**Ianthelliformisamines A-C: Antibacterial Bromotyrosine-derived Metabolites
from the Marine Sponge *Suberea ianthelliformis***

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A high-throughput screening campaign using a prefractionated natural product library and an in vitro *Pseudomonas aeruginosa* (PAO200 strain) assay identified two antibacterial fractions derived from the marine sponge *Suberea ianthelliformis*. Mass-directed isolation of the CH₂Cl₂/CH₃OH extract from *S. ianthelliformis* resulted in the purification of three new bromotyrosine-derived metabolites, ianthelliformisamines A-C (**1-3**), together with the known natural products aplysamine 1 (**4**) and araplysillin I (**5**). The structures of **1-3** were determined following analysis of 1D and 2D NMR and MS spectroscopic data. This is the first report of chemistry from the marine sponge *S. ianthelliformis*. Ianthelliformisamine A (**1**) showed inhibitory activity against the Gram-negative bacterium *P. aeruginosa* with an IC₅₀ value of 6.8 μM (MIC = 35 μM).

Bacterial infections are associated with significant mortality and morbidity amongst immunocompromised and hospitalized patients, particularly those with pre-existing chronic ailments such as heart disease, diabetes, AIDS and cancer.^{1,2} Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus maltophilia*, and *Acinetobacter* spp. pose a particular threat to these patients since they are all opportunistic pathogens that possess multidrug resistance.² Multidrug resistance is genetically acquired by these pathogens and is primarily due to the active transport of drugs out of the cell by efflux pump systems.^{3,4} In addition, intrinsic resistance further decreases the efficacy of clinically used treatments, such as ampicillin, cephalosporins and macrolide antibiotics, mainly due to impermeability.^{1,5} Unfortunately, the increasing prevalence of resistance in bacteria has meant that most known antibacterial drugs now lack efficacy and this highlights the need for the discovery and development of new anti-infective drugs.⁶

In order to discover new antibacterial compounds from nature a high-throughput screening (HTS) campaign was undertaken using a prefractionated natural product library⁷ and an in vitro assay using a *P. aeruginosa* efflux pump knockout strain (PAO200, MexAB-OprM deficient mutant).⁸ The PAO200 strain was used in an effort to increase initial hits by decreasing efflux clearance. HTS hits were re-tested against the wild type strain PAO1 and a Gram-positive methicillin-resistant bacterium, *Staph. aureus* (MRSA strain 01A1095)⁹ for estimation of Gram selectivity. Two fractions derived from the Australian marine sponge *Suberea ianthelliformis* showed activity against *P. aeruginosa* PAO1. (+)-LRESIMS of the active fractions identified ions at m/z 519/521/523 and 462/464/466, which were predicted to correspond to the bioactive natural

products. Mass-directed isolation of the CH₂Cl₂/CH₃OH extract of *S. ianthelliformis* resulted in the purification of three new bromotyrosine-derived metabolites, ianthelliformisamines A-C (**1-3**), together with two known compounds aplysamine 1 (**4**)¹⁰ and araplysillin I (**5**).¹¹ Herein we report the isolation and structure elucidation of ianthelliformisamines A-C (**1-3**), as well as the antibacterial activities for compounds **1-5** against *P. aeruginosa* PAO1 and *Staph. aureus* 01A1095.

The freeze-dried and ground marine sponge *S. ianthelliformis* was sequentially extracted with *n*-hexane, CH₂Cl₂, and CH₃OH. The CH₂Cl₂/CH₃OH extracts were combined and chromatographed using C₁₈ bonded silica HPLC (CH₃OH/H₂O/0.1% TFA) to yield sixty fractions. Fractions 34, 36-38, and 45 afforded ianthelliformisamines A (**1**, 7.9 mg, 0.100% dry wt), B (**2**, 8.4 mg, 0.110% dry wt), and C (**3**, 3.1 mg, 0.040% dry wt), respectively. Further purification of fractions 23-25 and 39 using C₁₈ bonded silica HPLC (CH₃OH/H₂O/0.1% TFA) resulted in the isolation of two known bromotyrosine derivatives, aplysamine 1 (**4**, 0.9 mg, 0.010% dry wt)¹⁰ and araplysillin I (**5**, 0.6 mg, 0.008 % dry wt).¹¹

Ianthelliformisamine A (**1**) exhibited an ion cluster at *m/z* 519/521/523 (1:2:1) in the (+)-LRESIMS spectrum, indicating the presence of two bromine atoms. The molecular formula of C₂₀H₃₂Br₂N₄O₂ was determined on the basis of the (+)-HRESIMS and NMR data for **1** (Table 1). ¹H and gHSQC NMR data analysis suggested that **1** contained one methoxy group (δ_H 3.82, s, 3H), one isolated *trans* olefin (δ_H 7.36, d, *J* = 16.0 Hz, 1H and 6.66 d, *J* = 16.0 Hz, 1H), one isolated aromatic signal (δ_H 7.88, s, 2H), one amide proton (δ_H 8.31, t, *J* = 6.1 Hz, 1H), three exchangeable

protons (δ_{H} 8.80, brs, 1H; 8.64, brs, 1H, and 7.94, brs, 1H) and several mutually-coupled methylene signals. The ^{13}C NMR data of **1** displayed ten aliphatic carbons, one methoxy carbon (δ_{C} 60.5), one carbonyl (δ_{C} 164.8), and six aromatic carbons. The COSY correlation data (Figure 1) provided unambiguous connectivities for the polyamine chain: $-\text{NH}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_3-\text{NH}_2$. This unit is the known natural product spermine, which is a polyamine involved in eukaryotic cellular metabolism.¹² A symmetrical 1,3,4,5-tetrasubstituted benzene moiety was established by the gHMBC correlations from the aromatic methine at δ_{H} 7.88 (s, 2H) to the carbon to which it was directly attached at δ_{C} 131.2 (Figure 1). The position of the methoxy on the benzene ring was determined on the basis of the $^3J_{\text{CH}}$ correlations from the aromatic proton at δ_{H} 7.88 and methoxy protons at δ_{H} 3.82 to the oxygenated aromatic carbon at δ_{C} 153.9 (C-4). HMBC correlations from the two olefinic protons to the quaternary aromatic carbon at δ_{C} 134.5 and the carbonyl carbon at δ_{C} 164.8 indicated that an α,β -unsaturated carbonyl group was linked to the 1,3,4,5-tetrasubstituted aromatic ring, thus forming a cinnamyl-derived moiety. The gHMBC correlations from the methylene protons at δ_{H} 3.26 and the exchangeable proton at δ_{H} 8.31 to the carbonyl group at δ_{C} 164.8 allowed the linkage of the spermine unit to the substituted cinnamyl moiety. Thus, the structure for ianthelliformisamine A (**1**) was established.

The major metabolite ianthelliformisamine B (**2**) exhibited a cluster of ions at m/z 462/464/466 (1:2:1) in the (+)-LRESIMS spectrum, indicating the presence of two bromine atoms. The molecular formula of $\text{C}_{17}\text{H}_{25}\text{Br}_2\text{N}_3\text{O}_2$ was assigned to **2** on the basis of the (+)-HRESIMS spectrum and NMR data (Table 1). In a similar manner to **1**, the ^1H and gHSQC data of **2** (Table 1)

displayed seven mutually-coupled methylenes, one methoxy group, a *trans* olefin, a symmetrical benzene moiety, an amide proton, and two more downfield exchangeable protons. These data identified that **2** had the same cinnamyl derivative as **1**, but contained a different polyamine chain. Compound **2** lacked three methylenes and one nitrogen atom that were present in **1**; this was inferred from the ^1H NMR and HRESIMS data. The polyamine spin system in **2** was elucidated as -NH-(CH₂)₃-NH-(CH₂)₄-NH₂ by gCOSY correlation data. This unit is the known natural product spermidine, which is a precursor to spermine.¹² The gHMBC correlations from the methylene protons at δ_{H} 3.25 and the amide proton at δ_{H} 8.29 to the carbonyl group at δ_{C} 165.0, and from the methylene protons at δ_{H} 1.79 to the methylene carbon at δ_{C} 35.6 allowed the linkage of spermidine unit to the cinnamyl substituted moiety. Thus, the structure of ianthelliformisamine B was assigned to **2**.

The minor compound ianthelliformisamine C (**3**) showed an ion cluster at m/z 835/837/839/841/843 (1:4:6:4:1) in the (+)-LRESIMS spectrum, indicating the presence of four bromine atoms. The molecular formula of **3** was determined to be C₃₀H₃₈Br₄N₄O₄ on the basis of the (+)-HRESIMS and NMR data (Table 1). The ^{13}C NMR spectrum of **3** displayed only 15 carbon resonances, indicating this molecule was symmetrical. The ^1H and gHSQC data of **3** (Table 1) were very similar to those of compound **1**. The differences included the replacement of a terminal NH₂ moiety in **1** with an amide proton (δ_{H} 8.25) in **3**, a downfield chemical shift of the methylene protons from δ_{H} 2.90 in **1** to δ_{H} 3.25 in **3**, and the doubling of the integration values for the aromatic singlets and the *trans* olefinic protons. These data established the existence of an additional

cinnamyl moiety in **3**. The gHMBC correlations from the methylene protons at δ_{H} 3.25 and the amide proton at δ_{H} 8.25 to the carbonyl carbon at δ_{C} 164.9, and from the methylene protons at δ_{H} 1.80 to the methylene carbon at δ_{C} 35.9 (C-10/C-18) confirmed the linkage of the polyamine chain. Hence, ianthelliformisamine C was assigned to structure **3**.

Numerous bromotyrosine-derived secondary metabolites have been isolated from marine sponges of the order Verongida and related organisms.¹³⁻¹⁶ Much of the variation of this structure class is associated with the amine component, which is typically derived from ornithine, lysine, tyrosine, cystamine, and histamine.¹³ Very few bromotyrosine-derived marine natural products contain spermine or spermidine. Examples include tokaradine C (**6**),¹⁷ spermatinamine (**7**)¹⁸ and pseudoceramines A-D,¹⁹ which have all been isolated from marine sponges belonging to the genus *Pseudoceratina*.

Compounds **1-5** were tested for their antibacterial activity against Gram-negative *P. aeruginosa* PAO1 and compounds **1**, **3**, **4** and **5** were tested against the Gram-positive bacterium *Staph. aureus* 01A1095. Compound **1** displayed selective activity against *P. aeruginosa*, with an IC_{50} of 6.8 μM ($\text{MIC} = 35 \mu\text{M}$), and 77% inhibition against *Staph. aureus* at 175 μM . Compound **2** showed only minor inhibition (80% at 87.5 μM) against *P. aeruginosa* and was not tested against *Staph. aureus*, while **3** showed similar activity against both *P. aeruginosa* and *Staph. aureus* with IC_{50} values of 8.9 ($\text{MIC} = 17.5 \mu\text{M}$) and 4.1 μM ($\text{MIC} = 8.75 \mu\text{M}$), respectively. Aplysamine 1 (**4**) showed no activity against either bacterial strain at 350 μM , while araplysillin I (**5**) showed minor growth inhibition towards *P. aeruginosa* (65% at 175 μM) and *Staph. aureus* (60% at 87.5 μM).

Although limited numbers of compounds were tested, some structure activity relationships were observed. The spermine moiety associated with **1** and **3** appeared to be important for *P. aeruginosa* activity, since replacement of spermine by spermidine as in **2** reduced the activity significantly. Furthermore the addition of an extra cinnamyl derivative in **3** to the terminal amine of the spermine chain decreased the antibacterial selectivity between *P. aeruginosa* against *Staph. aureus*, however the observed selectivity may be due to differential cell permeability between the Gram negative and the Gram positive bacteria.

In conclusion, this paper reports the isolation and structure elucidation of three new marine natural products, ianthelliformisamines A-C (**1-3**), and two known metabolites aplysamine 1 (**4**) and araplysillin I (**5**). All the compounds were evaluated for their ability to inhibit the growth of the Gram-negative bacterium *P. aeruginosa* and ianthelliformisamine A (**1**) was found to be the most active compound with an IC₅₀ value of 6.8 μ M (MIC = 35 μ M). Ianthelliformisamine A also displayed some selectivity toward *P. aeruginosa* compared to *Staph. aureus*.

EXPERIMENTAL SECTION

General Experimental Procedures. UV and IR spectra were recorded on a Bruker Tensor 27 spectrometer and a Jasco V650 UV/Vis spectrophotometer, respectively. NMR spectra were recorded at 30 °C on either a Varian 500 MHz or 600 MHz Unity INOVA spectrometer. The latter spectrometer was equipped with a triple resonance cold probe. The ¹H and ¹³C NMR chemical shifts were referenced to the solvent peak for DMSO-*d*₆ at δ_{H} 2.50 and δ_{C} 39.5. LRESIMS spectra

were recorded on a Waters ZQ mass spectrometer. HRESIMS spectra were recorded on a Bruker Daltonics Apex III 4.7e Fourier-transform mass spectrometer. Alltech Davisil 40-60 μm 60 Å C₁₈ bonded silica was used for pre-adsorption work. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler were used for HPLC. A Thermo Electron C₁₈ Betasil 5 μm 143 Å column (21.2 mm $\times$ 150 mm) and a Phenomenex Luna C₁₈ 5 μm 143 Å column (21.2 mm $\times$ 250 mm) were used for semi-preparative HPLC separations. All solvents used for chromatography, UV, and MS were Lab-Scan HPLC grade, and the H₂O was Millipore Milli-Q PF filtered. A BIOLINE orbital shaker was used for the large-scale extraction of the sponge material.

Sponge Material. The sponge *Suberea ianthelliformis* (Order: Verongida Family: Aplysinidae) was collected by scuba diving (-5 m) at Manta Ray Bommie, North Stradbroke Island, Australia during February of 2005, and kept frozen prior to freeze-drying and extraction. A voucher sample (G322245) has been lodged at the Queensland Museum, Brisbane, Australia.

Extraction and Isolation The freeze-dried and ground sponge (10 g) was poured into a conical flask (1L), *n*-hexane (250 mL) was added and the flask was shaken at 200 rpm for 2 h. The *n*-hexane extract was filtered under gravity then discarded. CH₂Cl₂/CH₃OH (4:1, 250 mL) was added to the de-fatted marine sample in the conical flask and shaken at 200 rpm for 2 h. The resulting extract was filtered under gravity, and set aside. CH₃OH (250 mL) was added and the CH₃OH/sponge mixture was shaken for a further 2 h at 200 rpm. Following gravity filtration the sponge sample was extracted with another volume of CH₃OH (250 mL), while being shaken at 200 rpm for 16 h. All CH₂Cl₂/CH₃OH extractions were combined and dried down under reduced

pressure to yield a dark brown solid (1.01 g). Some of this material (0.8 g) was pre-adsorbed to C₁₈-bonded silica (1 g) then packed into a HPLC stainless steel guard cartridge (10 × 30 mm) that was subsequently attached to a C₁₈ semi-preparative HPLC column. Isocratic HPLC conditions of 90 % H₂O (0.1% TFA)/10 % CH₃OH (0.1% TFA) were initially employed for the first 10 min, then a linear gradient to CH₃OH (0.1% TFA) was run over 40 min, followed by isocratic conditions of CH₃OH (0.1% TFA) for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected then analyzed by (+)-LRESIMS. Fractions 34 (+MS, *m/z* 519/521/523), 36-38 (+MS, *m/z* 462/464/466) and 45 (+MS, *m/z* 417/418/419/420/421, 835/837/839/841/843) contained the ions of interest and following lyophilization yielded ianthelliformisamines A (**1**, 7.9 mg, 0.10% dry wt), B (**2**, 8.4 mg, 0.11% dry wt), and C (**3**, 3.1 mg, 0.04% dry wt), respectively. ¹H NMR analysis of the remaining fractions from the first step of C₁₈ semi-preparative HPLC identified that fractions 23-25 and 39 contained semi-pure bromotyrosine derivatives. We isolated these compounds in order to obtain potential structure activity relationships. Fractions 23-25 (6.0 mg) were combined and further purified on a C₁₈ HPLC column using a 50 min linear gradient from 55% H₂O (0.1% TFA)/45% CH₃OH (0.1% TFA) to CH₃OH (0.1% TFA), followed by a linear gradient to CH₃OH (0.1% TFA) in 10 min, at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected and fraction 16 yielded pure aplysamine 1 (**4**, 0.9 mg, 0.01% dry wt). Fraction 39 (3.0 mg) was also further purified using C₁₈ HPLC. A linear gradient from 55% H₂O (0.1% TFA)/45% CH₃OH (0.1% TFA) was initially employed for 50 min, and finally a linear gradient to

CH₃OH (0.1% TFA) in 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected and fraction 26 yielded araplysillin I (**5**, 0.6 mg, 0.008 % dry wt).

Tris-TFA Salt of Ianthelliformisamine A (1): brown amorphous powder; UV λ_{max} (CH₃OH) (log ϵ) 282 (3.11), 239 (3.19) nm; IR ν_{max} (KBr) 3300, 3023, 2956, 1675, 1629, 1546, 1468, 1262, 1198, 1133, 886 cm⁻¹; ¹H and ¹³C NMR data (DMSO-*d*₆) see Table 1; (+)-LRESIMS *m/z* 519 (50%), 521 (100%), 523 (50%); (+)-HRESIMS *m/z* 519.0967 [M + H]⁺ (calcd for C₂₀H₃₃⁷⁹Br₂N₄O₂, 519.0965).

Bis-TFA Salt of Ianthelliformisamine B (2): brown amorphous powder; UV λ_{max} (CH₃OH) (log ϵ) 283 (3.40), 225 (4.10) nm; IR ν_{max} (KBr) 3281, 1613, 1513, 1345, 1262, 1012, 839 cm⁻¹; ¹H and ¹³C NMR data (DMSO-*d*₆) see Table 1; (+)-LRESIMS *m/z* 462 (50%), 464 (100%), 466 (50%); (+)-HRESIMS *m/z* 462.0340 [M + H]⁺ (calcd for C₁₇H₂₆⁷⁹Br₂N₃O₂, 462.0386).

Bis-TFA Salt of Ianthelliformisamine C (3): brown amorphous powder; UV λ_{max} (CH₃OH) (log ϵ) 282 (5.07), 239 (4.66) nm; IR ν_{max} (KBr) 3276, 3019, 2585, 2358, 1673, 1623, 1543, 1467, 1260, 1198, 1133, 892 cm⁻¹; ¹H and ¹³C NMR data (DMSO-*d*₆) see Table 1; (+)-LRESIMS *m/z* 418 [M + 2H]²⁺ (15%), 419 (60%), 420 (100%), 421 (60%), 422 (15%), 835 [M + H]⁺ (15%), 837 (60%), 839 (100%), 841 (60%), 843 (15%); (+)-HRESIMS *m/z* 834.9692 [M + H]⁺ (calcd for C₃₀H₃₉⁷⁹Br₄N₄O₄, 834.9699).

***Pseudomonas aeruginosa* Optical Density (OD₆₂₀) Viability Assay.** *P. aeruginosa* PAO1 or PAO200 strain (supplied by Pfizer Global Research and Development) cultures were prepared at 3.5 × 10⁴ CFU/mL in cation-adjusted Mueller Hinton (caMH) broth (Difco), from concentrated

frozen stocks of culture. The final bacterial concentration in the assay was 1500 CFU/well. Diluted bacteria (45 μ L) were added to a 384 well lidded, sterile clear plate (Becton Dickinson) containing controls/fractions by a Multidrop liquid handler (Thermo Scientific). Plates were incubated at 37 °C in a humidified incubator for 18 h or until the wells reached an optical density (OD₆₂₀) of between 0.7-0.8, then allowed to cool for 30 min. Clear plate seals (Perkin Elmer) were placed over the plate surface before reading on a Multiskan Ascent reader (Thermo Scientific) at 620 nm. Test fractions, compounds or control samples (5 μ L) were added to the assay plate prior to the addition of bacteria. Samples were prepared by dilution of stock fractions/compounds/controls in DMSO into the assay plate with addition of 0.875 μ L of stock and 4.125 μ L of autoclaved Milli-Q filtered H₂O with a Minitrak (Perkin Elmer) liquid handler. The final concentration of DMSO in the assay was 1.75%. Each assay plate contained both positive and negative controls in columns 23 and 24, respectively. The positive control, for uninhibited growth, consisted of 5 μ L of DMSO/ Milli-Q H₂O to a final concentration of 1.75% and the negative control, or 100% cell death, was comprised of 5 μ L of the broad spectrum antibiotic ciprofloxacin at a final concentration of 5 μ g/mL. Whole control plates were included for each assay run, which consisted of duplicate dose response curves of ciprofloxacin, each in triplicate. Ciprofloxacin was shown to have an IC₅₀ value of 0.038 μ M against *P. aeruginosa* PAO1.

***Staph. aureus* Optical Density Viability Assay.** The *Staph. aureus* assay was carried out as per *P. aeruginosa* assay, with the following modifications: the final bacterial concentration used was 1980 CFU/well and ciprofloxacin for the internal assay control wells was at 500 μ g/mL.

Incubation was for 19 h, or until the OD₆₂₀ reached 0.45. Ciprofloxacin was shown to have an IC₅₀ value of 125 μ M against methicillin-resistant *Staph. aureus* 01A1095.

ASSOCIATED CONTENT

Supporting Information.

NMR and MS spectra for ianthelliformisamines A-C (**1-3**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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lanthelliformisamine A (1)

lanthelliformisamine B (2)

lanthelliformisamine C (3)

Alypsamine-1 (4)

Araplysillin I (5)

Tokaradine C (6)

Spermatinamine (7)

Figure 1. Selected HMBC ($\rightarrow$) and COSY (—) correlations of **1**

Table 1. ^1H and ^{13}C NMR Data of Ianthelliformisamines A-C (**1-3**).^a

position	1		2		3	
	δ_{C} mult	δ_{H} mult (<i>J</i> in Hz)	δ_{C} mult ^b	δ_{H} mult (<i>J</i> in Hz)	δ_{C} mult	δ_{H} mult (<i>J</i> in Hz)
1	134.5, C	-	134.0, C	-	134.2, C	-
2,6	131.2, CH	7.88, s	131.2, CH	7.89, s	131.5, CH	7.87, s
3,5	117.9, C	-	117.1, C	-	118.0, C	-
4	153.9, C	-	153.7, C	-	153.9, C	-
4-OCH ₃	60.5, CH ₃	3.82, s	60.0, CH ₃	3.82, s	60.5, CH ₃	3.81, s
7	135.4, CH	7.36, d (16.0)	135.0, CH	7.35, d (16.0)	135.4, CH	7.35, d (16.0)
8	124.1, CH	6.66, d (16.0)	123.6, CH	6.66, d (16.0)	124.1, CH	6.66, d (16.0)
9	164.8, C	-	165.0, C	-	164.9, C	-
10	35.9, CH	3.26, dt (6.1, 6.0)	35.6, CH	3.25, dt (6.1, 6.0)	35.9, CH	3.25, dt (6.1, 6.0)
11	26.0, CH	1.79, tt (6.1, 6.5)	25.8, CH	1.79, tt (6.1, 6.5)	26.0, CH	1.80, tt (6.1, 6.5)
12	44.6, CH ₂	2.90, m	44.5, CH ₂	2.92, m	44.6, CH ₂	2.92, m
13	46.3, CH ₂	2.90, m	44.5, CH ₂	2.92, m	46.0, CH ₂	2.92, m
14	22.6, CH ₂	1.63, m	22.7, CH ₂	1.59, m	22.6, CH ₂	1.61, m
15	22.6, CH ₂	1.63, m	22.7, CH ₂	1.59, m	22.6, CH ₂	1.61, m
16	46.3, CH ₂	2.90, m	37.3, CH ₂	2.82, tq (6.1, 6.2)	46.0, CH ₂	2.92, m
17	43.8, CH ₂	2.90, m	-	-	44.6, CH ₂	2.92, m
18	23.7, CH ₂	1.86, m	-	-	26.0, CH ₂	1.80 tt (6.1, 6.5)
19	36.2, CH ₂	2.90, dd (6.1, 6.5)	-	-	35.9, CH ₂	3.25, m
9-NH	-	8.31, t (6.1)	-	8.29, t (6.1)	-	8.25, t (6.1)
12-NH	-	8.80, brs	-	8.57, brs	-	8.48, brs
16-NH	-	8.64, brs	-	7.84, brs	-	8.48, brs
19-NH ₂	-	7.94, brs	-	-	-	-
1'	-	-	-	-	134.2, C	-
2',6'	-	-	-	-	131.5, CH	7.88, s
3',5'	-	-	-	-	118.0, CH	-
4'	-	-	-	-	153.9, C	-
4'-OCH ₃	-	-	-	-	60.5, CH ₃	3.81, s
7'	-	-	-	-	135.4, CH	7.35, d (16.0)
8'	-	-	-	-	124.1, CH	6.66, d (16.0)
9'	-	-	-	-	164.9, C	-
9'-NH	-	-	-	-	-	8.25 t (6.1)

^a Recorded in DMSO-*d*₆ at 30 °C, 500 MHz for ^1H NMR of **1-3** and 125 MHz for ^{13}C NMR of **1** and **3**.^b ^{13}C chemical shifts obtained from 2D NMR experiments.