Bioorganic & Medicinal Chemistry Letters xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of isomeric analogues of *S*-ribosylhomocysteine analogues with homocysteine unit attached to C2 of ribose

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ARTICLE INFO

Article history:
Received 25 January 2017
Revised 1 March 2017
Accepted 2 March 2017
Available online xxxx

ABSTRACT

LuxS (*S*-ribosylhomocysteinase; EC 4.4.1.21) is an enzyme that catalyzes the cleavage of the thioether linkage in the catalytic pathway of *S*-ribosylhomocysteine (SRH) which produces homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD). DPD is the precursor of the signaling molecules known as autoinducer 2 (AI-2) responsible for the bacterial quorum sensing (QS) identified as cell to cell communication. Inhibitors of LuxS should be able to interfere with its catalytic pathway thus preventing the formation of the autoinducer molecules. In this work, the synthesis of 2-deoxy-2-bromo-SRH analogues was attempted by the coupling of the corresponding 2-bromo-2-deoxypentafuranosyl sugars with the homocysteinate anion. The displacement of the bromide from C2 rather than the expected substitution of the mesylate group from C5 was observed leading to a novel isomeric analogue of SRH in which Hcy moiety is attached to a ribose ring via C2-sulfur bond.

Bacteria are known to release a large variety of small molecules. They also produce and respond to diffusible signal molecules (termed autoinducers or pheromones). One of the cell to cell communication subtypes is related to population density in bacteria known as quorum sensing (QS) (Fig. 1). It is shown to be accomplished through the exchange of extracellular signaling molecules called autoinducers.[1–4] 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.[5–9] Pei and coworkers in 2003 proposed the pathway for the catalytic mechanism of *S*-ribosylhomocysteine presented in Scheme 1.

Various *S*-ribosylhomocysteine [SRH] analogues have been designed as mechanistic probes and/or inhibitors of the LuxS enzyme.[10] The most important one among them is the family of SRH analogues that targets mechanistic steps of LuxS catalytic cycle by effecting the initial ring opening step e.g., 1-deoxy-SRH analogue[11] and [4-aza][12] or 4-[thio]-SRH analogues[13] or one of the tautomerization/isomerization steps. These included substrates lacking enolizable hydroxyl group at C3 (e.g., OMe),[14] including mechanistically significant C3 halogenated [3-Br or F]-SRH analogues.[15] Zhou and coworkers synthesized substrate analogue *S*-homoribosyl-L-cysteine[11] and Chbib and coworkers prepared 4-*C*-alkyl/aryl-SRH derivatives[16] which were designed to prevent the final mechanistic step of LuxS catalytic cycle. Moreover, brominated furanone derivatives were found to modify LuxS selectively leading to the covalent inhibition.[17]

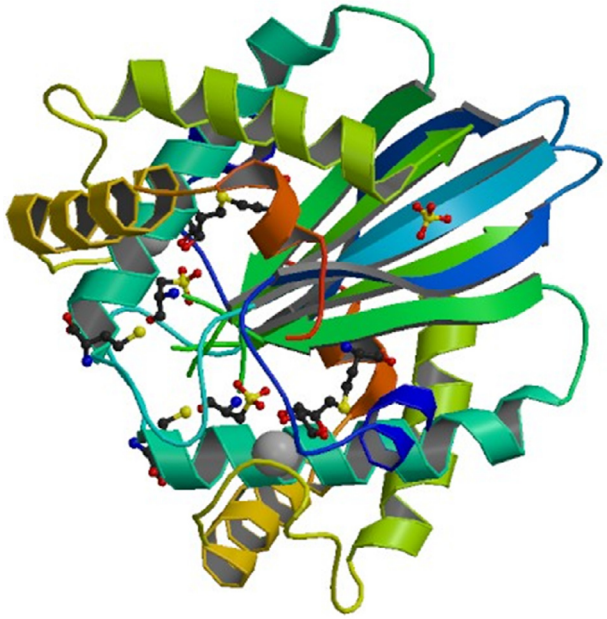

Fig. 1. X-ray crystallography of LuxS enzyme (Resolution: 2.2 Å, R-Value Free: 0.242, R-Value Work: 0.174).

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http://dx.doi.org/10.1016/j.bmcl.2017.03.004
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The initial objective of this research was the design and synthesis of novel LuxS inhibitors in which the hydroxyl group at C2 in the ribofuranose ring of the *S*-ribosylhomocysteine would be substituted by halogens such as bromide. The two main targets of the C2-substituted analogues were 2-deoxy-2-bromo-d-ribosylhomocysteine (2-[Br]-SRH, **10**) and its *arabino*-epimer **11** (Fig. 2). The targeted 2-halo-SRH analogues were 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 **2**, Scheme 1) during the interaction with LuxS. Herein we reported synthesis of a novel isomeric analogues of SRH in which Hcy moiety is attached to a ribose ring via C2-sulfur bond.

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* **10** and *arabino* **11** 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 **11** might prevent S_N2 displacement which requires a nucleophilic attack by Cys84 from the opposite site of the leaving group (bromide). The fact that targeted 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. 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 2) or opened ring form of the bromo-substrate (path 2, Scheme 2). The formation of enzyme-inhibitor complexes can be detected using mass spectroscopy.

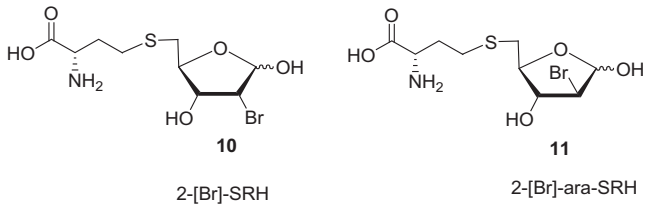
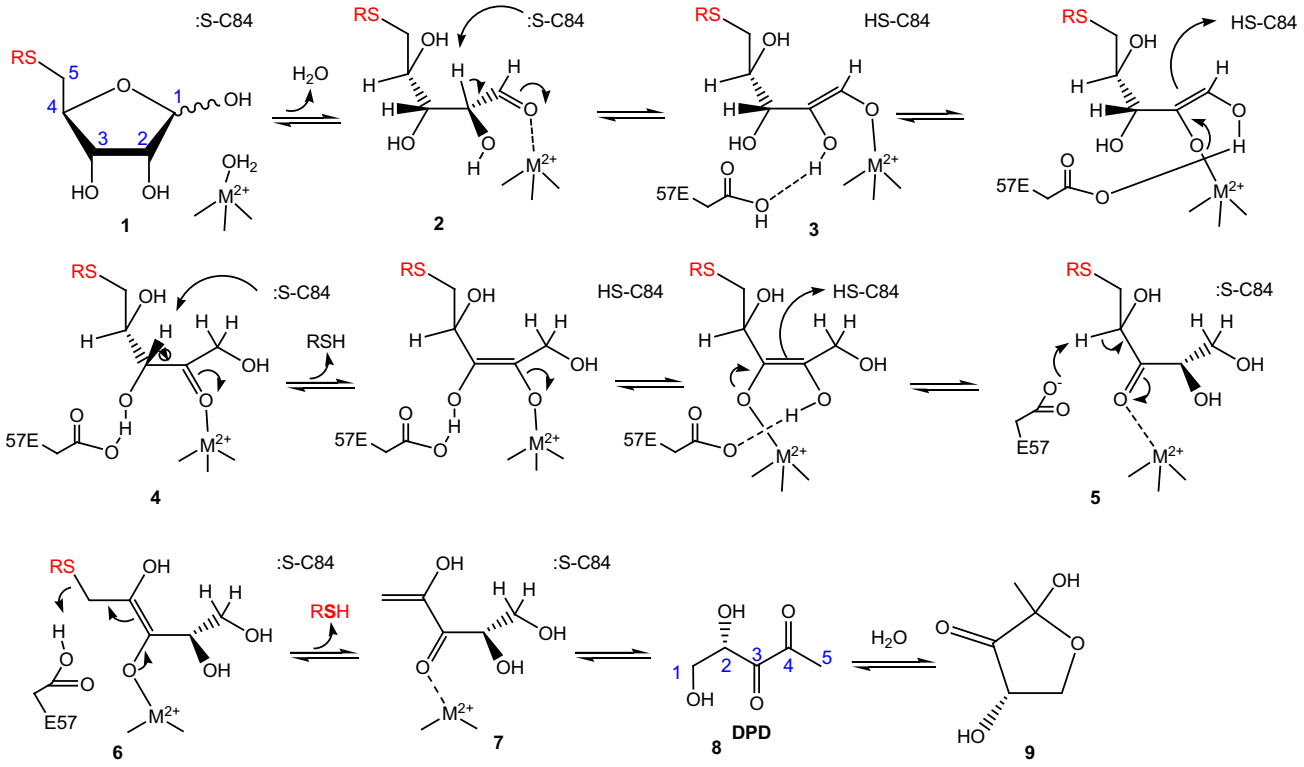


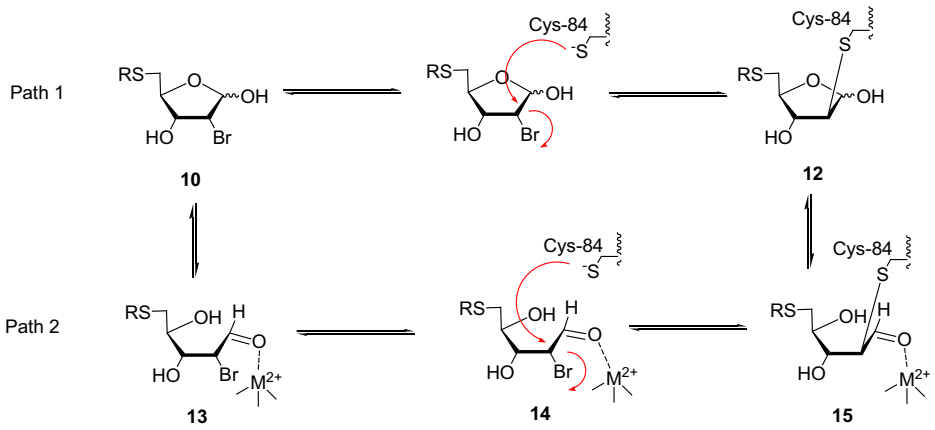
Fig. 2. Targeted 2-deoxy-2-bromo *S*-ribosylhomocysteine analogues.

Synthesis of 2-bromo-2-deoxy-ribono/arabinonolactones

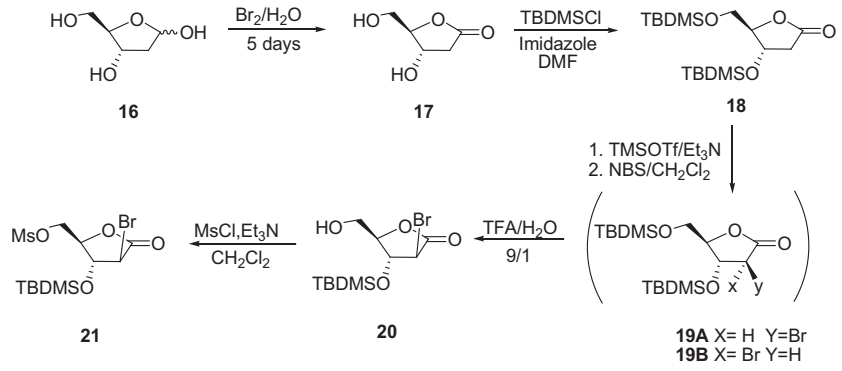
The synthesis of the 2-bromo-2-deoxy-SRH **10** was divided into two steps. In the first step the 2-bromo-2-deoxy precursor **21** was prepared (Scheme 3), while the second step was envisioned as the coupling between **21** and the homocysteine thiolate **25**. The bromo-sugar precursor **21** was prepared according to the literature report.[18] Thus, oxidation of the 2-deoxyribose **16** with Br_2 /H_2O yielded 2-deoxyribonolactone **17**. Treatment of the resulting **17** with TBDMSCl produced a disilylated ribonolactone **18** with 80% yield. Direct bromination of **18** with NBS, following the procedure developed by Sauve,[18] led to the formation of the bromo-lactone **19** as a mixture of *arabino*/*ribo* epimers in 2:1 ratio**.** The formation of **19A** and **19B** 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)[18] ratio. The *arabino*/*ribo* epimers of **19** were successfully separated using column chromatography. Compound **19** was considered as a precursor for the synthesis of the mesylated derivative **21** intended to be coupled with the homocysteine thiolate to afford the desired final product **26**. Thus, deprotection of **19** with TFA/H_2O (9:1) effected selective removal of TBDMS group from the



Scheme 1. Proposed mechanism of LuxS-catalyzed reaction.[5,6]



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

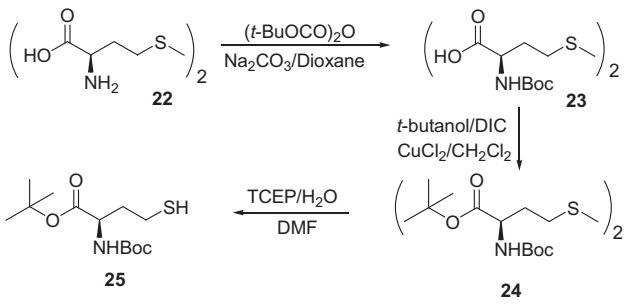


Scheme 3. Synthesis of the 2-bromo-2-deoxy-5-*O*-mesyl-ribono/arabinono lactone derivatives.

primary 5-hydroxyl group of **19** to afford **20** in 90% yield. Treatment of **20** with mesyl chloride in pyridine produced the desired 5-*O*-mesyl derivative **21** in 60% yield (Scheme 3).

Synthesis of protected homocysteine

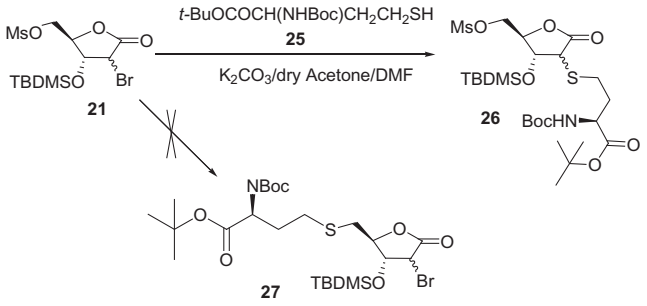
The protected homocysteine precursor **25** was prepared starting from the commercially available l-homocystine **22** following the literature protocol[19] (Scheme 4). Thus, treatment of the homocystine **22** with di-*t*-butyl dicarbonate gave *N*-Boc protected homocysteine **23**. Next, the carboxylic group in **23** was protected as *tert*-butyl ester upon treatment with diisopropylcarbodiimide (DIC) and *t*-butanol. The reduction of the disulfide bond in **24** with triscarboxyethylphosphine hydrochloride (TCEP) followed by aqueous workup afforded the homocysteine **25** of the appropriate purity for direct coupling with the sugar precursors. It is noteworthy that employing TCEP[14] as water soluble reducing agent rather than organic soluble trialkylphosphines[19,20] simplified the preparation of the protected homocysteine substrate **25**.



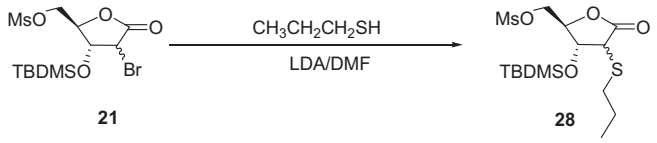
Scheme 4. Synthesis of homocysteine-thiolate.

Attempted couplings

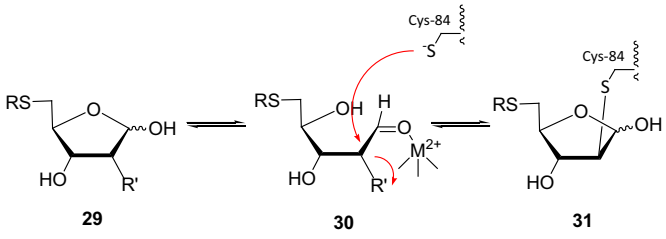
Two methods for the displacement of the mesylate group in the ribonolactone **21** with thiolate anion generated from homocysteine **25** were attempted**.** In the first approach, LDA was used as a base and DMF as a solvent. Thus, coupling of **25** and **21** in DMF resulted in the formation of the 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 a second approach K_2CO_3 was used as a base and dry acetone as solvent. The resulting complex reaction mixture was purified on column chromatography but desired product **27** was not isolated (Scheme 5). Proton NMR of the crude reaction mixture showed however the



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



Scheme 6. Model reaction of bromo-lactones with propylthiol



Scheme 7. Plausible inhibition of LuxS by 2-deoxy-2-propylthiol-*S*-ribosylhomocysteine.

characteristic pattern for the protons of homocysteine and sugar moieties. However, the mass spectra analysis of the major fraction, showed no presence of the typical pattern of bromine isotopes (M/M+2). The unexpected displacement reactions at C2 may be attributed to the fact that the bromine at the C2 is α to an ester carbonyl in the ribonolactone precursor **21** which is also a good leaving group and might be involved in nucleophilic substitution reaction with homocysteinate salts leading to **26**. To prove this hypothesis, I carried out a reaction between **21** with model alkylthiol.

In order to optimize conditions for the reactions of bromo-lactone **21** with thiols, I carried out reactions between **21** and propylthiol instead of homocysteine under different conditions**.** Thus, treatment of **21** (*R/S*, 60:40) with 1 equiv. of the propylthiolate generated from propylthiol/LDA in DMF at 0 °C resulted in the formation of new product and disappearance of substrate **21**. Purification of the crude reaction mixture on the silica column chromatography gave 1:1 mixture of the 2-*S*-propyl substituted 2-thiolactone **28** (as 1:1 mixture of the *arabino/ribo* epimers) in overall yield of 61% (Scheme 6). Structure for **28** 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 **28** 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.

A synthesis of novel class of 2-halo substituted *S*-ribosylhomocysteine (SRH) analogues designed as potential inhibitor of the *S*-ribosylhomocyteinase enzyme (LuxS) was attempted. The 2-bromo-2-deoxy pentafuranosyl precursors were obtained by direct bromination of the suitably protected 2-deoxyribono-1,4-lactone with *N*-bromosuccinimide in the presence of trimethylsilyl triflate. The 5-hydroxyl group in the resulting 2-bromo-2-deoxyarabinono-1,4-lactone was activated by the conversion to the methylsulfonate ester. Attempted 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 leading to a novel isomeric analogue of SRH in which Hcy moiety is attached to a ribose ring via C2-sulfur bond. 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 interaction of LuxS with novel isosteric isomer of SRH with Hcy unit attached to ribose at C2 position rather than C5 in my finding, could interact with LuxS via ring opening.

Inhibition of LuxS enzyme by the α-keto substituent analogue **29** might occur by the nucleophilic displacement of R′ (R′ = S-CH_2-CH_2-CH_3). Based on the previous studies with 3-halo-SRH analogues,[15] it is reasonable to expect that after ring opening the reactive α-keto substituent intermediate **30** might be a substrate for nucleophilic substitution by the active site Cys-84 leading to a covalent inhibition as depicted in Scheme 7**.**

Notes

LuxS PDB ID code 1IE0.

Acknowledgment

I would like to thank Dr. Stanislaw F. Wnuk from Florida International University for providing his laboratory facility and a helpful discussion during the preparation of this manuscript.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.03.004.

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Please cite this article in press as: Chbib C. Bioorg. Med. Chem. Lett. (2017), http://dx.doi.org/10.1016/j.bmcl.2017.03.004