ORGANIC LETTERS

2004
Vol. 6, No. 18
3043−3046

Synthesis of LuxS Inhibitors Targeting Bacterial Cell–Cell Communication

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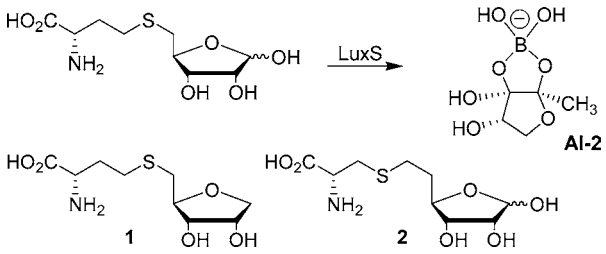
Received May 4, 2004

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 *β*-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.[1−3] Winas 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."[2] Quorum sensing is found in a broad spectrum of bacterial species and regulates toxin production, biofilm formation, sporulation, and virulence gene expression, for example.[1] 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.[1−3] Unlike other autoinducers that are species-specific, autoinducer-2 (AI-2, a furanosyl borate diester, see Scheme 1) mediates cross-species communication.[3−5] For instance, *Pseudomonas aeruginosa* does not produce AI-2. However, Surette and coworkers 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.[6] These findings underscore the impact of environment and microbiota shift in bacterial virulence.

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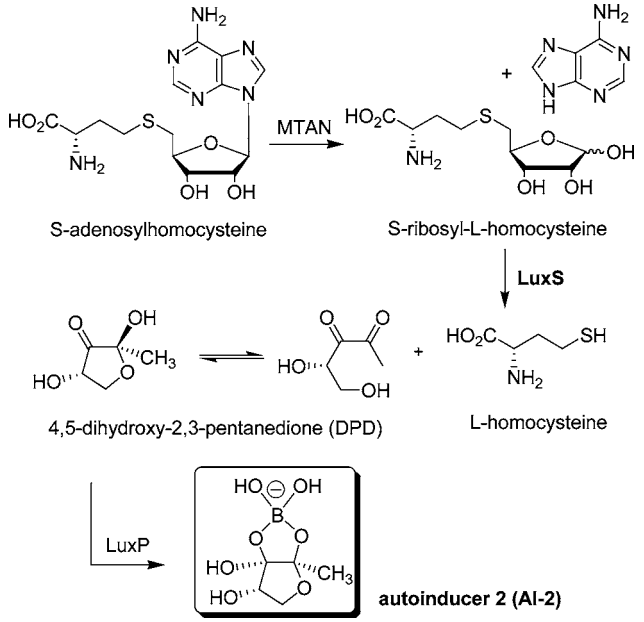
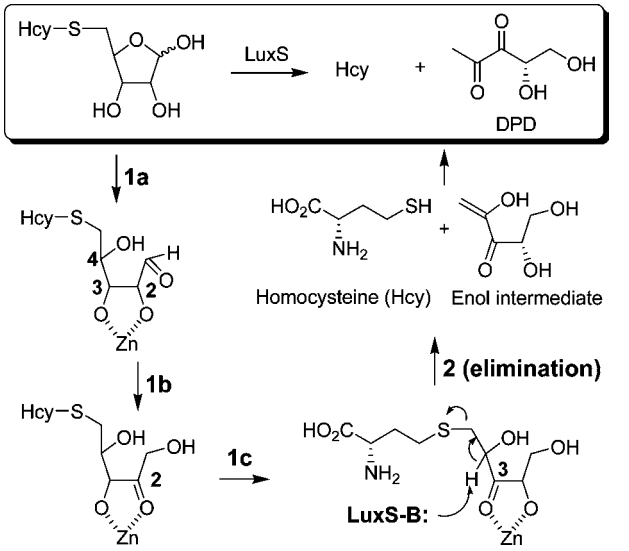
10.1021/ol049182i CCC: $27.50
Published on Web 08/04/2004

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



Scheme 2. Proposed Mechanism for LuxS



The ubiquitous AI-2 formation pathway is found in about half of bacterial species (see Scheme 1).[3,5] 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.[7] 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).[8−10] Then the enzyme LuxS cleaves *S*-ribosylhomocysteine to form L-homocysteine (Hcy) and 4,5-dihydroxy-2,3-pentanedione (DPD); the latter is the precursor of autoinducer-2.[11−15] LuxS is likely to be the enzyme ribosylhomocysteinase (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.[16,17]

As shown in Scheme 2, Pei's group and our group have proposed a mechanism for LuxS (the overall reaction is boxed).[15,18−20] 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.[21−23] However, aldose-ketose isomerization through two bonds, as proposed in the LuxS reaction, is unprecedented for enzyme-catalyzed transformations.[24,25] 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.[26,27] As proposed, both generate a C3-ketone intermediate before cleaving the carbon−sulfur bond via *β*-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,[26,27] 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 lysase.

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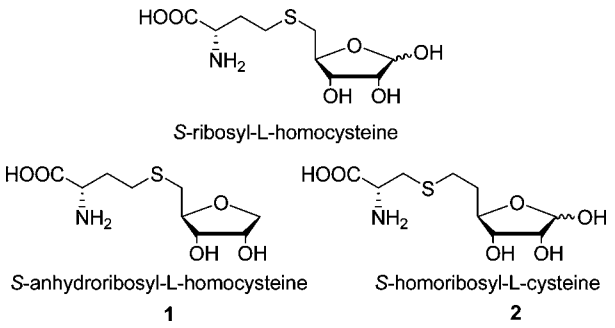
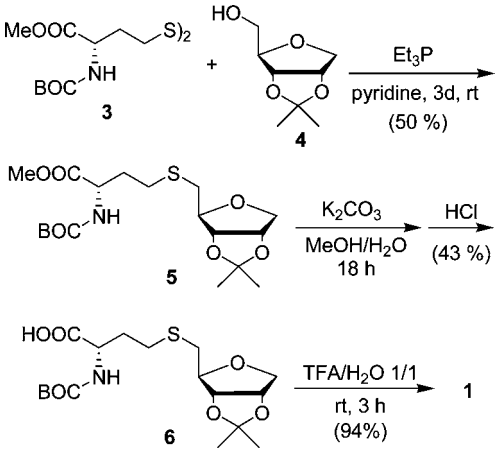


Figure 1. LuxS substrate and two substrate analogues.

Absent in humans, the LuxS enzyme is an attractive target for novel therapeutic agent development for bacterial infection.[3,5] 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*-anhydroribosyl-L-homocysteine (*S*-[1,4-anhydro-5-deoxy-D-ribitol-5-yl]-homocysteine). As depicted in Scheme 3,

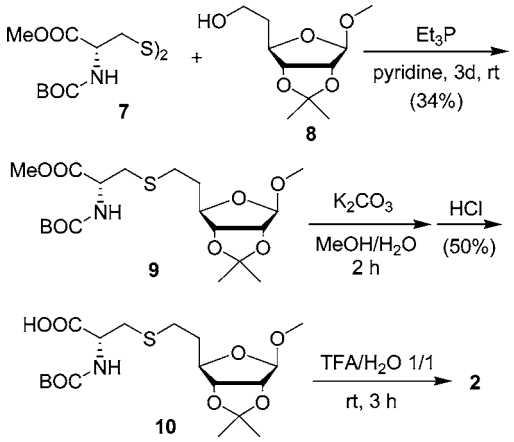
Scheme 3. Synthesis of *S*-Anhydroribosyl-L-homocysteine (**1**)



sulfide **5** was constructed via oxidation reduction condensation between the fully protected homocystine and anhydroribose;[28,29] 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.[15,18−20] On the other hand, *S*-anhydroribosyl-homocysteine still possesses the 2,3-diol for ligation to the active site metal ion.[22] 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, 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).[22,23] 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-

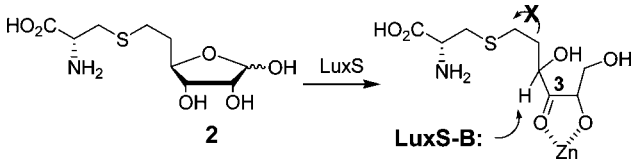
Scheme 4. Synthesis of *S*-Homoribosyl-L-cysteine (**2**)



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 β-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

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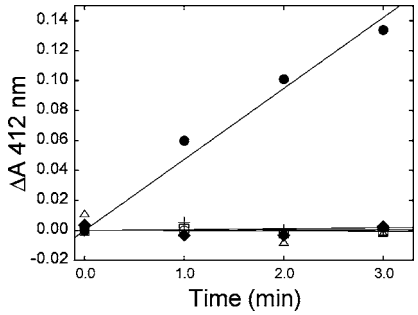

Figure 2. Time courses of reaction between *S*-ribosyl-homocysteine (100 μM) and LuxS (0.34 μM, ●); compound **2** (100 μM) and LuxS (0.34 μM, +; 3.4 μM, ♦); compound **2** (300 μM) and LuxS (3.4 μM, △); LuxS alone (0.34 μM, ○); and *S*-ribosyl-homocysteine alone (100 μM, □).

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

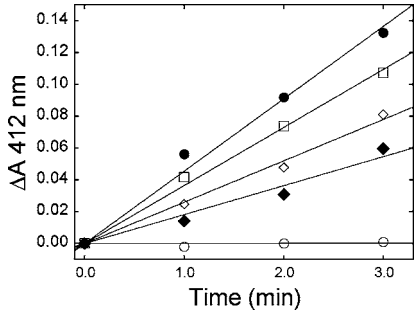

Figure 3. Reaction courses of *S*-ribosyl-homocysteine (100 μM) and LuxS (0.34 μM) in the presence of compound **1** (0 μM, ●; 100 μM, □; 300 μM, ◇; and 1000 μM, ♦); and LuxS alone (0.34 μM, ○).

Using our previously reported LuxS assay,[15,19] 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.

Acknowledgment. We are indebted to Drs. Martha L. Ludwig and Mark T. Hilgers at the University of Michigan for providing the LuxS expression strain and their support and Drs. Kenneth A. Cornell and Michael K. Riscoe at the Portland VA Medical Center for providing the AdoHcy nucleosidase expression strain. This work was supported by the National Institute of Allergy and Infectious Diseases (NIAID/NIH, 1R01AI058146 to Z.S.Z.) and the Herman Frasch Foundation (541-HF02 to Z.S.Z.). The WSU NMR Center equipment was supported by NIH grants RR0631401 and RR12948, NSF grants CHE-9115282 and DBI-9604689, and the Murdock Charitable Trust. J.F.A. was supported in part by the M.S. to Ph.D. transition award provided by the WSU Graduate School. D.P.W. was supported by a summer research fellowship from the Center for Integrated Biotechnology at Washington State University. E.L.K. was supported by the Summer Undergraduate Research Fellowship (SURF) program in Pharmacology and Toxicology at Washington State University. We thank Drs. Rob Ronald and Pat Meier for helpful comments on the manuscript and Dr. Greg Helms for assistance regarding NMR.

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.

OL049182I