Tasipeptins A and B: New Cytotoxic Depsipeptides from the Marine Cyanobacterium *Symploca* sp.

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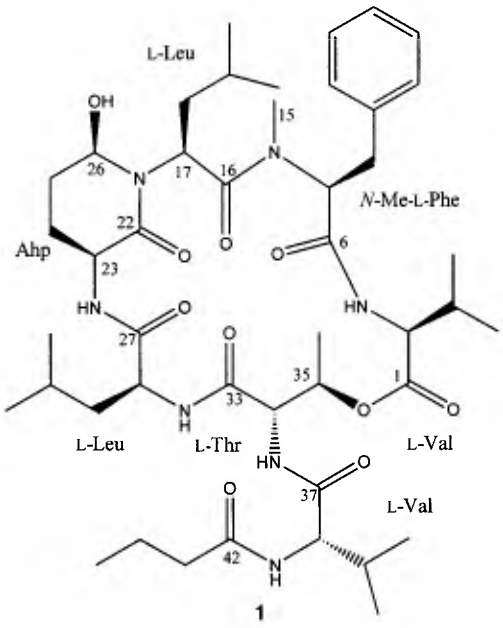
Two new depsipeptides have been isolated from a *Symploca* sp. collected in Palau. The gross structures of tasipeptins A (**1**) and B (**2**) were determined by standard spectroscopic techniques, and the absolute configuration of the amino acid units was determined by chiral HPLC. The relative stereochemistry of the 3-amino-6-hydroxy-2-piperidone (Ahp) moiety in both structures was determined by analysis of $^{2,3}J_{H,H}$ values. Oxidation with PCC and acid hydrolysis unmasked this latent glutamic acid moiety, allowing for elucidation of the total configuration of **1** and **2**. Tasipeptins A (**1**) and B (**2**) were cytotoxic toward KB cells with IC_{50} values of 0.93 and 0.82 μM, respectively.

Cyanobacteria are well-known sources of peptides and depsipeptides that display a variety of biological activities.[1] As part of a collaborative effort to discover new antitumor agents effective against solid and/or multidrug-resistant tumors, we began screening extracts of cyanobacteria collected in Micronesia.[2] The vast majority of these cyanobacteria belonged to the genus *Lyngbya*,[3] but among the samples collected in 1999 was a *Symploca* sp., the extracts of which displayed potent solid tumor selectivity.[4] Fewer reports have appeared in the literature on the chemistry and biological activity of this genus compared with *Lyngbya*;[5,6] however, recent investigations have hinted at the pharmaceutical potential of *Symploca*.[7−9]

Encouraged by these reports, a large collection of the cyanobacterium was made in the spring of 2000. From this *Symploca* sp., we have already described the isolation of tasiamide,[10] a cytotoxic acyclic peptide, and further examination of the aqueous extract has now led to the isolation and structure determination of the cyclic depsipeptides tasipeptins A (**1**) and B (**2**).



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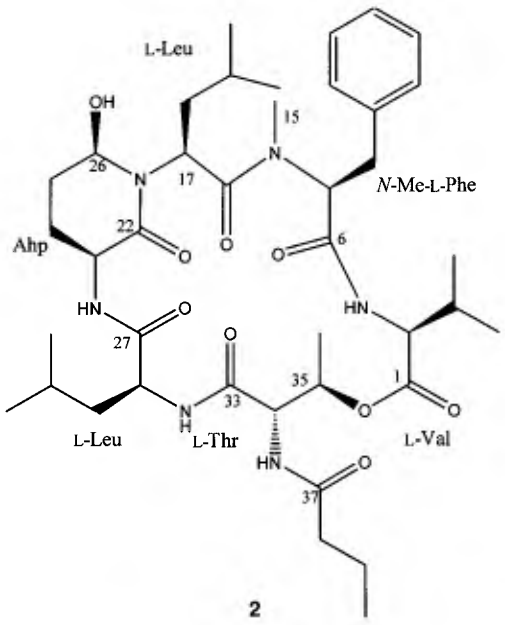
Results and Discussion

Solvent partitioning and gel permeation chromatography of the aqueous extract of *Symploca* sp. NIH304 yielded a series of cytotoxic fractions. One fraction after separation by repeated reversed-phase HPLC provided 4.3 and 2.2 mg of tasipeptins A (**1**) and B (**2**) in 0.24% and 0.12% yield based on the crude aqueous extract. Tasipeptins A (**1**) and B (**2**) were cytotoxic toward KB cells with IC_{50} values of 0.93 and 0.82 μM, respectively.

Tasipeptin A (**1**) was a colorless amorphous powder whose UV/vis spectrum showed end absorptions only. Examination of the ^{1}H and ^{13}C NMR spectra of **1** recorded in $CDCl_3$ indicated 14 sp^2 carbons, 13 methines, seven methylenes, and 11 methyl groups in accordance with a molecular weight of 892.5140 established by MADLI-TOF ($C_{45}H_{71}N_7O_{10}Na$, Δ 1.5 mmu). Based on chemical shifts, eight of the 14 sp^2 carbons were carbonyls (δ_C 174.0, 173.5, 172.5, 172.4, 171.2, 170.2, 169.6, and 168.8), and the remainder constituted a monosubstituted phenyl ring (δ_C 137.1, 129.2, 129.1, and 126.9). This accounted for a total of 12 of the 14 degrees of unsaturation implied by the molecular formula with the remaining two double bond equivalents in the form of rings. The thin-film IR spectrum of **1** suggested a depsipeptide with vibrations characteristic



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of amides, esters, and hydroxyl groups at 1643, 1731, and 3291 cm^{-1}, respectively. These conclusions were corroborated by five exchangeable proton signals—one hydroxyl and four secondary amide proton signals—deduced by the lack of correlations in the HSQC spectrum.

One-dimensional TOCSY experiments on the secondary amide proton signals in **1** established the presence of one threonine, two leucine, and two valine residues. Irradiation of the α-proton signal at δ_H 5.40 (H-7) revealed a pair of geminal methylene proton signals with a −14.3 Hz coupling (H-8) that denoted an adjacent π system; namely, H-7 and H-8 were part of a phenylalanine unit. A TOCSY experiment on the alcohol proton signal (26-OH) indicated a modified amino acid residue with initial magnetization transfer to a proton signal at δ_H 5.14 (H-26) and then to a series of diastereotopic methylene protons. Irradiation of a secondary amide proton signal at δ_H 7.23 (23-NH), in a TOCSY experiment, showed strong correlations to these diastereotopic protons as well as a methine at δ_H 4.53 (H-23) and served to linked these spin systems. HMBC correlations to C-24 from H-25_{ax} and from this amide proton (23-NH) along with a COSY cross-peak from H-25_{ax} to H-26 established this unusual moiety as 3-amino-6-hydroxypiperidone (Ahp) found in previously identified *Symploca* metabolites.[9]

The remaining protons were assigned from the DQF-COSY spectrum that showed a methylene multiplet at δ_H 1.71 (H-44) with cross-peaks to a methyl triplet at δ_H 0.98 (H-45) and to a methylene triplet at δ_H 2.35 (H-43). This aliphatic chain was expanded into a butyric acid moiety by HMBC correlations between a carbonyl signal at 173.5 ppm and both methylenes.

The sequence of **1** was determined primarily by $^2J_{C,H}$ HMBC correlations between the 2° amide proton signals and the carbonyl carbons. Specifically cross-peaks between C-27/23-NH, C-33/28-NH, C-37/34-NH, C-42/38-NH, and C-6/2-NH established two partial fragments: Ahp-Leu-Thr-Val-butyrate and Val-Phe. The latter was expanded by HMBC cross-peaks between the lone *N*-methylamide proton signal (H-15) and the α-carbon of phenylalanine (C-7) and between H-15 and the carbonyl of the remaining leucine residue (C-16). Finally, $^3J_{C,H}$ correlations between H-17 and C-22 of the Ahp unit and between C-1 and H-35 unambiguously established the cyclic structure of **1**.

The stereochemistry of all the proteogenic and *N*-methylated amino acids was determined by chiral HPLC analysis of the acid hydrolysate of **1**, which contained diagnostic peaks for L-Thr, L-Val, L-Leu, and *N*-Me-L-Phe. PCC oxidation of **1** prior to acid hydrolysis and chiral HPLC analysis led to L-glutamic acid from the Ahp unit. The absolute stereochemistry of C-23 in the Ahp unit was therefore *S*. Analysis of the $^{2,3}J_{H,H}$ coupling constants, obtained through one-dimensional TOCSY and selective decoupling experiments, established the relative stereochemistry around the piperidone ring. Two large $^3J_{H,H}$ couplings to H-23 and the small $^3J_{H,H}$ couplings to H-26 indicated an axial and equatorial orientation, respectively, for these protons. Therefore the absolute configuration of the Ahp moiety was 23*S*, 26*R*.

The NMR signals of **2** were almost superimposable on those of **1**, and this suggested a minor variation in the gross structure. High-resolution mass spectrometry data of the optically active (−13° c 0.7, MeOH) amorphous powder established a molecular formula of $C_{40}H_{62}N_6O_9$ (MNa^+ 793.4454), indicating a smaller compound than **1**. Analysis of the one-dimensional TOCSY data established all the isolated spin systems and indicated one of the valine residues had been eliminated in **2**, which accounted for all the differences in the molecular formula. Once again HMBC correlations between the *N*-methylamide and 2° amide proton signals provided two fragments, Val-(*N*-Me-Phe)-Leu and Ahp-Leu-Thr-butyrate, that could be linked by HMBC cross-peaks (H-17/C-22, H-17/C-26) to form a linear chain. Finally, a HMBC correlation between C-1 and H-35 of threonine confirmed the cyclic nature of **2**. The absolute configuration was deduced in a manner analogous to **1**, leading us to conclude the same stereochemistry in the remaining units (2*S*, 7*S*, 17*S*, 23*S*, 26*R*, 28*S*, 34*S*, and 35*R*).

Tasipeptins A and B display characteristics typical of many cyanobacterial metabolites. Features such as *N*-methylation, the incorporation of polyketide units, and modified amino acids are biosynthetic signatures of cyanobacteria and probably serve to enhance the biological efficacy.[11] To date, the unusual Ahp unit has appeared in approximately 50 secondary metabolites isolated primarily from terrestrial and marine cyanobacteria (*Microcystis*,[12] *Oscillatoria*,[13] *Anabaena*,[14] *Nostoc*,[15] *Microchaete*,[16] *Scyonema*,[17] *Lyngbya*,[18] and *Symploca*[9] spp.), although this moiety has appeared in natural products from *Streptomyces resistomicificus*.[19] Surprisingly, regardless of the source, all known Ahp-containing metabolites share the same basic structure of a 19-membered ring constructed from six amino acids cyclized through the alcohol oxygen of threonine, or in one case 3-hydroxy-4-methylproline,[14] with variable side chains attached to the amino terminus of threonine. Also the sequence of the six amino acids that comprise the macrocycle is to a large extent conserved in the form of cyclo(L-Val/Ile-*N*-Me-L-Phe/Tyr^{20}-X_1-(3*S*,6*R*)-Ahp-X_2-L-Thr) with X_1 a hydrophobic L-amino acid (Leu, Ile, Val, and Phe), although a few examples with Thr in this position have been reported.[16] The identity of X_2 appears to play a crucial role in determining the biological activity since compounds that possess a nonpolar amino acid in this position are often reported to be inhibitors of chymotrypsin, while those with polar residues generally inhibit trypsin but have little effect on chymotrypsin.[11,14,21–23] A crystal structure of a complex between trypsin and an Ahp-containing compound (A90720A; X_1 = L-Leu, X_2 = L-Arg) indeed showed that the guanadinium provided a number of key hydrogen bonds within the specificity pocket, while hydrogen bonds around the Ahp unit define the elliptical shape of A90720A.[24]

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. HRMALDI-MS data were recorded in the positive mode on a DE-STR spectrometer. The NMR spectra were recorded in $CDCl_3$ on a Varian Unity INOVA 500 operating at 500 and 125 MHz using the residual solvent signal as the internal reference. HPLC separations were performed on a Beckman 110B apparatus coupled to an Applied Biosystems 759A absorbance detector.

Biological Material. The cyanobacterium, designated NIH304, was collected at Short Drop-off in Palau during May of 2000. The organism was identified by V. J. Paul, and a voucher is maintained in formalin at the Smithsonian Marine Station, Fort Pierce, FL.

Extraction and Isolation. The freeze-dried cyanobacterium (250 g) was thrice extracted with 30% aqueous ethanol, and the extracts were combined and concentrated in vacuo. The crude extract was triturated with methanol, filtered, and

Table 1. NMR Spectral Data for Tasipeptin A (**1**) in $CDCl_3$

	C/H no.	δ_H[a] (*J* in Hz)	δ_C[b,c]	COSY[d]	HMBC[d,e]	ROESY
Val	1		172.5, s		3, 35	
	2	4.57, dd (7.8, 4.7)[f]	57.4, d	2-NH[f]	4, 5	
	2-NH	6.96, d (7.8)		2[f]		
	3	2.15, m	31.1, d	2[f]		
	4	0.92, d (6.6)	18.9, q	3		
	5	0.87, d (6.7)	18.1, q	3		15, 36
N-Me-Phe	6		169.6, s		2-NH, 7	
	7	5.40, dd (11.5, 2.5)	62.0, d	8	15	17
	8	3.45, dd (−14.3, 2.5)	34.0, t	7		
		2.91, dd (−14.3, 11.5)		7		
	9		137.1, s		7, 8	
	10/14	7.27, d (7.0)	129.1, d	11/13	8	
	11/13	7.18, t (7.0)	129.2, d	10/14, 12		
	12	7.22, t (7.0)	126.9, d	11/13		
	15	2.89, s	31.0, q		7	4
Leu	16		174.0, s		15, 17	
	17	4.84, dd (10.9, 4.2)	49.6, d	18		7
	18	1.68, m	37.4, t	17	17, 20, 21	26
		0.33, m		17		17
	19	0.97, m	24.2, d		20, 21	20, 21
	20	0.46, d (6.5)	21.6, q	19	21	17
	21	0.68, d (6.6)	23.6, q	19	20	
Ahp	22		170.2, s		17, 26	
	23	4.53, ddd (12.7, 8.0, 5.6)[f]	50.6, d	23-NH[f], 24	23-NH, 24_{ax}	
	23-NH	7.23, d (8.0)		23[f]		
	24_{ax}	2.43, qd (12.7, 2.2)	21.8, t	23, 25_{eq}	23-NH, 25_{ax}	24_{eq}, 25_{ax}, 23-NH
	24_{eq}	2.05, m				24_{ax}
	25_{eq}	2.04, m	29.5, t	26		26
	25_{ax}	1.83, td (13.5, 2.9)		24_{ax}, 26		26
	26	5.14, br d (5.0)	74.9, d	26-OH		18, 25, 26-OH
	26-OH	4.32, br d (5.0)		26		26
Leu	27		171.2, s		23-NH, 29a	
	28	4.48, br dd (9.4, 7.9)[f]	52.1, d	28-NH[f], 29a		
	28-NH	6.41, d (7.9)		28[f]	30	34
	29	1.91, ddd (−13.5, 9.4, 4.1)	38.8, t	28, 29b	28	
		1.57, m		28, 29a		
	30	1.53, m	24.7, d			
	31	0.90, d (6.1)	23.3, q	30	30, 32	
	32	0.83, d (6.6)	21.1, q	30	29, 30	
Thr	33		168.8, s		28-NH, 34, 35	
	34	4.74, d (9.2)	55.0, d	34-NH	35	28-NH, 35, 36
	34-NH	6.92, d (9.2)		34		38
	35	5.49, q (6.4)	71.6, d	36	1, 36	34, 36
	36	1.32, d (6.4)	18.3, q	35		4, 34, 35
Val	37		172.4, s		34, 34-NH	
	38	4.47, t (8.1)[f]	58.5, d	38-NH[f], 39		
	38-NH	6.36, d (8.1)		38[f]		43
	39	1.24, m	31.4, d	40, 41		
	40	0.96, d (7.4)	19.2, q	40	38	
	41	0.95, d (7.2)	18.1, q	40	38	
Butanoic	42		173.5, s		38-NH, 43, 44	
	43	2.35, t (7.1)	38.3, t	44	44, 45	38-NH, 44
	44	1.71, m	19.2, t	43, 45		43
	45	0.98, t (6.8)	13.3, q	44		38-NH

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Multiplicity deduced by HSQC. [d] Protons showing long-range correlation with indicated carbon. [e] Correlations were observed for $^nJ_{CH} = 7$ Hz. [f] Confirmed by 1D-TOCSY experiments on the appropriate 2° amide proton.

concentrated to afford approximately 2 g of extract from the organic layer. This mixture was chromatographed on a Sephadex LH-20 column (25 × 500 mm) and eluted with 5% MeOH in $CHCl_3$ (400 mL), 15% MeOH (700 mL), and pure MeOH (500 mL). The fractions eluting between 80 and 140 mL of 5% MeOH were combined and loaded on a C_{18} column and eluted with increasing amounts of aqueous MeCN. The 50% and 60% fractions were combined and subjected to reversed-phase HPLC (Ultracarb 5 ODS 30, 10 × 250 mm; flow rate 3 mL/min; detection at 220 nm) with 45% MeCN in H_2O to give tasipeptin B ($t_R = 47.6$ min) and tasipeptin A ($t_R = 56.3$ min). Both were subsequently repurified on a YMC-AQ column (10 × 250 mm; flow rate 2.5 mL/min; detection at 220 nm) with 50% aqueous MeCN to yield 2.2 mg ($t_R = 22.8$ min) of **2** and 4.3 mg ($t_R = 31.3$ min) of **1** in 0.12% and 0.24% yield, respectively, based on the crude aqueous extract.

Tasipeptin A (1): amorphous powder; $[\alpha]^{24}_D$ −23° (c 1.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.88) nm; IR (film) ν_{max} 3371, 3291, 1731, 1643, 1453 cm^{-1}; ^1H NMR, ^{13}C NMR, ^1H–^1H COSY, and HMBC data, see Table 1; HR-MALDI *m/z* [M + Na] $^+$ 892.5140 (calcd for $C_{45}H_{71}N_7O_{10}Na$ 892.5155).

Tasipeptin B (2): amorphous powder; $[\alpha]^{21}_D$ −13° (c 0.7, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.88) nm; IR (film) ν_{max} 3400, 3304, 1735, 1650, 1536, 1462, 1205 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 2; MALDI *m/z* [M + Na]$^+$ 793; HR-MALDI *m/z* [M + Na] $^+$ 793.4454 (calcd for $C_{40}H_{62}N_6O_9$-Na 793.4475).

Absolute Stereochemistry. To 0.3 mg of **1** in 0.1 mL of CH_2Cl_2 was added 2 mL of dry CH_2Cl_2 containing 2 mg of pyridinium chlorochromate. The reaction was stirred overnight before being partitioned between methylene chloride and water. The organic layer was removed under a stream of N_2 and dissolved in 0.3 mL of 6 N HCl. The solution was refluxed at 118 °C for 18 h, and then the solvent was evaporated. The hydrolysate was analyzed by chiral HPLC, and the retention times were compared with authentic standards [Column

Table 2. NMR Spectral Data for Tasipeptin B (**2**) in $CDCl_3$

	C/H no.	δ_H[a] (*J* in Hz)	δ_C[b,c]	COSY	HMBC[d,e]	ROESY
Val	1		172.4, s		3, 35	
	2	4.57, dd (7.2, 4.7)[f]	57.3, d	2-NH[f]	3, 4, 5	
	2-NH	6.94, d (7.2)		2[f]		7, 15
	3	2.13, m	31.1, d	2[f]		
	4	0.89, d (6.6)	18.8, q		3	
	5	0.86, d (6.7)	18.1, q		3	
N-Me-Phe	6		169.5, s		2-NH, 7	
	7	5.41, dd (8.9, 2.5)	62.1, d	8	15	2-NH
	8	3.45, dd (−14.7, 2.5)	33.9, t	7		17
		2.90, dd (−14.7, 8.9)		7		
	9		137.1, s		8, 11/13	
	10/14	7.27, d (5.9)	129.0, d	11/13	8, 10/14	
	11/13	7.19, dd (6.0, 5.9)	129.3, d	10/14, 12	11/13, 12	
	12	7.22, t (6.0)	126.8, d	11/13		
	15	2.86, s	31.0, q		7	
Leu	16		174.0, s		15, 17	
	17	4.82, t (9.5)	48.5, d	18b		7
	18	1.69, m	37.4, t	18b	17, 20, 21	
		0.31, dt (10.4, 9.5)				
	19	0.99, m	24.2, d	18b	20, 21	
	20	0.46, d (6.5)	21.7, q	19		17
	21	0.69, d (6.6)	23.6, q	19		
Ahp	22		170.3, s		17, 26	
	23	4.55, ddd (11.6, 8.6, 3.5)[f]	50.6, d	23-NH[f], 24		
	23-NH	7.21, d (8.6)		23[f]		24_{ax}
	24_{ax}	2.45, qd (11.6, 2.2)	21.8, t	23[f], 25	23	NH-23, 24_{eq}, 25_{eq}
	24_{eq}	2.04, m		25		
	25_{eq}	2.01, m	29.5, t	24	23	
	25_{ax}	1.85, td (13.5, 2.9)		24[f]		26
	26	5.10, br d (5.0)	74.8, d	25, 26-OH	17	26
	26-OH	4.42, br d (5.0)		26		
Leu	27		171.4, s		23-NH	
	28	4.50, br dd (9.3, 8.3)[f]	51.9, d	28-NH[f]		23
	28-NH	6.65, d (8.3)		28		29b, 34, 35
	29	1.97, ddd (−14.1, 9.3, 4.4)	39.2, t	28[f]	31, 32	26
		1.57, m				26
	30	1.53, m	24.7, d			
	31	0.90, d (6.6)	23.2, q	30	29a	
	32	0.85, d (6.6)	21.3, q	30	29a	
Thr	33		169.3, s		28-NH, 34	
	34	4.85, d (9.2)	54.7, d	34-NH[f]	36	28-NH, 34-NH
	34-NH	6.75, d (9.2)		34		38
	35	5.50, q (6.4)	71.8, d	36	36	34
	36	1.30, d (6.4)	18.2, q	35		34
Butanoic	37		174.1, s		34-NH, 38, 39	
	38	2.35, t (7.1)	38.3, t	39	39, 40	
	39	1.71, m	19.2, t	38, 40	38	
	40	0.98, t (6.8)	13.3, q	39		

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Multiplicity deduced by HSQC. [d] Protons showing long-range correlation with indicated carbon. [e] Correlations were observed for $^nJ_{CH} = 7$ Hz. [f] Confirmed by 1D-TOCSY experiments on the appropriate 2° amide proton.

Chirex Phase 3126 (D) (4.6 × 250 mm), Phenomenex; flow rate 0.8 mL/min; detection at 254 nm; solvent 2 mM $CuSO_4$ for Thr; 2 mM $CuSO_4$–MeCN (95:5) for Val, Leu, and Glu; and 85:15 for *N*-Me-Phe]. The retention times (t_R, min) of the standards were L-Thr (12.0), L-allo-Thr (14.9), D-Thr (15.0), D-allo-Thr (20.0), L-Val (21.8), D-Val (30.2), L-Leu (61.5), D-Leu (80.2), L-Glu (83.3), D-Glu (93.4), *N*-Me-L-Phe (38.1), *N*-Me-D-Phe (40.6). The retention times of the components in the hydrolysate of **1** were L-Thr (12.0), L-Val (21.8), L-Leu (61.5), L-Glu (83.3) and *N*-Me-L-Phe (38.1). The hydrolysate of **2** after PCC oxidation as described above was found to contain L-Thr (12.0), L-Val (21.8), L-Leu (61.5), L-Glu (83.3) and *N*-Me-L-Phe (38.1).

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