5-OHKF and NorKA, Depsipeptides from a Hawaiian Collection of *Bryopsis pennata*: Binding Properties for NorKA to the Human Neuropeptide Y Y_1 Receptor

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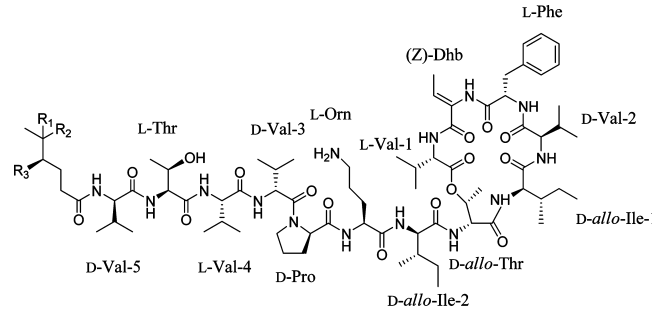
Two new cyclic depsipeptides, 5-OHKF (**1**) and norKA (**2**), together with the known congeners kahalalide F (**3**) and isokahalalide F ((4*S*)- methylhexanoic kahalalide F) (**4**) were isolated from the green alga *Bryopsis pennata*. The structures of the new compounds were established on the basis of extensive 1D and 2D NMR spectroscopic analysis and mass spectrometric (ESIMS) data. The absolute configuration of each amino acid of 5-OHKF (**1**) and norKA (**2**) was determined by chemical degradation and Marfey's analysis. The biological activities of these two compounds are also reported.

The green alga *Bryopsis pennata* and the sacoglossan mollusk *Elysia rufescens*, which feeds on the alga, have been extensively investigated for their biologically active natural products including depsipeptides.[1−9] Thus far, 17 cyclic depsipeptides, kahalalides A−F, isoKF, K, O−S, R′, S′, W, and Y, and five linear depsipeptides, kahalalides G, H, J, V, and X, have been isolated from the green alga *B. pennata* or the herbivorous marine mollusk *Elysia rufescens*, *E. ornata*, or *E. grandifolia*. The kahalalides show highly promising biological activities including antiviral, antimalarial, and primarily anticancer properties. KF (**3**) and isoKF (**4**) reveal significant in vitro and in vivo antitumor activity against various solid cell lines.[1,10] KF (**3**) and isoKF (**4**) have completed or are under phase I clinical trials for select solid tumors including androgen-refractory prostate cancer and colon cancer[12−14] and have completed or are under investigation in phase II clinical studies with patients having liver cancer, non-small-cell lung cancer, melanoma, and psoriasis.[11] The primary mechanism of action of KF (**3**) and isoKF (**4**) remains to be completely elucidated. However, early research indicated that KF (**3**) blocked the epidermal growth factor receptor (ErbB family), and recent findings have identified ErbB3 and PI3K-Akt as major determinants of the cytotoxic activity of KF (**3**) in vitro.[12] KF (**3**) and its derivatives[13,14] are potential candidates for inhibiting ErbB3 in tumor cells. Due to the promising therapeutic value of KF (**3**) and isoKF (**4**), we reinvestigated kahalalide derivatives from *B. pennata*.

The freeze-dried green alga sample was extracted three times with EtOH. The EtOH extracts were combined and concentrated, and then LC-MS analysis of the fractions indicated that this fraction contained kahalalide-related depsipeptides including KF (**3**). LC-MS-guided fractionation on a silica column followed by HPLC yielded 5-OHKF (**1**), norKA (**2**), KF (**3**), and isoKF (**4**).

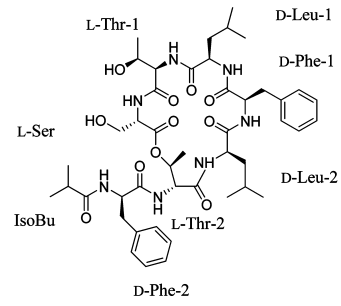
5-OHKF (**1**) was obtained as a white, amorphous powder. In the positive ESIMS, a pseudomolecular ion was detected at *m*/*z* 1493.9313 $[M + H]^+$ that was compatible with the molecular formula $C_{75}H_{124}N_{14}O_{17}$. Inspection of the ^1H and ^{13}C NMR spectra of **1** revealed structural features similar to KF (**3**). The mass difference of 16 Da and an additional oxygenated quaternary carbon suggested that the only difference between 5-OHKF (**1**) and KF (**3**) is one hydroxy group. COSY, HMBC, and TOCSY spectra of



1: R_1=OH R_2=CH_3 R_3=H

3: R_1=H R_2=CH_3 R_3=H

4: R_1=H R_2=H R_3=CH_3



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1 revealed that the fatty acid residue in **1** was 5-hydroxy-5-methylhexanoic acid.

Amino acid analysis by Marfey's method[18] revealed 12 amino acids: L-ornithine, D-*allo*-isoleucine (2), D-proline, L-threonine, D-*allo*-threonine, D-valine (3), L-valine (2), and L-phenylalanine. As Figure 1A, B show, the amino acid composition of 5-OHKF (**1**) was identical to that of KF (**3**). However, a single Marfey's analysis was not enough to determine the absolute configuration of the individual valine moieties and of the threonine units. As a result, KF (**3**) and 5-OHKF (**1**) were partially hydrolyzed into smaller units without the fatty acid residues, followed by comparison of their chromatographic properties.

5-OHKF (5 mg) was hydrolyzed with 4 N HCl/EtOH (1:1) at 70 °C for 8 h (Figure 2), and the fragments were separated by HPLC. Fragment 1 is the peptide KF-[5-MeHex-Val-5]. Mild acid

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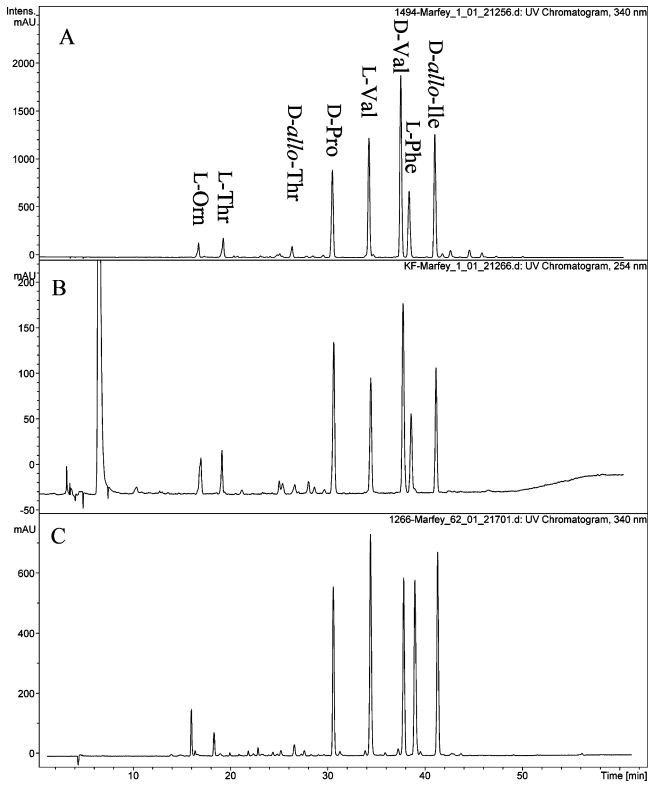

Figure 1. HPLC after total hydrolysis and FDAA derivatization of (A) 5-OHKF (**1**); (B) KF (**3**); and (C) fragment 1 from 5-OHKF (**1**) HPLC using an RP C8 column and a gradient from H_2O/MeCN/HCOOH (90/10/0.05) to H_2O/MeCN/HCOOH (40/60/0.05).

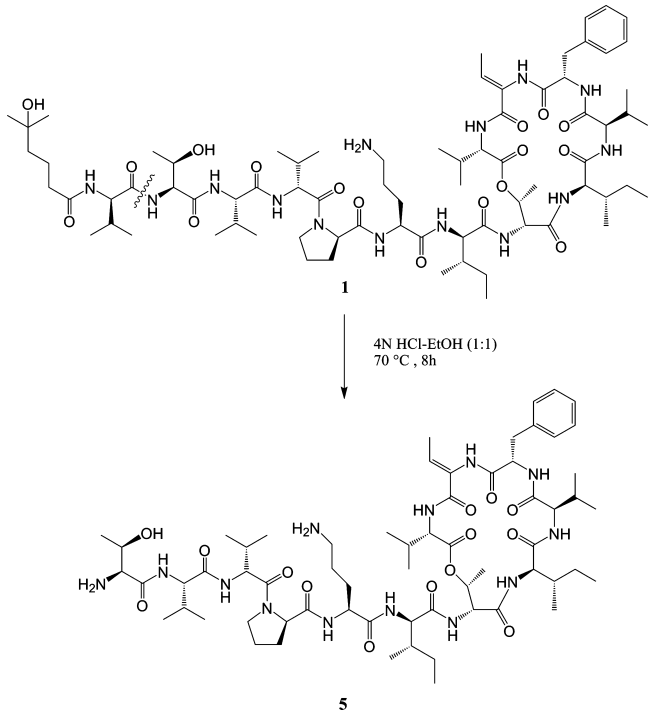

Figure 2. Mild acid hydrolysis of 5-OHKF (**1**) to fragment 1 (**5**) (the yield is 25%).

hydrolysis of KF (**3**) under the same conditions produced fragment 2, which has the same molecular weight as fragment 1.

Fragment 1 was analyzed by Marfey's method on LC-MS after total hydrolysis to amino acids (Figure 1C). Fragment 1 is KF less Val-5 and less 5-hydroxy-5-methylhexanoic acid. Val-5 must be D-Val because the D-Val peak is significantly more intense than the L-Val peak in the HPLC trace of intact 5-OHKF (**1**) relative to that of the fragment. We completely determined the absolute configuration of each amino acid moiety in 5-OHKF (**1**) by comparison with that of KF (**3**).

High-resolution ESIMS data furnished a molecular formula of $C_{45}H_{65}N_7O_{11}$ for norKA (**2**). This peptide is made up of seven amino acids, two D-Phe, two D-Leu, two L-Thr, and one L-Ser. Its ester linkage arises from the carbonyl group of L-serine and the hydroxy of L-threonine-2. The isobutyric acid, the fatty acid component, forms an amide with D-Phe-2 and represents a novel fatty acid component compared to other kahalalides. The structures of all amino acids were determined by 2D NMR methods including HMQC, HMBC, COSY, and TOCSY experiments. The amino acid sequence was determined by an HMBC experiment. The HMBC experiments provided a correlation between the NH of D-Phe-2 (δ_H 8.96) and the carbonyl of isobutyric acid (δ_C 180.0). There were also HMBC correlations between the NH of L-Thr-2 (δ_H 8.32) and the carbonyl of D-Phe-2 (δ_C 173.4), the NH of D-Leu-2 (δ_H 7.63) and the carbonyl of L-Thr-2 (δ_C 166.7), the NH of D-Phe-1 (δ_H 7.75) and the carbonyl of D-Leu-2 (δ_C 170.7), and the α-proton of L-Thr-1 (δ_H 3.81) and the carbonyl of D-Leu-1 (δ_C 174.4). The ester linkage was established by an HMBC correlation between the β-proton (δ_H 5.30) of L-Thr-2 and the carbonyl (δ_C 169.3) of L-Ser. The absolute configuration of the amino acids in norKA (**2**) was determined by Marfey's method. Both Thr and Ser residues are of L configuration, while the Phe and Leu residues are of D configuration. The only structure difference between norKA (**2**) and kahalalide A is the removal of a methylene (2-methylbutyric acid in kahalalide A to isobutyric acid in norKA).

Bioassay results showed that 5-OHKF (**1**) showed no antitumor, antifungal, or antibacterial activity but moderate in vitro antimalarial activity against D6 and W2 clones of *Plasmodium falciparum* with IC_{50} values of 1.5 and 1.2 μg/mL, respectively.[25] The aliphatic group in KF (**3**) is very important for its activity, and a minor change has a dramatic influence on its potency. KF (**3**) and isoKF (**4**) differ in only the aliphatic acid, but in vivo studies showed that **4** has enhanced efficacy against breast and prostate xenografts.[10] Our present results for 5-OHKF (**1**) support the conclusion that any inclusion of polar groups into the aliphatic chain in KF (**3**) will lead to decreased activity.[13] In 2006, two new natural KF analogues, kahalalide R and kahalalide S, were reported. The aliphatic group of kahalalide R is 7-methyloctanoic acid, which substitutes for the 5-methylhexanoic acid in KF (**3**), and the aliphatic group of kahalalide S is 5-hydroxy-7-methyloctanoic acid. The activity of kahalalide R is the same as that of KF (**3**); however, kahalalide S possesses little activity.[7]

NorKA (**2**) was tested within a group of marine natural products from diverse origin, using radio-receptor assays for its ability to inhibit the binding of [^{3}H]-BQ-123 and [^{3}H]-NPY to the endothelin ET_A (ET_A) and neuropeptide Y Y_1 (Y_1) receptors, respectively. Both receptors play an important role in vascular remodeling in cardiovascular diseases, and the Y_1 receptor is a promising target for novel treatments for anxiety and other psychiatric disorders.[15] In a preliminary screening of marine natural products (Table 3), 100 μM norKA (**2**) inhibited 82% of the specific binding of [^{3}H]-NPY to the Y_1 receptor, and it showed no inhibitory activity (only 4%) of the [^{3}H]-BQ-123 binding to the ET_A receptor. In this assay, at 100 μM norKA (**2**), 5,6-dibromo-*N*,*N*-dimethyltryptamine, 3-hydroxydihydrodiscorhabdin C, discorhabdins A and C, and sceptrin showed inhibitory activity only on the Y_1 receptor. To our knowledge, the selective ligands (agonist and antagonist) for the Y_1 receptor that are currently available are derivatives and fragments of the NPY molecule.[16] Furthermore, there are no Y_1 receptor antagonists or agonists derived from natural products. Our findings show interesting insights on structures developed by nature that can lead research on new selective ligands for the Y_1 receptor.

Table 1. NMR Spectroscopic Data (600 MHz, DMSO-d_6) for 5-OHKF (**1**)

amino acid	no.	δ_C, mult	δ_H (J in Hz)
L-Val-1	1	170.0, qC	(NH) 6.76, d (8.4)
	2	60.7, CH	3.82, t (9.6)
	3	29.9, CH	1.36, m
	4	19.2, CH_3	0.57, d (8.0)
	5	19.9, CH_3	0.61, m
(*Z*)-Dhb	1	163.9, qC	(NH) 9.71, s
	2	130.8, qC	
	3	130.5, CH	6.32, q (7.2)
	4	13.0, CH_3	1.25, m
L-Phe	1	171.9, qC	(NH)8.76, m
	2	56.3, CH	4.41, m
	3	36.7, CH_2	2.91, m
	4	137.5, qC	
	5,5′	129.9, CH	7.24, m
	6,6′	128.8, CH	7.24, m
	7	127.1, CH	7.17, m
D-Val-2	1	172.9, qC	(NH)7.59, d (8.4)
	2	56.4, CH	4.42, m
	3	33.1, CH	2.14, m
	4	17.1, CH_3	0.60, m
	5	20.3, CH_3	0.75, m
D-*allo*-Ile-1	1	170.8, qC	(NH) 8.78, d (8.4)
	2	57.8, CH	4.28, m
	3	38.7, CH	1.70, m
	4	26.3, CH_2	1.28,1.01, m,m
	5	15.2, CH_3	0.75, m
	6	12.1, CH_3	0.79, m
D-*allo*-Thr	1	169.2, qC	(NH) 8.51, d (7.8)
	2	57.0, CH	4.50, t (9.0)
	3	70.5, CH	4.93, m
	4	17.5, CH_3	1.05, d (6.0)
D-*allo*-Ile-2	1	171.8, qC	(NH) 7.87, m
	2	56.4, CH	4.31, m
	3	37.9, CH	1.67, m
	4	26.4, CH_2	1.26,1.01, m,m
	5	12.2, CH_3	0.77, m
	6	15.1, CH_3	0.75, m
L-Orn	1	172.0, qC	(NH) 7.94, d (7.8)
	2	51.8, CH	4.44, m
	3	30.9, CH_2	1.67, m
	4	24.0, CH_2	1.46, m
	5	38.9, CH_2	2.71, m
D-Pro	1	172.9, qC	
	2	60.1, CH	4.32, m
	3	30.1, CH_2	2.00, m
	4	25.1, CH_2	1.86,1.76, m
	5	47.8, CH_2	3.73,3.51, m
D-Val-3	1	170.5, qC	(NH) 8.01, d (7.2)
	2	56.6, CH	4.24, m
	3	30.4, CH	1.92, m
	4	19.9, CH_3	0.84, m
	5	19.2, CH_3	0.81, m
L-Val-4	1	171.3, qC	(NH) 7.56, d (9.0)
	2	58.1, CH	4.24, m
	3	31.4, CH	1.97, m
	4	18.3, CH_3	0.77, m
	5	19.9, CH_3	0.79, m
L-Thr	1	170.3, qC	(NH) 7.87, m
	2	58.5, CH	4.22, m
	3	66.9, CH	3.95, p
	4		4.83
	5	20.1, CH_3	0.97, d
D-Val-5	1	172.2, qC	(NH) 7.90, d (8.4)
	2	58.8, CH	4.19, m
	3	30.8, CH	1.93, m
	4	18.9, CH_3	0.82, m
	5	19.9, CH_3	0.82, m
5-Me-5-Hexol	1	173.2, qC	
	2	36.4, CH_2	2.10, m
	3	21.2, CH_2	1.46, m
	4	43.8, CH_2	1.27, m
	5	69.4, qC	
	6	29.9, CH_3	1.01, s
	7	29.8, CH_3	1.01, s

Table 2. NMR Spectroscopic Data (600 MHz, DMSO-d_6) for NorKA (**2**)

amino acid	no.	δ_C, mult	δ_H (J in Hz)
L-Ser	1	169.3, qC	(NH) 6.97, d (7.2)
	2	57.3, CH	3.91, m
	3	60.9, CH_2	3.41, 3.23, m
	4		OH 4.80
L-Thr-1	1	170.3, qC	(NH) 9.08, d (7.2)
	2	61.0, CH	3.81, d (6.0)
	3	65.3, CH	4.34, m
	4	21.1, CH_3	1.18, d (6.6)
	5		OH 5.08
D-Leu-1	1	174.4, qC	(NH) 7.21, m
	2	51.3, CH	4.85, m
	3	42.5, CH_2	1.36, 1.29, m
	4	24.8, CH	1.41, m
	5	23.3, CH_3	0.79, d (6.0)
	6	23.0, CH_3	0.76, d (6.0)
D-Phe-1	1	170.3, qC	(NH) 7.75, d (9.6)
	2	54.2, CH	4.70, dt (10.2, 4.8)
	3	38.8, CH_2	3.21, 2.71, dd (13.8,4.2)
	4	137.5, qC	
	5, 5′	129.5, CH	7.13, m
	6, 6′	128.7, CH	7.24, m
	7	127.2, CH	7.17, m
D-Leu-2	1	170.7, qC	(NH) 7.63, d (9.6)
	2	53.2, CH	4.24, q (9.6)
	3	41.9, CH_2	1.12, m
	4	24.4, CH	0.94, m
	5	22.1, CH_3	0.66, d (6.6)
	6	22.8, CH_3	0.60, d (6.6)
L-Thr-2	1	167.7, qC	(NH) 8.30, d (9.6)
	2	56.0, CH	4.30, dd (9.6,1.8)
	3	69.5, CH	5.30, dt (6.6,1.8)
	4	15.8, CH_3	0.54, d (6.6)
D-Phe-2	1	173.4, qC	(NH) 8.96, d (3.0)
	2	55.7, CH	4.88, m
	3	37.2, CH_2	2.94,2.85, dd (7.2, 6.0)
	4	137.0, qC	
	5, 5′	129.0, CH	7.12, m
	6, 6′	128.6, CH	7.17, m
	7	127.0, CH	7.18, m
isobutyric acid	1	179.9, qC	
	2	34.0, CH	2.57, m
	3	20.5, CH_3	1.03, d (7.8)
	4	19.4, CH_3	1.02, d (7.2)

Further studies will be carried out in due time to define the nature of these interactions.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a JASCO DIP 370 digital polarimeter. UV and IR spectra were respectively obtained using a Perkin-Elmer Lambda 3B UV/vis spectrophotomer and an AATI Mattson, Genesis Series FTIR. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 using Varian NMR spectrometers operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C. The HRESIMS spectra were measured using an Agilent MS TOF 1100 series with electrospray ionization.

Algal Material. A sample (20 kg wet weight) was collected from the waters of Kahala Bay near Black Point, Oahu, during March, April, and May of 2003. The collected sample is identical to voucher specimen #9507019 deposited at the Suntory Institute for Bioorganic Research, Osaka, Japan.

Extraction and Isolation. The freeze-dried sample was extracted three times with EtOH. The EtOH extracts were combined, concentrated, and then separated using VLC on a silica column with a stepwise gradient: hexanes, hexanes/EtOAc (1:1), EtOAc, EtOAc/MeOH (1:1), MeOH, and MeOH/H_2O (1:1). LCMS analysis of the fractions indicated that the EtOAc/MeOH (1:1) fraction (20 g) contained kahalalide-related depsipeptides including KF (**4**). This fraction was concentrated and further purified by HPLC using an RP C8 column and a gradient elution from H_2O/MeCN (1:1), yielding 5-OHKF (**1**) (15 mg, 7.5×10^{-5}% dry wt), norKA (**2**) (8.5 mg, 4.3×10^{-5}% dry wt), KF (**3**) (106.7 mg, 5.3×10^{-4}% dry wt), and isoKF (**4**) (53.3 mg, 2.7×10^{-4}% dry wt).

Table 3. Preliminary Screening of Marine Natural Products for Their Ability to Inhibit the Specific Binding of [^{3}H]-BQ-123 and [^{3}H]-NPY to the ET_A and Y_1 Receptors, Respectively[a]

		ET_A		Y_1	
no.	name	100 μg/mL	10 μg/mL	100 μg/mL	10 μg/mL
1	5-bromo-*N*,*N*-dimethyltryptamine[19]	89 ± 0 (2)[b]	97 ± 8 (2)	76 ± 3 (2)	100 ± 0 (2)
2	2-(13-carboxy-14,15-diacetoxyhexadecanyl)-2-penten-4-olide[20]	79 ± 0 (2)	98 ± 2 (2)	86 ± 1 (2)	100 ± 0 (2)
3	NorKA (**2**)	96 ± 0 (2)	99 ±1 (2)	18 ± 2 (2)	100 ±0 (2)
4	5,6-dibromo-*N*,*N*-dimethyltryptamine[19]	62 ± 5 (2)	100 ± 0 (2)	17 ± 3 (2)	100 ± 0 (2)
5	3-hydroxydihydrodiscorhabdin C[21]	100 ± 0 (2)	100 ± 0 (2)	29 ± 7 (2)	82 ± 2 (2)
6	discorhabdin C[21]	68 ± 1 (2)	94 ± 1 (2)	4 ± 1 (2)	73 ± 2 (2)
7	discorhabdin A[21]	79 ± 0 (2)	100 ± 0 (2)	40 ± 11 (2)	90 ± 2 (2)
8	plakortide N[22]	56 ± 6 (3)	85 ± 4 (3)	78 ± 3 (2)	100 ± 0 (2)
9	stevensine[24]	68 ± 2 (2)	78 ± 9 (2)	81 ± 4 (2)	100 ± 0 (2)
10	oridin[24]	79 ± 7 (2)	100 ± 0 (2)	81 ± 6 (2)	100 ± 0 (2)
11	plakortide F[23]	88 ± 0 (2)	100 ± 0 (2)	100 ± 0 (2)	100 ± 0 (2)
12	plakortide F (free acid)[23]	68 ± 7 (2)	100 ± 0 (2)	56 ± 0 (2)	100 ± 0 (2)
13	sceptrin[24]	80 ± 0 (2)	100 ± 0 (2)	12 ± 2 (2)	95 ± 1 (2)

[a] For the neuropeptide Y Y_1 receptor binding assay, the endogenous ligand NPY and the selective nonpeptide Y_1 antagonist BIBP3226 were used as positive controls, giving IC_{50} values of 1.2 and 8.8 nM, respectively. For the endothelin ET_A receptor binding assay, the endogenous ligands endothelin-1 and endothelin-2 were used as positive controls, giving IC_{50} values of 47 and 288 pM, respectively. [b] The results are expressed in percentage of specific radioligand binding (the number of independent experiments is given in parentheses).

5-OHKF (1): colorless, amorphous solid; $[\alpha]_D^{25}$ −12 (*c* 1.15, MeOH); UV (MeOH) λ_{max} 204 nm.^{1}H NMR (Table 1, 600 MHz, DMSO-d_6); ^{13}C NMR (Table 1, 150 MHz); HRESIMS *m*/*z* 1494. 9313 $[M + H]^+$ (calcd for $C_{75}H_{124}N_{14}O_{17,}$ 1494.9347).

NorKA (2): colorless, amorphous solid; $[\alpha]_D^{25}$ −16 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 204 nm; ^{1}H NMR (Table 1, 600 MHz, DMSO-d_6); ^{13}C NMR (Table 1, 150 MHz); HRESIMS *m*/*z* 880.4750 $[M + H]^+$ (calcd for $C_{45}H_{65}N_7O_{11}$, 880.4742).

Marfey's Analysis of Kahalalides.[18] A sample of 0.1 to 0.2 mg of peptide was hydrolyzed by treatment with 2 mL of 6 N HCl and left at 120 °C for 24 h in a sealed ampule. The hydrolysate was dried under N_2. In an Eppendorf tube, 3.6 μmol of a 1% acetone solution of FDAA (*N*-(5-flouro-2,4-dinitrophenyl)-L-alaninamide) and 20 μmol of a 1 M solution of $NaHCO_3$ were added to 2.5 μmol of amino acid. The reaction mixture was heated with frequent shaking over a hot plate at 40 °C for 1 h and then cooled to RT. Then, 20 μmol of 2 M HCl and 1 mL of MeOH were added to the reaction mixture. The samples were analyzed by LC-MS, by which molecular weights and retention times were compared with those of standard amino acids. Acetonitrile/water containing 0.05% HCOOH was used as the mobile phase under a linear gradient elution mode (MeCN, 10−60%, 40 min for 5-OHKF, 70 min for norKA) at a flow rate of 0.6 mL/min. Different conditions were used to analyze Phe and Leu in norKA (**2**): MeOH/water containing 0.05% HCOOH was used as the mobile phase under a linear gradient elution mode (MeOH, 20−85%, 90 min for norKA) at a flow rate of 0.8 mL/min. A mass range of *m*/*z* 100−3000 was covered with a scan time of 1 s, and data were collected in the positive ion mode. UV detection at 200−400 nm was performed by photodiode array detection.

Acid Hydrolysis of 5-OHKF (1) and KF (3) to Acquire Fragment 1. 5-OHKF (5 mg) was hydrolyzed with 4 N HCl/EtOH (1:1) at 70 °C for 8 h, and the fragments were separated by HPLC with solvents A (H_2O + 0.1% HCOOH) and B (MeCN + 0.1% HCOOH) and gradients thereof: A 70% to 60% in 10 min, linear 60% in 10 min. The separation yielded fragment 1. Fragment 1 is a peptide, KF-[5-MeHex-Val-5] (HRESIMS *m*/*z* $[M + H]^+$, 1266.7834; calcd for $C_{63}H_{104}N_{13}O_{14}$, 1266.7826). The mild acid hydrolysis of KF (**3**) under the same condition produced fragment 2, which has the same molecular weight as fragment 1.

[^{3}H]-NPY and [^{3}H]-BQ-123 Binding Assays.[17] For both assays, human neuroblastoma SK-N-MC cells (HTB-10, ATCC) naturally expressing the Y_1 and the ET_A receptors were grown in 24-well plates until the cell layer became confluent. Before the experiment, the cells were washed with 0.5 mL of PBS. Then 0.4 mL of binding buffer (DMEM containing 0.5% BSA for NPY binding and 0.1% BSA for BQ-123 binding) was added, and the mixture was left for 15 min at room temperature. The plate was preincubated with 50 μL of buffer (for the total binding) or of test substance (for competition binding) or 0.1 μM of either human NPY or human endothelin 1 (for nonspecific binding). Incubation started after addition of 50 μL of [^{3}H]-NPY or [^{3}H]-BQ-123 (final concentration 1 nM). Preincubation and incubation was for 30 min at 37 °C. At the end of the 60 min the plate was placed on ice, the binding buffer was removed, and the cells were washed with 0.5 mL of PBS buffer. To measure total cell binding of the radioligand, 0.2 mL of 1 M NaOH was added to the 24 wells and left for 30 min. Then 0.3 mL of distilled H_2O was added, and the resulting solution was placed into scintillation vials with scintillation liquid (Ready Safe, Beckman Coulter) and the radioactivity counted on a Beckman Coulter model LS 6500.

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Supporting Information Available: HRESIMS and 1D and 2D NMR spectra of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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