Polydiscamides B−D from a Marine Sponge *Ircinia* sp. as Potent Human Sensory Neuron-Specific G Protein Coupled Receptor Agonists

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Polydiscamides B, C, and D (**1**−**3**) were isolated from a sponge *Ircinia* sp. The structures of **1** to **3** were elucidated by the comparison of their NMR and HRESIMS spectroscopic data with that of a structurally related compound, polydiscamide A. All compounds showed potent agonist activity against human sensory neuron-specific G protein couple receptor (SNSR), a receptor involved in the modulation of pain, and they are the first examples of nonendogenous human SNSR agonists.

G protein coupled receptors (GPCRs) are involved in a broad array of signaling pathways and have long been a target in drug discovery. This superfamily of membrane-bound proteins transmit signals across the plasma membrane through heterotrimeric G proteins.[1] Despite the ongoing interest in GPCRs for drug discovery, there are still an enormous number of receptors for which the cognate ligand remains unknown, so-called "orphan" GPCRs. A group of such orphan receptors are the sensory neuron-specific G protein coupled receptors (SNSRs), which are expressed solely in dorsal root ganglia.[2] Previous studies showed that SNSRs are key players in both acute and persistent pain.[3] Due to the fact that SNSRs possess a highly restricted distribution in the body, ligands that interact with this receptor may potentially modulate pain with very few side effects.

To date, there are few ligands that have been identified for this family of GPCRs. Dong et al. originally described that arginine and phenylalanine containing neuropeptides worked as agonists for SNSRs in mice.[2] However, it was only recently, that the role of SNSRs in nociception was confirmed in a rat model.[4] Selective SNSR agonists were shown to evoke a behavioral profile similar to established pain-provoking agents, confirming their apparent role in pain transmission or modulation.

High-throughput screening identified an extract from a sponge *Ircinia* sp. that displayed potent agonist activity against SNSRs. Bioassay-guided fractionation and purification led to the isolation of polydiscamides B, C, and D (**1**−**3**).

Results and Discussion

Freeze-dried sponge sample (5 g) was extracted exhaustively with CH_2Cl_2 and MeOH. The combined extract (0.9 g) was chromatographed on a reversed-phase HPLC column using a gradient from TFA in H_2O (1%) to TFA in MeOH (1%) to yield three cyclic depsipeptides, polydiscamide B (**1**) (6 mg, 0.1% dry weight), polydiscamide C (**2**) (30 mg, 0.6% dry weight), and polydiscamide D (**3**) (16 mg, 0.32% dry weight).

Polydiscamide B (**1**) was obtained as an amorphous solid. The UV spectrum of **1** displayed absorption maxima at 207, 220 (sh), and 279 nm, suggesting the presence of aromatic systems in the molecule. IR peaks at 3307 (br) and 1642 cm^{-1} indicated the presence of alcohol, amine, and amide or ester functionalities. The ES positive ion mass spectrum of **1** revealed a $M + H^+$ ion at *m/z* 1709 and 1711 (∼1:1). The characteristic splitting pattern suggested the presence of a bromine atom in the molecule. High-resolution mass measurement on the $M + H^+$ ion (*m/z* 1709.700315), in combination with ^1H and ^{13}C NMR data (Table 1), supported the molecular formula $C_{75}H_{109}BrN_{18}O_{21}S$.

The molecular formula of **1** corresponded closely to the molecular formula reported for polydiscamide A ($C_{75}H_{110}BrN_{19}O_{20}S$).[5] The peptide nature of **1** was confirmed by the presence of a large number of carbonyl signals (δ 160–174) in the ^{13}C NMR spectrum and amide proton doublets (δ 7.0–8.4) in the ^1H NMR spectrum (Table 1). Comparison of the complete ^1H and ^{13}C NMR spectroscopic data of **1** (Table 1) with the published values for polydiscamide A revealed that **1** contained amino acid residues identical to those of polydiscamide A for alanine (Ala), *p*-bromo-phenylalanine (BrPhe), two prolines (Pro), *tert*-leucine (*t*-Leu), β-methylisoleucine (β-MeIle), tryptophan (Trp), arginine (Arg), cysteic acid (Cys), threonine (Thr), *N*-methylglutamine (NMeGln), and valine (Val). The only structural difference between **1** and polydiscamide A was the replacement of an asparagine with an aspartic acid (Asp). Detailed analysis of 2D NMR spectroscopic data (COSY, TOCSY, HSQC, HMBC, and ROESY) and HRESIMS supported structure **1**.

Acid hydrolysis of **1** followed by derivatization with Marfey's reagent and HPLC analysis[6] indicated that the common amino acids in **1** and polydiscamide A[5] had the same absolute configuration; that is, they both had D-Ala, L-BrPhe, L-Pro, D-*t*-Leu, D-Trp, L-Arg, D-Cys, L-Thr, D-Val, and D-Asp. We were unable to determine the absolute configuration of β-MeIle and NMeGln. We speculate that **1** had L-β-MeIle and L-NMeGln like that of polydiscamide A on the basis of their same biogenesis and the analogy of their NMR spectroscopic data.

Polydiscamide C (**2**) was also isolated as an amorphous solid. The comparison of the ^1H and ^{13}C NMR spectroscopic data (Table 1) between **1** and **2** suggested that **2** had two extra methyl doublets at δ 0.77 and 0.82 instead of a three-methyl singlet at 0.87. Detailed analysis of the TOCSY data revealed that the two extra methyl doublets belonged to a Val residue, suggesting the replacement of a *t*-Leu residue with a Val. This was supported by the molecular formula $C_{74}H_{107}BrN_{18}O_{21}S$ from HRESIMS measurement and confirmed by the detailed analysis of 2D spectroscopic data (COSY, HSQC, HMBC, and ROESY). Acid hydrolysis followed by derivatization with Marfeys reagent and HPLC analysis[6] indicated that **2** had the same absolute stereochemistry as that of **1**, and the extra Val had a D-configuration.

Polydiscamide D (**3**) also possessed spectroscopic properties very similar to those of **1** and **2**. The molecular formula of **3** was determined to be $C_{73}H_{105}BrN_{18}O_{21}S$ from HRESIMS measurement. Comparison of ^1H and ^{13}C spectroscopic data between **2** and **3** (Table 1) showed that the three β-MeIle methyl signals in **2** (δ 0.62, 0.60, and 0.71) were replaced by a methyl singlet in **3** (δ

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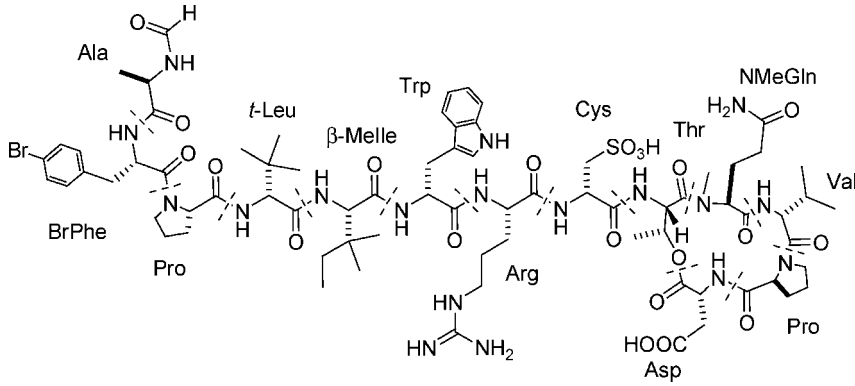
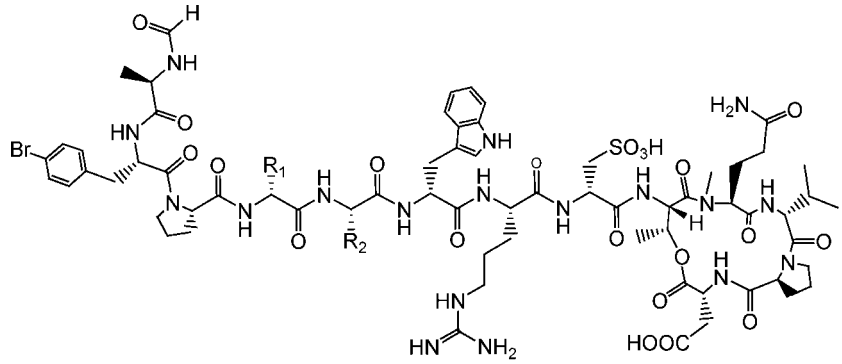


Figure 1. Constituent amino acids for **1**.

Chart 1. Chemical Structure for **1**, **2**, and **3**



1 $R_1=(CH_3)_3C$ $R_2=CH_3CH_2C(CH_3)_2$

2 $R_1=(CH_3)_2CH$, $R_2=CH_3CH_2C(CH_3)_2$

3 $R_1=(CH_3)_2CH$, $R_2=(CH_3)_3C$

0.72, 3 × CH_3). Further analysis of the 2D spectroscopic data (COSY, HSQC, HMBC, and ROESY) suggested that the β-MeIle in **2** was replaced by a *t*-Leu in **3**. Acid hydrolysis of **1** followed by derivatization with Marfey's reagent and HPLC analysis[6] indicated that **3** had the same absolute stereochemistry as that of **1**, and the extra *t*-Leu had an L-configuration.

Polydiscamides B−D (**1**−**3**) are structurally related to a number of compounds, such as polydiscamide A,[5] halicyclindramides,[7] and discodermins.[8,9] These compounds have been reported to possess a variety of activities, including antimicrobial, antifungal, and cytotoxicity. The three depsipeptides described here are potentially able to act as pain modulators by activating SNSR. These compounds were shown to be potent SNSR agonists, resulting in intracellular calcium mobilization in a HEK293 cell line as determined by bioassay using a fluorometric imaging plate reader (FLIPR). The three compounds showed potent agonist activity against human SNSR with EC_{50} values of 1.26, 3.57, and 2.80 μM, respectively. So far, the only known human SNSR agonists are the opioid peptide precursor proenkephalin A and a number of enkephalin fragments.[1] Polydiscamides B−D (**1**−**3**) are the first examples of nonendogenous compounds with human SNSR agonist activity and could potentially be modified for therapeutic use as pain modulators.

Experimental Section

General Experimental Procedures. UV and IR were measured with a Camspec M501 single beam scanning UV/vis spectrophotometer and Bruker Tensor 27 FT-IR spectrometer, respectively. Optical rotations were measured by a JASCO P-1020 polarimeter. NMR spectra were recorded at 30 °C on a Varian Inova 600 MHz spectrometer in d_6-DMSO (^{1}H δ 2.50 and ^{13}C δ 39.5). Mass spectra were acquired using a Mariner TOF biospectrometer. High-resolution mass measurement was acquired on a Bruker Daltonics Apex III 4.7e Fourier transform mass spectrometer fitted with an Apollo API source. The HPLC system included a Waters 600 pump equipped with a 996 photodiode array detector and a Gilson FC204 fraction collector. A Hypersil Betasil C_{18} 5 μm column (150 × 21.2 mm) was used for semipreparative HPLC. Solvents for extraction and chromatography were Omnisolv HPLC grade.

Animal Material. The sponge *Ircinia* sp. was collected by scuba diving at 30 m at the south side of the Porpoise Cay, the Great Barrier Reef, Australia. A voucher sample (G306512) is deposited at the Queensland Museum, Brisbane, Australia.

Extraction and Isolation. Freeze-dried sponge (5 g) was extracted exhaustively with CH_2Cl_2 and MeOH. The combined extract (0.9 g) was chromatographed on a semipreparative C_{18} HPLC column with a flow rate of 9 mL/min. A gradient elution from TFA in H_2O (1%) to TFA in MeOH (1%) over 34 min was used. Sixty fractions were collected, and fractions 33, 32, and 31 yielded polydiscamide B (**1**) (6 mg, 0.1% dry weight), polydiscamide C (**2**) (30 mg, 0.6% dry weight), and polydiscamide D (**3**) (16 mg, 0.32% dry weight), respectively.

Polydiscamide B (1): amorphous solid; $[\alpha]^{25}_D$ +3.65 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 207 (4.71), 220 (sh) (4.67), 279 (3.76) nm; IR (film) ν_{max} 3307 (br), 2971, 1642 (br), 1535, 1442, 1202, 1139, 1044, 802, 722, 599 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m/z* M + H$^+$ 1709.700315 (calcd for $C_{75}H_{110}BrN_{18}O_{21}S$, 1709.699146).

Polydiscamide C (2): amorphous solid; $[\alpha]^{25}_D$ +4.00 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 207 (4.71), 220 (sh) (4.67), 279 (3.76) nm; IR (film) ν_{max} 3294 (br), 2964, 1648 (br), 1541, 1448, 1202, 1043, 1011, 669 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m/z* M + H$^+$ 1695.685699 (calcd for $C_{74}H_{108}BrN_{18}O_{21}S$, 1695.683495).

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for **1**−**3**

	1		**2**		**3**	
amino acid	δ_H (mult., J Hz)[a]	δ_C[b]	δ_H (mult., J Hz)[a]	δ_C[b]	δ_H (mult., J Hz)[a]	δ_C[b]
	Ala		**Ala**		**Ala**	
α	4.28 (dq, 6.6, 7.8)	46.0	4.30 (dq, 6.6, 7.8)	46.1	4.31 (dq, 7.2, 7.2)	46.1
β	0.92 (d, 6.6)	18.0	0.92 (d, 7.2)	18.2	0.94 (d, 7.2)	18.2
NCHO	7.88 (br s)	160.0	7.90 (s)	160.2	7.90 (s)	160.2
CO		171.9		171.0		171.2
NH	8.10 (d, 7.8)		8.12		8.13 (d, 7.2)	
	BrPhe		**BrPhe**		**BrPhe**	
α	4.68 (ddd, 3.6, 8.4, 10.8)	51.0	4.72 (ddd, 3.6, 8.4, 10.8)	51.0	4.73 (m)	51.1
β	2.68 (dd, 10.8, 14.4), 2.90 (dd, 3.6, 14.4)	35.5	2.70 (dd, 9.6, 14.5), 2.97 (dd, 3.6, 14.5)	35.7	2.72 (dd, 10.8, 13.2), 3.00 (m)	35.9
C4		137.0		136.6		137.1
C5/C9	7.19 (d, 8.4)	131.5	7.21 (d, 8.4)	131.2	7.24 (d, 7.8)	131.5
C6/C8	7.36 (d, 8.4)	130.3	7.41 (d, 8.4)	130.3	7.42 (d, 7.8)	130.4
C7		119.0		119.2		119.1
CO		169.5		169.2		168.9
NH	8.33 (d, 8.4)		8.37 (d, 8.4)		8.37 (d, 8.4)	
	Pro		**Pro**		**Pro**	
α	4.50 (m)	60.0	4.47 (dd, 3.6, 8.4)	59.6	4.46 (m)	59.6
β	1.97 (m)	29.0	2.06 (m)	29.3	2.08 (m), 1.82 (m)	29.3
γ	1.86 (m)	23.0	1.86 (m)	23.9	1.89 (m)	24.2
δ	3.60 (m)	46.0	3.64 (m)	46.4	3.65 (m)	46.6
CO		170.3		170.9		171.0
	***t*-Leu**		**Val**		**Val**	
α	4.53 (d, 9.6)	59.0	4.46 (dd, 7.2, 7.2)	56.6	4.45 (dd, 6, 8.4)	56.7
β		36.0	2.07 (m)	29.3	2.09 (m)	29.5
γ	0.87 (br s)	26.0	0.82 (d, 6.6)	18.9	0.84 (d, 6.0)	19.2
δ			0.77 (d, 6.6)	17.0	0.78 (d, 7.2)	17.1
CO		170.6		170.9		170.9
NH	7.68 (d, 9.6)		7.83 (d, 9.0)		7.84 (d, 8.4)	
	***β*-MeIle**		***β*-MeIle**		***t*-Leu**	
α	4.12 (d, 7.8)	60.0	4.18 (d, 9.0)	59.7	4.15 (d, 8.4)	60.0
β		36.0		36.0		33.7
γ	1.05 (m)	31.0	1.04 (q, 7.2)	30.7	0.72 (br s)	26.2
δ	0.60 (t, 6.6)	8.0	0.62 (t, 7.2)	8.4		
χ′	0.60 (s)	22.0	0.60 (s)	22.3		
χ″	0.71 (s)	23.0	0.71 (s)	22.6		
CO		170.0		169.7		170.6
NH	7.76 (d, 7.2)		7.74 (d, 8.4)		7.77 (d, 8.4)	
	Trp		**Trp**		**Trp**	
α	4.61 (ddd, 7.8, 7.8, 5.4)	53.0	4.58 (m)	53.3	4.60 (m)	53.4
β	2.81 (dd, 7.8, 16.2), 3.13 (dd, 5.4, 16.2)	27.0	2.91 (dd, 6.5, 16.2), 3.17 (dd, 5.4, 16.2)	27.1	2.95 (dd, 7.8, 14.4), 3.14 (m)	28.1
C4		110.0		109.7		109.7
C5		127.0		126.7		127.1
C6	7.54 (d, 8.4)	118.0	7.59 (d, 8.4)	118.0	7.59 (d, 8.4)	118.1
C7	6.93 (dd, 8.4, 8.4)	120.0	6.96 (dd, 7.2, 7.8)	119.0	6.95 (dd, 7.2, 7.8)	118.7
C8	7.01 (dd, 8.4, 8.4)	122.0	7.02 (dd, 7.2, 7.2)	121.2	7.04 (dd, 7.2, 8.4)	121.3
C9	7.29 (d, 8.4)	113.0	7.32 (d, 7.8)	111.9	7.32 (d, 8.4)	111.2
C10		137.0		136.1		135.9
C11	7.11 (d, 1.2)	126.0	7.14 (br s)	124.8	7.14 (br s)	124.7
CO		171.5		171.2		171.0
NH	8.15 (d, 7.8)		8.22 (d, 6.6)		8.23 (d, 7.2)	
NH-indole	10.60 (d, 1.2)		10.61		10.62	
	Arg		**Arg**		**Arg**	
α	4.29 (m)	51.7	4.38 (m)	51.3	4.35 (m)	51.9
β	1.59 (m)	28.5	1.62 (m)	28.4	1.62 (m)	28.3
γ	1.26 (m), 1.33 (m)	22.5	1.25 (m)	23.1	1.30 (m)	23.2
δ	2.90 (m)	40.0	2.98 (m)	39.9	2.97 (m)	39.9
NHCNNH$_2$	7.36 (t, 7.2)				7.41 (t, 7.8)	
NHCNNH$_2$		156.0		156.3		156.3
NHCNNH$_2$	6.75 (br s)				6.69 (br s)	
CO		170.3		170.4		170.5
NH	7.98 (d, 6.6)		7.95 (d, 7.2)		7.99 (d, 7.2)	
	Cys		**Cys**		**Cys**	
α	4.61 (m)	50.5	4.60 (m)	50.8	4.60 (m)	50.8
β	2.90 (m)	39.0	2.93 (m)	39.9	2.99 (m)	39.9
CO		171.0		170.3		170.1
NH	8.20 (d, 6.6)		8.34 (d, 8.4)		8.31 (d, 6.6)	
	Thr		**Thr**		**Thr**	
α	4.90 (br d, 6.6)	51.5	4.93 (brd, 7.8)	51.5	4.93 (brd, 7.2)	51.6
β	5.07 (m)	70.0	5.11 (m)	69.3	5.11 (m)	69.5
γ	1.14 (d, 6.0)	16.0	1.16 (d, 6.6)	15.6	1.16 (d, 6.0)	15.8
CO		169.0		169.2		168.9
NH	7.89 (d, 6.6)		7.90 (d, 6.6)		7.89 (d, 6.6)	
	NMeGln		**NMeGln**		**NMeGln**	
α	5.10 (m)	55.0	5.10 (m)	54.4	5.11 (m)	54.5
β	1.95 (m)	24.0	1.90 (m)	23.6	1.90 (m)	24.1
γ	1.95 (m)	31.0	1.98 (m)	31.2	1.98 (m)	31.5
CO		171.0		171.3		170.5

Table 1. Continued

	1		**2**		**3**	
amino acid	δ_H (mult., *J* Hz)[a]	δ_C[b]	δ_H (mult., *J* Hz)[a]	δ_C[b]	δ_H (mult., *J* Hz)[a]	δ_C[b]
NMe	3.00 (s)	30.0	3.03 (s)	30.0	3.03 (s)	30.1
CONH$_2$		172.0		173.4		173.6
CONH$_2$	6.63 (br s), 7.22 (br s)				6.68 (br s), 7.7.25 (br s)	
	Val		**Val**		**Val**	
α	4.35 (dd, 7.8, 7.8)	55.0	4.34 (m)	55.0	4.36 (m)	54.9
β	1.90 (m)	30.0	1.95 (m)	29.3	1.96 (m)	29.5
γ	0.80 (d, 6.0)	17.0	0.82 (m)	17.3	0.81 (d, 6.0)	17.1
δ	0.91 (d, 6.6)	19.0	0.93 (d, 7.2)	18.2	0.85 (d, 6.0)	19.2
CO		172.0		171.1		170.6
NH	7.68 (d, 7.8)		7.79 (d, 8.4)		7.84 (d, 8.4)	
	Pro		**Pro**		**Pro**	
α	4.18 (dd, 2.2, 8.4)	60.0	4.21 (br d, 8.4)	59.9	4.21 (br d, 8.4)	59.9
β	2.00 (m)	29.0	2.02 (m)	29.3	2.03 (m)	29.3
γ	1.90 (m), 2.00 (m)	23.0	1.90 (m), 2.00 (m)	23.6	1.90 (m), 2.00 (m)	24.3
δ	3.52 (m), 3.86 (m)	46.0	3.66 (m), 3.90 (m)	46.7	3.55 (m), 3.89 (m)	46.6
CO		171.3		170.9		170.6
	Asp		**Asp**		**Asp**	
α	4.50 (m)	48.0	4.55 (m)	47.8	4.56 (m)	47.9
β	2.43 (br d, 13.8), 2.81 (m)	35.0	2.43 (br d, 16.2), 2.85 (dd, 7.2, 16.8)	34.6	2.43 (br d, 16.2), 2.84 (dd, 6.6, 16.2)	34.8
CO		170.0		170.3		170.5
NH	7.25 (d, 9.6)		7.22 (d, 8.4)		7.25 (d, 8.4)	
COOH		171.0		170.9		171.3

[a] Spectra were recorded at 600 MHz for ^{1}H using d_6-DMSO as solvent. [b] Spectra were recorded at 150 MHz for ^{13}C using d_6-DMSO as solvent.

Polydiscamide D (3): amorphous solid; $[\alpha]^{25}_D$ +1.90 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205 (4.73), 218 (sh) (4.49), 284 (3.58) nm; IR (film) ν_{max} 3306 (br), 2966, 1650 (br), 1535, 1445, 1201, 1043, 721 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m/z* M + Na$^+$ 1703.652888 (calcd for $C_{73}H_{105}BrN_{18}O_{21}SNa$, 1703.649806).

Stereochemistry of Polydiscamides B−D. In a typical hydrolysis a peptide (1.0 mg) was heated in 6 N HCl (10 mL) at 105 °C for 40 h. The resulting hydrolysate was dried, dissolved in distilled water (200 μL), and derivatized with 1-fluoro-2,4-dinitrophen-5-yl-L-alanine amide (FDAA) (1.5 mg) in acetone (500 μL) and 1 N sodium bicarbonate (100 μL) at 50 °C for 2 h. Upon completion of the reaction, the solution was acidified with 2 N HCl (500 μL) and stored in the dark until it was analyzed. HPLC analysis (C18 Supelco column, 25 cm × 4.6 mm, 5 μm; linear gradient elution, triethylammonium phosphate (50 mM, pH 3.0)/acetonitrile, 80:20–50:50 in 40 min, and then held at 50:50 for 20 min; 1.5 mL/min; PDA detection at 340 nm) of the FDAA-derivatized hydrolysates established the stereochemistry of the constituent amino acids. Each peak in the chromatographic trace was identified by comparing its retention time with that of the FDAA derivative of the pure amino acid standard and by co-injection. The acid hydrolysis of polydiscamide B (**1**) showed peaks at 5.32, 7.59, 12.29, 24.18, 27.34, 28.85, and 29.51 min and a broad peak between 14.41 and 15.42 min. Polydiscamides C and D (**2**, **3**) showed peaks at the same retention time except for 28.85 min. Polydiscamide D (**3**) also showed a peak at 23.45 min. The amino acid standards gave the following retention times: 5.35 for L- and 14.66 for D-Arg; 7.87 for L- and 15.50 for D-Thr; 12.01 for L- and 14.30 for D-Pro; 10.79 for L- and 14.87 for D-Ala; 4.61 for L- and 14.92 for D-Cys; 6.91 for L- and 15.40 for D-Asp; 18.10 for L- and 24.41 for D-Val; 22.83 for L- and 27.00 for D-Trp; 23.45 for L- and 29.23 for D-*t*-Leu; and 29.23 for L- and 34.53 for D-BrPhe. Co-injection confirmed L-Arg, L-Thr, L-Pro, D-Val, D-Trp, D-*t*-Leu, and L-BrPhe as well as confirming D-Ala, D-Cys, and D-Asp in the broad peak at 14.41–15.42 min in **1**−**3**.

Bioassay. Hek293s cells transfected with human SNSR were plated at 12 000 cells/well and grown for 24 h at 37 °C in UltraCulture media, without selection antibiotics. The medium was removed by inversion and blotting on paper towels. The cells were then "loaded" with nonwash calcium dye (20 μL) containing 1.67% DMSO (FLIPR calcium assay kit available from Molecular Devices). The plates were incubated at 5% CO_2 and 37 °C for 45 min and analyzed using the fluorescent imaging plate reader (FLIPR) for intracellular calcium mobilization. The fluorescence signals were measured using a 520–545 nm bandpass. A baseline reading was taken for 10 s, and then the active compounds (20 μL) were added to the cell plate. Data was collected every second for 60 s and then every 6 s for 18 s. EC_{50} values were determined using PRISM 3.02 (GraphPad, San Diego).

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Supporting Information Available: Photograph of the sponge *Ircinia* sp. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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