

# Salvadenosine, a 5'-Deoxy-5'-(methylthio) Nucleoside from the Bahamian Tunicate *Didemnum* sp.

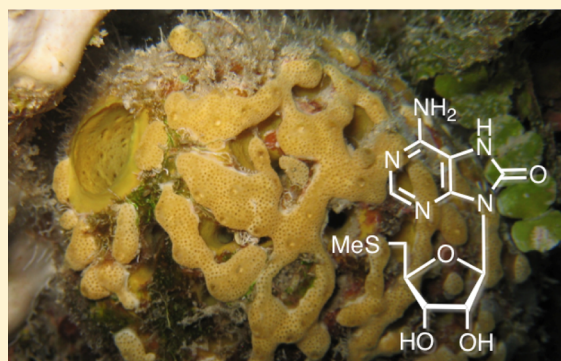
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**S** Supporting Information

**ABSTRACT:** Salvadenosine, (**1**) a rare 5'-deoxy-5'-(methylthio) nucleoside, was isolated from the deep-water Bahaman tunicate *Didemnum* sp. The structure was solved by integrated analysis of MS and 1D and 2D NMR data. We revise the structure of the known natural product, hamiguanosinol, which is a constitutional isomer of **1**, to **5** by interpretation of the spectroscopic data and comparison with synthesized nucleosides.

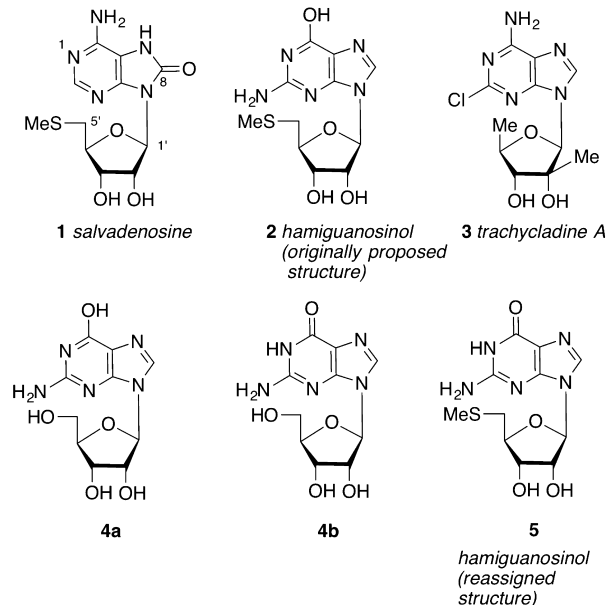


## INTRODUCTION

Modified nucleosides are relatively rare from marine organisms, but they have a long history. Early pioneering work<sup>1</sup> by Bergmann on Caribbean sponges of the genera *Tethya* and *Cryptotethya* resulted in the isolation of spongosine, spongothymidine,<sup>2</sup> and spongouridine<sup>2b,c</sup> (the first *arabino*-nucleosides) and others. Predating the modern era of marine natural products chemistry, these seminal discoveries were the inspiration<sup>3</sup> for development of the clinically important antitumor drugs Ara A<sup>4</sup> and Ara C.<sup>5</sup> In our investigations of antifungal and antitumor compounds using “nanomole-scale” techniques,<sup>6</sup> we examined several extracts of rare tunicates that displayed antifungal activity against a panel of *Candida* spp. and *Cryptococcus* spp. Here, we report salvadenosine (**1**), an uncommon 5'-deoxy-5'-(methylthio) nucleoside from an encrusting deep-water tunicate *Didemnum* sp. (Figure 1). In addition, we revise the structure of the known compound, hamiguanosinol (**2**), reported by Proksch and co-workers from the Pacific sponge *Hamigera hamigera*.<sup>7</sup> Salvadenosine (**1**) joins the family of rare marine-derived nucleosides that include Bergmann's *arabino*-nucleosides from *Cryptotethya* sp.<sup>2</sup> and the antiproliferative trachycladine A (**3**) from the sponge *Trachycladus laevispirulifer*.<sup>8</sup>

## RESULTS

The *n*-BuOH soluble partition of the methanol extract of *Didemnum* sp. was separated by reversed-phase HPLC to give **1** in addition to tryptamine and the known natural product 6-bromotryptamine.<sup>9</sup> The molecular formula of **1**, C<sub>11</sub>H<sub>15</sub>N<sub>5</sub>O<sub>4</sub>S, established from HRMS (ESI-TOF *m/z* 312.0777 [M - H]<sup>-</sup>), was isomeric with **2** (Table 1). COSY correlations confirmed



**Figure 1.** Structures of marine nucleosides (**1–3**), the tautomers of guanosine (**4a**, **4b**), and original and revised structures of hamiguanosinol (**2** and **5**).

the 5'-deoxy-5'-(methylthio)ribose moiety. Cross peaks arising from a modified ribose corresponded to the following contiguous spin system: anomeric proton H-1' ( $\delta_{\text{H}}$  5.87, d, J

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Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for **1** (Formate Salt,  $\text{CD}_3\text{OD}$ )

atom	$1 \delta^{13}\text{C}^a$	$1 \delta^1\text{H}$ (mult, $J$ , integ) <sup>b</sup>	gHMBC ( $^1\text{H} \rightarrow ^{13}\text{C}$ )	COSY ( $^1\text{H} \rightarrow ^1\text{H}$ )
1				
2	152.3 <sup>c</sup>	8.08 (s, 1H) <sup>c</sup>	4, 5	
3				
4	148.7			
5	105.2			
6	148.7			
7				
8	154.0			
1'	87.9	5.87 (d, $J = 4.9$ Hz, 1H)	2', 4, 6, 8	2'
2'	72.0	5.12 (t, $J = 4.9$ Hz, 1H)		1', 3'
3'	74.5	4.42 (t, $J = 4.9$ Hz, 1H)	1', 5'	2', 4'
4'	85.1	4.07 (ddd, $J = 4.9, 5.5, 7.2$ Hz, 1H)	3'	3', 5'a, 5'b
5'a	37.4	2.88 (dd, $J = -14.0, 5.5$ Hz, 1H)	3', 4', 6'	4'
5'b		2.81 (dd, $J = -14.0, 7.2$ Hz, 1H)		
6'	16.1	2.10 (s, 3H)	5'a, 5'b	

<sup>a</sup>125 MHz. <sup>b</sup>600 MHz. <sup>c</sup> $J_{\text{CH}} = 203.4$  Hz.

= 4.9 Hz) to H-2' ( $\delta_{\text{H}}$  5.12, t,  $J = 4.9$  Hz) to H-3' ( $\delta_{\text{H}}$  4.42, t,  $J = 4.9$  Hz) to H-4' ( $\delta_{\text{H}}$  4.07, ddd,  $J = 4.9, 5.5, 7.2$  Hz) and the diastereotopic protons H-5'a and H-5'b of a methylene attached to S instead of O ( $\delta_{\text{H}}$  2.88, dd,  $J = -14.0, 5.5$  Hz; 2.81, dd,  $J = -14.0, 7.2$  Hz). An HMBC correlation from the three-proton singlet ( $\delta_{\text{H}}$  2.10, s,  $\delta_{\text{C}}$  16.1,  $J_{\text{CH}} = 138.4$  Hz) to C-5' established the location of the MeS group.

Distinct differences were apparent between the  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts of **1** and those published for hamiguanosinol (**2**, Table 2), particularly the  $\text{sp}^2$   $^{13}\text{C}$  NMR signals. An HSQC

Table 2. Comparison of  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for **1**<sup>a</sup> and **2** (DMSO- $d_6$ )

atom	$1 \delta^1\text{H}^a$	$1 \delta^{13}\text{C}^b$	$2 \delta^1\text{H}^c$	$2 \delta^{13}\text{C}^c$	$\Delta\delta^1\text{H}$ (1-2)	$\Delta\delta^{13}\text{C}$ (1-2)
1						
2	8.00	150.8		153.5		-2.7
3						
4		146.7		153.0		-6.3
5		103.6		118.0		-14.4
6		147.2		157.2		-10.0
7						
8		151.7	7.90	135.0		16.7
1'	5.65	85.8	5.60	87.0	0.05	-1.2
2'	4.96	69.9	4.50	74.0	0.46	-4.1
3'	4.19	72.9	3.95	71.5	0.24	1.4
4'	3.88	83.0	4.05	84.0	-0.17	-1.0
5'a	2.80	36.1	2.80	35.0	0.0	1.1
5'b	2.69		2.70		-0.01	
6'	2.03	15.5	2.05	16.0	-0.02	-0.5

<sup>a</sup>Formate salt, 600 MHz. <sup>b</sup>125 MHz. <sup>c</sup>Reference 7.

correlation from the sole downfield nonexchangeable aromatic signal ( $\delta_{\text{H}}$  8.00,  $\delta_{\text{C}}$  150.8,  $J_{\text{CH}} = 203.4$  Hz) revealed a  $^{13}\text{C}$  chemical shift that was deshielded ( $\Delta\delta$  15.8 ppm) compared to that of the H-8 of **2** ( $\delta_{\text{H}}$  7.90,  $\delta_{\text{C}}$  135.0). Another notable difference was observed for the most shielded  $\text{sp}^2$   $^{13}\text{C}$  signal of **1** ( $\delta_{\text{C}}$  103.6, s) and **2** ( $\delta_{\text{C}}$  118.0, s, C-8;  $\Delta\delta$  14.4 ppm). HMBC correlations of **1** were observed from the anomeric proton H-1' signal, and  $\text{sp}^2$   $^{13}\text{C}$  signals were also inconsistent with guanine; for example, no correlation was observed between the downfield  $^1\text{H}$  NMR signal ( $\delta_{\text{H}}$  8.08, s, 1H) and the anomeric

carbon, C-1', a correlation common to guanosine nucleosides.<sup>10</sup> The HSQC (DMSO- $d_6$ ) showed the aforementioned downfield proton was attached to a carbon with a  $^{13}\text{C}$  signal ( $\delta_{\text{C}}$  151.7, s) more compatible with an imidazolone C=O group than the imidazole C-8 chemical shift of **2** ( $\delta_{\text{C}}$  135.0, s). A better match for the NMR data of **1** was obtained by replacement of guanine with 8-oxoadenine, a nucleobase isomeric with the former. The downfield  $^1\text{H}$  NMR signal ( $\delta_{\text{H}}$  8.08, s, 1H) was assigned to H-2, and all inconsistencies were resolved. For example, the expected long-range correlation between C-1' and H-8 for a guanine ring system—but missing in **2**—is replaced by  $^2J$  correlations of H-2 to C-4 and C-5 in an 8-oxoadenine, respectively. The  $^{13}\text{C}$  chemical shifts (DMSO- $d_6$ , Table 2) of the quaternary ring junction carbons C-4 and C-5 ( $\delta_{\text{C}}$  146.7, s; 103.6, s) of **1** are more polarized ( $\Delta\delta$  [C-4 - C-5] = 43.1 ppm) than the corresponding signals of **2** ( $\Delta\delta = 35$  ppm), guanosine, or adenosine but closer in magnitude than the 8-oxoadenosines, apudiamine, erinacean, and caissarone.<sup>11</sup> Additionally, we measured the heteronuclear coupling constants of the downfield  $\text{sp}^2$   $^1\text{H}$  NMR singlets in **1** and several purine nucleosides (Table 3), revealing a better match between 8-oxoadenosine and the natural product (**1**: H-2,  $J_{\text{CH}} = 203.4$  Hz; 8-oxoadenosine: H-2  $J_{\text{CH}} = 201.5$  Hz; guanosine: H-8  $J_{\text{CH}} = 213.5$  Hz).

The natural products salvadenosine (**1**) and hamiguanosinol are clearly not identical, but isomeric (Figure 1). Naturally, the

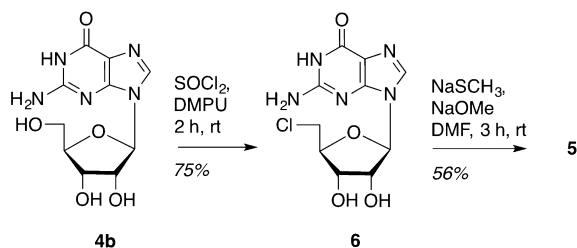
Table 3.  $^1\text{H}$ ,  $^{13}\text{C}$ , and  $J_{\text{CH}}$  NMR Data for Downfield  $^1\text{H}$  NMR Singlets of Purine Nucleosides

compd	atom	$\delta^1\text{H}^a$	$\delta^{13}\text{C}^b$	$J_{\text{CH}}^c$
adenosine ( <b>8</b> )	2	8.13 <sup>d</sup>	152.9 <sup>d</sup>	199.1
<b>8</b>	8	8.35 <sup>d</sup>	140.4 <sup>d</sup>	213.4
guanosine ( <b>4b</b> )	8	7.94 <sup>d</sup>	135.6 <sup>d</sup>	213.5
Hamiguanosinol ( <b>2</b> )	8	7.93 <sup>d</sup>	135.8 <sup>d</sup>	213.3
8-oxoadenosine ( <b>10</b> )	2	8.01 <sup>d</sup>	150.7 <sup>d</sup>	201.5
5'-chloro-5'-deoxy-8-oxoadenosine ( <b>11</b> )	2	8.03 <sup>d</sup>	150.9 <sup>d</sup>	203.5
salvadenosine ( <b>1</b> ) <sup>f</sup>	2	8.08 <sup>e</sup>	152.3 <sup>e</sup>	203.4
<b>1</b> <sup>g</sup>	2	8.06 <sup>e</sup>	152.1 <sup>e</sup>	202.4

<sup>a</sup>500 MHz. <sup>b</sup>125 MHz. <sup>c</sup>Measured from  $^{13}\text{C}$  satellites in the  $^1\text{H}$  NMR spectrum (500 MHz). <sup>d</sup>DMSO- $d_6$ . <sup>e</sup> $\text{CD}_3\text{OD}$ . <sup>f</sup>Formate salt. <sup>g</sup>Free base.

question of validity of the structure of **2** arises. The nucleobase in hamiguanosinol (incorrectly named “6-hydroxyguanine”) <sup>7</sup> was assigned as the enol tautomer (cf. enol form of guanine **4a**, Figure 1) based on a <sup>13</sup>C shift for C-6 of  $\delta_C$  157.2 “instead of a ca.170 ppm for a keto amide function”; <sup>7</sup> however, we find this less convincing for three reasons. Keto groups in purines (guanine, inosine, etc.) are not electronically equivalent to simple amides and often exhibit relatively high field chemical shifts in the range observed for **3**; the C-6 <sup>13</sup>C chemical shift reported for hamiguanosinol is not incompatible with the 6-keto tautomer **4b**. Numerous studies have shown the keto form of guanosine (**4b**) and deoxyguanosine are the naturally stable tautomers in protic solvents: in fact, Watson–Crick pairing of G–C in DNA is crucially dependent upon it. Given that electronic differences between guanosine and **2** are insignificant, there are no compelling reasons to expect that **2** would be “locked” in the enol form and unable to spontaneously tautomerize to the keto form in protic solvent. Lastly, compound **5** has been synthesized by van Tilburg and co-workers <sup>12</sup> who characterized it as the keto tautomer. We repeated the synthesis of **5** (Scheme 1) from guanosine (**4b**)

**Scheme 1. Synthesis of 5'-Deoxy-5'-(methylthio)guanosine (5)**



and showed the <sup>13</sup>C chemical shifts of the product were essentially the same as those reported for natural hamiguanosinol (Supporting Information, Table S2). <sup>13</sup> Therefore, the structure of hamiguanosinol is the keto tautomer **5**, <sup>14</sup> not the enol **2**: synthetic **5** and hamiguanosinol are identical.

In order to verify the structure of salvadenosine (**1**), the natural product was synthesized by extension of the sequence of reactions used to prepare **5**. *N,O*-Protected adenosine <sup>15</sup> was subjected to bromination (Scheme 2, saturated Br<sub>2</sub>–H<sub>2</sub>O, pH 4 NaOAc buffer), but only the cyclized product **7** was formed presumably through intramolecular attack by the 5'-OH group after bromination at C-8. <sup>16</sup> The same result was observed under aprotic conditions (NBS, DMF; 5,5-dimethyl-1,3-dibromohydantoin, DBH, DMF); <sup>17</sup> consequently, we turned to a “protecting-group free” strategy (Scheme 2). Adenosine (**8**) was converted to 8-bromoadenosine (**9**) (saturated Br<sub>2</sub>–H<sub>2</sub>O, pH 4 buffer) followed by an efficient conversion to the 8-oxoadenosine (**10**) under Chatgililoglus conditions. <sup>18</sup> Compound **10** was converted to primary chloride **11** with improved yield (SOCl<sub>2</sub>, DMPU, 70%); the latter, in turn, was subjected to nucleophilic substitution with sodium thiomethoxide to yield **1** in four steps and 17% yield from adenosine. Since the sample of natural **1** was purified as the formate salt, synthetic **1** was also converted to the formate salt for comparison. The <sup>1</sup>H and <sup>13</sup>C NMR spectra (Supporting Information, Table S3) as well as UV, IR, and CD data of the two samples **1**·HCO<sub>2</sub>H matched in every way. Co-injection of natural **1** with synthetic **1** by HPLC resulted in a single peak (see the Supporting Information,

**Scheme 2. Synthesis of 5'-Deoxy-5'-(methylthio)-8-oxoadenosine (1)**

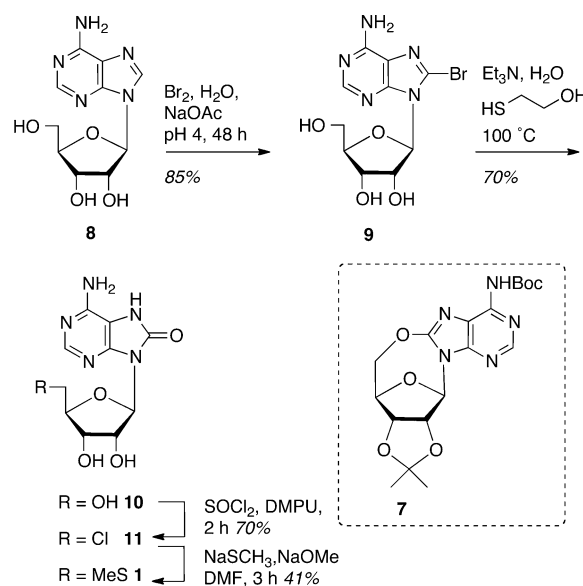
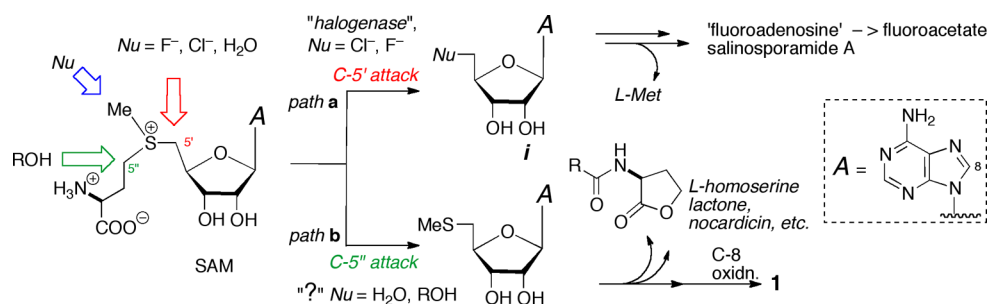


Figure S1). Therefore, salvadenosine is assigned the structure **1** with high confidence.

## DISCUSSION

The nature of the 5'-(methylthio) group in **1** and **5** deserves some comment. One plausible origin of **1** is from *S*-adenosyl methionine (SAM). The two CH<sub>2</sub> groups and one CH<sub>3</sub> group bonded to the S in SAM are electrophilic in nature. Common biological methylation involves S<sub>N</sub>2 nucleophilic substitution of the electrophilic Me-S bond through attack by C-, N-, O-, S-centered nucleophiles, generating *S*-adenosylhomocysteine: the latter is cycled back to adenosine and homocysteine. In the biosynthesis of both the chlorinated antitumor drug salinosporamide A <sup>19</sup> and rare fluorinated natural products, substantial biochemical and structural evidence supports participation of the 5'-CH<sub>2</sub>-S bond of SAM in S<sub>N</sub>2 substitutions by halide ions (Figure 2). For example, *Streptomyces cattleya* produces 5'-fluoroadenosine through nucleophilic attack at the 5'-CH<sub>2</sub> of SAM by F<sup>-</sup> (path a), catalyzed by the enzyme fluorinase; <sup>20</sup> the former in turn is catabolized to fluoroacetate. A homologous “chlorinase” catalyzes displacement at 5'-CH<sub>2</sub> of SAM by Cl<sup>-</sup> (path a) in salinosporamide A biosynthesis. <sup>19</sup> Phylogenetic and biochemical studies <sup>21</sup> have shown a bifurcation of this biosynthetic motif. The gene *duf62*, represented in Nature in about 100 bacterial and archaeal genomes, expresses a protein, DUF62, that has high structural homology to the halogenases. DUF62, a “protein of unknown function,” lacks halogenase activity, but carries out hydrolysis of SAM by nucleophilic attack at the 5'-CH<sub>2</sub> group of adenosine group by HO<sup>-</sup> (path b) to liberate L-methionine and adenosine. Attack at the 5'-CH<sub>2</sub> of SAM is similarly rare, with the best-characterized example occurring in the biosynthesis of nocardicin. <sup>22</sup> Transfer of the 3-amino-3-carboxypropyl group from SAM has also been proposed in the biosynthesis of modified bases for bacterial and yeast tRNAPhe, <sup>23</sup> the natural product discadenine, <sup>24</sup> nicotianamine (a precursor of plant siderophores), <sup>25</sup> and homoserine-based betaine lipids. <sup>26</sup> In addition, transfer of the 3-amino-propyl group from decarboxylated SAM is involved in



**Figure 2.** Hypothetical biosynthetic origin of **1** from *S*-adenosylmethionine (SAM). See text for discussion.

polyamine biosynthesis.<sup>27</sup> Common to these biological reactions is the release of 5'-deoxy-5'-(methylthio)adenosine.

We speculate that **1** may arise through displacement of 5'-deoxy-5'-(methylthio)adenosine from SAM (path b), possibly via a LuxI-type mechanism with release of homoserine lactone.<sup>28,29</sup> The product may then be converted to **1** by electrophilic or free-radical attack at C-8 with reactive oxygen species (ROS), a reaction known from purine metabolism and the biology of DNA damage.<sup>30</sup> The anomaly is **5**; no guanosine analogue of SAM has been demonstrated yet, but **5** has been detected in human urine as a byproduct of nucleotide catabolism.<sup>14</sup>

In conclusion, we confirmed the structure of a new nucleoside salvadenosine (**1**, 5'-deoxy-5'-(methylthio)-8-oxoadenosine) from the tunicate *Didemnum* sp. through integrated analysis of spectroscopic data and total synthesis. Re-evaluation of the published structure of hamiguanosinol and its synthesis from guanosine requires revision of the structure of hamiguanosinol<sup>7</sup> from the enol tautomer **2** to the keto form **5**.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Inverse detected 2D NMR spectra were measured on a 500 MHz spectrometer equipped with a 5 mm <sup>1</sup>H{<sup>13</sup>C} 5 mm probe or a 600 MHz NMR spectrometer with a 1.7 mm <sup>1</sup>H{<sup>13</sup>C} microcryoprobe. <sup>13</sup>C NMR spectra were collected on a 125 MHz spectrometer equipped with a 5 mm <sup>13</sup>C{<sup>1</sup>H} cryoprobe. NMR spectra were referenced to residual solvent signals, CD<sub>3</sub>OD ( $\delta_{\text{H}}$  3.31,  $\delta_{\text{C}}$  49.00), (CD<sub>3</sub>)<sub>2</sub>SO ( $\delta_{\text{H}}$  2.50,  $\delta_{\text{C}}$  39.52). High-resolution mass data were obtained with an ESI-TOF system. Low-resolution MS measurements were made on a UHPLC coupled to an MSD single-quadrupole detector. Automated medium-pressure chromatography was carried out using a 30 g C<sub>18</sub> cartridges under specified gradient elution conditions. Optical rotations were measured at the D-double emission line of Na. FTIR spectra were collected on a ZnSe plate. CD spectra were measured in quartz cells (1 or 2 mm path length). DMPU and triethylamine were distilled from CaH<sub>2</sub> under N<sub>2</sub>. Other solvents were dried by passage through activate alumina or molecular sieves under Ar. Reactions were performed under N<sub>2</sub>.

**Animal Material.** Two samples of the tunicate *Didemnum* sp. (11-25-039 and 11-14-018) were collected in July 2011 off Little San Salvador Island, Bahamas, at a depths of -28 and -32 m. Voucher samples of the tunicates (11-25-039 and 11-14-018) are archived at UC San Diego.

**Extraction and Isolation.** The MeOH extract (5 mL) of *Didemnum* sp. (11-14-018, 1.21 g dry) was separated by progressive solvent partition. The MeOH extract was adjusted to approximately 1:9 H<sub>2</sub>O/MeOH and was extracted repeatedly with hexane (5 mL  $\times$  3) to yield fraction A (7.7 mg). The aqueous layer was then adjusted to 2:3 H<sub>2</sub>O/MeOH followed by extraction with CH<sub>2</sub>Cl<sub>2</sub> (5 mL  $\times$  3) to yield fraction B (7.2 mg). The aqueous layer was adjusted to approximately 9:1 H<sub>2</sub>O/MeOH before the solution was extracted with *n*-BuOH (5 mL  $\times$  3) to yield fraction C (18.5 mg). Removal of the volatiles from the remaining aqueous phase provided fraction D (20

mg). Fraction C was further purified by reversed-phase HPLC (phenylhexyl column, 10  $\times$  250 mm, linear gradient (initial conditions 90:10 H<sub>2</sub>O (0.1% HCO<sub>2</sub>H)-CH<sub>3</sub>CN to 50:50 over 20 min) to yield 5'-deoxy-5'-(methylthio)-8-oxoadenosine (1·HCO<sub>2</sub>H, 0.8 mg,  $t_{\text{R}}$  = 10.2 min), 6-bromotryptamine (2.2 mg,  $t_{\text{R}}$  = 12.6 min), and tryptamine (0.43 mg,  $t_{\text{R}}$  = 9.4 min). Fraction B was purified under identical conditions to obtain additional **1** (0.46 mg) and 6-bromotryptamine (0.34 mg). The combined samples of **1** were repurified by HPLC over the same column (90:10 H<sub>2</sub>O-MeOH, 0.5% HCO<sub>2</sub>H) to yield 1·HCO<sub>2</sub>H (0.42 mg, calculated through NMR quantification of <sup>13</sup>C satellite peaks<sup>31</sup>). Extraction of sample 11-14-039 and purification was carried out in a similar manner to provide additional 1·HCO<sub>2</sub>H (~0.3 mg).

**Salvadenosine (1):** pale yellow powder, HCO<sub>2</sub><sup>-</sup> salt; [ $\alpha$ ]<sub>D</sub> +11.3 ( $c$  0.3, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  210 nm ( $\epsilon$  log<sub>10</sub> 4.49), 255 (3.95), 270 (4.00); FTIR (ATR, ZnSe plate):  $\nu$  3356, 3188, 2920, 2850, 1710, 1662, 1633, 1590, 1379, 1350, 1131, 1092, 1038, 1006 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR see Table 1 (CD<sub>3</sub>OD) and Supporting Information, Table S1 (DMSO-*d*<sub>6</sub>); ESI-TOF  $m/z$  312.0777 [M - H]<sup>-</sup> (calcd for C<sub>11</sub>H<sub>14</sub>N<sub>5</sub>O<sub>4</sub>S 312.0767).

**6-Bromotryptamine:** colorless powder; <sup>1</sup>H, <sup>13</sup>C, and HRMS data were consistent with previously published data.<sup>9</sup>

**Tryptamine:** colorless powder; MS and <sup>1</sup>H NMR spectra were identical to those of an authentic sample.

**HPLC Comparison of Synthetic and Natural 1.** Samples of natural and synthetic **1** (see below) were prepared in MeOH as equimolar solutions (0.025 mg/mL), and aliquots of each solution, along with an admixture of both (equivolume), were analyzed by HPLC (Polar-RP column, 4  $\mu$ m, 80 Å, 150  $\times$  4.6 mm, gradient elution; (H<sub>2</sub>O + 0.1% HCO<sub>2</sub>H/CH<sub>3</sub>CN, 0–5 min hold at 5% CH<sub>3</sub>CN, 5–18 min ramp to 50% CH<sub>3</sub>CN, 1 mL/min, 40 °C column oven). The following retention times were obtained: natural **1**,  $t_{\text{R}}$  = 10.48 min; synthetic **1**,  $t_{\text{R}}$  = 10.47 min; combined natural and synthetic samples,  $t_{\text{R}}$  = 10.48 min, single peak. (Supporting Information, Figure S1).

**5'-Chloro-5'-deoxyguanosine (6).** A protocol with improved yield was modeled after a literature procedure.<sup>12</sup> Guanosine (0.60 g, 2.12 mmol) was suspended in dry DMPU (10.6 mL) and dissolved upon heating. After the mixture was cooled in an ice bath, thionyl chloride (770  $\mu$ L, 10.6 mmol, 5 equiv) was slowly added with stirring and the mixture was warmed to 23 °C over 2 h. The mixture was cooled in an ice bath, diluted with cold H<sub>2</sub>O (10 mL), and absorbed onto a column of Dowex 50  $\times$  2–400 resin (200–400 mesh, H<sup>+</sup> form). The column was washed with water and the compound eluted with 5% aqueous ammonia. The volatiles were removed from the eluate under reduced pressure to yield **6** (478 mg, 1.58 mmol, 75% yield). The <sup>1</sup>H NMR spectrum of **6** was consistent with published data.<sup>7</sup>

**5'-Deoxy-5'-(methylthio)guanosine (5).** 5'-Chloro-5'-deoxyguanosine (0.10 g, 0.33 mmol) was dissolved in dry DMF with sodium thiomethoxide (232 mg, 3.3 mmol, 10 equiv) at 23 °C. To this, NaOMe in DMF (1.8 mg, 33  $\mu$ mol, 0.1 equiv) was added and the mixture stirred for 3 h. The reaction was neutralized with HCl (1 M) and extracted with ether three times. The aqueous layer was dried, and the residue was preabsorbed on C<sub>18</sub> stationary phase for solid-phase loading and purified by automated medium-pressure chromatography (gradient elution: 0.1% HCO<sub>2</sub>H/MeOH/H<sub>2</sub>O, 10–50% over 20 min).

The volatiles were evaporated under reduced pressure, and the aqueous residue was lyophilized to yield **5** (60 mg, 0.19 mmol, 56% yield) as a fluffy, white powder.  $^1\text{H}$  and  $^{13}\text{C}$  NMR data: Supporting Information, Table S2.

***N*-(*tert*-Butoxycarbonyl)-5'-*O*,8-cyclo-2',3'-*O*-isopropylideneadenosine (**7**).** A solution of *N*-(*tert*-butoxycarbonyl)-2',3'-*O*-isopropylideneadenosine (1.30 g, 3.19 mmol) in MeOH (25 mL) and NaOAc buffer (25 mL, 0.5 M, pH 4) was treated by slow addition of saturated  $\text{Br}_2$ -water (32.5 mL) and the resulting mixture stirred at room temperature for 48 h. The mixture was decolorized by addition of  $\text{NaHSO}_3$  (5 M) and adjusted to pH 7 with NaOH aqueous (2 M) to give a precipitate which was filtered, washed with water, and dried under reduced pressure. The residue was preabsorbed onto  $\text{C}_{18}$  stationary phase for solid-phase loading and purified by automated medium-pressure chromatography (gradient elution, 10–80% 0.1%  $\text{HCO}_2\text{H}/\text{MeOH}/\text{H}_2\text{O}$  over 20 min). The volatiles were evaporated under reduced pressure and the aqueous phase was lyophilized to yield **7** (290 mg, 0.72 mmol, 23% yield) as an off-white powder:  $[\alpha]_{\text{D}} -33.9$  (*c* 0.1, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  266 nm ( $\epsilon$ ,  $\log_{10}$  4.24), 210 (4.42); FTIR (ATR, ZnSe plate)  $\nu$  3336, 3187, 2990, 2932, 2363, 2337, 1710, 1645, 1606, 1462, 1377, 1292, 1084, 849  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta_{\text{H}}$  8.51 (s, 1H), 6.33 (s, 1H), 5.18 (d, 1H, *J* = 5.7 Hz), 4.87 (d, 1H, *J* = 5.7 Hz), 4.77 (d, 1H, *J* = 1.0 Hz), 4.70 (dd, 1H, *J* = 2.1, -13.0 Hz), 4.30 (d, 1H, *J* = -13.0 Hz), 1.57 (s, 9H), 1.53 (s, 3H), 1.36 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta_{\text{C}}$  157.3, 152.6, 152.4, 151.2, 149.4, 120.0, 114.1, 88.3, 87.5, 86.7, 82.6, 82.6, 76.4, 28.5, 26.4, 24.7; ESI-TOF  $m/z$  406.1723  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{18}\text{H}_{24}\text{N}_5\text{O}_6$  406.1721). Attempted bromination of *N*-(*tert*-butoxycarbonyl)-2',3'-*O*-isopropylideneadenosine (18.0 mg, 0.058 mmol) in DMF (300  $\mu\text{L}$ ) with 5,5-dimethyl-1,3-dibromohydantoin (DBH, 25.3 mg, 0.089 mmol, 1.5 equiv)<sup>17</sup> or NBS (16.2 mg, 0.089 mmol, 1.5 equiv) resulted in rapid loss of starting material (~30 min, TLC), no detection of starting material, and only **7** as the product (LCMS).

**8-Bromoadenosine (**9**).** Kohyama's protocol was used to yield **9** (1.04 g, 3.01 mmol, 85% yield).  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and MS data of **9** were identical to literature values.<sup>32</sup>

**8-Oxoadenosine (**10**).** The title compound was prepared according to a published method<sup>18</sup> and purified by automated flash chromatography (0.1%  $\text{HCO}_2\text{H}/\text{MeOH}/\text{H}_2\text{O}$ , gradient elution 10–50% over 25 min) to yield **10** (428 mg, 1.51 mmol, 70% yield).  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and MS data of **10** were identical with literature values.<sup>18</sup>

**5'-Chloro-5'-deoxy-8-oxoadenosine (**11**).** The improved protocol used in the synthesis of **6** was employed.<sup>12</sup> 8-Oxoadenosine (0.32 g, 1.1 mmol) was dissolved in dry DMPU (5.7 mL). The solution was cooled in an ice bath before slow addition of  $\text{SOCl}_2$  (400  $\mu\text{L}$ , 5.51 mmol, 5 equiv). The reaction was warmed to 23  $^\circ\text{C}$ , stirred for 2 h, cooled in an ice bath, diluted with cold water (10 mL), adsorbed on Dowex 50  $\times$  2–400 resin (200–400 mesh,  $\text{H}^+$  form), washed with water, and eluted with 5% aqueous ammonia. The eluant was dried under reduced pressure and lyophilized to yield **11** (136 mg, 0.45 mmol, 70% yield) as an off-white solid.  $^1\text{H}$ ,  $^{13}\text{C}$ , and MS data were consistent with literature data.<sup>33</sup>

**5'-Deoxy-5'-(methylthio)-8-oxoadenosine (**1**) free base.** Following the protocol described in the synthesis of **5**, compound **11** (0.10 g, 0.33 mmol) and sodium thiomethoxide (232 mg, 3.3 mmol, 10 equiv) were dissolved in dry DMF at 23  $^\circ\text{C}$  and treated with NaOMe in DMF (1.8 mg, 0.033 mmol, 0.1 equiv). The mixture was stirred for 3 h and then neutralized with HCl (1 M) and extracted with diethyl ether ( $\times 3$ ). The aqueous layer was concentrated under reduced pressure and the residue triturated with hot, anhydrous  $\text{CH}_3\text{CN}$  to yield, after removal of solvent, **1** free base (42 mg, 0.13 mmol, 41% yield) as a colorless powder:  $[\alpha]_{\text{D}} -147.0$  (*c* 0.1, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  210 nm ( $\epsilon$ ,  $\log_{10}$  4.45), 255 (3.89), 270 (3.96); UV (MeOH, NaOH, pH 12)  $\lambda_{\text{max}}$  210 nm ( $\epsilon$ ,  $\log_{10}$  4.43), 277 (4.03); FTIR (ATR, ZnSe plate)  $\nu$  3390, 3141, 2920, 1696, 1628, 1566, 1355, 1126, 1030, 1011  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta_{\text{H}}$  8.06 (s, 1H), 5.88 (d, 1H, *J* = 4.9 Hz), 5.13 (t, 1H, *J* = 4.9 Hz), 4.42 (t, 1H, *J* = 4.9 Hz), 4.07 (ddd, 1H, *J* = 7.2, 5.5, 0.9 Hz), 2.88 (dd, 1H, *J* = -14.0, 5.5 Hz), 2.80 (dd, 1H, *J* = -14.0, 7.2 Hz), 2.10 (s, 3H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta_{\text{C}}$  154.5,

152.1, 148.8, 148.8, 105.9, 87.9, 85.0, 74.5, 72.0, 37.4, 16.1; ESI-TOF  $m/z$  312.0773  $[\text{M} - \text{H}]^-$  (calcd for  $\text{C}_{11}\text{H}_{14}\text{N}_5\text{O}_4\text{S}$  312.0767).

**Preparation of Salvadenosine Formate salt (**1**- $\text{HCO}_2\text{H}$ ).** Free base **1** (10.88 mg) was dissolved in  $\text{CD}_3\text{OD}$  (500  $\mu\text{L}$ ),  $\text{HCO}_2\text{H}$  (90% aq, 1 equiv) was added, and the solution was dried under nitrogen to give **1**- $\text{HCO}_2\text{H}$ : white powder;  $[\alpha]_{\text{D}} +20.6$  (*c* 0.1, MeOH); FTIR (ATR, ZnSe plate)  $\nu$  3343, 3212, 1710, 1658, 1592, 1475, 1429, 1364, 1129, 1096, 1037  $\text{cm}^{-1}$ ; UV-vis was identical to free base **1**;  $^1\text{H}$  and  $^{13}\text{C}$  NMR, see the Supporting Information, Table S3.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

$^1\text{H}$ ,  $^{13}\text{C}$ , and 2D NMR spectra of **1** and **7** as well as  $^1\text{H}$  NMR spectra of **6** and **11**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### 📄 Notes

The authors declare no competing financial interest.

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