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Nahuoic Acid A Produced by a *Streptomyces* sp. Isolated From a Marine Sediment Is a Selective SAM-Competitive Inhibitor of the Histone Methyltransferase SETD8

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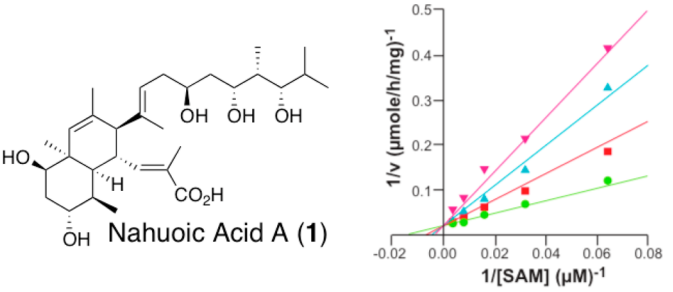
ABSTRACT



The histone lysine monomethyltransferase SETD8 is an epigenetic regulator of cell cycle progression. Nahuoic acid A (1), a polyketide produced in culture by a *Streptomyces* sp. obtained from a tropical marine sediment, is the first known selective SAM-competitive inhibitor of SETD8. The structure of nahuoic acid A (1) has been elucidated by chemical transformation and detailed analysis of spectroscopic data.

Protein methyltransferases (PMTs) transfer methyl groups from *S*-adenosylemethionine (SAM) to lysine or arginine residues in histones and other chromatin-associated substrates.[1] These proteins play essential roles in epigenetic regulation of gene expression and chromatin-dependent signaling. Mutation and aberrant regulation of PMTs are linked to many diseases, especially cancer,[2] and finding PMT inhibitors is considered to be a new frontier for drug discovery. Targeting the common (SAM) cofactor binding site of PMTs is a particularly attractive strategy for cancer therapy.[1]

SETD8, a PMT that monomethylates lysine 20 of histone H4 (H4K20), plays a central role in the silencing of euchromatic genes.[3,4] SETD8 also monomethylates lysine 382 of p53/TP53, thereby repressing p53/TP53-target genes,[5] and monomethylates lysine 248 of proliferating cell

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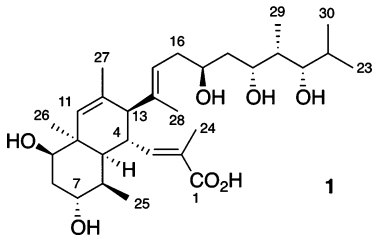
nuclear antigen (PCNA), enhancing the interaction between PCNA and the flap endonuclease FEN1.[6] SETD8 is overexpressed in various types of cancer, and aberrant monomethylation by SETD8 may lead to human carcinogenesis.[6] Potent SETD8 inhibitors would serve as useful chemical probes[7] to further investigate the cellular effects of SETD8 inhibition in both normal and diseased cells and as lead strucures for development of anticancer therapeutics. However, no SETD8 inhibitors that target the SAM cofactor site have been reported in the literature to date.

A screen of a library of marine organism extracts and pure marine natural products revealed that the highly hydroxylated polyketide nahuoic acid A (**1**) is a selective SAM-competitive SETD8 inhibitor *in vitro*. Nahuoic acid A (**1**) is produced in culture by a *Streptomyces* sp. (isolate RJA2928) obtained from a marine sediment collected near the passage Padana Nahua in Papua New Guinea. The highly modified tetrapeptides padanamide A (actinoramide A[8]) and padanamide B are produced by cultures of the same *Streptomyces* sp. (RJA2928).[9] Details of the isolation, structure elucidation, and SETD8 inhibitory activity of nahuoic acid A (**1**) are reported below.



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Production cultures of RJA2928 were grown as lawns on solid agar containing marine medium at rt for 14 days (see Supporting Information). Mature cultures were extracted by soaking cells and medium together in two separate aliquots of EtOAc. Concentration of the combined EtOAc extracts in vacuo, followed first by partition of the residue between H_2O and EtOAc and then Sephadex LH-20 chromatography (eluent, 4:1 MeOH/CH_2Cl_2) of the EtOAc soluble material, gave fractions containing the padanamides and nahuoic acid A (**1**). Further purification by step gradient Si gel flash chromatography gave fractions highly enriched in the ^1H NMR signals for nahuoic acid A. Pure nahuoic acid A (**1**) was obtained initially from C_8 reversed-phase HPLC by elution with 18:7 H_2O/MeCN. Subsequently, it was found that HPLC using the same column but with 7:3 (0.05% TFA/H_2O)/MeCN as eluent was more efficient.

Nahuoic acid A (**1**) was obtained as an optically active oil that gave a $[M + Na]^+$ ion in the HRESIMS at m/z 545.3467 appropriate for a molecular formula of $C_{30}H_{50}O_7$, requiring six sites of unsaturation. The ^1H and ^{13}C NMR spectra obtained for the samples of nahuoic acid A (**1**) obtained via C_8 HPLC with or without added TFA (Table 1, Supporting Information) were identical in all respects except that five exchangeable proton resonances seen as distinct peaks in the ^1H NMR spectrum of the first C_8 HPLC sample (eluent, H_2O/MeCN 18:7) coalesced into broad signals in the second HPLC sample due to the presence of trace amounts of TFA from the eluent. Therefore, the initial structural analysis of nahuoic acid A (**1**) was carried out using the NMR data obtained for the sample of **1** with distinct OH resonances and the structural arguments were subsequently confirmed on the sample containing TFA.

The $^1H/^{13}C$/gCOSY60/gHSQC/gHMBC NMR data obtained for **1** (Table 1, Supporting Information) identified resonances that could be assigned to 30 carbon atoms, with 44 protons bound to carbon (5 × C, 14 × CH, 3 × CH_2, 8 × CH_3) and six exchangeable protons, in agreement with the ESIHRMS measurement. Five of the six exchangeable proton signals were assigned to secondary OH protons [δ 4.36 (bs, OH-9), 4.30 (d, J = 4.9 Hz, OH-21), 4.29 (d, J = 4.7 Hz, OH-19), 4.26 (bd, J = 4.7 Hz, OH-17), and 4.21 (d, J = 5.8 Hz, OH-7)] that each correlated to a methine resonance [δ 3.47 (H-9), 3.12 (H-21), 3.77 (H-19), 3.56 (H-17), and 3.51 (H-7)] in the gCOSY60 experiment. The five methine proton resonances correlated in the gHSQC to carbon resonances with chemical shifts appropriate for carbinol methines [δ 72.8 (C-9), 78.6 (C-21), 70.0 (C-19), 67.1 (C-17), and 66.3 (C-7)]. A final exchangeable proton resonance at δ 11.78 was assigned to a carboxylic acid, whose carbon appeared at δ 169.1 (C-1).

Three trisubstituted alkenes [δ $^1H/^{13}C$ 6.41 (d, J = 10.7 Hz, H-3)/ 124.4 (C-2); 149.0 (C-3); 5.15 (bs, H-11)/132.5 (C-11); 132.7 (C-12); 4.97 (m, H-15)/ 135.1 (C-14); 125.4 (C-15)], four methyl doublets ($^1H/^{13}C$ δ 0.85 (J = 7.2 Hz, H-25)/18.2 (C-25); 0.83 (J = 6.5 Hz, H-30)/18.5 (C-30); 0.76 (J = 6.5 Hz, H-23)/19.5(C-23); 0.76 (J = 6.8 Hz, H-29)/7.2 (C-29)], four methyl singlets (δ $^1H/^{13}C$ 1.58 (H-24)/13.0 (C-24); 1.46 (H-27)/21.8 (C-27); 1.01 (H-26)/27.6 (C-26); 1.40 (H-28)/12.4 (C-28)], three aliphatic methylenes [δ $^1H/^{13}C$ 2.04 (H-16), 1.91 (H-16′)/37.0(C-16); 1.87 (H-8_{eq}), 1.62 (H-8_{ax})/40.2 (C-8); 1.18 (H-18), 1.42 (H-18′)/ 41.7 (C-18)], six additional methines [$\delta^1H/^{13}C$ 3.54 (H-4)/ 36.1 (C-4); 2.23 (H-13) /56.8 (C-13); 1.92 (H-6)/38.3 (C-6); 1.63 (H-22)/30.3 (C-22); 1.52 (H-5)/50.1 (C-5); 1.39 (H-20)/39.9 (C-20)], and one aliphatic quaternary carbon (δ 41.1, C-10) could also be readily identified from the NMR data. The carboxylic acid and three alkenes accounted for only four of the six sites of unsaturation required by the molecular formula, requiring that nahuoic acid A (**1**) had to contain two rings.

Six fragments shown in bold in Figure 1A could be unambiguously identified from the gCOSY60 data. These fragments could be connected via the series of HMBC correlations [Me-29 (δ 0.76) to C-19 (δ 70.0); Me-28 (δ 1.40) to C-13 (δ 56.8); Me-27 (δ 1.46) to C-13 (δ 56.8);

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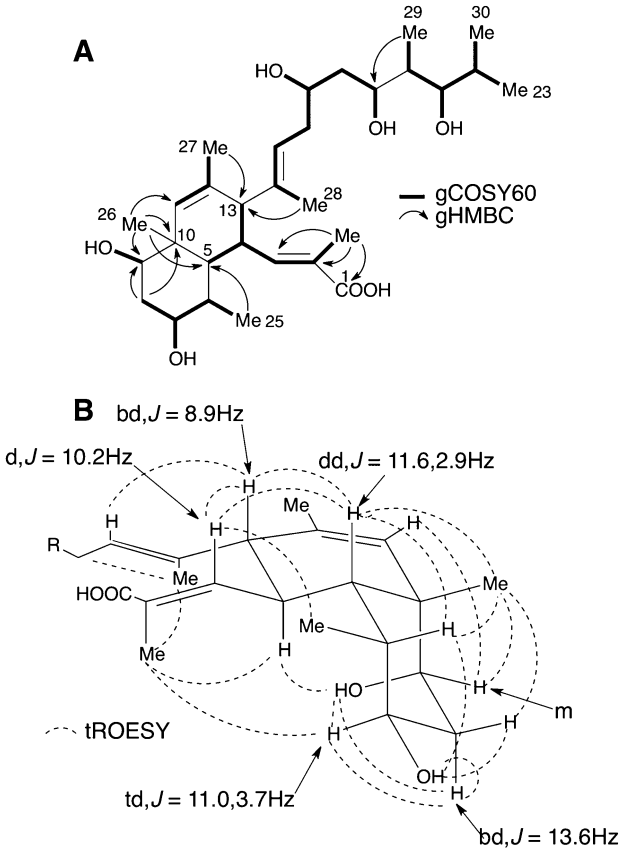
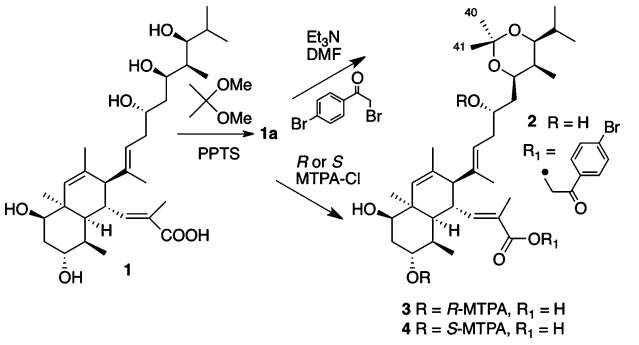


Figure 1. Selected gCOSY60, gHMBC, and tROESY correlations observed for Nahuoic acid A (**1**).

Me-26 (δ 1.01) to C-11 (δ 132.5), C-10 (δ 41.1), C-9 (δ 72.8); H-8_{eq} (δ 1.87) to C-9 (δ 72.8), C-10 (δ 41.1); Me-25 (δ 0.85) to C-5 (δ 50.1); Me-24 (δ 1.58) to C-1 (δ 169.1)] summarized with arrows in Figure 1A, establishing the linear carbon backbone of the polyketide chain from C-1 to C-23 and the locations of the three trisubstituted alkenes, the branching methyls, the carboxylic acid, and the secondary alcohols. Vicinal coupling observed between H-4 (δ 3.54) and H-13 (δ 2.23) in the gCOSY60 identified a bond between C-4 and C-13. gHMBC correlations observed between Me-26 (δ 1.01) and both C-5 (δ 50.1) and C-10 (δ 41.1) established a bond between C-10 and C-5. The C-4/C-13 and C-5/C-10 bonds created the two rings of a decalin ring system from the linear polyketide backbone.

The relative configuration about the decalin ring system in **1** was determined from the *J* coupling data obtained for **1** containing TFA, in which no coupling to the exchangeable OH protons was observed, and the series of tROESY correlations illustrated in Figure 1B. A tROESY correlation between Me-26 (δ 1.01) and H-5 (δ 1.52) established that the decalin ring junction was *cis*. The H-7 resonance at δ 3.51 appeared as a triplet of doublets with the large coupling of 11.0 Hz, indicating that the ring encompassing C-5 to C-10 was in a chair conformation with H-6, H-7, and H-8_{ax} all axial as shown in Figure 1B. tROESY correlations

Scheme 1. Derivatization of Nahuoic Acid A (**1**)



observed between Me-26 (δ 1.01) and each of H-6 (δ 1.92), H-8_{ax} (δ 1.62), and H-9 (δ 3.47) were consistent with a chair conformation and established that Me-26 was axial, H-5 was equatorial, Me-25 was equatorial, OH-7 was equatorial, and OH-9 was axial on the C-5 to C-10 ring. H-4 (δ 3.54) was coupled to each of H-3 (δ 6.41), H-5 (δ 1.52), and H-13 (δ 2.23) with a $J \geq 8.9$ Hz indicating diaxial relationships between H-4 and both H-5 and H-13, and an *anti* relationship between H-4 and H-3. tROESY correlations between the three methine protons H-3 (δ 6.41), H-5 (δ 1.52) and H-13 (δ 2.23) confirmed the diaxial relationship between H-5 and H-13 and the *cis* relationship between both H-5 and H-13 and C-3. tROESY correlations observed between Me-24 (δ 1.58) and H-4 (δ 3.54), between Me-28 (δ 1.40) and H-16/H-16′ (δ 1.91/2.04), and between H-15 (δ 4.97) and H-13 (δ 2.23) demonstrated that the $\Delta^{2,3}$ and $\Delta^{14,15}$ olefins both had the *E* configuration.

In order to facilitate the determination of the relative configuration of the C-17 to C-23 segment of nahuoic acid A, the natural product **1** was treated with 2,2-dimethoxypropane and PPTS, and without purification, the putative acetonides **1a** were subsequently reacted with *p*-bromophenacylbromide and Et_3N in DMF to give a major product ester **2** (Scheme 1) (see Supporting Information for MS and NMR assignments). The geminal methyl carbons (Me-40 and Me-41) in the C-19/C-21 acetonide had chemical shifts of δ 19.9 and 30.4 demonstrating that the C-19 and C-21 alcohols in **1** were *syn*.[10]

Analysis of the *J* couplings using a series of homonuclear decoupling experiments, combined with the tROESY and gCOSY60 NMR data for ester **2**, yielded the relative configurations at C-17, C-19, C-20, and C-21 as shown in Figure 2. tROESY correlations observed between the axial geminal methyl (C-40: δ 1.41) of the acetonide and both H-19 (δ 4.31) and H-21 (δ 3.21), and between H-19 and H-21, confirmed that the conformation of the dioxane ring, as predicted by Rychnovsky et al., was a chair[10] and that H-19 and H-21 were axial and C-18 and C-22 were equatorial. Small couplings (< 2 Hz) were observed between H-20 (δ 1.33) and both H-19 and H-21, indicating

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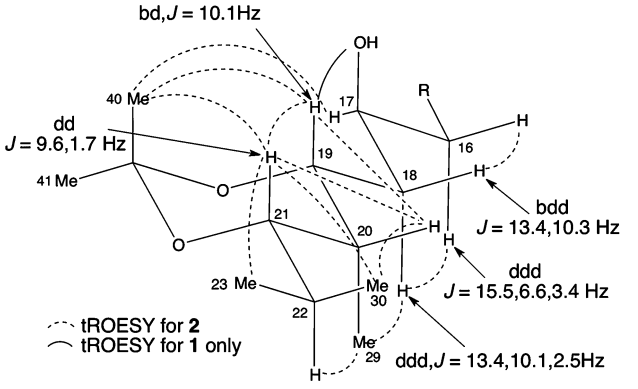

Figure 2. tROESY data for **1** and **2**.

that H-20 was equatorial. H-19 (δ 4.31) had a small coupling ($J < 2$ Hz) to the H-18b resonance (δ 1.38) and their correlation in the gCOSY60 spectrum was weak. In contrast, the coupling constant between H-19 and H-18a (δ 1.84) was 10.1 Hz and their gCOSY60 correlation was strong, indicating that these protons were anti. Similarly a 9.6 Hz coupling between H-21 and H-22 (δ 1.74) required these to be anti. Me-29 (δ 0.99) showed tROESY correlations to both H-18a and H-22 in agreement with the above assignment. A 2.5 Hz coupling between H-18a and H-17 (δ 4.10) required a dihedral angle of near 60° and tROESY correlations between H-16a (δ 2.15) and H-18a and between H-16b (δ 2.28) and H-18b (δ 1.38) were consistent with the extended zigzag conformation and the relative configurations for the carbon chain extending from C-17 to C-21 as shown in Figure 2. A weak tROESY correlation observed between Me-40 and H-17, and the small coupling ($J = 3.4$ Hz) between H-16a and H-17 provided additional support for the assigned relative configurations. tROESY correlations observed between H-19 (δ 3.77), and both H-21 (δ 3.12) and 17-OH (δ 4.26) in nahuoic acid A (**1**) suggests that the underivatized natural product adopts an analogous extended zigzag conformation.

The complete relative configuration and the absolute configuration of nahuoic acid A (**1**) was determined by a modified Mosher ester analysis. Nahuoic acid A (**1**) was first converted into the Mosher esters **3** and **4** as shown in Scheme 1. A standard Mosher ester analysis of the ^{1}H NMR data collected for **3** and **4** showed that the absolute configuration of **1** is 4*R*,5*S*,6*R*,7*R*,9*R*,10*R*,13*R*,17*S*,19*R*,20*S*,21*S*. The Mosher ester absolute configuration assignment was confirmed by calculating the CD spectrum for **1**, which matched the experimentally obtained data (see Supporting Information for details).

Nahuoic acid A (**1**) inhibited SETD8 activity with an IC_{50} value of 6.5 ± 0.5 μM and a Hill slope of 1.6 (Supporting Information). Interestingly, nahuoic acid A (**1**) had no significant inhibitory effect on the activity of other protein methyltransferases such as G9a, EHMT1, SETD7, SUV39H2, SUV420H1, SUV420H2, DOT1L, PRMT3, and PRMT5 and MLL complexes. Kinetic analysis of the inhibition of SETD8 activity by nahuoic acid A (**1**) indicated that it is a competitive inhibitor of SAM binding with a K_i value of 2 ± 0.3 μM (Supporting Information), but a noncompetitive inhibitor with respect to the binding of the peptide substrate (Supporting Information).

The pentahydroxylated and hexamethylated linear C-1 to C-23 carbon backbone of nahuoic acid A (**1**) appears to have a PKS origin, but its carbon skeleton is without precedent among known natural products.[11] A Diels–Alderase[12] may be involved in the formation of the decalin ring fragment. To the best of our knowledge, nahuoic acid A (**1**) is the first natural product known to inhibit SETD8[13] and the first selective SETD8 inhibitor known to be a competitive inhibitor of SAM binding. Nahuoic acid A (**1**) has the potential to be a useful tool to aid the investigation of the roles of SETD8 in cells and to act as a probe for defining a selective SAM-competitive binding site on SETD8 that can guide the design of more potent synthetic inhibitors for therapeutic[1] or cell biology research applications.

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Supporting Information Available. Experimental details; 1D and 2D NMR spectra for **1** - **4**; bioactivity data for **1**; details of Mosher ester analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.