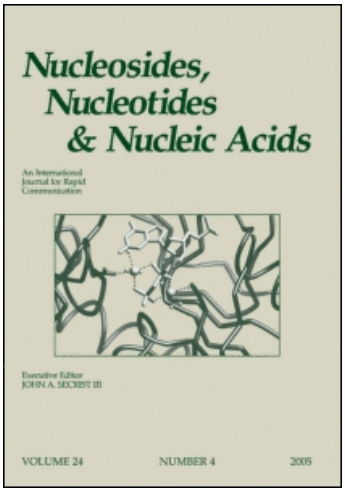

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Novel S-Ribosylhomocysteine Analogues as Potential Inhibitors of LuxS Enzyme

Stanislaw F. Wnuk[a]; Jennifer Lalama[a]; Jenay Robert[a]; Craig A. Garmendia[a]
[a] Department of Chemistry & Biochemistry, Florida International University, Miami, Florida, USA

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NOVEL *S*-RIBOSYLHOMOCYSTEINE ANALOGUES AS POTENTIAL INHIBITORS OF LUXS ENZYME

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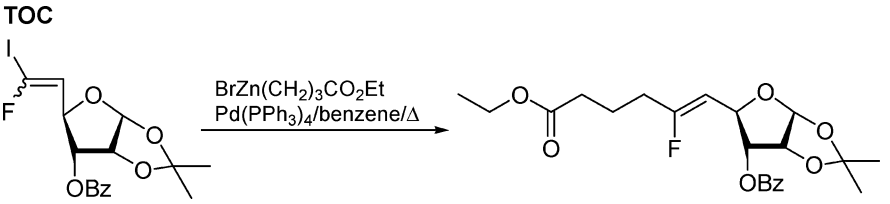
□ *Department of Chemistry & Biochemistry, Florida International University, Miami, Florida, USA*

TOC



□ *Selective cross-coupling of the protected 6-fluoro-6-iodo-α-D-*ribo*-hex-5-enofuranose with 2 equivalents of 4-ethoxy-4-oxobutylzinc bromide in the presence of Pd[P(Ph)$_3$]$_4$ followed by de-protections gave methyl 5,6,7,8,9-pentadeoxy-6-fluoro-α/β-D-*ribo*-dec-5(Z)-enofuranuronate; a* S*-ribosylhomocysteine analogue with the sulfur and carbon-5 atoms replaced by the fluoro(vinyl) unit.*

Keywords *S*-Ribosylhomocysteine; Negishi cross-coupling; vinyl fluorides; luxS enzyme

INTRODUCTION

Hydrolytic cleavage of the *S*-adenosyl-L-homocysteine (SAH) by SAH hydrolase produced adenosine (Ado) and L-homocysteine (Hcy).[1] The cellular levels of SAH are critical because SAH is a potent feedback inhibitor of crucial transmethylation enzymes. Alternatively, hydrolysis of SAH by nucleosidase pfs yields adenine and *S*-ribosyl-L-homocysteine (SRH). *S*-Ribosylhomocysteinase (LuxS) enzyme catalyzes the cleavage of the thioether linkage in SRH to produce Hcy and 4,5-dihydroxy-2,3-pentanedione (DHPD) (Figure 1).[2] DHPD spontaneously cyclizes to **A** and complexes with borate to form a furanosyl borate diester, a small signaling

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Address correspondence to Stanislaw F. Wnuk, Department of Chemistry & Biochemistry, Florida International University, Miami, FL 33199, E-mail: wnuk@fiu.edu

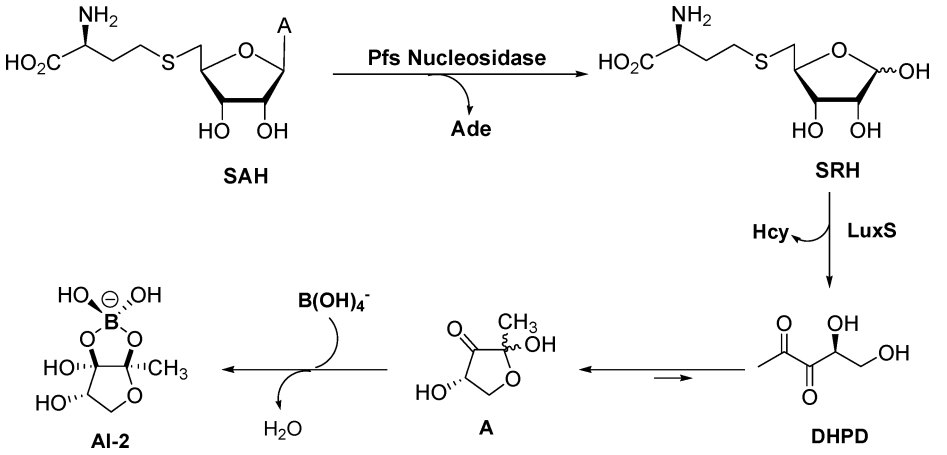


FIGURE 1 Enzymatic conversion of SAH by Pfs nucleosidase and LuxS: Biosynthetic pathway to AI-2.

molecule called autoinducer (AI) of type 2. Autoinducers function in *interspecies* communications and mediate a quorum sensing process of cell-cell communication that bacteria use to coordinate gene expression in response to fluctuation in cell density.[3]

LuxS is a metalloenzyme containing an Fe^{2+} ion coordinated by His-54, His-58, and Cys-126 and a water molecule. The native enzyme is unstable under aerobic conditions however substitution of Co^{2+} for the native metal ion produces a highly stable variant with wild-type catalytic activity. In the proposed catalytic mechanism of LuxS, the metal ion acts as a Lewis acid, facilitating two consecutive aldose-ketose (C1 → C2) and ketose-ketose (C2 → C3) isomerization steps and a final β-elimination of Hcy from 3-keto intermediates.[4]

Two substrate analogues *S*-anhydroribosyl-L-homocysteine (**B**) and *S*-homoribosyl-L-cysteine (**C**) which were found to prevent the initial step and the final step of the mechanism have been recently synthesized (Figure 2).[5] Pei's laboratory prepared a series of structural analogues in which the unstable enediolate moiety formed during isomerizations was replaced with a planar hydroxamate group. The stable isostere **D** showed submicromolar inhibition of the enzyme ($K_I = 0.72\ \mu M$).[6] We report herein synthesis

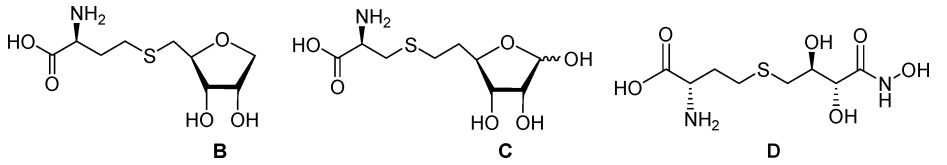
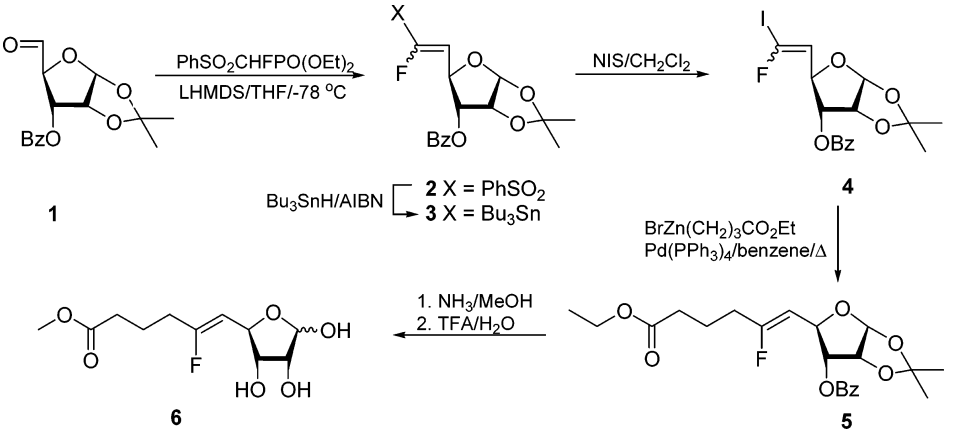


FIGURE 2 LuxS inhibitors.

of the SRH analogues with the sulfur and carbon-5 atoms replaced by a fluoro(vinyl) unit (e.g., **6**), which are not capable to undergo the final elimination step. These ribosyl (depurinated) analogues of SAH were also designed as probes to evaluate similarities between SAH hydrolase and SRHase (LuxS).

RESULTS AND DISCUSSION

Treatment of the diacetone 3-*O*-benzoylallose with periodic acid effected the regioselective removal of 5,6-*O*-isopropylidene group, and sequential oxidative cleavage of the exposed vicinal diol[7] gave the ribose 5-aldehyde **1** (Scheme 1). Wittig-Horner treatment of **1** with sulfonyl-stabilized fluorophosphonate[8] gave 6-(fluoro)homovinyl sulphones **2**. Stannyldesulfonylation followed by iododestannylation[9] of the resulting vinyl stannanes **3** afforded the protected 5,6-dideoxy-6-fluoro-6-iodo-α-D-*ribo*-hex-5-enofuranose **4** (*E/Z*, 3:2). *Trans* selective Negishi cross-coupling[10] of **4** using 2 equivalents of 4-ethoxy-4-oxobutylzinc bromide gave fluoro(vinyl) SRH analogue **5** (*Z*, 54%; 90% based on the conversion of the *E* isomer only).[11] Treatment of **5** with NH_3/MeOH affected debenzoylation and transesterification and subsequent deacetonization with aqueous trifluoroacetic acid (TFA) gave methyl 5,6,7,8,9-pentadeoxy-6-fluoro-α/β-D-*ribo*-dec-5(*Z*)-enofuranuronate **6** (40%; α/β, 3:7). To investigate whether the stereochemistry of the 3-OH group in the SRH analogues can effect a second enolization step,[4a] the *xylo* analogue of **6** was prepared similarly from diacetone 3-*O*-benzoylglucose. Moreover, debenzoylation of **3** (*E/Z*, 7:3) with NH_3/MeOH and subsequent concomitant protiodestannylation and deacetonization with TFA/H_2O afforded 5,6-dideoxy-6-fluoro-D-*ribo*-hex-5-enofuranose (*E/Z*, ~3:1; α/β ~1:6).



SCHEME 1

The 5,6,7,8,9-pentadeoxy-6-fluoro-D-*ribo*-dec-5(*Z*)-enofuranuronate **6** and 5,6-dideoxy-6-fluoro-D-*ribo*-hex-5-enofuranose and their *xylo* epimers were evaluated as potential inhibitors of *Bacillus subtilis S*-ribosylhomocysteinase (LuxS) using the inhibition assays as described previuosly.[6] None of the compounds showed significant activity. These results might indicate that LuxS has more rigid requirements for binding than SAH hydrolase and that the intact Hcy unit in substrate/inhibitor is required for proper binding.

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11. Typical Procedure: $Pd[P(Ph)_3]_4$ (22 mg, 0.01 mmol) was added to a stirred solution of **4** (42 mg, 0.097 mmol; *E/Z*, 3:2) in anhydrous benzene (4 mL) under N_2 at ambient temperature. After 2 minutes, 4-ethoxy-4-oxobutylzinc bromide (0.5M/THF; 0.30 mmol, 0.60 mL) was added and the resulting mixture was heated at 55°C for 5 hours. EtOAc (30 mL) and $NaHCO_3/H_2O$ (10 mL) were added and the separated organic layer was washed with H_2O (10 mL), $NaCl/H_2O$ (10 mL), dried (Na_2SO_4), and then was evaporated. Column chromatography (10 → 30% EtOAc/hexanes) gave **5**(*Z*) (22 mg, 54%; 90% based on the conversion of *E*-isomer): ^1H NMR δ 1.24 (t, $J = 7.1$ Hz, 3,

CH_3), 1.37 & 1.60 (2 × s, 2 × 3, 2 × CH_3), 1.84 ("quint", $J_{8\text{-}7/7'/9/9'}$ = 7.4 Hz, 2, H8/8′), 2.25 (dt, $J_{7\text{-}F}$ = 17.6 Hz, $J_{7\text{-}8/8'}$ = 7.5 Hz, 2, H7/7′), 2.32 (t, $J_{9\text{-}8/8'}$ = 7.4 Hz, 2, H9/9′), 4.09 (q, J = 7.1 Hz, 2, CH_2), 4.72 (dd, $J_{3\text{-}4}$ = 9.2 Hz, $J_{3\text{-}2}$ = 4.7 Hz, 1, H3), 4.75 (dd, $J_{5\text{-}F}$ = 35.0 Hz, $J_{5\text{-}4}$ = 8.9 Hz, 1, H5), 4.95 (t, $J_{2\text{-}1/3}$ = 4.3 Hz, 1, H2), 5.19 (t, $J_{4\text{-}3/5}$ = 9.1 Hz, 1, H4), 5.89 (d, $J_{1\text{-}2}$ = 3.8 Hz, 1, H1), 7.48–8.09 (m, 5, Ar); ^{19}F NMR δ −102.14 (dt, J_{F-H5} = 34.1 Hz, $J_{F\text{-}H7/7'}$ = 17.6 Hz); HRMS (AP-ESI) *m/z*: calcd for $C_{22}H_{28}FO_7$ (MH^+) 423.1814; found 423.1815.