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Inhibition of LuxS by *S*-ribosylhomocysteine analogues containing a [4-aza]ribose ring

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ABSTRACT

LuxS (*S*-ribosylhomocysteinase) catalyzes the cleavage of the thioether linkage of *S*-ribosylhomocysteine (SRH) to produce homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD), the precursor to a small signaling molecule that mediates interspecies bacterial communication called autoinducer 2 (AI-2). Inhibitors of LuxS should interfere with bacterial interspecies communication and potentially provide a novel class of antibacterial agents. In this work, SRH analogues containing substitution of a nitrogen atom for the endocyclic oxygen as well as various deoxyriboses were synthesized and evaluated for LuxS inhibition. Two of the [4-aza]SRH analogues showed modest competitive inhibition (K_I ~40 μM), while most of the others were inactive. One compound that contains a hemiaminal moiety exhibited time-dependent inhibition, consistent with enzyme-catalyzed ring opening and conversion into a more potent species (K_I^* = 3.5 μM). The structure–activity relationship of the designed inhibitors highlights the importance of both the homocysteine and ribose moieties for high-affinity binding to LuxS active site.

1. Introduction

Quorum sensing (QS) is a type of bacterial cell-to-cell communication mediated through the production, release and detection of the small signaling molecules called autoinducers (AIs).[1–3] Such communication allows bacterial control of crucial functions in united communities for enhancement of symbiosis, virulence, antibiotic production, biofilm formation, and many other processes.[4,5] Hence, there have been great interests in the synthesis of small molecules that can modulate QS pathways.[6–8] *S*-Ribosylhomocysteinase (LuxS) is a key enzyme in the biosynthetic pathway of type II autoinducer, which mediates the interspecies quorum sensing among both Gram-positive and Gram-negative bacteria.

The biosynthesis of AI-2 starts with the dual substrate-specific microbial enzyme 5′-methylthioadenosine/AdoHcy nucleosidase (MTAN), which catalyzes the depurination of *S*-adenosyl-L-homocysteine (SAH), a byproduct of many *S*-adenosyl-L-methionine-dependent methyltransferases reactions, to form *S*-ribosyl-L-homocysteine (SRH, Fig. 1).[9,10] SRH is subsequently converted to L-homocysteine and 4,5-dihydroxy-2,3-pentadione (DPD) by the LuxS enzyme.[11] DPD undergoes spontaneous cyclization to **1e** and complexation with borate to form a furanosyl borate diester, which acts as the AI-2 in some bacteria.[11,12] Chemical synthesis of the unstable DPD has been accomplished recently by the groups of Janda[13] and Semmelhack,[14] which allowed the vital complexation properties of DPD with borate[15] to be studied and provided DPD as a reliable standard for investigation of AI-2 regulated QS processes.

LuxS is a small metalloenzyme (157 amino acids in the *Bacillus subtilis* enzyme) containing Fe^{2+} coordinated by His-54, His-58, Cys-126, and a water molecule. The native enzyme is unstable under aerobic conditions, but substitution of Co^{2+} for Fe^{2+} gives a highly stable variant with essentially wild-type catalytic activity.[16–18] In the proposed catalytic mechanism, LuxS catalyzes consecutive aldose–ketose (**1a** → **1b**) and ketose–ketose (**1b** → **1c**) isomerization steps and then β-elimination of Hcy from a 3-keto intermediate (**1c** → **1d**) to form DPD.[11,19] LuxS-catalyzed cleavage of the C5–S thioether bond in SRH is analogous to that of SAH hydrolase, which effects cleavage of an equivalent thioether bond in SAH by first oxidizing the C3′ secondary alcohol into a ketone with an NAD^+ cofactor.[20,21]

Zhou and co-workers designed and synthesized two LuxS substrate analogues, the *S*-(anhydroribosyl)-L-homocysteine (**2**) and *S*-(homoribosyl)-L-cysteine compounds, which blocked initial and final mechanistic steps, respectively (Fig. 2).[22] Pei and co-workers have prepared a series of stable analogues of the putative enediolate intermediate, some of which showed submicromolar inhibition of the enzyme (e.g., K_I = 0.72 μM for isostere **3**).[23] Zhang et al. found that the brominated furanones **4** covalently modify and inactivate LuxS.[24] Recognizing structural similarities between substrates of mammalian AdoHcy hydrolase and bacterial

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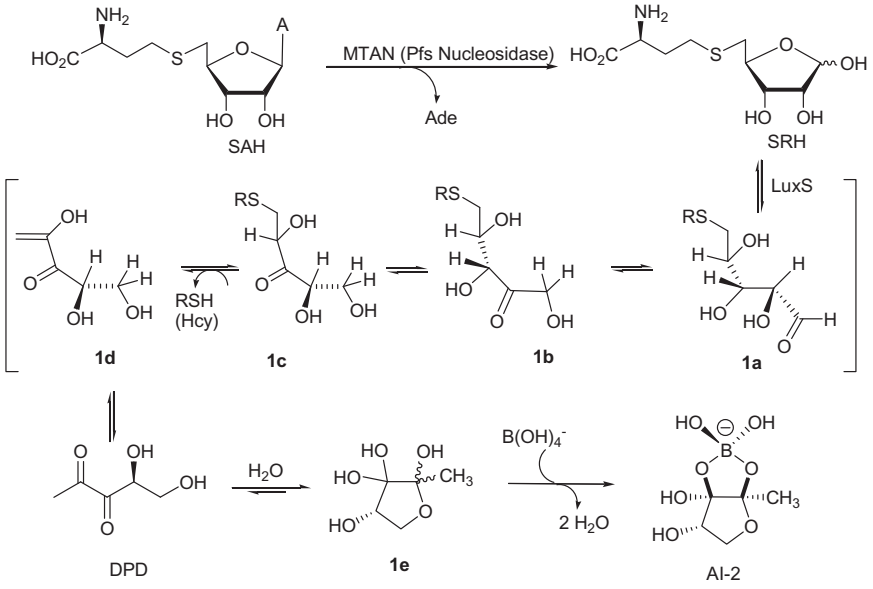


Figure 1. Biosynthetic pathway to AI-2. Enzymatic conversion of SRH to DPD by LuxS.

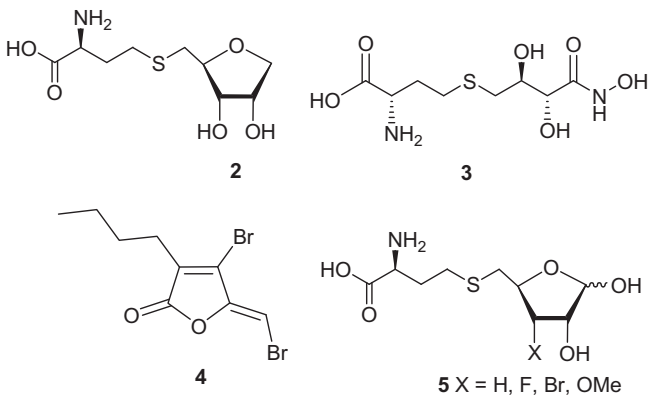


Figure 2. LuxS inhibitors.

S-ribosylhomocysteine (SRH) hydrolase (LuxS enzyme), we designed and synthesized SRH analogues with 6-(fluoro)vinyl moiety in place of the C5 and sulfur atoms which acted as weak/moderate inhibitors of LuxS enzyme.[25] The SRH analogues **5** lacking enolizable hydroxyl group at C3 were found to be competitive substrate of LuxS.[26,27] The time dependence inhibition with C3 halogenated substrates was caused by enzyme-catalyzed elimination of halide ions.[27]

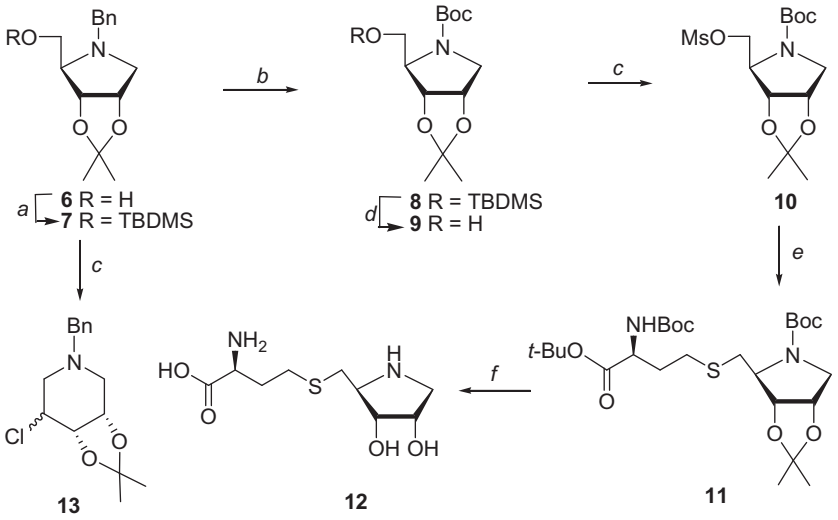
Here, we report synthesis of [4-aza]SRH mimics in which the furanose ring oxygen has been replaced by a nitrogen atom. The resulting hemiaminals should have different stabilities[28] relative to the *O,O*-hemiacetals present in SRH and as a result different rates of metabolic alteration. The higher basicity of the aza analogues is expected to have different binding strengths and rates for productions of the open chain aldehyde form–necessary for the first isomerization to occur. Also, the aminosugars can be protonated at physiological pH and the corresponding positive charge may have an effect on binding to the enzymatic active site. Azasugars[29] have been found to be potent inhibitors of glycosidases and glycosyltransferases[30,31] and have been targeted as transition-state models.[32,33] The 4′-azanucleosides[34] function as transition-state inhibitors of MTAN at the femtomolar level.[35,36]

2. Results and discussion

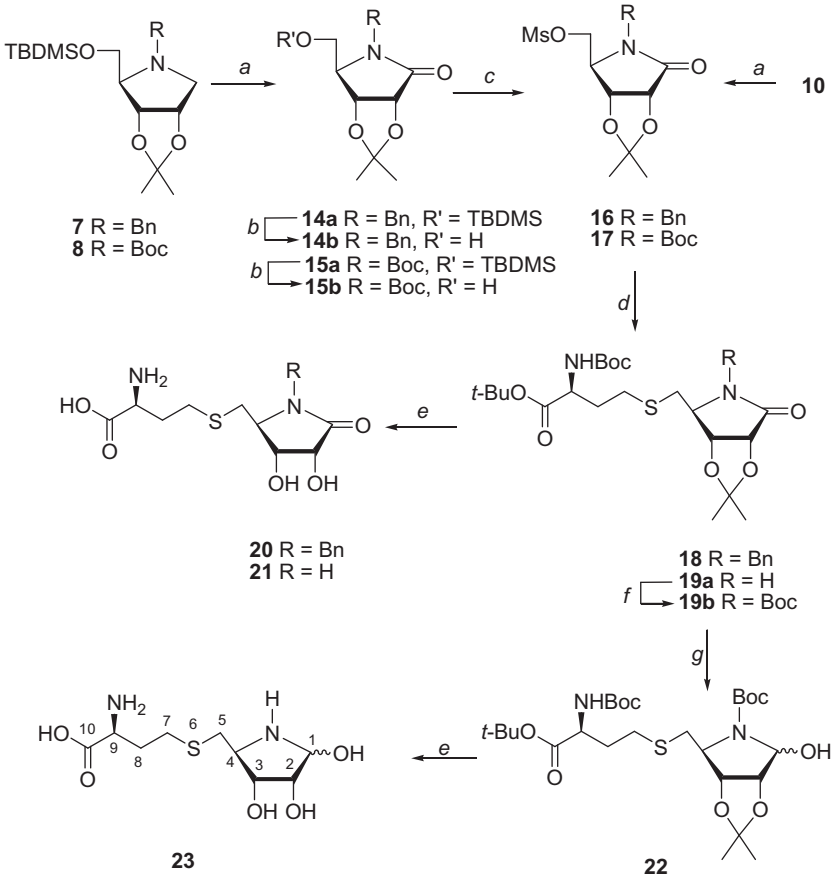
2.1. Chemistry

Our first target was 1,4-dideoxy-[4-aza]SRH **12** lacking the hydroxyl group at C1 (Scheme 1). Compound **12** cannot undergo ring opening (which will preclude the initial step of the LuxS-catalyzed reaction) and may act as a competitive inhibitor of LuxS. Synthesis of **12** started with the protected 1-amino-1,4-anhydro-1-deoxy-D-ribitol **6**, which was readily prepared from the commercially available D-gulonic acid γ-lactone.[37] However, attempted mesylation of the *N*-benzyl protected **6** resulted in the formation of piperidine derivative **13** as a mixture of two diastereomers (~3:1). Presumably, the mesylated pyrrolidine underwent a rearrangement reaction into the piperidines through an aziridine intermediates.[38,39] We found that replacement of the benzyl protecting group at ring nitrogen of **6** with a Boc group suppressed the nucleophilicity of the nitrogen and prevented ring expansion, allowing the formation of stable 5-*O*-mesyl derivatives. Thus, silylation of **6** with TBDMSCl and subsequent hydrogenation (5% Pd/C) in the presence of $(Boc)_2O$[39,40] yielded **8** (97% from **5**). Desilylation of **8** (70%) followed by mesylation gave **10** as a stable compound (96%). Displacement of the mesylate group with a thiolate, generated by reduction of properly protected L-homocystine[19] with water soluble tris(2-carboxyethyl)phosphine hydrochloride,[26] gave thioether **11** (86%). Treatment of **11** with TFA effected simultaneous removal of the *N*-Boc, acetonide and *t*-butyl ester protection groups to give the desired [4-aza]SRH analogue **12** in good yields (66%).

The second target was γ-lactam **21**, which contains an amide carbonyl at C1 and nitrogen as a replacement of the ring oxygen (Scheme 2). It is noteworthy that, as opposed to the [4-aza]SRH analogue **12** (or **23**), the lactam nitrogen cannot be protonated at physiological pH. Selective oxidation of the 5-*O*-TBDMS-azasugar **7** at C1 with $RuO_2/NaIO_4$ under $EtOAc/H_2O$ biphasic conditions[41] produced *N*-benzyl lactam **14a** (65%) and a small amount (18%) of the corresponding *N*-benzoylpyrrolidinone byproduct, resulted from oxidation of the benzylic carbon of the N-protecting group. Desilylation of **14a** with TBAF, followed by mesylation and displacement of the mesylate group with protected Hcy gave



Scheme 1. Reagents and conditions: (a) TBDMSCl/imidazole/DMAP/CH_2Cl_2/rt; (b) H_2/Pd-C/$(Boc)_2O$/Et_3N/EtOH/rt; (c) MsCl/Et_3N/CH_2Cl_2/rt; (d) TBAF/THF/rt; (e) BocNHCH(CH_2CH_2SH)CO_2t-Bu/LDA/DMF; (f) (i) TFA, (ii) TFA/H_2O.



Scheme 2. Reagents and conditions: (a) $NaIO_4/RuO_2$ x H_2O/EtOAc/H_2O/rt; (b) TBAF/THF/rt; (c) MsCl/Et_3N/CH_2Cl_2/rt; (d) BocNHCH(CH_2CH_2SH)CO_2t-Bu/LDA/DMF; (e) (i) TFA, (ii) TFA/H_2O; (f) $(Boc)_2O$/Et_3N/CH_2Cl_2/rt; (g) $LiEt_3BH$/THF/−78 °C.

thioether **18**. Treatment of **18** with TFA removed all of the acid-labile protection groups to yield *N*-benzyl protected [4-aza]SRH lactam **20** (48%). However, all attempts to remove the *N*-benzyl group from **18** or **20** (to yield **21**) were unsuccessful [e.g., H_2/Pd-C or $Pd(OH)_2$-C, Na/NH_3(liq.), BCl_3]. Our attempt to mesylate the *N*-Boc protected **15b** (prepared by RuO_2-catalyzed oxidation of **8** and desilylation of the resulting **15a**) failed to produce **17**, yielding only the starting material **15b**. Fortunately, oxidation of the 5-*O*-mesyl and *N*-Boc protected pyrrolidine **10** with $RuO_2/NaIO_4$ afforded **17** efficiently (95%). Coupling of **17** with homocysteinate

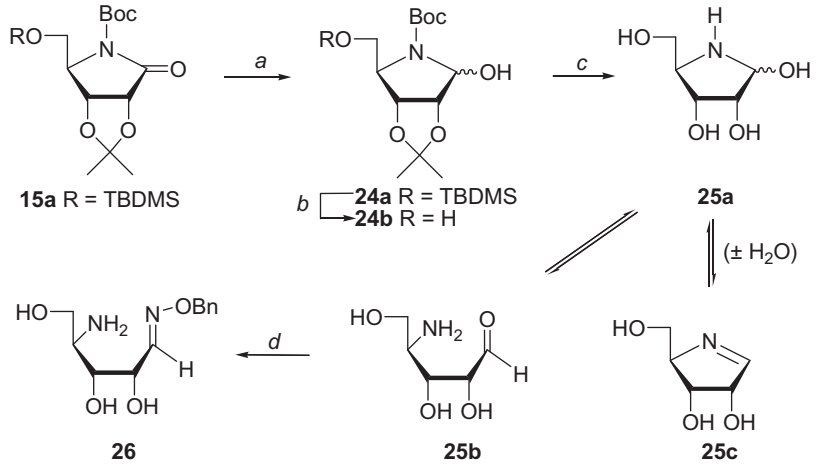
afforded thioether **19a** with concomitant loss of Boc group at ring nitrogen. Subsequent deprotection with TFA followed by TFA/H_2O gave [4-aza]SRH lactam **21** (58%).

Our next target was hemiaminal **23**. Since only the open aldehyde form of SRH is catalytically active, we were interested in the effect of the nitrogen substitution on the ring opening. The existence of azahemiacetals in equilibrium with dehydrated form (imine) as well as with open aldehyde and dimeric forms was reported for 4-azapentofuranoses.[42,43] It is noteworthy that sugar *N,O*-acetals were found to be stable enough to undergo coupling with nucleoside bases,[41] or transformation to proline.[44] Although direct reduction of lactam **21** (or **19a**) with $LiBEt_3H$ failed to yield hemiaminal **23** (or **22**), the protection of the ring nitrogen with a Boc group facilitated the reduction reaction.[44] Thus, treatment of **19a** with $(Boc)_2O$/DMAP gave *N*-Boc protected lactam **19b** (93%) which upon treatment with $LiBEt_3H$ produced hemiaminal **22** (92%) as a mixture of two anomers. Deprotection of **22** with TFA followed by TFA/H_2O gave desired [4-aza]SRH (*N,O*-acetal) analogue **23** (72%) as a mixture of α/β anomers. Interestingly, no free aldehyde or imine proton peaks were visible on ^1H NMR spectra. Compound **23** is stable when kept at 4 °C but decomposes slowly in solution at ambient temperature especially at basic pH.

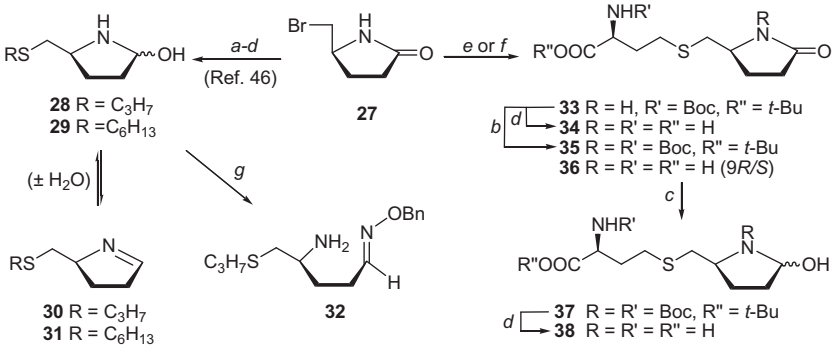
To determine whether the cyclic [4-aza]SRH exists in equilibrium with the open chain aldehyde form, we carried out a limited model study. Thus, *N*-Boc protected lactam **15a**[44] was reduced with $LiBEt_3H$ to afford protected hemiaminal **24a** (Scheme 3). Desilylation with TBAF yielded **24b**, which was treated with TFA to give deprotected hemiaminal **25a** as a mixture of anomers susceptible to dehydration at pH higher than 7 to form imine **25c**.[43] Subsequent treatment of **25a** with *O*-benzylhydroxylamine gave expected oxime **26** as the only product. The formation of oxime **26** indicates that azasugar **25a** exists in equilibrium with the open aldehyde form (**25b**) and that the equilibrium could be shifted by subsequent transformations.

2,3,4-Trideoxy-[4-aza]SRH **38** lacking the enolizable hydroxyl groups at C2 and C3 was next prepared to examine the importance of C2 and C3-OH groups for LuxS binding and catalysis. The key starting material (*S*)-5-(bromomethyl)-2-pyrrolidone (**27**) was conveniently prepared from L-pyroglutamic acid[45] (Scheme 4). Displacement of the bromide in **27** with the L-homocysteinate afforded thioether **33** (79%), which was deprotected with TFA quantitatively to give 2,3-dideoxy-4-azaSRH analogue **34** as a trifluoroacetate. Displacement with the unprotected D/L-homocysteine produced racemic **36** (75%) as a sodium salt, which upon treatment with TFA was also converted to its trifluoroacetate salt. As expected, ^1H NMR spectrum of **34** showed only one set of peaks which are present in the spectrum of racemic **36**. Treatment of **33** with $(Boc)_2O$/DMAP gave the *N*-Boc protected lactam **35**, which was reduced with $LiBEt_3H$ to give hemiaminal **37**. Subsequent deprotection with TFA produced **38**.

The 5-*S*-alkyl-2,3-dideoxy-[4-aza]SRH (e.g., **28/29**) and the 5-*S*-alkyl-[4-aza]SRH analogues with different length of the alkylthio chain were also prepared.[46] These cyclic azahemiacetals and their ancestor lactams were found to modulate *Pseudomonas aeruginosa*



Scheme 3. Reagents and conditions: (a) $LiEt_3BH$/THF/-78 °C; (b) TBAF/THF/rt; (c) (i) TFA/0 °C, (ii) TFA/H_2O/0 °C; (d) $BnONH_2$/pyr/rt.



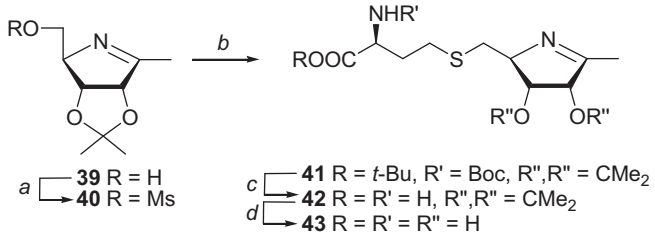
Scheme 4. Reagents and conditions: (a) RSH/NaH/DMF; (b) $(Boc)_2O$/DMAP/CH_2Cl_2; (c) $LiEt_3BH$/THF/CH_2Cl_2/−78 °C; (d) TFA; (e) $BocNHCH(CH_2CH_2SH)CO_2t$-Bu/LDA/DMF; (f) D/L-Hcy/NaH/DMF; (g) $BnONH_2$/pyr

QS.[46] The alkylthiomethyl azahemiacetal **28**/**29** existed in solution as an equilibrium mixture of anomers along with the open chain aldehydes [5–25%, ^{1}H NMR (δ 8.90), ^{13}C NMR (δ 180.8)] and the corresponding imines **30**/**31** [3–30%; ^{1} H NMR (δ 7.63), ^{13}C NMR (δ 167.0)].[46] Treatment of **28** with *O*-benzylhydroxylamine also produced the expected oxime **32**,[46] as observed for **25a**.

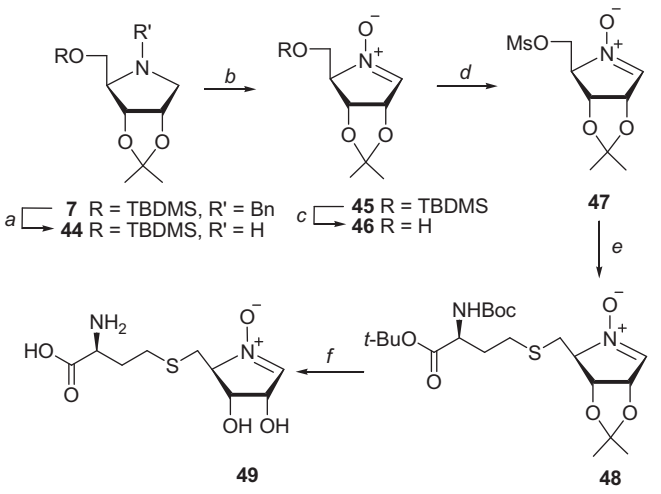
To explore the possibility of the LuxS-mediated addition of water across carbon–nitrogen double bond, we synthesized an imine-type analogue **43** (Scheme 5). The precursor 1-methyliminocyclitol **39** was prepared by the Moriarty rearrangement[47] of the *exo*-imino to *endo*-iminocyclitol, which involves inversion at C4 of the L-lyxo sugar to give the D-ribo azasugar. The imine **39**[47] was mesylated at the primary alcohol to give **40** (85%), which was coupled with protected L-Hcy to give **41** (85%, Scheme 5). Treatment of **41** with TFA for a short time gave only isopropylidene protected **42**. We found that the protons at the C1-methyl group are exchangeable with deuterium within few hours when compound **42** is dissolved in D_2O. Treatment of **42** with aqueous TFA (9:1) yielded fully deprotected **43** in quantitative yield. Protons at C1-methyl group of **43** were also exchangeable with deuterium. These exchange indicate that 1,4-ketimine-SRH analogue **43** might be expected to undergo enzyme-catalyzed hydrolysis to generate a [4-aza]SRH analogue with a methyl ketone rather than an aldehyde at C1. This change might affect the regioselectivity and rate of the first isomerization step in the LuxS-catalyzed reaction. We also proved that the methyl group protons in **39** are not susceptible to exchange even if **39** was dissolved in D_2O for several hours. Additionally, we noticed that observed low rate of exchange in **39** (relatively to **42** and **43**) can be enhanced exclusively in the presence of acid or amino acid (TFA and glycine were used, respectively). Attempted, one-step deprotection of **41** with BCl_3 led to a partial loss of chirality at C9 giving **43** as a mixture of diastereomers (2:3).

Our attempt to prepare the imine derivative of [4-aza]SRH was unsuccessful. Thus, debenzylation of **7** and treatment of the aminoribitol **44** with *N*-chlorosuccinimide (NCS) followed by dehydrochlorination of the resulting *N*-chloroamine with lithium tetramethylpiperidine gave unstable aldoimine of type **39** (H instead of CH_3), as reported.[48] However, couplings of such aldoimine with Hcy to give the imine SRH analogue failed. Acid-catalyzed hydrolysis of such imine analogue could serve as an alternative route to 4-azaSRH **23**. Also, enzyme-mediated protonation of the imine nitrogen atom and the addition of water might generate **23** and/or new species with an 'amino group' within the enzyme active site.

A nitrone analogue of SRH **49** was also targeted. Since nitrones are more electrophilic than imines such analogue might act as irreversible inhibitors by forming a covalent adduct(s) with enzyme. It is noteworthy that nitrones are overall neutral and cannot be protonated at physiological pH. Thus, treatment of the aminoribitol **44** with SeO_2/H_2O_2 gave nitrone **45**[49] (74%; Scheme 6). Desilylation and subsequent mesylation gave **47** (56%). Coupling of **47** with



Scheme 5. Reagents and conditions: (a) $MsCl/Et_3N/CH_2Cl_2$; (b) $BocNHCH(CH_2CH_2SH)CO_2t$-Bu/LDA/DMF; (c) TFA/rt; (d) TFA/H_2O.



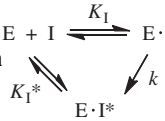
Scheme 6. Reagents and conditions: (a) H_2/Pd-C/EtOH/rt; (b) $SeO_2/H_2O_2/Me_2CO/-4$ °C; (c) TBAF/THF; (d) $MsCl/Et_3N/CH_2Cl_2/-4$ °C; (e) $BocNHCH(CH_2CH_2SH)CO_2t$-Bu/LDA/DMF/−20 °C; (f) (i) TFA/−4 °C, (ii) $TFA/H_2O/-4$ °C.

protected Hcy afforded a nitrone-SRH derivative **48** (43%). Deprotection of **48** with TFA produced unstable nitrone derivative **49** (40%).

2.2. Inhibition of LuxS

Compounds **12**, **20**, **21**, **23**, **28**, **36**, **38**, and **43** were evaluated as potential inhibitors of Co(II)-substituted *B. subtilis* LuxS. Compound **12** inhibited LuxS in a concentration-dependent manner that is consistent with competitive inhibition (Fig. 3a), with a K_I value of 48 μM (Table 1). Similarly, lactam **21** also behaved as a competitive inhibitor with K_I value of 37 μM. As expected, the lactam **20**, which contains a bulky benzyl group at the ring nitrogen, was found to be inactive, likely due to steric reasons. Compounds **36** and **38** were both inactive toward LuxS, highlighting the importance of the ribose hydroxyl groups for enzyme binding. The proposed mechanism predicts that the C2 and C3 hydroxyl groups directly coordinate with the catalytic metal ion during different catalytic steps (Fig. 1). The lack of activity of compound **43**, which contains a methyl group instead of a hydroxyl group at the C1 position, may be caused by both loss of favorable interactions with the OH group and the bulky size of the methyl group. Collectively, these results suggest that proper interactions between the ribose ring and the enzyme active site critically contribute to the formation of a productive E–S complex and subsequent catalysis.

Unlike the other analogues described above, inhibition of LuxS by the hemiaminal-containing analogue **23** was time dependent (Fig. 3b). Its inhibition kinetics can be described by the slow-binding equation



where K_I is the equilibrium constant for the formation of the initial E·I complex, k is the rate constant for the conversion of the E·I complex to the tighter E·I* complex, and K_I^* represents the dissociation constant of the E·I* complex. To assess its potency, different concentrations of compound **23** were preincubated with LuxS for 30 min at 4 °C and the residual enzymatic activity was measured. Plot of the residual activity against the inhibitor concentration resulted in an IC_{50} value of 60 μM, from which a K_I^* value of 3.5 μM was estimated (Table 1). Unfortunately, the complex inhibition kinetics precluded an accurate determination of the K_I value. While further work is clearly

necessary to determine the exact mechanism of inhibition by **23**, we propose a working hypothesis to explain the observed time dependence (Fig. 4). Since compounds **12** and **21**, which are structurally similar to **23**, did not exhibit time-dependent inhibition and our model study shows that the hemiaminal **25a** exists in equilibrium with the free aldehyde form (**25b**), we propose that hemiaminal **23** may undergo ring opening to form aldehyde **23a**. Due to its structural similarity to catalytic intermediate **1a** (Fig. 1), **23a** may undergo the aldose-ketose isomerization reaction to form 2-ketone **23b**, which presumably binds to the LuxS active site with higher affinity than the ribose analogue **23**. This behavior is very similar to that of a class of halogenated SRH analogues (e.g., [3-F]SRH and [3-Br]SRH), which have been shown to undergo LuxS-catalyzed ring opening to form open-chain species that are more potent LuxS inhibitors than the initial ribose analogues.[27]

The remaining compound **28** and its ancestor lactam showed no significant inhibition of LuxS.

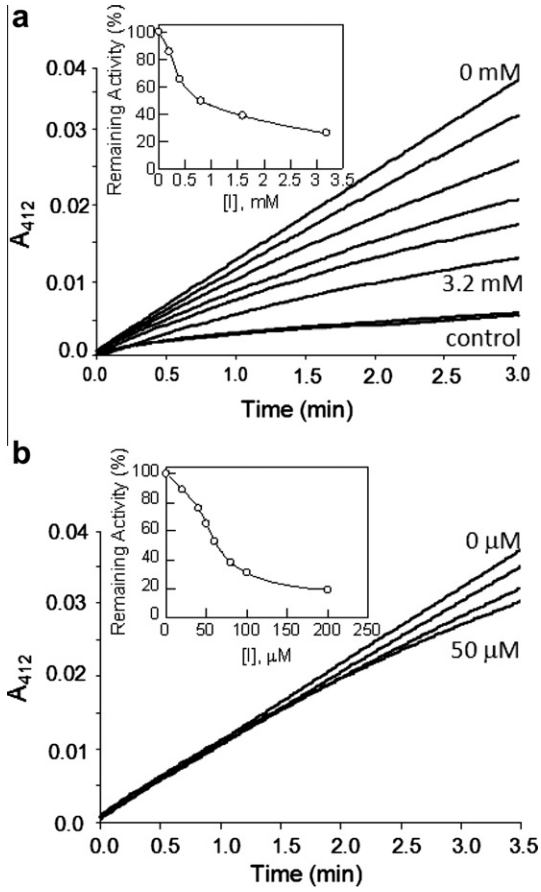


Figure 3. Inhibition of LuxS by compounds **12** and **23**. (A) Reaction progress curves in the presence of increasing concentrations of inhibitor **12** (0, 200, 400, 800, 1600, and 3200 μM). The last two curves were control reactions in the absence of LuxS. Inset, plot of remaining LuxS activity as a function of inhibitor **12** concentration. (B) Reaction progress curves of LuxS in the presence of increasing concentrations of inhibitor **23** (0, 20, 40, and 50 μM) (without preincubation). Inset, plot of remaining LuxS activity as a function of inhibitor **12** concentration (after 30 min preincubation).

Table 1
Inhibition constants of [4-aza]SRH analogues against *B. subtilis* LuxS

Compound	K_I or K_I^* (μM)
12	48
21	37
23	3.5

3. Conclusions

We have synthesized [4-aza] *S*-ribosylhomocysteine analogues in which the furanose ring oxygen has been substituted by a nitrogen atom having also the additional modifications at anomeric carbon. Coupling of the protected 4-amino-5-*O*-methanesulfonyl-4-deoxy-D-ribono-1,4-lactam with homocysteinate and subsequent deprotection with TFA gave [4-aza]SRH with an amide carbonyl at anomeric carbon. Reduction of the *N*-Boc protected lactam with $LiBEt_3H$ and acid catalyzed deprotection produced [4-aza]SRH hemiaminal analogue. The [4-aza]SRH analogue lacking the hydroxyl group at C1 and the corresponding lactam derivative showed modest competitive inhibition (K_I ~40–50 μM) of LuxS. The hemiaminal analogue exhibited time-dependent inhibition (K_I^* = 3.5 μM), consistent with the enzyme-catalyzed ring opening and generation of 2- and/or 3-ketone intermediates, which presumably bind to the LuxS active site with higher affinity than the ribose natural substrate.

4. Experimental procedure

The ^1H (400 or 600 MHz) and ^{13}C (100 MHz) NMR spectra were determined with solutions in $CDCl_3$ unless otherwise noted. Mass spectra (MS) were obtained with atmospheric pressure chemical ionization (APCI) technique and HRMS in AP-ESI or TOF-ESI mode. TLC was performed with Merck kieselgel 60-F_{254} sheets products were detected with 254 nm light or by visualization with $Ce(SO_4)_2/(NH_4)_6Mo_7O_{24}{\cdot}4H_2O/H_2SO_4/H_2O$ reagent. Merck kieselgel 60 (230–400 mesh) was used for column chromatography. HPLC purifications were performed using XTerra® preparative RP_{18} OBD™ column (5 μm 19 × 150 mm) with gradient program using CH_3CN/H_2O as a mobile phase. Reagent grade chemicals were used, and solvents were dried by reflux over and distillation from CaH_2 (except for THF/potassium) under argon.

4.1. 1-Amino-1,4-anhydro-*N*-benzyl-5-*O*-*tert*-butyldimethylsilyl-1-deoxy-2,3-*O*-isopropylidene-D-ribitol (7)

To a stirred solution of **6**[37] (150 mg, 0.57 mmol) in anhydrous CH_2Cl_2 (5 mL) at rt under Ar atmosphere were added DMAP (7 mg, 0.05 mmol) and imidazole (93 mg, 1.36 mmol) followed by TBDMSCl (103 mg, 0.68 mmol). The mixture was then stirred for

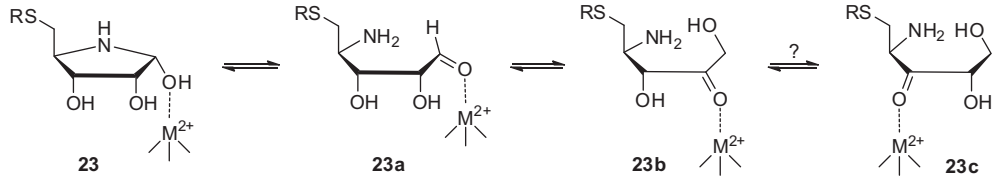


Figure 4. Proposed mechanism for the time-dependent inhibition of LuxS by [4-aza]SRH hemiaminal **23**.

10 h and partitioned (CH_2Cl_2//·$NaHCO_3$/H_2O). The organic layer was washed (brine), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography (15% EtOAc/hexane) to give **7**[50] (204 mg, 98%) as a colorless oil. ^{1}H NMR δ −0.13 (s, 3, CH_3), 0.00 (s, 3, CH_3), 0.83 (s, 9, *t*-Bu), 1.26 (s, 3, CH_3), 1.49 (s, 3, CH_3), 2.65 (dd, *J* = 2.7, 10.3 Hz, 1, H1), 2.94 ('q', *J* = , 2.2 Hz, 1, H4), 3.04 (dd, *J* = 5.5, 10.3 Hz, 1, H1′), 3.57 (dd, *J* = 4.1, 10.6 Hz, 1, H5), 3.64 (d, *J* = 13.4 Hz, 1, Bn), 3.71 (dd, *J* = 4.3, 10.6 Hz, 1, H5′), 3.94 (d, *J* = 13.4 Hz, 1, Bn), 4.49 (dd, *J* = 2.0, 6.5 Hz, 1, H3), 4.58 ('dt', *J* = 2.7, 6.2 Hz, 1, H2), 7.13–7.23 (m, 5, Bn); ^{13}C NMR δ −5.5, (CH_3), −5.4, (CH_3), 18.2 (*t*-Bu), 25.2 (C*Me*$_2$), 25.7 (CH_3), 25.9 (CH_3), 27.2 (C*Me*$_2$), 56.9 (Bn), 59.3 (C1), 63.2 (C5), 68.9 (C4), 79.4 (C2), 83.3 (C3), 111.9 (*C*Me$_2$), 128.2, 128.5, 126.8, 139.4 (Bn); MS (APCI) *m*/*z* 378 (100, MH^+).

4.2. 1-Amino-1,4-anhydro-*N*-*tert*-butoxycarbonyl-5-*O*-*tert*-butyldimethylsilyl-1-deoxy-2,3-*O*-isopropylidene-D-ribitol (8)

A solution of **7** (145 mg, 0.38 mmol), triethylamine (0.105 mL, 0.76 mmol), di-*tert*-butyldicarbonate (126 mg, 0.57 mmol) and Pd/C (5%, 300 mg) in ethanol (6 mL) was stirred under an atmosphere of hydrogen at room temperature for 6 h. The reaction mixture was filtered through Celite to remove the catalyst. The Celite was washed with ethanol (5 mL) and washings and the filtrate were combined and evaporated. The residue was partitioned (EtOAc//·$NaHCO_3$/H_2O). The organic layer was washed (brine), dried ($MgSO_4$) and evaporated. The residue was column chromatographed (20 → 30% EtOAc/hexane) to give **8** (147 mg, 99%) with spectral properties as reported.[44]

4.3. 1-Amino-1,4-anhydro-*N*-*tert*-butoxycarbonyl-1-deoxy-2,3-*O*-isopropylidene-D-ribitol (9)

TBAF (1 M/THF; 0.25 mL, 0.25 mmol) was added to a stirred solution of **8** (66 mg, 0.17 mmol) in THF (5 mL) at ambient temperature. After stirring for 30 min, the reaction mixture was partitioned (EtOAc//·$NaHCO_3$/H_2O). The organic layer was washed (brine), dried ($MgSO_4$) and evaporated. The residue was column chromatographed (50 → 60% EtOAc/hexane) to give **9** (32 mg, 70%) with spectral properties as reported.[51]

4.4. 1-Amino-1,4-anhydro-*N*-*tert*-butoxycarbonyl-1-deoxy-2,3-*O*-isopropylidene-5-*O*-methanesulfonyl-D-ribitol (10)

Procedure A. Triethylamine (99 μL, 0.71 mmol) and MsCl (25 μL, 0.33 mmol) were added dropwise to stirred solution of **9** (60 mg, 0.22 mmole) in anhydrous CH_2Cl_2 (6 mL) at 0 °C (ice-bath). After 5 min, ice-bath was removed and the reaction mixture was allowed to stir at ambient temperature for 30 min. The reaction mixture was quenched with saturated $NaHCO_3$/H_2O and was extracted with CH_2Cl_2. The organic layer was washed (brine), dried ($MgSO_4$) and evaporated to give **10** (73 mg, 96%) as a mixture (~3:2) of two rotamers of sufficient purity to be directly used in next step: ^{1}H NMR δ 1.28 (s, 3, CH_3), 1.42 (s, 12H, *t*-Bu, CH_3), 2.96 (s, 1.2, Ms), 2.98 (s, 1.8, Ms), 3.39 (dd, *J* = 4.8, 12.5 Hz, 0.4, H1), 3.46 (dd, *J* = 4.8, 12.5 Hz, 0.6, H1), 3.69 (d, *J* = 12.5 Hz, 0.6, H1'), 3.82 (d, *J* = 12.5 Hz, 0.4, H1'), 4.10–4.14 (m, 0.4, H4), 4.22–4.30 (m, 0.6, H4), 4.22–4.29 (m, 1.4, H5,5'), 4.45 (dd, *J* = 4.1, 10.1 Hz, 0.6, H5), 4.65 ('d', *J* = 5.9 Hz, 1, H3); 4.72 ('t', *J* = 5.3 Hz, 1, H2); ^{13}C NMR (major rotamer) δ 24.9 (C*Me*$_2$), 26.9 (C*Me*$_2$), 29.6 (*t*-Bu), 37.1 (Ms), 52.5 (C1), 62.4 (C4), 68.9 (C5), 79.2 (C2), 80.4 (*t*-Bu), 81.7 (C3), 112.1 (*C*Me$_2$),154.2 (NHCO); ^{13}C NMR (minor rotamer) δ 24.9 (C*Me*$_2$), 26.9 (C*Me*$_2$), 29.6 (*t*-Bu), 37.5 (Ms), 53.1 (C1), 62.6 (C4), 68.6 (C5), 78.5 (C2), 80.6 (*t*-Bu), 82.5 (C3), 112.1 (*C*Me$_2$), 153.6 (NHCO); MS (APCI) *m*/*z* 352 (10, MH^+), 252 (100, [MH_2-Boc]$^+$).

4.5. *S*-(1-Amino-1,4-anhydro-*N*-*tert*-butoxycarbonyl-1,5-dideoxy-2,3-*O*-isopropylidene-D-ribitol-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (11)

Procedure B. ***Step a***. H_2O (0.4 mL) and tris(2-carboxyethyl)phosphine hydrochloride (140 mg, 0.5 mmol) were added to a stirred solution of *N,N'*-di(*tert*-butoxycarbonyl)-L-homocystine di(*tert*-butyl) ester[19] (250 mg, 0.4 mmol) in anhydrous DMF (4 mL) at ambient temperature under Ar atmosphere. After 24 h, the reaction mixture [TLC (EtOAc/hexane, 2:8) showed conversion of disulfide (R_f 0.55) into thiol (R_f 0.65)] was partitioned between EtOAc and saturated $NaHCO_3$/H_2O. Aqueous layer was extracted with EtOAc, and the combined organic layer was washed with brine, dried ($MgSO_4$) and concentrated to give *N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester[19] (240 mg, 99%) as colorless oil of sufficient purity to be directly used in next step. ***Step b***. LDA (85 μL, 2.0 M/THF and heptane, 0.17 mmol) was added dropwise (10 min) to a stirred solution of freshly prepared thiol from step a (200 mg, 0.6 mmol) in anhydrous DMF (5 mL) under a vigorous stream of argon at 0 °C (ice-bath). After an additional 10 min, **10** (100 mg, 0.2 mmol) in anhydrous DMF (5 mL) was added via a syringe. After 15 min ice-bath was removed and the reaction mixture was stirred for 24 h at ambient temperature. Ice–cold saturated NH_4Cl/H_2O was added and the resulting suspension was diluted with EtOAc. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layer was washed (brine), dried ($MgSO_4$) and was evaporated. The residue was column chromatographed (40 → 50% EtOAc/hexane) to give **11** (130 mg, 86%) as a mixture of rotamers (~1:1): ^{1}H NMR δ 1.29 (s, 3, CH_3), 1.41 (s, 12H, *t*-Bu, CH_3), 1.44 (s, 18H, *t*-Bu), 1.79–1.92 (m, 2, H8,8'), 2.39–2.80 (m, 4, H5,5',7,7'), 3.37 (dd, *J* = 4.2, 11.7 Hz, 0.5, H1), 3.43 (dd, *J* = 4.5, 11.7 Hz, 0.5, H1), 3.70 (d, *J* = 12.6 Hz, 0.5, H1'), 3.84 (d, *J* = 12.8 Hz, 0.5, H1'), 3.99–4.05 (m, 0.5, H4), 4.11–4.17 (m, 0.5, H4), 4.18–4.29 (m, 1, H9), 4.56 (dd, *J* = 5.6, 10.4 Hz, 0.5, H3), 4.60 (dd, *J* = 5.6, 10.4 Hz, 0.5, H3), 4.69 (d, *J* = 4.8 Hz, 0.5, H2), 4.71 (d, *J* = 4.8 Hz, 0.5, H2), 5.06 (br d, *J* = 7.3 Hz, 0.5, NH), 5.29 (br d, *J* = 6.1 Hz, 0.5, NH); ^{13}C NMR δ 25.0 (C*Me*$_2$), 26.9 (C*Me*$_2$), 27.9 (C7), 28.0 (*t*-Bu), 28.3 (*t*-Bu), 28.4 (*t*-Bu), 32.2 (C5), 32.6 (C8), 32.9 (C8), 33.2 (C5), 51.7 (C1), 52.4 (C1), 53.5 (C9), 62.8 (C4), 63.2 (C4), 78.5 (C2), 78.5 (*t*-Bu), 79.2 (C2), 79.2 (*t*-Bu), 80.0 (*t*-Bu), 80.1 (*t*-Bu), 83.5 (C3), 84.1 (C3), 111.9 (*C*Me$_2$), 154.1 (CO), 154.9 (CO), 155.4 (CO), 171.2 (C10), 171.5 (C10); MS (APCI) *m*/*z* 547 (100, MH^+); HRMS (AP-ESI) *m*/*z* calcd for $C_{26}H_{47}N_2O_8S$ $[MH]^+$ 547.3048; found 547.3042.

4.6. *S*-(1-Amino-1,4-anhydro-1,5-dideoxy-D-ribitol-5-yl)-L-homocysteine (12)

Procedure C. ***Step a***. Compound **11** (39 mg, 0.07 mmol) dissolved in TFA (4.0 mL) was stirred at 0 °C for 3 h. Volatiles were coevaporated with toluene to give an oily residue, which was used directly in next step. *Step b*. Product from ***step a*** was treated with TFA/H_2O (9:1, 4.0 mL) for 1 h at 0 °C. Volatiles were evaporated and the crude product was purified on RP-HPLC (5% CH_3CN/H_2O at 2.5 mL/min; t_R = 12 min) to give **12** (12 mg, 66%) as a colorless oil: ^{1}H NMR (D_2O) δ 2.10–2.25 (m, 2, H8,8'), 2.71–2.81 (m, 2, H7,7'), 2.87 (dd, *J* = 10.4, 14.5 Hz, 1, H5), 3.15 (dd, *J* = 4.3, 14.5 Hz, 1, H5'), 3.34 (dd, *J* = 1.9, 13.0 Hz, 1, H1), 3.53 (dd, *J* = 4.0, 13.0 Hz, 1, H1'), 3.69 (ddd, *J* = 4.3, 8.7, 10.4 Hz, 1, H4), 3.86 (t, *J* = 6.2 Hz, 1, H9), 4.14 (dd, *J* = 4.1, 8.7 Hz, 1, H3), 4.39 (ddd, *J* = 1.9, 4.0, 4.1 Hz, 1, H2); ^{13}C NMR (D_2O) δ 26.8 (C8), 30.1 (C7), 30.6 (C5), 49.2 (C1), 53.6 (C9), 59.5 (C4), 69.5 (C2), 74.3 (C3), 174.0 (C10); MS *m*/*z* 251 (100, MH^+); HRMS (TOF MS-ESI) *m*/*z* calcd for $C_9H_{19}N_2O_4S$ $[M+H]^+$ 251.1060; found 251.1063

4.7. 1-Benzyl-5-chloro-3,4-dihydroxy-3,4-*O*-isopropylidenepiperidine [13(3*S*,4*S*,5*R*/*S*)]

Treatment of **6** (50 mg, 0.19 mmol) with MsCl (21.9 μL, 0.28 mmol) by Procedure A [column chromatography (20 → 30% EtOAc/hexane)] gave **13** (25 mg, 46%) as a 3:1 mixture of diastereomers: The major isomer had: ^{1}H NMR δ 1.30 (s, 3, CH_3), 1.50 (s, 3, CH_3), 2.13–2.19 (m, 1, H2), 2.49 (dd, J = 3.7, 13.3 Hz, 1, H6), 2.87 (dq, J = 1.7, 12.0 Hz, 1, H2'), 3.05 ('dt', J = 2.1, 13.3 Hz, 1, H6'), 3.50 (d, J = 13.4 Hz, 1, Bn), 3.61 (d, J = 13.4 Hz, 1, Bn), 3.93–3.99 (m, 2, H3,5), 4.19 (dd, J = 3.7, 7.5 Hz, 1, H4), 7.20–7.25 (m, 5, Bn); ^{13}C NMR δ 26.2 (C*Me*$_2$), 28.4 (C*Me*$_2$), 53.5 (C6), 56.5 (C2), 58.5 (C5), 61.5 (Bn), 73.8 (C4), 79.5 (C3), 112.8 (*C*Me$_2$), 127.3, 128.4, 128.9, 137.2 (Bn); MS m/z 282 (100, MH$^+$[^{35}Cl]), 284 (40, MH$^+$ [^{37}Cl]); HRMS (TOF MS-ESI) m/z calcd for $C_{15}H_{20}{}^{35}ClNO_2$ [M+H]$^+$ 282.1255; found 282.1259.

4.8. 4-Amino-*N*-benzyl-5-*O*-*tert*-butyldimethylsilyl-4-deoxy-2,3-*O*-isopropylidene-D-ribono-1,4-lactam (14a)

Procedure D. $RuO_2\cdot\times H_2O$ (4.3 mg, 0.032 mmol) was added to a stirred solution of $NaIO_4$ (83 mg, 0.39 mmol) in H_2O (1 mL) at ambient temperature. After 5 min, a solution of **7** (50 mg, 0.13 mmol) in EtOAc (1 mL) was added dropwise and the reaction mixture was continued to stir for 12 h. H_2O (10 mL) and EtOAc (10 mL) were added and the separated aqueous layer was furthermore extracted with EtOAc (2 × 10 mL). The combined organic layers were washed (brine), dried ($MgSO_4$) and evaporated. The residue was column chromatographed (50 → 60% EtOAc/hexane) to give **14a** (33 mg, 65%) and *N*-benzoylated byproduct (10 mg, 18%). Compound **14a** had: ^{1}H NMR δ 0.01 (s, 6, 2× CH_3), 0.84 (s, 9, *t*-Bu), 1.35 (s, 3, CH_3), 1.42 (s, 3, CH_3), 3.51 (t, J = 2.1 Hz, 1, H4), 3.62 (dd, J = 2.0, 10.9 Hz, 1, H5), 3.69 (dd, J = 2.3, 10.9 Hz, 1, H5'), 3.93 (d, J = 15.2 Hz, 1, Bn), 4.50 (d, J = 5.6 Hz, 1, H3), 4.69 (d, J = 5.6 Hz, 1, H2), 5.00 (d, J = 15.2 Hz, 1, Bn), 7.22–7.32 (m, 5, Bn); ^{13}C NMR δ −5.7, (CH_3), −5.6, (CH_3), 18.1 (*t*-Bu), 25.8 (*t*-Bu), 25.8 (C*Me*$_2$), 27.3 (C*Me*$_2$), 44.2 (Bn), 60.2 (C5), 62.0 (C4), 76.7 (C3), 78.0 (C2), 111.7 (*C*Me$_2$), 127.7, 128.2, 128.7, 135.6 (Bn), 171.9 (C1); MS (APCI) m/z 392 (100, MH$^+$). The 4-amino-*N*-benzoyl-5-*O*-*tert*-butyldimethylsilyl-4-deoxy-2,3-*O*-isopropylidene-D-ribono-1,4-lactam byproduct had: ^{1}H NMR δ 0.01 (s, 3, CH_3), 0.05 (s, 3, CH_3), 0.87 (s, 9, *t*-Bu), 1.40 (s, 3, CH_3), 1.51 (s, 3, CH_3), 3.83 (dd, J = 1.5, 10.7 Hz, 1, H5), 4.19 (dd, J = 2.2, 10.7 Hz, 1, H5'), 4.58 ('t', J = 1.8 Hz, 1, H4), 4.65 (d, J = 5.5 Hz, 1, H3), 4.76 (d, J = 5.5 Hz, 1, H2), 7.38–7.43 (m, 2, Bn), 7.51 ('dt', J = 1.3, 6.7 Hz, 2, Bn), 7.54–7.58 (m, 1, Bn); ^{13}C NMR δ −5.7, (CH_3), −5.6, (CH_3), 18.2 (*t*-Bu), 25.3 (C*Me*$_2$), 25.8 (*t*-Bu), 27.2 (C*Me*$_2$), 61.7 (C4), 62.1 (C5), 76.3 (C3), 78.7 (C2), 112.1 (*C*Me$_2$), 127.9, 128.7, 132.2, 134.1 (Bn) 170.6 (CO), 171.7 (C1); MS (APCI) m/z 406 (100, MH$^+$).

4.9. 4-Amino-*N*-benzyl-4-deoxy-2,3-*O*-isopropylidene-D-ribono-1,4-lactam (14b)

TBAF (1 M/THF; 0.18 mL, 0.18 mmol) was added dropwise to a stirred solution of **14a** (49 mg, 0.12 mmol) in THF (10 mL) at 0 °C. After 5 min, the ice-bath was removed and reaction mixture was allowed to stir at ambient temperature for 2 h. The reaction mixture was quenched with water and volatiles were evaporated. The residue was partitioned (EtOAc//·$NaHCO_3$/H_2O) and the organic layer was washed (brine), dried ($MgSO_4$) and evaporated. The residue was column chromatographed (80 → 90% EtOAc/hexane) to give **14b**[52] as a white solid (33 mg, 97%): ^{1}H NMR δ 1.37 (s, 3, CH_3), 1.47 (s, 3, CH_3), 3.35 (s, 1, OH), 3.54 ('t', J = 1.8 Hz, 1, H4), 3.64 (d of m, J = 11.8 Hz, 1, H5), 3.85 (br d, J = 12.0 Hz, 1, H5'), 4.08 (d, J = 15.2 Hz, 1, Bn), 4.64 (d, J = 5.6, Hz, 1, H3), 4.77 (d, J = 5.2 Hz, 1, H2), 5.04 (d, J = 15.2 Hz, 1, Bn), 7.29–7.37 (m, 5, Bn); MS (APCI) m/z 278 (100, MH$^+$).

4.10. 4-Amino-*N*-*tert*-butoxycarbonyl-5-*O*-*tert*-butyldimethylsilyl-4-deoxy-2,3-*O*-isopropylidene-D-ribono-1,4-lactam (15a)

Oxidation of **8** (90 mg, 0.23 mmol) with $NaIO_4$ (126 mg, 0.7 mmol) and $RuO_2\cdot xH_2O$ (8 mg, 0.05 mmol) by Procedure D [column chromatography (50 → 60% EtOAc/hexane)] gave **15a** (56 mg, 60%) as a colorless oil with data as reported.[44]

4.11. 4-Amino-*N*-*tert*-butoxycarbonyl-4-deoxy-2,3-*O*-isopropylidene-D-ribono-1,4-lactam (15b)

Desilylation of **15a** (50 mg, 0.12 mmol) with TBAF (1 M/THF, 0.14 mL, 0.14 mmol), as described for **14b**, [column chromatography (70 → 80% EtOAc/hexane)] gave **15b** (27 mg, 77%) as a colorless oil: ^{1}H NMR δ 1.36 (s, 3, CH_3), 1.46 (s, 12, *t*-Bu, CH_3), 3.88 ('dt', J = 3.9, 5.3 Hz, 1, H4), 4.03 (dd, J = 4.1, 11.4 Hz, 1, H5), 4.19 (dd, J = 3.8, 11.4 Hz, 1, H5'), 4.57 (dd, J = 5.3, 5.7 Hz, 1, H3), 4.63 (d, J = 5.7 Hz, 1, H2); ^{13}C NMR δ 25.7 (C*Me*$_2$), 27.0 (C*Me*$_2$), 27.6 (*t*-Bu), 56.7 (C4), 66.6 (C5), 76.7 (C2), 77.0 (C3), 83.3 (*t*-Bu), 112.4 (*C*Me$_2$), 153.0 (CO), 173.7 (C1); MS (APCI) m/z 288 (100, MH$^+$); HRMS (AP-ESI) m/z calcd for $C_{13}H_{22}NO_6$ [MH]$^+$ 288.1442; found 288.1437.

4.12. 4-Amino-*N*-benzyl-4-deoxy-2,3-*O*-isopropylidene-5-*O*-methanesulfonyl-D-ribono-1,4-lactam (16)

Mesylation of **14b** (67 mg, 0.24 mmol) with MsCl (27 μL, 0.36 mmol) by Procedure A gave **16** (83 mg, 97%) as a colorless oil of sufficient purity to be used directly in next step: ^{1}H NMR δ 1.30 (s, 3, CH_3), 1.36 (s, 3, CH_3), 3.07 (s, 3, Ms), 3.65 (t, J = 2.7 Hz, 1, H4), 4.06 (d, J = 15.2 Hz, 1, Bn), 4.16 (dd, J = 2.2, 11.0 Hz, 1, H5), 4.22 (dd, J = 3.0, 11.0 Hz, 1, H5'), 4.51 (d, J = 5.6 Hz, 1, H3), 4.69 (d, J = 5.6 Hz, 1, H2), 4.92 (d, J = 15.2 Hz, 1, Bn), 7.18–7.30 (m, 5, Bn); MS (APCI) m/z 388 (100, [MH+MeOH]$^+$), 356 (40, MH$^+$).

4.13. 4-Amino-*N*-*tert*-butoxycarbonyl-5-*O*-methanesulfonyl-4-deoxy-2,3-*O*-isopropylidene-D-ribono-1,4-lactam (17)

Oxidation of **10** (80 mg, 0.32 mmol) with $NaIO_4$ (172 mg, 0.96 mmol) and $RuO_2\cdot xH_2O$ (8.5 mg, 0.064 mmol) by procedure D [column chromatography (EtOAc)] gave **17** (78 mg, 95%) as a colorless oil: ^{1}H NMR δ 1.37 (s, 3, CH_3), 1.44 (s, 3, CH_3), 1.54 (s, 9H, *t*-Bu) 3.01 (s, 3, Ms), 4.39–4.43 (m, 2, H4,5), 4.58 (d, J = 5.5 Hz, 1, H3), 4.64 (dd, J = 3.1, 11.2 Hz, 1, H5'), 4.70 (d, J = 5.5 Hz, 1, H2); ^{13}C NMR δ 25.6 (C*Me*$_2$), 27.0 (C*Me*$_2$), 28.0 (*t*-Bu), 37.7 (Ms), 59.2 (C4), 67.0 (C5), 74.5 (C3), 77.5 (C2), 84.7 (*t*-Bu), 112.8 (*C*Me$_2$), 149.7 (CO), 170.2 (C1); MS (APCI) m/z 366 (5, MH$^+$), 297 (100, [MH$_2$-Boc+MeOH]$^+$).

4.14. *S*-(4-Amino-*N*-benzyl-4,5-dideoxy-2,3-*O*-isopropylidene-D-ribono-1,4-lactam-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (18)

Treatment of **16** (85 mg, 0.24 mmol) with lithium homocysteinate (104 mg, 0.36 mmol) by Procedure B [column chromatography (60 → 70% EtOAc/hexane)] gave **18** (94 mg, 70%) as a colorless oil. ^{1}H NMR δ 1.28 (s, 3, CH_3), 1.37 (s, 12, *t*-Bu, CH_3), 1.39 (s, 9, *t*-Bu), 1.74–1.86 (m, 1, H8), 1.95–2.17 (m, 1, H8'), 2.46–2.57 (m, 2, H7,7'), 2.69–2.73 (m, 2, H5,5'), 3.59–3.71 (m, 1,

H4), 3.86 (d, J = 15.0 Hz, 1, Bn), 4.17–4.18 (m, 1, H9), 4.43 (d, J = 5.7 Hz, 1, H3), 4.79 (d, J = 5.5 Hz, 1, H2), 4.97 (d, J = 15.0 Hz, 1, Bn), 5.05 (br d, J = 7.1 Hz, 1, NH), 7.17–7.27 (m, 5, Bn); ^{13}C NMR δ 25.6 (CMe_2), 27.0 (CMe_2), 28.0 (t-Bu), 28.3 (t-Bu), 29.0 (C7), 33.1 (C5), 33.2 (C8), 44.4 (Bn), 53.1 (C9), 60.3 (C4), 77.2 (C3), 77.5 (C2), 79.9 (t-Bu) 82.4 (t-Bu), 112.1 CMe$_2$, 127.9, 128.2, 128.8, 135.2 (Bn), 153.0 (CO), 171.1 (C10), 171.4 (C1); MS m/z 551 (100, MH$^+$). HRMS (AP-ESI) m/z calcd for $C_{28}H_{43}N_2O_7S$ [MH]$^+$ 551.2785; found 551.2792.

4.15. *S*-(4-Amino-4,5-dideoxy-2,3-*O*-isopropylidene-D-ribono-1,4-lactam-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (19a)

Treatment of **17** (70 mg, 0.19 mmol) with lithium homocysteinate (83 mg, 0.28 mmol) by Procedure B [column chromatography (70 → 80% EtOAc/hexane)] gave **19a** (40 mg, 45%) as a light yellow oil. ^{1}H NMR δ 1.36 (s, 3, CH$_3$), 1.43 (s, 9, t-Bu), 1.45 (s, 12, t-Bu, CH$_3$), 1.82–1.90 (m, 1, H8), 1.97–2.06 (m, 1, H8'), 2.58 ('t', J = 7.4 Hz, 2, H7,7'), 2.64–2.72 (m, 2, H5,5'), 3.83 ('t', J = 5.8 Hz 1, H4), 4.23–4.24 (m, 1, H9), 4.48 (d, J = 4.4 Hz, 1, H3), 4.69 (d, J = 4.6 Hz, 1, H2), 5.21 (br d, J = 7.9 Hz, 1, NH); ^{13}C NMR δ 25.6 (CMe_2), 27.0 (CMe_2), 28.0 (t-Bu), 28.4 (t-Bu), 29.2 (C7), 32.9 (C8), 33.8 (C5), 53.2 (C9), 60.4 (C4), 75.9 (C3), 77.5 (C2), 80.0 (t-Bu) 82.4 (t-Bu), 112.4 CMe$_2$, 155.4 (CO), 170.4 (C1), 171.2 (C10); MS (APCI) m/z 461 (100, MH$^+$).

4.16. *S*-(4-Amino-*N*-*tert*-butoxycarbonyl-4,5-dideoxy-2,3-*O*-isopropylidene-D-ribono-1,4-lactam-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (19b). Procedure E

DMAP (18.8 mg, 0.15 mmol) and (Boc)$_2$O (46.4 mg, 0.21 mmol) were added to a stirred solution of compound **19a** (27 mg, 0.06 mmol) in CH_2Cl_2 (2 mL) at ambient temperature under Ar atmosphere. After 48 h, the reaction mixture was quenched with H_2O (5 mL) and partitioned between CH_2Cl_2//$NaHCO_3$/H_2O. The organic layer was washed (brine), dried ($MgSO_4$) and evaporated. The residue was column chromatographed (30 → 40% EtOAc/hexane) to give **19b** (30 mg, 93%) as a colorless oil: ^{1}H NMR $\delta\delta$ 1.36 (s, 3, CH$_3$), 1.43 (s, 9, t-Bu), 1.45 (s, 3, CH3), 1.46 (s, 9, t-Bu), 1.54 (s, 9, t-Bu), 1.82–1.87 (m, 1, H8), 2.02–2.04 (m, 1, H8'), 2.49–2.55 (m, 1, H7), 2.58–2.62 (m, 1, H7'), 2.70 (dd, J = 7.1, 14.2 Hz, 1, H5), 2.95 (dd, J = 2.5, 14.2 Hz, 1, H5'), 4.22–4.24 (m, 1, H9), 4.23 (dd, J = 2.5, 7.1 Hz, 1, H4), 4.46 (d, J = 5.5 Hz, 1, H3), 4.80 (d, J = 5.5 Hz, 1, H2), 5.08 (br d, J = 7.9 Hz, 1, NH); ^{13}C NMR δ 25.6 (CMe_2), 26.9 (CMe_2), 28.0 (t-Bu), 28.3 (t-Bu), 28.4 (t-Bu), 28.9 (C7), 33.2 (C8), 36.6 (C5), 53.2 (C9), 58.0 (C4), 76.7 (C3), 79.1 (C2), 82.3 (2× t-Bu), 84.1 (t-Bu), 112.7 CMe$_2$, 149.9 (CO), 155.5 (CO), 171.2 (C10), 174.0 (C1); MS (ESI) m/z 583 (100, [M+Na]$^+$).

4.17. *S*-(4-Amino-*N*-benzyl-4,5-dideoxy-D-ribono-1,4-lactam-5-yl)-L-homocysteine (20)

Treatment of **18** (40 mg, 0.07 mmol) with TFA by Procedure C (step *a*, 3 h) gave crude **20** as colorless oil. RP-HPLC purification (5% CH_3CN/H_2O for 30 min followed by gradient 5 → 90% CH_3CN/H_2O for 30 min at 2.5 mL/min; t_R = 45 min) gave **20** (12 mg, 48%): ^{1}H NMR (D_2O) δ 1.98–2.11 (m, 2, H8,8'), 2.57–2.61 (m, 2, H7,7'), 2.71 (dd, J = 8.4, 14.0 Hz, 1, H5), 2.82 (dd, J = 3.9, 14.0 Hz, 1, H5'), 3.57 (dd, J = 3.6, 8.3 Hz, 1, H4), 3.78 ('t', J = 5.8 Hz, 1, H9), 4.36 (d, J = 5.3 Hz, 1, H3), 4.37 (d, J = 14.7 Hz, 1, Bn), 4.72 (d, J = 5.2 Hz, 1, H2), 4.78 (d, J = 14.7 Hz, 1, Bn), 7.33–7.46 (m, 5, Bn); ^{13}C NMR (D_2O) δ 27.7 (C7), 30.3 (C8), 30.8 (C5), 45.2 (Bn), 53.6 (C9), 63.9 (C4), 70.0 (C3), 70.1 (C2), 128.0, 128.1, 128.0, 135.1 (Bn), 173.9 (C1), 174.7 (C10); MS (APCI) m/z 355 (100, MH$^+$); HRMS (AP-ESI) m/z calcd for $C_{16}H_{22}N_2NaO_5S$ [M+Na]$^+$ 377.1142; found 377.1156.

4.18. *S*-(4-Amino-4,5-dideoxy-D-ribono-1,4-lactam-5-yl)-L-homocysteine (21)

Treatment of **19a** (30 mg, 0.065 mmol) with TFA by Procedure C (step *a*, 3 h) gave crude **21** as colorless oil. RP-HPLC purification (5% CH_3CN/H_2O at 2.5 mL/min; t_R = 16 min) gave **21** (10 mg, 58%): ^{1}H NMR (D_2O) δ 2.10–2.28 (m, 2, H8,8'), 2.75–2.77 (m, 3, H5',7,7'), 2.83–2.84 (m, 1, H5), 3.73 ('t', J = 6.5 Hz, 1, H4), 3.95 ('t', J = 5.8 Hz, 1, H9), 4.29 ('d', J = 5.2 Hz, 1, H3), 4.57 (d, J = 5.2 Hz, 1, H2); ^{13}C NMR (D_2O) $\delta\delta$ 26.2 (C7), 28.5 (C8), 32.7 (C5), 58.8 (C4), 51.1 (C9), 69.7 (C2), 70.5 (C3), 170.9 (C1), 176.1 (C10); MS (ESI) m/z 264 (100, M$^+$); HRMS (AP-ESI) m/z calcd for $C_9H_{17}N_2O_5S$ [MH]$^+$ 265.0853; found 265.0859.

4.19. *S*-(4-Amino-*N*-*tert*-butoxycarbonyl-4,5-dideoxy-2,3-isopropylidene-α/β-D-ribofuranos-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (22)

Procedure F. LiEt$_3$BH (1 M/THF; 125 μL, 0.125 mmol) was added to a stirred solution of **19b** (28 mg, 0.05 mmol) in anhydrous THF (1 mL) at −78 °C under N_2 atmosphere. After 30 min, the solution was quenched with water and volatiles were evaporated. The residue was partitioned (EtOAc//·$NaHCO_3$/H_2O), washed (brine) and dried ($MgSO_4$). The resulting oil was chromatographed (40% EtOAc/hexane) to give **22** [26 mg, 92%; mixture of anomers (3:2) which appear as a set of rotamers as colorless oil: ^{1}H NMR δ 1.30 (s, 3, CH$_3$), 1.43 (s, 12, t-Bu, CH$_3$), 1.46 (s, 9, t-Bu), 1.48 (s, 9, t-Bu), 1.83–1.94 (m, 1, H8), 2.03–2.10 (m, 1, H8'), 2.51–2.62 (m, 3, H7,7',5), 2.83 (dd, J = 3.7, 13.5 Hz, 0.6, H5'), 2.92 (dd, J = 3.5, 13.7 Hz, 0.4, H5'), 3.45 (dd, J = 3.5, 10.6 Hz, 0.3, H4), 3.99–4.28 (m, 1.7, H4,9), 4.57 (d, J =5.8 Hz, 0.6, H3), 4.59 (d, J = 5.9 Hz, 0.4, H3), 4.66 (d, J = 6.7 Hz, 0.4, H2), 4.72 (d, J = 5.8 Hz, 0.6, H2), 5.09 (br d, J = 7.2 Hz, 0.6, NH), 5.32 (br d, J = 7.8 Hz, 0.4, NH), 5.39 (s, 0.4, H1), 5.50 (s, 0.6, H1); ^{13}C NMR δ (major isomer) 24.8 (CMe_2), 26.7 (CMe_2), 28.0 (t-Bu), 28.3 (t-Bu), 28.4 (t-Bu), 29.7 (C7), 32.9 (C8), 34.7 (C5), 53.2 (C9), 63.9 (C4), 81.2 (2 × t-Bu), 82.3 (t-Bu), 82.8 (C2), 84.4 (C3), 87.1 (C1), 112.7 (CMe$_2$), 154.3 (CO), 155.4 (CO), 171.3 (C10); MS (ESI) m/z 585 (100, [M+Na]$^+$).

4.20. *S*-(4-Amino-4,5-dideoxy-α/β-D-ribofuranos-5-yl)-L-homocysteine (23)

Treatment of **22** (24 mg, 0.04 mmol) with TFA by Procedure C (step *a*, 1 h; step *b*, 10 h at 0 °C) gave crude **23**. RP-HPLC (5% CH_3CN/H_2O at 2.5 mL/min; t_R = 14 min) yielded **23** (8 mg, 72%) as a light yellow oil of a mixture of anomers (3:2): ^{1}H NMR (D_2O) δ 2.15–2.24 (m, 1, H8), 2.26–2.37 (m, 1, H8'), 2.75–2.82 (m, 2, H7,7'), 2.84–2.98 (m, 1, H5,5'), 3.09–3.17 (m, 1, H5,5'), 3.55–3.69 (m, 0.6, H4), 3.76–3.83 (m, 0.4, H4), 4.15–4.21 (m, 2, H2,3,9), 4.23–4.25 (m, 0.4, H2), 4.34 (dd, J = 4.8, 6.3 Hz, 0.6, H3), 5.27 (d, J = 2.6 Hz, 0.6, H1), 5.41 (d, J = 2.2 Hz, 0.4, H1); ^{13}C NMR δ (major isomer) 25.8 (C7), 28.7 (C8), 30.7 (C5), 51.3 (C9), 58.8 (C4), 72.0 (C3), 73.0 (C2), 86.5 (C1), 171.2 (C10); MS (ESI) m/z 267 (50, MH$^+$), 249 (100, [M-17]$^+$).

4.21. 4-Amino-*N*-*tert*-butoxycarbonyl-5-*O*-*tert*-butyldimethylsilyl-4-deoxy-2,3-*O*-isopropylidene-α/β-D-ribofuranose (24a)

Reduction of **15a**[44] (68 mg, 0.17 mmol) with LiEt$_3$BH (1 M/THF; 0.43 mL, 0.43 mmol) in anhydrous THF (2 mL) at -78 °C by the procedure F gave **24a**[44] (68 mg, 100%) as a colorless oil with data as reported.

4.22. 4-Amino-*N*-*tert*-butoxycarbonyl-4-deoxy-2,3-*O*-isopropylidene-α/β-D-ribofuranose (24b)

Desilylation of **24a** (55 mg, 0.13 mmol) with TBAF (1 M/THF, 0.19 mL, 0.19 mmol), as described for **14b**, [column chromatography (50 → 60% EtOAc/hexane)] gave **24b** (39 mg, 96%) as a colorless oil of a mixture of anomers (3:2): ^{1}H NMR δ 1.30 (s, 3, CH_3), 1.40 (s, 3, CH_3), 1.46 (s, 5.4, *t*-Bu), 1.49 (s, 3.6, *t*-Bu), 2.87 (br s, 0.4, OH), 3.15 (br s, 0.6, OH), 3.60–3.77 (m, 1.4, H5,5'), 3.84–3.90 (m, 0.8, H5,OH), 4.10 (br s, 0.6, H4), 4.27 (br s, 0.4, H4), 4.30 (br s, 0.6, OH), 4.55 (d, *J* = 5.9 Hz, 1, H3), 4.73 (d, *J* = 5.9 Hz, 0.4, H2), 4.77 (d, *J* = 5.9 Hz, 0.6, H2), 5.36 (d, *J* = 5.7 Hz, 0.4, H1), 5.51 (s, 0.6, H1); ^{13}C NMR (major anomer) δ 24.6 (C*Me*$_2$), 26.6 (C*Me*$_2$), 28.3 (*t*-Bu), 62.6 (C5), 65.8 (C4), 81.9 (C2), 81.3 (*t*-Bu), 85.3 (C3), 86.4 (C1), 111.4 (*C*Me$_2$), 154.3 (CO); ^{13}C NMR (minor anomer) δ 24.7 (C*Me*$_2$), 26.7 (C*Me*$_2$), 28.3 (*t*-Bu), 62.6 (C5), 65.8 (C4), 81.2 (C2), 81.3 (*t*-Bu), 86.4 (C3), 86.5 (C1), 111.4 (*C*Me$_2$), 153.5 (CO); MS (APCI) *m/z* 272 (50, [M-OH$^+$]), 213 [100, [MH-Boc-OH+CH_3CN]$^+$).

4.23. 4-Amino-4-deoxy-α/β-D-ribofuranose (25a)

Treatment of **24b** (27 mg, 0.09 mmol) with TFA by Procedure C (step *a*, 5 h; step *b*, 6 h at 0 °C) gave crude **25a**[43] (13 mg, 92%) as a light yellow oil of a mixture of anomers (α/β, 0.65:0.35): ^{1}H NMR (D_2O; pD = 5–6) 3.52–3.53 (m, 0.35, H4), 3.56–3.59 (m, 0.65, H4), 3.76–3.78 (m, 0.65, H3), 3.85 (dd, *J* = 6.0, 13.1 Hz, 0.35, H5), 3.86 (dd, *J* = 2.1, 12.8 Hz, 0.65, H5), 3.97–4.00 (m, 0.35, H3), 4.06 (dd, *J* = 3.0, 4.0 Hz, 0.35, H2), 4.10 (dd, *J* = 2.8, 13.5 Hz, 0.35, H5'), 4.16 (dd, *J* = 3.0, 12.8 Hz, 0.65, H5'), 4.20 ('t', *J* = 3.4 Hz, 0.65, H2), 4.84 (d, *J* = 1.3 Hz, 0.35, H1), 5.12 (d, *J* = 4.0 Hz, 0.65, H1); ^{13}C NMR (major anomer) δ 50.1 (C4), 58.9 (C5), 65.6 (C2), 69.8 (C3), 94.1 (C1); ^{13}C NMR (minor anomers) δ 49.9 (C4), 61.5 (C5), 63.6 (C2), 69.8 (C3), 94.1 (C1); MS (APCI) *m/z* 150 (100, MH$^+$).

The ^{1}H NMR (D_2O) at pD = 11 showed a singlet at 7.76 ppm which suggested formation of imine **25c**.

4.24. 4-Amino-4-deoxy-α/β-D-ribose *O*-benzyloxime (26)

A solution of the crude **25a** (13 mg. 0.09 mmol) and *O*-benzylhydroxylamine hydrochloride (43 mg, 0.27 mmol) in anhydrous pyridine (4 mL) was stirred under an atmosphere of nitrogen at room temperature for 12 h. Pyridine was evaporated to afford **26** of sufficient purity (~95%) for spectroscopic characterization together with the excess of $BnONH_2$ used: ^{1}H NMR (MeOH-d_4) δ 3.52 ('dt', *J* = 4.1, 8.4 Hz, 1, H4), 3.79 (dd, *J* = 3.8, 11.5 Hz, 1, H5), 3.85 (dd, *J* = 4.4, 8.7 Hz, 1, H3), 3.94 (dd, *J* = 8.4, 11.5 Hz, 1, H5'), 4.13 (dd, *J* = 6.8, 8.7 Hz, 1, H2), 4.92–5.16 (2H, Bn; signals for benzylic protons were within the envelope of the solvent peak but cross peaks between them were observed in COSY), 7.41 (1, H1; signal for H1 was within the envelope of protons from benzyl group but cross peaks of H1 to H2 were observed in COSY), 7.35–7.47 (m, 5H, Bn); ^{13}C NMR δ 56.2 (C4), 58.8 (C5), 71.2 (C3), 71.6 (C2), 77.0 (Bn, confirmed by HETCOR), 128.9, 129.3, 129.5, 139.1 (Bn), 151.9 (C1); MS (ESI) *m/z* 255 (100, MH$^+$).

4.25. *S*-(4-Amino-2,3,4,5-tetradeoxy-D-*glycero*-pentono-1,4-lactam-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (33)

Treatment of **27**[45] (5*S*; 18 mg, 0.1 mmol) with protected L-Hcy (35 mg, 0.12 mmol) by Procedure B (step *a* and *b*, 48 h) gave 50.5 mg of the yellow oil. This material was column chromatographed (EtOAc/MeOH, 19:1) to give **33** (31 mg, 79%) as a colorless oil: ^{1}H NMR δ 1.45 (s, 9), 1.48 (s, 9), 1.77–1.92 (m, 2, H3,8), 2.02–2.14 (m, 1, H8'), 2.27–2.47 (m, 3, H2,2',3'), 2.51–2.57 (dd, *J* = 8.1, 13.3 Hz, 1, H5), 2.57–2.65 (m, 2, H7,7'), 2.72 (dd, *J* = 5.1, 13.2 Hz, 1, H5'), 3.80 ('quin', *J* = 6.4 Hz, 1, H4), 4.28 (br 'd', *J* = 3.7 Hz, H9), 5.20 (br d, *J* = 7.1 Hz, 1, NHBoc), 6.45 (br s, 1, CONH); ^{13}C NMR δ 26.7 (C3), 28.0 (*t*-Bu), 28.3 (*t*-Bu), 28.3 (C7), 30.1 (C2), 33.2 (C8), 38.7 (C5), 53.3 (C9), 53.7 (C4), 79.9 (*t*-Bu), 82.3 (*t*-Bu), 155.4 (CO), 171.2 (C10), 177.7 (C1); HRMS (AP-ESI) *m/z* calcd for $C_{18}H_{33}N_2O_5S$ [MH]$^+$ 389.2105; found 389.2110.

4.26. *S*-(4-Amino-2,3,4,5-tetradeoxy-D-*glycero*-pentono-1,4-lactam-5-yl)-L-homocysteine (34)

Compound **33** (9 mg, 0.02 mmol) was dissolved in TFA (0.7 mL), and the resulting mixture was stirred at ambient temperature for 60 min. The reaction mixture was evaporated, and coevaporated with toluene to give a trifluoroacetate of **34** (7.5 mg, 95%) as a colorless oil: ^{1}H NMR (D_2O) δ 1.84–1.94 (m, 1, H3), 2.12–2.23 (m, 1, H8), 2.23–2.31 (m, 1, H8'), 2.31–2.37 (m, 1, H3',), 2.37–2.52 (m, 2, H2,2'), 2.73 (dd, *J* = 6.5, 13.6 Hz, 1, H5), 2.76 (t, *J* = 7.6 Hz, 2, H7,7'), 2.82 (dd, *J* = 5.4, 13.6 Hz, 1, H5'), 3.99 ('quin', *J* = 6.3 Hz, 1, H4), 4.17 (t, *J* = 6.4 Hz, 1, H9); ^{13}C NMR (D_2O) δ 25.3 (C3), 27.1 (C7), 29.7 (C8), 29.9 (C2), 36.8 (C5), 51.9 (C9), 54.3 (C4), 172.0 (C10), 181.5 (C1); HRMS (AP-ESI) *m/z* calcd for $C_9H_{17}N_2O_3S$ [MH]$^+$ 233.0954; found 233.0957.

4.27. *S*-(4-Amino-*N*-*tert*-butoxycarbonyl-2,3,4,5-tetradeoxy-D-*glycero*-pentono-1,4-lactam-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (35)

Treatment of **33** (40 mg, 0.1 mmol) in CH_2Cl_2 (2 mL) with DMAP (20 mg, 0.16 mmol), and $(Boc)_2O$ (63 mg, 0.29 mmol) by procedure E [column chromatography (30 → 35% EtOAc/hexane)] gave **35** (42 mg, 83%) as a colorless oil: ^{1}H NMR (isomers ratio ~3:2) δ 1.45 (s, 9H), 1.47 (s, 9H), 1.55 (s, 9H), 1.82–1.94 (m, 1, H8), 1.99–2.22 (m, 3, H3,3',8'), 2.44 & 2.45 (2× ddd, *J* = 2.5, 9.6, 17.9 Hz, 1, H2), 2.54–2.70 (m, 4, H2',5,7,7'), 2.94 ('dt', *J* = 3.5, 13.4 Hz, 1, H5'), 4.22–4.32 (m, 2, H4,9), 5.11 & 5.16 (2× br d, *J* = 7.9 Hz, 1, NHBoc); ^{13}C NMR δ 22.0 (C3), 28.0 (*t*-Bu), 28.1 (*t*-Bu), 28.3 (*t*-Bu), 28.8 (C7), 31.1 & 31.2 (C2), 33.0 & 33.2 (C8), 35.1 & 35.5 (C5), 53.2 & 53.3 (C9), 57.2 & 57.5 (C4), 79.8 (*t*-Bu), 82.1 & 82.2 (*t*-Bu), 83.2 & 83.3 (*t*-Bu), 149.9 & 150.0 (CO), 155.5 (CO), 171.3 (C10), 173.7 & 173.8 (C1); MS (APCI) *m/z* 489 (30, MH$^+$), 389 (100, [MH_2-Boc]$^+$).

4.28. *S*-(4-Amino-2,3,4,5-tetradeoxy-D-*glycero*-pentono-1,4-lactam-5-yl)-D/L-homocysteine (36)

Treatment of **27**[45] (62 mg, 0.35 mmol) with D/L-Hcy (52 mg, 0.385 mmol)/NaH (44 mg, 1.1 mmol; 60%/ mineral oil) by procedure G gave crude **36**. RP-HPLC purification (5% CH_3CN/H_2O at 2.5 mL/min; t_R = 14 min) gave **36** (60.5 mg, 75%) as a Na salt: ^{1}H NMR (D_2O) δ 1.83–1.95 (m, 2, H3,8), 1.95–2.04 (m, 1, H8'), 2.29–2.38 (m, 1, H3'), 2.38–2.53 (m, 2, H2,2'), 2.65 (t, *J* = 7.7 Hz, 2, H7,7'), 2.72 (dd, *J* = 6.5, 13.6 Hz, 1, H5), 2.81 (dd, *J* = 5.6, 13.6 Hz, 1, H5'), 3.47 (br s, 1, H9), 3.99 ('quin', *J* = 6.3 Hz, 1, H4); ^{13}C NMR (D_2O) δ 25.4 (C3), 27.9 & 28.0 (C7), 29.9 & 29.9 (C2), 33.5 & 33.6 (C8), 36.8 (C5), 54.4 (C4), 54.8 (br, C9), 180.4 (br, C10), 180.5 (C1); MS (APCI) *m/z* 233 (100, MH$^+$); (ESI) *m/z* 233 (100, MH$^+$).

Stirring sodium salt of **36** (10 mg) in TFA (1 mL) for 1 h at ambient temperature and evaporation of volatiles gave trifluoroacetate of **36** as a mixture of diastereomers (9*R*/*S*, ~1:1): ^{1}H NMR (D_2O) δ 1.79–1.89 (m, 1, H3), 2.09–2.19 (m, 1, H8), 2.21–2.28 (m, 1, H8'), 2.28–2.33 (m, 1, H3',), 2.34–2.48 (m, 2, H2,2'), 2.678 (dd, *J* = 6.5, 13.6 Hz, 0.5, H5, 9*R*), 2.683 (dd, *J* = 6.5, 13.6 Hz, 0.5, H5, 9*S*), 2.72 (t, *J* = 7.6 Hz, 2, H7,7'), 2.776 (dd, *J* = 5.4, 13.6 Hz, 0.5, H5', 9*R*), 2.781 (dd, *J* = 5.4, 13.6 Hz, 0.5, H5', 9*S*), 3.94 ('quin', *J* = 6.3 Hz, 1, H4), 4.181 (t, *J* = 6.4 Hz, 1, H9*R*), 4.186 (t, *J* = 6.4 Hz, 1, H9*S*).

Chemical shifts observed for TFA salt of **36**(9*R*/*S*) were different from its sodium salt but parallel the signals for the trifluoroacetate of **34**(9*S*) derived from Hcy.

4.29. *S*-(4-Amino-*N*-*tert*-butoxycarbonyl-2,3,4,5-tetradeoxy-α/β-D-*glycero*-pentofuranos-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (37)

Treatment of 35 (42 mg, 0.086 mmol) in CH_2Cl_2 (2 mL) with $LiEt_3BH$ (1 M/THF, 0.22 mL, 0.22 mmol), by procedure F [quenched with MeOH (3 mL) at low temp., column chromatography (30 → 40% EtOAc/hexane)] gave 37 (31 mg, 73%; colorless oil) as a complex mixture of isomers (^1H, and ^{13}C NMR): MS (APCI) *m/z* 473 (100, $[M-17]^+$), 373 (55, $[M-Boc-18]^+$); (ESI) *m/z* 473 (50, $[M-17]^+$), 373 (100, $[M-Boc-18]^+$).

4.30. *S*-(4-Amino-2,3,4,5-tetradeoxy-α/β-D-D-*glycero*-pentofuranos-5-yl)-L-homocysteine (38)

Treatment of **37** (20 mg, 0.04 mmol) with an excess of TFA (1 mL) by Procedure C (step a, 2 h at ambient temperature) gave trifluoroacetate of **38** (13 mg, 95%; light yellow oil) as a complex mixture of isomers accompanied (~10%) by the open aldehyde form [^1H NMR δ 8.89 (s)]: MS (APCI) *m/z* 217 (100, $[M-17]^+$).

4.31. 1-Amino-1,4-anhydro-1,*N*-didehydro-2,3-*O*-isopropylidene-5-*O*-methanesulfonyl-1-methyl-D-ribitol (40)

Treatment of **39**[47] (48.5 mg, 0.26 mmol) with MsCl (0.031 mL, 45 mg, 0.39 mmol) in the presence of Et_3N (0.11 mL, 80 mg, 0.79 mmol) by Procedure A [3 h; column chromatography (EtOAc → 10% MeOH/EtOAc)] gave **40** (59 mg, 85%) as a colorless oil: ^1H NMR δ 1.38 (s, 3), 1.39 (s, 3), 2.16 (d, *J* = 1.0 Hz, 3, N=CCH$_3$), 3.00 (s, 3, Ms), 4.38 (s, 1, H4), 4.39 (dd, 1, *J* = 3.6, 11.2 Hz, 1, H5), 4.53 (dd, *J* = 4.2, 11.3 Hz, 1, H5'), 4.64 (d, *J* = 5.8 Hz, 1, H3), 4.95 ('quin', *J* = 5.6 Hz, 1, H2); ^{13}C NMR δ 17.1 (N=C*Me*), 25.7 (C*Me*$_2$), 26.8 (C*Me*$_2$), 37.4 (Ms), 69.7 (C5), 75.0 (C4), 79.8 (C3), 87.4 (C2), 112.4 *C*Me$_2$, 177.0 (C=N); MS (APCI) *m/z* 264 (100, MH^+).

4.32. *S*-(1-Amino-1,4-anhydro-5-deoxy-1,*N*-Didehydro-2,3-*O*-isopropylidene-1-methyl-D-ribitol-5-yl)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (41)

Treatment of **40** (49.5 mg, 0.19 mmol) with protected L-Hcy (82 mg, 0.284 mmol) by Procedure B (step *a* and *b*, 36 h) gave 124 mg of yellow oil. Crude product was chromatographed (50 → 60% EtOAc/hexane) to give 41 as an colorless oil (73 mg, 85%): ^1H NMR δ 1.35 (s, 3), 1.35 (s, 3),1.43 (s, 9), 1.46 (s, 9), 1.84 ('sx', *J* = 7.3 Hz, 1, C8), 1.98–2.07 (m, 1, H8'), 2.11 (d, *J* = 1.1 Hz, 3, N=C*Me*), 2.54 ('t', *J* = 7.6 Hz, 2, H7,7'), 2.65 (dd, *J* = 6.4, 13.5 Hz, 1, H5), 2.87 (dd, *J* = 4.4, 13.5 Hz, 1, H5'), 4.17–4.27 (m, 1, H9), 4.33 (br 't', *J* = 4.6 Hz, 1, H4), 4.48 (d, *J* = 5.6 Hz, 1, H2) 4.96 (d, *J* = 5.6 Hz, 1, H3) 5.13 (br d, *J* = 7.7 Hz, 1, NH); ^{13}C NMR δ 17.0 (N=C*Me*), 25.7 (C*Me*$_2$), 26.8 (C*Me*$_2$), 28.0 (*t*-Bu), 28.3 (*t*-Bu), 29.2 (C7), 33.1 (C8), 35.3 (C5), 53.3 (C9), 76.5 (C4), 79.8 (*t*-Bu), 81.8 (C2), 82.1 (*t*-Bu), 87.3 (C3), 111.9 (*C*Me$_2$), 155.3 (CO), 171.2 (C10), 174.7 (N=C); MS (APCI) *m/z* 459 (100, MH^+); HRMS (TOF MS-ESI) *m/z* calcd for $C_{22}H_{39}N_2O_6S$ $[MH]^+$ 459.2523; found 459.2523.

4.33. *S*-(1-Amino-1,4-anhydro-5-deoxy-1,*N*-didehydro-2,3-*O*-isopropylidene-1-methyl-D-ribitol-5-yl)-L-homocysteine (42)

Treatment of **41** (62 mg, 0.136 mmol) with TFA by Procedure C (step *a*, ambient temperature) gave **42** (41 mg, 99%) as a colorless oil: ^1H NMR (D_2O) δ 1.38 (s, 3), 1.41 (s, 3), 2.10–2.20 (m, 1, H8), 2.22–2.31 (m, 1, H8'), 2.57 (br s, 3 → 0, exch. with deuterium within few hours, N=C*Me*), 2.75 (t, *J* = 7.3 Hz, 2, H7,7'), 2.99 (d, *J* = 6.2 Hz, 2, H5,5'), 4.17 (t, *J* = 6.4 Hz, 1, H9), 4.73 ('t', *J* = 6.3 Hz, 1, H4), 4.91 (d, *J* = 5.4, 1, H2), 5.64 (d, *J* = 5.3, 1, H3); ^1H NMR (DMSO) δ 1.28 (s, 3), 1.30 (s, 3), 1.95–2.07 (m, 2, H8,8'), 2.08 (s, 3, N=C*Me*), 2.60 (dd, *J* = 7.4, 13.6 Hz, 1, H5) 2.65 ('t', *J* = 7.9 Hz, 2, H7,7'), 2.82 (dd, *J* = 5.2, 13.7 Hz, 1, H5'), 4.01 (br s, 1, H9), 4.21 ('t', *J* = 5.8 Hz, 1, H4), 4.51 (d, *J* = 5.5, 1, H2), 5.10 (d, *J* = 5.5, 1, H3), 7.28 (br s, 3, $^+NH_3$); ^{13}C NMR (D_2O) δ 15.5 ('quin', *J* = 20 Hz, N=C*C*D$_3$), 24.0 (C*Me*$_2$), 25.4 (C*Me*$_2$) 27.4 (C7), 29.5 (C8), 31.6 (C5), 51.4 (C9), 71.2 (C4), 79.0 (C2), 84.0 (C3), 114.2 (*C*Me$_2$), 171.5 (C10), 191.7 (N=*C*CD$_3$); MS (APCI) *m/z* 303 (100, MH^+).

4.34. *S*-(1-Amino-1,4-anhydro-5-deoxy-1,*N*-didehydro-1-methyl-D-ribitol-5-yl)-L-homocysteine (43)

Treatment of **42** (41 mg, 0.136 mmol) with TFA/H_2O by Procedure C (step *b*, TFA/H_2O 4:1, ambient temperature, 12 h) gave homogenous **43** (36 mg, 98%) as a colorless oil: ^1H NMR (D_2O) δ 2.05–2.16 (m, 1, H8), 2.17–2.27 (m, 1, H8'), 2.45 (br s, 3 → 0, exch. with deuterium within few hours, N=C*Me*), 2.73 (dt, *J* = 3.3, 7.3 Hz, 2, H7,7'), 2.78 (dd, *J* = 8.3, 14.4 Hz, 1, H5), 2.92 (dd, *J* = 6.0, 14.3 Hz, 1, H5') 4.14 (t, *J* = 6.4 Hz, 1, H9), 4.35 ('t', *J* = 7.1 Hz, 1, H4), 4.37 (d, *J* = 5.3, 1, H2), 5.12 (d, *J* = 5.5, 1, H3); ^{13}C NMR (D_2O) δ 15.4 ('quin', *J* = 21 Hz, N=C*C*D$_3$), 27.0 (C7), 29.3 (C8), 30.7 (C5), 51.3 (C9), 71.6 (C2), 71.9 (C4), 76.8 (C3), 171.4 (C10), 195.8 (N=*C*CD$_3$); MS (APCI) m/*z* 263 (100, MH^+); HRMS (AP-ESI) *m/z* calcd for $C_{10}H_{19}N_2O_4S$ $[MH]^+$ 263.1060; found 263.1065.

4.35. 1-Amino-1,4-anhydro-5-*O*-*tert*-butyldimethylsilyl-1-deoxy-2,3-*O*-isopropylidene-D-ribitol (44)

To a solution of **7** (109 mg, 0.28 mmol) in EtOH (6 mL) was added 5% Pd/C (300 mg) and stirred under an atmosphere of H_2 at room temperature for 6 h. The mixture was filtered through Celite to remove the catalyst. The Celite bed was washed with ethanol (5 mL) and the filtrate and washings were combined and evaporated. The residue was column chromatographed (30% EtOAc/hexane) to give **44**[50] as a colorless oil (70 mg, 80%). ^1H NMR δ 0.04 (s, 3, CH$_3$), 0.04 (s, 3, CH$_3$), 0.87 (s, 9, *t*-Bu), 1.32 (s, 3, CH$_3$), 1.46 (s, 3, CH$_3$), 2.33 (s, 1, NH), 2.98 ('d', *J* = 2.6 Hz, 2, H1,1'), 3.20 ('dt', *J* = 0.6, 5.8 Hz, 1, H4), 3.52 (dd, *J* = 5.9, 10.3 Hz, 1, H5), 3.62 (dd, *J* = 5.1, 10.3 Hz, 1, H5'), 4.63 (dd, *J* = 0.8, 5.8 Hz, 1, H3), 4.68 ('dt', *J* = 2.6, 5.8 Hz, 1, H2); MS (APCI) *m/z* 288 (100, MH^+).

4.36. 1-Amino-1,4-anhydro-5-*O*-*tert*-butyldimethylsilyl-1,*N*-didehydro-2,3-*O*-isopropylidene-D-ribitol *N*–oxide (45)

A stirred solution of **44** (70 mg, 0.24 mmol) and SeO_2 (0.01 mmol, 1.1 mg) in acetone (3 mL) was cooled to −4 °C under N_2 atmosphere and H_2O_2 (25%) was added slowly (3–4 h) until the reaction was completed (as judged by TLC). Volatiles are evaporated and the residue was partitioned (EtOAc//$NaHCO_3$/H_2O).The organic layer was collected, washed (brine) and dried ($MgSO_4$). The resulting solid was chromatographed (50% EtOAc/hexane) to give **45** (54 mg, 73%) as a white solid with data as reported.[49]

4.37. 1-Amino-1,4-anhydro-1,*N*-didehydro-2,3-*O*-isopropylidene-D-ribitol *N*-oxide (46)

Desilylation of **45** (155 mg, 0.51 mmol) with TBAF (1 M/THF, 0.77 mL, 0.77 mmol) at −4 °C as described for **14b** [column chromatography (10 → 20% MeOH/$CHCl_3$)] gave **46** (40 mg, 42%) as a white solid: ^1H NMR δ 1.34 (s, 3, CH$_3$), 1.41 (s, 3, CH$_3$), 3.87 (dd, *J* = 2.6, 11.9 Hz, 1, H5), 4.02–4.03 (m, 1, H4), 4.14 (dd, *J* = 2.3,

11.9 Hz, 1, H5'), 4.96 (d, J = 6.2 Hz, 1, H3), 5.21 ('dt', J = 1.4, 6.2 Hz, 1, H2), 6.90 (s, 1, H1); ^{13}C NMR δ 25.7 (CMe_2), 27.2 (CMe_2), 58.9 (C5), 77.2 (C3), 78.9 (C4), 80.8 (C2), 111.5 (CMe_2), 134.6 (C1); MS (ESI) m/z 186 (100, M^+).

4.38. 1-Amino-1,4-anhydro-1,*N*-didehydro-2,3-*O*-isopropylidene-5-*O*-methanesulfonyl-D-ribitol *N*–oxide (47)

Treatment of **46** (40 mg, 0.21 mmol) at −4 °C with MsCl (26 μL, 0.32 mmol) by Procedure A [column chromatography (80% → 90% EtOAc/hexane)] gave **47** (24 mg, 56%) as a colorless oil: ^{1}H NMR δ 1.37 (s, 3, CH_3), 1.46 (s, 3, CH_3), 3.04 (s, 3, Ms), 4.23–4.24 (m, 1, H4), 4.55 (dd, J = 1.8, 11.1 Hz, 1, H5), 4.83 (dd, J = 2.4, 11.1 Hz, 1, H5'), 4.91 (dd, J = 1.1, 6.3 Hz, 1, H3), 5.25 ('dt', J = 1.5, 6.4 Hz, 1, H2), 6.99 (s, 1, H1); ^{13}C NMR δ 25.6 (CMe_2), 27.1 (CMe_2), 37.4 (Ms), 65.5 (C5), 75.8 (C3), 77.8 (C4), 78.4 (C2), 112.6 (CMe_2), 134.1 (C1); MS (APSI) m/z 266 (100, MH^+); HRMS (AP-ESI) m/z calcd for $C_9H_{16}NO_6S$ $[MH]^+$ 266.0693; found 266.0682.

4.39. *S*-(1-Amino-1,4-anhydro-5-deoxy-1,*N*-didehydro-2,3-*O*-isopropylidene-D-ribitol-5-yl *N*–oxide)-*N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester (48)

Treatment of **47** (24 mg, 0.09 mmol) with L-homocysteine (38 mg, 0.13 mmol) by Procedure B (step *a* and *b*, 8 h at -20 °C) and purification by column chromatography (80% → 90% EtOAc/hexane) gave **48** as a colorless oil (24 mg, 43%): ^{1}H NMR δ 1.37 (s, 3, CH_3), 1.44 (s, 9, *t*-Bu), 1.45 (s, 3, CH_3), 1.46 (s, 9, *t*-Bu), 1.81–1.90 (m, 1, H8), 1.96–2.09 (m, 1, H8'), 2.52–2.70 (m, 2, H7,H7'), 3.07 (dd, J = 3.5, 14.4 Hz, 1, H5), 3.14 (dd, J = 5.2, 14.4 Hz, 1, H5'), 4.20–4.31 (m, 2, H4,9), 4.71 (d, J = 6.2 Hz, 1, H3), 5.06 (m, 1, NH), 5.31 ('dt', J = 1.4, 6.4 Hz, 1, H2), 6.97 (s, 1, H1); ^{13}C NMR δ 25.6 (CMe_2), 26.1 (CMe_2), 28.0 (*t*-Bu), 28.3 (*t*-Bu), 29.1 (C7), 32.0 (C5), 32.8 (C8), 53.1 (C9), 77.9 (C3), 79.0 (C2), 79.1 (C4), 79.8 (*t*-Bu), 82.2 (*t*-Bu), 112.0 (CMe_2), 133.4 (C1), 155.4 (CO), 171.1 (C10); MS (APCI) m/z 461 (100, MH^+).

4.40. *S*-(1-Amino-1,4-anhydro-5-deoxy-1,*N*-didehydro-D-ribitol-5-yl *N*–Oxide)-L-homocysteine (49)

Treatment of **48** (72 mg, 0.15 mmol) with TFA by Procedure C (step *a*, 5 h; step *b*, 6 h at 0 °C) gave crude **49**. Purification on HPLC (5% CH_3CN/H_2O at 2.5 mL/min; t_R = 10–14 min) afforded **49** (16 mg, 40%) as a white solid: ^{1}H NMR (D_2O) δ 2.01–2.17 (m, 2, H8,8'), 2.83–2.89 (m, 2, H7,7'), 2.97 (dd, J = 6.3, 14.4 Hz, 1,H5), 3.06 (dd, J = 3.8, 14.4 Hz, 1, H5'), 3.73–3.77 (m, 1, H9), 4.08–4.22 (m, 1, H4), 4.40 (dd, J = 3.2, 6.0 Hz, 1, H3), 4.89–4.96 (m, 1, H2), 7.25 (s, 1, H1); ^{13}C NMR δ 27.6 (C7), 29.9 (C5), 30.5 (C8), 53.7 (C9), 70.4 (C3), 78.3 (C4), 80.7 (C2), 141.8 (C1), 173.9 (C10); MS (APCI) m/z 265 (100, MH^+); HRMS (TOF MS-ESI) m/z calcd for $C_9H_{16}N_2O_5SNa$ $[M+Na]^+$ 287.0672; found 287.0664.

4.41. LuxS Inhibition Assay

SRH was prepared by incubating SAH (typically 10 mM) with nucleosidase Pfs (2 μM) overnight at 4 °C and the completion of the reaction was monitored spectrophotometrically by the absorption difference between SAH and adenine ($\Delta\varepsilon_{276} = -1.4\ mM^{-1}\ cm^{-1}$). A typical LuxS reaction (total volume = 1.0 mL) contained 50 mM HEPES (pH 7.0), 150 mM NaCl, 17.8 μM SRH, and 150 μM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). The reaction was initiated by the addition of LuxS (final concentration 0.8 μM) and monitored continuously at 412 nm (ε = 14 000 $M^{-1}\ cm^{-1}$) in a Perkin-Elmer λ20 UV–vis spectrophotometer at room temperature. For compounds that showed time dependent inhibition, the inhibitor and LuxS (1.6 μM) were preincubated for 30 min at 4 °C and the reaction was then initiated by addition of SRH.

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