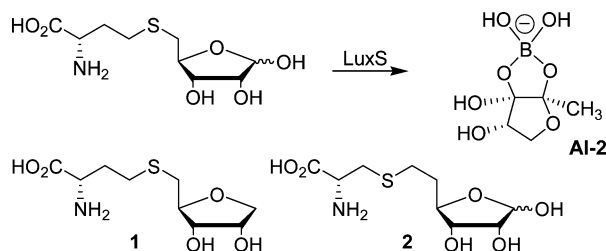


Synthesis of LuxS Inhibitors Targeting  
Bacterial Cell–Cell CommunicationJoshua F. Alfaro,<sup>†</sup> Tiao Zhang,<sup>†</sup> DonRaphael P. Wynn,<sup>†,‡,§</sup>  
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## ABSTRACT

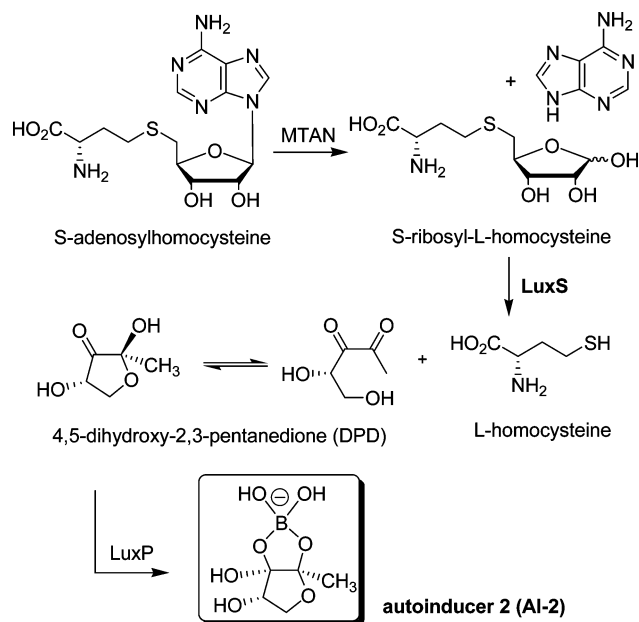


Quorum sensing is a process by which bacteria sense cell density. This cell–cell communication process is mediated by autoinducers. A cross-species messenger, autoinducer-2 (AI-2) is produced from *S*-ribosyl-L-homocysteine by the LuxS enzyme. A proposed mechanism for LuxS is an aldose-ketose isomerization of *S*-ribosylhomocysteine followed by a  $\beta$ -elimination. We report here the synthesis of two substrate analogues, *S*-anhydroribosyl-L-homocysteine and *S*-homoribosyl-L-cysteine, which prevent the initial and final step of the mechanism, respectively.

Although bacteria are monocellular organisms, they communicate actively within their own species and across species boundaries, allowing them to form highly diverse communities, such as biofilms. One of the cell–cell communication processes by which they sense cell density is termed quorum sensing.<sup>1–3</sup> Winans and Bassler called quorum sensing “Mob Psychology”. They wrote, “the (external) signal allows bacteria to sense when they have achieved a ‘quorum’. The quorum contains a sufficient number of bacteria to carry out processes that necessitates the cooperation of a large number of cells in order to be effective.”<sup>2</sup> Quorum sensing is found in a broad spectrum of bacterial species and regulates toxin

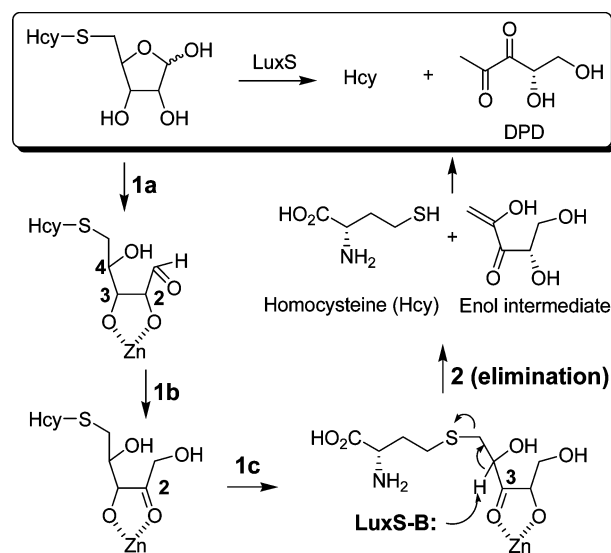
production, biofilm formation, sporulation, and virulence gene expression, for example.<sup>1</sup> Quorum sensing is mediated by autoinducers (AIs), small signaling molecules generated by bacteria. In general, Gram-negative bacteria produce *N*-acyl-homoserine lactones as autoinducers, whereas Gram-positive species generate peptides as signals.<sup>1–3</sup> Unlike other autoinducers that are species-specific, autoinducer-2 (AI-2, a furanosyl borate diester, see Scheme 1) mediates cross-species communication.<sup>3–5</sup> For instance, *Pseudomonas aeruginosa* does not produce AI-2. However, Surette and co-workers found that AI-2 induces several virulence genes in *P. aeruginosa*; moreover, AI-2 was generated in the oropharyngeal flora in sputum samples obtained from cystic fibrosis patients.<sup>6</sup> These findings underscore the impact of environment and microbiota shift in bacterial virulence.

<sup>†</sup> Department of Chemistry.<sup>‡</sup> School of Molecular Biosciences.<sup>§</sup> Center for Integrated Biotechnology.<sup>||</sup> Graduate Program in Pharmacology and Toxicology.<sup>⊥</sup> Departments of Chemistry and Biology, Lycoming College, Williamsport, PA 17701.(1) Fuqua, C.; Parsek, M. R.; Greenberg, E. P. *Annu. Rev. Genet.* **2001**, 35, 439.(2) Winans, S. C.; Bassler, B. L. *J. Bacteriol.* **2002**, 184, 873.(3) Xavier, K. B.; Bassler, B. L. *Curr. Opin. Microbiol.* **2003**, 6, 191.(4) Chen, X.; Schauder, S.; Potier, H.; Dorselaer, A. V.; Pelczar, I.; Bassler, B. L.; Hughson, F. M. *Nature* **2002**, 415, 545.(5) Winzer, K.; Hardie, K. R.; Williams, P. *Adv. Appl. Microbiol.* **2003**, 53, 291.(6) Duan, K.; Dammel, C.; Stein, J.; Rabin, H.; Surette, M. G. *Mol. Microbiol.* **2003**, 50, 1477.

**Scheme 1.** Formation of Autoinducer 2 (AI-2)

The ubiquitous AI-2 formation pathway is found in about half of bacterial species (see Scheme 1).<sup>3,5</sup> It begins with *S*-adenosyl-L-homocysteine (AdoHcy), a common product of *S*-adenosyl-L-methionine (AdoMet)-dependent methylation, a large family of transformations present in all organisms.<sup>7</sup> AdoHcy is hydrolyzed to *S*-ribosyl-L-homocysteine (SRH) and adenine by *S*-adenosyl-L-homocysteine/5'-methylthioadenosine nucleosidase (SAHN or MTAN, EC 3.2.2.9).<sup>8–10</sup> Then the enzyme LuxS cleaves *S*-ribosyl-L-homocysteine to form L-homocysteine (Hcy) and 4,5-dihydroxy-2,3-pentanedione (DPD); the latter is the precursor of autoinducer-2.<sup>11–15</sup> LuxS is likely to be the enzyme ribosyl-L-homocysteinase (EC 3.2.1.148; formerly *S*-ribosyl-L-homocysteine hydrolase, EC 3.3.1.3), which was first described by Duerre and co-workers in the 1960s.<sup>16,17</sup>

As shown in Scheme 2, Pei's group and our group have proposed a mechanism for LuxS (the overall reaction is

**Scheme 2.** Proposed Mechanism for LuxS

boxed).<sup>15,18–20</sup> In the initial steps (1a to 1c), an aldose-ketose isomerization generates a ketone at the C3 position on the carbohydrate moiety. Similar to other aldose-ketose isomerases, LuxS also contains a divalent metal ion in the active site.<sup>21–23</sup> However, aldose-ketose isomerization through two bonds, as proposed in the LuxS reaction, is unprecedented for enzyme-catalyzed transformations.<sup>24,25</sup> Thus, LuxS sports an intriguing mechanism on its own. In the final step of the proposed mechanism (2 in Scheme 2), a base in LuxS abstracts the C4 proton and eliminates the homocysteinyl thiol, and then the enol intermediate formed spontaneously rearranges into the DPD product. Hence, from a mechanistic point of view, LuxS is analogous to *S*-adenosylhomocysteine hydrolase.<sup>26,27</sup> As proposed, both generate a C3-ketone intermediate before cleaving the carbon–sulfur bond via  $\beta$ -elimination. On the other hand, the two enzymes differ in the way ketone intermediates are generated. The AdoHcy hydrolase produces the C3 ketone via nicotinamide adenine dinucleotide (NAD)-dependent redox chemistry,<sup>26,27</sup> whereas LuxS may produce the putative C3 ketone intermediate via an aldo-ketose isomerization. In essence LuxS possesses the function of both an aldo-ketose isomerase and a lyase.

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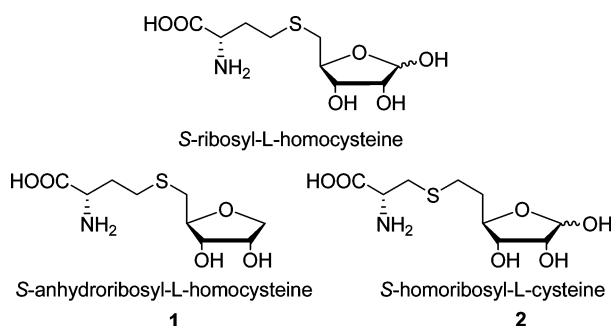
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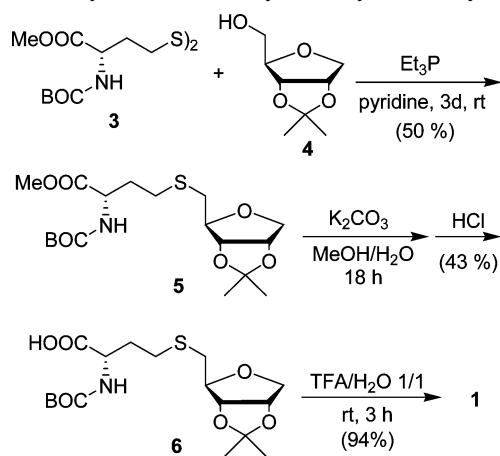


**Figure 1.** LuxS substrate and two substrate analogues.

Absent in humans, the LuxS enzyme is an attractive target for novel therapeutic agent development for bacterial infection.<sup>3,5</sup> Prior to this report, however, there was no report of a specific LuxS inhibitor. Herein we report the synthesis of two LuxS substrate analogues that function as inhibitors and mechanistic probes.

The first substrate analogue (compound **1** in Figure 1) is *S*-[1,4-anhydro-5-deoxy-D-ribitol-5-yl]-homocysteine). As depicted in Scheme 3,

**Scheme 3.** Synthesis of *S*-Anhydribose-L-homocysteine (**1**)



sulfide **5** was constructed via oxidation reduction condensation between the fully protected homocysteine and anhydribose,<sup>28,29</sup> both were prepared by literature procedures. Standard deprotection methods afforded the desired product. We rationalized that replacement of the hemiacetal in the *S*-ribosylhomocysteine substrate by an ether in compound **1** would prevent initial aldo-ketose isomerization.<sup>15,18–20</sup> On the other hand, *S*-anhydribose-L-homocysteine still possesses the 2,3-diol for ligation to the active site metal ion.<sup>22</sup> Thus, compound **1** is likely to bind to LuxS in a similar fashion as the substrate and could serve as a LuxS inhibitor. Up to now,

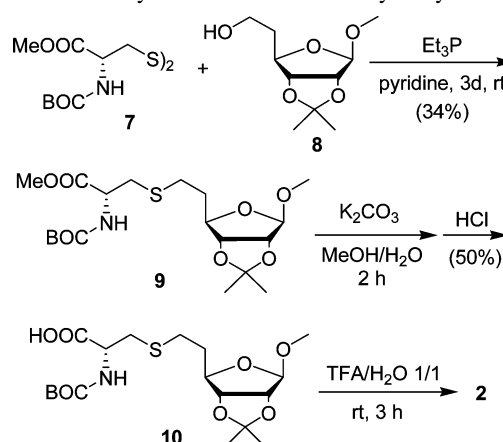
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the only substrate-enzyme complex structure was observed with an inactive form of the enzyme, in which a conserved cysteine residue was oxidized (Cys84 in the *B. subtilis* protein).<sup>22,23</sup> Because compound **1** is capable of forming a stable complex with the active form of the LuxS enzyme, the structural information gleaned using this substrate analogue will shed light on the interaction between the substrate and the active enzyme and assist structure-based inhibitor design as well.

The second substrate analogue (compound **2** in Figure 1) is *S*-homoribosyl-L-cysteine (*S*-(5,6-dideoxy-D-ribo-hexofuranos-6-yl)-L-cysteine). As illustrated in Scheme 4, oxida-

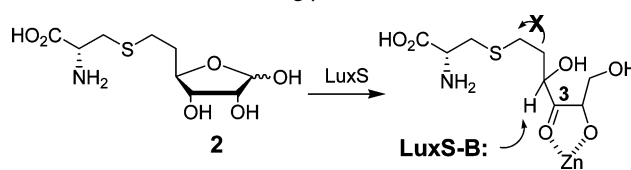
**Scheme 4.** Synthesis of *S*-Homoribosyl-L-cysteine (**2**)



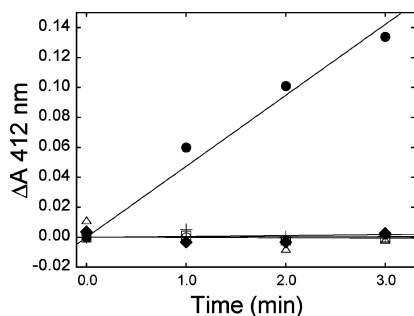
tion reduction condensation followed by standard deprotection procedures afforded the target molecule.

In compound **2**, the C5–C6 carbon–carbon bond replaces the C5 carbon–sulfur bond of the *S*-ribosylhomocysteine substrate, effectively making carbon–sulfur bond cleavage impossible (Scheme 5). On the other hand, the ribose moiety

**Scheme 5.** Accumulation of Ketone Intermediate by Preventing  $\beta$ -Elimination

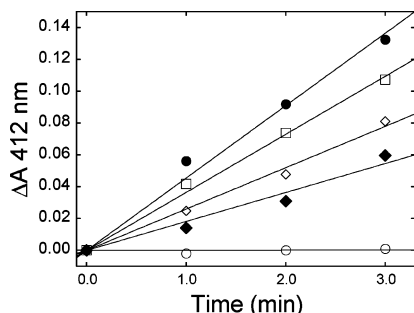


and the amino acid moiety of the substrate and compound **2** are connected by the same number of C–C and C–S bonds. As a result, this substrate analogue is expected to be able to bind to LuxS in a productive orientation, i.e., the compound should still be able to undergo initial aldo-ketose isomerizations to form a ketone at the C3 position (see Scheme 5). At this point, however, the C3 ketone cannot undergo further elimination reaction, which might allow direct observation of the keto intermediate via X-ray crystallography or by trapping with chemical reagents. This would provide direct



**Figure 2.** Time courses of reaction between *S*-ribosyl-homocysteine (100  $\mu$ M) and LuxS (0.34  $\mu$ M, ●); compound **2** (100  $\mu$ M) and LuxS (0.34  $\mu$ M, +; 3.4  $\mu$ M, ◆); compound **2** (300  $\mu$ M) and LuxS (3.4  $\mu$ M, △); LuxS alone (0.34  $\mu$ M, ○); and *S*-ribosyl-homocysteine alone (100  $\mu$ M, □).

support for the proposed involvement of the keto intermediate in LuxS catalysis and, again, structural information for rational inhibitor design.



**Figure 3.** Reaction courses of *S*-ribosyl-homocysteine (100  $\mu$ M) and LuxS (0.34  $\mu$ M) in the presence of compound **1** (0  $\mu$ M, ●; 100  $\mu$ M, □; 300  $\mu$ M, ◇; and 1000  $\mu$ M, ◆); and LuxS alone (0.34  $\mu$ M, ○).

Using our previously reported LuxS assay,<sup>15,19</sup> the preliminary studies show that LuxS does not cleave the C–S bond of compounds **1** and **2**; see Figure 2 (see Supporting Information for compound **1** data). Moreover, both compounds (**1** and **2**) inhibit the LuxS enzyme, see Figure 3 (see Supporting Information for compound **2** data).

With the ready availability of the first generation of specific LuxS inhibitors, we are now poised to investigate the effects of LuxS inhibition in both in vitro and in vivo studies. The results of our continuing work in this area will be reported in due course.

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**Supporting Information Available:** Experimental procedures for the synthesis of compounds **1** and **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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