Pyridine Nucleosides Neopetrosides A and B from a Marine Neopetrosia sp. Sponge. Synthesis of Neopetroside A and Its β-Riboside Analogue

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JUNE 2015

Impact Factor: 3.8 · DOI: 10.1021/acs.jnatprod.5b00256 · Source: PubMed

JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Pyridine Nucleosides Neopetrosides A and B from a Marine *Neopetrosia* sp. Sponge. Synthesis of Neopetroside A and Its β-Riboside Analogue

Larisa K. Shubina,[†] Tatyana N. Makarieva,*[,†] Dmitry V. Yashunsky,[‡] Nikolay E. Nifantiev,[‡] Vladimir A. Denisenko,[†] Pavel S. Dmitrenok,[†] Sergey A. Dyshlovoy,[†] Sergey N. Fedorov,[†] Vladimir B. Krasokhin,[†] Seung Hun Jeong,[§] Jin Han,[§] and Valentin A. Stonik[†]

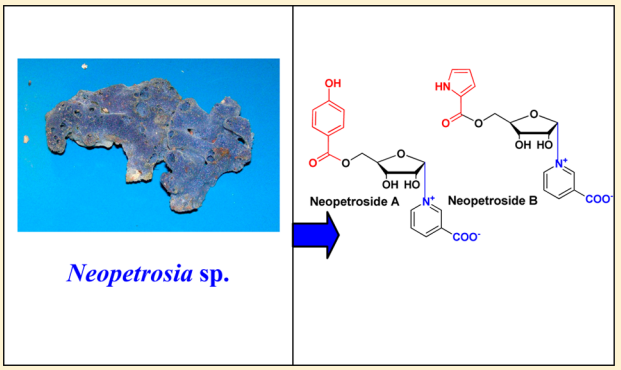
[†]G. B. Elyakov Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, Prospect 100-let Vladivostoku 159, Vladivostok 690022, Russian Federation

[‡]N.D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Leninsky Prospect, 47, 119991, Moscow, Russian Federation

[§]National Research Laboratory Mitochondrial Signaling, Cardiovascular and Metabolic Disease Center (CMDC), Department of Physiology, College of Medicine, Inje University, Busan 614-735, South Korea

Supporting Information

ABSTRACT: Neopetrosides A (**1**) and B (**2**), new naturally occurring ribosides of nicotinic acid with extremely rare α-*N*-glycoside linkages and residues of *p*-hydroxybenzoic and pyrrole-2-carboxylic acids attached to C-5′, were isolated from a marine *Neopetrosia* sp. sponge. Structures **1** and **2** were determined by NMR and MS methods and confirmed by the synthesis of **1** and its β-riboside analogue (**3**). Neopetroside A (**1**) upregulates mitochondrial functions in cardiomyocytes.



Marine sponges of the genus *Neopetrosia* have been reported to be a rich source of secondary metabolites with diverse chemical structures and biological activities.[1] This group consists of tetrahydroisoquinolines,[2] polycyclic alkaloids,[3−6] tricyclic peptides,[7] pentacyclic hydroquinones,[8] and sesquiterpene benzoquinones[9] found in different species of the genus. The isolated compounds demonstrate a broad spectrum of biological activities, including potent cytotoxic effects against cancer cells,[2,4,5] inhibitory activity against pathogenic microbes,[4] and inhibition of indoleamine-2,3-dioxygenase[3] and amoeboid invasion.[7] As part of our continuing search for bioactive secondary metabolites from marine organisms,[10,11] new pyridine α-ribosides (**1**, **2**) from a *Neopetrosia* sp. sponge have been isolated. Similar pyridine nucleosides, having both an α-glycosidic bond and an acyl substituent at C-5′, have never been isolated from natural sources or synthesized.

Pyridine nucleosides are well-known biosynthetic precursors of nicotinamide adenine dinucleotides, NAD^+ and NADH, important regulators of energy production, metabolism, redox reactions, some ion channels, and processes of cell survival and death under normal and pathological conditions.[12] It is known that the recently studied vitamin nicotinamide riboside may be used as a nutritional supplement to ameliorate metabolic and age-related disorders, characterized by defective mitochondrial functions, and as a protector against high-fat-diet-induced obesity.[13] It was also revealed that pyridine nucleosides and nucleotides play an important role in regulating a wide variety of functions in cardiovascular tissues. Deregulation of pyridine nucleotides is immediately involved in the pathogenesis of heart failure, arrhythmia, and age-associated abnormalities of the heart.[12] In addition, modified pyridine nucleotides and nucleosides can provide therapeutic benefits. For instance, tiazofurin and benzamide riboside, synthetic nucleosides, are used clinically as anticancer agents. They form cytotoxic NAD^+ analogues in the metabolism of humans.[14−16]

As natural products, modified pyridine nucleosides are exceedingly rare. So far, only three natural modified pyridine nucleosides have been discovered, namely, clitidine (3-carboxy-4-imino-l-(β-D-ribofuranosyl)-1,4-dihydropyridine) from the poisonous mushroom *Clitocybe acromelalga*,[17] 1-α-D-ribofuranosyl-4-pyridone-3-carboxamide from the urine of normal human individuals and leukemic patients,[18] and α-nicotinamide riboside from the marine sponge *Protophlitaspongia aga*.[19]

Received: March 24, 2015
Published: June 2, 2015

DOI: 10.1021/acs.jnatprod.5b00256
J. Nat. Prod. 2015, 78, 1383−1389

The occurrence of an oxidized nicotinamide adenine dinucleotide containing an α-riboside linkage in nature was first reported by Kaplan and co-workers.[20] A biological role for α-NAD$^+$ and α-NADP$^+$, however, was not uncovered. The function of naturally occurring α-NADPH anomers in mammalian physiology was first demonstrated later, after identifying the catalytic activity of renalase as an α-NADPH oxidase/anomerase.[21]

Herein we describe the structure elucidation of two new modified pyridine α-ribosides, named as neopetrosides A (**1**) and B (**2**), along with the synthesis and biological activity of neopetroside A.

RESULTS AND DISCUSSION

The EtOH extracts of the sponge were concentrated and partitioned between H_2O and *n*-BuOH. The *n*-BuOH-soluble materials, after concentration in vacuo were partitioned between aqueous EtOH and *n*-hexane. The EtOH-soluble materials were subjected to chromatographic separation on a YMC gel column, and further purification using reversed-phase HPLC afforded neopetrosides A and B.

Neopetroside A (**1**) was obtained as a light yellow, amorphous solid. The HRESIMS spectrum of **1** showed a protonated molecule at *m/z* 376.1033 $[M + H]^+$ and an adduct ion at *m/z* 398.0854 $[M + Na]^+$ consistent with a molecular formula $C_{18}H_{17}NO_8$. Analysis of ^{1}H, ^{13}C, HSQC, and COSY NMR data (Table 1) of **1** showed the presence of a ribofuranoside ring and aromatic carbons at δ_C 147.9, 144.3, 143.7, 139.5, 133.6, 127.8, 122.3, and 117.0; one oxygenated methylene (δ_H 4.50, 4.59 and δ_C 65.3), and three downfield nonprotonated carbons at δ_C 164.5, 167.2, and 168.2. GC analysis of the acetylated methyl acetal derivative, obtained as a result of methanolysis of **1** followed by acetylation, confirmed the presence of a ribose unit in this compound. The chemical shifts at δ_H 9.28 (s), 8.94 (dd, *J* = 8.0, 1.4 Hz), 8.06 (dd, *J* = 8.0, 6.2 Hz), and 8.96 (dd, *J* = 6.2, 1.4 Hz) and coupling constants values in the ^{1}H NMR spectrum (Table 1), together with HMBC and COSY data (Figure 2), strongly indicated the presence of the 3-substituted pyridinium ring. The HMBC correlations of the anomeric proton at δ_H 6.50 (H-1′) with C-2 and C-6 aromatic carbons indicated that the pyridinium ring is linked to the sugar moiety through a nitrogen atom. The presence of a carboxylate group was inferred by the IR absorption band at 1645 cm^{-1} and a singlet at δ_C 167.2. Moreover, both aromatic signals at δ_H 9.28 (H-2) and 8.94 (H-4) were coupled to the carboxylate carbon at δ_C 167.2 by HMBC. These data located the carboxylate function at C-3. On the whole, the ^{1}H and ^{13}C NMR data for atoms C-1−C-7 (Table 1) were in agreement with those of the betaine alkaloids trigonelline, pyridinebetaine A, and agelongine[22] and strongly indicated the presence of nicotinic acid as the aglycone in **1**.

The remaining aromatic signals exhibited an AA′XX′ spin system with δ_H 7.93 (d, *J* = 8.8 Hz, 2H) and 6.86 (d, *J* = 8.8 Hz, 2H), suggesting the presence of a 1,4-disubstituted aromatic moiety. This was further corroborated by COSY and HMBC data (Figure 2), while the HMBC correlations from the H-2″ and H-6″ to a carbonyl carbon with δ_C 168.2 as well as downfield shifted signals of methylene protons in the ribosyl moiety (Table 1) together with MS data were consistent with the presence of a *p*-hydroxybenzoic acid unit. This was supported by comparing the spectroscopic data with those reported in the literature for *p*-hydroxybenzoyloxy-containing natural compounds.[23] The HMBC correlations of the

Table 1. NMR Data of Neopetrosides A (1) and B (2) in CD_3OD[a]

	1		2	
position	δ_C,[b] type	δ_H mult (*J* in Hz)	δ_C,[b] type	δ_H mult (*J* in Hz)
2	144.3, CH	9.28, s	144.3, CH	9.27, s
3	139.5, C		139.0, C	
4	147.9, CH	8.94, dd (8.0, 1.4)	148.0, CH	8.94, m
5	127.8, CH	8.06, dd (8.0, 6.2)	127.8, CH	8.06, m
6	143.7, CH	8.96, dd (6.2, 1.4)	143.7, CH	8.95, m
7	167.2, C		168.0, C	
1′	98.6, CH	6.50, d (5.3)	98.7, CH	6.48, d (5.3)
2′	74.4, CH	4.79, m	74.4, CH	4.81, m
3′	73.3, CH	4.33, dd (3.8, 4.5)	73.3, CH	4.33, dd (3.4, 4.5)
4′	88.0, CH	4.96, ddd (3.8, 3.6, 4.6)	88.2, CH	4.93, ddd (3.4, 3.6, 4.6)
5′	65.3, CH_2	4.50, dd (4.6,12.2)	64.7, CH_2	4.48, dd (4.6,12.2)
		4.59, dd (3.6, 12.2)		4.57, dd (3.6, 12.2)
1″	122.3, C			
2″	133.6, CH	7.93, d (8.8)	123.6, C	
3″	117.0, CH	6.86, d (8.8)	117.7, CH	6.95, dd (1.7, 3.7)
4″	164.5, C		111.6, CH	6.21, dd (2.5, 3.7)
5″	117.0, CH	6.86, d (8.8)	125.9, CH	7.01, dd (1.7, 2.5)
6″	133.6, CH	7.93, d (8.8)	163.4, C	
7″	168.2, C			

[a]Spectra were recorded at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. [b]^{13}C NMR assignments were supported by HSQC and HMBC data.

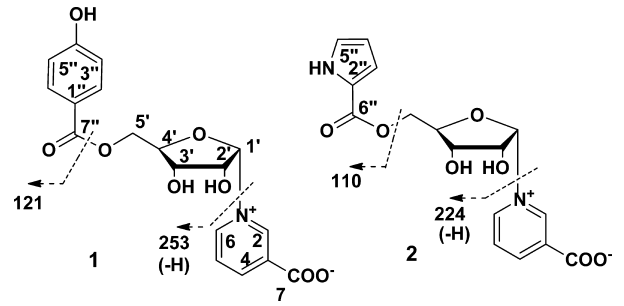


Figure 1. Structures and ESIMS/MS analysis of neopetrosides A (**1**) and B (**2**).

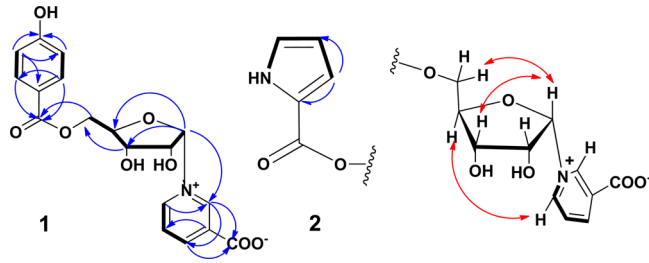
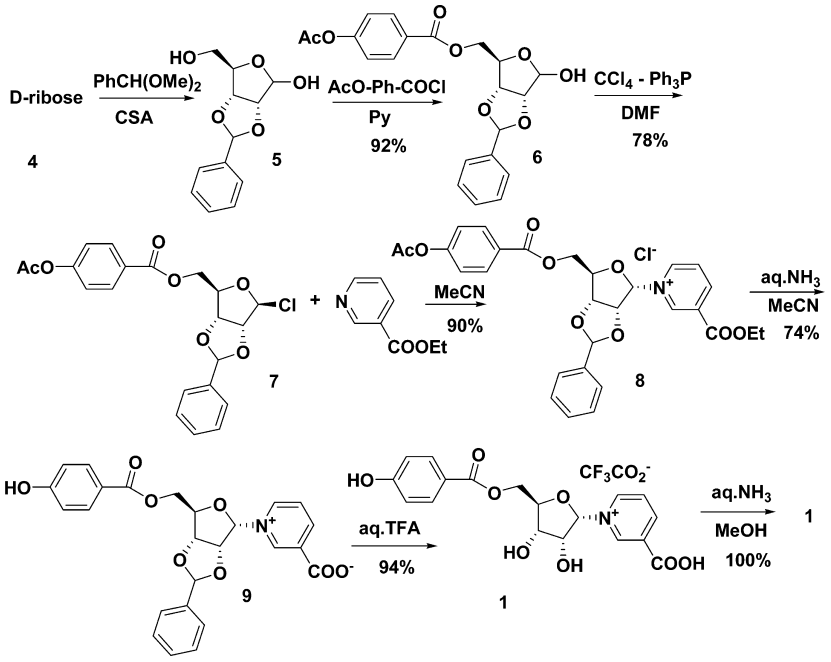


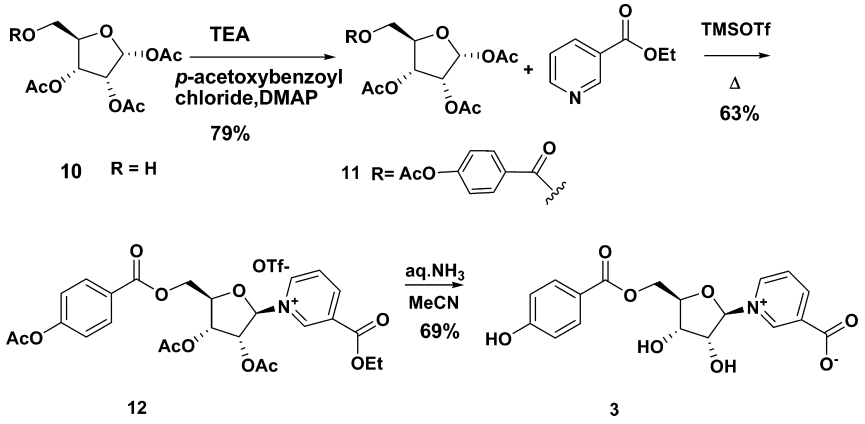
Figure 2. Key COSY (bold), HMBC (blue arrows), and NOE (red arrows) correlations for the structural assignment of **1** and **2**.

DOI: 10.1021/acs.jnatprod.5b00256
J. Nat. Prod. 2015, 78, 1383−1389

Scheme 1. Synthesis of 1



Scheme 2. Synthesis of 3



methylene protons H_2-5′ to the carbonyl C-7″, which in turn showed correlations with the aromatic protons H-2″/H-6″, indicated that the *p*-hydroxybenzoyloxy group was attached to C-5′ of the sugar moiety. The structure of **1** was further confirmed by ESIMS/MS analysis, showing fragmentations with the loss of nicotinic acid or a *p*-hydroxybenzoyl group (Figure 1). The ribofuranoside ring configuration was established on the basis of NOESY correlations between H-1′/H-3′, H-5′ and H-4′/H-2, H-6 (Figure 2). This assignment was further supported by the chemical shifts and coupling constants of the pentose unit that were close to those in α-nicotinamide riboside.[24]

The determination of the absolute configuration of the sugar unit was carried out after acid hydrolysis of **1** with 2 M TFA followed by preparation of acetylated (+)-2-octylglycosides. GC analysis of these compounds and comparison with authentic samples according to the procedure of Leontein et al.[25] revealed the D-configuration of the sugar.

Neopetroside B (**2**) was obtained as an amorphous solid. Its molecular formula was established as $C_{16}H_{16}N_2O_7$ from a prominent $[M - H]^-$ ion peak at *m*/*z* 347.0887 $[M - H]^-$ in its HRESIMS spectrum The spectroscopic properties of **2** were similar to those of **1** (Table 1). The difference between the NMR spectra of **1** and **2** was in the signals belonging to acyl substituents. The ^{1}H NMR spectrum of **2** showed three proton signals at δ_H 6.21 (dd, *J* = 2.5, 3.7 Hz), 6.95 (dd, *J* = 1.7, 3.7 Hz), and 7.01 (dd, *J* = 1.7, 2.5 Hz) and an exchangeable proton at δ_H 11.97 (br s, in the spectrum recorded in DMSO) instead of two aromatic proton doublets of the *p*-hydroxybenzoyl fragment in **1**. The corresponding ^{13}C chemical shifts (δ_C 111.6, 117.7, 125.9) and COSY connectivities from H-3″ to H-5″ and from NH to H-5″ as well as HMBC data (Figure 2) were consistent with a substituted pyrrole.[26]

Moreover, the base peak at *m*/*z* 110.0243 in the HRESIMS/MS spectrum (negative ion mode) corresponding to a $C_5H_4NO_2$ fragment was in agreement with the presence of pyrrole-2-carboxylic acid unit in the structure. Therefore, compound **2** is a new pyridine nucleoside esterified at C-5′ with a pyrrole-2-carboxylic acid residue.

5′-*O*-*p*-Hydroxybenzoylated and 5′-*O*-pyrrole-2-carboxylated ribosides of both nicotinic acid and nicotinamide are unprecedented among natural products. Until now only 5′-*O*-

DOI: 10.1021/acs.jnatprod.5b00256
J. Nat. Prod. 2015, 78, 1383−1389

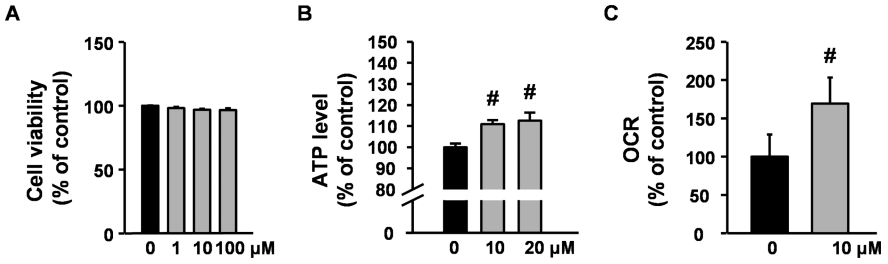


Figure 3. Evaluation of mitochondrial function. (A) Action of **1** on C2C12 cells after 24 h of treatment. (B) ATP levels in C2C12 cells after 1 h of treatment by **1**. (C) OCR in C2C12 cells after 1 h of treatment by **1**. Four independent in vitro experiments were performed. $^{\#}p < 0.05$ vs control group.

benzoyl-β-ribosides of nicotinic acid and nicotinamide have been synthesized as cofactors for DT-diaphorase and nitroreductase.[27,28]

To confirm the unusual structure of **1**, we have synthesized this compound and its β-analogue (**3**) from 2,3-*O*-benzylidene-D-ribose (Scheme 1) and 1,2,3-tri-*O*-acetyl-D-ribose (Scheme 2), respectively. Synthesis of **1** included the selective benzylidene formation (**4** → **5**)[29] and the introduction of the *p*-hydroxybenzoic acid residue at the C-5′ position (**5** → **6**). The corresponding ribofuranose derivative (**7**) as a pure β-isomer was obtained by treatment of **6** with the Ph_3P-CCl_4 system in DMF. Nucleophilic displacement of the Cl group with the ethyl ester of nicotinic acid proceeded with completed inversion to give the required derivative **8**. The synthesis was completed by the removal of protective groups by aqueous NH_3 in MeCN (**8** → **9**) and then with aqueous TFA to give pure **1** in its salt form. Treatment of the TFA salt form of **1** with aqueous NH_3 in MeOH gave **1** as a betaine. All the spectroscopic data of synthetic **1** were identical to those of the natural product (Supporting Information S19, S20, S21, and Table S1).

The synthesis of β-riboside analogue **3** was carried out from the D-ribose triacetate derivative **10**[30] by introduction of the *p*-acetoxybenzoyl group into the 5′-position (**10** → **11**) followed by treatment with the ethyl ester of nicotinic acid (**11** → **12**) with TMSOTf as the catalyst. Removal of the acetate groups and hydrolysis of the ethyl ester by aqueous NH_3 in CH_3CN provided **3**. The comparison of the NMR spectra of **1** and **3** revealed substantial differences in chemical shifts and spin-coupling constants of the ribofuranose fragments (Table S2).

Compound **1** was not cytotoxic against the C2C12 mouse myoblast cell line up to 100 μM concentration. However, compound **1** in its salt form modulated mitochondrial functions in C2C12 cells. To evaluate mitochondrial functions, mitochondrial ATP levels and oxygen consumption rate (OCR) in C2C12 cells treated with 10 μM **1** were measured, and these values were increased in comparison with those in the control experiments (Figure 3). It is known that nicotinic acid and its derivatives may provide beneficial effects on blood lipid and cholesterol profiles,[31] and we intend to study similar effects of the obtained compounds in the future.

In summary, neopetrosides A (**1**) and B (**2**) are polyfunctional biomolecular systems[32] and the first reported representatives of a novel class of pyridine nucleosides possessing an α-riboside linkage, acylated by aromatic acids, and containing a carboxylate group in the aglycone moieties. The noncytotoxic compound **1** upregulates mitochondrial functions and, therefore, may be a useful lead structure for drug development.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-360 polarimeter. UV spectra were recorded on a Shimadzu UV-1601 PC spectrophotometer. CD spectra were recorded with an Applied Photophysics Chirascan Plus spectropolarimeter. IR spectra were recorded using a Bruker Vector 22 spectrophotometer. The ^1H and ^{13}C NMR spectra were obtained using Bruker Avance III-700, Bruker Avance 600, and Bruker Avance III HD-500 spectrometers. Chemical shifts were referenced to the corresponding residual solvent signal (δ_H 7.26/δ_C 77.20 for $CDCl_3$, δ_H 3.30/δ_C 49.60 for CD_3OD, and δ_H 2.50/δ_C 39.60 for DMSO-d_6). ESI mass spectra (including HRESIMS) were measured on a MicrOTOF II (Bruker Daltonics) instrument and an Agilent 6510 Q-TOF LC-MS spectrometer by direct infusion in MeOH. GC analysis was conducted on an Agilent 6580 Series apparatus, equipped with a capillary column (HP-5 MS, 30 m × 0.25 mm) with helium carrier gas (1.7 mL/min) over the temperature range 100−250 °C at 5 deg/min. The temperatures of the injector and detector were 150 and 280 °C, respectively. Low-pressure column chromatography was performed using YMC gel ODS-A (Japan) and silica gel 60 (40−63 μm, E. Merck). HPLC was performed using a Shimadzu Instrument equipped with a UV−vis detector and a Develosil ODS-UG-5 (250 × 4.6 mm) column. TLC was performed on silica gel 60 F254 plates (E. Merck), and visualization was accomplished using UV light or by charring at 150 °C with 10% (v/v) H_3PO_4 in EtOH. All air- or moisture-sensitive reactions were carried out using dry solvents under dry argon. Chemicals were purchased from Acros, Fluka, or Aldrich and used without further purification.

Animal Material. A *Neopetrosia* sp. sponge was collected by scuba during the 38th scientific cruise of R/V *Academic Oparin*, May 2010, near Con Son Island (08°40,9 N; 106°44,4 E, depth 6−15 m) in Vietnamese waters. The shape of the sponge varies from a 1−2 cm thick-lobed encrustation up to 15 cm in the lateral dimension to massive. The surface is usually even and smooth or slightly velvety, with oscules scattered up to 5 mm in diameter, flush or on top with a conical elevation up to 5 mm high. The color is dull blue external and cream internal, distinguishing this animal from other *Neopetrosia* spp. The consistency is hard and brittle. The choanosome is dense. The ectosome is a tangential isodictial regular reticulation of single spicules. The skeleton is a rather regular reticulation of ascending and interconnecting multispicular spicule tracts, up to 150 μm thick, forming rounded meshes up to 400 μm in diameter. In deeper parts of the choanosome the skeleton is rather more compact and irregular. Erect spicule brushes extend outwardly through the surface. The spicules are oxeas, curved with an acute ending reaching 220 μm long by 10 μm wide. Until now about 30 species of *Neopetrosia* have been described. Definition of these sponges to species level is difficult. The morphologies of the skeleton and spicules are important taxonomic characteristics, but clearly insufficient in this case because of their ecological plasticity. This is a new undescribed species. A very similar sponge was noted in Singapore waters.[33] A voucher specimen is deposited under registration number O38-059 in the marine invertebrates collection of the Pacific Institute of Bioorganic Chemistry (Vladivostok, Russia).

DOI: 10.1021/acs.jnatprod.5b00256
J. Nat. Prod. 2015, 78, 1383−1389

Extraction and Isolation. Animal material (dry weight 120 g) was cut and extracted with EtOH immediately after collection (3 × 3 L). The EtOH extract after evaporation in vacuo was partitioned between H_2O and *n*-BuOH. The *n*-BuOH-soluble materials were partitioned with aqueous EtOH and *n*-hexane. The EtOH-soluble layer was fractionated by flash column chromatography on YMC gel ODS-A (75 μm), eluting with a step gradient of H_2O and EtOH (100:0 to 20:80) with monitoring by HPLC. The fractions that eluted with 20% EtOH were further purified by reversed phase HPLC (Develosil ODS-UG-5, 0.7 mL/min, 25 min, UV = 280 nm) using a gradient solvent system from 18% to 80% CH_3CN to afford neopetrosides A (**1**, 5 mg, 0.004% from dry weight) and B (**2**, 3 mg 0.002% from dry weight).

*Neopetroside A (**1**):* light yellow, amorphous solid; UV (EtOH) λ_{max} (log ε) 260 (3.54); ECD (EtOH, c 2.66 × 10^{-4} M) λ_{max} ($\Delta\varepsilon$) 275 (+0.21) nm; IR (KBr) ν_{max} 3417, 1708, 1642, 1608, 1385 cm^{-1}; ^1H, ^{13}C NMR, Table 1; HRESIMS m/z 376.1033 $[M + H]^+$ (calcd for $C_{18}H_{17}NO_8$, 376.1027) and 398.0854 $[M + Na]^+$ (calcd for $C_{18}H_{17}NNaO_8$, 398.0846).

*Neopetroside B (**2**):* light yellow, amorphous solid; UV (EtOH) λ_{max} (log ε) 266 (3.33); ECD (EtOH c 2.04 × 10^{-4}M) λ_{max} ($\Delta\varepsilon$) 275 (+0.18) nm; ^1H, ^{13}C NMR (CD_3OD), Table 1; ^1H NMR (500 MHz, DMSO-d_6) δ_H 11.97 (1H, s, H-1), 9.14 (1H, s, H-2), 8.80 (1H, m, H-4), 8.02 (1H, m, H-5), 8.89 (1H, m, H-6), 6.56 (1H, d, J = 5.3 Hz, H-1′), 4.63 (1H, m, H-2′), 4.23 (1H, dd, J = 3.4, 4.5 Hz, H-3′), 4.77 (1H, m, H-4′), 4.38 (1H, dd, J = 4.6, 12.2 Hz, H-5′) 4.49 (1H, dd, J = 3.6, 12.2 Hz, H-5′), 6.89 (1H, dd, J = 1.7, 3.7 Hz, H-3″), 6.21 (1H, dd, J = 2.5, 3.7 Hz, H-4″), 7.08 (1H, dd, J = 1.7, 2.5 Hz, H-5″); HRESIMS m/z 347.0887 $[M - H]^-$ (calcd for $C_{16}H_{16}N_2O_7$, 347.0885); HRESIMS/MS m/z 110.0243 $[C_5H_4NO_2]^-$ (calcd for $C_5H_4NO_2$, 110.0248).

Monosaccharide Analysis. Acetyl chloride in MeOH (1:10 v/v, 100 μL) was added to **1** (0.8 mg), and the solution was heated during 3 h at 100 °C. The solvent was removed by a stream of argon, and a mixture of pyridine/acetic anhydride (1:1, v/v, 0.4 mL) was added. After stirring overnight at room temperature (rt) the mixture was concentrated in vacuo. GC analysis of the obtained residue was carried out by comparison with authentic samples of standard sugars prepared by the same procedure. The retention times for the sample (7.59, 7.95, 8.30, 8.34 min) matched those for ribose (7.58, 7.97, 8.34, 8.37 min).

Acid Hydrolysis and Determination of the Absolute Configuration of the Monosaccharide. A solution of **1** (0.8 mg) in 1 M TFA (0.5 mL) in a sealed vial was heated at 100 °C for 2 h. The reaction mixture was then washed with $CHCl_3$ (3 × 0.5 mL) and evaporated to dryness. One drop of concentrated TFA and 0.3 mL of (+)-2-octanol (Aldrich) were added to the residue, and the reaction mixture was heated in a glycerol bath at 130 °C for 6 h. The solution was evaporated and treated with a mixture of pyridine/acetic anhydride (1:1, 0.4 mL) for 20 h at rt. The acetylated (+)-2-octylglycosides were analyzed by GC using the corresponding authentic samples prepared by the same procedure. The peaks of four tautomeric forms of ribose were detected in the hydrolysate at 24.04, 24.64, 24.93, and 24.94 min. The retention times for authentic samples were 24.04, 24.64, 24.93, and 24.95 min (D-Rib) and 24.52, 24.75, 24.83, and 25.06 min (L-Rib). The retention times of the L-isomer derivatives were determined in accordance with the procedure of Leontein et al.[25]

Syntheses of Neopetroside A (1) and β-Riboside 3. *2,3-O-Benzylidene-5-O-(4-acetoxybenzoyl)-β-D-ribofuranosyl Chloride (**7**).* To a stirred solution of 2,3-*O*-benzylidene-D-ribose **5**[29] (200 mg, 0.840 mmol) in pyridine (3 mL) were added *p*-acetoxybenzoyl chloride (200 mg, 1.02 mmol, 1.2 equiv) and DMAP (5 mg). The mixture was stirred at rt for 30 min, diluted with CH_2Cl_2, washed with aqueous 1 M HCl, H_2O, and saturated aqueous $NaHCO_3$, dried, and concentrated to give riboside **6** (310 mg) as a mixture with a 1,5-diacylated derivative. The above mixture was treated with triphenylphosphine (406 mg, 1.55 mmol, 2 equiv) and CCl_4 (0.15 mL) in DMF (3 mL) at rt for 2 h, diluted with EtOAc, washed with H_2O and brine, dried, concentrated, and subjected to silica gel column chromatography in CH_2Cl_2 to provide 276 mg (78%) of riboside **7**: colorless syrup; R_f 0.56 (10% EtOAc/$PhCH_3$); HRESIMS m/z 441.0706 $[M + Na]^+$ (calcd for $C_{21}H_{19}ClNaO_7$, 441.0712).

*Ethyl 1-[2,3-O-Benzylidene-5-O-(4-acetoxybenzoyl)-α-D-ribofuranosyl]pyridinium-3-carboxylate Chloride (**8**).* A solution of ribosyl chloride **7** (270 mg, 0.645 mmol) and ethyl nicotinate (2.5 mL) in CH_3CN (2.5 mL) was kept at room temperature for 10 h, concentrated under vacuum, and triturated two times with ether to give 330 mg (90%) of nicotinoyl riboside **8**: amorphous solid; R_f 0.32 (15% MeOH/$CHCl_3$); HRESIMS m/z 534.1750 $[M]^+$ (calcd for $C_{29}H_{28}NO_9$ 534.1759).

Synthetic Neopetroside A (TFA Salt). To a stirred solution of nicotinoyl riboside **8** (100 mg, 0.175 mmol) in CH_3CN (2.5 mL) was added 1.4 N aqueous ammonia solution (2.2 mL). The mixture was stirred at rt for 5 h, diluted with H_2O, and concentrated to dryness. The residue was subjected to silica gel column chromatography (MeOH/CH_2Cl_2 15% → 30%) to give 60 mg (74%) of nicotinoyl riboside **9**. The latter was dissolved in 90% aqueous TFA, and the mixture was kept at rt for 1 h, concentrated in vacuo, dissolved in H_2O, and freeze-dried to give 60 mg (94%) of riboside **1** in its salt form: solid; R_f 0.36 ($CHCl_3$/MeOH/H_2O, 10:10:1); $[\alpha]^{19}_D$ +20 (c 1, H_2O); ^1H NMR (600 MHz, CD_3OD) δ_H 4.37 (dd, 1H, J = 3.8, 4.6 Hz, H-3′), 4.51 (dd, 1H, J = 4.5, 12.3 Hz, H-5′a), 4.59 (dd, 1H, J = 3.5, 12.3 Hz, H-5′b), 4.80 (m, 1H, H-2′), 4.98 (m, 1H, H-4′), 6.54 (d, 1H, J = 5.2 Hz, H-1′), 6.86 (d, 2H, J = 8.5 Hz, H-3″, H-5″), 7.93 (d, 2H, J = 8.5 Hz, H-2″, H-6″), 8.17 (dd, 1H, J = 6.8, 7.9 Hz, H-5), 9.02 (dd, 1H, J = 1.3, 8.0 Hz, H-4), 9.12 (dd, 1H, J = 1.4 and 6.2 Hz, H-6), 9.38 (s, 1H, H-2); ^{13}C NMR (150 MHz, CD_3OD) δ_C 64.8 (C-5′), 72.8 (C-3′), 74.0 (C-2′), 88.0 (C-4′), 98.3 (C-1′), 116.5 (C-3″, C-5″), 127.8 (C-5), 133.1 (C-2″, C-6″), 139.9 (C-3), 144.2 (C-6), 145.0 (C-2), 147.8 (C-4), 164.0 (C-4″), 165.3 (C-7), 167.7 (C-7″); HRESIMS m/z 398.0849 $[M + Na]^+$ (calcd for $C_{18}H_{17}NNaO_8$, 398.0846).

*Synthetic Neopetroside A (**1**).* To a solution of 2 mg of neopetroside A (TFA salt) in MeOH (0.5 mL) was added a 1.4 N aqueous ammonia solution (0.1 mL). The mixture was kept at rt for 20 min and concentrated in vacuo. ^1H NMR, Supporting Information S19; ^{13}C NMR, Supporting Information S20.

*1,2,3-Tri-O-acetyl-5-O-(4-acetoxybenzoyl)-D-ribofuranose (**11**).* To a stirred solution of riboside **10**[30] (117 mg, 0.424 mmol) and triethylamine (0.2 mL) in CH_2Cl_2 (2 mL) were added *p*-acetoxybenzoyl chloride (100 mg, 0.51 mmol, 1.2 equiv) and DMAP (5 mg). The mixture was stirred at rt for 30 min, diluted with CH_2Cl_2, washed with aqueous 1 M HCl, H_2O, and saturated aqueous $NaHCO_3$, dried, and concentrated. The residue was subjected to silica gel column chromatography in EtOAc/toluene (1:4) to provide 147 mg (79%) of riboside **11**: syrup; R_f 0.76 (10% Me_2CO/$CHCl_3$); $[\alpha]^{19}_D$ +55 (c 1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ_H 2.10 (s, 3H, Ac), 2.13 (s, 6H, 2Ac), 2.32 (s, 3H, Ac), 4.46 (dd, 1H, J = 3.8, 12.0 Hz, H-5′a), 4.57 (m, 1H, H′-4), 4.60 (dd, 1H, J=3.2, 12.0 Hz, H-5′b), 5.33 (dd, 1H, J = 4.8, 6.4 Hz, H′-2), 5.38 (dd, 1H, J = 3.0, 6.7 Hz, H′-3), 6.46 (d, 1H, J = 4.5 Hz, H′-1), 7.20 (d, 2H, J = 8.5 Hz, C-3″, C-5″), 8.07 (d, 2H, J = 8.5 Hz, C-2″, C-6″); ^{13}C NMR (150 MHz, $CDCl_3$) δ_C 20.3 (Ac), 20.6 (Ac), 21.0 (Ac), 21.1 (Ac), 63.9 (C-5′), 69.9 (C-2′),70.1 (C-3′), 81.7 (C-4′), 94.1 (C-1′), 121.8 (C-3″, C-5″), 131.3 (C-2″, C-6″), 126.9 (C-1″), 154.6 (C-4″), 165.2 (C-7″), 168.7 (Ac), 169.3 (Ac), 169.6 (Ac), 170.1 (Ac); HRESIMS m/z 461.1053 $[M + Na]^+$ (calcd for $C_{20}H_{22}NaO_{11}$, 461.1054).

*Ethyl 1-[2,3-Di-O-acetyl-5-O-(4-acetoxybenzoyl)-β-D-ribofuranosyl]pyridinium-3-carboxylate Trifluoromethanesulfonate (**12**).* To a stirred solution of riboside **11** (117 mg, 0.267 mmol) and ethyl nicotinate (0.056 mL, 0.4 mmol, 1.5 equiv) in CH_2Cl_2 (1.5 mL) was added TMSOTf (0.084 mL, 0.267 mmol). The mixture was stirred under reflux for 1 h, diluted with toluene, and subjected to silica gel column chromatography (CH_2Cl_2 → 97:3 CH_2Cl_2/MeOH) to give 114 mg (63%) of nicotinoyl riboside **12**: colorless, amorphous solid; R_f 0.52 (15% MeOH/$CHCl_3$); $[\alpha]^{18}_D$ +1 (c 1, MeOH); ^1H NMR (600 MHz, CD_3OD) δ_H 1.36 (t, 3H, J = 7.1 Hz, $COOCH_2C\underline{H}_3$), 2.02 (s, 3H, Ac), 2.16 (s, 3H, Ac), 2.22 (s, 3H, Ac), 2.32 (s, 3H, Ac); 4.49 (q, 2H, J = 7.1 Hz, $COOC\underline{H}_2CH_3$), 4.76 (dd, 1H, J = 3.9, 12.9 Hz, H-5′a), 4.88 (br d, H-5′b), 4.97 (m, 1H, H-4′), 5.61 (t, 1H, J = 5.6 Hz, H-3′), 5.71 (dd, 1H, J = 4.7, 5.6 Hz, H-2′), 6.65 (d, 1H, H-1′), 7.20 (d, 2H, J = 8.5 Hz, H-3″, H-5″), 8.02 (d, 2 H, J = 8.5 Hz, H-2″, H-6″), 8.24 (m, 1H, H-5), 9.10 (d, 1H, J = 8.0 Hz, H-4), 9.34 (d, 1H, J = 6.2 Hz, H-6),

DOI: 10.1021/acs.jnatprod.5b00256
J. Nat. Prod. 2015, 78, 1383−1389

9.58 (s, 1H, H-2); ^{13}C NMR (150 MHz, CD_3OD) δ_C 14.8 ($COOCH_2\underline{C}H_3$), 20.8 (2 Ac) and 21.4 (Ac), 64.8 (C-5′), 64.9 ($COO\underline{C}H_2CH_3$), 71.3 (C-3′), 78.1 (C-2′), 85.1 (C-4′), 99.7 (C-1′), 123.8 (C-3″,C-5″), 128.6 (C-1″), 130.5 (C-5), 132.8 (C-2″,C-6″, C-3), 143.4 (C-6), 145.5 (C-2) 149.4 (C-4), 157.0 (C-4″), 162.8 (COOEt), 167.1 (C-7″), 171.0 (Ac), 171.7 (Ac), 172.2 (Ac); HRESIMS *m/z* 530.1641 $[M]^+$ (calcd for $C_{26}H_{28}NO_{11}$, 530.1657).

*1-[5-O-(4-Hydroxybenzoyl)-β-D-ribofuranosyl]pyridinium-3-carboxylate (**3**).* To a stirred solution of nicotinoyl riboside **12** (55 mg, 0.081 mmol) in CH_3CN (1.15 mL) was added a 1.4 N aqueous ammonia solution (1.6 mL). The mixture was stirred at rt for 4 h, diluted with H_2O, and concentrated to dryness. The residue was subjected to silica gel column chromatography (90% MeOH/H_2O–CH_2Cl_2 25% → 50%] to give 21 mg (69%) of nicotinoyl β-riboside **3**: colorless solid; R_f 0.36 ($CHCl_3$/MeOH/H_2O, 10:10:1); $[\alpha]^{19}_D$ −21 (*c* 1, H_2O); ^{1}H, ^{13}C NMR (500 MHz,CD_3OD), Table S1; ^{1}H NMR (600 MHz, D_2O) δ_H 4.45 (dd, 1H, *J* = 2.7, 12.7 Hz, H-5′a), 4.48 (dd, 1H, *J* = 3.5, 5.0 Hz, H-3′), 4.60 (t, 1H, *J* = 4.9 Hz, H-2′), 4.78 (q, 1H, *J* = 2.8 Hz, H-4′), 4.83 (dd, 1H, *J* = 2.8, 12.7 Hz, H-5′b), 6.65 (d, 1H, *J* = 4.3 Hz, H-1′), 6.72 (d, 2H, *J* = 9.7 Hz, H-3″, H-5″), 7.52 (d, 2H, *J* = 9.7 Hz, H-2″,6″), 7.92 (dd, 1H, *J* = 6.3, 7.8 Hz, H-5), 8.67 (dd, 1H, *J* = 1.3, 7.9 Hz, H-4), 8.94 (d, 1H, *J* = 6.2 Hz, H-6), 9.20 (s, 1H, H-2); ^{13}C NMR (150 MHz, D_2O) δ_C 66.3 (C-5′), 73.9 (C-3′), 80.6 (C-2′), 88.9 (C-4′), 101.9 (C-1′), 118.3 (C-3″, C-5″), 123.0 (C-1″), 130.6 (C-5), 134.5 (C-2″ C-6″), 139.9 (C-3), 142.9 (C-6), 143.3 (C-2), 149.2 (C-4), 163.9 (C-4″), 169.5 (C-7), 170.1 (C-7″); HRESIMS *m/z* 376.1030 $[M + H]^+$ (calcd for $C_{18}H_{18}NO_8$, 376.1027).

Cell Culture. Mice myoblast C2C12 cells (American Type Culture Collection) were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 50 U/mL penicillin, and 50 μg/mL streptomycin (Lonza).

Measurement of Cytotoxicity. C2C12 cells were cultured at 2 × 10^4 cells/well in 96-well tissue culture plates. After 16 h, cells were treated with 1, 10, and 100 μM **1** for 24 h. Cytotoxicity was assessed by a quantitative fluorescence assay with the CellTox Green cytotoxicity assay (Promega). This cytotoxicity assay measures changes in membrane integrity that occur as a result of cell death. They were quantified by measuring fluorescence (excitation/emission = 485 nm/530 nm) using a microplate reader (Molecular Devices).

Measurement of ATP Levels. ATP levels were measured using the Mitochondrial ToxGlo assay (Promega) according to the manufacturer's protocol. Briefly, C2C12 cells were cultured at 2 × 10^5 cells/well in 60-mm tissue culture plates. After 16 h, cells were treated with 10 and 20 μM **1** for 1 h. Harvested treated cells were resuspended by pipetting until cells were evenly dispersed. Resuspended cells were plated at 2 × 10^4 cells/well in white, clear-bottom 96-well culture plates. Cells were separated by centrifugation at 200*g* for 10 min, and 50 μL of fresh medium containing 10 mM glucose (cellular ATP; glycolysis and mitochondrial ATP) or galactose (instead of glucose; only mitochondrial ATP) was added to each well. Plates were incubated at 37 °C in a humidified and CO_2-supplemented incubator for 90 min. Assay solution (100 μL) was added to each well, and plates were then incubated at room temperature for 30 min. Luminescence was measured using a luminometer (Molecular Devices).

Measurement of Oxygen Consumption Rate. OCR was measured as previously described.[34] Briefly, C2C12 cells were cultured at 2 × 10^4 cells/well in XF24 cell culture plates (Seahorse Bioscience). After 16 h, cells were treated with 10 μM **1** for 1 h. After 1 h, the media was changed with 500 μL of XF assay medium-modified DMEM (Seahorse Bioscience) and then incubated at 37 °C without CO_2 for 1 h. OCR was measured using an XF24 analyzer and software (Seahorse Bioscience). Assay results were normalized by cell number, which was counted in each well using a Luna automated cell counter (Logos).

■ ASSOCIATED CONTENT

Supporting Information

Tables and NMR spectra for synthetic and natural compounds, 1D and 2D NMR spectra, HRESIMS spectra for compounds **1** and **2**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00256.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 7 (4232) 31-11-68. Fax: 7 (4232) 31-40-50. E-mail: makarieva@piboc.dvo.ru.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The research described in this publication was supported by Grant 148.2014.4 from the President of RF, Grant 13-03-00986 from RFBR, Grant 14-50-00126 from RSF, and the National Research Foundation Joint Research Program (2014K2A1A2048550) from the Republic of Korea. The authors are thankful to Dr. N. Yu. Kim for providing UV and CD spectroscopic data.

■ REFERENCES

(1) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2015**, *32*, 116–211 and other reviews of this series.

(2) Oku, N.; Matsunaga, S.; van Soest, R. W. M.; Fusetani, N. *J. Nat. Prod.* **2003**, *66*, 1136–1139.

(3) Brastianos, H. C.; Vottero, E.; Patrick, B. O.; van Soest, R.; Matainaho, T.; Mauk, A. G.; Andersen, R. J. *J. Am. Chem. Soc.* **2006**, *128*, 16046–16047.

(4) Wei, X.; Nieves, K.; Rodriguez, A. D. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5905–5908.

(5) Li, Y.; Qin, S.; Guo, Y. W.; Gu, Y. C.; van Soest, R. W. M. *Planta Med.* **2011**, *77*, 179–181.

(6) Sorek, H .; Rudi, A.; Benayahu, Y.; Kashman, Y. *Tetrahedron Lett.* **2007**, *48*, 7691–7694.

(7) Williams, D. E.; Austin, P.; Diaz-Marrero, A. R.; Van Soest, R.; Matainaho, T.; Roskelley, C. D.; Roberge, M.; Andersen, R. J. *Org. Lett.* **2005**, *7*, 4173–4176.

(8) Leone, P. D.; Carroll, A. R.; Towerzey, L.; King, G.; McArdle, B. M.; Kern, G.; Fisher, S.; Hooper, J. N. A.; Quinn, R. J. *Org. Lett.* **2008**, *10*, 2585–2588.

(9) Winder, P. L.; Baker, H. L.; Linley, P.; Guzman, E. A.; Pomponi, S. A.; Diaz, M. C.; Reed, J. K.; Wright, A. E. *Bioorg. Med. Chem.* **2011**, *19*, 6599–6603.

(10) Makarieva, T. N; Ogurtsova, E. K.; Denisenko, V. A.; Dmitrenok, P. S.; Tabakmakher, K. M.; Guzii, A. G.; Pislyagin, E. A.; Es'kov, A. A.; Kozhemyako, V. B.; Aminin, D. L.; Wang, Y.-M.; Stonik, V. A. *Org. Lett.* **2014**, *16*, 4292–4295.

(11) Shubina, L. K.; Kalinovsky, A. I.; Makarieva, T. N.; Fedorov, S. N.; Dyshlovoy, S. A.; Dmitrenok, P. S.; Kapustina, I. I.; Mollo, E.; Utkina, N. K.; Krasokhin, V. B.; Denisenko, V. A.; Stonik, V. A. *Nat. Prod. Commun.* **2012**, *7*, 487–490.

(12) Nakamura, M.; Bhatnagar, A.; Sadoshima, J. *Circ. Res.* **2012**, *111*, 604–610.

(13) Canto, C.; Houtkooper, R. H.; Pirinen, E.; Youn, D. Y.; Oosterveer, M. H.; Cen, Y.; Fernandez-Marcos, P. J.; Yamamoto, H.; Andreux, P. A.; Cettour-Rose, P.; Gademann, K.; Rinsch, C.; Schoonjans, K.; Sauve, A. A.; Auwerx, J. *Cell Metab.* **2012**, *15*, 838–847.

(14) Belenky, P.; Bogan, K. L.; Brenner, C. *Trends Biochem. Sci.* **2007**, *32*, 12–19.

(15) Bieganowski, P.; Brenner, C. *Cell* **2004**, *117*, 495–502.

DOI: 10.1021/acs.jnatprod.5b00256
J. Nat. Prod. 2015, 78, 1383–1389

(16) Pankiewicz, K. W. *Pharmacol. Ther.* **1997**, *76*, 89−100.
(17) Konno, K.; Hayano, K.; Shirahama, H.; Sato, H.; Matsumoto, T. *Tetrahedron* **1982**, *38*, 3281−3284.
(18) Chheda, G. B.; Patrzyc, H. B.; Tworek, H. A.; Dutta, S. P. *Nucleosides Nucleotides* **1995**, *14*, 1519−1537.
(19) Hattori, T.; Matsuo, S.; Adachi, K.; Shizuri, Y. *Fish. Sci.* **2001**, *67*, 690−693.
(20) Kaplan, N. O.; Ciotti, M. M.; Stolzenbach, F. E.; Bachur, N. R. *J. Am. Chem. Soc.* **1955**, *77*, 815−816.
(21) Beaupre, B. A.; Carmichael, B. R.; Hoag, M.; Shah, D. D.; Moran, G. R. *J. Am. Chem. Soc.* **2013**, *135*, 13980−13987.
(22) (a) Sciuto, S.; Chillemi, R.; Piattelli, M. *J. Nat. Prod.* **1988**, *51*, 322−325. (b) Cafieri, F; Fattorusso, E; Taglialatela-Scafati, O. *J. Nat. Prod.* **1998**, *61*, 1171−1173. (c) Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O.; Carnuccio, R. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 799−804.
(23) (a) Miyase, T.; Noguchi, H.; Chen, X.-M. *J. Nat. Prod.* **1999**, *62*, 993−996. (b) Li, J. L.; Xiao, B.; Park, M.; Yoo, E. S.; Shin, S.; Hong, J.; Chung, H. Y.; Kim, H. S.; Jung, J. H. *J. Nat. Prod.* **1999**, *62*, 993−996.
(24) Oppenheimer, N. J.; Kaplan, N. O. *Biochemistry* **1976**, *15*, 3981−3989.
(25) Leontein, K.; Lindberg, B.; Lonngren, J. *Carbohydr. Res.* **1978**, *62*, 359−362.
(26) (a) Macherla, V. R.; Liu, J. N.; Bellows, C.; Teisan, S.; Nicholson, B.; Lam, K. S.; Potts, B. C. M. *J. Nat. Prod.* **2005**, *68*, 780−783. (b) Lian, X.-Y.; Zhang, Z. *Nat. Prod. Res.* **2013**, *27*, 2161−2167.
(27) Davies, L. C. *Nucleosides Nucleotides* **1995**, *14*, 311−312.
(28) Friedlos, F.; Jarman, M.; Davies, L. C.; Boland, M. P.; Knox, R. J. *Biochem. Pharmacol.* **1992**, *44*, 25−31.
(29) Grindey, T. B.; Szarek, W. A. *Carbohydr. Res.* **1972**, *25*, 187−195.
(30) Ebner, A.; Stutz, A. E. *Carbohydr. Res.* **1998**, *305*, 331−336.
(31) Karple, F.; Frayn, K. N. *Lancet* **2004**, *363*, 1892−1894.
(32) Ananikov, V. P.; Khokhlova, E. A.; Egorov, M. P.; Sakharov, A. M.; Zlotin, S. G.; Kucherov, A. V.; Kustov, L. M.; Gening, M. L.; Nifantiev, N. E. *Mendel. Commun.* **2015**, *25*, 75−82.
(33) Lim, S. C.; de Voogd, N.; Tan, K. S. *A Guide to Sponges of Singapore*; Singapore Science Centre: Singapore, 2008; p 173.
(34) Jeong, S. H.; Song, I. S.; Kim, H. K.; Lee, S. R.; Song, S.; Suh, H.; Yoon, Y. G.; Yoo, Y. H.; Kim, N.; Rhee, B. D.; Ko, K. S.; Han, J. *Mol. Cells* **2012**, *34*, 357−365.

DOI: 10.1021/acs.jnatprod.5b00256
J. Nat. Prod. 2015, 78, 1383−1389