Isolation and Synthesis of Pulmonarins A and B, Acetylcholinesterase Inhibitors from the Colonial Ascidian Synoicum pulmonaria

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Supporting Information

ABSTRACT: Pulmonarins A and B are two new dibrominated marine acetylcholinesterase inhibitors that were isolated and characterized from the sub-Arctic ascidian Synoicum pulmonaria collected off the Norwegian coast. The structures of natural pulmonarins A and B were tentatively elucidated by spectroscopic methods and later verified by comparison with synthetically prepared material. Both pulmonarins A and B displayed reversible, noncompetitive acetylcholinesterase inhibition comparable to several known natural acetylcholinesterase inhibitors. Pulmonarin B was the strongest inhibitor, with an inhibition constant ($K_i$) of 20 μM. In addition to reversible, noncompetitive acetylcholinesterase inhibition, the compounds displayed weak antibacterial activity but no cytotoxicity or other investigated bioactivities.

Marine invertebrates and their associated bacterial symbionts represent organisms with great potential for the discovery of novel bioactive compounds and chemical scaffolds.1–3 The sessile benthic lifestyle promotes the production of defensive secondary metabolites to provide an evolutionary advantage. To investigate their potential as sources for bioactive compounds, several hundred different Arctic marine invertebrates, algae, and microorganisms have been collected during a systematic study of Arctic marine organisms along the Norwegian coast.4 The Arctic waters provide a significantly less studied biotope in comparison to warmer waters; however they are still rich in biodiversity.4–6 One such Arctic organism, the colonial ascidian Synoicum pulmonaria, has been shown to contain a family of dibrominated bioactive compounds known as the synoxazolidinones.7–9 In the current paper, further studies of the extract from $S$. pulmonaria revealed additional novel compounds named pulmonarins A (1) and B (2).

The pulmonarins are structurally related to the synoxazolidinones (3–5), which are heavily modified antimicrobial dipeptide derivatives displaying a rare 4-oxazolidinone core linking a dibrominated tyrosine derivative to an arginine/agmatine side chain. The pulmonarins do not display the 4-oxazolidinone core or the guanidine group of the synoxazolidinones, but instead they contain quaternary ammonium groups implying potential acetylcholinesterase (AChE)-inhibiting properties. AChE inhibitors are a class of drugs used for the treatment of Alzheimer’s disease, glaucoma, myasthenia gravis (an autoimmune disorder), and the recovery of neuromuscular block after surgery.10,11 Some well-known AChE inhibitors in clinical use today include physostigmine (6), a naturally occurring carbamate from the Calabar bean, used for the treatment of glaucoma.10,12 Examples of other current AChE-inhibitor-based drugs are galanthamine (7) and donepezil, both used in most countries for the treatment of Alzheimer’s disease. Several toxins such as nerve agents, insecticides, and antiparasitic chemicals used in agriculture and aquaculture also act by inhibiting AChE and consist mostly of organophosphorus compounds and carbamates.13,14 At least three

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other marine natural products with similar structures to 1 and 2 have been reported in the literature. Compound 8, with significant structural similarities to 1, was isolated by Fattorusso et al. from the Caribbean sponge *Pseudoceratina crassa* (later reclassified as *Aiolochroia crassa*) in 1994.\(^{15}\) Compound 8 was recently studied by Munoz and co-workers and shown to display weak antiparasitic properties.\(^{16}\) Compound 9 was isolated from another Caribbean sponge *Verongula sp.*, but no biological activity was reported for the compound.\(^{17}\) A derivative of 9, compound 10, was isolated from the sponge *Verongia fistularia*.\(^{18}\)

The current paper describes the isolation, structural characterization, and syntheses of 1 and 2 and the evaluation of their biological activities including AChE inhibition employing electric eel AChE.

### RESULTS AND DISCUSSION

Stepwise fractionation of an aqueous extract of *S. pulmonaria* resulted in the isolation of two compounds. The aqueous extract was loaded on a solid-phase extraction (SPE) cartridge and eluted with 40/60 CH\(_3\)CN/H\(_2\)O. The eluate was loaded on a semipreparative RP-HPLC C\(_{18}\) column. MS analysis revealed compounds with isotope patterns indicating dibrominated compounds. Compounds 1 and 2 were isolated as yellow gels with the molecular formula C\(_{17}\)H\(_{27}\)Br\(_{2}\)O\(_{2}\)N\(_{2}\) for 1 and C\(_{17}\)H\(_{27}\)Br\(_{2}\)O\(_{2}\)N\(_{2}\) for 2. Database searching suggested new compounds, and their tentative structures were solved on the basis of a number of 1D and 2D NMR experiments and mass spectrometric analyses. Spectroscopic similarities with the synoxazolidinones were rapidly established.\(^{7}\) Table 1 lists proton and carbon NMR shift values and both gCOSY and gHMBC correlations of natural 1 and 2.

An initial inspection of the 2D NMR data revealed similar motifs in the aromatic region to those found in the synoxazolidinones.\(^{1}\) The carbon shifts (Table 1) used for the structure elucidation were obtained from the gHSQC experiment, while the quaternary carbon signals are derived from gHMBC data by comparison with the structurally related synoxazolidinones isolated from the same organism. The tentative structure of 1 was solved based on the following correlations: The gHMBC correlation from the H-1 methoxy protons to C-2 indicated the position of the methoxy group on the substituted aromatic ring; the shift value of C-3/3' (\(\delta_\text{C}^1 118.5\)) was indicative of two bromine atoms at these positions; and protons H-4/4' showed a gHMBC correlation to the ester carbonyl C-6. Furthermore, a gCOSY correlation was observed between the two adjacent methylene protons H-7 and H-8, and the high chemical shift of H-7 (\(\delta_H 4.66\)) placed it adjacent to the ester oxygen. The gHMBC correlation from protons H-9/9' to C-8 supported the terminal trimethylated quaternary ammonium. The shift values of C-9/9' (\(\delta_\text{C} 53.3\)) and H-4/4' (\(\delta_H 133.9\)) were consistent with literature values.\(^{19−21}\) The assignment of the terminal trimethylated ammonium was also supported by a loss of 59 mass units from the parent ion as indicated by a prominent peak at \(m/z 334\) in the mass spectrum of 1.

Table 1. 1D and 2D NMR Data (600/175 MHz DMSO-\(d_6\)) of Natural 1 and 2

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<th>no.</th>
<th>(\delta_\text{C}^a) type(^b)</th>
<th>(\delta_\text{H}^b) (mult., (J) in Hz)</th>
<th>gCOSY</th>
<th>gHMBC</th>
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\(^a\)Carbon shifts obtained from gHSQC and gHMBC experiments.

\(^b\)Not observed.
acetamide derivative of orthidine F.22 C-9 was placed next to the amide nitrogen based on gCOSY correlations between amide protons H-8 and H-9. This spin system further involved H-7 to H-10, generating a saturated five-carbon segment between the amide and terminal trimethyl ammonium, which was further verified by the gHMBC correlations from protons H-14/14′ to C-13 in analogy with 1. This spin system matched well with the chemical shifts of the analogous spin system reported for clavatadine D, isolated from the Australian sponge Suberea clavata.23 Figure 1 shows key gHMBC and gCOSY correlations of natural 1 and 2.

Because the purification led to sample loss and the incomplete NMR data of the compounds, total synthesis was required to generate sufficient material for structural verification and biological testing. Compound 1 was prepared in one step. Dicyclohexylcarbodiimide (DCC) was used as coupling agent to react 3,5-dibromo-4-methoxybenzoic acid and choline chloride in the presence of a catalytic amount of 4-(Dimethylamino)pyridine (DMAP) in CH₂Cl₂. The reaction was slow, and sufficient amounts of 1 could only be obtained after an extended reaction time followed by HPLC purification. Several different solvent systems were evaluated, but only CH₂Cl₂ generated 1 due to solubility issues. Compound 2 was synthesized in three steps from 4-methoxyphenylacetic acid, which was initially dibrominated with Br₂/FeBr₃ to produce 3,5-(dibromo-4-methoxyphenyl)acetic acid (2a) according to Weller et al.24 The bromination reaction was followed by MS, and a complete conversion to the desired dibrominated product required additional Br₂/FeBr₃ after 24 h. Compound 2a was then coupled with 5-(dimethylamino)amylamine using 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as coupling agent to generate 2-(3,5-dibromo-4-methoxyphenyl)-N-(5-(dimethylamino)pentyl)-acetamide (2b). N-Methylation of 2b with CH₃I completed the synthesis of 2. The synthetic routes to 1 and 2 are displayed in Scheme 1.

Figure 1. Key gHMBC (H→C) and gCOSY (—) correlations of 2.

Both ¹H and ¹³C NMR shifts of the natural and synthetic compounds are analogous, thus validating their proposed structures (Table S3). This is further supported by comparing the 2D NMR data.

The para-methoxy dibrominated phenyl group moiety is a common structural motif among marine secondary metabolites and particularly those that are tyrosine derivatives.25 The isolated compounds bear structural resemblances with not only the synoxazolidinones,7,8 and 8, 9, and 1015,17,18 but also ianthelline26 and subereamine B27 among others.

As both pulmonarins are terminally trimethylated ammonium compounds, they were evaluated as AChE inhibitors in addition to the standard screens routinely performed during our screening process.4 Electric eel AChE, sharing a high degree of sequence homology and having a 3D structure very similar to human AChE,28 was employed as a model vertebrate AChE system, and the well-established method of Ellman was employed to study the inhibition kinetics.29,30 Synthetic 1 induced 50% inhibition of electric eel AChE at 42 μg/mL (105 μM) in the presence of acetylthiocholine chloride as a substrate (Figure 2). The inhibitory constant (Kᵢ) was determined from the Dixon plot31 and revealed 1 to be a reversible, noncompetitive inhibitor with a Kᵢ of 36 μg/mL (90 μM) (Figure 3). Compound 2 showed a stronger reversible, noncompetitive inhibition, and 50% inhibition was obtained at 16 μg/mL (36 μM) with a Kᵢ of 9 μg/mL (20 μM) (Figures 2 and 3).

A Kᵢ of 9 μg/mL (20 μM) for 2 indicates moderate binding affinities for vertebrate AChE, similar to that of the FDA-
approved drug galanthamine.\textsuperscript{32} The affinity of 1 for AChE is 4 times weaker. AChE inhibitors with significantly varying binding affinities are used in approved medications. Physostigmine is a strong AChE inhibitor with an IC\textsubscript{50} value of approximately 30 nM,\textsuperscript{33} while galanthamine, used to combat Alzheimer’s disease, displays a moderate \( K_i \) of 2–20 \( \mu \)M against AChE from different vertebrate species.\textsuperscript{34} The \( K_i \) values obtained here are therefore within the pharmaceutically interesting regime. For competitive inhibitors of AChE, studies based on molecular modeling and quantitative structure–activity relationship (QSAR) analysis have shown that a degree of hydrophobicity and the presence of an ionizable nitrogen are prerequisites for the inhibitor–enzyme interactions.\textsuperscript{10} Considering this, it was expected that the more hydrophobic 1, as determined by HPLC retention times, would be more active. As the observed AChE inhibition contradicts this, it is clear that further investigations are required for a mechanistic explanation.

As an allosteric inhibitor of AChE, the presence of a quaternary nitrogen in both compounds suggests binding through electrostatic interactions at the peripheral anionic site (PAS) of the enzyme, which is located on the surface of the protein at the entrance of the active site cleft. This site is implicated in a number of nonclassical functions such as amyloid deposition, cell adhesion, and neurite outgrowth.\textsuperscript{34} The PAS is thus a potential target in rational drug design for the development of novel and improved inhibitors and therapeutics for the treatment of neural cancers, nerve regeneration, and neurodegenerative disorders such as Alzheimer’s disease.\textsuperscript{11,34} QSAR studies of 1 and 2 could potentially contribute to future development of new drug candidates in various therapeutic fields.

We have previously reported that the 40/60 (v/v) \( \text{CH}_2\text{CN}/\text{H}_2\text{O} \) SPE fraction of \( S. \) \textit{pulmonaria} exhibited antibacterial activity against a number of bacteria, showing an activity at 160 \( \mu \)g/mL against \textit{Corynebacterium glutamicum} and at 80 \( \mu \)g/mL against \textit{Staphylococcus aureus}.\textsuperscript{55} These compounds were therefore also included in the extensive screening performed at MabCent to investigate other potential bioactivities.\textsuperscript{4} A lack of activity at concentrations above 100 \( \mu \)g/mL classified the compounds as “inactive” in all assays apart from the antibacterial screen. Compound 1 displayed only very mild activity against one bacterial strain, \textit{C. glutamicum}, at a concentration of 500 \( \mu \)g/mL, indicating a possible synergistic effect in the active fraction. Compound 2 was slightly more active against \( C. \) \textit{glutamicum}, displaying a minimal inhibitory concentration of 250 \( \mu \)g/mL. No activities were seen in the other bacterial screens in our panel (test strains used were \( S. \) \textit{aureus} (ATCC 9144), \( E. \) \textit{coli} (ATCC 25922), \( P. \) \textit{aeruginosa} (ATCC 27853), and \( C. \) \textit{glutamicum} (ATCC 13032)) (data not included). As well as testing negative against other bacteria, both 1 and 2 also tested negative for cytotoxic activities against three adherent cancer cell lines (A2058 melanoma, HT29 colon carcinoma, and MCF7 breast carcinoma cells) and against adherent, nonmalignant lung fibroblasts (MRC-5 cells) (data not included). They were also inactive in an anti-inflammatory assay targeted against TNF-\( \alpha \) and in a biofilm formation inhibition assay using \( S. \) \textit{epidermidis} (ATCC 35984) (data not included). Finally 1 and 2 were shown to be inactive as cellular antioxidants (data not included). Compounds 1 and 2 thus appear to possess a highly specific action as reversible noncompetitive AChE inhibitors displaying no apparent cellular toxicity. As reported for the synoxazolidinones (3–5), it was not possible to determine whether symbiotic microorganisms are responsible for the biosynthesis of 1 and 2, which is a common case for marine natural products.\textsuperscript{1} On the basis of the key structural differences between the synoxazolidinones and the compounds in the present study, it seems unlikely that they share a common biosynthetic route in the organism apart from being tyrosine derivatives. Compound 1 can be considered as a dibrominated version of \( p \)-hydroxybenzoylcholine, which has been isolated from the seeds of \textit{Sinapis albae}, and it was proposed to originate biosynthetically from a \( p \)-hydroxy benzyl alcohol derivative.\textsuperscript{36,37} An analogous biosynthetic route toward 1 is therefore plausible. The proposed biosynthesis of 2 is similar (see Supporting Information) and is suggested to be derived from amide formation between methylated 2-(4-hydroxy-3,5-dibromophenyl)acetic acid and ascosphylline, both previously described in extracts of marine organisms.\textsuperscript{38,39}

\section*{CONCLUSIONS}

Two new marine bioactive compounds have been isolated and characterized from \( S. \) \textit{pulmonaria}. Curtailed by the lack of the natural compounds, total synthesis provided enough material for structure verification and biological testing. Both compounds turned out to be nontoxic, reversible, noncompetitive inhibitors of AChE. The isolation of 1 and 2 yet again demonstrates the chemical diversity of the cold-water marine

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**Figure 3.** Determination of the type of inhibition and the inhibition constant \( K_i \) for 1 (left graph) and 2 (right graph) by Dixon plot analysis. The concentrations of the substrate acetylthiocholine were 0.25 (●), 0.5 (□), and 1 mM (■). \( K_i \) was determined to 36 \( \mu \)g/mL (90 \( \mu \)M) for 1 and 9 \( \mu \)g/mL (20 \( \mu \)M) for 2.
organism S. pulmonaria and the potential of the species for the discovery of new structural motifs for further lead compound development in a number of therapeutic areas.

**EXPERIMENTAL SECTION**

**General Experimental Procedures.** UV spectra were determined on a Lambda 25 spectrometer from Perkin-Elmer Instruments. Infrared spectra were recorded on an Avatar 320 FT-IR spectrometer from Nicolet. 1D and 2D NMR spectra of natural products were recorded on a Varian VNMRS 600 MHz spectrometer in DMSO-d<sub>6</sub>. Chemical shifts were referenced to the residual solvent peaks, δ<sub>H</sub> 2.50 and δ<sub>C</sub> 39.5. Mass spectra were acquired on an LTQ Orbitrap XL Hybrid Fourier Transform mass spectrometer from Thermo Scientific and the Thermo Scientific Accela HPLC-LTQ Ion Trap-Orbitrap Discovery system. HPLC of synthetic compounds was performed on a 1200 series instrument with a binary pump and a photodiode array detector from Agilent Technologies. All starting materials were purchased from Sigma-Aldrich except for methyl iodide, which was distilled and stored over Cu<sub>2</sub>O prior to use. Electric eel AChE (6.25 U/ml) was provided by Sigma-Aldrich.

**Extraction and Purification of Pulmonarins A and B.** Specimens of *Synocium pulmonaria* (Ellis and Solander, 1786) were collected off the coast of Tromsø in northern Norway and identified by Professor Bjørn Gulilksen (Department of Arctic and Marine Biology, University of Tromsø, Tromsø, Norway). The organisms are stored at the Norwegian College of Fishery Science, University of Tromsø, Norway. Specimens (80 g, wet weight) of the organism were collected apart from 5-(dimethylamino)aniline, which was provided by Matrix Scientific. Chemicals were used without further purification except for methyl iodide, which was distilled and stored over Cu<sub>2</sub>O prior to use. Electric eel AChE (6.25 U/ml) was provided by Sigma-Aldrich.

**Syntheses.** 2-(3,5-Dibromo-4-methoxybenzoyl)oxy)-N,N,N-trimethylthylammonium Chloride (1). DCC (1.12 g, 3 equiv, 5.40 mmol), 1.68 g of 3,5-dibromo-4-methoxybenzoic acid (1.5 equiv, 5.40 mmol), and 0.16 g of DMAP (0.2 equiv, 1.28 mmol) were added to 10 mL of CH<sub>2</sub>Cl<sub>2</sub>. The mixture was stirred at room temperature (rt) for 6 days. A white solid was obtained, which was washed with H<sub>2</sub>O (3 × 20 mL), filtered, and dried. An aliquot of the dried filtrate (713 mg) was dissolved in 5% CH<sub>3</sub>CN to a concentration of 25 mg/mL and purified by HPLC using a gradient of 0% to 40% CH<sub>3</sub>CN/0.05% TFA in H<sub>2</sub>O. The 40% CH<sub>3</sub>CN SPE eluate was subsequently loaded on a C<sub>18</sub> solid phase. The aqueous phase was loaded on a C<sub>18</sub> solid phase. The aqueous phase was loaded on a C<sub>18</sub> solid phase. The aqueous phase was loaded on a C<sub>18</sub> solid phase. The aqueous phase was loaded on a C<sub>18</sub> solid phase.

**Acetylcholinesterase Inhibition Assay.** AChE activity was measured by Ellman’s method<sup>29,30</sup> using acetylthiocholine chloride (0.25, 0.5, or 1 mM) as a substrate in 100 mM potassium phosphate buffer pH 7.4 at 25 °C and electric eel AChE was used as a source of enzyme (final concentration in the test 0.0075 U/mL). Hydrolysis of acetylthiocholine chloride was followed on a kinetic microplate reader (Dynex Technologies) at 405 nm. Stock solutions (2 mg/mL) of 1 and 2 were prepared in deionized H<sub>2</sub>O and DMSO, respectively. AChE inhibition by 1 and 2, progressively diluted in H<sub>2</sub>O, was monitored for 5 min. All readings were corrected for their appropriate blanks, and a run with only acetylthiocholine chloride served as assay positive control. In the case of the blank, the lack reactions without the inhibitor were run in the presence of the appropriate dilution of DMSO. Every measurement was repeated at least three times.

**Antibacterial Assay.** Test strains used were *Staphylococcus aureus* (ATCC 9144), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Corynebacterium glutamicum* (ATCC 13032). All isolates were grown at 37 °C in Mueller Hinton broth (Difco Laboratories). Bacterial growth was continuously monitored with an Envision plate reader (Perkin-Elmer). The test was performed in 96-well Nunc microtiter plates, in which 50 μL of test fractions dissolved in H<sub>2</sub>O was incubated with 50 μL of a suspension of an actively growing (log phase) culture of bacteria diluted to a starting concentration of approximately 5 × 10<sup>8</sup> cells per well. The antimicrobial peptide cecropin B (25 μM) was used as a positive control. The minimum inhibitory concentration was defined as the minimum concentration resulting in no change in optical density after incubation for 24 h at 37 °C. Compounds were tested at concentrations ranging from 8 to 500 μg/mL.

3,5-(Dibromo-4-methoxyphenyl)acetic Acid (2a). Compound 2a was prepared in 21% yield according to the method described by Weller et al.<sup>24</sup> Spectral data matched those previously reported. 2-(3,5-Dibromo-4-methoxyphenyl)-N-(5(dimethylaminopentyl)-acetamide (2b). A 0.25 mg amount of 5-(dimethylamino)aniline (1.51 mmol, 1.05 equiv) was added to a stirred solution of 2a (494.0 mg, 1.53 mmol) and HBTU (580 mg, 1.53 mmol, 1.0 equiv) in 5 mL of DMF and 1.06 mL of DIEPA (6.12 mmol, 4 equiv). The reaction mixture was stirred overnight at rt before being diluted with 20 mL of EtOAc and washed with 2 × 25 mL of 10% citric acid, 25 mL of 10% NaHCO<sub>3</sub>, and 25 mL of saturated brine. The EtOAc extract was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated in vacuo to yield a yellow oil (57% yield). No further purification was performed. H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.36 (s, 2H), 3.79 (s, 3H), 3.34 (s, 2H), 1.34 (q, J = 6.0 Hz, 2H), 2.28 (m, 2H), 2.24 (s, 6H), 1.47 (m, 2H), 1.40 (m, 2H), 1.24 (m, 2H) ppm; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 170.1 (C), 153.0 (C), 134.2 (C), 133.5 (C), 118.2 (C), 60.7 (CH<sub>3</sub>), 59.1 (CH<sub>2</sub>), 45.1 (CH<sub>3</sub>), 41.8 (CH<sub>2</sub>), 39.7 (CH<sub>3</sub>), 29.1 (CH<sub>2</sub>), 24.3 (CH<sub>3</sub>) ppm; HRESIMS m/z 435.0279 [M + H]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>27</sub>Br<sub>2</sub>O<sub>2</sub>N<sub>2</sub>, 435.0283).

5-(2-(3,5-Dibromo-4-methoxyphenyl)acetamidino)-N,N,N-trimethylylammonium Chloride (2). A 380 mg amount of 2b (0.87 mmol) was dissolved in 5 mL of CH<sub>2</sub>Cl<sub>2</sub>, and 5 mL of a 1 M K<sub>2</sub>CO<sub>3</sub> solution was added. The resulting mixture was stirred at room temperature for 15 min before 0.41 mL of methanol (10 equiv) was added and the reaction was stirred overnight. The pH of the reaction was adjusted to 7 with 2 M HCl. All volatiles were evaporated, and the crude solid material (537 mg) was dissolved in CH<sub>2</sub>Cl<sub>2</sub>. Silica was added, and the crude material was adsorbed onto the silica under vacuum. Silica column purification was performed using a mixture of HOAc/H<sub>2</sub>O/MeOH/EtOAc (3:2:3:3) and EtOAc 1:9 as eluent. A final purification of the isolated 237 mg of 2 with semipreparative reversed-phase HPLC was necessary. The isolated 2 was dissolved in 5% CH<sub>3</sub>CN to a concentration of 25 mg/mL, and HPLC purification was performed employing a Waters Sunfire Prep C<sub>18</sub> column (10 μm, 90 Å, 10 × 250 mm) using a gradient of 16–40% CH<sub>3</sub>CN in H<sub>2</sub>O over 40 min until 50 mg (13% yield) of 2 was collected. IR <sup>max</sup> 3036, 1688, 1473, 1200, 1125 cm<sup>−1</sup>; UV (MeOH) <sup>λ</sup><sub>max</sub> 220 nm; <sup>1</sup>H NMR and <sup>13</sup>C NMR, see Table S3 in the Supporting Information; HRESIMS m/z 449.0439 [M<sup>+</sup>] (calcd for C<sub>21</sub>H<sub>27</sub>Br<sub>2</sub>O<sub>2</sub>N<sub>2</sub>, 449.0440).

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ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR and HREIMS data of both natural and synthetic 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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