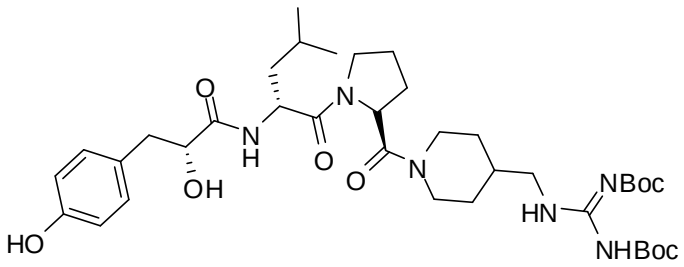
40a:



Yield 58%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.45 (s, 18H), 1.65 (m, 8H), 1.81 (m, 2H), 2.01 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.31 (m,

8H), 4.03 (m, 2H), 4.26 (m, 1H,) 4.45 (m, 1H), 4.51 (m, 1H), 5.03 (s, 2H), 6.91 (d, 2H, *J* = 8.7 Hz), 7.17 (d, 2H, *J* = 8.7 Hz), 7.35 (m, 5H).

40b:



Yield 87%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.45 (s, 18H), 1.65 (m, 8H), 1.81 (m, 2H), 2.01 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.31 (m, 8H), 4.03 (m, 2H), 4.26 (m, 1H,) 4.45 (m, 1H), 4.51 (m, 1H), 5.03 (s, 2H), 6.91 (d, 2H, *J* = 8.7 Hz), 7.17 (d, 2H, *J* = 8.7 Hz), 8.42 (s, 1H), 11.41 (s, 1H).

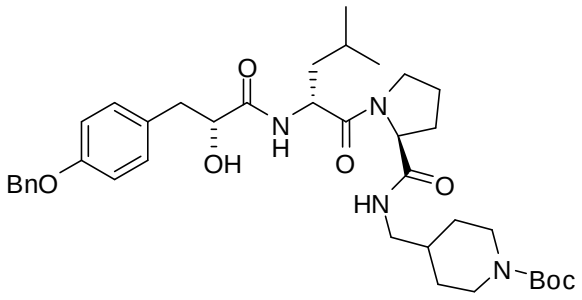
Compound 40:

Colorless gummy material. Yield 95%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.45 (s, 18H), 1.65 (m, 8H), 2.01 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.31 (m, 8H), 4.03 (m, 2H), 4.26 (m, 1H,) 4.45 (m, 1H), 4.51 (m, 1H), 5.03 (s, 2H), 6.91 (d, 2H, *J* = 8.5 Hz), 6.96 (d, 2H, *J* = 8.6 Hz); MS calcd for $C_{27}H_{42}N_6O_5$+H 530.66, found 531.60.

Preparation of teterapeptide 41

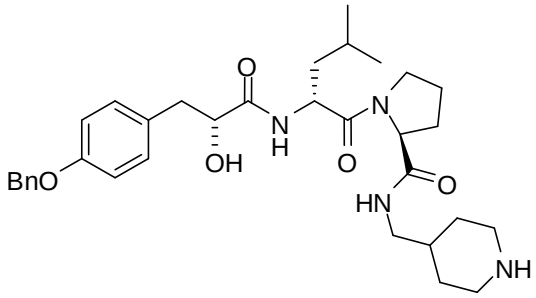
The intermediates for the preparation of compound **41**:

41a:



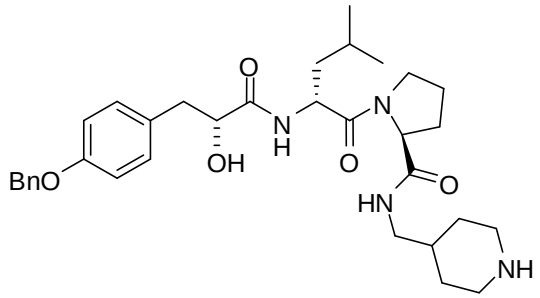
Yield 74% ,^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.45 (s, 9H), 1.65 (m, 8H), 1.81 (m, 2H), 2.01 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.31 (m, 8H), 4.03 (m, 2H), 4.26 (m, 1H,) 4.45 (m, 1H), 4.51 (m, 1H), 5.03 (s, 2H), 6.91 (d, 2H, *J* = 8.7 Hz), 7.17 (d, 2H, *J* = 8.7 Hz), 7.35 (m, 5H).

41b:



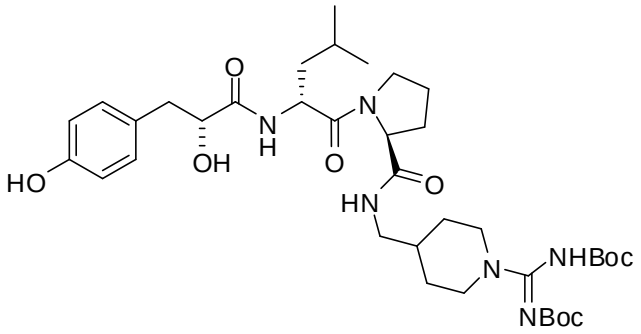
Yield 99%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.65 (m, 8H), 1.81 (m, 2H), 2.01 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.31 (m, 8H), 4.03 (m, 2H), 4.26 (m, 1H,) 4.45 (m, 1H), 4.51 (m, 1H), 5.03 (s, 2H), 6.91 (d, 2H, *J* = 8.7 Hz), 7.17 (d, 2H, *J* = 8.4 Hz), 7.37 (m, 5H).

41c:



Yield 75%, ^{1}H NMR (CD_3OD, 400 MHz) δ 0.87 (m, 6H), 1.45 (s, 18H), 1.65 (m, 8H), 1.81 (m, 2H), 2.01 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.31 (m, 8H), 4.03 (m, 2H), 4.26 (m, 1H,) 4.45 (m, 1H), 4.51 (m, 1H), 5.03 (s, 2H), 6.91 (d, 2H, *J* = 8.7 Hz), 7.17 (d, 2H, *J* = 8.6 Hz), 7.37 (m, 5H)**,** 10.21 (s, 1H).

41d:



Yield 88%, ^{1}H NMR (CD_3OD, 400 MHz) δ 0.87 (m, 6H), 1.45 (s, 18H), 1.65 (m, 8H), 1.81 (m, 2H), 2.01 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.31 (m, 8H), 4.03 (m, 2H), 4.26 (m, 1H,) 4.45 (m, 1H), 4.51 (m, 1H), 5.03 (s, 2H), 6.91 (d, 2H, *J* = 8.7 Hz), 7.17 (d, 2H, *J* = 8.6 Hz).

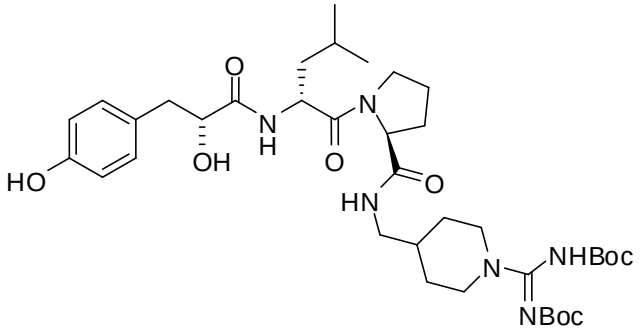
Compound 41:

Light brown gummy material. Yield 98%, ^{1}H NMR (CD_3OD, 400 MHz) δ 0.87 (m, 6H), 1.65 (m, 8H), 1.81 (m, 2H), 2.01 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.31 (m, 8H), 4.03 (m, 2H), 4.26 (m, 1H,) 4.45 (m, 1H), 4.51 (m, 1H), 5.03 (s, 2H), 6.91 (d, 2H, *J* = 8.7 Hz), 7.17 (d, 2H, *J* = 8.6 Hz). HRMS calcd for $C_{27}H_{42}N_6O_5$+H 531.3295, found 531.3182.

Preparation of teterapeptide 42

The intermediates for the preparation of compound **42**:

42b:



Yield 87%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.45 (s, 9H), 1.65 (m, 8H), 1.81 (m, 2H), 2.01 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.31 (m, 8H), 4.03 (m, 2H), 4.26 (m, 1H,) 4.45 (m, 1H), 4.51 (m, 1H), 5.03 (s, 2H), 6.91 (d, 2H, *J* = 8.7 Hz), 7.17 (d, 2H, *J* = 8.7 Hz).

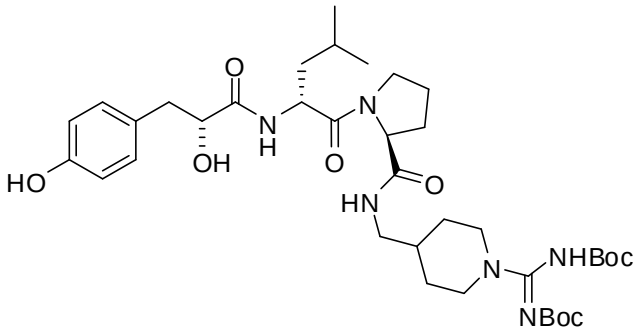
Compound 42:

Colorless gummy material. Yield 95%, ^{1}H NMR (CD_3OD, 400 MHz) δ 0.87 (m, 6H), 1.65 (m, 8H), 1.81 (m, 2H), 2.01 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6Hz), 3.31 (m, 8H), 4.03 (m, 2H), 4.26 (m, 1H,) 4.45 (m, 1H), 4.51 (m, 1H), 5.03 (s, 2H), 6.91 (d, 2H,

J = 8.7 Hz), 7.17 (d, 2H, *J* = 8.7 Hz); HRMS calcd for $C_{24}H_{38}N_6O_5$+H 500.2873, found 500.2856.

Preparation of teterapeptide 43

43a:



Yield 85%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.21 (s, 2H), 1.25 (t, 3H, *J* = 7.3Hz), 2.01 (m, 2H), 2.10 (m, 2H), 2.58 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.02 (t, 2H, *J* = 7.2 Hz) 4.35 (m, 1H), 4.29 (m, 1H), 4.42 (m, 2H) 4.58 (m, 1H), 5.03 (s, 2H), 6.93 (d, 2H, *J* = 8.0 Hz), 6.94 (s, 1H) 7.17 (d, 2H, *J* = 8.4 Hz), 7.38 (m, 5H), 7.82 (s, 1H).

Compound 43:

White solid. Yield 92%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (m, 6H), 1.21 (s, 2H), 1.25 (t, 3H, *J* = 7.3Hz), 2.01 (m, 2H), 2.10 (m, 2H), 2.58 (m, 2H), 2.81 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.02 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.02 (t, 2H, *J* = 7.2 Hz) 4.35 (m, 1H), 4.29 (m, 1H), 4.42 (m, 2H) 4.58 (m, 1H), 5.03 (s, 2H), 6.93 (d, 2H, *J* = 8.0 Hz), 6.94 (s, 1H) 7.17 (d, 2H, *J* = 8.4 Hz), 7.82 (s, 1H); HRMS calcd for C26H37N5O5+H 500.2873, found 500.2856.

Preparation of teterapeptide 44

The intermediates for the preparation of compound **44**:

Compound 44:

White solid, yield 85%, NMR: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.25 (t, 3H, *J* = 7.3 Hz), 2.01 (m, 2H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.35 (m, 1H), 4.29 (m, 1H), 4.74 (m, 1H), 4.98 (s, 2H), 6.93 (d, 2H, *J* = 8.1 Hz), 7.17 (d, 2H, *J* = 8.4 Hz), 7.33 (d, 2H, *J* = 8.4 Hz), 7.47 (d, 2H, *J* = 8.3 Hz); HRMS calcd for $C_{28}H_{34}N_4O_5$+H 507.2607, found 507.2609.

Preparation of teterapeptide 45

Compound 45:

White solid. Yield 75%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.25 (t, 3H, *J* = 7.3 Hz), 2.01 (m, 2H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.35 (m, 1H), 4.29 (m, 1H), 4.74 (m, 1H), 4.79 (s, 2H), 6.93 (d, 2H, *J* = 8.1 Hz), 7.17 (d, 2H, *J* = 8.4 Hz), 7.33 (d, 2H, *J* = 8.1 Hz), 7.52 (d, 2H, *J* = 8.1 Hz); HRMS calcd for $C_{27}H_{34}N_4O_7$+H 527.2506, found 527.2493.

Preparation of teterapeptide 46

Compound 46:

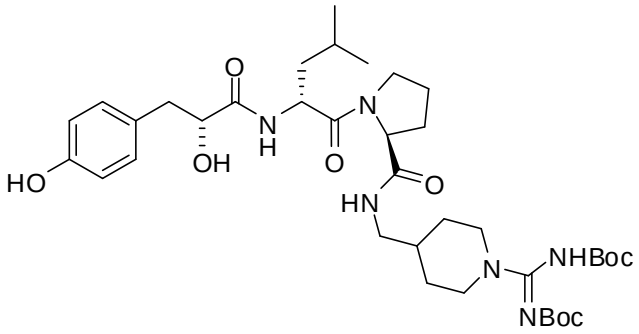
Light yellow sticky semisolid. Yield 57%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.25 (t, 3H, *J* = 7.3 Hz), 2.01 (m, 2H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.02 (s, 2H), 4.35 (m, 1H), 4.29 (m, 1H),

4.74 (m, 1H), 6.93 (d, 2H, *J* = 8.0 Hz), 7.17 (d, 2H, *J* = 8.4 Hz), 7.8 (d, 2H, *J* = 7.2 Hz), 8.15 (d, 2H, *J* = 7.3Hz); HRMS Calcd for $C_{27}H_{34}N_4O_7$+H is 482.2506, found 482.2519.

Preparation of teterapeptide 47

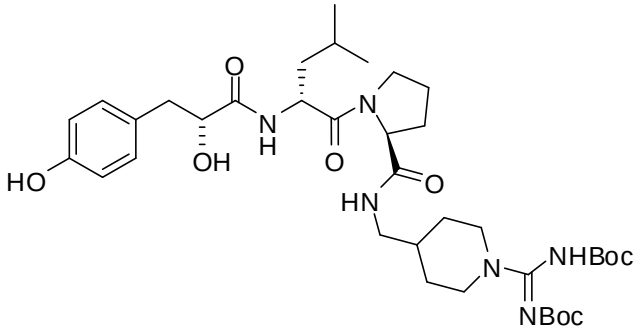
The intermediates for the preparation of compound **47**:

47a:



Yield 85%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.25 (t, 3H, *J* = 7.3Hz), 2.01 (m, 2H), 2.07 (s, 3H), 2.10 (s, 3H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 2.89 (s, 3H), 2.91 (s, 3H), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.02 (s, 2H), 4.35 (m, 1H), 4.29 (m, 1H), 4.74 (m, 1H), 6.93 (d, 2H, *J* = 8.0 Hz), 7.17 (d, 2H, *J* = 8.4 Hz), 8.00 (s, 1H).

47b:



Yield 78%, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.25 (t, 3H, *J* = 7.3 Hz), 2.01 (m, 2H), 2.07 (s, 3H), 2.10 (s, 3H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.02 (s, 2H), 4.35 (m, 1H), 4.29 (m, 1H), 4.74 (m, 1H), 5.05 (s, 2H), 6.93 (d, 2H, *J* = 8.0 Hz), 7.17 (d, 2H, *J* = 8.4 Hz), 7.30 (m, 3H).

Compound 47:

White powder, yield 95%**,** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (m, 6H), 1.1 (m, 1H), 1.25 (t, 3H, *J* = 7.3 Hz), 2.01 (m, 2H), 2.10 (m, 2H), 2.85 (dd, 1H, *J* = 14.2, 6.9 Hz), 3.05 (dd, 1H, *J* = 14.2, 3.6 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 4.02 (s, 2H), 4.35 (m, 1H), 4.29 (m, 1H), 4.74 (m, 1H), 5.05 (s, 2H), 6.93 (d, 2H, *J* = 8.0 Hz), 7.17 (d, 2H, *J* = 8.4 Hz), 7.30 (m, 3H). HRMS calcd for $C_{27}H_{33}Cl_2N_3O_5$+H 550.2087, found 550.2087.

3. General procedures for enzyme inhibition assays

Thrombin from bovine plasma, trypsin from bovine pancreas, trypsin inhibitor from chicken egg white, and trypsin substrate N-benzoyl-DL-arginine-4-nitroanilide hydrochloride were purchased from Sigma; chromogenic thrombin substrate, H-D-HHTAla-Arg-pNA.2AcOH (SPECTROZYME), was obtained from American Diagnostica Inc. Other common chemicals and reagents were purchased from Aldrich or Lancaster. The known thrombin inhibitor, PPACK, was purchased from Calbiochem (EMD bioscience). Standard enzyme assay procedures and chromogenic substrates were used for both enzymes. UV absorbance of the cleaved p-nitroaniline was monitored at 405 nm on a UV spectrophotometer. Typical inhibitor concentrations are 1.0, 0.50, 0.25, 0.13, 0.063, 0.031 mg/mL, and 0 (buffer was used instead of

inhibitor). Further serial dilutions were performed for compounds that showed above 90% inhibition at the lowest concentration tested. In general, test compounds were dissolved in distilled water or a small amount of DMSO and then diluted with distilled water. Thrombin buffer is composed of 10 mM Tris–HCl (pH = 8), 150 mM of NaCl, 10 mM of HEPES, 0.10% of PEG6000 in distilled water. Enzyme assays were performed in 96-well microtiter plates using a microtiter plate reader (Biotek Powerwave XS Spectrophotometer). A mixture of 50 mL of inhibitor solution, 20 mL of bovine thrombin solution (0.25 NIH units/mL), and 30 mL thrombin buffer solution was incubated at 37 °C for 10 min, then 50 mL of substrate Spectrozyme (0.032 mg/mL) was added to each cell, this final mixture was then incubated for 30 min at 37 °C with slow shaking. The total volume in the cell was 150 mL. The UV absorbance at 405 nm before adding substrate was recorded as the blank. The absorbance at 405 nm was measured immediately after the incubation. The IC_{50} value was obtained from the curve of inhibitor concentration versus absorbance, the final data was obtained as an average of triplicate experiments.

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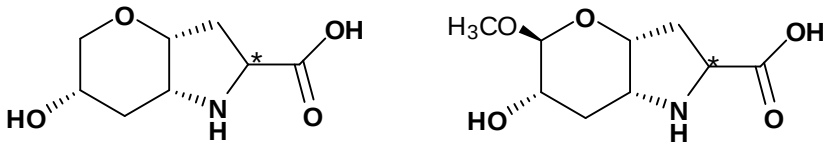
Chapter 3

Synthesis of aeruginosin 298-A analogs: modification at P2 using O-Choi (bicyclic unnatural amino acids) derived from sugars

3.1 Abstract

Aeruginosins are a family of marine natural products containing mostly non-proteogenic amino acids. These compounds contain a common 2-carboxy-6-hydroxy-octaindole (Choi) rigid bicyclic structure. Many aeruginosins are inhibitors for enzymes involved in the blood coagulation cascade, such as thrombin and Factor VIIa. In order to understand the structure activity relationship (SAR) of the aeruginosins and to discover novel anticoagulants with potentially improved inhibitory and pharmacokinetic properties, we have synthesized a series of novel analogs of aeruginosin 298-A, in which the Choi will be replaced with oxygenated Choi analogs, and the Argol is replaced with various other functionalities. These new O-Choi have been derived from sugar and are unnatural amino acids which have been replaced with the Choi (P2) moiety of aeruginosin. The preparation of oxygenated Choi analogs starting from glucose using a new method will be presented.



3.2. Introduction

Thrombin (factor IIa) is a key trypsin like serine protease of the blood coagulation and is also a potent activator of platelet aggregation.[1-4] Thrombin cause thrombosis and if not treated, it can lead to heart stroke, pulmonary diseases and many life threatening deaths. Hence design and synthesis of thrombin inhibitor can be very important in treating or preventing thrombosis. In recent years many thrombin inhibitors have been design and synthesized based on the crystal structure of thrombin.[5]

Despite much research effort, it is still very challenging to find an orally active anticoagulant which is safe and effective agent for treating or preventing thrombosis disorders. The major anticoagulants heparin and warfarin were introduced about 50 years ago. Currently recombinant hirudin and argatroban are approved for the treatment of thrombosis associated with heparin induced thrombocytopenia (HIT). Hirudin (66 amino acids) and its analogue hirulog (20 amino acids) are polypeptides isolated from medicinal leeches.9 Argatroban is a synthetic small molecule thrombin inhibitor which was recently approved for use as an anticoagulant for treating prophylaxis, HIT, and associated thrombotic complications.10 Like heparin, both hirudin and argatroban are administered parenterally. Ximelagatran is an orally active thrombin inhibitor and drug.11 In recent years, a new class of marine nature products, the aeruginosins, were isolated from cyanobacterial blooms.[6-15] They are small linear peptides containing unnatural amino acids and they are found to be serine protease inhibitors. The approximately 16 compounds in this family share a common new bicyclic amino acid core structure 2-carboxy-6-hydroxyoctahydroindole (Choi).

The X-ray crystal structures of thrombin being inhibited by several aeruginosins have been obtained through the inhibitor and thrombin-hirugen complexes.[5] The binding mode of aeruginosin 298-A resembles closely that of D-Phe-Pro-Arg chloromethyl ketone and other serine protease inhibitors. Aeruginosin 298-A binds to the active site of thrombin in a monovalent way forming an antiparallel strand with thrombin.[15] The five-membered ring of the Choi residue occupies the hydrophobic binding site, while its six-membered ring projects out and loosely interacts with Try 60 and Tyr 60 from thrombin. The active site where the Choi group interacts has many degrees of freedom. The crystal structures of oscillarin and dysinosin-A thrombin complexes revealed similar binding patterns as aeruginosin 298-A. Dysinosin-A forms a hydrogen bonding network with thrombin complex, but the 5,6-dihydroxyoctahydroindole group appears not to have hydrogen bonding interactions with the P2 binding pocket even though there are polar amino acid residues in the pocket 25 The crystal structure of oscillarin with thrombin complex is shown in Figure 3.2. The amide NH from the octahydroindole carboxamide forms a hydrogen bond with Ser 256, however no interactions were observed with the 6-hydroxyl group of the Choi moiety. The terminal phenyl group has no interaction with the enzyme.22 In all the crystal structures of aeruginosin-thrombin complexes so far, Choi moieties of the aeruginosins which occupy the S2 binding site generally have a good degree of freedom. Thrombin generally can tolerate imprecise binding from different molecules. The rigid bicyclic amino acid structure is very important in defining the conformation of the molecule and it is essential in their antithrombin activity. The 6-hydroxyl group projects out and the Choi core is not interacting strongly with residues at the active site. Thus modifications on the core structure in this area should not impact negatively on binding to the protein while some changes will increase contact and enhance binding. Such changes include introducing an oxygen at the C-4.

As pharmacokinetics are very unpredictable, these changes will provide other possibilities that might provide scope for improving the pharmacokinetic profile of this compound class if required. The availability of different stereoisomers is also important in elucidating structure-activity relationships. Studies to rationalize electronic and steric influence of different inhibitors have not given good correlations so far. Modification from the natural analogues can allow us to understand the stereoelectronic factors that determine the activity of these inhibitors. In recent years, a new class of marine natural products, the aeruginosin came into the picture. More than 20 compounds have been isolated in the Aeruginosin series. They are a class of serine protease inhibitors isolated from cyanobacterial blooms. They are a linear peptide containing a new bicyclic amino acid core structure known as 2-carboxy-6-hydroxyotahydroinhole (Choi). . These proposed Choi analogues are novel structures and will have great potential in discovering better thrombosis inhibitors. The 14 initially reported members mostly contain the configuration of 2*S*,3a*S*,6*R*,7a*S* in their azabicyclic core. The structure below represents one of the aeruginosin series compound named 298-A. It shows a value of inhibition against thrombin (IC_{50} = 0.3 μM) and trypsin (IC_{50} = 1.0 μM)

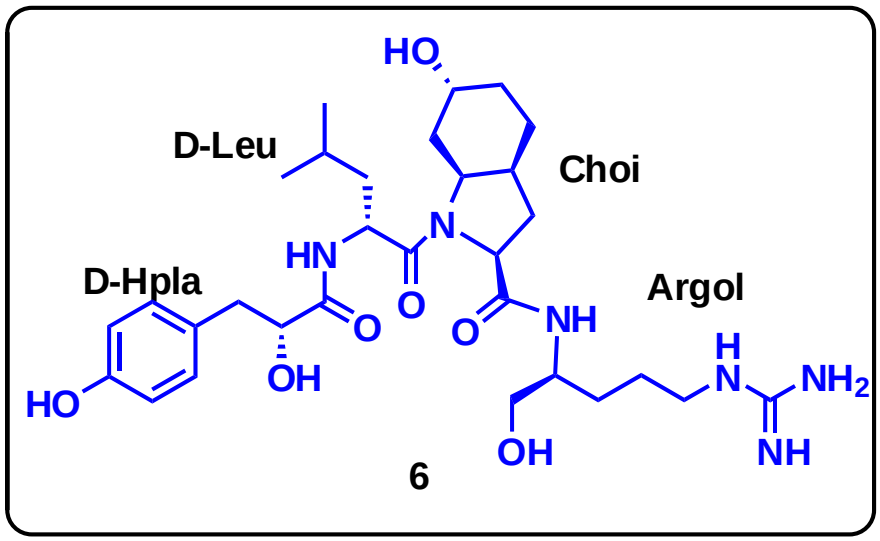


Figure 3.1. Structure of Aeruginosin 298-A.

The structures of aeruginosin 298-A is shown in Figure 3.1. Most of them exhibited high potency against thrombin making them attractive small-molecules targets in the search for new anticoagulents. These molecules are divided into three parts- P1, P2 and P3 subunits which correspond to the hydroxylphenyllactic acid (Hpla), Leucine, (P1), 2-carboxy-6-hydroxyotahydroinhole (Choi) (P2), and argininal (Argal) at P3.

The unusual structure and interesting biological activities of this class of molecules have attracted several research groups to carry out their total syntheses.[15-19] Several methods are also available for synthesizing the Choi core structure and its analogs.[20-23] Besides the synthesis of the aeruginosins, there is also a great interest in preparing analogs of the aeruginosin natural products and understanding the structure–activity relationship (SAR) pattern for these serine protease inhibitors. While several aeruginosin 298-A analogs have been synthesized, most of the modifications or variations of structures are on the units other than the Choi.[24-25] These include the preparation of a combinatorial library and the study of stereochemical influences of the

amino acid residues at P3, P4 or P1 to the trypsin inhibition activity[24] and several other analogs of aeruginosins with different amino acid residues.[25]

Our group has worked previously on the modification of p2 unit and has synthesized new bicyclic amino acids which can be derived from sugars.[21, 26-27] Different bicyclic unnatural amino acids can be derived from different sugars. However it's always been a challenge to synthesize them.

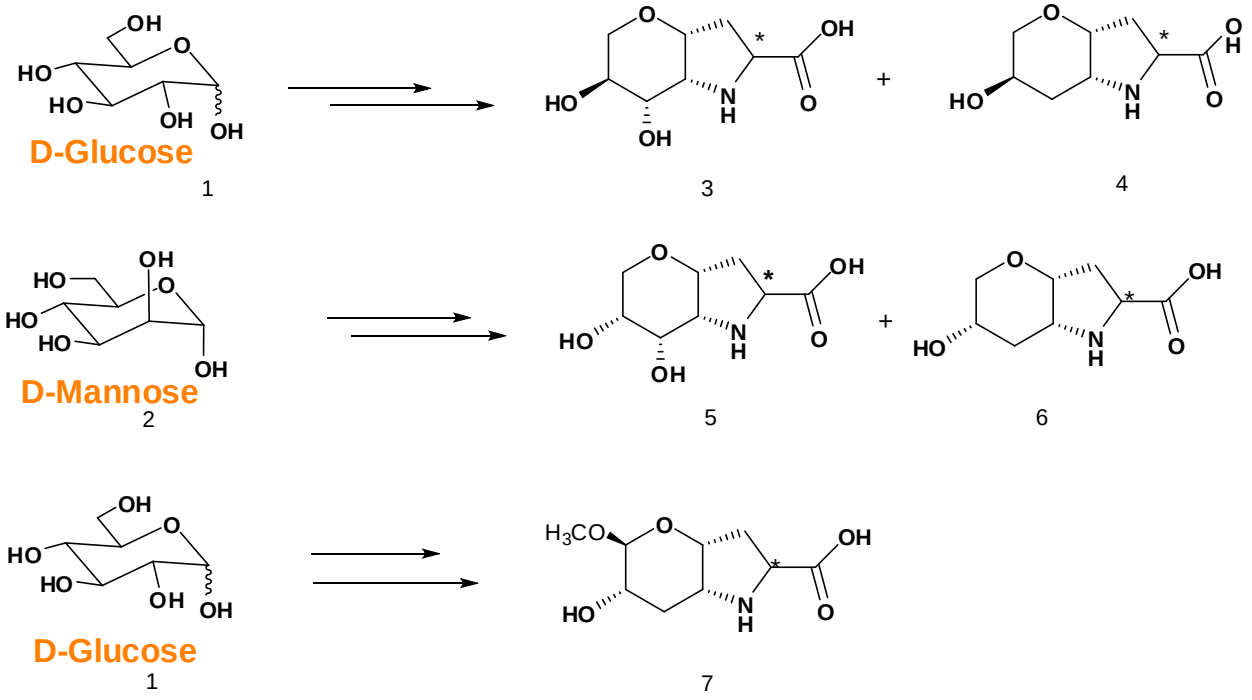
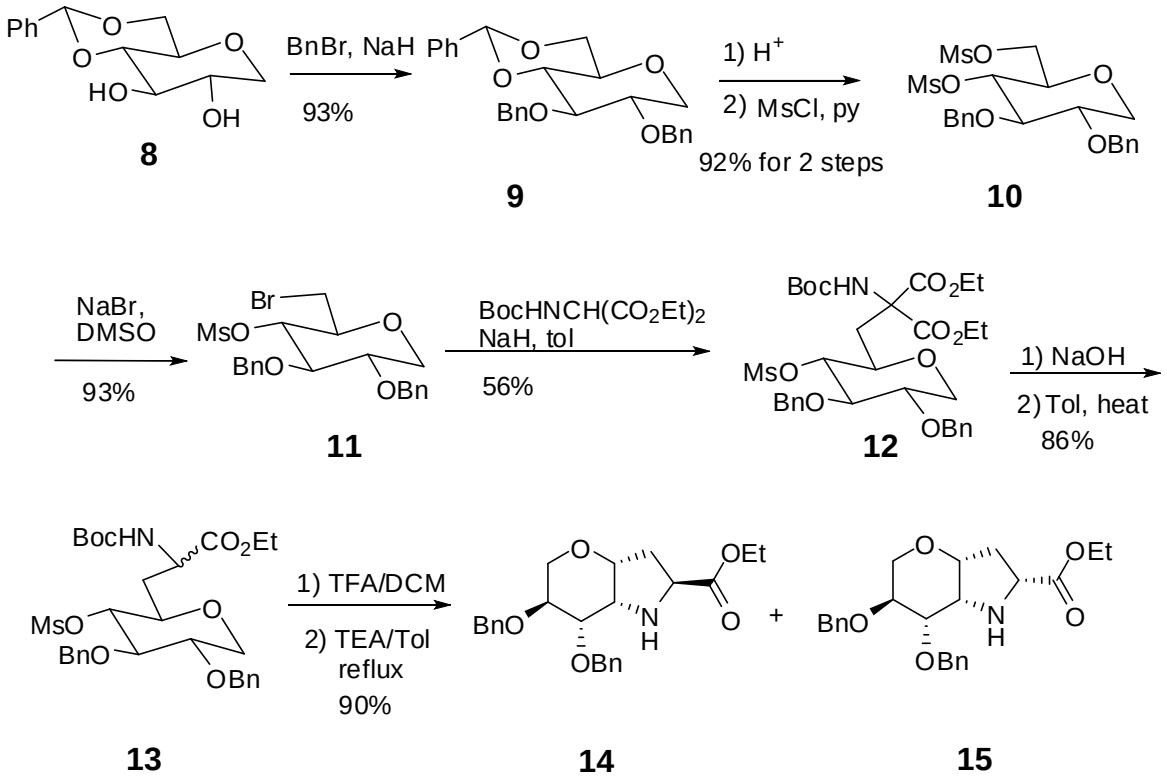


Figure 3.2. Different possible derived structure from different sugar

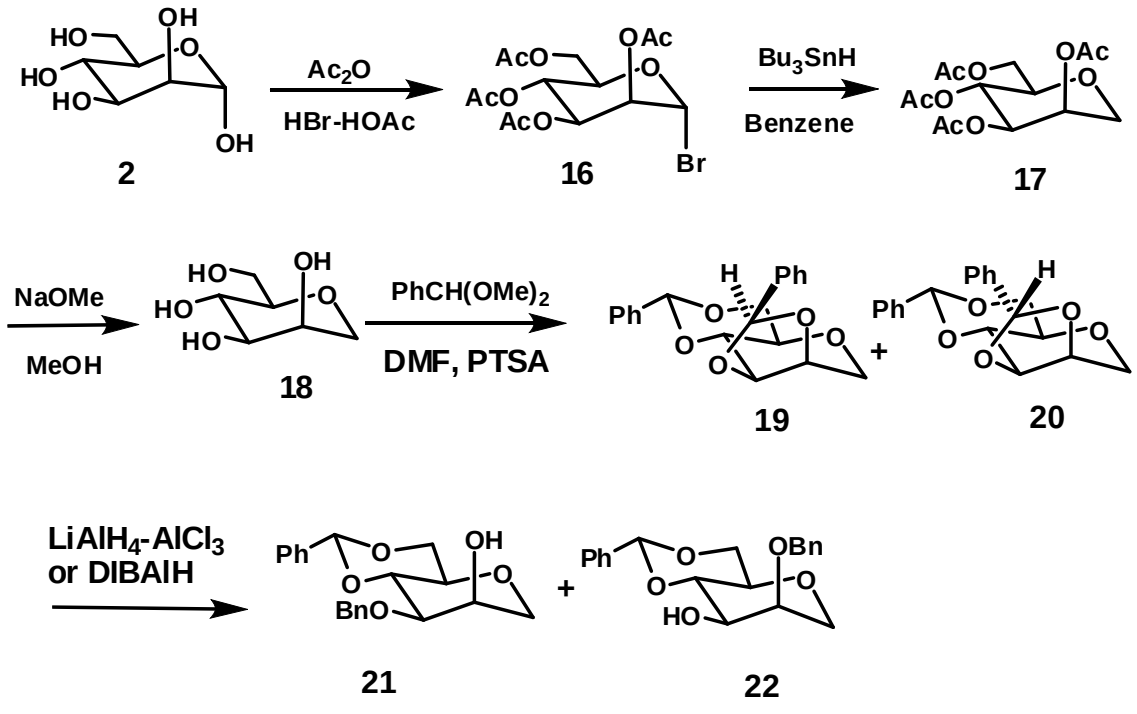
Synthesis of bicyclic amino acids form glucose has been achieved previously.[21] The general strategy to synthesize these structure has been shown in the Figure 3.2.

Starting from D-glucose, after several steps we can obtain the protected version of ring oxygenated Choi analogs **14** and **15.** Compound **8** can be prepared in 2-3 steps**.** It is then dibenzylated using sodium hydride and benzyl bromide, followed by deprotection of the acetal using acidic conditions and then mesylation to give the dimesylate **10**, this is then converted to the bromomesylate **11**, a key intermediate in the synthesis. The important step in this synthesis is the alkylation step to add a two carbon unit to the molecule to give compound **12**, which is then converted to the cyclized products smoothly. This method is applicable for other sugar starting material as well, this allows us to synthesize a series of choi analogs with different stereochemistry or substituents.



Scheme 3.1. Synthetic approach towards o-Choi synthesis

To do further structure activity relationship we wanted to see how stereochemistry would play a role in the activities of thrombin. We designed o-choi with a mannose configuration and designed our strategy in the same way as we did for the glucose. The D- mannose **2** was acetylated and anomeric group was substituted using bromo in one pot reaction to get compound **16**. The bromo group was reduced to obtain tetra acetate **17** and then the acetyl groups were hydrolysed and then it was protected using benzylidine acetal to get a mixture of two products **19** and **20**. In case of mannose 2, 3 hydroxyl and 4, 6 hydroxyl on sugar are cis to each other and its difficult to protect 4,6-OH with benzylidine acetal. The reaction worked fine but all four hydroxyl groups were protected. And then we tried to reduce the 5 membered acetal group, we get a mixture of 2 products, which is difficult to purify.

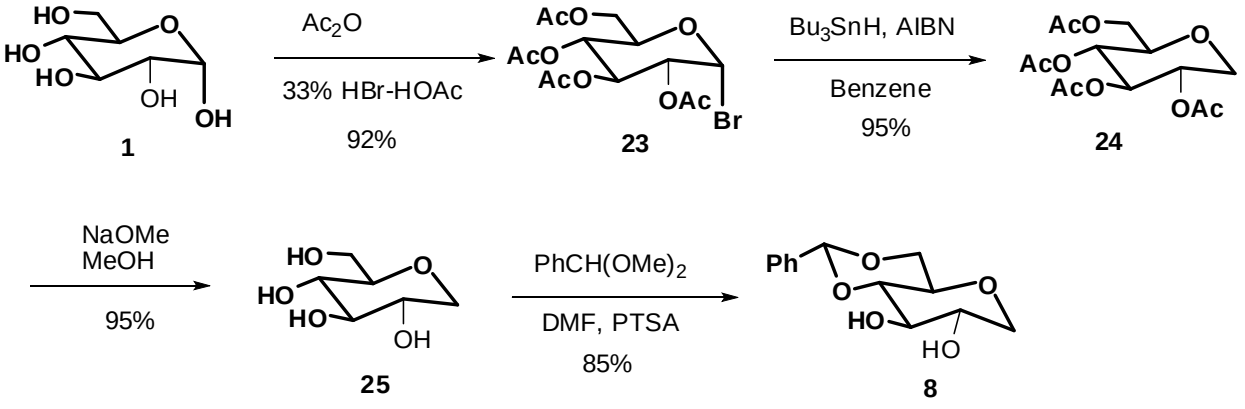


Scheme 3.2. Synthetic approach for O-Choi starting from D-mannose

The above scheme in not a good way to synthesize, as its very difficult to do the benzylidine protection at 4,6-position.

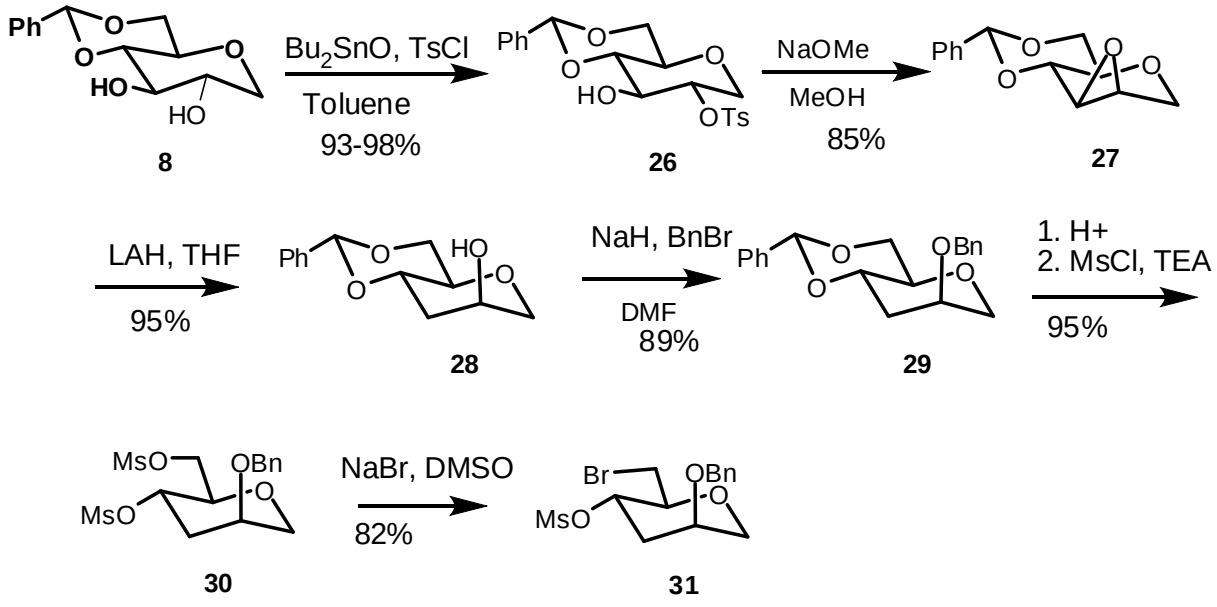
Our group recently published a paper where it is reported that using mitsunobu reaction the stereochemistry of 2-OBn can be transformed into mannose configuration.[28] However we wanted to comeup with a new idea where we could explore new chemistry and can comeup with high yield reactions.

Keeping that in mind we synthesized various aeruginosin analogs where we replaced Choi moiety at P2 by a new kind of bicyclic unnatural amino acid which was derived from sugar and the P1 and P3 moieties were also replaced by other functionalities. We modified our strategy and started our synthesis starting from d-glucose.



Scheme 3.3. Synthetic approach towards O-Choi (mannose configuration)

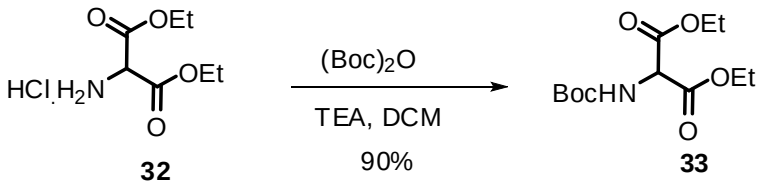
First 1-deoxy-4,6-benzylidine-2,3-hydroxy compound was synthesized starting from D-mannose. The D-glucose was aceylated and in one pot anomeric –OAc group was replaced by bromo. The bromo group at anomeric position was then reduced using *N*-dibutyl tin hydride to get tetra acetate sugar compound. All acetyl groups were hydrolyzed using sodium methoxide in methanol and the compound was dried using azeotorope toluene. Further using Benzylidine dimethyl acetal and a catalytic amount of PTSA 2,3-dihydroxy-4,6-benzylidine deoxy glucose was afforded.



Scheme 3.4. Synthetic approach towards O-Choi (mannose configuration contd.)

Further, the tosylation reaction was performed at 2-hydroxy using different conditions. Tosylation performed using pyridine and p-toluene sulfonyl chloride in different equivalents we always ended up with a mixture of 2-tosyl, 3-tosyl and 2,3-ditosyl product. Replacing pyridine by dibutyl tin oxide, the 2-tosyl product was formed predominately which was isolated using recrystallization on a small scale to large scale. And by this reaction its easy to scale up for **26**.

Further epoxidation ring formation reaction was performed in methanol using 4 equivalents of sodium methoxide to give clean product **27**. The epoxide ring was opened using lithium aluminum hydride in THF as a solvent to obtain 2-hydoxy compound with a mannose configuration **28**. The hydroxy group at C-2 was benzylated using sodium hydride and benzyl bromide in DMF to obtain **29**.The 4,6-benzylidine group was deprotected using 65% acetic acid at 65 °C. The 4,6-dihydroxyl were mesylated using methane sulfonyl chloride and triethyl amine in dichloro methane to compound **30**. The primary mesylate was replaced by bromo using sodium bromide in DMSO to obtain **31**.



32 **33**

Scheme 3.5. Boc-protection of diethyl malonate

For the cyclization purpose, the diethyl amino malonate-HCl **32** was protected using boc-anhydride and triethyl amine in dichloromethane to obtain compound **33**. The bromo compound was treated with mono boc-diethyl malonate ester using sodium hydride and TBAI and refluxed in toluene for 24 hours.

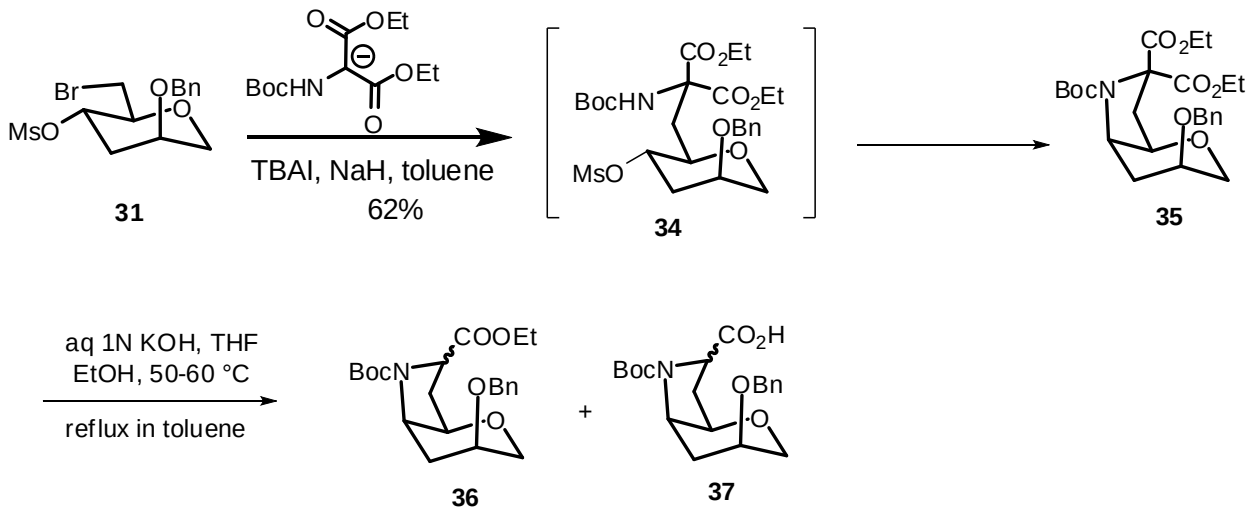
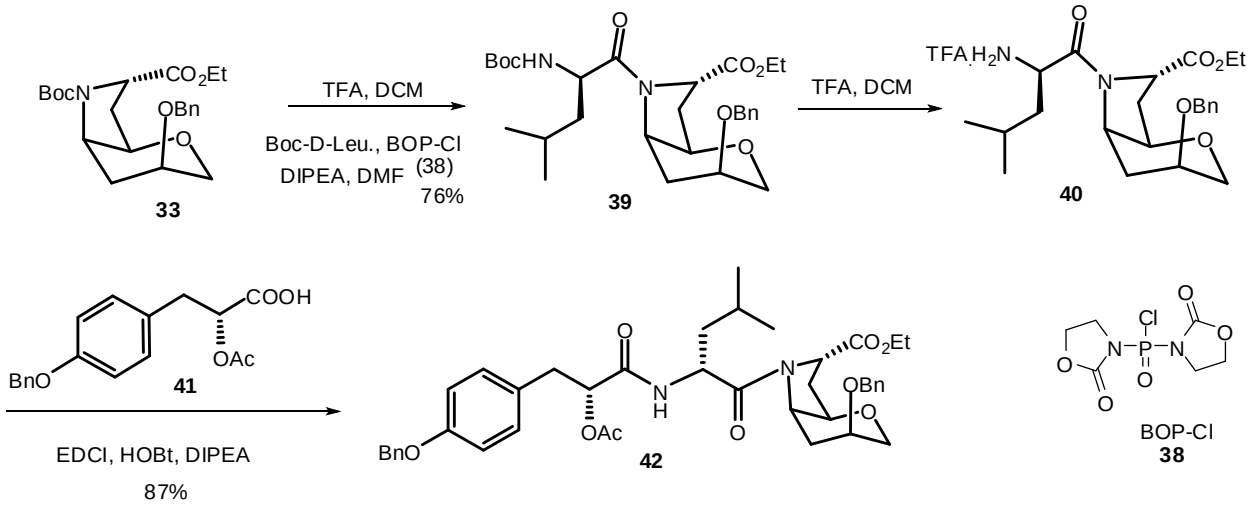


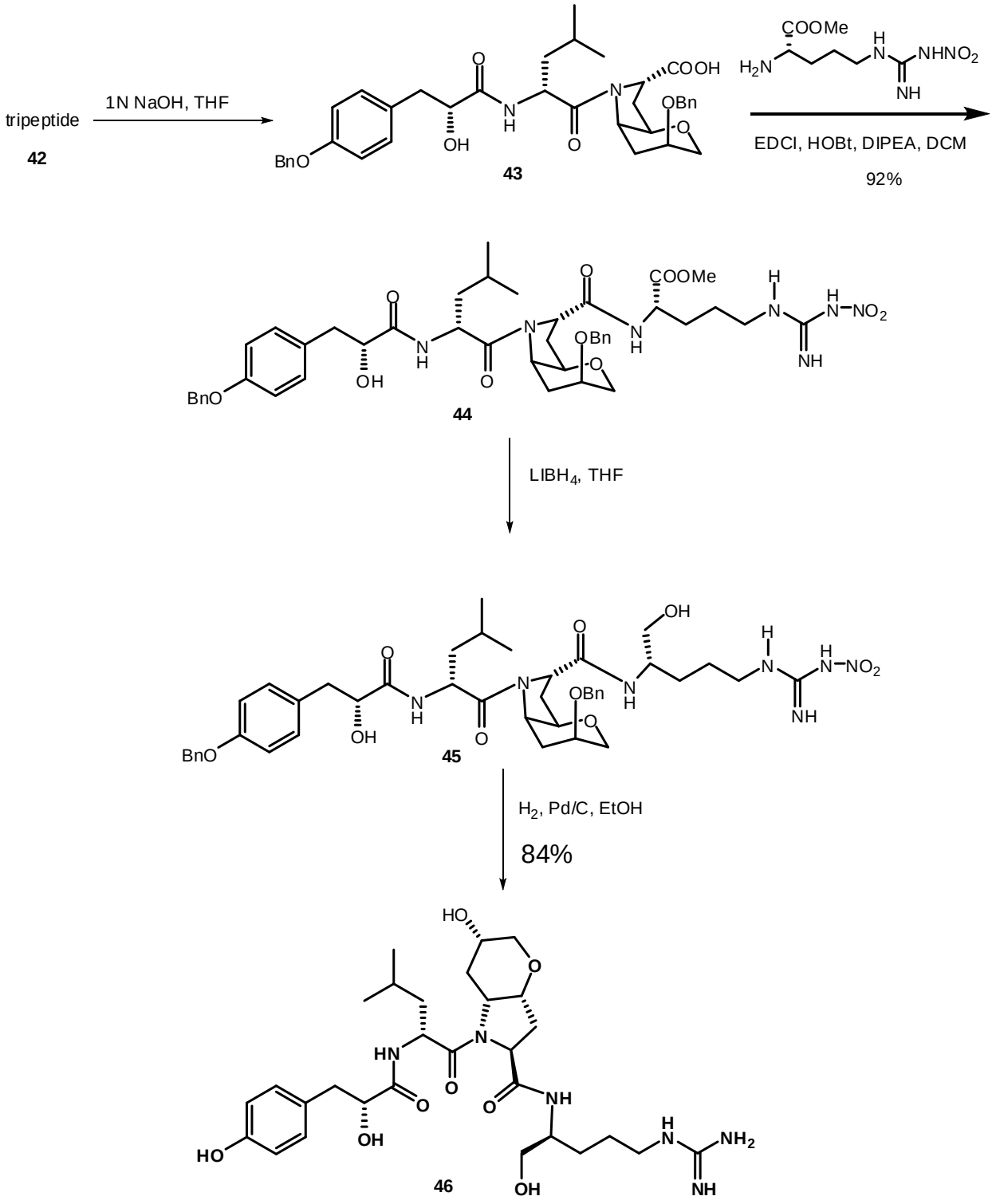
Figure 3.3. Synthetic approach towards O-Choi (mannose configuration)

Both the displacement and cyclized reaction were done in one pot to get the cyclized material **35**. It was further hydrolyzed using sodium hydroxide and decarboxylation was done in refluxing toluene to get diastereomers of bicyclic amino acids.



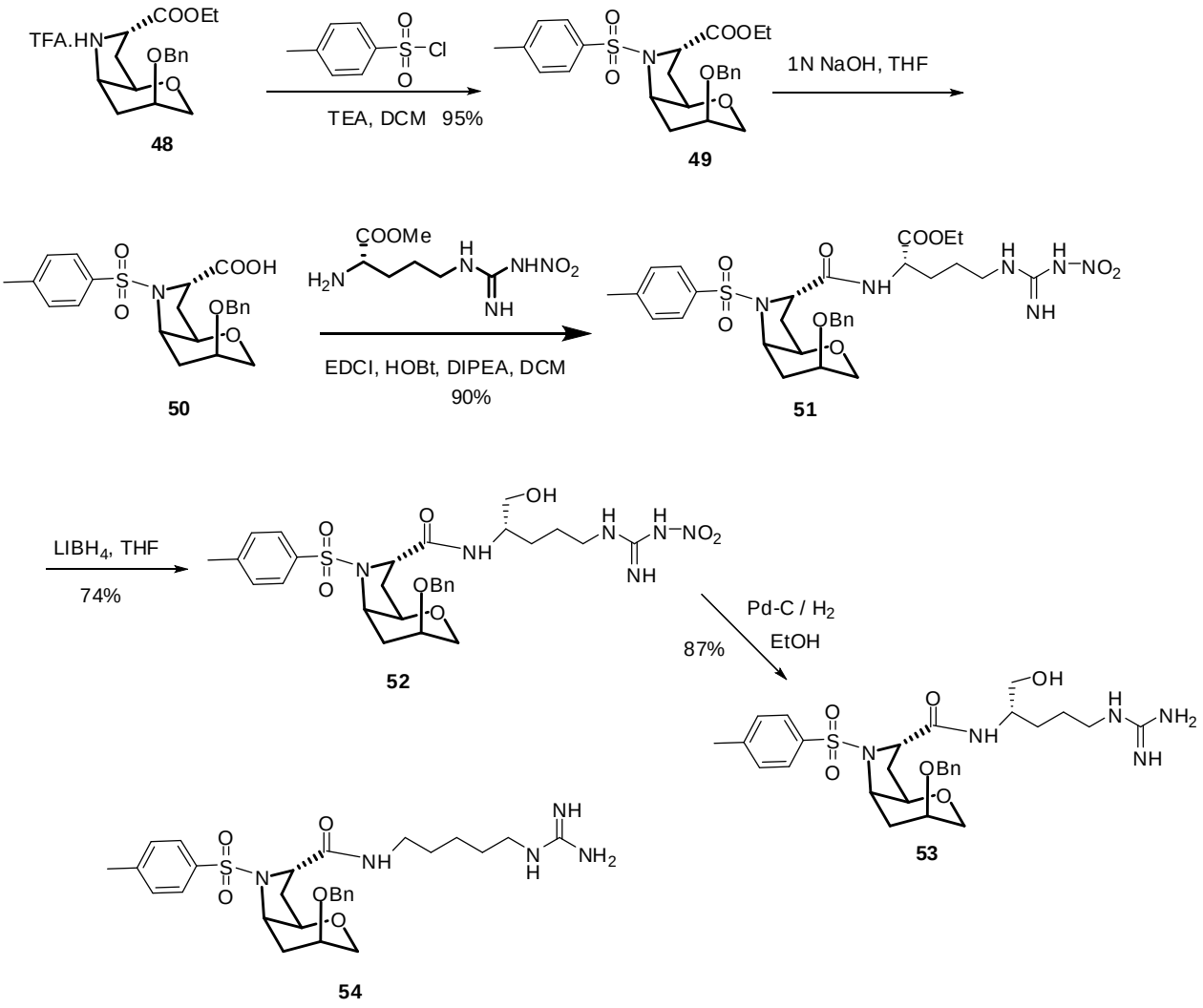
Scheme 3.6. Synthetic approach for the tertapeptide using O-Choi (mannose configuration)

The boc group was cleaved using acidic conditions and then it was coupled to Boc-D-Leucine using BOP-Cl as a coupling reagent to give **39**. The other coupling reagents like EDCI, HATU and PyBOP were also used. However, we could not get the desired product or with very less yield. After column purification, Boc-protection of the dipeptide was cleaved using TFA and was further coupled to protected D-HPLA derived from D-tyrosine using EDCI, HOBt to get compound **42**. The tripeptide ester was hydrolyzed using LiOH to free acid **43** and then coupled with nitro arginine-methyl ester using EDCI, HOBt to give tertapeptide **44**. The ester group of arginine was reduced to primary alcohol using lithium Borohydride to get **45** and finally nitro arginine and benzyl groups were reduced using Pd-C / H_2 to give aeruginosin-298 analog **46**.



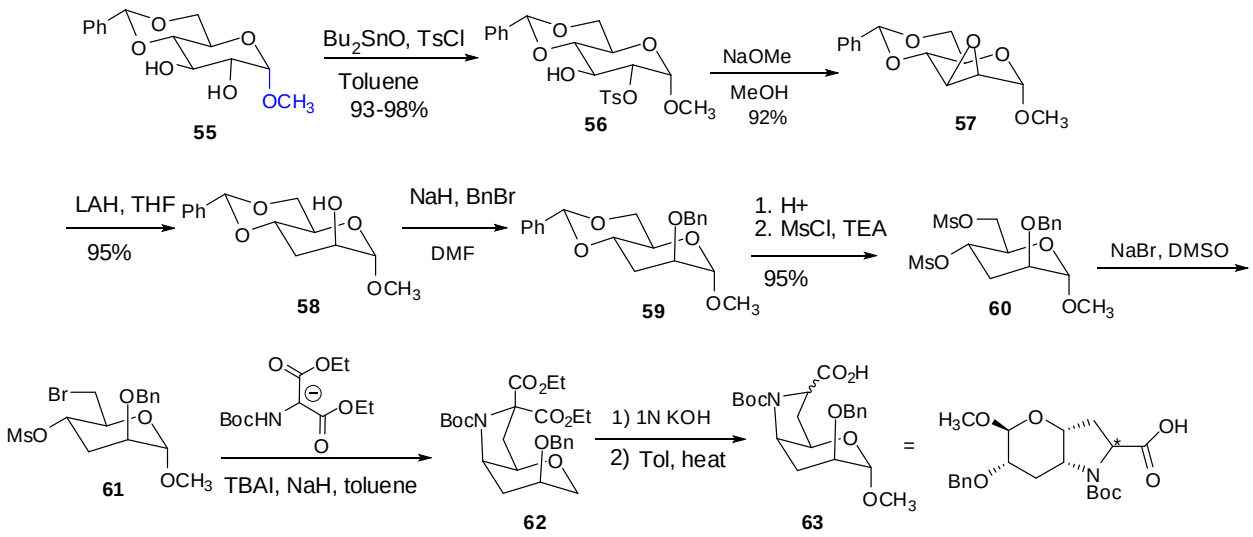
Scheme 3.7. Synthetic approach for the tertapeptide using O-Choi (mannose configuration contd.)

After synthesizing the analog with p2 moiety, we were also interested in changing the p3 moiety. In place of D-HPLA-D-Leu, we replace the dipeptide with p-toluene sulfonyl chloride. The free amino group of bicyclic compound **48** was treated with p-toluene sulfonyl chloride to get sulfonamide **45** in quantitative yield. and then the ester of dipeptide was hydrolyzed using 1N NaOH solution to get free acid **50** and then it was treated with nitro-arginine methyl ester using coupling reagent like EDCI, HOBt to give tripeptide **51**. The ester group of the tripeptide was reduced using lithium borohydride to give an alcohol **52**. And then finally the reduction of nitro group of arginine and debenzyaltion was performed using Pd-C / H2 to give aeruginosin analog **53**. In the same way the compound **54** was also synthesized.



Scheme 3.8. Synthetic approach for the aeruginosin analogs

To do further structure modification and to make synthesis more short we make another analog starting from D-glucose where we did not remove the anomeric position –OH group. The idea behind this to have one more hydroxyl available at sugar ring which can be further used in synthesis of various analogs of aeruginosin as well as for other peptidomemtics.



Scheme 3.9. Synthetic approach for the Choi analogs

4,6- position of Methoxy-D-glucose was protected using benzylidine acetal and then selective tosylation was performed at 2-OH position using dibutyl tin oxide and tosyl chloride. Further epoxide ring formation was carried out using sodium methoxide in methanol. Epoxide ring was opened using LAH and then it was benzylated using benzyl bromide and sodium hydride as a base. Further benzylididne group was removed using acidic conditions and then both hydroxyl groups were mesylated and then primary mesytyl group was converted to bromo group where coupling reaction was performed using diethyl mono-boc malonate to get the cyclized product.

3.3. Conclusion

We have developed an efficient synthesis for aeruginosin 298-A analogs in which the P2 Choi unit is modified by replacing a methylene group in the six-membered ring with an oxygen and different configurations. The ring oxygenated Choi (mannose configuration) variants were

synthesized from D-glucose in an very efficient way. During this process a new highly yielding procedure to convert D-glucose to D-mannose has been established. The crucial step for the formation of the bicyclic Choi includes the alkylation and cyclization steps in a one-pot. These O-Choi were coupled with arginine, HPLA and D-leucine resulted in the tetrapeptides in good yields, which were then converted to the target compounds. We have also modified the P3 position where we used p-toluene sulfonyl chloride. Biological studies for these compounds are ongoing.

3.4. Experimental section

General methods

Reagents, solvents, and starting materials were purchased from Aldrich, VWR, or Lancaster unless otherwise specified. Anhydrous solvents were purchased from Aldrich insure-seal bottles and used directly without further treatment. ^{1}H and ^{13}C NMR spectra were acquired on a Varian 400 MHz and 500 MHz machine. Melting point was measured using a Fisher-Johns melting point apparatus. Typically thin layer chromatography (60 A pore, UV254, phosphomolybdic acid or Ninhydrin as the staining agent) was used to monitor reactions. Silica gel (230–400 meshes) was used for flash chromatography.

Acetobromo-α-D-glucopyranose 23.

The compound was synthesized according to literature procedure. To a magnetically stirred solution of D-glucose **7** (10.0 g) in 50 ml acetic anhydride was added 10 ml 33% HBr-HOAc

solution. The reaction mixture was stirred at room temperature for 5 hours. Another 50 ml 33% HBr- HOAc solution was then introduced, and after stirring at room temperature for another 6 hours, the clear solution is diluted with 250 ml DCM and poured into 250 ml of ice-water. The phase was separated and the aqueous layer was extracted with DCM (150 ml x3). The organic phases were combined and neutralized with cold saturated $NaHCO_3$ solution carefully. Then the DCM phase was washed with water and brine and dried over Na_2SO_4. After evaporation and recrystallization (ether-hexane), 20.4 g (89% yield) of white crystals was obtained as pure product. Mp 87-89 ^oC, $[\alpha]_D$ +196.0 (c 1.9, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.56 (d, 2H, *J* = 4.0 Hz), 6.50 (t, 1H, *J* = 9.7 Hz), 5.10 (t, 1H, *J* = 9.7 Hz), 4.79 (dd, 1H, *J* = 9.99, 4.0 Hz), 4.29-4.23 (m, 2H), 2.37 (dd, 1H, *J* = 12.1, 0.9 Hz,), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.74, 169.67, 169.4, 86.5, 72.0, 70.5, 70.1, 67.1, 60.8, 20.6, 20.53, 20.4, 20.4.

2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-glucitol 24.

Bu_3SnH (14.4 g, 49.6 mmol) was added to a magnetically stirred solution of acetobromo-α-D-glucopyranose **1** (20.4 g, 49.6 mmol) in 150 ml benzene. A few crystals of AIBN were then added to the solution. The reaction mixture was immersed immediately in a pre-heated oil-bath and stirred at 80 ^oC for one hour. After cooled down to room temperature, the reaction mixture was diluted with 150 ml ether and followed by adding a solution of 8.6g KF dissolved in 30 ml water. The mixture was rapidly stirred for 30 min. The insoluble Bu_3SnF was collected and washed thoroughly with ether. The water was separated from the combined filtrate and the organic layer were washed with water and brine and dried over Na_2SO_4. After evaporation and recrystallization (ether-hexane), 14.9g of pure product was obtained in the form of colorless

crystals. The mother liquid was purified by flash chromatography and another 1.1g of pure product was recovered. The total weight of pure product was 16.0g (97% yields). Mp 71-73 ^{o}C, $[\alpha]_D$ +42.6 (c 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.04 (t, 1H, *J* = 9.5 Hz), 4.87-4.81 (m, 2H), 4.04 (dd, 1H, *J* = 4.9, 12.4 Hz), 3.99-3.94 (m, 2H), 3.47-3.44 (m, 1H), 3.15 (t, 1H, *J* = 10.9 Hz), 1.92 (s, 3H), 1.86 (s, 6H), 1.85 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 169.9, 169.4, 169.2, 76.1, 73.4, 68.6, 68.1, 66.5, 61.9, 20.34, 20.27.

1, 5-anhydro-4, 6-*O*-benzylidene-D-glucitol 8.

Compound **23** (16.0 g, 48.1 mmol) was dissolved in 100 ml absolute ethanol. A solution of NaOEt in ethanol (21 wt% in ethanol, 0.37 ml, 10 mol%) was added to the flask. The reaction mixture was then stirred at room temperature under the protection of anhydrous $CaCl_2$ till all starting material was consumed (monitored by TLC). The solvent was then completely evaporated followed by co-evaporation with toluene twice, after which, the product was dried again under high vacuum for 2 hours to afford the anhydrous 1,5-anhydro-D-glucitol **25**. The dried compound **25** was then dissolved in 100 ml DMF, followed by the addition of benzaldehyde dimethyl acetyl (9.0 ml, 60 mmol) and *p*-TsOH (1.14 g, 6.0 mmol) at room temperature. The reaction mixture was stirred at 60^{o}C for 7-10 hours, and then it was concentrated on a rotary evaporator to remove the methanol formed. The reaction was monitored by ^{1}H NMR spectrum. Repeat the heating and evaporation till crude NMR shows all starting material consumed. DMF was then removed under reduced ressureusing an oil pump, and the residue was diluted with EtOAc, washed with aqueous saturated $NaHCO_3$. The water phase was extracted with EtOAc three times, and the combined organic phase was washed with water and brine and dried over Na_2SO_4. After evaporation and recrystallization (EtOAc-hexane), 6.4g of

pure product was obtained in the form of colorless crystals. The mother liquor was purified again by flash chromatography and another 2.7g of pure product was recovered. The total yield for **8** was 9.1g (75% yield); mp 163-165^{o}C, $[\alpha]_D$ -20.5 (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48-7.46 (m, 2H), 7.37-7.35 (m, 3H), 5.51 (s, 1H), 4.31 (dd, 1H, *J* = 10.4, 4.9 Hz), 4.04 (dd, 1H, *J* = 11.3, 5.5 Hz), 3.81- 3.76 (m, 1H), 3.73-3.65 (m, 2H), 3.45 (t, 1H, *J* = 9.2 Hz), 3.41-3.32 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.0, 129.3, 128.4, 126.3, 101.8, 81.0, 75.4, 71.0, 70.3, 69.9, 68.7

Synthesis of 1,5-anhydro-2-O-tosyl-4,6-O-benzylidene-D-glucitol 26:

To a round bottom flask compound 1,5-anhydro-4,6-O-benzylidene-D-glucitol **3** (1 g, 3.96 mmol) was dissolved in 10 ml of toluene. Dibutyl tin oxide (1.08 g, 4.35 mmol) was added to the reaction mixture. The reaction mixture was heated to reflux conditions for 3-4 hrs and then toluene was evaporated using rotavaporator. P-toluene sulfonyl chloride (0.83 g, 4.35 mmol) and toluene (15 ml) was added to the reaction mixture and it was heated at 60 °C for another 3-4 hrs. The reaction was monitored using NMR. For work up the toluene was removed using rotavaporator and reaction mixture was quenched using 20 ml of saturated sodium bicarbonate solution. The reaction mixture was also diluted using ethyl acetate (50 ml). Then it was filtered using celite bed and then extracted using EtOAc: water. The water layer was again extracted using (2 x 50 ml) of EtOAc. The organic layer was combined and then it was washed with brine and dried over sodium sulphate. The organic layer was concentrated on rotavaporator. The compound was purified using EtOAc: Hexane (1:4) to get (1.57 g, 97.5%) compound as a white solid, mp 174.2-175.0 °C $[\alpha]_D^{25}$ = -33.0 ($CHCl_3$, c = 1.2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, 2H, *J* = 8.4 Hz), 7.46–7.44 (m, 2H), 7.36-7.34 (m, 5H), 5.47 (s, 1H), 4.47-4.41 (m, 1H), 4.30

(dd, 1H, *J* = 10.6, 5.1 Hz), 4.11 (q, 1H, *J* = 11.3, 5.8 Hz), 3.85 (t, 1H, *J* = 9.1 Hz), 3.64 (t, 1H, J = 10.3 Hz), 3.39-3.34 (m, 2H), 3.36-3.33 (m, 1H), 2.45 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.3, 136.7, 132.8, 129.8, 129.2, 128.3, 128.0, 126.1, 101.8, 80.6, 78.1, 72.3, 70.9, 68.4, 67.7, 21.7; HRMS calcd for $C_{20}H_{22}O_7S$+H, 407.1165, found 407.1162

Experimetal procedure for 27 by epoxide ring formation: Compound 1,5-anhydro-2-O-tosyl-4,6-O-benzylidene-D-glucitol 2 (0.5 g, 1.23 mmol) was dissolved in dry methanol (5 ml) and then it was heated at 45 °C for 15 min. Sodium methoxide (0.39 g, 7.38 mmol) was added to it and then the reaction mixture was heated to reflux for 45 minutes. The reaction was monitored by NMR. For work up the methanol was removed using rotavaporator and then reaction mixture was diluted using EtOAc and water. The organic layer was extracted and water layer was again extracted using ethyl acetate (2 times). The organic layer was combined and a brine wash was given. The compound was dried on sodium sulphate and it was concentrated on rotavaporator. The compound was purified using column chromatography using EtOAc: Hexane (1:6) to get (0.26 g, 90%) compound as a white solid. Mp 122.4-123.2 °C, $[\alpha]_D^{25}$ = -15.8 ($CHCl_3$, c = 1.5). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.49 (m, 2H), 7.40–7.37 (m, 3H), 5.58 (s, 1H), 4.30-4.28 (m, 1H), 4.25 (d, 1H, *J* = 13.9 Hz), 3.97 (d, 1H, *J* = 13.5 Hz), 3.73-3.63 (m, 2H), 3.51 (d, 1H, *J* = 13.5 Hz), 3.25 (d, 1H, *J* = 4.0 Hz), 3.20-3.14 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.0, 129.2, 128.3, 126.1, 102.3, 75.5, 69.4, 69.4, 65.3 HRMS calcd for $C_{13}H_{14}O_4$+Na 257.0790, found 257.0791.

Experimental procedure for 28 by epoxide ring opening:

To a stirred solution of **27** (2.0 g, 8.53 mmoles) in THF (20 ml) at 0 °C the LAH (0.65 g, 17.07 mmoles) was added slowly. The reaction mixture was stirred at room temperature for 30 min. The reaction was monitored by TLC. For work up first the unreacted LAH was quenched using saturated sodium sulphate solution and then the RM was filtered using celite. The reaction mixture was diluted with EtOAc and was extracted using water. The organic layer was extracted and the water layer was again extracted using ethyl acetate. The organic layer was combined and was washed using brine. The organic layer was dried on sodium sulphate and was concentrated using rotavaporator. The compound was purified on column using EtOAc: Hexane (1:4) to get compound 6 as white solid in (1.99 g, 99%) yield. Mp 87.4-88.0 °C, $[\alpha]_D^{25}$ = -28.7 ($CHCl_3$, c = 2.0) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52-7.46 (m, 2H), 7.38–7.34 (m, 3H), 5.58 (s, 1H), 4.30-4.28 (m, 1H), 4.06 (s, 1H),4.0- 3.96 (m, 1H),3.90 (d, 1H, *J* = 12.5 Hz), 3.77 (t, 1H, *J* = 10.2 Hz), 3.65 (d, 1H, *J* = 12.5 Hz), 3.40-3.34 (m, 1H), 2.26 (d, 1H, *J* = 12.8 Hz), 1.84-1.77 (m, 1H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 129.3, 128.6, 126.4, 102.3, 74.6, 74.5, 72.8, 69.3, 67.3, 35.9 HRMS calcd for $C_{13}H_{16}O_4$+Na, 259.0946, found 259.0948.

Experimental procedure for 29 by benzylation reaction: Compound **28** (1.9 g, 8.04 mmoles) was dissolved in DMF (10 ml). NaH (0.23 g, 9.65 mmoles) was added to the reaction mixture at 0 °C. Reaction mixture was stirred for 45 min at 0 °C. Benzyl bromide (1.65 ml, 9.65 mmoles) was added and then the reaction mixture was stirred at room temperature for 2 hrs. The reaction was monitored by TLC. For work up first reaction mixture was quenched using saturated ammonium chloride and then it was extracted using ether and water. The organic phase was extracted and water layer was again extracted using ether (2 times). The organic layer was combined and was washed with brine. The organic phase was dried on sodium sulphate and was

concentrated on rotavapor. Compoud **29** was purified on column chromatography using EtOAc: Hexane (1:7) to give an oily material (2.3 g, 87%). $[\alpha]_D^{25}$ = -28.5 ($CHCl_3$, c = 1.0). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50-7.47 (m, 2H), 7.39-7.29 (m, 8H), 5.61 (s, 1H), 4.62 (2, 2H), 4.31-4.27 (m, 1H), 3.81 (t, 1H, J = 10.3 Hz), 3.74 (s, 1H), 3.56 (d, 1H, *J* = 12.5 Hz), 3.43-3.36 (m, 1H), 2.44 (d, 1H, *J* = 13.1 Hz), 1.76 (t, 1H, *J* = 12.1 Hz) ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 137.9, 129.3, 128.7, 128.6, 128.0, 127.9, 126.4, 102.1, 74.7, 73.3, 70.7, 69.9, 69.3, 33.0 HRMS calcd for $C_{20}H_{22}O_4$+H, 327.1596; found, 327.1601.

Experimental Procedure for 30 by mesylation reaction: Compound **29** (1.4 g, 5.87 mmoles) was dissolved in 20 ml of dry dichloromethane. The reaction mixture was cooled at 0 °C. Triethyl amine (1.9 ml, 2.5 mmoles) was added to the reaction mixture. After 10 min methyl sulfonyl chloride (1.68 ml, 2.5 mmoles) was added to the reaction mixture. The reaction mixture was stirred for overnight at room temperature. For workup the reaction mixture was diluted using DCM and was extracted using water. The water phase was again extracted using DCM (2 x 50 ml). The organic phase was combined and dried over sodium sulphate. The crude was purified over column chromatography using EtOAc: Hexane (9:1) to give pure compound as a gummy material (2.20 g, 95%). $[\alpha]_D^{25}$ = + 17.5 ($CHCl_3$, c = 1.5) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.32 (m, 4H), 7.30-7.26 (m, 1H), 4.91-4.85 (m, 1H), 4.55 (s, 2H), 4.49 (dd, 1H, *J* = 11.7 Hz, 1.83 Hz), 4.37-4.33 (m, 1H), 4.07 (d, 1H, *J* = 12.4 Hz), 3.69 (s, 1H), 3.59-3.55 (m, 1H), 3.407(d, 1H, *J* = 11.4 Hz), 3.04 (s, 3H), 3.03 (s, 3H), 2.76-2.70 (m, 1H), 1.87-1.80 (m, 1H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.6, 128.4, 127.7, 127.6, 76.6, 71.9, 71.7, 70.2, 68.9, 68.2, 38.3, 37.6, 34.4 HRMS calcd for $C_{15}H_{22}O_8S_2$+H, 395.0834; found, 395.0832.

Experemental procedure for 31 by substitution of primary Mesylate by bromo:

Dimesylate compound **30** (1.3 g, 3.29 mmoles) was dissolved in 10 ml of DMSO. Sodium bromide (2.034 g, 19.77 mmoles) was added to the reaction mixture. Then TBAB (0.12 g, 0.823 mmoles) was added and the reaction mixture was heated at 60 °C for overnight. Reaction was monitored by TLC and NMR. The reaction was diluted with ethyl acetate and was washed with water. The water layer was again washed with ethyl acetate and the combined organic layer was washed with brine and was dried over sodium sulphate. After concentrating at rotavaporator the compound was purified over column chromatography EtOAc: Hexane (8:1) to give a gummy material of (1.12 g, 89%). $[\alpha]_D^{25}$ = + 14.5 ($CHCl_3$, c = 1.2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.32 (m, 4H), 7.29-7.27 (m, 1H), 4.90-4.89 (m, 1H), 4.62-4.51 (m, 2H), 4.15-4.08 (m, 1H), 3.71-3.66 (m, 2H), 3.53-3.48 (m, 3H), 3.06 (s, 3H), 2.72-2.64 (m, 1H), 1.82-1.79 (m, 1H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 128.7, 128.0, 127.9, 78.3, 75.1, 72.3, 70.6, 69.1, 38.9, 34.9, 32.1 HRMS calcd for $C_{14}H_{19}BrO_5S$+Na, 401.0034; found, 401.0036.

Synthesis of Bicyclic compound 35:

Monoboc diethyl malonate compound **33** (0.2 g, 0.726 mmoles) was dissolved in dry toluene (10 ml) under nitrogen was treated using NaH (20.93 mg, 0.872 mmoles). Reaction mixture was stirred at RT for 30 minutes before **31** was added to it. The reaction mixture was stirred for 1 hour at room temp and then TBAI was added and the reaction mixture was refluxed for 17 hours. For work up the RM was quenched by dil. HCl and diluted with Ethyl acetate. The organic layer was separated and the aqueous layer. The organic layer was washed with brine and was dried over sodium sulphate. The compound was purified using column chromatography using EtOAc: Hexane (6:1) to give an oily compound (0.171 g, 68%). $[\alpha]_D^{25}$ = + 27.4 ($CHCl_3$, c = 2.4) ^{1}H

NMR (400 MHz, $CDCl_3$) δ 7.36-7.32 (m, 4H), 7.30-7.26 (m, 1H), 4.91-4.85 (m, 1H), 4.55 (s, 2H), 4.49 (dd, 1H, *J* = 11.7 Hz, 1.83 Hz), 4.37-4.33 (m, 1H), 4.07 (d, 1H, *J* = 12.4 Hz), 3.69 (s, 1H), 3.59-3.55 (m, 1H), 3.40 (d, 1H, *J* = 11.4 Hz), 3.04 (s, 3H), 3.03 (s, 3H), 2.76-2.70 (m, 1H), 1.87-1.80 (m, 1H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.6, 128.4, 127.7, 127.6, 76.6, 71.9, 71.7, 70.2, 68.9, 68.2, 38.3, 37.6, 34.4 HRMS calcd for $C_{30}H_{45}N_2O_9$+H, 478.2441; found, 478.2240.

Synthesis of O-Choi 36:

Compound **35** was dissolved in THF: MeOH (1:1). Cool it to 0 degree and then add 1N LiOH to it and stir for 4-5 hrs. Reaction mixture was extracted using ether and then ether layer was washed with water. The water layer was acidified and then it was extracted using EtOAc (4 times) using 50 ml of ethyl acetate. The ethyl acetate was washed with brine and concentrated on vacuum. Then it was dissolved in toluene and was heat to reflux for 18 hours for decarboxylation. Completion of reaction gives 3 products which can be further esterified using ethyl Iodide to give only two products which can be further purified using column chromatography. $[\alpha]_D^{25}$ = + 17.6 ($CHCl_3$, c = 1.0) ^1H NMR (400 MHz, $CDCl_3$) δ 7.39–7.21 (m, 5H), 4.58–4.38 (m, 2H), 4.19–4.07 (m, 2H), 4.05–3.98 (m, 1H), 3.97–3.70 (m, 3H), 3.22 (dd, 1H, *J* = 9.3, 9.0 Hz),1.76–1.93 (m, 1H), 1.61 (s, 9 H), 1.44 (s, 5 H), 1.17 (t, 3H, *J* = 4.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) d 172.0, 171.8, 154.2, 138.3, 128.1, 127.4, 80.1, 79.8, 76.0, 75.3, 70.6, 70.2, 69.5, 69.4, 68.7, 59.0, 57.5, 35.2, 30.5, 29.4, 13.9 HRMS calcd for $C_{22}H_{31}NO_6$+Na, 428.2049; found, 428.2052.

Procedure for synthesis of dipeptide 39:

BOP-Cl (0.16 g, 0.62 mmoles) and DIPEA (.16 g, 1.25 mmoles) were added to a stirred solution of Boc-D-Leucine (.1 g, .417 mmoles) in 10 ml of DCM: CH_3CN (1:1) at 0 °C. After stirring for 15 minutes amine (.14 g, .45 mmoles) were added. The reaction mixture was stirred for 15 hours at room temperature. The reaction was diluted using 100 ml of ethyl acetate and then washed using NaHCO3, water and then by 1N HCl and then finally using brine. The organic layer was dried over sodium sulphate and it was purified using column chromatography to get dipeptide as a oily material. $[\alpha]_D^{25}$ = +14.5 ($CHCl_3$, c = 0.8) ^{1}H NMR (400 MHz, $CDCl_3$, mixture of conformers) : 7.36-7.28 (m, 5H), 4.59-4.57 (m, 2H), 4.45-4.39 (m, 1H), 4.36-4.28 (m, 2H), 4.23-4.15 (m, 2H), 3.80-3.74 (m, 2H), 3.60-3.55 (m, 1H), 3.48-3.43 (m, 1H), 2.29 (t, 2H, *J* = 8.0 Hz), 1.90-1.77 (m, 2H), 1.40 (s, 9H), 1.26 (t, 3H, *J* = 7.3 Hz), 1.02-0.96 (m, 6H) ^{13}C NMR (100 MHz, $CDCl_3$): 172.3, 171.1, 155.5, 137.8, 128.3, 127.7, 127.5, 127.4, 79.6, 73.1, 70.7, 70.6, 64.2, 61.1, 56.4, 53.8, 49.6, 43.3, 42.0, 32.4, 28.1, 24.5, 23.3, 21.7, 13.9 HRMS calcd for $C_{28}H_{42}N_2O_7$+H, 519.3070; found, 519.3067.

Procedure for synthesis of tripeptide 42:

Boc protected dipeptide was stirred in DCM at 0 °C and then TFA was added to the reaction mixture. Reaction was monitored using TLC. The reaction mixture concentrated on rotavaporator and can be dried on high vacuum. EDCI (66.9 mg, 0.34 moles), HOBt (42.9 mg, .318 mmoles) and DIPEA (123 mg, .954 mmoles) were added subsequently to a stirred solution of (R)-β-[4-(benzyloxy) phenyl lactic acid (100 mg, .318 mmoles) and dipeptide (159.7 mg, .381 mmoles) were added in 10 ml of DCM at 0 °C. The reaction mixture was stirred for overnight at room temperature. The reaction mixture was diluted using 100 ml of ethyl acetate and then it was washed using saturated $NaHCO_3$, water, 1N HCl, and brine subsequently. The organic phase

was dried on sodium sulphate and it was purified using column chromatography to give pure tripeptide. $[\alpha]_D^{25}$ = +7.5 ($CHCl_3$, c = 0.75) ^{1}H NMR (400 MHz, $CDCl_3$, mixture of conformers) : δ 7.44-7.28 (m, 10H), 6.91 (d, 2H, *J* = 8.8 Hz), 6.91 (d, 2H, *J* = 8.4 Hz), 6.79 (d, 1H, *J* = 10.9 Hz), 5.0 (s, 2H), 4.55 (q, 2H, J = 9.2, 20.8 Hz), 4.44 (t, 1H, J = 9.2 Hz), 4.36-4.29 (m, 1H), 4.28-4.22 (m, 2H), 4.20-4.18 (m, 1H), 4.12 (q, 2H, J = 6.9, 14.0 Hz), 3.83-3.78 (m, 2H), 3.63-3.43 (m, 3H), 3.16-3.08 (m, 1H), 2.84-2.76 (m, 1H), 2.52-2.46 (m, 1H), 2.34-2.25 (m, 2H), 2.04 (s,3H), 1.56 (s, 9H), 1.29-1.23 (m, 4H), 1.02-0.96 (m, 6H) ^{13}C NMR (100 MHz, $CDCl_3$): 172.8, 171.9, 171.5, 171.1, 157.8, 137.8, 136.9, 130.5, 130.4, 128.8, 128.5, 128.5, 128.3, 127.9, 127.8, 127.5, 127.4, 114.9, 73.15, 72.6, 70.9, 70.7, 69.9, 64.3, 62.3, 61.3, 57.8, 56.5, 54.0, 51.2, 48.2, 43.3, 41.7, 41.1, 40.4, 39.9, 33.3, 32.5, 28.7, 28.1, 24.7, 23.4, 22.8, 21.9, 21.7, 14.0

HRMS calcd for $C_{41}H_{50}N_2O_7$+H, 715.8340; found, 715.8342.

Experiment to synthesize tertapeptide 44:

A solution of **42** (145 mg, 0.35 mmol) in THF (7.5 mL) was treated with 0.2 N LiOH (7.5 mL) at room temperature. The reaction mixture was stirred overnight and quenched with 1 N HCl. The mixture was extracted with EtOAc, washed with brine, and dried with Na_2SO_4. After evaporation of the solvent, the crude acid was dried under vacuum for 2 h. To a solution of compound 23a (170 mg, 0.29 mmol) in DCM (8 mL) was added TFA (2 mL) at 0 °C. The reaction mixture was warmed up to room temperature and stirred for 2 h before the solvent was evaporated under reduced pressure. The TFA salt 24 was dried under vacuum for 2 h. HATU (135 mg, 0.35 mmol) and DIEA (0.15 mL, 0.87 mmol) were added subsequently to a stirred solution of acid 27 and TFA salt 24 in DMF (2.5 mL) at 0 °C. After stirred for 24 h at room temperature, the reaction was quenched with saturated $NaHCO_3$, extracted with EtOAc (20 mL x 5), washed with

brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (2% MeOH in chloroform) to get pure tetrapeptide (192 mg, 77%), $[\alpha]_D^{25}$ = -18.5 ($CHCl_3$, c = 0.8) ^{1}H NMR (400 MHz, $CDCl_3$, mixture of conformers) 8.54 (d, 1H, *J* = 8.4 Hz), 7.45–7.09 (m, 12H), 6.89 (d, 2H, *J* = 8.2 Hz), 5.0 (s, 2H), 4.63–4.35 (m, 6H), 4.30–4.13 (m, 1H), 3.98 (br, 1H), 3.90-3.88 (m, 1H), 4.03–3.82 (m, 2H), 3.69 (s, 3H), 3.57–3.30 (m, 2H), 3.13–3.02 (m, 2H), 2.90-2.82 (m, 1H), 2.40–2.12 (m, 2H), 1.82-1.75 (m, 1H), 1.61-1.23 (m, 6H), .93-.83 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 174.4, 173.9, 172.9, 172.2, 172.0, 171.8, 159.3, 159.2, 157.9, 157.6, 137.6, 137.4, 137.0, 136.9, 130.7, 130.6, 129.2, 128.5, 128.4, 128.1, 127.9, 127.8, 127.6, 127.5, 114.9, 114.6, 76.0, 75.1, 72.8, 72.6, 71.3, 70.3, 69.9, 69.8, 68.7, 65.2, 61.8, 58.2, 55.1, 52.3, 52.1, 51.6, 51.1, 49.9, 48.2, 42.2, 40.2, 39.5, 37.0, 31.5, 28.7, 25.4, 25.3, 24.5, 24.3, 23.5, 21.3, 21; HRMS calcd for $C_{44}H_{57}N_7O_{11}$ + H, 860.4194; found, 860.4199.

Synthesis of compound 45 by reduction using $LiBH_4$*:*

Freshly prepared 0.2 N $LiBH_4$ solution in THF (1.0 mL, 0.20 mmol) was added via syringe to a N_2 protected solution of tetrapeptide **44** (120 mg, 0.13 mmol) in dry THF (5 mL) at 0 °C. The resulting solution was stirred at 0 °C for 30 min, another 1.0 mL 0.2 N $LiBH_4$ solution was added, and continued stirring at 0 °C for 30 min. The reaction was quenched with water followed by saturated ammonium chloride. The resulting mixture was extracted with MeOH: $CHCl_3$ (1:10). The extracted solution was dried over Na_2SO_4, concentrated, and the crude product was purified by chromatography (5% MeOH in $CHCl_3$) to give pure material (84.4 mg, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of conformers) δ 8.63 (br, 1H), 8.07 (d, 1H, J = 9.1 Hz), 7.74 (br, 2H), 7.39–7.25 (m, 10H), 7.18 (br, 1H), 7.15 (d, 2H, *J* = 6.4 Hz), 6.89 (d, 2H, *J* = 8.2 Hz), 4.99 (m, 2H), 4.74–4.51 (m, 2H), 4.51–4.41 (m, 1H), 4.41–4.25 (m, 3H), 4.25–4.14 (m,

1H), 4.14–4.00 (m, 2H), 3.99–3.79 (m, 1H), 3.77–3.59 (m, 2H), 3.58–3.38 (m, 2H), 3.36–2.99 (m, 3H), 2.83 (br, 2H), 2.71–2.46 (m, 1H), 2.42–1.97 (m, 3H), 1.82–1.11 (m, 7H), 1.00–1.73 (complex, 6H, J = 5.5, 5.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 159.4, 157.9, 136.9, 136.8, 130.6, 128.7, 128.6, 128.3, 127.9, 127.5, 115.0, 76.1, 72.9, 70.6, 69.9, 69.8, 68.9, 64.9, 61.7, 58.2, 51.9, 50.4, 40.8, 39.9, 37.4, 29.7, 27.9, 25.7, 24.5, 24.2, 23.4, 21.0; HRMS calcd for $C_{43}H_{57}N_7O_{10}$ + H, 832.4245; found, 832.4252.

Synthesis of final aeruginosin analog 46:

Compound tertapeptide **45** (30 mg, 0.036 mmol) dissolved in 5.0 mL methanol containing 0.2 mL TFA was treated with Pd/C (10.0 mg). The suspension was sequentially evacuated and purged with H_2, stirred under atmosphere of H_2 (1 atm) for 24 h. The reaction mixture was filtered through a pad of Celite, which was washed with several portion of methanol. The combined solution was concentrated and dried under vacuum to afford aeruginosin 298-A analog as colorless amorphous solid (22 mg, 95%), $[\alpha]_D$ + 37.6 (c 0.3, MeOH). 1H NMR (300 MHz, CD3OD, mixture of conformers) d 8.03 (d, 0.6H, J = 8.5 Hz), 7.96–7.79 (br, 1H), 7.06 (d, 2H, *J* = 8.0 Hz), 6.69 (d, 2H, *J* = 8.2 Hz), 4.73–4.57 (m, 1H), 4.57–4.39 (m, 1H), 4.39–4.11 (m, 3H), 4.10–3.98 (m, 2H), 3.96 (s, 1H), 3.92–3.82 (m, 1H), 3.82–3.72 (m, 1H), 3.72–3.46 (m, 1H), 3.38 (d, 2.4H, *J* = 5.2 Hz), 3.27–3.08 (m, 2H), 3.03 (t,1.6H, *J* = 9.6 Hz), 2.90–2.63 (m, 2H), 2.54 (dd,1H, *J* = 8.5, 4.4 Hz), 2.43–2.05 (m, 2H),1.79–1.41 (m, 6H), 1.41–1.22 (m, 1H), 0.90 (d, 3H, *J* = 6.0 Hz), 0.85 (d, 3H, *J* = 6.3 Hz); ^{13}C NMR (75 MHz, CD3OD, major conformer) d 174.9, 173.8, 162.3, 157.5, 155.5, 132.2, 131.0, 130.64, 127.8, 115.7, 114.67, 75.9, 70.2, 63.7, 63.0, 62.3, 61.8, 60.4, 51.2, 41.5, 37.2, 37.0, 29.7, 28.2, 27.8, 25.1, 24.7, 22.7,20.6; HRMS calcd for $C_{29}H_{46}N_6O_8$+H, 607.3455; found, 607.3452.

Synthesis of 56:

To a round bottom flask compound **55** (10 g, 35.34 mmol) was dissolved in 50 ml of toluene. Dibutyl tin oxide (9.67g, 38.88 mmol) was added to the reaction mixture. The reaction mixture was heated to reflux conditions for 3-4 hrs and then toluene was evaporated using rotavaporator. P-toluene sulfonyl chloride (7.41g, 38.88 mmol) and toluene (50 ml) was added to the reaction mixture and it was heated at 60 °C for another 3-4 hrs. The reaction was monitored using NMR. For work up the toluene was removed using rotavaporator and reaction mixture was quenched using 100 ml of saturated sodium bicarbonate solution. The reaction mixture was also diluted using ethyl acetate (200 ml). Then it was filtered using celite bed and then extracted using EtOAc: water. The water layer was again extracted using (2 x 100 ml) of EtOAc. The organic layer was combined and then it was washed with brine and dried over sodium sulphate. The organic layer was concentrated on rotavaporator. The compound was purified using EtOAc: Hexane (1:4) to get (14.7 g, 95%) compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, 2H, *J* = 8.4 Hz), 7.46–7.44 (m, 2H), 7.36-7.34 (m, 5H), 5.47 (s, 1H), 4.83 (d, 1H, *J* = 3.7 Hz), 4.44-4.38 (m, 1H), 4.27-4.23 (m, 1H), 4.15-4.10 (m, 1H), 3.83-3.87 (m, 1H), 3.70 (t, 1H, *J* = 10.2 Hz), 3.45 (t, 1H, *J* = 9.5 Hz), 3.39-3.34 (m, 2H), 3.33 (s, 3H), 2.45 (s, 3H), 1.25 (t, 1H, *J* = 6.9 Hz) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.1, 136.9, 133.1, 129.7, 129.1, 128.2, 128.0, 126.2, 101.8, 98.1, 80.9, 79.4, 68.6, 68.2, 61.9, 55.6, 21.6.

Synthesis of 57:

Compound **56** (10.0 g, 22.91 mmol) was dissolved in dry methanol (50 ml) and then it was heated at 45 °C for 15 min. Sodium methoxide (7.42 g, 137.46 mmol) was added to it and then the reaction mixture was heated to reflux for 45 minutes. The reaction was monitored by NMR.

For work up the methanol was removed using rotavaporator and then reaction mixture was diluted using EtOAc and water. The organic layer was extracted and water layer was again extracted using ethyl acetate (2 times). The organic layer was combined and a brine wash was given. The compound was dried on sodium sulphate and it was concentrated on rotavaporator. The compound was purified using column chromatography using EtOAc: Hexane (1:6) to get (5.57 g, 92%) compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.48 (m, 2H), 7.40–7.36 (m, 3H), 5.58 (s, 1H), 4.90 (s, 1H), 4.29-4.23 (m, 1H), 3.73-3.63 (m, 3H), 3.47 (s, 3H), 3.17 (d, 1H, *J* = 3.7 Hz) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.0, 129.2, 128.3, 126.1, 102.3, 96.8, 74.8, 69.8, 69.4, 61.6, 55.7, 53.8, 50.5.

Experimental procedure for epoxide ring opening 58:

To a stirred solution of **57** (2.0 g, 6.79 mmoles) in THF (20 ml) at 0 °C the LAH (0.52 g, 13.58 mmoles) was added slowly. The reaction mixture was stirred at room temperature for 30 min. The reaction was monitored by TLC. For work up first the unreacted LAH was quenched using saturated sodium sulphate solution and then the RM was filtered using celite. The reaction mixture was diluted with EtOAc and was extracted using water. The organic layer was extracted and the water layer was again extracted using ethyl acetate. The organic layer was combined and was washed using brine. The organic layer was dried on sodium sulphate and was concentrated using rotavaporator. The compound was purified on column using EtOAc: Hexane (1:4) to get compound **6** as white solid in (1.91 g, 95%) yield ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.47 (m, 2H), 7.39–7.34 (m, 3H), 5.57 (s, 1H), 4.50 (s, 1H), 4.27-4.22 (m, 1H), 3.99- 3.91 (m, 2H), 3.87-3.83 (m, 2H), 3.40 (s, 3H), 3.65 (d, 1H, *J* = 12.5 Hz), 3.40-3.34 (m, 1H), 2.61-2.59 (m, 2H),

2.07-2.92 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 129.0, 128.2, 126.1, 102.0, 100.6, 73.8, 69.2, 68.2, 64.8, 54.7, 31.8, 74.5, 72.8, 69.3, 67.3, 35.9.

Experimental procedure for 59 by benzylation reaction: Compound **58** (5.0 g, 18.77 mmoles) was dissolved in DMF (30 ml). NaH (0.7 g, 28.16 mmoles) was added to the reaction mixture at 0 °C. Reaction mixture was stirred for 45 min at 0 °C. Benzyl bromide (2.45 ml, 20.64 mmoles) was added and then the reaction mixture was stirred at room temperature for 2 hrs. The reaction was monitored by TLC. For work up first rexn mixture was quenched using saturated ammonium chloride and then it was extracted using ether and water. The organic phase was extracted and water layer was again extracted using ether (2 times). The organic layer was combined and was washed with brine. The organic phase was dried on sodium sulphate and was concentrated on rotavapor. Compoud was purified on column chromatography using EtOAc: Hexane (1:7) to give a oily material (6.02 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57-7.50 (m, 2H), 7.42-7.37 (m, 8H), 5.58 (s, 1H), 4.72-4.62 (m, 2H), 4.31-4.27 (m, 1H), 4.09-4.02 (m, 1H), 3.89-3.84 (m, 2H), 3.74-3.70 (m, 1H), 3.51-3.48 (m, 1H), 3.42 (s, 3H), 2.31-2.25 (m, 1H), 2.07-1.97 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 137.5, 129.1, 128.9, 128.3, 128.2, 128.0, 127.7, 127.6, 126.0, 101.9, 98.7, 74.9, 74.0, 71.1, 69.2, 69.1, 64.9, 54.6, 29.1.

Experimental Procedure for 60 by mesylation reaction: Compound **59** (3.0 g, 11.18 mmoles) was dissolved in 30 ml of dry dichloromethane. The reaction mixture was cooled at 0 °C. Triethyl amine (4.83 ml, 33.54 mmoles) was added to the reaction mixture. After 10 min methyl sulfonyl chloride (3.84 ml, 33.54 mmoles) was added to the reaction mixture. The reaction mixture was stirred for overnight at room temperature. For workup the reaction mixture was

diluted using DCM and was extracted using water. The water phase was again extracted using DCM (2 x 100 ml). The organic phase was combined and dried over sodium sulphate. The crude was purified over column chromatography using EtOAc: Hexane (9:1) to give pure compound as a gummy material (3.81 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.31 (m, 5H), 4.91-4.85 (m, 1H), 4.64-4.55 (m, 4H), 4.40-4.36 (m, 1H), 3.96-3.92 (m, 1H), 3.61 (s, 1H), 3.38 (s, 3H), 3.04 (s, 6H), 2.55-2.49 (m, 1H), 2.1-2.04 (m, 1H); ^{13}C NMR (100 MHz, CDCl3) δ 137.4, 128.5, 127.9, 127.7, 98.2, 74.0, 71.4, 71.1, 68.2, 55.2, 38.5, 37.7, 30.4

Experemental procedure for 61 by substitution of primary Mesylate by bromo:

Dimesylate compound **60** (2.0 g, 4.71 mmoles) was dissolved in 10 ml of DMSO. Sodium bromide (2.90 g, 28.26 mmoles) was added to the reaction mixture. Then TBAB (0.18 g, 1.18 mmoles) was added and the reaction mixture was heated at 60 °C for overnight. Reaction was monitored by TLC and NMR. The reaction was diluted with ethyl acetate and was washed with water. The water layer was again washed with ethyl acetate and the combined organic layer was washed with brine and was dried over sodium sulphate. After concentrating at rotavaporator the compound was purified over column chromatography EtOAc: Hexane (8:1) to give a gummy material of (1.61 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.28 (m, 5H), 4.86-4.79 (m, 1H), 4.69 (s, 1H), 4.59 (s, 2H), 3.93-3.88 (m, 1H), 3.71-3.68 (m, 1H), 3.61-3.60 (m, 1H), 3.53-3.49 (m, 1H), 3.43 (s, 3H), 3.04 (s, 3H), 2.48-2.43 (m, 1H), 2.10-2.03 (m, 1H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.5, 128.5, 127.9, 127.7, 98.2, 74.5, 74.2, 71.1, 69.6, 55.1, 38.7, 32.0, 30.7

Synthesis of Bicyclic compound 62:

Monoboc diethyl malonate compound **33** (0.2 g, 0.726 mmoles) was dissolved in dry toluene (10 ml) under nitrogen was treated using NaH (20.93 mg, 0.872 mmoles). Reaction mixture was stirred at RT for 30 minutes before **61** (140 mg, .363 mmol) was added to it. The reaction mixture was stirred for 1 hour at room temp and then TBAI (25 mg) was added and the reaction mixture was refluxed for 17 hours. For work up the RM was quenched by dil. HCl and diluted with Ethyl acetate. The organic layer was separated and the aqueous layer. The organic layer was washed with brine and was dried over sodium sulphate. The compound was purified using column chromatography using EtOAc: Hexane (6:1) to give an oily compound (0.117 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.32 (m, 4H), 7.30-7.26 (m, 1H), 4.91-4.85 (m, 1H), 4.55 (s, 2H), 4.49 (dd, 1H, *J* = 11.7 Hz, 1.83 Hz), 4.37-4.33 (m, 1H), 4.07 (d, 1H, *J* = 12.4 Hz), 3.69 (s, 1H), 3.59-3.55 (m, 1H), 3.407(d, 1H, *J* = 11.4 Hz), 3.04 (s, 3H), 3.03 (s, 3H), 2.76-2.70 (m, 1H), 1.87-1.80 (m, 1H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.6, 128.4, 127.7, 127.6, 76.6, 71.9, 71.7, 70.2, 68.9, 68.2, 38.3, 37.6, 34.4.

Synthesis of O-Choi 36:

Compound **62** (50 mg, 0.10 mmol) was dissolved in THF: MeOH (1:1) 5 ml. Cool it to 0 ° C and then add 1N LiOH (5 mg, .20 mmol) to it and stir for 4-5 hrs. Reaction mixture was extracted using ether and then ether layer was washed with water. The water layer was acidified and then it was extracted using EtOAc (4 times) using 50 ml of ethyl acetate. The ethyl acetate was washed with brine and concentrated on vacuum. Then it was dissolved in toluene and was heat to reflux for 18 hours for decarboxylation. Completion of reaction gives 3 products which can be further esterified using ethyl Iodide to give only two products which can be further purified using column chromatography to give a mixture of compounds. Final compound was purified to get a

oily compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.28 (m, 5H), 4.6 (s, 2H), 4.45-4.42 (m, 1H), 4.26 (s, 1H), 3.69 (s, 1H), 3.44 (s, 1H), 3.38 (s, 3H), 2.29-2.24 (m, 2H), 1.95 (d, 2H, *J* = 15.7 Hz), 1.42 (s, 9H), 1.24 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 136.2, 133.4, 128.5, 128.3, 128.0, 97.6, 81.2, 71.5, 69.4, 61.2, 55.5, 55.2, 35.8, 29.6, 28.1, 21.4

3.5. References

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Chapter 4: Self-assembly of D-glucosamine derivatives

4.1. Abstract

Carbohydrate-based low molecular weight gelators are an interesting class of molecules with many potential applications. Previously, we have found that certain esters and carbamates of 4,6-O-benzylidene-α-D-methyl-glucopyranoside are low molecular weight gelators for a variety of solvents, including water. In order to obtain effective and robust sugar-based organogelators and understand the structure and gelation relationship, we extended our studies using 4,6-O-benzylidene-α-D-methyl-2-deoxy-2-amino-glucopyranoside as well as 4,6-O-p-methoxy-benzylidene-α-D-methyl-2-deoxy-2-amino-glucopyranoside the headgroup and a series of amides and ureas were prepared from the protected D-glucosamine and the corresponding isocyanates or acid chlorides, in good yields. The self-assembling properties of these compounds were studied in several solvents, including water and aqueous solutions. Comparing to the ester and carbamate derivatives previously prepared from D-glucose, the amides and urea derivatives afforded more robust gels at lower concentrations typically. Most of these compounds were found to be efficient low molecular weight hydrogelators (LMHGs) for aqueous solutions at concentrations lower than 0.5 wt %. The preparation and characterization of these compounds are reported here.

4.2. Intoduction:

This chapter is adapted from paper: Goyal, N.; Cheuk, S.; Wang, G. *Tetrahedron*. **2010**, *66*, 5962-5971.

Low molecular weight organogelators and hydrogelators are an interesting new class of compounds that have drawn great attention over the past few decades. LMWGs are small molecules that can self-assemble and form 3-dimensional network which allows the immobilization of solvents. [1-6] Based on the solvent system they are divided into two categories. Low molecular weight hydro gelator (LMHGs) and low molecular weight organo gelator (LMOGs). The resulting gels are usually called supramolecular gels or physical gels to differentiate from polymer gels. Formation of a compound to form gel depends on many factors including π-π interaction, hydrophobic interaction and Van der Waal force attractions. [7-9]

The preparation of organogels or hydrogels using LMOGs is pretty straightforward. In general, a certain amount of a LMOG is placed in a vial containing the testing solvent. Then it is heated above the sol-gel temperature (Tgel) to dissolve the compound. Gels are formed when the solution is cooled below the Tgel.[10-11] The resulting solidification or increase of the viscosity of the solvent(s) is caused by the formation of a fibrous network, which is created by the noncovalent inter and intramolecular forces. The minimum gelation concentration (MGC) is obtained through serial dilution and heating until a stable gel is no longer formed.

LMWGs have gained considerable amount of interest due to their implications in supramolecular chemistry, and their potential applications as advanced soft materials in biomedical and materials research. For instance, organogelators have been explored as optical electronic devices and have

found applications in semiconductors and photovoltaic cells. They have also been explored as sequestering agent for oil and chemical spill cleanup. As for hydrogels, they are also being explored for biomedical applications, such as matrix for cell growth, enzyme assays, drug delivery, and tissue engineering. The supramolecular gels are reversible and can be engineered to be stimuli-responsive.[12-15]

Because the main driving force for supramolecular hydrogel is hydrophobic interactions, the structure of hydrogelators mainly consists of contrasting polar and nonpolar regions, in which polar groups mainly include amino acids or sugars,[16-25] while the nonpolar regions mainly include long alkyl chains or aromatic rings.[26-28] Figure 3.1 shows the structures of several compounds (**1-5**) that can form gels in water. Compounds **3** and **4** are ambidextrous (they are able to gelate both water and organic solvents).[29]

Compound **5** has a unique bisoxalyl group with a phenyl side chain. It forms gels in organic solvents and its *trans* isomer can also form a gel in water. [30]

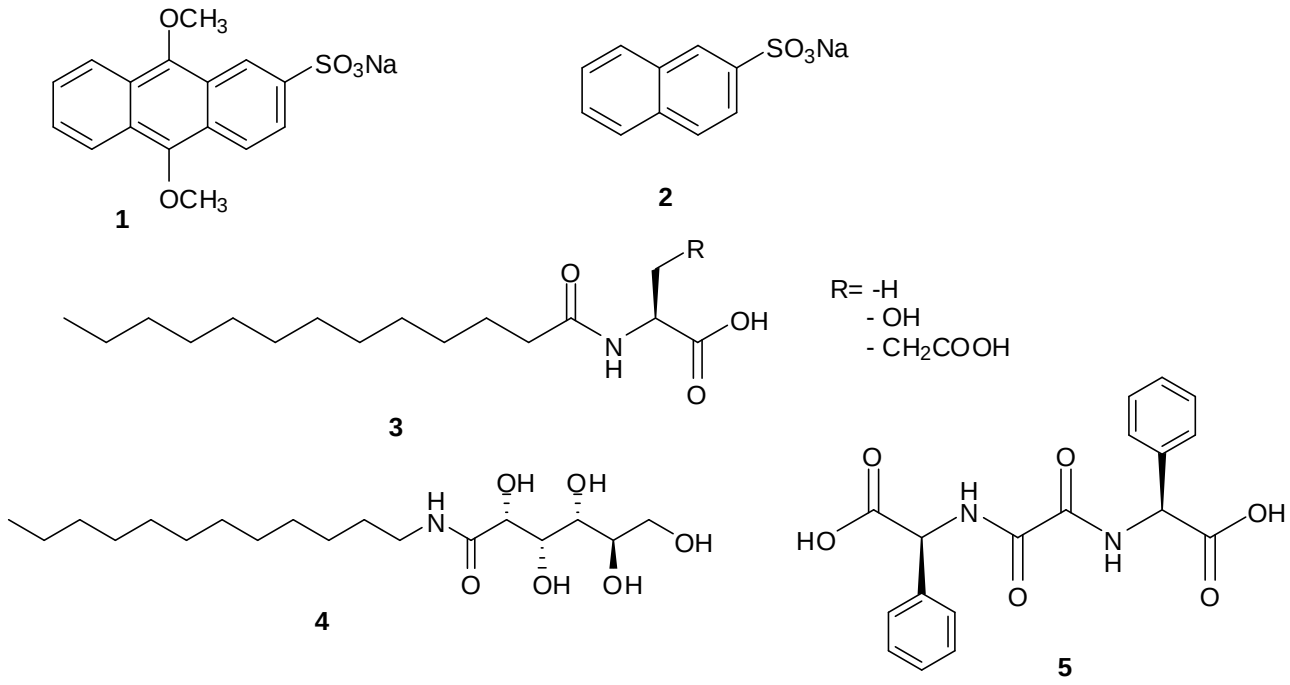


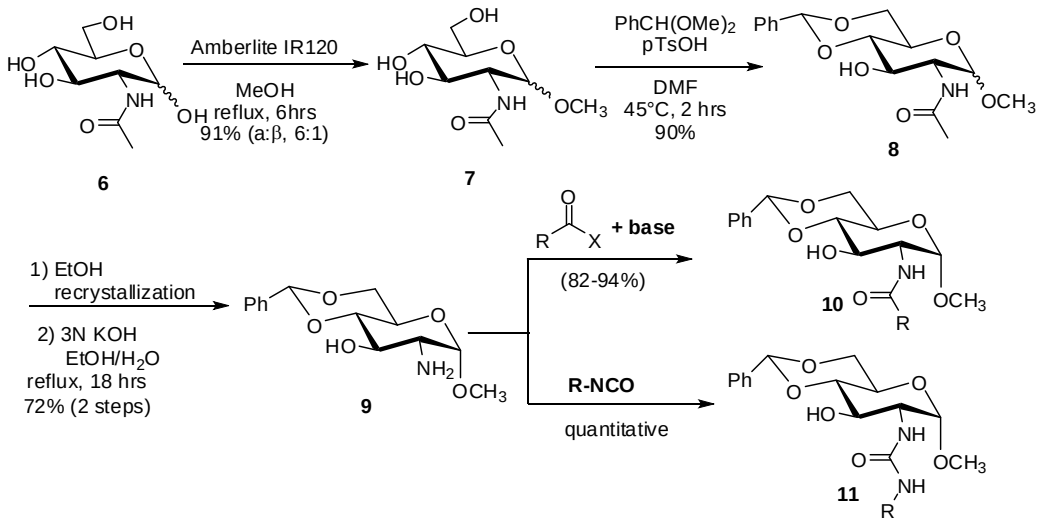
Figure 4.1. Structures of some hydrogelators.[28-30]

Many studies have been directed towards designing hydrogelators. Most of them are discovered serendipitously and improved analogs are found by modifying the parent structure. To design a hydrogel, there must be a balance between the polar and nonpolar groups. Furhop and coworkers synthesized a library of LMHGs by introducing the alkyl amine chain into several aldonic acids.[29] Hamilton's group has shown that ureas with short alkyl chains act as hydrogelators, while those with larger chains act as organogelators.[21] Urea derivatives are placed in a separate category, because they exhibit strong hydrogen bonding, even in water.[21] Suzuki and coworkers synthesized many compounds with positively charged nitrogen atoms in the structure and tested for their gelation activity, many of them form gel in pure water.[24]

Carbohydrates are naturally abundant renewable resources, which are useful in the preparation of chiral intermediates and advanced materials.[31] Carbohydrate-based low-molecular-weight gelators have been reported by several research groups.[32-50] The dense chiral hydroxyl groups present in carbohydrates can be regioselectively functionalized to form interesting self-assembled supramolecular structures including LMWGs. The modification of carbohydrate derivatives to obtain effective low molecular weight hydrogelators and organogelators is of great interest to us.[35-40] Previously, we had found that various derivatives of 4,6-O-benzylidene-methyl-α-D-glucopyranose exhibited good gelation properties.[47-49]

4.3. Results and Discussion

In order to understand the structure requirements for the glucosamine derivatives to form stable and efficient hydro/organogels at lower gelation concentrations, we synthesized and characterized two new series of compounds, the amides **10** and ureas **11**. The amides and ureas can be synthesized by derivatizing the amino group in compound **9**, which is obtained in four steps from N-acetyl-glucosamine **6** as shown in Scheme 4.1.[51] First, N-Acetyl-*D*-glucosamine **6** was methylated in the presence of acidic resin, giving a mixture of anomers with the anomer as the major product. The resulting intermediate **7** was converted to the actamide **8** using benzylidene dimethyl acetal. The major isomer was separated via recrystallization in ethanol, followed by hydrolysis using KOH afforded the head group **9**.



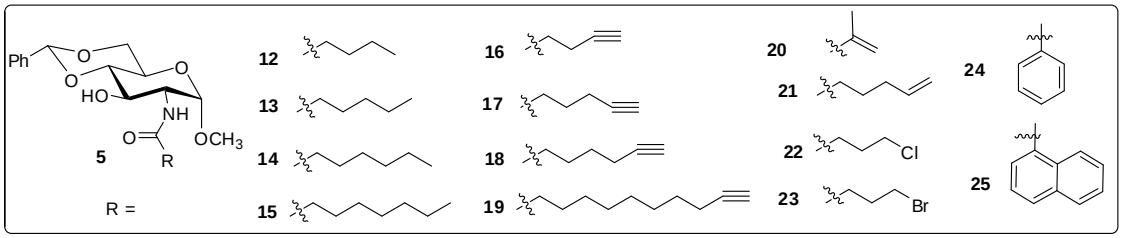
Scheme 4.1. Synthesis of the amides and ureas from *N* -acetyl-*D*-glucosamine.

Amide derivatives

The amides **10** were prepared by the method shown in the last step of Scheme 4.1. The amino group from **9** was typically functionalized with an acid chloride in the presence of pyridine or triethylamine to give the amide in good yield. The structures of the amides synthesized and their gelation properties in several solvents are summarized in Table 4.1. These functional groups were chosen partially based on the structural information obtained from the ester derivatives. We expected that the additional hydrogen bonding unit would allow a wider range of the hydrophobic tail R groups to afford gels because of the potential for enhanced hydrogen bonding interactions. Therefore, a broader series of acyl groups were selected here. These include the straight chain analogs with 5-8 carbons **12-15**, compounds with terminal acetylene functional groups **16-19**, compounds with alkene groups **20** and **21**, compounds with halogens **22** and **23**, and aromatic derivatives **24** and **25**. After their preparation, these compounds were screened for their gelation properties (Table 4.1). From Table 4.1, most of the compounds were able to form

stable gels in 33% aqueous solutions of DMSO or ethanol at concentrations lower than 5.0 mg/mL. Several compounds also formed gels in ethanol and fewer formed gels in hexane or water. These results are different than the ones obtained from the glucose ester derivatives, in which a majority of the alkynyl 2-esters were able to gelate both hexane and water. The presence of the amide bonds, especially the NH bond, is important in the formation of the self-assembled networks. Similar to the glucose ester derivatives, the alkyl chain plays an important role in gelation. We found that amides containing chains that were 6-8 carbons in length formed very stable gels at relatively low concentrations. Typically, the amides formed robust and translucent gels. For example, the hexylamide formed gels that were stable for several months in closed containers. The gels can be reformed repeatedly by reheating and sonication of the solution phases. The ability of these various amides to form stable gels in aqueous mixtures might arise from a hydrogen bonding interaction of the amide bonds with the solvents. Ethanol and DMSO can aid in self-assembly by solvating the hydrophobic regions of the LMOGs, disrupting the crystalline packing, and thus forming gels. In addition, the compounds and the solvents have a certain ‘matched’ hydrophobicity and hydrophilicity, in that ethanol and DMSO can help to stabilize the gels by interacting with both regions in the amides.

Table 4.1. Library of amide derivatives of **9** and their corresponding minimum gelation concentrations (MGCs) in mg/mL.



Compd	Structure	Hexane	Water	EtOH	Water:DMSO (2:1)	Water:EtOH (2:1)	THF	Isoprop.	DCM
12		I	I	G 5.0	G 4.0	4.0	S	S	S
13		C	C	S	G 1.3	G 1.3	S	S	S
14		G 1.7	I	S	G 1.0	G 2.0	S	S	S
15		G* 2.0	I	S	G 1.2	G 1.6	S	S	S
16		C	I	G 10.0	G 10.0	G 5.0	S	G 20	S
17		C	I	S	G 5.0	G 2.2	S	G10	S
18		C	C	S	G 2.0	G 0.7	C	C	S
19		G 4.0	I	S	G 1.7	G 1.2	P	C	S
20		C	I	S	C	G 10	S	S	S
21		C	I	G 10	G 5	G 2.5	P	S	S
22	Cl	C	I	G 10	G 6.6	G 5.0	S	S	S
23	Br	I	I	G 20	G 5	G 20	S	S	S
24		I	G 2	S	G 5.0	G 3.3	C	S	S
25		C	I	S	C	G 10	C	S	S

G*unstable gel; G, gel at room temperature; the numbers in the table are the minimum gelation concentrations in mg/mL; I, insoluble; C, crystallization; S, soluble at ~20 mg/mL, P, precipitation.

For the aliphatic derivatives, the amides with saturated alkyl chains (**12-15**) are quite versatile and efficient LMOGs that are able to efficiently gelate a range of solvents, including hexane and aqueous mixtures of ethanol and DMSO, but not pure water. The compounds with 6-8 carbon chains (**13-15**) are the most efficient gelators. The alkynyl derivatives exhibit a similar trend, in that a longer chain is somewhat more favorable than a shorter chain; compounds **17-19** are good gelators for aqueous solutions of DMSO and EtOH. The hexynyl compound **16** proved to be the most efficient, forming stable gels in ethanol/water at 0.7 mg/mL. For the alkenyl derivatives, the pentenyl derivative **21** is a versatile gelator, but the methacrylate derivative **20** is less efficient. The halogenated compounds **22** and **23** also showed positive results. For the two aryl derivatives, the phenyl amide **24** formed stable gels in water, and aqueous DMSO and ethanol, while the naphthyl amide **25** only formed gels in aqueous DMSO at higher concentrations.

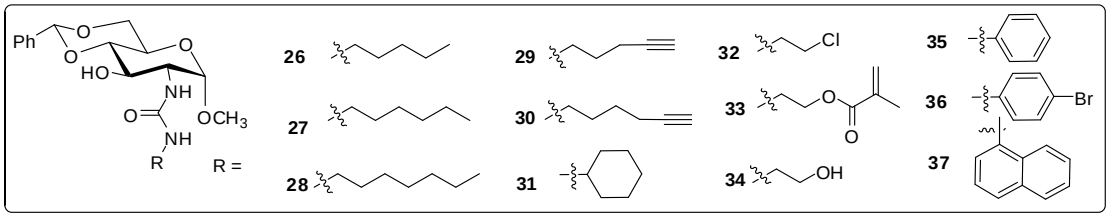
Urea derivatives

Several urea derivatives were prepared by reacting compound **9** with a stoichiometric amount of the corresponding isocyanate in THF. For the compounds with terminal acetylenes, terminal alkynyl acids were converted to the corresponding isocyanates in situ by Curtis rearrangement using DPPA and triethylamine. The reactions generally proceeded with close to quantitative yields and the products can be purified on silica gel by flash chromatography using a polar solvent. The structures of the ureas synthesized and their gelation properties are shown in Table 4.2. The selection of the R groups is based on the results from the carbamate and amide series and the availability of starting materials. These include compounds **26-28**, with saturated 5-7 carbon alkyl chains, and **29** and **30**, which are 5-6 carbon terminal alkynyl derivatives. The

cyclohexyl urea 31, several compounds with terminal substituents **32-34**, and aryl ureas **35-37** were also prepared. The gelation test results are shown in Table 4.2. From the screening of the gelation results of these compounds, we can determine how the structure of the alkyl or aryl groups affects self assembling.

For the sugar derivatives here, the urea analogs showed gelation tendencies similar to the amides, with the aliphatic derivatives with 5-7 carbons being most versatile gelators for aqueous mixtures. Compounds **26-29** formed gels in EtOH/water and DMSO/water at concentrations lower than 0.2 wt %. The presence of a terminal acetylene group does not seem to affect gelation, and the compounds with the same chain length as their saturated counterparts gave similar gelation results. Interestingly, the chloroethyl and methacryloyl ethyl ureas (**32**, **33**) were also able to form gels in aqueous solutions, while the carbamate and ester analogs were not able to form gels in these solvents. The methacrylate **32** can form gels in water at 10 mg/mL. After hydrolysis of the methacrylate, the alcohol **34** can also form stable clear gels in water. Aromatic ureas are somewhat less effective for aqueous solutions compared to the aliphatic ureas; this may be due to the extra rigidity in the molecule. The aromatic urea derivatives **35-37** contain hydrogen bonding functions plus the aromatic rings necessary for π-π stacking. These aromatic interactions reinforce the molecular packing and result in stronger intermolecular forces. Therefore, they tend to be insoluble or crystallize in polar solvents.

Table 4.2. Library of urea derivatives of **2** and corresponding MGCs (mg/mL).



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Cmpd	R =	Hexane	Water	EtOH	Water: DMSO	Water: EtOH	THF	Isoprop.	DCM
26		I	I	S	G 1.2	G 1.3	S	S	S
27		I	I	S	G 1.0	G 1.5	S	S	S
28		I	P	G 10	G 1.0	G 1.6	S	G20	S
29		P	I	G 6.6	G 2.8	G 1.2	S	G20	S
30		C	I	S	G 2.8	G 1.3	P	S	S
31		I	I	G 10	G 3.3	G 1.3	S	S	S
32	Cl	I	I	S	G 10	G 10	S	S	S
33	O O	P	G 10	S	G 6.7	G 2.2	S	S	S
34	OH	I	G 2.2	G 20	G 10	G 4.0	S	G20	S
35		I	I	G 10	G 5	G 20	S	S	S
36	Br	I	I	I	G 2.5	G 2.0	C	S	S
37		I	I	G 15	G 2.2	P	S	S	S

G, stable gel at room temperature, the numbers are the minimum gelation concentrations in mg/mL; I, insoluble; C, crystallization; S, soluble at ~20 mg/mL, P, precipitate.

We analyzed several gels in their gel states using optical microscopy, in an attempt to reveal the supramolecular assembly of the gels with the solvents still trapped inside. The dried xerogels were also studied by optical microscopy and scanning electron microscopy. These results are

shown in Figures 4.2-4.4. The optical micrographs of the wet gels formed by compound **19** are shown in Figures 4.2. The gel of **19** in ethanol/water showed bundled fibrous assemblies, as shown in Figures 4.2 a-c. At lower magnification (200x), we can see the long fibers floating in the gel solvent matrix. Figures 4.2c is a picture of the bundled fibers in Figure 4.2b at a higher magnification, and it is evident that the dark regions are composed of many tubular types of aggregates and smaller, softer feather like fibers spanning from the center. In DMSO/water solutions, we also observed tubular types of structures, as shown in Figure 4.2d-f. The fibers or tubules emerged from the gel media or were trapped in the gel media (Figure 4.2d and e). At higher magnification (500x), we can observe the embedded fibrous network at the surface of the gel droplet (Figure 4.2f).

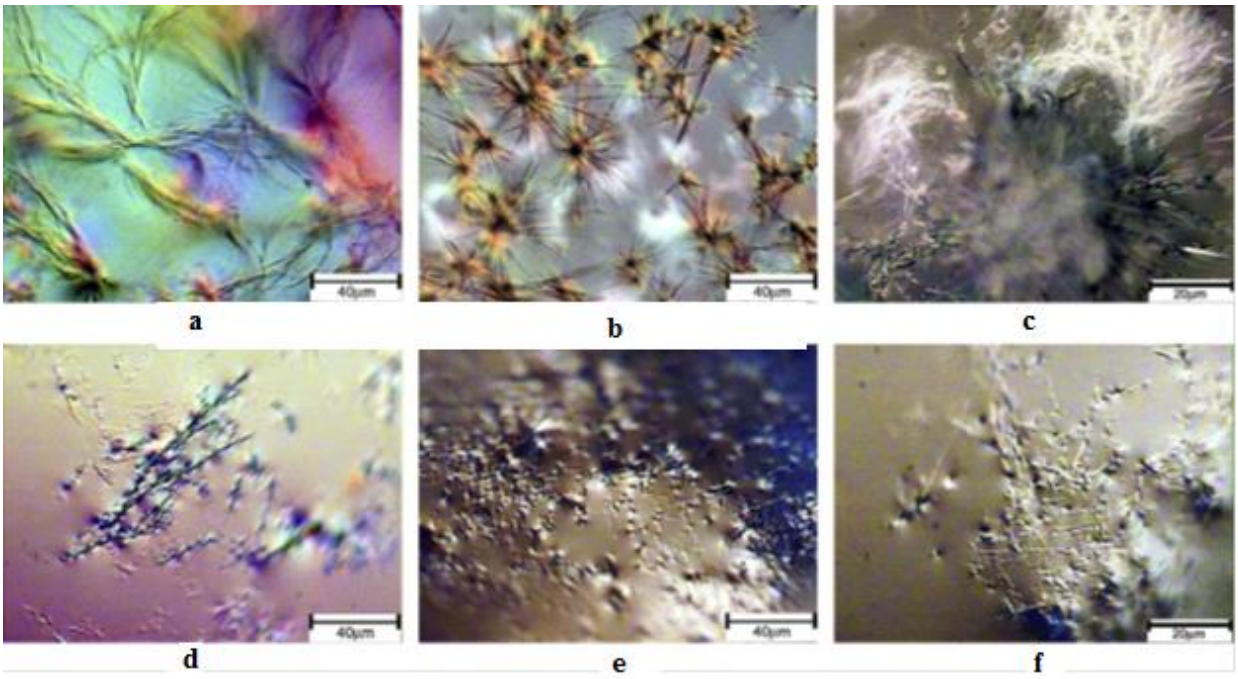


Figure 4.2. Optical micrographs of the gels formed by compound **19** in wet gel state. a-c are gels in EtOH/H2O (1:2) at 3 mg/mL; d-f are gels in DMSO/H2O (1:2) at 2 mg/mL. a,b,d and e are obtained at 200x magnification; c and f are at 500x magnification.

The gels formed by compound **21** also showed somewhat similar morphologies, as shown in Figure 4.3. In EtOH/H2O, we can clearly observe the fibrous assemblies at low magnification (Figure 4.3a), while at higher magnification, more detailed features of the fibrous networks can be observed (Figure 4.3b-d), including birefringent fibers and tubules. In DMSO/water, long fibers were also the predominant morphologies observed in the gel (Figure 4.3e-f). These were more difficult to see since the gel is three-dimensional. In many regions, we were only able to focus on one slice of the gels.

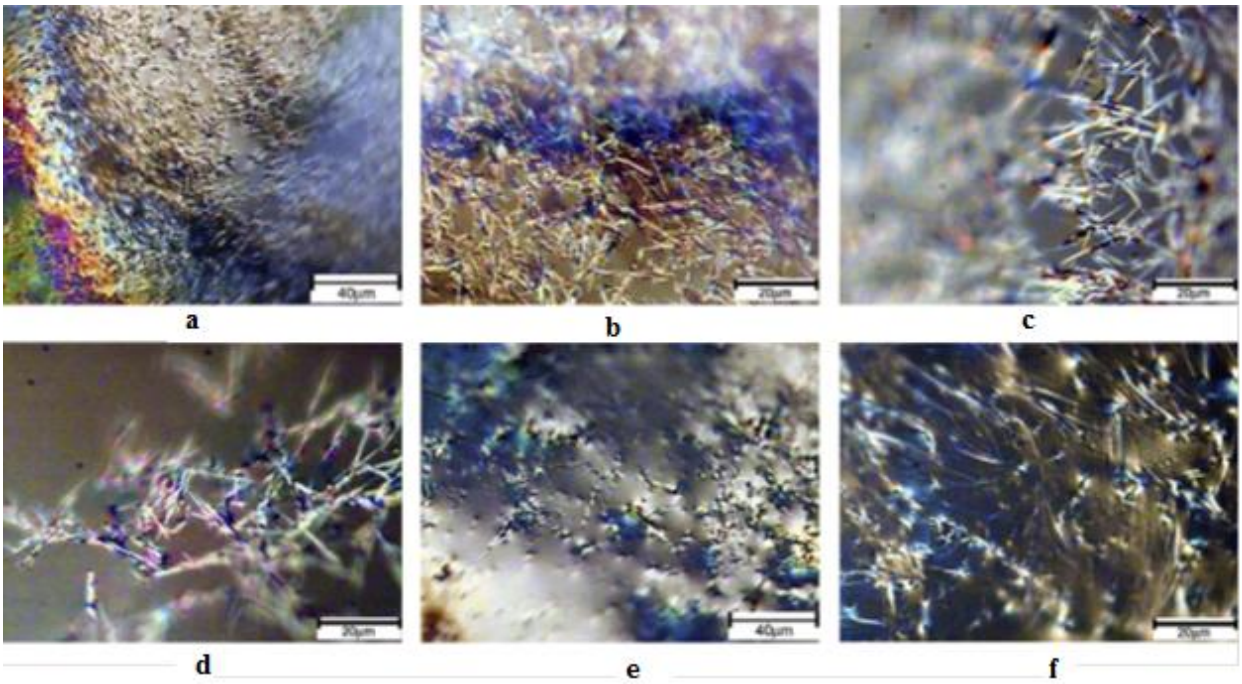


Figure 4.3. Optical micrographs of the gels formed by compound **21** in wet gel state. a and d are gels in EtOH/H_2O (1:2) at 2.5 mg/mL; e and f are gels in DMSO/H2O (1:2) at 3 mg/mL.
These images were obtained under crossed polarizers in phase contrast mode. a and e are 200x, b,c,d and f are at 500x magnification.

We also obtained the optical micrographs of the ureas in the gel phase, and these are shown in Figure 4.4. They formed somewhat different morphologies compared to those of the amides. Compound **30** formed a stable gel in ethanol/water (1:2). The gel surface was smooth and

contained some contiguous fibrous assemblies, though it was difficult to obtain better-quality pictures since, typically, they were not isolated fibers (Figure 4.4a). In DMSO/water (1:2), it formed a similar morphology, and a slice of the gel showed some intertwined fibrous networks (Figure 4.4b). The gel formed by compound **26** showed different morphologies as well. In EtOH/H_2O, in thinner regions, we could observe the soft tubular networks (Figure 4.4c), while in the denser regions, when the solvent evaporated, we were able to observe densely packed fibrous structures (Figure 4.4d). In DMSO/H_2O, we again were able to see some continuous intertwined fibrous assemblies at low magnification (Figure 4.4e) and the tubular fibrous networks were observed more clearly at higher magnifications (Figure 4.4f). Again, it was difficult to obtain the images of the gels since they are three-dimensional, so we obtained the micrographs of different cross-sections of the gels. These images showed that at different surfaces of the gels similar features of the fibrous assemblies can be observed.

For the dried gels, it was easier to obtain their morphologies using OM or EM, and several of these are shown in Figure 4.5. The gels exhibit different morphologies, depending on their structures. The optical microscopy of the dried gels revealed that flexible fibers lead to more effective gelators, and that the derivatives with straight alkyl chains (as opposed to aromatic groups) tend to form these morphologies, especially in aqueous solvents. The gel formed by **18** in ethanol/water showed long uniform fibrous features (Figure 4.5a). The gel of compound **13** in DMSO/water showed presence of very thin fibrous network structures (Figure 4.5d).

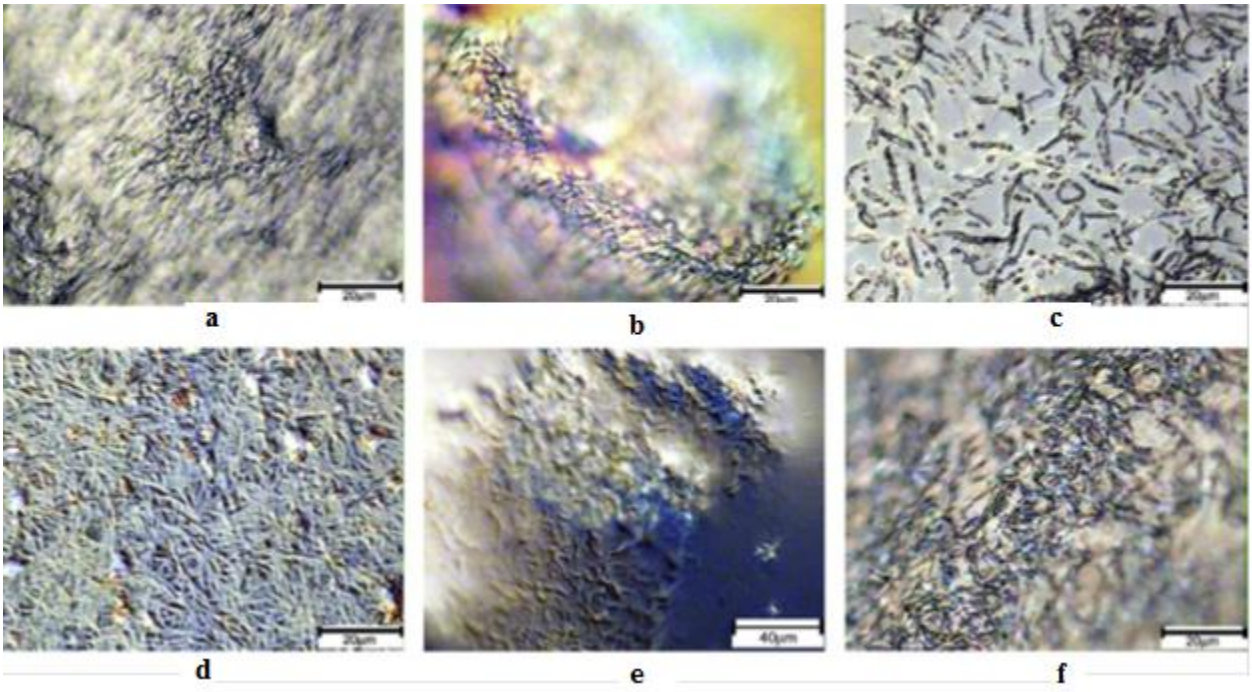


Figure 4.4. Optical micrographs of the gels formed by compounds **26** and **30** in gel phases. a, gel by **30** in EtOH/H_2O (1:2) at 1.5 mg/mL; b, gel by **30** in DMSO/H2O (1:2) at 3.0 mg/mL; c and d, gel by **28** in EtOH/H_2O (1:2) at 1.5 mg/mL; e and f, gel by **28** in DMSO/H_2O (1:2) at 1.2 mg/mL. Magnification for e is 200x, and for the rest is 500x.

Both the OM and SEM of the gel formed by heptyl amide **14** in hexane showed the formation of entangled fibrous networks (Figure 4.5b-c). The urea derivative **34** also formed fibrous or tubular type of structures (Figure 4.5e-f). The SEM indicated that the tubular structures typically have diameters less than 1 μm.

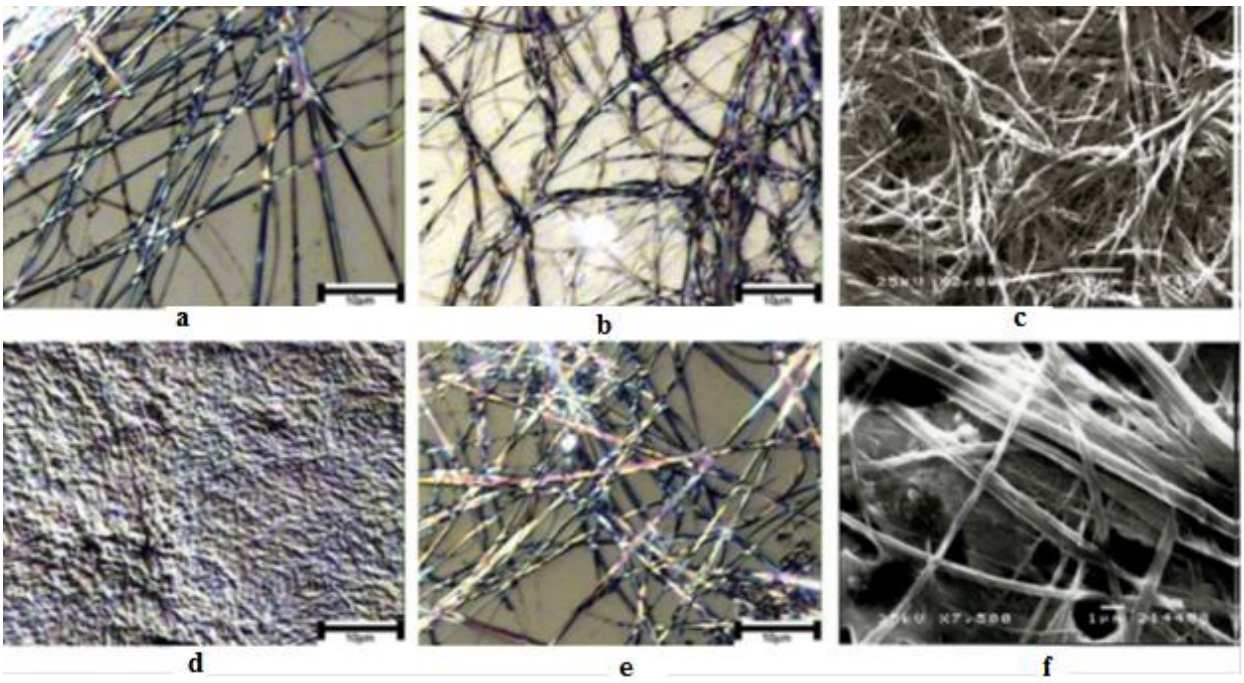
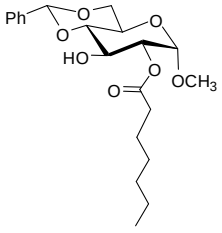


Figure 4.5. Optical micrographs (a, b, d, e) and scanning electron micrographs (c, f) of several dried gels. A, gel of compound **18** in EtOH/H_2O (1:2) at 0.7 mg/mL, b and c, gel of compound **14** in hexane at 1.7 mg/mL; d, gel formed by compound **13** in DMSO/H_2O (1:2) at 1.0 mg/mL, e and f, gel formed by compound **34** in water at 2.5 mg/mL. Magnifications for a, b, c, and e are 1000x.

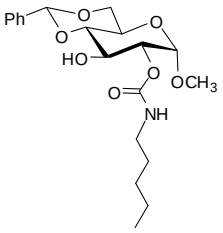
From Tables 4.1 and 4.2, we can compare the gelation properties of amides and ureas containing similar alkyl chain derivatives. The compounds containing similar alkyl chain lengths are shown in Table 4.3, where the headgroup linkages are ester **38**, carbamate **39**, amide **14**, or urea **26**. The gelation ability of the compounds increases with the addition of an NH hydrogen bond donor. The solvents also affect the results, since hydrophobic forces are dominant in hydrogels, whereas hydrogen bonding is the primary force in organogels. In aqueous solutions of DMSO and ethanol, the presence of organic soluble components makes the hydrogen bonding somewhat more important and the hydrophobic interactions less important. The notable difference in the gelation ability between the ester and carbamate derivatives of compound **9** indicates that the -NH is essential. This hydrogen bond donor is likely involved in the formation of a one-

dimensional hydrogen bonding network. When the hydrogen bond donor NH group is closer to the sugar pyranoside ring as in compounds **15** and **19**, the gelation capabilities are similar to that of the carbamates. From esters to carbamates, amides, and ureas, the number of hydrogen bond donors in the molecules increases. It seems that the increase in intermolecular interactions is necessary to extend the network. Typically, the amides and ureas formed more robust gels than the esters.

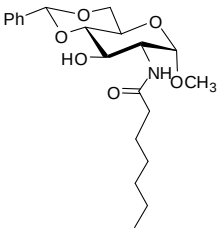
Table 4.3. Comparing LMOGs with similar alkyl chain lengths. Positive gelation results are noted in mg/mL; P, precipitate; I, insoluble.



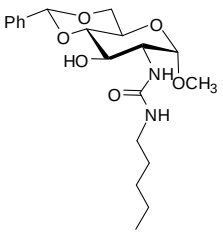
38



39



14



26

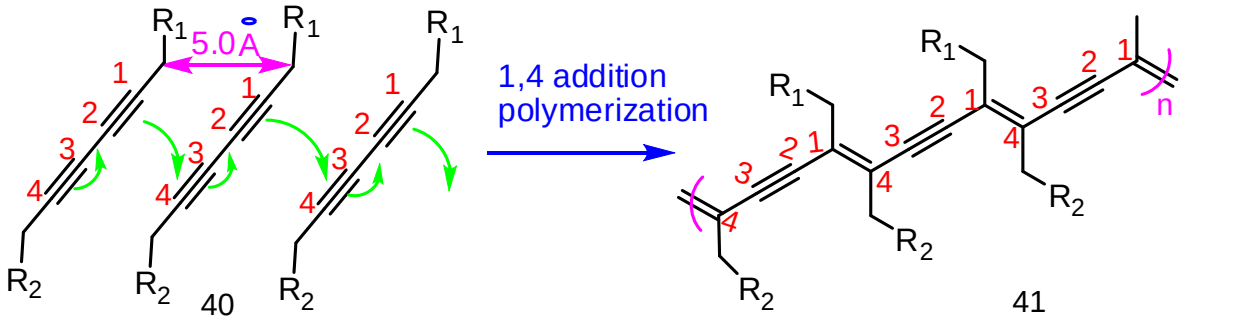
Compounds	**38**	**39**	**14**	**26**
H_2O	I	20	I	I
H_2O:DMSO 2:1	P	3.0	1.0	1.2
H_2O:EtOH 2:1	P	1.4	2.0	1.3

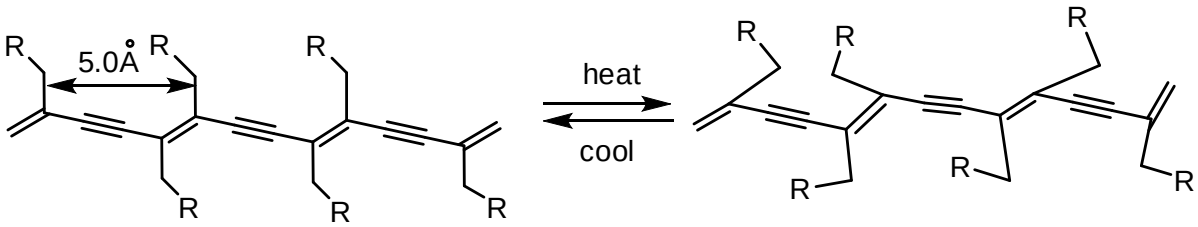
4.4. Polymerizable diacetylenes

Polydiacetylenes (PDAs) are important conjugate polymers that have drawn great attention over the past few decades. PDAs exhibit a unique blue to red color transition in the presence of heat, mechanical stress, pH change, and binding to biological agents. [52-54] Polydiacetylenes are interesting polymers exhibiting a typically blue to red color transitions in response to

environmental changes and binding to biomolecules, they also have non-linear optical properties and show other important optical effects. Extensive studies of polymerizable diacetylenes have been carried out in crystals, [55,56] thin films,[57,58] and at air-water interfaces. The optical electronic properties and the unique color transitions of polydiacetylenes lend themselves to many applications in optical electronic devices, chemosensors, and biosensors.[59-64]

To cross-link diacetylene groups, the monomer diacetylenes must be aligned at specific distances and orientations to their neighbors. It is generally accepted that the color transition is due to the conformation change of the PDA side chains (Scheme 4.2).

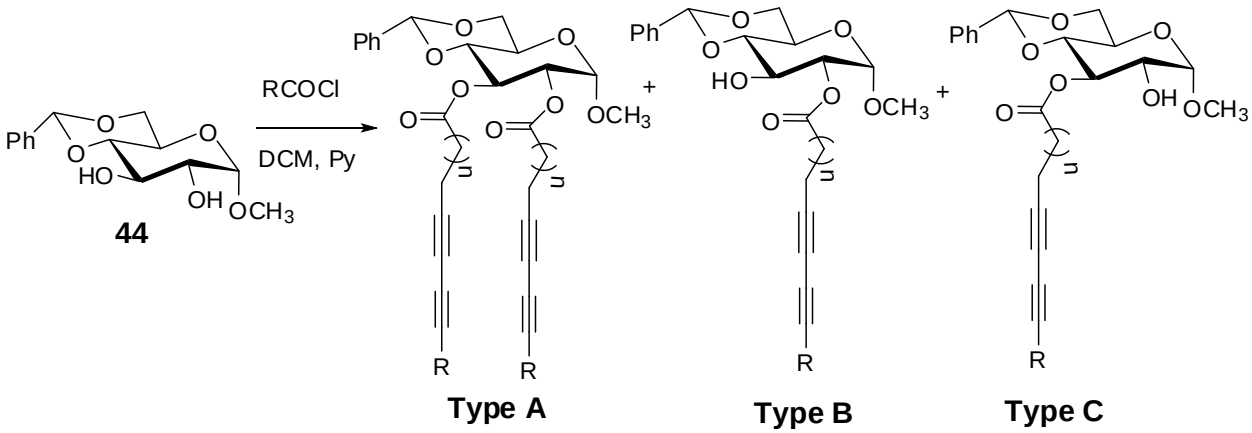




Scheme 4.2. The topochemical polymerization of diacetylene and the color transition mechanism

The diacetylene-containing organogels may have interesting properties of color transition combined with gel-solution phase transition in response to external stimuli. These compounds can be useful in designing biosensors or chemosensors. Sugar containing amphiphilic molecules are cell membrane mimics and are expected to be biocompatible. As part of our goal to discover carbohydrate-based stimuli-responsive functional materials that are useful in enzyme purification, protein and DNA immobilization, drug and gene delivery carriers, and as scaffolding material for tissue engineering.

We have previously studied a series of diacetylene containing glycolipids using D-glucose as the headgroup, a small library of glycolipids were synthesized and screened, we found that a majority of the 2-monoacylated derivatives were effective low molecular weight gelators for ethanol and ethanol aqueous mixtures.



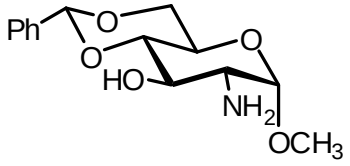
n = 2, 5, 7

R = H, Ph, Et, CH_2CH_2Ph, $(CH_2)_7CH_3$, $(CH_2)_5CH_3$

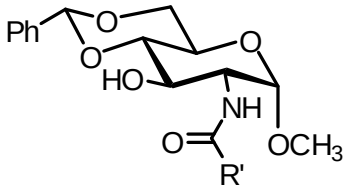
Scheme 4.3. Synthesis of diacetylene containing lipids type **A**, type **B**, and type **C**.

These diacetylene containing glycolipids with long chain fatty acyl tails are effective gelators for organic solvents, including ethanol, and ethanol/water mixtures, but not pure water. The gels formed by some of our diacetylene ester compounds can be polymerized, and depending on the structure, some of them showed the typical blue to red color transition upon heating.[47]

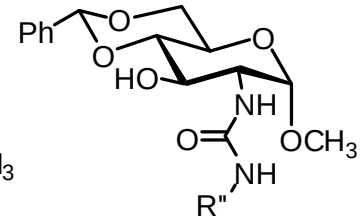
Recently we also found that amide and urea analogs (**10, 11**) using the protected D-glucosamine 2 as headgroup are excellent gelators for polar solvents and aqueous mixtures.[50] The additional hydrogen bonding capacity in amides and ureas can potentially produce more stable gels than ester derivatives. Therefore, we synthesized a series of diacetylene derivatives using glucosamine headgroup.



9



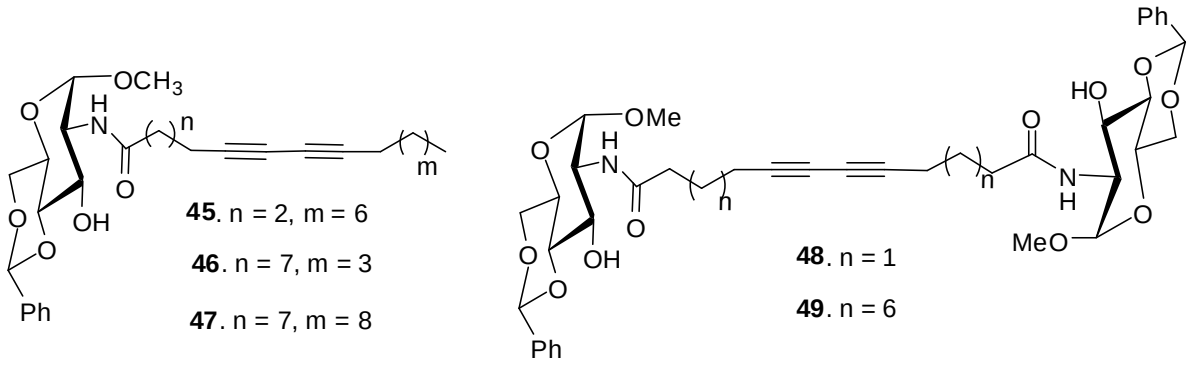
10



11

4.5. Results and Discussion

Previously, we have found that diacetylene containing glycolipids with long chain fatty acyl tails are effective gelators, however, these esters may be susceptible to hydrolysis under basic or strong acid conditions.[47] Also, the gels produced typically required concentrations around 1wt%. Recently we also found that amide and urea analogs using the protected D-glucosamine as headgroup are excellent gelators for polar solvents and aqueous mixtures at concentrations well below 1wt%. The additional hydrogen bonding capacity in amides and ureas can potentially produce more stable gels than ester derivatives. We are interested in producing effective gels that are responsive to environmental changes, this could be used as chemosensors or biosensors. In this study, we synthesized a series of diacetylene containing compounds using the D-glucosamine headgroup, these are shown in Schemes 4.2-4.3. Here we prepared similar monoacyl chain derivatives which can be viewed as analogs to the type B ester gelators. The preparation of these compounds is straightforward with generally high yields. Besides these, we also synthesized a few bis-polar derivatives (Figure 4.6) with two polar headgroups at each end. The dimeric systems (**48**, **49**, **54**) are interesting compounds that may produce different self-assembling morphology and properties than the monomeric systems (**45-47**, **50-53**) and form important and interesting materials.



Scheme 2. Synthesis of urea derivatives

Figure 4.6. Structures of diacetylene containing amide derivatives synthesized.

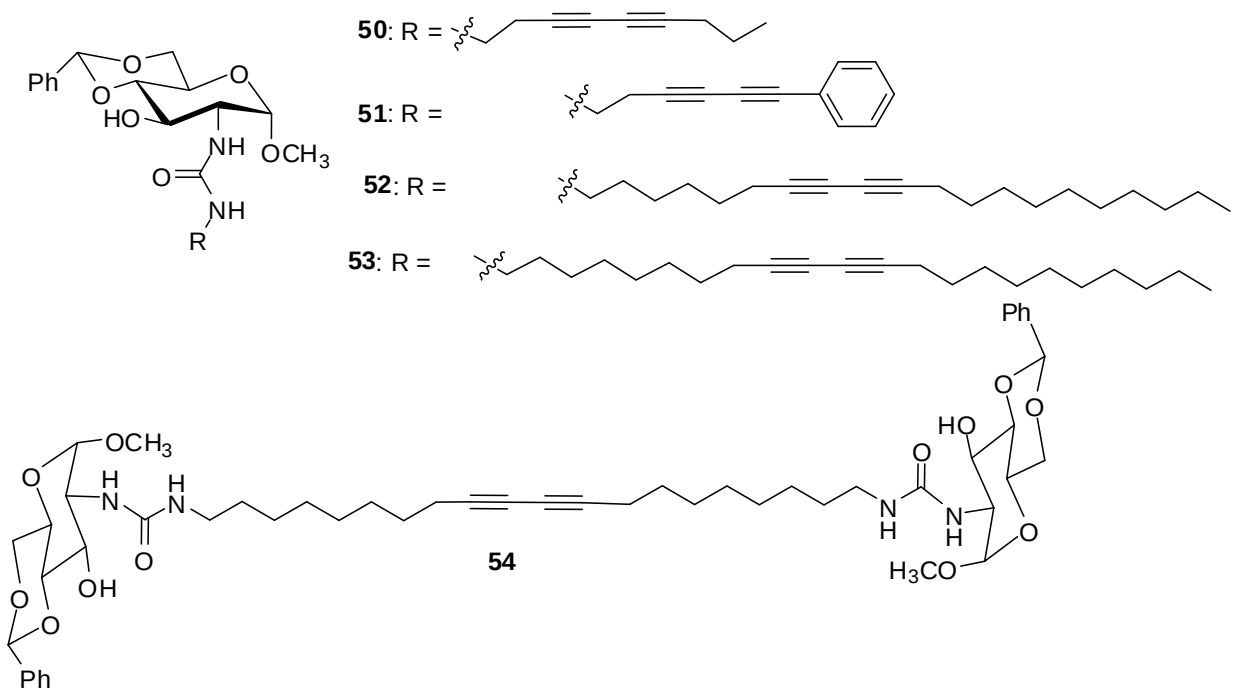


Figure 4.7. Structures of diacetylene containing urea derivatives synthesized.

These compounds were then screened for their gelation in a series of solvents, the result is shown in Table 4.4. Nearly all the compounds synthesized here are effective gelators for ethanol at

concentration less than 20 mg/mL, (2 wt%) except compound **45** which is soluble in ethanol. Most of the compounds are not soluble in either water or hexane or water/DMSO mixture. However, while many compounds were able to gelate water and ethanol mixture, most of them are efficient gelator for toluene. Several of them are also effectively solidify isopropanol. Among these compounds, the amide **47** is the most efficient gelator, forming gel in ethanol at 0.08 wt%, and 0.3 wt% in isopropanol. For the bispolar compound, they are as effective as or better than the monomeric derivatives.

Table 4.4. Library of diacetylene containing amides and ureas derivatives of D-glucosamine and their corresponding minimum gelation concentrations (MGCs) in mg/mL.

Compound	Hexane	toluene	EtOH	iPr-OH	H_2O:EtOH (2:1)	H_2O:EtOH (3:1)	Water	H_2O: DMSO
45	I	20	S	S	1.0	20	I	I
46	I	10	20.0	20	1.6	C	I	C
47	I	10	0.8	3.0	C	ND	I	I
48	I	I	10.0	10	2.5	I	I	I
49	I	20	3.0	4.0	C	ND	I	I
50	I	5	5.0	10	2.5	10	I	I
51	I	10	5.0	5	2.5	C	I	I
52	I	6.6	5.0	S	I	ND	I	I
53	I	20	5.0	S	I	ND	I	I
54	I	10	1.2	6.6	I	ND	I	I

U, unstable gel; G, gel at room temperature; I, insoluble; C, coaservate at 10 mg/mL; Cr, crystallization; S, soluble at ~20 mg/mL

4.6. Conclusions

A series of acyl derivatives of the protected D-glucosamine were prepared and analyzed for their gelation properties. These include amides and ureas, which were synthesized via straightforward reactions with high selectivity. For both libraries, alkyl derivatives are the most efficient LMOGs and they form very robust gels in aqueous ethanol or aqueous DMSO solutions. These two classes of compounds contain extra hydrogen bond donors in comparison to the esters and carbamates with similar acyl functional groups. The amino group at the 2-postion easily allows for the preparation of these library compounds and the reactions can be scaled up for large scale application studies. These small molecules can be useful for entrapping large biomolecules such as enzymes and provide a good media for enzymatic reactions. The gels formed by several compounds were analyzed using optical microscopy and scanning electronic microscopy. Several excellent low molecular gelators were obtained in this study, and the correlation between the structure and gelation can be used for the design of other functional organo/hydrogelators. We have also used this glucosamine to synthesis the diacetylene based amides and ureas. The self-assembling properties of these compounds were studied in several solvents. We identified several excellent organogelators for ethanol and ethanol water mixture. The color transition properties of the polydiacetylene gels can be used to predict changes in environmental conditions, such as in the event of binding to a biological agent.

4.7. Experimental Procedures

General Methods

Gelation Testing. The compounds were tested in a 1 dram vial with a rubber lined screw cap from Kimble. A starting concentration of 20 mg/mL was used. The mixture was heated and sonicated until the sample was fully dissolved compounds are dissolved. The solution was allowed to cool at r.t. for 20-30 minutes. If a gel is formed, then the vial is inverted and if no solvent flows while the gel is inverted, then it is called a stable gel. If the gel falls apart during inversion and by gentle shaking, then it is called an unstable gel. If a stable gel is formed, serial dilution is performed until the gels resulted are no longer stable. The concentration prior to the unstable gel was recorded as the minimum gelation concentration (MGC).

Optical Microscopy. The slides were prepared after a stable gel has formed. A small amount of the gel was placed on a clean glass slide and air dried over night. The xerogels were observed with an Olympus BX60M optical microscope using a DSP Color Hi-Res EXvision camera and an Olympus U-TV1X lens. The program used to acquire and store the photos was Corel Photo-Paint 7.

Scanning Electron Microscopy. Samples were prepared by drying the gel on an aluminum pellet in a desiccator under reduced pressure for several days. A thin layer of platinum was deposited on to the pellet by a Denton Vacuum (model Desk II) at a reduced pressure of ~30

mtorr and a current of 45 mA for 60 sec. The sample was analyzed using a JEOL JSM 5410 scanning microscope with an EDAX Detecting Unit.

Acknowledements: Thanks to Prof. Guijun Wang and Dr. Sherwin Cheuk for help with for OM and SEM images.

General procedure for the synthesis of amides

4,6-*O*-Benzylidene-2-amino-2-deoxy-methyl-α-D-glucopyranoside **4** and pyridine or DIEA (2 eq) were mixed in anhydrous THF at 0°C. The corresponding acyl halide (1.1 eq) was added dropwise. After 4-6 hrs the reaction was diluted with DCM and washed with H_2O and then dilute HCl (~0.1N). The organic layer was dried with anhydrous Na_2SO_4. The crude products are purified using flash chromatography with a gradient of solvent system hexane and acetone.

General procedure for the synthesis of ureas

The urea library was synthesized by mixing compound **4** and the corresponding isocyanate in stoichiometric quantities in anhydrous THF. The solution was stirred at r.t. for 6-8 hours and the crude products are formed. These are purified by chromatography if the ^{1}H NMR spectrum indicates the product is not pure. Typically DCM/MeOH gradient solvent system is used for the chromatography separation if needed.

Pentyl amide 12

The compound was obtained as a white solid at a yield of 87.6%, m.p. 195.2-196.0 °C. ^{1}H NMR (400MHz, $CDCl_3$), δ (ppm) 7.47-7.54 (m, 2H), 7.32-7.40 (m, 3H), 5.83 (d, 1H, J = 8.1 Hz), 5.57

(s, 1H), 4.72 (d, 1H, *J* = 4.0 Hz), 4.20-4.33 (m, 2H), 3.90 (dt, 1H, *J* = 3.3, 9.5 Hz), 3.78 (m, 2H), 3.59 (m, 1H), 3.41 (s, 3H), 3.13 (d, 1H, *J* = 3.3 Hz, OH), 2.26 (t, 2H, *J* = 7.3 Hz), 1.64 (m, 2H), 1.36 (sext, 2H, *J* = 7.3Hz), 0.92 (t, 3H, *J* = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 137.1, 129.1, 128.2, 126.3, 101.8, 98.8, 82.0, 70.7, 68.8, 62.3, 55.3, 54.0, 36.3, 27.6, 22.2, 13.7. HRMS Cacld for $C_{19}H_{28}NO_6$ $[M+H]^+$ 366.1917, found 366.1920.

Hexyl amide 13

The hexyl amide was obtained as a white solid at a yield of 86%, m.p. 192.3-193.1°C. ^{1}H NMR, (400 MHz, $CDCl_3$) δ (ppm) 7.47-7.52 (m, 2H), 7.33-7.39 (m, 3H), 5.85 (d, 1H, *J* = 8.4 Hz), 5.57 (s, 1H), 4.72 (d, 1H, *J* = 3.7 Hz), 4.19-4.32 (m, 2H), 3.91 (dd~t, 1H, *J* = 9.7 Hz), 3.74-3.83 (m, 2H), 3.59 (m, 1H), 3.41 (s, 3H), 2.25 (t, 2H, *J* = 7.3), 1.65 (p, 2H, *J* = 7.3 Hz), 1.32 (m, 4H), 0.90 (t, 3H, *J* = 7.2 Hz). ^{13}C NMR, (100 MHz, $CDCl_3$) δ (ppm) 174.9,137.0, 129.1, 128.2, 126.2, 101.8, 98.9, 82.0, 69.7, 68.7, 62.4, 55.2, 53.8, 36.3, 31.2, 25.1, 22.2, 13.8. HRMS Calcd for $C_{20}H_{30}NO_6$ $[M+H]^+$ 380.2073, found 380.2071.

Heptyl amide 14

The product was obtained as a white crystalline solid at a yield of 87%. m.p. 197.8-198.1°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.44-7.51 (m, 2 H), 7.30-7.38 (m, 3 H) 6.08 (d, 1 H, *J* = 8.8 Hz), 5.54 (s, 1 H), 4.69 (d, 1 H, *J* = 3.7 Hz), 4.25 (m, 1H), 4.15 (d, 1 H, *J* = 3.7, 9.9 Hz), 3.83 (t, 1H, J = 9.7 Hz), 3.69-3.78 (m, 2 H), 3.55 (m, 1 H), 3.37 (s, 3 H), 2.13-2.26 (m, 3H), 1.60 (m, 2H), 1.27 (s, 6 H), 0.85 (t, 3H, *J* = 6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 137.0, 129.1, 128.2, 126.2, 101.9, 98.9, 82.1, 70.0, 68.8, 62.4, 55.3, 53.8, 36.5, 31.4, 28.7, 25.5, 22.4, 14.0. HRMS Calcd for $C_{21}H_{32}NO_6$ $[M+H]^+$ 394.2230, found 394.2237.

Octyl amide 15

The product was obtained as a white crystalline solid at a yield of 88%, m.p. 183.8-185.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.46-7.52 (m, 2H), 7.33-7.39 (m, 3H), 5.85 (d, 1H, *J* = 8.8 Hz), 5.57 (s, 1H), 4.72 (d, 1H, *J* = 4.0 Hz), 4.28 (m, 1H), 4.23 (m, 1H), 3.90 (t, 1H, *J* = 9.5 Hz), 3.74-3.83 (m, 2H), 3.59 (m, 1H), 3.41 (s, 3H), 3.15 (sb, 1H), 2.25 (m, 2H), 1.64 (m, 2H), 1.19-1.40 (m, 8H), 0.87 (t, 3H, *J* = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 174.7, 137.0, 129.2, 128.2, 126.3, 101.9, 98.8, 82.1, 70.9, 68.8, 62.3, 55.3, 54.0, 36.6, 31.7, 29.1, 29.0, 25.6, 22.6, 14.0. HRMS Calcd for $C_{22}H_{34}NO_6$ $[M+H]^+$ 408.2386, found 408.2395.

4-pentynyl amide 16

The compound was obtained as a white solid at a yield of 79%, m.p. 180.0-180.7 °C. ^{1}H NMR (400MHz, $CDCl_3$), δ (ppm) 7.46-7.53 (m, 2H), 7.32-7.40 (m, 3H), 6.04 (d, 1H, *J* = 8.4 Hz), 5.57(s, 1H), 4.72 (d, 1H, *J* = 4.0 Hz), 4.22-4.33 (m, 2H), 3.91 (t, 1H, *J* = 9.5 Hz), 3.73-3.85 (m, 2H), 3.59 (m, 1H), 3.41 (s, 3H), 2.99 (sb, 1H), 2.52-2.59 (m, 2H), 2.45-2.50 (m, 2H), 2.02 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 136.9, 129.1, 128.1, 126.2, 101.8, 98.8, 82.6, 81.9, 69.5, 69.2, 68.7, 62.4, 55.2, 53.9, 35.0, 14.7. HRMS Cacld for $C_{19}H_{24}NO_6$ $[M+H]^+$ 362.1604, found 366.1604.

5-Hexynyl amide 17.

Flash chromatography using solvent CH_2Cl_2:MeOH 9.3: 0.7 to obtain the product in 70% yield as a white solid. M.p. 176.1-177.0 °C. ^{1}H NMR (400MHz, $CDCl_3$), δ (ppm) 7.47-7.52 (m, 2H), 7.33-7.40 (m, 3H), 5.92 (d, 1H, *J* = 8.8 Hz), 5.57(s, 1H), 4.72 (d, 1H, *J* = 3.7 Hz), 4.20-4.31 (m,

2H), 3.90 (dt, 1H, *J* = 3.3, 9.9 Hz), 3.73-3.83 (m, 2H), 3.59 (m, 1H), 3.41 (s, 3H), 3.06 (d, 1H, *J* = 3.3 Hz), 2.41 (t, 2H, *J* = 7.1 Hz), 2.28 (m, 2H), 1.99 (t, 1H, *J* = 2.6 Hz), 1.88 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 137.0, 129.2, 128.3, 126.3, 101.9, 98.8, 83.4, 82.0, 70.8, 69.3, 68.8, 62.3, 55.3, 53.9, 34.9, 23.9, 17.6. HRMS Cacld for $C_{20}H_{26}NO_6$ $[M+H]^+$ 376.1760, found 376.1756.

6-Heptynyl amide 18.

The compound was isolated as a white solid at a yield of 70%, m.p. 174.4-175.1 °C. ^{1}H NMR (400MHz, $CDCl_3$), δ (ppm) 7.47-7.53 (m, 2H), 7.33-7.40 (m, 3H), 5.85 (d, 1H, *J* = 8.4 Hz), 5.57 (s, 1H), 4.72 (d, 1H, *J* = 3.7 Hz), 4.29 (m, 1H), 4.24 (ddd, 1H, *J* = 3.7, 4.0, 8.8 Hz), 3.90 (dt, 1H, *J* = 3.3, 9.5 Hz), 3.74-3.85 (m, 2H), 3.59 (m, 1H), 3.41 (s, 3H), 3.03 (d, 1H, *J* = 3.3 Hz), 2.29 (t, 2H, *J* = 7.3 Hz), 2.22 (dt, 1H, *J* = 2.6, 7.0 Hz), 1.96 (t, 1H, *J* = 2.6 Hz), 1.79 (m, 2H), 1.58 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 137.0, 129.1, 128.2, 126.3, 101.8, 98.8, 84.0, 82.0, 70.6, 68.8, 68.6, 62.3, 55.3, 53.9, 35.9, 27.7, 24.6, 18.1. HRMS Cacld for $C_{21}H_{28}NO_6$ $[M+H]^+$ 390.1917, found 390.1917.

10-Undecynyl amide 19

The product was obtained as a white solid at a yield of 84%. Mp 184.5-185.3°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.46-7.52 (m, 2H), 7.32-7.39 (m, 3H), 5.92 (d, 1H, *J* = 8.4 Hz), 5.54 (s, 1H), 4.71 (d, 1H, *J* = 3.7 Hz), 4.26 (m, 1H), 4.20 (ddd, 1H, *J* = 3.7, 4.0, 8.8 Hz), 3.87 (t, 1H, *J* = 9.6 Hz), 3.71-3.82 (m, 2H), 3.57 (t, 1H, *J* = 9.0 Hz), 3.38 (s, 3H), 2.22 (m, 2H), 2.16 (dt, 2H, *J* = 2.6, 7.1 Hz), 1.93 (t, 1H, *J* = 2.6 Hz), 1.62 (m, 2H), 1.50 (pentet, 2H, *J* = 7.0), 1.37 (m, 2H), 1.29 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 174.6, 137.1, 129.1, 128.2, 126.3, 101.8, 98.8,

84.6, 82.0, 70.5, 68.7, 68.1, 62.3, 55.2, 53.9, 36.5, 29.1, 29.0, 28.8, 28.5, 28.3, 25.5, 18.3. HRMS Calcd for $C_{25}H_{36}NO_6$ $[M+H]^+$ 446.2543, found 446.2524.

Methacrylamide 20

The compound was obtained as a white solid at a yield of 85%. m.p. 187.2-187.9 °C. ^{1}H NMR (400MHz, $CDCl_3$), δ (ppm) 7.45-7.55 (m, 2H), 7.30-7.42 (m, 3H), 6.21 (d, 1H, *J* = 8.4 Hz), 5.76 (s, 1H), 5.56 (s, 1H), 5.39 (s, 1H), 4.76 (d, 1H, *J* = 3.7 Hz), 4.23-4.33 (m, 2H), 3.94 (t, 1H, *J* = 9.5 Hz), 3.72-3.84 (m, 2H), 3.60 (t, 1H, *J* = 8.8 Hz), 3.40 (s, 3H), 3.29 (s, 1H), 1.99 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 139.2, 137.1, 129.1, 128.2, 126.3, 120.7, 101.8, 98.8, 82.0, 70.6, 68.8, 62.3, 55.3, 54.1, 18.5. HRMS Cacld for $C_{18}H_{24}NO_6$ $[M+H]^+$ 350.1604, found 355.1588.

5-hexenyl amide 21

The compound was obtained as a white solid at a yield of 76%, m.p.178.1-179.0 °C. ^{1}H NMR (400MHz, $CDCl_3$), δ (ppm) 7.46-7.52 (m, 2H), 7.33-7.39 (m, 3H), 5.86 (d, 1H, *J* = 8.4 Hz), 5.77 (m, 1H), 5.56 (s, 1H), 5.03 (d, 1H, *J* = 17.9 Hz), 4.99 (d, 1H, J = 10.6 Hz), 4.71 (d, 1H, *J* = 3.7 Hz), 4.28-4.19 (m, 2H), 3.88 (t, 1H, *J* = 9.5 Hz), 3.77 (m, 2H), 3.58 (m, 1H), 3.40 (s, 3H), 3.19 (sb, 1H), 2.25 (t, 2H, *J* = 7.5 Hz), 2.10 (q, 2H, *J* = 7.0 Hz), 1.76 (pentet, 2H, *J* = 7.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 137.8, 137.1, 129.2, 128.2, 126.3, 115.5, 101.9, 98.8, 82.0, 70.8, 68.8, 62.3, 55.3, 54.0, 35.7, 33.0, 24.5. HRMS Cacld for $C_{20}H_{28}NO_6$ $[M+H]^+$ 378.1917, found 378.1899.

4-Chlorobutyl amide 22

The compound was obtained as a white solid at a yield of 89%, m.p. 195.2-196.1. °C. ^{1}H NMR (400MHz, $CDCl_3$), δ (ppm) 7.46-7.53 (m, 2H), 7.34-7.40 (m, 3H), 5.89 (d, 1H, *J* = 8.4 Hz), 5.57 (s, 1H), 4.72 (d, 1H, *J* = 4.0 Hz), 4.21-4.34 (m, 2H), 3.91 (dt, 1H, *J* = 2.9, 9.9 Hz), 3.73-3.84 (m, 2H), 3.55-3.68 (m, 3H), 3.42 (s, 3H), 2.91 (d, 1H, *J* = 2.9 Hz), 2.46 (t, 2H, *J* = 7.0 Hz), 2.14 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 136.9, 129.0, 128.1, 126.1, 101.7, 98.8, 81.8, 69.3, 68.7, 62.4, 55.2, 53.8, 44.2, 33.0, 27.9. HRMS Cacld for $C_{18}H_{25}NO_6Cl$ $[M+H]^+$ 386.1370, found 386.1355.

5-Bromopenyl amide 23

The compound was obtained as a white solid at a yield of 80.5%. m.p. 169.2-170.0 °C. ^{1}H NMR (400MHz, $CDCl_3$), δ (ppm) 7.45-7.52 (m, 2H), 7.33-7.39 (m, 3H), 5.88 (d, 1H, *J* = 8.4 Hz), 5.56 (s, 1H), 4.72 (d, 1H, *J* = 4.0 Hz), 4.28 (m, 1H), 4.22 (m, 1H), 3.89 (dd~t, 1H, *J* = 9.7 Hz), 3.77 (m, 2H), 3.58 (m, 1H), 3.41 (t, 2H, *J* = 6.6 Hz), 3.40 (s, 3H), 2.38 (t, 2H, *J* = 7.1 Hz), 1.90 (m, 2H), 1.81 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 136.9, 129.0, 128.1, 126.1, 101.7, 98.8, 81.8, 69.2, 68.6, 62.4, 55.1, 53.8, 44.2, 32.9, 27.9. HRMS Cacld for $C_{19}H_{27}BrNO_6$ $[M+H]^+$ 444.1022, found 444.1014.

Benzoyl amide 24

The product was obtained as a white crystalline solid at a yield of 89%, m.p. 247.2-247.8 ^{1}H NMR (400MHz, $CDCl_3$-MeOH), δ (ppm) 7.74 (d, 2H, *J* = 8.1 Hz), 7.40-7.47 (m, 3H), 7.33-7.38 (m, 3H), 7.26-7.32 (m, 3H), 5.52 (s, 1H), 4.78 (d, 1H. *J* = 3.7 Hz), 4.27 (dd, 1H, *J* = 3.3, 10.3 Hz), 4.22 (m, 1H), 3.91 (t, 1H, *J* = 9.5 Hz), 3.70-3.82 (m, 2H), 3.57 (t, 1H, *J* = 8.8 Hz), 3.33 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$-MeOH) δ (ppm) 168.7, 137.0, 133.6, 131.8, 129.0, 128.4,

128.1, 127.0, 126.1, 102.1, 99.1, 81.9, 69.1, 68.7, 62.5, 55.2, 54.4. HRMS Calcd for $C_{21}H_{24}NO_6$ $[M+H]^+$ 386.1604, found 386.1595.

Naphthyl amide 25

The compound was obtained as a white solid at a yield of 84%, m.p. 206.8-207.4 °C. ^{1}H NMR (400MHz, $CDCl_3$, CD_3OD), δ (ppm) 8.34 (d, 1H, *J* = 8.1 Hz), 7.93 (d, 1H, *J* = 8.4 Hz), 7.88 (d, 1H, *J* = 7.3 Hz), 7.67 (d, 1H, *J* = 7.0 Hz), 7.43-7.59 (m, 5H), 7.32-7.40 (m, 3H), 6.38 (d, 1H, *J* = 8.8 Hz), 5.60 (s, 1H), 4.93 (d, 1H, *J* =3.7 Hz), 4.53 (m, 1H), 4.31 (m, 1H), 4.02 (m, 1H), 3.75-3.88 (m, 2H), 3.63 (t, 1H, *J* = 9.2 Hz), 3.41 (s, 3H), 3.25 (d, 1H, *J* = 3.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, CD_3OD) δ 170.7, (170.6 minor rotamer), 136.9, 133.6 (133.62, minor rotamer), 133.4, 130.6, 129.8, 129.0, 128.1, 128.06, 127.0, 126.2, 126.1, 125.1, 124.9, 124.6, 101.7, 98.9, 81.9, 69.1, 68.7, 62.5, 55.2, 54.4 (54.5, minor tautomer). HRMS Cacld for $C_{25}H_{26}NO_6$ $[M+H]^+$ 436.1760, found 436.1742.

Pentyl urea 26

The compound was obtained as a white solid at a yield of 96%, m.p. 186.2-187.0. ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 7.41-7.49 (m, 2H), 7.34-7.41 (m, 3H), 6.06 (t, 1H, *J* = 5.5 Hz), 5.79 (d, 1H, *J* = 8.8 Hz), 5.59 (s, 1H), 5.21 (d, 1H. *J* = 5.1 Hz), 4.61 (d, 1H, *J* = 3.7 Hz), 4.16 (dd, 1H, *J* = 4.8, 9.9 Hz), 3.63-3.77 (m, 2H), 3.41-3.62 (m, 3H), 3.28 (s, 3H), 2.95 (q, 2H, *J* = 6.3 Hz), 1.35 (pentet, 2H, *J* = 7.0 Hz), 1.24 (m, 4H), 0.85 (t, 3H, *J* = 6.6 Hz). ^{13}C NMR, (100 MHz, d_6-DMSO) δ (ppm) 158.1, 137.8, 129.0, 128.1, 126.5, 101.0, 99.6, 82.0, 68.6, 68.1, 62.6, 54.8, 54.7, 39.2, 29.6, 28.7, 21.9, 14.0. HRMS Calcd for $C_{20}H_{31}N_2O_6$ $[M+H]^+$ 395.2182, found 395.2196.

Hexyl urea 27

Isolated as a light yellow solid in quantitative yield, m.p. 183.8-185.0°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 7.42-7.49 (m, 2H), 7.34-7.41 (m, 3H), 6.07 (t, 1H, *J* = 5.5 Hz), 5.80 (d, 1H, *J* = 8.4 Hz), 5.60 (s, 1H), 5.22 (sb, 1H), 4.62 (d, 1H, *J* = 3.7 Hz), 4.17 (dd, 1H, *J* = 4.8, 9.9 Hz), 3.73 (t, 1H, *J* = 10.3 Hz), 3.69 (dd, 1H, *J* = 3.7, 8.8 Hz), 3.55-3.62 (m, 1H), 3.49 (m, 2H), 3.30 (s, 3H), 2.98 (q, 2H, *J* = 6.2 Hz), 1.35 (m, 2H), 1.25 (sb, 6H), 0.86 (t, 3H, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, d_6-DMSO) δ (ppm) 157.9, 137.7, 128.8, 127.9, 126.3, 100.8, 99.5, 81.9, 68.5, 68.0, 62.4, 54.6, 54.55, 39.1, 31.0, 29.8, 26.0, 22.0, 13.8. HRMS Calcd for $C_{21}H_{33}N_2O_6$ $[M+H]^+$ 409.2339, found 409.2355.

Heptyl urea 28

Isolated as an off white solid in quantitative yield, m.p. 201.3-202.2 °C. ^{1}H NMR (400 MHz, DMSO) δ (ppm) 7.40-7.46 (m, 2H), 7.32–7.39 (m, 3H), 6.04 (t, 1H, *J* = 5.5 Hz), 5.78 (d, 1H, *J* = 8.4 Hz), 5.58 (s, 1H), 5.21 (d, 1H, *J* = 5.1 Hz), 4.60 (d, 1H, *J* = 3.2 Hz), 4.15 (dd, 1H, *J* = 4.6, 9.8 Hz), 3.63-3.74 (m, 2H), 3.57 (m, 1H), 3.42-3.51 (m, 2H), 3.28 (s, 3H), 2.96 (q, 2H, *J* = 6.2 Hz), 1.33 (m, 2H), 1.23 (bs, 8H), 0.84 (t, 3H, *J* = 6.4 Hz). ^{13}C NMR (100 MHz, DMSO) δ 157.8, 137.7, 128.8, 127.9, 126.3, 100.8, 99.4, 81.9, 68.4, 68.0, 62.4, 54.6, 54.5, 39.1, 31.2, 29.9, 28.4, 26.3, 22.0, 13.9. HRMS Calcd for $C_{22}H_{35}N_2O_6$ $[M+H]^+$ 423.2495, found 423.2500.

4-Pentynyl urea 29

The compound was obtained as a white solid at a yield of 90.5%, m.p. 210.0-211.0 °C. ^{1}H NMR (400MHz, DMSO), δ (ppm) 7.42-7.48 (m, 2H), 7.34-7.41 (m, 3H), 6.13 (t, 1H, *J* = 5.6

Hz), 5.78 (d, 1H, *J* = 8.8 Hz), 5.60 (s, 1H), 5.20 (d, 1H, *J* = 5.5 Hz), 4.62 (d, 1H, *J* = 3.7 Hz), 4.17 (dd, 1H, *J* = 4.8, 9.9 Hz), 3.73 (m, 1H), 3.69 (m, 1H), 3.59 (dt, 1H, *J* = 4.8, 9.9 Hz), 3.44-3.55 (m, 2H), 3.30 (s, 3H), 3.04 (pseudo q, 2H, *J* = 6.0 Hz), 2.78 (t, 1H, *J* = 2.6 Hz), 2.15 (dt, 2H, *J* = 2.6, 7.0 Hz), 1.54 (pentet, 2H, *J* = 7.0 Hz), ^{13}C NMR (100 MHz, DMSO) δ 157.8, 137.7, 128.8, 127.9, 126.3, 100.8, 99.4, 83.9, 82.0, 71.3, 68.5, 68.0, 62.4, 55.6, 54.5, 38.2, 28.8, 15.2 HRMS Cacld for $C_{20}H_{27}N_2O_6$ $[M+H]^+$ 391.1869, found 391.1855.

5-Hexynyl urea 30

The compound was obtained as a white solid at a yield of 87%, m.p. 212.0-213.0 °C. ^{1}H NMR (400MHz, $CDCl_3$), δ (ppm) 7.47-7.52 (m, 2H), 7.33-7.39 (m, 3H), 5.58 (s, 1H), 4.72 (d, 1H, *J* = 3.7 Hz), 4.62-4.81 (m, 2H), 4.28 (m, 1H), 3.84-3.96 (m, 2H), 3.73-3.83 (m, 2H), 2.53-3.61 (m, 1H), 3.40 (s, 3H), 3.21 (m, 2H), 2.22 (dt, 2H, *J* = 2.6, 6.9 Hz), 1.96 (t, 1H, *J* = 2.6 Hz), 1.51-1.68 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$-1drop of CD_3OD) δ 159.2, 137.0, 129.0, 128.1, 126.2, 101.8, 99.5, 84.1, 82.0, 70.5, 68.8, 68.5, 62.4, 55.2, 54.9, 39.5, 28.9, 25.5, 18.0. HRMS Cacld for $C_{21}H_{29}N_2O_6$ $[M+H]^+$ 405.2026, found 405.2034.

Cyclohexyl urea 31

Isolated as a light yellow solid in quantitative yield, m.p. 210°C (dec). ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 7.42-7.47 (m, 2H), 7.35-7.40 (m, 3H), 6.04 (d, 1H, *J* = 8.1 Hz), 5.74 (d, 1H, *J* = 8.4 Hz), 5.60 (s, 1H), 5.23 (d, 1H, *J* = 4.8 Hz), 4.61 (d, 1H, *J* = 3.3 Hz), 4.17 (dd, 1H, *J* = 4.8, 9.9 Hz), 3.64-3.76 (m, 2H), 3.58 (m, 1H), 3.44-3.54 (m, 2H), 3.36 (m, 1H), 3.34 (s, 3H), 1.68-1.78 (m, 2H), 1.57-1.67 (m, 2H), 1.46-1.55 (m, 1H), 1.19-1.32 (m, 2H), 1.00-1.18 (m, 3H). ^{13}C NMR (100 MHz, d_6-DMSO) δ (ppm) 157.1, 137.7, 128.8, 127.9, 126.3, 100.8, 99.4, 81.9, 68.5, 68.0,

62.4, 54.6, 54.5, 47.6, 33.2, 25.2, 24.3. HRMS Calcd for $C_{21}H_{31}N_2O_6$ $[M+H]^+$ 407.2182, found 407.2191.

2-Chloroethyl urea 32

The compound was obtained as a white solid at a yield of 95%, m.p. 184.1°C ^{1}H NMR, (400 MHz, d_6-DMSO) δ (ppm) 7.40-7.47 (m, 2H), 7.33-7.39 (m, 3H), 6.34 (t, 1H, *J* = 5.5 Hz), 6.07 (d, 1H, *J* = 8.8 Hz), 5.58 (s, 1H), 5.20 (d, 1H, *J* = 5.5 Hz), 4.61 (d, 1H, *J* = 3.3 Hz), 4.15 (dd, 1H, *J* = 4.8, 9.9 Hz), 3.64-3.75 (m, 2H), 3.53-3.61 (m, 3H), 3.42-3.53 (m, 2H), 3.32 (m, 2H), 3.28 (s, 3H). ^{13}C NMR, (100 MHz, d_6-DMSO) δ (ppm) 157.6, 137.7, 128.9, 128.0, 126.4, 100.8, 99.4, 81.9, 68.3, 68.0, 62.5, 54.7, 54.6, 44.7, 41.4. HRMS calcd for $C_{17}H_{24}N_2O_6Cl$ $[M+H]^+$ 387.1323, found 387.1335.

2-Methyl-acrylic acid 2-ureido-ethyl ester 34

The compound was obtained as a white solid in quantitative yield, m.p. 198.8-200.0°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 7.42-7.48 (m, 2H), 7.34-7.40 (m, 3H), 6.24 (t, 1H, *J* = 5.7 Hz), 6.08 (sb, 1H), 5.99 (d, 1H, *J* = 8.4 Hz), 5.70 (m, 1H), 5.60 (s, 1H), 5.20 (d, 1H, *J* = 5.5 Hz), 4.62 (d, 1H, *J* = 3.7 Hz), 4.17 (dd, 1H, *J* = 4.8, 9.9 Hz), 4.06 (m, 2H), 3.66-3.77 (m, 2H), 3.65-3.63 (m, 1H), 3.43-3.53 (m, 2H), 3.22-3.32 (m, 5H), 1.89 (s, 3H). ^{13}C NMR (100 MHz, d_6-DMSO) δ (ppm) 166.5, 157.7, 137.7, 135.8, 128.8, 127.9, 126.4, 125.9, 100.8, 99.4, 81.9, 68.3, 68.0, 64.2, 62.5, 54.62, 54.57, 38.1, 17.9. HRMS Calcd for $C_{21}H_{29}N_2O_8$ $[M+H]^+$ 437.1924, found 437.1944.

(2-Hydroxy-ethyl)-urea 35

Isolated as a white solid solid in quantitative yield, m.p. 232.3-233.0 °C. ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 7.42-7.47 (m, 2H), 7.34-7.39 (m, 3H), 6.26 (t, 1H, *J* = 5.5 Hz), 6.13 (d, 1H, *J* = 8.1 Hz), 5.59 (s, 1H), 5.33 (sb, 1H), 4.78 (sb, 1H), 4.61 (d, 1H, *J* = 3.3 Hz), 4.16 (dd, 1H, *J* = 4.8, 9.9 Hz), 3.72 (m, 1H), 3.66 (m, 1H), 3.58 (m, 1H), 3.44-3.55 (m, 2H), 3.32-3.40 (m, 2H, overlap), 3.29 (s, 3H), 3.05 (q, 2H, *J* = 5.5 Hz). ^{13}C NMR (100 MHz, d_6-DMSO) δ ppm 158.2, 137.7, 128.8, 128.0, 126.4, 100.8, 99.5, 81.9, 68.3, 68.0, 62.5, 60.7, 54.7, 42.0. HRMS Calcd for $C_{17}H_{25}N_2O_7$ $[M+H]^+$ 369.1662, found 369.1677.

Phenyl urea 36

Isolated as a light yellow solid solid in quantitative yield, m.p. >300°C. ^{1}H NMR, (400 MHz, d_6-DMSO) δ (ppm) 8.65 (s, 1H), 7.43-7.49 (m, 2H), 7.43-7.42 (m, 5H), 7.22 (m, 2H), 6.89 (m, 1H), 6.15 (d, 1H, *J* = 8.8 Hz), 5.63 (s, 1H), 5.34 (sb, 1H), 4.71 (d, 1H, *J* = 3.3 Hz), 4.20 (dd, 1H, *J* = 4.8, 9.9 Hz), 3.72-3.83 (m, 2H), 3.63 (m, 1H), 3.49-3.60 (m, 2H), 3.34 (s, 3H). ^{13}C NMR (100 MHz, d_6-DMSO) δ (ppm) 154.8, 140.3, 137.7, 128.8, 128.6, 127.9, 126.3, 121.0, 117.3, 100.8, 99.2, 81.7, 68.3, 68.0, 62.6, 54.7, 54.2. HRMS Calcd for $C_{21}H_{25}N_2O_6$ $[M+H]^+$ 401.1713, found 401.1726.

4-Bromophenyl urea 37

The compound was obtained as a white solid at a yield of 92%, m.p. unstable after 265 °C. ^{1}H NMR, (400 MHz, d_6-DMSO) δ (ppm) 8.77 (sb, 1H), 7.43-7.48 (m, 2H), 7.33-7.42 (m, 7H), 6.16 (d, 1H, *J* = 8.4 Hz), 5.62 (s, 1H), 5.33 (d, 1H, *J* = 5.5Hz), 4.70 (d, 1H, *J* = 3.7Hz), 4.19 (dd, 1H, *J* = 4.8, 9.9 Hz), 3.72-3.82 (m, 2H), 3.49-3.66 (m, 3H), 3.33 (s, 3H). ^{13}C NMR (100 MHz, d_6-

DMSO) δ (ppm) 154.6, 139.7, 137.7, 131.4, 128.8, 128.0, 126.4, 119.3, 112.3, 100.8, 99.2, 81.7, 68.3, 68.0, 62.6, 54.7, 54.2. HRMS Calcd for $C_{21}H_{24}N_2O_6Br$ $[M+H]^+$ 479.0818, found 479.0836.

Napthyl urea 38

Isolated as a light yellow solid in quantitative yield, m.p. 253.8-255.0 °C. ^{1}H NMR, (400 MHz, d_6-DMSO) δ (ppm) 8.73 (s, 1H), 8.14 (d, 1H, *J* = 8.1 Hz), 8.09 (d, 1H, *J* = 7.7 Hz), 7.87 (d, 1H, *J* = 8.1 Hz), 7.49-7.56 (m, 3H), 7.44-7.49 (m, 2H), 7.39-7.43 (m, 1H), 7.34-7.39 (m, 3H), 6.81 (d, 1H, *J* = 8.4 Hz), 5.63 (s, 1H), 5.42 (sb, 1H), 4.76 (d, 1H, *J* = 3.3 Hz), 4.20 (dd, 1H, *J* = 4.4, 9.9 Hz), 3.88 (m, 1H), 3.77 (t, 1H, *J* = 10.1 Hz), 3.60-3.71 (m, 2H), 3.56 (m, 1H), 3.36 (s, 3H). ^{13}C NMR, (100 MHz, d_6-DMSO) δ 155.4, 137.7, 135.1, 133.7, 128.9, 128.4, 128.1, 126.4, 126.0, 125.8, 125.4, 125.0, 121.9, 121.2, 115.7, 101.0, 99.4, 81.9, 68.5, 68.1, 62.7, 54.8, 54.5. HRMS Calcd for $C_{25}H_{27}N_2O_6$ $[M+H]^+$ 451.1869, found 451.1882.

Amide 45 from 5,7-Hexadecadiynoic acid

87% yield ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.54 (m, 2H), 7.32-7.41 (m, 3H), 5.97 (d, 1H, *J* = 8.4 Hz), 5.55 (s, 1H), 4.71 (d, 1H, *J* = 4.0 Hz), 4.26 (m, 1H), 4.2 (m, 1H), 3.88 (dt, 1H, *J* = 2.9, 9.8 Hz), 3.71-3.83 (m, 2H), 3.57 (t, 1H, *J* = 9.0 Hz), 3.40 (s, 3H), 3.28 (d, 1H, *J* = 3.3 Hz), 2.34 (m, 4H), 2.24 (t, 2H, *J* = 7.0 Hz), 1.84 (q, 2H, *J* = 7.0 Hz), 1.51 (q, 2H, *J* = 7.1 Hz), 1.18-1.43 (m, 10H), 0.86 (t, 3H, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 137.1, 129.1, 128.2, 126.3, 101.8, 98.8, 82.0, 78.1, 76.0, 70.5, 68.8, 66.3, 65.0, 62.3, 55.3, 53.9, 34.9, 31.7, 29.1, 29.0, 28.8, 28.2, 23.8, 22.6, 19.1, 18.4, 14.1. HRMS calcd for $C_{30}H_{42}NO_6$ $[M+H]^+$ 512.3102, found 512.2999.

Amide 46 from 10,12-octadecadiynoic acid

The product was obtained as a white crystal at a yield of 83%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.46-7.54 (m, 2H), 7.33-7.40 (m, 3H), 5.85 (d, 1H, *J* = 8.8 Hz), 5.56 (s, 1H), 4.72 (d, 1H, *J* = 3.7 Hz,), 4.28 (m, 1H), 4.23 (ddd ~dt,1H, *J* = 3.7, 8.8, 9.8 Hz), 3.90 (ddd ~dt,1H, *J* = 3.3, 9.8 Hz), 3.73-3.83 (m, 2H), 3.58 (m, 1H), 3.40 (s, 3H), 3.15(d, 1H, *J* = 3.3 Hz), 2.19-2.29 (m, 6H), 1.64 (m, 2H), 1.51 (m, 4H), 1.22-1.42 (m, 12H), 0.89 (t, 3H, *J* = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 100MHz) not pure contain acid δ 174.7, 137.1, 129.1, 128.2, 126.3, 101.9, 98.8, 82.1, 77.6, 77.4, 70.8, 68.7, 68.1, 65.3, 62.3, 55.3, 54.0, 36.6, 31.0, 29.1, 29.0, 28.9, 28.7, 28.2, 28.0, 25.5, 22.1, 19.1, 13.8. HR ESIMS calcd for $C_{32}H_{46}NO_6$ [M+H]+ 540.3325, found 540.3308.

Amide 47 from 10,12-Tricosadiynoic acid

yield = 83.5% ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48-7.53 (m, 2H), 7.33-7.39 (m, 3H), 5.84 (d, 1H, *J* = 8.4 Hz), 5.57 (s, 1H), 4.72 (d, 1H, *J* = 3.7 Hz), 4.23 (ddd~dt, 1H, *J* = 3.6, 9.8, 9.9 Hz), 3.90 (ddd~dt, 1H, *J* = 2.9, 9.5 Hz), 3.74-3.84 (m, 2H), 3.59 (m, 1H), 3.41 (s, 3H), 3.12 (d, 1H, *J* = 3.7 Hz), 2.20-2.29 (m, 6H), 1.64 (m, 2H), 1.50 (m, 4H), 1.20-1.42 (m, 22H), 0.88 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 137.1, 129.1, 128.2, 126.3, 101.9, 98.8, 82.1, 77.6, 77.4, 70.9, 68.8, 65.3, 65.2, 62.3, 55.3, 54.0, 36.6, 31.8, 29.5, 29.4, 29.3, 29.1, 29.0, 28.9, 28.8, 28.7, 28.3, 28.2, 25.5, 22.6, 19.2, 19.1, 14.1. HRMS $[M+1]^+$ $C_{37}H_{56}NO_2$ calcd 610.4108, found 610.4108.

Amide 48 from using 5,7-dodecadiyndioic acid

yield = 83% ^{1}H NMR (400 MHz, DMSO) δ 7.91-7.82 (d, 1H, *J* = 8.4 Hz), 7.45-7.42 (m, 4H), 7.36-7.34 (m, 6H), 5.59 (s, 2H), 5.13 (d, 2H, *J* = 3.7 Hz), 4.60 (d, 2H, *J* = 3.7 Hz), 4.17-4.09

(m, 2H), 3.85-3.80 (m, 2H), 3.74-3.55 (m, 6H), 3.46 (t, 2H, *J* = 9.1 Hz), 3.27 (s, 6H), 3.15 (d, 1H, *J* = 5.4 Hz), 2.47 (m, 8H), 2.47 (m, 1H), 2.28 (t, 4H, *J* = 6.9Hz), 2.20 (t, 4H, *J* = 7.3 Hz), 1.70-1.63 (m, 4H); ^{13}C NMR (100 MHz, DMSO) δ 172.6, 138.3, 129.5, 128.7, 127.0, 101.5, 99.3, 82.5, 78.4, 68.0, 66.2, 63.1, 55.5, 54.7, 34.6, 24.7, 18.5 ; HRMS $[M+1]^+$ $C_{40}H_{49}N_2O_{12}$ calcd 749.3286, found 749.3278.

Amide 49 from using 10,12-docosadiyndioic acid

yield = 78% ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.4-7.52 (m, 4H), 7.31-7.39 (m, 6H), 5.91 (d, 2H, *J* = 8.8 Hz), 5.55 (s, 2H), 4.72 (d, 2H, *J* = 3.7 Hz), 4.27 (m, 2H), 4.20 (m, 2H), 3.88 (m, 2H), 3.71-3.82 (m, 4H), 3.57 (m, 2H), 3.39 (s, 6H), 3.32 (d, 2H, *J* = 2.9 Hz), 2.17-2.28 (m, 8H), 1.62 (m, 4H), 1.50 (m, 4H), 1.20-1.41 (m, 16H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 137.1, 129.1, 128.2, 126.3, 101.8, 98.8, 82.0, 77.4, 70.6, 68.8, 65.3, 62.3, 55.3, 54.0, 36.5, 29.1, 29.0, 28.8, 28.6, 28.2, 25.5, 19.1 ; HRMS $[M+1]^+$ $C_{50}H_{69}N_2O_{12}$ Calcd 889.4851, found 889.4857.

Synthesis of urea (50)

Yield = 90% ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47-7.52 (m, 2H), 7.32-7.42 (m, 3H), 5.56 (s, 1H), 5.12 (m , peusdo triplet, 1H), 4.91 (d, 1H, *J* = 8.1 Hz), 4.72 (d, 1H, *J* = 3.3 Hz), 4.28 (m, 1H), 3.85-3.97 (m, 2H), 3.73-3.83 (m, 2H), 3.55-3.63 (m, 2H), 3.41 (s, 3H), 3.24-3.39 (m, 2H), 2.46 (m, 2H), 2.23 (t, 2H, *J* = 7.0 Hz), 1.54 (hex, 2H, *J* = 7.3 Hz), 0.98 (q, 3H, *J* = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.4, 137.1, 129.2, 128.3, 126.3, 101.9, 99.2, 82.0, 78.3, 74.6, 71.5, 68.8, 66.8, 65.1, 62.2, 55.3, 39.1, 21.7, 21.1, 20.9, 13.5. HRMS Calcd for $C_{24}H_{31}N_2O_6$ M+H HRMS: $[M+H]^+$ $C_{24}H_{31}N_2O_6$ Calcd. 443.2179, found 443.2182

Synthesis of urea (51)

Yield = 90% ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.51 (m, 4H), 7.26-7.37 (m, 6H), 5.55 (s, 1H), 5.26 (m , peusdo triplet, 1H), 5.03(d, 1H, *J* = 7.7 Hz), 4.72 (d, 1H, *J* = 3.3 Hz), 4.27 (m, 1H), 3.86-3.97 (m, 2H), 3.70-3.83 (m, 2H), 3.55-3.62 (m, 1H), 3.40 (s, 3H), 3.28-3.45 (m, 2H), 2.56 (td, 2H, *J* = 2.2, 6.3 Hz).^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 137.1, 132.5, 129.2, 129.1, 128.4, 128.3, 126.3, 121.6, 101.9, 99.2, 82.0, 81.96, 75.4, 74.0, 71.4, 68.8, 66.5, 62.2, 55.3, 39.0, 21.3. HRMS: $[M+H]^+$ $C_{27}H_{29}N_2O_6$ Calcd 477.2014, found 477.2026

Synthesis of urea (52)

yield = 90% ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.50 (m, 2H), 7.30-7.37 (m, 3H), 5.54 (s, 1H), 5.07-5.20 (m, 2H), 4.69 (d, 1H, *J* = 3.7 Hz), 4.28 (m, 1H), 3.81-4.00 (m, 2H), 3.71-3.81 (m, 2H), 3.54-3.63 (m, 1H), 3.37 (s, 3H), 2.98-3.24 (m, 2H), 2.22 (t, 4H, *J* = 7.0 Hz), 2.06 (bs, 1H), 1.18-1.56 (m, 24H), 0.87 (t, 3H, *J* = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.0, 137.1, 129.1, 128.2, 126.3, 101.9, 99.4, 82.0, 77.7, 77.2, 71.2, 68.8, 65.4, 65.1, 62.3, 55.2, 55.1, 40.4, 31.8, 29.8, 29.5, 29.4, 29.2, 29.0, 28.9, 28.4, 28.3, 28.1, 26.3, 22.6, 19.1, 19.0, 14.1. HRMS, $[M+H]^+$ $C_{35}H_{53}N_2O_6$ Calcd 597.3904, found 597.3907

Synthesis of urea (53)

Yield = 87% ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47-7.54 (m, 2H), 7.32-7.40 (m, 3H), 5.56 (s, 1H), 4.83 (d, 1H, *J* = 8.1 Hz), 4.73 (m, 1H), 4.71 (d, 1H, *J* = 3.7 Hz), 4.28 (m, 1H), 3.84-4.00 (m, 2H), 3.72-3.83 (m, 2H), 3.55-3.63 (m, 1H), 3.40 (s, 3H), 3.15 (m, 2H), 2.24 (t, 4H, *J* = 7.0 Hz), 1.15-1.57 (m, 28H), 0.88 (t, 3H, *J* = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.8, 137.1, 129.2, 128.3, 126.3, 101.9, 99.2, 82.0, 77.6, 77.4, 71.6, 68.8, 65.3, 65.2, 62.2, 55.3, 40.1, 31.8, 29.9,

29.5, 29.4, 29.3, 29.1, 28.9, 28.8, 28.7, 28.3, 28.2, 26.7, 22.6, 19.2, 19.1, 14.1. HRMS, $[M+H]^+$ $C_{37}H_{56}N_2O_6$ Calcd 625.4217, found 625.4220.

Synthesis of urea (54)

Yield = 90% as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.48 (m, 4H), 7.34-7.39 (m, 6H), 6.04 (t, 2H, *J* = 5.4 Hz), 5.76 (d, 2H, *J* = 8.4 Hz), 5.60 (s, 2H), 5.19 (d, 2H, *J* = 5.1 Hz), 4.61 (d, 2H, *J* = 3.7 Hz), 4.16 (dd, 2H, *J* = 4.4, 9.9 Hz), 3.43-3.75 (m, 10H), 3.30 (s, 6H), 2.97 (m, 4H), 2.27 (t, 4H, *J* = 6.8 Hz), 1.17-1.48 (m, 24H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.9, 137.7, 128.8, 128.0, 126.4, 100.8, 99.5, 81.9, 78.0, 68.5, 68.0, 65.3, 62.4, 54.7, 54.6, 40.2, 29.9, 28.6, 28.4, 28.1, 27.7, 26.3, 18.2. HRMS: Calcd. For $C_{50}H_{71}N_4O_{12}$ $[M+H]^+$ 919.5068, found 919.5067.

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Vita

The author was born in Dhand (Haryana), India in 1982. He received his B.Sc (Honors) in 2002 and M.Sc (Honors) in 2004 from Panjab University, Chandigarh, India. After that he worked for 2 years (2004-2006) at Aurigene Discovery Technology, a pharmaceutical company at Bangalore, India. In 2006 he joined the research group of Prof. Guijun Wang at University of New Orleans. He received his M.S in 2010 and completed his PhD in 2011**.**