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

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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 by Bergmann on Caribbean sponges of the genera Tethya and Cryptotethya resulted in the isolation of spongosine, spongouridine, and others. Predating the modern era of marine natural products chemistry, these seminal discoveries were the inspiration for development of the clinically important antitumor drugs Ara A4 and Ara C.5 In our investigations of antitumor compounds using “nanomole-scale” techniques,6 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.7 Salvadenosine (1) joins the family of rare marine-derived nucleosides that include Bergmann’s arabino-nucleosides from Cryptotethya sp.5 and the antiproliferative trachycladine A (3) from the sponge Trachycladus laevispiralifer.8

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. The molecular formula of 1, C11H15N5O4S, established from HRMS (ESI-TOF m/z 312.0777 [M − H]−), was isomeric with 2 (Table 1). COSY correlations confirmed the 5′-deoxy-5′-(methylthio)ribose moiety. Cross peaks arising from a modified ribose corresponded to the following contiguous spin system: anomic proton H-1′ (δH 5.87, d, J

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

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correlation from the sole downfield nonexchangeable aromatic signal (δ_H 8.00, δ_C 150.8, δ_{1H} = 203.4 Hz) revealed a δ^{13}C chemical shift that was deshielded (Δδ = 15.8 ppm) compared to that of the H-8 of 2 (δ_H 7.90, δ_C 135.0). Another notable difference was observed for the most shielded sp^2 δ^{13}C signal of 1 (δ_C 103.6, s) and 2 (δ_C 118.0, s, C-8; Δδ = 14.4 ppm). HMBC correlations of 1 were observed from the anomeric proton H-1’ signal, and sp^2 δ^{13}C signals were also inconsistent with guanine; for example, no correlation was observed between the downfield δ^{13}C NMR signal (δ_C 8.08, s, 1H) and the anomic carbon, C-1’, a correlation common to guanosine nucleosides.\(^1^\)

The HSQC (DMSO-d_6) showed the aforementioned downfield proton was attached to a carbon with a δ_{1H} signal (δ_C 151.7, s) more compatible with an imidazole C═O group than the imidazole C-8 chemical shift of 2 (δ_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 δ^{13}C NMR signal (δ_C 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 δ_{1H} correlations of H-2 to C-4 and C-5 in an 8-oxoadenine, respectively. The δ^{13}C chemical shifts (DMSO-d_6) of the quaternary ring junction carbons C-4 and C-5 (δ_C 146.7, s, 2C-3, 6) of 1 are more polarized (Δδ [C-4 − C-5] = 43.1 ppm) than the corresponding signals of 2 (Δδ = 35 ppm), guanosine, or adenosine but closer in magnitude than the 8-oxoadenosines, alpinidin, ericain, and caissonare.\(^1^\)

Additionally, we measured the heteronuclear coupling constants of the downfield sp^2 δ^{1H} NMR singlets in 1 and several purine nucleosides (Table 3), revealing a better match between 8-oxoadenosine and the natural product (1: H-2, δ_{1H} = 203.4 Hz; 8-oxoadenosine: H-2 δ_{1H} = 201.5 Hz; guanosine: H-8 δ_{1H} = 213.5 Hz).

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

| Compd    | Atom | δ_{1H} | δ_{13}C | δ_{1H} | δ_{13}C |
|----------|------|--------|---------|--------|---------|
| adenosine (8) | 2    | 8.13   | 152.9   | 2     | 199.1   |
| 8        | 2    | 8.35   | 140.4   | 2     | 213.4   |
| guanosine (4b) | 8    | 7.94   | 135.6   | 2     | 213.5   |
| Hamiguanosinol (2) | 8    | 7.93   | 135.8   | 2     | 213.3   |
| 8-oxoadenosine (10) | 2    | 8.01   | 150.7   | 2     | 201.5   |
| S′-chloro-S′-deoxy-8-oxoadenosine (11) | 2    | 8.03   | 150.9   | 2     | 203.5   |
| salvadenosine (1) | 1    | 8.09   | 152.3   | 2     | 203.4   |
| 1′       | 2    | 8.09   | 152.1   | 2     | 202.4   |

Table 3. δ^{1H}, δ^{13}C, and δ_{1H} NMR Data for Downfield δ^{1H} NMR Singlets of Purine Nucleosides

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**Table 1.** δ^{1H} and δ^{13}C NMR Data for 1 (Formate Salt, CD3OD)

| Atom | δ^{13}C | δ^{1H} | J{1H} | Integ |
|------|---------|--------|-------|-------|
| 1    | 152.3   | 8.08   | 1H    | 4, 5  |
| 2    | 150.8   | 8.30   | 2H    | 4, 5  |
| 3    | 148.7   | 8.30   | 2H    | 4, 5  |
| 4    | 148.7   | 8.30   | 2H    | 4, 5  |
| 5    | 150.8   | 8.30   | 2H    | 4, 5  |
| 6    | 150.8   | 8.30   | 2H    | 4, 5  |
| 7    | 150.8   | 8.30   | 2H    | 4, 5  |
| 8    | 150.8   | 8.30   | 2H    | 4, 5  |
| 1′   | 85.1    | 5.87   | 2H    | 4, 5  |
| 2′   | 72.0    | 5.12   | 2H    | 4, 5  |
| 3′   | 74.5    | 4.42   | 2H    | 4, 5  |
| 4′   | 85.1    | 4.07   | 2H    | 4, 5  |
| 5′a  | 37.4    | 2.88   | 2H    | 4, 5  |
| 5′b  | 37.4    | 2.88   | 2H    | 4, 5  |
| 6′a  | 16.1    | 2.10   | 2H    | 4, 5  |
| 6′b  | 16.1    | 2.10   | 2H    | 4, 5  |

*125 MHz. b600 MHz. cδ_{1H} = 203.4 Hz.

= 4.9 Hz) to H-2′ (δ_{1H} 5.12, t, J = 4.9 Hz) to H-3′ (δ_{1H} 4.42, t, J = 4.9 Hz) to H-4′ (δ_{1H} 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 (δ_{1H} 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 (δ_{1H} 2.10, s, δ_{1C} 16.1, δ_{1C} = 138.4 Hz) to C-5′ established the location of the MeS group.

Distinct differences were apparent between the δ^{1H} and δ^{13}C chemical shifts of 1 and those published for hamiguanosinol (Table 2), particularly the sp^2 δ^{13}C NMR signals. An HSQC
question of validity of the structure of 2 arises. The nucleobase in hamiguanosinol (incorrectly named “6-hydroxyguanine”) was assigned as the enol tautomer (cf. enol form of guanosine 4a, Figure 1) based on a 13C shift for C-6 of δ 157.2 “instead of a ca.170 ppm for a keto amide function”; 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 13C 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 coworkers12 who characterized it as the keto tautomer. We repeated the synthesis of 5 (Scheme 1) from guanosine (4b) and showed the 13C chemical shifts of the product were essentially the same as those reported for natural hamiguanosinol (Supporting Information, Table S2).13 Therefore, the structure of hamiguanosinol is the keto tautomer 5,14 not the enol 2: synthetic 5 and natural 5 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 adenosine15 was subjected to bromination (Scheme 2, saturated Br2–H2O, pH 4 NaOAc buffer), but only the cyclized product 7 was formed presumably through intramolecular attack by the S′-OH group after bromination at C-8.16 The same result was observed under aprotic conditions (NBS, DMF; 5,5-dimethyl-1,3-dibromohydantoin, DBH, DMF);17 consequently, we turned to a “protection-group-free” strategy (Scheme 2). Adenosine (8) was converted to 8-bromoadenosine (9) (saturated Br2–H2O, pH 4 buffer) followed by an efficient conversion to the 8-oxoadenosine (10) under Chaitgialialogus conditions.18 Compound 10 was converted to primary chloride 11 with improved yield (SOCl2, 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 1H and 13C NMR spectra (Supporting Information, Table S3) as well as UV, IR, and CD data of the two samples 1-HCO2H matched in every way. Co-injection of natural 1 with synthetic 1 by HPLC resulted in a single peak (see the Supporting Information, 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 CH3 groups and one CH2 group bonded to the S in SAM are electrophilic in nature. Common biological methylation involves S1,2 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 A19 and rare fluorinated natural products, substantial biochemical and structural evidence supports participation of the 5′-CH2–S bond of SAM in S1,2 substitutions by halide ions (Figure 2). For example, Streptomyces castellii produces 5′-fluoroadenosine through nucleophilic attack at the 5′-CH2 of SAM by F− (path a), catalyzed by the enzyme fluorinase,20 the former in turn is catabolized to fluoroacetate. A homologous “chlorinase” catalyzes displacement at 5′-CH2 of SAM by Cl− (path a) in salinosporamide A biosynthesis.19 Phylogenetic and biochemical studies21 have shown a bifurcation of this biosynthetic motif. The gene duf62, represented in Nature in about 100 bacterial and archael 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′-CH2 group of adenosine group by HO− (path b) to liberate l-methionine and adenosine. Attack at the 5′-CH2 of SAM is similarly rare, with the best-characterized example occurring in the biosynthesis of nocardiadin.22 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,23 the natural product discacidenine,24 nicotianamine (a precursor of plant siderophores),25 and homoserine-based betaine lipids.26 In addition, transfer of the 3-amino-propyl group from decarboxylated SAM is involved in
polyamine biosynthesis.$^{27}$ Common to these biological reactions is the release of $5'$-deoxy-$5'$-(methylthio)adenosine. We speculate that it 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.\(^{28,29}\) 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.\(^{30}\) The anomaly is $S$; no guanosine analogue of SAM has been demonstrated yet, but $S$ has been detected in human urine as a byproduct of nucleotide catabolism.$^{14}$

In conclusion, we confirmed the structure of a new nucleoside salvadenosine (1, $5'$-deoxy-$5'$-(methylthio)-8-oxo-adenosine) 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\(^1\) 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 $^1$H ($^13$C) 5 mm probe or a 600 MHz NMR spectrometer with a $1.7$ mm $^1$H ($^13$C) microprobe. \(^{13}$C NMR spectra were collected on a 125 MHz spectrometer equipped with a 5 mm $^1$H($^13$C) cryoprobe. NMR spectra were referenced to residual solvent signals, CD$_2$OD ($\delta$$_H$ 3.31, $\delta$$_C$ 49.00), (CD$_3$)$_2$SO ($\delta$$_H$ 2.50, $\delta$$_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$_{18}$ 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 elution conditions. Optical rotations were measured at the D-double emission line of Na. FTIR spectra were collected on a ZnSe plate. CD elution conditions. Optical rotations were measured at the D-double emission line of Na. FTIR spectra were collected on a ZnSe plate. CD elution conditions. Optical rotations were measured at the D-double emission line of Na. FTIR spectra were collected on a ZnSe plate. CD elution conditions. Optical rotations were measured at the D-double emission line of Na. FTIR spectra were collected on a ZnSe plate. CD elution conditions.

**Salvadenosine (1):** pale yellow powder, HCO$_2$H salt; \([\alpha]_D ^{20} +11.3$ (c 0.3, MeOH); UV (MeOH) \(\lambda_{max} 210$ nm (c 4.49), 255 (3.95), 270 (4.00); FTIR (ATR, ZnSe plate): 3356, 3188, 2920, 2850, 1710, 1662, 1633, 1590, 1379, 1350, 1131, 1092, 1030, 1006 cm$^{-1}$; \(^{1}$H and \(^{13}$C NMR see Table 1 (CD$_3$OD) and Supporting Information, Table S1 (DMSO-$d_6$); ESI-TOF m/z 312.0777 \([M−H]^−$) (calc'd for C$_{11}$H$_{14}$N$_5$O$_4$S 312.0777). Extraction of sample 11-14-039 and puriﬁcation was carried out in a similar manner to provide additional 1: HCO$_2$H ($−0.3$ mg).

**Tryptamine:** colorless powder; \(^{1}$H, \(^{13}$C, and HRMS data were consistent with previously published data.\(^{2}$

**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 (equimole), were analyzed by HPLC (Polar-RP column, 4 mm, 80 Å, 150 × 4.6 mm, gradient elution; \(H_2O + 0.1% HCO_2H/CH_3CN, 0$–$5$ min hold at %$5$CH$_3CN, 5$–$18$ min ramp to 50$%$CH$_3CN, 1$ mL/min, 40 °C column oven). The following retention times were obtained: natural 1, \(t_R = 10.48$ min; synthetic 1, \(t_R = 10.47$ min; combined natural and synthetic samples, \(t_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.\(^{12}$ 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 µL, 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$_2$O (10 mL), and absorbed onto a column of Dowex 50 × 2–400 resin (200–400 mesh, H$^+$ 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 \(^1$H NMR spectrum of 6 was consistent with published data.\(^{7}$

**Tryptamine:** colorless powder; MS and \(^1$H NMR spectra were identical to those of an authentic sample.
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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. 1H and 13C NMR data: Supporting Information, Table S2.

N-(tert-Butyloxycarbonyl)-5′-O-(2-cyclo-2′-O-isopropylidene)adenosine (7). A solution of N-(tert-butyloxycarbonyl)-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 Br2−water (32.5 mL) and the mixture stirred at room temperature for 48 h. The mixture was decolorized by addition of NaHSO3 (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 C18 stationary phase for solid-phase loading and purified by automated medium-pressure chromatography (gradient elution, 10–80% 0.1% HCO3H/MeOH/H2O 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: [α]D = −33.9 (c 0.1, MeOH); UV (MeOH) λmax 210 nm (ε 47,210), 228. (b) The same compound was reported from Didemnum sp. and Y. Su and A. Mrse (UCSD) for MS data and help with NMR measurements, respectively. The 500 MHz NMR spectrometer and the HPLC TOFMS were purchased with funding from the NSF (Chemical Research Instrument Fund, CHE0741968) and the NIH Shared Instrument Grant (S10RR025636) programs, respectively. This work was supported by grants from NIH (AI1007786 to TFM) and NSERC (CBN).

ASSOCIATED CONTENT

SUPPORTING INFORMATION

1H, 13C, and 2D NMR spectra of 1 and 7 as well as 1H 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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