Semiplenamides A−G, Fatty Acid Amides from a Papua New Guinea Collection of the Marine Cyanobacterium *Lyngbya semiplena*

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Semiplenamides A (**1**) to G (**7**), a series of new anandamide-like fatty acid amides, were isolated from a 1997 Papua New Guinea collection of the marine cyanobacterium *Lyngbya semiplena*. The planar structures of these lipids were determined using standard 1D and 2D NMR methods. The relative stereochemistry of the aliphatic portion of the new metabolites was deduced from 1D NOE data and ^{1}H-decoupling experiments, while the absolute stereochemistry of the amino alcohol moieties was assigned by chemical derivatization and chiral GC−MS methods. All of these new metabolites displayed toxicity in the brine shrimp model system, while semiplenamides A, B, and G showed weak affinity for the rat cannabinoid CB_1 receptor and semiplenamide A was a moderate inhibitor (IC_{50} = 18.1 μM) of the anandamide membrane transporter (AMT).

Marine cyanobacteria have emerged over the past few years as one of the richest groups of marine organisms for their bioactive and structurally complex natural products. Particularly prevalent among these natural products is a rich elaboration upon a cyclic peptide template using a diversity of standard as well as modified amino acids.[1] However, a growing trend from our investigations of these life forms is the production of fatty acid amides that combine unusual fatty acids with a variety of "biogenic amines". Examples include the hermitamides[2] and grenadamide,[3] two categories of toxic malyngamide-type natural products from the marine cyanobacterium *Lyngbya majuscula*. In this regard, they structurally resemble anandamide and other endocannabinoids of importance to mammalian physiology,[3] a finding that has stimulated our investigation of their pharmacological properties in several relevant bioassays.

Results and Discussion

Collections of a shallow water (1−3 m) strain of *L. semiplena* were made in Wewak Bay, Papua New Guinea. Preliminary bioassay of the crude organic extract showed good activity in the brine shrimp toxicity model at 10 ppm.[4] Guided by this assay, the natural products semiplenamides A (**1**) to G (**7**) were isolated and purified by sequential vacuum liquid chromatography (VLC) and HPLC in 0.1−1.5 mg/g crude extract yield.

Semiplenamide A (**1**) showed an $[M + H]^+$ peak at *m/z* 366.3372 for a molecular formula of $C_{23}H_{44}NO_2$ by HR-FABMS (three degrees of unsaturation). The structure of **1** was established mainly from analysis of 1D (^{1}H, ^{13}C, and NOE) and 2D NMR spectra (COSY, HSQC, and HMBC). The ^{1}H NMR spectrum of **1** was indicative of a fatty acid amide-type metabolite, with the presence of proton signals for a long aliphatic chain in the δ 1.25−1.35 envelope (18H), and a poorly defined terminal $-CH_3$ triplet (δ 0.88), as well as a broad amide singlet (δ 6.25) (Table 1). The three degrees of unsaturation were accounted for by an amide carbonyl carbon resonance (δ 171.5; IR absorption at 1659 cm^{-1}) and two olefinic bonds (δ 130.7, 137.4, 129.2, 132.3). In the ^{1}H NMR spectrum of **1**, a deshielded methyl singlet at δ 1.89 (H_3-21) was assigned to a vinyl methyl moiety because it showed two- and three-bond HMBC correlations to two olefinic carbons (δ 130.7, C-2 and 137.4, C-3) as well as to the amide carbonyl carbon (δ 171.5, C-1). An ethanolamine moiety could be deduced from two mutually coupled (COSY) midfield proton signals at δ 3.54 (dt, 6.0, 4.0 Hz) and 3.82 (t, 5.0 Hz), each of which integrated to two protons and which were correlated by HSQC with two midfield carbon resonances at δ 43.6 and 63.8, respectively. The former proton signal was also coupled to the broadened amide NH proton (δ 6.25). The relative position of the two olefinic bonds was determined by HMBC (Table 1), which showed two- and three-bond coupling from two mutually coupled methylene resonances (δ 2.23, H_2-4; 2.14, H_2-5) to all four olefinic carbons. Additionally, the olefinic proton resonance at δ 6.45 (H-3) showed a three-bond correlation to the amide carbonyl carbon resonance (δ 171.5, C-1), thus positioning this partial structure relative to the amide motif. The linkage of the ethanolamine moiety to this amide was shown by an HMBC connectivity between the midfield methylene protons (δ 3.54, H_2-1′) and the C-1 carbonyl. Combining these partial structures accounted for all atoms and thus completed the planar structure of semiplenamide A (**1**).

The geometry of the $\Delta^{6,7}$ disubstituted olefin was determined by a ^{1}H NMR decoupling experiment in which the signals for the associated protons were simplified by decoupling H_2-5 and H_2-8 (δ 2.14 and 1.98, respectively). From this latter spectrum it was possible to measure $^3J_{H-6/H-7}$ as 15.0 Hz and thus to assign the C-6 olefin as *trans*. The geometry of the $\Delta^{2,3}$ olefin in **1** was assigned using 1D NOE experiments. Reciprocal NOE enhancements were seen from the vinyl methyl protons (δ 1.88, H_3-21) to the methylene protons (δ 2.23, H_2-4) as well as to the amide NH proton, thus defining the geometry of the C-2 olefin as *E*.

The ^{1}H NMR data for semiplenamide B (**2**) were similar to those obtained for **1**, except for an additional methyl singlet at δ 2.08. HRFABMS data for **2** showed an $[M + H]^+$ ion at *m/z* 408.3473, consistent with a molecular

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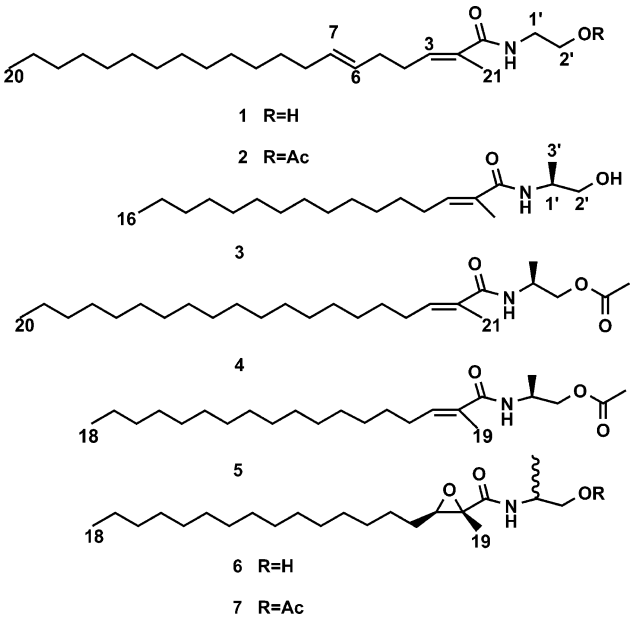
Table 1. ^{1}H and ^{13}C NMR Assignments for Semiplenamides A (**1**) and B (**2**) ($CDCl_3$, 300 MHz)

	semiplenamide A (**1**)			semiplenamide B (**2**)		
atom number	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1		171.5[a]			169.8	
2		130.7			131.2	
3	6.45 (t, 7.0)	137.4	C-21, C-1	6.36 (t, 7.0)	136.4	C-1, C-21
4	2.23 (dm, 7.0)	29.0	C-2, C-3, C-5, C-6	2.23 (dm, 7.0)	29.0	C-3, C-5
5	2.14 (dm, 7.0)	32.3	C-3, C-4, C-6, C-7	2.14 (dm, 7.0)	32.3	C-3, C-4, C-7
6	5.42 (m)	129.2	C-8	5.42 (m)	129.2	C-8
7	5.46 (m)	132.3	C-5, C-8	5.46 (m)	132.3	C-5, C-8
8	1.98 (dm, 7.5)	33.4	C-6, C-7, C-9	1.98 (dm, 7.5)	33.4	C-6, C-7, C-9
9	1.33 (m)	30.6	C-8, C-10	1.33 (m)	30.6	C-8, C-10
10−17	1.27 (m)	30.3	C-9	1.27 (m)	30.3	C-9
18	1.27 (m)	32.8		1.27 (m)	32.8	
19	1.30 (m)	23.8		1.30 (m)	23.8	
20	0.88 (t, 6.5)	14.9	C-18, 19	0.88 (t, 6.5)	14.9	C-18, C-19
21	1.89 (s)	12.5	C-1, C-2, C-3	1.84 (s)	13.1	C-1, C-2, C-3
1′	3.54 (dt, 6.0, 4.0)	43.6	C-1, C-2′	3.58 (dt, 6.0, 4.0)	39.6	C-2′, C-1
2′	3.82 (t, 5.0)	63.8		4.21 (t, 5.0)	63.8	C-1′, -*C*(O)CH_3
OAc				2.08 (s)	21.30	
					171.5[a]	
N-H	6.25 (br s)			6.03 (br s)		

[a] Data obtained from HMBC spectrum.



formula of $C_{25}H_{46}NO_3$, indicating a structure with four degrees of unsaturation. In concurrence with the ^{1}H NMR data (Table 1), the additional degree of unsaturation was attributed to a terminal acetate motif linked to the ethanolamine; this was confirmed by ^{1}H, ^{13}C, and 2D NMR data (Table 1).

HRFABMS of semiplenamide C (**3**) gave an $[M + H]^+$ peak at 326.3053, yielding a molecular formula of $C_{20}H_{40}NO_2$ with two degrees of unsaturation. As for **1**, the planar structure of **3** was elucidated using an assemblage of 1D and 2D NMR experiments (COSY, HSQC, and HMBC). The ^{1}H NMR spectrum of **3** had several features strikingly similar to that of **1** (Table 2), including a deshielded olefinic ^{1}H signal (δ 6.38), a broad amide NH resonance (δ 5.81), and a deshielded methyl singlet (δ 1.84), as well as proton signals for a long aliphatic chain in the δ 1.25−1.35 envelope and a terminal methyl triplet (δ 0.88). However, semiplenamide C (**3**) lacked resonances for the $\Delta^{6,7}$ double bond found in **1** and **2** and was therefore a more saturated derivative. An additional difference between the ^{1}H NMR spectra for **1** and **3** was the presence of a CH resonance at δ 4.13 (H-1′) and CH_2 resonances at δ 3.72 and 3.58 (H_2-2′) in **3** instead of the two CH_2 resonances at δ 3.54 (H_2-1′) and 3.82 (H_2-2′) in the spectrum of **1**. This methine resonance (δ 4.13 m) was coupled to two mutually coupled methylene resonances (δ 3.72 dd, 3.58 dd), a broad amide NH resonance (δ 5.81), and a methyl doublet (δ 1.22) in the COSY spectrum of **3**. Two- and three-bond HMBC correlations (Table 2) from H_3-3′ to C-1′ and C-2′ confirmed the connectivity deduced from COSY and thus established the structure of an alaninol moiety in **3** versus the ethanolamine moiety in **1**. An L configuration for this group was determined by chiral GC−MS comparison of the corresponding PFPA-Ac ester derivative with similarly prepared alaninol standards.[5]

Table 2. ^{1}H and ^{13}C NMR Assignments for Semiplenamide C (**3**) ($CDCl_3$, 400 MHz)

atom number	δ_H	δ_C	HMBC
1		170.9[a]	
2		131.1[a]	
3	6.38 (t, 7.2)	137.7	C-1, C-17, C-4
4	2.13 (dm, 7.2)	29.1	C-3, C-5, C-2
5	1.41 (m)	29.4	C-4
6−13	1.25−1.35 (m)	30.1−29.7	
14	1.25 (m)	32.0	
15	1.31 (m)	22.8	
16	0.88 (t, 7.0)	14.8	C-14, C-15
17	1.84 (s)	12.8	C-1, C-2, C-3
1′	4.13 (m)	48.7	
2a′	3.58 (dd, 5.5, 3.5)	68.3	C-1′, C-3′
2b′	3.72 (dd, 5.5, 6.0)	68.3	C-1′, C-3′
3′	1.22 (d, 7.0)	17.3	C-1′, C-2′
N-H	5.81 (br s)		

[a] Data obtained from HMBC spectrum.

HRFABMS data for semiplenamide D (**4**) showed an $[M + H]^+$ ion at *m*/*z* 424.3802, consistent with a molecular formula of $C_{26}H_{50}NO_3$, indicating a structure with three degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra for **4** displayed signals very similar to those of **3** (Table 3), except for an additional methyl singlet (δ_H 2.08, δ_C 21.2). Detailed analysis of the ^{13}C and 2D NMR data assigned this additional methyl signal to a terminal acetate motif linked to the alaninol (as in **2**), which also accounted for the additional degree of unsaturation. Consistent with HRFABMS data, compounds **3** and **4** also differed in the lengths of their "fatty acid" chains (16 and 20 carbons, respectively).

While the NMR data for semiplenamide E (**5**, Table 3) were essentially identical to those obtained for **4**, integra-

Table 3. ^{1}H and ^{13}C NMR Assignments for Semiplenamides D (**4**) and E (**5**) ($CDCl_3$, 400 MHz)

	semiplenamide D (**4**)			semiplenamide E (**5**)		
atom number	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1		169.3[a]			169.3	
2		130.9[a]			130.9	
3	6.33 (t, 7.0)	137.1		6.33 (t, 7.0)	137.1	C-1, C-2, C-19
4	2.13 (dm, 7.0)	28.8		2.11 (dm, 7.0)	28.8	C-3, C-5
5	1.41 (m)	29.2		1.41 (dm, 7.0)	29.2	C-4
6−15	1.25 (m)	30.1		1.25 (m)	30.1	
16	1.25 (m)	32.3		1.25 (m)	32.3	
17	1.29 (m)	23.1		1.29 (m)	23.1	
18	1.29 (m)	23.1		0.88 (t, 6.0)	14.5	C-17, C-16
19	1.29 (m)	23.1		1.82 (s)	13.0	C-1, C-3
20	0.88 (t, 6.0)	14.5	C-19, C-18			
21	1.82 (s)	13.0	C-1, C-2, C-3			
1′	4.33 (m)	45.1		4.33 (m)	45.1	C-2′, C-3′
2a′	4.20 (dd, 5.5, 5.5)	67.5		4.20 (dd, 5.5, 5.8)	67.5	C-1′, C-3′, *C*OCH$_3$
2b′	4.05 (dd, 5.5, 4.0)	67.5		4.05 (dd, 5.5, 4.0)	67.5	C-1′, C-3′, *C*OCH$_3$
3′	1.20 (d, 7.0)	17.8	C-1′, C-2′	1.20 (d, 7.0)	17.8	C-1′, C-2′
OAc	2.08(s)	21.2		2.08 (s)	21.2	
		171.8[a]			171.8	
N-H	5.82 (br s)			5.82 (br s)		

[a] Data obtained from HMBC spectrum.

Table 4. ^{1}H and ^{13}C NMR Assignments for Semiplenamides F (**6**) and G (**7**) ($CDCl_3$, 400 MHz)

	semiplenamide F (**6**)			semiplenamide G (**7**)		
atom number	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1		173.2[a]			173.2[a]	
2		60.3			60.4	
3	2.89 (t, 6.2)	64.4	C-4	2.84 (t, 6.2)	64.4	C-1, C-2, C-19
4	1.58 (m)	28.4	C-3	1.58 (m)	28.4	C-3, C-5
5	1.26 (m)	32.0		1.26 (m)	32.0	C-4
6−15	1.27 (m)	30.2		1.27 (m)	30.2	
16	1.30 (m)	23.1		1.30 (m)	23.1	
17	1.41 (m)	26.6		1.41 (m)	26.6	
18	0.88 (t, 6.0)	14.5	C-17	0.88 (t, 6.0)	14.5	C-17, C-16
19	1.55 (s)	13.4	C-1, C-2, C-3	1.51 (s)	13.4	C-1, C-3
1′	4.01 (m)	48.1	C-1, C-2′, C-3′	4.20 (m)	45.1	C-2′, C-3′
2a′	3.68 (dd, 6.0, 6.5)	67.5	C-1′, C-3′	4.04 (dd, 2.0, 1.0)	67.5	C-1′, C-3′
2b′	3.58 (dd, 4.0, 6.5)	67.5	C-1′, C-3′	4.04 (dd, 2.0, 1.0)	67.5	C-1′, C-3′
3′	1.16 (d, 7.2)	17.8	C-1′, C-2′	1.13 (d, 7.2)	17.8	C-1′, C-2′
OAc				2.08 (s)	21.2	
					171.8	
N-H	6.49 (br s)			6.40 (br s)		

[a] Data obtained from HMBC spectrum.

tion of the aliphatic chain proton envelope was slightly less for **5** than for **4** (24H vs 28H), suggesting that **5** had a shorter aliphatic chain. HRFABMS data for **5** showed an $[M + H]^+$ ion at *m/z* 396.3584, consistent with a molecular formula of $C_{24}H_{46}NO_3$ and an 18-carbon chain, in contrast to the 20-carbon chain of **4**.

HRFABMS of semiplenamide F (**6**) gave an $[M + H]^+$ peak at 370.3803, yielding a molecular formula of $C_{22}H_{44}NO_3$ with two degrees of unsaturation. In the ^{1}H NMR spectrum of **6** (Table 4), chemical shifts of the alaninol residue protons were apparent as well as a broad amide NH resonance (δ 6.49). Notably, the deshielded olefinic proton resonance and vinyl methyl singlet in the spectra of **1**−**5** were replaced by an upfield methine triplet (δ 2.89, $J = 6.2$ Hz) and methyl singlet (δ 1.55). The appearance of two midfield signals (δ 60.3, 64.4) in the ^{13}C NMR spectrum, taken together with the HMBC data for **6** (Table 4), led to the assignment of an epoxide ring at C-2 (δ 60.3) and C-3 (δ 64.4).

The ^{1}H NMR data for semiplenamide G (**7**) were similar to those obtained for **6** (Table 4), with the exception again of an additional methyl singlet at δ 2.08. HRFABMS data for **7** showed an $[M + H]^+$ ion at *m/z* 412.3427, consistent with a molecular formula of $C_{24}H_{46}NO_4$, indicating a structure with three degrees of unsaturation. From examination of the HMBC data as well as the ^{13}C NMR spectrum (Table 4), the additional degree of unsaturation in **7** was attributed to a terminal acetate group linked to the alaninol motif. While in $CDCl_3$ the H_3-19 and H_2-4 signals were overlapped, in MeOH-d_4 they were resolved and a 1D NOE irradiation of H_3-19 produced a significant enhancement in the H_2-4 resonance (δ 1.58). Therefore, a relative stereochemistry of 2*S**, 3*R** was assigned to the epoxide in **7**. Because the optical rotation values for semiplenamides F and G (**6**, **7**) were very similar (−5.0° and −3.0°, respectively), we conclude that they likely possess the same relative and absolute stereochemistry. However, while it is likely that the alaninol moieties of **6** and **7** are of the same L-stereochemistry as in semiplenamides C−E (**3**−**5**), the small amount isolated of these compounds precluded this assignment.

The semiplenamides were evaluated for their biological activity in several systems. In the brine shrimp (*Artemia salina*) toxicity assay,[4] semiplenamides A (**1**) to G (**7**) showed LD_{50} values of 1.4, 2.5, 1.5, 18, 19, 1.4, and 2.4 μM, respectively. Semiplenamides E (**5**) and G (**7**) are structurally very close except for the replacement of the olefin moiety in **5** by an epoxide ring in **7**; however, the LD_{50} value of **5** is 8-fold higher than that of **7**. Apparently, the epoxide ring produces an enhanced toxicity in this series of metabolites.

Due to the structural resemblance of the novel ethanolamide derivatives with anandamide (*N*-arachidonoylethanolamine), an endogenous agonist of cannabinoid receptors,[6] we tested them on the best characterized proteins of the endocannabinoid system: (1) the "central" cannabinoid CB_1 receptors; (2) the anandamide membrane transporter (AMT), which is responsible for anandamide cellular uptake; and (3) the fatty acid amide hydrolase (FAAH), which catalyzes anandamide hydrolysis.[7] When the semiplenamides were tested for their capability to displace the high-affinity CB_1 ligand [^{3}H]SR141716A from cannabinoid CB_1 receptors in rat brain membranes, three semiplenamides (A (**1**), B (**2**), and G (**7**)) exhibited some affinity. The K_i values were 19.5 ± 7.8, 18.7 ± 4.6, and 17.9 ± 5.2 for compounds A, B, and G, respectively (means ± SD, $n = 3$). Under the same conditions, anandamide exhibited a K_i = 0.4 μM, and therefore, these three *Lyngbya* metabolites should be considered as weak CB_1 agonists. This was not surprising because CB_1 ligand recognition requires the presence of at least one *cis* double bond situated at the middle of the fatty acid carbon chain, indicating a preference for ligands whose hydrophobic tail can adopt a bent U-shaped conformation.[8] Moreover, fatty acid ethanolamides need to contain at least three homoconjugated double bonds in order to interact in an optimal way with CB_1 receptors.[6] However, it was interesting to note that (1) semiplenamide B (**2**) was as active as its nonacetylated analogue, semiplenamide A (**1**), in agreement with the previous finding that the 2′-hydroxy group in acylethanolamides is not necessary for the interaction with CB_1 receptors;[6] (2) semiplenamide G (**7**) was more potent than its nonepoxide analogue, compound E (**5**); this is the first time that the affinity for CB_1 receptors of a 2,3-epoxide-acyl-ethanolamide derivative has been investigated.

Next, we tested the effect of the novel compounds on [^{14}C]-anandamide hydrolysis, by using membranes from mouse N18TG2 neuroblastoma cells, which express high levels of FAAH (K_m for anandamide = 15 μM).[9] No appreciable inhibitory effect was found for any of the tested compounds. This was not surprising because previous experiments had shown that both 2-methyl and 1′-methyl groups on anandamide lessen its quality as a substrate or inhibitor of FAAH.[6]

Finally, we tested the effect of the semiplenamides on the uptake of [^{14}C]anandamide by intact RBL-2H3 cells, a cell type in which an anandamide membrane transporter (AMT) has been partially characterized (K_m for anandamide ranges from 9.3 to 33 μM).[10,11] Only semiplenamide A inhibited the AMT (IC_{50} = 18.1 ± 3.2 μM). Although more potent AMT inhibitors have been described in the literature (with IC_{50} values ranging between 0.8 and 10 μM), semiplenamide A (**1**) is the first of this class of inhibitors to contain both 6(*E*) and 2-methyl-2(*Z*) double bonds. As these two features should confer increased metabolic stability, the discovery of semiplenamide A extends the possible chemical features that acylethanolamide derivatives might possess in order to inhibit anandamide cellular uptake. In conclusion, these data support the importance of natural products as a possible reservoir for new molecules capable of interacting with proteins of the endocannabinoid system.[12]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. IR and UV spectra were recorded on Nicolet 510 and Beckman DU640B spectrophotometers, respectively. NMR spectra were recorded on Bruker Avance DPX 400 MHz and Bruker Avance 300 MHz spectrometers with the solvent $CDCl_3$ used as an internal standard (δ_H at 7.26, δ_C at 77.4). Mass spectra were recorded on a Kratos MS50TC mass spectrometer. Chiral GC−MS analysis was accomplished on a Hewlett-Packard gas chromatograph 5890 Series II with a Hewlett-Packard 5971 mass selective detector using an Alltech capillary column (CHIRASIL-VAL phase 25 m × 0.25 mm). HPLC isolations were performed using a Waters 515 HPLC pump and Waters 996 photodiode array detector.

Collection. The marine cyanobacterium *Lyngbya semiplena* (voucher specimen available from WHG as collection number PNGE12-7Dec99-3) was collected from shallow waters (1−3 m) in Wewak Bay, Papua New Guinea, on December 7, 1999. The material was stored in 2-propanol at −20 °C until extraction.

Extraction and Isolation. Approximately 138 g (dry wt) of the alga was extracted repeatedly with CH_2Cl_2/MeOH (2:1) to produce 3.05 g of crude organic extract. The extract (3.0 g) was fractionated by silica gel vacuum liquid chromatography using a stepwise gradient solvent system of increasing polarity starting from 10% EtOAc in hexanes to 100% MeOH. Fractions eluting with 50%, 65%, 75%, and 100% EtOAc in hexanes were found to be the most active at 10 ppm in the brine shrimp toxicity assay. These fractions were further chromatographed on Mega Bond RP_{18} solid-phase extraction (SPE) cartridges using a stepwise gradient solvent system of decreasing polarity starting from 80% MeOH in H_2O to 100% MeOH. The most active fractions after SPE (85% toxicity at 1 ppm to brine shrimp) were then purified by HPLC [Phenomenex Sphereclone 5 μm ODS (250 × 10.00 mm), 9:1 MeOH/H_2O, detection at 211 nm], giving compounds **1** (1.3 mg), **2** (0.5 mg), **3** (4.5 mg), **4** (0.3 mg), **5** (2.5 mg), **6** (0.3 mg), and **7** (2.0 mg).

Semiplenamide A (1): white amorphous solid; UV (MeOH) λ_{max} 206 nm (ϵ 5100); IR (neat) 3330, 2918, 2850, 1659, 1616, 965 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* [M + H]$^+$ 366.3372 (calcd for $C_{23}H_{44}NO_2$, 366.3375).

Semiplenamide B (2): colorless oil; UV (MeOH) λ_{max} 203 nm (ϵ 5900); IR (neat) 3389, 2920, 2851, 1741, 1660, 966 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* [M + H]$^+$ 408.3473 (calcd for $C_{25}H_{46}NO_3$, 408.3469).

Semiplenamide C (3): white amorphous solid; $[\alpha]^{26}_D$ −5.0° (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} 213 nm (ϵ 6500); IR (neat) 3282, 2915, 2847, 1658, 1622, 966 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRFABMS *m/z* [M + H]$^+$ 326.3053 (calcd for $C_{20}H_{40}NO_2$, 326.3047).

Semiplenamide D (4): white amorphous solid; $[\alpha]^{26}_D$ −10.6° (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} 207 nm (ϵ 5200); IR (neat) 3279, 2916, 2849, 1721, 1622, 955 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; HRFABMS *m/z* [M + H]$^+$ 424.3802 (calcd for $C_{26}H_{50}NO_3$, 424.3814).

Semiplenamide E (5): white amorphous solid; $[\alpha]^{26}_D$ −7.1° (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} 207 nm (ϵ 4300); IR (neat) 3280, 2916, 2849, 1719, 1623, 966 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; HRFABMS *m/z* [M + H]$^+$ 396.3584 (calcd for $C_{24}H_{46}NO_3$, 396.3578).

Semiplenamide F (6): white amorphous solid; $[\alpha]^{26}_D$ −5.0° (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} 205 nm (ϵ 5500); IR (neat) 3295, 2916, 2849, 1646, 1535, 1080 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 4; HRFABMS *m/z* [M + H]$^+$ 370.3803 (calcd $C_{22}H_{44}NO_3$,370.3808).

Semiplenamide G (7): white amorphous solid; $[\alpha]^{26}_D$ −3.0° (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max} 207 nm (ϵ 6100); IR (neat) 3283, 2917, 2849, 1723, 1650, 1539, 1273 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 4; HRFABMS *m/z* [M + H]$^+$ 412.3427 (calcd for $C_{24}H_{46}NO_4$, 412.3428).

Chiral GC−MS Analyses of the Alaninol Moiety in 3. Approximately 0.2 mg of compound **3** was hydrolyzed with 6 N HCl at 110 °C (20 h). The hydrolysate was evaporated to dryness and resuspended in HCl (0.2 N, 100 μL) and heated (110 °C, 5 min). The residue was reduced to dryness using a stream of N_2, and the reaction mixture was esterified with acetyl chloride (10 μL) at 100 °C (1 h) and reduced to dryness.

To the ice-cooled esterified mixture were added successively pyridine (0.5 μL), CH_2Cl_2 (10 μL), and pentafluoropropionic anhydride (1.5 μL). Finally, the mixture was heated in a sealed vial (2 h, 100 °C), evaporated to dryness, and resuspended in hexane. Racemic mixtures as well as optically pure L- or D-alaninol standards were derivatized in similar fashion. Capillary GC−MS analyses were conducted using a Chirasil-Val column (Alltech, 25 m × 0.25 mm) with the following conditions: initial oven temperature of 50 °C (4 min), a 5 °C/min ramp from 50 to 150 °C, and concluding with a 20 °C/min ramp from 150 to 180 °C. The fragment derivatized from compound **3** and the derivatized L-alaninol standard both eluted at 13.02 min. The derivatized D-alaninol standard eluted at 13.58 min.

Brine Shrimp Toxicity Bioassay. Brine shrimp *Artemia salina* toxicity was measured as previously described.[4] After a 24 h hatching period, aliquots of a 10 mg/mL stock solution of compounds A−G were added to test wells containing 5 mL of artificial seawater and brine shrimp to achieve a range of final concentrations from 0.1 to 100 ppm. After 24 h the live and dead shrimp were tallied.

Anandamide Cellular Uptake Assay. The effect of compounds on the uptake of [^{14}C]AEA by intact rat basophilic leukemia (RBL-2H3) cells was studied by using 5.0 μM (20 000 cpm) of [^{14}C]AEA as described previously.[7,11] Cells were incubated with [^{14}C]AEA for 5 min at 37 °C, in the presence or absence of varying concentrations of the inhibitors. Residual [^{14}C]AEA in the incubation media after extraction with $CHCl_3$/CH_3OH (2:1, by vol.), determined by scintillation counting of the lyophilized organic phase, was used as a measure of the AEA that was taken up by cells. Previous studies[11] had shown that, after a 5 min incubation, the amount of [^{14}C]AEA that disappeared from the medium of RBL-2H3 cells is found mostly (>90%) as unmetabolized [^{14}C]AEA in the cell extract. Nonspecific binding of [^{14}C]AEA to cells and plastic dishes was determined in the presence of 100 μM AEA and was never higher than 30%. Data are expressed as the concentration exerting 50% inhibition of AEA uptake (IC_{50}).

CB_1 Receptor Binding Assay. Displacement assays for CB_1 receptors were carried out by using [^{3}H]SR141716A (0.4 nM, 55 Ci/mmol, Amersham) as the high-affinity ligand, and the filtration technique described previously,[7] on membrane preparations (0.4 mg/tube) from frozen male CD rat brains (Charles River, Wilmington, MA) and in the presence of 100 μM PMSF. Specific binding was calculated with 1 μM SR141716A and was 84.0%. Data are expressed as the K_i, calculated using the Cheng−Prusoff equation from the concentration exerting 50% inhibition of [^{3}H]SR141716A specific binding (IC_{50}).

Fatty Acid Amide Hydrolase Assay. The effect of compounds on the enzymatic hydrolysis of AEA was studied as described previously,[9,12] using membranes prepared from mouse neuroblastoma N18TG2 cells, incubated with the test compounds and [^{14}C]AEA (10 μM, 40 000 cpm) in 50 mM Tris-HCl, pH 9, for 30 min at 37 °C. [^{14}C]Ethanolamine produced from [^{14}C]AEA hydrolysis was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volumes of $CHCl_3$/CH_3OH (2:1, by vol.). Data are expressed as the concentration exerting 50% inhibition of [^{14}C]AEA hydrolysis (IC_{50}).

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra in $CDCl_3$ for semiplenamides A (**1**) to G (**7**); 1D NOE data for semiplenamide A (**1**) in $CDCl_3$ and for semiplenamide G (**7**) in MeOH-d_4. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. In *Alkaloids*: *Chemistry and Biology*; Cordell, G. A., Ed.; Academic Press: New York, 2001; Vol. 57, pp 75−184.
(2) Tan, L. T.; Okino, T.; Gerwick, W. H. *J. Nat. Prod.* **2000**, *63*, 952−955.
(3) Sitachitta, N.; Gerwick, W. H. *J. Nat. Prod.* **1998**, *61*, 681−684.
(4) Meyer, B. N.; Ferrigni, N. R.; Putnam, L. B.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31−33.
(5) Gerard, J. M.; Haden, P.; Kelly, M. T.; Andersen, R. J. *J. Nat. Prod.* **1999**, *62*, 80−85.
(6) Di Marzo, V.; Bisogno, T.; De Petrocellis, L.; Melck, D.; Martin, B. R. *Curr. Med. Chem.* **1999**, *6*, 715−738.
(7) Melck, D.; Bisogno, T.; De Petrocellis, L.; Chang, H.; Julius, D.; Bifulco, M.; Di Marzo, V. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 275−284.
(8) Sheskin, T.; Hanus, L.; Slager, J.; Vogel, Z.; Mechoulam, R. *J. Med. Chem.* **1997**, *40*, 659−667.
(9) Maurelli, S.; Bisogno, T.; De Petrocellis, L.; Di Marzo, V. *FEBS Lett.* **1995**, *377*, 82−86.
(10) Fowler, C. J.; Jacobsson, S. O. *Prostaglandins Leukot. Essent. Fatty Acids* **2002**, *66*, 193−200.
(11) Bisogno, T.; Maurelli, S.; Melck, D.; De Petrocellis, L.; Di Marzo, V. *J. Biol. Chem.* **1997**, *272*, 3315−3323.
(12) Bisogno, T.; Melck, D.; De Petrocellis, L.; Bobrov, M. Yu.; Gretskaya, N. M.; Bezuglov, V. V.; Sitachitta, N.; Gerwick, W. H.; Di Marzo, V. *Biochem. Biophys. Res. Commun.* **1998**, *248*, 515−522.

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