Microspinosamide, a New HIV-Inhibitory Cyclic Depsipeptide from the Marine Sponge *Sidonops microspinosa*[1]

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Microspinosamide (**1**), a new cyclic depsipeptide incorporating 13 amino acid residues, was isolated from extracts of an Indonesian collection of the marine sponge *Sidonops microspinosa*. Its structure was elucidated by extensive NMR and mass spectral analyses, and by chemical degradation and derivatization studies. The tridecapeptide **1** incorporates numerous uncommon amino acids, and it is the first naturally occurring peptide to contain a *β*-hydroxy-*p*-bromophenylalanine residue. Microspinosamide (**1**) inhibited the cytopathic effect of HIV-1 infection in an XTT-based in vitro assay with an EC_{50} value of approximately 0.2 *μ*g/mL.

Marine sponges have provided a wide range of structurally unique, biologically active nonribosomal peptide derivatives.[2,3] Sponge peptides, including discodermins A−H from *Discodermia kiiensis*,[4−8] polydiscamide A (**2**) from *Discodermia* sp.,[9] and halicylindramides A−C from *Halichondria cylindrata*[10] constitute a family of structurally related tridecapeptides and tetradecapeptides. These cyclic depsipeptides share many uncommon amino acid residues, and they reportedly possess antimicrobial and cytotoxic properties.[4−10] We have broadened the known taxonomic distribution of this series of peptides with the isolation of microspinosamide (**1**) from an Indonesian collection of the sponge *Sidonops microspinosa* Wilson (Geodiidae). To the best of our knowledge, microspinosamide (**1**) is the first metabolite described from a *Sidonops* species, and it contains the amino acid residue *β*-hydroxy-*p*-bromophenylalanine, which has not been reported previously from a naturally occurring peptide.

Both the aqueous and organic extracts of *S. microspinosa* exhibited activity in the NCI's primary anti-HIV screen.[11] A portion of the aqueous extract (5 g) was dissolved in H_2O and applied to a wide-pore C_4 vacuum liquid chromatography (VLC) column. Sequential elution with 100% H_2O, H_2O−MeOH (2:1), H_2O−MeOH (1:2), and 100% MeOH concentrated the anti-HIV activity into the H_2O−MeOH (1:2) eluent. This material was further purified by gradient elution C_{18} reversed-phase HPLC, with increasing concentrations of CH_3CN in H_2O, to give 14.7 mg of microspinosamide (**1**). The positive-ion FABMS of **1** exhibited pseudomolecular ion pairs at *m/z* 1725.7 and 1727.7 for $[M + H]^+$ and *m/z* 1747.7 and 1749.7 for $[M + Na]^+$, while negative-ion FABMS revealed $[M - H]^-$ peaks at *m/z* 1723.7 and 1725.7. These MS data revealed the presence of one bromine atom in **1** and established its nominal molecular weight as 1724.7 and 1726.7. The molecular formula for **1** was determined to be $C_{75}H_{109}BrN_{18}O_{22}S$ by high-resolution FABMS analysis of a CsI-doped sample ($[M + Cs]^+$ *m/z* 1857.6169, calcd for $C_{75}H_{109}{}^{79}BrN_{18}O_{22}SCs$, 1857.5919) and by detailed analysis of the ^1H and ^{13}C NMR data. The molecular formula of **1** corresponded closely to the molecular formula ($C_{75}H_{109}BrN_{19}O_{20}SNa$) reported for polydiscamide A (**2**),[9] which suggested a close structural relationship between these two compounds. The peptide nature of **1** was confirmed by the presence of 15 carbonyl signals (*δ* 171−178) in its ^{13}C NMR spectrum and 10 secondary amide proton resonances between *δ* 7.48 and 8.45 in its ^1H NMR spectrum. Comparison of the complete ^1H and ^{13}C NMR spectral data of **1** (Table 1) with the values published for polydiscamide A (**2**)[9] confirmed the structural similarity of these two peptides.

Extensive 1D and 2D NMR analyses of microspinosamide (**1**) established the presence of alanine, tryptophan, arginine, threonine, aspartic acid, valine, and two proline residues. It was also possible to identify the uncommon amino acid residues *tert*-leucine, *β*-methylisoleucine, *N*-methylglutamine, and cysteic acid. A new residue, *β*-hydroxy-*p*-bromophenylalanine, was inferred from interpretation of the NMR data. A proton spin system comprising an amide NH (*δ* 8.42), an α-methine proton (δ_H 4.92, δ_C 57.3), and a *β*-oxymethine group (δ_H 4.80, δ_C 75.7) was evident from COSY and HSQC correlations. A pair of two-proton doublets (*δ* 7.36 and 7.47, $J = 8.0$ Hz) and aromatic carbon resonances at *δ* 122.8, 130.5 (2C), 132.2 (2C), and 141.3 revealed the presence of a 1,4-disubstituted benzene ring. HMBC correlations between both the α- and *β*-methine protons and the C-1 aromatic carbon resonating at *δ* 141.3 established that the aromatic ring was attached to the *β*-carbon. A close similarity between the aromatic proton and carbon NMR data reported for the *p*-bromophenylalanine residue in polydiscamide A (**2**)[9] and the corresponding resonances observed in **1**, indicated the presence of a *para*-bromine substituent. Thus, it was evident that microspinosamide (**1**) contains a *β*-hydroxy-*p*-bromophenylalanine amino acid residue, which has not previously been reported in a naturally occurring peptide.

Another difference between microspinosamide (**1**) and polydiscamide A (**2**) was that NMR analysis of **1** indicated the presence of aspartic acid and *N*-methylglutamine

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Table 1. NMR Spectral Data for Microspinosamide (**1**) in CD_3OH

amino acid	$^{13}C^a$	^1H	mult *J* (Hz)	COSY[b]	TOCSY	HSQC−TOCSY[c]	HMBC[c]	NOESY
D-alanine (Ala)								
α	48.7 (d)	4.28	q (7.5)	0.99, 8.17	0.99, 8.17		18.2, 163.4, 173.6	8.42
β	18.2 (q)	0.99	d (7.5)	4.28		48.7	48.7, 173.6	
C═O	173.6 (s)							
NH		8.17	d (7.5)	4.28	0.94, 4.28			4.80 4.91, 7.97
HC═O	163.4 (s)	7.97	s					
β-hydroxy-*p*-bromophenylalanine (*β*-OH-BrPhe)								
α	57.3 (d)	4.92	m	4.80, 8.42	4.80, 8.42		75.7, 141.3, 171.6, 173.6	
β	75.7 (d)	4.80	m	4.92			57.3, 130.5, 141.3, 171.6	1.99, 3.78, 3.96
C-1	141.3 (s)							
C-2/C-6	130.5 (d)	7.36	d (8.0)	7.47		132.2	75.7, 122.8, 132.2	
C-3/C-5	132.2 (d)	7.47	d (8.0)	7.36		122.8, 130.5	122.8, 141.2	
C-4	122.8 (s)							
C═O	171.6 (s)							
NH		8.42	m	4.92	4.80, 4.98		173.6	4.28
L-proline I (Pro I)								
α	61.8 (d)	4.64	dd (8.5, 3.0)	2.19	1.99, 2.19, 3.78, 3.96	25.4, 48.9, 30.7		7.93
β	30.7 (t)	2.19	m	1.99, 4.64	1.99, 3.78, 3.96	25.4, 48.9	25.5, 173.9	
		2.19	m					
γ	25.4 (t)	1.98	m	4.64	3.78, 3.96, 4.64	30.7, 48.9, 61.8	61.8	
		1.98	m					
δ	48.9 (t)	3.78	dd (19.5, 8.5)	1.99	1.99, 2.19, 3.96, 4.64	25.4, 30.7, 61.8	25.5, 171.6	4.92
		3.96	m	1.99	1.99, 2.19, 3.78, 4.64	25.4, 30.7, 61.8		4.92
C═O	173.9 (s)							
L-*t*-Leucine (*t*-Leu)								
α	62.2 (d)	4.49	d (8.5)	7.93	7.93		27.4, 36.0, 173.2	7.93
β	36.0 (s)							
γ	27.4 (q)	1.01	s				36.0, 62.2	
NH		7.93	d (8.5)	4.49	4.49		173.1, 173.2	1.01, 4.49, 4.64
C═O	173.2 (s)							
L-*β*-methylisoleucine (*β*-MeIle)								
α	62.1 (d)	4.21	d (8.0)	7.68	7.68		23.6, 37.2, 173.2	4.21, 8.21
β	37.2 (s)							
γ	32.7 (t)	1.15	m	0.73	0.73	8.1	8.1, 23.0, 23.6, 37.2, 61.9	
		1.15	m					
δ	8.1 (q)	0.73	t	1.15	1.15	32.7		
γ′	23.2 (q)	0.72	s				23.6, 32.7, 37.2, 62.1	
γ″	23.6 (q)	0.83	s				23.2, 32.7, 37.2, 62.1	
NH		7.68	m	4.21	4.21	61.9	173.1, 173.2	1.01, 4.49
C═O	173.1 (s)							
D-tryptophan (Trp)								
α	56.0 (d)	4.68	m	3.14, 3.33, 8.21	3.14, 3.33, 8.21		28.5, 110.9, 173.7	8.21
β	28.5 (t)	3.14	m	4.68	4.68, 8.21	56.0	125.0, 128.5, 173.7	8.21
		3.33	m	4.68	4.68, 8.21	56.0	56.0, 125.0, 128.5	
C-2	125.0 (d)	7.15	s				110.9, 128.5, 138.1	
C-3	110.9 (s)							
C-3a	128.5 (s)							
C-4	119.3 (d)	7.53	br d (8.0)	6.97, 7.07	6.97, 7.07	112.3, 119.8. 122.4	110.9, 122.4, 138.1	
C-5	119.8 (d)	6.97	br. t (8.0)	7.07, 7.53	7.07, 7.31, 7.53	112.3, 119.3, 122.4	112.3, 128.5	
C-6	122.4 (d)	7.07	br. t (8.0)	6.97, 7.31	6.97, 7.31, 7.53	112.3, 119.3, 119.8	119.3, 138.1	
C-7	112.3 (d)	7.31	br d (8.0)	6.97, 7.07	6.97, 7.07	119.3, 119.8, 122.4		
C-7a	138.1 (s)							
NH-indole		10.27	s					
NH		8.21	d (8.0)	4.68	3.14, 3.33, 4.71	56.0	173.1, 173.7	4.21
C═O	173.7 (s)							
L-arginine (Arg)								
α	54.6 (d)	4.34	m	1.60, 8.45	1.31, 1.60, 1.76, 3.02		171.9, 173.7	8.38
β	29.6 (t)	1.60	m	1.31, 4.34		25.6, 29.6, 41.9, 54.6		
		1.76	m	1.31		25.6, 29.6, 41.9		
γ	25.6 (t)	1.31	m	1.60, 1.76, 3.02	1.31, 1.60, 1.76, 4.34	29.6, 41.9, 54.6	54.6	
δ	41.9 (t)	3.02	m	1.31	1.31, 1.60, 1.76, 4.34	25.6, 29.6, 54.6	25.6, 29.6, 158.6	
guanidine	158.6 (s)							
NH		8.45	m	4.34	4.34			3.33 (weak), 4.34

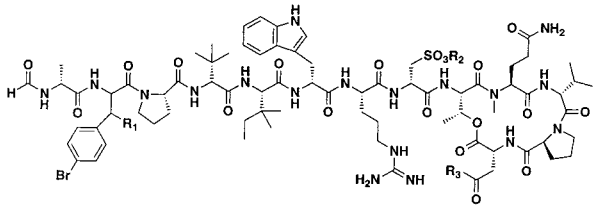
Table 1 (Continued)

amino acid	$^{13}C^a$	^1H	mult *J* (Hz)	COSY[b]	TOCSY	HSQC−TOCSY[c]	HMBC[c]	NOESY
C═O	171.9 (s)							
D-cysteic acid (Cys)								
α	52.4 (d)	4.85	m	8.38			171.9	3.34, 8.38
β	52.2 (t)	3.32	m	4.85	4.85, 8.38	52.4	52.4	
		3.34	m					4.85
NH		8.38	m	4.85	3.33, 3.34, 4.85	52.4		3.34, 4.34, 4.85
C═O	172.8 (s)							
L-threonine (Thr)								
α	54.4 (d)	4.99	d (9.5)	8.16	8.16, 5.43		171.2, 172.8	
β	70.3 (d)	5.43	m	4.99	1.50, 4.99	17.2, 70.3	171.2, 172.6	3.13
γ	17.2 (q)	1.27	d (6.5)	5.43	4.99, 5.43	70.3	54.4, 70.3	
NH		8.16	d	4.99	4.99, 5.43	17.2, 54.4		4.85
C═O	171.2 (s)							
L-*N*-methylglutamine (NMeGln)								
α	57.3 (d)	5.12	m	2.17		24.9, 32.6, 57.3	24.9, 32.6	7.99
β	24.9 (t)	2.17	m	1.99, 2.08, 5.12		32.6, 57.3	32.6, 177.3	
		2.17	m					
γ	32.6 (t)	1.99	m	2.17		57.3		
		2.08	m	2.17				
N-Me	31.5 (q)	3.15	s				172.3, 57.3	1.27, 4.99, 7.99
C═O	172.3 (s)							
O═C−NH_2		6.75	bs	7.34			32.6	
		7.34	bs	6.75			177.3	
O=*C*-NH_2	177.3 (s)							
D-valine (Val)								
α	57.7 (d)	4.44	m	7.99	2.07, 7.99	18.8, 31.7	18.8, 31.7, 172.5	7.99
β	31.7 (d)	2.07	m	4.44	0.94, 0.97, 4.44	18.8, 31.7, 57.7	19.3, 57.7	4.05
γ	18.8 (q)	0.97	d	2.07	2.07, 4.44	31.7, 57.7		
δ	19.3 (q)	0.94	d	2.07	2.07, 4.44	31.7, 57.7	18.8, 31.7, 57.7	
NH		7.99	d	4.44	0.94, 2.07, 4.44	18.8, 31.7, 57.7	172.3, 172.5	2.17, 3.15, 5.12
C═O	172.5 (s)							
L-proline II (Pro II)								
α	62.4 (d)	4.42	m	2.19	1.99, 2.19, 3.67, 4.05	25.6, 30.9, 48.8	25.6, 30.9, 172.5	
β	30.9 (t)	2.19	m	4.42			25.6, 174.0	
		2.05	m	4.42		25.6, 62.4		
γ	25.6 (t)	1.99	m	3.67				
		2.19	m	3.67		30.9, 48.8, 62.4		
δ	48.8 (t)	3.67	m	1.99, 2.19	2.19, 4.42	25.6, 30.9, 62.4		4.44
		4.05	m	2.19, 4.42	25.6, 30.9, 62.4	30.9		4.44
C═O	174.0 (s)							
D-aspartic acid (Asp)								
α	48.7 (d)	4.66	m	2.57, 2.98, 7.48	2.57, 2.98		172.6, 174.0	
β	35.8 (t)	2.57	m	4.66	4.66	48.7	172.6	
		2.98	m	4.66	4.66		172.6, 172.4	
COOH	172.4 (s)							
NH		7.48	m	4.66			174.0	2.18, 4.05, 4.42
C═O	172.6 (s)							

[a] Multiplicities determined using the DEPT pulse sequence. [b] Geminal couplings not shown. [c] Carbons correlated to proton resonances in the ^1H column.

residues, while compound **2** reportedly contained asparagine and *N*-methylglutamine.[9] Compound **1** contained only a single primary amide group, as only one pair of mutually coupled NH protons, *δ* 6.75 and 7.34, were observed in its ^1H NMR spectrum. The location of this amide group was facilitated by an HMBC correlation from the *δ* 6.75 proton to the *γ*-carbon (*δ* 32.6) of the *N*-methylglutamine residue. In an effort to confirm the presence of an aspartic acid residue in **1**, the parent peptide was treated with diazomethane to give the monomethyl ester derivative **3**. An HMBC correlation in **3** was observed between the methyl ester OMe group (*δ* 3.62, 3H, s) and the ester carbonyl carbon (*δ* 172.1). This unambiguously established the presence of an aspartic acid residue in **1** and revealed that treatment of **1** with diazomethane resulted in methylation of this residue, and not the cysteic acid.

The sequence of the amino acids in **1** was established from inter-amino acid NOESY and HMBC correlations (Figure 1). The ester link between the aspartic acid residue and the threonine hydroxyl group was defined by HMBC data. The downfield chemical shift of the threonine *β*-oxymethine proton (*δ* 5.43) supported the assignment of an ester link at this position. Placement of the formamide group



1 R_1 = OH, R_2 = H, R_3 = OH
2 R_1 = H, R_2 = Na, R_3 = NH_2
3 R_1 = OH, R_2 = H, R_3 = OCH_3

(δ_H 7.97 and δ_C 163.4) on the N-terminal alanine residue followed from an HMBC correlation between the alanine α-methine proton (*δ* 4.28) and the formamide carbonyl carbon. Thus, the macrocyclic ring and amino acid sequence of microspinosamide (**1**) are the same as those reported for polydiscamide A (**2**), except for the substitution in **1** of *β*-hydroxy-*p*-bromophenylalanine and aspartic acid for the *p*-bromophenylalanine and asparagine residues in **2**.

The absolute stereochemistries of the constituent amino acids of **1** were assigned by acid hydrolysis, treatment of the hydrolysate with Marfey's reagent (FDAA),[12] and LC−MS comparison with appropriate amino acid standards.[13] Additional LC−MS analysis of the amino acid derivatives

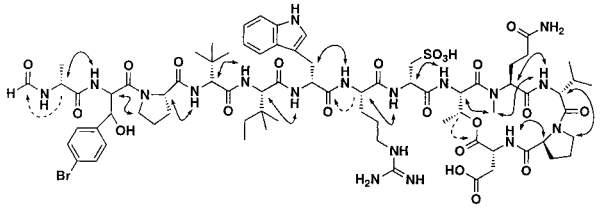


Figure 1. Arrows indicate key HMBC (dashed arrow) and NOESY (↔) correlations in **1**.

generated by reaction of the peptide hydrolysate with 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate (GITC)[14−16] confirmed these assignments. By a combination of these two techniques, it was possible to define the presence of D-alanine, L-proline, D-*tert*-leucine, L-*β*-methylisoleucine, D-tryptophan, L-arginine, D-cysteic acid, L-threonine, L-*N*-methylglutamine, D-valine, and D-aspartic acid residues in **1**. The stereochemistry of the *β*-hydroxy-*p*-bromophenylalanine residue was not assigned; however, mass selective LC detection revealed the presence of FDAA and GITC derivatives of *β*-hydroxy-*p*-bromophenylalanine with $[M + H]^+$ pseudomolecular ion pairs at *m*/*z* 494/496 and 649/651, respectively.

Microspinosamide (**1**) was evaluated for anti-HIV properties in a cell-based in vitro assay, details of which have been described previously.[17] It effectively inhibited the cytopathic effect of HIV-1 infection in CEM-SS target cells, with an EC_{50} value of approximately 0.2 *μ*g/mL. However, **1** was only cytoprotective over a modest concentration range because of cytotoxic effects toward the target cells (IC_{50} value of approximately 3.0 *μ*g/mL). Microspinosamide (**1**) represents the latest of a small group of cyclic depsipeptides from diverse marine sponges, which have been reported to exhibit anti-HIV activity.[18−20]

Experimental Section

General Experimental Procedures. NMR spectra were acquired in CD_3OH on a Varian Unity INOVA spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C and referenced to the residual nondeuterated solvent. FABMS were recorded on a JEOL SX102 spectrometer using glycerol or nitrobenzyl alcohol as matrix. Chiral GC−MS analyses were also performed on the JEOL SX102 mass spectrometer. Electrospray mass spectra were acquired on a Hewlett-Packard HP1100 integrated LC−MS system. IR spectra were obtained from neat samples on KCl disks in a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter; and UV spectra were recorded on a Beckman DU 640 spectrophotometer.

Animal Material. Samples of *S. microspinosa* were collected in May 1993, at a depth of 33 feet around a mangrove island located near Sulawesi, Indonesia (longitude 125 18.3° E; latitude 02 20.66° N). Freshly collected sponge was frozen on site and transported to Frederick, Maryland, over dry ice. A voucher specimen (voucher ID # 0CDN1503) is maintained at the Smithsonian Institution, Washington, D.C.

Extraction and Isolation. Frozen sponge samples (1246 g wet wt) were mixed with dry ice, ground to a fine powder, and extracted with H_2O. Lyophilization of the resulting solution provided 56 g of aqueous extract. The ground sponge material was lyophilized and further extracted with CH_2Cl_2−MeOH (1:1) and then 100% MeOH to give 5.9 g of combined organic extract. Both the aqueous and organic extracts exhibited activity in the NCI's primary in vitro anti-HIV screen.[11] A 5-g aliquot of the aqueous extract was redissolved in H_2O, applied to a wide-pore C_4 column (5 × 8 cm), and sequentially eluted with H_2O, H_2O−MeOH (2:1), H_2O−MeOH (1:2), and MeOH. The anti-HIV activity was concentrated into the fraction (320 mg) eluted with H_2O−MeOH (1:2). Final purification of this material by reversed-phase C_{18} HPLC, eluted with a gradient of increasing concentrations of CH_3CN in H_2O (from 20 to 56% CH_3CN over 25 min, 0.5% TFA vol/vol), provided 14.7 mg of microspinosamide (**1**).

Microspinosamide (1): white amorphous powder; $[\alpha]_D$ +2.4° (*c* 0.5, MeOH); UV (EtOH) λ_{max} (log ϵ) 211 (4.51), 218 (4.49), 278 (3.52), 289 (3.45) nm; IR (film, KCl) ν_{max} 3317, 1651, 1525, 1203 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRFABMS of a CsI-doped sample, $[M + Cs]^+$, *m*/*z* 1857.6169 (calcd for $C_{75}H_{109}{}^{79}BrN_{18}O_{22}SCs$, 1857.5919).

Preparation of Methylated Derivative (3). A 1.0-mg aliquot of microspinosamide (**1**) was dissolved in 1.0 mL of MeOH and treated with an excess of ethereal diazomethane. After standing for 30 min at room temperature, the solvents were evaporated under a stream of N_2 to provide virtually pure microspinosamide methyl ester (**3**): FABMS $[M + H]^+$ *m*/*z* 1739 and 1741, appropriate for $C_{76}H_{112}N_{18}O_{22}BrS$; the ^1H NMR spectrum (CD_3OD) contained a 3H singlet at δ 3.62, in addition to resonances that corresponded to all of those assigned in compound **1**.

Determination of Absolute Stereochemistry. (a) Hydrolysis. The peptide (200 *μ*g) was dissolved in degassed 6 N HCl (0.5 mL) in an evacuated glass tube and heated at 110 °C for 16 h. The solvent was removed in vacuo and the residue placed under high vacuum.

(b) LC−MS Analysis of FDAA Derivatives.[12] An aqueous solution of the peptide hydrolysate (20 *μ*g in 20 *μ*L) was treated with 6% triethylamine (10 *μ*L) and 1% *N*-(3-fluoro-4,6-dinitrophenyl)-L-alaninamide in Me_2CO (20 *μ*L) at 40 °C for 1 h. The reaction mixture was diluted with 50 *μ*L of H_2O, and an aliquot was applied to a C_{18} HPLC column eluted with CH_3CN−0.05% aqueous TFA using a linear gradient from 10% CH_3CN to 50% CH_3CN over 20 min. Derivatized amino acids were detected by absorption at 340 nm and by MSD (mass range 300−800 Da) and compared with similarly derivatized amino acid standards.[13] Retention times (min) are given in parentheses: L-Ala (10.13), D-Ala (11.76), L-Arg (8.13), D-Arg (7.71), L-Asp (8.31), D-Asp (8.94), L-Cys (OH) (14.93), D-Cys-(OH) (17.22), L-*β*-MeIle (22.35),* D-*β*-MeIle (25.71),* L-*t*-Leu (14.94), D-*t*-Leu (17.22), L-Pro (10.66), D-Pro (11.29), L-Thr (8.29), D-Thr (10.11), l-Trp (14.73), D-Trp (16.16), L-Val (13.23), D-Val (15.56); D- and L-MeGlu were not resolved at (9.18). *These samples were analized on a separate run with a slightly slower HPLC flow rate.

(c) LC−MS Analysis of GITC[14−16] Derivatives. An aqueous solution of the peptide hydrolysate (20 *μ*g in 20 *μ*L) was treated with 6% triethylamine (10 *μ*L) and 1% GITC in Me_2CO (20 *μ*L) at room temperature for 5 min. The reaction mixture was applied directly to a C_{18} HPLC column eluted as described above for the FDAA derivatives. Derivatized amino acids were detected by absorption at 254 nm and by MSD (mass range 400−950). Retention times (min) are given in parentheses: L-Ala (10.33), D-Ala (11.08), L-Asp (9.91), D-Asp (10.33), L-Cys (OH) (6.99), d-Cys(OH) (7.24), L-*t*-Leu (14.41), D-*t*-Leu (15.39), L-Pro (10.15), L-*β*-MeIle (19.81), D-*β*-MeIle (20.99), D-Pro (10.93), L-Thr (9.91), D-Thr (10.76), L-Trp (15.91), D-Trp (16.46), L-Val (13.18), d-Val (14.09); D- and L-Arg were not resolved at (8.93).

Anti-HIV Screening. Chromatographic fractions and purified peptide samples were dissolved in DMSO, diluted to the desired concentration, and tested in an in vitro XTT-based assay, the experimental details of which have been reported previously.[17]

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of trade names, commercial products, or organization imply endorsement by the U.S. Government.

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