Micromide and Guamamide: Cytotoxic Alkaloids from a Species of the Marine Cyanobacterium *Symploca*

Philip G. Williams,[†] Wesley Y. Yoshida,[†] Richard E. Moore,*[,†] and Valerie J. Paul[‡,§]

Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822, and University of Guam Marine Laboratory, UOG Station, Mangilao, Guam 96913

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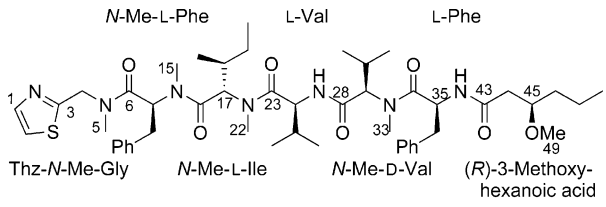
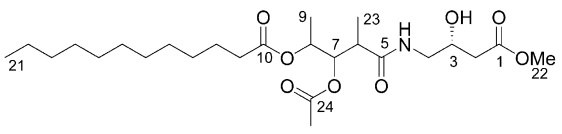
Two new cytotoxins have been isolated from a species of marine cyanobacterium belonging to the genus *Symploca* that was collected in Guam. These new compounds, micromide (**1**) and guamamide (**2**), were accompanied by the known lipopeptides apramides A (**3**), B (**4**), and G (**5**). The planar structures of both alkaloids were elucidated by standard 2D NMR techniques, and the configurations of the amino acid-derived units in **1** were determined by chiral HPLC. The stereochemistry of the *β*-methoxyhexanoic acid in **1** was determined by derivatization with methyl D-mandelate, after acid hydrolysis, and comparison with synthetic standards.

Historically cyanobacterial extracts have been known for their toxic properties, but more recent chemical investigations have revealed that they are a promising group of organisms from which to isolate a wide variety of biologically active natural products.[1] In particular, marine cyanobacteria have yielded a diverse array of cytotoxins,[2] immunosuppressives,[3] antibiotics,[4] ichthyotoxins,[5] tumor promoters,[6] and proteinase inhibitors.[7−9]

Our efforts in recent years have focused on screening marine cyanobacteria[10] for natural products[8] that are effective against multi-drug-resistant solid tumors. Of the extracts screened in 2001, two displayed significant selectivity for solid-tumor cell lines when compared to the leukemia and control cell lines. Here we report the results of the bioassay-guided fractionation of one of these solid-tumor selective extracts[11] that has led to the isolation and structure determination of two new alkaloids, micromide (**1**) and guamamide (**2**), along with three known lipopeptides, apramides A (**3**), B (**4**), and G (**5**).[12] Micromide (**1**) and guamamide (**2**) displayed IC_{50} values against KB cells at 260 and 1200 nM, respectively.



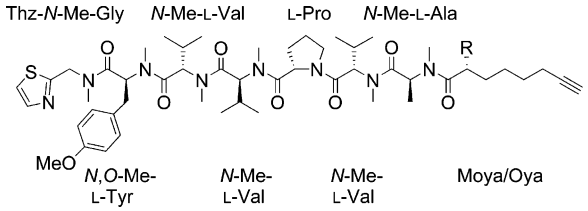
Micromide (**1**)



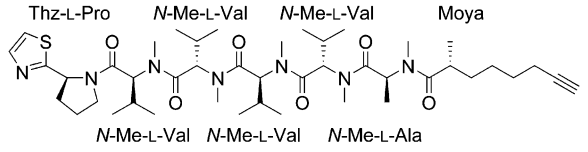
Guamamide (**2**)

Results and Discussion

The lipophilic extract of VP727 was separated by successive gel permeation and repeated reversed-phase chromatography to afford **1** in 0.12% yield, based on the crude extract.



Apramide A (3) R = Me
Apramide B (4) R =H



Apramide G (**5**)

The initial spectral data of the amorphous powder showed IR and UV/vis absorptions at 1680 cm^{-1} and 225 nm that suggested **1** was a peptide containing a heteroaromatic ring. HRMS (MALDI) established the elemental composition of the optically active compound as $C_{49}H_{73}N_7O_7S$, despite the presence of only 44 carbon signals in the ^{13}C NMR spectrum. Distinctive carbon resonances at δ_C 136.8, 135.9, 129.3, 128.5, 128.4, 126.9, and 126.6 suggested the presence of two monosubstituted phenyl rings and brought the total carbon count from the ^{13}C NMR spectrum into agreement with the mass spectrometry data. This also accounted for eight of the 17 double-bond equivalents implied by the molecular formula. On the basis of the number of sp^2 carbons and their chemical shifts, the remaining degrees of unsaturation could be ascribed to seven carbonyl equivalents, one carbon−carbon double bond, and one more ring system.

Examination of the spectral data recorded in $CDCl_3$ (Table 1) established a series of partial structures. In conjunction with the UV/vis and MS data, a pair of doublets at δ_H 7.64 and 7.26 in the HSQC spectrum with $^1J_{C,H}$

* To whom correspondence should be addressed. Tel: (808) 956-7232. Fax: (808) 956-5908. E-mail: moore@gold.chem.hawaii.edu.
[†] University of Hawaii at Manoa.
[‡] University of Guam Marine Laboratory.
[§] Present address: Smithsonian Marine Station, Fort Pierce, FL 34949.

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Table 1. NMR Spectral Data for **1** in $CDCl_3$

	C/H no.	δ_H(J in Hz)	δ_C[a]	^{1}H−^{1}H COSY	HMBC[b]
N-Me-Gly-thiazole	1	7.64, d (3.3)	142.0, d	2	
	2	7.26, d (3.3)	120.1, d	1	
	3		165.6, s		4
	4	4.88, d (−15.1)	48.9, t	4_u	5
		4.38, d (−15.1)			
	5	2.56, s	34.8, q		4
N-Me-Phe	6		169.8, s		4, 5, 7
	7	5.34, dd (10.4, 4.2)	53.8, d	8	8, 15
	8	3.11, dd (−13.1, 10.4)	35.1, t	7	7
		2.22, dd (−13.1, 4.2)			
	9		136.8, s		8, 11/13
	10/14	7.02, d (7.7)	129.3, d		8, 14/10
	11/13	7.22, t (7.7)	128.5, d		12, 13/11
	12	7.13, t (7.7)	126.9, d		
	15	2.94, s	30.8, q		7
N-Me-Ile	16		171.4, s		7, 15, 17
	17	5.22, d (10.9)	57.0, d	18	21, 22
	18	2.19, m	33.3, d	17, 19, 21	17
	19	1.24, m	23.9, t	18, 20	20, 21
		1.00, m		18, 20	
	20	0.87, t (6.6)	10.9, q	19	
	21	0.84, d (6.0)	15.4, q	18	17
	22	3.24, s	30.3, q		17
Val	23		171.3, s		22, 24
	24	4.77, dd (8.3, 4.1)	54.0, d	24-NH, 25	26, 27
	24-NH	6.95, d (8.3)		24	
	25	1.88, m	30.5, d	24, 26, 27	24, 26, 27
	26	0.93, d (6.9)	19.9, q	25	24, 27
	27	0.81, d (6.8)	16.9, q	25	24, 26
N-Me-Val	28		170.0, s		24-NH, 24, 29
	29	4.65, d (11.2)	62.4, d	30	31, 32, 33
	30	2.34, m	25.0, d	29, 31, 32	31, 32
	31	1.01, d (6.4)	19.8, q	30	32
	32	0.84, d (5.9)	18.7, q	30	30, 31
	33	2.90, s	30.7, q		29
Phe	34		171.6, s		33, 35
	35	5.19, ddd (8.0, 7.2, 6.0)	50.1, d	36	36
	35-NH	6.82, d (8.0)			
	36	3.22, dd (−13.5, 6.0)	36.9, t	35, 36_u	
		2.80, dd (−13.5, 7.2)		36_d	
	37		135.9, s		36, 39/41
	38/42	6.83, d (7.9)	129.3, d		36, 40
	39/41	7.12, t (7.9)	128.4, d		
	40	7.17, t (7.9)	126.6, d		
3-methoxyhexanoic acid	43		170.5, s		35-NH, 44, 45
	44	2.30, d (6.1)	40.7, t	45	45
	45	3.45, m	77.4, d	44, 46	44, 49
	46	1.40, m	35.4, t	45	44, 45, 48
		1.28, m			
	47	1.27, m	18.3, t	48	48
	48	0.86, t (6.6)	14.1, q	47	
	49	3.27, s	56.6, q		45

[a] Multiplicity deduced by HSQC. [b] Protons showing long-range correlation with indicated carbon.

correlations to sp^2 carbon signals at δ_C 142.0 and 120.1 suggested the presence of a thiazole ring. HMBC correlations to a carbon at δ_C 165.6 from a pair of diastereotopic methylene protons (H-4) expanded this fragment into a thiazole-glycine unit. A second fragment was constructed starting from an oxygenated methine at δ_H 3.45. A series of $^3J_{C,H}$ correlations from this proton (H-45) to C-43, C-44, C-46, and C-49 revealed the majority of this unit. Further HMBC cross-peaks from a methyl triplet (H-48) to C-47 and C-46 established this last unusual moiety as 3-methoxyhexanoic acid. The remaining fragments, assembled from COSY and HMBC correlations, were one isoleucine, two valine, and two phenylalanine units. The sequence of **1** was determined through HMBC correlations. Two fragments, (Thz-*N*-Me-Gly)-(*N*-Me-Phe)-(*N*-Me-Ile)-Val (C-1 through C-27) and (*N*-Me-Val)-Phe (C-28 through C-40), were constructed by HMBC correlations from the *N*-methylamide proton signals. To be specific, cross-peaks were observed from H-5 to C-4 and C-6, from H-15 to C-7 and C-16, and from H-22 to C-17 and C-23. HMBC correlations to the secondary amide proton signals provided the remaining connectivites needed to establish unambiguously the planar structure depicted.

The absolute stereochemistry of the units in **1** was determined by a combination of chiral and RP-HPLC. Ozonolysis and acid hydrolysis liberated the amino acid-derived centers in **1** that were subsequently analyzed by chiral HPLC. This established the stereocenters derived from *N*-Me-L-Ile, *N*-Me-L-Phe, *N*-Me-D-Val, L-Val, and L-Phe. The configuration of the final stereocenter (C-45) was determined by comparison with synthetic standards.[13] Using a chiral column, racemic methyl 3-hydroxyhexanoate was resolved and the enantiomers were identified based on comparison of their optical rotations with the reported values.[14] In the successful procedure (Figure 1), the hydroxyester was methylated[15] in petroleum ether with dimethyl sulfate, potassium hydroxide, and a catalytic amount of triethylamine, before saponification of the ester.

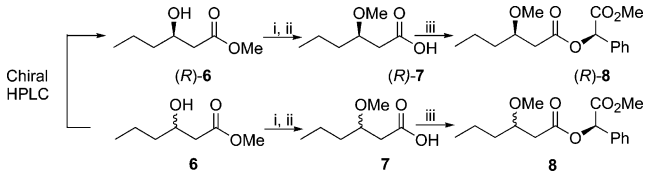

Figure 1. Synthesis of methyl D-mandelate derivatives.

Unfortunately, attempts to resolve these methoxyacids (**7**) failed on the OD or OJ chiral columns that were available to us. In the end, separation was achieved of the methyl D-mandelate derivatives (**8**) by C_{18} HPLC. The remaining quantity of **1** was therefore hydrolyzed with 6 N HCl at 118 °C for 24 h and the residue exhaustively extracted with CH_2Cl_2. Subsequent derivatization of this residue with methyl D-mandelate, followed by purification and analysis by C_{18} HPLC, established the configuration of C-45 as *R*. Thus the absolute stereochemistry of **1** is 7*S*,17*S*,18*S*,24*S*,-29*R*,35*S*,45*R*.

Examination of other fractions from the Sephadex LH-20 separation of the crude extract led to the isolation of guamamide (**2**). This optically active amorphous powder ($[\alpha]^{21}_D$ +6°) was less cytotoxic than **1**, with an IC_{50} value against KB cells of 1200 nM. High-resolution FABMS established the molecular formula of **2** as $C_{25}H_{45}NO_8$ based on a pseudo-molecular ion peak at 488.3225 ($[M + H]^+$ Δ 0.2 mDa) and revealed four degrees of unsaturation. These could be accounted for by the ^{13}C NMR spectrum, which along with three oxygenated methine signals (δ_C 74.1, 69.5, and 67.1) contained four carbonyl signals (δ_C 173.3, 173.1, 172.6, and 170.3). From the proton NMR spectrum, it was apparent that **2** contained one acetate (δ_H 2.11), one secondary amide (δ_H 6.17), one methoxy (δ_H 3.72), and two methyl doublet proton signals (δ_H 1.23 and 1.17). These fragments were expanded with the aid of the 2D NMR data (Table 2) to provide the gross structure depicted for **2**.

Only the configuration of C-3 in **2** was established. After derivatization with α-methoxyphenylacetic acid (MPA) the difference in the δ_H values of the (*R*)-MPA and (*S*)-MPA adducts (Figure 2) indicated C-3 possessed an *R* absolute configuration.

Micromide and guamamide are part of an ever-increasing class of polyketide-derived compounds that have been isolated from marine cyanobacteria. The most common of these metabolites are the malyngamides, characterized by a (−)-7-methoxy-4(*E*)-enoic acid moiety of varying length, appended through an amide linkage to a functionalized cyclohexane ring.[16] Micromide (**1**) contains structural features common to many cyanobacterial metabolites, including *N*-methylated amino acids, a D-amino acid, and a modified cysteine unit in the form of a thiazole ring. It should be noted that despite the structural similarity between micromide and the apramides, the IC_{50} of the former compound against KB cells was an order of magnitude greater than the latter series.

Experimental Section

General Experimental Procedures. The optical rotations were measured on a Jasco-DIP-700 polarimeter at the sodium D line (589 nm). The UV spectra were determined on a Hewlett-Packard 8453 spectrophotometer, and the IR spectra were recorded on a Perkin-Elmer 1600 FTIR instrument as a film on a NaCl disk. The NMR spectra of **1** and **2** were recorded in $CDCl_3$ on a Varian Inova 500 operating at 500 and 125 MHz using the residual solvent signal as the internal reference (δ_H 7.26, δ_C 77.00). FABMS and HRFABMS were recorded in the positive mode on a VG ZAB2SE spectrometer, and the MALDI spectra were recorded on a DE-STR. HPLC separations were performed on a Beckman 110B apparatus coupled to an Applied Biosystems 759A absorbance detector. The chiral OD and OJ silica columns, derivatized with cellulose tris(3,5-dimethylphenyl carbamate) and cellulose tris(4-methylbenzoate), respectively, were purchased from Chiral Technologies, Inc. Racemic methyl 3-hydroxyhexanoate was purchased from CTC Organics. HMBC experiments were optimized for $^nJ_{CH}$ = 7 Hz. All synthetic yields are unoptimized.

Table 2. NMR Spectral Data for **2** in $CDCl_3$

C/H no.	δ_H (*J* in Hz)	δ_C[a]	$^1H-^1H$ COSY	HMBC[b]
1		172.6, s		2, 22
2	2.50, d (4.3)	38.6, t	3	3
3	4.14, m	67.1, d	2, 4_d	2, 4_d
4	3.62, ddd (−11.6, 7.4, 4.4)	44.5, t	3, 4, NH	2
	3.08, ddd (−11.6, 7.4, 4.4)		4_d, NH	
NH	6.17, t (4.4)		4	
5		173.1, s		4_u, NH, 7, 23
6	2.55, p (7.1)	42.4, d	7, 23	7, 23
7	5.25, dd (7.1, 4.4)	74.1, d	6, 8	6, 9, 8, 23
8	5.00, qd (6.5, 4.4)	69.5, d	7, 9	6, 7, 9
9	1.23, d (6.5)	14.4, q	8	7, 8
10		173.3, s		8, 11
11	2.26, t (7.4)	34.4, t	12	12
12	1.58, m	24.8, t	11, 13	11
13	1.20, m	24.0, t	12	
14	1.20, m	29.6, t[c]		
15	1.20, m	29.6, t[c]		
16	1.20, m	29.5, t[c]		
17	1.20, m	29.3, t[c]		
18	1.20, m	29.3, t[c]		
19	1.20, m	31.9, t		21
20	1.21, m	22.6, t	21	21
21	0.87, t (6.9)	14.1, q	20	
22	3.72, s	52.0, q		
23	1.17, d (7.1)	13.5, q	6	6, 7
24		170.3, s		7, 25
25	2.11, s	20.8, q		

[a] Multiplicity deduced by HSQC. [b] Protons showing long-range correlation with indicated carbon. [c] These carbons may be interchanged.

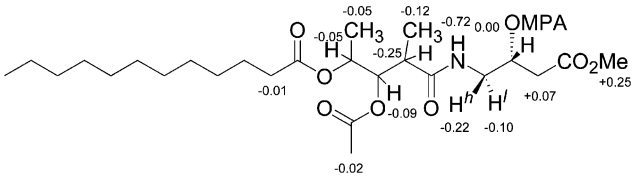

Figure 2. Δδ ($\delta_R - \delta_S$) values in ppm for the MPA esters of **2**.

Biological Material. The cyanobacterium VP727 was collected in the spring of 2001 in Guam at Fingers Reef. A voucher is maintained in formalin at the Smithsonian Marine Station, Fort Pierce, FL.

Extraction and Isolation. The cyanobacterium was exhaustively extracted with 4:1 $CH_3CN-CH_2Cl_2$ to afford 1.23 g of lipophilic extract. After initial partitioning between hexane and 80% aqueous MeOH, the organic residue from a second partitioning with *n*-BuOH and water was loaded onto a Sephadex LH-20 column (360 × 20 mm) and eluted with 5% MeOH in $CHCl_3$. The bioactive fraction that eluted between 120 and 140 mL was further purified by C_{18} chromatography, and the two fractions that eluted with 50 and 70% CH_3CN were combined. Separation by RP-HPLC [Ultracarb ODS, 250 × 10 mm, 3 mL/min, detection at 254 nm] afforded apramide A (t_R 22 min, 1.3 mg), apramide B (t_R 15 min, 1.0 mg), and a mixture of apramide G and micromide (t_R 41 min). This latter fraction was further purified by a linear gradient of 80 to 100% aqueous MeOH [YMC-AQ ODS column, 250 × 10 mm, 3 mL/min, PDA detection] to afford the former (t_R 14.2 min) and **1** (t_R 15.9 min) in 1.8 and 1.7 mg, respectively. The Sephadex

fraction eluting between 180 and 200 mL was purified by RP-HPLC with a linear gradient of 65 to 75% aqueous CH_3CN over 30 min [YMC-AQ ODS, 250 × 10 mm, 3 mL/min, PDA detection] to afford 1.7 mg of **2** (t_R 23 min).

Micromide (1): amorphous powder; $[\alpha]^{21}_D$ −28° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.40), 225 (3.80), 274 (2.40) nm; IR (film) ν_{max} 3311, 1680, 1462, 1379 cm^{-1}; ^1H NMR, ^{13}C NMR, $^1H-^1H$ COSY, and HMBC data, see Table 1; MALDI *m/z* [M + Na]$^+$ 926.5; HRMALDI *m/z* [M + Na]$^+$ 926.5155 (calcd for $C_{49}H_{73}N_7O_7SNa$ 926.5184).

Guamamide (2): amorphous powder: $[\alpha]^{21}_D$ +6° (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (3.71) nm; IR (film) ν_{max} 3382, 1739, 1651, 1371, 1233, 1095 cm^{-1}; ^1H NMR, ^{13}C NMR, $^1H-^1H$ COSY, and HMBC data, see Table 2; FAB *m/z* [M + Na]$^+$ 510; HRFAB *m/z* [M + H]$^+$ 488.3225 (calcd for $C_{25}H_{46}NO_8$ 488.3223).

Ozonolysis and Acid Hydrolysis of 1. Ozone was bubbled through a solution of 0.3 mg of **1** in 3 mL of CH_2Cl_2 for 15 min until a blue color persisted. The solvent was then removed and the residue hydrolyzed with 6 N HCl for 24 h at 118 °C. After removal of the acid under a stream of N_2, the residue was dissolved in 10% aqueous methanol and passed over a small C_{18} column. The components of this mixture were then analyzed by chiral HPLC [Column Chirex Phase 3126 (D), 250 × 4.6 mm, Phenomenex, flow rate 0.8 mL/min, detection at 254 nm]. The retention times (t_R, min) of the standards with CH_3CN-2 mM $CuSO_4$ (5:95) were *N*-Me-L-Val (14.0), *N*-Me-D-Val (19.2), L-Val (20.3), D-Val (27.1), *N*-Me-L-allo-Ile (27.0), *N*-Me-L-Ile (28.1), *N*-Me-D-Ile (42.0), and *N*-Me-D-allo-Ile (42.1). The retention times of the standards with CH_3CN-2 mM $CuSO_4$ (15:85) were *N*-Me-L-Phe (33.4), *N*-Me-D-Phe (36.2), L-Phe (41.0), and D-Phe (42.5). The retention times of the components of the hydrolyzate were *N*-Me-D-Val (19.2), L-Val (20.3), *N*-Me-L-Ile (28.1), *N*-Me-L-Phe (33.4), and L-Phe (41.0), as confirmed by co-injection.

Chiral Separation of Methyl 3-Hydroxyhexanoate. A racemic mixture of methyl 3-hydroxyhexanoate was separated by chiral HPLC [OD gold, 250 × 10 mm, 15% *i*-PrOH in hexane, 1 mL/min, PDA detection] to afford methyl (*R*)- and (*S*)-3-hydroxyhexanoate at 21.4 and 27.6 min with $[\alpha]^{23}_D$ of −22° and +23° (*c* 0.8, MeOH), respectively.[15]

Racemic and (*R*)-3-Methoxyhexanoic Acid (6). To 0.5 mL of racemic methyl 3-hydroxyhexanoate in 20 mL of petroleum ether (bp 40−60 °C) were added KOH (4 pellets), 14 μL of triethylamine, and 0.39 mL of dimethyl sulfate. The solution was allowed to stir at room temperature for 5 h before removal of the solvent under a stream of N_2. To this mixture were then added 5 mL of 0.5 N NaOH and 5 mL of MeOH, and the solution was stirred overnight. The mixture was then acidified to pH 2 with 1 N HCl and extracted into diethyl ether. The organic layer was dried with $MgSO_4$ and concentrated in vacuo to afford racemic **6** in approximately 80% yield. Treatment of methyl (*R*)-3-hydroxyhexanoate in a similar manner provided (*R*)-**6**. ^1H NMR of (*R*)-**6** ($CDCl_3$, 300 MHz): δ_H (integration, multiplicity, *J* in Hz) 3.64 (1H, m), 3.39 (3H, s), 2.53 (2H, d, *J* = 5.9), 1.61 (1H, m), 1.49 (1H, m), 1.40 (2H, m), 0.93 (3H, t, *J* = 7.3).

Mandelate Derivatives (8). To 10 mg of **6** in 0.2 mL of CH_2Cl_2 were added 2 mg of DMAP, 12 mg of methyl D-mandelate, and 70 μL of 1 M *N*,*N*′-dicyclohexylcarbodiimide (DCC) in CH_2Cl_2. The mixture was stirred at room temperature for 3 h before filtration and evaporation. The crude residue was purified by silica chromatography eluting with a mixture of 6:1 hexane−*i*-PrOH. Evaporation of the solvent afforded a mixture of (methyl D-mandelyl) (*R*)- and (*S*)-3-methoxyhexanoate [(*R*)- and (*S*)-**8**] in 85% yield. (*R*)-**6** was treated in the same manner to afford enantiomerically pure (*R*)-**8**. ^1H NMR of (*R*)-**8** ($CDCl_3$, 300 MHz): δ_H (integration, multiplicity, *J* in Hz) 7.46 (5H, m), 5.95 (1H, s), 3.72 (3H, s), 3.70 (1H, m), 3.34 (3H, s), 2.67 (1H, dd, *J* = −15.3, 7.3), 2.57 (1H, dd, *J* = −15.3, 5.6), 1.6−1.4 (4H, m), 0.88 (3H, t, *J* = 6.5).

Acid Hydrolysis and Mandelate Derivatization of 1. To 0.5 mg of **1** was added 0.3 mL of 6 N HCl, and the solution was refluxed at 118 °C for 24 h. After removal of the acid under a stream of N_2, the residue was dissolved in water and exhaustively extracted with CH_2Cl_2. The organic extracts were combined and dried over $MgSO_4$, and the solvent was removed. This residue was then dissolved in 0.1 mL of CH_2Cl_2, and to this were added 1 crystal of DMAP, 1.2 mg of methyl D-mandelate in 0.5 mL of CH_2Cl_2, and 0.02 mL of 1 M DCC. After stirring for 3 h the mixture was purified by HPLC [Bondclone Si, 250 × 10 mm, a linear gradient of 1 to 15% *i*-PrOH in hexane, 2 mL/min, PDA detection]. The fraction eluting between 9 and 10 min was subsequently repurified by HPLC [Phenosphere Silica, 150 × 4.6 mm, 0.5% *i*-PrOH in hexane, 0.75 mL/min, PDA detection].

HPLC Analysis of (Methyl D-Mandelyl) 3-Methoxyhexanoate. The derivatized hydrolyzate of **1** was analyzed by HPLC [Phenosphere Silica, 150 × 4.6 mm, 0.5% *i*-PrOH in hexane, 0.75 mL/min, PDA detection] to establish the configuration of C-45 of **1** by comparison with the synthetic standards (*SR*)-**8** and (*R*)-**8**. The derivatized hydrolyzate was found to contain (methyl D-mandelyl) 3*R*-methoxyhexanoate (27.3 min), which was verified by co-injection of the appropriate standard. The retention times of the synthetic standards under the same HPLC conditions were 24.1 and 27.3 min for (*S*)- and (*R*)-**8**, respectively.

MPA Derivatives of 2: (*R*)-MPA-2. To 0.5 mg of **2** in 0.3 mL of CH_2Cl_2 were added 1 crystal of DMAP, 4 mg of (*R*)-methoxyphenylacetic acid (MPA), and 4 mg of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride. The solution was stirred at room temperature overnight and then quenched with cold 1 N HCl (0.4 mL). After partitioning between ethyl acetate and water, the organic layer was dried over $MgSO_4$. The residue was dissolved in 20% EtOAc in hexanes and applied to a prepacked silica column (500 mg). (*R*)-MPA-**2** eluted in the 40% EtOAc in hexane fraction (4 mL) after 2 mL of hexane and 2 mL of 25% EtOAc had passed through the column. ^1H NMR ($CDCl_3$, 500 MHz): δ_H (position, multiplicity, *J* in Hz) 7.47−7.37 (Ph-MPA), 5.29 (NH, t, *J* = 6.2), 5.31 (H-3, ddt, *J* = 6.2, 3.7, 1.5), 5.15 (H-7, dd, *J* = 7.1, 5.6), 4.93 (H-8, qd, *J* = 6.6, 5.6), 4.78 (α-MPA, s), 3.65 (H-22, s), 3.45 (H-4_L, ddd, *J* = −13.9, 6.2, 3.7), 3.25 (H-4_H, dt, *J* = −13.9, 6.2), 3.42 (OMe-MPA, s), 2.60 (H-2, d, *J* = 1.5), 2.26 (H-11, t, *J* = 7.5), 2.18 (H-6, p, *J* = 7.1), 2.08 (H-25, s), 1.51 (H-12, m), 1.28 (H-13 to H-20, m), 1.15 (H-9, d, *J* = 6.6), 0.98 (H-23, d, *J* = 7.1), 0.88 (H-21, t, *J* = 6.9).

(*S*)-MPA-2. This derivative was prepared in the same manner as (*R*)-MPA-**2** except using (*S*)-methoxyphenylacetic acid. ^1H NMR ($CDCl_3$, 500 MHz): δ_H (position, multiplicity, *J* in Hz) 7.44−7.32 (Ph-MPA), 6.01 (NH, t, *J* = 6.4), 5.31 (H-3, ddt, *J* = 6.4, 4.4, 1.2), 5.24 (H-7, dd, *J* = 7.1, 5.1), 4.98 (H-8, qd, *J* = 6.6, 5.1), 4.79 (α-MPA, s), 3.55 (H-4_L, ddd, *J* = −13.4, 6.4, 4.4), 3.47 (H-4_H, dt, *J* = −13.4, 6.4), 3.42 (OMe-MPA, s), 3.40 (H-22, s), 2.53 (H-2, d, *J* = 1.2), 2.43 (H-6, p, *J* = 7.1), 2.27 (H-11, t, *J* = 7.4), 2.10 (H-25, s), 1.51 (H-12, m), 1.28 (H-13 to H-20, m), 1.20 (H-9, d, *J* = 6.6), 1.10 (H-23, d, *J* = 7.1), 0.88 (H-21, t, *J* = 6.5).

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References and Notes

(1) Burja, A. M.; Banaigs, B.; Abou-Mansour, E.; Burgess, J. G.; Wright, P. C. *Tetrahedron* **2001**, *57*, 9347−9377.

(2) Nogle, L. M.; Gerwick, W. H. *Org. Lett.* **2002**, *4*, 1095−1098.
(3) Koehn, F. E.; Longley, R. E.; Reed, J. K. *J. Nat. Prod.* **1992**, *55*, 613−619.
(4) Jaki, B.; Heilmann, J.; Sticher, O. *J. Nat. Prod.* **2000**, *63*, 1283−1285.
(5) Orjala, J.; Nagle D. G.; Hsu, V.; Gerwick, W. H. *J. Am. Chem. Soc.* **1995**, *117*, 8281−8282.
(6) Mynderse, J. S.; Moore, R. E. *J. Org. Chem.* **1978**, *43*, 2301−2303.
(7) Sano, T.; Kaya, K. *J. Nat. Prod.* **1996**, *59*, 90−92.
(8) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Mooberry, S. L. *J. Nat. Prod.* **2000**, *63*, 611−615.
(9) Mitchell, S. S.; Faulkner, D. J.; Rubins, K.; Bushman, F. D. *J. Nat. Prod.* **2000**, *63*, 279−282.
(10) Moore, R. E.; Corbett, T. H.; Patterson, G. M. L.; Valeriote, F. A. *Curr. Pharm. Des.* **1996**, *2*, 317−330.
(11) For the compounds isolated from the other solid-tumor selective extract see: Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2003**, *63*, 620−624, and references therein.
(12) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2000**, *63*, 1106−1112.
(13) Attempts to demethylate **1** with BBr_3 failed to liberate the secondary alcohol needed for Mosher analysis. Bhatt, M. V.; Kulkarni, S. U. *Synthesis* **1983**, *4*, 249−282.
(14) Lemieux, R. U.; Giguere, J. *Can. J. Chem.* **1951**, *29*, 678−690.
(15) Fafadia, N. J.; Wakankar, D. M.; Nambudiry, M. E. N. *Chem. Ind.* **1988**, *6*, 203−204.
(16) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: San Diego, 2001; Vol. 57, pp 75−184.

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