Bioorganic & Medicinal Chemistry 17 (2009) 6699–6706

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

# Inhibition of *S*-ribosylhomocysteinase (LuxS) by substrate analogues modified at the ribosyl C-3 position

Stanislaw F. Wnuk [a,*], Jenay Robert [a], Adam J. Sobczak [a,‡], Brandon P. Meyers [a], Venkata L. A. Malladi [a], Jinge Zhu [b], Bhaskar Gopishetty [b], Dehua Pei [b,†]

[a] Department of Chemistry and Biochemistry Florida International University, Miami, FL 33199, USA
[b] Department of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, OH 43210, USA

ARTICLE INFO

*Article history:*
Received 2 June 2009
Revised 22 July 2009
Accepted 23 July 2009
Available online 26 July 2009

*Keywords:*
Enzyme inhibition
Fluoro sugars
LuxS enzyme
*S*-Ribosylhomocysteine

ABSTRACT

*S*-Ribosylhomocysteinase (LuxS) catalyzes the cleavage of the thioether bond of *S*-ribosylhomocysteine (SRH) to produce homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD), which is the precursor of type 2 autoinducer for bacterial cell–cell communication. In this work, we have synthesized several SRH analogues modified at the ribose C3 position as potential inhibitors of LuxS. While removal or methylation of the C3–OH resulted in simple competitive inhibitors of moderate potency, inversion of the C3 stereochemistry or substitution of fluorine for C3–OH resulted in slow-binding inhibitors of improved potency. The most potent inhibitor showed a $K_I^*$ value of 0.43 μM.



## 1. Introduction

The bacterial enzyme 5′-methylthioadenosine/*S*-adenosylhomocysteine (SAH) nucleosidase (MTAN, EC 3.2.2.9) catalyzes the depurination of SAH to form adenine and *S*-ribosyl-L-homocysteine (SRH) (Fig. 1).[1] Encoded by the *pfs* gene, MTAN is involved in several bacterial processes including polyamine biosynthesis, quorum sensing (QS), methyl-transfer reactions, and adenine and methionine salvage.[2] SRH is subsequently converted to L-homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD) by the enzyme LuxS (or *S*-ribosylhomocysteinase).[3] DPD is in equilibrium with its cyclic hemiketal **A** and forms several QS molecules, which function as bacterial interspecies signaling molecules called autoinducers 2 (AI-2), and are detected by a wide range of bacteria.[4–6] The (2*S*,4*S*)-isomer of the hemiketal **A** undergoes complexation with borate to form a furanosyl borate diester as a distinct AI-2.[7] Recent chemical synthesis of the unstable DPD and their analogues has made it possible to assess the complexation properties of DPD with borate and provided DPD samples for investigation of AI-2-regulated processes.[8–15] QS regulates many crucial bacterial functions such as symbiosis, virulence, antibiotic production, biofilm formation.[16–19] Therefore, proteins involved in QS including LuxS are being pursued as targets for designing novel antibacterial agents.

LuxS is a small metalloenzyme containing a $Fe^{2+}$ ion 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 wild-type catalytic activity.[20,21] In the proposed catalytic mechanism of LuxS, the metal ion acts as a Lewis acid and catalyzes two consecutive aldose–ketose (C1→C2) and ketose–ketose (C2→C3) isomerization steps and the β-elimination of Hcy from a 3-keto intermediate to give DPD.[22–24] Cleavage of the C5–S thioether bond of SRH by LuxS is mechanistically distinct from the reversible reaction catalyzed by SAH hydrolase, which cleaves the equivalent thioether bond in SAH. The latter process is initiated by oxidation of the C3′ alcohol of SAH with an $NAD^+$ cofactor to give a 3′-keto intermediate that undergoes β-elimination of Hcy.[25,26]

Recently, Zhou and co-workers designed two substrate analogues, the *S*-(anhydroribosyl)-L-homocysteine (**B**) and *S*-(homoribosyl)-L-cysteine (**C**) compounds (Fig. 2). The initial mechanistic step was prevented by **B**, and the final step by **C**.[27] Pei and coworkers[28] prepared a series of structural analogues of the enediolate intermediates, in which the unstable enediolate moiety was replaced with a stable hydroxamate group. Among them, stable isostere **D** inhibited LuxS at submicromolar concentrations ($K_I$ = 0.72 μM). The ribosyl and xylosylhomocysteines in which the carbon-5 and sulfur atoms were replaced by a vinyl or (fluoro)vinyl unit did not show significant inhibition of LuxS.[29] We now describe the synthesis and evaluation of several carbon-3

* Corresponding author. Tel.: +1 305 348 6195; fax: +1 305 348 3772.
*E-mail address:* wnuk@fiu.edu (S.F. Wnuk).
† Correspondence concerning enzymatic studies please direct to pei.3@osu.edu.
‡ Faculty leave from the Department of Chemistry, Poznan University of Life Sciences, Poland.

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.07.057

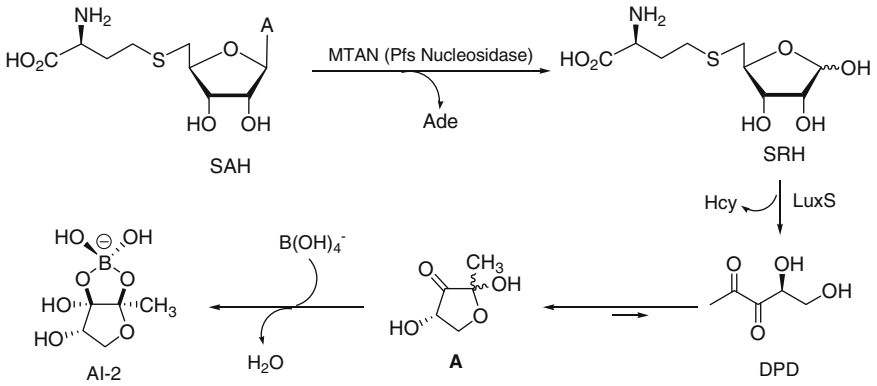



**Figure 1.** Enzymatic conversion of SAH by MTAN and LuxS: biosynthetic pathway to AI-2.

modified SRH analogues that lack a hydroxyl group which can be converted into an enol. These compounds act as mechanism-based inhibitors of LuxS and could impede the production of autoinducers of type 2, and disrupt quorum sensing and cell–cell communication in bacteria.

## 2. Results and discussion

### 2.1. Chemistry

We initially targeted 3-deoxy-SRH analogue **10** because it cannot undergo the second enolization step to produce the 3-keto intermediate.[3,22] Treatment of diacetone 3-deoxyglucose[30] with periodic acid selectively removed the 5,6-*O*-isopropylidene group and oxidatively cleaved the resulting vicinal 5,6-diol[31] to give the corresponding 5-aldehyde, which was reduced with $NaBH_4$ to give 3-deoxy furanoside **1** (Scheme 1). Mesylation at C5, displacement with thiolate derived from D/L-homocysteine,[32] and treatment with aqueous trifluoroacetic acid gave the desired *S*-(3-deoxyribosyl)homocysteine **10** (α/β, 1:3; 25% from **1**).

Next, the 3-*O*-methyl xylo- (**11**) and ribofuranosyl analogues (**12**) were prepared as potential LuxS inhibitors, because the 3-methoxy group was expected to prevent the second enolization step as well. We were also interested to see whether the LuxS active site can tolerate both *R* and *S* epimers at the C3 position. Thus, mesylation of l,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranose **2** afforded ester **5**. Nucleophilic displacement with the thiolate group of D/L-homocysteine afforded thioether **8**. Deprotection ($TFA/H_2O$) and purification by reversed-phase HPLC gave *S*-(3-*O*-methylxylosyl)homocysteine **11** as a mixture of two anomers (α/β, 1:1; 50%). *S*-(3-*O*-Methylribosyl)homocysteine **12** (α/β, 3:7) was similarly prepared from the corresponding ribose **3**, except that the C5–OH group was activated by conversion into 5-*O*-tosylate **6** (instead of 5-*O*-mesylate) because treatment of the latter with Hcy/NaOH led to the hydrolysis of the mesylate and recovery of alcohol **3**.

*S*-Xylosylhomocysteine (SXH, compound **16**) was synthesized from 1,2-isopropylidene-α-D-xylofuranose **13**[33] (Scheme 2). The diol of **13** was converted into cyclic sulfate **14** by treatment with thionyl chloride in the presence of $Et_3N$ in DCM and followed by oxidation of the resulting sulfite with $RuCl_3$ and $NaIO_4$. The cyclic sulfate **14** was readily opened[22] with the protected L-homocysteine in the presence of BuLi in DMF to give desired thioether **15**, which was converted into the target compound SXH **16** by treatment with TFA.

*S*-(3-Deoxy-3-fluoroxylosyl)homocysteine **21a** and the corresponding glycoside **21b** were prepared from the readily available diacetone 3-deoxy-3-fluoroglucose **17**.[34] Thus, sequential treatment of **17** with $H_5IO_6$ and $NaBH_4$[31] gave 3-deoxy-3-fluoro-l,2-*O*-isopropylidene-α-D-xylofuranose (**18**, 60%), which was then converted into 5-*O*-mesylate **19b** (Scheme 3). Displacement of the mesylate group with thiolate generated from *N*-Boc-L–Hcy–$CO_2t$-Bu (LDA/DMF) gave thioether **20b** in 92% yield. It is noteworthy that the use of water soluble tris(2-carboxyethyl)phosphine for the generation of the protected homocysteine free thiol from *N,N′*-di(*tert*-butoxycarbonyl)-L-homocystine di(*tert*-butyl) ester not only improved the reaction yield but also simplified the purification process, as compared to the more commonly used tributyl-[22] and triethylphoshines[27] for the reduction of homocystine analogues. Deprotection (TFA followed by $TFA/H_2O$) of **20b** gave *S*-(3-deoxy-3-fluoroxylosyl)homo-cysteine **21a**, which showed two sets of peaks at the $^{19}F$ NMR spectrum at $\delta$ −200.55 (ddd, $J$ = 12.6, 28.0, 50.6 Hz, 0.6F; β) and −202.41 (ddd, $J$ = 18.7, 25.4, 52.1 Hz, 0.4F; α). Treatment of thioether **20b** with $BCl_3$ at −50 °C followed by quenching of the reaction mixture with MeOH resulted in the removal of all protection groups and the formation of methyl glycoside **21b** as a 1:1.1 mixture of α/β anomers (29%) as well as small amounts of **21a** (7%). Displacement of the tosylate group from **19a** with D/L-homocysteinate thiolate produced **20a** (9*R/S*, 1:1) and subsequent acid-catalyzed removal of the acetonide group afforded **21a** (9*R/S*, 1:1; α/β, 2:3), which showed four sets of peaks at the $^{19}F$ NMR spectrum.

Synthesis of 3-deoxy-3-fluoro-SRH **22** and 3-bromo-3-deoxy-SRH analogue **23** have recently been described.[35]

### 2.2. Inhibition of LuxS

The SRH analogues **10–12, 16**, **21a**, and **21b** were evaluated as potential inhibitors of *Bacillus subtilis* LuxS. Compound **10** acted as a simple, competitive inhibitor of moderate potency, with a $K_I$

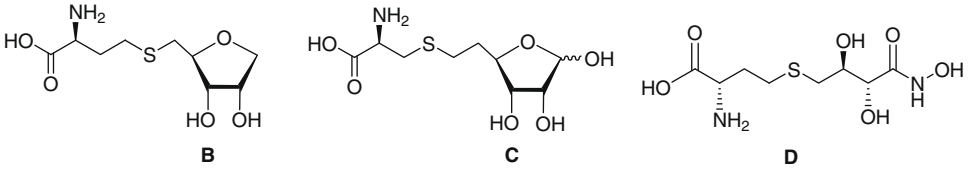
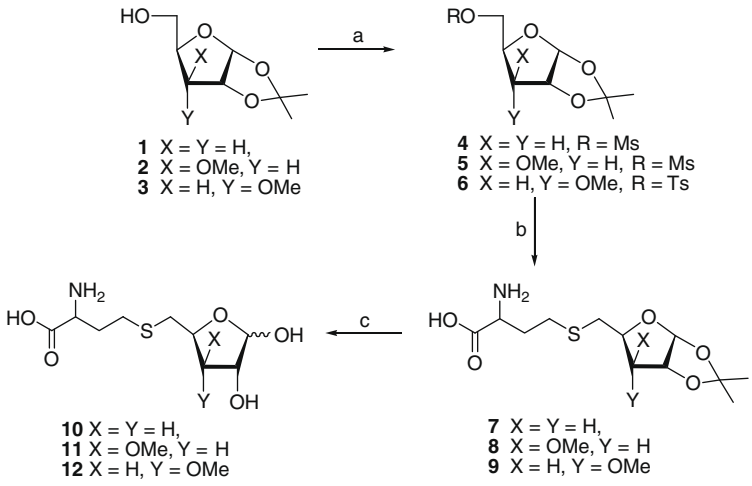
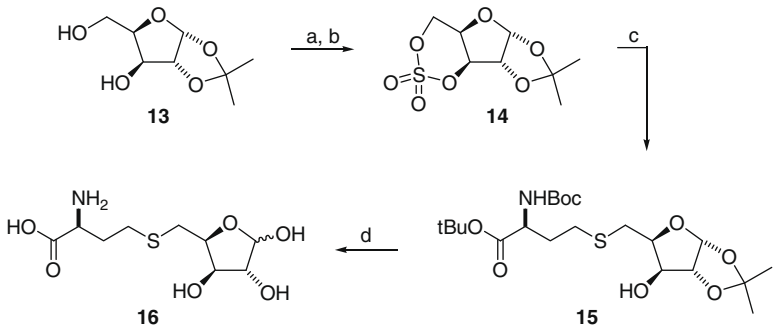


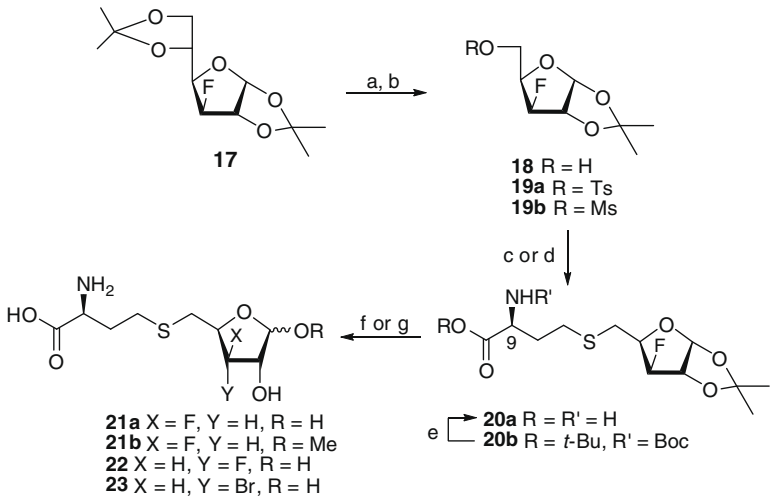

**Figure 2.** LuxS inhibitors.




**Scheme 1.** Reagents: (a) MsCl or TsCl/$Et_3N$; (b) Hcy/NaOH/MeOH/$H_2O$; (c) TFA/$H_2O$.




**Scheme 2.** Reagents and conditions: (a) $SOCl_2/Et_3N/DCM/-78$ °C to rt; (b) $RuCl_3/NaIO_4/CCl_4/CH_3CN/H_2O/0$ °C to rt; (c) (i) BocNH–CH($CH_2CH_2SH$)–$CO_2t$-Bu/BuLi/DMF/0 °C to rt, (ii) THF/$H_2SO_4$/$H_2O$/0 °C; (d) TFA/$H_2O$/0 °C to rt.




**Scheme 3.** Reagents: (a) (i) $H_5IO_6$/EtOAc, (ii) $NaBH_4$/EtOH; (b) TsCl/$C_5H_5N$ or MsCl/$Et_3N$/$CH_2Cl_2$; (c) Hcy/NaOH/MeOH/$H_2O$; (d) BocNH–CH(CHCHSH)–$CO_2t$-Bu/LDA/DMF; (e) TFA; (f) TFA/$H_2O$; (g) $BCl_3/CH_2Cl_2$/MeOH.

value of 55 ± 6 μM (Table 1 and Fig. 3). We also tested whether compound **10** can act as a substrate of LuxS. In particular, we were interested in whether it could be converted into the corresponding 2-ketone intermediate in the LuxS reaction. Since the intermediate cannot be converted into DPD product due to its lack of the 3-hydroxyl group, this would provide an alternative substrate to study the detailed mechanism of the first catalytic step. The $^{13}C$ NMR spectrum of an overnight reaction mixture of **10** and LuxS failed to show any signal in the $\delta$ 180–230 region, indicating that no significant amount of the 2-keto intermediate was generated under the equilibrium conditions. Unfortunately, this result does not reveal whether removal of the C3–OH prevented the formation of the 2-ketone intermediate or simply shifted the equilibrium position between the substrate and the 2-ketone intermediate. Like

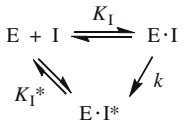
**Table 1**
Inhibition constants of C3-modified SRH analogues against *Bacillus subtilis* LuxS[a]

| Compound | $K_I$ (μM) | $K_I^*$ (μM) |
|---|---|---|
| **10** | 55 ± 6 | — |
| **11** | 66 ± 10 | — |
| **12** | 42 ± 8 | — |
| **16** | 4.2 ± 1.2 | 0.43 ± 0.04 |
| **21a** | 7.7 ± 1.3 | 2.8 ± 0.3 |
| **21b** | 42 ± 1 | 8.8 ± 1.6 |
| **22** | 10.6 ± 2.4 | 0.70 ± 0.26 |
| **23** | 7.9 ± 1.6 | 1.4 ± 0.4 |

[a] Inhibition constants for compounds **10**–**12** were determined using an epimeric mixture (9*R*/*S*), while for the remaining compounds the pure 9*S* isomers were used.

the 3-deoxy analogue **10**, the 3-methoxy analogues **11** and **12** also acted as competitive inhibitors, with $K_I$ values of 66 and 42 μM, respectively, against BsLuxS.

On the other hand, compounds **16**, **21a**, and **21b** all exhibited time-dependent inhibition of LuxS. Figure 4 shows the example of inhibition of *Vibrio harveyi* LuxS (VhLuxS) by compound **21a**. Preincubation of LuxS with the inhibitor resulted in gradual loss of LuxS activity with time; the activity decrease continued for ~60 min, when there was no further change in the residual activity. The compounds were also incubated with BsLuxS and the reaction mixtures were analyzed by electrospray ionization mass spectrometry to detect any covalent adduct between the enzyme and the inhibitors. We only observed signals that correspond to the unmodified BsLuxS protein, indicating that compounds **16**, **21a**, and **21b** do not covalently modify the LuxS protein. Thus, these compounds act as slow-binding inhibitors of LuxS and their inhibition kinetics may be described by equation




where E·I represents the initial enzyme–inhibitor complex, E·I* is the final, tighter enzyme–inhibitor complex, $K_I$ 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. The $K_I$ values were determined by initiating the enzymatic reactions with the LuxS enzyme (no preincubation), whereas the $K_I^*$ values were obtained by incubating the enzyme with the inhibitors for 30 min at room temperature prior to initiating the reactions by

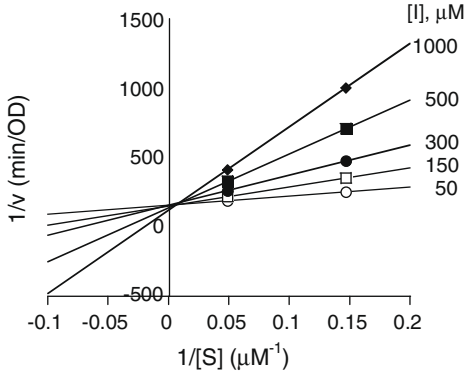



**Figure 3.** Lineweaver–Burk plot showing competitive inhibition of 3-deoxy-SRH **10** against $Co^{2+}$-substituted BsLuxS.

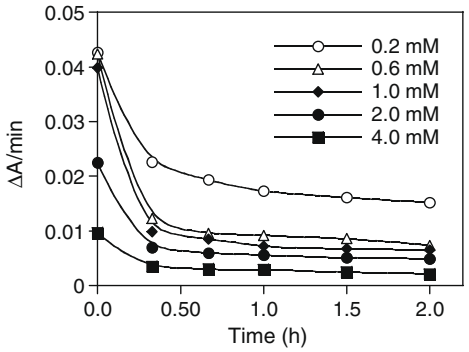



**Figure 4.** Time-dependent inhibition of VhLuxS by compound **21a**. Co-VhLuxS (96 μM) was preincubated with the indicated concentrations of compound **21a** (0.2–4.0 mM) at room temperature. At varying time points (0–2 h), 10-μL aliquots were withdrawn and rapidly diluted into a 1.0-mL solution containing 20 μM SRH and the rate of homocysteine formation was monitored with DTNB (absorbance change at 412 nm).

the addition of SRH ($K_M$ = 2.4 μM) as the last component. The rate constant $k$ could not be determined accurately, because the relatively fast E·I to E·I* conversion makes the $K_I$ measurement less reliable. Among the three compounds, SXH (**16**) was most potent, having $K_I$ and $K_I^*$ values of 4.2 and 0.43 μM, respectively (Table 1). The 3-fluorinated **21a** had $K_I$ and $K_I^*$ values of 7.7 and 2.8 μM, respectively. The glycoside was least potent ($K_I$ = 42 μM and $K_I^*$ = 8.8 μM), probably because the methyl group at the C1 position creates steric clashes with the active-site residues.

The observed slow-binding behavior of compounds **16**, **21a**, and **21b** is very similar to that of [3-F]SRH (**22**) and [3-Br]SRH (**23**), both of which exhibited time-dependent inhibition of LuxS and their time dependency was caused by enzyme-catalyzed release of halide ions.[35] Because compounds **16** and **21a** are structurally similar to **22** and **23**, we hypothesize that compounds **16** and **21a** may undergo similar structural changes in the LuxS active site (e.g., the formation of 2-ketone intermediate-like species). The inverted stereochemistry at the C3 position in SXH would prevent the conversion of the intermediate into products. Unfortunately, all of our attempts to isolate/detect the altered inhibitor species (I*) failed. Thus, we cannot rule out the possibility that the observed slow-binding inhibition was caused by the conversion of E·I into E*·I, where E* is LuxS in an alternative conformation induced by inhibitor binding. The origin of time dependency of glycoside **21b** is less clear, as it cannot undergo ring opening and therefore any catalyzed transformation.

## 2.3. Conclusion

In this work, we have synthesized several SRH analogues that lack an enolizable hydroxyl group at the carbon-3 position. Their evaluation against LuxS showed that removal or methylation of the C3–OH resulted in simple competitive inhibitors of moderate potency, while inversion of the C3 stereochemistry or substitution of fluorine for C3–OH resulted in slow-binding inhibition with the improved potency.

## 3. Experimental section

$^1H$ ($Me_4Si$) NMR spectra were determined at 400 or 600 MHz, $^{13}C$ ($Me_4Si$) at 100.6 MHz and $^{19}F$ ($CFCl_3$) at 376.5 MHz with solution in $CDCl_3$ unless otherwise noted. Mass spectra (MS) were obtained by atmospheric pressure chemical ionization (APCI) and HRMS by electron impact techniques unless otherwise noted. Reagent grade chemicals were used and solvents were dried by reflux over and distillation from $CaH_2$ under an argon atmosphere

except THF (K/benzophenone). TLC was performed on Merck kieselgel 60-$F_{254}$ precoated aluminium sheets, and products were detected by visualization with $Ce(SO_4)_2/(NH_4)_6Mo_7O_{24}{\cdot}4H_2O/H_2SO_4/H_2O$ reagent or with 254 nm light. 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 program using $CH_3CN/H_2O$ as a mobile phase.

### 3.1. 3-Deoxy-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (1)

$H_5IO_6$ (922 mg, 4.1 mmol) was added to a stirred solution of diacetone 3-deoxyglucose[30] (500 mg, 2.05 mmol) in dried EtOAc (40 mL) at ambient temperature. A precipitate appeared within the first 5 min and after 15 min, the somehow unstable 3-deoxy-1,2-*O*-isopropylidene-α-D-*erythro*-pentodialdo-1,4-furanose aldehyde can be detected on TLC and $^1$H NMR spectra ($\delta$ 9.75 (d, $J_{5-4}$ = 4.8 Hz, ~0.9, H5). The white precipitate was filtered off and was washed with EtOAc (3 × 5 mL). The combined organic layer was evaporated, and the residue was quickly dissolved in EtOH (30 mL). Sodium borohydride (115 mg, 3.0 mmol) and water (10 mL) was added until all of the sodium borohydride was dissolved. After 45 min, the reaction mixture was evaporated, and the resulting residue was dissolved in EtOAc and washed with $NaHCO_3$. The organic layer was dried ($Na_2SO_4$), evaporated and flash column chromatographed (40→60% EtOAc/hexane) to give **1**[36] (217 mg, 61%).

### 3.2. 3-Deoxy-l,2-*O*-isopropylidene-5-*O*-methylsulfonyl-α-D-*erythro*-pentofuranose (4)

Triethylamine (125 μL, 90.9 mg, 0.9 mmol) and $CH_3SO_2Cl$ (35 μL, 51.3 mg, 0.45 mmol) were added to a stirred solution of **1** (52 mg, 0.3 mmol) in anhydrous $CH_2Cl_2$ (10 mL) under $N_2$ at 0 °C. After 10 min, the reaction mixture was partitioned ($NaHCO_3/H_2O//CHCl_3$), and the organic layer was washed (brine), dried ($Na_2SO_4$) and evaporated to yield **4**[37] (73 mg, 97%) of sufficient purity to be used in the next step: $^1$H NMR $\delta$ 1.34 (s, 3H), 1.53 (s, 3H), 1.80 (ddd, *J* = 4.7, 10.7, 13.5 Hz, 1H), 2.14 (dd, *J* = 4.2, 13.2 Hz, 1H), 3.08 (s, 3H), 4.27 (dd, *J* = 4.9, 11.4 Hz, 1H), 4.45 (dd, *J* = 2.8, 11.2 Hz, 1H), 4.44–4.53 (m, 1H), 4.79 (t, *J* = 4.2 Hz, 1H), 5.85 (d, *J* = 3.6 Hz, 1H).

### 3.3. l,2-*O*-Isopropylidene-3-*O*-methyl-5-*O*-methylsulfonyl-α-D-xylofuranose (5)

Treatment of **2**[38] (50 mg, 0.25 mmol; prepared in 77% yield by dehomologation[31] of diacetone 3-*O*-methylglucose[39] as described for **1**) with triethylamine (104 μL, 76 mg, 0.75 mmol) and $CH_3SO_2Cl$ (30 μL, 43 mg, 0.38 mmol), as described for **4**, gave **5** (68 mg, 96%) of sufficient purity to be used in the next step: $^1$H NMR $\delta$ 1.35 (s, 3H), 1.55 (s, 3H), 3.08 (s, 3H), 3.42 (s, 3H), 3.81 (d, *J* = 3.2 Hz, 1H), 4.37–4.51 (m, 3H), 4.62 (d, *J* = 3.8 Hz, 1H), 5.94 (d, *J* = 3.7 Hz, 1H); HRMS (ESI) *m/z* calcd for $C_{10}H_{19}O_7S$ $[M+H]^+$ 283.0851, found 283.0844.

### 3.4. l,2-*O*-Isopropylidene-3-*O*-methyl-5-*O*-*p*-toluenesulfonyl-α-D-ribofuranose (6)

TsCl (93 mg, 0.49 mmol) was added to a stirred solution of **3**[40](90 mg, 0.45 mmol; prepared in 67% yield by dehomologation[31] of diacetone 3-*O*-methylallose[40] as described for **1**) in pyridine (1 mL) at 0 °C. After 12 h, an additional TsCl (93 mg, 0.49 mmol) was added and the stirring was continued for 8 h. Pyridine was evaporated/coevaporated with toluene (3×), and the residue was dissolved in EtOAC and washed with $NaHCO_3$ and NaCl. The organic layer was evaporated and column chromatographed (hexane/EtOAc, 1:1) to give **6**[40] (117 mg, 72%) of sufficient purity to be used in the next step: $^1$H NMR $\delta$ 1.34 (s, 3H), 1.52 (s, 3H), 2.44 (s, 3H), 3.44 (s, 3H), 3.64 (dd, *J* = 4.2, 9.0 Hz, 1H), 4.07 (dt, *J* = 2.4, 9.1 Hz, 1H), 4.18 (dd, *J* = 3.3, 11.2 Hz, 1H), 4.27 (dd, *J* = 2.1, 11.2 Hz, 1H), 4.64 (t, *J* = 3.9 Hz, 1H), 5.66 (d, *J* = 3.6 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 8.4 Hz, 2H).

### 3.5. *S*-(3,5-Dideoxy-l,2-*O*-isopropylidene-α-D-*erythro*-pentofuranos-5-yl)homocysteine (7)

Compound **4** (45.6 mg, 0.18 mmol) was added to 1 M NaOH/$H_2O$ (5 mL) and degassed with $N_2$ for 30 min. D/L-Homocysteine (36.5 mg, 0.27 mmol) was then added, and the suspension was heated to 60 °C. After 12 h, the reaction mixture was cooled to room temperature, neutralized with dilute HCl to pH ~ 7 and washed with EtOAc (3×). The water layer was evaporated and purified by HPLC (25% $CH_3CN/H_2O$ for 35 min at 2.5 mL/min; $t_R$ = 12.6 min) to give **7** (16 mg, 31%): $^1$H NMR ($D_2O$) $\delta$ 1.39 (s, 3H), 1.55 (s, 3H), 1.83 (ddd, *J* = 4.7, 10.9, 14.0 Hz, 1H), 2.08–2.21 (m, 2H), 2.24 (dd, *J* = 4.3, 14.0, 1H), 2.72–2.78 (m, 2H), 2.81 (dd, *J* = 6.8, 13.9 Hz, 1H), 2.93 (dd, *J* = 4.5, 13.8 Hz, 1H), 3.88 (t, *J* = 6.3 Hz, 1H), 4.46 (dddd, *J* = 4.3, 4.5, 6.8, 10.9 Hz, 1H), 4.98 (t, *J* = 4.1 Hz, 1H), 5.94 (d, *J* = 3.7 Hz, 1H); MS *m/z* 292 (100%, $MH^+$).

### 3.6. *S*-(5-Deoxy-l,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-yl)homocysteine (8)

Treatment of **5** (35 mg, 0.12 mmol) with D/L-homocysteine (25 mg, 0.19 mmol), as described for **7**, and purification by HPLC (5% $CH_3CN/H_2O$ for 45 min at 2.5 mL/min; $t_R$ = 30 min) gave **8** (11 mg, 28%): $^1$H NMR ($D_2O$) $\delta$ 1.36 (s, 3H), 1.53 (s, 3H), 1.90–2.10 (m, 2H), 2.65–2.73 (m, 2H), 2.82 (dd, *J* = 7.5, 13.6 Hz, 1H), 2.87 (dd, *J* = 6.6, 13.6 Hz, 1H), 3.46 (s, 3H), 3.57 ('t', *J* = 6.9 Hz, 1H), 3.91 (d, *J* = 2.9 Hz, 1H), 4.38 (dt, *J* = 2.9, 7.2 Hz, 1H), 4.88 (d, *J* = 3.9 Hz, 1H), 5.98 (d, *J* = 3.9 Hz, 1H); $^{13}$C NMR ($D_2O$) $\delta$ 24.9, 25.4, 27.9, 28.0, 28.42, 32.71, 54.54, 57.42, 79.8, 79.9, 81.0, 83.3, 104.3, 113.8, 174.0; HRMS (AP-ESI) *m/z* calcd for $C_{13}H_{24}NO_6S$ $[M+H]^+$ 322.1325, found 322.1321.

### 3.7. *S*-(5-Deoxy-l,2-*O*-isopropylidene-3-*O*-methyl-α-D-ribofuranos-5-yl)homocysteine (9)

Treatment of **6** (48 mg, 0.14 mmol) with D/L-homocysteine (28 mg, 0.06 mmol), as described for **7**, and purification by HPLC (5% $CH_3CN/H_2O$ for 45 min at 2.5 mL/min; $t_R$ = 35.0 min) gave **9** (13 mg, 30%): $^1$H NMR ($D_2O$) $\delta$ 1.40 (s, 3H), 1.56 (s, 3H), 2.03–2.23 (m, 2H), 2.72 (t, *J* = 7.6 Hz, 2H), 2.79 (dd, *J* = 7.1, 14.3 Hz, 1H), 3.03 (dd, *J* = 3.2, 14.3 Hz, 1H), 3.48 (s, 3H), 3.80 (dd, *J* = 4.1, 9.0 Hz, 1H), 3.78–3.83 (m, 1H), 4.12 (ddd, *J* = 3.1, 7.1, 9.3 Hz, 1H), 4.97 (t, *J* = 3.9 Hz, 1H), 5.90 (d, *J* = 3.7, 1H); MS *m/z* 322 (100%, $MH^+$).

### 3.8. *S*-(3,5-Dideoxy-D-*erythro*-pentofuranos-5-yl)homocysteine (10)

A solution of **7** (9 mg, 0.03 mmol) in TFA/$H_2O$ (9:1, 3 mL) was stirred for 45 min. at 0 °C (ice-bath). The reaction mixture was evaporated and coevaporated [toluene (3×), $CH_3CN$ (3×)] and the residue was purified by HPLC (5% $CH_3CN/H_2O$ for 25 min at 2.5 mL/min) to yield **7** (7 mg, 90%, α/β, ~1:3; $t_R$ = 12.6 min): $^1$H NMR ($D_2O$) $\delta$ 2.02 (ddd, *J* = 4.7, 9.4, 14.0 Hz, 1H), 2.06–2.25 (m, 3H), 2.69–2.78 (m, 2H), 2.78–2.91 (m, 2H), 3.87 ('t', *J* = 6.1 Hz, 1H), 4.26 (d, *J* = 4.6 Hz, 0.75H), 4.35 ('dt', *J* = 4.1, 6.8 Hz, 0.25H), 4.44–4.53 (m, 1H), 5.26 (s, 0.75H), 5.35 (d, *J* = 4.0 Hz, 0.25H); HRMS

(LCT-ESI) *m/z* calcd for $C_9H_{17}O_5NSNa$ $[M+Na]^+$ 274.0725, found 274.0725.

### 3.9. *S*-(5-Deoxy-3-*O*-methyl-D-xylofuranos-5-yl)homocysteine (11)

Treatment of **8** (22 mg, 0.07 mmol) with TFA/$H_2O$, as described for **10**, and purification by HPLC (5% $CH_3CN/H_2O$ for 45 min at 2.5 mL/min; $t_R$ = 20 min) gave **11** (12 mg, 60%; α/β, ~1:1): $^1H$ NMR ($D_2O$) $\delta$ 2.06–2.24 (m, 2H), 2.68–2.76 (m, 2H), 2.78–2.85 (m, 1.5H), 2.91 (dd, *J* = 5.9, 13.7 Hz, 0.5H), 3.41 (s, 1.5H), 3.44 (s, 1.5H), 3.80 (dd, *J* = 1.7, 4.7 Hz, 0.5H), 3.83–3.89 (m, 1H), 3.92 (dd, *J* = 4.0, 4.8 Hz, 0.5H), 4.20 (s, 0.5H), 4.24 (t, *J* = 4.1 Hz, 0.5H), 4.38–4.47 (m, 1H), 5.20 (s, 0.5H), 5.39 (d, *J* = 4.4 Hz, 0.5H); HRMS (LCT-ESI) *m/z* calcd for $C_{10}H_{19}O_6NSNa$ $[M+Na]^+$ 304.0831, found 304.0829.

### 3.10. *S*-(5-Deoxy-3-*O*-methyl-D-ribofuranos-5-yl)homocysteine (12)

Treatment of **9** (10 mg, 0.03 mmol) with TFA/$H_2O$, as described for **10**, and purification by HPLC (5% $CH_3CN/H_2O$ for 45 min at 2.5 mL/min; $t_R$ = 20 min) gave **12** (5 mg, 59%; α/β, ~3:7): $^1H$ NMR ($D_2O$) $\delta$ 2.07–2.26 (m, 2H), 2.70–2.79 (m, 2H), 2.79–2.99 (m, 2H), 3.44 (s, 0.9H), 3.47 (s, 2.1H), 3.78 (t, *J* = 5.0 Hz, 0.3H), 3.84 (t, *J* = 5.5 Hz, 0.3H), 3.86 (dd, *J* = 5.5, 7.0 Hz, 0.7H), 3.94 (dd, *J* = 4.5, 6.5 Hz, 0.7H), 4.07–4.14 (m, 1H), 4.23 (dd, *J* = 1.3, 4.3 Hz, 0.7H), 4.29 ('t' *J* = 4.6 Hz, 0.3H) 5.26 (s, 0.7H), 5.37 (d, *J* = 4.0 Hz, 0.3H); HRMS (LCT-ESI) *m/z* calcd for $C_{10}H_{19}O_6NSNa$ $[M+Na]^+$ 304.0831, found 304.0827.

### 3.11. 1,2-*O*-Isopropylidene-3,5-*O*-sulfonyl-α-D-xylofuranose (14)

To a stirred solution of diol **13**[33] (0.4 g, 2.11 mmol) in $CH_2Cl_2$ (12 mL) were added TEA (1.2 mL, 8.42 mmol) and $SOCl_2$ (2 M/DCM; 2.1 mL, 4.22 mmol) at −78 °C under argon atmosphere. The mixture was allowed to warm to 0 °C and stirred until the reaction was completed (1 h). The reaction mixture was diluted in DCM (50 mL), washed with $H_2O$ (20 mL), dried over $MgSO_4$, and concentrated to dryness. The residue was dissolved in $CCl_4$ (6 mL) and $CH_3CN$ (6 mL). To the resulting solution was added $H_2O$ (9 mL) followed by $RuCl_3$ hydrate (15 mg) and $NaIO_4$ (0.9 g, 4.22 mmol) at 0 °C. The mixture was stirred for 2 h, diluted in EtOAc (50 mL), washed with $H_2O$ (20 mL), dried over $MgSO_4$, and concentrated in reduced pressure. The crude product was purified by silica gel chromatography (EtOAc/hexane, 1:2) to afford the cyclic sulfate **14** (0.39 g, 74%) as a white solid: $^1H$ NMR $\delta$ 1.33 (s, 3H), 1.49 (s, 3H), 4.24 (m, 1H), 4.70 (d, *J* = 3.6 Hz, 1H), 4.81 (d, *J* = 12.8 Hz, 1H), 4.95 (dd, *J* = 2.0, 12.8 Hz, 1H), 5.16 (d, *J* = 2.0 Hz, 1H), 6.03 (d, *J* = 4.0 Hz, 1H); $^{13}C$ NMR $\delta$ 26.1, 26.5, 69.3, 72.4, 82.8, 86.5, 104.7, 113.1; HRMS (ESI) *m/z* calcd for $C_8H_{12}O_7SNa^+$ $[M+Na^+]$ 275.0201, found 275.0212.

### 3.12. *N-tert*-Butoxycarbonyl-*S*-(5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl)-L-homocysteine *tert*-Butyl ester (15)

To a solution of BocNH–CH($CH_2CH_2SH$)–$CO_2t$Bu (1.1 g, 3.77 mmol) in DMF (15 mL) was added BuLi (2.5 M/hexane; 0.9 mL, 2.26 mmol) at 0 °C under argon atmosphere. After stirring for 5 min, a solution of cyclic sulfate **14** (0.38 g, 1.51 mmol) dissolved in DMF (10 mL) was added. The mixture was stirred for 4 h, diluted with EtOAc (75 mL), washed with $H_2O$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$ and concentrated to dryness. The residue was dissolved in THF (3 mL) and concentrated $H_2SO_4$ (2 μL) and $H_2O$ (2 μL) were added at 0 °C. The mixture was stirred for 1 h at 0 °C and then partitioned between EtOAc (100 mL) and 5% $NaHCO_3$ solution (40 mL). The organic layer was collected, washed with $H_2O$ (20 mL) and brine (20 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by silica gel chromatography (EtOAc/hexane, 2:3) to afford alcohol **15** (0.47 g, 67%) as a white solid: $^1H$ NMR $\delta$ 1.29 (s, 3H), 1.43 (s, 9H), 1.45 (s, 9H), 1.48 (s, 3H), 1.87 (m, 1H), 2.09 (m, 1H), 2.50 (d, *J* = 5.6 Hz, 1H), 2.56–2.69 (m, 2H), 2.81 (dd, *J* = 8.4, 13.2 Hz, 1H), 2.87 (dd, *J* = 5.2, 13.2 Hz, 1H), 4.21–4.30 (m, 3H), 4.51 (d, *J* = 3.6 Hz, 1H), 5.10 (d, *J* = 5.6 Hz, 1H), 5.90 (d, *J* = 3.6 Hz, 1H); $^{13}C$ NMR $\delta$ 26.2, 26.7, 28.0, 28.3, 28.6, 29.2, 33.0, 53.2, 74.9, 79.7, 80.0, 82.4, 85.2, 104.7, 111.6, 155.5, 171.4; HRMS (ESI) *m/z* calcd for $C_{21}H_{37}NO_8SNa^+$ $[M+Na^+]$ 486.2138, found 486.2141.

### 3.13. *S*-(5-Deoxy-D-xylofuranos-5-yl)-L-homocysteine (16)

Alcohol **15** (40 mg, 0.09 mmol) was treated with TFA containing 10% anisole and 10% water (5 mL) at room temperature for 6 h. The volatile solvents were removed by evaporation, and the residue was dissolved in $H_2O$ (3 mL) and washed with $CH_2Cl_2$ (3 × 2 mL). The aqueous solution was lyophilized to give the desired SXH **16** (20 mg, 61%) as a trifluoroacetate salt: $^1H$ NMR (250 MHz, $D_2O$) $\delta$ 1.85–2.10 (m, 2H), 2.35–2.70 (m, 4H), 3.60–4.40 (m, 3H), 4.96 (s, 1H), 5.20 (d, *J* = 4.3 Hz, 1H); HRMS (ESI) *m/z* calcd for $C_9H_{17}NO_6SNa^+$ $[M+Na^+]$ 290.0674, found 290.0684.

### 3.14. 3-Deoxy-3-fluoro-l,2-*O*-isopropylidene-α-D-xylofuranose (18)

Treatment of **17**[34] (75 mg, 0.29 mmol) with $H_5IO_6$ (77 mg, 0.34 mmol) and $NaBH_4$ (16.5 mg, 0.43 mmol), as described for **1**, followed by flash column chromatography (EtOAc/hexane, 1:1) gave **18** (39 mg, 71%) with data as reported:[41] $^{19}F$ NMR $\delta$ −208.66 (ddd, *J* = 11.2, 30.1, 50.4 Hz).

### 3.15. 3-Deoxy-3-fluoro-l,2-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-α-D-xylofuranose (19a)

Treatment of **18** (66 mg, 0.34 mmol) with TsCl (130 mg, 0.68 mmol) in pyridine (1 mL), as described for **6**, followed by flash column chromatography (EtOAc/hexane, 25:75) gave **19a**[42] (72 mg, 62%): $^1H$ NMR $\delta$ 1.32 (s, 3H), 1.48 (s, 3H), 2.47 (s, 3H), 4.17–4.28 (m, 2H), 4.41 (dtd, *J* = 2.2, 6.3, 28.2 Hz, 1H), 4.67 (dd, *J* = 3.8, 10.6 Hz, 1H), 4.96 (dd, *J* = 2.2, 50.1 Hz, 1H), 5.93 (d, *J* = 3.6 Hz, 1H), 7.38 (d, *J* = 8.3 Hz, 2H), 7.83 (d, *J* = 8.3 Hz, 2H); $^{19}F$ NMR $\delta$ −208.85 (ddd, *J* = 10.1, 27.7, 49.7 Hz).

### 3.16. 3-Deoxy-3-fluoro-1,2-*O*-isopropylidene-5-*O*-methanesulfonyl-α-D-xylofuranose (19b)

$Et_3N$ (0.115 mL, 84 mg, 0.83 mmol) and MsCl (0.032 mL, 47 mg, 0.41 mmol) were added dropwise to a stirred solution of **18** (53 mg, 0.27 mmol) in anhydrous $CH_2Cl_2$ (5 mL) at ambient temperature. After 15 min, the reaction mixture was quenched with saturated $NaHCO_3/H_2O$, and extracted with $CHCl_3$. The combined organic layer was washed with brine, dried ($Na_2SO_4$) and was evaporated and column chromatographed (20→40% EtOAc/hexane) to give **19b** (54 mg, 75%) as a white solid: $^1H$ NMR $\delta$ 1.36 (s, 3H), 1.52 (s, 3H), 3.10 (s, 3H), 4.42–4.48 (m, 2H), 4.52 (dddd, *J* = 2.3, 5.5, 7.1, 28.5 Hz, 1H), 4.74 (dd, *J* = 3.7, 10.8 Hz, 1H), 5.04 (dd, *J* = 1.8, 50.0 Hz, 1H), 6.03 (d, *J* = 3.7 Hz, 1H); $^{13}C$ NMR $\delta$ 26.2, 26.7, 37.6, 65.9 (d, *J* = 11.0 Hz) 77.4 (d, *J* = 18.7 Hz), 82.4 (d, *J* = 31.7 Hz), 93.7 (d, *J* = 184.8 Hz), 105.1, 112.8; $^{19}F$ NMR $\delta$ −208.7 (ddd, *J* = 10.6, 27.7, 50.2 Hz); MS *m/z* 271 ($MH^+$).

### 3.17. *S*-(3,5-Dideoxy-3-fluoro-l,2-*O*-isopropylidene-α-D-xylofuranos-5-yl)homocysteine [20a(9*R*/*S*)]

A solution of **19a** (18.5 mg, 0.05 mmol) in 2 M NaOH/$H_2O$–EtOH solution (4 mL, 1:1) was degassed with $N_2$ for 30 min. D/L-Homocysteine (11 mg, 0.08 mmol) was added, and the solution was heated to 60 °C. After 36 h, the reaction mixture was cooled to room temperature, neutralized with concd HCl and washed with EtOAc (3×). The water layer was evaporated and purified by HPLC (5% $CH_3CN/H_2O$ for 60 min at 2.5 mL/min; $t_R$ = 30 min) to give **20a** (5 mg, 32%; 9*R*/*S*, ~1:1): $^1$H NMR ($D_2O$) $\delta$ 1.37 (s, 3H), 1.52 (s, 3H), 2.05–2.26 (m, 2H), 2.72–2.78 (m, 2H), 2.86–2.97 (m, 2H), 3.83 (dd, *J* = 5.7, 7.0 Hz, 1H), 4.45 (dtd, *J* = 2.0, 6.6, 28.7 Hz, 1H), 4.94 (dd, *J* = 3.9, 10.8 Hz, 1H), 5.12 (dd, *J* = 2.0, 49.1 Hz, 1H), 6.10 (d, *J* = 4.0 Hz, 1H); $^{19}$F NMR ($D_2O$) $\delta$ −208.45 (ddd, *J* = 10.7, 28.9, 49.1 Hz, 0.5F; **20a**-9*S*), −208.43 (ddd, *J* = 10.7, 28.9, 49.1 Hz, 0.5F; **20a**-9*R*); MS (APCI) *m*/*z* 310 ($MH^+$).

### 3.18. *N*-(*tert*-Butoxycarbonyl)-*S*-(3,5-dideoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl)-L-homocysteine *tert*-Butyl ester (20b)

*Step a*: $H_2O$ (0.24 mL) and tris(2-carboxyethyl)phosphine hydrochloride (88 mg, 0.31 mmol) were added to a stirred solution of *N*,*N*′-di(*tert*-butoxycarbonyl)-L-homocystine di(*tert*-butyl) ester (160 mg, 0.28 mmol) in anhydrous DMF (2.4 mL) at ambient temperature under Ar atmosphere. After 32 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 $NaHCO_3/H_2O$. After separation, the aqueous layer was extracted with EtOAc, and the combined organic layer was washed with brine, dried ($Na_2SO_4$), and concentrated to give *N*-*tert*-butoxycarbonyl-L-homocysteine *tert*-butyl ester[22] (159 mg, 99%) as colorless oil of sufficient purity to be directly used in next step. *Step b*: A freshly prepared homocysteine (80 mg, 0.275 mmol) was dissolved in anhydrous DMF (0.5 mL), and was stirred under a vigorous stream of argon for 10 min at 0 °C (ice-bath). Next, a solution of LDA (138 μL, 2.0 M/THF–heptane) was added dropwise and after an additional 10 min **19b** (31 mg, 0.115 mmol) in anhydrous DMF (0.5 mL) was added via syringe. After 15 min, ice-bath was removed and the reaction mixture was stirred for 28 h at ambient temperature (TLC showed consumption of **19b**). 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 with brine, dried ($Na_2SO_4$) and was evaporated to give 94 mg of yellowish oily residue. This crude product was column chromatographed on silica gel (hexane/EtOAc, 4:1) to give **20b** as an colorless oil (49 mg, 92%): $^1$H NMR $\delta$ 1.33 (s, 3H), 1.45 (s, 9H), 1.48 (s, 9H), 1.51 (s, 3H), 1.84–1.95 (m, 1H), 2.05–2.16 (m, 1H), 2.63 (t, *J* = 7.7 Hz, 2H), 2.78 (dd, *J* = 8.4, 13.7 Hz, 1H), 2.86 (ddd, *J* = 1.4, 6.2, 13.7 Hz, 1H), 4.23–4.32 (m, 1H), 4.31 (dm, *J* = 28.2 Hz, 1H), 4.70 (dd, *J* = 3.8, 10.7 Hz, 1H), 4.98 (dd, *J* = 1.9, 49.9 Hz, 1H), 5.12 (br. d, *J* = 6.7 Hz, 1H), 5.96 (d, *J* = 3.9 Hz, 1H); $^{13}$C NMR $\delta$ 26.2, 26.7, 28.0, 28.3, 28.6, 28.7, 33.2, 53.3, 79.8, 80.1 (d, *J* = 19.4 Hz), 82.2, 82.41 (d, *J* = 32.7 Hz), 93.78 (d, *J* = 185.0 Hz), 104.8, 112.2, 155.3, 171.3; $^{19}$F NMR $\delta$ −208.16 (ddd, *J* = 10.4, 28.4, 49.8 Hz); HRMS (AP-ESI) *m*/*z* calcd for $C_{21}H_{37}FNO_7S$ $[M+H]^+$ 466.2269; found 466.2265.

### 3.19. *S*-(3,5-Dideoxy-3-fluoro-D-xylofuranos-5-yl)homocysteine [21a(9*R*/*S*)]

Treatment of **20a** (6 mg, 0.02 mmol; 9*R*/*S*, ~1:1) with TFA/$H_2O$ (9:1, 5 mL), as described for **10**, and purification by HPLC (preparative RP-C18 column, 5% $CH_3CN/H_2O$ for 45 min at 2.5 mL/min; $t_R$ = 16.5 min) gave **21a** (3 mg, 55%; 9*R*/*S*, ~1:1, α/β, ~2:3): $^{19}$F NMR ($D_2O$) $\delta$ −202.41 (ddd, *J* = 18.7, 25.4, 52.1 Hz, 0.2F; α, **21a**-9*S*), −202.38 (ddd, *J* = 18.7, 25.4, 52.1 Hz, 0.2F; α, **21a**-9*R*), −200.55 (ddd, *J* = 12.6, 28.0, 50.6 Hz, 0.3F; β, **21a**-9*S*), −200.52 (ddd, *J* = 12.6, 28.0, 50.6 Hz, 0.3F; β, **21a**-9*R*); $^1$H NMR spectra as reported below for **21a**-9*S*; MS (APCI) *m*/*z* 270 ($MH^+$); HRMS (LCT-ESI) *m*/*z* calcd for $C_9H_{16}FNO_5S$ $[M+H]^+$ 270.0811; found 270.0798.

### 3.20. *S*-(3,5-Dideoxy-3-fluoro-D-xylofuranos-5-yl)-L-homocysteine (21a)

Compound **20b** (17 mg, 0.037 mmol) was dissolved in TFA (1 mL) and the resulting mixture was stirred at ambient temperature for 4 h and was evaporated and coevaporated with toluene. The crude mixture [$^{19}$F NMR ($D_2O$) $\delta$ −208.45 (0.82F, **20a**-9*S*), −202.41 (0.07F; α, **21a**-9*S*), −200.55 (0.11F; β, **21a**-9*S*)] was treated (2 h) with TFA/$H_2O$ (9:1, 2 mL), as described for **10**, and purified on HPLC (preparative RP-C18 column, 5% $CH_3CN/H_2O$ for 45 min at 2.5 mL/min; $t_R$ = 16.5 min) to give **21a** (4.7 mg, 46% overall; α/β, ~2:3): $^1$H NMR ($D_2O$) $\delta$ 2.10–2.35 (m, 2H), 2.72–2.82 (m, 2H), 2.82–3.01 (m, 2H), 4.01 ('t' , *J* = 6.1 Hz, 1H), 4.33 (d, *J* = 12.6 Hz, 0.6H), 4.40 (ddd, *J* = 2.4, 4.3, 8.6 Hz, 0.4H), 4.39–4.55 (m, 1H), 5.03 (dd, *J* = 3.3, 50.6 Hz, 0.6H), 5.10 (ddd, *J* = 2.5, 3.6, 52.1 Hz, 0.4H), 5.30 (s, 0.6H), 5.53 (dd, *J* = 1.0, 4.3 Hz, 0.4H); $^{19}$F NMR ($D_2O$) $\delta$ −202.41 (ddd, *J* = 18.7, 25.4, 52.1 Hz, 0.4F; α), −200.55 (ddd, *J* = 12.6, 28.0, 50.6 Hz, 0.6F; β); MS (APCI) *m*/*z* 270 ($MH^+$). HRMS (TOF MS-ESI) *m*/*z* calcd for $C_9H_{16}FNNaO_5S$ $[M+Na]^+$ 292.0631; found 292.0611.

### 3.21. *S*-(3,5-Dideoxy-3-fluoro-1-*O*-methyl-D-xylofuranos-5-yl)-L-homocysteine (21b)

$BCl_3$ solution (1.0 M/$CH_2Cl_2$; 0.2 mL) was added dropwise to a stirred solution of **20b** (25 mg, 0.054 mmol) in $CH_2Cl_2$ (3 mL) at −50 °C, under Ar atmosphere. The reaction mixture was stirred at same temperature for 20 min, and reaction was quenched with MeOH (2 mL). The volatiles were evaporated and resulting crude product was purified on RP-HPLC (as described for **21a**) to give **21a** (1 mg, 7%; α/β 1:3; $t_R$ = 16.5 min), and **21b** (4.5 mg, 29%; α/β 45:55; $t_R$ = 24.5 min). Compound **21b** had: $^1$H NMR ($D_2O$) $\delta$ 2.07–2.28 (m, 2H), 2.76 (q, *J* = 7.1 Hz, 2H), 2.80–3.00 (m, 2H), 3.43 (s, 0.55H), 3.46 (s, 0.45H), 3.89 ('t', *J* = 6.1 Hz, 1H), 4.30–4.75 (m, 1H), 4.38 (d, *J* = 12.2 Hz, 0.55H), 4.47 (ddd, *J* = 2.9, 4.5, 23.2 Hz, 0.45H), 4.97 (s, 0.55H), 5.05 (dd, *J* = 4.0, 51.2 Hz, 0.55H), 5.09 (ddd, *J* = 3.0, 4.6, 53.5 Hz, 0.45H), 5.12 (d, *J* = 4.6 Hz, 0.45H); $^{13}$C NMR ($D_2O$) $\delta$ 27.3, 27.4, 29.2, 29.3, 30.2, 30.2, 53.6, 55.4, 55.6, 75.6 (d, *J* = 27.1 Hz), 76.9 (d, *J* = 19.7 Hz), 77.3 (d, *J* = 27.1 Hz), 81.2 (d, *J* = 19.5 Hz), 94.8 (d, *J* = 185.2 Hz), 96.9 (d, *J* = 184.2 Hz), 102.1 (d, *J* = 6.0 Hz), 108.7, 173.9; $^{19}$F NMR ($D_2O$) $\delta$ −201.21 (dt, *J* = 22.8, 53.6 Hz, 0.45F; α), −199.51 (ddd, *J* = 11.9, 27.1, 50.8 Hz, 0.55F; β); MS (AP-CI) *m*/*z* 284 $(MH)^+$. HRMS (TOF MS-ESI) *m*/*z* calcd for $C_{10}H_{18}FNNaO_5S$ $[M+Na]^+$ 306.0787; found 306.0775.

### 3.22. LuxS assay

Inhibition assays were performed in a buffer containing 50 mM HEPES (pH 7.0), 150 mM NaCl, 150 μM 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) and various concentrations of SRH (0–55 μM) and inhibitors (0–1 mM). The reactions were initiated by the addition of Co-BsLuxS (final concentration 0.4–0.5 μM) and monitored continuously at 412 nm ($\varepsilon$ = 14150 $M^{-1}$ $cm^{-1}$) in a Perkin–Elmer λ25 UV–vis spectrophotometer at room temperature (23 °C). The initial rates recorded from the early regions of the progress curves were fitted into the Lineweaver–Burk equation $1/V = K'_M/(k_{cat}\ [E]_0) \times 1/[S] + 1/(k_{cat}\ [E]_0)$ and the Michaelis–Menten equation $V = k_{cat}\ [E]_0\ [S]/(K'_M + [S])$ using KaleidaGraph 3.5 to determine

the inhibition pattern. $K_I$ values were calculated from the equation ($K'_M = K_M \times (1 + [I]/K_I)$, where $K_M = 2.2$ μM.

## Acknowledgments

This work was supported by grants from the NIH (SC1CA138176 and AI062901). J.R. was sponsored by MBRS RISE program (NIH/NIGMS; R25GM61347).

## References and notes

1. Lee, J. E.; Cornell, K. A.; Riscoe, M. K.; Howell, P. L. *Structure* **2001**, *9*, 941.
2. Singh, V.; Shi, W.; Almo, S. C.; Evans, G. B.; Furneaux, R. H.; Tyler, P. C.; Painter, G. F.; Lenz, D. H.; Mee, S.; Zheng, R.; Schramm, V. L. *Biochemistry* **2006**, *45*, 12929.
3. Pei, D.; Zhu, J. *Curr. Opin. Chem. Biol.* **2004**, *8*, 492.
4. Schauder, S.; Shokat, K.; Surette, M. G.; Bassler, B. L. *Mol. Microbiol.* **2001**, *41*, 463.
5. Winzer, K.; Hardie, K. R.; Burgess, N.; Doherty, N.; Kirke, D.; Holden, M. T. G.; Linforth, R.; Cornell, K. A.; Taylor, A. J.; Hill, P. J.; Williams, P. *Microbiology* **2002**, *148*, 909.
6. Miller, S. T.; Xavier, K. B.; Campagna, S. R.; Taga, M. E.; Semmelhack, M. F.; Bassler, B. L.; Hughson, F. M. *Mol. Cell* **2004**, *15*, 677.
7. Chen, X.; Schauder, S.; Potier, N.; Van Dorsselaer, A.; Pelczer, I.; Bassler, B. L.; Hughson, F. M. *Nature* **2002**, *415*, 545.
8. Meijler, M. M.; Hom, L. G.; Kaufmann, G. F.; McKenzie, K. M.; Sun, C.; Moss, J. A.; Matsushita, M.; Janda, K. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 2106.
9. Lowery, C. A.; Park, J.; Kaufmann, G. F.; Janda, K. D. *J. Am. Chem. Soc.* **2008**, *130*, 9200.
10. Semmelhack, M. F.; Campagna, S. R.; Federle, M. J.; Bassler, B. L. *Org. Lett.* **2005**, *7*, 569.
11. Semmelhack, M. F.; Campagna, S. R.; Hwa, C.; Federle, M. J.; Bassler, B. L. *Org. Lett.* **2004**, *6*, 2635.
12. De Keersmaecker, S. C. J.; Varszegi, C.; van Boxel, N.; Habel, L. W.; Metzger, K.; Daniels, R.; Marchal, K.; De Vos, D.; Vanderleyden, J. *J. Biol. Chem.* **2005**, *280*, 19563.
13. Frezza, M.; Soulere, L.; Queneau, Y.; Doutheau, A. *Tetrahedron Lett.* **2005**, *46*, 6495.
14. Frezza, M.; Balestrino, D.; Soulère, L.; Reverchon, S.; Queneau, Y.; Forestier, C.; Doutheau, A. *Eur. J. Org. Chem.* **2006**, 4731.
15. Frezza, M.; Soulere, L.; Balestrino, D.; Gohar, M.; Deshayes, C.; Queneau, Y.; Forestier, C.; Doutheau, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1428.
16. Waters, C. M.; Bassler, B. L. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 319.
17. Bassler, B. L.; Losick, R. *Cell* **2006**, *125*, 237.
18. Camilli, A.; Bassler, B. L. *Science* **2006**, *311*, 1113.
19. Lebeer, S.; De Keersmaecker, S. C. J.; Verhoeven, T. L. A.; Fadda, A. A.; Marchal, K.; Vanderleyden, J. *J. Bacteriol.* **2007**, *189*, 860.
20. Zhu, J.; Dizin, E.; Hu, X.; Wavreille, A.-S.; Park, J.; Pei, D. *Biochemistry* **2003**, *42*, 4717.
21. Rajan, R.; Zhu, J.; Hu, X.; Pei, D.; Bell, C. E. *Biochemistry* **2005**, *44*, 3745.
22. Zhu, J.; Hu, X.; Dizin, E.; Pei, D. *J. Am. Chem. Soc.* **2003**, *125*, 13379.
23. Zhu, J.; Patel, R.; Pei, D. *Biochemistry* **2004**, *43*, 10166.
24. Zhu, J.; Knottenbelt, S.; Kirk, M. L.; Pei, D. *Biochemistry* **2006**, *45*, 12195.
25. Turner, M. A.; Yang, X.; Yin, D.; Kuczera, K.; Borchardt, R. T.; Howell, P. L. *Cell Biochem. Biophys.* **2000**, *33*, 101.
26. Wnuk, S. F. *Mini-Rev. Med. Chem.* **2001**, *1*, 307.
27. Alfaro, J. F.; Zhang, T.; Wynn, D. P.; Karschner, E. L.; Zhou, Z. S. *Org. Lett.* **2004**, *6*, 3043.
28. Shen, G.; Rajan, R.; Zhu, J.; Bell, C. E.; Pei, D. *J. Med. Chem.* **2006**, *49*, 3003.
29. Wnuk, S. F.; Lalama, J.; Garmendia, C. A.; Robert, J.; Zhu, J.; Pei, D. *Bioorg. Med. Chem.* **2008**, *16*, 5090.
30. Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059.
31. Xie, M.; Berges, D. A.; Robins, M. J. *J. Org. Chem.* **1996**, *61*, 5178.
32. Miles, R. W.; Nielsen, L. P. C.; Ewing, G. J.; Yin, D.; Borchardt, R. T.; Robins, M. J. *J. Org. Chem.* **2002**, *67*, 8258.
33. Moravcová, J.; Capková, J.; Stanek, J. *Carbohydr. Res.* **1994**, *263*, 61.
34. Tewson, T. J.; Welch, M. J. *J. Org. Chem.* **1978**, *43*, 1090.
35. Gopishetty, B.; Zhu, J.; Rajan, R.; Sobczak, A. J.; Wnuk, S. F.; Bell, C. E.; Pei, D. *J. Am. Chem. Soc.* **2009**, *131*, 1243.
36. Shiozaki, M.; Deguchi, N.; Mochizuki, T.; Wakabayashi, T.; Ishikawa, T.; Haruyama, H.; Kawai, Y.; Nishijima, M. *Tetrahedron* **1998**, *54*, 11861.
37. Bentley, N.; Dowdeswell, C. S.; Singh, G. *Heterocycles* **1995**, *41*, 2499.
38. Yadav, J. S.; Subba Reddy, B. V. *Carbohydr. Res.* **2000**, *329*, 885.
39. Yan, S.; Klemm, D. *Tetrahedron* **2002**, *58*, 10065.
40. Haga, M.; Takano, M.; Tejima, S. *Carbohydr. Res.* **1972**, *21*, 440.
41. Jeong, L. S.; Lim, B. B.; Marquez, V. E. *Carbohydr. Res.* **1994**, *262*, 103.
42. Tsoukala, E.; Agelis, G.; Dolinsek, J.; Botic, T.; Cencic, A.; Komiotis, D. *Bioorg. Med. Chem.* **2007**, *15*, 3241.