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The 4-aza-S-ribosyl-L-homocysteine Derivatives and the Related Gamma-lactam and Azahemiacetal Analogs: Synthesis, Inhibition and Quorum Sensing Activity

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FLORIDA INTERNATIONAL UNIVERSITY
Miami, Florida

THE 4-AZA-S-RIBOSYL-L-HOMOCYSTEINE DERIVATIVES AND THE RELATED GAMMA-LACTAM AND AZAHEMIACETAL ANALOGS:
SYNTHESIS, INHIBITION AND QUORUM SENSING ACTIVITY

A dissertation submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in CHEMISTRY by Venkata Malladi

2011
To: Dean Kenneth Furton  
College of Arts and Sciences

This dissertation, written by Venkata Malladi, and entitled The 4-aza-S-ribosyl-L-homocysteine Derivatives and the Related Gamma-lactam and Azahemiacetal Analogs: Synthesis, Inhibition and Quorum Sensing Activity, have been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

______________________________
Kevin O'Shea

______________________________
Watson Lees

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Kalai Mathee

______________________________
Jeffrey Joens

______________________________
Stanislaw F. Wnuk, Major Professor

Date of Defense: March 21, 2011

The dissertation of Venkata Malladi is approved.

______________________________
Dean Kenneth Furton  
College of Arts and Sciences

______________________________
Interim Dean Kevin O'Shea  
University Graduate School

Florida International University, 2011
DEDICATION

I dedicate this work to my beloved ‘SWAMI’ who is my guiding light and source of happiness.

Also to my parents and my husband for their unconditional love and support.
ACKNOWLEDGMENTS

I would like to extend my heartfelt gratitude to my mentor, Dr. Wnuk for teaching me valuable lessons in chemistry and beyond. His remarkable enthusiasm, knowledge and motivation guided me to become an independent researcher. I also am thankful for his care and help beyond professional development. I am grateful to my committee members for their valuable suggestions and intellectual support. I would like to acknowledge the chemistry department for the financial assistance. I am also grateful to all my lab members for all the joyful moments we shared and for giving me moral and intellectual support whenever I needed. I am indebted to my family and friends for their unconditional love and care throughout my studies. Last but not the least, I would like to thank GOD for this life and the experiences that came with it.
ABSTRACT OF THE DISSERTATION

THE 4-AZA-S-RIBOSYL-L-HOMOCYSTEINE DERIVATIVES AND THE RELATED GAMMA-LACTAM AND AZAHEMIACETAL ANALOGS: SYNTHESIS, INHIBITION AND QUORUM SENSING ACTIVITY

by

Venkata Malladi

Florida International University, 2011

Miami, Florida

Professor Stanislaw F. Wnuk, Major Professor.

Quorum sensing (QS) is a population-dependent signaling process bacteria use to control multiple processes including virulence, critical for establishing infection. There are two major pathways of QS systems. Type 1 is species specific or intra-species communication in which N-acylhomoserine lactones (Gram-negative bacteria) or oligopeptides (Gram-positive bacteria) are employed as signaling molecules (autoinducer one). Type 2 is inter-species communication in which S-4,5-dihydroxy-2,3-pentanedione (DPD) or its borate esters are used as signaling molecules. The DPD is biosynthesized by LuxS enzyme from S-ribosylhomocysteine (SRH).

Recent increase in prevalence of bacterial strains resistant to antibiotics emphasizes the need for the development of new generation of antibacterial agents. Interruption of QS by small molecules is one of the viable options as it does not affect bacterial growth but only virulence, leading to less incidence of microbial resistance. Thus, in this work, inhibitors of both N-acylhomoserine lactone (AHL) mediated intra-species and LuxS enzyme, involved in inter-species QS are targeted.

The γ-lactam and their reduced cyclic azahemiacetal analogs, bearing the additional alkylthiomethyl substituent, were designed and synthesized targeting AHL mediated QS systems in P. aeruginosa and Vibrio harveyi. The γ-lactams with nonylthio or dodecylthio chains acted as
inhibitors of las signaling in P. aeruginosa with moderate potency. The cyclic azahemicetal with shorter propylthio or hexylthio substituent were found to strongly inhibit both las and rhl signaling in P. aeruginosa at higher concentrations. However, lactam and their azahemicetal analogs were found to be inactive in V. harveyi QS systems.

The 4-aza-S-ribosyl-L-homocysteine (4-aza-SRH) analogs and 2-deoxy-2-substituted-S-ribosyl-L-homocysteine analogs were designed and synthesized targeting Bacillus subtilis LuxS enzyme. The 4-aza-SRH analogs in which oxygen in ribose ring is replaced by nitrogen were further modified at anomeric position to produce pyrrolidine, lactam, nitrone, imine and hemiaminal analogs. Pyrrolidine and lactam analogs which lack anomeric hydroxyl, acted as competitive inhibitors of LuxS enzyme with $K_I$ value of 49 and 37 µM respectively. The 2,3-dideoxy lactam analogs were devoid of activity. Such findings attested the significance of hydroxyl groups for LuxS binding and activity. Hemiaminal analog of SRH was found to be a time-dependent inhibitor with IC$_{50}$ value of 60 µM.
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<td>α</td>
<td>alpha</td>
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<tr>
<td>A</td>
<td>absorbance</td>
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<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>AHL</td>
<td>acylhomoserine lactone</td>
</tr>
<tr>
<td>AI</td>
<td>autoinducer</td>
</tr>
<tr>
<td>APCI</td>
<td>atmospheric pressure chemical ionization</td>
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<td>Bn</td>
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<td>delta chemical shift in parts per million (NMR)</td>
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<td>dA</td>
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<td>DMAP</td>
<td>4-(N,N-dimethylamino)pyridine</td>
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DMF \( N,N \)-dimethylformamide
DMSO dimethylsulfoxide
DPD 4,5-dihydroxy-2,3-pentanedione
DTNB 5,5’-dithio-bis-(2-nitrobenzoic acid)
\( \varepsilon \) epsilon
ESI electrospray ionization
Et ethyl
\( \gamma \) gamma
g gram(s)
gem geminal
h hour(s)
Hey homocysteine
HEPES \( N \)-(2-hydroxyethyl)piperazine-\( N' \)--(2-ethanesulfonic acid)
HPLC high performance liquid chromatography
HRMS high resolution mass spectroscopy
ICP-MS inductively coupled plasma mass spectroscopy
IPTG isopropyl \( \beta \)-D-thiogalactoside
\( J \) coupling constant in Hz (NMR)
L liter
LU light units
LuxS \( S \) -ribosylhomocysteinase
LuxR\(_{vh}\) LuxR present in \( V \). \( h \)arveyi
m milli; multiplet (NMR)
\( \mu \) micro
M moles per liter
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<td>5’-methylthioadenosine</td>
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<td>nano molar</td>
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<td>S-arabinosylhomocysteine</td>
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<td>SAM</td>
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<td>SRH</td>
<td>S-ribosylhomocysteine</td>
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<td>RP</td>
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<td>TNB</td>
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1. INTRODUCTION

1.1. Quorum sensing

Quorum sensing (QS) is a cell density dependent bacterial communication phenomenon. The term, ‘quorum’ is defined as “the minimum bacterial behavioral unit required inducing this communication”. Observation of autoinduction of luminescence in *V. fisheri* was the first example of involvement of QS. Later, many examples of bacteria employing QS (both Gram positive and Gram negative) for interspecies and intraspecies communication were reported. Such conversations allow bacteria which are single-celled organisms to control crucial functions in united communities such as symbiosis, motility, virulence, antibiotic production, biofilm formation, exchange of DNA and many other processes; and thereby receive some of the benefits of multicellular organisms. Bacteria secrete chemical molecules called autoinducers (AIs) to induce QS. At low cell density, the concentration of this chemical is also low. When cell density increases, the concentration increases to a point critical for induction of genes responsible for community behaviors.

There are two major pathways of QS systems. Type 1 is species specific or intra-species communication in which acyl homoserine lactones (Gram-negative bacteria) and oligopeptides (Gram-positive bacteria) are employed as autoinducers (referred as AI-1s). On the other hand type 2 is inter-species signaling system in which borate diester of cyclized dihydroxypentanedione or *R*-tetrahydroxytetrahydrofuran (*R*-THMF) are used as autoinducers (referred as AI-2) (Figure 2).

1.1.1. Intraspecies quorum sensing

1.1.1.1. Gram negative bacteria

Acyl homoserine lactones (AHLs)

The major intraspecies signaling molecules among Gram negative bacteria are AHL analogs. QS in marine luminescent *V. fisheri* was the first report of AHL involvement as
autoinducer molecules.\textsuperscript{7,8} These sensor molecules can be distinct to each species or could be shared among other bacteria.

The AHLs contain a long fatty acid chain connected to a homoserine lactone through an amide bond. The AHLs are synthesized from SAM and acylated acyl carrier protein (acyl-ACP) by inducer synthases (LuxI like proteins) at low basal levels.\textsuperscript{9} At high cell densities, as the AHL reaches the threshold concentration, it binds to the cognate receptors (LuxR-like proteins) which reside in cytoplasm. Thereafter the AHL:LuxR complex undergoes homodimerization followed by multimerization. Subsequently, the multimer binds to 20 bp-element called lux-type-box and activates transcription of target genes required for community behaviors (Figure 1).\textsuperscript{10,11} Details of different LuxI/LuxR type of systems present in various Gram negative bacteria along with their functions are included in Table 1.\textsuperscript{6}

\textbf{Figure 1.} Model of AHL biosynthesis and quorum sensing in a bacterial cell.\textsuperscript{10}
The LuxR type proteins share 18-23% sequence homology. The conserved residues required for binding were identified to be Ala38 (Gly in LasR and VanR), Tyr53 (Cys in AhyR), Tyr57, Asp70 and Thr129 (Ser in AhyR, LasR, LuxR, RhlR and VanR). Recently, the crystal structure of *Agrobacterium tumefaciens* TraR in complex with its inducing ligand, 3-oxo-C8-AHL bound to target DNA was solved by Allesandro et al. From the crystallographic studies, the binding pocket of TraR was found to have hydrophobic and aromatic residues to accommodate the fatty acid side chain. Also, hydrogen bonding between Trp57 and carbonyl of lactone, Asp70 and amide nitrogen and Tyr53 and side chain amide carbonyl were observed.

Table 1. Representative AHLs along with their regulators and corresponding functions

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Signala</th>
<th>LuxI/LuxR</th>
<th>QS phenotype</th>
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<tbody>
<tr>
<td><em>Agrobacterium hydrophilia</em></td>
<td>C4-AHL</td>
<td>AhyI/AhyR</td>
<td>Exoprotease production</td>
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<tr>
<td><em>A. tumefaciens</em></td>
<td>3-oxo-C8-AHL</td>
<td>TraI/R</td>
<td>Ti plasmid conjugation</td>
</tr>
<tr>
<td><em>Burkholderia cenocepacia</em></td>
<td>C8-AHL, C6-AHL</td>
<td>CciI/R;CepI/R</td>
<td>Biofilm, swarming motility</td>
</tr>
<tr>
<td><em>Erwinia carotovora</em></td>
<td>3-oxo-C6-AHL</td>
<td>ExplI/R; CarlI/R</td>
<td>Carbapenem, exoenzymes,virulence</td>
</tr>
<tr>
<td><em>Psuedomonas aeruginosa</em></td>
<td>3-oxo-C12-AHL</td>
<td>LasI/R</td>
<td>Exoenzymes, pyocyanin and biofilm</td>
</tr>
<tr>
<td></td>
<td>3-oxo-C4-AHL</td>
<td>RhlI/R</td>
<td>formation</td>
</tr>
<tr>
<td><em>Serratia liquefaciens</em></td>
<td>C4-AHL</td>
<td>SwrI/R</td>
<td>Extracellular proteases, swarming</td>
</tr>
<tr>
<td><em>Vibrio fisheri</em></td>
<td>3-oxo-C6-AHL</td>
<td>LuxI/R</td>
<td>bioluminescence</td>
</tr>
<tr>
<td><em>Vibrio harveyi</em></td>
<td>3-hydrdoxy-C4-AHL</td>
<td>LuxLM/N</td>
<td>bioluminescence</td>
</tr>
</tbody>
</table>

aAHL abbreviations/nomenclature used in the entire text

Recently, a third chemical class of QS signal (CAI-1as AI-1) in *V. cholerae* was also discovered. It is identified as (S)-3-hydroxytridecane-4-one (Figure 2). While other bacteria produce biofilms and virulence factors at high cell density through QS, *V. cholerae*, in contrast at
high population of bacteria, represses the factors regulating their production by employing CAI-1 and AI-2.\textsuperscript{14}

A fourth type of QS system was observed in \textit{P. aeruginosa} known as 4QS pathway (4-quinolone signal). It is modulated by using 2-heptyl-3-hydroxy-4-quinolone as an autoinducer (Figure 2) and detected by QscR receptor. Three signaling pathways exist in \textit{P. aeruginosa}. Two QS mediated systems (\textit{lasR} and \textit{rhlR}) use AHL and the third uses a 4-quinolone derivative. The LasI/R synthesizes and detects \textit{N}-(3-oxo-dodecanoyl)-\textit{L}-homoserine lactone (3-oxo-C\textsubscript{12}-AHL) while RhlI/R synthesizes and detects \textit{N}-butanoyl-\textit{L}-homoserine lactone (C\textsubscript{4}-AHL) (Figure 2). These two signaling systems regulate 6\% of the gene expression in \textit{P. aeruginosa}. The QscR is an orphan receptor which lacks LuxI of its own and recognizes AHL produced by LasI and RhlI systems. It represses QS activity.\textsuperscript{15} Together, the three QS systems act in a complex hierarchical partially redundant manner to control virulence, secondary metabolite production and various host immune responses.\textsuperscript{16}

\textbf{1.1.1.2. Gram positive bacteria}

Gram positive bacteria use oligopeptides as autoinducers to mediate two component QS regulatory system.\textsuperscript{17} These two components are: (i) receptor for binding to AI-1s and (ii) response-regulatory protein. The latter is involved in gene regulation upon activation along with AI-1 production through a positive feedback loop. When AI-1 binds to the sensor receptor, a histidine sensor kinase protein is activated which auto phosphorylate the histidine residue.\textsuperscript{18} The phosphorylated protein transfers the phosphoryl group to the conserved aspartate residue in the cognate response regulator leading to the activation of several genes responsible for bacterial QS.\textsuperscript{18,19}
A. Acylhomoserine lactones (AI-1)

\[
\text{R group}
\]

B. Oligopeptides (AI-1)

\[
\begin{align*}
\text{ADPITROWGD} & & \text{B. subtilis (ComX)} \\
\text{ERG} & & \text{B. subtilis (CSF)} \\
\text{EMRLSKFRDFILQKK} & & \text{S. pneumoniae (CSP)} \\
\end{align*}
\]

\[
\begin{align*}
P. \text{aeruginosa (LasI)} & & \text{AIP-I} & & \text{S. aureus (AgrD)} \\
P. \text{aeruginosa (RhlI)} & & \text{AIP-II} \\
V. \text{fisher} (\text{LuxI}) & & \text{YSTCDFIM} & & \text{GZNACSSLF} \\
A. \text{tumefaciens (TraI)} & & \text{AIP-III} & & \text{INCDFLL} \\
V. \text{harvey} (\text{LuxLM}) & & \text{AIP-IV} & & \text{YSTCYFIM} \\
\end{align*}
\]

C. Furanones (AI-2)

\[
\begin{align*}
\text{AI-2 in } V. \text{harvey} (\text{LuxS}) \\
\text{AI-2 in } S. \text{typhimurium (LuxS)}
\end{align*}
\]

D. Tridecane (AI-1)

\[
\begin{align*}
\text{CAI-1 in } V. \text{cholera} (\text{CqsA}) \\
\text{PQS in } P. \text{aeruginosa (PqsABCD)}
\end{align*}
\]

Figure 2. Representative structures of autoinducers (respective synthases mentioned in parenthesis) A. AHLs used by Gram negative bacteria B. Oligopeptides used by Gram positive bacteria. C. AI-2s recognized by \textit{V. harveyi} and \textit{S. typhimurium}. D. AI-1 autoinducer used in \textit{V. cholerae}. E. PQS autoinducer used by \textit{P. aeruginosa}.

1.1.2. Interspecies quorum sensing

While AHLs are the chemical lexicon of Gram negative bacteria and oligopeptides for Gram positive bacteria, for communication within the species, derivatives of 4,5-dihydroxy-2,3-
pentanedione (DPD), referred as AI-2 is the common signaling molecule for both Gram positive and negative bacteria.\textsuperscript{20-23}

\textit{Vibrio harveyi}, a Gram negative bioluminescent marine bacterium, is the paradigm for the interspecies communication, as involvement of AI-2 was extensively studied and demonstrated in this organism. It uses AHL mediated QS for regulation of bioluminescence. In a series of biochemical and genetic experiments conducted by Bassler \textit{et al.} to study QS in \textit{V. harveyi}, the bacterial mutant cultures, in spite of lacking the AHL synthase (LuxM) and cognate receptor (LuxN),\textsuperscript{24,25} were observed to induce bioluminescence. Such findings suggested a second class of signaling molecules, apart from AHL. That was the first example of association of AI-2 as QS mediator. However, apart from AHLS and AI-2 QS systems, another new class of QS molecule called CAI-1 (whose structure is unknown but speculated to be related to CAI-1 in \textit{V. cholerae} ((S)-3-hydroxytridecane-4-one)) was also shown to mediate QS (Figure 2).\textsuperscript{14} These three QS systems work convergently to control production of bioluminescence\textsuperscript{26} virulence factors such as metalloprotease production, type III secretion\textsuperscript{27} and siderophore production.\textsuperscript{28}

\textbf{An overview of QS system of \textit{V. harveyi}}

\textit{V. harveyi} recognizes and secretes three types of autoinducers: (1) the AI-1 (\textit{N}-(3-hydroxybutyryl)-\textit{L}-homoserine lactone) referred as HAI-1, is synthesized by LuxM, (2) the AI-2 (borate ester of 4,5-dihydroxy-2,3-pentanedione\textsuperscript{27} (DPD) is synthesized by LuxS (it will be discussed in detail in later sections), and (3) cholera autoinducer 1 (CAI-1) is synthesized by CqsA enzyme.\textsuperscript{14} Membrane bound histidine kinases detect the autoinducers (Figure 3).\textsuperscript{29} The HAI-1 is detected by the LuxN histidine kinase.\textsuperscript{27,30} The AI-2 is detected by the periplasmic protein LuxP in complex with the LuxQ histidine kinase,\textsuperscript{24,27,31} while CAI-1 is detected by the CqsS histidine kinase.\textsuperscript{14,27,32} These proteins are bifunctional as they can act as kinases and phosphatases depending on cell density.\textsuperscript{28} In the absence autoinducers, i.e., at low cell density, these proteins get phosphorylated and subsequently transfer the phosphate to LuxO via LuxU.
Phosphorylated LuxO along with σ^54 activates production of five sRNAs. These in turn bind with chaperon Hfq to destabilize mRNA encoding the transcriptional regulator, LuxR_{vh}. Therefore no LuxR_{vh} is produced at low cell density. At high cell density, when AIs are released, the cognate receptors turn themselves into phosphatases and reverse the sequence of phosphorylation leading to up regulation of LuxR_{vh} which regulates for more than 50 genes including those encoding luciferase and virulence factors (Figure 3).

1.1.2.1. The signaling pathway for auto inducer of type 2 (AI-2)

In order to study the enzymes involved in the AI-2 signaling, Schaudre et al. made mutants of *V. harveyi*, *E. coli* and *S. typhimurium*. In all the mutants of bacteria, luxS gene was observed to be highly conserved. The involvement of luxS in AI-2 signaling was confirmed by observation of the fact that bacterial cultures lacking luxS gene failed to produce AI-2 activity. The luxS gene was found to be present in more than 60 bacterial species (both Gram positive and negative) supporting the hypothesis of AI-2 as inter species signal.

**Figure 3.** A. *Vibrio harveyi* signal transduction at low cell density: B. *Vibrio harveyi* signal transduction at high cell density.

**Biosynthesis of AI-2**

The key to identification of biosynthetic pathway of AI-2 was unraveled when luxS gene was observed to be located downstream of *pfs* and *metk*. The gene *metk* encodes for enzyme, S-
adenosyl-L-methionine synthetase which catalyzes production of S-adenosyl-L-methionine (SAM) from ATP and methionine.\textsuperscript{40} The SAM acts as a methyl donor in the biosynthesis and/or modification of DNA, RNA and various proteins and produces S-adenosylhomocysteine (SA\textsubscript{d}H). Through negative feedback inhibition, SA\textsubscript{d}H inactivates SAM-dependent methyltransferase. Therefore, SA\textsubscript{d}H should be transformed into a nontoxic metabolite for the successful operation of SAM dependent methylation reactions.\textsuperscript{41} Eukaryotes employ the enzyme, SA\textsubscript{d}H hydrolase to convert SA\textsubscript{d}H to adenine and homocysteine (Hcy) whereas prokaryotes follow a two-step strategy using Pfs enzyme and LuxS.\textsuperscript{42} In the first step Pfs catalyzes depurination of SA\textsubscript{d}H to give adenine and S-ribosyl-L-homocysteine (SRH) (Scheme 1). In the second step, LuxS converts SRH to homocysteine and 4,5-dihydroxy- 2,3-pentanedione (DPD) by a nonredox pathway. Subsequently, homocysteine undergoes methylation and reenters the catalytic cycle.\textsuperscript{43} It was hypothesized that LuxS catalyzes the final step of converting SRH into DPD which ultimately leads to production of AI-2 activity. This hypothesis was proved by conducting series of experiments with the use of cell lysates, purifies Pfs, LuxS and substrates like SAM, SA\textsubscript{d}H and SRH.\textsuperscript{44}

\begin{equation}
\text{Scheme 1. Biosynthesis of 4,5-dihydroxy- 2,3-pentanedione (DPD) (precursor to AI-2)}
\end{equation}
The structure of AI-2

The DPD is a highly unstable molecule. It undergoes dehydration and cyclization reactions to give a mixture of interconvertible furanones. But none of these sugar derivatives, including DPD, showed bioluminescence comparable to AI-2 activity in *V. harveyi* bioassay. Such result clearly shows that LuxS in vitro mixtures contain an elusive chemical (AI-2) which is distinct from the rest of the furanones.\(^{44}\) Bassler et al. successfully solved the structure of AI-2 from the crystal structure of *V. harveyi* AI-2 sensing protein, LuxP in complex with AI-2 (depicted in Figure 4 along with network of hydrogen bonds) and \(^{11}\)B NMR studies. It was found to be borate diester of S-THMF (2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran). The proposed formation of AI-2 from DPD involves (i) cyclization and hydration of DPD to give the furanone derivative, S-THMF (ii) complexation of S-THMF with borate to form AI-2 (Scheme 2).\(^{22}\)

Scheme 2. Formation of AI-2 from 4,5-dihydroxy-2,3-pentanedione (DPD).\(^{45}\)
The crystal structure of *Salmonella typhimurium* LsrB (AI-2 binding protein) bound AI-2, revealed a chemically different form of AI-2 compared to *V. harveyi* (Scheme 2). While *V. harveyi* uses S-THMF-borate as AI-2, *S. typhimurium* uses R-THMF ((2R,4S)-2-methyl-2,3,3,4-tetrahydroxymethyltetrahydropyran). *S. typhimurium* being a gut pathogen has no access to boron, so it is conceivable that it uses nonboron compound as a sensing molecule. These findings support the validity of DPD as a precursor to AI-2 sensor and establish its role in interspecies communication.

Figure 4. Crystal structure of *V. harveyi* LuxP with AI-2 at the binding site (Figure 4A) and crystal structure of *Salmonella typhimurium* LsrB with AI-2 at the binding site (Figure 4B).

1.2.2.2. S-ribosylhomocysteinase (LuxS)

Currently four homologues of LuxS (*Deinococcus radiodurans, Haemophilus influenzae, Helicobacter pylori* and *Bacillus subtilis*) have been resolved by X-ray crystallography at resolution of 1.2 to 2.4 Å. All LuxS proteins are homodimers. Each subunit is a single polypeptide chain which share a novel α-β fold consisting of four-stranded antiparallel β-sheet bordered by 3 α helices on one side and a short 3₁₀ helix on the other side. The LuxS sequence
alignment from 26 species, revealed 23 repeated amino acid residues, some of which are part of the active center. In the crystal, as well as in solution, LuxS is present as symmetric homodimer with two symmetric active sites. The dimeric interface contains a complex network of H bonding, ionic and hydrophobic interactions.\(^47\)

The Lux\(_{\text{B}}\) (LuxS present in \textit{Bacillus subtilis}) is a single polypeptide with 157 amino acids per each monomer.\(^46\) Initially, the structure of LuxS was determined with Zn\(^{2+}\) at the active site, coordinated by three conserved residues viz His-54, His-58, Cys-126 and water molecule (Figure 5). Because of the presence of zinc, a molecule of water and the motif HXXEH, similar to the protease-binding motif found in thermolysin and other zinc dependent hydrolases, LuxS was proposed to perform a similar enzymatic role. The function of the metal as a Lewis acid was established from the crystal structure of LuxS with the 2-keto intermediate, \(3\) (Scheme 3).\(^49\) The proposed metal mediated catalysis was confirmed by the dramatic change in electronic absorption spectrum of cobalt ion when the natural substrate, SRH was added to the Co-Lux\(_{\text{B}}\) (from peaks at 530, 570, 650 nm to none).\(^50\)

Interestingly, Cys84 which is highly conserved in the active site, was found oxidized to cysteine sulfonic acid and cysteine sulfinic acids leading to rapid inactivation of LuxS, suggesting complexation of metal ion to Cys84 in catalysis.\(^46,47\) A ligand combination (two His, one Cys, one water molecule and Fe\(^{2+}\)) similar to LuxS was also reported in peptide deformylase which also underwent inactivation by an identical route.\(^51,52\) These findings along with the studies done by Pei \textit{et al.} on purified LuxS enzyme, suggested that native metal ion in LuxS was Fe\(^{2+}\) instead of Zn\(^{2+}\).\(^50\) However, the Zn and the Co coordinated proteins were found to be more stable to aerobic oxidation compared to native iron bound LuxS. Substitution of Co\(^{2+}\) or Fe\(^{2+}\) for Zn\(^{2+}\) gave a variant with a 10-fold increase in catalytic activity \textit{in vitro}.\(^50\)
The crystal structure of LuxS<sub>Bs</sub> in complex with SRH at 2.2 Å and homocysteine at 2.3 Å respectively, was determined by Baker <i>et al</i>.<sup>46</sup> The homocysteine moiety of SRH is bound in an extended conformation with the amino and carboxyl groups stabilized by hydrogen bonds with five neighboring residues from helix α1 and strand β3 (Arg65, Asp78, Ile79, Ser80 and Gly127 and Lys35 of neighboring subunit). The ribose moiety appears to be in the C2′-endo conformation and the ribose O2 and O3 hydroxyl groups can be seen involved in long ion-dipole interactions with the Zn<sup>2+</sup> ion.<sup>23</sup> In addition, the O2 and O3 ribose hydroxyl groups are stabilized by hydrogen bonds with the oxygen atoms of oxidized Cys84<sup>46,53</sup>.

**Mechanism of S-ribosylhomocysteinase (LuxS)**

The proposed catalytic mechanism of LuxS involves consecutive aldose-ketose and ketose-ketose isomerizations and subsequent β-elimination to release Hcy and DPD (Scheme 3).<sup>54</sup> In the

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**Figure 5.** Crystal structure of LuxS<sub>Bs</sub> in complex with 2-keto intermediate.<sup>49</sup>
first catalytic step, the aldehyde form of SRH binds to the metal ion, to increase the acidity at C2 so that, Cys84 in LuxSBS, acting as acid/base, could abstract the hydrogen at C2 to form enediolate, 2. Assisted by the second base /acid (probably Glu57), the metal ion migrates from C1 to C2 position, followed by reprotonation at C1 by Cys84 to give 2-keto intermediate 3 (Scheme 3). The second step is repetition of the isomerization reactions as in first step, to give 3-keto intermediate, 5. In the third step, base/acid abstracts proton at C4 with elimination of homocysteine to give an enol intermediate which ultimately tautomerizes to DPD.49,50,53,54 The LuxS-catalyzed cleavage of the C5–S thioether bond in SRH is dramatically different from that proposed in the reversible mechanism for SAdHase. While SAdHse cleaves the thioether bond in SAdH by using enzyme-bound NAD$^+$ (redox reaction), LuxS cleaves thioether bond in a nonredox fashion using enzyme bound water, acid-base pairs and a metal ion via a series of isomerization steps.41,55

The proposed mechanism was validated by conducting various studies: (i) existence of proton transfer reactions were confirmed by studies with D$_2$O,50 (ii) involvement of keto intermediates, 3 and 5 was established using real time by $^{13}$C NMR spectroscopy.54 Involvement of keto intermediates as chemically and kinetically competent substrates was further proved by the synthesis of 2 and 3-keto intermediates, 3 and 5 and testing against LuxS,54,56 (iii) the intermediacy of 3 in catalysis and the function of metal ion as Lewis acid was proved by the crystal structure of 2-ketone intermediate 3 bound to a catalytically inactivated LuxS mutant (Cys84),49 and (iv) the stereochemical and regiochemical specificity of the proton transfer reactions catalyzed by Cys84 and Glu57 was elucidated by deuterium labeled SRH studies in LuxSBS and the conversion of intermediate 3 to 5 was found to be partially rate limiting,53,54 and (v) the role of Glu57 and Cys84 as general acids/bases (Scheme 3) was confirmed by the site-directed mutagenesis.50
Recent studies done by Pei et al. using [3-F]SRH and [3-Br]SRH as substrates for LuxS Bs suggested involvement of Tyr-89 and His-11 along with Glu57 and Cys84 in keto-isomerization steps (part of proton relay system).56

Scheme 3. LuxS catalyzed conversion of SRH into Homocysteine and DPD as proposed by Pei et al.54

1.2. Applications of QS modulation

Recent increase in prevalence of bacterial strains resistant to antibiotics emphasizes the need for the development of new generation of antibacterial agents. Since some of the pathogenic bacteria use QS to regulate virulence, antibiotic resistance and biofilm formation, blocking bacterial QS offers a novel therapeutic strategy.6,19,57-63 Over the past decade, several new components in QS signal transduction pathways (both intra and interspecies) were identified expanding the range of new approaches available to modulate QS. Interruption of QS, which regulates virulence or biofilm formation, does not result in either bacteriostatic or bactericidal
effect. It only attenuates the virulence but does not affect bacterial growth. Such behavior has the advantage of putting less selective pressure on bacteria resulting in lower incidence of microbial resistance. An important bacterial behavior regulated by QS, is biofilm formation, which is one of the causes of antibiotic therapy failure. Biofilm can be described as “an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material” 64 which makes bacterial cells less permeable to antibiotics. *P. aeruginosa*, one of the human opportunistic pathogen survives in the host by formation of biofilms causing hindrance to the entry of antibiotics into the cells and makes therapy ineffective. Thus, inhibition of biofilm formation should make the bacteria more susceptible to antibiotics. Therefore, QS inhibitors can act as adjuvants to antibiotic therapy.65,66

The importance of QS inhibition in regulating bacterial pathogenicity and other community related behaviors can be further underscored by the natural evolution of bacterial quorum quenching enzymes to prevent these population based activities of bacteria competing for energy and nutrients.67,68

**General strategies for manipulation of QS include:**

(1) inhibition of synthesis of autoinducer molecules, (2) inhibition of binding of autoinducer to sensor proteins, (3) inhibition of molecular targets downstream of autoinducer synthesis, (4) sequestration of autoinducer via molecular traps,61 (5) degradation of autoinducer,67-69 and (6) inhibition of autoinducer transport and release.6

**1.2.1. Modulation of intraspecies communication mediated by acylhomoserine lactones (AHLs)**

Interception of the AHL (LuxI/LuxR) pathway can be achieved by three general approaches: (1) Inhibition of AHL synthases (LuxI), (2) degradation of AHLs, (3) inhibition of ligand-receptor binding (AHL-LuxR)

**1.2.1.1. Inhibitors targeting the synthases**

The AHLs are synthesized by proteins called LuxIs or its homologues from SAM and
acyl carrier proteins. Therefore inhibition of enzymes which utilize SAM can also inhibit AHL mediated QS. The SAM is also the substrate for methyl transferases which are involved in crucial biochemical processes. Consequently, AHL synthases which alter the SAM metabolism can have undesired effects on the ubiquitous SAM pathway. Hence, inhibitors of synthases were not developed until recently. With the recent availability of crystal structures of LuxI type of proteins, design of ligands which specifically target AHL synthases without interfering with other SAM requiring enzymes might be undertaken.70-72

1.2.1.2. Inhibitors targeting the ligand

The second method to interrupt AHL pathway is degradation of the AHL ligand itself by using several methods such as, specific enzymes (acylases or lactonases), oxidizing agents and pH (lactones are hydrolyzed at basic pH). So far the quorum quenching approach has been utilized in protecting plants from infectious bacterium.73,74

1.2.1.3. Inhibitors targeting the receptor

The final method of interruption of AHL mediated QS, is inhibiting the AHL binding to its transcriptional regulators or LuxR type proteins. Significant research has been done in designing AHL analogs as LuxR inhibitors.75 According to the review published by Wang et al.6 AHL inhibitors can be classified based on the structural modification on the AHL signal as: inhibitors (1) with alteration of the acyl side chain, (2) with modification on the γ-lactone ring, (3) with simultaneous modification on both acyl side chain and lactone ring, and (4) inhibitors structurally unrelated to AHL.6

(1) Inhibitors with modification on the acyl side chain of AHLs

As there is 70-80% sequence homology at the ligand binding domain of the LuxR-type of proteins and some species share identical AHLs, cross QS communication from one species to another is expected. The AHLs with short acyl chain length tend to act as antagonists in species
which use long chain AHL. The C₄-AHL, an agonist for RhlR (cognate receptor for \textit{P. aeruginosa}), AhyR (cognate receptor for \textit{A. hydrophilia}) and SwrR (cognate receptor for \textit{S. liquefaciens}) is an antagonist for LuxR (receptor for \textit{V. fisheri}).\textsuperscript{76} Winan \textit{et al.} studied activities of AHL mimics against TraR in \textit{A. tumefaciens WCF47Δ-traI} derivative harbouring \textit{lacZ} fusion as their reporter strain. The C₈-AHL, a natural ligand for CciR in \textit{Burkholderia cenocepacia}, was the most potent inhibitor identified in this study (Table 1).\textsuperscript{77}

Eberhard \textit{et al.}\textsuperscript{76} and Greenberg \textit{et al.}\textsuperscript{78} independently studied the effect of synthetic AHLs having various side chain and lactone ring modifications of 3-Oxo-C6-AHL which regulates LuxR system in \textit{V. fisheri}. Compound 7 with chain length of C7 and lacking carbonyl at C3 was found to be an inhibitor. From these studies, the authors suggested: (1) the 3-oxo group is required for agonistic activity, (2) chain length longer than C5 results in inhibitory activity, (3) introducing unsaturation into acyl chains results in only moderate agonistic activity, suggesting the requirement of flexibility for binding (Figure 6).

Douthea and co-workers have synthesized a series of AHL analogs by introducing steric bulk at the side chain\textsuperscript{79} and replacing the amide bond with either sulfonamide\textsuperscript{80} or urea\textsuperscript{81} functionality. They tested the compounds in a strain harboring LuxR and \textit{lux} box from \textit{V. fisheri} and the luciferase gene cluster \textit{luxCDABE} from \textit{Photorhabdus luminescens} which produces luminescence upon addition of exogenous AHL. In the first study, the effect of steric bulk on binding to LuxR was studied. The cyclopentyl analoge 8 with ED₅₀ = 0.25 µM (equal to the natural substrate) was the most potent agonist while the phenyl substituted 9 and 10 with IC₅₀ = 2 µM were the most potent antagonists identified in the study (Figure 6).\textsuperscript{79} Among sulfonamide group and urea series, none had agonistic activity. The most active inhibitors from sulfonamide series are 11 (IC₅₀ = 2 µM) and 12 (IC₅₀ = 3 µM) and from urea series are 13 (IC₅₀ = 1 mM) and 14 (IC₅₀ = 1.1 mM).
The data from Douthea and co-workers studies suggest: (1) addition of bulky substituent is well tolerated by LuxR leading to inhibition, but more than one ring can render the molecule inactive (napthyl is inactive), (2) replacement of amide with urea or sulfonamide results in loss of agonism and can confer antagonism, (3) reduction of acyl side chain close to C6 or less can render antagonistic properties, (4) presence of amide isosters, sulfonyl and urea groups are tolerated as they make hydrogen bond contacts at the LuxR binding domain, and (5) flattening of lactone ring (15) by introducing unsaturation results in loss of activity (Figure 6).\(^\text{80,81}\)

Neilson et al. reported a series of AHL mimics containing sulfonyl, sulfoxide and thioether groups on the acyl chain (Figure 6).\(^\text{82}\) As LuxR system in \emph{V. fisheri} accepts ligands with wider
structural diversity than *P. aeruginosa* LasR, initial screening was performed in *V. fisheri* using a quorum sensing inhibitor selector system (QSIS) constructed by Rasmussen *et al.* Compounds active in this screening were later tested in *P. aeruginosa* las system in which lasB promoter is fused to *gfp* (encodes for green fluorescence protein). Compounds with the thioether group were found to be potent inhibitors while sulfoxide analogs were weak inhibitors and sulfonyls were inactive. Among the synthesized thioether AHL mimics, compounds 16 and 17 displayed strong antagonism with IC\textsubscript{50} = 6 µM and IC\textsubscript{50} = 50 µM (Figure 6).

Blackwell *et al.* prepared a library of AHL derivatives having various long chain and phenyl substituents and tested in *V. fisheri* ES114 Δ-luxI reporter strain. Of the compounds tested, 3-nitro-phenyl acetyl lactone, 18 was found to be a super agonist with EC\textsubscript{50} = 0.3 µM in comparison to the natural ligand, 3-oxo-C12 with EC\textsubscript{50} = 3 µM (Figure 6). Interestingly phenyl acetyl lactones, 19 (IC\textsubscript{50} = 0.6 µM), 20 (IC\textsubscript{50} = 0.9 µM), 21 (IC\textsubscript{50} = 1.0 µM), 22 (IC\textsubscript{50} = 1.1 µM), 24 (IC\textsubscript{50} = 1.4 µM) and 23 (IC\textsubscript{50} = 3.7 µM) with electron-withdrawing groups (EWG) at the para position were identified to be inhibitors. They also reported indole derivative of AHL, 25 as antagonist of TraR in *A. tumefaciens* and LasR in *P. aeruginosa*, reduced biofilm formation in *P. aeruginosa* (Figure 6).

On the basis of these results, Blackwell *et al.* prepared various AHL analogs with diverse structural modifications in order to understand structure activity relationship of AHL analogs in QS systems of reporter strains viz., *A. tumefaciens* WCF47 (lacks traI) harboring a plasmid-born PتراI-lacZ fusion (pCF372), *E. coli* DH5R harboring the LasR expression vector pJN105L and a plasmid-born PlasI-lacZ fusion (pSC11) and *V. fisheri* ES114 lacking luxI. Compounds 26 to 31 are the most potent antagonists, identified from this study (Figure 7A). The structure and activity correlations observed can be summarized as: (1) TraR ligand binding site is most compact and lipophilic of the three tested. Hence subtle changes in the ligand structure results in dramatic change in activities, (2) AHLs with acyl chain length of not more than eight atoms and
aromatic group with electron withdrawing groups or long chains can antagonize all three receptors, (3) carbon spacer length between lactone moiety and aromatic ring should not be less than one, to confer inhibitory activity, (4) D-AHL 31 was also capable of inducing antagonism in LasR (as the natural ligand was L-AHL), and (5) LuxR antagonists derived from phenyl AHLs are agonists at higher concentrations (compound 21). 

\[ \text{IC}_{50} = 0.57 \mu M \text{ against TraR} \]

\[ \text{IC}_{50} = 0.46 \mu M \text{ against TraR} \]

\[ \text{IC}_{50} = 0.61 \mu M \text{ against TraR} \]

\[ \text{IC}_{50} = 0.74 \mu M \text{ against LuxR} \]

\[ \text{IC}_{50} = 0.04 \mu M \text{ against LasR} \]

\[ \text{IC}_{50} = 0.54 \mu M \text{ against LasR} \]

**Figure 7.** (A) AHL antagonists identified across *A. tumefaciens, E. coli* and *V. fisheri* strains and their corresponding IC\(_{50}\) values. (B) Isothiocyanate analogs as irreversible inhibitors of LasR.

Meijler *et al.* designed and synthesized electrophilic AHL analogs as probes to covalently modify Cys79, found at the active site of LasR. Among the analogs tested isothiocyanate analogs (32-34) were found to modify the binding pocket covalently and inhibit QS controlled activities.
(Figure 7B). When tested for inhibition in reporter strain PAO-JP2 (lasI/rhlI deleted) harboring plasmid pKD201 containing a lasI coupled to luxCDBE luminescence system, compound 33 displayed partial agonism.85

(2) Inhibitors with modification of the lactone ring

In order to explore the effect of modification of the lactone ring functionality on the AHL mediated LuxR type of receptors, analogs with replacement of the lactone ring with saturated and unsaturated rings of various sizes were designed.

Suga et al. reported a library of antagonists of LasR and RhlR in P. aeruginosa fluorescent strain PAO-JP2 which lacks genes encoding AHL syntheses (LasI for 3-oxo-C12-AHL and RhlI for 3-oxo-C4-AHL).87 All of the inhibitors had acyl side chain identical to the natural ligand (3-oxo-C12-AHL in case of LasR and 3-oxo-C4-AHL in case of RhlR) and lactone carbonyl group replaced with amine, alcohol and ketones groups which are capable of hydrogen bonding (Figure 8). The 3-oxo-C12-AHL cyclopentanone derivative 35 had agonistic activity against LasR signaling protein, comparable to native substrate at 400 µM and its C4 side chain counterpart 36 activated RhlR at 100 µM. The cyclopentanol analog 37 had agonistic activity comparable to 3-oxo-C12-AHL at 50 µM when tested against LasR. The 3-oxo-C12-cyclohexanone derivative 39 inhibited LasR at 100 µM by 35% and its C4 counterpart, 40 inhibited RhlR systems by 60% at 50 µM when competing with 1µM 3-oxo-C12-AHL and 10 µM 3-oxo-C4-AHL respectively. Cyclohexanone analog 39 also inhibited QS mediated pyocyanin, elastase B and biofilm production.87 Based on the initial results, eight non-AHL analogs with lactone ring replaced by aromatic group were reported. Compound 43 (Figure 9) was found to inhibit LasR system and also elastase B production in P. aeruginosa.88
Moroshi and co-workers reported acyl cyclopentane derivatives as inhibitors of *Serratia marcescens* QS.\(^8^9\) *Serratia marcescens* is a Gram negative pathogen which regulates prodigiosin production, swarming motility and biofilm formation using AHL mediated QS.\(^9^0,9^1\) It uses \(N\)-hexanoyl homoserine lactone (C\(_6\)-AHL) and \(N\)-(3-oxo-hexanoyl) homoserine lactone (3-oxo-C\(_6\)-AHL) as QS signals.\(^9^2\) The \(N\)-nonanoyl-cyclopetylamine 44\(a\) lacking the lactone ring was found to inhibit prodigiosin production at four fold concentration relative to the natural agonist (Figure 9). Kato *et al.* reported \(N\)-decanoyl cyclopentyl amide, 44\(b\) as inhibitor of *P. aeruginosa* QS. It inhibited 3-oxo-C\(_{12}\)-AHL mediated lasB-lac\(Z\) expression with IC\(_{50}\) value of 80 \(\mu\)M and 3-oxo-C\(_{4}\)-AHL mediated rhl\(A\)-lac\(Z\) expression with 90 \(\mu\)M (Figure 9).\(^9^3\)

**Figure 8.** *P. aeruginosa* AHLs and their ring modified analogs.\(^8^7\)

![Figure 8](image)

**Figure 9.** Cyclcopentanone, phenol analogs (43-44\(b\))\(^8^9\) and thiolactone and caprolactam analogs (45-46).\(^7^8\)
Eberhard et al.\textsuperscript{76} and Greenberg et al.\textsuperscript{78} independently studied the effect of synthetic AHLs having various side chain and lactone ring modifications in \textit{V. fisheri} LuxR system. The AHL mimics with replacement of lactone ring with thiolactone 45 and caprolactam 46 were prepared (Figure 9). Thiolactone 45 had only moderate activity but caprolactam 46 was inactive, probably because of the lack of sensor binding. However, the detailed studies on lactam analogs with various ring sizes were not explored in this study.

(3) Inhibitors with modifications on the lactone ring and acyl side chain

In an effort to find more potent QS inhibitors, AHL analogs modified at both lactone and acyl side chain were also prepared. \textit{Psedomonas aeruginosa} produces 3-oxo-C\textsubscript{12}-AHL as the AI-1 to coordinate QS related behaviors like virulence factor production and also host immunomodulatory responses. Spring and co-workers described the total synthesis of 3-oxo-C\textsubscript{12}-AHL and also reported four 3-oxo-C\textsubscript{12}-AHL analogs 47-48b having various nonhydrolysable cyclic ketone and lactone rings along with changes at the acyl side chain as LasR inhibitors.\textsuperscript{94,95} Cyclic ketones, 48a, 48b were found to reduce immunomodulatory responses (for e.g., TNFα factor and nitric oxide production) (Figure 10).

![Figure 10. P. aeruginosa AHLs analogs with modification at lactone ring and acyl side chain.\textsuperscript{95}](image)

Muh \textit{et al.} screened a library of 200,000 compounds using ultra-high-throughput-cell based screening and identified two LasR inhibitors having a phenyl (49) and tetrazole ring (50) connected to 12 carbon aliphatic chain similar to 3-oxo-C\textsubscript{12}-AHL (Figure 11).\textsuperscript{96} Phenyl analog 49 and tetrazole analog 50 inhibited both production of pyocyanin and elastase B in strain PAO-1-MW1 (which lacks \textit{lasI} and \textit{rhlI} genes) with IC\textsubscript{50} value of 10 µM and 30 nM, respectively.
Figure 11. Inhibitors of LasR identified through high-throughput-cell based screening.96

4. Inhibitors structurally unrelated to AHL

Muh et al. discovered the triphenyl compound, 51 and its analog 52 (which is structurally distinct to the natural ligand) mimicking the activity of 3-oxo-C12-AHL against LasR protein (51 had EC$_{50}$ of 0.014 µM and 52 had EC$_{50}$ of 0.054 µM) from the ultra-high-throughput-cell based screening (Figure 12).97

Figure 12. Triphenyl, nonnative agonists of 3-oxo-C12-AHL mediated QS.97

De Nys et al. isolated halogenated furanones 53-60 from red alga Delisisa pulchra (Figure 13).98 These furanones were the first example of small molecules identified to inhibit AHL mediated QS.99 Compounds 53 and 54 were found to reduce QS-mediated behaviors like swarming motility in S. liquefaciens mutants lacking AHL synthase activity.99 Bromofuranones were proposed to inhibit QS by destabilization of LuxR protein.100 Compound 56 displayed concentration-dependent inhibition of bioluminescence production in E. coli (pSB403) by interrupting binding of its auto inducer, 3-oxo-C$_{6}$-AHL to LuxR. Compound 58 and 60 were able to reduce P. aeruginosa infection in pulmonary model.101,102
Several butenolides found in *Streptomyces antibioticus* demonstrated antimicrobial activity against *P. aeruginosa* and weak inhibition of chitinase in *Serratia marcescens*. On the basis of preliminary biological results and their structural resemblance to furanones, Nielsen *et al.* prepared synthetic butenolides and tested for anti QS activity. From the library of compounds tested on GFP-fused E. coli (pJBA89) QS (that responds to 3-oxo-C12-AHL to induce QS-mediated fluorescence), compounds with short side chain displayed maximum inhibition compared to long chain derivatives. Compound 61 was the most effective inhibitor with IC$_{50}$ = 2.4 µM, while 62 had IC$_{50}$ = 3.5 µM and 63 had IC$_{50}$ = 3.1 µM (Figure 14).

Using QS inhibitor selector system (QSISI), Nielsen *et al.* discovered anti-QS properties of garlic extracts. Garlic components 64 and 65 were shown to have LuxR antagonistic activities (Figure 14).

![Figure 13. Halogenated bromofuranones as AHL mediated QS modulators.](image)

![Figure 14. Butenolide analogs (61-63) and inhibitors from garlic (64-65).](image)
1.2.2. Modulation of interspecies communication

Inhibition of AHL mediated intraspecies communication among Gram negative bacteria have been studied over two decades. With recent discovery of AI-2 pathway which was found in more than half of the bacterial species, interspecies communication can also be inhibited giving unique advantage of simultaneous inhibition of communication among multiple species. Moreover, bacteria which are devoid of AI-2 pathway, can still recognize the foreign AI-2 and induce QS.\(^{104}\)

Interspecies regulation of QS can be achieved by: (1) inhibiting LuxS which synthesizes DPD, (2) inhibiting the sensor receptor for AI-2 (LuxP in case of \textit{V. harveyi} and LsrB in case of \textit{S. typhimurium} and \textit{E. coli}), (3) inhibiting enzymes downstream of AI-2 biosynthesis (e.g., 5’-Methylthioadenosine/AdoHcy nucleosidase (MTAN) inhibitors).

1.2.2.1. Inhibition of LuxS enzyme

The \textit{luxS} gene is conserved in more than 60 microbial species including many Gram positive and negative human pathogenic bacteria such as \textit{Vibrio cholerae} (causative agent of cholera), \textit{Salmonella typhimurium} (salmonellosis), \textit{Shigella flexneri} (shigellosis), \textit{Streptococcus pneumonia} (pneumonia, meningitis and arthritis), \textit{Neisseria meningitidis} (meningitis), \textit{Clostridium perfringens} (food poisoning) and \textit{Borrelia burgdorferi} (lyme disease).\(^6\) The LuxS enzyme is reported to be involved in cross talk among bacteria for regulating various community related behaviors like virulence factor production, biofilm formation, toxin production etc.\(^5\) Many examples or cases of involvement of LuxS/AI-2 in bacterial pathogenicity are reported recently and the list is growing. Mutations of \textit{luxS} gene resulted in interference in expression of virulence factors and in some cases rendering the bacteria avirulent.\(^{105-107}\) As LuxS enzyme is a bacterial enzyme inhibition of which might cause less toxicity in humans. All of these factors/facts validate LuxS inhibitors as viable targets, to serve as broad spectrum antibacterial agents.
Reported LuxS Inhibitors

As SRH is a most important intermediate in the synthesis of AI-2 and LuxS is present in many species, researchers aimed at making SRH analogs as inhibitors of LuxS. The crystal structure and mechanism of LuxS catalysis was identified recently. However, the inhibitors of LuxS are limited. Recently Zhou et al. reported the first examples of LuxS inhibitors, S-(anhydroribosyl)-L-homocysteine (66) and S-(homoribosyl)-L-cysteine (67). The 1-deoxySRH (66) was reported to act by inhibiting the first mechanistic step in enzyme catalysis and compound (67) by inhibiting the final step of β-elimination (Figure 15). Pei et al. prepared a series of structural analogs with planar hydroxamate group which mimic the unstable enediolate intermediate formed during the isomerizations. Hydroxamates (68) and (69) were the most potent inhibitors reported to date with $K_i$ values of 0.72 and 0.37 $\mu$M respectively. These results also proposed that homocysteine moiety is essential for binding and activity. On the basis of the similarities between substrates of mammalian SAHase and bacterial LuxS enzyme, as well as differences in mechanism of the two enzymes, our lab designed and synthesized SRH analogs with 6-(fluoro)vinyl unit in place of the C5 and sulfur atoms which acted as weak/moderate inhibitor of LuxS enzyme. Compound (70) acted as a competitive inhibitor with $K_i$ value of 96 $\mu$M. Recently our lab in collaboration with Pei lab reported [3-F]SRH, (71) and [3-Br]SRH, (72) as time-dependent reversible inhibitors of LuxS. It was rationalized that covalent inhibition will be observed by nucleophilic attack of active site cysteine at the C3 position, with removal of halide. But time-dependent inhibition was observed because of the enzyme catalyzed elimination of halide ions via E1cb mechanism (Figure 15). Recent studies in our laboratory focused on design and synthesis of 3-deoxy and 3-deoxy-3-substituted SRH analogs. These 3-fluoro and 3-O-methyl SRH analogs (lacking enolizable hydroxyl group at C3) were designed as mechanism based LuxS inhibitors. Among the 3-modified SRH analogs, xylo analog, (73) was found to be most potent slow binding inhibitor with $K_i$ value of 4.2 and $K_i^*$ value of 0.43, while compound (74) and (75)
were moderately potent slow binding inhibitors with $K_i^*$ value of 2.8 and 8.8, respectively. These findings are consistent with the time-dependent inhibition proposed for [3-F] SRH, 71 and [3-Br] SRH, 72 (Figure 15).\textsuperscript{109}

\begin{align*}
\text{Figure 15. Reported LuxS enzyme inhibitors} \\
\text{Halogenated furanones isolated from red alga } D. \text{ pulchra } \text{ were reported to clear } P. \text{ aeruginosa } \\
\text{infection in mouse pulmonary infection models and inhibit } B. \text{ anthracis } \text{ growth and expression of virulence genes. However the mechanism of QS antagonism was elusive.}\textsuperscript{110} \text{ Recently Zhou et al. reported the mechanism of their action to be the time-dependent modification of LuxS enzyme.} \\
\text{On the basis of experimental findings, an addition elimination mechanism was proposed for the covalent inhibition of LuxS by bromofuranone 53 (Figure 15).}\textsuperscript{111}
\end{align*}
1.2.2.2. 5'-Methylthioadenosine/AdoHcy nucleosidase (MTAN) inhibitors

The 5'-Methylthioadenosine/AdoHcy nucleosidase (MTAN) is a dual substrate-specific microbial enzyme encoded by pfs gene. It catalyzes deadenylation of SAH to form adenine and SRH, the substrate for LuxS and also deadenylation of 5'-methylthioadenosine (MTA) to 5'-methylthioribose (MTR) (Scheme 4). The MTA is synthesized from S-adenosylmethionine and acylated acyl carrier protein by AHL synthase. It is a feedback inhibitor of AHL synthase. Thus MTAN plays a vital role in both AI-1 and AI-2 biosynthesis. According to the MTAN catalytic mechanism as proposed by Schramm group, it undertakes catalysis through an early and late transition states (TS) involving formation of ribosyl oxacarbenium ions. Based on the oxacarbenium ion transition state of MTAN catalyzed reactions, early and late TS analogs were synthesized. The 5'-thio-substituted immucillin (ImmA) mimic the early TS where the glycosylic bond is partially broken whereas the second generation DADMe-immucillin analogs mimic a late TS in which ribose moiety is completely broken from adenine (Scheme 4).

Schramm and coworkers designed and synthesized a series of TS analogs (Scheme 4). The TS analogs inhibit MTAN with in nM range when tested in Streptococcus pneumoniae (Figure 16). Early TS analogs 6 and 7 inhibited with of 60 nM and 40 nM, respectively. The DADMe-Immucillin analogs 80 and 84, mimicking the late TS inhibited with values of 24 nM and 2.3 nM, respectively. In order to improve the potency of TS analogs, the Schramm group synthesized a series of azetidine analog resembling late TS. Methylthio azetidine 85 inhibits E. coli and S. pneumonia MTAN with value of 0.84 nM and 150 nM, respectively.
Scheme 4. (A) 5'-Methylthioadenosine/AdoHcy nucleosidase catalyzed hydrolysis of 5'-methylthioadenosine and S-adenosylmethionine (B) Proposed transition state of 5'-Methylthioadenosine/AdoHcy nucleosidase along with aza TS analogs.113
The compound 86 was shown to have $K_I$ of 0.45 and 84 nM with *E. coli* and *S. pneumoniae* MTAN, respectively. When 80 (MT-DADMe-Immucillin-A), 81 (EtT-DADMe-Immucillin-A) and 82 (BuT-DADMe-Immucillin-A) were tested in *V. cholerae* MTAN, they were found to be slow-onset, tight-binding inhibitors of with IC$_{50}$ values of 27, 31 and 6 nM, respectively. The DADMe-Immucillins 80 and 82 also inhibited *E. coli* MTAN with IC$_{50}$ in nM range. These results clearly validated the use of MTAN inhibitors as potential drug molecules.

![Chemical Structures](image)

**Figure 16.** Transition state analogs of 5’-Methylthioadenosine/AdoHcy nucleosidase

### 1.2.2.3. Modulation of AI-2 receptor (LuxP/LsrB)

As described earlier, many bacteria use AI-2 as interspecies signaling molecule but different bacteria recognize different forms of AI-2. For example, *V. harveyi* recognizes borate ester of $S$-THMF while *S. typhimurium* recognizes $R$-THMF as AI-2. The sensor protein for *V. harveyi* is LuxP and LsrB in case of *S. typhimurium*. The crystal structures of the binding proteins along with their AI-2s revealed a substantial difference in the residues at the binding pocket between LuxP and LsrB explaining the selective preference of LuxP for the anionic borate ester and LsrB for the neutral $R$-THMF. As AI-2 binding proteins play a significant role in QS cascade,
compounds which can inhibit LuxP or LasrB receptors or which can mimic the structures of AI-2 will provide substantial information on the binding site. This might open new doors for development of novel QS inhibitors.

**AI-2 agonists**

The crystal structure of LuxP with AI-2 was solved by Bassler et al.\textsuperscript{44} Certain degree of flexibility within the binding pocket of LuxP was observed. Encouraged by such results, the Janda group synthesized eight DPD structural analogs \textsuperscript{87-94} and tested in \textit{V. harveyi} strain MM30 (a LuxS mutant that is unable to synthesize DPD) (Figure 17).\textsuperscript{115} The results of the activity studies suggested a cleft in the binding pocket responsible for accommodation of structurally diverse molecules. Boron binding, presence, position and stereochemistry of hydroxyl group were suggested to be critical for optimum activity.

The DPD is an unstable molecule and difficult to isolate and purify. Because of this reason studies on AI-2 QS were performed using LuxS mutants as the source of DPD. Employment of mutants lacks the accurate control on concentrations of DPD used. To overcome these practical limitations and to study binding of AI-2 at the receptor, many groups have obtained DPD and its analogs by synthetic route.\textsuperscript{116-119} Recently Doutheau et al. synthesized bis-(\textit{O})-acetylated DPD (Ac\textsubscript{2}-DPD), a precursor to DPD, \textsuperscript{96} (Figure 17). The Ac\textsubscript{2}-DPD is stable at physiological pH and also in concentrated solutions. The esterases present in vivo hydrolyze \textsuperscript{96} to provide DPD. However, Ac\textsubscript{2}-DPD was found to be less potent than DPD in BB170 strain of \textit{V. harveyi} bioluminescence assay and MET844 strain of \textit{S. typhimurium} $\beta$-galactosidase assay, presumably because of the difference in rates of hydrolysis to release DPD. It has comparable activity to DPD in biofilm formation when assayed in the \textit{B. cereus} strain 407.\textsuperscript{120} As replacement of hydrogen atoms with fluorine atom often results in modulation of biological and metabolic properties, the Doutheau lab also reported a trifluoro methyl analog of DPD, \textsuperscript{95} (Figure 17). However, \textsuperscript{95} was found to be less potent than the natural substrate.\textsuperscript{121}
Figure 17. Various DPD agonists resembling DPD, furanosyl DPD or its borate ester AI-2

Janda et al. found that S-THMF-carbonate, 97 produced from the complexation of furanosyl form of S-DPD with carbonate, acts as AI-2 agonists by inducing bioluminescence in V. harveyi strain MM30 (Figure 17). Among the several metal salts tested to identify the potential of metal
ions complexes of furanosyl DPD other than boron to act as QS modulators, cesium carbonate was able to induce bioluminescence. Interestingly, carbonate anion rather than the metal ion was found responsible for the QS modulatory activity. The agonistic activity of S-THMF-carbonate was conceivable, as it fits perfectly in the binding pocket of LuxP mimicking the borate complex.122

Steinberg et al. synthesized several oxaborazolidine analogs resembling the structure of AI-2. Compounds 98 and 99 (Figure 17) displayed co-agonistic activity in *V. harveyi* strain BB170 (which lacks luxN, the sensor for AI-1) and also in strain BB77 (which lacks luxS and luxM, the synthases for AI-1 and 2, respectively). These results demonstrated the involvement of oxaborazolidines specifically in AI-2 signal transduction by their ability to act synergistically with DPD or AI-2.123

Recently Janda et al. reported C1-Alkyl substituted DPD analogs as modulators of AI-2 QS (Figure 17). These compounds were evaluated in *S. typhimurium* strain Met844 with a lacZ-lsr fusion (lacks luxS, the DPD synthase) for the induction of β-galactosidase activity, with and without external DPD (lacZ encodes for the enzyme β-galactosidase which is under the control of promoter lsr. The lsr activation is, in turn influenced by AI-2). Propyl-DPD, 101 was found to be an antagonist while ethyl derivative, 100 has no activity. Interestingly when tested in *V. harveyi* MM32 (lacks AI-1 sensor, LuxN and DPD synthase, LuxS) all the derivatives showed agonistic activity only in presence of DPD by inducing luminescence. Propyl-DPD, 101 and azido-DPD, 103 (Figure 17) were found to be active by seven fold when compared to DPD.124 Meijler et al. and Sintim et al. also synthesized alkyl DPDs independently and also observed similar agonistic activity in *V. harveyi* assays, supporting the results observed by the Janda group.125,126 However, no definite trend was observed with respect to the effect of C1-chain length or ring size on synergism with AI-2 induced bioluminescence.126
The Janda group also performed a short and efficient synthesis of natural product fimbrolide, a known QS inhibitor (53) and studied its inhibitory effects on AI-2 mediated QS in comparison with the C1-substituted DPD analogs. Hexyl-DPD, 102 was found to be more potent than fimbrolide 53, in inhibiting bioluminescence of *V. harveyi* (Figure 17)

**AI-2 antagonists**

Recently Wang *et al.* reported para-substituted phenylboronic acid derivatives, 104-108 (Figure 18) as antagonists of AI-2 mediated QS in *V. harveyi* strain MM32 which shows bioluminescence only with addition of external DPD. Only para substituted phenyl boronic acids were found to exhibit strong antagonism (IC$_{50}$ in single digit µM range) whereas derivatives substituted with amino, carboxyl and alkyl groups possessed no comparable activity. Analogs with no ionizable functional groups and low pK$_a$ values were found to possess strong antagonism for LuxP receptor. It was proposed that, analogs which existed as tetrahedral borates at neutral pH, mimic the natural substrate and exhibit antagonism. 60

Wang group also reported pyrogallols as antagonists to AI-2 signal protein in *V. harveyi* strain MM32. 128 Since the natural substrate, AI-2 in *V. harveyi* is a diol-borate complex, it was rationalized that diols which could bind to borate tightly and mimic the structural geometry of AI-2 and LuxP complex, could be antagonists. 129-131 Among the fifteen aromatic polyols tested, pyrogallols, 109-113 possessing nonionizable functional groups were found to be more potent inhibitors with IC$_{50}$ values in single digit µM range (Figure 18). From the structure based virtual screening of a library of compounds against the crystal structure of LuxP heterocyclic sulfones, 114 and 115 were identified as AI-2 QS inhibitors. 132
Coenye et al. reported nucleoside analogs as AI-2 signal transduction inhibitors. The most active compound found from their screening was an adenosine derivative substituted at C3 by \textit{p}-methoxyphenylpropionamide moiety, 116 and its deadenylated derivative 117 (Figure 19). Interestingly, though structurally 116 resembles SAdH, the microbiological assays performed on \textit{V. harveyi} mutant strains revealed the molecular target of 116 to be LuxPQ which is involved in AI-2 signal transduction (which is upstream of AI-2 production). Adenosine derivative, 116 also
reduced the AI-2 mediated QS behaviors like starvation response, biofilm formation, pigment and protease production in various *Vibrio* species.

![Chemical structures](image)

**Figure 19.** 3-Amino-3-deoxy sugar and nucleoside inhibitors.\textsuperscript{133}

Using *V. harveyi* strain BB170 which lacks the sensor for AI-1, Pillai *et al.* discovered a series of fatty acids obtained from poultry meat wash to be inhibitors of AI-2 mediated QS.\textsuperscript{134} Linoleic acid, \textbf{118a} oleic acid, \textbf{118b} palmitic acid, \textbf{118c} and stearic acid, \textbf{118d} (Figure 20) at 0.1, 1, and 10 mM concentrations resulted in AI-2 inhibition ranging from approximately 25% to 99%.

![Fatty acids](image)

**Figure 20.** Fatty acids as AI-2 mediated QS inhibitors in *V. harveyi*.\textsuperscript{134}

Recently Bentley *et al.* investigated inhibition of interspecies QS by sequestration of AI-2 using LsrK. The LsrK is a AI-2 kinase enzyme which phosphorylates AI-2 into phosphoAI-2 which in turn modulates QS by activating *lsr* regulated gene expression in *S. typhimurium* and
E. coli. It was rationalized that by providing LsrK externally, Al-2 could be phosphorylated ex in vivo and should be incapable of entering the cells to induce QS. Quorum quenching was observed, when LsrK was added to cultures of E. coli which generates Al-2 and S. typhimurium which detects Al-2, suggesting the utility of LsrK as broad range quorum quencher.

1.3. Aza sugars

As part of my dissertation deals with aza sugar analogs of SRH in which ribosyl oxygen is replaced with nitrogen atom, I would like also to summarize recent developments in the chemistry of biologically active aza sugars. Synthetic strategies pertaining to the synthesis of 4-aza-SRH analogs will be discussed (Detailed synthesis of aza sugars was reviewed by Pearson et al. 135)

Aza sugars are polyhydroxy alkaloids that mimic natural sugars. They are carbohydrate analogs in which the ring oxygen is replaced by a nitrogen atom. They are also called the imino sugars. Naturally available aza sugars identified so far are classified based on the type of ring structure as: (1) polyhydroxy piperidines (e.g., nojirimycin), (2) pyrrolidines (e.g., CYB 3, nectricin), (3) indolizidines, (e.g., swaianosonine) (4) pyrrolizidines, (e.g., alexin) and (5) nortropanes (e.g., calystegine A3). Representative structures belonging to each class are depicted in Figure 21. 135

Aza sugars are found to act as glycosidase inhibitors because of their resemblance to natural sugars. Glycosidase and glycotransferases are set of enzymes which control metabolism and biosynthesis of oligosaccharides and glycoconjugates. Such carbohydrate derivatives are essential for vital biological functions like cell adhesion, cell-cell recognition, cell growth, maintenance of immune system, oncogenesis, tumor metastasis etc. Therefore, inhibitors of glysosidases are widely sought after, as they can provide novel antiviral, antibacterial, anticancer, antidiabetic, antiHIV-AIDS agents.
For example, N-hydroxyethyl-1-deoxynojirimycin (Miglitol)\textsuperscript{138} and N-butyl-1-deoxynojirimycin (Miglustat)\textsuperscript{139} are approved drugs against type-2 diabetes and Gaucher’s disease, respectively (Figure 22). Furthermore, castanospermine is found to show potent antiviral activity,\textsuperscript{140} swainsonine\textsuperscript{141} displays anticancer activity, and the bicyclic indoline derivative, lentiginosine\textsuperscript{142} is an amyloglucosidase inhibitor.

\textbf{Figure 22.} Examples of aza sugars with proven biological application.\textsuperscript{138-140,142}

\textit{1.3.1. Transition state analogs of glycoside hydrolases}

According to TS theory, the catalytic proficiency of an enzyme is derived from its capacity to lower activation energy and stabilization of the TS. Altered substrates which mimic the TS of the enzyme bind to the enzyme with greater affinity than the natural substrate as they are proposed to
catch all the binding interactions available. Such non-natural substrates also assist in understanding the mechanism of enzyme catalysis and thereby facilitate the development of potent inhibitors. As a result of the wide utility of glycoside hydrolases in vital biological functions, there has been huge interest in developing inhibitors.

Glycoside hydrolases catalyze cleavage of glycosidic bond in sugar via formation of an oxacarbenium ion which is in the half chair conformation at the anomeric center (at the TS) leading to either retention (retaining glycosidases) or inversion of configuration (inverting glycosidases) of anomeric center. Two carboxylic residues at the active site acting as acid/base and a molecule of water catalyze hydrolysis.

1.3.1.1. Cleavage mechanism of glycosidases

Retaining glycosidases

The proposed mechanism of catalysis for retaining glycosidases of sugars (119a) involves: (i) protonation of anomeric oxygen with acidic groups present at the active site (119b, Figure 23), (ii) generation of oxocarbenium ion 119c after removal of ROH from the anomeric carbon (the glycosyl cation 119c is proposed to exist as oxocarbenium ion 119h in half chair conformation), (iii) subsequent formation of an ester bond at the anomeric center with the active site carboxylate (119d from Figure 23), and (iv) formation of hemiacetal product 119e with the retention of anomeric configuration by nucleophilic displacement of ester group with the enzyme bound water molecule (Figure 23).137

Inverting glycosidases

The first step of catalysis is similar to the retaining glycosidases where general acid protonates the anomeric oxygen. In the later step, however inverting glycosidases differ from the retaining glycosidases. In case of retaining glycosidases, ester intermediate precedes nucleophilic attack by naked water molecule generating the product, while inverting glycosidases act by
nucleophilic attack of the carboxylate directed water molecule at the anomeric center to generate the hemiacetal product 119f, hence inversion of configuration (Figure 23).

Figure 23. Cleavage mechanism of (a) retaining and (b) inverting glycosidases. (c) Resonance forms of oxocarbenium ion in half chair confirmation.

1.3.1.2. Aza sugars as transition state analogs

The design of TS inhibitors focuses on either emulating the supposed half chair geometry or the charge at the TS or both. Since the charge build up occurs in different places at the TS,
mimics with charge at various positions were designed. TS analogs are broadly categorized according to the charge build up and geometry.\textsuperscript{137}

(a) Inhibitors mimicking positive charge at anomeric oxygen

Since the proposed glycosidases mechanism of catalysis was shown to have protonated exocyclic oxygen in early TS (\textsuperscript{119b} in Figure 23), analogs mimicking the charge at the anomeric oxygen were synthesized. Most potent inhibitors in this category are sugars which possess a nitrogen atom in place of anomeric oxygen.\textsuperscript{137} The protonated amine can mimic the early TS. Trehalase inhibitor, trehazolin \textsuperscript{120a} and chitinase inhibitor, allosimidine \textsuperscript{120b} (Figure 24) are examples of this class of inhibitors.\textsuperscript{144}

(b) Inhibitors mimicking positive charge at ring oxygen

As late TS involves formation of glucosyl oxacarbenium ion in half chair conformation with charge either at anomeric carbon (\textsuperscript{119g}) or at ring oxygen (\textsuperscript{119h}), TS analogs mimicking either geometry or charge at the ribocarboxenium ion were designed. Most potent $\alpha$-glucosidase inhibitor, 1-deoxynojirimycin\textsuperscript{145} \textsuperscript{120c} ($K_I = 18 \mu M$) which contains nitrogen in place of ring oxygen (resembles oxacarbenium ion upon protonation) and mannosidase inhibitor, imidazolo pentose\textsuperscript{146} \textsuperscript{120d} (Figure 24) (resembles half chair geometry) are examples of this class of inhibitors.

(c) Inhibitors mimicking positive charge at anomeric carbon

The TS analogs resembling intermediates \textsuperscript{119e} or \textsuperscript{119a} which possess charge at the anomeric carbon were also developed.\textsuperscript{137} The most potent inhibitors of glycoside hydrolases fall into the category of \textsuperscript{119c} mimics. For example, $\beta$-glycosidase inhibitor, isofagomine \textsuperscript{120e} ($K_I = 0.11 \mu M$) contains nitrogen at the psuedo anomeric position (Figure 24). The difference in selectivity of isofagomines and 1-deoxynojirimycin by $\alpha$ and $\beta$-glucosidases was attributed to the location of their active site residues. In case of $\alpha$-retaining glucosidase TS, active site carboxylate group is at the top of the sugar ring and charge is located at the endocyclic oxygen where as in case of $\beta$-
retaining TS, active site carboxylate group is present beneath the sugar ring and charge is located at the anomeric carbon.\textsuperscript{147}

**Figure 24.** Aza sugar based transition state analogs of glycosylases.\textsuperscript{137}

(d) Inhibitors mimicking positive charge at multiple places
Inhibitors with charge distributed over to multiple atoms at the TS (Figure 23) combining the electronic properties of the charged TS intermediates 119b, 119c and 119h as well as half chair confirmation were also developed. Such TS analogs mimic TS more closely because of the charge at various positions:

(i) The amidine analog 120f resembling both endo cyclic and exocyclic charge on oxygen (119b and 119h) was found to be strong β-glucosidase inhibitor with $K_I$ of 8 µM.

(ii) The imiadazole derivative 120g which mimic both half chair confirmation and charge at the anomeric center was also developed. As tetrazoles have $pK_a$ value of -4 and cannot be expected to be fully protonated in the active site. Compound 120g inhibited α-glucosidase and α-galactosidase with a $K_I$ of about 1.5 µM.

(iii) The 1-azafagomine 120h possessing endocyclic nitrogen similar to 1-deoxynojirimycin and exocyclic nitrogen at the pseudo anomeric position like isofagomine displayed strong inhibition of both α- and β-glucosidases (α-glucosidase with $K_I = 6.9$ µM and β-glucosidase with $K_I = 0.32$ µM, respectively) (Figure 24).

(iv) The hydrazine analog 120i, a hybrid of isogalactofogamine and and aminogalactose was found to be inhibitor of galactosidase (α-glactosidase with $K_I = 73$ µM and β-galactosidase with $K_I = 410$ µM) (Figure 24). Such analog mimics glucosyl cation 119c and intermediate with protonated anomeric oxygen 119b.

(e) Inhibitors which does not imitate charge

From the kinetic isotope effects and TS structure, it was suggested that the anomeric carbon is sp²-hybridized. Moreover such observation was attested by potent inhibition observed by lactam and lactone analogs although they only mimic the hybridization but not the charge. However except for lactam analog 120j and 120k which inhibit xylanase with $K_I = 0.34$ µM and with $K_I = 18$ nM, respectively, in general, this class of inhibitors are the weakest of all the
classes of inhibitors described earlier. The weak inhibition was attributed to the mimicry of geometry alone rather than the charges at the TS (Figure 24).

Recently Schramm et al. prepared various femtomolar and picomolar TS mimics of 5’-methylthioadenosine nucleosidase (MTAN), which also bear ring nitrogen in place of oxygen and mimic the oxacarebenium ion formed at the TS. For more description of these MTAN inhibitors refer to section 1.2.2.2.

### 1.3.2. Hemiaminal sugar chemistry

As the proposed 4-aza-SRH analogs are structurally hemiaminal congeners (N,O-acetal or azahemiacetal) of ribose sugar (polyhydroxylated pyrrolidine), in this section I will discuss in brief, the chemistry of the biologically relevant hemiaminal sugars. Hemiaminals as hemiacetals and hemithiols exist in equilibrium as a mixture of open chain aldehyde and closed ring forms whose individual percentage varies depending on pH, conformation of sugar, leaving group strengths, anomeric effect and ionic strength of the solvent medium (Scheme 5). The chemistry of N,O acetals of sugars is found to be complex.

![Scheme 5: Structures of ribose, 4-thioribose and 4-azaribose in their respective open (aldehyde) and closed ring forms](image)
4-amino-4-deoxyribofuranoside (4-epi-nectricine)

Nectricine (Figure 21) is a fungal metabolite isolated from *Nectria lucida*. It exists solely as an imine sugar while its ribose epimer 4-epi-nectricine exist as a complex equilibrium mixture consisting of dimer, imine and hemiaminal forms. These isomers transform interchangeably depending on the pH and concentration of the aqueous solution (Scheme 6). At pH 7-7.5, in 1M solution, it exists as a mixture of 4-aza-4-deoxyribopyranoside 121a and its dimeric form 121d in equal ratio. At pH 9-11, dimer 121d was observed as a single entity. While at more basic pH (13-14) dimer 121d and dehydrated hemiaminal 121c (imine) forms coexist in equilibrium. Interestingly when the 1 M solution was diluted by 20 times at pH 7-7.5, hemiaminal 121b appeared along with imine 121c and pyranoside 121a.

\[ \text{H}_{3}N^{+} \text{CH} \rightarrow \text{OH} \]
\[ \text{HO} \]
\[ \text{4-amino-4-deoxyribofuranoside} \quad 121a \]

\[ \text{HO} \]
\[ \text{N} \]
\[ \text{4-aza-ribose (hemiaminal)} \quad 121b \]

\[ \text{HO} \]
\[ \text{N} \]
\[ \text{epi-nectricine} \quad (\text{dehydrated hemiaminal}) \quad 121c \]

\[ \text{HO} \]
\[ \text{N} \]
\[ \text{dimer} \quad 121d \]

Scheme 6. 4-Amino-4-deoxyribofuranoside in its equilibrium forms at variable pH.

Nojiricemycin

The naturally occurring hemiaminal sugar nojiricemycin is a highly potent glycosidase inhibitor relative to its 1-deoxy analog (Figure 21 and 24). It is weakly basic with pKₐ value of 5.3 in water. As it exists purely in its closed ring form (hemiaminal), it does not react with...
ninhydrin reagent. The chemical shifts and J values of anomeric proton signals of nojirimycin in D$_2$O are 5.3 ($J = 2.5$) for $\alpha$-anomer and 5.79 ($J = 10.0$) for $\beta$-anomer. Because of the complex $^1$H NMR, nojirimycin is characterized by converting it into its 1-deoxy analog using catalytic reduction.

**gem-Diamine 1-N-imino sugars**

Siastatin B (gem-diamine, 1-N-imino sugar) (122a, Scheme 7) is a glycosidase inhibitor in which anomeric hydroxyl is replaced by nitrogen.\textsuperscript{157} The protonated form of siastatin B 122a was proposed to mimic the hexopyranosyl cation formed at the TS. As a result of the potent inhibitory activity of siastatin B, trifluoromethyl derivative of siastatin B, 122b was synthesized and tested for enzymatic inhibition at various pH.\textsuperscript{158} A time-dependent alteration of 122b was observed at pH 5. From the spectral studies, the hemiaminal 122c, imine 122d, enolate 122e and ketone 122f formation was revealed (Scheme 7). The similar equilibrium mixture was also observed previously in case of hemiaminal 4-epi-necticine.

![Scheme 7](image.png)

**Scheme 7.** 1-N-imino sugar of galacturonic acid 122b in its equilibrium forms at pH 5.0.\textsuperscript{157}
2. RESEARCH OBJECTIVE

The overall aim of this study is the design, synthesis and biological evaluation of (a) novel 4-aza-S-ribosyl-L-homocysteine (4-aza-SRH) analogs and (b) 2-deoxy-2-substituted-S-ribosyl-L-homocysteine analogs as potential inhibitors of LuxS enzyme which is involved in interspecies QS. Also targeted are γ-lactam and their reduced cyclic hemiaminal analogs, bearing the additional alkylthiomethyl substituent as AHL mediated intraspecies QS antagonists in *P. aeruginosa* and *V. harveyi*.

QS is a population dependent bacterial communication phenomenon utilized by number of pathogenic bacteria to direct symbiosis, virulence and biofilm formation. The recent increase in prevalence of bacterial strains resistant to antibiotics emphasizes the need for the development of new generation of antibacterial agents. Interruption of QS, as a strategy to combat pathogenesis is a viable option as it does not affect bacterial growth but only virulence, leading to less incidence of microbial resistance. The LuxS is a bacterial enzyme present in more than 60 species and responsible for biosynthesis of AI-2, a sensor molecule utilized for intraspecies bacterial communication. As a result, inhibitors of LuxS enzyme should inhibit AI-2 synthesis and consequently AI-2 mediated QS, giving rise to a new generation of antibacterial agents. Intraspecies communication in Gram negative bacteria is mediated largely by AHLs using “R” type sensor proteins. Human opportunistic Gram negative pathogen, *Pseudomonas aeruginosa* regulates virulence factor production, biofilm formation via *las* and *rhl* mediated QS. Thus, inhibition of these QS systems can result in attenuation of virulence. For these reasons, inhibition of AHL sensors protein, LasR, can serve as attractive target for the design of antimicrobial therapy.

Initially, I have taken effort to synthesize 4-aza-S-ribosyl-L-homocysteine (SRH) analogs with sugar ring oxygen replaced by nitrogen atom (e.g., 143). The nitrogen atom in 4-aza-SRH compound should have the ability to mimic the interactions created by oxygen atom present in
natural substrate with the LuxS enzyme. The 4-aza-SRH will allow to study of the effect of \( N,O \)-acetal function vs \( O,O \)-acetal present in the natural substrate. Since the hemiaminal sugar analogs are weak bases at physiological pH, they exist predominantly in cyclic hemiaminal form. I envisaged that upon enzyme mediated protonation of anomeric hydroxyl group and dehydration, the resulting imine derivatives might act as suicide inhibitors by reacting with proximal nucleophiles in the protein. The 4-aza-SRH may also undergo LuxS mediated catalysis and release aza analogs of 4,5-dihydroxy-2,3-pentanedione (DPD), a precursor to the novel aza-AI-2 analog.

In order to interfere with the enzyme mediated sugar ring opening, 4-aza-SRH is further modified at the anomeric carbon to afford analogs bearing pyrrolidine (e.g., 129), lactam (e.g., 141), endocyclic imine (e.g., 158), and cyclic nitrone (e.g., 153) functionality (Figure 24). I rationalized that 1-deoxy-4-aza-SRH 129, could inhibit the enzyme mediated ring opening because of the lack of hydroxyl group at the anomeric carbon and hence can act as a competitive inhibitor. In the case of the lactam analog of SRH 141 in which C1-hydroxyl is replaced by carbonyl group, I rationalized that its interaction with LuxS might also result in competitive inhibition because of the increase in stability of amide bond towards the hydrolysis. Moreover since carbonyl group has the ability to act as good hydrogen bond acceptor and can tautomerize into the enolic form, new insights into LuxS binding should be unraveled. In order to attest the hypothesis of the covalent adduct formation between 4-aza-SRH derived imine intermediates and the enzyme, an imine analog of 4-aza-SRH bearing methyl group at C1 (158) is also targeted. Since the nitrone group is a better electrophile than imine, the proposed covalent adduct formation is also investigated using the nitrone analog of SRH (153).
In my second approach towards design of LuxS inhibitors, I aimed at designing and synthesizing SRH analogs modified at C2. Such analogs might prevent the first aldo-keto isomerization and hence inhibit formation of 2-keto intermediate. In this series, I plan to target 2-deoxy-SRH, 2-deoxy-2-fluoro-S-arabinosyl-L-homocysteine ([2-F]SAH) and S-arabinosyl-L-homocysteine (SAH) (Figure 25). The [2-F]SAH might also undergo nucleophilic attack at C2 by the active site Cys-84 residue, to form a covalent adduct with the enzyme, leading to irreversible inhibition. In case of SAH, because of the reversal of stereochemistry at C2-hydroxyl, proton abstraction from C2 by Csy84 might be forbidden since proton transfers were proved to occur stereochemically. Moreover, such mechanism based inhibitors might also provide information on structural features necessary for inhibition and further explain the role of active site residues in catalysis.
The next goal of my research was to develop modulators of intraspecies QS regulated by AHLs. Though several AHL mimics were discovered to be potent QS inhibitors, the utility of such compounds to act as drug targets is limited because of the instability of lactone ring. For this reason, the development of non-native AHL ligands has emerged as a promising new strategy to regulate QS in Gram-negative bacteria. Only a few examples of inhibitors with the altered lactone ring structure of AHL have been reported. Though the effect of caprolactam analogs were explored before, detailed studies on \( \gamma \)-lactams were not conducted. In order to study effect of nonnative \( \gamma \)-lactam ring on QS and also to find common structural requirements for inhibition of LuxS and AHL-mediated QS, I undertook an effort to synthesize \( \gamma \)-lactam and their reduced cyclic hemiaminal analogs, bearing the additional alkylthiomethyl substituent (from \( C_3-C_{12} \)) (e.g., 160). I envisioned that lactam ring being an isoster of lactone ring, will be more stable to basic environments when compared to AHLs. Thus, these analogs are expected to act as potential QS inhibitors in Gram negative bacteria.
3. RESULTS AND DISCUSSION

3.1. Design and synthesis of 4-aza-S-ribosyl-L-homocystiene analogs

3.1.1. Pyrrolidine analog

The first targeted azasugar analog of SRH was pyrrolidine analog 129 lacking the hydroxyl group at C1 (Scheme 8). Since the analog 129 could be protonated but could not undergo ring opening, I predicted that the initial step of the LuxS-catalyzed reaction could be precluded. The protected 1,4-dideoxy-1,4-imino-D-ribitol 123 served as a convenient starting material which was prepared from commercially available D-gulonic-\(\gamma\)-lactone.\(^{159}\) However, attempted mesylation of the N-benzyl protected 123 instead of providing 5-\(O\)-mesyl precursor for coupling with homocysteine resulted in the formation of piperidine byproduct 130 as a mixture of two diastereomers at C5 (~3:1). Analogous rearrangements of pyrrolidines into piperidines through ring enlargement via aziridine type intermediates are known.\(^{160,161}\) We found that either replacement of the benzyl protection group with the Boc group at ring nitrogen or oxidation of the iminoribitol to ribonolactam (e.g., 131, \textit{vide infra}) reduced basicity of nitrogen enough to prevent ring enlargement providing stable 5-\(O\)-mesyl derivatives. Thus, silylation of 123 with \textit{tert}-butyl-dimethylsilyl chloride (TBDMSCl) afforded 124 and catalytic hydrogenolysis (5% Pd/C) of 124 in the presence of (Boc)\(_2\)O affected removal of benzyl group and protection with Boc in a one-pot reaction\(^{161,162}\) yielding 125 (99% from 123). Desilylation of 125 produced 126 (70%), which upon mesylation gave stable 127 (96%). Nucleophilic displacement of mesylate with thiolate generated from protected L-homocystine\(^{54}\) with tris(2-carboxyethyl)phosphine hydrochloride afforded 128 (86%). Using water soluble tris(2-carboxyethyl)phosphine for the generation of homocysteine reagent not only improved the yields but also simplified purification as compared to the previously described analogous substitution which employed tributyl\(^{54}\) and triethylphosphines\(^{59}\) for the reduction of homocystine. Treatment of 128 with TFA effected simultaneous removal of the \(N\)-Boc, acetonide and \textit{t}-butyl ester protection groups to give 4-azaSRH analog 129 (66%).
Reagents and conditions (a) TBDMSCl/imidazole/DMAP/CH2Cl2/rt; (b) H2/Pd-C/(Boc)2O/Et3N/EtOH/rt; (c) MsCl/Et3N/CH2Cl2/rt; (d) TBAF/THF/rt; (e) BocNHCH(CH2CH2SH)CO2t-Bu/LDA/DMF/rt; (f) (i) TFA/0 °C (ii) TFA/H2O/0 °C.

Scheme 8. Synthesis of pyrrolidine analog of 4-aza-SRH

3.1.2. Lactam analog

Next, lactam-type SRH analog 141 with the amide oxygen at C1 was targeted. As the flat planar carbonyl group in amides might mimic the keto intermediate and also fail to undergo ring opening, lactam 141 can act as competitive inhibitor. I also speculated that the lactim tautomer can mimic the anomeric hydroxyl resulting in binding affinity comparable to the natural substrate. Thus, regioselective oxidation of the 5-O-TBDMS-azasugar 124 (hydroxypyrrolidine) at C1 with RuO2/NaIO4 under EtOAc/H2O biphasic conditions produced lactam 131 (65%). Minor quantity (18%) of the corresponding N-benzoylpyrrolidinone byproduct was also formed because of the additional oxidation of the benzylic protons (Scheme 9). Desilylation of 131 with TBAF produced 132 (97%). Treatment with MsCl and replacement of 5-mesylate group from 135 with protected Hcy gave 137 (70%). Treatment of 137 with TFA effected removal of all acid-labile
protection groups to yield 140 (48%). Attempts to remove N-benzyl protection [e.g., H2/Pd-C or Pd(OH)2-C, Na/NH3(liq), BCl3] from 137 were unsuccessful.

Reagents and conditions (a) NaIO4/Hydrated RuO2/EtoAc/H2O/rt; (b) TBAF/THF/rt; (c) MsCl/Et3N/CH2Cl2/rt; (d) BocNHCH(CH2CH2SH)CO2t-Bu/LDA/DMF/rt; (e) (i) TFA/0 °C (ii) TFA/H2O/0 °C; (f) (Boc)2O/Et3N/CH2Cl2/rt (g) LiEt3BH/THF/-78 °C.

Scheme 9. Synthesis of lactam and hemiaminal analogs of 4-aza-SRH

To overcome this setback the synthetic approach was undertaken with acid-labile Boc protection group at nitrogen. Thus, RuO2-catalyzed oxidation of 125 gave 133 (60%) as a sole product and subsequent desilylation with TBAF afforded 134 (77%). Although mesylation of 134 to provide
was unsuccessful we were able to prepare 136 by oxidation of 127 with RuO₂/NaIO₄. Coupling of 136 with thiolate generated from L-Hcy resulted in 138 with loss of Boc group at sugar nitrogen. Deprotection with TFA followed by TFA/H₂O yielded lactam 141 (58%).

3.1.3. Hemiaminal analog

The hemiaminal 143 (Scheme 9) which possess ring nitrogen in place of oxygen in SRH, was synthesized to explore its effect on LuxS binding, LuxS catalyzed ring opening and degradation. Thus, direct reduction of the unprotected amide 138 (or 141) with super hydride¹⁶⁴ failed to yield 143. However, treatment of 138 with (Boc)₂O/DMAP gave fully protected lactam 139 which upon treatment with LiBEt₃H produced hemiaminal 142. Deprotection with TFA and TFA/H₂O gave desired 4-azaSRH 143 as a mixture of complex mixture of several isomers. Interestingly, analogous N,O-acetals were reported to be stable under conditions required for coupling with nucleoside bases.¹⁶³

3.1.3.1. Benzyloxime derivative of hemiaminal sugar

In order to support our hypothesis that the hemiaminal analog 143 could be converted to the open aldamine form during the first step of LuxS catalysis, limited model studies were performed with hemiaminal 4-amino-4-deoxy-α/β-D-ribofuranose 146a¹⁵⁶ (Scheme 10). Thus, previously described N-Boc protected lactam 133 was reduced with LiBEt₃H to afford protected hemiaminal 144. Desilylation with TBAF yielded 145, which was treated with TFA to give deprotected hemiaminal 146a as a mixture of α/β anomers. Subsequent treatment of 146a with O-benzylhydroxylamine gave desired oxime derivative 147 as a single product. The formation of oxime 147 suggested that though hemiaminal 146a is the major isomer at neutral pH, equilibrium could be shifted towards open aldehyde form 146b by derivatization. Such results clearly supported the plausible conversion of hemiaminal analog 143 to its open aldehyde form.
Reagents and conditions (a) LiEt₃BH/THF, THF/-78 °C; (b) TBAF/THF/rt; (c) (i) TFA/0 °C (ii) TFA/H₂O/0 °C; (d) Bn-OH/Pyridine/rt.

Scheme 10. Benzyl oxime derivative of 4-azaribose

3.1.4. Nitrore analog

A nitrore analog of SRH was also targeted. As nitrores are more electrophilic than imines such analog might act as a suicide inhibitor by forming a covalent adduct with proximal nucleophilic groups in the protein. Thus, treatment of the 1,4-iminoribitol 148 with SeO₂/H₂O₂ gave nitrore 149\textsuperscript{165} (73%) as a white crystalline solid (Scheme 11). Desilylation and subsequent mesylation yielded 151 (56%). Treatment of the mesylated sugar 151 with protected Hcy afforded a nitrore-type SRH analog 152 (43%). Deprotection of 152 with TFA produced unstable homocysteine derivative 153 (40%).

3.1.5. Imine analog

To explore possibility of the LuxS-mediated addition of water across carbon-nitrogen double bond an imine-type analog of SRH 158 (Scheme 12) was targeted. It is noteworthy that addition of water to imine 158 might result in the \textit{in situ} generation of the hemiaminal of type 143. Thus,
the precursor, 1-methyl iminocyclitol 154 was prepared by the Moriarty rearrangement\textsuperscript{166} of L-lyxo sugar to give the D-riboazasugar.

\begin{align*}
\text{RO} & \quad \text{b} \quad \text{RO} \\
\text{N} & \quad \text{O} \\
\text{R} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\underline{124} & \quad \text{R'} = \text{TBDMS, R = Bn} \\
\underline{148} & \quad \text{R'} = \text{TBDMS, R = H (80\%)} \\
\underline{149} & \quad \text{R' = TBDMS (73\%)} \\
\underline{150} & \quad \text{R' = H (42\%)} \\
\underline{151} & \quad \text{R' = TBDMS (56\%)} \\
\underline{152} & \quad \text{R' = TFA/-4\ºC; (ii) TFA/H_2O/-4 \ºC}. \\
\end{align*}

Reagents and conditions (a) H_2/Pd-C/EtOH/rt; (b) SeO_2/H_2O_2/Me_2CO/-4 \ºC; (c) TBAF/THF/-4 \ºC; (d) MsCl/Et_3N/CH_2Cl_2/-4 \ºC; (e) BocNHCH(CH_2CH_2SH)CO_2t-Bu/LDA/DMF/-20 \ºC; (f) (i) TFA/-4\ºC; (ii) TFA/H_2O/-4 \ºC.

Scheme 11. Synthesis of nitrone analog of 4-aza-SRH

The imine 154\textsuperscript{166} was converted into 5-O-mesyl derivative 155 (85\%), which was coupled with protected L-Hcy to give 156 (Scheme 12, 85\%). Treatment of 156 with TFA gave isopropylidene protected 157. The protons at the C1-methyl group were found to be exchangeable with deuterium within a few hours when compound 157 was dissolved in D_2O. Treatment of 157 with aqueous TFA (9:1) yielded fully deprotected 158 in quantitative yield. As in the case of 157, protons at C1-methyl group of 158 were exchangeable with deuterium. These exchange indicate that 1,4-ketimine-SRH analog 158 might undergo enzyme-catalyzed hydrolysis to generate a 4-aza-SRH analog with a methyl ketone rather than an aldehyde at C1. This change might affect the regioselectivity and rate of the first isomerization step in the LuxS-catalyzed reaction. I also
showed that the methyl group protons in 154 are not susceptible to exchange even if 154 was dissolved in D$_2$O for several hours.

![Chemical structure](image)

Reagents: (a) MsCl/Et$_3$N/CH$_2$Cl$_2$; (b) BocNHCH(CH$_2$CH$_2$SH)CO$_2$t-Bu/LDA/DMF; (c) TFA; (d) TFA/H$_2$O

**Scheme 12.** Synthesis of imine analog of 4-aza-SRH

### 3.1.6. γ-lactams with alkylthiomethyl substitution at carbon γ and their N,O-acetal counterparts

As part of the second objective of my dissertation, I have designed and synthesized γ-lactams and cyclic azahemiacetals, bearing alkylthiomethyl substituent with different length of carbon chain (C$_3$-C$_{12}$) as AHL mediated QS inhibitors. Although, previously ε-lactam (caprolactam) analogs were reported to lack LuxR binding$^7$, studies on γ-lactams were not performed. In order to further investigate the effects of non-native lactam scaffold on QS, novel γ-lactam ligands were designed. As lactam rings are more stable to basic conditions than lactones, these nonnative lactam analogs might prove to be potent inhibitors. Moreover, the γ-lactam and cyclic azahemiacetal ligands were further modified in a way that they resemble SRH.

Initially, 5-$S$-alkylthio derivatives of lactam 160 differing in alkyl chain length were prepared (Scheme 13). The key substrate (S)-5-(bromomethyl)-2-pyrrolidone (159) was conveniently derived from L-pyroglutamic acid$^{167}$ (Scheme 13). Displacement of bromide in 159 with sodium...
propanethiolate produced γ-lactam with propylthiomethyl substitution at carbon γ (160a, 96%).

As it was demonstrated previously that the length of side chain is also critical for determining the agonistic and antagonistic activity,168 I also synthesized analogs containing various chain lengths. Higher homologated analogs 160b-d with C6, C9 and C12 alkyl straight chains were prepared analogously to 160a. A set of reduced form of lactam analogs which contain a hydroxyl group instead of a keto group at C2 were also prepared. Attempted reduction of lactams 160 with LiBEt₃H were unsuccessful, however reduction of the N-Boc protected lactams 161a-d proceeded smoothly to afford hemiaminals (N,O-acetals) 162a-d. Subsequent deprotection with trifluoroacetic acid gave N,O-acetal counterparts of γ-lactams (163a-d) as a mixture of at least four isomers along with the open chain aldehyde form (25%; ¹H NMR). The existence of hemiaminal moiety is proved by treatment of this mixture with O-benzylhydroxylamine which produced the desired oxime derivative.

![Scheme 13](image)

Reagents: (a) R'SH/NaH/DMF; (b) (Boc)₂O/DMAP/CH₂Cl₂; (c) LiEt₃BH/THF/CH₂Cl₂; (d) TFA

**Scheme 13.** Synthesis of γ-lactams and the corresponding hemiaminal analogs with thioalkyl side chain

**3.1.7.** (5S)-(hexyl- or nonylthiomethyl)-3,4-dihydroxypropyrrolidin-2-ones and their hemiaminal congeners

To increase the polarity/solubility of the lactam and azahemiacetal analogs in the testing media, aza analogs with hydroxyl groups at C3 and C4 were also prepared. The N-Boc protected 1,4-dideoxy-1,4-imino-D-ribitol 126, readily prepared from the commercially available D-gulonic-γ-lactone, served as a convenient starting material for the synthesis of dihydroxy lactams
Mesylation of compound 126 with MsCl and triethylamine gave 127. Coupling of the mesylate 127 with sodium hexane- and nonanethiolate produced 5-S-alkyl thioethers 164 and 165 in high yields which were deprotected with TFA to give (5S)-(hexyl- or nonylthiomethyl)-3,4-dihydroxypyrrolidin-2-ones 166 and 167 (Scheme 14). Reduction of 164 and 165 with LiBEt₃H afforded hemiaminals 168 and 169 and subsequent deprotection with TFA afforded 5-S-(hexyl or nonyl)-3,4-dihydroxypyrrolidin-2-ols 170 and 171 as a complex mixture of azahemiacetals existing in equilibrium with dehydrated form (imine) as well as with open aldehyde and dimeric forms, as reported for such class of 4-azaribofuranoses.156

3.2. Design and synthesis of S-ribosyl-L-homocysteine and its C2 modified analogs

3.2.1. S-Ribosyl-L-homocysteine

The SRH, required for our enzymatic study, was synthesized according to the methods described previously with few modifications that made the synthesis more efficient and bench friendly.170 First of all, I have used ribose sugar derivative (required for coupling to homocysteine) with bromide as a leaving group instead of the activated hydroxyl. Secondly homocysteine thiolate was generated by using our previously published method109 employing triscarboxyethylphosphine hydrochloride (TCEP) as water soluble reducing agent of disulfide bond rather than in situ generated homocysteine thiolate by Mitsunobu reaction between sugar and homocysteine.170 Thus, bromo ribose 172171 upon treatment with protected Hcy afforded 173 in 72% yield (Scheme 15). Subsequent standard deprotection yielded natural substrate SRH 174 in 50% yield.
Reagents: (a)MsCl/NEt3/CH2Cl2/rt; (b) NaIO4/hydrated RuO2/EtOAc/H2O/rt; (C)RSH/NaH/DMF; (d) TFA/H2O; (e) LiEt3BH/THF/CH2Cl2

Scheme 14. Synthesis of ribolactams and their hemiaminal congeners

Reagents and conditions (a) BocNHCH(CH2CH2SH)CO2t-Bu/LDA/DMF/rt; (b) (i) TFA/0 °C (ii) TFA/H2O/0 °C.

Scheme 15. Synthesis of S-ribosyl-L-homocysteine
3.2.2. 2-Deoxy S-ribosyl-L-homocysteine

The 2-deoxySRH 180 (Scheme 16), because of the lack of hydroxylic group at the second position, should not undergo the first aldose-ketose isomerization step and hence block 2-keto intermediate formation and DPD release. Thus, the 2-deoxyribose was converted to methyl 2-deoxy-α/β-D-erythro-pentofuranoside, 175 with methanol and H₂SO₄.¹⁷² Treatment of 175 with MsCl and NEt₃ gave compound 176 (43%) which upon protection with the benzoyl group at C₃ afforded 177 (95%) (Scheme 16). Coupling of mesylated derivative 177 with protected Hcy gave 2-deoxySRH derivative 178. However, attempted removal of of Boc protection under acidic conditions failed to produce the desired 2-deoxySRH. Nonetheless, the methyl 2-deoxySRH analog, 179 (37%) was obtained by coupling of the mesylated sugar 175 with racemic D/L-homocysteine in aqueous NaOH.¹⁰⁹

Reagents and conditions (a) MsCl/Et₃N/CH₂Cl₂/ 0 °C; (b) BzCl/2,6-lutidine/DMAP/CH₂Cl₂/ 35 °C; (c)BocNHCH(CH₂CH₂SH)CO₂t-Bu/LDA/DMF/rt; (d) NH₂CH(CH₂CH₂SH)CO₂H/NaOH/H₂O/65 °C (e) TFA/H₂O/0 °C or AcOH, HCl or TMSOTf

Scheme 16. Synthesis of 2-deoxy-S-ribosyl-L-homocysteine
3.2.3. S-Arabinosyl-L-homocysteine

I next explored synthesis of S-arabinosyl-L-homocysteine (SAH) 187 (Scheme 17). As it was found earlier that the xylo analog 73,109 with the inversion of stereochemistry at C3 was the most potent of C3 modified sugars (as the proton shuttle is stereospecific), it will be interesting to explore the effect of inversion of stereochemistry at C2 (SAH). Thus, selective mesylation of acetonide protected 181173 gave 182 (33%). Subsequent benzylation of the free hydroxyl at C3 afforded 183 in 63% yield. However, coupling of the mesylated sugar 183 with protected homocysteine was unsuccessful. In another approach, the mesylated sugar 183 was converted to bromo compound 184 (47%) by treatment with NaBr/DMF171 (Scheme 17). Subsequently, bromo sugar 184 was coupled to the thiolate of Hcy to afford protected SAH intermediate 185 in a very low yield. However, such reaction was not reproducible and the targeted SAH 187 was not obtained in desired purity to perform enzymatic study.

3.2.4. 2-Fluoro-2-deoxy-S-arabinosyl-L-homocysteine

As it is well known that introduction of fluorine atom brings change in physical, chemical and biological properties of a molecule, I next focused on synthesis of [2-F]SAH 194 (Scheme 18). Such a compound can potentially act as competitive inhibitor as it could bind to LuxS but because of the lack of hydroxyl group at C2, should not undergo carbonyl shift from C1 to C2 to form 2-keto intermediate. Alternatively [2-F]SAH might undergo nucleophilic attack by Cys-84 forming a covalent adduct between inhibitor and protein, resulting in irreversible inhibition of the enzyme. Thus, benzylation of the triisopropylsilyl (TIPS) protected 2-deoxy-2-fluoroarabinose 188174 at anomeric hydroxyl, produced 189 (77%).
Reagents and conditions (a) MsCl/Et$_3$N/CH$_2$Cl$_2$/ 0°C; (b) BzCl/2,6-lutidine/DMAP/CH$_2$Cl$_2$/35 °C; (c) NaBr/ DMF/CH$_2$Cl$_2$/120 °C; (d) BocNHCH(CH$_2$CH$_2$SH)CO$_2$t-Bu/LDA/DMF/rt; (e) TFA/H$_2$O/0 °C; (f) NH$_3$/MeOH

**Scheme 17.** Synthesis of S-arabinosyl-L-homocysteine (SAH)

Desilylation of 189 with NH$_4$F gave the corresponding 2-deoxy-2-fluoroarabinose 190 (37%) which upon mesylation afforded 191 (65%) (Scheme 18). However, attempted coupling of mesylated sugar 191, under various conditions failed to produce desired protected 2-fluoro analog of SRH 192. In another approach, selective acid mediated mono desilylation of 189 with TFA/H$_2$O and subsequent mesylation afforded 5-O-mesyl-3-O-TIPS protected mesyl sugar. Unfortunately, attempts to couple this sugar with Hcy were unsuccessful. Therefore, desired [2-F]SAH 194 was not obtained.
Reagents and conditions (a) BzCl/2,6-lutidine/DMAP/CH$_2$Cl$_2$/ 35 °C; (b) NH$_4$F/MeOH/60 °C; (c) MsCl/Et$_3$N/CH$_2$Cl$_2$/0 °C; (d)BocNHCH(CH$_2$CH$_2$SH)CO$_2$-Bu/LDA/DMF/rt; (e) TFA/H$_2$O/0 °C; (f) NH$_3$/MeOH.

Scheme 18. Synthesis of 2-deoxy-2-fluoro-S-arabinosyl-L-homocysteine

3.3. Biological evaluation of targeted quorum sensing modulators

3.3.1. Quorum sensing assays of γ-lactams with alkylthiomethyl substitution at carbon γ and their N,O-acetal counterparts

3.3.1.1. Screening against las and rhl signaling in P. aeruginosa

To determine the effect of the γ-lactam (160, 166 and 177) (Figure 26) and their corresponding hemiaminal analogs (163, 170 and 171) (Figure 26) on the P. aeruginosa las and rhl AHL-mediated pathways, las- and rhl-dependent β-galactosidase reporters were independently expressed with their respective receptor proteins in E. coli as previously described by Dr. Kalai Mathee’s research group at FIU.$^{15}$ As expected and in agreement with published data, exogenous 3-oxo-C$_{12}$-AHL and C$_4$-AHL activated the las and rhl β-galactosidase reporters, respectively. The AHL treated controls (3-oxo-C$_{12}$-AHL in case of las and C$_4$-AHL in case of rhl)
in absence of inhibitor are considered as 100% active (Figure 26). Also, no activity was observed in absence of exogenous AHL. The effect of the synthesized γ-lactam and their corresponding hemiaminal analogs on the las and rhl reporter activity was also compared with the effect of addition of the DMSO as a solvent (Figure 26).

**Screening against las signaling**

Compounds 160a, 163a were initially screened at a concentration of 100 µg/mL (0.57 mM) for their activity against the las system (Figure 26). Hemiaminal 163a was found to enhance las reporter activity by 15% while lactam 160a inhibited las activity approximately by 28%. Inhibition was dependent upon the addition of 2 µM of 3-oxo-C12-AHL. However, hemiaminal 163a significantly stimulated (approximately 2.3-fold) las reporter activity at 50 µg/mL and inhibited las reporter activity by 69% and 89% at 150 and 200 µg/mL, respectively. In contrast, lactam 160a inhibited las activity at all concentrations tested. Cell growth was not inhibited by the addition of lactam and hemiaminal compounds at the tested concentrations.

Among other lactam analogs tested, percent inhibition increased in a concentration dependent manner. Inhibition potency also increased as the alkylthio chain length increased. Specifically, nonylthio lactam 160c and dodecylthio lactam 160d were found to possess greatest inhibition at all concentrations tested. At the lowest concentration tested (50 µg/mL), 160c inhibited 28% while 160d inhibited 48%. On the contrary, among the cyclic hemiaminals analogs, no general trend was observed between chain length and percent inhibition. As with 163a, hemiaminal 163b also stimulated QS at 50 µg/mL but with much lesser potency, however inhibited 100% at all higher concentrations tested. Hemiaminals containing nonyl side chain (163c) and dodecyl chain (163d) showed moderate activity. The ribolactam analogs (166 and 167) and their cyclic hemiaminal counterparts (170 and 171) were found to inhibit las activity at all concentrations tested but only with moderate potency with hemiaminals being slightly more active.
Figure 26. Effect of lactam and hemiaminal analogs on $P_{lacI}$-lacZ expression in *Escherichia coli*. The compounds were tested at 50 -200 µg/mL concentrations against 2 µM of 3-oxo-C12-AHL. $P_{lacI}$-lacZ activity is depicted as percent activity relative to the DMSO treated control in presence of AHL. Significance was determined by a paired two-tailed Student $t$-test and is denoted as follows: *$p$-value < 0.05, † $p$-value < 0.02, ‡ $p$-value < 0.01. The $p$ value < 0.05 correspond to 5% ($< 0.02$ corresponds to 2% and, $< 0.01$ corresponds to 1%) chance of rejecting the null hypothesis when it is true. Rejection of null hypothesis infers that the result is statistically significant. Each trial was performed as a triplicate.
Screening against rhl signaling

The hexylthio lactam 160b and nonylthio lactam 160c stimulated rhl QS activities at higher concentrations with moderate potency with hexylthio lactam 160b being most active (Figure 27; Table 2). Propylthio lactam 160a and dodecylthio lactam 160d were inactive at the concentrations tested. In contrast, cyclic hemiaminals with shorter alkylthio chain (163a and 163b) inhibited rhl activity, while analogs with longer alkyl chain (163c and 163d) were inactive. The hexylthio hemiaminal 163b completely inhibited rhl signaling at concentrations of 100 µg/mL and higher. The strong inhibition observed with propylthio 163a and hexylthio 163b hemiaminal analogs having the side chain lengths similar to C4-AHL is in agreement with the structure activity relationship reported for various synthetic AHL mimetics targeting RhlR.75,175 Except for ribolactam 166 which stimulated rhl signaling, all the ribolactam analogs (167) and their cyclic hemiaminal counterparts (170/171) were inactive in rhl signaling system (Figure 27; Table 2)

Thus, in this dissertation novel lactam and the corresponding hemiaminal analogs as las and rhl signaling modulators were reported. Studies of structural features other than the AHL scaffold as tools to understand the R type protein interaction with AHLs are limited, only a few examples of inhibitors with the altered lactone ring structure of AHL have been reported (for more discussion on AHL mimics with altered lactone ring, refer to section 1.2.1.3.). For example, Smith et al. reported 3-oxo-C12-(2-aminocyclohexanone) (Figure 8, 39) as a strong antagonist of LasR system,87 while Muh et al. identified two LasR inhibitors having a phenyl and tetrazole ring (Figure 11, 49 and 50, respectively), with IC50 in nM range (Figure 11).96 It is noteworthy that γ-thiolactone analogue of 7b (Figure 9) showed inhibition of LuxR while the corresponding ε-lactam (caprolactam) analog was reported to lack LuxR binding.78 The targeted optically pure γ-lactams and cyclic hemiaminals were capable of either inhibiting or, in some cases, inducing P. aeruginosa QS pathways. Though, such analogues had moderate antagonism (dodecylthio lactam 160d had 48% inhibition at 0.16 mM and hexylthio hemiaminal 163b had 100% inhibition at 0.38
mM) when compared to previously reported inhibitors (with IC$_{50}$ values in submicromolar), the novel lactam scaffold might serve as stable alternative to lactone present in AHL and further facilitate future generation of AHL mimics.

**Figure 27.** Effect of lactam and cyclic hemiaminal analogs on P$_{rhlA}$-lacZ expression in *E. coli*. The compounds were tested at 50-200 µg/mL concentrations against 2 µM of C$_4$-AHL. P$_{rhlA}$-lacZ activity is depicted as percent activity relative to the DMSO treated control in presence of AHL. Significance was determined by a paired two-tailed Student $t$-test and is denoted as follows: *p*-value < 0.05, †p*-value < 0.02, ‡p*-value < 0.01. The p*-value < 0.05 corresponds to 5%, (< 0.02 corresponds to 2% and < 0.01 corresponds to 1%) chance of rejecting the null hypothesis when it is true. Rejection of null hypothesis infers that the result is statistically significant.
Table 2. Effect of lactam and cyclic hemiaminal analogs on rhl signaling

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No inhibitor</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>No AHL</td>
<td>0.29</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. viminalis</em></td>
<td>33± 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>160a</td>
<td>104± 24</td>
<td>118± 0.8</td>
<td>104± 13</td>
<td>108± 17</td>
</tr>
<tr>
<td>160b</td>
<td>126± 8</td>
<td>126± 29</td>
<td>130± 31</td>
<td>145± 19†</td>
</tr>
<tr>
<td>160c</td>
<td>95± 3</td>
<td>115± 30</td>
<td>108± 12</td>
<td>137± 46</td>
</tr>
<tr>
<td>160d</td>
<td>103± 7</td>
<td>106± 6</td>
<td>109± 8</td>
<td>106± 5</td>
</tr>
<tr>
<td>163a</td>
<td>121± 6</td>
<td>138± 3</td>
<td>41± 1†</td>
<td>9± 1†</td>
</tr>
<tr>
<td>163b</td>
<td>26± 0.2</td>
<td>0.2± 0.3†</td>
<td>0± 0†</td>
<td>0± 0†</td>
</tr>
<tr>
<td>163c</td>
<td>93± 12</td>
<td>80± 13</td>
<td>100± 11</td>
<td>99± 3</td>
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</tr>
<tr>
<td>166</td>
<td>132± 36</td>
<td>129± 10</td>
<td>148± 9</td>
<td>161± 7†</td>
</tr>
<tr>
<td>167</td>
<td>106± 3</td>
<td>117± 8‡</td>
<td>115± 11</td>
<td>135± 5‡</td>
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<td>109± 12</td>
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</tr>
<tr>
<td>171</td>
<td>98± 5</td>
<td>127± 14†</td>
<td>116± 9</td>
<td>121± 14</td>
</tr>
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</table>

* Effect of lactam and cyclic hemiaminal derivatives on P_{rhlA-lacZ} expression in E. coli tested at 50-200 µg/mL concentrations against 2 µM of C4-AHL. P_{rhlA-lacZ} activity is depicted as percent activity relative to the DMSO treated control in presence of AHL. †p-value < 0.05, ‡p-value < 0.02, §p-value < 0.01 according to a paired, two-tailed Student t-test. * Activity in absence of AHL was considered negligible. † 20 µL water extract of *Callistemon viminalis* was shown to have anti-QS activity as expected and reported previously. 191

3.3.1.2. Screening against bioluminescence in *V. harveyi*

The lactam (**160, **166 and **167**) and hemiaminal derivatives (**163, 170** and **171**) were also tested for their effect on QS mediated luminescence of wild type *V. harveyi* MAV. None of the analogs had any effect on growth (Table 3). In case of luminescence assay, γ-lactams and their hemiaminal counterparts displayed two patterns showing (i) inhibition of luminescence as found for lactam compounds **160b, 166** and **167** and hemiaminals **163b, 163c and 170**, and (ii) no effect at the concentrations tested, as observed with propylthio γ-lactam **160a** as well as with **160d, 163c, 163d** and nonylthio ribohemiaminal **171** (Table 3). In order to identify the pathway
of inhibition of luminescence (as three QS systems operate in \textit{Vibrio harveyi}), the synthetic ligands were also tested against \textit{V. harveyi} strains BB170 and BB152 lacking AI-1 (AHL) sensor. Such tests revealed similar luminescence data as found for wild type. The analogs which showed bioluminescence inhibition (160b, 160c, 163b, 166, 167 and 170) when tested against light producing enzyme, luciferase displayed identical trend in reduction of bioluminescence (personal communication of Dr. Makemson at FIU). In conclusion, luminescence and lucifersase assays revealed that the inhibition of luminescence was not mediated by QS but by luciferase.

\textbf{Table 3. Effect }\gamma\text{-lactam and hemiaminal analogs on }\textit{V. harveyi} \textit{MAV} bioluminescence and growth

<table>
<thead>
<tr>
<th>Aza analogs</th>
<th>% Inhibition Luminescence</th>
<th>% Inhibition of Growth</th>
<th>Conc X 10^{-4}M</th>
</tr>
</thead>
<tbody>
<tr>
<td>160a</td>
<td>0</td>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td>160b</td>
<td>99.8</td>
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<td>160c</td>
<td>64</td>
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<td>160d</td>
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<td>163a</td>
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<tr>
<td>163c</td>
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<td>0</td>
<td>0</td>
<td>2.7</td>
</tr>
<tr>
<td>166</td>
<td>29</td>
<td>0</td>
<td>3.2</td>
</tr>
<tr>
<td>167</td>
<td>80</td>
<td>0</td>
<td>2.8</td>
</tr>
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<td>170</td>
<td>52</td>
<td>0</td>
<td>3.2</td>
</tr>
<tr>
<td>171</td>
<td>0</td>
<td>0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Compounds dissolved in water or DMSO served as a control and % inhibition is calculated relative to the solvent control. Bioluminescence was recorded in light units (1 LU = 1.08 \times 10^8 q/s/mL) and all measurements were obtained in a single trial.

3.3.2. \textit{Inhibition studies of 4-aza-SRH analogs against LuxS enzyme}

\textit{Bacillus subtilis} LuxS preparation and preliminary inhibition studies of selected 4-aza-SRH analogs against LuxS\textsubscript{bs} were performed by Dr. Pei’s research group at Ohio State University using published protocols.\textsuperscript{54,58} The LuxS activity assay was based on quantitative detection of the
liberated homocysteine released from the natural substrate SRH which is readily detected by Ellman’s reagent (DTNB). The DTNB reacts with free thiol (homocysteine) rapidly and quantitatively to form a chromophore, 5-thionitrobenzoic acid (TNB) whose absorbance can be measured at 412 nm ($\varepsilon_{412} = 14,150 \text{ M}^{-1}\text{cm}^{-1}$) (Scheme 19). From activity assay, LuxS was found to show saturation kinetics toward SRH with a $K_M$ value of 1.05 $\mu$M and a $V_{\text{max}}$ value of $1.8 \times 10^{-4}$ $\mu$M/min (fitted into Michaleis-Menten equation $V = (V_{\text{max}}[S])/(K_M + [S])$ (Table 4; Figure 28).

**Table 4. Rate of LuxS catalysis at various concentrations of natural substrate**

<table>
<thead>
<tr>
<th>[SRH] $\mu$M</th>
<th>dA/min (rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44.5</td>
<td>0.0185</td>
</tr>
<tr>
<td>26.7</td>
<td>0.0178</td>
</tr>
<tr>
<td>8.9</td>
<td>0.0153</td>
</tr>
<tr>
<td>4.4</td>
<td>0.0141</td>
</tr>
<tr>
<td>2.2</td>
<td>0.0126</td>
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</tbody>
</table>

Inhibition of 4-Aza-SRH analogs was determined in a manner similar to the activity assay except for addition of various concentrations of inhibitors to the reaction mixture besides DTNB, SRH and LuxSBs. Rate of the reaction and $K_M$ values in presence of aza analogs were calculated and mechanism of inhibition was determined. Percentage activity of LuxS in presence of inhibitors was reported relative to the percentage activity of SRH alone (i.e., without inhibitor activity percentage was considered 100%). From the 4-aza-SRH derivatives tested, pyrrolidine analog 129, lactam analog 140, and propythio $\gamma$-lactam analog 160a were found to act as competitive inhibitors of moderate potency.
Scheme 19. LuxS activity assay for detection of the released homocysteine employing DTNB

Figure 28. Lineweaver-Burk plot of Co-LuxS enzyme with SRH (data were fitted into the Michaelis-Menten equation $V = V_{\text{max}}[S]/(K_M + [S])$)
Because of the absence of the hydroxylic group at C1, 1-deoxy-4-aza-SRH 129 (Figure 30) should not undergo enzyme mediated ring opening which is the very first step in the LuxS-mediated catalysis, but might still be able to bind to LuxS (because of the ability to bind at homocysteine pocket and chelate to the metal ion via C2 and C3 hydroxyls) hence act as competitive inhibitor. Supporting our hypothesis, pyrrolidine analog 129 acted as a competitive inhibitor with $K_I$ value of 49 µM and IC$_{50}$ value of 0.8 mM (Figure 29). Weak inhibition of 129 was probably the result of loss of the binding energy derived from C1-hydroxyl. This hydroxylic group was originally shown to interact with several active site residues (Ser-6, His-11 and Arg-39) via hydrogen bonding in the co-crystal structure of C84A-LuxS$_{bs}$ with 2-ketone intermediate.$^{49}$ Because of the positively charged ring nitrogen ($\sim$\(pK_a = 6.6\)), the conformational change in sugar ring might have also resulted in improper fit into the active site.

![Figure 29. Dose response curve of pyrrolidine analog 129 against LuxS enzyme](image-url)
Lactam-type SRH analog 141 (Figure 30) with the amide oxygen at C1 was tested next as LuxS inhibitor. Apart from its inability to open the sugar ring, the lactam ring was chosen mainly because the planar amide group resembles the carbonyl group existing in aldose form of SRH or the C2 keto group present in 2-keto intermediate. Secondly the carbonyl of lactam might also form new interactions at the active site via hydrogen bonding. Thirdly, the carboxamide moiety

Figure 30. Activity of the selected 4-aza-SRH analogs against LuxS enzyme
has the potential to tautomerize to a lactim form so as to generate a hydroxylic group at C1 which mimics the natural substrate.\textsuperscript{177,178} Lactam 141 was found to be a competitive inhibitor with $K_i$ value of 37 µM and IC\textsubscript{50} value of 0.66 mM (Figure 31; Table 5). As expected, the corresponding lactam analog 140 with benzylic group at the ring nitrogen was found to be inactive. This may be the result of the steric bulkiness caused by benzylic group preventing access to LuxS active site.

Table 5. Rate and percentage activity of LuxS at different concentrations of analogs 129 and 141

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µM)</th>
<th>dA/min (rate)</th>
<th>% Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>129</td>
<td>0</td>
<td>0.01012</td>
<td>100</td>
</tr>
<tr>
<td>129</td>
<td>200</td>
<td>0.00865</td>
<td>85.5</td>
</tr>
<tr>
<td>129</td>
<td>400</td>
<td>0.00659</td>
<td>65.1</td>
</tr>
<tr>
<td>129</td>
<td>800</td>
<td>0.00500</td>
<td>49.4</td>
</tr>
<tr>
<td>129</td>
<td>1600</td>
<td>0.00392</td>
<td>38.7</td>
</tr>
<tr>
<td>129</td>
<td>3200</td>
<td>0.00261</td>
<td>25.8</td>
</tr>
<tr>
<td>141</td>
<td>0</td>
<td>0.00936</td>
<td>100</td>
</tr>
<tr>
<td>141</td>
<td>80</td>
<td>0.00833</td>
<td>89</td>
</tr>
<tr>
<td>141</td>
<td>160</td>
<td>0.00728</td>
<td>78</td>
</tr>
<tr>
<td>141</td>
<td>320</td>
<td>0.00578</td>
<td>62</td>
</tr>
<tr>
<td>141</td>
<td>655</td>
<td>0.00462</td>
<td>49</td>
</tr>
<tr>
<td>141</td>
<td>1310</td>
<td>0.00306</td>
<td>33</td>
</tr>
</tbody>
</table>

Figure 31. Dose response curve of lactam analog 141 against LuxS enzyme
As the lactam analog 141 was found to inhibit LuxS, further modification of the lactam analog by removing hydroxylic groups at C2 and C3 was performed to provide 2,3-dideoxy lactam analog of SRH (e.g., 195; Figure 30). Lactam 195 was found to be inactive. The lack of the vicinal hydroxyl groups might have totally eradicated LuxS binding ability, probably because of the large difference in polarity and absence of metal chelation. The hemiaminal analog 196 (Figure 30) was also devoid of activity confirming the significance of C2 and C3 hydroxylic groups for LuxS binding. These findings are consistent with the structural requirements for LuxS binding and activity as reported in literature.

Hemiaminal analog of 4-aza-SRH 143 (Figure 30) was tested to study the effect of $N,O$-acetal function vs $O,O$-acetal present in natural substrate on the LuxS catalyzed reaction. As only open aldehyde form of SRH is catalytically active, ring opening of SRH should serve as an important step to study catalysis of LuxS enzyme. The LuxS was reported to bind to cyclic ribofuranose form of SRH ($O,O$-hemiacetal) and catalyze ring opening. I envisaged that because of the intrinsic difference in leaving group property of amine when compared to hydroxylic group (existing in natural substrate), the hemiaminal analog 143 can interfere with enzyme mediated ring opening and therefore act as LuxS inhibitor. In case of natural substrate SRH, ($O,O$-hemiacetal), the enzyme catalyzes protonation of the ring oxygen, leading to the sugar ring opening. On the other hand, as the $pK_a$ of hemiaminal 143 is around 5.8 at physiological pH, the enzyme mediated protonation of 143 is speculated to occur preferentially at anomeric hydroxyl as opposed to ring nitrogen, resulting in loss of water to give imine 197. The imine intermediate 197 might act as suicide inhibitor (path (a) in Scheme 20) by forming a covalent adduct with proximal nucleophilic groups (for example Cys84). Alternatively it can also tautomerize to give 2-keto-4-aza-SRH 200 (path (b) in Scheme 20) via formation of enamine 199 (Amadori rearrangement). The keto byproduct 200 might also serve as competitive inhibitor because of its structural resemblance to the 2-keto SRH intermediate. The LuxS-mediated $N,O$-
acetal ring opening leading to the open chain aldamine and ultimately to the formation of aza analog of DPD 201 ((S)-1,2-dihydroxy-4-iminopentan-3-one) has to be also considered (path (c) in Scheme 20). The acyclic imine analog of DPD 201 may cyclize to give furan derivative 203a (aza analog of pro-AI-2). The imine analog of pro-AI-2 might also tautomerize to give enamine 202 or can hydrolyze to provide DPD. Because of the electron withdrawing nature of α-substituents, it is speculated that the imine tautomer is less favorable when compared to enamine.181 A time-dependent inhibition of this N,O-acetal analog with IC_{50} value of 60 µM was observed with 4-aza-SRH 143 (Figure 32; Table 6). Chemical equilibration among hemiaminal, open aldehyde, imine and enamine forms of 143 might be responsible for time-dependent inhibition. The complex kinetics of this analog precluded determination of K_I value. Further work is necessary to determine the exact mechanism of inhibition and to support the formation of the aza DPD analog as well as the detection of enzyme substrate complex.

**Figure 32.** Dose response curve of hemiaminal analog 143 against LuxS enzyme
Scheme 20. Plausible mechanism of interaction of hemiaminal analog of SRH 143 with LuxS. Production and complexation of aza-DPD analogs.
Table 6. Rate and percentage activity of LuxS at different concentrations of analogs 143 and 160a

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µM)</th>
<th>dA/min (rate)</th>
<th>% Activity</th>
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<td>0.01028</td>
<td>100</td>
</tr>
<tr>
<td>143</td>
<td>20</td>
<td>0.00917</td>
<td>89</td>
</tr>
<tr>
<td>143</td>
<td>40</td>
<td>0.00777</td>
<td>76</td>
</tr>
<tr>
<td>143</td>
<td>50</td>
<td>0.00665</td>
<td>65</td>
</tr>
<tr>
<td>143</td>
<td>60</td>
<td>0.00548</td>
<td>53</td>
</tr>
<tr>
<td>143</td>
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<td>0.00395</td>
<td>38</td>
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<td>200</td>
<td>0.00196</td>
<td>19</td>
</tr>
<tr>
<td>160a</td>
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</tr>
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<td>160a</td>
<td>100</td>
<td>0.00935</td>
<td>97</td>
</tr>
<tr>
<td>160a</td>
<td>200</td>
<td>0.00896</td>
<td>93</td>
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<td>400</td>
<td>0.00906</td>
<td>94</td>
</tr>
<tr>
<td>160a</td>
<td>800</td>
<td>0.00862</td>
<td>89</td>
</tr>
<tr>
<td>160a</td>
<td>1600</td>
<td>0.00830</td>
<td>86</td>
</tr>
<tr>
<td>160a</td>
<td>3200</td>
<td>0.00720</td>
<td>74</td>
</tr>
<tr>
<td>160a</td>
<td>6069</td>
<td>0.00638</td>
<td>66</td>
</tr>
<tr>
<td>160a</td>
<td>12138</td>
<td>0.00457</td>
<td>47</td>
</tr>
</tbody>
</table>

In order to further investigate the effect of imine group on the inhibition of LuxS, I also synthesized 1-methyl imino SRH analog 158. Imino sugars with endocyclic C=N structural motif were described to act as potential suicide inhibitors because of the strong electrophilic nature of imine bond. Three plausible ways of interaction of this analog with LuxS are speculated. Firstly, nucleophilic residues at the active site might covalently bond to imine carbon, resulting in irreversible inhibition (pathway (a) in Scheme 21). Secondly, imine analog 158 can also tautomerize to give methyl 2-keto-4-aza-SRH 208 analogous to path (b) described for hemiaminal 143 in Scheme 20. As a third plausible pathway, 158 can transform in situ into the ketohemiaminal 209 by nucleophilic attack of the enzyme bound water (pKₐ = 10) across imine bond. Ring opening of the hemiaminal should generate ketone 208 which might be coordinated by Co²⁺ and converted to an aza analog of DPD 211 with an additional methyl group at C1 (path
(c) in Scheme 20. The imine 209 might also tautomerize to enamine 212 or cyclize to give furan derivative 213. Unfortunately, inhibition assay determined no activity for the imine 158. This may be because of its misfit into the polar active site of LuxS owing to hydrophobic CH₃ at C1.

Scheme 21. Plausible mechanism of interaction of cyclic imine analog of SRH 158 with LuxS
In order to find structural requirements for inhibition of both intra species (AHL mediated) and inter species QS (AI-2 mediated), the propylthio γ-lactam analog 160a and its hemiaminal counterpart 163a, which were earlier found to be AHL mediated QS inhibitors in *P. aeruginosa* (Section 3.4.1.2), were tested for LuxS inhibition as well. The propylthio-lactam analog 160a displayed very weak inhibition with $K_I$ value of 0.71 mM and $IC_{50}$ value of 11 mM (Figure 33). The reduced hemiaminal counterpart 163a was devoid of inhibition (Table 6). These data were consistent with the LuxS inhibition results found for 2,3-dideoxy SRH analogs, 195 and 196 which were also inactive. Depletion of inhibition might be because of the loss of binding energy derived from: (i) binding of C1-hydroxylic group at the active site, (in case of lactam analog), (ii) homocysteine moiety, and (iii) metal ligation to hydroxylic groups owing to missing C1, C2 and C3 hydroxylic groups and homocysteine. However, no common structural motif was found as propylthio compounds were inactive, probably owing to the large intrinsic differences in the active sites of LasR and LuxS.

**Figure 33.** Dose response curve of propylthio lactam analog 160a against LuxS enzyme

The LuxS inhibition studies with 4-aza-SRH analogs were also attempted in our laboratory with histidine-tagged LuxS protein. *E. coli* BL21(DE3)/pLuxS strain was kindly provided by Dr. Zhou from Northeastern University. The protein was isolated and purified by Dr. Wang’s
research group at FIU by following published methods. Such purified HTCo-LuxS$_{Bs}$ protein showed characteristics identical to the properties described in the literature. For example, presence of purple color and UV-VIS absorption bands at 630, 580 and 530 nm. Moreover cobalt incorporation into apo-enzyme was confirmed by determination of cobalt concentration (12.87 mM) using ICP-MS analysis in the laboratory of Dr.Cai at FIU. However, testing results of this protein are still not conclusive. The inhibition studies with 2-deoxy analog 179 and nitrone analog 153 will be investigated in future.

3.3.3. Complexation studies with aza-DPD

With $^{11}$B NMR

Since 4-aza-SRH 143 was proposed to release aza analog of DPD 201 upon LuxS mediated catalysis (pathway (c), Scheme 20), an additional model study was performed to investigate the possibility of formation of aza-DPD analog 201 and its plausible cyclisation and binding with borate to form aza-AI-2, 203c or diol complex 203d (Scheme 22). The differential affinity of borate binding towards amino alcohol (as in pro-aza-AI-2 203b) vs cis-diols (as in pro-AI-2, S-THMF, Scheme 2) was measured employing $^{11}$Boron NMR binding studies. The aminoethanol 205, cis-2-aminocyclopentanol and ethanediol 204 in borate solution were used as model compounds, to simulate the complexation of 203b. Treatment of either 2-aminoethanol or cis-2-aminocyclopentanol with saturated borate solution in D$_2$O at pH 7.3 or in benzene solution showed broad peaks on $^{11}$B NMR spectra which were inconclusive of complexation as reported for the related vicinal aminoalcohols with borate. On the contrary, $^{11}$B NMR data for D-ribose was consistent with literature values for 2:1 and 1:1 sugar/borate complexes (Figure 34). Taylor et al. showed that NH$_2$C(CH$_2$OH)$_3$ has less tendency to form borate complexes than the corresponding triols with alkyl substituents (instead of amino group), although mono-chelate and bis-chelate borate complexes with vicinal hydroxyl groups were formed.
Scheme 22. Borate binding study of ethanediol and aminoethanol

Figure 34. $^{11}$Boron NMR spectrum of D-ribose in saturated borate solution in D$_2$O at pH 7.8. Peaks at 18.92 ppm (C), 12.92 ppm (D) and 1.01 (E) ppm correspond to borates $\text{B(OH)}_4^{-}$, $\text{B}_3\text{O}_3(\text{OH})_4^{-}$ and $\text{B}_5\text{O}_6(\text{OH})_4^{-}$, respectively.\textsuperscript{184}
Energy minimization calculations [density functional theory (DFT)] were performed between 2,3-borate ester (Al-2) and 3,4-borate ester of the cyclic DPD by Dr. Mebel from FIU (Figure 35). The relative energy of 2,3-diester (Al-2) was found to be lower than that of the corresponding 3,4-diester. However, in case of aza analogues, DFT calculations between aza-Al-2 \textbf{203c} and 3,4-borate ester \textbf{203d} showed that complex \textbf{203} had lower energy than aminoalcohol complex \textbf{203c}. Such results indicate once more the preferential affinity of boron for diols as compared to aminoalcohols.

\textbf{Figure 35.} DFT calculations for borate esters of \textbf{203b} (Figure 35A) and S-THMF (Figure 35B)
4. EXPERIMENTAL PROCEDURES

4.1. General procedures

The $^1$H (400 or 600 MHz) and $^{13}$C (100 MHz) spectra were determined with solutions in CDCl$_3$ unless otherwise noted. The $^{11}$B (192.6 MHz) NMR spectra were recorded in D$_2$O. Mass spectra (MS) were obtained with atmospheric pressure chemical ionization (APCI) or ESI technique and HRMS in AP-ESI or TOF-ESI mode. The TLC was performed with Merck kieselgel 60-F$_{254}$ sheets products were detected with 254 nm light or by visualization with Ce(SO$_4$)$_2$($\text{NH}_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O/H$_2$SO$_4$/H$_2$O reagent. Merck kieselgel 60 (230-400 mesh) was used for column chromatography. The HPLC purifications were performed using XTerra® preparative RP$_{18}$ OBD™ column (5μm 19 x 150 mm) with gradient program using CH$_3$CN/H$_2$O as a mobile phase. Reagent grade chemicals were used, and solvents were dried by reflux over and distillation from CaH$_2$ (except THF/potassium) under argon. Using SPSS analytical software two-tailed Student $t$-test was performed and p values were generated.

The aza sugars synthesized in this dissertation are treated as SRH analogs throughout the manuscript and therefore numbering of carbons starts at anomeric position. However, in the experimental section, the numbering of carbons for aza sugars either follows sugar nomenclature or heterocyclic nomenclature depending on the nature of the sugar (Figure 36).
4.2. Synthesis of the inhibitors

*N-Benzyl-5-O-tert-butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol* (124). To a stirred solution of 123$^{159}$ (150 mg, 0.57 mmol) in anhydrous CH$_2$Cl$_2$ (5 mL) at rt under Ar atmosphere was added DMAP (7 mg, 0.05 mmol) and imidazole (93 mg, 1.36 mmol) followed by TBDMSCl (0.68 mmol, 103 mg). The mixture was then stirred for 10 h and partitioned (CH$_2$Cl$_2$//NaHCO$_3$/H$_2$O). The organic layer was washed (brine), dried (MgSO$_4$) and evaporated. The residue was column chromatographed (15% EtOAc/hexane) to give 124$^{187}$ (204 mg, 98%) as a colorless oil.$^1$H NMR δ -0.13 (s, 3, CH$_3$), 0.00 (s, 3, CH$_3$), 0.83 (s, 9, t-Bu), 1.26 (s, 3, CH$_3$), 1.49 (s, 3, CH$_3$), 2.65 (dd, $J$ = 2.7, 10.3 Hz, 1, H1), 2.94 (‘q’, $J$ = 2.2 Hz, 1, H4), 3.04 (dd, $J$ = 5.5, 10.3 Hz, 1, H1’), 3.57 (dd, $J$ = 4.1, 10.6 Hz, 1, H5), 3.64 (d, $J$ = 13.4 Hz, 1, Bn), 3.71 (dd, $J$ = 10.6, 4.3 Hz, 1, H5’), 3.94 (d, $J$ = 13.4 Hz, 1, Bn), 4.49 (dd, $J$ = 2.0, 6.5 Hz, 1, H3), 4.58 (‘dt’, $J$ = 2.7, 6.2 Hz, 1, H2), 7.13-7.23 (m, 5, Bn); $^{13}$C NMR δ -5.50, (CH$_3$), -5.43, (CH$_3$), 18.16 (t-Bu), 25.20 (CMe$_2$), 25.72 (CH$_3$), 25.91 (CH$_3$), 27.20 (CMe$_2$), 56.91 (Bn), 59.28 (C1), 63.15 (C5), 68.93 (C4), 79.44 (C2), 83.33 (C3), 111.86 (CMe$_2$), 128.22, 128.47, 126.83, 139.41 (Bn); MS (APCI) m/z 378 (100, MH$^+$).

*N-tert-Butoxycarbonyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-5-O-tert-butyldimethylsilyl-D-ribitol* (125). A solution of 124 (145 mg, 0.38 mmol), triethylamine (76.7 mg, 0.106 mL, 0.76 mmol), di-tert-butyldicarbonate (126 mg, 0.57 mmol) and 5% Pd/C (300 mg) in ethanol (6 mL) was stirred under an atmosphere of hydrogen at room temperature for 6 h. The reaction mixture was filtered through Celite to remove the catalyst. The Celite was washed with ethanol (5 mL) and washings and the filtrate were combined and evaporated. The residue was partitioned (EtOAc//NaHCO$_3$/H$_2$O). The organic layer was washed (brine), dried (MgSO$_4$) and evaporated. The crude product was column chromatographed (20 → 30% EtOAc/hexane) to give 125 (147 mg, 99%) with data as reported.$^{164}$
**N-tert-Butoxycarbonyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol** (126).

TBAF (1 M/THF, 0.25 mL, 0.25 mmol) was added to a stirred solution of 125 (66 mg, 0.17 mmol) in THF (5 mL) at ambient temperature. After stirring for 30 min, the reaction mixture was partitioned (EtOAc/NaHCO₃/H₂O). The organic layer was washed (brine), dried (MgSO₄) and evaporated. The residue was column chromatographed (50→60% EtOAc/hexane) to give 126 (32 mg, 70%) with data as reported.⁶⁹

**N-tert-Butoxycarbonyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-5-O-methanesulfonyl-D-ribitol** (127). **Procedure A.** Triethylamine (66 mg, 92 µL, 0.66 mmol) and MsCl (37.6 mg, 25 µL, 0.33 mmol) were added drop wise to stirred solution of 126 (60 mg, 0.22 mmole) in anhydrous CH₂Cl₂ (6 mL) at 0°C (ice-bath). After 5 min, ice-bath was removed and the reaction mixture was allowed to stir at ambient temperature for 30 min. The reaction mixture was quenched with saturated NaHCO₃/H₂O and was extracted with CH₂Cl₂. The organic layer was washed (brine), dried (MgSO₄) and evaporated to give 127 (73 mg, 96%) as a mixture (~3:2) of two rotamers of sufficient purity to be directly used in next step: ¹H NMR δ 1.28 (s, 3, CH₃), 1.42 (s, 12H, t-Bu, CH₃), 2.96 (s, 1.2, Ms), 2.98 (s, 1.8, Ms), 3.39 (dd, J = 4.8, 12.5 Hz, 0.4, H1), 3.46 (dd, J = 4.8, 12.5 Hz, 0.6, H1), 3.69 (d, J = 12.5 Hz, 0.6, H1'), 3.82 (d, J = 12.5 Hz, 0.4, H1'), 4.10-4.14 (m, 0.4, H4), 4.22-4.30 (m, 0.6, H4), 4.22-4.29 (m, 1.4, H5,5'), 4.45 (dd, J = 4.1, 10.1 Hz, 0.6, H5), 4.65 (d', J = 5.9 Hz, 1, H3); 13C NMR (major rotamer) δ 24.93 (CMe₂), 26.93 (CMe₂), 29.59 (t-Bu), 37.09 (Ms), 52.51 (C1), 62.39 (C4), 68.94 (C5), 79.20 (C2), 80.43 (t-Bu), 81.68 (C3), 112.07 (CMe₂), 154.24 (CO); ¹³C NMR (minor rotamer) δ 24.93 (CMe₂), 26.93 (CMe₂), 29.59 (t-Bu), 37.48 (Ms), 53.11 (C1), 62.64 (C4), 68.59 (C5), 78.54 (C2), 80.64 (t-Bu), 82.53 (C3), 112.07 (CMe₂), 153.63 (CO); MS (APCI) m/z 252 [100, (MH₂-Boc)⁺], 352 (10, MH⁺)

**S-(N-tert-Butoxycarbonyl-1,4-imino-2,3-O-isopropylidene-1,4,5-trideoxy-D-ribitol-5-yl)-N-tert-butoxycarbonyl-L-homocysteine tert-butyl ester** (128). **Procedure B. Step a.** H₂O (0.4
mL) and tris(2-carboxyethyl)phosphine hydrochloride (140 mg, 0.5 mmol) were added to a stirred solution of \(N,N'\text{-di(tert-butoxycarbonyl)-L-homocystine di(tert-butyl) ester}\) (250 mg, 0.4 mmol) in anhydrous DMF (4 mL) at ambient temperature under Ar atmosphere. After 24 h, the reaction mixture [TLC (EtOAc/hexane, 2:8) showed conversion of disulfide (R\(_f\) 0.55) into thiol (R\(_f\) 0.65)] was partitioned between EtOAc, and saturated NaHCO\(_3\)/H\(_2\)O. Aqueous layer was extracted with EtOAc, and the combined organic layer was washed with brine, dried (MgSO\(_4\)), and concentrated to give \(N\text{-tert-butoxycarbonyl-L-homocysteine tert-butyl ester}\) (240 mg, 99%) as colorless oil of sufficient purity to be directly used in next step. 

**Step b.** LDA (2.0 M/THF and heptanes; 85 µL, 0.17 mmol) was added drop wise (10 min) to a stirred solution of freshly prepared thiolate from step a (200 mg, 0.6 mmol) in anhydrous DMF (5 mL) under a vigorous stream of argon at 0 ºC (ice-bath). After an additional 10 min, 127 (100 mg, 0.2 mmol) in anhydrous DMF (5 mL) was added via a syringe. After 15 min ice-bath was removed and the reaction mixture was stirred for 24 h at ambient temperature. Ice-cold saturated NH\(_4\)Cl/H\(_2\)O was added and the resulting suspension was diluted with EtOAc. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layer was washed (brine), dried (MgSO\(_4\)) and evaporated. The residue was column chromatographed (40 → 50% EtOAc/hexane) to give 128 (130 mg, 86%) as a mixture of rotamers (~1:1): \(^1\)H NMR δ 1.29 (s, 3, CH\(_3\)), 1.41 (s, 12H, t-Bu, CH\(_3\)), 1.44 (s, 18H, t-Bu), 1.79-1.92 (m, 2, H8,8'), 2.39-2.80 (m, 4, H5,5',7,7'), 3.37 (dd, \(J = 4.2, 11.7 \text{ Hz}, 0.5, \text{H1}\)), 3.43 (dd, \(J = 4.5, 11.7 \text{ Hz}, 0.5, \text{H1}\)), 3.70 (d, \(J = 12.6 \text{ Hz}, 0.5, \text{H1'}\)), 3.84 (d, \(J = 12.8 \text{ Hz}, 0.5, \text{H1'}\)), 3.99-4.05 (m, 0.5, H4), 4.11-4.17 (m, 0.5, H4), 4.18-4.29 (m, 1, H9), 4.56 (dd, \(J = 5.6, 10.4 \text{ Hz}, 0.5, \text{H3}\)), 4.60 (dd, \(J = 5.6, 10.4 \text{ Hz}, 0.5, \text{H3}\)), 4.69 (d, \(J = 4.8 \text{ Hz}, 0.5, \text{H2}\)), 4.71 (d, \(J = 4.8 \text{ Hz}, 0.5, \text{H2}\)), 5.06 (br. d, \(J = 7.3 \text{ Hz}, 0.5, \text{NH}\)), 5.29 (br. d, \(J = 6.1 \text{ Hz}, 0.5, \text{NH}\)); \(^1\)C NMR δ 24.97 (CMe\(_2\)), 26.93 (CMe\(_2\)), 27.91 (C7), 28.01 (t-Bu), 28.34 (t-Bu), 28.43 (t-Bu), 32.18 (C5), 32.57 (C8), 32.92 (C8), 33.22 (C5), 51.73 (C1), 52.42 (C1), 53.48 (C9), 62.76 (C4), 63.16 (C4), 78.47 (C2), 78.50 (t-Bu), 79.21 (C2), 79.23 (t-Bu), 80.00 (t-Bu), 80.10 (t-Bu), 83.49
(C3), 84.07 (C3), 111.85 (CMe2), 154.09 (CO), 154.93 (CO), 155.36 (CO), 171.25 (CO), 171.46 (CO); MS (APCI) m/z 547 (100, MH+).

**S-(1,4,5-Trideoxy-1,4-imino-D-ribitol-5-yl)-L-homocysteine (129). Procedure C. Step a.** Compound 128 (39 mg, 0.07 mmol) in TFA (4.0 mL) was stirred at 0 ºC for 3 h. Volatiles were evaporated and coevaporated with toluene to give an oily residue, which was used in next step.

**Step b.** Product from step a was treated with TFA/H2O (9:1, 4.0 mL) for 1 h at 0 ºC. Volatiles were evaporated and the crude product was purified on RP-HPLC (5% CH3CN/H2O for 50 min at 2.5 mL/min; tR = 12 min) to give 129 (12 mg, 66%) as a colorless oil: 1H NMR (D2O) δ 2.00-2.15 (m, 2, H8,8'), 2.61-2.71 (m, 2, H7,7'), 2.77 (dd, J = 10.4, 14.5 Hz, 1, H5), 3.05 (dd, J = 4.3, 14.5 Hz, 1, H5'), 3.24 (dd, J = 1.9, 13.0 Hz, 1, H1), 3.43 (dd, J = 4.0, 13.0 Hz, 1, H1'), 3.59 (dd, J = 4.3, 8.7, 10.4 Hz, 1, H4), 3.76 (t, J = 6.2 Hz, 1, H9), 4.04 (dd, J = 4.1, 8.7 Hz, 1, H3), 4.29 (ddd, J = 1.9, 4.0, 4.1 Hz, 1, H2); 13C NMR (D2O) δ 26.81 (C8), 30.06 (C7), 30.62 (C5), 49.16 (C1), 53.54 (C9), 59.48 (C4), 69.48 (C2), 74.34 (C3), 174.02 (COOH); MS m/z 251 (100, MH+).

HRMS (TOF MS-ESI) m/z calculated for C9H18N2O4S [M+H]+ 251.106; found 251.1063.

1-Benzyl-5-chloro-3,4-dihydroxy-3,4-O-isopropylidinepiperidine [130(3S,4S,5R/S)].

Treatment of 122 (50 mg, 0.19 mmol) with MsCl (31.9 mg, 21.9 μL, 0.28 mmol) by Procedure A [column chromatography (20 → 30% EtOAc/hexane)] gave 130 (25 mg, 46%) as a 3:1 mixture of diastereomers: The major isomer had: 1H NMR δ 1.30 (s, 3, CH3), 1.50 (s, 3, CH3), 2.13-2.19 (m, 1, H2), 2.49 (dd, J = 3.7, 13.3 Hz, 1, H6), 2.87 (dd, J = 1.7, 12.0 Hz, 1, H2'), 3.05 ('dt', J = 2.1, 13.3 Hz, 1, H6'), 3.50 (d, J = 13.4 Hz, 1, Bn), 3.61 (d, J = 13.4 Hz, 1, Bn), 3.93-3.99 (m, 2, H3,5), 4.19 (dd, J = 3.7, 7.5 Hz, 1, H4), 7.20-7.25 (m, 5, Bn); 13C NMR δ 26.18 (CMe2), 28.43 (CMe2), 53.54 (C6), 56.53 (C2), 58.48 (C5), 61.52 (Bn), 73.77 (C4), 79.52 (C3), 112.80, 127.26, 128.40, 128.92, 137.16 (Bn); MS m/z 282 (100, MH+ [35Cl]), 284 (40, MH+ [37Cl]); HRMS (TOF MS-ESI) m/z calculated for C15H2035ClNO2 [M+H]+ 282.1255; found. 282.1259.
**N-Benzyl-5-(tert-butyldimethylsiloxyethyl)-3,4-dihydroxy-3,4-O-isopropylideneypyrrrolidin-2-one** [131(3R,4R,5S)]. Procedure D. RuO$_2 \times $H$_2$O (4.3 mg, 0.032 mmol) was added to a stirred solution of NaIO$_4$ (83 mg, 0.39 mmol) in H$_2$O (1 mL) at ambient temperature. After 5 min, a solution of 124 (50 mg, 0.13 mmol) in EtOAc (1 mL) was added drop wise and the reaction mixture was continued to stir for 12 h. Water (10 mL) and EtOAc (10 mL) were added and the separated aqueous layer was furthermore extracted with EtOAc (2 × 10 mL). The combined organic layers were washed (brine), dried (MgSO$_4$) and evaporated. The residue was column chromatographed (50→60% EtOAc/hexane) to give 131 (33 mg, 65%) and N-benzozylpyrrolidinone as a byproduct (10 mg, 18%). Compound 131 had: $^1$H NMR δ 0.01 (s, 6, CH$_3$), 0.84 (s, 9, t-Bu), 1.35 (s, 3, CH$_3$), 1.42 (s, 3, CH$_3$), 3.51 (t, $J = 2.1$ Hz, 1, H5), 3.62 (dd, $J = 2.0$, 10.9 Hz, 1, H6), 3.69 (dd, $J = 2.3$, 10.9 Hz, 1, H6'), 3.93 (d, $J = 15.2$ Hz, 1, Bn), 4.50 (d, $J = 5.6$ Hz, 1, H4), 4.69 (d, $J = 5.6$ Hz, 1, H3), 5.00 (d, $J = 15.2$ Hz, 1, Bn), 7.22-7.32 (m, 5, Bn); MS m/z 392 (100, MH$^+$).$^{13}$C NMR δ -5.67, (CH$_3$), -5.56, (CH$_3$), 18.11 (t-Bu), 25.79 (t-Bu), 25.81 (CMe$_2$), 27.26 (CMe$_2$), 44.19 (Bn), 60.22 (C6), 62.02 (C5), 76.66 (C4), 78.01 (C3), 111.69 (CMe$_2$), 127.71, 128.18, 128.73, 135.59 (Bn), 171.89 (CO). The N-Benzoyl-5-(tert-butyldimethylsiloxyethyl)-3,4-dihydroxy-3,4-O-isopropylidene-2-pyrrolidinone byproduct had: $^1$H NMR δ 0.01 (s, 6, CH$_3$), 0.05 (s, 3, CH$_3$), 0.87 (s, 9, t-Bu), 1.40 (s, 3, CH$_3$), 1.51 (s, 3, CH$_3$), 3.83 (dd, $J = 1.5$, 10.7 Hz, 1, H6), 4.19 (dd, $J = 2.2$, 10.7 Hz, 1, H6'), 4.58 (d, $J = 1.8$ Hz, 1, H5), 4.65 (d, $J = 5.5$ Hz, 1, H4), 4.76 (d, $J = 5.5$ Hz, 1, H3), 7.38-7.43 (m, 2, Bn), 7.51 (d, $J = 1.3$, 6.7 Hz, 2, Bn), 7.54-7.58 (m, 1, Bn), $^{13}$C NMR δ -5.72, (CH$_3$), -5.58, (CH$_3$), 18.16 (CH$_3$), 25.34 (CMe$_2$), 25.82 (t-Bu), 27.22 (CMe$_2$), 61.68 (C5), 62.12 (C6), 76.30 (C4), 78.67 (C3), 112.09 (CMe$_2$), 127.87, 128.73, 132.15, 134.11 (Bn) 170.56 (CO), 171.66 (CO); MS (APCI) m/z 406 (100, MH$^+$).

**N-Benzyl-3,4-dihydroxy-5-(hydroxymethyl)-3,4-O-isopropylideneypyrrrolidin-2-one** [132b(3R,4R,5R)]. TBAF (1 M/THF, 0.18 mL, 0.18 mmol) was added drop wise to a stirred
solution of 131 (49 mg, 0.12 mmol) in THF (10 mL) at 0 °C. After 5 min, the ice-bath was removed and reaction mixture was allowed to stir at ambient temperature for 2 h. The reaction mixture was quenched with water and volatiles were evaporated. The residue was partitioned (EtOAc//NaHCO3/H2O). The organic layer was washed (brine), dried (MgSO4) and evaporated. The residue was column chromatographed (80 → 90% EtOAc/hexane) to give 132 as a white solid (33 mg, 97%): ^1H NMR δ 1.37 (s, 3, CH3), 1.47 (s, 3, CH3), 3.35 (s, 1, OH), 3.54 (t', J = 1.8 Hz, 1, H5), 3.64 (d of m, J = 5.0, 11.8 Hz, 1, H6), 3.85 (br. d, J = 12.0 Hz, 1, H6'), 4.08 (d, J = 15.2 Hz, 1, Bn), 4.64 (d, J = 5.6, Hz, 1, H4), 4.77 (d, J = 5.2 Hz, 1, H3), 5.04 (d, J = 15.2 Hz, 1, Bn), 7.29-7.37 (m, 5, Bn); MS (APCI) m/z 310 (100, [MH+MeOH] +).

N-(tert-Butoxycarbonyl)-5-(tert-butyldimethylsiloxymethyl)-3,4-dihydroxy-3,4-O-isopropylidene-pyrrolidin-2-one [133(3R,4R,5R)]. Oxidation of 125 (90 mg, 0.23 mmol) with NaIO4 (126 mg, 0.7 mmol) and RuO2•H2O (8 mg, 0.05 mmol) by Procedure D [column chromatography (50 → 60% EtOAc/hexane)] gave 133 (56 mg, 60%) as a colorless oil with data as reported.164

N-(tert-Butoxycarbonyl)-3,4-dihydroxy-5-(hydroxymethyl)-3,4-O-isopropylidene-pyrrolidin-2-one [134(3R,4R,5R)]. Desilylation of 133 (50 mg, 0.12 mmol) with TBAF (1 M/THF, 0.14 mL, 0.14 mmol), as described for 132, [column chromatography (70→ 80% EtOAc/hexane)] gave 134 (27 mg, 77%) as a colorless oil: ^1H NMR δ 1.36 (s, 3, CH3), 1.46 (s, 12, t-Bu, CH3), 3.88 (dt, J = 3.9, 5.3 Hz, 1, H5), 4.03 (dd, J = 4.1, 11.4 Hz, 1, H6), 4.19 (dd, J = 3.8, 11.4 Hz, 1, H6'), 4.57 (dd, J = 5.3, 5.7 Hz, 1, H4), 4.63 (d, J = 5.7 Hz, 1, H3); 13C NMR δ 25.66 (CMe2), 26.98 (CMe2), 27.60 (t-Bu), 56.68 (C5), 66.63 (C6), 76.70 (C3), 77.02 (C4), 83.28 (t-Bu), 112.37 (CMe2), 152.98 (CO), 173.71 (CO); MS (APCI) m/z 320 (100, [MH+MeOH] +).

N-Benzyl-3,4-dihydroxy-3,4-O-isopropylidene-5-[(methanesulfonyloxy)methyl]pyrrolidin-2-one [135(3R,4R,5R)]. Mesylation of 132 (67 mg, 0.24 mmol) with MsCl (41.0 mg, 27 µL, 0.36 mmol) by Procedure A gave 135 (83 mg, 97%) as a
colorless oil of sufficient purity to be used directly in next step: $^1$H NMR $\delta$ 1.30 (s, 3, CH$_3$), 1.36 (s, 3, CH$_3$), 3.07 (s, 3, CH$_3$), 3.65 (t, $J$ = 2.7 Hz, 1, H5), 4.06 (d, $J$ = 15.2 Hz, 1, Bn), 4.16 (dd, $J$ = 2.2, 11.0 Hz, 1, H6), 4.22 (dd, $J$ = 3.0, 11.0 Hz, 1, H6'), 4.51 (d, $J$ = 5.6 Hz, 1, H4), 4.69 (d, $J$ = 5.6 Hz, 1, H3), 4.92 (d, $J$ = 15.2 Hz, 1, Bn), 7.18-7.30 (m, 5, Bn); MS (APCI) m/z 388 (100, [MH+ MeOH]$^+$), 356 (40, MH$^+$).

$N$-(tert-Butoxycarbonyl)-3,4-dihydroxy-3,4-O-isopropylidene-5-[(methanesulfonyloxy)methyl]pyrrolidin-2-one [136(3$R$,4$R$,5$R$)]. Oxidation of 127 (80 mg, 0.32 mmol) with NaIO$_4$ (172 mg, 0.96 mmol) and RuO$_2$$\times$H$_2$O (8.5 mg, 0.064 mmol) by procedure D [column chromatography (EtOAc)] gave 136 (78 mg, 95%) as a colorless oil: $^1$H NMR $\delta$ 1.37 (s, 3, CH$_3$), 1.44 (s, 3, CH$_3$), 1.54 (s, 9, t-Bu), 3.01 (s, 3, Ms), 4.39-4.43 ('m', 2, H5,6), 4.58 (d, $J$ = 5.4 Hz, 1, H4), 4.64 (dd, $J$ = 3.1, 11.2 Hz, 1, H6'), 4.70 (d, $J$ = 5.5 Hz, H3); $^{13}$C NMR $\delta$ 25.62 (CMe$_2$), 27.04 (CMe$_2$), 27.97 (t-Bu), 37.70 (Ms), 59.18 (C5), 67.03 (C6), 74.54 (C4), 77.54 (C3), 84.72 (t-Bu), 112.75 (CMe$_2$), 149.66 (CO), 170.20 (CO); MS (APCI) m/z 297 [100, (MH$_2$-Boc+MeOH)$^+$].

$S$-[(N-Benzyl-3,4-dihydroxy-3,4-O-isopropylidene-pyrrolidin-2-one-5-yl)methyl]-$N$-tert-butoxycarbonyl-L-homocysteine tert-butyl ester [137(3$R$,4$R$,5$S$)]. Treatment of 135 (85 mg, 0.24 mmol) with lithium homocysteinate (104 mg, 0.36 mmol) by Procedure B [column chromatography (60 $\rightarrow$ 70% EtOAc/hexane)] gave 137 (94 mg, 70%) as a colorless oil. $^1$H NMR $\delta$ 1.28 (s, 3, CH$_3$), 1.37 (s, 12, t-Bu, CH$_3$), 1.39 (s, 9, t-Bu), 1.74-1.86 (m, 1, H8), 1.95-2.17 (m, 1, H8'), 2.46-2.57 (m, 2, H7,7'), 2.69-2.73 (m, 2, H6,6'), 3.59-3.71 (m, 1, H5), 3.86 (d, $J$ = 15.0 Hz, 1, Bn), 4.17-4.18 (m, 1, H9), 4.43 (d, $J$ = 5.7 Hz, 1, H4), 4.79 (d, $J$ = 5.5 Hz, 1, H3), 4.97 (d, $J$ = 15.0 Hz, 1, Bn), 5.05 (br. d, $J$ = 7.1 Hz, 1, NH); 7.17-7.27 (m, 5, Bn); $^{13}$C NMR $\delta$ 25.63 (CMe$_2$), 27.04 (CMe$_2$), 27.99 (t-Bu), 28.31 (t-Bu), 29.02 (C7), 33.13 (C6), 33.18 (C8), 44.40 (Bn), 53.08 (C9), 60.34 (C5), 77.19 (C4), 77.48 (C3), 79.90 (t-Bu) 82.35 (t-Bu), 112.09 (CMe$_2$), 127.94,
128.16, 128.80, 135.18 (Bn), 152.98 (CO), 171.11 (CO), 171.35 (CO); MS m/z 551 (100, MH+).

HRMS (AP-ESI) m/z calculated for C_{28}H_{43}N_{2}O_{7}S [MH]+ 551.2785; found 551.2792.

**S-[(3,4-Dihydroxy-3,4-O-isopropylidene)pyrrolidin-2-one-5-yl]methyl]-N-tert-butoxycarbonyl-L-homocysteine tert-butyl ester [138(3R,4R,5S)].** Treatment of 136 (70 mg, 0.19 mmol) with lithium homocysteinate (83 mg, 0.28 mmol) by Procedure B [column chromatography (70% → 80% EtOAc/hexane)] gave 138 (40 mg, 45%) as a light yellow oil. \(^1\)H NMR δ 1.36 (s, 3, CH₃), 1.43 (s, 9, t-Bu), 1.45 (s, 12, t-Bu, CH₃), 1.82-1.90 (m, 1, H8), 1.97-2.06 (m, 1, H8'), 2.58 ('t', J = 7.4 Hz, 2, H7,7'), 2.64-2.72 (m, 2, H6,6'), 3.83 ('t', J = 5.8 Hz 1, H5), 4.23-4.24 (m, 1, H9), 4.48 (d, J = 4.4 Hz, 1, H4), 4.69 (d, J = 4.6 Hz, 1, H3), 5.21 (br. d, J = 7.9 Hz, 1, NH); \(^{13}\)C NMR δ 25.55 (CMe₂), 26.98 (CMe₂), 27.98 (t-Bu), 28.43 (t-Bu), 29.16 (C7), 32.88 (C8), 33.78 (C6), 53.15 (C9), 60.38 (C5), 75.90 (C4), 77.52 (C3), 79.98 (t-Bu) 82.36 (t-Bu), 112.41 (CMe₂), 149.88 (CO), 155.94 (CO), 170.37 (CO), 171.15 (CO); MS (APCI) m/z 461 (100, MH+).

**S-[N-(tert-Butoxycarbonyl)-3,4-dihydroxy-3,4-O-isopropylidene]pyrrolidin-2-one-5-yl]methyl]-N-tert-butoxycarbonyl-L-homocysteine tert-butyl ester [139(3R,4R,5S)].**

**Procedure E.** DMAP (18.8 mg, 0.15 mmol) and (Boc)₂O (46.4 mg, 0.21 mmol) were added to a stirred solution of compound 138 (27 mg, 0.06 mmol) in CH₂Cl₂ (2 mL) at ambient temperature under Ar atmosphere. After 48 h, the reaction mixture was quenched with H₂O (5 mL) and partitioned (CH₂Cl₂/NaHCO₃/H₂O). The organic layer was washed (brine), dried (MgSO₄) and evaporated. The residue was column chromatographed (30→ 40% EtOAc/hexane) to give 139 (30 mg, 93%) as a colorless oil: \(^1\)H NMR δ 1.36 (s, 3, CH₃), 1.43 (s, 9, t-Bu), 1.45 (s, 3, CH₃), 1.46 (s, 9, t-Bu), 1.54 (s, 9, t-Bu), 1.82-1.87 (m, 1, H8), 2.02-2.04 (m, 1, H8'), 2.49-2.55 (m, 1, H7), 2.58-2.62 (m, 1, H7'), 2.70 (dd, J = 7.1, 14.2 Hz, 1, H6), 2.95 (dd, J = 2.5, 14.2 Hz, 1, H6'), 4.22-4.24 (m, 1, H9), 4.23 (dd, J = 2.5, 7.1 Hz, 1, H5), 4.46 (d, J = 5.5 Hz, 1, H4), 4.80 (d, J = 5.5 Hz, 1, H3), 5.08 (br. d, J = 7.9 Hz, 1, NH); \(^{13}\)C NMR δ 25.56 (CMe₂), 26.93 (CMe₂), 27.98 (t-Bu),
28.33 (t-Bu), 28.42 (t-Bu), 28.91 (C7), 33.21 (C8), 36.58 (C6), 53.15 (C9), 60.39 (C5), 77.52 (C4), 75.90 (C3), 82.28 (2x t-Bu), 84.11 (t-Bu), 112.41 (CMe2), 149.88 (CO), 155.50 (CO), 170.37 (CO), 171.15 (CO); MS (ESI) m/z 583 (100, [M+Na]+).

S-[N-Benzyl-3,4-dihydroxypropyrrolidin-2-one-5-yl)methyl]-L-homocysteine [140(3R,4R,5S)]. Treatment of 137 (40 mg, 0.07 mmol) with TFA by Procedure C (step a, 3 h) gave crude 140 as colorless oil. RP-HPLC purification (5% CH3CN/H2O for 30 min followed by gradient 5 → 90% CH3CN/H2O for 30 min at 2.5 mL/min; tR = 45 min) gave 140 (12 mg, 48%): 1H NMR (D2O) δ 1.88-2.01 (m, 2, H 8,8'), 2.47-2.51 (m, 2, H 9,9'), 2.61 (dd, J = 8.4, 14.0 Hz, 1, H6), 2.72 (dd, J = 3.9, 14.0 Hz, 1, H6'), 3.47 (dd, J = 3.6, 8.3 Hz, 1, H5), 3.68 ('t', J = 5.8 Hz, 1, H9), 4.26 (dd, J = 5.3 Hz, 1, H4), 4.27 (d, J = 14.7 Hz, 1, Bn), 4.62 (d, J = 5.2 Hz, 1, H3), 4.68 (d, J = 14.7 Hz, 1, Bn), 7.23-7.36 (m, 5, Bn); 13C NMR (D2O) δ 27.71 (C7), 30.31 (C8), 30.75 (C6), 45.18 (Bn), 53.58 (C9), 63.87 (C5), 70.03 (C4), 70.14 (C3), 128.03, 128.07, 128.02, 135.12 (Bn), 173.88 (CO), 174.73 (CO); MS (APCI) m/z 355 (50, MH+). HRMS (AP-ESI) m/z calculated for C16H22N2O5S [M+Na]+ 377.1147; found 377.1156.

S-[N-(Benzyl)-3,4-dihydroxypropyrrolidin-2-one-5-yl)methyl]-L-homocysteine [141(3R,4R,5S)]. Treatment of 138 (30 mg, 0.065 mmol) with TFA by Procedure C (step a, 3 h) gave crude 141 as colorless oil. RP-HPLC purification (5% CH3CN/H2O at 2.5 mL/min; tR = 16 min) gave 141 (10 mg, 58%) : 1H NMR (D2O) δ 2.00-2.18 (m, 2, H8,8'), 2.65-2.67 (m, 3, H6,7,7'), 2.73-2.74 (m, 1, H6'), 3.63 ('t', J = 6.5 Hz, 1, H5), 3.85 ('t', J = 5.8 Hz, 1, H9), 4.19 ('d', J = 5.2 Hz, 1, H4), 4.47 (d, J = 5.2 Hz, 1, H3); 13C NMR (D2O) δ 26.21 (C7), 28.53 (C8), 32.70 (C6), 58.83 (C5), 51.07 (C9), 69.71 (C3), 70.46 (C4), 170.87 (CO), 176.10 (CO); MS (ESI) m/z 265 (100, MH+).

S-[N-(tert-Butoxycarbonyl)-3,4-dihydroxy-3,4-O-isopropylidenepyrrolidin-2-ol-5-yl)methyl]-N-tert-butoxycarbonyl-L-homocysteine tert-butyl ester [142(3R,4R,5S)]. Procedure F. LiEt3BH (1 M/THF; 125 μL, 0.12 mmol) was added to a stirred solution of 139 (28 mg, 0.05 mmol) in anhydrous THF (1 mL) at -78°C under N2 atmosphere. After 30 min, the
solution was quenched with water and volatiles were evaporated. The residue was partitioned (EtOAc//NaHCO₃/H₂O), washed (brine) and dried (MgSO₄). The resulting oil was chromatographed (40% EtOAc/hexane) to give 142 [26 mg, 92%; mixture of anomers (α/β, 3:2) which appear as a set of rotamers (1:1)] as colorless oil: ¹H NMR δ 1.30 (s, 3, CH₃), 1.43 (s, 12, t-Bu, CH₃), 1.46 (s, 9, t-Bu), 1.48 (s, 9, t-Bu), 1.83-1.94 (m, 1, H₈), 2.03-2.10 (m, 1, H₈'), 2.51-2.62 (m, 3, H₇,H₇',6), 2.83 (dd, J = 3.7, 13.5 Hz, 0.6, H₆'), 2.92 (dd, J = 3.5, 13.7 Hz, 0.4, H₆'), 3.45 (dd, J = 3.5, 10.6 Hz, 0.3, H₅), 3.99-4.28 (m, 2.7, H₅), 4.57 (d, J = 5.8 Hz, 0.6, H₄), 4.59 (d, J = 5.9 Hz, 0.4, H₄), 4.66 (d, J = 6.7 Hz, 0.4, H₃), 4.72 (d, J = 5.8 Hz, 0.6, H₃), 5.09 (br. d, J = 7.2 Hz, 0.6, NH); 5.32 (br. d, J = 7.8 Hz, 0.4, NH), 5.39 (s, 0.4, H₂), 5.50 (s, 0.6, H₂); ¹³C NMR δ (major isomer) 24.82 (C₂Me₂), 26.68 (C₂Me₂), 27.99 (tBu), 28.31 (tBu), 28.38 (tBu), 29.68 (C₇), 32.85 (C₈), 34.68 (C₆), 53.21 (C₉), 63.86 (C₅), 82.76 (C₃), 84.44 (C₄), 87.13 (C₂), 81.22 (2×t-Bu), 82.31 (t-Bu), 112.65 (C₂Me₂), 154.31 (CO), 155.39 (CO), 171.25 (CO); MS (ESI) m/z 585 (100, [M+Na]+).

S-([3,4-Dihydroxy-pyrroloidin-2-ol-5-yl)methyl]-L-homocysteine 143(3R,4R,5S)]. Treatment of 142 (24 mg, 0.04 mmol) with TFA by Procedure C (step a, 1 h; step b, 10 h at 0 °C) gave crude 143. The RP-HPLC (5% CH₃CN/H₂O at 2.5 mL/min; tᵣ = 14 min) yielded 143 [8 mg, 72%; as mixture of anomers, (α/β, 3:2)] as a slightly yellow oil: ¹H NMR (D₂O) δ 2.15-2.24 (m, 1, H₈), 2.26-2.37 (m, 1, H₈'), 2.75-2.82 (m, 2, H₇,H₇'), 2.84-2.98 (m, 1, H₆,6'), 3.09-3.17 (m, 1, H₆,6'), 3.55-3.69 (m, 0.6, H₅), 3.76-3.83 (m, 0.4, H₅), 4.15-4.21 (m, 2, H₃,4,9), 4.23-4.25 (m, 0.4, H₃), 4.34 (dd, J = 4.8, 6.3 Hz, 0.6, H₄), 5.27 (d, J = 2.6 Hz, 0.6, H₂); 5.41 (d, J = 2.2 Hz, 0.4, H₂); ¹³C NMR δ (major isomer) 25.83 (C₇), 28.66 (C₈), 30.72 (C₆), 51.28 (C₉), 58.76 (C₅), 71.98 (C₄), 72.96 (C₃), 86.54 (C₂), 171.15 (CO); MS (ESI) m/z 267 (50, MH⁺), 249 (100, [M-17]⁺).

N-(tert-Butyloxycarbonyl)-5-(tert-butyldimethylsiloxyl)-2,3-O-isopropylidene-4-amino-4-deoxy-α/β-D-ribofuranose (144). Reduction of 133 [68 mg, 0.17 mmol] with LiEt₃BH (1
M/THF; 0.43 mL, 0.43 mmol) in anhydrous THF (2 mL) at -78 ºC by the procedure F gave 144\(^{164}\) (68 mg, 100%) as a colorless oil with data as reported.

\textbf{N-(tert-Butoxycarbonyl)-2,3-O-isopropylidene-4-amino-4-deoxy-\alpha/\beta-D-ribofuranose (145).} Desilylation of 144 (55 mg, 0.13 mmol) with TBAF (1 M/THF, 0.19 mL, 0.19 mmol), as described for 132, [column chromatography (50 → 60% EtOAc/hexane)] gave 145 (\(\alpha/\beta\), 3:2; 39 mg, 96%) as a colorless oil: \(^1\)H NMR \(\delta\) 1.30 (s, 3, CH\(_3\)), 1.40 (s, 3, CH\(_3\)), 1.46 (s, 5.4, t-Bu), 1.49 (s, 3.6, t-Bu), 2.87 (br. s, 0.4, OH), 3.15 (br. s, 0.6, OH), 3.6-3.77 (m, 1.6, H5,\(^5\)), 3.84-3.90 (m, 0.8, H5',OH), 4.10 ('s', 0.6, H4), 4.27 ('s', 0.4, H4), 4.30 (br. s, 0.6, OH), 4.55 (d, \(J = 5.9\) Hz, 1, H3), 4.73 (d, \(J = 5.9\) Hz, 0.4, H2), 4.77 (d, \(J = 5.9\) Hz, 0.6, H2), 5.36 (d, \(J = 5.7\) Hz, 0.4, H1), 5.51 (s, 0.6, H1); \(^{13}\)C NMR (\(\alpha\) anomer) \(\delta\) 24.58 (C\(_\text{Me2}\)), 26.58 (C\(_\text{Me2}\)), 28.33 (t-Bu), 62.61 (C5), 65.84 (C4), 81.87 (C2), 81.34 (t-Bu), 85.31 (C3), 86.40 (C1), 111.39 (CMe2), 154.29 (NHCO); \(^{13}\)C NMR (\(\beta\) anomer) \(\delta\) 24.69 (C\(_\text{Me2}\)), 26.66 (CMe2), 28.33 (t-Bu), 62.61 (C5), 65.84 (C4), 81.21 (C2), 81.34 (t-Bu), 86.40 (C3), 86.53 (C1), 111.39 (CMe2), 153.53 (CO); MS (APCI) \(m/z\) 272 (50, \([\text{M-17}]^+\)), 213 (100, \([\text{MH-Boc-OH+CH}_3\text{CN}]^+\)).

\textbf{4-Amino-4-deoxy-\alpha/\beta-D-ribofuranose (146a).} Treatment of 145 (27 mg, 0.09 mmol) with TFA by Procedure C (step a, 5 h; step b, 6 h at 0°C) gave crude 146a (13 mg, 92% as a light yellow oil of a mixture of anomers (\(\alpha/\beta\), 0.65:0.35): \(^1\)H NMR (D\(_2\)O) 3.37-3.38 (m, 0.35, H4), 3.41-3.44 (m, 0.65, H4), 3.61-3.63 (m, 0.65, H3), 3.70 (dd, \(J = 6.0\), 13.1 Hz, 0.35, H5), 3.71 (dd, \(J = 2.1\), 12.8 Hz, 0.65, H5), 3.83 (m, 0.35, H3), 3.91 (dd, \(J = 3.0\), 4.0 Hz, 0.35, H2), 3.95 (dd, \(J = 2.8\), 13.5 Hz, 0.35, H5'), 4.01 (dd, \(J = 3.0\), 12.8 Hz, 0.65, H5'), 4.05 (t', \(J = 3.4\) Hz, 0.65, H2), 4.69 (d, \(J = 1.3\) Hz, 0.35, H1), 4.97 (d, \(J = 4.0\) Hz, 0.65, H1); \(^{13}\)C NMR (major rotamer) \(\delta\) 50.13 (C4), 58.86 (C5), 65.61 (C2), 69.84 (C3), 94.05 (C1); \(^{13}\)C NMR (minor rotamer) \(\delta\) 49.85 (C4), 61.50 (C5), 63.56 (C2), 69.79 (C3), 94.05 (C1); MS (APCI) \(m/z\) 150 (100, MH\(^+\)).

\textbf{4-Amino-4-deoxy-D-ribose O-Benzylxime (147).} A solution of 146a (13 mg, 0.09 mmol) and O-benzylhydroxylamine hydrochloride (43 mg, 0.27 mmol) in anhydrous pyridine (4 mL)
was stirred under an atmosphere of nitrogen at room temperature for 12 h. Pyridine was evaporated to give 147 of sufficient purity for spectroscopic analysis: $^1$H NMR (MeOD) $\delta$ 3.52 ('dt', $J = 4.1, 8.4$ Hz, 1, H4), 3.79 (dd, $J = 3.8, 11.5$ Hz, 1, H5), 3.85 (dd, $J = 4.4, 8.7$ Hz, 1, H3), 3.94 (dd, $J = 8.4, 11.5$ Hz, 1, H5'), 4.13 (dd, $J = 6.8, 8.7$ Hz, 1, H2), 4.92-5.16 (Bn, 2H, signal within the envelope of the solvent peaks but cross peaks to each other protons observed in COSY and confirmed by HETCOR), 7.41 (H1, signal within the envelope of Bn protons but cross peaks to H2 observed in COSY), 7.35-7.47 (m, 5H, Bn); $^{13}$C NMR $\delta$ 56.20 (C4), 58.81 (C5), 71.22 (C3), 71.56 (C2), 77.01 (Bn), 128.91, 129.30, 129, 139.07 (Bn); MS (ESI) $m/z$ 255 (60, MH$^+$).

5-O-tert-Butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol (148). To a solution of 124 (109 mg, 0.28 mmol) in EtOH (6 mL) was added 5% Pd/C (300 mg) and stirred under an atmosphere of H$_2$ at room temperature for 6 h. The mixture was filtered through celite to remove the catalyst. The celite bed was washed with ethanol (5 mL) and washings and the filtrate were combined and evaporated. The residue was column chromatographed (30% EtOAc/hexane) to give 148 as a colorless oil (70 mg, 80%): $^1$H NMR $\delta$ 0.04 (s, 3, CH$_3$), 0.04 (s, 3, CH$_3$), 0.87 (s, 9, t-Bu), 1.32 (s, 3, CH$_3$), 1.46 (s, 3, CH$_3$), 2.33 (s, 1, NH), 2.98 ('dt', $J = 2.6$ Hz, 2, H1,1'), 3.20 ('dt', $J = 0.6, 5.8$ Hz, 1, H4), 3.52 (dd, $J = 5.9, 10.3$ Hz, 1, H5), 3.62 (dd, $J = 5.1, 10.3$ Hz, 1, H5'), 4.63 (dd, $J = 0.8, 5.8$ Hz, 1, H3), 4.68 ('dt', $J = 2.6, 5.8$ Hz, H2) MS (APCI) $m/z$ 288 (100, MH$^+$).

5-O-tert-Butyldimethylsilyl-1,N-didehydro-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol N–Oxide (149). A stirred solution of 148 (70 mg, 0.24 mmol) and SeO$_2$ (0.01 mmol, 1.1 mg) in acetone (3 mL) was cooled to -4 °C under N$_2$ atmosphere and H$_2$O$_2$ (25%) was added slowly (3-4 h) until the reaction was completed (monitored by TLC). Volatiles were evaporated and the residue was partitioned (EtOAc//NaHCO$_3$/H$_2$O). The organic layer was collected, washed (brine) and dried (MgSO$_4$). The resulting solid was chromatographed (50% EtOAc/hexane) to give 148 (54 mg, 73%) as a white crystalline solid with data as reported.
1,N-Didehydro-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol N-Oxide (150).

Desilylation of 149 (155 mg, 0.51 mmol) with TBAF (1 M/THF, 0.77 mL, 0.77 mmol) at -4 °C as described for 132 [column chromatography (10%→20% MeOH/CHCl₃)] gave 150 (40 mg, 42%) as a white crystalline solid: ¹H NMR δ 1.34 (s, 3, CH₃), 1.41 (s, 3, CH₃), 3.87 (dd, J = 2.1, 19.6 Hz, 1, H₅), 4.02-4.03 (m, 1, H₄), 4.14 (dd, J = 2.3, 11.9 Hz, 1, H₅'), 4.96 (d, J = 6.2 Hz, 1, H₃), 5.21 ('dt', J = 1.4, 6.2 Hz, 1, H₂), 6.90 (br. s, 1, H₁); ¹³C NMR δ 25.66 (C₆Me₂), 27.15 (C₆Me₂), 58.87 (C₅), 77.16 (C₃), 78.85 (C₄), 80.78 (C₂), 111.50 (C₆Me₂), 134.63 (C₁); MS (ESI) m/z 186 (100, [M-1]+).

1,N-Didehydro-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-5-O-methanesulfonyl-D-ribitol N–Oxide (151). Treatment of 150 (40 mg, 0.21 mmol) at -4 °C with MsCl (26 µL, 0.32 mmol) by Procedure A [column chromatography (80% → 90% EtOAc/hexane)] gave 151 (24 mg, 56%) as a colorless oil: ¹H NMR δ 1.37 (s, 3, CH₃), 1.46 (s, 3, CH₃), 3.04 (s, 3, Ms), 4.23-4.24 (m, 1, H₄), 4.55 (dd, J = 1.8, 11.1 Hz, 1, H₅), 4.83 (dd, J = 2.4, 11.1 Hz, 1, H₅'), 4.91 (dd, J = 1.1, 6.3 Hz, 1, H₃), 5.25 ('dt', J = 1.5, 6.4 Hz, 1, H₂), 6.99 (s, 1, H₁); ¹³C NMR δ 25.56 (C₆Me₂), 27.07 (C₆Me₂), 37.42 (Ms), 65.54 (C₅), 75.78 (C₃), 77.75 (C₄), 78.43 (C₂), 112.64 (C₆Me₂), 134.10 (C₁); MS (APSI) m/z 266 (100, MH⁺).

S-(1,N-Didehydro-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol-5-yl N-Oxide)-N-tert-butoxycarbonyl-L-homocysteine tert-butyl ester (152). Treatment of 151 (24 mg, 0.09 mmol) with L- homocysteinate (38 mg, 0.13 mmol) by Procedure B (step a and b, 8 h at -20°C) and purification by column chromatography (80% → 90% EtOAc/Hexane) gave 152 as a colorless oil (24 mg, 43%): ¹H NMR δ 1.44 (s, 9, t-Bu ), 1.45 (s, 3, CH₃), 1.46 (s, 9, t-Bu), 1.81-1.90 (m, 1, H₈), 1.96-2.09 (m, 1, H₈'), 2.52-2.70 (m, 2, H₇,H₇'), 3.07 (dd, J = 3.5, 14.4 Hz, 1, H₅), 3.14 (dd, J = 5.2, 14.4 Hz, 1, H₅'), 4.20-4.31 (m, 2, H₄,9), 4.71 (d, J = 6.2 Hz, 1, H₃), 5.06 (br. s, 1, NH), 5.31 ('dt', J = 1.4, 6.4 Hz, 1, H₂), 6.97 (s, 1, H₁); ¹³C NMR δ 25.64 (C₆Me₂), 26.07 (C₆Me₂), 28.00 (t-Bu), 28.32 (t-Bu), 29.07 (C₇), 32.00 (C₅), 32.81 (C₈), 53.07 (C₉), 77.94 (C₃),
S-(1,N-Didehydro-1,4-dideoxy-1,4-imino-D-ribitol-5-yl N-Oxide)-L-homocysteine (153). Treatment of 152 (72 mg, 0.15 mmol) with TFA by Procedure C (step a, 5 h; step b, 6 h at 0 °C) gave crude 152. Purification by HPLC (5% CH3CN/H2O for at 2.5 mL/min; tR = 10-14 min) afforded 153 (16 mg, 40 %) as a white solid: 1H NMR (D2O) δ 2.01-2.17 (m, 2, H8,8'), 2.83-2.89 (m, 2, H7,7'), 2.97 (dd, J = 6.3, 14.4 Hz, 1,H5), 3.06 (dd, J = 3.8, 14.4 Hz, 1, H5'), 3.73-3.77 (m, 1, H9), 4.08-4.22 (m, 1, H4), 4.40 (dd, J = 3.2, 6.0 Hz, 1, H3), 4.89-4.96 (m, 1, H2), 7.25 (s, 1, H1); 13C NMR δ 27.63 (C7), 29.87 (C5), 30.51 (C8), 53.73 (C9), 70.42 (C3), 78.26 (C4), 80.71 (C2), 141.80 (C1), 173.92 (CO); MS (APCI) m/z 265 (50, MH+). HRMS (AP-ESI) m/z calculated for C9H16N2NaO5S [MH+Na]+ 287.0678; found 287.0664.

1,N-Didehydro-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-5-O-methanesulfonyl-1-methyl-D-ribitol (155). Treatment of 154166 (48.5 mg, 0.26 mmol) with MsCl (0.031 mL, 45 mg, 0.39 mmol) in the presence of Et3N (0.11 mL, 80 mg, 0.79 mmol) by Procedure A [3 h; column chromatography (EtOAc → 10% MeOH/EtOAc)] gave 155 (59 mg, 85%) as a colorless oil: 1H NMR δ 1.38 (s, 3, CH3), 1.39 (s, 3, CH3), 2.16 (d, J = 1.0 Hz, 3, N=CMe), 3.00 (s, 3, Ms), 4.38 (s, 1, H4), 4.39 (dd, 1, J = 3.6, 11.2 Hz, 1, H5), 4.53 (dd, 1, J = 4.2, 11.3 Hz, 1, H5'), 4.64 (d, J = 5.8 Hz, 1, H3), 4.95 ('q', J = 5.6 Hz, 1, H2); 13C NMR δ 17.08 (N=CMe), 25.70 (CMe2), 26.84 (CMe2), 37.37 (Ms), 69.68 (C5), 74.97 (C4), 79.77 (C3), 87.42 (C2), 112.37 (CMe2), 177.04 (C=N); MS (APCI) m/z 264 (100, MH+).

S-(1,N-Didehydro-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-1-methyl-D-ribitol-5-yl)-N-(tert-butoxycarbonyl)-L-homocysteine tert-butyl ester (156). Treatment of 155 (49.5 mg, 0.19 mmol) with protected L-Hcy (82 mg, 0.284 mmol) by Procedure B (step a and b, 36 h) gave 124 mg of yellowish oily residue. Crude product was column chromatographed (50→ 60% EtOAc/hexane) to give 156 as an colorless oil (73 mg, 85%): 1H NMR δ 1.35 (s, 3, CH3 ), 1.35 (s,
S-(1,N-Didehydro-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-1-methyl-D-ribitol-5-yl)-L-homocysteine (157). Treatment of 156 (62 mg, 0.136 mmol) with TFA by Procedure C (step a, ambient temperature) gave 157 (41 mg, 99%) as a colorless oil: \(^1\)H NMR (D\(_2\)O) \(\delta\) 1.38 (s, 3, CH\(_3\)), 1.41 (s, 3, CH\(_3\)), 2.10-2.20 (m, 1, H8), 2.22-2.31 (m, 1, H8'), 2.57 (br. s, 3 \(\rightarrow\) 0, exchanged with D\(_2\)O within few hours, N=CMe), 2.75 (t, \(J = 7.3\) Hz, 2, H7,7'), 2.99 (d, \(J = 6.2\) Hz, 2, H5,5'), 4.17 (t, \(J = 6.4\) Hz, 1, H9), 4.73 ('t', \(J = 6.3\) Hz, 1, H4), 4.91 (d, \(J = 5.4\) Hz, 1, H2), 5.64 (d, \(J = 5.3\) Hz, 1, H3); \(^1\)H NMR (DMSO) \(\delta\) 1.28 (s, 3, CH\(_3\)), 1.30 (s, 3, CH\(_3\)), 1.95-2.07 (m, 2, H8,8'), 2.08 (s, 3, N=CMe), 2.60 (dd, \(J = 7.4\), 13.6 Hz, 1, H5) 2.65 ('t', \(J = 7.9\) Hz, 2, H7,7'), 2.82 (dd, \(J = 5.2, 13.7\) Hz, 1, H5'), 4.01 (br. s, 1, H9), 4.21 ('t', \(J = 5.8\) Hz, 1, H4), 4.51 (d, \(J = 5.5\) Hz, 1, H2), 5.10 (d, \(J = 5.5\) Hz, 1, H3), 7.28 (br. s, 3, N\(^3\)NH\(_3\)); \(^{13}\)C NMR (D\(_2\)O) \(\delta\) 15.45 ('q', \(J = 20\) Hz, N=C=CD\(_3\)), 23.99 (CMe\(_2\)), 25.44 (CMe\(_2\)) 27.41 (C7), 29.46 (C8), 31.63 (C5), 51.36 (C9), 71.15 (C4), 78.97 (C2), 83.97 (C3), 114.23 (CMe\(_2\)), 171.45 (COOH), 191.73 (N=C=CD\(_3\)); \(^{13}\)C NMR (DMSO) \(\delta\) 16.53 (N=C=CD\(_3\)), 25.38 (CMe\(_2\)), 26.59 (CMe\(_2\)), 27.47 (C7), 30.07 (C8), 33.24 (C5), 50.94 (C9), 74.75 (C4), 80.76 (C2), 86.05 (C3), 111.13 (CMe\(_2\)), 170.67 (CO), no signal (N=C=CD\(_3\)); MS (APCI) m/z 303 (100, MH\(^+\)).

S-(1,N-Didehydro-1,4-dideoxy-1,4-imino-1-methyl-D-ribitol-5-yl)-L-homocysteine (158). Treatment of 157 (41 mg, 0.136 mmol) with TFA/H\(_2\)O by Procedure C (step b, TFA/H\(_2\)O 4:1,
ambient temperature, 12 h) gave homogenous 158 (36 mg, 98%) as a colorless oil: $^1$H NMR (D$_2$O) $\delta$ 2.05-2.16 (m, 1, H8), 2.17-2.27 (m, 1, H8'), 2.45 (br. s, 3→0, exchanged with D within few hrs, N=CMe), 2.73 (dt, $J = 3.3$, 7.3 Hz, 2, H7,7'), 2.78 (dd, $J = 8.3$, 14.4 Hz, 1, H5), 2.92 (dd, $J = 6.0$, 14.3 Hz, 1, H5') 4.14 (t, $J = 6.4$ Hz, 1, H9), 4.35 ('t', $J = 7.1$ Hz, 1, H4), 4.37 (d, $J = 5.3$ Hz, 1, H2), 5.12 (d, $J = 5.5$ Hz, 1, H3); $^{13}$C NMR (D$_2$O) $\delta$ 15.36 ('q', $J = 21$ Hz, N=CMe), 26.95 (C7), 30.73 (C5), 51.31 (C9), 71.62 (C2), 71.92 (C4), 76.75 (C3), 171.36 (CO), 195.79 (N=CCD$_3$); MS (APCI) $m/z$ 263 (100,MH$^+$).

5-(Propylthiomethyl)pyrrolidin-2-one [160a(5S)]. Procedure G. Propanethiol (50 μL, 42 mg, 0.55 mmol) was added dropwise to a stirred suspension of NaH (35 mg, 0.875 mmol, 60% mineral oil) in dry DMF (1 mL) under Ar atmosphere at 0°C. After 10 min (till gas evolution has ceased), solution of compound 159$^{167}$ [(5S), 82 mg, 0.46 mmol] in dry DMF (1 mL) was added dropwise, and after 15 min the reaction mixture was allowed to warm to ambient temperature. After 12 h the resulting mixture was quenched with water at 0 °C, volatiles were evaporated, and the residue was column chromatographed (EtOAc→ 10% MeOH/EtOAc) to give 160a(5S) (77 mg, 96%) as a colorless oil: $^1$H NMR 0.98 (t, $J = 7.3$ Hz, 3, H9), 1.60 (sx, $J = 7.3$ Hz, 2, H8,8'), 1.76-1.87 (m, 1, H4), 2.25-2.34 (m, 1, H4'), 2.34-2.45 (m, 2, H3,3'), 2.52 (t, $J = 7.3$ Hz, 2, H7,7'), 2.54 (dd, $J = 7.7$, 13.2 Hz, 1, H6), 2.68 (dd, $J = 5.5$, 13.2 Hz, 1, H6'), 3.80 ('q', $J = 5.5$ Hz, 1, H6), 6.73 (br. s, 1, NH); $^{13}$C NMR $\delta$ 13.39 (C9), 23.05 (C8), 26.64 (C4), 30.16 (C3), 34.65 (C7), 38.56 (C6), 53.90 (C5), 177.95 (CO); MS (APCI) $m/z$ 174 (MH$^+$). HRMS (AP-ESI) $m/z$ calculated for C$_8$H$_{15}$NNaOS [M+Na]$^+$ 196.0772; found 196.0779.

5-(Hexylthiomethyl)pyrrolidin-2-one [160b(5S)]. Treatment of 159$^{167}$ [(5S), 823 mg, 4.62 mmol] in dry DMF (6 mL) with a thiolate soln in dry DMF (6 mL) generated from hexanethiol (682 μL, 573 mg, 4.86 mmol), and NaH (204 mg, 5.09 mmol, 60%/mineral oil) by Procedure G [column chromatography (80% EtOAc/hexane→ 5% MeOH/EtOAc)] gave 160b(5S) (932 mg, 94%) as a colorless oil. $^1$H NMR $\delta$ 0.90 (t, $J = 7.0$ Hz, 3, H12), 1.24-1.33 (m, 4, H10,H11), 1.33-
1.42 (m, 2, H9), 1.58 ('q', $J = 7.4$ Hz, 2, H8,8'), 1.78-1.87 (m, 1, H4), 2.27-2.46 (m, 3, H4',H3,3'), 2.53 (dd, $J = 8.0$, 13.4 Hz, 1, H6), 2.54 (t, $J = 7.3$ Hz, 2, H7,7'), 2.70 (dd, $J = 5.3$, 13.2 Hz, 1, H6'), 3.81 ('q', $J = 6.6$ Hz, 1, H5), 6.47 (br. s, 1, NH); $^{13}$C NMR $\delta$ 14.00 (C12), 22.51 (C11), 26.75 (C4), 28.47 (C9), 29.72 (C8), 30.10 (C3), 31.37 (C10), 32.67 (C7), 38.74 (C6), 53.77 (C5), 177.65 (CO); MS (APCI) m/z 248 (75, [MH+MeOH]+), (ESI) m/z 216 (100, MH+).

5-(Nonylthiomethyl)pyrrolidin-2-one [160c(5S)]. Treatment of 159\textsuperscript{167} [(5S), 458 mg, 2.58 mmol] in dry DMF (3 mL) with thiolate soln in dry DMF (7 mL) generated from nonanethiol (433 mg, 510 μL, 2.71 mmol) and NaH (114 mg, 2.84 mmol, 60%/mineral oil) in dry DMF (7 mL) by Procedure G [column chromatography (80% EtOAc/hexane→ EtOAc)] gave 160c(5S) (652 mg, 98%) as a colorless oil. $^1$H NMR $\delta$ 0.86 (t, $J = 7.0$ Hz, 3, H15), 1.19-1.29 (m, 10, H10-H14), 1.29-1.38 (m, 2, H9), 1.54 ('q', $J = 7.3$ Hz, 2, H8,8'), 1.75-1.85 (m, 1, H4), 2.23-2.43 (m, 3, H4',H3,3'), 2.51 (t, $J = 7.5$ Hz, 2, H7,7'), 2.54 (dd, $J = 7.4$, 13.2 Hz, 1, H6), 2.65 (dd, $J = 5.8$, 13.2 Hz, 1, H6'), 3.78 ('q', $J = 6.5$ Hz, 1, H5), 6.97 (br. s, 1, NH); $^{13}$C NMR $\delta$ 14.05 (C15), 22.61 (C14), 26.59 (C3), 28.78 (C9), 29.17, 29.20, 29.43 (C11-C13), 29.76 (C8), 30.14 (C3), 31.81 (C10), 32.70 (C7), 38.63 (C6), 53.93 (C5), 177.97 (CO); MS (ESI) m/z 258 (100, MH+).

5-(Dodecylthiomethyl)pyrrolidin-2-one [160d(5S)]. Treatment of 159\textsuperscript{167} [(5S), 448 mg, 2.52 mmol] in dry DMF (3 mL) with thiolate soln in dry DMF (7 mL) generated from dodecanethiol (634 μL, 535 mg, 2.65 mmol), and NaH (110 mg, 2.77 mmol, 60%/mineral oil) in dry DMF (7 mL) by Procedure G [column chromatography (80% EtOAc/hexane→ EtOAc)] gave 160d (5S) (637 mg, 85%) as a colorless oil. $^1$H NMR $\delta$ 0.88 (t, $J = 7.0$ Hz, 3, H18), 1.21-1.32 (m, 16, H10-H17), 1.32-1.40 (m, 2, H9), 1.57 ('q', $J = 7.4$ Hz, 2, H8,8'), 1.77-1.87 (m, 1, H4), 2.26-2.45 (m, 3, H4',H3,3'), 2.53 (t, $J = 7.6$ Hz, 2, H7,7'), 2.54 (dd, $J = 7.8$, 13.2 Hz, 1, H6), 2.68 (dd, $J = 5.4$, 13.2 Hz, 1, H6'), 3.80 ('q', $J = 6.6$ Hz, 1, H5), 6.64 (br. s, 1, NH); $^{13}$C NMR $\delta$ 14.08 (C18), 22.66 (C17), 26.70 (C3), 28.81 (C9), 29.19, 29.32, 29.50, 29.57, 29.60, 29.62 (C11-
C16), 29.77 (C8), 30.11 (C3), 31.89 (C10), 32.69 (C7), 38.71 (C5), 53.83 (C5), 177.74 (CO); MS (ESI) m/z 300 (100, MH+).

**N-tert-Butoxycarbonyl-5-(propylthiomethyl)pyrrolidin-2-one [161a(5S)].** Procedure E: DMAP (114 mg, 0.93 mmol), and (Boc)₂O (398 mg, 1.82 mmol) were added to a stirred solution of compound 160a (77 mg, 0.445 mmol) in CH₂Cl₂ (2 mL) at ambient temperature under Ar atmosphere. After 48 h, the reaction mixture was quenched with H₂O (5 mL) and partitioned between CH₂Cl₂/NaHCO₃/H₂O. The organic layer was washed (brine), dried (MgSO₄) and evaporated. The residue was column chromatographed (30 → 40% EtOAc/hexane) to give 161a (5S) (107 mg, 88%) as a colorless oil: ¹H NMR δ 0.95 (t, J = 7.3 Hz, 3, H9), 1.50 (s, 9, t-Bu), 1.58 (sx, J = 7.3 Hz, 2, H8,8'), 1.96-2.04 (m, 1, H4), 2.06-2.17 (m, 1, H4'), 2.40 (ddd, J = 2.6, 9.6, 17.9 Hz, 1, H3), 2.50 ('dt', J = 4.9, 7.3 Hz, 2, H7,7'), 2.58-2.67 (m, 1, H3'), 2.60 (dd, J = 9.2, 13.5 Hz, 1, H6), 2.86 (ddd, J = 0.5, 2.8, 13.5 Hz, 1, H6'), 4.20-4.27 (m, 1, H5); ¹³C NMR δ 13.31 (C9), 21.91 (C3), 23.12 (C8), 28.03 (t-Bu), 21.91 (C3), 23.12 (C8), 28.03 (t-Bu), 149.75 (CO), 174.17 (CO); MS (ESI) m/z 274 (10, MH+), 215 (100, [MH-59]+).

Structure of compound 160a was additionally confirmed by conversion to the corresponding O-benzylxime derivative with benzylhydroxylamine hydrochloride (6 equiv.) in anhydrous pyridine: MS (ESI) m/z 281 (60, MH⁺), 158 (100, [M-BnOH]+), (APCI) m/z 281 (100, MH+).

**N-tert-Butoxycarbonyl-5-(Hexylthiomethyl)pyrrolidin-2-one [161b(5S)].** Treatment of 160b (311 mg, 1.45 mmol) in CH₂Cl₂ (6 mL) with DMAP (185 mg, 1.52 mmol), and (Boc)₂O (746 mg, 3.42 mmol) by procedure E [column chromatography (20 → 40% EtOAc/hexane)] gave 161b (5S) (429 mg, 94%) as a colorless oil. ¹H NMR δ 0.89 (t, J = 7.0 Hz, 3, H12), 1.25-1.33 (m, 4, H10,H11), 1.34-1.42 (m, 2, H9), 1.55 (s, 9, t-Bu), 1.59 ('q', J = 7.4 Hz, 2, H8,8'), 2.01-2.08 (m, 1, H4), 2.10-2.21 (m, 1, H4'), 2.45 (ddd, J = 2.5, 9.6, 17.9 Hz, 1, H3), 2.56 ('dt', J = 2.9, 7.3 Hz, 2, H7,7'), 2.62-2.72 (m, 1, H3'), 2.63 (dd, J = 9.3, 13.5 Hz, 1, H6), 2.91 (dd, J = 2.7, 13.5 Hz, 1, H6'), 4.24-4.31 (m, 1, H5); ¹³C NMR δ 13.99 (C12), 21.97 (C3), 22.51 (C11), 28.07 (t-Bu), 28.42
(C9), 29.80 (C8), 31.21 (C3), 31.37 (C10), 32.93 (C7), 35.53 (C6), 57.53 (C5), 83.10 (t-Bu), 149.82 (CO), 174.10 (CO); MS (APCI) m/z 316 (5, M-1\(^{+}\)), (ESI) m/z 315 (15, M\(^{+}\)), 256 (100, [M-59 \(^{+}\)])

\textit{N-tert-Butoxycarbonyl-5-(nonylthiomethyl)pyrrolidin-2-one [161c(5S)]}. Treatment of 160c (250 mg, 0.97 mmol) in CH\(_2\)Cl\(_2\) (5 mL) with DMAP (125 mg, 1.02 mmol), and (Boc)\(_2\)O (712 mg, 3.27 mmol) by procedure E [column chromatography (20 → 25% EtOAc/hexane)] gave 161c(5S) (345 mg, 99\%) as a colorless oil. \(^1\)H NMR \(\delta\) 0.85 (t, \(J = 7.0\) Hz, 3, H15), 1.19-1.29 (m, 10, H10-H14), 1.29-1.38 (m, 2, H9), 1.51 (s, 9, t-Bu), 1.55 (‘q’, \(J = 7.3\) Hz, 2, H8,8’), 1.96-2.04 (m, 1, H4), 2.06-2.17 (m, 1, H4’), 2.40 (ddd, \(J = 2.5, 9.6, 17.8\) Hz, 1, H3), 2.52 (‘dt’, \(J = 3.0, 7.4\) Hz, 2, H7,7’), 2.58-2.67 (m, 1, H3’), 2.60 (dd, \(J = 9.3, 13.5\) Hz, 1, H6), 2.86 (dd, \(J = 2.6, 13.5\) Hz, 1, H6’), 4.21-4.27 (m, 1, H5); \(^{13}\)C NMR \(\delta\) 14.04 (C15), 21.94 (C4), 22.60 (C14), 28.03 (t-Bu), 28.71 (C9), 29.14, 29.18, 29.40 (C11-C13), 29.80 (C8), 31.16 (C3), 31.80 (C10), 32.87 (C7), 35.50 (C6), 57.48 (C5), 82.99 (t-Bu), 149.77 (CO), 174.01 (CO); MS (APCI) m/z 358 (10, MH\(^{+}\)), (ESI) m/z 357 (10, M\(^{+}\)), 299 (100, [MH-59 \(^{+}\)])

\textit{N-tert-Butoxycarbonyl-5-(dodecylthiomethyl)pyrrolidin-2-one [161d(5S)]}. Treatment of 160d (234 mg, 0.78 mmol) in CH\(_2\)Cl\(_2\) (5 mL) with DMAP (100 mg, 0.82 mmol), and (Boc)\(_2\)O (600 mg, 2.75 mmol) by procedure E [column chromatography (15 → 20% EtOAc/hexane)] gave 161d(5S) (302 mg, 97\%) as a solidifying oil. \(^1\)H NMR \(\delta\) 0.87 (t, \(J = 7.0\) Hz, 3, H15), 1.21-1.31 (m, 16, H10-H17), 1.31-1.40 (m, 2, H9), 1.53 (s, 9, Boc), 1.58 (‘q’, \(J = 7.5\) Hz, 2, H8,8’), 1.99-2.06 (m, 1, H4), 2.08-2.20 (m, 1, H4’), 2.43 (ddd, \(J = 2.5, 9.6, 17.9\) Hz, 1, H3), 2.54 (‘dt’, \(J = 2.9, 7.4\) Hz, 2, H7,7’), 2.60-2.70 (m, 2, H3’), 2.62 (dd, \(J = 9.2, 13.5\) Hz, 1, H6), 2.89 (dd, \(J = 2.6, 13.5\) Hz, 1, H6’), 4.23-4.29 (m, 1, H5); \(^{13}\)C NMR \(\delta\) 14.08 (C15), 21.96 (C4), 22.65 (C17), 28.06 (t-Bu), 28.75 (C9), 29.18, 29.31, 29.48, 29.56, 29.59, 29.61 (C11-C16), 29.83 (C8), 31.19 (C3), 31.88 (C10), 32.91 (C7), 35.52 (C6), 57.51 (C5), 83.04 (t-Bu), 149.81 (t-Bu), 174.04 (CO); MS (APCI) m/z 300 (25, [M-Boc\(^{+}\)]), (ESI) m/z 400 (10, MH\(^{+}\)), 341 (100, [MH-59 \(^{+}\)])
5-(Propylthiomethyl)pyrrolidin-2-ol [162a(S)]. Procedure F. LiEt3BH (1M soln in THF, 0.98 mL, 0.98 mmol) was added to a stirred solution of 161a (107 mg, 0.39 mmol) in CH2Cl2 (3 mL) at -78 °C under N2 atmosphere. After 30 min, the solution was quenched with MeOH (4 mL) and allowed to warm to ambient temperature. Volatiles were evaporated. The residue was partitioned (EtOAc// NaHCO3/H2O), washed (brine) and dried (MgSO4). The resulting oil was chromatographed (30 → 40% EtOAc/hexane) to give N-tert-butoxycarbonyl-5-(propylthiomethyl)pyrrolidin-2-ol 162a(S); 104 mg, 96% as a colorless oil of the mixture of 4 anomers/rotamers: MS (APCI) m/z 274 (10, [M -1]+), 258 (90, [M -17]+), (ESI) m/z 258 (65, [M -17]+).

Procedure D. Compound 162a (104 mg, 0.37 mmol) in TFA (4.0 mL) was stirred at rt for 2 h. Volatiles were evaporated to give 163a as light yellow oil having spectral properties of a complex mixture [4 isomers of aza-furanose forms accompanied by 25% of aldehyde (chain form), as indicated by 1H and 13C NMR spectra]: MS (ESI) m/z 158 (10, [M-17]+).

5-(Hexylthiomethyl)pyrrolidin-2-ol [163b(S)]. Treatment of 161b (178 mg, 0.56 mmol) in CH2Cl2 (3 mL) with LiEt3BH (1 M soln in THF, 1.41 mL, 1.41 mmol), by procedure F [column chromatography (30 → 40% EtOAc/hexane)] gave N-tert-butoxycarbonyl-5-(hexylthiomethyl)pyrrolidin-2-ol 162b(S); 170 mg, 95% as a colorless oil as a mixture of 4 isomers: MS (ESI) m/z 316 (100, [M-1]+), 300 (20, [M -17]+). Treatment of 162b with an excess of TFA by Procedure D (step a, 2 h at rt) gave crude 163b as light yellow oil as amixture of 4 isomers of the aza-furanose form accompanied by 25% of aldehyde (chain form); MS (APCI) m/z 200 (100, [M-17]+) (ESI) m/z 200 (10, [M-17]+).

5-(Nonylthiomethyl)pyrrolidin-2-ol [163c(S)]. Treatment of 161c (227 mg, 0.64 mmol) in CH2Cl2 (4 mL) with LiEt3BH (1 M soln in THF, 1.59 mL, 1.59 mmol), by procedure F [column chromatography (20 → 30% EtOAc/hexane)] gave tert-Butoxycarbonyl-5-(nonylthiomethyl)pyrrolidin-2-ol 162c(S); 220 mg, 96%; as a colorless oil of a complex mixture of 4 isomers: MS (ESI) m/z 358 (10, [M-1]+), 342 (100, [M-17]+). Treatment of 162c
with an excess of TFA by Procedure D (step a, 2 h at rt) gave crude 163c as light yellow oil as a mixture of 4 isomers of the aza-furanose forms accompanied by 25% of the aldehyde (chain form): MS (APCI) m/z 258 (15, [M-1]+), 242 (100, [M-17]+).

5-(Dodecylthiomethyl)pyrrolidin-2-ol [163d(5S)]. Treatment of 162d (224 mg, 0.56 mmol) in CH₂Cl₂ (4 mL) with LiEt₃BH (1 M soln in THF, 1.4 mL, 1.4 mmol), by procedure F [column chromatography (20 → 30% EtOAc/hexane)] gave N-tert-butoxycarbonyl-5-(dodecylthiomethyl)pyrrolidin-2-ol [162d(5S) (219 mg, 97%) as a solidifying oil of the mixture of 4 isomers]: MS (APCI) m/z 400 (50, [M-1]+), 384 (100, [M -17]+). Treatment of 162d with an excess of TFA by Procedure D (step a, 2 h at rt) gave crude 163d as light yellow oil as a mixture of 4 isomers of aza-furanose form accompanied by 25% of aldehyde (chain form): MS (APCI) m/z 301 (5, M⁺), 300, (20, [M-1]+), 284 (100, [M-17]+).

N-(tert-Butoxycarbonyl)-3,4-dihydroxy-3,4-O-isopropylidene-5-(hexylthiomethyl)pyrrolidin-2-one [164 (3R,4R,5S)]. Treatment of 136 (60 mg, 0.16 mmol) in dry DMF (0.5 mL) with sodium hexathiolate (generated from hexanethiol (46.8 μL, 0.33 mmol)/NaH (14 mg, 0.35 mmol, 60%/mineral oil) in dry DMF (0.5 mL)) by Procedure G [column chromatography (5% → 10% MeOH/EtOAc)] gave 164 (25 mg, 40%) as a colorless oil and N-Boc deprotected 164 (24 mg, 38%) as a white crystalline solid. Compound 164 had: ¹H NMR δ 0.81 (t, J = 7.0 Hz, 3, H12), 1.16-1.27 (m, 6, H9, 10, 11), 1.30 (s, 3, CH₃), 1.39 (s, 3, CH₃), 1.44-1.59 (m, 11, H8,8', t-Bu), 2.36-2.50 (m, 2, H7,7'), 2.76 (dd, J = 6.2, 14.4 Hz, 1, H6), 2.82 (dd, J = 2.7, 14.4 Hz, 1, H6'), 4.31 (dd, J = 2.7, 6.2 Hz, 1, H5), 4.38 (d, J = 5.5 Hz, 1, H4), 4.78 (d, J = 5.5 Hz, 1, H3); ¹³C NMR δ 14.00 (C12), 22.49 (C10), 24.38 (CMe₂), 25.46 (CMe₂), 27.97 (t-Bu), 28.30 (C9), 29.63 (C8), 31.33 (C11), 33.67 (C7), 33.85 (C6), 60.84 (C5), 76.09 (C4), 77.63 (C3), 83.94 (t-Bu), 112.27 (CMe₂), 170.59 (CO) 149.80 (CO); MS (APCI) m/z 288 (100, [MH-Boc]+).

N-Boc deprotected 164 had: ¹H NMR δ 0.88 (t, J = 7.0 Hz, 3, H12), 1.25-1.36 (m, 6, H9,10,11), 1.38 (s, 3, CMe₂), 1.48 (s, 3, CMe₂), 1.56-1.62 (m, 2, H8,8'), 2.52-2.75 (m, 3, H6,7,7'), 2.73 (dd,
$J = 5.9, 13.4 \text{ Hz, 1, H}6'), 3.81 ('t', J = 6.1 \text{ Hz, 1, H}5), 4.50 (d, J = 5.9 \text{ Hz, 1, H}4), 4.69 (d, J = 5.9 \text{ Hz, 1, H}3), 5.94 (s, 1, NH)$; $^{13}$C NMR $\delta$ 14.01 (C12), 29.66 (C8), 22.52, 28.49, 31.36 (C9, C10, C11), 25.57 (CMe$_2$), 26.94 (CMe$_2$), 33.19 (C7), 33.66 (C6), 58.01 (C5), 76.60 (C4), 79.15 (C3), 112.73 (CMe$_2$), 173.23 (CO); MS (APCI) $m/z$ 288 (100, [MH$^+$]).

$N$-(tert-Butoxycarbonyl)-3,4-dihydroxy-3,4-O-isopropylidine-5-(nonylthiomethyl)pyrrolidin-2-one [165(3R,4R,5S)]. Treatment of 136 (60 mg, 0.16 mmol) in dry DMF (0.5 mL) with nonathiolate [generated from nonanethiol (62 $\mu$L, 0.33 mmol)/NaH (14 mg, 0.35 mmol, 60% mineral oil) in dry DMF (0.5 mL)] by Procedure G (column chromatography (5% → 10% MeOH/EtOAc) gave 165 (30 mg, 42%) as a colorless oil: $^1$H NMR $\delta$; 0.87 (t, $J = 7.0 \text{ Hz, 3, H}15), 1.25-1.31 (m, 12, H9-H14), 1.36 (s, 3, CMe$_2$), 1.45 (s, 3, CMe$_2$), 1.56-1.57 (m, 11, H8,8', t-Bu), 2.44-2.54 (m, 2, H7,7'), 2.82 (dd, $J = 6.2, 14.4 \text{ Hz, 1, H}6), 2.91 (dd, $J = 2.7, 14.4 \text{ Hz, 1, H}6'), 4.38 (dd, $J = 2.7, 6.2 \text{ Hz, 1, H}5), 4.45 (d, J = 5.5 \text{ Hz, 1, H}4), 4.85 (d, J = 5.5 \text{ Hz, 1, H}3); ^{13}$C NMR $\delta$ 14.07 (C15), 22.64, 28.68, 29.15, 29.22, 29.43, 31.83 (C9-14), 29.66 (C8), 24.38 (CMe$_2$), 25.46 (CMe$_2$), 26.99 (C9), 27.97 (t-Bu), 33.67 (C7), 33.87 (C6), 60.84 (C5), 76.09 (C4), 77.61 (C3), 83.93 (t-Bu), 112.27 (CMe$_2$), 149.80 (CO), 170.58 (CO); MS (APCI) $m/z$ 330 (100, [MH$_2$-Boc$^+$]).

3,4-Dihydroxy-5-(hexylthiomethyl)pyrrolidin-2-one [166(3R,4R,5S)]. TFA/H$_2$O (1 mL, 9:1) was added to N-Boc deprotected 164 (22 mg, 0.07 mmol) or 164 and the resulting solution was stirred at 0 ºC 3 h. Evaporation of volatiles gave light yellow oil that was column chromatographed (5 → 10% MeOH/EtOAc to give 166 (12 mg, 63%) as a colorless oil: $^1$H NMR $\delta$ 0.87 (t, $J = 7.0 \text{ Hz, 3, H}12), 1.24-1.39 (m, 6, H9, 10, 11), 1.52-1.59 (m, 2, H8,8'), 2.50-2.55 (m, 3, H5,7,7'), 2.73 (dd, $J = 5.5, 13.6 \text{ Hz, 1, H}6'), 3.71 ('t', $J = 6.4 \text{ Hz, 1, H}5), 4.21 (d, J = 5.0 \text{ Hz, 1, H}4), 4.44 (d, J = 5.0 \text{ Hz, 1, H}3), 7.11 ($s$, 1, NH); $^{13}$C NMR $\delta$ 14.01 (C12), 29.63 (C8), 14.11, 22.55, 31.42 (C9,C10,C11), 32.73 (C7), 35.27 (C6), 59.85 (C5), 69.77 (C4), 71.75 (C3), 175.97 (CO); MS (APCI) $m/z$ 248 (100, [MH$^+$]).
3,4-Dihydroxy-5-(nonylthiomethyl)pyrrolidin-2-one [167(3R,4R,5S)]. Treatment of 165 (20 mg, 0.04 mmol) with TFA/H₂O (1 mL, 9:1) as described for 164 gave 167 (10 mg, 85%) as a colorless oil. 


1H NMR 0.88 (t, J = 6.8 Hz, 3, H15), 1.20-1.38 (m, 12, H9- H14), 1.52-1.59 (m, 2, H8,8'), 2.49-2.54 (m, 3, H 6,7,7'), 2.70 (dd, J = 5.4, 13.5 Hz, 1, H6'), 3.71 (t, J = 5.6 Hz, 1, H5), 4.25 (d, J = 4.9 Hz, 1, H4), 4.49 (d, J = 4.6 Hz, 1, H3), 7.15 ( br. s, 1, NH); 13C NMR δ 14.07 (C15), 22.66, 28.84, 29.24, 29.27, 29.65, 31.86 (C9-14), 29.50 (C8), 26.99 (C9), 32.61 (C7), 34.99 (C6), 60.66 (C5), 69.74 (C4), 72.50 ( C3), 176.24 (CO); MS (APCI) m/z 290 (100, [MH]+).

3,4-Dihydroxy-5-(hexylthiomethyl)pyrrolidin-2-ol [170(3R,4R,5S)]. Treatment of 164 (40 mg, 0.1 mmol) in THF (1 mL) with LiEt₃BH (1 M/THF, 0.26 mL, 0.26 mmol), by procedure F [column chromatography (10→ 20% EtOAc/hexane)] gave N-(tert-butoxycarbonyl)-3,4-dihydroxy-3,4-O-isopropylidene-5-hexylthiomethyl-pyrrolidine-2-ol [168 (3R,4R,5S); 39 mg, 97%] as a colorless oil of the mixture of 4 isomers: MS (ESI) m/z 389 (100, [M−17]+). Treatment of 168 (39 mg, 0.1 mmol) with TFA/H₂O (0.9:0.1 mL) at 0 ºC for 3 h gave a light yellow oil that was column chromatographed (5→ 10% MeOH/EtOAc) to give 170 (22 mg, 88%) as a light yellow oil. 1H NMR showed a complex mixture of four isomers of the aza-furanose form also accompanied by aldehyde (chain form); MS (APCI) m/z 250 (20, [MH]+), 232 (60, [M−17]+).

3,4-Dihydroxy-5-(nonylthiomethyl)pyrrolidin-2-ol [171(3R,4R,5S)]. Treatment of 165 (40 mg, 0.09 mmol) in THF (1 mL) with LiEt₃BH (1 M/THF, 0.22 mL, 0.22 mmol), by procedure F [column chromatography (10→ 20% EtOAc/hexane)] gave N-(tert-butoxycarbonyl)-3,4-dihydroxy-3,4-O-isopropylidene-5-nonylthiomethyl-pyrrolidine-2-ol [168(3R,4R,5S); 30 mg, 75%] as a colorless oil as a mixture of 4 isomers: MS (APCI) m/z 414 (100, [M−17]+), 314 (95 [MH-Boc-H₂O]+). Treatment of 169 (28 mg, 0.06 mmol) with an excess of TFA/H₂O (0.9:0.1 mL) at 0 ºC for 3 h gave a light yellow oil that was column chromatographed (MeOH/EtOAc) to give 171 (18 mg, 96%) as a light yellow oil. 1H NMR showed a complex
mixture of four isomers of the aza-furanose form also accompanied by aldehyde (chain form); MS (APCI) m/z 292 (80, [MH]+), 274 (60, [M-17]+).

*S-(Methyl 2,3-0-Isopropylidene-β-D-ribofuranos-5-yl)-N-(tert-butoxycarbonyl)-L-homocysteine tert-butyl ester (173).* Treatment of 172 (170 mg, 0.64 mmol) (prepared according to reported methods171 with NMR data as reported189 with lithium homocysteinate (260 mg, 0.89 mmol) by Procedure B [column chromatography (15 → 20% EtOAc/hexane)] gave 173 (220 mg, 72%) as a light yellow oil: 1H NMR δ 1.32 (s, 3, CH₃), 1.45 (s, 9, t-Bu), 1.47 (s, 12, t-Bu, CH₃), 2.07-2.21 (m, 2, H8,8'), 2.54-2.59 (m, 3, H5,7,7'), 2.77 (dd, J = 6.1, 13.4 Hz, 1, H5'), 4.21-4.25 (m, 1, H9), 4.23 (dd, J = 6.1, 9.5 Hz, 1, H4), 4.60 (d, J = 5.9 Hz, 1, H3), 4.70 (d, J = 5.9 Hz, 2, H2), 5.10 (s, 1, H1), 5.30 (br. s, 1, NH); 13C NMR δ 24.94 (CMe₂), 26.42 (CMe₂), 27.99 (t-Bu), 28.31 (t-Bu), 27.73 (C7), 33.18 (C8), 35.58 (C5), 53.77 (C9), 54.93 (OCH₃), 83.32 (C2), 85.26 (C3), 85.78 (C4), 109.59 (C1), 82.17 (t-Bu), 82.21 (t-Bu), 112.44 (CMe₂), 155.32 (CO), 171.05 (CO); MS m/z 500 (100, [M+ Na]+).

*S-Ribosyl-L-homocysteine.* Treatment of 173 (30 mg, 0.06 mmol) with TFA/H₂O (9:1, 3 mL) by Procedure C at 0 °C for 12 h gave SRH 174 (8.4 mg, 50%) as a colorless oil with data as reported.170

**Methyl 2-Deoxy-5-O-methanesulfonyl-α/β-D-erythro-pentofuranoside (176).** Treatment of 175172 (550 mg, 3.73 mmol) with MsCl (0.290 mL, 316 mg, 4.1 mmol) in the presence of Et₃N (1.56 mL, 1.13 g, 11.19 mmol) by Procedure A [3 h; column chromatography (EtOAc→ 10% MeOH/EtOAc)] gave 176 (α/β, 3:2; 367 mg, 43%): 1H NMR δ 1.99-2.18 (m, 2, H2), 3.06 (s, 1.8, Ms), 3.09 (s, 1.2, Ms), 3.39 (s, 1.2, OMe), 3.41 (s, 1.8, OMe), 4.19-4.25 (m, 0.6, H3), 4.25-4.32 (m, 2.6, H4,5,5'), 4.08-4.14 (m, 0.4, H4), 4.44 (dt, J = 4.5, 5.6 Hz, 0.4, H3), 5.09 (dd, J = 1.6, 5.2 Hz, 0.4, H1), 5.11 (d, J = 4.6 Hz, 0.6, H1); 13C NMR δ 37.50 (Ms), 37.53 (Ms), 41.05 (C2), 41.31 (C2), 55.06 (OCH₃), 55.16 (OCH₃), 69.31 (C5), 70.21 (C5), 71.87 (C4), 72.30 (C4), 83.14 (C3), 83.99 (C3), 105.49 (C1), 105.55 (C1); MS (APCI) m/z 240 (100, [M+H₂O]+).
**Methyl 3-O-Benzoyl-2-deoxy-5-O-methanesulfonyl-α/β-D-erythro-pentofuranoside (177).**

**Procedure H.** A mixture of 176 (300 mg, 1.3 mmol), 2,6-lutidine (0.30 mL, 278 mg, 2.6 mmol) and catalytic amount of DMAP (7.5 mg, 0.65 mmol) in CH$_2$Cl$_2$ was heated to 35 °C and BzCl (0.180 mL, 218 mg, 1.56 mmol) was added to this mixture and stirred overnight. The mixture was cooled to room temperature and partitioned (CH$_2$Cl$_2$/NaHCO$_3$/H$_2$O). The organic layer was washed (brine), dried (MgSO$_4$) and evaporated. The residue was column chromatographed (25% EtOAc/hexane) to give 177 (α/β, 3:2; 440 mg, 95%) isolated by column chromatography. The α–Anomer had: $^1$H NMR $\delta$ 2.36 (dt, $J$ = 5.3, 10.7, 14 Hz, 1, H2), 2.50 (ddd, $J$ = 2.1, 7.3, 14 Hz, 1, H2'), 3.10 (s, 3, Ms), 3.41 (s, 3, OMe), 4.35-4.44 (m, 3, H4,5), 4.49 ( dd, $J$ = 4.4, 9.5 Hz, 1, H5'), 5.22 (dd, $J$ = 2.1, 5.4 Hz, 1, H1), 5.45-5.49 (m, 1, H3), 7.45-7.96 (m, 5, H$_{arom}$); $^{13}$C NMR $\delta$ 37.72 (Ms), 39.02 (C2), 55.52 (OCH$_3$), 69.80 (C5), 75.01 (C3), 81.73 (C4), 105.49 (C1), 105.87 (C1), 128.51, 129.70, 133.49 (C$_{arom}$), 166.45 (OCOPh); MS (APCI) $m/z$ 147 ([MH-Bz-Ms]$^+$). β–Anomer had: $^1$H NMR $\delta$ 2.21 (dd, $J$ = 1.2, 14.4 Hz, 1, H2), 2.48 (ddd, $J$ = 5.3, 8.3, 14.4 Hz, 1, H2'), 3.08 (s, 3, Ms), 3.41(s, 1.0, OMe), 4.37 (q, 1, H4), 4.54 (d, $J$ = 5.0 Hz, 1, H5), 5.18 (d, $J$ = 5.0 Hz, 1, H1), 5.30-5.33 (m, 1, H3), 7.45-8.06 (m, 5, H$_{arom}$).

**S-(Methyl 3-O-Benzoyl-2-deoxy-β-D-erythro-pentofuranos-5-yl)-N-(tert-butoxycarbonyl)-L-homocysteine tert-butyl ester (178).** Treatment of β-anomer of 177 (427 mg, 1.29 mmol) with protected L-Hcy (530 mg, 1.8 mmol) by Procedure B (step a and b, 36 h) gave 950 mg of oily residue. The crude was column chromatographed (20 → 30% EtOAc/hexane) to give 178 as a colorless oil (440 mg, 64%). β–Anomer had: $^1$H NMR $\delta$ 1.39 (s, 9, t-Bu), 1.42 (s, 9, t-Bu), 1.84-1.89 (m, 1, H8), 2.03-2.09 (m, 1, H8'), 2.10 (dd, $J$ = 1.5, 14.5, 1,H2), 2.51 (ddd, $J$ = 5.3, 8.4, 14.3 Hz, 1, 2'), 2.63 (t', $J$ = 7.8 Hz, 2, H7,7'), 2.84 (dd, $J$ = 5.7, 14.0 Hz, 1, H5), 2.92 (dd, $J$ = 4.4, 13.8 Hz, 1, H5'), 3.36 (s, 3, OMe), 4.17-4.26 (m, 1, H9), 4.35 (dt, $J$ = 4.3, 5.4 Hz, 1, H4),5.10 (d, $J$ = 5.0 Hz, 1, H1), 5.13 (br. d, $J$ = 7.9 Hz, 1, NH), 5.20 (ddd, $J$ = 2.3, 4.0, 8.3 Hz, 1, H3), 7.42-8.02 (m, 5, H$_{arom}$); $^{13}$C NMR $\delta$ 27.88 (t-Bu), 28.31 (t-Bu), 29.03
(C7), 33.15 (C8), 34.86 (C5), 39.25 (C2), 54.96 (C9), 55.48 (OCH3), 79.73 (C3), 82.53 (C4), 104.79 (C1), 82.06 (t-Bu), 83.68 (t-Bu), 128.36, 129.75, 129.86, 133.15 (C arom), 155.36 (CO), 166.45 (COPh), 171.12 (CO); MS (APCI) m/z 426 (60, [MH2-Boc]+). Treatment of 178 with TFA/H2O resulted in a complex mixture indicating decomposition of sugar. Therefore, 2-deoxy-SRH could not be synthesized from 178.

*S-(Methyl 2-Deoxy-α/β-D-erythro-pentofuranos-5-yl)homocysteine* (179) Compound 176 (65 mg, 0.28 mmol) was added to 1 M NaOH/ H2O (5 mL) and degassed with N2 for 30 min, followed by addition of D/L-Homocysteine (56.7 mg, 0.42 mmol) and the suspension was heated to 60 °C. After 12 h, the reaction mixture was cooled to room temperature, neutralized with dilute HCl to pH 7 and washed with EtOAc (3 × 10 mL). The water layer was evaporated and purified by HPLC (5% CH3CN/H2O for 35 min at 2.5 mL/min; tR = 13.6 min) to give 179 (α/β, 3:1; 30 mg, 37%). β–Anomer had: 1H NMR (D2O) δ 1.8-1.84 (m, 1, H2), 2.30-2.37 (m, 1, H2'), 2.02-2.15 (m, 2, H8), 2.61-2.78 (m, 4, H5,5',7,7'), 3.76 ('t', J = 5.8 Hz, 1 H9), 4.05-4.09 (m, 1, H4), 4.11-4.15 (m, 1, H3), 3.28 (s, 3, OMe), 5.10 (d, J = 5.0 Hz, 1, H1), 5.13 (br. d, J = 7.9 Hz, 1, NH), 5.06 (d, J = 4.7 Hz, 1, H1); 13C NMR δ 27.49 (C7), 30.38 (C8), 39.25 (C2), 53.78 (C9), 54.79 (OCH3), 79.60 (C4), 84.20 (C3), 105.48 (C1), 174.10 (COOH); MS (APCI) m/z 266 (100, MH+).

1,2-**O**-Isopropylidene-5-**O**-methanesulfonyl-β-D-arabinofuranoside (182). Treatment of 181 (85 mg, 0.44 mmol) with MsCl (0.03 mL, 37.7 mg, 0.49 mmol) in the presence of Et3N (0.186 mL, 135 mg, 1.34 mmol) by Procedure A [3 h; column chromatography (60 → 70% EtOAc/hexane) gave 182 (40 mg, 33%) as a colorless oil: 1H NMR δ 1.32 (s, 3, CH3), 1.55 (s, 3, CH3), 3.12 (s, 3, Ms), 4.30 (br. s, 1, H3), 4.25 (ddd, J = 1.9, 5.4, 7.3 Hz, 1, H4), 4.36 (dd, J = 5.4 10.9 Hz, 1, H5), 4.47 (dd, J = 7.3, 10.9 Hz, 1, H5'), 4.60 (d, J = 3.8 Hz, 1, H2), 5.98 (d, J = 3.8 Hz, 1, H1); 13C NMR δ 25.81 (CMe2), 26.59 (CMe2) 37.94 (Ms), 68.81 (C5), 75.50 (C3), 85.10 (C4), 105.49 (C1), 86.38 (C2), 106.13 (C1), 113.0 (CMe2).
3-O-Benzoyl-1,2-O-isopropylidene-5-O-methanesulfonyl-β-D-arabinofuranoside (183). Treatment of 182 (35 mg, 0.13 mmol) with 2,6-lutidine (0.030 mL, 27.8 mg, 0.156 mmol), DMAP (0.75 mg, 0.065 mmol) and BzCl (0.018 mL, 21.8 mg, 0.156 mmol) in CH₂Cl₂ by Procedure H [12 h; column chromatography (50 → 60% EtOAc/hexane)] afforded 183 (52 mg, 63%): ¹H NMR δ 1.32 (s, 3, CH₃), 1.62 (s, 3, CH₃), 3.13 (s, 3, Ms), 4.45-4.56 (m, 3, H₄,₅,₅'), 4.80 (d, J = 3.8 Hz, 1, H2), 5.30 (s, 1, H3), 6.05 (d, J = 3.8 Hz, 1, H1), 7.46-8.01 (m, 5, Harom); ¹³C NMR δ 25.75 (CMe₂), 26.49 (CMe₂), 38.05 (Ms), 68.63 (C₅), 77.43 (C₃), 84.14 (C₂), 83.32 (C₄), 106.34 (C₁), 113.23 (CMe₂), 128.48, 128.84, 129.81, 133.80 (Carom), 165.41 (OCOPh); MS (APCI) m/z 373 (10, MH⁺).

3-O-Benzoyl-5-bromo-1,2-O-isopropylidene-β-D-arabinofuranoside (184). Compound 183 (40 mg, 0.107 mmol) and NaBr (12 mg, 0.117 mmol) were heated in DMF (2 mL) under reflux for 12 h. The reaction mixture was poured into ice water (20 mL). The mixture was partitioned (CH₂Cl₂/NaHCO₃/H₂O). The organic layer was washed (brine), dried (MgSO₄) and evaporated. The residue was column chromatographed (5 → 10% EtOAc/hexane) to give 184 (18 mg, 47%): ¹H NMR δ 1.34 (s, 3, CH₃), 1.59 (s, 3, CH₃), 3.62 (dd, J = 6.3, 10.2 Hz, 1, H₅), 3.71 (dd, J = 8.4, 10.3 Hz, 1, H₅'), 4.48 ('t', J = 7.6 Hz, 1, H₄), 4.74 (d, J = 3.8 Hz, 1, H₂), 5.49 (br. s, 1, H3), 6.03 (d, J = 3.8 Hz, 1, H1), 7.46-8.03 (m, 5, H arom); ¹³C NMR δ 25.83 (CMe₂), 26.85 (CMe₂), 31.35 (C₅), 78.43 (C₃), 83.32 (C₄), 84.47 (C₂), 85.39 (C₄), 106.32 (C₁), 113.14 (CMe₂), 128.54, 129.08, 129.83, 133.64 (Carom), 165.30 (OCOPh).

S-(3-O-Benzoyl-1,2-O-isopropylidene-β-D-arabinofuranos-5-yl)-N-(tert-butoxycarbonyl)-L-homocysteine tert-butyloxycarbonyl-L-homocysteine tert-butyl ester (185). Treatment of 184 (7 mg, 0.019 mmol) with protected L-Hcy (6.6 mg, 0.02 mmol) by Procedure B (step a and b, 36 h) [column chromatographed (20 → 25% EtOAc/hexane)] gave 185 as a colorless oil (5 mg, 18%): ¹H NMR δ 1.32 (s, 3, CH₃), 1.37 (s, 9, t-Bu), 1.38 (s, 9, t-Bu), 1.80-1.87 (m, 1, H₈), 1.88-2.02 (m, 1, H₈'), 2.52-2.63 (m, 2, H7,7'), 2.86 (dd, J = 6.9, 13.8 Hz, 1, H₅), 2.95 (dd, J = 8.0, 13.8 Hz, 1, H₅'), 4.29
(br. s, 1, H9), 4.30 (t', J = 6.6 Hz, 1, H4), 4.66 (d, J = 3.9 Hz, 1, H2), 5.13 (br. d, J = 7.6, 1, NH), 5.29 (br. s, 1, H3), 5.93 (d, J = 3.9 Hz, 1, H1), 7.37-7.96 (m, 5, H\textsubscript{arom}); MS m/z 590 (45, [M+Na]\textsuperscript{+}).

\textbf{1-O-Benzoyl-2-deoxy-2-fluoro-3,5-di-O-triisopropylsilyl-\alpha/\beta-D-arabinofuranoside (189).}

\textbf{Procedure G.} Treatment of 188\textsuperscript{74} (540 mg, 1.16 mmol) with 2,6-lutidine (0.26 mL, 248 mg, 2.32 mmol), DMAP (6.7 mg, 0.58 mmol) and BzCl (0.16 mL, 194 mg, 1.39 mmol) by Procedure H [column chromatographed (3 \rightarrow 5\% EtOAc/hexane)] gave 189 (\alpha/\beta, 1:1; 500 mg, 77 \%): \textsuperscript{1}H NMR δ 0.78-1.21 (m, 42H, TIPS), 3.77-3.93 (m, 2, H5,5'), 4.26-4.35 (m, 1, H4), 4.78-4.86 (m, 1, H3), 5.16-4.96 (m, 1, H2), 6.46-6.53 (m, 0.5, H1), 6.56-6.62 (m, 0.5, H1), 7.40-7.96 (m, 5, H\textsubscript{arom}); \textsuperscript{19}F NMR δ -188.58 (ddd, J = 11.2, 20.8, 44.7 Hz, 1, F2), -203.26 (dd, J = 15.5, 52.2 Hz, 1, F2); (APCI) m/z 569 (100, MH\textsuperscript{+}).

\textbf{1-O-Benzoyl-2-deoxy-2-fluoro-\alpha/\beta-D-arabinofuranoside (190).} A mixture of 189 (100 mg, 0.17 mmol) and NH\textsubscript{4}F (51.8 mg, 1.4 mmol) was refluxed in methanol for 12 h at 60 °C. The reaction mixture was evaporated and column chromatographed to give (75 \rightarrow 80\% EtOAc/hexane) 190 (\alpha/\beta, 1:1; 17 mg, 37 \%): β-Anomer had: \textsuperscript{1}H NMR δ 3.85 (dd, J = 3.4, 12.4 Hz, 1, H5), 3.69 (dd, J = 4.1, 12.6, Hz, 1, H5'), 3.99 (dt, J = 3.8, 7.4 Hz, 1, H4), 4.71 (dt, J = 7.2, 17.0 Hz, 1, H3), 5.12 (dd, J = 1.7, 50.7 Hz, 1, H2), 6.43 (d, J = 1.3 Hz, 1, H1), 7.37-8.01 (m, 5, H\textsubscript{arom}); \textsuperscript{13}C NMR δ 62.09 (C5), 72.31 (d, J = 21.5 Hz, 1, C3), 82.42 (d, J = 9.6 Hz, 1, C4), 99.83 (d, J = 38.5 Hz, 1, C1), 100.55 (d, J = 200.9 Hz, 1, C2), 128.60 (d, J = 3.5 Hz), 129.21 (d, J = 7 Hz), 129.90 (d, J = 7.1 Hz), 133.72 (d, J = 4.3 Hz) (C\textsubscript{arom}), 171.32 (OCOPh); \textsuperscript{19}F NMR δ -190.63 (ddd, J = 11.3, 23.2, 50.6 Hz, 1, F2). α-Anomer had \textsuperscript{1}H NMR δ 3.81 (ddd, J = 1.74, 3.7, 12.5 Hz, 1, H5), 3.73 (dd, J = 4.0, 12.6, Hz, 1, H5'), 4.22 (dt, J = 3.8, 5.8 Hz, 1, H4), 4.45 (ddd, J = 1.7, 5.8, 23.3 Hz, 1, H3), 5.07 (ddd, J = 5.0, 7.3, 52.6 Hz, 1, H2), 6.49 (d, J = 4.5 Hz, 1, H1), 7.37-8.01 (m, 5, H\textsubscript{arom}); \textsuperscript{13}C NMR δ 61.77 (C5), 75.35 (d, J = 22 Hz, 1, C3), 86.00 (d, J = 4 Hz, 1, C4), 114
93.26 (d, $J = 18.1$ Hz, 1, C1), 94.53 (d, $J = 184.5$ Hz, 1, C2), 128.46, 129.49, 130.16, 133.61 (C$_{arom}$), 165.1 (OCOPh); $^{19}$F NMR $\delta$ -190.63 07 (ddd, $J = 11.3, 23.2, 50.6$ Hz, 1, F2).

1-O-Benzoyl-2-deoxy-2-fluoro-5-O-methanesulfonyl-$\alpha$/\$\beta$-D-arabinofuranoside (191).

Treatment of 190 (15 mg, 0.05 mmol) with MsCl (5.0 $\mu$L, 7.3 mg, 0.06 mmol) in the presence of Et$_3$N (21 $\mu$L, 15.1 mg, 0.15 mmol) by Procedure A [3 h; column chromatography (60 $\rightarrow$ 70% EtOAc/hexane) gave 191 ($\alpha$/\$\beta$, 1:1; 13 mg, 65%) as a crystalline solid: $^{1}$H NMR $\delta$ 2.98 (s, 3, Ms), 3.13 (s, 3, Ms), 4.41 ('br. s', 4, H5,5'), 4.46 (dd, $J = 4.1, 6.8$ Hz, 1, H4), 4.60 ('q', $J = 4.0$ Hz, 1, H4), 5.13 (dd, $J = 4.2, 19.6$ Hz, 1, H3), 5.26-5.40 (m, 3, H2,3), 6.55 (d, $J = 8.0$ Hz, 1, H1), 6.56 (d, $J = 5.8$ Hz, 1, H1), 7.39-8.10 (m, 10, Harom); $^{13}$C NMR $\delta$ 37.82 (Ms), 38.31 (Ms), 66.40 (C5), 67.28 (C5), 77.77 (d, $J = 8$ Hz, 1,C4), 78.90 (d, $J = 23$ Hz, 1, C3), 79.35 (d, $J = 31$ Hz, 1, C3), 91.79 (d, $J = 204$ Hz, 1,C2), 92.57 (d, $J = 18$ Hz, 1,C1), 97.05 (d, $J = 37$ Hz, 1,C1), 97.07 (d, $J = 186$ Hz, 1,C2), 128.31, 128.71, 128.90, 129.97, 130.08, 130.55, 134.06, 134.57 (C$_{arom}$), 165.41 (d, $J = 31$ Hz, 1, OCOPh), 171.13 (OCOPh): $^{19}$F NMR $\delta$ -190.14 (ddd, $J = 10.0, 19.7, 48.8$ Hz, 1, F2).

4.3. Borate complexation study

The $^{11}$B NMR spectra were recorded following the published methods described for the study of DPD binding to borate. The $^{11}$B NMR spectra were recorded at 25 °C using Bruker ultrashield™ spectrophotometer equipped with TBI probe $^{1}$H/$^{13}$C/D-BB Z-GRD at 192.6 MHz and referenced to Et$_2$O.BF$_3$. The NMR sample tubes (10 mm o.d.) made of poly(tetrafluoroethylene) (PTFE) were used for all measurements. All the spectra were recorded by collecting 16 scans. The boron binding affinities of aminoaethols and cis-diols were estimated using $^{11}$B NMR either by taking compounds of interest and borate in 1:1, 1:2 and 2:1 molar ratios in 0.5 mL of NaHCO$_3$ in D$_2$O solution at pH 7.8 or 5 mg of compound in saturated borate at pH 7.8 in 0.5 mL of D$_2$O solution.
4.4. Biological screening methods

4.4.1. Antiquorum sensing assay

4.4.1.1. β-galactosidase assay

An overnight culture of *E. coli* DH5α harboring the plasmids pSC11, which contains a P_{las} -lacZ translational fusion\(^\text{15}\) and pJN105L, which contains a P_{BAD}-lasR expression plasmid\(^\text{190}\) grown in LB media (10 g tryptone, 5 g yeast extract, 5 g sodium chloride per liter) supplemented with ampicillin (100 μg/ ml) and gentamycin (15 μg/ ml), was diluted to an OD\(_{600}\) of 0.150. At this time, arabinose (0.2 % w/v), N-3-(oxododecanoyl)homoserine lactone (3-oxo-C\(_{12}\)-AHL; 2 μM), and either the compound under analysis, water extract from *Conocarpus erectus* or *Callistemon viminalis*\(^\text{191}\) or solvent (water or DMSO), were added to the culture (1.5 mL). A negative control containing only solvent and arabinose (0.2% w/v) was also assayed. The cultures were incubated with shaking for three hours at 37 °C.

The conditions for the rhl biomonitor *E. coli* DH5α harboring pECP61.5 plasmid\(^\text{192}\), were essentially same except that the LB medium was only supplemented with ampicillin (100 μg/ ml), the overnight culture was diluted to an OD\(_{600}\) of 0.150, induced with 1 mM isopropyl β-D-thiogalactoside (IPTG), 2 μM C\(_4\)-AHL and the compounds or the controls added when the OD\(_{600}\) reached 1.0. After incubation at 37 °C for 4 hours with shaking, β-galactosidase activity was assayed as described previously.\(^\text{193}\) Assays were repeated at least twice. For each biological replicate, experimental triplicates were performed and the average percent activity calculated by dividing the average Miller units from the samples containing compound or extract by the average Miller units from the sample containing solvent and multiplying by 100. Significance of inhibition was determined using a paired two-tailed Student *t*-test.

4.4.1.2. Growth and luminescence measurement drop assay

Growth and luminescence measurement drop assays were conducted by Professor Makemson at FIU. The *V. harveyi* strain B392 (MAV) (or strain BB170 (AI-1\(^-\), AI-2\(^+\)) or strain BB152 (AI-1\(^-\))...
was grown in AB medium (75% artificial sea water, 0.3% glycerol, 0.2% casamino acids, 0.02% glycerol-phosphate, 25 mM HEPES buffer) at a pH 7.5. Growth was measured in a Spectronic 20 as OD660nm and luminescence was measured in a calibrated photometer and recorded in light units (1 LU = 1.08 x 10^8 q/s/mL). An overnight culture of V. harveyi was added to 45 mL of AB medium to an OD660nm of ~0.03. When the culture was rapidly growing (OD660nm ~0.05 to 0.07), the culture was split (2.5 mL/13x100mm culture tubes) and small amounts (2.5 µL and 10 µL) of each compound were added to each tube. The compound, dissolved in water or DMSO served as a control. Each measurement was obtained from a single trial and luminescence without inhibitor was considered 100%.

4.4.2. LuxS enzyme inhibition studies with 4-aza-SRH analogs

4.4.2.1. LuxS activity assay

The DTNB assay was performed in a buffer containing 50 mM HEPES (pH 7.0), 150 mM NaCl and 150 µM DTNB. The concentration of substrate (SRH) varied from 2.2 to 50 µM. The reaction was initiated by the addition of 0.8 µM of Co-BsLuxS and monitored continuously at 412 nm (ε = 14150 M^{-1} cm^{-1}) in a Perkin-Elmer λ25 UV-VIS spectrophotometer at room temperature. The initial rates were recorded from the early regions of the progress curves (3 min for BsLuxS reactions) and fitted against Michaelis-Menten equation \( V = \frac{k_{cat}[E]_0 [S]}{K_M + [S]} \) to obtain \( K_M \) and \( k_{cat} \) values.

4.4.2.2. LuxS inhibition assay

The LuxS inhibition assay was carried out in a similar manner as the activity assay but with varying concentrations of inhibitors and 17.8 µM of SRH (to a total volume of 1.0 mL). The inhibitors were dissolved in DMSO and the final DMSO concentration was kept less than 5% as to not to interfere with the enzyme activity. Kinetics was started by the addition of 0.8 µM B. subtilis LuxS coordinated with cobalt (Co-BsLuxS) and monitored at 412 nm. Slope was determined from 0-3 min. To calculate the \( K_I \) values, the relative activity \( (V/V_0) \) (\( V \) and \( V_0 \) are the
reaction rates in the presence and absence of inhibitors, respectively) was plotted against inhibitor concentrations \([I]\) and fitted against the equation: 
\[
\frac{V}{V_0} = \frac{(K_M + [S])}{K_M(1 + [I]/K_I) + [S]}. 
\]
In case of hemiaminal 143 which displayed time-dependent inhibition, Co-BsLuxS (1.6 \(\mu\)M) was incubated with 143 in buffer (without DTNB) for 30 min at room temperature. The reaction was then started by adding SRH and DTNB. The \(K_I^*\) values were obtained in the same way from a similar equation: 
\[
\frac{V}{V_0} = \frac{(K_M + [S])}{K_M(1 + [I]/K_I^*) + [S]}. 
\]
5. CONCLUSION

I have designed and synthesized various LuxS inhibitors and LasR QS modulators. The common synthetic approach involved activation of C5 hydroxyl group of the suitable sugar precursor and coupling with either thiolate derived from homocysteine (in case of LuxS inhibitors) or alkyl thiols (in case of γ-lactam and hemiaminal analogs). The \(N\)-Boc-L-Hcy-CO\(_2\)-t-Bu was synthesized from commercially available L-homocysteine. Reduction of the protected homocysteine with water soluble tris(2-carboxyethyl)phosphine hydrochloride generated protected Hcy thiol. The \(N\)-tert-butoxycarbonyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol served as the common sugar precursor for the synthesis of 4-aza-SRH analogs. Thus, the synthesis of pyrrolidine analog was achieved by coupling of the mesylated \(N\)-Boc protected iminoribitol with thiolate of \(N\)-Boc-L-Hcy-CO\(_2\)-t-Bu and subsequent deprotection of acid labile groups with TFA. For the synthesis of lactam analog, the mesylated ribolactam required for coupling to Hcy was produced by selective oxidation of the mesylated aza sugar with RuO\(_2\)/NaIO\(_4\). The hemiaminal analog was accessed conveniently by the super hydride® promoted reduction of the protected lactam analog and subsequent TFA treatment. Oxidation of TBDMS protected imino ribitol with SeO\(_2\)/H\(_2\)O\(_2\) produced nitrone based sugar which upon subsequent standard desilylation and mesylation yielded desired activated nitrone sugar. In case of methyl imino analog, the key sugar substrate, D-riboiminocyclitol was synthesized from L-lyxo sugar by \(exo\)-imino to \(endo\) iminocyclitol rearrangement. Such imino sugar was subsequently mesylated and coupled to the thiolate of \(N\)-Boc-L-Hcy-CO\(_2\)-t-Bu to give protected methyl imine analog which upon TFA treatment gave imine analog. The 2-deoxy-SRH analog was synthesized by condensation of the mesylated methyl 2-deoxy-\(\alpha/\beta\)-D-ribose with Hcy. The mesylated-arabinose intermediate, required for coupling to Hcy was conveniently accessed from D-arabinose. However, coupling of the mesyl sugar with protected Hcy provided protected S-arabinosyl-L-homocysteine (SAH) in very low yields. In
case of [2-F]SAH 194, the key sugar precursor 5-O-mesyl-2-fluoroarabinose 188 was synthesized from reported 2-fluoroarabino lactone. However coupling of the -O-mesyl derivative 188 with Hey was unsuccessful.

The γ-lactams with alkylthiomethyl substitution at carbon γ and their N,O-acetal counterparts were prepared from the key substrate (S)-5-(bromomethyl)-2-pyrrolidone 159 (derived from L-pyroglutamic acid). Displacement of bromide in 159 with sodium alkylthiolate produced 5-S-alkylthio lactams 160 with different lengths of alkyl chain (C₃-C₁₂). Reduction with super hydride® and subsequent deprotection gave hemiaminal analogs 163. The hexylthio ribolactam 166 and nonylthio ribolactam 167 were synthesized analogously. Reduction of the protected ribolactams with super hydride and subsequent deprotection with TFA provided the hemiaminal counterparts 170 and 171.

The optically pure γ-lactams and corresponding hemiaminals were evaluated for their effect on P. aeruginosa AHL-dependent las and rhl QS pathways isolated in E. coli. Lactam analogs 160 showed selectivity between two QS systems, acting as inhibitors against lasR and moderate activators against rhlR, presumably because of the differences in the active sites of their cognate R proteins. Antagonism of lasR activity increased with the length of the alkylthio chain. Interestingly, the cyclic hemiaminal derivatives with shorter alkylthio chain (163a and 163b) were found to stimulate lasr QS systems at lower concentrations while strongly inhibit both QS systems at higher concentrations. The ribolactam 166/167 and the corresponding cyclic hemiaminal 170/171 analogs inhibited lasR and stimulated rhlR moderately. Although, the mechanism of inhibition is still unknown, it is plausible that the compounds act as competitive inhibitors by binding to the QS sensor or affect events downstream, such as the binding of LasR (or in some cases, RhlR) to the promoter. Affecting downstream events would require that the compound enter the cells, which is unknown. Alternatively, it is established that although the rhl signaling molecule, C₄-AHL diffuses freely, transport of the las signaling molecule is more
complex, involving partitioning into the membrane and transport out of the cell by the MexAB-OprM efflux pump. Thus, it is also possible that the compounds with longer side chains affect the membrane and that the las pathway is more sensitive to these changes.

The pyrrolidine analog 129, lacking C1-hydroxyl exhibited weak inhibition against LuxS enzyme with an IC$_{50}$ value of 0.8 mM and $K_I$ of 49 µM. Similarly, lactam analog 141, which also lacked anomeric hydroxyl displayed weak competitive inhibition with IC$_{50}$ value of 0.6 mM and $K_I$ of 37 µM, suggesting the significance of anomeric hydroxyl for binding or the requirement of substrate to exist in open form. This data were also in agreement with the weak inhibition observed previously for 1-deoxy-SRH. However, as the inhibitors 129 and 141 possess $K_I$ values in similar range (40-60 µM), the inhibition might be because of the binding of the homocystiene moiety alone. As expected, the N-benzyl lactam analog 140 had no measurable activity. The large nonpolar benzylic group at ring nitrogen might have prevented access of 140 into the active site. Similarly 2,3-dideoxy lactam analogs of SRH, 195 and 196 were devoid of activity at tested concentrations, underscoring the importance of hydroxyl groups for metal chelation and binding at the active site.

The 4-aza-SRH 143 was found to be a time-dependent inhibitor of moderate potency with an IC$_{50}$ value of 60 µM. However, because of the complex kinetics of 143 observed with the enzyme, the $K_I$ value could not be determined and the mechanism of time-dependent inhibition was not identified. Nonetheless, in order to support the hypothesis of covalent inhibition by 4-aza-SRH 143 via the dehydrated imine 197, methyl imine analog of 4-aza-SRH 158 was independently synthesized and tested. Unfortunately, the1-methyl imine analog of SRH 158 was found to be inactive. Lack of anomeric hydroxyl or misfit of iminocyclitols at the active site or lack of stability of the compound at the testing conditions might have contributed to the loss of activity. However, without further enzymatic studies, the precise effect of imine functionality on
LuxS activity could not be concluded at the present time. Also, despite the attempts from various research groups, correlation between LuxS enzymatic activity and QS was not found so far.

In this dissertation, two approaches for identification of QS inhibitors in both interspecies (LuxS inhibitors) as well as intraspecies (human pathogen, *P. aeruginosa*) were addressed. The structural and biological data provided by these analogs might contribute to the development of more effective QS inhibitors which in turn might impact drug discovery and health care.

**Future directions**

As a result of this dissertation, a future work towards understanding the function of S-ribosylhomocysteinase (LuxS) should mainly concentrate on synthesis of the aza-DPD analogs as well as novel inhibitors of LuxS with modifications at C2 or C4 of the ribose ring of the natural substrate.

Aza-DPD analogs should be prepared to evaluate their modulation of QS activity and might also allow study of their complexation with borate. Borate complexation experiments with aminoalcohols related to aza-AI-2 should shed the light on understanding the binding preferences of borate and might further facilitate the understanding of the mechanism of action of hemiaminal 143.

Synthesis and evaluation of C2 modified SRH derivatives as potential LuxS inhibitors might assist in understanding the role of acid/base residues at the active site. As [3-F]SRH and [3-Br]SRH were previously found to be time-dependent LuxS inhibitors due to the LuxS catalyzed elimination of halide ion, it will be interesting to study if the mechanism of interaction of [2-F]SRH and [2-Br]SRH with LuxS follows the nucleophilic substitution at C2 resulting in covalent inhibition or elimination mechanism as observed with C3 analogs. So far, SRH analogs with modifications at C1 and C2 and C3 were targeted as LuxS inhibitors. Such substrate analogs were proposed to inhibit sugar ring opening, first and second keto-enol tautomerization steps associated with LuxS catalysis, respectively. Design and synthesis of C4 modified SRH analogs
should permit to study the β-elimination reaction in LuxS catalysis (which is the last step leading to the release of DPD and Hcy). Modification of SRH with long alkyl side chains either at C4 or C7 might inhibit homodimerization of LuxS and thereby prevent LuxS catalysis. As it is known from literature that Hcy binding pocket is far from the sugar binding active site, such SRH analogs with hydrophobic alkyl chains should fit into the binding pockets for both sugar and Hcy moieties.\(^49\) However, the presence of hydrophobic alkyl chain might prevent dimerization and hence LuxS catalysis.

Moreover, structural basis for the time-dependent inhibition observed by 143 can be estimated by cocrystallizing LuxS with 143. As this analog exists as a complex mixture of several isomers, such study will also reveal the identity of the isomer responsible for inhibition. Conformational changes, if any occurred due to the replacement of ring oxygen of SRH with nitrogen (as in hemiaminal 143) can also be studied using crystal structural data.

To confirm the LasR inhibition observed by lactam/hemiaminal analogs, inhibition of LasR regulated extracellular virulence factors (pyocyanin and elastase production) in culture fluids of \(P.\ aeruginosa\) can be estimated. Lactam/hemiaminal analogs with alkylthiomethyl substitution at carbon γ were targeted as QS modulators in this study. As a step forward, analogs with alkylthiomethyl substitution at α position can also be synthesized. Since similar α linkage of side chain to the lactone ring is found in the native AHL, such analogs might prove to be better QS inhibitors than γ substituted analogs. Similar thiolactam analogs can also be prepared and targeted as QS modulators.
BIBLIOGRAPHY


179. Compounds 195 and 196 were prepared by Dr. Adam Sobczak from Dr. Wnuk's research group. Synthesis of these compounds will be published elsewhere.


APPENDIX: $^1$H NMR spectra of the selected compounds. For peak assignments refer to the experimental section.
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