Paltolides A−C, Anabaenopeptin-Type Peptides from the Palau Sponge *Theonella swinhoei*†

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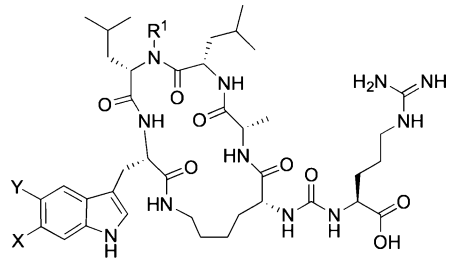
Three new anabaenopeptin-like peptides, named paltolides A−C, were isolated from a deep-water specimen of the marine sponge *Theonella swinhoei* from Palau. Paltolides belong to a rare subgroup of sponge-derived anabaenopeptins that have in common a C-terminal tryptophan residue linked to the ε-amine of a lysine bearing a D configuration. The structures of paltolides A−C were determined by NMR and tandem MS techniques. Paltolide A is the first anabaenopeptin structure where a non-*N*-methylated amino acid precedes the C-terminal residue.

Sponges belonging to the genus *Theonella* have proven to be one of the most prolific sources of bioactive natural products, some of which are almost certainly biosynthesized by symbiotic bacteria or cyanobacteria.[1] Among this group, the sponge *Theonella swinhoei* (order Lithistida, family Theonellidae) in particular has yielded a number of unusual peptides with unique structures including the antifungals cyclolithistide A,[2a] theonegramides,[2b] and theopalauamide,[2c] the HIV inhibitors papuamides,[3] and the cytotoxic polytheonamides.[4] In our continuing studies of lithistid demosponges, we identified a deep-water Palauan specimen of *T. swinhoei* with a red-purple ectosome and cream endosome that showed activity in HIV-1 neutralization assays. Fractionation of its methanol extract led to the isolation of the known glycopeptide theopalauamide[2c] and six anabaenopeptin-like peptides (**1**−**6**). Paltolides A−C (**1**−**3**) are new peptides, while compounds **4**−**6** have been previously isolated from the Australian sponge *Melophlus* sp.[5] Their structures were elucidated by extensive spectroscopic methods including 1D (^{1}H and ^{13}C) and 2D (DQF-COSY, HOHAHA, HMBC, HSQC, and ROESY) NMR experiments as well as ESIMS^n analysis. The absolute configurations of the paltolides were established by LC-MS analysis of their advanced Marfey's derivatives.[6]



1	X = H	Y = H	R^1 = H
2	X = H	Y = OH	R^1 = Me
3	X = Br	Y = H	R^1 = Me
4	X = Cl	Y = OH	R^1 = Me
5	X = Cl	Y = H	R^1 = Me
6	X = H	Y = H	R^1 = Me

Following lyophilization of the frozen sponge, extracts were prepared through sequential extraction of the freeze-dried sponge with hexanes, $CHCl_3$, and MeOH. The MeOH extract was partitioned with *n*-BuOH−H_2O, and the organic layer was fractionated on Sephadex LH-20. C12 reversed-phase HPLC of the fractions containing peptides yielded compounds **1**−**6**. The most polar compound among this group was paltolide A (**1**). Its HRESIMS showed a major ion peak at *m*/*z* 812.4775 $[M + H]^+$, corresponding to a molecular formula of $C_{39}H_{61}N_{11}O_8$. An IR band at ν 1649 cm^{-1} and HSQC correlations between six α-proton signals at δ 3.90−4.70 and six carbon resonances at δ 51.0−56.5 indicated that **1** was a peptide containing six amino acid residues. Detailed analysis of the 2D NMR data obtained from HSQC, HMBC, 2D-HOHAHA, and DQF-COSY experiments revealed the presence of arginine, lysine, alanine, and two leucine residues (see Table 1). Moreover the downfield region of the ^{1}H NMR spectrum contained a singlet signal at δ 7.11 (1H, s), along with proton signals characteristic of a1,2-disubstituted benzene at δ 7.57 (1H, br d, J = 8.1 Hz), 7.31 (1H, br d, J = 8.1 Hz), 7.07 (1H, t, J = 7.3 Hz), and 7.00 (1H, t, J = 7.3 Hz). These data together with diagnostic HMBC correlations (see Table 1) from the proton signals at δ 7.11 (H-2′$_{Trp}$) and 7.57 (H-4′$_{Trp}$) to the carbon resonances at δ 111.5 (C-3′$_{Trp}$), 128.6 (C-9′$_{Trp}$), and 138.0 (C-8′$_{Trp}$) indicated the presence of an indole ring. Subsequently, long-range correlations from the β-methylene protons at δ 3.48 and 3.20 to the carbon resonances at δ 111.5, 124.5, 128.6, and 173.7 (C-1$_{Trp}$) allowed us to assign the sixth residue as tryptophan.

The amino acid sequence of **1** could be established from interpretation of a single HMBC experiment where long-range correlations between α-protons and carbonyl carbons belonging to adjacent amino acids provided the partial sequence Lys-Ala-Leu1-Leu2-Trp (Table 1). Cyclization between the C-terminal tryptophan and the lysine ω-amine was apparent from HMBC correlations between the ε-methylene protons of lysine (δ_H 3.51, 3.11, δ_C 40.1) and the carbonyl resonance at δ 173.7 (C-1$_{Trp}$). An unassigned carbon resonance at δ 160.1 remained, and HMBC correlations from α-protons at δ 4.32 (H-2$_{Arg}$) and 4.08 (H-2$_{Lys}$) to this carbon were indicative of the presence of a ureido moiety connecting the arginine and N-terminal lysine residues, establishing the structure of paltolide A as depicted in **1**.

Additional evidence confirming this peptide sequence was obtained from ESIMS^n experiments. Fragmentation of the major ion peak at *m*/*z* 812 $[M + H]^+$ displayed an intense ion at *m*/*z* 612 $[M + H - Arg - CO]^+$ and two fragments of small intensity at *m*/*z* 484 $[M + H - Arg - CO - Lys]^+$ and *m*/*z* 413 $[M + H - Arg - CO - Lys - Ala]^+$. Further MS^3 fragmentation of the daughter ion at *m*/*z* 612 yielded ion fragments at *m*/*z* 499 $[M + H - Arg - CO - Leu]^+$, *m*/*z* 386 $[M + H - Arg - CO - Leu - Leu]^+$, and *m*/*z* 200 $[M + H - Arg - CO - Leu - Leu - Trp]^+$. Therefore the MS^n fragmentation patterns were in total agreement with the structure proposed for **1** by NMR.

† Dedicated to the late Dr. John W. Daly of NIDDK, NIH, Bethesda, Maryland, and to the late Dr. Richard E. Moore of the University of Hawaii at Manoa for their pioneering work on bioactive natural products.

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Table 1. NMR Spectroscopic Data for Paltolides A (**1**) and B (**2**) (CD_3OD)

	1			**2**		
position	δ_C[a]	δ_H[b] (*J* in hz)	HMBC[c]	δ_C[a]	δ_H[b] (*J* in Hz)	HMBC[c]
	Lys			**Lys**		
1	175.8			175.8		
2	56.0	4.08, t (4.7)	1, 3, 4, 1_{urea}	56.1	4.13, br t (4.8)	1, 3, 4, 1_{Urea}
3	32.4	1.84, 1.73, m	2	32.5	1.92, 1.68, m	2
4	22.5	1.64, 1.41, m		21.5	1.58, 1.32, m	
5	30.0	1.56, 1.50, m		28.8	1.58, m	
6	40.1	3.51, 3.11, m	4, 5, 1_{trp}	39.6	3.79, 2.93, m	4, 5, $1_{5'\text{-OHTrp}}$
NH-6					7.95, br d (7.6)	$1_{5'\text{-OHTrp}}$
	Ala			**Ala**		
1	176.0			175.4		
2	51.4	4.20,q (6.8)	1, 3, 1_{lys}	50.7	4.26[d]	1, 3, 1_{Lys}
3	17.3	1.39, d (6.8)	1, 2	17.1	1.48, d (6.8)	1, 2
	Leu1			**Leu**		
1	176.0			175.4		
2	54.5	4.12, dd (8.5, 6.5)	1, 3, 4, 1_{ala}	49.6	4.92, dd (11.9, 2.8)	3, 4
3	41.7	1.54, m	1, 2, 5, 6	41.4	1.80, 1.32, m	1, 2, 4, 5, 6
4	25.9	1.69, m	3, 5, 6	26.3	1.94, m	3, 5, 6
5	23.3	0.99, d (6.6)	3, 4, 6	23.8	1.03, d (6.8)	3, 4, 6
6	22.2	0.96, d (6.6)	3, 4, 5	22.4	1.01, d (6.8)	3, 4, 5
NH					8.71, br s	1_{Ala}
	Leu2			**NMeLeu**		
1	174.4			172.1		
2	55.7	3.87, dd (10.4, 4.7)	1, 3, 4, 1_{leu1}	60.0	4.95, t (7.3)	1, 3, 4, *N*Me, 1_{Leu1}
3	39.5	1.75, 1.39, m	1, 2, 5, 6	38.3	1.77, 1.30, m	1, 2, 4, 5, 6
4	26.0	1.49, m	3, 5, 6	25.8	1.47, m	3, 5, 6
5	23.4	0.86, d (6.5)	3, 4, 6	23.7	0.95, d (6.4)	3, 4, 6
6	21.5	0.82, d (6.5)	3, 4, 5	23.7	0.93, d (6.4)	3, 4, 5
NMe				29.0	1.89, s	2, 1_{Leu}
	Trp			**5′-OHTrp**		
1	173.7			174.7		
2	56.1	4.57, dd (9.8, 4.0)	1, 3, 5, 1_{leu2}	55.5	4.67, m	1, 3, 3′, 9′, 1_{NMeLeu}
3a	27.8	3.48, dd (14.6, 4.0)	1, 2, 2′, 3′, 9′	30.1	3.44, dd (15.0, 2.8)	1, 2, 2′, 3′, 9′
3b		3.20[d]	1, 2, 2′, 3′, 9′		2.92[d]	1, 2, 2′, 3′, 9′
NH-1′					9.96, s	
2′	124.5	7.11, s	3, 3′, 8′, 9′	125.6	6.70, s	2, 3, 3′, 4′, 8′, 9′
3′	111.5			110.4		
4′	119.1	7.57, br d (8.1)		103.5	6.98, d (1.9)	2′, 5′, 7′, 8′
5′	119.7	7.00, t (7.3)	3′, 6′, 7′, 8′, 9′	150.9		
6′	122.3	7.07, t (7.3)	6′, 7′, 9′	112.6	6.63, dd (8.8, 1.9)	4′, 5′, 8′
7′	112.3	7.31, br d (8.1)	4′, 8′	112.7	7.12, d (8.8)	4′, 5′, 9′
8′	138.0		4′, 5′, 9′	132.6		
9′	128.6			128.5		
NH					8.97, d (8.5)	2, 3, 1_{NMeLeu}
	urea			**urea**		
1	160.1			160.1		
	Arg			**Arg**		
1	176.0			176.3		
2	53.6	4.32, m	1, 3, 4, 1_{Urea}	54.3	4.25, m	1, 3, 4, 1_{Urea}
3	30.7	1.94, 1.70, m	2, 4	30.4	1.94, 1.70, m	2, 4
4	26.2	1.72, 1.65, m	5	26.0	1.72, m	5
5	41.1	3.25, 3.21, m	3, 4, 6	41.9	3.23, m	3, 4, 6
6	158.3			158.5		

[a] Recorded at 125 MHz; referenced to residual CD_3OD at δ 49.1 ppm. [b] Recorded at 500 MHz; referenced to residual CD_3OD at δ 3.30 ppm. [c] Proton showing HMBC correlation to indicated carbon. [d] Overlapped signal.

Acid hydrolysis of paltolide A followed by derivatization with L- and D-FDLA (1-fluoro-2,4-dinitrophenyl-5-L/D-leucinamide) and subsequent LC-MS analysis[6] in comparison to respective standards allowed us to establish the absolute configurations of Arg, Ala, Trp, and the two Leu residues as L, while the configuration of Lys was established as D.

The molecular formula of paltolide B (**2**) was determined to be $C_{40}H_{63}N_{11}O_9$ by HRESIMS (*m*/*z* 842.4880 $[M + H]^+$, calcd for $C_{40}H_{64}N_{11}O_9$, 842.4888). Its ^1H NMR spectrum showed signals characteristic of a peptide including exchangeable NH signals at δ 7.70−10.00 and a signal at δ 1.89 corresponding to an upfield-shifted methylamide. Furthermore, HSQC correlations and the coupling patterns of its aromatic signals at δ 6.63−7.12 suggested the presence of a 2′,5′-disubstituted indole ring (see Table 1). Analysis of the 2D NMR data established the presence of Lys, Ala, Leu, *N*MeLeu, 5′-hydroxytryptophan (5′-OHTrp), Arg, and a urea moiety. Additionally, the HMBC spectrum indicated paltolide B has a very similar amino acid sequence with respect to that of the previously reported compound **4** (see Table S1 for NMR data of **4**). In fact comparison between their NMR data showed that the only difference between these two compounds was the absence of chlorine at the 6′ position of the tryptophan residue in paltolide B, thereby establishing **2** as the des-chloro analogue of **4**. The presence of bromine in paltolide C (**3**) was apparent from the isotopic pattern of 1:1 observed for the major ion peaks at 904 $[M + H]^+$ and 906 $[M + 2H]^+$, and its molecular formula was assigned as $C_{40}H_{62}BrN_{11}O_8$ by HRESIMS (*m*/*z* 904.4030 $[M + H]^+$). Comparison of the 2D NMR spectra of paltolide C with the spectra of paltolide B showed signals assigned to 5′-hydroxytryptophan (5′-OHTrp) to be replaced with signals belonging to 6′-bromotryp-

tophan (6′-BrTrp). LC-MS analysis of the L/D-FDLA-derivatized hydrolysates of **2** and **3** revealed L configurations for *N*MeLeu, Leu, Arg, and Ala and D configuration for Lys. Due to insufficient quantities of peptide and poor yields of the substituted Trp residues following acid hydrolysis and derivatization, we were unable to determine the absolute configuration of the substituted tryptophan residues by LC-MS. However, the NMR data and chemical composition of **2** and **3** are highly similar to those of the known compound **4**, whose absolute configuration was determined by X-ray crystallography to include the D configuration of Lys and L configuration for all other amino acids, including the substituted tryptophan.[5] On the basis of these data and the coexistence of paltolides A−C with the known compound **4** in this *T. swinhoei* sample, we suggest that the 5′-OHTrp residue in paltolide B (**2**) and 6′-BrTrp residue in paltolide C (**3**) also possess an L configuration.

Paltolide B and compound **4** did not inhibit HIV-1 entry or show cytotoxicity toward HCT-116 or a control mammalian cell line at concentrations up to 100 μg/mL. Instead, the HIV-1 neutralizing activity of the extract was traced to the known glycopeptide theopalauamide.

Anabaenopeptins, which have been isolated from cyanobacteria and sponges, are a well-described family of ureido-containing hexapeptides characterized by a five-residue lactam ring formed by cyclization between the C-terminal acid and the ε-amine of an invariant N-terminal lysine residue. Exocyclic to the lactam, a sixth amino acid is linked to the α-amine of Lys through a urea bridge. On the basis of their structural features, paltolides A−C (**1**−**3**) can be placed within a rare subgroup of anabaenopeptins that have in common a tryptophan residue at the C-terminus linked to the ε-amine of the N-terminal lysine. The remaining members of this subgroup include six carboxypeptidase U inhibitors from *Melophlus* sp. described in a patent by researchers in Australia,[5] and konbamide,[7] a calmodulin antagonist isolated from an Okinawan collection of *Theonella* sp. Another signature of all anabaenopeptins regardless of source is the presence of an *N*-methylated amino acid appearing adjacent to and before the C-terminal residue.[8−10] Surprisingly, paltolide A (**1**) possesses a standard leucine residue at this position, presenting the first example where the amino acid at this position lacks an *N*-methyl group. Paltolides A−C also represent the first example of anabaenopeptin-type peptides coming from *T. swinhoei*. Other sponge-derived anabaenopeptins have come from collections of *Theonella* spp. (konbamide,[7] keramamides A[11a] and L,[11b] and mozabamides A and B[12]), *Psammocinia* aff. *bulbosa* (psymbamide A[8]), and *Melophlus* sp.[5]

It is also interesting to note that the lysine residue in each of the paltolides has a D configuration and the remaining amino acids an L configuration. All 29 cyanobacterial-derived anabaenopeptins contain a D-Lys, and the remaining amino acids have L configurations, while anabaenopeptins isolated from sponges typically show L configurations for all their amino acids including Lys.[10,13] Similar to the configuration seen in paltolides A−C, the sponge-derived compounds psymbamide A and anabaenopeptins from *Melophlus* sp. also contain a D-lysine with L configurations for the other five amino acids. This distribution is remarkable given that several of the L-Lys-containing peptides were isolated from sponges of the genus *Theonella* and that to date anabaenopeptins have not been found in numerous other Palauan *T. swinhoei* samples collected at shallower depths (less than 100 ft). These findings extend the diversity of chemistry associated with *T. swinhoei* and probably indicate that cyanobacteria or other prokaryotes are involved in the synthesis of these peptides. Indeed, Piel, Crews, and their coworkers recently showed a prokaryotic origin for psymberrin, an antitumor natural product found in some *P.* aff. *bulbosa* chemotypes such as those that yield psymbamide A, another anabaenopeptin-like peptide.[14]

Although paltolides A−C did not show inhibition of HIV-1 entry nor cytotoxicity toward HCT-116, their structural features warrant further biological evaluation. As mentioned above, paltolides A−C are closely related to a group of anabaenopeptins that are submicromolar inhibitors of carboxypeptidase U with greater than 50-fold selectivity over other carboxypeptidases.[5]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Jasco P-2000 polarimeter, UV spectra were recorded on an Agilent 8453 spectrophotometer, and IR spectra were recorded on a Perkin-Elmer FT-IR Spectrum One spectrometer. NMR spectra were recorded in CD_3OD on Bruker spectrometers operating at 600 and 500 (cryoprobe) MHz for ^1H and 150 and 125 MHz for ^{13}C NMR. DQF-COSY, 2D-HOHAHA, HSQC, HMBC, and ROESY experiments were recorded using standard pulse programs. HSQC experiments were optimized for $^1J_{C-H} = 145$ Hz, and HMBC spectra were optimized for $^{2,3}J_{C-H} = 5$, 6, and 8 Hz. The accurate mass electrospray ionization (ESI) mass spectra were measured on a Waters LCT Premier time-of-flight (TOF) mass spectrometer. The instrument was operated in ω-mode at a nominal resolution of 10 000. The electrospray capillary voltage was set at 2 kV and the sample cone voltage at 60 V. The desolvation temperature was set to 275 °C, and nitrogen was used as the desolvation gas with a flow rate of 300 L/h. Accurate masses were obtained using the internal reference standard method. ESIMS/MS data were obtained using a Thermo-Scientific (San Jose, CA) LTQ ion trap mass spectrometer. Sample was infused into the mass spectrometer using an Advion BioSciences (Ithaca, NY) Triversa chip-based nanoelectrospray ionization system. The nitrogen gas pressure was 0.25 psi, and the electrospray tip voltage was 1.4 kV. The CID MS/MS collision energy was 35 V, and the parent ion isolation width was 3 Da. The maximum injection time for parent ions was 700 ms, and 500 ms for daughter ions. The maximum AGC ion target setting was $1 \times 10e^5$ for parent ions and $5 \times 10e^4$ for daughter ions. QTOF-MS/MS data were obtained using a Waters (Milford, MA) Q-TOF-2 mass spectrometer operated in positive ion mode. The ESI capillary voltage was 3.5 kV, the desolvation gas was nitrogen with a flow of 300 L/h, and the desolvation temperature was 250 °C. The collision gas was argon, and the collision energy was 45 V. The parent fragment ion was generated by in-source fragmentation with a cone voltage of 40 V.

Sponge Material. The marine sponge *Theonella swinhoei* (lithistid Demospongiae: family Theonellidae) was collected by hand using scuba on Uchelbeluu Reef in Palau at a depth of 330 feet in June 2008 and frozen within 2 h. Samples were flown to Maryland frozen and stored at −80 °C until freeze-drying prior to extraction.

Isolation. The lyophilized sponge (30 g) was sequentially extracted with hexanes, $CHCl_3$, and MeOH. The MeOH extract (4 g) was partitioned between *n*-BuOH−H_2O (1:1), and the organic layer (1 g) was fractionated on Sephadex LH-20. Fractions containing peptides (95 mg) were purified by reversed-phase HPLC (Jupiter Proteo C12, 250 × 10 mm, 4 μ, DAD at 220 and 280 nm) eluting with a linear gradient of 50−80% MeOH in 0.05% TFA in 50 min to afford compounds **1** (0.5 mg, $t_R = 19.0$ min), **2** (1.5 mg, $t_R = 20.7$ min), **3** (0.6 mg, $t_R = 36.5$ min), **4** (6.8 mg, $t_R = 25.2$ min), **5** (0.3 mg, $t_R = 35.3$ min), **6** (24.8 mg, $t_R = 26.3$ min), and theopalauamide (0.9 mg, $t_R = 39.8$ min).

Paltolide A (1): colorless, amorphous powder; $[\alpha]^{23}_D$ −22 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.29), 262 (3.95) nm; IR (film) ν_{max} 3318, 2956, 1649, 1619, 1550, 1458, 1203, 1139, 1086, 1026, 838, 721 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 812.4775 $[M + H]^+$ (calcd for $C_{39}H_{62}N_{11}O_8$, 812.4783).

Paltolide B (2): colorless, amorphous powder; $[\alpha]^{23}_D$ −71 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.88), 260 (4.58) nm; IR (film) ν_{max} 3321, 2960, 1649, 1641, 1553, 1468, 1203, 1183, 1139 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 842.4880 $[M + H]^+$ (calcd for $C_{40}H_{64}N_{11}O_9$, 842.4888).

Paltolide C (3): colorless, amorphous powder; $[\alpha]^{23}_D$ −19 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.39), 260 (4.08) nm; IR (film) ν_{max} 3318, 2965, 1669, 1637, 1548, 1458, 1204, 1135 cm^{-1}; ^1H and ^{13}C NMR data for Lys, Ala, Leu, *N*MeLeu, urea, and Arg are identical to those reported for **2** in Table 1; ^1H NMR (CD_3OD, 500 MHz) 6′-BrTrp, δ 4.72 (1H, m, H-2d), 3.50 (1H, dd, $J = 14.4$, 3.6 Hz H-3a), 2.96 (1H, overlapped, H-3b), 6.77 (1H, s, H-2′), 7.54 (1H, d, $J = 8.4$ Hz, H-4′), 7.11 (1H, dd, $J = 8.2$, 1.3 Hz, H-5′), 7.46 (1H, d, $J = 1.3$

Hz, H-7′), 9.01 (1H, d, J = 8.5 Hz, NH); ^{13}C NMR (CD_3OD, 125 MHz) 6′-BrTrp, δ 174.7 (C-1), 29.4 (C-2), 125.5 (C-2′), 112.0 (C-3′), 120.5 (C-4′), 122.9 (C-5′), 115.5 (C-6′), 115.0 (C-7′), 138.4 (C-8′), 127.4 (C-9′); HRESIMS m/z 904.4030 $[M + H]^+$ (calcd for $C_{40}H_{63}BrN_{11}O_8$, 904.4044).

LC/MS Analysis of L/D-FDLA Derivatives. Approximately 0.4 mg of compounds **1**−**3** was separately hydrolyzed with 5 N HCl (LabChem Inc., traceable to NIST) (0.8 mL) in an Ace high-pressure tube for 16 h at 90 °C, dried, and dissolved in H_2O (100 μL). To a 50 μL aliquot of each was added 1 N $NaHCO_3$ (20 μL) and 1% 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (L-FDLA or D-FDLA solution in acetone, 100 μL), and the mixtures were heated to 40 °C for 40 min, allowed to cool to rt, neutralized with 2 N HCl (20 μL), and evaporated to dryness. Residues were dissolved in CH_3CN and analyzed by LC-MS. Analyses of the L- and L/D-FDLA (mixture of D- and D-FDLA) derivatives were performed using a Phenomenex Jupiter Proteo C12 column (4 μm, 150 × 4.6 mm); aqueous CH_3CN containing 0.01% TFA was used as a mobile phase eluting with a linear gradient of 25−70% CH_3CN in 45 min at a flow rate of 0.5 mL/min. An Agilent Series 1100 MSD mass spectrometer was used for detection in negative ESI mode. The fragmentor and capillary voltage were kept at 70 and 1000 V, respectively, and the ion source was kept at 350 °C. A mass range of m/z 100−1000 was scanned in 0.1 min. Retention times (t_R, min) of the FDLA-derivatized amino acids for compounds **1**−**3**: L-Lys 38.3, D-Lys 38.8 m/z 735 $[M + H]^-$ (bis derivative); L-Trp 30.0, D-Trp 32.8 m/z 497 $[M - H]^-$; L-*N*MeLeu 32.8, D-*N*MeLeu 35.5 m/z 438 $[M - H]^-$; L-Ala 24.5, D-Ala 27.0 m/z 382 $[M - H]^-$; L-Leu 30.6, D-Leu 36.8 m/z 424 $[M - H]^-$; L-Arg 15.9, D-Arg 13.9 m/z 467 $[M - H]^-$.

Biological Assays. Cytotoxicity assays were carried out using an MTT cell proliferation assay kit (American Type Culture Collection) according to the instructions provided. Briefly, HCT-116, BSC-1, or TZM-BL cells were seeded in 96-well tissue culture plates at a density of 2×10^4 cells/well in 50 μL of growth media and allowed to adhere for 18 h. Attached cells were incubated with inhibitors for 24 h (as controls for the neutralization assay), after which time the media was either replaced or diluted 3-fold with fresh growth media. Following an additional 48 h incubation period, cell viability was assessed upon treatment with MTT (A_{570}, Molecular Devices 96-well absorbance plate reader). Single round HIV-1 neutralization assays were performed with viruses pseudotyped with SF162 envelope using published conditions.[15] At least two separate experiments were performed for each assay, and all assays were performed in duplicate.

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Supporting Information Available: 1D and 2D NMR spectra for compounds **1**−**3** and NMR spectroscopic data for compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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