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Design and Synthesis of S-ribosylhomocysteine Analogues

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DESIGN AND SYNTHESIS OF *S*-RIBOSYLHOMOCYSTEINE ANALOGUES

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by

Christiane Chbib

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To: Dean Kenneth G. Furton
College of Arts and Sciences

This dissertation written by Christiane Chbib and entitled Design and Synthesis of S-ribosylhomocysteine Analogues, having 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.

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Florida International University, 2014

DEDICATION

I dedicate this dissertation to my parents and my husband for their unconditional love, support and encouragement. Also to the LORD who is always my guide and my source of happiness.

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I would like to extend my heartfelt gratitude to my mentor Dr Wnuk for the opportunity you gave me to be part of his lab. I want to thank you for your patience, your assistance and your care in my professional development and beyond. You left a major impact in my life. Thank you for setting the example of how an expert and professor should be and thank you for all the help you provided me in every chance you had.

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ABSTRACT OF THE DISSERTATION

DESIGN AND SYNTHESIS OF *S*-RIBOSYLHOMOCYSTEINE ANALOGUES

by

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Florida International University, 2014

Miami, Florida

Professor Stanislaw Wnuk, Major Professor

Bacteria are known to release a large variety of small molecules known as autoinducers (AI) which effect quorum sensing (QS) initiation. The interruption of QS effects bacterial communication, growth and virulence.

Three novel classes of *S*-ribosylhomocysteine (SRH) analogues as potential inhibitors of *S*-ribosylhomocysteinase (LuxS enzyme) and AI-2 modulators of QS were developed. The synthesis of 2-deoxy-2-bromo-SRH analogues was attempted by coupling of the corresponding 2-bromo-2-deoxypentafuranosyl precursors with the homocysteinate anion. The displacement of the bromide from C2 rather than the expected substitution of the mesylate from C5 was observed. The synthesis of 4-C-alkyl/aryl-S-ribosylhomocysteine analogues involved the following steps: (i) conversion of the D-ribose to the ribitol-4-ulose; (ii) diastereoselective addition of various alkyl or aryl or vinyl Grignard reagents to 4-ketone intermediate; (iii) oxidation of the primary hydroxyl group at C1 followed by the intramolecular ring closure to the corresponding 4-C-alkyl/aryl-substituted ribono-1,4-lactones; (iv) displacement of the activated 5-hydroxyl group with the protected homocysteinate. Treatment of the 4-C-alkyl/aryl-substituted SRH analogues with lithium triethylborohydride effected reduction of the ribonolactone

to the ribose (hemiacetal) and subsequent global deprotection with trifluoroacetic acid provided 4-C-alkyl/aryl-SRHs.

The 4-[thia]-SRH were prepared from the 1-deoxy-4-thioribose through the coupling of the α-fluoro thioethers (thioribosyl fluorides) with homocysteinate anion. The 4-[thia]-SRH analogues showed concentration dependent effect on the growth on *las (*50% inhibitory effect at 200 μg/mL). The most active was 1-deoxy-4-[thia]-SRH analogue with sufur atom in the ring oxidized to sulfoxide decreasing *las* gene activity to approximately 35% without affecting *rhl* gene. Neither of the tested compounds had effect on bioluminescence nor on total growth of *V. harveyi*, but had however slight inhibition of the QS.

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LIST OF ABBREVIATIONS

Å	Angstrom
ABC	ATP-binding cassette
Ac	Acetyl
AHL	*N*-acyl homoserine lactone
AI	Autoinducer
AIP	Autoinducing peptides
Aq	Aqueous
ATP	Adenosine triphosphate
BHL	*N*-butyryl-HSL
Bn	Benzyl
Boc	*tert*-butyloxycarbonyl
CAI-1	Cholerae autoinducer 1
CH_2Cl_2	Dichloromethane
$COCl_2$	Oxalyl chloride
CPS	4-chlorophenylsulfonamide group
CrO_3	Chromium trioxide
Cys84	Cysteine 84
d	Doublet
DAST	Diethylaminosulfur trifluoride
DCC	*N,N′*-dicyclohexylcarboiimide
dd	Doublet of a doublet

DHFR	Dihydrofolate reductase
DHMF	(2*R*,4*S*)- and (2*S*,4*S*)-2,4-dihydroxy-2-methyldihydrofuran-3-one
DIC	Diisopropylcarbodiimide
DMAP	4-(*N,N*-dimethylamino)pyridine
DMF	*N,N*-dimethylformamide
DMSO	Dimethyl sulfoxide
DOP	Dioxopentanal
DPD	4,5-dihydroxy-2,3pentanedione
E.coli	*Escherichia coli*
E1cB	Elimination unimolecular conjugate base reaction
E-57	Glutamine 57
^{19}F NMR	Fluorine-19 nuclear magnetic resonance
g	Gram
h	Hour
Hcy	Homocysteine
Her-2	Human epidermal growth factor receptor-2
HHQ	4-hydroxy-2-heptylquinoline
His	Histidine
HIV-1	Human immunodeficiency virus
HKPH	Histidine kinase protein H
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectroscopy

HTH	Helix-turn-helix motif
IC50	The half maximal inhibitory concentration
J	Coupling constant in Hz (NMR)
K_2CO_3	Potassium carbonate
KDA	KiloDalton
K_i	Inhibition constant
LDA	Lithium diisopropylamide
$LiEt_3BH$	Lithium triethylborohydride
LiHMDS	Lithium bis(trimethylsilyl)amide
$LuxR_{vi}$	LuxR in *V. harveyi*
m	Multiplet (NMR)
M	Moles per Liter
m/z	Mass to charge ratio (MS)
*m*BTL	*meta*-Bromothiolactone
MCPBA	*meta*-Chloroperoxybenzoic acid
*m*CTL	*meta*-Chlorothiolactone
min	Minutes
mL	Milliliter
Mol	Mole(s)
Ms	Mesyl
MS	Mass spectroscopy
MTAN	5'-methylthioadenosine nucleosidase

NADH	Nicotinamide adenine dinucleotide
$NaIO_4$	Sodium periodate
NBS	*N*-Bromosuccinimide
NFSi	*N*-fluorobenzene sulfonamide
NH_3	Ammonia
NMO	*N*-methylmorpholine *N*-oxide
NNRTI	Non-nucleoside reverse transcriptase inhibitor
^oC	Degree Celsius
OdDHL	*N*-(3-oxododecanoyl)-HSL
P. aeruginosa	*Pseudomona aeruginosa*
PG	2-Phosphoglycolic acid
Ph	Phenyl
PQS	Pseudomonas Quinolone Signal
p-TSA	*p*-Toluenesulfonic acid
q	Quartet (NMR)
QQ	Quorum quenching
QS	Quorum sensing
QSSM	Quorum sensing signaling molecule
RHKs	Receptor-histidine kinases
rt	Room temperature
s	Singlet (NMR)
S. typhimurium	*Salmonella typhimurium*

SAH	*S*-Adenosyl-L-homocysteine
SAM	*S*-Adenosyl-L-methionine
$SbCl_3$	Antimony trichloride
SRH	*S*-Ribosylhomocysteine
t	Triplet (NMR)
TBAF	*Tetra-n*-butylammonium fluoride
TBDMS	*t*-Butyldimethylsilyl
t-Bu	*tert*-Butyl
TCEP	Tris(2-carboxyethyl)phosphine
TEA or Et_3N	Triethylamine
TFA	Trifuoroacetic acid
THF	Tetrahydrofuran
THMF	(2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TPAP	Tetrapropylammonium perrenthenate
Tr	Trityl
V. harveyi	*Vibrio harveyi*
α	Alpha
β	Beta
γ	Gamma
δ	Delta chemical shift in parts per million (NMR)
μ	Micro

1. INTRODUCTION

1.1. Quorum sensing (QS)

Bacteria are known to release a large variety of small molecules. They also produce and respond to diffusible signal molecules (termed autoinducers or pheromones). Cell to cell communication in bacteria is shown to be accomplished through the exchange of extracellular signaling molecules called autoinducers.[1-4] This process is defined as QS where bacteria are allowed to coordinate gene expression. The concept of quorum sensing (QS) was discovered in the luminescent marine bacterium *Vibrio fischeri*.[5] Light emission (the Luciferase enzyme) was only expressed when the organisms were associated with their fish or squid symbiotic hosts. The mechanism behind it was uncovered demonstrating that the expression of luciferase-encoding genes is related to a factor secreted by the cells and accumulates as cell density increases. Bioluminescence, a QS dependent behavior in V*ibrio*, was observed as a light production that was controlled by the secretion and the detection of small signal molecules.[6-9] When a specific threshold is reached (Figure 1), programmed changes in gene expression coordinate population-dependent behavior.[5] Some of the changes that occur include biofilm formation, toxin and virulence secretion in addition to sporulation and swarming ability. QS was employed as a novel target for drug design especially in relation to pathogenic bacteria as *Pseudomonas aeruginosa*, *Salmonella typhimutium*, *Yersinia enterocolitica* and others.[10] Autoinducer (AI) molecules are molecule facilitators that are conventionally divided into two groups. Acyl homoserine lactones (AHL, AI-1),[11] used in Gram negative bacteria and oligopeptides used in Gram positive bacteria (Figure 1). However the QS process is not limited to the previous two groups only, but include a universal group of autoinducer-

2 (AI-2) found in both Gram positive and negative bacteria.[12-16] It has been proposed that AI-2 represent a possible means by which different bacteria communicate with each other contrary to the intraspecies communication mediated by other autoinducers.[12,17]

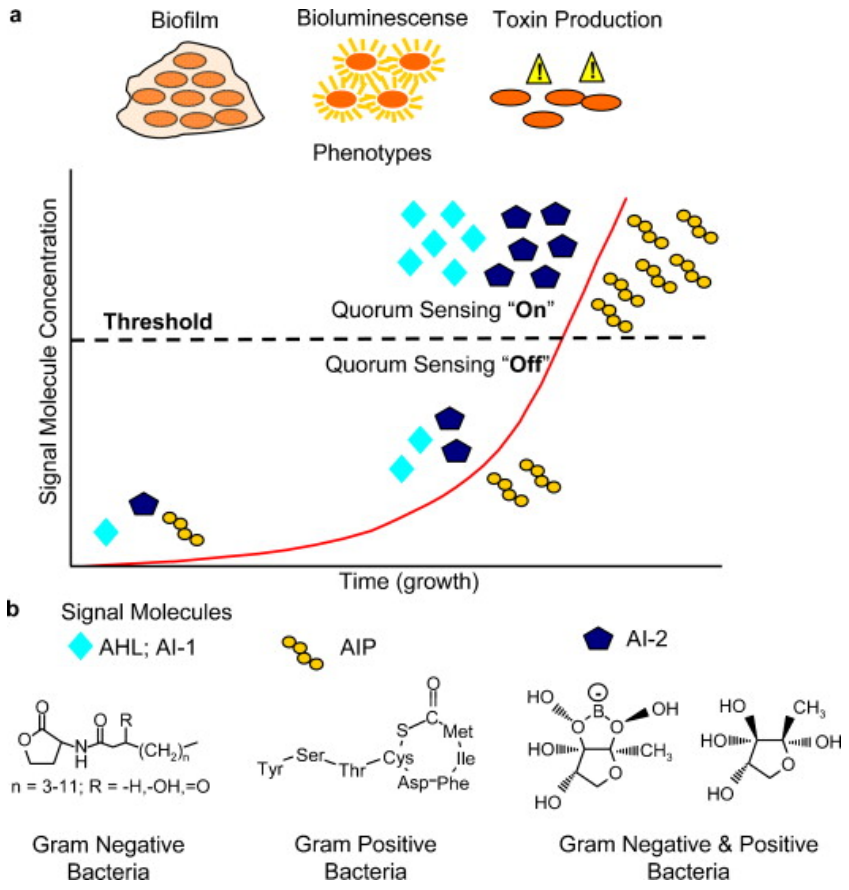


Figure 1. The signal molecules in quorum sensing process in Gram positive and negative bacteria.[18]

QS was demonstrated to regulate many important traits including biofilm formation, virulence and gene transfer between bacteria.[4,19] Understanding QS may simplify the means to inhibit or silence QS pathway and weaken bacterial pathogenicity. Most new antibiotics are the derivatives of older generations which present a universal threat when it comes to bacterial resistance. This leads to the necessity of the research for new antimicrobials with different mechanism of actions. Quorum sensing, is the process of

population dependent bacterial cell-cell signaling, which has a major impact on accelerating the bacterial virulence. It is considered an interesting target for new research in antibiotics. Antibacterials are divided into either bacteriostatic (inhibit bacterial growth) or bactericidals (cause bacterial cell death).[17] The rise of a new family of antibacterial that belongs to neither but in fact operates by reducing selective pressure like quorum sensing mechanism has generated major interest.[19]

The process of quorum sensing involves three major characters[4] (Figure 2): (1) synthase, (2) signal, and (3) sensor. The synthase is the basic part of the model, since it initiates the process of quorum sensing by synthesizing small-molecule signals called autoinducers (AI) which when detected by the sensors at a threshold level generate gene expression.

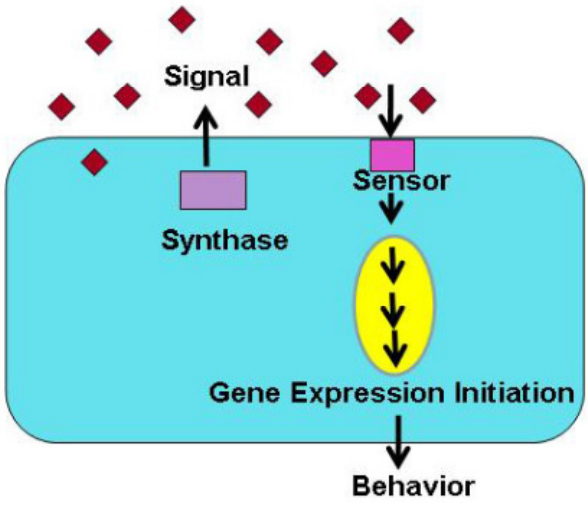


Figure 2. Quorum sensing model: Cell to cell communication.[4]

QS signaling molecules (QSSM) or autoinducers are categorized into three major classes[20,21] as illustrated in Table 1 and also shown in Figure 1. They are (a) *N*-acylhomoserine lactone (AHLs) produced by Gram negative bacteria, (b) autoinducing

oligopepetide derivatives (AIP; see also Figure 6) employed by Gram positive bacteria, and (c) autoinducer-2 (AI-2) derived from the precursor 4,5-dihydroxy-2,3pentanedione (DPD) used by both Gram positive and negative bacteria (Figure 3). Other recognized QSSM consist of alkyl quinolones, for example, Pseudomonas Quinolone Signal (PQS or HHQ), autoinducer-3[22] and cholerae autoinducer 1 (CAI-1).[23]

Pathway	Signal molecule	Bacteria
AI-1	various AHLs	Gram negative
PQS and HHL	*Pseudomonas* quinolone signal 4-hydroxy-2-heptylquinoline	Gram negative
AI-3	Epinephrine/Norepinephrine	Gram negative
AI-2	Furanosyl borate esters of DPD	Gram negative and positive
AIP	various oligopeptides	Gram positive
CAI-1	(*S*)-3-hydroxytricedan-4-one	Gram negative (*V. Cholerae*)

Table 1. Different Quorum sensing pathways in bacteria.[17]

AHLs (**1**, Figure 3) vary in the length and the oxidation state of the acyl side chain.[24] Blackwell and co-workers[21] found that the length of the acyl chain is critical for the activity of AHL. In the unique case of *P.aeruginosa* a specific signal molecule having quinolone motifs such as PQS and HHQ is released from the cell.[18] AI-2, a furanosyl borate ester of DPD in *V. harveyi* **2**, exists as non-borated in *S. typhinium* **2a** (Figure 3).

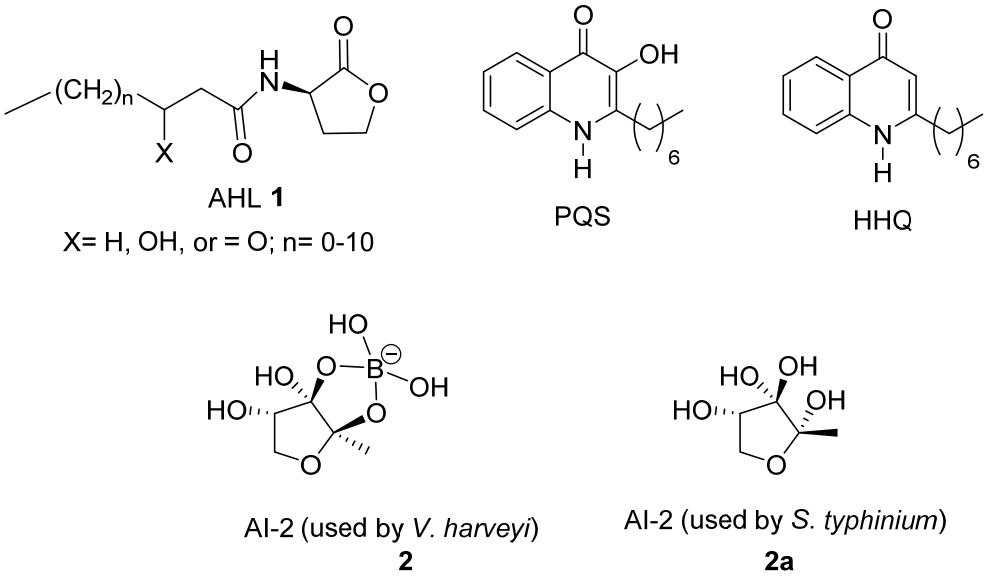


Figure 3. Chemical structures of QS signaling molecules (QSSM).[24]

1.1.1. Intraspecies quorum sensing

In Gram positive bacteria, signaling pheronomones are generally peptides. They signal through receptor-histidine kinases (RHKs) that are embedded in the membrane.[25] The intraspecies communication in Gram-positive bacteria is through autoinducing peptides (AIP)[17] in the form of oligopeptides consisting of 5-20 residues, whereas in Gram negative bacteria, acetylated homoserine lactones (AHL) are used as autoinducers which consist of small molecules diffusible across the cytoplasmic membrane and able to bind to the regulatory proteins within the cell to trigger transcriptional changes.[12,25]

In Figure 4 *part A*, peptide signaling through receptor-histidine kinases (RHKs) in Gram positive bacteria shows that the extracellular signaling molecules bind to the sensor domain of the RHK leading to the activation via phosphorylation and dephosphorylation of the HK domain. Then gene expression is controlled at the level of transcription. In Gram negative bacteria (Figure 4 *part B)*, small signaling molecules cross to the

intracellular receptor protein R. Upon binding, R is stabilized in a form that binds to DNA leading to the modulation of gene expression.

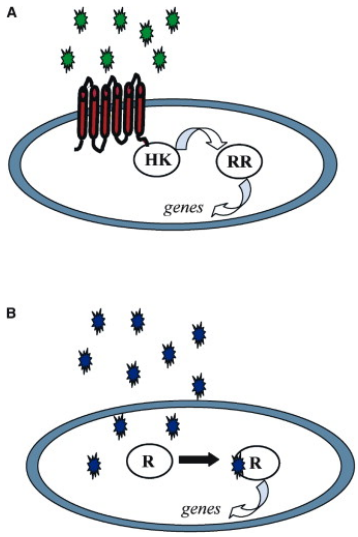


Figure 4. The different signaling in Gram positive (A) and negative bacteria (B).[25]

1.1.1.1. Gram positive bacteria

Intraspecies communication in Gram positive bacteria as *S. aureus*, is related to the secretion of an extracellular peptide AIP and to its accumulation.[25] Upon reaching a threshold, AIP binds to the sensor protein (histidine kinase protein H, HKPH) located in the cell membrane of the bacteria and triggers the activation of the receptor-histidine kinase AgrC. HKPH is part of a two-component signal transduction system. The QS system includes the sensing protein (receptor) and a response-regulatory protein which controls gene activation and positive feedback regulation of AIP (Figure 5). Kinase protein H is activated when binding to AIP, leading to its autophosphorylation. The phosphoryl group is transferred to an aspartate residue D that undergoes subsequent phosphorylation leading to the activation of the response regulatory protein (peptide-pheromone precursors). The latter is cleaved to afford the autoinducer signal exported by

the ATP-binding cassette (ABC)[26]. Once exported, AIP can be sensed by self and other bacteria.

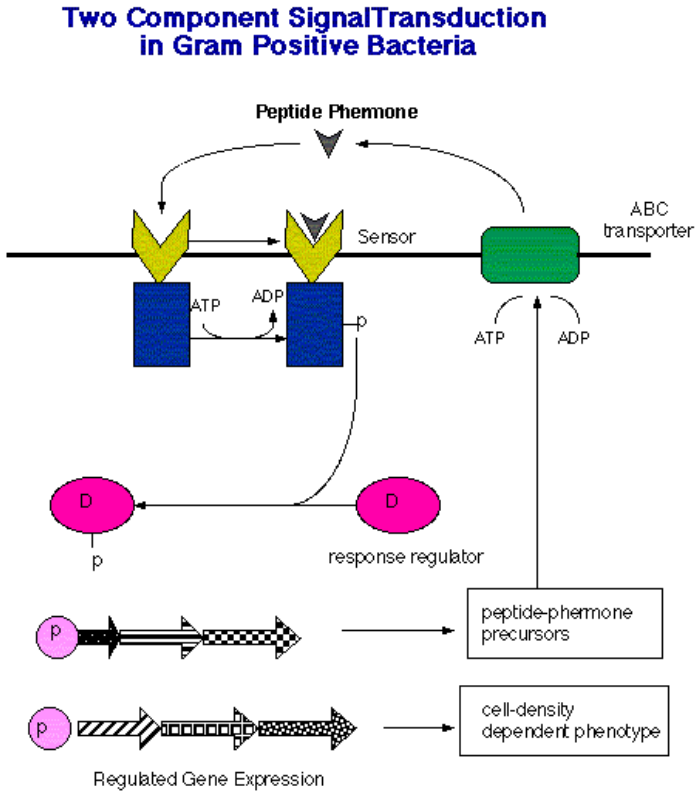


Figure 5. QS mediated by peptides pheromones and two-component regulatory systems in Gram-positive bacteria.[26]

The autoinducing peptides AIP are the short oligopeptide fragments[17] that are post-translationally modified through the insertion of a lactone, thiolactone, lanthionine or isoprenyl group to yield diverse structures (Figure 6). They could differ in binding selectivities and activation in different Gram positive bacteria.

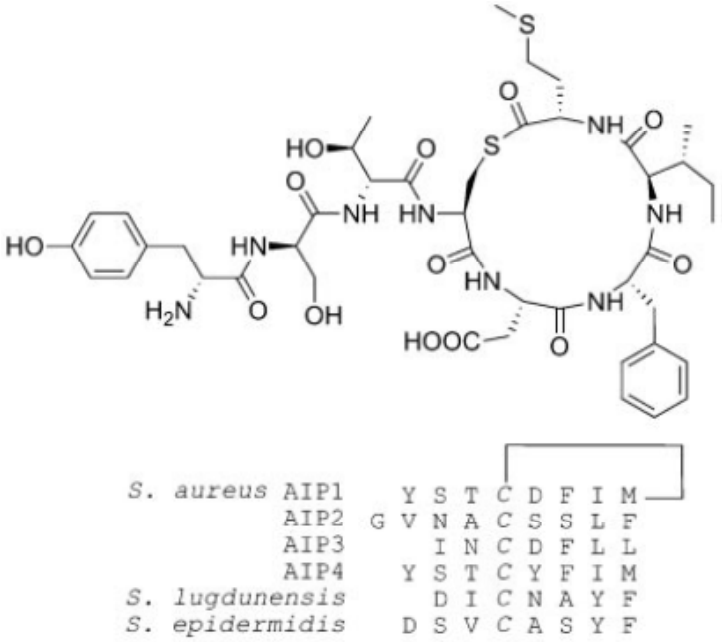


Figure 6. Structures of the selected AIPs.[17]

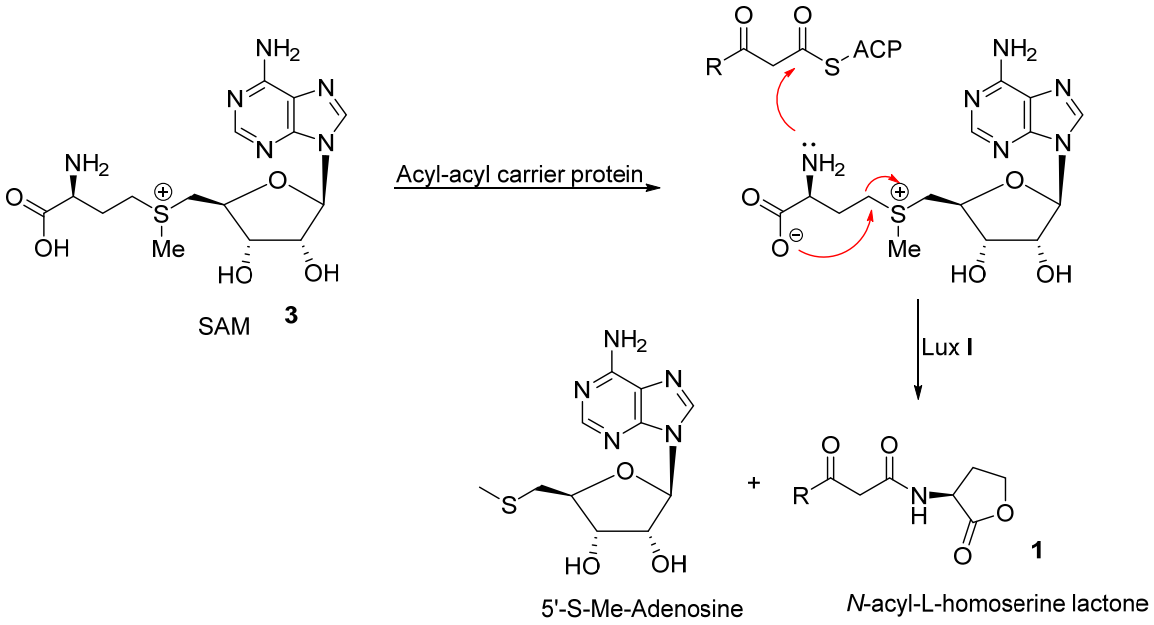
1.1.1.2. Gram negative bacteria

The mechanism of QS in Gram negative bacteria starts with the secretion of autoinducers or pheromones (AHL). When the inducer binds to the receptor, it activates the transcription of certain genes. As the population of bacteria grows a positive feedback is started.[27] The AHL molecules are composed of fatty acyl chain linked to a lactonised homoserine through an amide bond.[28] Different structures of AHLs which modulate different regulatory proteins in different bacteria are presented in Table 2. They are the subject of vast literature reports.[17,29-41] Their structure and composition vary in the acyl chain and lead to different biochemical and physiological functions.[17]

Organism	Signal	Regulatory proteins	Phenotype
A.hydrophila	C_4-HSL	Ahy1/AhyR	Exoprotease production
A.salmonicida	C_4-HSL	AsaI/AsaR	Extracellular protease
A.tumedaciens	3-oxo-C_8-HSL	TraI/TraR	Ti plasmid conjugation
B.cepacia	C_8-HSL	CepI/CepR	Protease siderophores
C.violaceum	C_6-HSL	CviI/CviR	Exoenzyme, antibiotics
E.carotovora	C_6-HSL	ExpI/ExpR	Exoenzyme, carbapenem antibiotic
E. chrysanthemi	3-oxo-C_6-HSL	ExpI/ExpR	Pectate lyase
P. aureofaciens	C_6-HSL	PhzI/PhzR	Phenazine antibiotics
P. aeroginosa	3-oxo-C_{12}-HSL	LasI/LasR RhII/RhIr	Multiple extracellular enzyme, biofilm formation
R.sphaeroides	C_{14}-HSL	CerI/RhIR	Dispersal from bacterial aggregate
S.liquefaciens	C_4-HSL	SwrI/SwrR	Extracellular protease
V.fischeri	C_6-HSL	LuxI/LuxR	Bioluminescence
V.harveyi	3-hydroxy-C_4-HSL	LuxM/N	Bioluminescence
Y. pseudoruberculosis	C_6-HSL	YpsR/I YthR/I	Regulation of clumping and motility
Y. pestis	C_{10}-HSL/C_6-HSL	YspI/YspR YpeI/YspR	Regulation of clumping and motility

Table 2. Representative AHLs and their function in Gram negative bacteria.[17,29-41]

The *N*-acyl-L-homoserine lactones (**1**, AHLs) are derived from S-adenosylmethionine (SAM, **3,** Scheme 1) with the assistance of the enzymatic action of LuxI[25,42] as shown in Scheme 1. Intramolecular cyclization of the homocysteine fragment with the elimination of the 5'-*S*-methyl-5'-thioadenosine and the concomitant acylation of the amino group with the common acyl carriers provided various AHL analogues.



Scheme 1. Biosynthesis of *N*-(acyl)-L-homoserine lactone.[25]

The mechanism of action of AHL QS starts with the diffusion of the latter in the cell (Figure 7). The interaction with the LuxR-type protein leads to acyl-HSL bond formation. It is divided into two domains: first, the conserved one which includes the *N*-terminal of LuxR from acyl-HSL-binding region; second, the C-terminus with a helix-turn-helix motif (HTH) required for DNA binding. The binding between *N*-terminal and acyl-HSL leads to conformation changes followed by a DNA binding.[17]

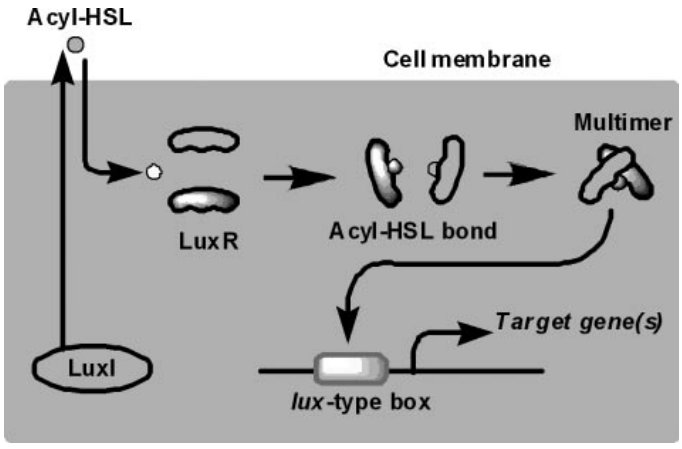


Figure 7. AHL quorum sensing mechanism.[17]

E. coli and *S. typhimurium* are common causes of food-bourne illness.[43] They are believed to have homologous QS pathways.[44] In solution, DPD undergoes rapid cyclisation (see below Scheme 4) and exists in equilibrium with several different isoforms. Thus AI-2 is not a specific molecular structure but a family of isomers.[45] The recruitment of boron is needed for the case of *V. harveyi.*[18] As shown in Figure 8a below, the AI-2 QS process in both *E. coli* and *S. typhimurium* is regulated by the same LuxS-regulated operon (*lsr*). After DPD cyclizes to form AI-2, it gets exported extracellularly where it accumulates. Once a threshold is reached, the bacterial QS is initiated and signal transduction is activated. AI-2 is imported by *lsr* inside the cell, via *lsr* transporter,[46] where it gets phosphorylated by kinase to form phospho-AI-2. This form allows it to bind to LsrR known as a repressor which effects it to be released. A positive feedback is used causing increased expression of the transporter and resulting in more importation of AI-2.

In case of *V. harveyi* and *V. cholerae* [47] (Figure 8b), the three cognate receptors CqsS (mediated by CAI-1),[48] LuxN (mediated by AI-1) and LuxQ (which interacts with the complex LuxP-AI-2) are membrane-bound histidine sensor kinases.[49] The sensory mechanism is a two-component regulatory path that produces LuxR.[50-53] The role of LuxR is to control the expression of bioluminescence related genes. At low concentrations, the cognate receptors act as kinases and lead to the formation of phosphor-LuxO and then the synthesis of sRNA that leads to the degradation of luxR mRNA. At high concentrations, the cognates switch from kinases to phosphorylases and remove phosphate from LuxO to LuxU which permits translation of luxR mRNA to LuxR and expression of bioluminescence.[4]

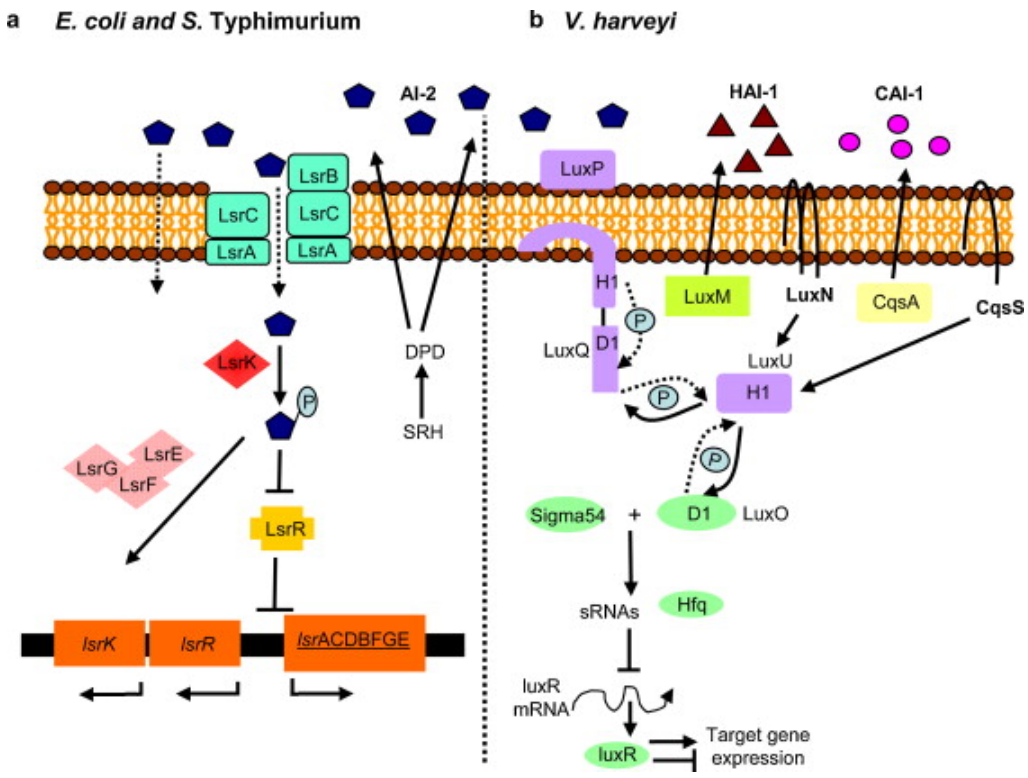


Figure 8. Quorum sensing pathways in *E.* coli and *S.* typhimurium and *V.* harveyi.[18]

An important example of Gram negative bacteria is *Pseudomonas aeroginosa*, capable of infecting insects, plants, animals and humans. As an opportunistic pathogen, it colonizes immunocompromised hosts.[54,55] It is most prominent in patients with cystic fibrosis (a common lethal, genetic disease where the patient often dies because of impaired lung function). A key factor that contributes to the pathogenesis of *P. aeroginosa* and antibiotic resistance is its ability to form a biofilm that attaches either to an interface or to each other, inhabit an extracellular polymeric matrix and exhibit a phenotype distinct from the planktonic cells in terms of gene expression, growth and protein production.[56]

P. aeruginosa is unique from the other pathogens described here since it does not make its own AI-2.[16] *P. aeruginosa* utilizes two LuxI/R systems: LasI/R and RhII/R,

which work in coordination to control the expression of various QS associated virulence factors[57] (Figure 9). Both LasI and RhII produce different AHL autoinducers, N-(3-oxododecanoyl)-HSL (OdDHL) and N-butyryl-HSL (BHL), respectively.[58] Once activated by OdDHL, LasR boosts the expression of the *rhl* regulon through *mvfR*, which causes a subsequent increase in 4-hydroxy-2-heptylquinoline (HHQ, Figure 3) and *Pseudomomas* quinoline signal (PQS, Figure 3) production. The PQS signal is released through the extracellular membrane due to its hydrophobic properties, whereas HHQ stays intracellular. By regulating *rhl* and *mvfR*, LasR controls the production of the virulence factors and biofilm formation.[59] *P. aeruginosa* QS is capable of sensing both AHL signaling and AI-2.[16]

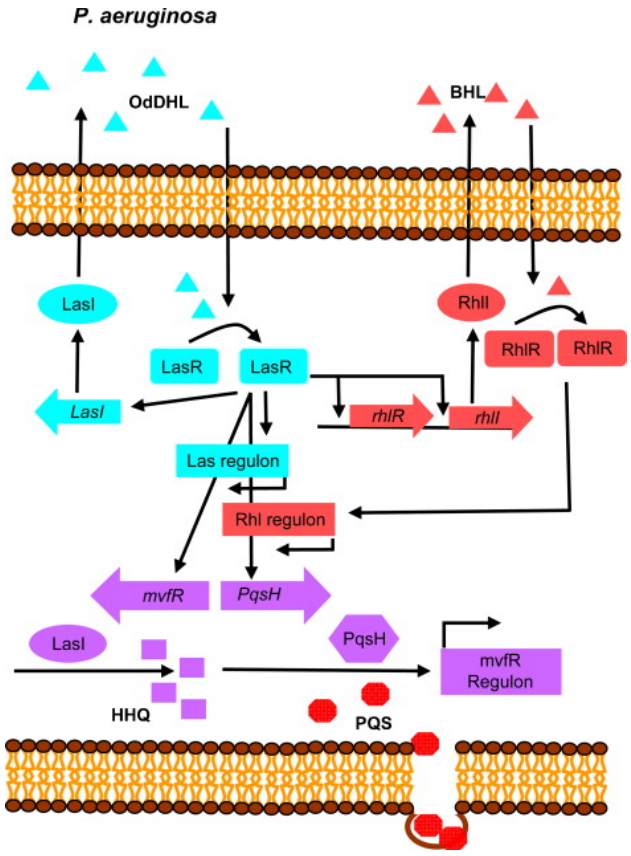


Figure 9. Quorum sensing circuit in *P. aeruginosa*.[18]

1.1.2. Interspecies quorum sensing

1.1.2.1. S-Ribosylhomocysteinase (LuxS): Structure and functions

LuxS is a small metalloenzyme containing Fe^{2+} ion tetrahedrally coordinated by His-54 and His-58, Cys-126 and a water molecule. A highly conserved Cys-84 is located 4.86 Å from the metal ion. It is widely preserved among Gram negative and Gram positive bacteria.[60-64] The structure determination of LuxS protein revealed an α and β fold[64] featuring a four-stranded β-sheet partially surrounded by five helices. The β-sheet is composed of sheets 1, 2, 4, 3 and a 3_{10} helix. Helix α1 is adjacent to β1 and β3. Helices α2, 3 and 4 are perpendicular to α1 (Figure 10A). Crystal structure of the protein revealed that LuxS is a homodimer with the subunits related by crystallographic symmetry. The central part is a dimeric interface that consists of β-sheets coming from each monomer.[64] The dimeric interface has an extensive surface burying 2,125 $Å^2$ of solvent-accessible area per monomer compared to 1,685 $Å^2$ as a mean surface for other dimers.[65] In Figure 10B, the letters A and B designate the two chains of the dimer of LuxS.

Crystal structure

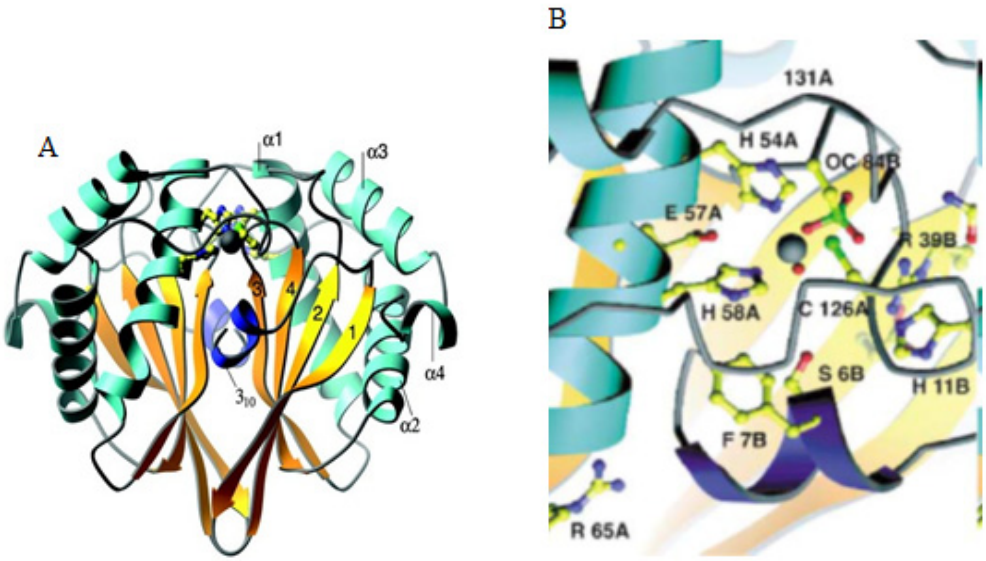
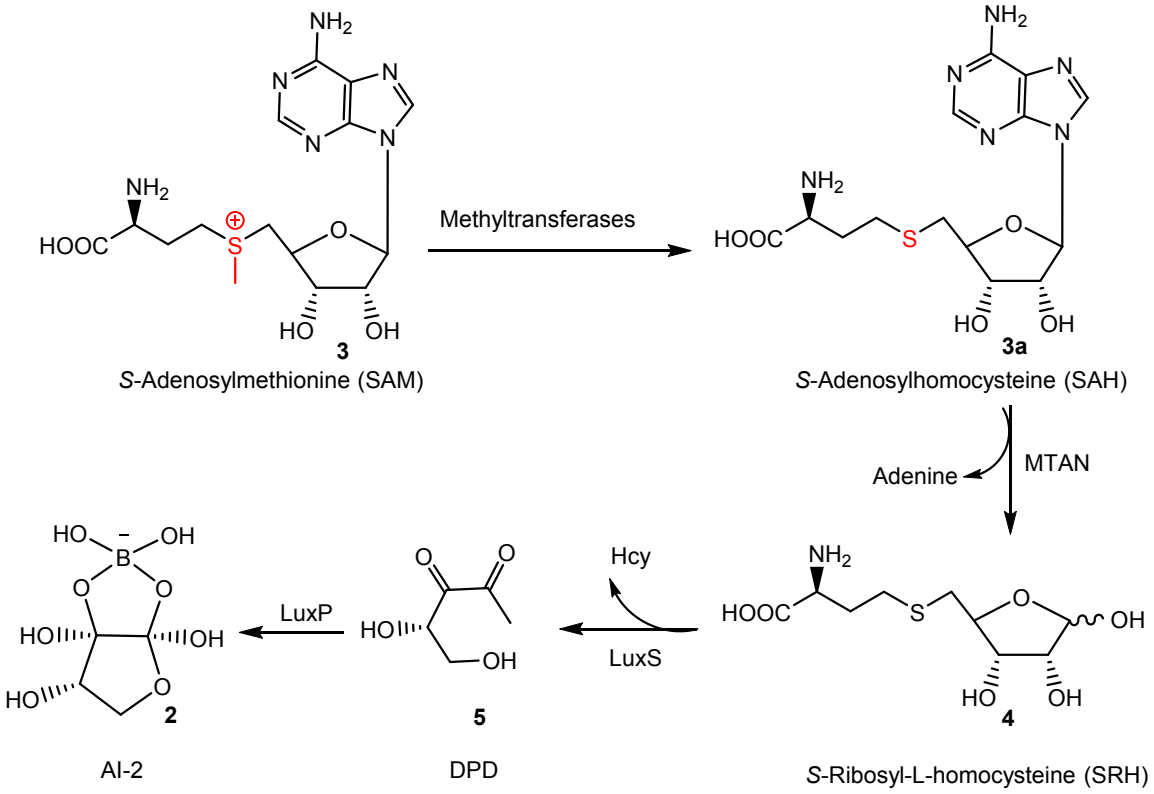


Figure 10. (A) Crystal structure of LuxS[64]; (B) A stereoview of the active site of LuxS.[66]

LuxS mechanism of catalysis

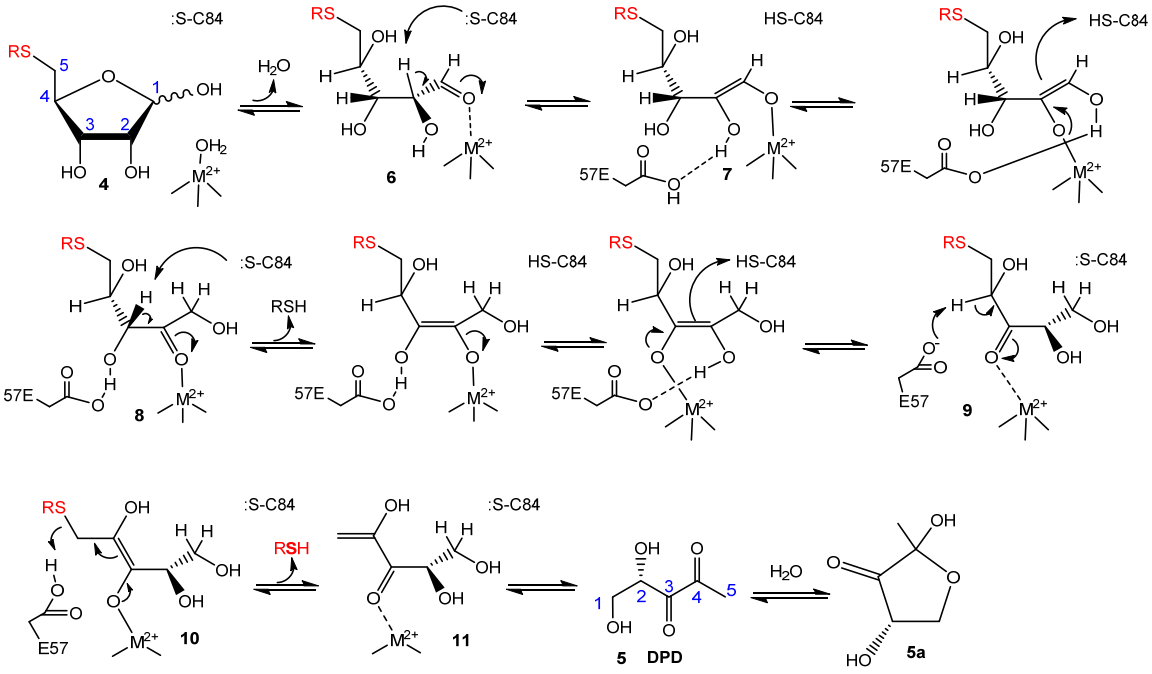
The formation of AI-2 (**2**) starts with S-adenosyl-L-homocysteine (**3a**, SAH, Scheme 2) which is a product of S-adenosyl L-methionine (**3**, SAM)-dependent methylation. The hydrolysis of SAH by 5'-methylthioadenosine nucleosidase (MTAN) leads to adenine molecule and S-ribosyl-L-homocysteine (**4,** SRH). Afterwards, The SRH is cleaved by LuxS enzyme into L-homocysteine (Hcy) and 4,5-dihydroxy-2,3-pentanedione (DPD) which is the precursor of AI-2[1] (**2**, Scheme 2). The formation of AI-2 molecule is the result of a straightforward path that involves DPD (**5**) and boronic acid. The cyclization of DPD was shown to be thermodynamically favorable leading to the tetrahydrofuranone derivative pro-AI-2 (see also Scheme 5) that will complex with boronic acid to form the cyclic borate diester (AI-2).



Scheme 2. Pathway for the conversion of SAM to AI.[18]

Pei and coworkers in 2003 proposed the pathway for the catalytic mechanism of *S*-ribosylhomocysteine [61,67] presented in Scheme 3. Based on the kinetic, spectroscopic and labeling experiments they proposed that in the first initial step, coordination between the metal in LuxS and a water molecule occurs, making the newly formed open sugar complex **6** act as a Lewis acid. The acidic proton at C2 position in SRH complex **6** is then abstracted by the basic Cys-84 present at the β-face of the SRH substrate at the active site of LuxS. The formed enediolate **7** undergoes ligand exchange with the C2-OH group assisted by a Glu57. Tautomerisation to keto form generates a carbonyl at C2 position (**8**). The repetition of this enolization-tautomerization step leads to the formation of the 3-keto SRH complex (**9**), which is prone to a β-elimination (**10** → **11**) of homocysteine catalyzed by Glu57. The release of the homocysteine as a free thiol molecule occurs

simultaneously with the formation of DPD in its enol form which will immediately tautomerize into the more stable keto form (**5**). DPD will then undergo cyclization to form a tetrahydrofuranone derivative (**5a**) which upon further complexation with boronic acid will lead to the formation of borate complex AI-2. Therefore, LuxS enzyme catalysis can be divided into three main steps: First the migration of carbonyl group from C1 to C2 position, then the shift of the carbonyl to C3 position and finally the β-elimination Hcy and formation of DPD.



Scheme 3. Proposed mechanism of LuxS- catalyzed reaction.[60,61]

1.1.2.2. The signaling pathway of autoinducer-2 (AI-2)

Structure of AI-2.

AI-2 (**2**) is the universal signal molecule[68-70] generated by LuxS synthase enzyme. Due to the fact that the differentiation in AI-2 signaling occurs at the transduction level,

different bacteria respond to AI-2 differently. The structure of AI-2 contains two fused five-membered rings located within the LuxP binding site. The hydrogen binding network is the main cause that stabilizes AI-2 molecule in the binding site (Figure 11). AI-2 interacts with different amino acids, the two arginines 215 and 310 present in the active site which are the positively charged residues that form hydrogen bonding with three borate oxygens in AI-2 and therefore stabilize the negative charge of the borate.

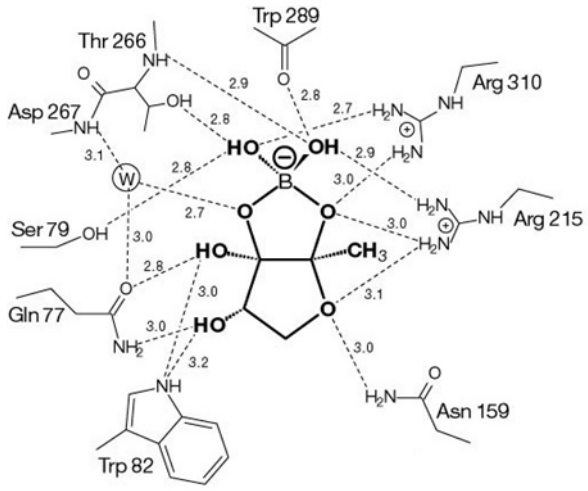


Figure 11. Interaction of the furanosyl borate diester (AI-2) with the amino acids at the binding site.[1]

AI-2: A common language among bacteria:

Unlike other autoinducers, AI-2 does not resemble any other signaling molecules.[42] It is proposed to serve as a universal signal for inter-species communication (Figure 12). It includes a group of signal molecules formed from a common precursor 4,5-dihydroxy-2,3-pentanedione (DPD) (**5** ; Scheme 2). In its turn, DPD is a bacterial by-product of the activated methyl cycle in a reaction catalyzed by LuxS.[71] In a co-culture, the *E. coli* produces AI-2, which is sensed by *S. typhimurium.*

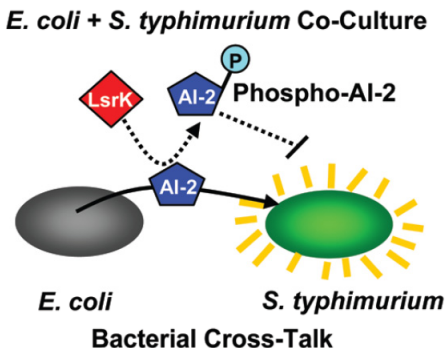


Figure 12. Bacterial cross-talk through AI-2[42]

The crystal structure of AI-2 (Figure 13) in complex with its required signaling partner, LuxP, was revealed by Bassler and coworkers[72] containing the furanosyl borate diester.

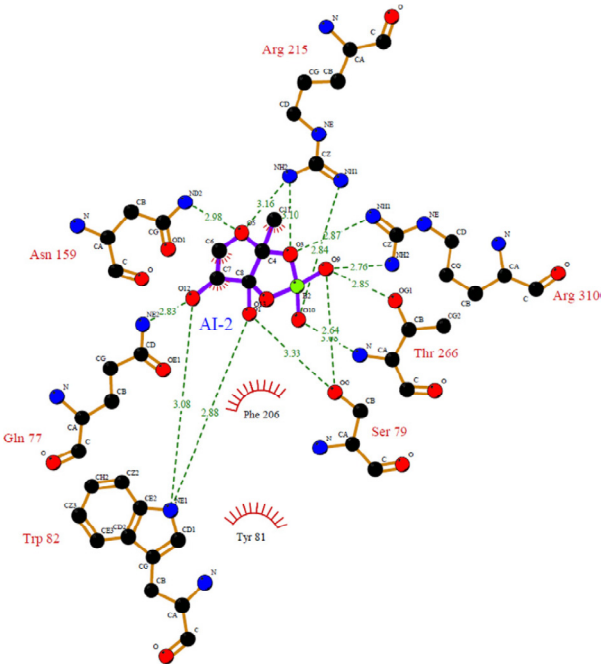


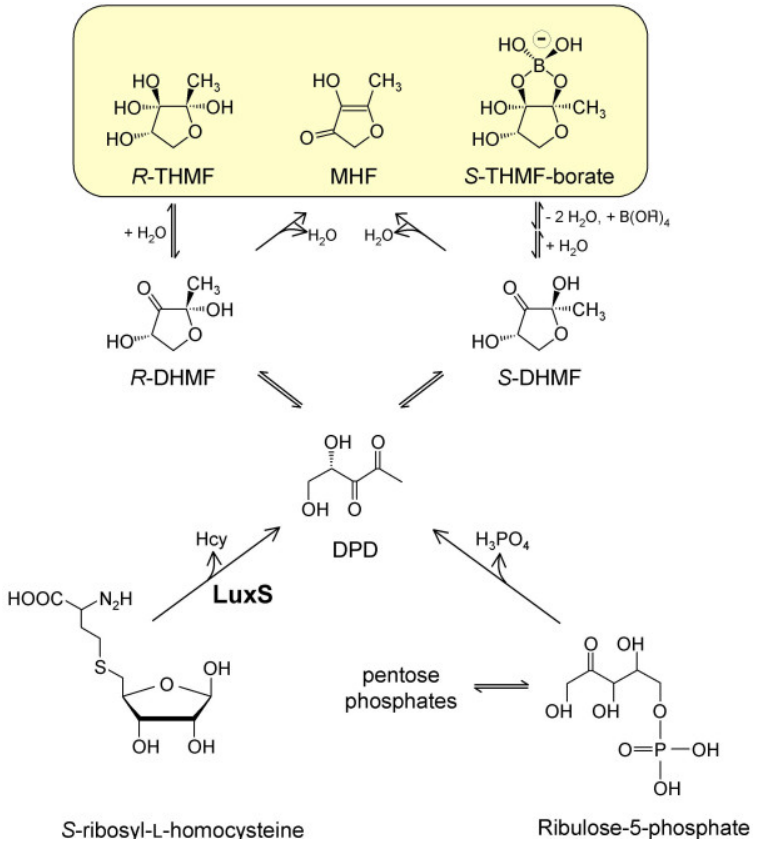
Figure 13. A schematic illustration of AI-2 with LuxP.[1]

To date, two classes of AI-2 specific receptors have been identified: LuxP family found in *vibrio sp*. and the LsrB family identified in S. *Typhimurium.*[3] Miller *et al.* [73] identified a non-borated enantiomer of DPD (2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate, *R*-THMF, Scheme 4) ligand in a crystal structure of

an AI-2 receptor *LsrB* from the *Salmonella enterica* species. These studies demonstrated that bacteria have the ability to detect different molecules based on the stereochemical assignment including differentiation of enantiomers.

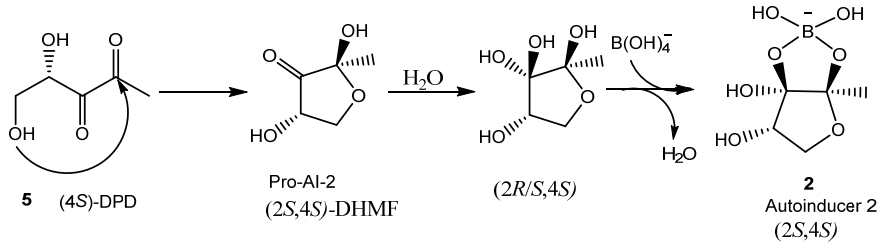
Formation of DPD:

AI-2 is a collective name for a group of signaling molecules formed from 4,5-dihydroxy-2,3-pentanedione (DPD, **5**).[44,71,74-76] DPD is an enigmatic molecule that has been known since 1971 as a bacterial byproduct[45] of the activated methyl cycle in a reaction catalyzed by LuxS.[71] DPD is the product of the cleavage of *S*-ribosylhomocysteine (SRH) (Scheme 3) which spontaneously cyclizes into furanone derivatives (Scheme 4): (2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate (*S*-THMF-borate) and (2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate (*R*-THMF) which are known by specific periplasmic binding proteins in *V. harveyi* and *S. enterica* respectively.[1,73] DPD can be also synthesized from ribulose-5-phosphate and although this pathway is not clinically significant it has been proved to be an important metabolic passageway for DPD.[77,78] DPD has recently been synthesized[45,79] by Semmelhack and Meijler and their coworkers. It exists in an equilibrium mixture of two cyclic tetrahydrofuranone isomers: *S*-DHMF ((2*S*,4*S*)-2-methyl-2,4-dihydroxytetrahydrofuranone) and *R*-DHMF ((2*R*,4*S*)-2-methyl-2,4-dihydroxytetrahydrofuranone) (Scheme 4) with the respective hydrated analogues *S*-THMF and *R*-THMF. When complexed to boronic acid, only the *S*-DHMF forms *S*-THMF-borate known as AI-2[1] (**2,** Scheme **2**).



Scheme 4. Biosynthetic pathways for the formation of DPD and AI-2.[3]

DPD undergoes spontaneously cyclization to the corresponding tetrahydrofuranose derivative known as pro-AI-2. Hydration reactions in solution produced *R*-THMF diastereoisomers. In the presence of Boronic acid, the *S*-THMF undergoes complexation to form *S*-THMF-borate known as AI-2 (Scheme 5).



Scheme 5. Cyclization pathway of DPD to AI-2.[80]

1.1.3. Strategies for quorum quenching (QQ)

The disturbance of quorum sensing should reduce pathogenicity.[5,81] The approach of interfering with or destroying the QS signal is referred to as Quorum quenching (QQ). Strategies for quorum quenching include competing with the native signaling process either by creating signal molecules analogs, limiting signal generation or destroying the native molecules by sequestering traps. Organization of the quorum quenching is based on the location of the quenching in the track. It could either occur at the signal generator, the signal itself or the signal receptor and transducer (Figure 14). Currently there are three models being followed for the development of QQ agents. First, inhibition of QSSM synthase, for example LuxS in case of AI-2 QS[82-84] or LuxI type in case of AHL-based systems.[85] Second, neutralization of QSSM by protein based strategies as QSSM degrading enzyme[86] or sequestrating antibodies.[87-89] Third, imitation of the ligand at the receptor level through QSSM analogs.[56,90-92] Other strategies have been employed including inhibition of the signal secretion[93,94] or interference with the downstream signal.[95]

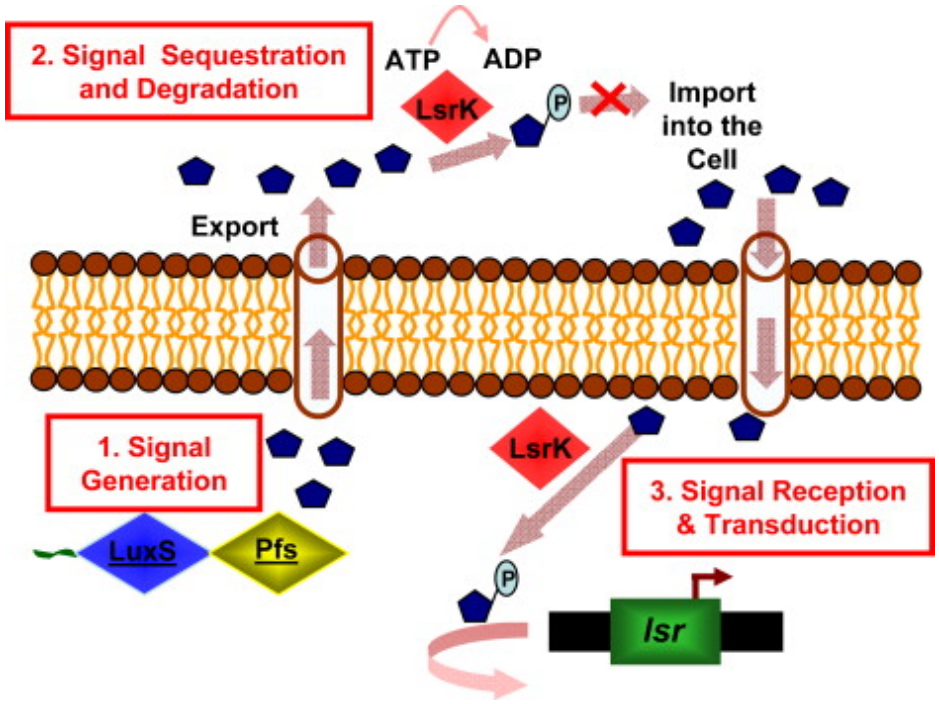


Figure 14. Strategies for quorum quenching.[18]

The QS mechanism in *E. coli* consists of various luxS regulated *lsr* genes.[46] Signal transduction by *lsr* gene expression, characterizes the native AI-2 QS response (Figure 15a). By binding to the transcriptional repressor LsrR, phospho-AI-2 induces transcription of the *lsr* genes. Roy and coworkers,[42] tried to quench the cross communication among different bacteria (Figure 15b) using LsrK, an enzyme that phosphorylates AI-2 upon uptake into the cell, using ATP. LsrK and ATP are delivered outside the cell and phosphorylation of AI-2 leads to the unstable phosphor-AI-2 that auto-degrades to 2-phosphoglycolic acid (PG) overnight, which is then prevented from being carried into the cells. They demonstrated that when LsrK and ATP where added ex-vivo to bacteria, the native QS response significantly diminished.

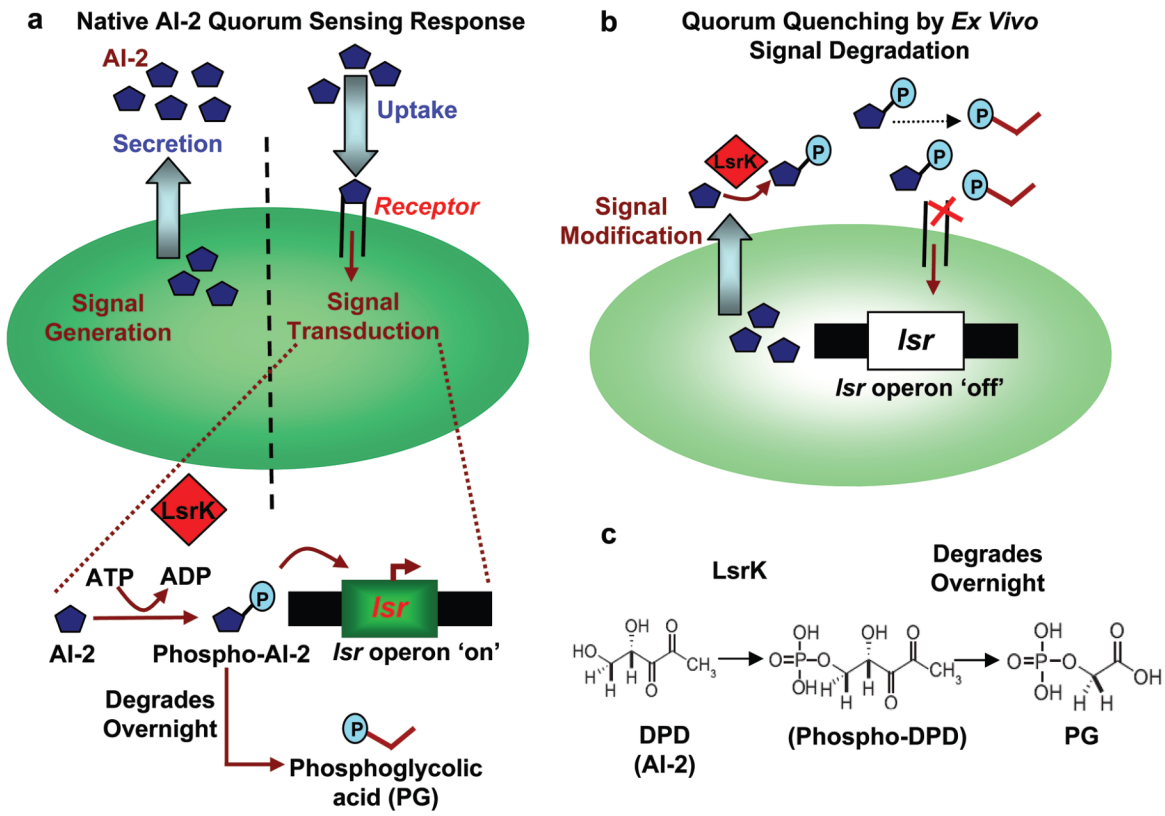


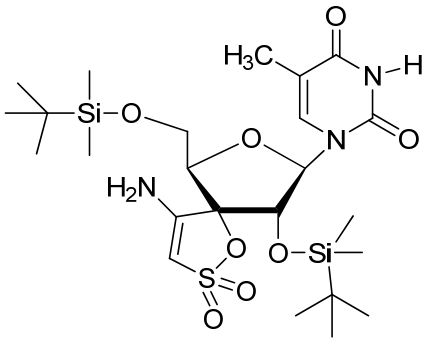
Figure 15. Schematic LsrK-mediated quenching.[42]

1.2. Dimerisation inhibition

LuxS enzyme is a metalloenzyme with a symmetrical dimer structure that catalyzes the non-redox cleavage of the stable thioether bond in SAM cycle with no need of NADH cofactor for the chemical reaction.[60] Introducing a dimer inhibitor molecule to LuxS might be considered as a new strategy for the inhibition of the LuxS enzyme which has not been studied before. Some other dimer inhibitors that have been designed in litterature act on different dimer enzymes. Examples of dimer inhibitors include Herceptin® [96] (Trastuzumab) and TSAO-T.[97]

1.2.1. Examples of dimer inhibitors of other enzymes

Since many enzymes are homodimers or multimers, the disturbance of the stereochemical orientation between protein subunits offers another rational way for enzyme inhibition leading the ability to alter the function of the enzyme.[65] One important example would be Trastuzumab, a new anticancer agent (monoclonal antibody) that binds to the juxtamembrane region of the human epidermal growth factor receptor 2 known as HER 2. Trastuzumab deactivates the kinase, providing a steric barrier to the interaction of the transmembrane region and consequently disrupts HER 2 interactions with the protein.[96] Also, a thymidine nucleoside analogue modified at the C3 position with a spiro sulfonate ring belonging to TSAO family (**12,** Figure 16), which is a part of the non-nucleoside reverse-transcriptase inhibitors known as NNRTI, is used as a selective inhibitor against HIV-1 virus, by binding to the p66/p51 dimer subunit interface.[98]



12

Figure 16. TSAO-T, selective inhibitor of HIV-1.[99]

1.2.2. Dimerization process in LuxS enzyme

LuxS enzyme is a small (35 kDa) homodimeric protein.[100] In theory, SRH is positioned in between the two monomers at the active site of LuxS. The stereoview of the atomic interactions between LuxS and the 2-ketone intermediate reveals that the subunit

A of the dimer has the metal ion of the active site (in gold color in Figure 17), whereas the subunit B atoms shown in cyan color,[101] hold the catalytic residue Cys84 and contribute less to the substrate binding residues (Notice that the oxygen atom of the 2-ketone position of the intermediate is coordinated to the cobalt ion. Also the Ala-84 of the Cys-84-ala LuxS protein is positioned near the C2 and C3 atoms). Thus, the insertion of a dimer inhibitor in between the two monomers would theoretically prevent their interactions. The lack of coordination between subunits A and B should lead to the inactivation of the LuxS enzyme, since the catalytic role of subunit B which includes the cleavage of SRH to DPD would not be associated with subunit A due to the steric insertion between the two subunits.[101]

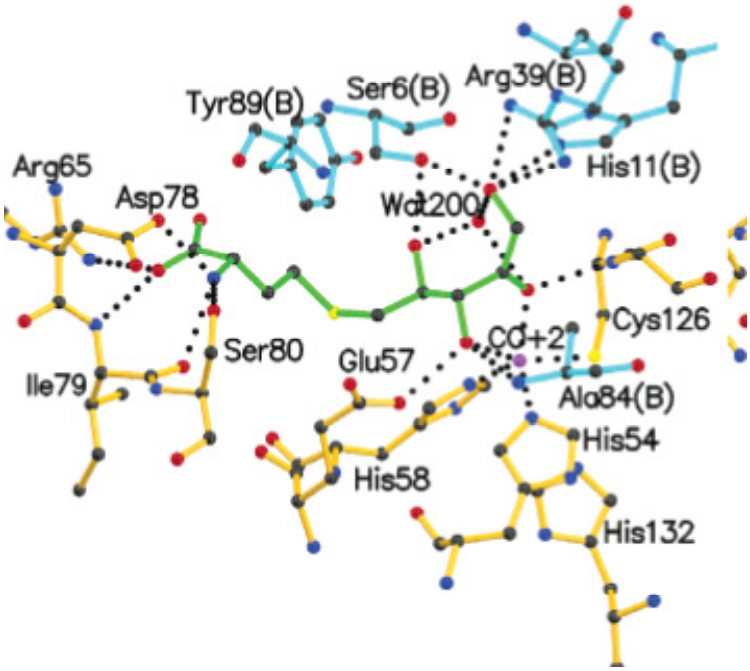


Figure 17. LuxS dimer active site.[101]

Studies have been conducted to explain the constituent of the interface of LuxS. They aim to show the mechanism of ligand binding and chain connectivity in protein

dihydrofolate reductase (DHFR). The dimeric state of LuxS enzyme of four organisms which were available to study demonstrated a distinctive fold to the alpha-beta family of proteins.[102] The LuxS interface includes three vertices of an isosceles triangle. Two of the active clusters have the active site residue while the third one, apex cluster, located at the apex of the dimer with the display loop residues in a mini-triad form arrangement. The active site clusters are made up of three histidines and one glutamine from one subunit and a phenylalanine residue from the other subunit. Thus the active site of subunit A is supported from phenylalanine of subunit B and vice versa.[102] This triad is repetitive between the two subunits of the dimeric protein. The mutation of any of the components of the triad has been showed to inactivate LuxS enzyme.[102]

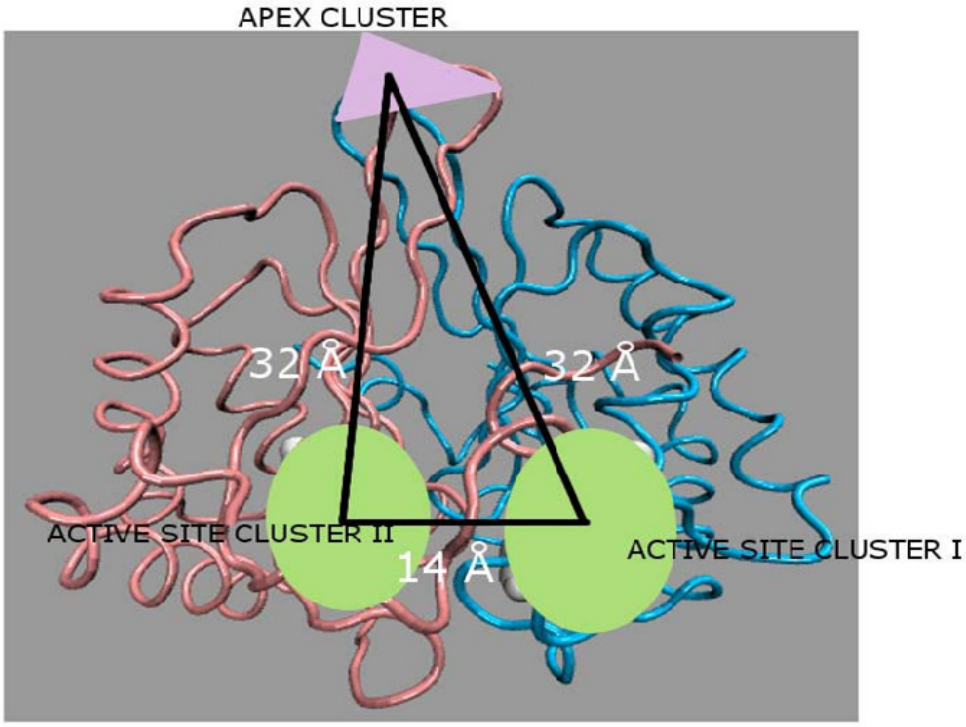
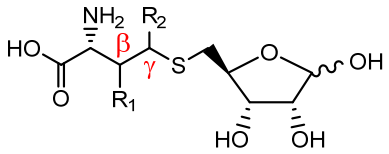


Figure 18. Schematic representation of isoceles orientation of the LuxS.[102]

Recently SRH analogues modified at the homocysteine unit (**4a**) were proposed as potential dimer inhibitors of LuxS (Figure 19).[103,104] It was hypothesized that inhibitors with the inserted long alkyl/aryl chain at either the β-carbon or the γ-carbon of Hcy unit should interfere with two monomers of the protein, which might alter the proximity between the monomers, which in turn should lead to a change in enzyme activity. The steric hindrance at the dimerization site caused by **4a** would result in dimer disruption and subsequently a change in the proximity of the LuxS monomers. It was also proposed that fluorescence proximity assay might be used for the detection of the dimerization inhibition of LuxS by measuring the change in fluorescence upon monomer separation[105-107] resulting from the supposed interaction of inhibitors **4a** with LuxS protein.



4a

R_1= alkyl, aryl; R_2=H
R_1= H; R_2= alkyl, aryl
R_1= R_2= alkyl, aryl

Figure 19. SRH analogues proposed as potential dimerization inhbitors.[103,104]

1.3. Inhibition of LuxS enzyme by substrate analogues

General approaches towards the modulations of QS, and AI-2 inhibition strategies in particular, are presented in Table 3. As can be concluded based on the biosynthetic pathway for the production of DPD and consequently AI-2 presented in Scheme 3, the major target responsible for QS inhibition is the LuxS enzyme which controls the production of AI-2.

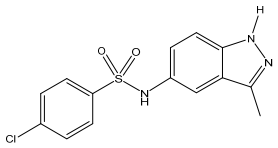
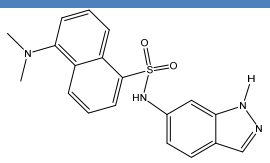
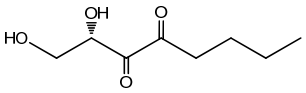
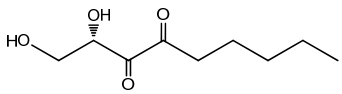
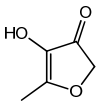
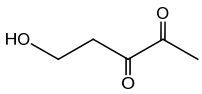
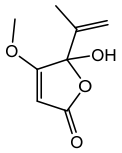
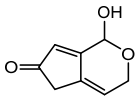
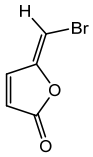
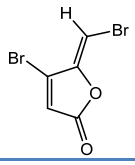
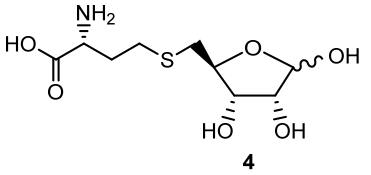
Inhibitor Target	Active site	Inhibitors
Signal generators	SAH/MTA nucleosidase (Pfs)	 4-CPS derivative Ki = 4 μM[108]  Dansyl derivative Ki = 1 μM[108]
	LuxS enzyme	Competitive substrate inhibitors and SRH analogues
Signal molecules	AI-2	Introducing LsK and ATP to phosphorylate AI-2[42] and prevent its intracellular uptake
Signal receptor/ transduction	DPD analogues[109]	  Substrate inhibitor designed by Ganin et al.  MHF  Laurencione
Various	Brominated furanones[110-112]	Natural product  Penicillic acid  Patulin Synthetic bromo-furanones  

Table 3. Overview of QS inhibition strategies[18,42,60,84,109,113-119]

1.3.1 *S*-ribosylhomocysteine substrate analogues

Absent in humans, the LuxS enzyme is considered a smart target for novel antibacterials. Zhou and coworkers[19] were the first to develop synthetic analogues of SRH which act by competing with the natural substrate SRH **4** (Figure 20). The first

competitive substrate analogue was *S*-anhydroribosyl-L-homocysteine **13a**[19] in which the hemiacetal group has been replaced by an ether functional group. Compound **13a** should prevent the first step in the catalysis of LuxS enzyme known as the aldo-ketose isomerization.[83] The presence of the 2,3-diol functional group was expected to ligate to the active site metal ion which allows it to bind to LuxS in a similar fashion as SRH. The second substrate analogue *S*-homoribosyl-L-cysteine **13b**[19] had a replacement of the carbon–sulfur bond (C5-Sulfur) in SRH **3** by a carbon-carbon bond (C5 and C6). Substituting the sulfur atom by a carbon at position 6 should allow **13b** to bind properly to LuxS enzyme and undergo aldo-ketose isomerization to form a ketone at C3 of **13b**. Further elimination reaction would however be prohibited since the cleavage of the carbon–sulfur bond cannot occur, preventing elimination of Hcy.



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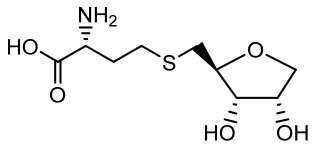
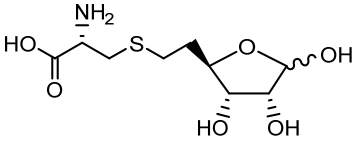
S-ribosyl-L-homocysteine (SRH)



13a

S-anhydroribosyl-L-homocysteine



13b

S-homoribosyl-L-cysteine

Figure 20. Substrate inhibitors of LuxS enzyme designed by Zhou and coworkers.[19]

Pei and coworkers[60] designed and synthesized substrates for LuxS enzyme with the an open ribonic acid moiety (Figure 21). Thus, the replacement of the enediolate moiety with a planar hydroxamate group in compound **14**[120,121] was envisioned to produce

a stable isostere with high affinity to LuxS. The metal ion in LuxS which was proposed to give the stability of the enediolate intermediate e.g. (**4**, Scheme 2) was expected to bind to the planar hydroxamate group in **14**. As a stereoisomer of **14** at the β-carbon of the hydroxamate, compound **15**[122] was expected to have similar LuxS inhibition. Compound **16** (without a Hcy moiety) was also tested to check the importance of the Hcy moiety in the proper binding of the inhibitor to the active site of LuxS. Pei *etal.* also proposed compound **17** in which the amino acid portion and a metal chelating group (*N*-formylhydroxylamine) are linked. These compounds were tested against co-(II)-substituted *B.subtilis* LuxS, compounds **14** and **15** acted as potent competitive inhibitors with respective K_i = 0.72 and 0.37 μM. Compound **16** had a lower affinity of K_i > 150 μM which reinforced the importance of the amino acid moiety in the affinity to LuxS. Compound **17** exhibited slight inhibition with K_i = 68 μM whereas the SRH analogues **18** and **19** having Hcy amino acid unit modified did not show inhibitory activity.

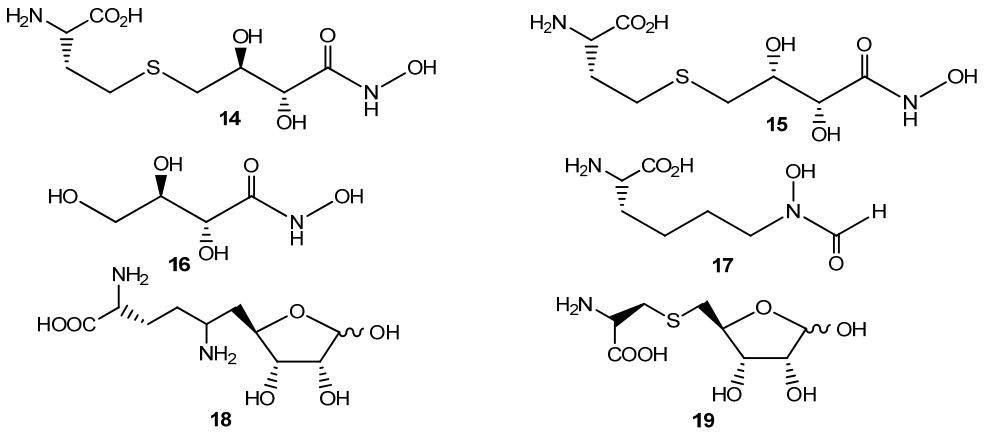


Figure 21. Substrate inhibitors of LuxS enzyme designed by Pei and coworkers[60]

Wnuk and coworkers investigated two groups of potential LuxS inhibitors.[123,124] One group had a vinyl or halovinyl moiety incorporated at C5 of SRH as depicted in compounds **20**-**23** (Figure 22). In compounds **20**-**22**, the incorporation of the fluoro-vinyl unit was envisioned to target the final step in the LuxS mechanism preventing the β-elimination to occur. These compounds tested also a possible hydrolytic activity of LuxS enzyme since it was shown that a related *S*-adenosylhomocysteine hydrolase which also catalyzes C5-sulfur bond in the *S*-adenosylhomocysteine, was able to add water across the isolated C5'-C6' bond.[123,124] As for **23**,[123] the lack of the hydroxyl group at C3 of the sugar should prevent the second enolization step of the LuxS catalyzed reaction. No significant inhibition has been reported for the above compounds except **23** (Table 4). The second group of SRH analogues had carbon-3 modification in the ribose ring. These compounds should prevent the second enolisation step in the LuxS catalysis because of (i) the lack of the hydroxyl group as in 3-deoxy-analogue **24**, (ii) the lack of the enolizable proton in 3-methoxy analogues **25** and **26,** (iii) the inversion of the stereochemistry at C3 as in xylo-analogue **27**, or (iv) the replacement of the 3-hydroxyl group by a halogen atom as in **28** and **29** (Figure 22). Compounds **28** and **29** showed time-dependent inhibition of LuxS, while compounds **20-22** did not show any inhibitory activity. The loss of fluoride ion via the postulated E1cB elimination reaction in **28** instead of the nucleophilic substitution in the bromo atom in **29** led to the faster conversion of the initial enzyme-inhibitor complex E·I in **28** into a tighter enzyme-inhibitor complex $E \cdot I^*$ than in **29**.

	Compound	K_i
Competitive inhibitors	**23**	96 μM
	24	55 μM
	25	66 μM
	26	42 μM
	28	10.6 μM
	29	7.9 μM

Table 4. Ki values of different substrate inhibitors of LuxS.[124,125]

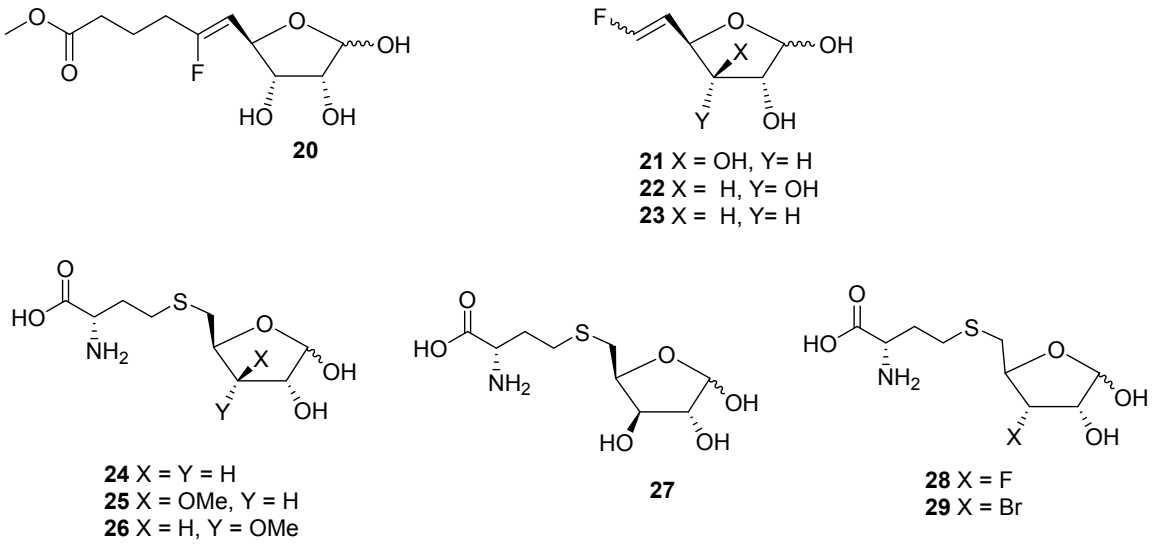
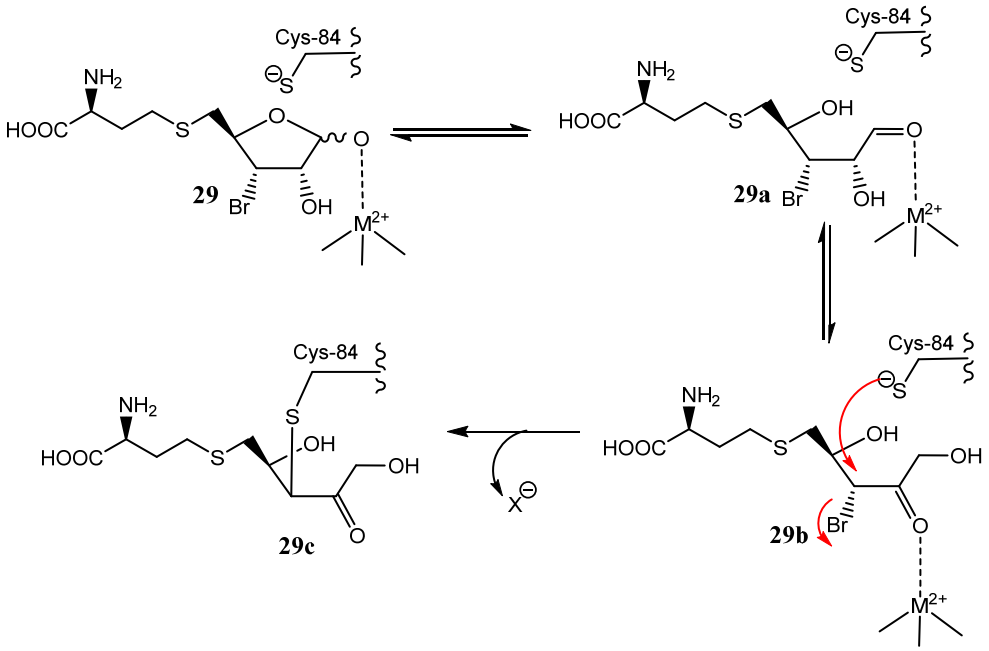


Figure 22. Substrates of LuxS enzyme synthesized by Wnuk and coworkers.[123,124]

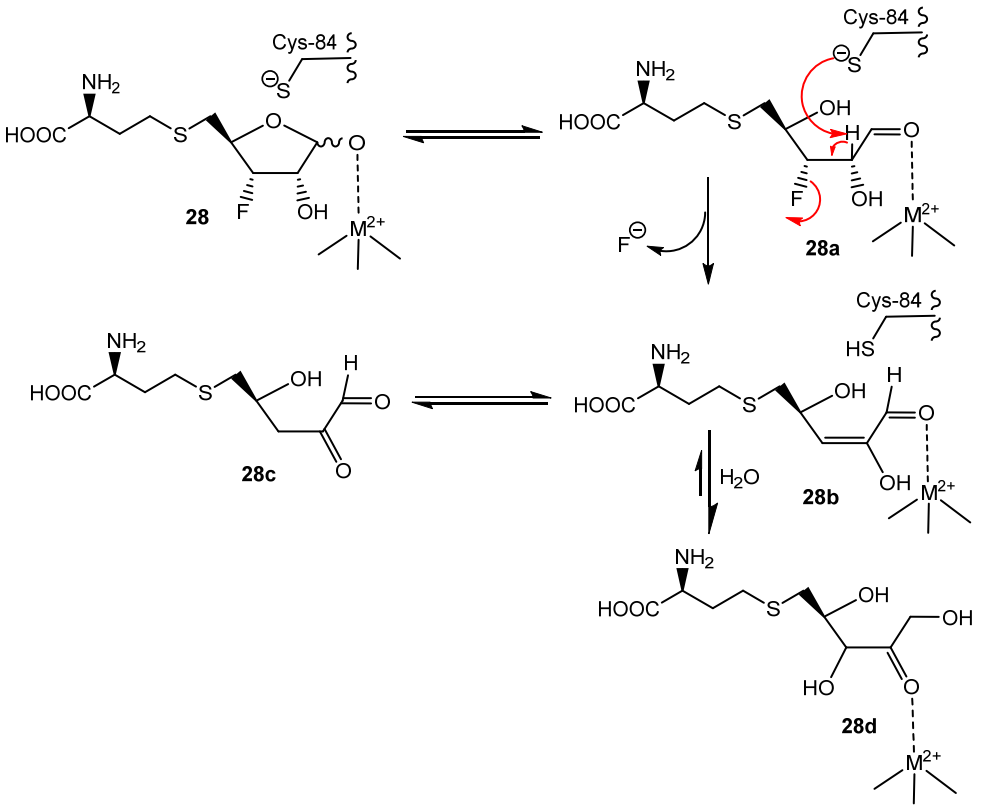
Two 3-halogeno-substituted SRH analogues (3-Br-SRH; **29** and 3-F-SRH**; 28**) acted as potential suicide inhibitors for LuxS.[125] Since both molecules have a halogen (either bromide or fluoride) at carbon C3 instead of a hydroxyl group, they were expected to

bind to the active site of LuxS and to alert the enolization reaction that occurs at C3 to prevent the ketone formation at C3 (see Scheme 3). Detailed mechanistic studies performed in Professor Pei's laboratory demonstrated that 3-[Br]-SRH **29** and 3-[F]-SRH **28** inhibit LuxS by different mechanisms.[125] In the case of **29**, the negatively charged Cys-84 nucleophilically attacks C3 in **29b** (reactive α-bromoketone) leading to the loss of bromide ion and the formation of a new covalent adduct between C3 of the ribose ring and the sulfur atom of Cys-84 as shown in **29c** (Scheme 6)**.**



Scheme 6. Proposed mechanism for the inhibition of LuxS by 3-Br-SRH.[125]

In the case of **28**, the negatively charged Cys-84 abstracts the acidic α proton at C2 of the hydroxy-aldehyde **28a** leading to the loss of fluoride ion via the postulated E1cB elimination reaction. The formed enol **28b** could exist in equilibrium with the diketone **28c** or undergo hydration to form **28d** (Scheme 7).



Scheme 7. A plausible mechanism for the inhibition of LuxS by 3-F-SRH.[125]

Wnuk and coworkers also designed the 4-aza-SRH analogues, in which nitrogen replaces the oxygen atom of the furanose ring (Figure 23).[126] The hemiaminal derivatives (e.g. **32**) were expected to have different stabilities compared to the *O*,*O*-hemiacetals present in SRH **3** which should result in different rates of metabolism. The binding strength of the aza analogues were expected to be different than the hemiacetals mainly because of the higher basicity of the nitrogen atom in the aza compounds as compared to the oxygen atom in the natural substrate SRH. Thus the binding strengths and rate of production of the open chain aldehyde was expected to be different. In addition, the aminosugars are more prone of protonation at physiological pH which should also alter the enzymatic activity.

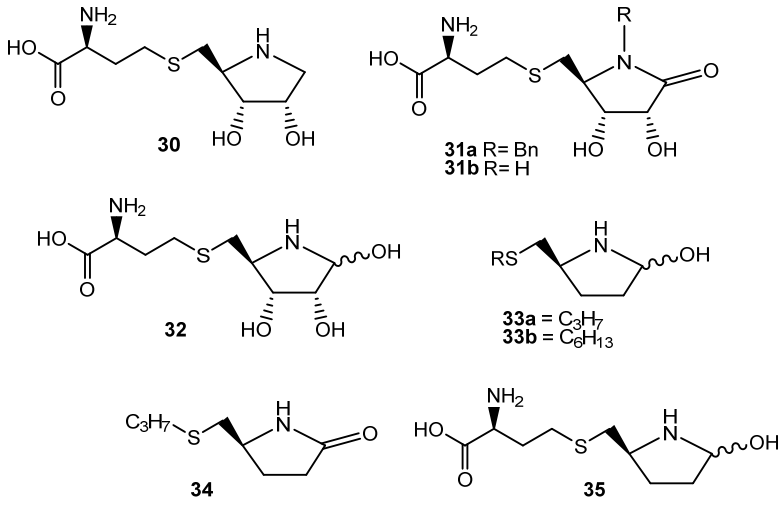
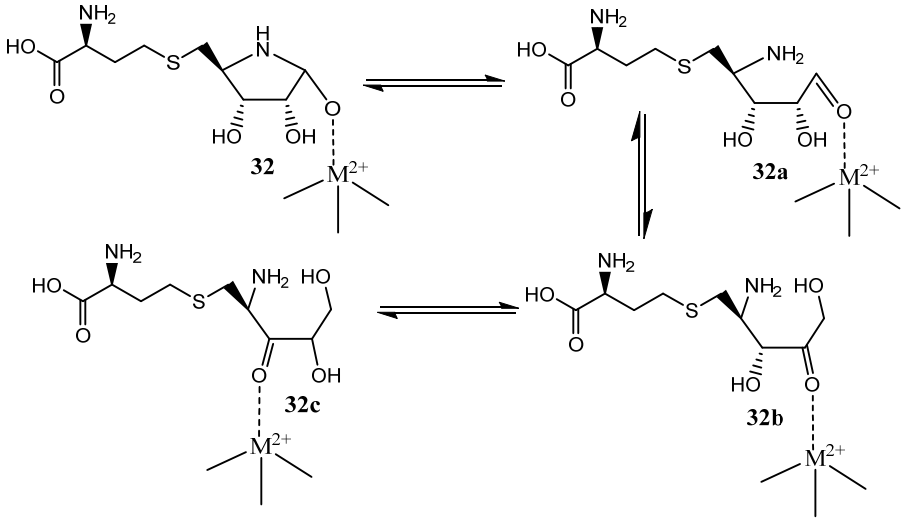


Figure 23. 4-[aza]-SRH analogues.[126,127]

The aza-analogue **30** lacking a hydroxyl group at C1 showed a concentration – dependent inhibition of LuxS that was consistent with competitive inhibition (K_i = 48 μM). The lactam **31b** had a K_i value of 37 μM while the *N*-benzyl protected lactam **31a** was found to be inactive mostly due to the steric reasons. The aza analogues lacking hydroxyl groups at C2 and C3 as in **33-35** were inactive mainly due to the absence of the hydroxyl groups which are important to coordinate with the catalytic metal ion in the LuxS protein. Unlike other aza-analogues, the hemiaminal **32** was found to be a time-dependant inhibitor of LuxS with IC_{50} value of 60 μM and K_i value of 3.5 μM. The time-dependent inhibition of LuxS by **32** might indicate that the hemiaminal exists in the equilibrium with the free aldehyde form **32a** as depicted in Figure 23. The aldehyde **32a** was proposed to undergo aldose-ketose isomerization reaction to generate 2-keto intermediate **32b** and consequently 3-keto intermediate **32c**. However, no spectroscopic proofs for the presence of the formation of the 2 or 3-keto intermediate were found.[126]

The mechanism of inhibition of LuxS by aza-analogues appears to be similar to the initial step of the inhibition of LuxS by the halogenated SRH analogues such as [3Br-SRH] and [3-F-SRH] which also showed to undergo LuxS-catalysed ring opening.[125]



Scheme 8. Plausible mechanism for the time-dependent inhibition of LuxS by 4-aza-SRH hemiaminal 32.[126]

The aza analogues **33** (aza-hemiacetal) and the corresponding lactam **34** in which the Hcy unit was replaced by an alkyl-thio group bearing a long alkyl chain, acted as strong inhibitors against AHL-dependent *las* (activity 43% at [1.16 mM] inhibitor concentration) and weaker ones against *rhl* (activity 104% at [1.16 mM] inhibitor concentration) QS pathways in *P. aeruginosa*. Antagonism of *las* activity has been increased with the length of the alkyl-thio chain. The hexylthio-analogue **33b** appeared to be the strongest inhibitor among the tested compounds (*las* activity 0%; *rhl* activity 0%) against both *las* and *rhl* systems.[127]

The other strategies for modulating QS and effecting production of AI-2, which were presented in Table 3, include the novel synthetic analogues of DPD[128,129] or the

brominated furanoses[110-112] and other small molecules that have been recently reviewed.[129]

An interesting approach for the inhibition of bacterial QS was recently developed by Meijler's group which is based on the covalent modification of a QS receptor as a new tool to study molecular mechanism of bacterial group behavior and to target bacterial virulence. Meijler's group developed isothiocyanate and α-halomethyl carbonyl probes which are modified AHL analogues (Figure 24).[91] These very reactive isothiocyanate and haloacetamide analogues were envisioned to react with proximal nucleophiles via "azido free" click-chemistry leading to covalent inhibition and molecule labeling. These analogues were shown to strongly inhibit *P. aeruginosa* QS-related gene expression (*las*) resulting in the inhibition of the transcriptional activator LasR.

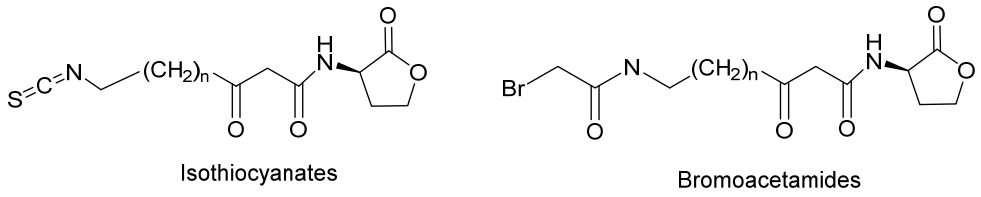


Figure 24. AHL analogues modified with isothiocyanates and bromoacetamides.[91]

2. RESEARCH OBJECTIVE

The objective of this dissertation is the design, synthesis and biological evaluation of novel LuxS inhibitors which should interfere with quorum sensing processes. My initial targets were *S*-ribosylhomocysteine [SRH] analogues in which the hydroxyl group at C2 in the ribofuranose ring of the SRH would be substituted by halogens such as bromide or fluoride. The two main targets of the C2-substituted sugar analogs are 2-deoxy-2-fluoro-D-ribosylhomocysteine **36a** and its corresponding *arabino*-epimer **36b** as well as 2-deoxy-2-bromo-D-ribosylhomocysteine **37a** and its *arabino*-epimer **37b** (Figure 25). The targeted 2-halo-SRH analogues are envisioned to interact with the LuxS protein differently than the natural substrate *S*-ribosylhomocysteine does, upon binding to the LuxS enzyme. Thus, 2-halo-SRH analogues, lacking the 2-hydroxyl group, should prevent the formation of the 2-keto intermediates (e.g., complex **6,** Scheme 3) during the interaction with LuxS.

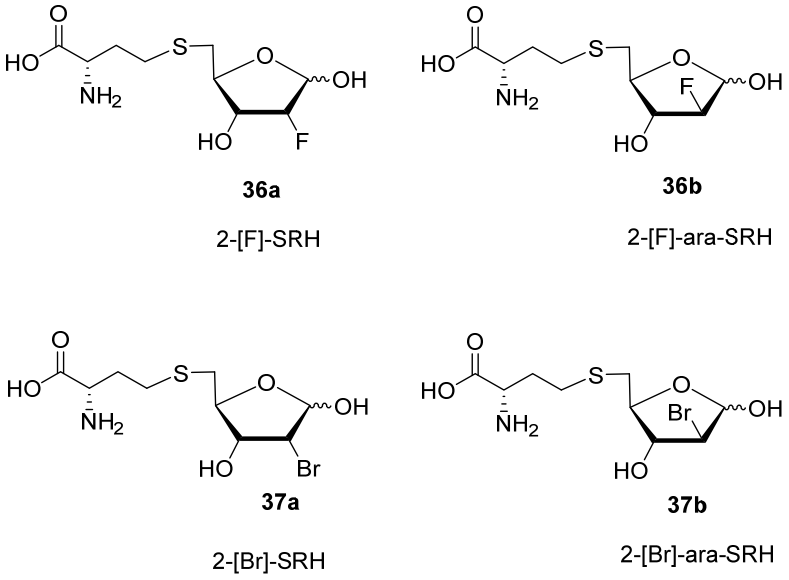
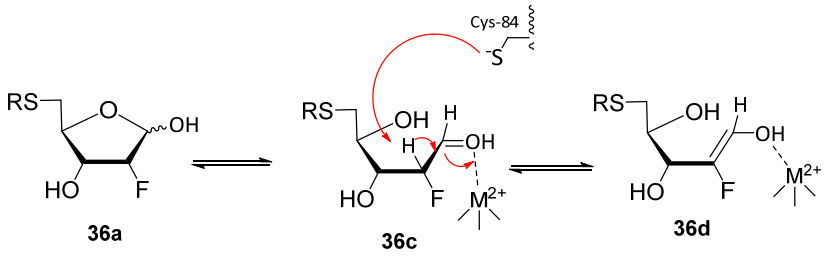


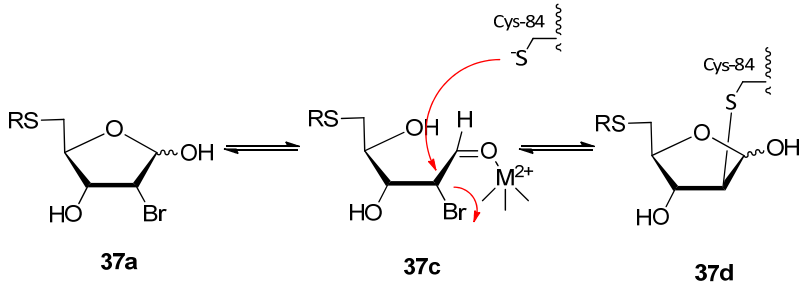
Figure 25. Targeted 2-deoxy-2-halo-*S*-ribosylhomocysteine analogues.

It is postulated that the 2-[F]-SRH analogue **36a** after ring opening and abstraction of the acidic H2 proton by proximal Cys84 (from the β face) should lead to the formation of the fluoro-enol intermediate **36d** rather than the 2-keto intermediate which is formed during metabolism of the natural SRH (see Scheme 3).



Scheme 9. A possible mechanism of inhibition of LuxS by 2-[F]-SRH.

The interaction of LuxS with 2-[Br]-SRH analogue **37a** might occur by the formation of the analogous bromo-enol intermediates or by the nucleophilic displacement of bromide. Based on the previous studies with 3-halo-SRH analogues[125] (see Scheme 6), it is reasonable to expect that after ring opening the reactive α-bromo-ketone intermediate **37c** might be a substrate for nucleophilic substitution by the active site Cys-84 leading to a covalent inhibition as depicted in Scheme 10.

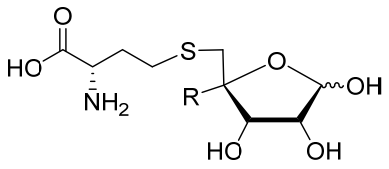


Scheme 10. Plausible inhibition of LuxS by 2-[Br]-SRH.

The displacement of the bromide by cysteinate thiol could occur at either closed or opened form of the ribose ring. However, because of the different stereochemical

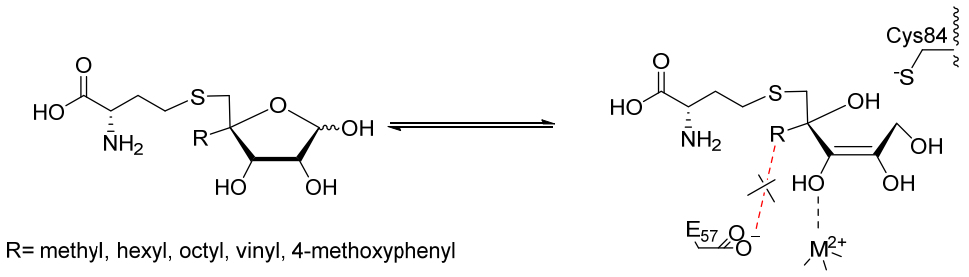
requirements of the bromo substituent in *ribo* **37a** and *arabino* **37b** substrate, inhibition might also shed some light on the leniency of the active site for the stereochemical regimen of the substituent at the C2 of the ribose ring. For example, the bromo substituent at the β face in the *arabino* substrate **37b** might prevent S_N2 displacement which requires a nucleophilic attack by Cys84 from the opposite site of the leaving group (bromide). The fact that all four 2-halo-2-deoxy-SRH analogues lack the 2-hydroxyl group, the first tautomerization step and the generation of the 2-keto-SRH intermediate, which is critical for the enzymatic activity of LuxS might be prevented.

My second targets were SRH analogues in which the hydrogen at C4 of the ribose ring is substituted by an alkyl group of varying length (e.g. alkyl, vinyl and aryl; Figure 26). These 4-*C*-alkyl-SRH analogues are targeted for two reasons. First, I anticipated that 4-*C*-alkyl-[SRH] analogues because of the lack of hydrogen at C4 would prevent the elimination of Hcy (as depicted in Scheme 3, conversion **7** → **8**) effecting the overall production of DPD (Scheme 11). Secondly, since LuxS is a biologically active homodimer with a triad interface,[102] the addition of an alkyl group could lead to the destabilization of the LuxS dimer.



R= methyl, hexyl, octyl, vinyl, 4-methoxyphenyl

Figure 26. 4-*C*-alkyl-substituted SRH analogues (4-[R]-SRH).



Scheme 11. A plausible mechanism for the inhibition of LuxS by 4-[R]-SRH analogues.

The next goal of my dissertation was the synthesis of 4-[thia]-SRH mimics (e.g. **39**) in which the furanose ring oxygen has been replaced by a sulfur atom (Figure 27). The resulting thiohemiacetal (*O*,*S*-acetals) should have different stabilities relative to the *O*,*O*-acetals present in SRH and as a result different rates of metabolic alteration. The expected increased stability of the glycosyl bond should therefore result in decreased rates of metabolic degradation and effect production of the open chain aldehyde form necessary for the first LuxS-catalyzed isomerization to occur. Moreover, the [4-thia]-SRH analogues can be converted to a thio-analogue of DPD **39a** which could interfere with QS because of the different ability for the ring closure (e.g, **39b**) and consequently different capability of the resulting thiol group to form covalent bonds with borate to generate possible thio-analogues of AI-2 signaling molecules.

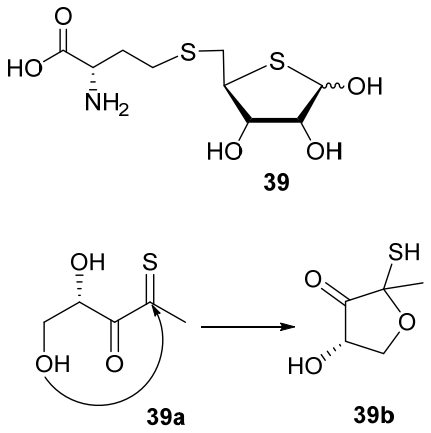


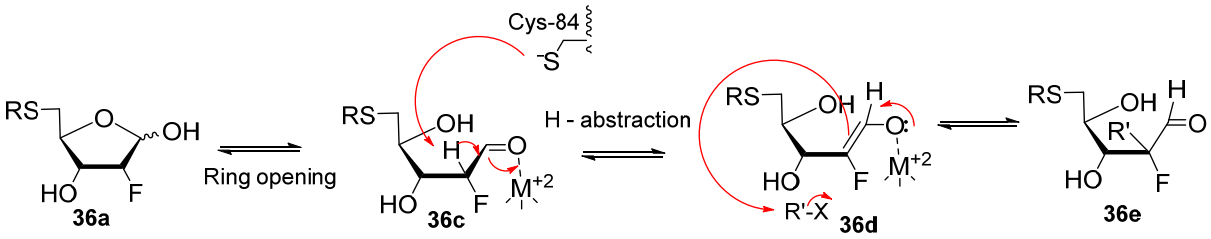
Figure 27. The SRH analogues with furanose ring oxygen substituted by sulfur ([4-thia]-SRH) and the expected thio-analogues of DPD

The last goal in my dissertation is to evaluate the activity of the target compounds against LuxS enzyme and to test them as possible quorum sensing modulators of *P. aeuroginosa* and *V. harveyi*. The significance of this work is to design and synthesize new molecules with the capability to interfere with the quorum sensing pathway considered as the main communication method among most Gram positive and Gram negative bacteria. *In vitro* and *in vivo* studies will be necessary to evaluate the molecules as an emerging class of antibiotics.

3. RESULTS AND DISCUSSION

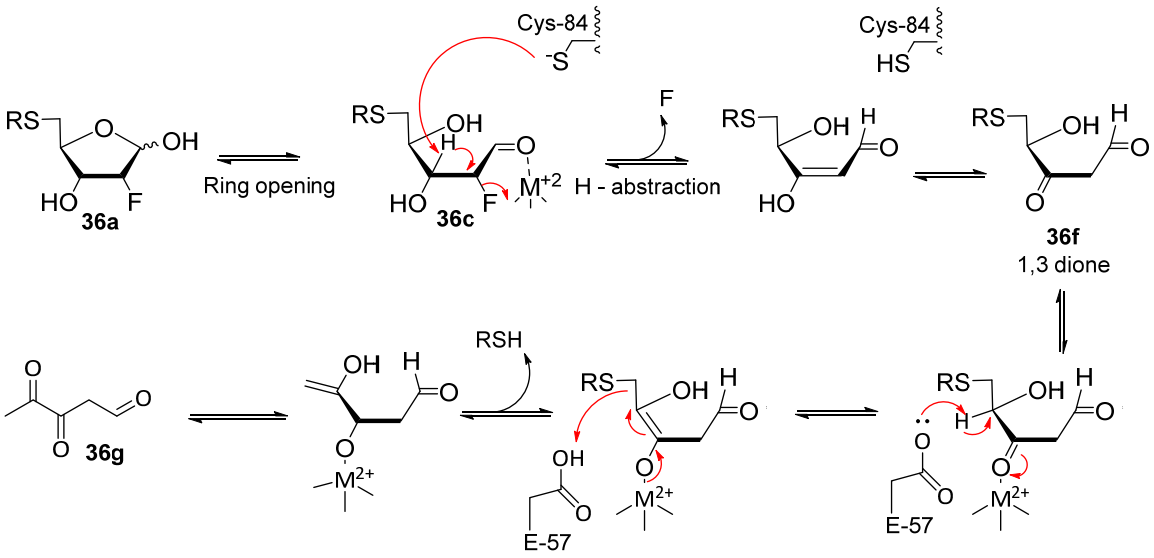
3.1 Design and synthesis of 2-halo substituted *S*-ribosylhomocysteine analogues

LuxS enzyme might interact with 2-halo-SRH analogues (e.g., **36** or **37**) through the novel mode of actions. Thus, ring opening of 2-fluoro-SRH substrate **36a** and complexation of the metallo-Lewis acid with the carbonyl group will make H2 susceptible to the abstraction by the proximal Cys-84 as presented in complex **36c** (Scheme 12). The generated *in situ* fluoroenol **36d** might interact with electrophiles leading to the alkylation at C2 as depicted in **36e**.



Scheme 12. A plausible inhibition of LuxS by 2-[F]-SRH via H2 abstraction.

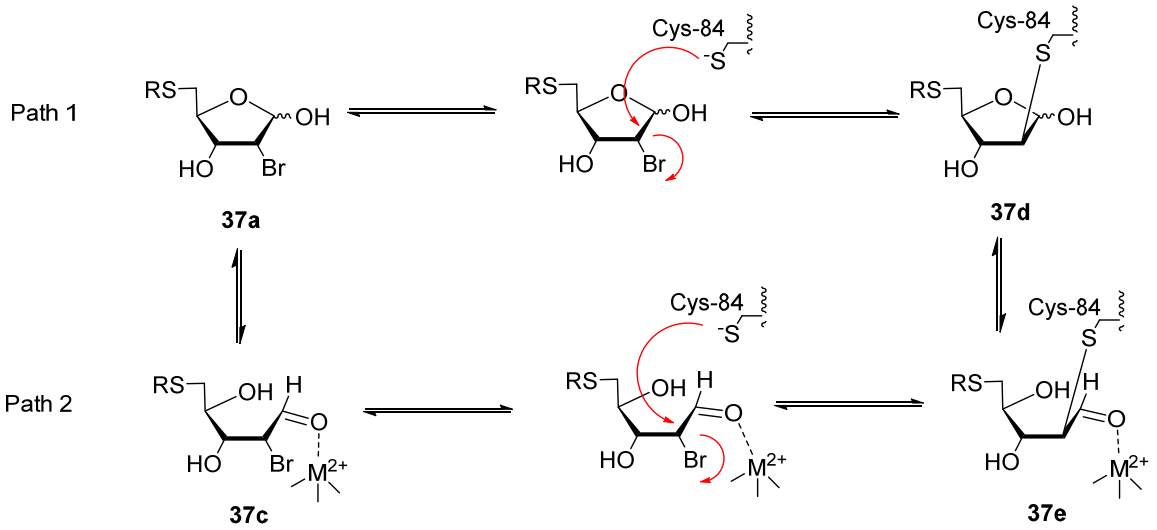
In a second mode of action, after a ring opening, Cys-84 might abstract H3 from the intermediate **36c** which will result in elimination of fluorine in E1cB fashion to produce an enone which could further tautomerize to 1,3-dicarbonyl intermediate **36f** (Scheme 13). This 3-keto intermediate could subsequenly undergo β-elimination to release homocysteine and produce 3,4-dioxopentanal (DOP) **36g** as a highly oxidized analogue of DPD (precursor of AI-2). Conversely, abstraction of H3 might happen prior to the ring opening leading however to same 1,3-dicarbonyl intermediate.



Scheme 13. A plausible inhibition of LuxS by 2-[F]-SRH via H3 abstraction.

Different stereochemistry at C2 in 2-[F]-*arabino*-SRH **36b** (F is in β-face) might preclude abstraction of H2 by Cys-84, which is known from X-ray studies on LuxS to be positioned on the β-face of the substrate, therefore I would expect the attack at C3 to be a major pathway of interaction of 2-[F]-*arabino*-SRH with LuxS.

In the case of 2-[Br]-SRH, since the bromine is a much better leaving group than fluorine, I expect that direct replacement of bromine by Cys-84 via S_N2 mechanism might lead to the covalent inhibition by the attack of Cys-84 at C2. The attack might happen at the hemiacetal (path 1, Scheme 14) or opened ring form of the bromo-substrate (path 2, Scheme 14). The formation of enzyme-inhibitor complexes can be detected using a mass spectroscopy technique.

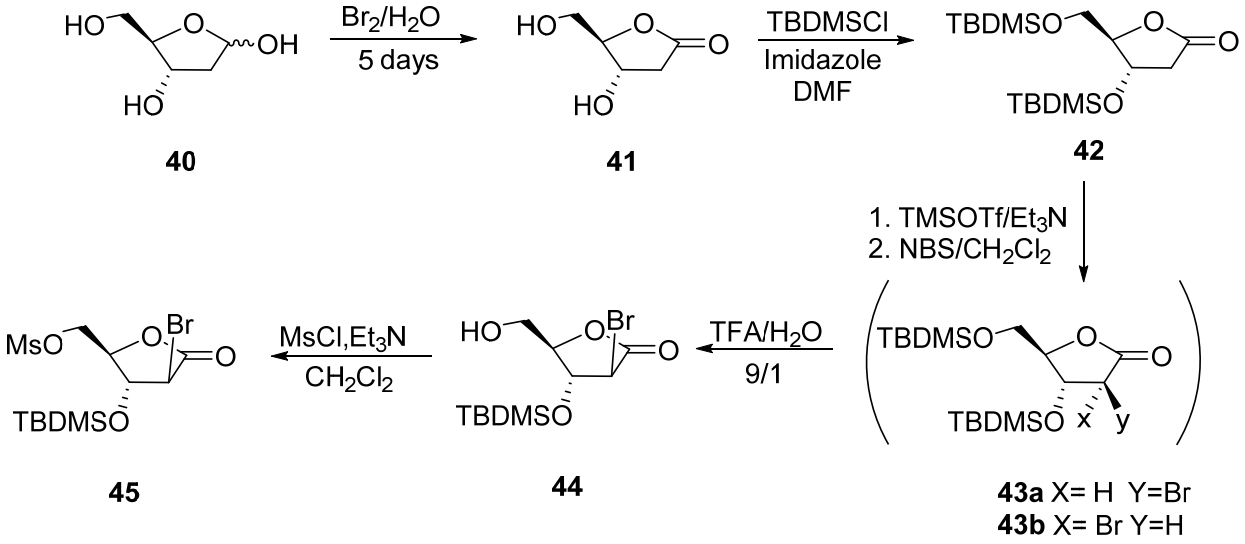


Scheme 14. A plausible inhibition of LuxS by 2-[Br]-SRH via S_N2 mechanism.

3.1.1. Synthesis of 2-bromo-2-deoxy-S-ribosylhomocysteine analogues

The synthesis of the 2-bromo-2-deoxy-SRH **37a** was divided into two steps. In the first step the 2-bromo-2-deoxyribose precursor **45** was prepared (Scheme 15), while the second step was envisioned as the coupling between **45** and the homocysteine thiolate **49**. The bromo-sugar precursor **45** was prepared according to the literature report.[130] It started with the oxidation of the 2-deoxyribose **40** with Br_2 /H_2O to 2-deoxyribonolactone **41**. The treatment of the resulting **41** with TBDMSCl produced a disilylated ribonolactone **42** with 80% yield. Direct bromination of **42** with NBS, following the procedure developed by Sauve,[130] led to the formation of the bromo-lactone **43** as a mixture of *arabino/ribo* epimers in 2:1 ratio**.** The formation of **43a** and **43b** was consistent with the bromination of the intermediate enolate. However, the obtained mixture of the *arabino* and *ribo*-epimers differed from the reporting in literature (*arabino/ribo*, 1:1.4)[130] ratio. The *arabino/ribo* epimers of **43** were successfully separated using column chromatography.

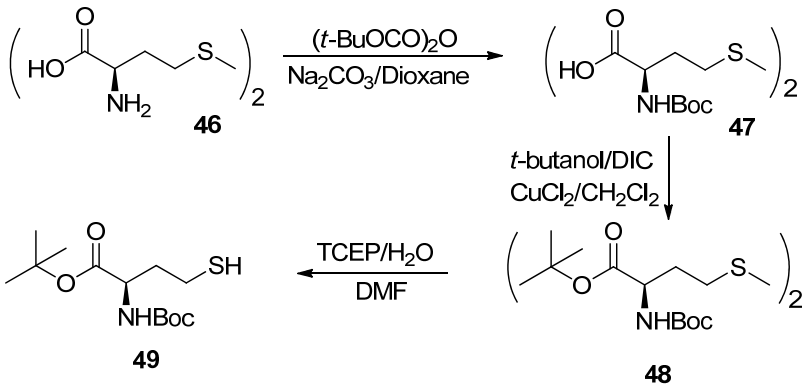
Compound **43** was considered as a precursor for the synthesis of the mesylated derivative **45** intended to be coupled with the homocysteine thiolate to afford the desired final product **50**. Thus, deprotection of **43** with TFA/H_2O (9:1) effected selective removal of TBDMS group from the primary 5-hydroxyl group of **43** to afford **44** in 90% yield. Treatment of **44** with mesyl chloride in pyridine produced the desired 5-*O*-mesyl derivative **45** in 60% yield (Scheme 15).



Scheme 15. Synthesis of the 2-bromo-2-deoxy-5-*O*-mesyl-ribono/arabinono lactone derivatives.

The protected homocysteine precursor **49** was prepared starting from the commercially available L-homocystine **46** following the literature protocol[67] (Scheme 15). Thus, treatment of the homocystine **46** with di-*t*-butyl dicarbonate gave *N*-Boc protected homocysteine **47.** Next, the carboxylic group in **47** was protected as *tert*-butyl ester upon treatment with diisopropylcarbodiimide (DIC) and *t*-butanol. The reduction of the disulfide bond in **48** with triscarboxyethylphosphine hydrochloride (TCEP) followed by aqueous workup afforded the homocysteine **49** of the appropriate purity for the

coupling with sugar precursors. It is noteworthy that employing TCEP[124] as water soluble reducing agent rather than organic soluble trialkylphosphines[67,83] simplified the preparation of the protected homocysteine substrate **49**.

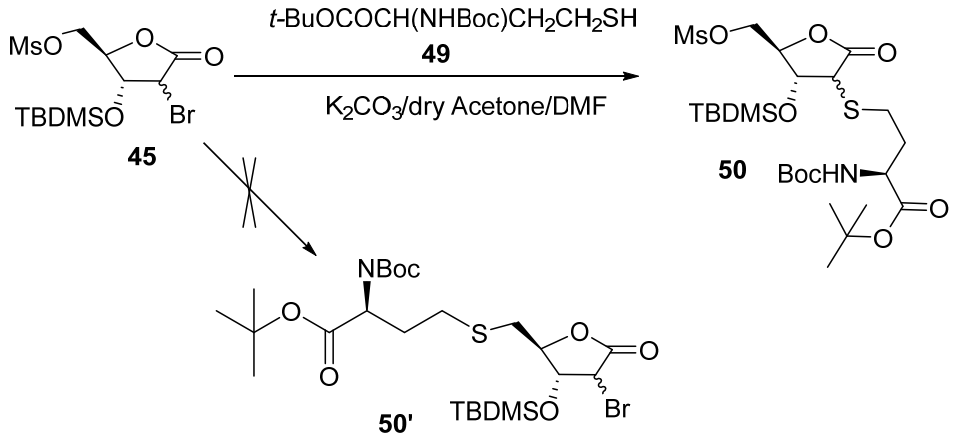


Scheme 16. Synthesis of homocysteine-thiolate.

Two methods for the displacement of the mesylate group in the ribonolactone **45** with thiolate anion generated from homocysteine **49** were attempted**.** In the first approach, LDA was used as a base and DMF as a solvent. Thus, coupling of **49** and **45** in DMF resulted in the formation of the complex reaction mixture. Careful purification of the crude reaction mixture on the column chromatography resulted in the isolation of few products in low yields which showed no presence of bromine in their mass spectra.

In the second approach K_2CO_3 was used as a base and dry acetone as solvent. The complex reaction mixture was purified on column chromatography and desired product **50'** was not isolated (Scheme 17). Proton NMR of the crude reaction mixture showed however the characteristic pattern for the protons of homocysteine and sugar moieties. Mass spectra analysis of the major fraction, showed no presence of the typical pattern of bromine isotopes (M/M+2).

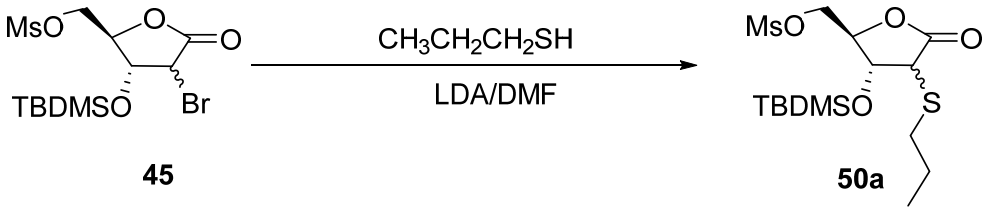
The complexity for the displacement reactions maybe attributed to the fact that the bromine at C2 in the ribonolactone precursor **45** (at Cα to ester carbonyl) also is a good leaving group and might be involved in nucleophilic substitution reaction with homocysteinate salts leading to **50**. To prove this hypothesis, I carried out a reaction between **45** with model alkyl-thiol.



Scheme 17. Coupling between the 5-*O*-mesyl-2-bromopentafuranose lactone and homocysteine thiolate.

In order to optimize conditions for the reactions of bromo-lactone **45** with thiols, I carried out reactions between **45** and propylthiol instead of homocysteine under different conditions**.** Thus, treatment of **45** (*R/S*, 60:40) with 1 equiv. of the propylthiolate generated from propylthiol/LDA in DMF at 0 ^{0}C resulted in the formation of new product and disappearance of substrate **45**. Purification of the crude reaction mixture on the silica column chromatography gave 1:1 mixture of the 2-*S*-propyl substituted 2-thioribonolactone **50a** (as 1:1 mixture of the *arabino/ribo* epimers) in overall yield of 61% (Scheme 18). Structure for **50a** was established based on spectroscopic data. Thus,

^{1}H NMR spectra shows the singlet at 3.06 ppm indicative of the presence of the mesyl group in addition to the characteristic peaks for the propylthiol moiety. On ^{13}C NMR, peaks at 171.47/172.74 ppm indicate the presence of the carbonyl groups in the lactone **50a** for the *arabino-ribo* epimers, while the lack of the bromine patterns (M/M+2) on mass spectra analysis suggested the substitution at C2 and the loss of the bromine substituent. These results indicate that the secondary bromine at the α-position to the carbonyl group in ribonolactones is a better leaving group than the mesylate at the primary hydroxyl group at C5.

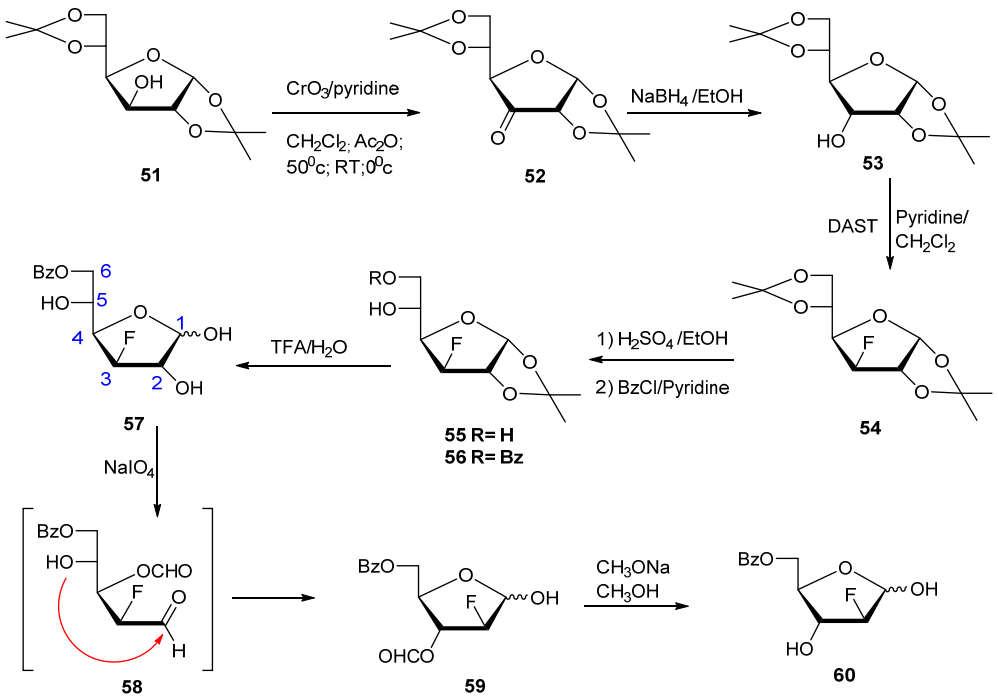


Scheme 18. Model reaction of bromo-lactones with propylthiol

3.1.2. Synthesis of 2-deoxy-2-fluoro-*S*-ribosylhomocysteine analogues

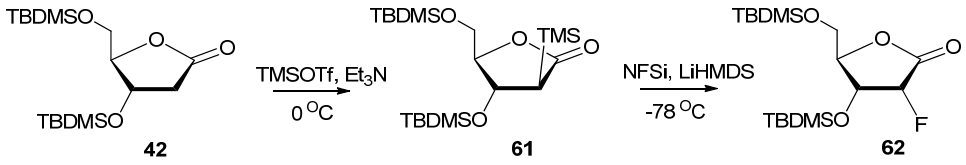
Two literature procedures were considered for the preparation of 2-fluoro-2-deoxypentafuranosyl precursors (e.g., **60,** Scheme 19) for the coupling with homocysteine. One approach was based on Fox's methodology[131] presented in Scheme 19. It involves the fluorination of allose **53** to fluoroglucose **55** followed by an intramolecular rearrangement to yield to **60**. The overall synthesis of **60**, started with the oxidation of 1,2,5,6-di-*O*-isopropylidene-α-D-glucofuranose **51** with chromium trioxide – pyridine complex to afford its corresponding ketone **52**. Reduction of **52** with sodium borohydride is expected to deliver hydride anion at the β-face[132] of the sugar ring because of stereo-hindrance of the isopropylidene group present at C1 and C2 to give allose **53**.

Treatment of **53** with DAST resulted in a nucleophilic displacement of the hydroxyl group at C3 by fluoride with the inversion of configuration at C3 position to give 3-fluoroglucose **54**.[133] Selective deprotection of the isopropylidene group from diol at C5 and C6 yielded **55** in high yield. Treatment of the latter with benzoyl chloride effected selective protection of the primary hydroxyl group at C6 to give **56**. Subsequent removal of the isopropylidene group in **57** from C1 and C2 by treatment with TFA/H_2O gave 6-*O*-benzoyl-3-fluoroglucose **57** as an anomeric mixture (α/β, ~1:3). Treatment of **57** with $NaIO_4$ effected cleavage of *cis*-vicinal diol at C1 and C2 to give open sugar **58** which underwent simultaneous rearrangement involving a ring closure between the hydroxyl group at C5 with the aldehyde group generated at the former C2 (as depicted in **58**) resulting in overall repositioning of fluorine atom from C3 to C2 leading to **59**. Treatment of **59** with sodium methoxide yielded the fluoroarabinose **60**.



Scheme 19. Synthesis of 2-deoxy-2-fluoro-α,D-arabinofuranose

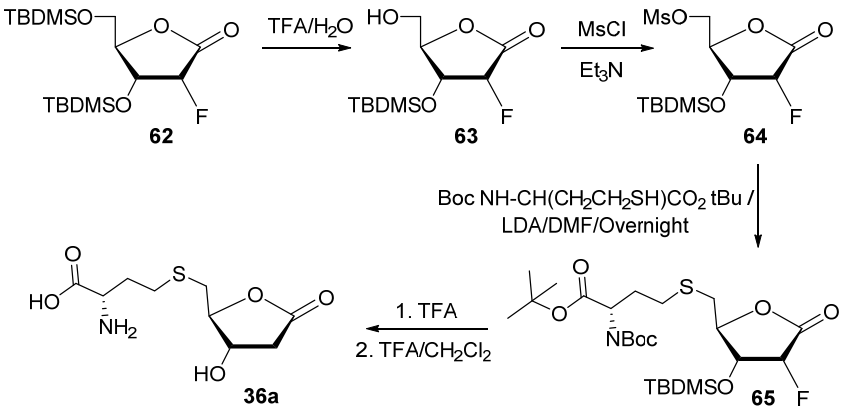
The second approach for the synthesis of 2-fluoropentofuranose was based on Cen and Sauve[130] protocol. In this approach, the fluorination of the protected ribonolactone **42** is reported to give 2-fluoro-2-deoxy-ribo-lactone **62.** The protocol involved enolization of lactone **42** with TMSOTf followed by fluorination of the resulting α-silyl lactone **61** intermediate with NFSi (*N*-fluorobenzene sulfonamide) to give 2-deoxy-fluororibonolactone **62** as a single *ribo*-epimer. The stereoselectivity of the reaction is attributed to the diasteroselective formation of α-silyl lactone **61** due to sterically bulky TBDMS that prevents the *syn* approach to the generated in situ enolate. In our hands, treatment of **42** with TMSOTf/NFSi gave fluorolactone **62** in lower yields than reported.



Scheme 20. Synthesis of 2-Deoxy-2-fluororibonolactone

Having in hand the fluoropentafuranoses sugar precursors **60** and **62,** I focused on the preparation of the fluoro analogues with (a) 5-hydroxyl group activated for the replacement with homocysteine unit and (b) proper protection of other hydroxyl group which would be suitable for the coupling procedures which employs bases. Treatment of fluororibonolactone **62** with TFA/H_2O effected regioselective removal of the silyl protection group from the primary hydroxyl group at C5 to give **63.** Subsequent mesylation of the primary hydroxyl at C5 gave **64**; a suitable precursor for the displacement reaction with thiolate anion generated from the protected homocysteine. However, coupling of **64** with homocysteine **49** (in LDA/DMF) produced a complex reaction mixture from which I was not able to isolate a desired product **65.** Attempted

deprotection of the crude and contaminated **65** with TFA followed by RP-HPLC purification failed to yield the desired 2-[F]-SRH **36a** (Scheme 21).



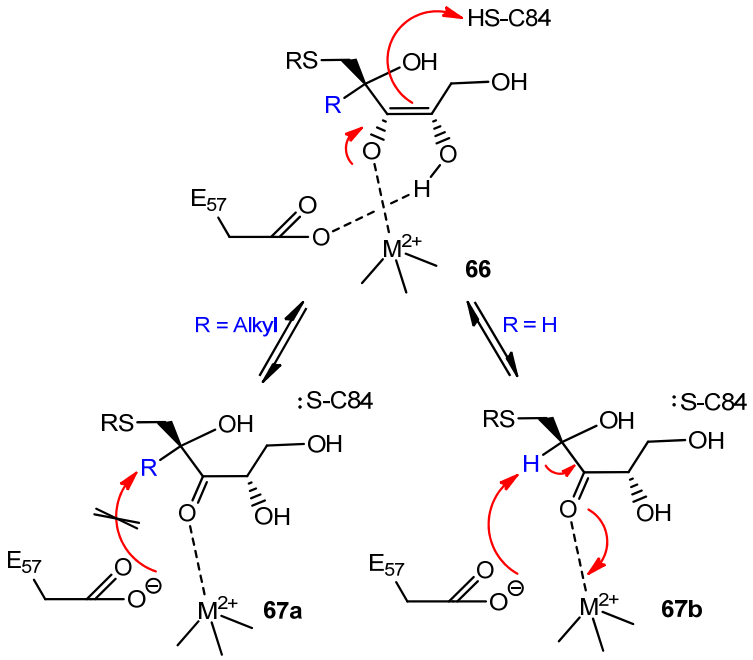
Scheme 21. The proposed plan for the synthesis of 2-deoxy-2 fluoro-D-arabinofuranose.

3.2. Design and synthesis of 4-*C*-alkyl/or aryl-*S*-ribosylhomocysteine analogues

3.2.1. Rationale for the synthesis of the 4-substituted SRH analogues:

The substitution of the hydrogen at C4 of the 4-alkyl/or aryl-[SRH] derivatives by an alkyl or aryl group should impede the third tautomerization in the LuxS-catalyzed reaction and therefore prevent the elimination of the homocysteine molecule by the β-elimination process (Scheme 22). Thus, inhibition of DPD production should theoretically be shut down effecting also the QS communication in bacteria. The abstraction of H-atom from C4 by the glutamine amino acid should not occur in the case of 4-*C*-substituted [SRH] derivative (complex **67a**). I was also interested in checking the differences in the inhibitory activity based on the alkyl group length and chemical nature such as alkyl, vinyl, and aryl groups incorporated at C4 of ribose ring. The length of the alkyl group might also play an additional role in imposing the dimerization inhibition,

especially since LuxS is a dimer protein. Thus, the alteration of the active site of the enzyme may lead to an allosteric inhibition. In theory, the longer the alkyl chain incorporated a more potent dimerization inhibition of LuxS should be observed by making the inhibitor at ease to reach both homodimer parts of the protein. The inhibitor might block one monomer leading to the alteration of the activity and as a consequence conformational changes of the second monomer.



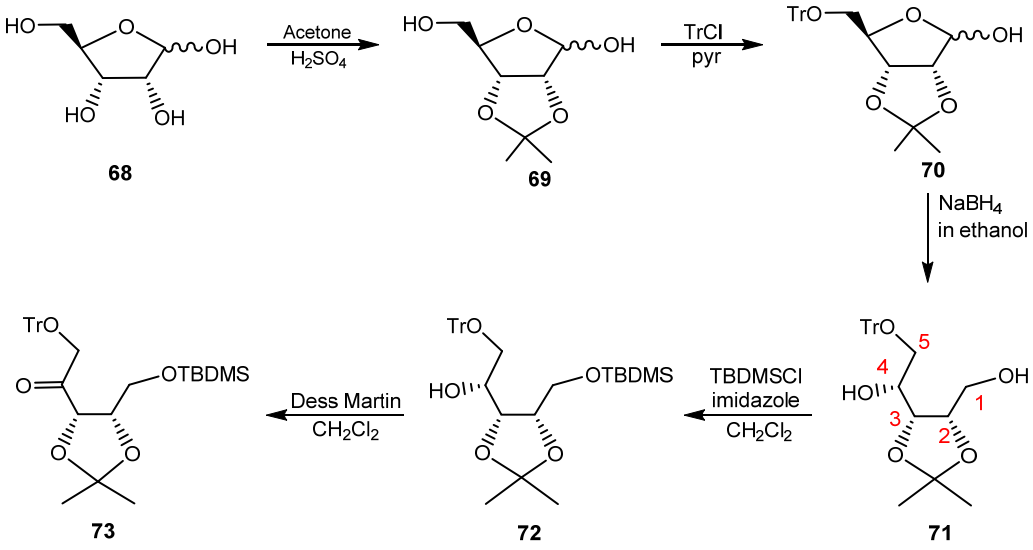
Scheme 22. A plausible inhibition of LuxS by 4-alkyl/or aryl-[SRH] substrate.

3.2.2. Synthesis of 4-*C*-substituted SRH analogs:

3.2.2.1 Synthesis of 4-C-substituted ribono-1,4-lactones

Preparation of 4-*C*-alkyl/aryl-*S*-ribosylhomocysteine analogues was envisioned to proceed through a keto-substrate **73**[134] suitable for the alkylation with varying Grignard reagents. The ribitol-4-ulose **73** was prepared from ribose **68** employing the procedure reported by Maddaford.[134] Thus, treatment of ribose **68** with acetone in the presence of

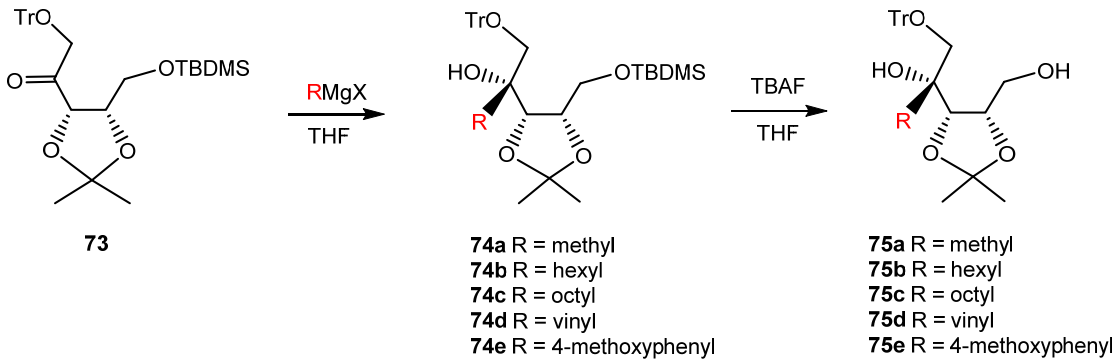
H_2SO_4 gave the isopropylidene protected ribofuranose **69** (Scheme 23).[135] Tritylation of the primary 5-hydroxyl group **69** with TrCl yielded **70**. Reduction of the hemiacetal **70** with $NaBH_4$ provided the acyclic ribitol **71**. Subsequent silylation of **71** with TBDMSCl effected regioselective protection of the primary hydroxyl group at C1 to give **72.** Oxidation of the secondary hydroxyl group at C4 of **72** with Dess-Martin periodinane reagent provided the 4-ulose derivative **73**, as a suitable precursor for the subsequent alkylation reactions with Grignard-based reagents. The overall yield for the conversion of the ribose **68** to ribitol-4-ulose **73** was 75% (5 steps).



Scheme 23. Preparation of the precursors for the synthesis of 4-*C*-alkyl-SRH analogs.[134]

Diastereoselective addition of different Grignard reagents to ketone **73** was accomplished following Pryde and coworkers procedure.[134] Alkyl, vinyl and aryl substituents were successfully inserted at the 4-position of the ribose ring to give a variety of 4-substituted ribitols **74a-e** (Scheme 24). Thus treatment of ketone **73** with methylmagnesium bromide at -78 ^{o}C for 15 min produced the corresponding 4-*C*-methyl-

ribitol **74a** in 88% yield as single isomer after purification on silica gel column. The ^{1}H and ^{13}C NMR spectra were diagnostic for the structure of **74a.** On the ^{1}H NMR spectrum, a new singlet for the methyl group at 1.35 ppm was observed along with the disappearance of the signal for H4 and simplification of the multiplets signals for H5 and H5' to doublets (as compared to **72**) with geminal coupling constants between them of 8.7 Hz. Addition of hexylmagnesium bromide or octylmagnesium bromide to ketone **73** gave the corresponding 4-*C*-hexyl and 4-*C*-octyl ribitols **74b** and **74c** in 64% and 60% isolated yields, respectively. Also addition of the vinyl magnesium bromide to ketone **73** proceeded smoothly to give the 4-*C*-vinyl-ribitol **74d** in 55% yield as a single isomer. The characteristic pattern of the peaks for the terminal monosubstituted olefin was observed in the ^{1}H NMR spectrum of **74d.** The addition of 4-methoxyphenylmagnesium bromide to ketone **73** also proceeded efficiently to provide 4-*C*-aryl ribitol **74e** in 96% yield.



73

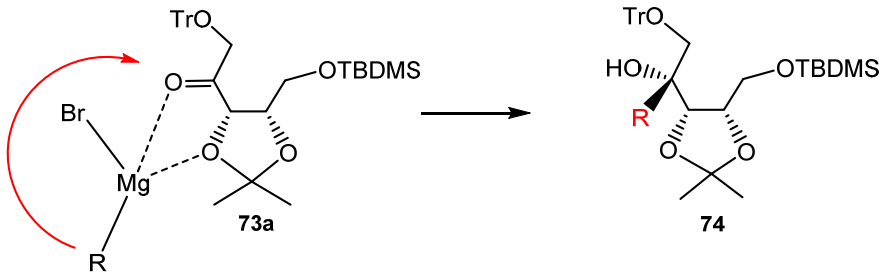
74a R = methyl
74b R = hexyl
74c R = octyl
74d R = vinyl
74e R = 4-methoxyphenyl

75a R = methyl
75b R = hexyl
75c R = octyl
75d R = vinyl
75e R = 4-methoxyphenyl

Scheme 24: Diasteroselective addition of Grignard reagents to the 4-ketoribitol precursors.

The stereoselective formation of 4-*C*-substituted ribitols **74** can be explained by the Cram's rule for the chelation of heteroatom on the α-carbon to a carbonyl group (Scheme 25). The Grignard reagent addition to the ribitol-4-ulose **73**, which is an α-alkoxy ketone,

proceeds via a 5-membered cyclic chelate **73a**. Crams's rule stated that nucleophilic addition with either organometallic or metal hydride reagent leads to chelation control in diastereoselective carbonyl reduction. The carbonyl is flanked by two smaller groups S and M. The larger group L is eclipsed to the carbonyl group. The metal part gets complexed with the carbonyl group and the alkanide carbanion is transferred to the trigonal carbon from the side of small group in preference to medium group or in the anti-fashion to the α-hydroxyl group at C3.



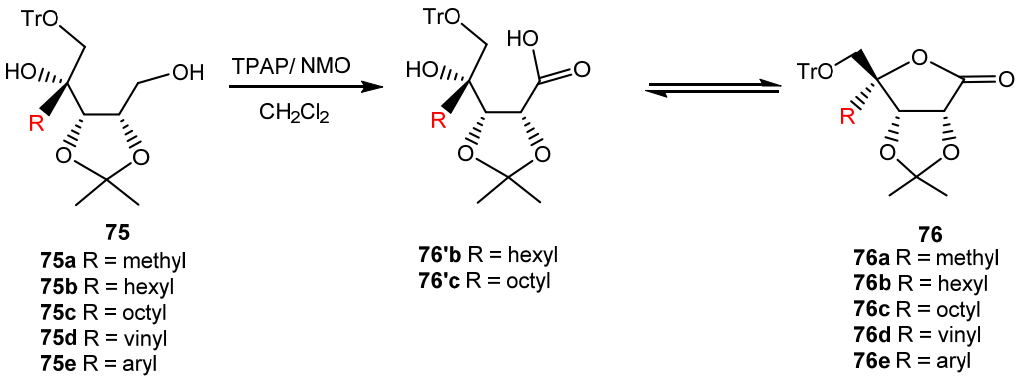
Scheme 25. Proposed model for the diastereoseletive addition of the Grignard reagent to ketone 73.

Treatment of 4-*C*-methylribitol **74a** with TBAF in THF at 0 ^{o}C for 30 min effected selective removal of the TBDMS protection group to give the ribitol **75a** in 77% yield. Analogous desilylation of **75b-e** produced the corresponding 4-*C*-substituted ribitols in 77-87% yield for **75b-d** and 59% yield for **75e** having a primary hydroxyl group at C1 and a tertiary hydroxyl group at C3.

With the 4-*C*-substituted ribitols **75** in hands, I investigated few options for the ring closure in order to prepare the suitable 4-substituted ribose precursors for the coupling with Hcy. The first option involved oxidation of the primary hydroxyl at C1 in **75** to the carboxylic acid with the concomitant ring closure to the corresponding ribono-1,4-lactone. Thus, the Ley-Griffith oxidation of the primary hydroxyl group at C1 in **75a** with

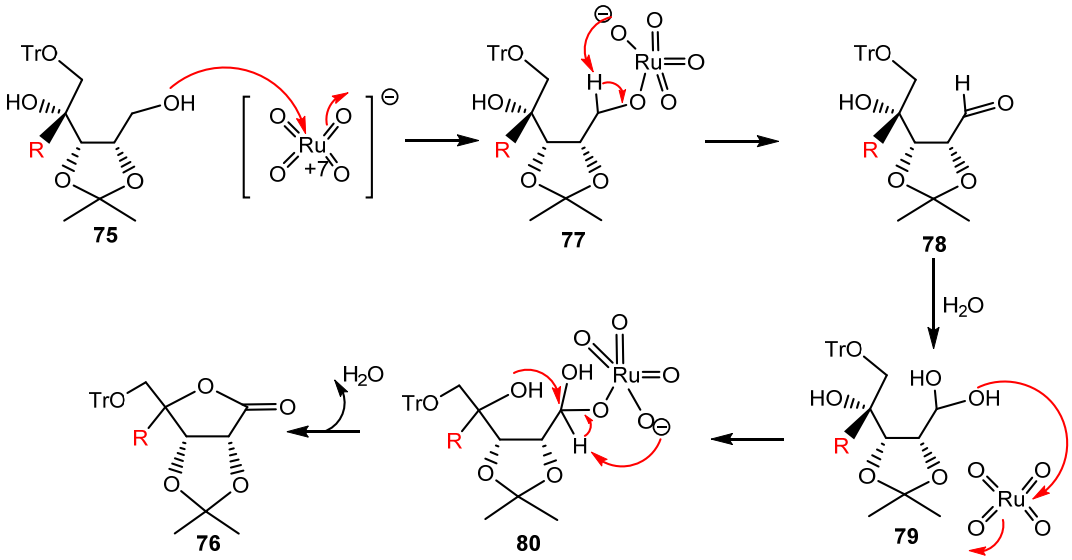
tetrapropylammonium perrenthenate (TPAP)/*N*-methylmorpholine *N*-oxide (NMO) followed by the intramolecular ring closure gave the corresponding 4-*C*-methyl ribono-1,4-lactone **76a** in 80% yield after silica gel column chromatography. The oxidation of **75a** occurred with catalytic amount of TPAP in the presence of a stoichiometric amount of co-oxidant NMO that regenerates the catalyst. The ^{1}H and ^{13}C NMR spectra were diagnostic for the structure of **76a.** On the ^{1}H NMR spectrum, the disappearance of the signals for H1 and H1' and simplification of the signal for H2 to a doublet with vicinal coupling constants of 5.6 Hz to H3 was indicative of the oxidation of the hydroxyl group at C1 and the formation of lactone. The formation of lactone was also supported by the appearance of a peak at 172.07 ppm for the carbonyl carbon at C1 on ^{13}C NMR spectrum.

Oxidation of 4-*C*-hexyl and 4-*C*-octyl ribitols **75b** or **75c** with TPAP/NMO also proceeded smoothly to give the 4-*C*-hexyl- and 4-*C*-octylribonolactones **76b** and **76c** in 90-95% yield as single isomers. On ^{1}H NMR spectra, the appearance of the doublet of H2 at 4.1 ppm with a coupling constant of 5.6 Hz was indicative of the ring closure and formation of lactones **76b** or **76c** (Scheme 26). Similarly to **76a** a new peak at 174.41 ppm appeared on ^{13}C NMR proving the presence of the carbonyl group at C1. However, depending on the reaction workup conditions, especially for the ribonolactones with long alkyl chain, the open form of the ribonolactones **76'b** and **76'c** with the free carboxylic group at C1 were detected. During the oxidation of **75c** to **76c** the ratio of **76c** to **76'c** in equilibrium was found to be 3:1. The ^{1}H NMR spectrum of the equilibrium mixture of the ribonolactone **76c** and its opened carboxylic acid form **76'c** have two distinctive sets of peaks of the ribosylic protons H2, H3, H5 and H5' (see experimental part). The MS spectra which differ by 18 units (H_2O) were indicative of ring opening of **76'c** to **76c**.



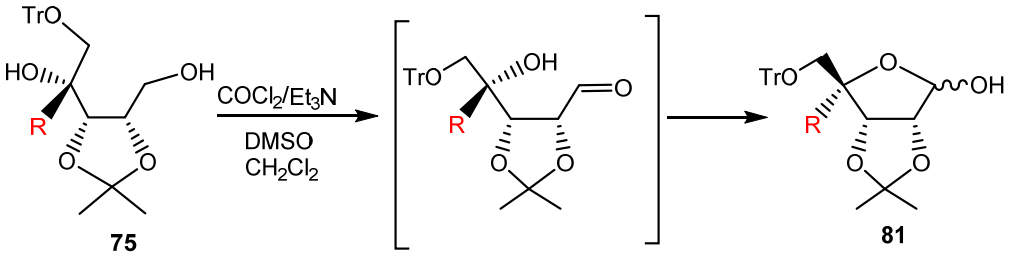
Scheme 26. Oxidation of 4-*C*-substituted ribitols with TPAP and NMO

The oxidation of the riitols **75** and intramolecular ring closure to lactone **76** is believed to be initiated by the attack of the primary hydroxyl group at C1 on the ruthenium oxidant.[136] The resulting oxide **77** further abstracts the hydrogen atom from C1 to form aldehyde **78** (Scheme 27). Hydration of the aldehyde to **79** and the repetition of the oxidation cycle leads to the ring closure and formation of the 4-*C*-alkylribonolactone **76**, which occurred by a nucleophilic attack of the C4 hydroxyl group on C1, as depicted in complex **80,** leading to the release of the ruthenium molecule.



Scheme 27: Mechanism of oxidation with NMO/TPAP and ring closure to ribonolactone.

In a second approach towards ring closure of the 4-*C*-substituted ribitols **75**, I examined the possibility of the oxidation of the primary hydroxyl at C1 to an aldehyde, which should subsequently cyclizes to the hemiacetal **81** via the intramolecular ring closure (Scheme 28). The advantage of this method is that it leads to an already reduced sugar **81** instead of a lactone precursor **76**. However, attempts to oxidize **75b** or **75c** employing Swern reaction condition (oxalic chloride/DMSO/Et_3N) gave a complex reaction mixture with low yields and hemiactal **81** has never been isolated from the reaction mixture in a pure form (Scheme 28).



Scheme 28. Attempted synthesis of hemiacetals 81 by Swern oxidation.

3.2.2.2 Coupling of 4-C-substituted 1,4-ribonolactones and Hcy

With the 4-*C*-substituted ribonolactones **76a-e** in hands, I considered options to prepare the suitable 4-substituted ribose precursors for coupling with Hcy (Scheme 29). Thus, detritylation of **76a** (16 h, rt) with TFA/CH_2Cl_2 gave **84a** in 66% yield after purification on silica gel column chromatography. On ^{1}H NMR spectrum of **84a**, the absence of the aromatic hydrogens between 7.25-7.38 ppm was indicative of the trityl group removal. HRMS showed a molecular ion $[M+Na]^+$ peak at 225.0734 verifying a molecular formula $C_9H_{14}O_5Na^+$.

Analogous treatment of the hexyl **76b** and octyl **76c** with TFA (5 h, rt) gave **84b** and **84c** in 80% and 75% yields, respectively. Interestingly, when the reaction mixture (**76b**

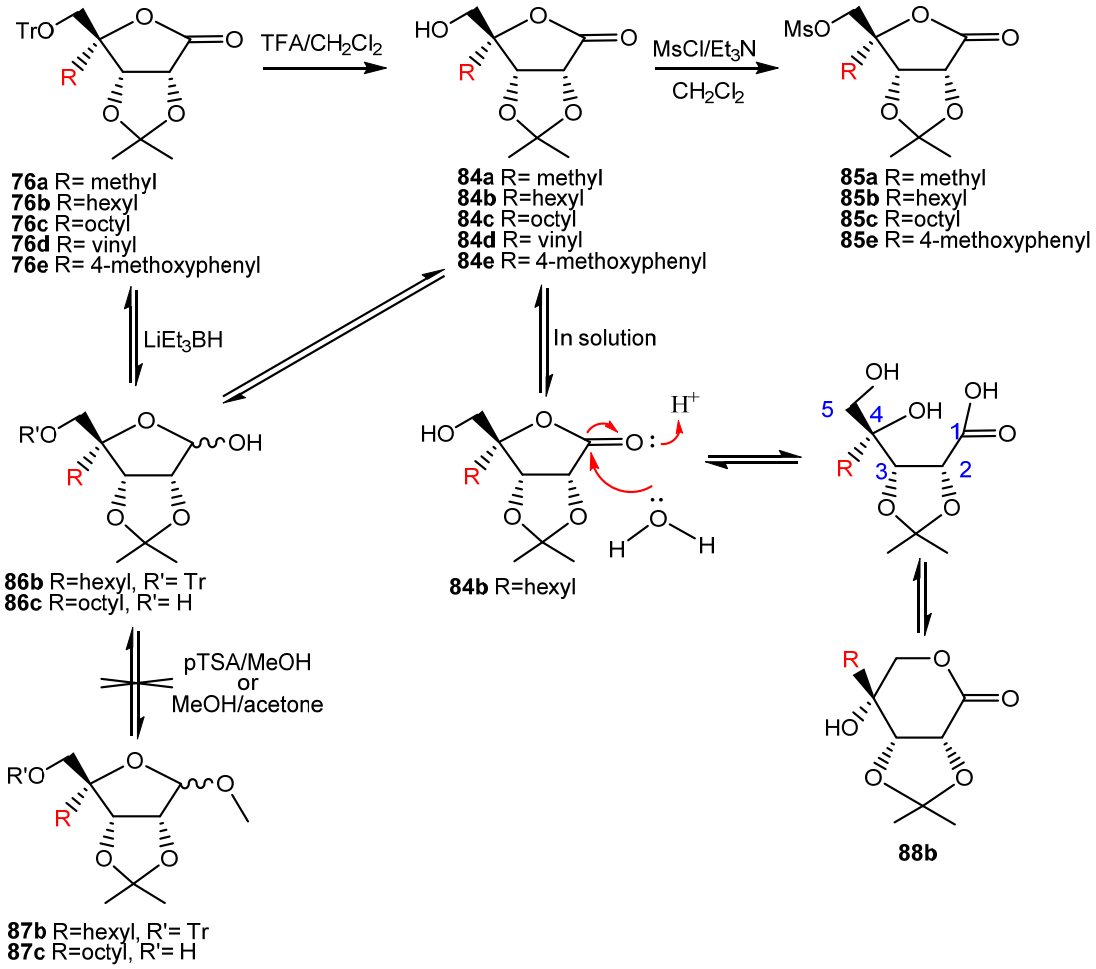
→ **84b**) was left stirring overnight, a second product was formed in a ratio of 1:1 to **84b**. The new product is believed to be the corresponding 4-*C*-hexylribopyranose **88b** formed via the ring opening of the ribofuranose **84b** and the subsequent ring closure with C5 hydroxyl group**.** ^{1}H NMR and COSY spectra of the 1:1 mixture of **84b**/**88b** showed two sets of distinctive peaks. A doublet for H2 of **88b** was shifted downfield from 4.55 ppm to 4.22 ppm. Also, the vicinal coupling constant between H2 and H3 was changed from 5.6 Hz in **84b** to 2.5 Hz in **88b**. The magnitude of the geminal coupling constants between protons H5 and H5' in **88b** (12.7 Hz) were larger than the one observed in **84b** **(**11.6 Hz). Treatment of the vinyl analogue **76d** with TFA (5 h, rt) also gave detritylated ribonolactone **84d** but in only 35% yield after purification on silica gel column chromatography. Similarly, **76e** was converted to **84e** with 60% yield. HRMS spectra of compounds **84a** to **84e** mass spectra were consistent with the theoretical molecular mass of respective compounds, which verified that detrytilation occurred.

In the next step, I considered reduction of the 4-*C*-substituted fully protected **76b** or detrytilated **84c** ribofuranoses with $LiEtBH_3$ as a valuable approach for the synthesis of the cyclic hemiacetal **86,** especially since our previous attempts to convert ribitol **75** to hemiacetal **81** employing the Swern reaction were unsuccessful as described above. Thus, treatment of the 4-*C*-hexyl **76b** with $LiEtBH_3$ in CH_2Cl_2 at 0 ^{o}C for 30 min gave the corresponding hemiacetal **86b** as an anomeric mixture (α/β, 1:3) in 54% yield after column chromatography. The chemical shift and vicinal $^{3}J_{H1\text{-}H2}$ coupling constant was diagnostic for the composition of α/β anomers. For the α-isomer the signal for H1 was observed as a doublet of a doublet with splitting to H2 and OH group at C1 with respective coupling constants of 4.2, 11.6 Hz. For the β-isomer, the signal for H1

appeared as doublet with coupling constant of 8.5 Hz to hydroxyl group only. In addition, H2 appeared as a doublet of doublet with couplings to both H1 and H3 in the α-isomer, whereas for the β-isomer, the signal fo H2 is simplified to a doublet with a coupling constant of 6.0 Hz to H3 only.

Analogous treatment of the 4-*C*-octyl **84c** with $LiEtBH_3$ gave the hemiacetals **86c** as an anomeric mixture: **86c** (α/β, 1:1; 63%) after column chromatography (Scheme 29). Attempted conversion of the hemiacetals **86b** and **86c** to the respective methyl riboside acetals **87b** and **87c** upon treatment with either (i) MeOH/acetone for **86b** or (ii) pTSA/MeOH for **86c** failed to produce the desired acetal **87b** or **87c.** Since acetals are stable in basic conditions, they were envisioned as good substrates (after activation of the 5-hydroxyl group) for the condensation with Hcy which required basic conditions.

In the next approach for the coupling of 4-*C*-alkyl riboses with Hcy, I turned my attention to the *direct* coupling of ribono-1,4-lactones with Hcy as a plausible alternative. Thus, treatment of **84a**, **84b**, **84c** and **84e** with mesyl chloride gave the corresponding 5-*O*-mesylribonolactone **85a** with 63% yield, **85b** and **85c** with approximately 50% yield, and **85e** with 83% yield after column chromatography. On ^{1}H NMR spectra, a singlet at 3.01 ppm was diagnostic for the presence of the mesyl group at C5 in all compounds. A shift of H5 and H5' signals from 3.75 and 3.85 ppm in **84b** and **84c** to 4.20 and 4.32 ppm in **85b** and **85c** indicated the formation of the mesylate ester. Similarly, a comparable downfiled shift of H5 and H5' occurred on ^{1}H NMR spectra in **85a** and **85e** as a result of the introduction of methylsulfonate EWG.



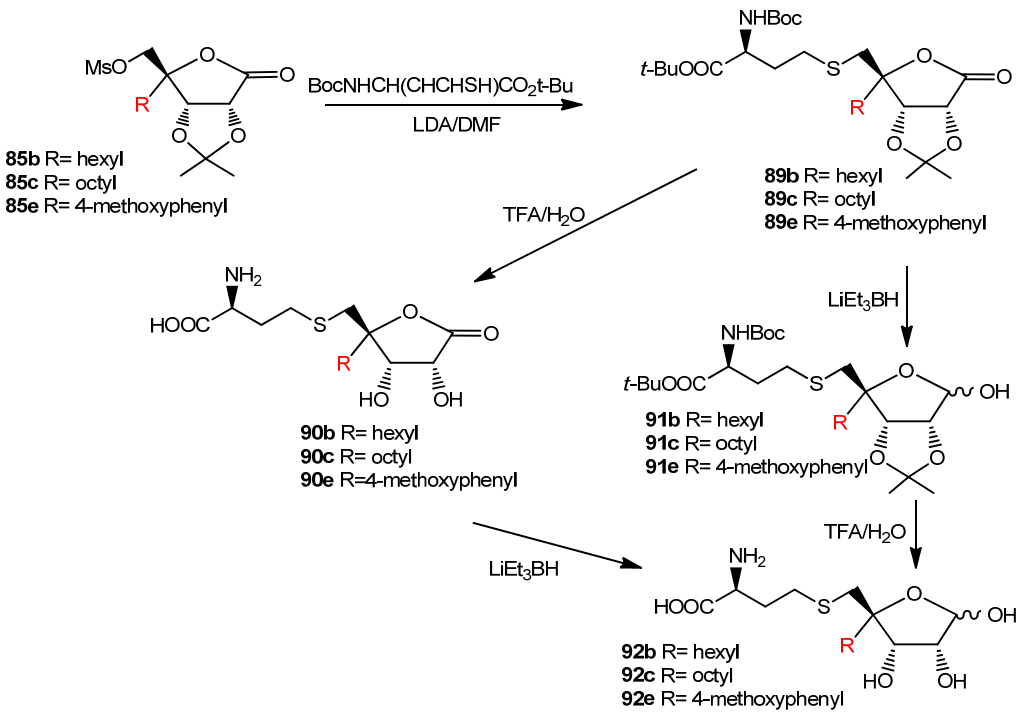
Scheme 29. Different strategies of 4-substituted ribose precursors

Nucleophilic displacement of the mesylate in **85b** with Hcy thiolate (3 equiv.), generated from the protected L-homocysteine **49** and LDA in DMF, afforded **89b** in 65% yield after silica gel column purification (Scheme 30). The structure of the **89b** was established by the spectroscopic techniques. HRMS (AP-ESI) proved the molecular formula of $C_{27}H_{48}NO_8S^+$. Analogous coupling of **85c** with Hcy gave **89c** (~ 30%) contaminated with the protected Hcy **49** substrate (~50%) which was used in excess in the reaction. The upfield shift of H5 and H5' signals on ^{1}H NMR spectra from 4.20 and 4.32 ppm in **85b** and **85c** to 2.72 and 2.80 in **89b** and **89c** indicated the formation of the

new C-S bond. The 4-*C*-aryl mesylate **85e** was coupled with homocysteine thiolate **49** to give **89e** with 48% yield.

Treatment of the crude **89c** with TFA effected global deprotection of all acid label groups to give crude **90c.** Purification of the mixture on Sepak column gave 4-*C*-octyl-SRH lactone **90c** as a 95% pure product in 15% overall yield from **85c**. The molecular formula for **90c** [$C_{17}H_{31}NO_6S^+$] was verified by HRMS. Deprotection of **89b** with TFA gave **90b** in 60% yield after HPLC purification. Analogous deprotection of 4-*C*-4-methoxyphenyl SRH lactone **89e** with TFA and its purification gave **90e.** The molecular formula for **90b** [$C_{15}H_{27}NO_6S^+$] and **90e** [$C_{16}H_{21}NO_7S^+$] were verified by HRMS.

Treatment of **89b** with $LiEt_3BH$/THF (5 equiv.) in CH_2Cl_2 at 0 ^{0}C effected reduction of the lactone to the hemiacetal yielding protected SRH analogue **91b** as a mixture of α/β anomers (1:4). Deprotection of **91b** with TFA and TFA/H_2O effected removal of all the protection groups to give anomers of 4-*C*-hexyl-SRH **92b**. Similarly, deprotection of **91e** yielded **92e**. Subjection of **89e** to the reduction and deprotection sequence afforded **92e**. Alternatively, the deprotected lactone **90c** was reduced with $LiEt_3BH$/THF to afford hemiacetal **92c.**



Scheme 30. Coupling of mesyl-ribonolactone derivative with homocysteine thiolate and deprotection

3.3. Design and synthesis of 4-[thia]-*S*-ribosylhomocysteine analogues

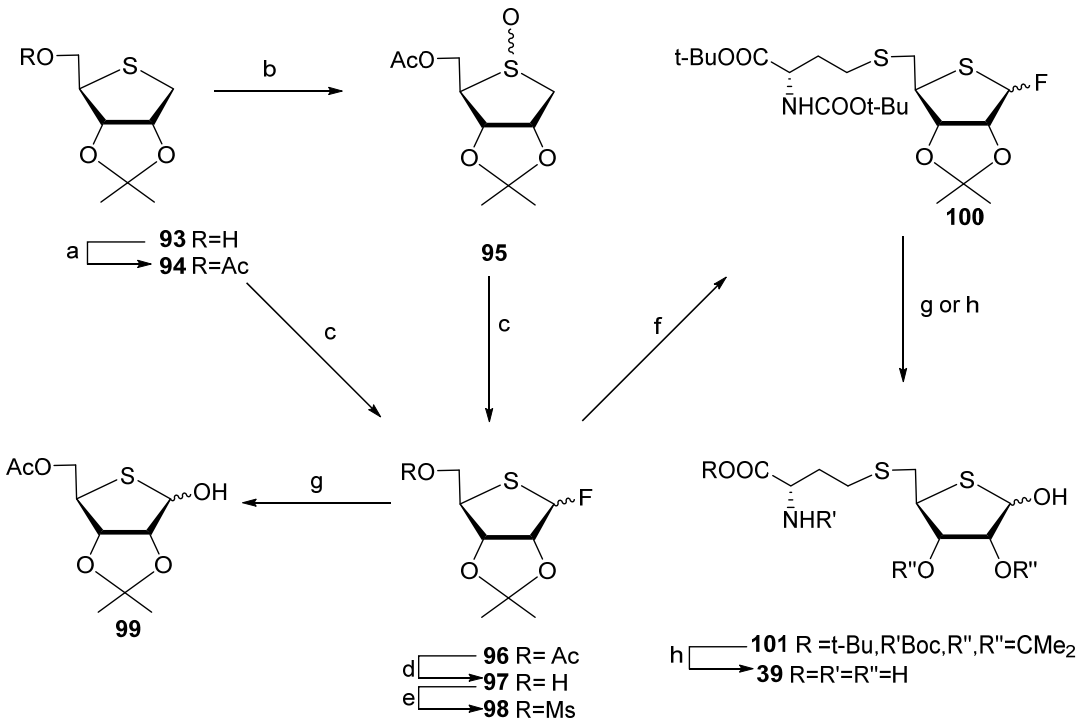
Replacement of the sugar ring oxygen by sulfur provides close mimics of natural sugars, it is noteworthy, that the sulfur atom is larger and more polarizable than oxygen and the carbon –sulfur bond is longer (C-S, 0.182 nm *vs* C-O, 0.143nm), weaker and less polar than a carbon-oxygen bond (electronegativities on the Sanders scale: O, 3.65; S, 2.96; C, 2.75).[137] Such differences are responsible for significant alterations in the anomeric effect, conformational behavior, chemical reactivity, molecular recognition by proteins, and metabolic stability of thiosugars analogues in comparison with their oxygen counterparts. Since only the open aldehyde form of SRH is catalytically active, I was interested in the effect of the sulfur substitution on the ring opening, especially since previously has been shown that for 4-[aza]-SRH analogue acted as time-dependent

inhibitors of LuxS as discussed in section 1.1.5 (Scheme 27 and 28). The existence of the thiohemiacetals in equilibrium with open aldehyde form is expected for 4-thioriboses under basic and/or acidic conditions.[138] In my efforts to synthesize thiohemiacetal **39** (Scheme 29), I explored the possibility of employing thioribosyl fluorides as precursors for the preparation of the *O*,*S*-acetals analogues of SRH.

The synthetic approach started from 1-deoxy thiosugar precursor **93**, which was prepared from D-ribose or gulonolactone following the procedures of Mastuda[139] or Jeong.[140,141] Thus, acetylation of the 5-hydroxyl group in **93** gave **94** in 95% yield (Scheme 29). Subsequent oxidation afforded sulfoxides **95** as a 4:1 mixture of diastereomers at sulfur. Treatment of sulfoxide **95** with DAST/$SbCl_3$ combination[142] resulted in fluoro-Pummerer rearrangement to give α-fluoro thioether **96** (α/β~ 1:9) in 65% combined yield after flash column chromatography. Reactions of sulfide **94** with DAST/$SbCl_3$[143] also produced thioribosyl fluorides **96** (β-anomer only in 49% yield), demonstrating that oxidation of sulfide to sulfoxide is unnecessary step in the conversion of thioethers to α-fluoro thioethers. On ^{1}H NMR spectrum the thioribosyl fluorides **96** showed a geminal coupling constant between F and H1 (J = 54.0 Hz). ^{19}F NMR spectrum of **96** (β-anomer) showed a ddd at δ -140.85 (J = 2.3, 9.5, 54.0 Hz) whereas ^{19}F NMR spectrum of **96** (α-anomer) showed a dd at δ -157.24 (J = 15.2, 56.7 Hz).

Since α-fluoro thioethers are at the carbonyl oxidation level, these thioacetals were found to be sensitive to acidic conditions.[142] I found out that under slightly acidic conditions, such as silica gel, a spontaneous hydrolysis of α-fluoro thioethers **96** occurred to yield thiohemiacetals **99**, as was proven by the loss of the fluoride signal in the ^{19}F NMR. Since on the other hand α-fluoro thioethers are relatively stable to basic

conditions, in parallel with acetals, I discovered that treatment of **96** (β-anomer) with methanolic ammonia effected deacetylation providing **97** as a single β-anomer. The fluoride **97** was then mesylated at primary hydroxyl to give reasonable stable **98**. I was fortunate to find that coupling of **98** with protected Hcy (scheme 29) indeed afforded relatively stable 4-[thia]-SRH fluoride **100**. Since the presence of moisture and slightly acidic conditions cause hydrolysis of thioribosyl fluoride to thiohemiacetals, attempted purification of the crude **100** on silica gel column yielded separable mixture of **100** (25% yield) and protected 4-[thia]-SRH derivative **101** as a single β anomer (27% yield). The structure of the 4-[thia]-SRH fluoride **100** was established by spectroscopic techniques. HRMS (AP-ESI) proved the molecular formula of $C_{21}H_{36}FNNaO_6S_2$ $[M+Na]^+$ (*m/z* 504.1847). Treatment of **100** with aqueous TFA effected hydrolysis of fluoride as well as removal of the *N*-Boc, acetonide, and *t*-butyl ester protection groups in a single step to give 4-[thia]-SRH derivative **39** in 91% yield as a mixture of anomers whose α:β anomeric ratio changed when stored in D_2O from 1:1 → 1:4. Also acid catalyzed deprotection of **101** also led to the formation of **39**. The structure of the 4-[thia]-SRH **39** was established by spectroscopic techniques. HRMS (AP-ESI) proved the molecular formula of $C_9H_{16}NO_5S_2$ $[M-H]^-$ (*m/z* 282.0484).



Reagents and conditions (*a*) Ac_2O/DMAP; (*b*) MCPBA/CH_2Cl_2/-78°C; (*c*) DAST/SbCl3/CH2Cl2/55°C; (*d*) NH3/MeOH; (*e*) MsCl/Et3N/CH2Cl2; (*f*) BocNHCH(CHCHSH)CO2*t*-Bu/LDA/DMF; (*g*) H2O/H+; (*h*) TFA/H2O (9:1).

Scheme 31. Synthesis of [4-thia]-SRH analogue.

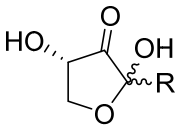
3.4 Biological activities

3.4.1. Inhibition of LuxS enzyme

Interaction of the 4-*C*-alkyl/aryl-SRH lactose **90** and hemiacetals **92** will be studied in the collaboration with Professor Pei in Ohio State University and the results will be published elsewhere.

3.4.2. Screening against *rhl* and *las* in *P. aeruginosa* QS

AI-2, known as the universal signaling molecule in bacteria[144] with a known X-ray crystallographic[1] structure in complex with LuxP, has been targeted by different researchers. Meijler and coworkers.[109] studied a panel of DPD analogues with 1 carbon increment in the alkyl chain at C2 (Figure 28). The extension in the alkyl group unit was thought to alter ligand induced conformational changes without changing the receptor-ligand affinities. Results showed that the presence of DPD, the synthesized DPD analogues acted as agonists, increasing luminescence in the *V. Harveyi*. Two theories were drawn from these findings: either (i) the analogues are allosterically interacting with the AI-2 receptor LuxP but only when DPD is present or (ii) they bind to an alternative site resulting in increased gene expression and DPD increased DPD induction. In order to further examine the preceding results, Ganin and coworkers focused on *P. aeruginosa* as a bacterium of choice to test their compounds since it is a bacterium that responds to AI-2 but does not synthesize DPD by itself. Results showed that significant inhibition of luminescence occurred with butyl and pentyl-DPD with a maximum of 40% inhibition at 200 μM for both.



R= methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl

Figure 28. Alkyl-DPD analogues.[109]

To search for the effects of non-native AHL scaffold on QS, I have also tested the 4-*C*-alkyl/aryl-SRH and 4-[thia]-SRH analogues against the *P. aeruginosa* QS. The different analogues were designed in a way to resemble SRH known to regulate QS through the LuxS-mediated biosynthesis of AI-2. In 4-[thia]-SRH analogues (**39**, Figure 29), the oxygen in the ribose unit is replaced by a sulfur atom or the sulfoxide moeity.

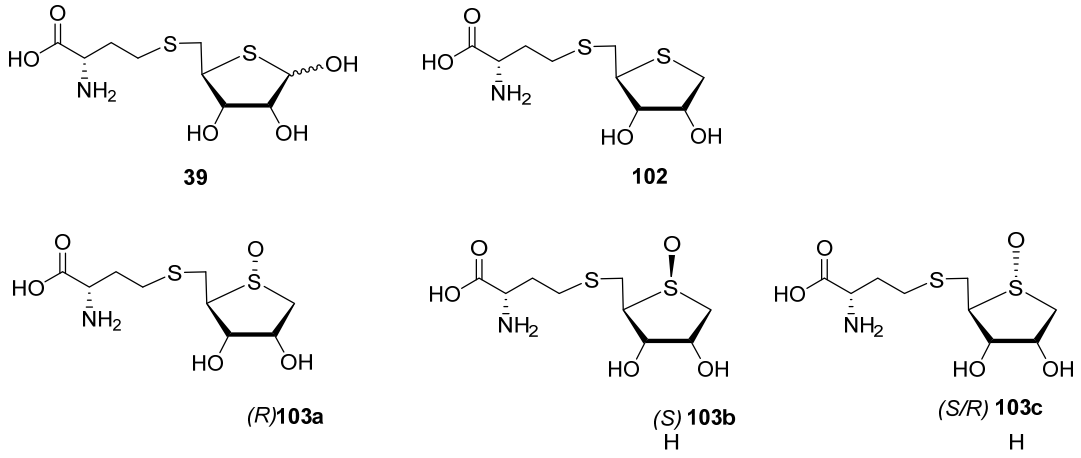


Figure 29. Thia-*S*-ribosyl-L-homocysteine analogues screened for their anti-QS activity and bacterial growth.

It is noteworthy to add that very recently a team of Dr Bassler and Dr Semmelhack reported novel thio-homoserine analogues: *meta*-chloro-thiolactone (mCTL) **104a** and *meta*-bromo-thiolactone (mBTL) **104b** (Figure 29) which were able to act as inhibitors of *Pseudomonas aeruginosa* QS and prevent biofilm formation and production of virulence

factor.[145] These compounds have similar structure to the lactams prepared by Malladi or the thiolactone analogues synthesized in this dissertation. The main difference is that the compounds synthesized by Malladi[126] and in this dissertation, have a bulky Hcy fragment for 5-*S*-alkyl unit at C4 of the sugar ring while Bassler's new thiolactone analogues has the modified acetylated amino-fragment attached to C2.

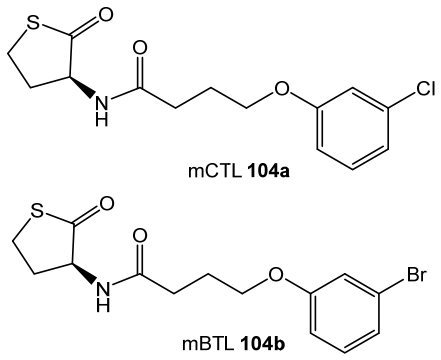


Figure 30. Structure of meta-chloro/or bromo-thiolactone

To determine the effect of the 4-[thia]-SRH analogues on the *P. aeruginosa las* and *rhl* AHL-mediated pathways, *las* and *rhl*- dependent β-galactosidase were expressed with their respective receptor proteins by Dr Kalai Mathee's research group at FIU.[146] With the collaboration with Dr Mathee's laboratory, 4-[thia]-SRH compounds were tested against *las* and *rhl*, the AHL treated controls (3-oxo-C_{12}-AHL in case of *las* and C_4-AHL in case *rhl*) were in absence of inhibitors as 100% active (no activity was observed when AHL was absent).

Screening against rhl signaling

I found that 4-[thia]-SRH (**39**) did not show any effect on *rhl* gene function (110% *rhl* activity at 200 μg/mL). The same lack of inhibitory effect was observed for the 1-deoxy-[thia]-SRH analogue (**102**). From a pair of diasterioselectively pure sulfoxide, it was found that isomer (*S*) at the sulfur atom in **103b** has more inhibitory potential than the

isomer (*R*). At 100 µg/mL the *rhl* activity was 85% compared to 125% for **103a.** The racemic mixture showed a median effect of 110% activity for 100 µg/mL of **103c** concentration.

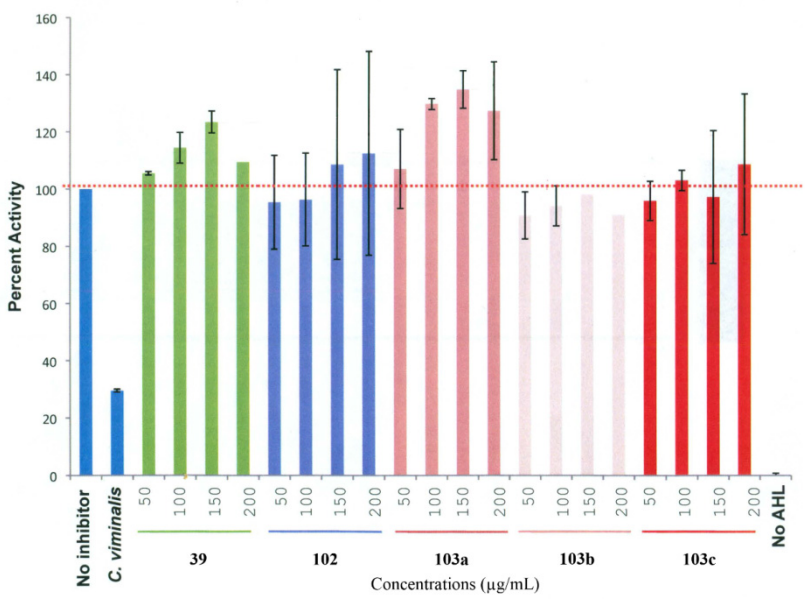


Figure 31. Effect of 4-thio-SRH analogues on *rhl* expression in *P.$_{lasI}$-lacZ* expression in E.coli (p- value ‹ 0.05).

Screening against las signaling

As for their effect on *las* gene, all the preceding 4-thiofuranosides showed in general a significant concentration dependent inhibitory effect. The most potent among others was sulfoxide **103b** that decreased the activity of *las* to 40% at 200 µg/mL concentration. Similar result was observed for the racemic mixture **103c** which exhibited 35% *las* activity for 200 µg/mL concentration. The thioethers **39** and **102** had comparable inhibitory activity (55% *las* activity at 200 µg/mL concentration).

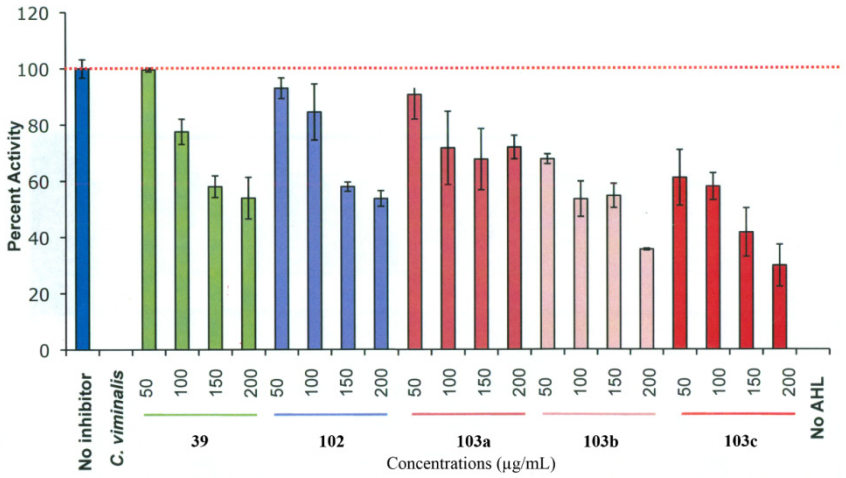


Figure 32. Effect of 4-thio-SRH analogues on las expression in *P.*$_{rhlAI}$*lacZ* expression in E.coli (p- value ‹ 0.05).

With the collaboration with Dr Makemson, the 4-[thia]-SRH analogues were also tested for their effect on QS mediated luminescence of wild-type *V. harveyi*. It was found that none of the tested compounds had any effect on bioluminescence. Most of the compounds had slight inhibition of the QS but not on total growth of the bacteria. In fact, 103b and 103c had a slight stimulatory effect.

	P. aeruginosa		*V. harveyi*		
Compound	Anti-QS activity against LasR	Anti-QS activity against Rhl	Bioluminescence	QS	Growth
39	inhibition	no effect	no effect	slight inhibition	no effect
102	inhibition	no effect	no effect	less than 50% inhibition	no effect
103a	slight inhibition	no effect	-	-	-
103b	inhibition	no effect	no effect	slight inhibition	slight stimulatory
103c	inhibition	no effect	no effect	slight inhibition	slight stimulatory

Table 5. Anti-QS activity and bacterial growth of 4-[thia]-SRH derivatives.

4. EXPERIMENTAL SECTION

4.1. General Procedures

The ^{1}H (400 MHz),^{13}C (100 MHz), or ^{19}F (376 MHz) NMR spectra were recorded at ambient temperature in solutions of $CDCl_3$ or MeOH-d_4 or D_2O, as noted. Mass spectra (MS) were obtained with atmospheric pressure chemical ionization (APCI) or ESI technique and HRMS in AP-ESI or TOF-ESI mode. The reactions were followed by TLC with Merck Kieselgel 60-F_{254} sheets and products were detected with a 254 nm light or with Hanessian's stain. Column chromatography was performed using Merck Kieselgel 60 (230-400 mesh). The purity of the newly synthesized compounds was determined to be ≥95% by elemental analysis (C, H, N) and/or HPLC on X-terra® RP-C18 with gradient program using CH_3CN/H_2O as mobile phase. Reagent grade chemicals were used and solvents were dried by reflux distillation over CaH_2 under nitrogen gas, unless otherwise specified, and reactions carried out under Ar atmosphere.

The compounds synthesized in my dissertation are considered, where possible, as sugar derivatives or SRH analogs thus the numbering of carbons followed carbohydrate nomenclature with anomeric carbon (or the precursor to anomeric carbon) designated as C1 (Figure 33).

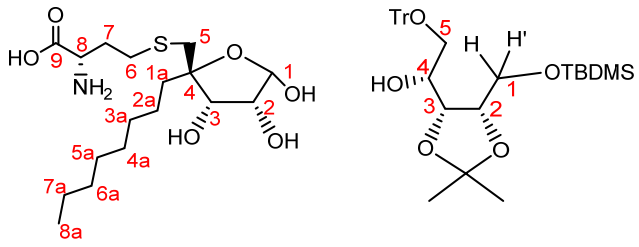


Figure 33. Numbering of the carbons in *S*-ribosylhomocysteine analogues and the corresponding sugar precursors used for the nomenclature of compounds and description of their NMR spectra.

The following *General Procedures* have been used to describe synthesis of targeted compounds:

Procedure A: Regioselective mesylation of the primary 5-hydroxyl group

Procedure B: *Step a*: Preparation of the protected homocysteine; *Step b:* Coupling of the homocysteine with the sugar precursor in the presence of K_2CO_3

Procedure C: Addition of the Grignard reagents to the ribitol-4-ulose

Procedure D: Deprotection of TBDMS group with TBAF

Procedure E: Oxidation of the primary hydroxyl with NMO/TPAP and ring closure to the lactones,

Procedure F: Removal of the 5-trityl protection group

Procedure G: Coupling of the homocysteine with the sugar precursors in the presence of LDA

Procedure H: Removal of the Boc, *t*-BuO ester and isopropylidene protection groups with TFA and TFA/H_2O

Procedure I: Reduction of the lactones to hemiacetals with $LiEt_3BH$

4.2. Synthesis of the inhibitors and their spectroscopic characteristization

2-Deoxy-D-ribonolactone (41). The 2-deoxyribose **40** (1.03 g, 7.45 mmol) was added to a solution of Br_2 (2 mL) in water (6 mL). The mixture was kept stirring at ambient temperature for 5 days and then silver carbonate was added to neutralize the mixture to pH = 7. The mixture was filtered and the filtrate was concentrated under reduced pressure to yield to yellow oil**.** The volatiles were evaporated and the sample was dried under vacuum to give **41**[130] (1.02 g, 90%): ^{1}H NMR ($CDCl_3$) δ 2.59 (dd, *J* = 3.0, 18.6 Hz, 1H, H2'), 3.05 (dd, *J* = 3.0, 18.6 Hz, 1H, H2), 3.81 (dd, *J* = 4.5, 12.9 Hz, 1H, H5'), 3.92 (dd, *J* = 3.1, 12.9 Hz, 1H, H5), 4.50-4.60 (m, 2H , H3, H4).

2-Deoxy-3,5-di-*O*-*(*tert-butyldimethylsilyl)-D-ribonolactone (42). Imidazole (2.5 g, 37.3 mmol) and TBDMSCl (4.5 g, 29.8 mmol) were added to compound **41** (1.0 g, 7.57 mmol) dissolved in anhydrous DMF (20 mL). The mixture was stirred at ambient temperature for 1 day and quenched by the addition of water. The water layer was extracted with EtOAc (3 x 10 mL) and the organic layers were combined, washed with brine and dried with anhydrous Mg_2SO_4 and evaporated. The remaining residue was column chromatographed (90% hexane/EtOAc) to give **42**[130] (750 mg, 80%): ^{1}H NMR ($CDCl_3$) δ 0.08-0.10 (m, 12H), 0.80-0.91 (s, 18H), 2.35 (dd, *J* = 2.6, 17.6 Hz, 1H, H2), 2.80 (dd, *J* = 6.7, 17.6 Hz, 1H, H2'), 3.74 (dd, *J* = 2.5, 11.4 Hz, 1H, H5), 3.78 (dd, *J* = 3.2, 11.5 Hz, 1H, H5'), 4.30-4.32 (m, 1H, H3), 4.46-4.81 (m, 1H, H4).

2-Bromo-2-deoxy-3,5-di-*O*-(tert-butyldimethysilyl)-D-ribonolactone (43a) and **2-bromo-2-deoxy-3,5-di-*O*-(tert-butyldimethysilyl)-D-arabinonolactone (43b).** Triethylamine (0.42 mL, 303 mg, 3.0 mmol) and TMSOTf (333 mg, 1.5 mmol) were added to a stirred solution of **42** (180 mg, 0.50 mmol) in CH_2Cl_2 (6 mL) at 0 ^{o}C for 30

min. A solution NBS (134 mg, 0.75 mmol) in 1.5 mL of CH_2Cl_2 was added and the mixture was kept stirring for 1 h at 0 ^{0}C. The volatiles were evaporated and the residue was washed with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried with anhydrous Mg_2SO_4 and silica gel column chromatographed (85% hexane/EtOAc) to give a mixture of *ribo:arabino* epimers **43a**/**43b**[130] (1:2; 167 mg, 76%). *Ribo*-epimer had: ^{1}H NMR ($CDCl_3$) δ 0.08-0.10 (m, 12H), 0.80-0.91 (s,18H), 3.75 (dd, *J* = 2.0, 6.4 Hz, 1H, H5), 3.95 (dd, *J* = 2.3, 4.9 Hz, 1H, H5'), 4.17-4.22 (m, 1H, H4), 4.38-4.40 (m, 1H, H3), 4.65 (t, *J* = 6.0, 12.7 Hz, 1H, H2). *Arabino*-epimer had: ^{1}H NMR ($CDCl_3$) 0.08-0.10 (m, 12H), 0.80-0.91 (m, 18H), 3.77 (dd, *J* = 3.0, 6.2 Hz, 1H, H5), 3.90 (dd, *J*= 2.2, 4.8 Hz, 1H, H5'), 4.27-4.32 (m, 1H, H4), 4.36 (d, 1H, *J* = 4.9 Hz, 1H, H3), 4.46 (d, *J* = 5.6 Hz, 1H, H2). MS (ESI$^+$) *m/z* 456 (98, M+NH_4^+, [^{79}Br]), 458 (100, M+NH_4^+, [^{81}Br]).

2-Bromo-2-deoxy-3-*O*-*tert*-butyldimethysilyl-arabinonolactone (44). A solution of **43b** (25 mg, 0.05 mmol) in TFA/H_2O (2 mL, 9:1) was stirred for 1 h at 0 ^{o}C. Volatiles were evaporated and the residue was co-evaporated with toluene (0.1 mL). Purification of the residue on flash column chromatography column (hexane/EtOAc, 85:15) gave **44** (19 mg, 90%): ^{1}H NMR ($CDCl_3$) δ 0.17 & 0.20 (2 x s, 2 x 3H), 0.90 (s, 9H), 3.76 (d, *J* = 12.8 Hz, 1H, H5), 4.08 (d, *J* = 13.0 Hz, H5'), 4.20-4.31 (m, 1H, H4), 4.44 (t, *J* = 5.8, 11.5 Hz, 1H, H3), 4.48 (d, *J* =5.6 Hz, 1H, H2); ^{13}C NMR ($CDCl_3$) δ -5.78, -6.06 (Si-*Me*$_2$), 22.16 (C-*Me*$_3$), 25.71 (*C*-Me_3), 44.85 (C2), 58.41 (C5), 67.26 (C3), 83.23 (C4), 169.55 (C1). MS (ESI$^+$) *m/z* 342 (98, M+NH_4^+, [^{79}Br]), 344 (100, M+NH_4^+, [^{81}Br]).

2-Bromo-2-deoxy-3-*O*-*tert*-butyldimethysilyl-5-*O*-mesyl-arabinonolactone (45). Procedure A: TEA (42 μL, 30 mg, 0.29 mmol) and MsCl (9.3 μL, 13 mg, 0.11 mmol)

were added to a stirred solution of **44** (34 mg, 0.104 mmol) in dry CH_2Cl_2 (1.75 mL) at 0 ^oC (ice-bath) under N_2 atmosphere. After 5 min, the ice bath was removed and the reaction mixture was partitioned between CH_2Cl_2 and diluted HCl. The separted organic layer was then washed with aqueous solution of $NaHCO_3$ and brine and was then dried over anhydrous $MgSO_4$. Column chromatography (85% hexane/EtOAc) gave **45** (26 mg, 60%): ^1H NMR ($CDCl_3$) δ 0.17 & 0.20 (2 x s, 2 x 3H), 0.90 (s, 9H), 3.06 (s, 3H), 4.28 (t, *J* = 5.8, 12.7 Hz, 1H, H3), 4.36 (d, *J* = 5.9 Hz, H2), 4.40 (dd, *J* = 3.0, 12.0 Hz, 1H, H5), 4.47-4.50 (m, 1H, H4), 4.56 (dd, *J* = 2.2, 12.1 Hz, 1H, H5'); MS (ESI^+) *m/z* 451.9 (98, $M+NH_4^+ +CH_3OH$, [^{79}Br]), 453.9 (100, $M+NH_4^+ +CH_3OH$, [^{81}Br]).

2-Deoxy-3-*O*-*tert*-butyldimethysilyl-2-[(*tert*-butoxycarbonyl)-L-homocysteine *tert*-butyl ester]arabinonolactone (50). Procedure B. *Step a*. H_2O (0.45 mL) and tris(2-carboxyethyl)phosphine hydrochloride (70 mg, 0.24 mmol), were added to a stirred solution of *N*',*N*'-di(*tert*-butoxycarbonyl)-L-homocystine di(*tert*-butyl) ester[67] **48** (100 mg, 0.17 mmol) in anhydrous DMF (5 mL) at room temperature under Ar atmosphere. After 24 hours, the reaction mixture showed on TLC (80% hexane/EtOAc) the conversion of the disulfide moiety into a thiol **49**. The reaction mixture was partitioned between $NAHCO_3/H_2O$ and ethyl acetate. The organic layer was separated and was washed with brine, dried ($MgSO_4$) and was evaporated to give **49**[67] (98 mg, 99%) as a colorless oil of sufficient purity to be used in the next step: ^1H NMR ($CDCl_3$) δ 1.40 (s, 10H), 1.51 (s, 10H), 1.9 (m, 1H), 2.09-2.12 (m, 1H), 2.6 (m, 1H), 4.30 (d, *J* = 5.2 Hz, 1H), 5.10 (d, *J* = 7.6 Hz, 1H, NH). *Step b.* K_2CO_3 (40 mg, 0.29 mmol) was added to a stirred solution of **45** (20 mg, 0.05 mmol) and **49** (14.2 mg, 0.04 mmol) in dry acetone (5 mL). The heterogeneous mixture was refluxed for 1 h under argon atmosphere. Volatiles were

evaporated and the residue was partitioned between EtOAc (10 mL) and $NaHCO_3$ (10 mL). The combined organic layer was evaporated and the residue was column chromatographed (80% hexane/EtOAc) to give **50** (5 mg, 20%): ^{1}H NMR ($CDCl_3$) δ 0.08-0.10 (m, 6H), 0.80-0.91 (s, 9H), 1.39 (2 x s, 18 H), 1.90 - 2.11 (m, 2H, H7, H7'), 2.25 - 2.40 (m, 2H, H6, H6'), 3.01 (s, 3H, Ms), 4.15- 4.25 (m, 1H, H8), 4.70 (d, *J* = 3.2 Hz, 1H, H2), 5.00 (d, *J* =2.5, NH), 5.30 ("t", 1H, H3); ^{13}C NMR ($CDCl_3$) δ 8.62 (Si*Me*$_2$), 14.16 (NC*Me*$_3$), 21.00 (O*Me*$_3$), 22.65 (C7), 23.09 (C6), 29.57 (Si*C*Me$_3$), 32.12 (SiC*Me*$_3$), 37.00 (Ms), 46.03 (*C*H-NBoc), 46.76 (C2), 60.38 (C5), 66.69 (C3), 163.73(CO), 171.15 (CO), 180.0 (CO).

5-*O*-Mesyl-3-*O*-tert-butyldimethylsilyl-2-*S*-propyl-2-thioribonolactone (50a). LDA (2 M/THF and heptanes, 20 µl, 0.04 mmol) was added slowly to a stirred solution of propylthiol (3.8 mg, 0.05 mmol) in anhydrous DMF (2 ml) under Ar atmosphere at 0 ^{o}C (ice bath). After 30 min, a solution of **45** (10 mg, 0.02 mmol) in DMF (2 mL) was added by a syringe and the mixture was continued to stirr for 1 h at 0 ^{o}C then at room temperature for 24 hours. The TLC showed the formation of new less polar product. The reaction was quenched with NH_4Cl and the volatiles were evaporated under high vaccum. The residue was partitioned between EtOAc and $NaHCO_3$, and the organic layer was washed with brine and dried with anhydrous $MgSO_4$. The resulting oil was column chromatographed (7:3, hexane/EtOAc) to give **50a** as a mixture of two isomers (α:β, 1:1; 6 mg, 61%): ^{1}H NMR ($CDCl_3$) δ 0.08-0.10 (m, 12H), 0.80-0.91 (s, 18H), 1.10 (t, *J* =6.6 Hz, 6H, 2 x Me), 1.45-1.47 (m, 4H, 2 x 2H), 2.45-2.48 (m, 4H, 2 x CH_2S), 3.06 (s, 6H, 2 x Me), 3.20 (d, *J* = 5.5 Hz, 1H, H2), 3.45 (d, *J* = 6.4 Hz, 1H, H2), 4.05 (t, *J* = 3.2 Hz, 1H, H3), 4.17 (dd, *J* = 3.6, 12.1 Hz, 1H, H5), 4.22-4.25 (m, 1H, H4), 4.26-4.33 (m, 2H, H5

and H5'), 4.34 (t, *J*= 2.7 Hz, H5'), 4.38 (t, *J* = 2.2 Hz, 1H, H4), 4.40 (t, *J* = 6.8 Hz, 1H, H3). ^{13}C NMR -5.18 & -4.25 (Si-(*Me*)$_2$), 14.15 & 14.21 (Me), 17.84 & 18.11 (CH_2), 21.09 & 22.67 (Si-*C*-(Me)$_3$), 25.60 (Si-C-(*Me)$_3$*), 31.61 & 33.71 (CH_2), 37.78 & 37.89 (S-Me), 48.81 & 60.43 (C2), 66.09 & 66.38 (C3), 69.61 & 73.81 (C5), 8.73 & 82.25 (C4), 171.47 &172.74 (CO).

6-Deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (55). A solution of **54**[131,133] (0.02 g, 0.076 mmol) in dioxane (0.24 mL) and ethanol (0.12 mL) in the presence of H_2SO_4 (5 M, 0.04 mL) was stirred at room temperature for 4 h. The reaction was then neutralized with Na_2CO_3 and extracted with CH_2Cl_2. The organic layer was washed with brine, dried and evaporated to give **55**[131] (19 mg, 90%) of sufficient purity to be directly used in the next step: ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 3H), 1.50 (s, 3H), 3.81 (dd, *J* = 5.0, 11.3 Hz, H6'), 3.90 (dd, *J* = 3.2, 11.4 Hz, H6), 3.95-4.01 (m, 1H, H5), 4.20 (ddd, *J* = 2.2, 8.5, 29.4 Hz, 1H, H4), 4.70 (dd, *J* = 3.7, 10.8 Hz, 1H, H2), 5.10 (dd, *J* = 2.2, 50.2 Hz, 1H, H3), 5.90 (d, *J* = 3.7 Hz, 1H, H2); ^{19}F NMR δ -208.11 (ddd, *J* = 10.9, 29.3, 50.0 Hz).

6-*O*-Benzoyl-3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (56). Benzoyl chloride (0.1 mL, 130 mg, 0.92 mmol) was added dropwise to a stirred solution of **55** (200 mg, 0.9 mmol) in dry pyridine (2.5 mL) and CH_2Cl_2 (0.9 mL) at -15 ^{o}C under N_2 atmosphere. After 3 h, volatiles were removed and the residue was dissolved in $CHCl_3$ (2 mL). The resulting solution was washed with saturated $NaHCO_3$ aquous solution (3 x 1.5 mL), water (2 x 1.5 mL), dried (Na_2SO_4) and evaporated to give **56**[131] (61 mg, 34%) as colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 3H), 1.50 (s, 3H), 4.10-4.15 (m, 1H, H5),

4.18-4.21 (m, 1H, H4), 4.40 (dd, *J* = 10.0, 11.8 Hz, 1H, H6), 4.61-4.67 (m, 2H, H2, H6'), 5.10 (dd, *J* = 2.0, 49.4, 1H, H3), 6.01 (d, *J* = 3.7 Hz, 1H, H1), 7.41-7.92 (m, 5H, Ar).

6-*O*-Benzoyl-3-deoxy-3-fluoro-D-glucofuranose (57). TFA/H_2O (9/1, 1.5 mL), was added to dry **56** (31 mg, 0.97 mmol) and left stirring for half hour at 0 ^oC (ice-bath) then left for an additional half hour at ambient temperature. The volatiles were co-evaporated with toluene then dried to give a mixture of α:β isomers compound **57a**/**57b**[131] (1:3; 26 mg, 92%): ^1H NMR ($CDCl_3$) δ 3.70-3.80 (m, 2H, H2, H6'), 4.10-4.17 (m, H5, 1H), 4.45-4.75 (m, 2H, H3, H6), 4.65 ("t", *J* = 9.1, 18.0, H4, 1H), 5.30 (s, 0.5H, H1; β-isomer), 6.01 (d, *J* = 3.1 Hz, 0.5H, H1; α-isomer), 7.41-7.92 (m, 5H, Ar). ^{19}F NMR δ -195.65 (dt, *J* = 4.7, 54.7 Hz), -200.60 (dt, *J* = 11.8, 53.6 Hz).

6-*O*-Benzoyl-2-deoxy-2-fluoro-D-arabinofuranose (60). *Step a.* $NaIO_4$ (23 mg, 0.10 mmol) was added to a stirred solution of **57**[131] (23 mg, 0.08 mmol) in water (3 mL) at ambient temperature and left overnight. The mixture was then extracted with chloroform (30 mL), the organic layer was dried over anhydrous $MgSO_4$. *Step b.* The crude material from *Step a* (25 mg) was dissolved in MeOH (2 mL) and MeONa (3 mg, 0.05 mmol) was added. The resulting mixture was stirred at ambient temperature for 1 h. The volatile were evaporated and the residue was column chromatographied (30% hexane/EtOAc) gave a mixture of α:β isomers **60a/b** (1:9; 20 mg, 86%). The major anomer had: ^1H NMR ($CDCl_3$) δ 4.25 (d, *J* = 9.2, 1H, H4), 4.45 (m, 2H, H6, H6'), 4.6 (s, 1H), 5.01 (d, *J* = 49.8, H2, 1H), 5.40 (dd, H3, *J* = 3.3, 20.7 Hz, 1H), 5.65 (d, *J* = 10.8 Hz, H1), 7.41-7.92 (m, 5H, Ar). ^{19}F NMR δ -190.58 (ddd, *J* = 11.1, 21.8, 49.2 Hz, 0.9F) and δ -190.94 (ddd, *J* = 10.8, 19.6, 54.0 Hz, 0.1F).

2,3-*O*-Isopropylidene-5-*O*-trityl-D-ribitol (71). $NaBH_4$ (4.0 mg, 0.10 mmol) was added to a stirred solution of **70**[134,147] (30 mg, 0.07 mmol) in EtOH (1 mL) at 0 ^oC (ice-bath) under N_2 atmosphere. After 1 h, the reaction mixture was partitioned between $NaHCO_3/H_2O$ and EtOAc (15 mL).The organic layer was dried over anhydrous $MgSO_4$ and evaporated. The residue was column chromatographied (30% hexane/EtOAc) to give **71**[134] (25 mg, 83%): ^1H NMR ($CDCl_3$) δ 0.08 (s, 6H, 2 x CH_3), 2.96 (d, *J* = 3.6 Hz, 1H), 3.08 (dd, *J* = 5.0, 8.4 Hz, 1H), 3.34 (dd, *J* = 6.9, 9.8 Hz, 1H), 3.50 (dd, *J* = 2.9, 9.8 Hz, 1H), 3.75-7.81 (m, 1H), 3.83-3.91 (m, 2H), 4.10-4.75 (m, 1H), 4.33-4.40 (m, 1H), 7.25-7.38 (m, 15H, Ar).

1-*O*-*tert*-Butyldimethysilyl-2,3-*O*-isopropylidene-5-*O*-trityl-D-ribitol (72). TBSMSCl (139 mg, 0.92 mmol) and imidazole (93.9 mg, 1.38 mmol) were added to a solution of **71** (200 mg, 0.46 mmol) in DMF (4 mL) at room temperature and stirring was continued for 72 h. The volatiles were evaporated and the residue was partitioned between saturated NH_4Cl/H_2O and EtOAc. The separated organic layer was then washed with $NaHCO_3/H_2O$, dried over Mg_2SO_4, evaporated and the resulting residue was column chromatographed (50% hexane/EtOAc) to give **72**[134] (180 mg, 71%) as an amorphous white powder: ^1H NMR ($CDCl_3$) δ 0.08 (s, 6H, Si*Me*$_2$), 0.81 (s, 9H, Si-C-*Me*$_3$), 1.20 (s, 6H, 2 x Me), 3.20 (dd, *J* = 5.3, 9.7 Hz, 1H, H5), 3.25 (dd, *J* = 2.8, 9.7 Hz, 1H, H5'), 3.49 (dd, *J* = 4.1, 10.6 Hz, 1H, H1), 3.68 (dd, *J* = 8.7, 10.5 Hz, 1H, H1'), 3.79-3.81 (m, 1H, H4), 4.13-3.15 (m, 1H, H2), 4.22 (dd, *J* = 5.5, 9.2 Hz, 1H, H3), 7.25-7.38 (m, 15H, Ar).

1-*O*-*tert*-Butyldimethysilyl-2,3-*O*-isopropylidene-5-*O*-trityl-D-ribitol-4-ulose (73). Dess-Martin reagent (15 wt% solution/CH_2Cl_2; 3.0 mL, 492 mg, 1.16 mmol) was added to a solution of **72** (320 mg, 0.58 mmol) in CH_2Cl_2 (8 mL) at room temperature and

stirred for 3 h. The reaction mixture was partitioned between 0.5 mL $Na_2S_2O_3$ in 10 mL of H_2O and 10 mL $NaHCO_3$ and CH_2Cl_2 (15 mL). The organic layer was dried over anhydrous $MgSO_4$ and evaporated. The residue was column chromatographied (85% hexane/EtOAc) to give **73**[134] (290 mg, 91%) as an oil: ^{1}H NMR ($CDCl_3$) δ 0.08 (s, 6H, Si*Me*$_2$), 0.81 (s, 9H, Si-C-*Me*$_3$), 1.35 (s, 3H, Me), 1.37 (s, 3H, Me), 3.68 (dd, *J* = 4.2, 11.2 Hz, 1H, H1), 3.76 (dd, *J* = 4.0, 11.2 Hz, 1H, H1'), 4.04 (d, *J* =17.7 Hz, 1H, H5), 4.20 (d, *J* = 17.8 Hz, 1H, H5'), 4.51-4.53 (m, 1H, H2), 4.71 (d, *J* = 7.8 Hz, 1H, H3), 7.25-7.38 (m, 15H, Ar).

1-*O*-*tert*-Butyldimethysilyl-2,3-*O*-isopropylidene-4-*C*-methyl-5-*O*-trityl-D-ribitol (74a). **Procedure C.** MeMgBr (1 M/THF, 2 mL, 2.0 mmol) was added to a stirred solution of **73** (205 mg, 0.37 mmol) in anhydrous THF (4 mL) at -78 ^{o}C under N_2 atmosphere. After 15 min, the reaction mixture was allowed to warm up to ambient temperature and was kept stirring for 1 h. The reaction was then quenched by the addition of MeOH (1 mL) and diluted with EtOAc (10 mL). The resulting mixture was washed with 0.1N HCl and the organic layer dried over anhydrous $MgSO_4$. Volatiles were evaporated and the crude residue was then purified by flash column chromatography (90% hexane/EtOAc) to give **74a**[134] (180 mg, 85%) as an clear oil: ^{1}H NMR ($CDCl_3$) δ 0.08 (s, 6H, Si*Me*$_2$), 0.81 (s, 9H, Si-C-*Me*$_3$), 1.35 (s, 3H, Me, C1a), 1.40 (2 x s, 2 x 3H, 2 x Me), 3.01 (d, *J* = 8.7 Hz, 1H, H5), 3.12 (d, *J* = 8.7 Hz, 1H, H5'), 3.25 (dd, *J* = 3.8, 10.9 Hz, 1H, H1), 3.70 (dd, *J*= 3.8, 10.9 Hz, 1H, H1'), 3.90-3.95 (m,1H, H2), 4.40 (d, *J* = 5.5 Hz, 1H, H3), 7.25-7.38 (m, 15H, Ar). HRMS calcd for $C_{34}H_{46}O_5NaSi^+$ $[M+Na]^+$ 585.3007, found 585.3009.

1-*O-tert*-Butyldimethysilyl-4-*C*-hexyl-2,3-*O*-isopropylidene-5-*O*-trityl-D-ribitol (74b). Treatment of **73** (160 mg, 0.29 mmol) with hexyl-MgBr (0.8 M/THF; 1 mL, 0.80 mmol) by Procedure C (flash column chromatography; 80% hexane/EtOAc) gave **74b** (120 mg, 64%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 0.08 (s, 6H, Si*Me*$_2$), 0.81 (s, 9H, Si-C-*Me*$_3$), 0.89 (t, *J*= 6.6 Hz, CH_3, C6a), 1.30-1.40 (m, 8H, C2a-C5a), 1.40 (s, 3H, Me), 1.50 (s, 3H, Me), 1.50-1.60 (m, 2H, C1a), 3.06 (d, *J* = 8.9 Hz, 1H, H5), 3.22 (d, *J* = 8.9 Hz, 1H, H5'), 3.28 (dd, *J* = 3.8, 11.1 Hz, 1H, H1), 3.72 (dd, *J* = 7.6, 11.0 Hz, 1H, H1'), 3.80-3.85 (m, 1H, H2), 4.40 (d, *J* = 5.2 Hz, 1H, H3), 7.25-7.38 (m, 15H, Ar).

1-*O-tert*-Butyldimethysilyl-2,3-*O*-isopropylidene-4-*C*-octyl-5-*O*-trityl-D-ribitol (74c). Treatment of **73** (480 mg, 0.87 mmol) with octyl-MgBr (2 M/THF; 7.5 mL, 15 mmol) by Procedure C gave **74c** (350 mg, 60%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 0.08 (s, 6H, Si*Me*$_2$), 0.81 (s, 9H, Si-C-*Me*$_3$), 0.89 (t, *J*= 6.6 Hz, Me, C8a), 1.28-1.30 (m, 12H, C2a-C7a), 1.38 (s, 3H, Me), 1.42 (s, 3H, Me), 1.50-1.60 (m, 2H, C1a), 3.05 (d, *J* = 8.9 Hz, 1H, H5), 3.21 (d, *J* = 8.9 Hz, 1H, H5'), 3.26 (dd, *J* = 3.8, 11.0 Hz, 1H, H1), 3.70 (dd, *J* = 3.9, 7.6 Hz, 1H, H1'), 3.80-3.85 (m, 1H, H2), 4.40 (d, *J* = 5.2 Hz, 1H, H3), 7.25-7.38 (m, 15H, Ar).

1-*O-tert*-Butyldimethysilyl-2,3-*O*-isopropylidene-5-*O*-trityl-4-*C*-vinyl-D-ribitol (74d). Treatment of **73** (120 mg, 0.21 mmol) with vinyl-MgBr (1 M/THF; 0.63 mL, 0.63 mmol) by Procedure C gave **74d** (70 mg, 55%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 0.08 (s, 6H, Si*Me*$_2$), 0.81 (s, 9H, Si-C-*Me*$_3$), 1.35 (s, 3H, Me), 1.37 (s, 3H, Me), 2.92 (d, *J* = 9.0 Hz, 1H, H5), 3.12 (d, *J* = 9.0 Hz, 1H, H5'), 3.42 (dd, *J* = 4.5, 10.7 Hz, 1H, H1), 3.80 (dd, *J*= 8.5, 10.7 Hz, 1H, H1'), 4.00-4.13 (m, 1H, H2), 4.69 (d, *J* = 6.1 Hz, 1H, H3), 5.16 (dd, *J*= 1.7, 10.9 Hz, 1H, CH=CH*H*), 5.40 (dd, *J* = 1.8, 17.4 Hz, 1H, CH=C*H*H), 6.20 (dd, *J*

= 10.9, 17.4 Hz, 1H, C*H*=CHH), 7.25-7.38 (m, 15H, Ar); ^{13}C NMR δ -5.40 (Si*Me*$_2$), 21.02 & 25.29 (C*Me*$_2$), 25.77 (Si-C-*Me*$_3$), 31.58 (Si-*C*-Me$_3$), 60.47 (*C*-Ph$_3$), 61.98 (C1), 69.13 (C5), 74.87 (C4), 78.15 (C2), 78.45 (C3), 107.87 (CH=*C*HH), 114.84 (*C*-Me$_2$), 126.91 & 127.71 & 128.87 & 143.40 (Ar), 138.71 (*C*H=CHH), 146.89; HRMS calcd for $C_{35}H_{46}O_5NaSi^+$ $[M+Na]^+$ 597.3007, found 597.3006.

1-*O*-*tert*-Butyldimethysilyl-2,3-*O*-isopropylidene-4-*C*-4-methoxyphenyl-5-*O*-trityl-D-ribitol (74e), Treatment of **73** (165 mg, 0.30 mmol) with methoxyphenyl-MgBr (1 M/THF; 0.89 mL, 0.89 mmol) by Procedure C gave **74e** (190 mg, 96%) as a clear oil:^{1}H NMR ($CDCl_3$) δ 0.08 (s, 6H, Si*Me*$_2$), 0.81 (s, 9H, Si-C-*Me*$_3$), 1.35 (s, 3H, Me), 1.37 (s, 3H, Me), 3.01 (d, *J* = 9.1 Hz, 1H, H5), 3.15 (d, *J* = 9.1 Hz, 1H, H5'), 3.41 (dd, *J* = 4.5, 10.8 Hz, 1H, H1), 3.80 (s, 3H, CH_3O), 3.85 (dd, *J* = 4.5, 10.8 Hz, 1H, H1'), 4.19-4.22 (m, 1H, H2), 5.05 (d, *J* = 6.4 Hz, 1H, H3), 6.85 (d, *J* = 6.9 Hz, 2H, Ar), 7.61 (d, *J* = 8.9 Hz, 2H, Ar), 7.25-7.38 (m, 15H, Ar); HRMS calcd for $C_{40}H_{50}O_6NaSi^+$ $[M+Na]^+$ 677.3269, found 677.3267.

2,3-*O*-Isopropylidene-4-*C*-methyl-5-*O*-trityl-D-ribitol (75a). **Procedure D.** TBAF (1 M/THF; 0.5 mL, 0.5 mmol) was added to a stirred solution of **74a** (183 mg, 0.32 mmol) in THF (6 mL) at 0 °C (ice-bath). After 30 min, the volatiles were evaporated and the resulting residue was washed with $NaHCO_3/H_2O$ and extracted with EtOAc. The organic layer was then dried over Mg_2SO_4 and evaporated to give crude residue which was column chromatographed (80 → 70% hexane/EtOAc) to give **75a** (100 mg, 68%): ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H, CH_3, C1a), 1.40 (s, 3H, Me), 1.49 (s, 3H, Me), 3.05 (d, *J* = 9.0 Hz, 1H, H5), 3.32 (d, *J* = 9.0 Hz, 1H, H5'), 3.58 (dd, *J* = 5.2, 12.0 Hz, 1H, H1), 3.75 (dd, *J*= 5.5, 12 Hz, 1H, H1'), 4.10- 4.20 (m,1H, H2), 4.30 (d, *J* = 6.2 Hz, 1H, H3), 7.25-

7.38 (m, 15H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.21 (C1a), 25.17 & 27.28 (C*Me*$_2$), 60.42 (C5), 61.43 (C1), 67.95 (*C*-Ph_3), 68.38 (C4), 77.61 (C2), 79.10 (C3), 107.67 (*C*Me_2), 127.27 & 127.97 & 128.62 & 143.43 (Ar).

2,3-*O*-Isopropylidene-4-*C*-hexyl-5-*O*-trityl-D-ribitol (75b). Treatment of **74b** (280 mg, 0.44 mmol) with TBAF by Procedure D gave **75b** (145 mg, 87%) as an viscous oil: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, *J*= 6.6 Hz, Me, C6a), 1.30-1.40 (m, 8H, C2a-C5a), 1.25 (s, 3H, Me), 1.40 (s, 3H, Me), 1.50-1.60 (m, 2H, C1a), 2.91 (d, *J* = 9.3 Hz, 1H, H5), 3.20 (d, *J* = 9.3 Hz, 1H, H5'), 3.30 (dd, *J* = 5.0, 12.2 Hz, 1H, H1), 3.40 (dd, *J* = 5.0, 12.0 Hz, 1H, H1'), 3.79-3.82 (q, *J* = 5.8 Hz, 1H, H2), 4.15 (d, *J* = 5.8 Hz, 1H, H3), 7.25-7.38 (m, 15 Hz, Ar); ^{13}C NMR ($CDCl_3$) δ 14.14 (C6a), 22.66, 23.54, 25.45, 27.57 (C2a-C5a), 29.74 & 31.83 (C*Me*$_2$), 36.05 (C1a), 61.71 (C5), 65.39 (C1), 72.06 (*C*-Ph_3), 77.55 (C4), 78.99 (C2), 86.92 (C3), 107.21 (*C*Me_2), 127.31 & 127.96 & 128.65 & 145.26 (Ar); HRMS calcd for $C_{33}H_{42}O_5Na^+$ $[M+Na]^+$ 541.2924, found 541.2924.

2,3-*O*-Isopropylidene-4-*C*-octyl-5-*O*-trityl-D-ribitol (75c). Treatment of **74c** (350 mg, 0.52 mmol) with TBAF by Procedure D gave **75c** (200 mg, 84%) as an viscous oil. ^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, CH_3, C8a), 1.20 (s, 12H, C2a-C7a), 1.29 (s, 3H, Me), 1.40 (s, Me), 1.50-1.60 (m, 2H, C1a), 2.95 (d, *J*= 9.4 Hz, 1H, H5), 3.25 (d, *J*= 9.3 Hz, 1H, H5'), 3.35 (dd, *J*= 4.92, 12.2 Hz, 1H, H1), 3.45 (dd, *J*= 5.4, 12.2 Hz, 1H, H1'), 3.80 (m, 1H, H2), 4.10 (d, *J* = 5.8 Hz, 1H, H3), 7.25- 7.38 (m, 15H, Ar); ^{13}C NMR ($CDCl_3$) δ 14.11 (C8a), 22.68, 23.09, 25.43, 27.55, 28.42, 29.24 (C2a-C7a), 29.56 & 30.08 (C*Me*$_2$), 36.06 (C1a), 61.89 (C5), 64.24 (C1), 66.95 (*C*-Ph_3), 74.36 (C4), 77.61 (C2), 79.18 (C3), 107.20 (*C*Me_2), 126.91 & 127.71 & 128.87 & 143.40 (Ar); HRMS calcd for $C_{35}H_{46}O_5Na^+$ $[M+Na]^+$ 569.3237, found 569.3237.

2,3-*O*-Isopropylidene-4-*C*-vinyl-5-*O*-trityl-D-ribitol (75d). Treatment of **74d** (200 mg, 0.34 mmol) with TBAF by Procedure D gave **75d** (124mg, 77%) as a viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H, Me), 1.37 (s, 3H, Me), 3.38 (d, *J* = 11.2 Hz, 1H, H5), 3.54 (d, *J* = 11.2 Hz, 1H, H5'), 3.58 (dd, *J* = 5.1, 11.3 Hz, 1H, H1), 3.90 (d, *J* = 8.6, 11.3 Hz, 1H, H1'), 4.25-4.30 (m, 1H, H2), 4.32 (d, *J* = 6.5 Hz, 1H, H3), 5.20 (dd, *J* = 1.2, 11.0 Hz, 1H, CH=CH*H*), 5.40 (dd, *J* = 1.5, 17.6 Hz, 1H, CH=C*H*H), 6.20 (dd, *J* = 11.0, 17.5 Hz, 1H, C*H*=CHH), 7.25-7.38 (m, 15H, Ar). ^{13}C NMR ($CDCl_3$) δ 24.65 & 27.07 (C-*Me*$_2$), 60.29 (*C*Ph_3), 60.62 (C1), 68.07 (C5), 77.90 (C2), 78.10 (C4), 82.01 (C3), 115.48 (*C*-Me_2), 116.03 (CH=*C*HH), 126.82 & 127.65 & 128.75 & 143.28 (Ar), 146.88 (*C*H=*C*HH).

2,3-*O*-Isopropylidene-4-*C*-4-methoxyphenyl-5-*O*-trityl-D-ribitol (75e). Treatment of **74e** (190 mg, 0.29 mmol) with TBAF by Procedure D gave **75e** (92 mg, 59%) as a viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H, Me), 1.37 (s, 3H, Me), 3.01 (dd, *J* = 6.0, 11.7 Hz, 1H, H1), 3.03 (d, *J* = 9.0 Hz, 1H, H5), 3.23 (dd, *J* = 6.0, 11.7 Hz, 1H, H1'), 3.45 (d, *J* = 9.0 Hz, 1H, H5'), 3.72 (s, 3H, CH_3O), 4.19-4.22 (m, 1H, H2), 4.75 (d, *J* = 6.6 Hz, 1H, H3), 6.85 (d, *J* = 6.9 Hz, 2H, Ar), 7.61 (d, *J* = 8.9 Hz, 2H, Ar), 7.25-7.38 (m, 15H, Ar); ^{13}C NMR δ 24.58 & 27.02 (C-*Me*$_2$), 55.19 (CH_3O), 60.04 (*C*Ph_3), 61.28 (C1), 69.73 (C5), 78.08 (C2), 79.05 (C3), 82.02 (C4), 113.48 (*C*-Me_2), 127.25, 127.37, 127.92, 159.10 (Ar), 128.57 & 129.68 & 132.91 & 146.88 (Ar).

2,3-*O*-Isopropylidene-4-*C*-methyl-5-*O*-trityl-D-ribono-1,4-lactone (76a). **Procedure E.** *N*-methylmorpholine *N*-oxide (NMO; 50 mg, 0.42 mmol), tetrapropylammonium perrenthenate (TPAP; 1 mg, 0.002 mmol) and 4 Å molecular sieves (100 mg) were added to a stirred solution of **75a** (51 mg, 0.11 mmol) in CH_2Cl_2

(3.7 mL) at ambient temperature under N_2 atmosphere. After 6 h, the reaction mixture was filtered off and the filtrate was dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography (75 → 50% hexane/EtOAc) to give **76a** (40 mg, 80%): ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 3H, C1a), 1.35 (s, 3H, Me), 1.40 (s, 3H, Me), 2.91 (d, *J* = 10.2 Hz, 1H, H5), 3.50 (d, *J* = 10.2 Hz, H1, H5'), 4.20 (d, *J*= 5.6 Hz, 1H, H2), 5.01 (d, *J* = 5.6 Hz, 1H, H3), 7.25-7.38 (m, 15 Hz, Ar). ^{13}C NMR ($CDCl_3$) δ 16.40 (C1a) 25.90 & 26.78 (C-*Me*$_2$), 66.65 (*C*Ph_3), 67.63 (C5), 77.70 (C2), 79.77 (C3), 88.56 (C4), 126.07 (*C*-Me_2), 127.46 & 128.25 & 128.67 & 146.87 (Ar), 172.07 (C1).

2,3-*O*-Isopropylidene-4-*C*-hexyl-5-*O*-trityl-D-ribono-1,4-lactone (76b). Treatment of **75b** (37 mg, 0.09 mmol) with NMO/TPAP by Procedure E gave **76b** (35 mg, 94%): ^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, 3H, C6a), 1.19-1.21 (m, 8H, C2a-C5a), 1.24 (s, 3H, Me), 1.40 (s, 3H, Me), 1.50-1.60 (m, 2H, C1a), 2.85 (d, *J* =10.2 Hz, 1H, H5), 3.51 (d, *J* = 10.2 Hz, 1H, H5'), 4.10 (d, *J*= 5.6 Hz, 1H, H2), 5.01 (d, *J*= 5.6 Hz, 1H, H3), 7.25-7.38 (m, 15 Hz); ^{13}C NMR ($CDCl_3$) δ 13.98 (C6a), 23.33 & 26.76 (C-*Me*$_2$), 29.69, 31.38, 29.42, 29.52 (C2a-C5a), 31.60 (C1a), 66.15 (*C*Ph_3), 66.29 (C5), 77.21 (C2), 80.11 (C3), 88.25 (C4), 112.92 (*C*-Me_2), 127.36 & 128.32 & 128.67 & 142.85 (Ar), 174.41 (C1).

2,3-*O*-Isopropylidene-4-*C*-octyl-5-*O*-trityl-D-ribono-1,4-lactone (76c). Treatment of **75c** (12 mg, 0.02 mmol) with NMO/TPAP by Procedure E gave **76c** (8 mg, 95%): ^{1}H NMR ($CDCl_3$) δ 0.80-0.84 (m, Me, C8a), 1.10-1.20 (s, 12H, C2a-C7a), 1.25 (s, Me), 1.40 (s, Me), 1.50-1.60 (m, C1a), 2.90 (d, *J* = 10.1 Hz, 1H, H5), 3.55 (d, *J* = 10.2 Hz, 1H, H5'), 4.10 (d, *J* = 5.6 Hz, 1H, H2), 5.01 (d, *J* = 5.6 Hz, 1H, H3), 7.25-7.38 (m, 15H, Ar); ^{13}C NMR ($CDCl_3$) δ 13.2 (C8a), 21.79 & 22.61 (C*Me*$_2$), 25.05, 25.07, 28.23, 28.39, 29.20, 29.25 (C2-C7), 30.98 (C1a), 64.91 (*C*Ph_3), 65.06 (C5), 65.44 (C2), 79.25 (C3),

87.41 (C4), 112.07 (*C*Me_2), 126.56 & 127.30 & 127.67 & 142.09 (Ar), 170.21 (C1); MS (ESI^+) *m/z* 560 ($M+NH_4$).

Note When **76c** is left in solution overnight, a second minor product **76'c** appeared to be formed with a ratio of **76'c**:**76c** (1:3); the ribosyl peaks for the **76c'** product were as followed: ^{1}H NMR ($CDCl_3$) δ 3.75 (d, *J* = 11.6 Hz, 1H, H5'), 3.91 (d, *J* = 11.6 Hz, 1H, H5),), 4.60 (d, *J* = 5.6 Hz, 1H, H2), 4.90 (d, *J* = 5.6 Hz, 1H, H3); MS (ESI^+) *m/z* 605 ($M+2Na^+$).

2,3-*O*-Isopropylidene-5-*O*-trityl-4-*C*-vinyl-D-ribono-1,4-lactone (76d). Treatment of **75d** (100 mg, 0.21 mmol) with NMO/TPAP by Procedure E gave **76d** (90 mg, 95%): ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 3H, Me), 1.26 (s, 3H, Me), 2.91 (d, *J* = 10.2 Hz, 1H, H5), 3.50 (d, *J* = 10.3 Hz, H1, H5'), 4.20 (d, *J*= 5.6 Hz, 1H, H2), 5.00 (d, *J* = 5.6 Hz, 1H, H3), 5.21 (d, *J* = 11.2 Hz, 1H, CH=CH*H*), 5.34 (d, *J* = 17.4 Hz, 1H, C*H*H), 5.61 (dd, *J* = 11.2, 17.4 Hz, C*H*=CHH), 7.25-7.38 (m, 15 H). ^{13}C NMR ($CDCl_3$) δ 25.92 & 26.64 (C*Me*$_2$), 53.50 (*C*Ph_3), 65.50 (C5), 80.01 (C2), 82.03 (C3), 88.87 (C4), 112.03 (CH=*C*HH), 118.50 (*C*-Me_2), 127.29 & 127.95 & 129.69 & 145.01 (Ar), 146.85 (*C*H=*C*HH), 174.14 (C1); HRMS (TOF) *m/z* calcd for $C_{29}H_{28}O_5^+$ $[M+Na]^+$ 479.1829, found 479.1829.

2,3-*O*-Isopropylidene-4-*C*-4-methoxyphenyl-5-*O*-trityl-D-ribono-1,4-lactone (76e). Treatment of **75e** (90 mg, 0.16 mmol) with NMO/TPAP by Procedure E gave **76e** (73 mg, 82%): ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 3H, Me), 1.25 (s, 3H, Me), 3.25 (d, *J* = 10.5 Hz, 1H, H5'), 3.35 (d, *J* = 10.5 Hz, 1H, H5), 3.80 (s, 3H, CH_3O), 4.48 (d, *J* = 5.5 Hz, 1H, H2), 5.20 (d, *J* = 5.5 Hz, 1H, H3), 6.82 (d, *J* = 8.8 Hz, 2H, Ar), 7.12 (d, *J* = 9.7 Hz, 2H, Ar), 7.25- 7.38 (m, 15H, Ar); ^{13}C NMR ($CDCl_3$) δ 25.92 & 26.64 (C*Me*$_2$), 55.18 (CH_3O),

62.94 (*C*Ph_3), 68.79 (C5), 80.77 (C2), 88.53 (C3), 88.87 (C4), 113.60 (*C*Me_2), 126.9 & 127.25 &128.75 & 159.10, (Ar), 128.57 & 129.68 & 132.91 &-146.40 (Ar), 174.14 (C1).

Attempts to synthesize 4-*C*-octyl-D-ribono-1,4-hemiacetal (81c) by the Swern oxidation of 4-*C*-alkyl-D-ribitol (75c): To a solution of oxalyl chloride (0.01 mg, 0.0001mmol, 0.013 mL) and DMSO (0.02 mg, 0.0002 mmol, 0.02 mL) in dry CH_2Cl_2 (0.14 mL) at -60 ^oC for 15 min, was added dropwise the solution of **75c** (70 mg, 0.13 mmol) in CH_2Cl_2 (0.14 mL). The reaction was stirred for 15 min and dry triethylamine (0.06 mg, 0.006 mmol, 95 μL) was over a period of 10 min at -60 ^oC. The mixture was extracted with H_2O and CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. Purification on silica gel column (hexane/EtOAc, 8:2) gave a mixture of products (≈10 mg) with distinguishable sugar peaks.

2,3-*O*-Isopropylidene-4-*C*-methyl-D-ribono-1,4-lactone (84a). Procedure F. TFA (0.22 mL) and CH_2Cl_2 (4 mL) were added to a stirred solution of **76a** (40 mg, 0.009 mmol) at ambient temperature for 5 h. The volatiles were evaporated and residue co-evaporated with toluene. The oily residue was partitioned between aqueous $NaHCO_3$ and CH_2Cl_2. The separated organic layer was washed with brine, dried $MgSO_4$, and evaporated. Purification on silica gel column (hexane/EtOAc, 8:2) gave **84a** (12 mg, 66%): ^1H NMR($CDCl_3$) δ 1.32 (s, 3H, C1a), 1.35 (s, 3H, Me), 1.42 (s, 3H, Me), 3.60 (d, *J* = 11.6 Hz, 1H, H5), 3.71 (d, *J* = 11.6 Hz, H1, H5'), 4.55 (d, *J*= 5.6 Hz, 1H, H2), 4.90 (d, *J* = 5.6 Hz, 1H, H3). ^{13}C NMR ($CDCl_3$) δ 16.42 (C1a), 25.81 & 26.76 (C*Me*$_2$), 67.61(C5), 77.82 (C2), 80.01 (C3), 86.35 (C4), 113.02 (*C*Me_2), 174.46 (CO); HRMS (TOF) *m/z* calcd for $C_9H_{14}O_5Na^+$ $[M+Na]^+$ 225.0733, found 225.0734.

2,3-*O*-Isopropylidene-4-*C*-hexyl-D-ribono-1,4-lactone (84b) and 2,3-*O*-Isopropylidene-4-*C*-hexyl-D-ribono-1,5-lactone (88b). Treatment of **76b** (30 mg, 0.058 mmol) with TFA by Procedure F (flash column chromatography; 80% hexane/EtOAc) gave **84b** (12 mg, 80%): ^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, CH_3, C6a), 1.19-1.21 (m, 8 H, C2a-C5a), 1.41 (s, 3H, Me), 1.50 (s, 3H, Me), 1.50-1.60 (m, 2H, C1a), 3.75 (d, *J*= 11.8 Hz, 1H, H5), 3.85 (d, *J* = 11.4 Hz, 1H, H5'), 4.65 (d, *J* = 5.6 Hz, 1H, H2), 4.96 (d, *J* = 5.6 Hz, 1H, H3). ^{13}C NMR ($CDCl_3$) δ 13.97 (C6a), 23.43 & 25.79 (C*Me*$_2$), 29.41, 29.56, 29.71, 31.14 (C2a-C5a), 31.91 (C1a), 77.20 (C5), 71.89 (C2), 80.63 (C3), 89.73 (C4), 112.85 (*C*Me$_2$), 172.45 (CO); HRMS (TOF) *m/z* calcd for $C_{14}H_{24}O_5Na^+$ $[M+Na]^+$ 295.1516, found 295.1516.

Note: When the reaction of **76b** with TFA was kept overnight, the mixture of **84b** and its D-ribono-1,5-lactone (**88b**) was isolated after column chromatography (**84b**/**88b,** 1:1, 12 mg, 80%). The ribosyl peaks for the **88b** product were as followed: ^{1}H NMR ($CDCl_3$) δ 3.95 (d, *J*= 12.7 Hz, 1H, H5), 4.20 (d, *J*= 12.7 Hz, 1H, H5'), 4.22 (d, *J*= 2.4 Hz, 1H, H2), 4.37 (dd, *J*= 0.9, 2.5 Hz, 1H, H3); ^{13}C NMR ($CDCl_3$) δ 14.08 (C6a), 22.67 & 23.54 (C*Me*$_2$), 26.78, 27.05, 31.6, 31.59 (C2a-C5a), 37.07 (C1a), 65.83 (C5), 69.06 (C2), 79.76 (C3), 87.97 (C4), 128.54 (*C*Me$_2$), 142.95 (C1).

2,3-*O*-Isopropylidene-4-*C*-octyl-D-ribono-1,4-lactone (84c). Treatment of **76c** (35 mg, 0.064 mmol) with TFA by Procedure F gave **84c** (16 mg, 75%): ^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, CH_3, C8a), 1.30-1.32 (m, 12H, C2a-C7a), 1.40 (s, 3H, Me), 1.50 (s, 3H, Me), 1.50-1.60 (m, 2H, C1a), 3.75 (d, *J* = 11.6 Hz, 1H, H5), 3.85 (d, *J* = 11.6 Hz, 1H, H5'), 4.65 (d, *J* = 5.6 Hz, 1H, H2), 4.95 (d, *J* = 5.6 Hz, 1H, H3); ^{13}C NMR ($CDCl_3$) δ 14.08 (CH_3, C8a), 22.63 & 23.46 (C*Me*$_2$), 25.81, 26.74, 29.12, 29.24, 30.04, 31.14 (C2a-

C7a), 31.82 (C1a), 65.89 (C5), 77.31 (C2), 79.74 (C3), 88.53 (C4), 112.07 (*C*Me$_2$), 174.57 (C1); HRMS (TOF) *m/z* calcd for $C_{16}H_{28}O_5Na^+$ $[M+Na]^+$ 323.1829, found: 323.1915.

2,3-*O*-Isopropylidene-4-*C*-vinyl-D-ribono-1,4-lactone (84d). Treatment of **76d** (30 mg, 0.065 mmol) with TFA by Procedure F (flash column chromatography; 80% hexane/EtOAc) gave somehow an unstable product **84d** (5 mg, 35%): ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 3H, Me), 1.26 (s, 3H, Me), 3.61 (d, *J* = 11.7 Hz, 1H, H5), 3.65 (d, *J* = 11.8 Hz, 1H, H5'), 4.60 (d, *J* = 5.6 Hz, 1H , H2), 4.82 (d, *J* = 5.6 Hz, 1H, H3), 5.24 (d, *J* = 11.3 Hz, 1H, CH=CH*H*), 5.34 (d, *J* =17.5 Hz, 1H, C=CH*H*), 5.71 (dd, *J* = 11.2, 17.5 Hz, C*H*=CHH); ^{13}C NMR ($CDCl_3$) δ 25.87, 26.70 (C*Me$_2$*), 66.37 (C5), 76.70 (C2), 80.01 (C3), 87.95 (C4), 113.29 (*C*Me$_2$), 118.21 (CH=*CH$_2$*), 130.53 (*CH*=CH$_2$), 174.06 (CO); HRMS (TOF) *m/z* calcd for $C_{10}H_{14}O_5Na^+$ $[M+Na]^+$ 237.0733, found 237.0733.

2,3-*O*-Isopropylidene-4-*C*-4-methoxyphenyl-D-ribono-1,4-lactone (84e). Treatment of **76e** (70 mg, 0.13 mmol) with TFA by Procedure F (flash column chromatography; 80% hexane/EtOAc) gave somehow an unstable product **84d** (20 mg, 60%): ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 3H, Me), 1.25 (s, 3H, Me), 3.80 (s, 3H, CH_3O), 3.85 (d, *J* = 12.3 Hz, 1H, H5), 3.95 (d, *J* = 12.5 Hz, 1H, H5'), 4.95 (d, *J* = 5.4 Hz, 1H, H2), 5.15 (d, *J* = 5.3 Hz, 1H, H3), 6.82 (d, *J* = 8.8 Hz, 2H, Ar), 7.12 (d, *J* = 9.0 Hz, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ 25.83 & 26.65 (C*Me$_2$*), 55.25 (CH_3O), 68.50 (C5), 77.41 (C2), 80.57 (C3), 90.12 (C4), 112.93 (*C*Me$_2$), 113.76, 126.17, 126.89, 159.33 (Ar); 174.86 (C1); HRMS (TOF) *m/z* calcd for $C_{15}H_{18}O_6Na^+$ $[M+Na]^+$ 317.0996, found 317.0982.

2,3-*O*-Isopropylidene-5-*O*-mesyl-4-*C*-methyl-D-ribono-1,4-lactone (85a). Treatment of **75a** (20 mg, 0.09 mmol) with MsCl by Procedure A (column

chromatography; hexane/EtOAc, 6:4) gave **76a** (17 mg, 63%): ^{1}H NMR ($CDCl_3$) δ-1.32 (s, 3H, C1a), 1.35 (s, 3H, Me), 1.42 (s, 3H, Me), 2.99 (s, 3H, Ms), 4.15 (d, *J* = 11.0 Hz, 1H, H5), 4.25 (d, *J* = 10.9 Hz, 1H, H5'), 4.60 (d, *J* = 5.7 Hz, 1H, H2), 4.85 (d, *J* = 5.7 Hz, 1H, H3).

2,3-*O*-Isopropylidene-4-*C*-hexyl-5,*O*-mesyl-D-ribono-1,4-lactone (85b). Treatment of **84b** (22 mg, 0.08 mmol) with MsCl by Procedure A (column chromatography; hexane/EtOAc, 7:3) gave **85b** (14 mg, 50%): ^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, 3H, C6a), 1.20-1.28 (m, 8H, C2a-C5a), 1.33 (s, 3H, Me), 1.41 (s, 3H, Me), 1.50-1.60 (m, 2H, C1a), 2.99 (s, 3H, Ms), 4.20 (d, *J* = 11.0 Hz, 1H, H5), 4.32 (d, *J* = 11.0 Hz, 1H, H5'), 4.60 (d, *J* = 5.7 Hz, 1H, H2), 4.88 (d, *J* = 5.7 Hz, 1H, H3); ^{13}C NMR ($CDCl_3$) δ 14.03 (CH_3, C6a), 22.46 & 23.37 (C*Me*$_2$), 25.78, 26.69, 29.55, 31.38 (C2a-C5a), 31.45 (C1a), 45.20 (Ms), 71.80 (C5), 77.91 (C3), 85.77 (C2), 113.79 (C4), 117.85 (*C*Me$_2$), 173.21 (C1).

2,3-*O*-Isopropylidene-5-*O*-mesyl-4-*C*-octyl-D-ribono-1,4-lactone (85c). Treatment of **84c** (32 mg, 0.1 mmol) with MsCl by Procedure A (column chromatography; hexane/EtOAc, 6:4) gave **85c** (14 mg, 50%): ^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, Me, C8a), 1.20-1.28 (m, 12H, C2a-C7a), 1.35 (s, 3H, Me), 1.41 (s, 3H, Me), 1.50-1.60 (m, 2H, C1a), 3.0 (s, 3H, Ms), 4.20 (d, *J* = 10.9 Hz, 1H, H5), 4.32 (d, *J* = 10.9 Hz, 1H, H5'), 4.60 (d, *J* = 5.6 Hz, 1H, H2), 4.84 (d, *J* = 5.6 Hz, 1H, H3); ^{13}C NMR ($CDCl_3$) δ 14.08 (CH_3, C8a), 22.63 & 23.46 (C*Me*$_2$), 25.81, 26.74, 29.12, 29.24, 30.04, 30.52 (C2a-C7a), 31.14 (C1a), 44.50 (Ms), 71.76 (C5), 77.31 (C3), 78.91 (C2), 113.76 (C4), 117.92 (*C*Me$_2$), 171.01 (C1).

2,3-*O*-Isopropylidene-5,*O*-mesyl-4-*C*-4-methoxyphenyl-D-ribono-1,4-lactone (85e). Treatment of **84e** (24 mg, 0.08 mmol) with MsCl by Procedure A (column chromatography; hexane/EtOAc, 6:4) gave **85e** (25 mg, 83%): ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H, Me), 1.41 (s, 3H, Me), 3.0 (s, 3H, Ms), 3.80 (s, 3H, CH_3O), 4.20 (d, *J* = 11.2 Hz, 1H, H5), 4.55 (d, *J* = 11.2 Hz, 1H, H5'), 5.01 (d, *J* = 5.5 Hz, 1H, H2), 5.15 (d, *J* = 5.5 Hz, 1H, H3), 6.82 (d, *J* = 8.8 Hz, 2H, Ar), 7.12 (d, *J* = 9.0 Hz, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ 25.90 & 26.58 (C*Me*$_2$), 44.10 (Ms), 55.33 (CH_3O), 73.26 (C5), 77.35 (C2), 79.71 (C3), 87.04 (C4), 113.79 (*C*Me_2), 114.10, 124.91, 127.00, 159.95 (Ar); 173.01 (C1).

2,3-*O*-Isopropylidene-4-*C*-hexyl-5-*O*-trityl-*D*-ribofuranose (86b). $LiEt_3BH$ (1M/THF, 0.18 mL, 0.18 mmol) was added dropwise to a solution of **84b** (37 mg, 0.07 mmol) in CH_2Cl_2 (0.5 mL). The mixture was stirred for 30 min at 0 ^oC (ice-bath) under N_2 atmosphere. The reaction was quenched with MeOH, the volatiles were then evaporated. The residue was partitioned between CH_2Cl_2 and N_aHCO_3, washed with brine and dried with anhydrous $MgSO_4$. The resulting oil was column chromatographed (75:15, hexane: ethyl acetate) to give a mixture of anomers α/β **86b** (α: β; 1:3; 20 mg, 54%): The major anomer had: ^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, 3H, C6a), 1.20-1.28 (m, 8H, C2a-C5a), 1.33 (s, 3H, Me), 1.41 (s, 3H, Me), 1.50-1.60 (m, 2H, C1a), 3.16 (d, *J* = 10.08 Hz, 1H, H5), 3.32 (d, *J* = 10.08 Hz, 1H, H5'), 3.74 (d, *J* = 8.8 Hz, OH), 4.50 (d, *J* = 6.0 Hz, 1H, H3), 4.75 (d, *J* = 6.0 Hz, 1H, H2), 5.15 (d, *J* = 8.4 Hz, 1H, H1). 7.25-7.38 (m, 15H, Ar); the minor anomer had: ^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, 3H, C6a), 1.20-1.28 (m, 8H, C2a-C5a), 1.33 (s, 3H, Me), 1.41 (s, 3H, Me), 1.50-1.60 (m, 2H, C1a), 2.94 (d, *J* = 9.9 Hz, 1H, H5), 3.25 (d, *J* = 9.9 Hz, 1H, H5'), 3.86 (d, *J* = 11.5 Hz, OH), 4.20 (d, *J* = 6.1 Hz, 1H, H3), 4.62 (dd, *J* = 4.2, 6.1 Hz, 1H, H2), 5.61 (dd, *J* = 4.2,

11.5 Hz, 1H, H1), 7.25-7.38 (m, 15H, Ar). α/*β*-anomers mixture had: ^{13}C NMR ($CDCl_3$) δ 14.01, 23.61 (C6a, α & β anomers), 23.94 (C6a), 24.57 & 24.98 (C*Me*$_2$), 25.99, 26.22, 29.65, 31.51 (C2a-C5a), 31.83 (C1a), 67.33 & 68.18 (C5), 80.01 & 82.78 (C2), 83.09 & 83.82 (C3), 88.05 & 88.12 (C4), 96.55 & 102.85(C1), 112.06 & 112.32 (*C*Me$_2$), 126.56, 127.30, 127.67, 142.09 (Ar), 139.0 & 143.1 (CO), 156.08 & 158.03 (CO), 174.81 (C1).

2,3-*O*-Isopropylidene-4-*C*-octyl-D-ribofuranose (86c). Treatment of **84c** (11 mg, 0.03 mmol) with $LiEt_3BH$, as described for **86b** gave a mixture of α/*β*-anomers **86c** (α:β; 1:1; 7 mg, 63%) ^{1}H NMR ($CDCl_3$) δ 0.80-0.82 (m, 2 x C8a), 1.20-1.28 (m, 24H, 6 x 2 x CH_2, C2a-C7a), 1.33 (s, 6H, 2 x Me), 1.41 (s, 6H, 2 x Me), 1.50-1.60 (m, 4H, 2 x C1a), 3.55 (d, *J* = 11.8 Hz, 2H, H5), 3.94 (d, *J* = 11.8 Hz, 2H, H5'), 3.96 (d, *J* = 7.9 Hz, 1H, H3, α-anomer), 4.10 (dd, *J* = 4.2, 7.9 Hz, 1H, H2, α-anomer), 4.28 (m, 1H, H2, β-anomer), 4.41 (d, *J* = 5.0 Hz, 1H, H3, β-anomer), 4.81 (s, 1H, H1, β-anomer), 5.09 (d, *J*= 4.1 Hz, 1H, H1, α-anomer).

***S*-(2,3-*O*-Isopropylidene-4-*C*-hexyl-D-ribono-1,4-lactone-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (89b). Procedure G.** LDA (2M/THF and heptanes, 40 μl, 0.08 mmol) was added slowly to a stirred solution of protected L-homocysteine **49** (27 mg, 0.093 mmol) (prepared by Procedure B, *step a*) in anhydrous DMF 1.5 ml under Ar atmosphere at 0 ^{o}C (ice bath). After 30 min, solution **85b** (11 mg, 0.031 mmol) in DMF(2 mL) was added by a syringe and the mixture was left stirring for 15 min at 0 ^{o}C then at room temperature for 24 hours. The TLC shows 5 different spots. The significant ones were the ones for disulfide (R_f = 0.55), the thiol (R_f = 0.65), the desired product at R_f = 0.50 and a byproduct at R_f = 0.30. The reaction was quenched with NH_4Cl and volatiles were evaporated on high vaccum. The residue was partitioned

between ethyl acetate and $NaHCO_3$, washed with brine and dried with anhydrous $MgSO_4$. The resulting oil was column chromatographed (8:2, hexane: ethyl acetate) to give **89b** (18 mg, 65%): ^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, 3H, C6a), 1.20-1.28 (m, 2 x 4 CH_2, C2a-C5a), 1.31 (s, 3H, Me), 1.39 &1.41 (2 x s, 18H), 1.42 (s, 3H, Me), 1.50-1.60 (m, 2H, C1a), 2.99 (s, 3H), 1.80 (m, 1H, H7), 1.90 (m, 1H, H7'), 2.50 (m, 2H, H6 and H6'), 2.72 (d, *J* = 14.68 Hz, 1H, H5), 2.80 (d, *J* = 14.72 Hz, 1H, H5'), 4.20-4.25 (m, 1H, C8), 4.40 (d, *J* = 5.92 Hz, 1H, H3), 5.00 (d, 1H, NH), 5.10 (d, *J* = 5.88 Hz, 1H, H2). ^{13}C NMR ($CDCl_3$) δ 14.01 (C6a), 22.5 & 22.7 (C*Me*$_2$), 23.45, 25.66, 26.56, 30.67 (C2a-C5a), 28.08 (*t*-Bu), 28.32 (*t*-Bu), 34.34 (C1a), 39.86 (C5), 77.0 (C2), 77.20 (C3), 80.59 (CO), 82.41 (CO), 88.98 (C4), 113.30 (*C*Me$_2$), 139.0 (CO), 156 (CO), 174 (C1). HRMS calcd for $C_{27}H_{48}NO_8S^+$ $[M+H]^+$ 546.3095, found 546.3104.

***S*-(2,3-*O*-Isopropylidene-4-*C*-octyl-D-ribono-1,4-lactone-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (89c).** Treatment of **85c** (24 mg, 0.063 mmol) with homocysteinate lithium salt as described by procedure G gave a mixture of Hcy and **89c** that couldn't be separated on column since they have the same R_f = 0.55 (5:1, 60 mg): ^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, Me, C8a), 1.20-1.25 (m, 12H, C2a-C7a), 1.21 (s, 3H, Me), 1.40 (s, 3H, Me), 1.39 &1.41 (2 x s, 90H), 1.50-1.60 (m, 2H, C1a), 1.85-1.89 (m, 6H, H7), 1.9-2.0 (m, 6H, H7'), 2.68-2.71 (m, 10H, H6 and H6'), 2.80-2.83 (m, H5, H5', 2H), 4.20-4.26 (m, C8, 9H), 4.40 (d, *J* = 5.92 Hz, 1H, H3), 5.10 (d, 1H, NH), 5.15 (d, *J* = 5.88 Hz, 1H, H2).

***S*-(2,3-*O*-Isopropylidene-4-*C*-4-methoxyphenyl-D-ribono-1,4-lactone-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (89e).** Treatment of **85e** (22 mg, 0.07 mmol) with homocysteinate lithium salt as described by procedure G gave a mixture of

Hcy and **89e** (20 mg, 48%): ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H, Me), 1.41 (s, 3H, Me), 1.39 &1.41 (2x s, 18H), 1.80 (m, 1H, H7), 1.90 (m, 1H, H7'), 2.50 (m, 2H, H6 and H6'), 2.85 (d, *J* = 14.8 Hz, 1H, H5), 3.20 (d, *J* = 15.1 Hz, 1H, H5'), 3.80 (s, 3H, CH_3O), 4.20-4.25 (m, 1H, C8), 4.80 (d, *J* = 5.8 Hz, 1H, H3), 5.01 (d, , *J* = 8.1 Hz 1H, NH), 5.30 (d, *J* = 5.8 Hz, 1H, H2), 6.82 (d, *J* = 8.8 Hz, 2H, Ar), 7.12 (d, *J* = 9.0 Hz, 2H, Ar); HRMS calcd for $C_{28}H_{41}NO_9SNa^+$ $[M+Na]^+$ 590.2394, found 590.2378.

***S*-(4-*C*-Hexyl-D-ribono-1,4-lactone-5-yl)-L-homocysteine (90b). Procedure H. Procedure H.** *Step a.* Compound **89b (**18 mg, 0.03 mmol) was stirred in TFA (2 mL) at 0 ^oC for 1 h then placed in ambient temperature for 3 h. *Step b.* H_2O (0.1 mL) was added to the reaction mixture from *Step a* and stirring was continued for 1 h at ambient temperature. Volatiles were evaporated and the residue was coevaporated with acetonitrile (0.1 mL) to give **90b** (7 mg, 60%): ^{1}H NMR ($CDCl_3$) δ 0.82 (t, *J* = 6.6 Hz, Me, C6a), 1.20-1.28 (m, 12H, C2a-C5a), 1.45-1.50 (m, 2H, C1a), 1.87-2.00 (m, 1H, H7), 2.05-2.12 (m, 1H, H7'), 2.45-2.55 (m, 2H, H6 & H6'), 2.82 (d, *J* = 13.6 Hz, 1H, H5), 2.87 (d, *J* = 13.6 Hz, 1H, H5'), 4.14 (d, *J* = 5.4 Hz, 1H, H2), 4.72 (d, *J* = 5.4 Hz, 1H, H3); ^{13}C NMR ($CDCl_3$) δ 15.01 (C6a), 23.20, 23.56, 23.90, 29.20 (C2a-C5a), 32.10 (C1a), 63.25 (C5), 72.55 (C2), 78.50 (C3), 88.50 (C4), 141.50 (C1). HRMS calcd for $C_{15}H_{27}NO_6S^+$ $[M+H]^+$ 350.163, found 350.182 and $[M+Na]^+$ 372.145, found 372.161.

***S*-(4-*C*-Octyl-D-ribono-1,4-lactone-5-yl)-L-homocysteine (90c).** Treatment of the crude **89c** contaminated by the protected homocysteine as described above for **89c** (1:5, 60 mg, 0.02 mmol) in TFA (2 mL) by Procedure H gave an oily residue that was partitioned between water and chloroform. The aqueous layer was evaporated. The residue (20 mg) was divided into two portions. Each portion was separately purified on

Sep-Pak column (classic C18) as follow. The portion of crude **90c** was dissolved in water/ACN (2.5 mL, 19:1, v/v) and was injected into the Sepak column. The column was eluted with 5 mL of water, a second portion of 5 mL of water and 5mL of ethanol. The first water eluent contained mainly Hcy (TLC and ^{1}H NMR) while the ethanol eluent was evaporated to give **90c** (5 mg, 76%): ^{1}H NMR ($CDCl_3$) δ 0.82 (t, *J* = 6.6 Hz, Me, C8a), 1.20-1.28 (m, 12H, C2a-C7a), 1.50-1.60 (m, 2H, C1a), 1.90-2.00 (m, 1H, H7), 2.05-2.12 (m, 1H, H7'), 2.55-2.65 (m, 2H, H6 & H6'), 2.80 (d, *J* = 13.8 Hz, 1H, H5), 2.87 (d, *J* = 13.9 Hz, 1H, H5'), 4.14 (d, *J* = 5.4 Hz, 1H, H2), 4.72 (d, *J* = 5.4 Hz, 1H, H3); ^{13}C NMR ($CDCl_3$) δ 15.01 (C8a), 23.00, 23.50, 23.85, 29.00, 30.67, 30.51 (C2a-C7a), 32.07 (C1a), 60.50 (C6), 61.20 (C7), 62.25 (C5), 71.54 (C2), 77.20 (C3), 84.59 (C4), 141.50 (C1). HRMS calcd for $C_{17}H_{31}NO_6S^+$ $[M+H]^+$ 378.1945, found 378.1964 and $[M+Na]^+$ 400.1764, found 400.1783.

***S*-(4-*C*-4-Methoxyphenyl-D-ribono-1,4-lactone-5-yl)-L-homocysteine (90e).** Treatment of **89e** (6.2 mg, 0.01 mmol) with in TFA (1 mL) as described by procedure H gave **90e** (3 mg, 75%): ^{1}H NMR ($CDCl_3$) δ ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H, CH_3O), 1.80 (m, 1H, H7), 1.90 (m, 1H, H7'), 2.50 (m, 2H, H6 and H6'), 2.85 (d, *J* = 14.8 Hz, 1H, H5), 3.20 (d, *J* = 15.1 Hz, 1H, H5'), 4.20 (d, *J* = 5.8 Hz, 1H, H2), 4.20-4.25 (m, 1H, C8), 4.70 (d, *J* = 5.8 Hz, 1H, H3), 6.82 (d, *J* = 8.8 Hz, 2H, Ar), 7.12 (d, *J* = 9.0 Hz, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ 15.01 (C8a), 55.33 (CH_3O), 63.20 (C5), 74.40 (C2), 78.20 (C3), 85.40 (C4), 117.20, 125.81, 127.00, 162.28 (Ar), 143.50 (C1); HRMS calcd for $C_{16}H_{21}NO_7S^+$ $[M+Na]^+$ 394.0931, found 393.2894.

2,3-*O*-Isopropylidene-5-[(*tert*-butoxycarbonyl)-L-homocysteine *tert*-butyl ester]-4-*C*-hexyl-D-ribofuranose (91b). Procedure I. $LiEt_3BH$ (1M/THF, 0.05 mL, 0.05

mmol) was added dropwise to a solution of **89b** (5 mg, 0.009 mmol) in CH_2Cl_2 (0.5 mL). The mixture was stirred for 30 min at 0 ^oC (ice-bath) under N_2 atmosphere. The reaction was quenched with MeOH, the volatiles were then evaporated. The residue was partitioned between CH_2Cl_2 and N_aHCO_3, washed with brine and dried with anhydrous $MgSO_4$. The resulting oil was column chromatographed (80:20, hexane: ethyl acetate) to give a **91b** mixture of anomers α/β (β major product, 4.5 mg, 90%): ^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, Me, C8a), 1.2-1.28 (m, 8H, C2a-C5a), 1.35-1.41 (2 x s, 18H), 1.35 (s, 6H), 1.50-1.60 (m, 2H, C1a), 1.9-2.0 (m, 2H, H7, H7'), 2.48-2.58 (m, 2H, H6, H6'), 2.60 (d, *J* = 12.8 Hz, 1H, H5), 2.94 (d, *J* = 12.6 Hz, 1H, H5'), 4.19-4.21 (m, 1H, H8), 4.44 (d, *J* = 5.9 Hz, 1H, H3), 4.64 (d, *J* = 5.9 Hz, 1H, H2), 5.19-5.21 (m, 1H, NH), 5.35 (s, 1H, β-H1).

2,3-*O*-Isopropylidene-5-[(*tert*-butoxycarbonyl)-L-homocysteine *tert*-butyl ester]-4-*C*-4-methoxyphenyl-D-ribofuranose (91e). Treatment of **89e** (6 mg, 0.01 mmol) with $LiEt_3BH$ (25 μL) following procedure I gave **92c** (4 mg, 66%): ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H, Me), 1.41 (s, 3H, Me), 1.39 &1.41 (2x s, 18H), 1.80 (m, 1H, H7), 1.90 (m, 1H, H7'), 2.50 (m, 2H, H6 and H6'), 3.01 (d, *J* = 14.8 Hz, 1H, H5), 3.20 (d, *J* = 15.1 Hz, 1H, H5'), 3.60 (d, *J* = 5.8 Hz, 1H, H3), 3.80 (s, 3H, CH_3O), 4.01 ("t", *J* = 5.8 Hz, 1H, H2), 4.20-4.25 (m, 1H, C8), 5.01 (d, , *J* = 8.1 Hz 1H, NH), 5.60 (s, H1, 1H, major isomer 99%), 5.80 (d, 1H, minor isomer), 6.82 (d, *J* = 8.8 Hz, 2H, Ar), 7.12 (d, *J* = 9.0 Hz, 2H, Ar); HRMS calcd for $C_{28}H_{43}NO_9S^+$ $[M+Na]^+$ 592.2551, found 592.2492.

***S*-(5-Deoxy-4-*C*-hexyl-D-ribofuranos-5-yl)-L-homocysteine (92b).** Treatment of **91b** (4.5 mg, 0.01 mmol) with in TFA (1 mL) as described by procedure H gave **92b** (2 mg, 75%):^{1}H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, Me, C6a), 1.2-1.28 (m, 8H, C2a-C5a),

1.50-1.60 (m, 2H, C1a), 1.9-2.0 (m, 2H, H7, H7'), 2.48-2.58 (m, 2H, H6, H6'), 2.60 (d, *J* = 12.8 Hz, 1H, H5), 2.94 (d, *J* = 12.6 Hz, 1H, H5'), 4.19-4.21 (m, 1H, H8), 4.44 (d, *J* = 5.9 Hz, 1H, H3), 4.64 ("t", *J* = 5.9 Hz, 1H, H2), 5.19-5.21 (m, 1H, NH), 5.35 (s, 1H, α or β-H1); MS (ESI^-) *m/z* 350 (MH^-).

***S*-(5-Deoxy-4-*C*-octyl-D-ribofuranos-5-yl)-L-homocysteine (92c).** Treatment of **90c** (6 mg, 0.01 mmol) with $LiEt_3BH$ (0.03 mL) following procedure I gave **92c** (4 mg, 60%): ^1H NMR ($CDCl_3$) δ 0.80 (t, *J*= 6.6 Hz, Me, C8a), 1.2-1.32 (m, 14H, C2a-C7a), 1.50-1.60 (m, 2H, C1a), 1.9-2.0 (m, 2H, H7, H7'), 2.48-2.58 (m, 2H, H6, H6'), 2.79 (d, *J* = 12.8 Hz, 1H, H5), 2.90 (d, *J* = 12.6 Hz, 1H, H5'), 4.19-4.21 (m, 1H, H8), 3.80 (d, *J* = 5.9 Hz, 1H, H3), 4.50 ("t", *J* = 5.9 Hz, 1H, H2), 5.19-5.21 (m, 1H, NH), 5.80 (s, 0.5H, β-H1); 5.82 (s, 0.5H, α-H); ^{13}C NMR δ 15.01 (C8a), 23.00, 23.50, 23.85, 29.00, 30.67, 30.51 (C2a-C7a), 32.07 (C1a), 40.50 (C6), 41.50 (C7), 41.99 (C5), 49.51 (C8), 62.77 (C3), 72.09 (C2), 87.16 (C4), 99.90 (C1), 174.50 (CO); HRMS calcd for $C_{17}H_{33}NO_6S^+$ $[M+Na]^+$ 396.2283, found 396.1862.

***S*-(5-Deoxy-4-*C*-4-methoxyphenyl-D-ribofuranos-5-yl)-L-homocysteine (92e).** Treatment of **91e** (4 mg, 0.01 mmol) with in TFA (1 mL) as described by procedure H gave **92e** (2 mg, 75%): ^1H NMR ($CDCl_3$) δ 1.80 (m, 1H, H7), 1.90 (m, 1H, H7'), 2.50 (m, 2H, H6 and H6'), 3.01 (d, *J* = 14.8 Hz, 1H, H5), 3.20 (d, *J* = 15.1 Hz, 1H, H5'), 3.60 (d, *J* = 5.8 Hz, 1H, H3), 3.80 (s, 3H, CH_3O), 4.01 ("t", *J* = 5.8 Hz, 1H, H2), 4.20-4.25 (m, 1H, C8), 5.60 (s, H1, 1H, major isomer 99%), 6.82 (d, *J* = 8.8 Hz, 2H, Ar), 7.12 (d, *J* = 9.0 Hz, 2H, Ar); ^{13}C NMR ($CDCl_3$) δ 15.01 (C8a), 55.33 (CH_3O), 63.20 (C5), 64.40 (C2), 65.20 (C3), 85.40 (C4), 117.20, 125.81, 127.00, 162.28 (Ar), 173.50 (C1).

5-*O*-Acetyl-1-deoxy-2,3-*O*-isopropylidene-4-thio-D-ribofuranose (94). DMAP (7 mg, 0.057 mmol) was added to a stirred solution of **93**[139] (117 mg, 0.62 mmol) in Ac_2O (6 mL) at ambient temperature. After 2.5 h, MeOH (15 mL) was added and resulting mixture was stirred for additional 1 h at 0° C. The volatiles were evaporated to give the crude **94** (136 mg, 95%), of sufficient purity to be directly used in next step: ^{1}H NMR δ 1.34 (s, 3H, Me), 1.54 (s, 3H, Me), 2.10 (s, 3H, Ac), 2.94 (dd, *J* = 1.2, 13.0 Hz, 1H, H1), 3.12 (dd, *J* = 4.7, 13.0 Hz, 1H, H1') 3.49 (ddd, *J* = 1.0, 5.9, 7.6 Hz, 1H, H4), 4.05 (dd, *J* = 8.4, 11.4 Hz, 1H, H5), 4.18 (dd, *J* = 5.8, 11.4 Hz, 1H, H5'), 4.70 (dd, *J* = 1.2, 5.6 Hz, 1H, H3), 4.94 ('dt', *J* = 1.3, 5.2 Hz, 1H, H2); ^{13}C NMR δ 20.92 (Ac), 24.66 (C*Me*$_2$), 26.46 (C*Me*$_2$), 37.70 (C1), 52.33 (C4), 64.82 (C5), 83.55 (C2), 85.72 (C3), 111.26 (*C*Me$_2$), 170.70 (Ac).

5-*O*-Acetyl-1-deoxy-2,3-*O*-isopropylidene-*S*-oxo-4-thio-D-ribofuranose [95(*R/S*)$_S$]. A solution of MCPBA (138 mg, 0.56 mmol, ~70% reagent) in CH_2Cl_2 (3 mL) was added dropwise to a solution of **94**[139] (136 mg, 0.59 mmol) in CH_2Cl_2 (2 mL) at -78 °C, under Ar atmosphere. The resulting suspension was stirred for 30 min or until disappearance of **94** on TLC (14 h, -78 °C → rt). The reaction mixture was quenched with saturated $NaHCO_3/H_2O$, and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The crude product was column chromatographed (50% EtOAc/hexane) to give unchanged **94** (7 mg, 5%) followed by (EtOAc → 10% MeOH/EtOAc) **95** (133 mg, 91%) as a separable mixture (4:1) of two diastereoisomers. Major isomer had: ^{1}H NMR δ 1.33 (s, 3H, Me), 1.49 (s, 3H, Me), 2.10 (s, 3H, Ac), 3.23 (dd, *J* = 4.1, 14.4 Hz, 1H, H1), 3.34 (dd, *J* = 6.4, 14.4 Hz, 1H, H1') 3.36 ('dt', *J* = 5.6, 8.9 Hz, 1H, H4), 4.49 (dd, *J* = 8.8, 12.0 Hz, 1H, H5), 4.61 (dd, *J* = 4.9, 12.0

Hz, 1H, H5'), 4.94 (t, *J* = 6.1 Hz, 1H, H3), 5.22 (dt, *J* = 4.2, 6.3 Hz, 1H, H2); ^{13}C NMR δ 20.80 (Ac), 24.75 (C*Me*$_2$), 27.29 (C*Me*$_2$), 56.13 (C1), 58.19 (C5), 63.93 (C4), 79.86 (C2), 82.75 (C3), 113.47 (*C*Me$_2$), 170.29 (Ac). Minor isomer had: ^{1}H NMR δ 1.37 (s, 3H, Me), 1.61 (s, 3H, Me), 2.09 (s, 3H, Ac), 3.22 (dd, *J* = 6.0, 14.8 Hz, 1H, H1), 3.42 ('td', *J* = 1.8, 14.8 Hz, 1H, H1') 3.75-3.79 (m, 1H, H4), 4.35 (dd, *J* = 5.3, 12.2 Hz, 1H, H5), 4.39 (dd, *J* = 4.5, 12.2 Hz, 1H, H5'), 4.86 (dd, *J* = 2.7, 5.9 Hz, 1H, H3), 5.14 (dt, *J* = 2.2, 6.0 Hz, 1H, H2); ^{13}C NMR δ 20.69 (Ac), 24.48 (C*Me*$_2$), 26.82 (C*Me*$_2$), 57.25 (C1), 60.93 (C5), 71.40 (C4), 82.87 (C2), 84.45 (C3), 112.91 (*C*Me$_2$), 170.00 (Ac); MS (ESI) *m/z* 249 (100, $[MH]^+$).

5-*O*-Acetyl-1-deoxy-1-fluoro-2,3-*O*-isopropylidene-4-thio-α/β-D-ribofuranose (96), and **5-*O*-Acetyl-2,3-*O*-isopropylidene-4-thio-β-D-ribofuranose (99)**. **Procedure J**. Deoxo-fluor (125 mg, 0.25 mL of 50% solution in THF, 0.57 mmol) was added to a stirred solution of sulfoxide **95** (15.5 mg, 0.11 mmol) in CH_2Cl_2 (0.5 mL) containing $SbCl_3$ (30 mg, 0.13 mmol) under Ar atmosphere at ambient temperature. Reaction mixture was heated at 55 ºC for 3 h, and was quenched with saturated $NaHCO_3/H_2O$. The resulting mixture was extracted with CH_2Cl_2 and the combined organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The crude product was column chromatographed (20 → 25% EtOAc/hexane) to give **96** (9 mg, 58%) as colorless oil of major β anomer. ^{1}H NMR δ 1.33 (s, 3H, Me), 1.48 (s, 3H, Me), 2.10 (s, 3H, Ac), 3.67 (ddd, *J* = 2.5, 5.3, 10.1 Hz, 1H, H4), 4.06 ('t', *J* = 10.8 Hz, 1H, H5), 4.27 (dd, *J* = 5.3, 11.6 Hz, 1H, H5'), 4.94 (d, *J* = 5.4 Hz, 1H, H3), 5.03 (dd, *J* = 5.4, 9.5 Hz, 1H, H2), 5.97 (d, *J* = 54.0 Hz, 1H, H1); ^{13}C NMR δ 20.81 (Ac), 24.57 (C*Me*$_2$), 26.33 (C*Me*$_2$), 54.86 (d,

J = 1.8 Hz, C4), 65.82 (C5), 84.81 (C3), 89.15 (d, *J* = 38.6 Hz, C2), 106.60 (d, *J* = 218.3 Hz, C1), 111.33 (*C*Me$_2$), 170.47 (Ac); ^{19}F NMR δ -140.85 (ddd, *J* = 2.3, 9.5, 54.0 Hz).

A further elution (25% EtOAc/hexane) afforded an analytical sample of the minor α anomer of **96** (1.1 mg, 7%): ^{1}H NMR δ 1.38 (s, 3, Me), 1.56 (s, 3, Me), 2.10 (s, 3, Ac), 3.98-4.03 (m, 1H, H4), 4.19 (dd, *J* = 6.3, 11.6 Hz, 1H, H5), 4.34 (dd, *J* = 6.2, 11.5 Hz, 1H, H5'), 4.67 (dd, *J* = 3.5, 6.7 Hz, 1H, H3), 4.83 (ddd, *J* = 4.4, 6.7, 15.2 Hz, 1H, H2), 5.81 (dd, *J* = 4.4, 56.7 Hz, 1H, H1); ^{13}C NMR δ 20.72 (Ac), 25.82 (d, *J* = 1.4 Hz, C*Me*$_2$), 25.94 (d, *J* = 2.3 Hz, C*Me*$_2$), 48.96 (C4), 64.42 (d, *J* = 2.1 Hz, C5), 83.21 (C3), 85.06 (d, *J* = 19.8 Hz, C2), 98.02 (d, *J* = 231.5 Hz, C1), 115.27 (*C*Me$_2$), 170.49 (Ac); ^{19}F NMR δ -157.24 (dd, *J* = 15.2, 56.7 Hz); MS (ESI) *m/z* 250 (100, M$^+$).

Additional elution (40% EtOAc/hexane) resulted in the separation of the thiohemiacetal **99** (β–anomer; 2.6 mg, 17%). ^{1}H NMR δ 1.34 (s, 3H, Me), 1.52 (s, 3H, Me), 2.13 (s, 3H, Ac), 2.50 (br. s, OH), 3.63-3.68 (m, 1H, H4), 4.17 (dd, *J* = 8.9, 11.4 Hz, 1H, H5), 4.41 (dd, *J* = 5.8, 11.4 Hz, 1H, H5'), 4.87 (d, *J* = 5.4 Hz, 1H, H3), 4.92 (d, *J* = 5.4 Hz, 1H, H2), 5.37 (br. s, 1H, H1); ^{13}C NMR δ 20.90 (Ac), 24.56 (C*Me*$_2$), 26.44 (C*Me*$_2$), 54.83 (C4), 66.53 (C5), 85.78 (C3), 86.98 (C1), 90.47 (C2), 110.82 (*C*Me$_2$), 170.57 (Ac).

Procedure K: Sulfide **94** (27.5 mg, 0.12 mmol) was treated with Deoxo-fluor (127 mg, 0.26 mL, 50% soln in THF, 0.59 mmol) and $SbCl_3$ (30 mg, 0.13 mmol) as described above (Procedure J, 4 h). The crude product (spectrally identical to the crude sample obtained by Procedure J) was column chromatographed (20 → 25% EtOAc/hexane) to give major β anomer of **96** (14.5 mg, 49%) as colorless oil.

1-Deoxy-1-fluoro-2,3-*O*-isopropylidene-4-thio-β-D-ribofuranose (97). Compound **96** (β–anomer; 45 mg, 0.18 mmol) was dissolved in saturated methanolic ammonia solution (4 mL) and the resulting mixture was stirred for 15 h at ambient temperature. The volatiles were evaporated to give crude product **97** (37 mg, 99%) as a light yellow oil of sufficient purity to be used for next step. ^{1}H NMR δ 1.32 (s, 3H, Me), 1.49 (s, 3H, Me), 2.02 (s, 1H, OH), 3.64-3.79 (m, 3H, H4, H5, H5'), 4.96 (d, *J* = 5.4 Hz, 1H, H3), 5.01 (dd, *J* = 5.4, 10.2 Hz, 1H, H2), 5.99 (d, *J* = 55.7 Hz, 1H, H1); ^{13}C NMR δ 24.43 (C*Me*$_2$), 26.37 (C*Me*$_2$), 59.38 (d, *J* = 1.9 Hz, C4), 64.97 (d, *J* = 1.4 Hz, C5), 85.22 (C3), 89.64 (d, *J* = 36.9 Hz, C2), 107.16 (d, *J* = 216.8 Hz, C1), 110.99 (*C*Me$_2$); ^{19}F NMR δ -137.16 (dd, *J* = 10.3, 55.8 Hz).

1-Deoxy-1-fluoro-2,3-*O*-isopropylidene-5-*O*-methanesulfonyl-4-thio-β-D-ribofuranose (98). Compound **97** (37 mg, 0.18 mmol) was treated with MsCl (0.16 mL, 237 mg, 3.31 mmol) in the presence of Et_3N (0.6 mL, 437 mg, 4.33 mmol) by Procedure A (18 h, 0 ºC → rt). The crude product (104 mg) was column chromatographed (20% → 40 % EtOAc/hexane) to give **98** (31 mg, 61%): ^{1}H NMR δ 1.33 (s, 3H, Me), 1.48 (s, 3H, Me), 3.07 (s, 3H, Ms), 3.76 (ddd, *J* = 2.2, 5.3, 10.2 Hz, 1H, H4), 4.17 ('t', *J* = 10.5 Hz, 1H, H5), 4.34 (dd, *J* = 5.3, 10.7 Hz, 1H, H5'), 5.01-5.03 (m, 1H, H3), 5.03-5.07 (m, 1H, H2), 5.99 (d, *J* = 53.6 Hz, 1H, H1); ^{13}C NMR δ 24.49 (C*Me*$_2$), 26.24 (C*Me*$_2$), 37.73 (Ms), 54.80 (C4), 69.85 (C5), 84.16 (C3), 88.84 (d, *J* = 38.6 Hz, C2), 106.58 (d, *J* = 218.0 Hz, C1), 111.58 (*C*Me$_2$); ^{19}F NMR δ -140.85 (ddd, *J* = 1.9, 8.7, 53.6 Hz).

***S*-(1,5-Dideoxy-1-fluoro-2,3-*O*-isopropylidene-4-thio-β-D-ribofuranos-5-yl)-*N*-(*tert*-butoxycarbonyl)-L-homocysteine *tert*-butyl ester (100)**, and ***S*-(5-Deoxy-2,3-*O*-**

isopropylidene-4-thio-β-D-ribofuranos-5-yl)-*N*-(*tert*-butoxycarbonyl)-L-homocysteine *tert*-butyl ester (101). Compound **98** (31 mg, 0.11 mmol) was treated with lithium homocysteinate (91 mg, 0.31 mmol) by Procedure G (3h). The crude product was column chromatographed (15 → 20% EtOAc/hexane) to give **100** (13 mg, 25%) as a colorless oil: ^{1}H NMR δ 1.34 (s, Me, 3H), 1.47 (s, 9H), 1.49 (s, 12H), 1.85-1.95 (m, 1H, H8), 2.04-2.16 (m, 1H, H8'), 2.60-2.66 (m, 2H, H7, H7'), 2.71 (dd, *J* = 11.1, 13.9 Hz, 1H, H5), 2.87 (dd, *J* = 5.7, 13.9 Hz, 1H, H5'), 3.55 (ddd, *J* = 2.3, 5.4, 10.9 Hz, 1H, H4), 4.26-4-34 (m, 1H, H9), 5.04 (d, *J* = 0.8 Hz, 1H, H3), 5.06 (d, 1, *J* = 2.9 Hz, 1H, H2), 5.11 (br. d, *J* = 6.9 Hz, 1H, NH), 6.00 (d, *J* = 53.7 Hz, 1H, H1); ^{13}C NMR δ 24.59 (C*Me*$_2$), 26.31 (C*Me*$_2$), 28.02 (t-Bu), 28.33 (C7, t-Bu), 33.11 (C8), 38.14 (C5), 53.32 (C9), 56.43 (d, *J* = 1.8 Hz, C4), 79.87 (t-Bu), 82.31 (t-Bu), 85.98 (C3), 88.86 (d, *J* = 39.2 Hz, C2), 107.09 (d, *J* = 216.9 Hz, C1), 111.21 (*C*Me$_2$), 155.35 (CO), 171.20 (C10); ^{19}F NMR δ -140.87 (dd, *J* = 3.8, 53.8 Hz); MS (ESI) *m/z* 462 (100, [M-19]$^{+}$). HRMS (AP-ESI) *m/z* calcd for $C_{21}H_{36}FNNaO_6S_2$ [M+Na]$^{+}$ 504.1860; found 504.1847.

Further elution (25 → 30% EtOAc/hexane) afforded **101** (14 mg, 27%) as a colorless oil of single β anomer: ^{1}H NMR δ 1.33 (s, 3H), 1.47 (s, 9H), 1.49 (s, 9H), 1.51 (s, 3H), 1.89-2.00 (m, 1H, H8), 2.07 (tdd, *J* = 5.8, 9.6, 13.7 Hz, 1H, H8'), 2.59-2.67 (m, 1H, H7), 2.68-2.77 (m, 1H, H7'), 2.85-2.94 (br., OH), 2.89 (dd, *J* = 8.7, 13.5 Hz, 1H, H5), 2.97 (dd, *J* = 6.8, 13.6 Hz, 1H, H5'), 3.56 (t, *J* = 7.9 Hz, 1H, H4), 4.27-4-35 (m, 1H, H9), 4.87 (d, *J* = 5.4 Hz, 1H, H2), 4.95 (d, 1, *J* = 5.4 Hz, 1H, H3), 5.18 (br. d, *J* = 6.9 Hz, 1H, NH), 5.41 (s, 1H, H1); ^{13}C NMR δ 24.61 (C*Me*$_2$), 26.48 (C*Me*$_2$), 28.03 (t-Bu), 28.26 (C7), 28.34 (t-Bu) 33.05 (C8), 38.34 (C5), 53.31 (C9), 56.92 (C4), 80.01 (t-Bu), 82.39 (t-Bu), 87.37 (C1), 87.65 (C3), 90.22 (C2), 110.67 (*C*Me$_2$), 155.39 (CO), 171.21 (C10); MS

(ESI) *m/z* 462 (100, $[M-17]^+$) 480 (30, $[MH]^+$); HRMS (ESI) *m/z* calcd for $C_{21}H_{37}NNaO_7S_2$ $[M+Na]^+$ 502.1909; found 502.1884.

***S*-(5-Deoxy-4-thio-D-ribofuranos-5-yl)-L-homocysteine (39)**. *Method A*. Compound **100** (13 mg, 0.027 mmol) was treated with aqueous TFA by Procedure H (4 h) to give colorless oil of **39** (7 mg, 91%) as a mixture of anomers whose ratio changed when stored as D_2O solution (1:1 → 1:4, α:β). Major β-isomer had: ^{1}H NMR (D_2O) δ 2.25-2.45 (m, 2H, H8, H8'), 2.78 (dd, *J* = 8.7, 13.4 Hz, 1H, H5), 2.87 (t, *J* = 7.3 Hz, 2H, H7, H7'), 3.20 (dd, *J* = 4.6, 13.4 Hz, 1H, H5'), 3.54 (ddd, *J* = 4.6, 7.8, 9.0 Hz, 1H, H4), 4.16-4.21 (m, 3H, H2, H3, H9), 5.16 (d, *J* = 1.6 Hz, 1H, H1); ^{13}C NMR (D_2O) δ 29.20 (C8), 32.32 (C7), 35.68 (C5), 48.81 (C4), 51.69 (C9), 76.75, 79.64 (C2, C3), 81.08 (C1), 171.75 (C10). Minor α-isomer had: ^{1}H NMR (D_2O) δ 2.25-2.45 (m, 2, H8, H8'), 2.67 (dd, *J* = 8.7, 13.5 Hz, 1H, H5), 2.87 (t, *J* = 7.3 Hz, 2H, H7, H7'), 3.03 (dd, *J* = 5.5, 13.4 Hz, 1H, H5'), 3.77 (dt, *J* = 5.6, 8.7, Hz, 1H, H4), 4.10 (dd, *J* = 3.7, 5.7 Hz, 1H, H3), 4.16-4.21 (m, 1H, H9), 4.24 (t, *J* = 4.0 Hz, 1H, H2), 5.45 (d, *J* = 4.3 Hz, 1H, H1); MS (ESI) *m/z* 284 (100, $[MH]^+$). HRMS (AP-ESI) *m/z* calculated for $C_9H_{16}NO_5S_2$ $[M-H]^-$ 282.0475; found 282.0484.

Method B. Compound **101** (6 mg, 0.012 mmol) was treated with aqueous TFA by Procedure H (4 h) to give **39** (3 mg, 85%) as a colorless oil with spectral data identical to the sample of **39** obtained from *Method A*.

4.3. Biological Assays

4.3.1 Anti-QS assay for 4-[thia]-SRH

4.4.1.1. β-galactosidase assay

An overnight culture of *E. coli* DH5α harboring the plasmids pSC11, which contains a P_{lasI}-*lacZ* translational fusion[148] and pJN105L, which contains a P_{BAD}-*lasR* expression plasmid[149] grown in LB media (10 g tryptone, 5 g yeast extract, 5 g sodium chloride per liter) supplemented with ampillicin (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*[150] 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[151], were essentially same except that the LB medium was only supplemented with ampillicin (100 μg/ ml), the overnight culture was diluted to an OD600 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.[152] 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.

4.3.1.2. 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$^-$, AI-2$^+$), 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[153] Growth was measured in a Spectronic 20 as OD_{660nm} 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 OD_{660nm} of ~0.03, When the culture was rapidly growing (OD_{660nm} ~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%.

4.3.2. Anti-QS assay for 4-*C*-alkyl/aryl-SRH

The 4-*C*-alkyl/aryl-[SRH] analogues and the 4-[thia]-SRH analogues synthesized in this dissertation will be evaluated for the inhibition of LuxS enzyme in the laboratoty of Professor Dehua Pei at the University of Ohio.

5. CONCLUSION

I have developed different strategies for the synthesis of the three novel classes of *S*-ribosylhomocysteine (SRH) analogues which were designed as potential inhibitors of the S-ribosylhomocyteinase enzyme (LuxS). The first class I attempted to prepare was 2-halo-SRH analogues. The 2-deoxy-2-fluoro pentafuranosyl precursors were prepared from the D-allose in a multistep procedure, which involved dehomologation and intramolecular rearrangement of the 3-deoxy-3-fluoroglucose to 2-deoxy-2-fluoroarabinose. The 2-bromo-2-deoxy pentafuranosyl precursors were prepared by direct bromination of the suitably protected 2-deoxyribono-1,4-lactone with *N*-bromosuccinimide in the presence of trimethylsilyl triflate resulting in the formation of 2-bromo-2-deoxyribono-1,4-lactones as a mixture of *arabino/ribo* epimers in 2:1 ratio. The 5-hydroxyl group in 2-bromo-2-deoxyarabinono-1,4-lactones was activated by the conversion to the methylsulfonate ester. However attempts of the displacement of the mesylate group with the suitably protected homocysteinate anion led to the substitution of the secondary bromine at the α-position to the carbonyl group in ribonolactones rather than the replacement of the primary mesylate group. Model reaction with propanethiol confirmed that the displacement of the bromide from C2 takes precedence over the substitution of the mesylate from the C5 leading to the formation 2-*S*-propyl substituted 2-thioribonolactone.

The second class was the 4-C-alkyl/aryl-S-ribosylhomocysteine analogues synthesis of which was accomplished from D-ribose. The preparation of the ribono-1,4-lactone analogues of 4-C-substituted SRH involved the following critical steps: (i) conversion of the D-ribose to the ribitol-4-ulose; (ii) diastereoselective addition of various alkyl or aryl

or vinyl Grignard reagents to 4-ketone intermediate; (iii) oxidation of the primary hydroxyl group at C1 followed by the intramolecular ring closure to the corresponding 4-C-alkyl/aryl-substituted ribono-1,4-lactones; (iv) displacement of the activated 5-hydroxyl group with the protected homocysteinate. Treatment of these 4-C-alkyl/aryl-substituted SRH analogues with lithium triethylborohydride effected reduction of the ribonolactone to the ribose (hemiacetal) and subsequent global deprotection with trifluoroacetic acid provided 4-C-alkyl/aryl-SRHs.

The third class was the 4-[thia]-SRH analogues synthesis which started from the 1-deoxy-4-thioribose which was in turns prepared from D-ribose. Treatment of the 1-deoxy-4-thioribose thioether or the corresponding sulfoxides with DAST/$SbCl_3$ combination resulted in the fluoro-Pummerer rearrangement to give the corresponding thioribosyl fluorides in high yields. These α-fluoro thioethers were sensitive to acidic conditions and underwent hydrolysis to the corresponding thiohemiacetals. However, the α-fluoro thioethers (thioribosyl fluorides) were relatively stable to basic conditions, in parallel with acetals. Thus, ribosyl fluorides were mesylated at primary 5-hydroxyl and subsequent coupling with the protected homocysteinate anion afforded the relatively stable 4-[thia]-SRH fluoride. Treatment of the latter with aqueous TFA effected hydrolysis of the fluoride as well as the global removal of the *N*-Boc, acetonide, and *t*-butyl ester protection groups in a single step to give 4-[thia]-SRH derivative as a mixture of α:β anomers.

The 4-[thia]-SRH analogues were evaluated for their effect on *P. aeruginosa* QS. All the tested 4-[thia] derivatives showed a general concentration-dependent (50-200 μG/mL) inhibition of *las* QS pathways, while none of them affected *rhl* gene. The most

active was 1-deoxy-4-[thia]-SRH analogue with the sufur atom in the ring oxidized to the sulfoxide decreasing *las* gene activity to approximately 35%. In addition 4-[thia]-SRH analogues were tested for their effect on QS, bioluminescence and growth of the *V. harveyi*. They did not display any significant effects. The interaction of the prepared SRH analogues with LuxS and their effect in other QS sytems are currently being evaluated in the collaboration with other research groups.

Future research direction will depend on the results generated from the interaction of the synthesized analogues of SRH with LuxS enzyme. It might, however, involve the synthesis of different thio-DPD and aza-DPD analogues and study their borate complexation to help us to understand better the mechanism of action and the binding properties. The bulky 4-C-alkyl/aryl SRH should be used to study dimer interface of LuxS and the disruption of the dimerization processes. The use of the fluorescence proximity assay, which is a technique used to test the inhibition of a dimeric protein in presence of an inhibitor by measuring the change in fluorescence upon monomer separation, can be used in future studies.

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