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Isolation and Total Synthesis of Bromoiesol sulfates, Antitrypanosomal arylethers from a *Salileptolyngbya* sp. Marine Cyanobacterium

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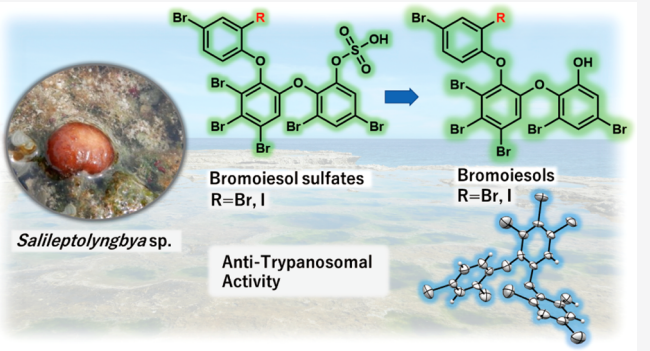
ABSTRACT: Bromoiesol sulfates A (**1**) and B (**2**), new polyhalogenated aryl sulfates, were isolated from a *Salileptolyngbya* sp. marine cyanobacterium along with their hydrolyzed compounds, bromoiesols A (**3**) and B (**4**). To pick up the candidates of their structures, we used Small Molecule Accurate Recognition Technology (SMART), an artificial intelligence-based structure-prediction tool, and their structures were elucidated on the basis of single-crystal X-ray diffraction analysis of bromoiesols (**3** and **4**). In addition, to verify the structures, the total synthesis of bromoiesol A sulfate (**1**) and bromoiesol A (**3**) was achieved. The bromoiesol family, especially bromoiesols (**3** and **4**), selectively inhibited the growth of the bloodstream form of *Trypanosoma brucei rhodesiense*, the causative agent of human African sleeping sickness.

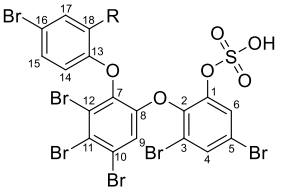


INTRODUCTION

Tropical diseases caused by *Trypanosoma* sp., such as African sleeping sickness and Chagas disease, are some of the most serious infectious diseases worldwide. They are endemic in developing countries, where they threaten the health of over 400 million people and slow economic development.[1] Although some drugs for these diseases are already available for use, they have limitations such as serious side effects and the emergence of drug resistance.[1] Therefore, new antiprotozoal drugs are needed.

Meanwhile, marine natural products are considered to be good sources for drug leads.[2] In particular, secondary metabolites produced by marine cyanobacteria have unique structures and versatile biological activities[3] including antiprotozoal activity. For example, gallinamide A[4] shows very potent antimalarial activity by inhibiting plasmodial cysteine proteases,[5] and almiramides[6] perturb the function of glycosome, that is, an essential organelle for survival of kinetoplastid parasites.[7] As for other activities, a number of important bioactive natural products have been discovered from marine cyanobacteria such as largazole,[8] carmaphycins,[9] and antillatoxin B.[10] Against this background, we have investigated the secondary metabolites of Japanese marine cyanobacteria and have discovered several novel natural products possessing antiparasitic activities such as ikoamide,[11] iheyamides,[12] and hoshinoamides.[13]

Recently, we found new antitrypanosomal aryl ethers, bromoiesol sulfates (**1** and **2**), and their hydrolysates, bromoiesols (**3** and **4**), from a *Salileptolyngbya* sp. marine cyanobacterium. Structurally, **1** and **2** are aryl ethers containing three polyhalogenated benzene rings and a sulfate group, which is rare in natural products. In addition, we clarified that the bromoiesol family (**1**−**4**), especially bromoiesols (**3** and **4**), exhibited antitrypanosomal activity. Here, we report the isolation and structure elucidation of the bromoiesol family (**1**−**4**), and the first total synthesis of **1** and **3**.


Bromoiesol A sulfate (**1**) R = Br
Bromoiesol B sulfate (**2**) R = I

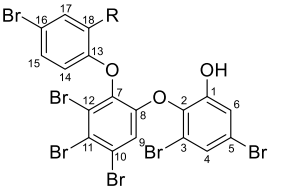

Bromoiesol A (**3**) R = Br
Bromoiesol B (**4**) R = I

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Table 1. ^{1}H and $^{13}C\{^{1}H\}$ NMR Data for Bromoiesol Family (1−4) in CD_3OD

	Bromoiesol A (3)		Bromoiesol B (4)		Bromoiesol A sulfate (1)		Bromoiesol B sulfate (2)	
position	δ_C,[a] type	δ_H[b] (J in Hz)	δ_C,[a] type	δ_H[b] (J in Hz)	δ_C,[a] type	δ_H[b] (J in Hz)	δ_C,[a] type	δ_H[b] (J in Hz)
1	153.0, C		154.5, C		147.2, C		-[c]	
2	139.3, C		139.0, C		140.8, C		141.0, C	
3	123.0, C		118.6, C		119.4, C		-[c]	
4	126.9, CH	7.27, d (2.3)	125.4, CH	7.16, d (2.3)	130.6, CH	7.62, d (2.3)	130.0, CH	7.62, d (2.3)
5	120.5, C		119.9, C		119.0, C		-[c]	
6	120.8, CH	7.07, d (2.3)	121.5, CH	7.02, d (2.3)	124.2, CH	7.88, d (2.3)	123.9, CH	7.89, d (2.3)
7	142.1, C		142.4, C		140.8, C		141.0, C	
8	151.0, C		151.3, C		149.9, C		149.6, C	
9	119.8, CH	6.96, s	119.9, CH	6.96, s	119.1, CH	7.00, s	118.9, CH	7.02, s
10	123.8, C		120.6, C		122.5, C		-[c]	
11	121.6, C		121.1, C		121.0, C		-[c]	
12	118.9, C		123.0, C		122.1, C		-[c]	
13	153.9, C		156.5, C		152.7, C		155.1, C	
14	117.9, C	6.66, d (9.1)	116.8, C	6.59, d (9.0)	117.3, CH	6.83, d (9.1)	115.9, C	6.73, d (9.0)
15	132.4, CH	7.36, dd (2.3, 9.1)	133.2, CH	7.38, dd (2.4, 9.0)	132.1, CH	7.43, dd (2.3, 9.1)	132.4, CH	7.44, dd (2.4, 9.0)
16	116.1, C		116.3, C		115.0, C		115.1, C	
17	136.7, CH	7.89, d (2.3)	142.5, CH	7.97, d (2.4)	135.4, CH	7.74, d (2.3)	140.7, CH	7.93, d (2.4)
18	113.4, C		86.7, C		111.9, C		84.8, C	

[a]Measured at 100 MHz. [b]Measured at 400 MHz. [c]Not observed.

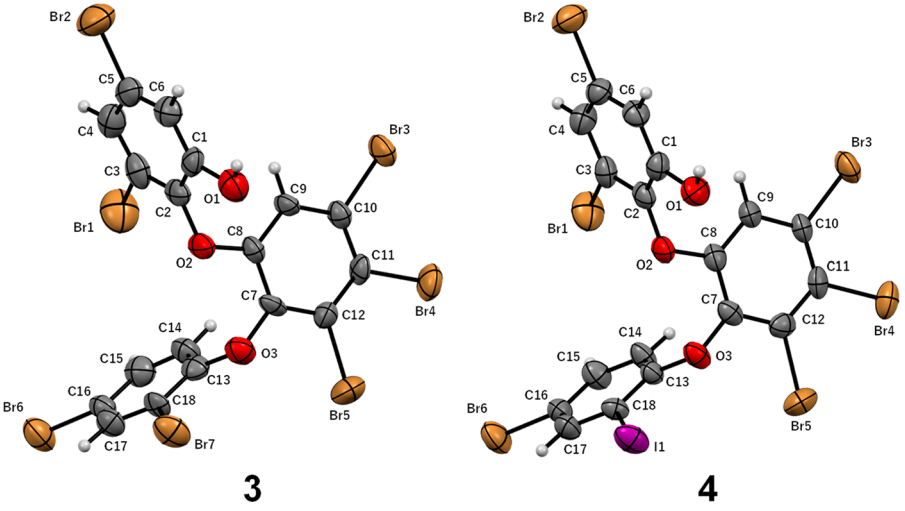

Figure 1. X-ray ORTEP drawings of bromoiesols 3 and 4.

RESULTS AND DISCUSSION

A *Salileptolyngbya* sp. marine cyanobacterium was collected at Ie Island, Okinawa, Japan, in July 2020. The collected cyanobacterium (650 g, wet weight) was extracted with EtOH, and the extract was filtered and concentrated. The residue was partitioned between EtOAc and H_2O. The material obtained from the organic layer was partitioned between 90% aqueous MeOH and hexane. The aqueous MeOH fraction was separated by reversed-phase column chromatography (ODS silica gel, MeOH-H_2O). Reversed-phase HPLC analysis of the fraction eluted with 80% aqueous MeOH showed the presence of two compounds with strong UV absorption (bromoiesol sulfates **1** and **2**). Since **1** and **2** were eluted as sharp peaks only with the use of an acidified mobile phase, we used ODS HPLC with 91% aqueous MeOH with 0.1% TFA as a solvent for the isolation of bromoiesol sulfates (**1** and **2**). However, after evaporation of the solvent, large percentages of **1** and **2** were converted to other compounds, bromoiesols (**3**, 8.6 mg, and **4**, 3.6 mg, respectively). Therefore, we first determined the structures of **3** and **4** and then elucidated the structures of the original compounds, **1** and **2**, based on the structures of bromoiesols (**3** and **4**).

Compared to **1** and **2**, **3** and **4** are much less polar (see isolation conditions in the Supporting Information) and 80 units smaller m/z in the mass spectrum. In addition, bromoiesols (**3** and **4**) showed a sharp peak in HPLC without the use of an acidified solvent. Therefore, **3** and **4** were easily purified by HPLC (Cosmosil 5PE-MS, MeCN-H_2O, 8.6 mg of **3** and 3.6 mg of **4**). The molecular formula of bromoiesol A (**3**) was determined on the basis of negative HRESIMS data (m/z 822.4606, calcd for $C_{18}H_6Br_7O_3$ $[M-H]^-$ 822.4606). The presence of seven bromine atoms was supported by octet isotopic $[M-H]^-$ peaks with a ratio of 1:7:21:35:35:21:7:1. The NMR data for **3** are summarized in Table 1. In the ^{1}H NMR spectrum in CD_3OD, **3** showed only six aromatic signals with a simple coupling pattern (δ_H 6.66, 6.96, 7.07, 7.27, 7.36, and 7.78), and the analyses of the coupling constants and the

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decoupling experiments clarified the presence of three aromatic rings as follows: 1, 2, 4-trisubstituted, 1, 3, 4, 5-tetrasubstituted, and 1, 2, 3, 4, 5-pentasubstituted benzene rings. Meanwhile, the $^{13}C\{^1H\}$ NMR spectrum revealed the existence of 18 sp^2 carbons (δ_C 113.4−153.9), supporting the result of the ^1H NMR analysis. To obtain clues about the structure, we analyzed HMQC spectral data of **3** using Small Molecule Accurate Recognition Technology (SMART), an artificial intelligence-based structure-prediction tool (see Supporting Information).[14] As a result, several oxygenated arylethers, such as spongiadioxin A[15] (Cosine score: 0.9803) and ambigol C[16] (cosine score: 0.9602), were proposed as natural products that have a similar structure to bromoiesol A (**3**), suggesting that the three aromatic rings in **3** are connected by ether bonds. However, the scarcity of protons in the structure prevented us from clarifying the connectivity of the three aromatic rings using 2D NMR data. Therefore, we tried to crystallize **3** for single-crystal X-ray diffraction analysis. Recrystallization was attempted using hexane, acetone, acetonitrile, and methanol, but only methanol gave a single crystal. The structure of bromoiesol A (**3**) was determined by single-crystal X-ray diffraction analysis, as shown in Figure 1.

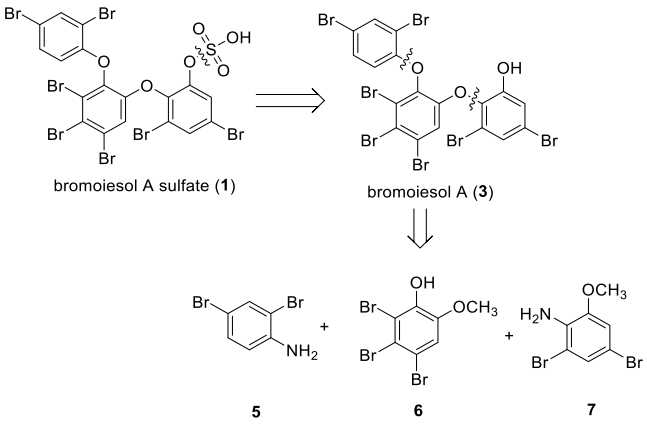
The molecular formula of bromoiesol B (**4**) was determined by HRESIMS (*m/z* 870.4491, calcd for $C_{18}H_6Br_6IO_3$ $[M-H]^-$ 870.4467). The NMR data for **4** are similar to those for bromoiesol A (**3**) except for a chemical shift at C-18, which is the typical value for an iodine-attached aromatic carbon (Table 1). Bromoiesol B (**4**) was crystallized in the same way, and the structure was determined by single-crystal X-ray diffraction analysis as shown in Figure 1.

Next, we deduced the structures of the original compounds, bromoiesol sulfates (**1** and **2**), based on the structures of bromoiesols (**3** and **4**). As we mentioned above, **1** and **2** have much higher polarity and 80 units greater *m/z*. In addition, HRESIMS analysis clarified that the difference in the molecular formulas between **1** and **3**, and **2** and **4** was one sulfur and three oxygens. On the basis of these experimental findings, we deduced that **1** and **2** were sulfates of bromoiesols (**3** and **4**). If so, **1** and **2** seem unstable under strong acidic conditions such as with TFA. Therefore, we modified the solvent for the HPLC purification of bromoiesol sulfates (**1** and **2**). As a result, we found that a solvent containing 20 mM AcOH/AcONa buffer at pH 4 was suitable for the isolation of **1** and **2** without hydrolysis of the sulfate group. We recollected the same cyanobacterium at Ie Island in September 2020 and successfully isolated bromoiesol sulfates (**1**, 1.8 mg, and **2**, 1.3 mg) without decomposition by using the modified condition. In this second isolation experiment, we obtained neither **3** nor **4**. The NMR data of **1** and **2** were summarized in Table 1.

To verify the structure of bromoiesol A sulfate (**1**), we achieved the total synthesis of **1** along with bromoiesol A (**3**). Our synthetic strategy is shown in Scheme 1. Bromoiesol A sulfate (**1**) could be synthesized from bromoiesol A (**3**) by condensation with sulfur trioxide. Bromoiesol A (**3**) could be obtained by sequential coupling of known fragments **5**, **6**, and **7**.[17−19] In these key coupling reactions, we used aryliodonium salts,[20,21] derived from **5** and **7** as an electrophile, respectively.

Our total synthesis commenced with the preparation of aryl iodonium salts **9** and **11** (Scheme 2). Known amine **5**[17] was converted to known iodide **8** by the Sandmeyer reaction.[22] The oxidation of **8** using *m*CPBA followed by the addition of trimethoxybenzene (TMB) afforded iodonium salt **9**.[20]

Scheme 1. Outline of Synthesis of Bromoiesol A sulfate (1)



Meanwhile, known amine **7**[19] was converted to iodonium salt **11** in the same way as **9**.

The first coupling reaction between iodonium salt **9** and known phenol **6**[18] under basic condition gave aryl ether **12** (Scheme 3).[21] After the demethylation of **12** by boron tribromide,[19] the second coupling reaction with **11** afforded arylether **14**. Demethylation of **14** provided bromoiesol A (**3**), and **3** was successfully converted to bromoiesol A sulfate (**1**) by sulfur trioxide. The spectral data of synthetic bromoiesol A sulfate (**1**) and bromoiesol A (**3**) were consistent with those of natural **1** and **3**. Therefore, we verified the correctness of our structure determination.

As for biological activity, the bromoiesol family (**1**−**4**) showed antitrypanosomal activity against the bloodstream form of *Trypanosoma brucei rhodesience* IL-1501 strain, the causative agent of African sleeping sickness, without affecting the growth of HeLa cells (Table 2) at 10 μM. In addition, although iodine substitution did not change the degree of activity of the bromoiesol family (**1**−**4**), hydrolysis of the sulfate group drastically increased the antiparasitic activity. Considering the instability of the sulfate group and the significant change in biological activities between the sulfates (**1** and **2**) and the hydrolysates (**3** and **4**), bromoiesol sulfates (**1** and **2**) may act as prodrug-like compounds in the natural environment.

In conclusion, bromoiesol sulfates (**1** and **2**), polyhalogenated aryl ethers, and their hydrolysates (**3** and **4**) were isolated from the marine cyanobacterium *Salileptolyngbya* sp. We determined their structures based on SMART analysis of the HMQC data of **3** and single-crystal X-ray diffraction analyses of **3** and **4**. In addition, to verify the structures, the total synthesis of bromoiesol A sulfate (**1**) and bromoiesol A (**3**) was achieved. The bromoiesol family, especially bromoiesols (**3** and **4**), showed selective growth-inhibitory activity against the causative agent of Human African trypanosomiasis. To date, several structurally related natural products, such as crossbyanols A−D,[23] have been reported. They also showed significant differences in biological activities between sulfates and the corresponding hydrolysates. The biosynthetic pathways of these polyhalogenated aromatic compounds are revealed by Moore's group.[24] Each aromatic ring is derived from 4-hydroxybenzoic acid with bromination by flavin-dependent halogenase Bmp5, and the 4-hydroxybenzoic acid is synthesized from chorismic acid by chorismate lyase Bmp6. Meanwhile, the aryl ether bonds are constructed by cytochrome P450 enzyme Bmp7. In fact, a recent study

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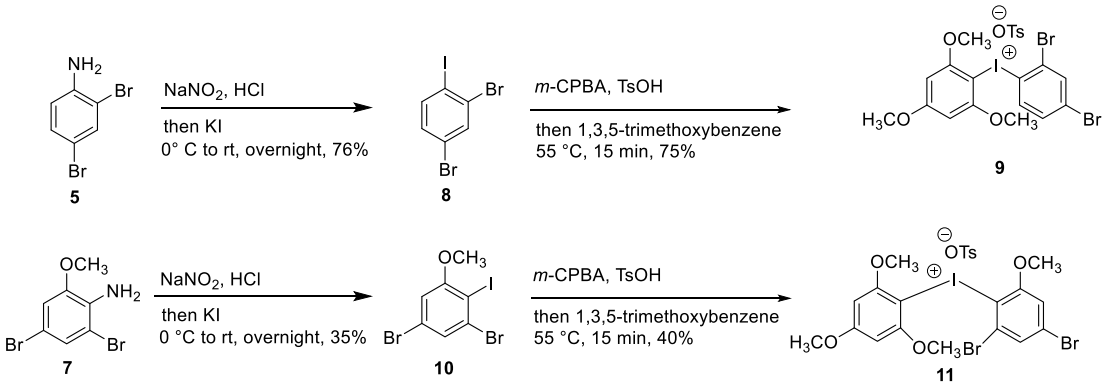
Scheme 2. Synthesis of Aryliodonium Salts 9 and 11



Scheme 3. Synthesis of Bromoiesol A (3) and Bromoiesol A sulfate (1)

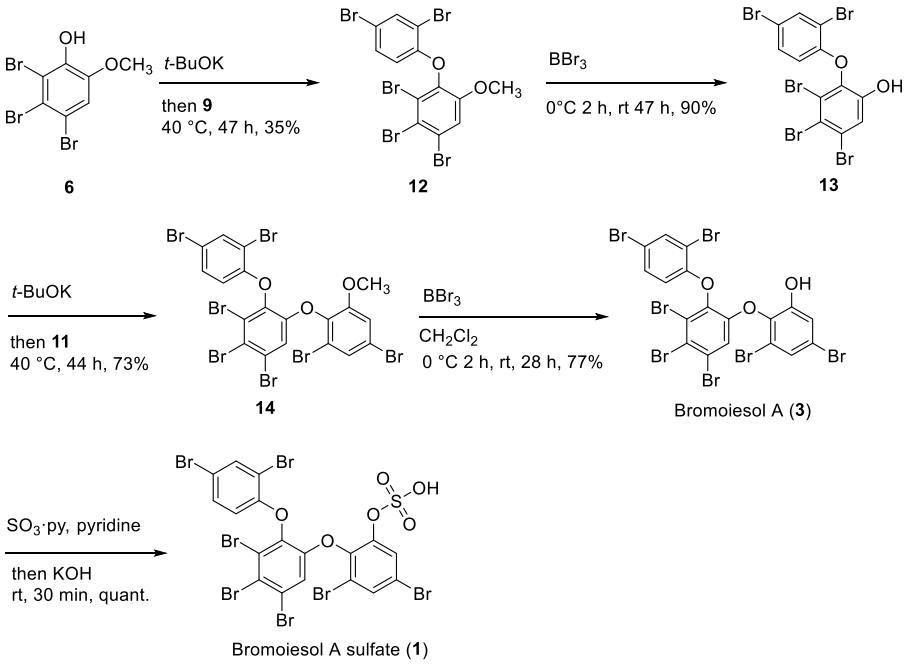


Table 2. Growth-Inhibitory Activities of the Bromoiesol Family (1−4)

compounds	IC_{50} (μM)	
	HeLa cells	*T. b. rhodesiense*
bromoiesol A (3)	21 ± 6	1.2 ± 0.1
bromoiesol B (4)	21 ± 5	0.70 ± 0.23
bromoiesol A sulfate (1)	>10[a]	8.8 ± 1.3
bromoiesol B sulfate (2)	>10[a]	7.9 ± 1.8

[a]Because of the scarcity of the sample, the exact IC_{50} value was not determined.

clarified that ambigols A−C, polyhalogenated triphenyls produced by the terrestrial cyanobacterium *Fischerella ambigua* 108b, are synthesized by the similar pathway as described above.[25] Therefore, it is likely that bromoiesols are constructed in the same way.

Now that we have developed a synthetic route for the bromoiesol family, a detailed study on the structure−activity relationships of synthetic analogs may contribute to the development of new antitrypanosomal drugs.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR spectra were recorded on a JASCO RT/IR-4200 instrument. All NMR spectral data were recorded on a JEOL JNM-ECS400 spectrometer for ^1H (400 MHz) and $^{13}C\{^1H\}$ (100 MHz). ^1H NMR chemical shifts (referenced to residual $CHCl_3$ and CHD_2OD observed at δ_H 7.26 and 3.31, respectively) were assigned using a combination of data from ^1H decoupling and HMQC experiments. Similarly, $^{13}C\{^1H\}$ NMR chemical shifts (referenced to $CDCl_3$ and CD_3OD observed at δ_C 77.16 and 49.0) were assigned based on HMBC and HMQC experiments. HRESIMS and HRAPCIMS spectra were obtained on an LCT Premier XE time-of-flight (TOF) mass spectrometer. Chromatographic analyses were performed using an HPLC system consisting of a pump (model PU-2080, JASCO) and a UV detector (model UV-2075, JASCO). All chemicals and solvents used in this study were the best grade and available from a commercial source (Nacalai Tesque). Reactions were monitored by thin-layer chromatography (TLC), and TLC plates were visualized by both UV detection and phosphomolybdic acid solution. Silica Gel 60N (Irregular, 63-212 μm) were used for open column chromatography unless otherwise noted. Automated Flash Chromatography System (AFCS, EPCLC AI-580, YAMAZEN) was also used for purification of synthesized compounds. X-ray crystal structure analysis was

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performed using a Bruker D8 VENTURE instrument and APEX3. All moisture-sensitive reactions were performed under an atmosphere of nitrogen, and the starting materials were azeotropically dried with toluene before use.

Identification of Marine Cyanobacterium. A cyanobacterial filament was isolated under a microscope and crushed with freezing and thawing. The 16S rDNA genes were PCR-amplified from the isolated DNA using the primer set CYA106F (a cyanobacterial-specific primer) and 16S1541R (a universal primer). The PCR reaction contained DNA derived from a cyanobacterial filament, 0.5 μL of KOD-Multi and Epi- (Toyobo), 0.25 μL of each primer, 12.5 μL of 2 × PCR Buffer for KOD-Multi and Epi-, and H_2O for a total volume of 24 μL. The PCR reaction was performed as follows: initial denaturation for 2 min at 94 °C, and amplification by 40 cycles of 10 s at 98 °C and 10 s at 58 °C, and 1 min at 66 °C. PCR products were analyzed on agarose gel (1%) in TBE buffer and visualized by ethidium bromide staining. The obtained DNA was sequenced with CYA106F and 16S1541R primers.

This sequence is available in the DDBJ/EMBL/Genbank databases under accession number LC603015. The obtained nucleotide sequence of 16S rRNA gene was used for phylogenetic analysis with the sequences of related cyanobacterial 16S rRNA genes. All sequences were aligned by the SINA web service (version 1.2.11)[26] with default settings. The poorly aligned positions and divergent regions were removed by Gblocks Server (version 0.91b),[27] implementing the options for a less stringent selection, including the 'Allow smaller final blocks', 'Allow gap positions within the final blocks', and 'Allow less strict flanking positions' options. The obtained 904 nucleotide positions were used for phylogenetic analysis. JModeltest (version 2.1.7)[28,29] with default settings was used to select the best model of DNA substitution for the maximum likelihood (ML) analysis and Bayesian analysis according to the Akaike information criterion (AIC). The ML analysis was conducted by PhyML (version 20131016) using the GTR+I+G model with a gamma shape parameter of 0.4850, a proportion of invariant sites of 0.5220, and nucleotide frequencies of F(A) = 0.2490, F (C) = 0.2307, F (G) = 0.3118, F(T) = 0.2084. Bootstrap resampling was performed on 1000 replicates. The ML tree was visualized with Njplot (version 2.3).[30] The Bayesian analysis was conducted by MrBayes (version 3.2.5)[31] using the GTR+I+G model. The Markov chain Monte Carlo process was set at 2 chains, and 1 000 000 generations were conducted. Sampling frequency was assigned at every 500 generations. After analysis, the first 100 000 trees were deleted as burn-in, and the consensus tree was constructed. The Bayesian tree was visualized with FigTree (version 1.4.0, http://tree.bio.ed.ac.uk/software/figtree). As a result, the cyanobacterium (accession no. LC603015) formed a clade with *Salileptolyngbya* sp. Therefore, the cyanobacterium was classified into *Salileptolyngbya* sp. The phylogenetic tree is shown in Figure S1 in the Supporting Information.

Collection, Extraction, and Isolation of Bromoiesols A and B (3 and 4). The *Salileptolyngbya* sp. cyanobacterium (650 g, wet weight) was collected at Ie-Island, Okinawa, Japan in July 2020. The collected cyanobacterium was extracted with EtOH (2 L) for 1 week at room temperature. The extract was filtered, and the residue was re-extracted with EtOH (2 L) at room temperature for 2 days. The extract was filtered, and the combined filtrates were concentrated. The residue was partitioned between EtOAc (3 × 300 mL) and water (300 mL). The combined organic layers were concentrated, and the residue was partitioned between 90% aqueous MeOH (300 mL) and hexane (3 × 300 mL). The aqueous MeOH layer was concentrated, and the obtained residue (387 mg) was separated by column chromatography on ODS (5 g) eluted with 25 mL of 40%, 60%, and 80% aqueous MeOH, followed by 25 mL of MeOH. The fraction eluted with 80% MeOH (96.3 mg) was subjected to HPLC [Cosmosil 5C_{18}-MS-II (ϕ 20 mm × 250 mm); solvent 91% aqueous MeOH with 0.1% TFA; flow rate 5 mL/min; detection UV 215 nm] in three batches to give a fraction that contained **3** and **4** (18 mg, t_R = 35−45 min). This fraction was further separated by HPLC [Cosmosil 5PE-MS (ϕ 20 mm × 250 mm); solvent 78% aqueous MeCN; flow rate 5 mL/min; detection UV 215 nm] to give bromoiesol A (**3**) (8.6 mg, t_R = 45 min) and bromoiesol B (**4**) (3.6 mg, t_R = 48 min).

Bromoiesol A (3). A white solid; IR (neat) 3094, 1576, 1460, 1427, 1348, 1283, 1213, 1036, 924, 892, 804 cm^{-1}; UV (MeCN) λ_{max} (log ε) 289 nm (3.54); melting point of crystal 128.0−129.5 °C; ^{1}HNMR, ^{13}C{^{1}H} NMR, and HMBC data, Table S1 in the Supporting Information; HRESIMS m/z 822.4606 [M-H]$^-$ (calcd for $C_{18}H_6{}^{79}Br_7O_3$ 822.4606).

Bromoiesol B (4). A white solid; IR (neat) 1572, 1461, 1429, 1351, 1277, 1213, 1029, 768 cm^{-1}; UV (MeCN) λ_{max} (log ε) 288 nm (3.75);melting point of crystal 140.0−147.5 °C;^{1}HNMR, ^{13}C{^{1}H} NMR, and HMBC data, Table S2 in the Supporting Information; HRESIMS m/z 870.4491 [M-H]$^-$ (calcd for $C_{18}H_6{}^{79}Br_6IO_3$ 870.4467).

Collection, Extraction, and Isolation of Bromoiesol sulfates A and B (1 and 2). The *Salileptolyngbya* sp. cyanobacterium (300 g, wet weight) was collected at Ie-Island, Okinawa, Japan in September 2020. The experiments before the ODS column chromatography step were conducted in the same way as the previous ones. The residue (108 mg) obtained by concentration of the 90% aqueous MeOH layer was separated by column chromatography on ODS (3 g) eluted with 15 mL of 40%, 60%, and 80% aqueous MeOH, followed by 25 mL of MeOH. Among them, the fraction eluted with 80% MeOH (24.9 mg) contained bromoiesol sulfates (**1** and **2**). We examined several conditions for the purification of bromoiesol sulfates (**1** and **2**), and the most successful procedure is described below.

One-third of the fraction eluted with 80% MeOH (8.3 mg) was separated by HPLC [Cosmosil 5C_{18}-MS-II (ϕ 20 mm × 250 mm); solvent 92% MeOH, 8% pH4 20 mM AcOH/AcONa buffer; flow rate 5 mL/min; detection UV 215 nm] to give a fraction that contained **1** and **2** (4.3 mg, t_R = 25−35 min). This fraction was further purified by HPLC [Cosmosil 5PE-MS (ϕ 20 mm × 250 mm); solvent 65% MeCN, 35% pH4 20 mM AcOH/AcONa buffer; flow rate 5 mL/min; detection UV 215 nm] to give two fractions that contained almost pure **1** (2.3 mg, t_R = 29−33 min) and almost pure **2** (1.1 mg, t_R = 33−40 min), respectively.

These fractions were combined with other fractions containing **1** or **2** that were purified by other methods to give two fractions containing **1** (3.0 mg) and **2** (2.6 mg), respectively. The fraction containing **1** (3.0 mg) was finally purified by column chromatography on ODS (0.2 g) with 80% aqueous MeOH to give 1.8 mg of bromoiesol A sulfate (**1**). The fraction containing **2** (2.6 mg) was purified by HPLC [Cosmosil 5PE-MS (ϕ 20 mm × 250 mm); solvent 65% MeCN, 35% pH 4 20 mM AcOH/AcONa buffer; flow rate 5 mL/min; detection UV 215 nm], and the resulting crude **2** (t_R = 33−40 min) was finally purified by column chromatography on ODS (0.2 g) with 80% aqueous MeOH to give 1.3 mg of bromoiesol B sulfate (**2**).

Bromoiesol A sulfate (1). A pale white solid; IR (neat) 1464, 1427, 1284, 1260, 1055, 927 cm^{-1}; UV (MeCN) λ_{max} (log ε) 283 nm (3.62); ^{1}H NMR, ^{13}C{^{1}H} NMR, and HMBC data, Table S3 in the Supporting Information; HRESIMS m/z 902.4178 [M-H]$^-$ (calcd for $C_{18}H_6{}^{79}Br_7O_6S$ 902.4174).

Bromoiesol B sulfate (2). A pale white solid; IR (neat) 1460, 1420, 1280, 1260, 1055, 920 cm^{-1}; UV (MeCN) λ_{max} (log ε) 285 nm (3.68); ^{1}H NMR, ^{13}C{^{1}H} NMR, and HMBC data, Table S4 in the Supporting Information; HRESIMS m/z 950.4043 [M-H]$^-$ (calcd for $C_{18}H_6{}^{79}Br_6IO_6S$ 950.4036).

X-ray Crystallographic Analysis of Bromoiesols A (3) and B (4). Bromoiesol A (**3**, 1.0 mg) MeOH solution (0.1 mL) was allowed to stand at −20 °C for 2 days. Single crystal of **3** was precipitated and was collected by a spatula. Single crystal of bromoiesol B (**4**) was also prepared in the same procedure. The crystals were kept at 300 K during data collection. The structures were solved with the ShelXT structure solution program using intrinsic phasing and refined with the ShelXL refinement package using least squares minimization. The data for **3** and **4** were summarized in Table S5 in the Supporting Information. Crystallographic data for **3** and **4** have been deposited at the Cambridge Crystallographic Data Center (CCDC 2082191 and CCDC 2082192).

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Total Synthesis Procedures. *2,4-Dibromo-1-iodobenzene (**8**).* This compound is a known compound, and we synthesized it in the same manner as in the previous report.[22]

*(2,4-Dibromophenyl)(2,4,6-trimethoxyphenyl)iodonium tosylate (**9**).* To a stirred solution of aryl iodide **8** (274 mg, 0.757 mmol) in dry acetonitrile (2.3 mL) were added *p*-toluenesulfonic acid monohydrate (158 mg, 0.83 mmol) and *m*CPBA (70% active oxidant, 205 mg, 0.83 mmol). The reaction mixture was stirred at 55 °C in an oil bath for 50 min before being treated with 1,3,5-trimethoxybenzene (140 mg, 0.83 mmol). The reaction mixture was stirred at 55 °C in an oil bath for another 15 min and concentrated under reduced pressure. The obtained crude residue was washed with Et_2O (4 × 1 mL) and dried in vacuo to give diaryliodonium salt **9** (397 mg, 0.57 mmol, 75%) as an orange solid; IR (neat)1696, 1694, 1592, 1457, 1346, 1203, 1125, 1051, 1009, 695, 681, 566 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ8.10 (d, J = 2.4 Hz, 1H), 8.00 (d, J = 8.9 Hz, 1H), 7.70 (d, J = 7.9 Hz, 2H), 7.59 (dd, J = 2.4, 8.9 Hz, 1H), 7.23 (d, J = 7.9 Hz, 2H), 6.42 (s, 2H), 3.97 (s, 6H), 3.90 (s, 3H), 2.37 (s, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD) δ 168.0, 160.5 (2C), 140.7, 140.0, 136.1, 133.4, 128.8 (2C), 128.0, 127.7, 126.0 (2C), 119.2, 91.9 (2C), 85.6, 56.6 (2C), 55.7, 20.3; HRESIMS m/z 526.8359 $[M]^+$ (calcd for $C_{15}H_{14}{}^{79}Br_2IO_3$ 526.8349).

*1,5-Dibromo-2-iodo-3-methoxybenzene (**10**).* To aryl amine **7** (1.00 g, 3.56 mmol) were added H_2O (7.1 mL) and 37% aq. HCl (7.1 mL), and the mixture was heated to 80 °C in an oil bath while stirring. The reaction mixture was stirred for 30 min followed by cooling to 0 °C on an iced water bath. To the reaction mixture was added $NaNO_2$ (270 mg, 3.92 mmol, 1.1 eq) maintaining the internal temperature below 10 °C. The mixture was stirred at 0 °C for 30 min followed by addition of KI (650 mg, 3.92 mmol, 1.1 equiv). Next, the reaction mixture was warmed to room temperature and was stirred overnight. The resulting mixture was extracted with CH_2Cl_2, and the organic layer was washed with 10% sodium hydroxide, 1 M sodium thiosulfate, 10% hydrochloric acid, water, and brine. After the removal of organic solvents, the obtained crude compound was purified by AFCS (gradient condition, Hexane/EtOAc, 100/0 → 81/19) to afford 493 mg (1.26 mmol, 35%) of aryl iodide **10** asa pale yellow solid; IR (neat) 1558, 1460, 1384, 1262, 1220, 1038, 1007, 833, 773 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 2.1 Hz, 1H), 6.84 (d, J = 2.1 Hz, 1H), 3.88 (s, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD) δ 160.4, 131.1, 127.5, 123.3, 112.7, 93.1, 57.2; HRESIMS m/z 374.7503 $[M\text{-}CH_3]^-$ (calcd for $C_6H_2{}^{79}Br_2IO$ 374.7517).

*(2,4-Dibromo-6-methoxyphenyl)(2,4,6-trimethoxyphenyl)-iodonium tosylate (**11**).* To a stirred solution of aryl iodide **10** (246 mg, 0.63 mmol) in acetonitrile (2.0 mL) were added *p*-toluene-sulfonic acid monohydrate (132 mg, 0.69 mmol) and *m*CPBA (70% active oxidant, 171 mg, 0.69 mmol). The reaction mixture was stirred at 55 °C in an oil bath for 50 min, and then 1,3,5-trimethoxybenzene (117 mg, 0.69 mmol) was added. The reaction mixture was stirred at 55 °C in an oil bath for another 15 min and was concentrated under reduced pressure. The obtained residue was washed with Et_2O (4 × 1 mL) and dried in vacuo to give a crude compound. The crude compound was purified by column chromatography (SiO_2, CH_2Cl_2/MeOH, 20/1→ 10/1) to afford 185 mg (0.25 mmol, 40%) of iodonium salt **11** as a pale purple solid; IR (neat) 1583, 1457, 1413, 1388, 1346, 1262, 1208, 1162, 1126, 1032, 1009, 868, 814, 755, 680, 566 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.68 (d, J = 8.3 Hz, 2H), 7.62 (d, J = 1.8 Hz, 1H), 7.33 (d, J = 1.8 Hz, 1H), 7.21 (d, J = 8.3 Hz, 2H), 6.36 (s, 2H), 3.92 (s, 6H), 3.90 (s, 3H), 3.87 (s, 3H), 2.36 (s, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD) δ 167.4, 160.7, 160.2, 142.4, 140.6, 129.7, 128.9, 128.8 (2C), 127.9, 125.9 (2C), 114.7, 110.4, 91.6 (2C), 84.6, 57.4, 56.5 (2C), 55.6, 20.4; HRESIMS m/z 556.8458 $[M]^+$ (calcd for $C_{16}H_{16}{}^{79}Br_2IO_4$ 556.8455).

*1,2,3-Tribromo-4-(2,4-dibromophenoxy)-5-methoxybenzene (**12**).* To a stirred solution of *t*-BuOK (70 mg, 0.63 mmol) in dry THF (3 mL) at 0 °C was added phenol **6** (206 mg, 0.57 mmol). The reaction solution was stirred at 0 °C for 15 min. After addition of the iodonium salt **9** (397 mg, 0.57 mmol), the reaction mixture was further stirred at 40 °C in an oil bath for 47 h and was diluted with H_2O (5 mL) at 0 °C. THF was removed in vacuo, and the residual aqueous mixture was extracted with Et_2O (2 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The obtained residue was purified by AFCS (gradient condition; Hexane/EtOAc, 100/0 → 82/18) to give aryl ether **12** (119 mg, 0.20 mmol, 35%) as a white solid; IR (neat) 1571, 1464, 1427, 1360, 1290, 1250, 1223, 1177, 1041, 864, 772, 726 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 2.4 Hz, 1H), 7.29 (s, 1H), 7.23 (dd, J = 2.4, 8.7 Hz, 1H), 7.23 (dd, J = 2.4, 8.7 Hz, 1H), 3.77 (s, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 152.7, 152.3, 141.1, 136.0, 131.2, 122.6, 122.4, 119.2, 116.7, 115.3, 115.1, 112.4, 56.8; HRAPCIMS m/z 589.6363 $[M]^+$ (calcd for $C_{13}H_7{}^{79}Br_5O_2$ 589.6369).

*3,4,5-Tribromo-2-(2,4-dibromophenoxy)phenol (**13**).* This compound is a known compound, and we synthesized it in the same manner as in the previous report.[19]

*1,2,3-Tribromo-5-(2,4-dibromo-6-methoxyphenoxy)-4-(2,4-dibromophenoxy)benzene (**14**).* To a stirred solution of *t*-BuOK (24 mg, 0.20 mmol) in dry THF (1 mL) was added phenol **13** (104 mg, 0.18 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 15 min. After addition of iodonium salt **11** (131 mg, 0.18 mmol), the reaction mixture was stirred at 40 °C in an oil bath for 44 h and was diluted with H_2O (5 mL) at 0 °C. THF was removed in vacuo, and then the residue was extracted with Et_2O (2 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The obtained residue was purified by AFCS (gradient condition, Hexane/EtOAc, 100/0 → 83/17) to give aryl ether **14** (110 mg, 0.13 mmol, 73%) as a white solid; IR (neat) 1579, 1468, 1433, 1394, 1350, 1259, 1218, 1181, 1035, 871, 834, 757 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.73 (d, J = 2.5 Hz, 1H), 7.36 (d, J = 2.2 Hz, 1H), 7.27 (dd, J = 2.5, 8.8 Hz, 1H), 7.04 (d, J = 2.2 Hz, 1H), 6.81 (s, 1H), 6.58 (d, J = 8.8 Hz, 1H), 3.73 (s, 3H); $^{13}C\{^1H\}$ NMR(100 MHz, CD_3OD) δ 153.1, 152.6, 149.6, 140.8, 138.7, 135.9, 130.8, 127.6, 123.1, 122.1, 121.2, 119.9, 118.5, 118.0, 116.4, 115.8, 115.3, 112.8, 56.6; HRMS data were not obtained probably due to the lack of the polarity. Therefore, we fully characterized a compound in the next step.

*Bromoiesol A (**3**).* To a stirred solution of aryl ether **14** (60 mg, 0.071 mmol) in CH_2Cl_2 (1 mL) was added dropwise a 1 M solution of boron tribromide (1.07 mL, 1.07 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h, followed by at room temperature for 28 h. Next, the reaction mixture was quenched with water at 0 °C. The organic layer was separated, and then the aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The obtained residue was purified by AFCS (gradient condition, Hexane/EtOAc, 73/27 → 52/48) to give bromoiesol A (**3**, 45 mg, 0.55 mmol, 77%) as a white solid; IR (neat) 3094, 1576, 1460, 1427, 1348, 1283, 1213, 1036, 924, 892, 804 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.78 (d, J = 2.3 Hz, 1H), 7.36 (dd, J = 2.3, 9.1 Hz, 1H), 7.27 (d, J = 2.3 Hz, 1H), 7.07 (d, J = 2.3 Hz, 1H), 6.96 (s, 1H), 6.66 (d, J = 9.1 Hz, 1H); $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD) δ 153.9, 153.0, 151.0, 142.1, 139.3, 136.7, 132.4, 126.9, 123.8, 123.0, 121.6, 120.8, 120.5, 119.8, 118.9, 117.9, 116.1, 113.4; HRESIMS m/z 822.4626 $[M\text{-}H]^-$ (calcd for $C_{18}H_6{}^{79}Br_7O_3$ 822.4606).

*Bromoiesol sulfate (**1**).* To a stirred solution of sulfur trioxide pyridine complex (57 mg, 0.36 mmol) in pyridine (500 μL) was added bromoiesol A (**3**, 10 mg, 0.012 mmol) at room temperature. The reaction mixture was stirred at 45 °C in an oil bath for 1 h, and then 5 mL of 1 M KOH aq. was added at 0 °C. The resulting mixture was applied on a ODS plug (5.0 g) and washed with H_2O and 40% aqueous MeOH to remove potassium sulfate and unreacted reagents. The elution with 80% aqueous MeOH afforded bromoiesol A sulfate (**1**, 13 mg, 0.014 mmol, quant.) as a pale white solid; IR (neat) 1464, 1427, 1284, 1260, 1055, 927 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ7.88 (d, J = 2.3 Hz, 1H), 7.74 (d, J = 2.3 Hz, 1H), 7.62 (d, J = 2.3 Hz, 1H), 7.43 (dd, J = 2.3, 9.1 Hz, 1H), 7.00 (s, 1H), 6.83 (d, J = 9.1 Hz, 1H); $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD) δ 152.7, 149.9, 147.2, 140.8, 135.4, 132.1, 130.6, 124.2, 122.5, 122.1, 121.0, 119.4, 119.4, 119.1, 119.0, 117.3, 115.0, 111.9; HRESIMS m/z 902.4178 $[M\text{-}H]^-$ (calcd for $C_{18}H_6{}^{79}Br_7O_6S$ 902.4174).

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Cell Growth Analysis. HeLa cells were cultured at 37 °C with 5% CO_2 in DMEM (Nissui, Japan) supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin, 300 μg/mL L-glutamine, and 2.25 mg/mL $NaHCO_3$. HeLa cells were seeded at 5×10^3 cells/well in 96-well plates (Iwaki, Japan) and cultured overnight. Various concentrations of compounds were then added, and cells were incubated for 72 h. Cell proliferation was measured by the MTT assay. The assay was performed in triplicate.

In Vitro Antitrypansomal Assay. The *Trypanosoma brucei rhodesiense* strain IL-1501[32] was cultured at 37 °C under a humidified 5% CO_2 atmosphere in HMI-9 medium[33] supplemented with 10% heat-inactivated fetal bovine serum (FBS). For in vitro studies, compounds were dissolved in DMSO and diluted in culture medium prior to being assayed. The maximum DMSO concentration in the in vitro assays was 1%. The compounds were tested in an AlamarBlue serial drug dilution assay[34] to determine the 50% inhibitory concentrations (IC_{50}). Serial drug dilutions were prepared in 96-well microtiter plates containing the culture medium, and wells were inoculated with 2.0×10^4 cells/mL *T. b. rhodesiense* IL-1501 parasites. Cultures were incubated for 69 h at 37 °C under a humidified 5% CO_2 atmosphere. After this time, 10 μL of resazurin (12.5 mg resazurin [Sigma] dissolved in 100 mL of phosphate-buffered saline) was added to each well. The plates were incubated for an additional 3 h. The plates were read in a SpectraMax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The assay was performed in duplicate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c01214.

^1H, $^{13}C\{^1H\}$, HMQC, and HMBC NMR spectra in CD_3OD for the bromoiesol family (**1**–**4**); ^1H and $^{13}C\{^1H\}$ NMR spectra for synthetic products; phylogenetic tree of cyanobacterial sample; results of SMART analysis (PDF)

Accession Codes

CCDC 2082191 and 2082192 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: + 44 1223 336033.

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Notes

The authors declare no competing financial interest.

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