Probing the Bioactive Constituents from Chemotypes of the Sponge *Psammocinia* aff. *bulbosa*

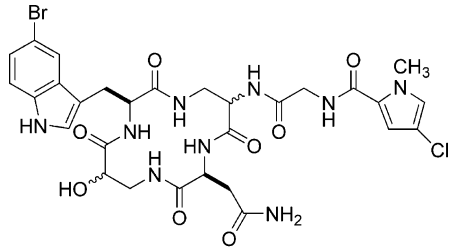
Sarah J. Robinson,[†] Karen Tenney,[†] Desiree F. Yee,[†] Lizabeth Martinez,[†] Joseph E. Media,[‡] Frederick A. Valeriote,[‡] Rob W. M. van Soest,[§] and Phillip Crews*[,†]

Department of Chemistry and Biochemistry and Institute for Marine Sciences, University of California, Santa Cruz, Santa Cruz, California 95064, Josephine Ford Cancer Center, Henry Ford Health System, Detroit, Michigan 48202, and Zoölogisch Museum, University of Amsterdam, P.O. Box 94766, 1090 GT, Amsterdam, The Netherlands

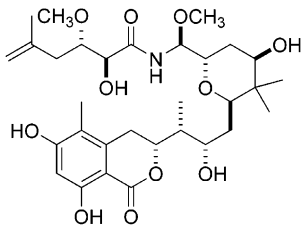
Received April 13, 2007

Since the report of (+)-psymberin (**2**) in 2004, many synthetic groups have pursued the synthesis of this compound, and our group has further collected *Psammocinia* aff. *bulbosa* to successfully isolate more **2**. With more (+)-psymberin (**2**) in hand, additional clonogenic studies, a therapeutic efficacy assessment, and the hollow fiber assay have been completed. The inconsistent production of (+)-psymberin (**2**) and the classification of six *Psammocinia* species are further discussed herein. The most recent of these six collections resulted in the isolation of a new brominated cyclic peptide, (−)-psymbamide A (**4**), which is the first report of a *Psammocinia*-derived compound in this peptide class. The planar structure was solved via dereplication with Marfey's method, HRESIMS, and 1D and 2D NMR techniques, and the absolute configuration determined using Marfey's method.

Ten years ago we disclosed the characterization of a bis-halogenated hexapeptide cytotoxin, (+)-cyclocinamide A (**1**), isolated from a relatively obscure sponge, *Psammocinia* sp. (Dictyoceratida, Irciniidae).[1] Perplexing results were obtained during a study that involved parallel examination of four *Psammocinia* collections,[2] each yielding the known sesterterpene variabilin (see Chart S1, Supporting Information), but only one was a source of (+)-cyclocinamide A (**1**).[1] Subsequent investigations of these samples plus an additional four[3] afforded other known compounds including (+)-swinholide A (see Chart S1, Supporting Information), mixtures of polybrominated phenol ethers, and the new astoundingly bioactive compound (+)-psymberin (**2**).[4] However, none of these additional collections were a source of (+)-cyclocinamide A (**1**), and in a retrospective analysis, it was not easy to detect the presence of (+)-psymberin (**2**).

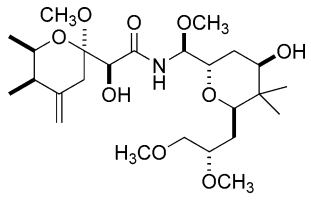


(+)-cyclocinamide A (**1**)[1]



(+)-psymberin (**2**)[4]

The unusual structure and significant biological properties of (+)-psymberin (**2**), identical to another independently reported highly cytotoxic substance, (+)-irciniastatin A (**2**) isolated from *Ircinia* cf. *ramosa* (Dictyoceratida, Irciniidae),[5] make this molecular framework and its sponge sources of high priority for further investigation. Impressive inhibitory activities against human cancer tumor cell lines have been described as illustrated by the data for (+)-psymberin (e.g., LC_{50} < 2.5 nM vs MDA-MB-435 breast cancer line),[4] (+)-irciniastatin (e.g., GI_{50} = 5.2 nM vs MCF-7 breast cancer line),[5] and synthetic (+)-psymberin (e.g., IC_{50} = 1 nM vs PC3 prostate cancer line).[6] There have been two total syntheses of **2**.[7,8] The first synthesis was accompanied by the preparation of 4-*epi*-(+)-psymberin (e.g., IC_{50} = 347 nM vs PC3) and 8-*epi*-(+)-psymberin (e.g., IC_{50} = 200 nM vs PC3), which finalized the assignment of the 4*S* chiral center of **2**.[7] Also of note are the three separate syntheses of (+)-psymberin substructures.[9−11] The bioactivity of these synthetic samples plus the evaluation of the designed compound psympederin (see Chart S1, Supporting Information, e.g., IC_{50} = 822 nM vs PC3)[6] revealed additional structure−activity relationships and underscores the robust activity associated with the unaltered (+)-psymberin structure. The biological properties of the (+)-psymberin family are distinct from those of the structurally related (+)-pederin (**3**),[12−14] and its analogues, yet both **2** and **3** may arise from a similar polyketide synthase−nonribosomal peptide synthetase (PKS/NRPS) biosynthetic pathway.[15,16] To date, no further natural (+)-psymberin (**2**) congeners have been described.

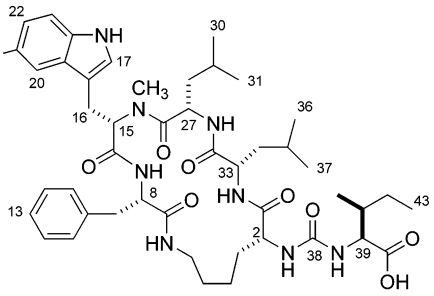


(+)-pederin (**3**)[11-13]

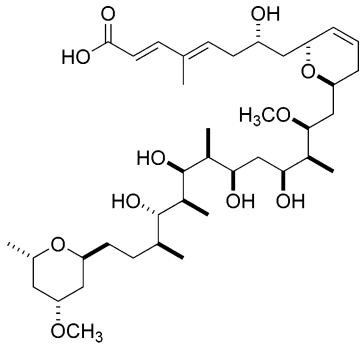
Our previous experience in linking the natural history of *Psammocinia* sponges to a predictable chemical outcome has been mixed, especially in regard to the isolation of new (+)-psymberin analogues.[17] The intent of the present account is to provide a progress report on research conducted along several parallel lines with the goal of interrogating new collections of "psymberin-containing" *Psammocinia* sponges. Described below are findings including (a) updated information for taxa now identified as *Psammocinia* aff. *bulbosa*, (b) the reisolation of (+)-psymberin (**2**) and its further evaluation against solid tumor cell lines, (c) new

* To whom correspondence should be addressed. Tel: 831-459-2603. Fax: 831-459-2935. E-mail: phil@chemistry.ucsc.edu.
[†] University of California, Santa Cruz.
[‡] Josephine Ford Cancer Center.
[§] University of Amsterdam.

10.1021/np070171i CCC: $37.00
Published on Web 06/09/2007



(-)-psymbamide A (**4**)



(-)-preswinholide A (**5**)[24]

insights on distinct sponge populations considered to be chemotypes, and (d) the isolation and characterization of a new polypeptide, (−)-psymbamide A (**4**).

Results and Discussion

The first step was to update the taxonomic record of sponge specimens identified as *Psammocinia* sp. that we considered as potential sources of (+)-psymberin (**2**). Prior to 1995 there were only five *Psammocinia* species described, and one included *P*. aff. *bulbosa*.[18] Currently, there are 19 such species assigned to that genus,[19] and we have found that it can be difficult to distinguish sponges suspected to be *Psammocinia* from members of the *Ircinia* and *Cacospongia* genera. The overall morphology of the sponges relevant to this study is discussed in Figure 1 for species I−VI, and the taxonomic status was concluded to be *Psammocinia* aff. *bulbosa*. Not evaluated here were the specimens designated as sample I, which was noted above to be a source of variabilin and (+)-cyclocinamide A (**1**), and sample II. The other four samples mentioned in Figure 1, samples III−VI, were concluded to be identical and possessed this identification. A visual illustration of the macrostructural differences for these six versus that of authentic *P*. aff. *bulbosa* can be gained by comparing our photographic records with that published for the holotype material QM-G304689.[20]

The disk diffusion assay profiles of semipure fractions expected to contain (+)-psymberin (**2**) provided an excellent tool to guide the selection of taxa for the reisolation efforts.[21] This approach was possible due to the phenomenal potency of (+)-psymberin (**2**) in our disk diffusion assay, as only 900 pg/disk gave a zone of inhibition size of 18 mm. Using these characteristics, the extracts of two *P*. aff. *bulbosa* specimens, samples II and III, were identified as promising for further isolation work. Additional confirmatory evidence came from LC-MS experiments using extracted ion chromatograph scans over the *m*/*z* range 577−579. Figure 2 illustrated that (+)-psymberin (**2**) was clearly present only in sample III and not samples V and VI. Thus, only two (II and III) of the six specimens discussed in Figure 1 emerged as containing (+)-psymberin (**2**).

Our chemical understanding of the major constituents of the other four sponge samples can be summarized as follows: (a) I: (+)-

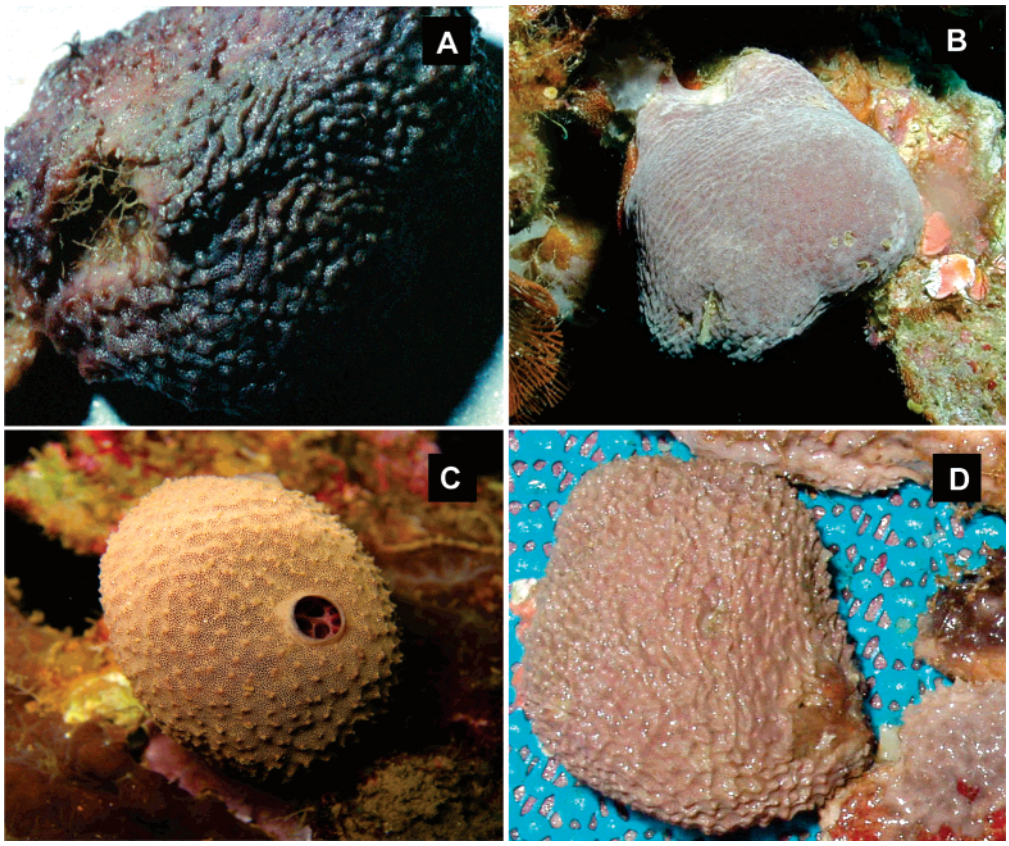


Figure 1. Varying morphologies for *Psammocinia* aff. *bulbosa*: (A) sample I (coll. no. 93145), (B) sample II (coll. no. 02135), (C) sample V (coll. no. 06121), (D) sample VI (coll. no. 06122). Underwater pictures of sample III (coll. no. 03526) and sample IV (coll. no. 05411) have been previously published.[17]

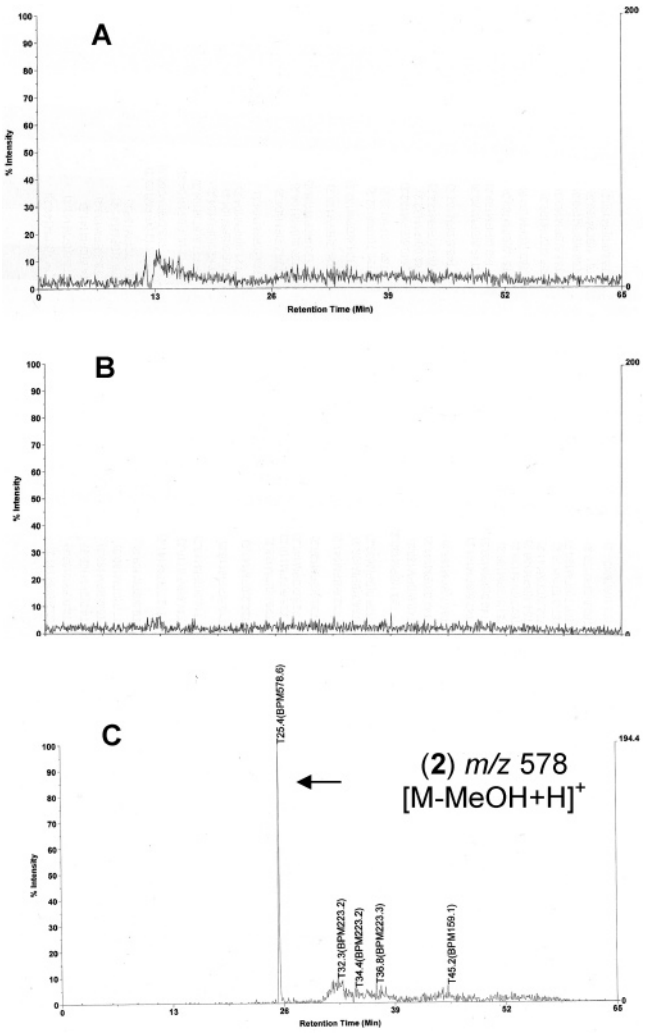


Figure 2. ESIMS extracted ion chromatograph scan at *m*/*z* 577−579 of *Psammocinia* aff. *bulbosa* crude extracts (see Figure 1): (A) sample V, (B) sample VI, and (C) sample III.

cyclocinamide A (**1**) and (−)-variabilin (see Chart S1, Supporting Information); (b) IV: (−)-preswinholide A (**5**) and (+)-swinholide A (see Chart S1, Supporting Information); (c) V: (−)-preswinholide A (**5**) and a new compound discussed below; and (d) VI: constituents still under investigation. Since the subsequent reisolation work to obtain **2** from sample II initially yielded (+)-swinholide A (see Chart S1, Supporting Information), we found that it was more productive to pursue sample III, of which a larger amount of extract was in hand. Eventually more than 40 mg of (+)-psymberin (**2**) (MW 609) was isolated along with miniscule amounts of two other analogues having molecular masses of 595 and 625. These data indicated that the new compounds, which have not been fully characterized, appeared relative to **2** as having one less methyl group in the former and one extra OH group in the latter. Significantly, the reisolation provided sufficient quantities of (+)-psymberin (**2**) to launch additional biological evaluations.

A multifaceted process was used to gain an understanding about the therapeutic potential of **2**. An important baseline experiment was to assess the cytotoxicity properties of the new sample, and an IC_{50} of 0.1 ng/mL was observed against HCT-116 cells. Next, a clonogenic dose−response study was conducted with the hypothesis being that a positive in vivo therapeutic effect will be observed only when the tumor cell kill is 90% or greater. This assessment involves measuring cytotoxicity effects at varying concentrations and at three different exposure durations. The outcome provides input to design an effective dose schedule for compound administration into tumor-bearing mice.[22] Promising results were obtained as shown in Figure 3, which plots HCT-116 cell survivability as a function of exposure and concentration. The dose−response studies for **2** were carried out at 2, 24, and 168 h. Results for 90% cell kill are as follows: 2 h ≥3 ng/mL, 24 h ≥2 ng/mL, and 168 h ≥120 pg/mL. These data predict that (a) a therapeutic effect could be observed in vivo against HCT-116 cells as either a bolus or chronic drug administration and (b) the exposure of tumor cells to **2** must be above 3 ng/mL for 2 h, 2 ng/mL for 24 h, or 120 pg/mL for 7 days.

The preceding encouraging results were used to design a test of the therapeutic efficacy of (+)-psymberin (**2**) using in vivo models. First, the MTD of **2** was determined to be about 125 μg/kg for SCID mice, and similar results of 25−50 μg/kg were provided by the NCI Developmental Therapeutics Program. Next, HCT-116 tumor-bearing SCID mice were treated with (+)-psymberin (**2**) with a bolus injection (125, 62, and 31 μg/kg), and the change in average tumor weight as a function of time following the inoculation is shown in Figure 4. The highest dose was toxic; the second and third doses gave T/C values of 75% and 86%, respectively, at 23 days, demonstrating minimal but encouraging therapeutic efficacy of **2** against HCT-116 tumors. Following the daily × 5 MTD determination, a second therapeutic assessment is planned and pharmacokinetic studies are in progress. Finally, **2** was evaluated in the NCI Developmental Therapeutics Program hollow fiber assay using multiple solid tumor cell lines.[23] A positive outcome was observed, as **2** attained an overall score of 34 (actives score ≥20) based on scores of 28 against the intraperatineal (ip) fibers and 6 against the subcutaneous (sc) fibers. The follow-up xenograft testing has also begun.

It was disappointing to observe, as noted above and shown in Figure 2, that two re-collections of *P.* aff. *bulbosa* (samples V and VI) were devoid of (+)-psymberin (**2**). Alternatively, a ^{1}H NMR spectrum of the CH_2Cl_2 semipure fraction from sample V was rich with signals in the upfield region, indicative of multiple methyl groups, and also in the low-field portion, diagnostic of aromatic groups. The former sample (V) was designated for additional isolation efforts, while the latter (VI) was set aside. A series of preparative and analytical HPLC runs eventually afforded the known (−)-preswinholide A[24] (**5**, 4.8 mg) and a new compound, **4** (7.7 mg), recognized as bromine-containing from the ESIMS pseudomolecular ion cluster at *m*/*z* 937, 939 (1:1 $[M + H]^+$).

The characterization of **4** ($C_{46}H_{65}BrN_8O_8$), named (−)-psymbamide A, began by considering a possible relationship of **4** to (+)-cyclocinamide A (**1**), of molecular formula $C_{29}H_{33}BrClN_9O_8$, previously isolated from *P.* aff. *bulbosa* (sample I). In fact, NMR data characteristic of a bromotryptophan were assigned in **4**, even though several peaks were overlapping in the aromatic region of the ^{1}H NMR spectrum, as shown in Figure 5. These data, also summarized in Table 1, consisted of signals for CH_2-16 (δ_C 22.2; δ_H 3.14, 2.83), CH-17 (δ_C 125.7; δ_H 7.01), CH-20 (δ_C 120.7; δ_H 7.80), CH-22 (δ_C 123.4; δ_H 7.15), and CH-23 (δ_C 113.4; δ_H 8.5). The position of the resonances for C-20, C-23, and C-21 (δ_C 111.4) each provided unmistakable support for the placement of the Br at C-21 and not C-22 (see Figure S10, Supporting Information).[25] The remaining low-field resonances (Figure 5) could be assigned to a phenylalanine (also supported by gCOSY data), an indole NH (δ_H 11.1), and six additional NH groups. Two other unusual signals in the ^{1}H NMR spectrum were those of a methyl group (δ_C 27.6; δ_H 1.91, s) and a very shielded diastereotopic $-CH_2$ moiety (δ_H 0.90, m; −0.61, dd, J = 12.0, 2.4 Hz).

Collectively, these preceding data guided the next two steps, consisting of establishing the molecular formula and pursuing dereplication efforts. The HRESIMS $[M + H]^+$ at *m*/*z* 937.4139 was consistent with 14 formulas that could be pruned to just that shown above employing the ^{13}C NMR-derived count of C_{46}. A partial formula range consisting of $C_{46}N_{7-11}O_{7-11}$ was the seed for dereplication searches, with the N and O count based on the

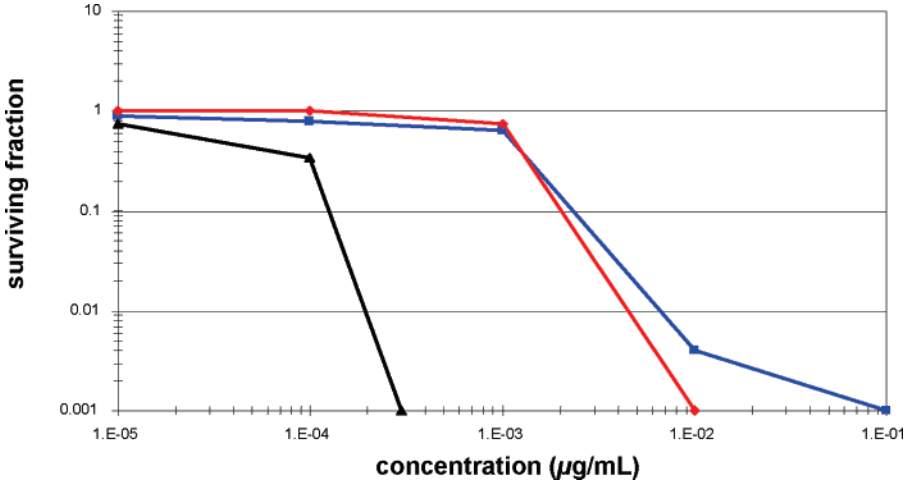


Figure 3. Clonogenic dose−response curve of HCT-116 cells exposed to (+)-psymberin (**2**) for 2 h (blue ■), 24 h (red ♦), and 168 h (black ▲) in vitro.

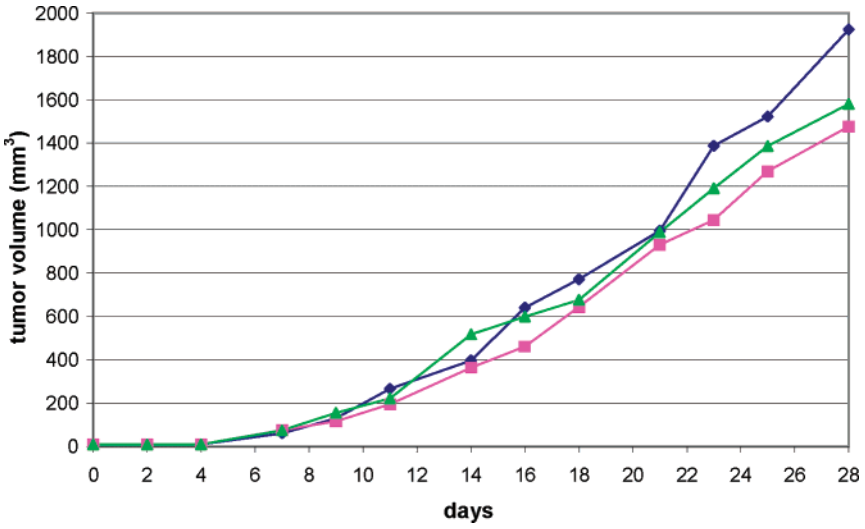
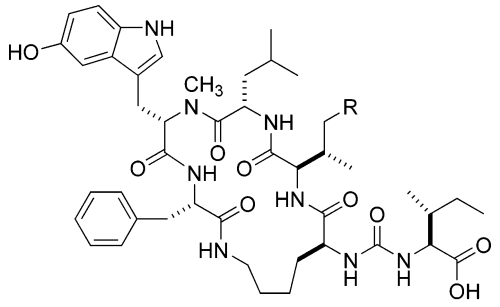


Figure 4. Therapeutic in vivo assessment of (+)-psymberin (**2**) in HCT-116 tumor-bearing SCID mice. Daily tumor weight measured per day after inoculation of (+)-psymberin (**2**) without **2** (black ♦), with 62 μg/kg (magenta ■), and with 31 μg/kg (green ▲).



mozamide A[27] R = H
mozamide B (**6**)[27] R = CH_3

assignment of six α-amino acid protons (δ_H 4.74, 4.55, 4.27, 4.05, 4.03, 3.87), plus the indole NH, and the remaining C═O to explain the δ_C = 173.9. An unsaturation number of 18 was required by the molecular formula, and a cyclic peptide structure seemed attractive to account for the remaining unassigned unsaturation unit. This partial formula search of Marinlit[26] yielded eight hits (see Figure S11, Supporting Information), each of which was a cyclic peptide, and the three most interesting were mozamide B (**6**) ($C_{46}H_{66}N_8O_9$),[27] anabaenopeptin H ($C_{46}H_{70}N_{10}O_{10}$),[28] and orbiculamide A ($C_{46}H_{62}$-BrN_9O_{10}).[29] The first and the last were sponge-derived and possessed Phe and Trp groups, and the last was halogenated. Choosing the best structure among this trio emerged from the delightful discovery that replacing the OH in the molecular formula of **6** by a Br gave the formula for **4**.

The NMR data of Table 1 enabled the identification of several additional substructures in **4**. These included two leucines, an isoleucine, and a lysine. An aggregate of ^{1}H and ^{13}C NMR data from both mozamides A and B (**6**) was used for comparison to **4**. The minor difference between mozamide A and mozamide B (**6**) is a methyl group: valine versus isoleucine. The unusual CH_3-25 NMR shift of **4** was identical to both mozamide A and B signals for the *N*-methyl-5′-hydroxytrophan. An upfield C-38 carbonyl shift was also a signature signal and was identical to the urea group of mozamide A. Due to insufficient material of mozamide B (**6**), only protonated carbons (32 out of 46) were detected via gHMQC. However, additional information to complete the structure of **4** came from a side-by-side comparison of the ^{13}C NMR δ values for **4** versus **6**, which showed all but five resonances for the reported 32 carbons to be ≤4 ppm of one another. The core pentapeptide planar structure was further confirmed by the 2D NMR correlations shown in Figure 6.

The structural changes in **4** versus **6** were additionally pinpointed by using the data in Figure 7, which plots the absolute value of the ^{13}C NMR shifts for the two regions showing variations. Reflecting the differences for these two moieties are shift changes of ≥4 ppm for (a) the differential C-21-substituted tryptophan by the data at C-20 and C22 and (b) the C-33 change of Leu for Ile by the data

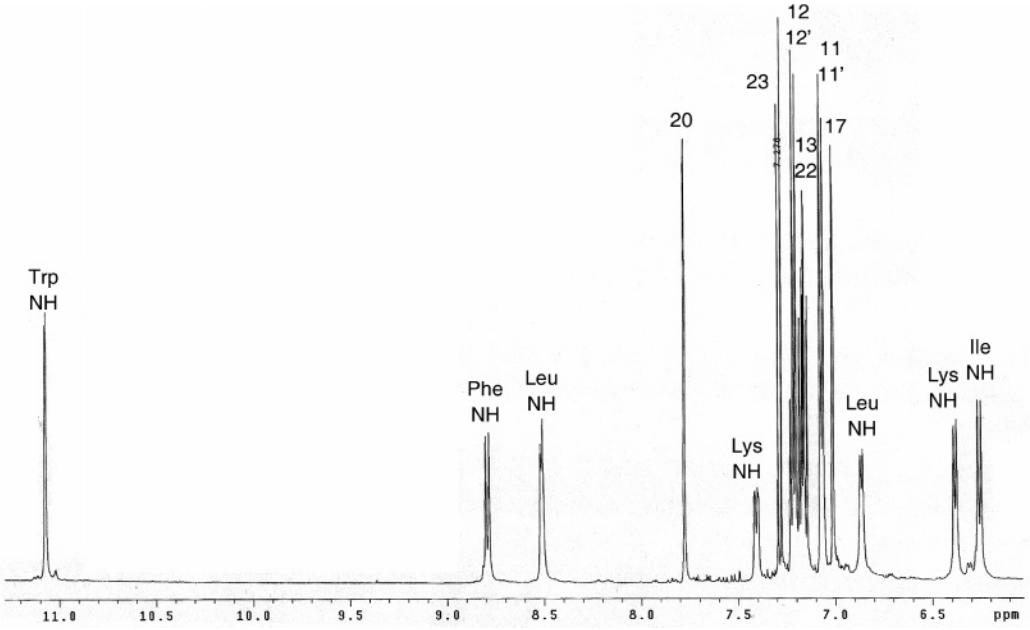


Figure 5. ^{1}H NMR 500 MHz (DMSO-d_6) downfield region of (−)-psymbamide A (**4**).

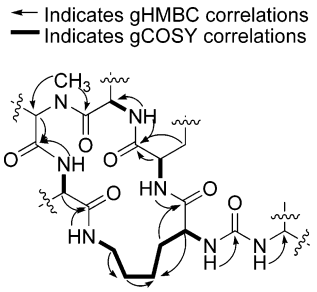


Figure 6. 2D NMR correlations providing connectivity relationships of (−)-psymbamide A (**4**).

at C-33, C-36, and C-37. Marfey's analysis of the **4** acid hydrolysate revealed L-Phe, L-Leu, L-Ile, and D-Lys, indicating identical absolute configurations between **4** and **6** at C-8 and C-27, but different at C-2 and C-41. The assignment of the 5-substituted-*N*-methyl-L-tryptophan position was based on the similar unusual upfield NMR shifts observed for both **4** and **6** at the *N*-Me-25 (**4**: $\delta_{C/H}$ 27.5/1.91; **6**: $\delta_{C/H}$ 28.2/1.96) and at the Leu$_1$-H-28 (**4**: δ −0.61; **6**: δ −0.42). A 1D NOE experiment involving irradiation at H-28 yielded correlations to several Trp protons, NH (δ_H 11.1), H-17 (δ_H 7.01), and H-20 (δ_H 7.80), consistent with facial proximity only possible by L−L or D−D configurations for these two amino acids. Additional data to support the L−L possibility presented in **4** came from observing similar upfield signals for the **4** and **6** Leu$_1$-Me-30 (**4**: $\delta_{C/H}$ 22.5/0.32; **6**: $\delta_{C/H}$ 23.4/0.38) and Leu$_1$-Me-31 (**4**: $\delta_{C/H}$ 19.2/0.25; **6**: $\delta_{C/H}$ 20.1/0.25).

Structures such as **4** and **6** are members of an uncommon peptide class. They contain a cyclic pentapeptide core shown in Table 2, which we call a CSCPP, with a uriedo side chain link to one additional amino acid. The entire set of such compounds discovered to date consists of 18 examples, with a majority (12) of cyanobacterial origin. Prior to our study, all of the five sponge-derived compounds had been reported from the genus *Theonella*. There is a striking parallel biosynthetic theme evident for all of the sponge metabolites as follows: (a) L-aromatic amino acids occur at AA#1, (b) L-Leu is present at AA#3, (c) a D- or L-Lys constitutes AA#5, and (d) the presence of *N*CH$_3$-25 is invariant. Some of these similarities are also seen among the structures produced by cyanobacteria including structural features noted above in points (a) with just two exceptions, (c) and (d). Given these extensive parallels, it is tempting to conclude that cyanobacteria may be important for the biosynthesis of the sponge-derived compounds. At this point it would appear that a parallel study of microorganism communities from any two different sponge species of Table 2 as well as the simultaneous examination of biosynthetic gene clusters from a sponge and cyanobacteria of Table 2 would be rewarding. Experiments along these lines are underway in our laboratory.

Even though a bioassay-guided isolation was not used to discover (−)-psymbamide A (**4**), there are precedents in the literature indicating that it deserves further study in biological screens. For example, bioactive structural types similar to (−)-psymbamide A (**4**) include (Table 2) the sponge-derived brunsvicamides A−C, with inhibitory activity (IC_{50} = 7−62 μM) against *Mycobacterium tuberculosis*[34] and anabaenopeptins from various cyanobacteria with action (IC_{50} = 5−8 ng/mL) against carboxypeptidase A.[28] An NCI evaluation of **4** (NSC #D743837-A) is currently pending.

Experimental Section

General Experimental Procedures. The 1D NMR spectra were obtained using a Varian Unity 500+ at 500 MHz for ^{1}H and 125 MHz for ^{13}C. 2D NMR spectra were obtained using both a Varian Unity 500+ and Varian Inova 600 at 600 MHz for ^{1}H and 150 MHz for ^{13}C. Multiplicities of ^{13}C NMR peaks were determined using DEPT and HMQC data. Optical rotation was determined on a Jasco DIP 370 polarimeter.

Low- and high-resolution mass spectrometry was performed on a benchtop Mariner electrospray ionization time-of-flight instrument (ESITOF). Preparative HPLC was carried out using a single-wavelength (λ = 254 nm) UV detector and evaporative light-scattering detector (ELSD) in series with a Waters RP C_{18}, 5 μm particle column.

Biological Material. The sponge samples I−VI were collected from Milne Bay, Papua New Guinea (PNG). A total of 3.4 kg of sample I (coll. no. 93145) was collected from the Normanby Islands, Papua New Guinea, in March 1993. Sample II (coll. no. 02135, 0.8 kg) was collected in May 2002 at 9°43.969′ S 150°44.488′ E, 10°13.126′ S 150°52.937′ E, and 10°13.832′ S 150°49.994′ E. Sample III (coll. no. 03526, 1.8 kg) was collected in December 2003 at 9°37.214′ S 150°57.332′ E, 9°14.008′ S 150°46.782′ E, 9°14.147′ S 150°47.173′ E, and 9°19.868′ S 150°43.906′ E. In November 2005 sample IV (coll. no. 05411, 1.4 kg) was collected at 11°26.421′ S 154°24.319′ E, 11°19.718′ S 154°16.722′ E, 11°16.260′ S 153°57.518′ E, and 10°41.613′ S 152°50.871′ E. In April 2006 sample V (coll. no. 06121, 2.7 kg) was collected at 10°15.836′ S 150°40.193′ E, 10°15.061′ S 150°41.878′ E, 10°15.184′ S 150°42.865′ E, and 9°58.998′ S 150°57.359′ E. Sample VI (coll. no. 06122, 0.1 kg) was collected at 10°15.061′ S

Table 1. ^{1}H, ^{13}C, and gCOSY NMR Data for (−)-Psymbamide A (**4**) in DMSO-d_6 at 500 MHz (^{1}H) and 125 MHz (^{13}C)

amino acid	position	^{13}C δ (type)	^{1}H δ (J in Hz)	gCOSY (δ_H to δ_H)	gHMBC (δ_H to δ_C)
D-lysine	1	172.2 (C)			
	2	54.5 (CH)	3.87 ddd (10.8, 6, 4.2)	Lys 2-NH, 3	3[a], 4[a], 32[a], 38[a]
	3	31.9 (CH_2)	1.55 m	2, 4	1[a], 2[a], 4[a]
	4	20.3 (CH_2)	1.34 m, 1.13 m	3, 5	
	5	28.4 (CH_2)	1.40 m, 1.23 m	4, 6	4[a]
	6	38.4 (CH_2)	3.62 m, 2.88 m	Lys 6-NH, 5	5[a]
	2-NH		6.38 d (7.5)	2	3[a], 4[a], 38[a]
	6-NH		7.40 d (9.0, 2.5)	6	7
L-phenylalanine	7	170.8 (C)			
	8	54.6 (CH)	4.55 ddd (12.6, 9.0, 3.6)	Phe NH, 9b	7, 9[a]
	9	37.7 (CH_2)	3.41 dd (14.0, 3.5), 2.79 m	8	10, 11
	10	138.5 (C)			
	11,11′	128.9 (CH)	7.06 m	12	12, 13
	12,12′	128.3 (CH)	7.21 m	11	10[a]
	13	126.1 (CH)	7.19 m		
	NH		8.79 d (9.0)	8	14
N-methyl-L-5′-bromotryptophan	14	169.7 (C)			
	15	60.3 (CH)	4.74 dd (11.0, 3.5)	16b	14, 25
	16	22.2 (CH_2)	3.14 dd (15.0, 3.5) 2.83 dd (15.0, 11.0)	15	15, 17, 18, 19
	NH	(CH)			
	17	125.7 (CH)	7.01 d (2.5)	Trp NH	18, 19, 24
	18	109.4 (C)			
	19	129.0 (C)			
	20	129.7 (CH)	7.80 d (1.5)		18, 21, 22, 24
	21	111.4 (C)			
	22	123.4 (CH)	7.15 dd (8.5, 1.5)	23	21, 23, 24
	23	113.4 (CH)	7.28 d (8.5)	22	19, 21, 22
	24	134.6 (C)			
	25	27.5 (CH_3)	1.91 s		26, 15
L-leucine	26	172.3 (C)			
	27	47.1 (CH)	4.27 m	Leu NH, 28	28b[a], 29[a]
	28	37.1 (CH_2)	0.90 m, −0.61 dd (12.0, 2.4)	27, 29	27[a], 29, 30[a]
	29	22.9 (CH)	1.46 m	28, 30, 31	28b[a], 30[a]
	30	22.5 (CH_3)	0.32 d (7.0)	29	28, 29
	31	19.2 (CH_3)	0.25 d (6.5)	29	29
	NH		8.51 d (5.5)	27	27, 32[a]
L-leucine	32	173.0 (C)			
	33	50.8 (CH)	4.05 dt (7.7, 5.5)	Leu NH, 34	32, 34, 35
	34	39.5 (CH_2)	1.49 m	33, 35	32[a], 33, 35, 36[a], 37[a]
	35	23.8 (CH)	1.68 m	34, 36, 37	33[a], 34[a], 36, 37[a]
	36	22.8 (CH_3)	0.90 d (6.5)	35	34, 35, 37[a]
	37	22.0 (CH_3)	0.84 d (6.5)	35	34, 35, 36[a]
	NH		6.86 d (6.5)	33	1[a]
urea	38	157.2 (C)			
L-isoleucine	39	56.9 (CH)	4.03 dd (9.5, 5.0)	I1e NH, 41	41, 42, 44[a]
	40	173.9 (C)			
	41	37.1 (CH)	1.72 m	40, 42, 44	39[a], 42[a], 43[a]
	42	24.6 (CH_2)	1.37 m, 1.16 m	41, 43	39[a], 41[a], 43[a]
	43	11.5 (CH_3)	0.86 t (7.8)	42	41, 42
	44	15.7 (CH_3)	0.83 d (6.6)	41	39, 41, 42
	NH		6.25 d (9.0)	40	

[a] gHMBC at 600 MHz optimized for 5 Hz correlations in CD_3CN.

150°41.878′ E in April 2006. The genus *Psammocinia* is characterized by thin threadlike fibers of clear uncolored spongin freely occurring in bundles/masses outside the fiber skeleton. This is similar to *Ircinia* but differs by a sand-coat, i.e., a continuous surface layer of tightly packed sand grains.[39] Collection depths ranged from 60 to 30 ft. Sponge samples III−VI were identified as *Psammocinia* aff. *bulbosa* with properties similar to the literature,[20] and voucher specimens have been deposited at the Zoological Museum of Amsterdam (sample III: ZMAPOR19842, sample IV: ZMAPOR19843, sample V: ZMAPOR19844, and sample VI: ZMAPOR19845). Pictures and voucher specimens are available from the corresponding author (P.C.).

Extraction and Isolation. Samples of the sponge sample V (coll. no. 06121) were preserved in the field according to our standard procedure[40] and transported back to the laboratory at ambient temperature. The preserved sponge was thoroughly dried, ground, and extracted with hexanes, then CH_2Cl_2, and last with MeOH under high pressure (1700 psi) at 110 °C. The dichloromethane extract was dried to a brown oil, 3.7702 g, and was subjected to preparative reversed-phase (RP) HPLC (45−73% CH_3CN in H_2O with 0.1% formic acid over 35 min) using a Waters C_{18}, 5 μm particle column to afford 13 fractions. Preparative fraction P3, 125.4 mg, was further separated by semi-preparative (RP) HPLC (45−100% CH_3CN in H_2O with 0.1% formic acid over 45 min and 70−90% CH_3CN in H_2O with 0.1% formic acid over 20 min) to afford 4.8 mg of (−)-preswinholide A (**5**). Preparative fraction P6, 295.1 mg, was fractionated by two consecutive rounds of semipreparative (RP) HPLC (60−80% CH_3CN in H_2O with 0.1% formic acid over 40 min and 63−74% CH_3CN in H_2O with 0.1% formic acid over 22 min) to give 7.7 mg of (−)-psymbamide A (**4**).

(−)-Psymbamide A (4): white powder (7.7 mg); $[\alpha]_D^{27}$ −156 (*c* 0.001, MeOH); 1D ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) and 2D ^{1}H NMR (600 MHz, DMSO-d_6 and CD_3CN) and ^{13}C NMR (150 MHz, DMSO-d_6 and CD_3CN), see Table 1; HRESIMS $[M + H]^+$ obsd *m*/*z* 937.4139, calcd for $C_{46}H_{66}N_8O_8Br$ 937.4181.

(−)-Preswinholide (5): fine white powder; HRESIMS $[M + H]^+$ obsd *m*/*z* 713.4758, calcd for $C_{39}H_{69}O_{11}$ 713.4756. NMR data identical with literature.[24]

Hydrolysis and Derivatization of (−)-Psymbamide A (4). (−)-Psymbamide A (0.5 mg) was hydrolyzed with 1 mL of 6 N HCl at 110 °C for 18 h. The hydrolysate was evaporated with nitrogen and treated with both 100 μL of 1 M $NaHCO_3$ and 50 μL of a 10 mg/mL

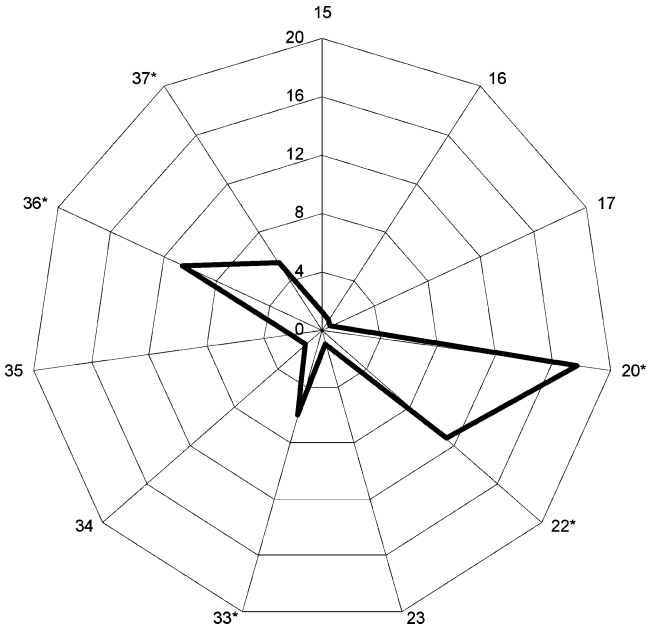


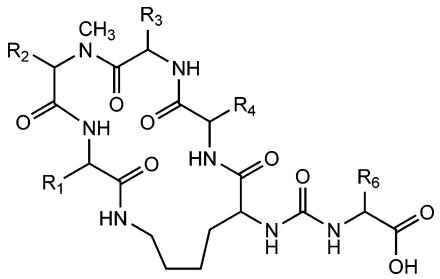
Figure 7. ^{13}C δ shift difference between (−)-psymbamide A (**4**) and (−)-mozamide B (**6**) (* indicates $\Delta\delta \geq 4$ ppm).

solution of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA). The mixture was heated for 30 min at 80 °C, cooled to room temperature, and then quenched with 50 μL of 2 N HCl. Then 300 μL of CH_3CN was added to the mixture to prepare it for LC-MS. Standard amino acids (0.5 mg) were derivatized following the same procedure. For amino acids excluding Lys, a linear gradient of CH_3CN with 0.01 M formic acid from 30 to 70% on a chiral RP Chiracel column (5 μm, 150 mm × 10 mm i.d.) and UV detection at 340 nm was used. A linear gradient of CH_3CN with 0.01 M formic acid from 0 to 60% on a RP Synergi column (45 μm, 150 mm × 4.60 mm) and UV detection at 340 nm was used for Lys. Retention times of standards were recorded and compared with the derivatized hydrolysate of **4**. Standards: D-Leu 13.9 min, L-Leu 12.7 min, D-*allo*-Ile 13.6 min, D-Ile 13.6 min, L-*allo*-Ile 12.0 min, L-Ile 12.3 min, D-Phe 15.1 min, L-Phe 14.5 min, D-Lys 18.9, and L-Lys 19.3. Hydrolysate: L-Phe 14.5 min, L-Leu 12.7 min, L-Ile 12.3 min, and D-Lys 18.8 min.

(+)-Psymberin IC_{50} Determinations. Cells were plated at 5×10^4 cells in T25 tissue culture flasks (BD Falcon Plastics, Franklin Lakes, NJ) with 5 mL of RPMI 1640 media (Cellgro, Herndon, VA) supplemented with 15% BCS (Hyclone, Logan, UT), 5% pen. strep., and 5% glutamine (Cellgro, Herndon, VA). Three days later (cells in logarithmic growth phase; 5×10^5 cells/flask), test compound was added to the flasks to achieve concentrations ranging from 10 to 10^{-5} μg/mL. At day 3, the flasks were washed, trypsinized, and spun down, and the cells counted for both viable and dead cells using 0.08% trypan blue (Gibco, Gaithersburg, MD). Viable cell number as a function of concentration was plotted, and the IC_{50} values were determined by interpolation and are as follows: MDA-MB-231 (breast) = 0.2 ng/mL; MCF-7 (breast) = 0.2 mg/mL; HT29 (colon) = 5 ng/mL; L1210 (leukemia) = 0.18 ng/mL; and H125 (lung) = 0.12 ng/mL.

(+)-Psymberin (2) HPLC Analysis. This analysis was carried out with a Waters 2690 separation module set at 4 °C and a model 2487 UV/vis detector set at 218 nm. An analytical 3.9 × 150 mm Symmetryshield C_{18}, 5 μm column (Waters, Milford, MA) was maintained at 30 °C using a Waters temperature control module. The mobile phase was 50% CH_3CN and 50% 0.1% acetic acid in deionized water with a flow rate of 1 mL/min and a run time of 10 min. The lower limit of quantitation was 100 ng/mL. Under these conditions, the (+)-psymberin (**2**) peak eluted near 7.0 min. Absolute values of (+)-psymberin (**2**) were obtained using a standard curve (five concentrations) of a stock (+)-psymberin (**2**) solution. Validation of each set of results was accomplished using run standards (stock drug solution dissolved in mobile phase at 1 mg/mL). (+)-Psymberin (**2**) was extracted from both plasma and tumor samples by solid-phase extraction. Briefly, 0.5 mL of MeOH was added to 250 μL of the sample followed by vigorous vortex and centrifuged at 14 000 rpm at 4 °C. To the supernatant, 1 mL of water was added, and the mixture was diluted to 3 mL with 0.1% acetic acid. Samples were passed through Waters Sep-pak vac 1 cm^3 (100 mg) C_{18} cartridges equilibrated with 1 mL of MeOH and 1 mL of deionized water. Cartridges were washed with 2 mL of 0.1% acetic acid containing 5% MeOH. (+)-Psymberin (**2**) was eluted from the cartridges by passing 1.5 mL of 2% formic acid in CH_3CN. Extract was evaporated in a Turbovap LV evaporator

Table 2. Absolute Stereochemistry of CSCPP[a] Class Metabolites



compound	R_1	R_2	R_3	R_4	Lys	R_6	source	species
anabaenopeptin I[30]	L-Leu	*N*-Me-L-Ala	L-Hty	L-Val	D	L-Ile	cyanobacteria	*Aphanizomenon flos-aquae*
anabaenopeptin J[30]	L-Phe	*N*-Me-L-Ala	L-Hty	L-Val	D	L-Ile	cyanobacteria	*Aphanizomenon flos-aquae*
schizopeptin 791[31]	L-Phe	*N*-Me-L-Ala	L-Hph	L-Ile	D	L-Ile	cyanobacteria	*Schizothrix* sp.
anabaenopeptin A[32]	L-Phe	*N*-Me-L-Ala	L-Hty	L-Val	D	L-Tyr	cyanobacteria	*Anabaena flos-aquae*
anabaenopeptin B[32]	L-Phe	*N*-Me-L-Ala	L-Hty	L-Val	D	L-Arg	cyanobacteria	*Anabaena flos-aquae*
anabaenopeptin E[33]	L-Phe	*N*-Me-L-Ala	Mhty	L-Val	D	L-Arg	cyanobacteria	*Oscillatoria agardhii*
anabaenopeptin F[33]	L-Phe	*N*-Me-L-Ala	Hty	L-Ile	D	L-Arg	cyanobacteria	*Oscillatoria agardhii*
anabaenopeptin G[28]	L-Ile	*N*-Me-L-Hty	L-Hty	L-Ile	D	L-Tyr	cyanobacteria	*Oscillatoria agardhii*
anabaenopeptin H[28]	L-Ile	*N*-Me-L-Hty	L-Hty	L-Ile	D	L-Arg	cyanobacteria	*Oscillatoria agardhii*
brunsvicamide A[34]	L-Phe	*N*-Me-L-Trp	L-Leu	L-Val	L	L-Ile	cyanobacteria	*Tychonema* sp.
brunsvicamide B[34]	L-Phe	*N*-Me-L-Trp	L-Leu	L-Ile	L	L-Ile	cyanobacteria	*Tychonema* sp.
brunsvicamide C[34]	L-Phe	*N*-Me-*N*′-formyL-D-kynu	L-Leu	L-Val	L	L-Ile	cyanobacteria	*Tychonema* sp.
mozamide A[27]	L-Phe	*N*-Me-L-5′-Htp	L-Leu	D-Val	L	L-*allo*-Ile	sponge	*Theonella* sp.
mozamide B[27]	L-Phe	*N*-Me-L-5′-Htp	L-Leu	D-Ile	L	L-*allo*-Ile	sponge	*Theonella* sp.
konbamide[35,36]	L-Br Trp	*N*-Me-L-Leu	L-Leu	L-Ala	L	L-Leu	sponge	*Theonella* sp.
keramamide A[37]	L-Phe	*N*-Me-L-Cht	L-Leu	L-Leu	L	L-Phe	sponge	*Theonella* sp.
keramamide L[38]	L-Phe	*N*-Me-L-CTrp	L-Leu	L-Leu	L	L-Phe	sponge	*Theonella* sp.
(−)-psymbamide A	L-Phe	*N*-Me-L-5′-Br-Trp	L-Leu	L-Leu	D	L-Ile	sponge	*Psammocinia* aff. *bulbosa*

[a] CSCPP, cyano-sponge cyclic pentapeptide.

(Zymark, Hopkinton, MA) to dryness under a stream of nitrogen at 45 °C and reconstituted in 100 μL of mobile phase for HPLC analysis.

(+)-Psymberin (2) Clonogenic Dose−Response Analysis against HCT-116 Cells. Concentration- and time-survival studies were carried out with HCT-116 cells seeded at 200 to 20 000 cells in 60 mm dishes. (+)-Psymberin (**2**) was added to the medium (RPMI + 10% FBS) to a final concentration of 10 μg/mL and 10-fold dilutions thereof. At either 2 or 24 h, the drug-containing medium was removed and fresh medium without drug was added. For continuous exposure to drug, it remained in contact with the cells for the entire incubation period. The dishes were incubated for up to 7 days, the medium was removed, and the colonies were stained with methylene blue. Colonies containing 50 cells or more were counted. The results were normalized to an untreated control. Plating efficiency for the untreated cells was about 90%. Repeat experiments were carried out to define the cell survival range between 100% and 0.1% survival, with results shown in Figure 3.

(+)-Psymberin (2) Therapeutic Assessment. The in vivo therapeutic assessment trial was carried out using the HCT-116 human colon tumor model as previously described.[22] Individual mouse body weights for each experiment were within 5 g, and all mice were over 17 g at the start of therapy. The mice were supplied food and water *ad libidum*. SCID mice were pooled, implanted subcutaneously with 10^6 tissue-culture-derived tumor cells, and pooled again before distribution to treatment and control groups (5 mice per group). Treatment with **2** was started 1 day after tumor inoculation. Mice were sacrificed after 30 days had elapsed from tumor inoculation. Tumor weights were estimated using two-dimensional caliper measurements done three times per week using the following formula: tumor weight (mg) = $(a \times b^2)/2$, where a and b are the tumor length and width, respectively, in mm. The median was calculated as an indication of antitumor effectiveness. The parameter, %T/C, is determined after each measurement and the minimum value reported as therapeutic efficacy. (+)-Psymberin (**2**) was prepared as a stock solution in DMSO, diluted 1:1 (v/v) with Cremophor−propylene glycol (40:60 v/v) and further diluted at least 20-fold with saline before injection. Drug was prepared at 10, 5, and 2 mg/mL for intravenous administration as 0.25 mL volumes via the tail vein. Drug was administered as a bolus injection, which corresponds to 125, 62, and 31 μg/kg respectively. The overall results are shown in Figure 4.

(+)-Psymberin (2) Hollow Fiber Assay Cells. (+) Psymberin was tested at 0.009 mg/kg/dose on a QD × 4, day 3 dose schedule. It was tested against the following cells: breast cancers (MDA-MB-231 and MDA-MB-435), non-small-cell lung cancers (NCI-H23 and NCI-H522), colon cancers (SW-620 and COLO 205), melanomas (LOX IMVI and UACC-62), ovarian cancers (OVCAR-3 and OVCAR-5), and CNS cancers (U251 and SF-295).

Acknowledgment. This work was supported by grants from the NSF (NSF-CHE-0617056) and NIH (R01 CA 047135), NMR equipment grants NSF-CHE-0342912 and NIH S10 RR19918, and MS equipment grant NIH S10-RR20939. D.Y. and L.M. were supported through ACCESS by the NIH (R25 GM 051765). Thanks to C. Boot for experimental assistance and intellectual contributions, to R. Chichewicz for work on samples I, II, and III and the isolation of **2**, and to N. Cotton for her work on sample III. Thanks to J. de Brabander for a synthetic sample of (+)-psymberin (**2**), to L. Matainaho for providing collection permit support, and to Captain C. de Wit and Crew of the *M. V. Golden Dawn* for their assistance in sponge collection.

Supporting Information Available: ^{1}H, ^{13}C, gCOSY, gHMBC, and HMQC NMR data for **4** and MS data and an isolation scheme for **4** and **5** are provided along with structures of background compounds, structures of Marinlit dereplication search hits, and reference data for assignment of the Br-substituted Trp are all available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Clark, W. D.; Corbett, T.; Valeriote, F. A.; Crews, P. *J. Am. Chem. Soc.* **1997**, *119*, 9285−9286.
(2) This involved material collected in the Milne Bay and Normanby regions of Papua New Guinea over a three-year period designated as sp 1 (coll. nos. 90135, 91113, and 93105) and sp 2 (see sample I, Figure 1, coll. no. 93145).
(3) Additional specimens obtained from PNG were coll. nos. 95033, 95075, 99159, and 01236.
(4) Cichewicz, R. H.; Valeriote, F. A.; Crews, P. *Org. Lett.* **2004**, *6*, 1951−1954.
(5) Pettit, G. R.; Xu, J.-P.; Chapuis, J.-C.; Pettit, R. K.; Tackett, L. P.; Doubek, D. L.; Hooper, J. N. A.; Schmidt, J. M. *J. Med. Chem.* **2004**, *47*, 1149−1152.
(6) Jiang, X.; Williams, N.; De Brabander, J. K. *Org. Lett.* **2007**, *9*, 227−230.
(7) Jiang, X.; Garcia-Fortanet, J.; De Brabander, J. K. *J. Am. Chem. Soc.* **2005**, *127*, 11254−11255.
(8) Shangguan, N.; Kiren, S.; Williams, L. J. *Org. Lett.* **2007**, *9*, 1093−1096.
(9) Kiren, S.; Williams, L. J. *Org. Lett.* **2005**, *7*, 2905−2908.
(10) Green, M. E.; Rech, J. C.; Floreancig, P. E. *Org. Lett.* **2005**, *7*, 4117−4120.
(11) Rech, J. C.; Floreancig, P. E. *Org. Lett.* **2005**, *7*, 5175−5178.
(12) Cardani, C.; Ghiringhelli, D.; Mondelli, R.; Quilico, A. *Tetrahedron Lett.* **1965**, *6*, 2537−2545.
(13) Furusaki, A.; Watanabe, T.; Matsumoto, T.; Yanagiya, M. *Tetrahedron Lett.* **1968**, 6301−6304.
(14) Matsuda, F.; Tomiyoshi, N.; Yanagiya, M.; Matsumoto, T. *Tetrahedron* **1988**, *44*, 7063−7080.
(15) Piel, J.; Butzke, D.; Fusetani, N.; Hui, D.; Platzer, M.; Wen, G.; Matsunaga, S. *J. Nat. Prod.* **2005**, *68*, 472−479.
(16) Piel, J. *Curr. Med. Chem.* **2006**, *13*, 39−50.
(17) Rubio, B. K.; van Soest, R. W. M.; Crews, P. *J. Nat. Prod.* **2007**, in press.
(18) Cook, S. D.; Bergquist, P. R. *N. Z. J. Mar. Freshwater Res.* **1996**, *30*, 19−34.
(19) van Soest, R. W. M.; Boury-Esnault, N.; Janussen, D.; Hooper, J. World Porifera database. http://www.vliz.be/vmdcdata/porifera (accessed on Feb 22, 2007).
(20) Cook, S. D. C.; Bergquist, P. R. *N. Z. J. Mar. Freshwater Res.* **1998**, *32*, 399−426.
(21) Valeriote, F.; Grieshaber, C. K.; Media, J.; Pietraszkewicz, H.; Hoffmann, J.; Pan, M.; McLaughlin, S. *J. Exp. Ther. Oncol.* **2002**, *2*, 228−236.
(22) Subramanian, B. N., A.; Tenney, K.; Crews, P.; Gunatilaka, L.; Valeriote, F. A. *J. Exp. Ther. Oncol.* **2006**, *5*, 195−204.
(23) Hall, L. A.; Krauthauser, C. M.; Wexler, R. S.; Hollingshead, M. G.; Slee, A. M.; Kerr, J. S. *Anticancer Res.* **2000**, *20*, 903−911.
(24) Todd, J. S.; Alvi, K. A.; Crews, P. *Tetrahedron Lett.* **1992**, *33*, 441−442.
(25) Segraves, N. L.; Crews, P. *J. Nat. Prod.* **2005**, *68*, 1484−1488.
(26) Blunt, J.; Munro, M. *Marinlit*, July 2006; University of Cantebury: Christchurch, NZ.
(27) Schmidt, E. W.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **1997**, *60*, 779−782.
(28) Itou, Y.; Suzuki, S.; Ishida, K.; Murakami, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1243−1246.
(29) Fusetani, N.; Sugawara, T.; Matsunaga, S.; Hirota, H. *J. Am. Chem. Soc.* **1991**, *113*, 7811−7812.
(30) Murakami, M.; Suzuki, S.; Itou, Y.; Kodani, S.; Ishida, K. *J. Nat. Prod.* **2000**, *63*, 1280−1282.
(31) Reshef, V.; Carmeli, S. *J. Nat. Prod.* **2002**, *65*, 1187−1189.
(32) Harada, K.; Fujii, K.; Shimada, T.; Suzuki, M.; Sano, H.; Adachi, K.; Carmichael, W. W. *Tetrahedron Lett.* **1995**, *36*, 1511−1514.
(33) Shin, H. J.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *J. Nat. Prod.* **1997**, *60*, 139−141.
(34) Muller, D.; Krick, A.; Kehraus, S.; Mehner, C.; Hart, M.; Kupper Frithjof, C.; Saxena, K.; Prinz, H.; Schwalbe, H.; Janning, P.; Waldmann, H.; Konig Gabriele, M. *J. Med. Chem.* **2006**, *49*, 4871−4878.
(35) Kobayashi, J.; Sato, M.; Murayama, T.; Ishibashi, M.; Walchi, M. R.; Kanai, M.; Shoji, J.; Ohizumi, Y. *J. Chem. Soc., Chem. Commun.* **1991**, 1050−1052.
(36) Schmidt, U.; Weinbrenner, S. *Angew. Chem., Int. Ed.* **1996**, *35*, 1336−1338.
(37) Kobayashi, J.; Sato, M.; Ishibashi, M.; Shigemori, H.; Nakamura, T.; Ohizumi, Y. *J. Chem. Soc., Perkin Trans.* **1991**, *1*, 2609−2611.
(38) Uemoto, H.; Yahiro, Y.; Shigemori, H.; Tsuda, M.; Takao, T.; Shimonishi, Y.; Kobayashi, J. *Tetrahedron* **1998**, *54*, 6719−6724.
(39) *Systema Porifera A guide to the Classification of Sponges*; Hooper, J. N. A., van Soest, R. W. M., Eds.; New York, 2002; Vol. 1, p 1026.
(40) Sperry, S.; Valeriote, F. A.; Corbett, T. H.; Crews, P. *J. Nat. Prod.* **1998**, *61*, 241−247.

NP070171I