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Molecular-size reduction of a Potent CXCR4-chemokine Antagonist Using Orthogonal Combination of Conformation-based and Sequence-based Libraries**

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Experimental

General

Exact mass (HRMS) spectra were recorded on a JEOL JMS-01SG-2 or JMS-HX/HX 110A mass spectrometer. Ion-spray (IS)-mass spectrum was obtained with a Sciex API*III*E triple quadrupole mass spectrometer. Optical rotations were measured in water with a Horiba high-sensitive polarimeter SEPA-200. A ^{1}H-NMR spectrum was recorded using a Bruker AM 600 spectrometer at 600 MHz frequency. Chemical shifts are calibrated to the solvent signal (2.50 ppm, s = singlet, d = doublet, dd = double doublet, m = multiplet). For HPLC separations, a Cosmosil 5C18-ARII analytical column (Nacalai Tesque, 4.6 ×•250 mm, flow rate 1 mL/min) or a Cosmosil 5C18-ARII preparative column (Nacalai Tesque, 20 ×•250 mm, flow rate 11 mL/min) was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% TFA solution (v/v, solvent A) and 0.1% TFA in MeCN (v/v, solvent B) were used for HPLC elution.

General procedure for synthesis of protected peptide resins.

Protected peptide-resins were manually constructed by Fmoc-based solid phase peptide synthesis (SPPS). *t*-Bu for L/D-Tyr, Pbf for L/D-Arg were used for side-chain protection. Fmoc deprotection were achieved by 20% piperidine in DMF (2 × 1 min, 1 × 20 min). Fmoc-amino acids were coupled by treatment with five equivalents of reagents [Fmoc-amino acid, *N*,*N*′-diisopropylcarbodiimide (DIPCDI),

and HOBt·H_2O] to free amino group (or hydrazino group) in DMF for 1.5 h.

Synthesis of cyclic peptides (method A):

p-Nitrophenyl carbonate Wang resin (108 mg, 0.100 mmol) was treated with $NH_2NH_2·H_2O$ (0.485 mL, 1.00 mmol) in DMF (5 mL) at room temperature for 2 h to give a hydrazino linker. After Fmoc-based SPPS by the general procedure, the protected peptide resin was treated with TFA for 1.5 h at room temperature, and the mixture was filtered. Concentration under reduced pressure followed by preparative HPLC (15% B in A) gave a peptide hydrazide. To a stirred solution of the peptide hydrazide in DMF (2 mL) were added a solution of 4M HCl in DMF (0.0664 mL, 0.300 mmol) and isoamyl nitrite (0.120 mL, 0.600 mmol) at – 40 °C. After stirring for 30 min at – 20 °C, the mixture was diluted with precooled DMF (100 mL). To the above solution was added (*i*-Pr)$_2$NEt (0.870 mL, 5.00 mmol) at – 40 °C, and the mixture was stirred for 24 h at – 20 °C. Concentration under reduced pressure and purification by preparative HPLC (25% B in A) gave a cyclic peptide.

Synthesis of cyclic peptides (method B):

The protected peptide resin (0.100 mmol), which was constructed on H-Gly-(2-chloro)trityl resin by general procedure, was subjected to AcOH/TFE/CH_2Cl_2 (1:1:3, 10 mL) treatment at room temperature for 2 h. After filtration of the residual resin, the filtrate was

concentrated under reduced pressure to give a crude protected peptide. To a stirred mixture of the protected peptide and $NaHCO_3$ (57.1 mg, 0.680 mmol) in DMF (41 mL) was added diphenylphosphoryl azide (DPPA, 0.0879 mL, 0.408 mmol) at – 40 °C. The mixture was stirred for 36 h with warming to room temperature and filtered. The filtrate was concentrated under reduced pressure to give an oily residue, which was subjected to solid phase extraction over basic alumina in $CHCl_3$-MeOH (9:1) to remove inorganic salts derived from DPPA. The resulting cyclic protected peptide was treated with 95% TFA solution for 1.5 h at room temperature. Concentration under reduced pressure and purification by preparative HPLC gave a cyclic peptide.

Synthesis of cyclic peptides (method C):

The protected peptide resin (0.100 mmol), which was constructed by the same procedure described in method A, was treated with 10% TFA in $CHCl_3$ at room temperature for 1.5 h, and the mixture was filtered. The filtrate was concentrated under reduced pressure and the residue was dissolved in DMF (2 mL). To a stirred solution of the protected peptide hydrazide in DMF were added a solution of 4M HCl in DMF (0.0664 mL, 0.300 mmol) and isoamyl nitrite (0.120 mL, 0.600 mmol) at – 30 °C, and the mixture was stirred for 20 min at – 10 °C. Precooled DMF (100 mL) and $(i\text{-Pr})_2NEt$ (0.870 mL, 5.0 mmol) were added to the above mixture at – 40 °C, and the mixture was stirred for 24 h at – 20 °C. Concentration under reduced pressure

gave an oily residue, which was treated with 95% TFA at room temperarture for 2 h. Concentration under reduced pressure followed by purification by preparative HPLC gave a cyclic peptide.

Synthesis of linear peptides:

The protected peptide resin (0.100 mmol), which was constructed on a Rink amide resin by general procedure, was treated with 95% TFA solution at room temperature for 2 h. After filtration of the residual resin, the filtrate was concentrated under reduced pressure. The crude product was purified by preparative HPLC (20% B in A) to yield a linear peptide as a freeze-dried powder.

***Cyclo*(-L-Nal-Gly-D-Tyr-L-Arg-L-Arg-) 8k**

$[\alpha]^{30}_{D}$ –67.4 (c = 0.089 in water); t_R = 24.8 min (linear gradient of B in A, 15 to 30% over 30 min); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 300 K): δ•= 1.22-1.37 (m, 2 H), 1.41 (m, 1 H), 1.55 (m, 1 H), 1.66 (m, 2 H), 2.74 (dd, ^3J(H,H) = 13.5 and 6.2 Hz, 1 H), 2.78 (dd, ^3J(H,H) = 13.5 and 8.6 Hz, 1 H), 3.02 (m, 4 H), 3.12 (dd, ^3J(H,H) = 13.4 and 7.3 Hz, 1 H), 3.20 (dd, ^3J(H,H) = 13.4 and 7.5 Hz, 1 H), 3.55 (dd, ^3J(H,H) = 15.6 and 4.8 Hz, 1 H), 3.75 (dd, ^3J(H,H) = 15.5 and 6.7 Hz, 1 H), 3.92 (m, 1 H), 4.11 (m, 1 H), 4.24 (m, 1 H), 4.34 (m, 1 H), 6.64 (d, ^3J(H,H) = 8.2 Hz, 2 H), 6.94 (d, ^3J(H,H) = 8.2 Hz, 2 H), 7.36 (d, ^3J(H,H) = 8.4 Hz, 1 H), 7.47 (m, 2 H), 7.50 (m, 1 H), 7.55 (m, 1 H), 7.67 (s, 1 H), 7.80-7.84 (m, 3 H), 7.86 (d, ^3J(H,H)

= 7.8 Hz, 1 H), 7.93 (d, ^{3}J(H,H) = 6.7 Hz, 1 H), 8.08 (m, 1 H), 8.27 (d, ^{3}J(H,H) = 6.7 Hz, 1 H), 8.37 (d, ^{3}J(H,H) = 7.3 Hz, 1 H); HRMS (FAB), *m/z* calcd for $C_{36}H_{48}N_{11}O_{6}$ (MH^{+}) 730.3789, found: 730.3765.

Evaluation of antagonistic activity against CXCR4 receptor.

The IC_{50} of candidate cyclic pentapeptides was determined by displacement of binding of [^{125}I]SDF-1 to CHO transfectants stably expressing CXCR4. Briefly, CXCR4 transfectants were incubated with [^{125}I]SDF-1 (0.15 nM) on a shaker at 4 °C for 1 hour in the presence or absence of candidate cyclic pentapeptides. Cell pellets were centrifuged through an oil cushion to separate unbound isotope and counted. The ability of candidate cyclic pentapeptides to inhibit [^{125}I]SDF-1 binding was analyzed at 0.01, 0.10, 1.0, and 10 μM concentrations. Ranges were determined in two independent experiments. Specific IC_{50} values were determined by Scatchard analysis if >50% inhibition was obtained at cyclic pentapeptide concentrations less than 0.01 μM. IC_{50} values were determined from binding assays with [^{125}I]SDF-1 and CXCR4 transfectants using concentrations ranging from 0.03 nM to 3.16 μM in half-log increments to displace the radioligand. Bound isotope was separated from free as described above. IC_{50} values were calculated with Prism software (GraphPad Software, Inc.) using standard approaches. The results are the mean of at least three independent experiments.

Anti-HIV-1 assay

Anti-HIV-1 activity was determined based on the protection against HIV-1-induced cytopathogenicity in MT-4 cells. Various concentrations of test compounds were added to HIV-1-infected MT-4 cells at a multiplicity of infection (MOI) of 0.01, and placed in wells of a flat-bottomed microtiter tray (1.5 × 104 cells/well). After 5 days' incubation at 37 °C in a CO_2 incubator, the number of viable cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (EC_{50}).[S1]

NMR Spectroscopy.

The peptide sample was dissolved in DMSO-d_6 at concentration of 5 mM. ^{1}H-NMR spectra of the peptides were recorded at 300 K. The assignments of the proton resonances were completely achieved by use of ^{1}H-^{1}H COSY spectra. $^3J(H^N,H^\alpha)$ coupling constants were measured from one-dimensional spectra. The mixing time for the NOESY experiments was set at 200, 300 and 400 ms. NOESY spectra were composed of 2048 real points in the F2 dimension and 512 real points, which were zero-filled to 1024 points in the F1 dimension, with 32 scans per t1 increment. The cross-peak intensities were evaluated by relative build-up rates of the cross-peaks.

Calculation of Structures.

The structure calculations were performed on a Silicon Graphics Origin 2000 workstation with the NMR-refine program within the Insight II/Discover package using the consistent valence force field (CVFF).[S2] Pseudoatoms were defined for the methylene protons

of Nal[1], Gly[2], D-Tyr[3], Arg[4], and Arg[5] prochiralities of which were not identified by ^{1}H-NMR data. The restraints, in which the Gly[2] α-methylene participated, were defined for the separate protons without definition of the prochiralities. The dihedral ϕ angle constraints were calculated based on the Karplus equation: $^3J(H^N,H^\alpha) = 6.7\cos^2(\theta - 60) - 1.3\cos(\theta - 60) + 1.5$, except for that of Arg[5] residue.[S3] Lower and upper angle errors were set to 15°. The NOESY spectrum with a mixing time of 200 ms was used for the estimation of the distance restraints between protons. The NOE intensities were classified into three categories (strong, medium and weak) based on the number of contour lines in the cross-peaks to define the upper-limit distance restraints (2.7, 3.5 and 5.0 Å, respectively). The upper-limit restraints were increased by 1.0 Å for the involved pseudoatoms. Lower bounds between nonbonded atoms were set to their van der Walls radii (1.8 Å). These 41 distance and 5 dihedral angle restraints were included with force constants of 25 – 100 kcal·mol^{-1}·Å^{-2} and 25 – 100 kcal·mol^{-1}·rad^{-2}, respectively. The 50 initial structures generated by the NMR refine program randomly were subjected to the simulated annealing calculations. The final minimization stage was achieved until the maximum derivative became less than 0.01 kcal·mol^{-1}·Å^{-2} by the steepest descents and conjugate gradients methods.

References

[S1] a) H. Nakashima, Y. Kido, N. Kobayashi, Y. Motoki, M. Neushul, N. Yamamoto, *Antimicrob. Agents Chemother.* **1986**, 31, 1524; b) R. Pauwels, B. M. Balzarini, R. Snoeck, D. Schols, P.

Herdewijn, J. Desmyter E. De Clercq, *J. Virol. Methods* **1988**, 20, 309.

[S2] a) K. Miyamoto, T. Nakagawa, Y. Kuroda, *J. Pept. Res.* **2001**, *58*, 193; b) K. Miyamoto, T. Nakagawa, Y. Kuroda, *Biopolymers* **2001**, *59*, 380, and references cited therein.

[S3] S. Ludvigsen, K. V. Andersen, F. M. Poulsen, *J. Mol. Biol.* **1991**, *217*, 731.

Table S1. Structure, synthetic method, chemical yield and FAB-mass of cyclic peptides.

	compound	method	yield (%)	FAB-MS (HR)[a]
1a	cyclo(-L-Arg-Gly-L-Tyr-D-Nal-L-Arg-)	A	29	730.3773
2a	cyclo(-L-Arg-Gly-L-Nal-D-Tyr-L-Arg-)	A	20	730.3773
3a	cyclo(-L-Arg-Gly-L-Arg-D-Tyr-L-Nal-)	A	36	730.3817
4a	cyclo(-L-Arg-Gly-L-Tyr-D-Arg-L-Nal-)	A	39	730.3804
5a	cyclo(-L-Arg-Gly-L-Nal-D-Arg-L-Tyr-)	A	57	730.3788
6a	cyclo(-L-Arg-Gly-L-Arg-D-Nal-L-Tyr-)	A	51	730.3813
7a	cyclo(-L-Nal-Gly-L-Arg-D-Tyr-L-Arg-)	A	41	730.3803
8a	cyclo(-L-Nal-Gly-L-Tyr-D-Arg-L-Arg-)	A	17	730.3763
9a	cyclo(-L-Nal-Gly-L-Arg-D-Arg-L-Tyr-)	A	32	730.3775
10a	cyclo(-L-Tyr-Gly-L-Nal-D-Arg-L-Arg-)	A	37	730.3804
11a	cyclo(-L-Tyr-Gly-L-Arg-D-Nal-L-Arg-)	A	32	730.3763
12a	cyclo(-L-Tyr-Gly-L-Arg-D-Arg-L-Nal-)	A	28	730.3809
1b	cyclo(-L-Arg-Gly-L-Tyr-L-Nal-D-Arg-)	B	19	730.3771
2b	cyclo(-L-Arg-Gly-L-Nal-L-Tyr-D-Arg-)	B	11	730.3777
3b	cyclo(-L-Arg-Gly-L-Arg-L-Tyr-D-Nal-)	B	20	730.3804
4b	cyclo(-L-Arg-Gly-L-Tyr-L-Arg-D-Nal-)	B	27	730.3798
5b	cyclo(-L-Arg-Gly-L-Nal-L-Arg-D-Tyr-)	B	34	730.3803
6b	cyclo(-L-Arg-Gly-L-Arg-L-Nal-D-Tyr-)	B	16	730.3768
7b	cyclo(-L-Nal-Gly-L-Arg-L-Tyr-D-Arg-)	B	23	730.3777
8b	cyclo(-L-Nal-Gly-L-Tyr-L-Arg-D-Arg-)	B	25	730.3801
9b	cyclo(-L-Nal-Gly-L-Arg-L-Arg-D-Tyr-)	B	24	730.3808
10b	cyclo(-L-Tyr-Gly-L-Nal-L-Arg-D-Arg-)	B	32	730.3771
11b	cyclo(-L-Tyr-Gly-L-Arg-L-Nal-D-Arg-)	B	33	730.3820
12b	cyclo(-L-Tyr-Gly-L-Arg-L-Arg-D-Nal-)	B	25	730.3771

[a] FAB-MS (HR), *m/z* calcd for $C_{36}H_{48}N_{11}O_6$ 730.3789.

Table S1. Structure, synthetic method, chemical yield and FAB-mass of cyclic peptides. (continued)

	compound	method	yield (%)	FAB-MS (HR)[a]
1c	cyclo(-D-Arg-Gly-D-Tyr-L-Nal-D-Arg-)	B	27	730.3810
2c	cyclo(-D-Arg-Gly-D-Nal-L-Tyr-D-Arg-)	B	22	730.3772
3c	cyclo(-D-Arg-Gly-D-Arg-L-Tyr-D-Nal-)	B	15	730.3774
4c	cyclo(-D-Arg-Gly-D-Tyr-L-Arg-D-Nal-)	B	22	730.3798
5c	cyclo(-D-Arg-Gly-D-Nal-L-Arg-D-Tyr-)	B	22	730.3784
6c	cyclo(-D-Arg-Gly-D-Arg-L-Nal-D-Tyr-)	B	14	730.3814
7c	cyclo(-D-Nal-Gly-D-Arg-L-Tyr-D-Arg-)	B	33	730.3761
8c	cyclo(-D-Nal-Gly-D-Tyr-L-Arg-D-Arg-)	B	39	730.3800
9c	cyclo(-D-Nal-Gly-D-Arg-L-Arg-D-Tyr-)	B	20	730.3773
10c	cyclo(-D-Tyr-Gly-D-Nal-L-Arg-D-Arg-)	B	27	730.3780
11c	cyclo(-D-Tyr-Gly-D-Arg-L-Nal-D-Arg-)	B	33	730.3809
12c	cyclo(-D-Tyr-Gly-D-Arg-L-Arg-D-Nal-)	B	41	730.3804
1d	cyclo(-D-Arg-Gly-D-Tyr-D-Nal-L-Arg-)	B	24	730.3774
2d	cyclo(-D-Arg-Gly-D-Nal-D-Tyr-L-Arg-)	B	45	730.3800
3d	cyclo(-D-Arg-Gly-D-Arg-D-Tyr-L-Nal-)	B	18	730.3798
4d	cyclo(-D-Arg-Gly-D-Tyr-D-Arg-L-Nal-)	B	20	730.3782
5d	cyclo(-D-Arg-Gly-D-Nal-D-Arg-L-Tyr-)	B	38	730.3771
6d	cyclo(-D-Arg-Gly-D-Arg-D-Nal-L-Tyr-)	B	22	730.3816
7d	cyclo(-D-Nal-Gly-D-Arg-D-Tyr-L-Arg-)	B	25	730.3814
8d	cyclo(-D-Nal-Gly-D-Tyr-D-Arg-L-Arg-)	B	37	730.3801
9d	cyclo(-D-Nal-Gly-D-Arg-D-Arg-L-Tyr-)	B	19	730.3767
10d	cyclo(-D-Tyr-Gly-D-Nal-D-Arg-L-Arg-)	B	29	730.3780
11d	cyclo(-D-Tyr-Gly-D-Arg-D-Nal-L-Arg-)	B	49	730.3794
12d	cyclo(-D-Tyr-Gly-D-Arg-D-Arg-L-Nal-)	B	29	730.3766

[a] FAB-MS (HR), *m/z* calcd for $C_{36}H_{48}N_{11}O_6$ 730.3789.

Table S1. Structure, synthetic method, chemical yield and FAB-mass of cyclic peptides. (continued)

	compound	method	yield (%)	FAB-MS (HR)[a]
8e	cyclo(-L-Nal-Gly-D-Tyr-L-Arg-D-Arg-)	C	3	730.3759
8f	cyclo(-D-Nal-Gly-L-Tyr-D-Arg-L-Arg-)	B	29	730.3762
8g	cyclo(-L-Nal-Gly-D-Tyr-D-Arg-L-Arg-)	B	39	730.3812
8h	cyclo(-D-Nal-Gly-L-Tyr-L-Arg-D-Arg-)	C	15	730.3764
8i	cyclo(-L-Nal-Gly-L-Tyr-L-Arg-L-Arg-)	C	13	730.3813
8j	cyclo(-D-Nal-Gly-D-Tyr-D-Arg-D-Arg-)	C	25	730.3817
8k	cyclo(-L-Nal-Gly-D-Tyr-L-Arg-L-Arg-)	C	12	730.3765
8l	cyclo(-D-Nal-Gly-L-Tyr-D-Arg-D-Arg-)	C	21	730.3773
8m	cyclo(-L-Nal-Gly-L-Tyr-D-Arg-D-Arg-)	C	40	730.3805
8n	cyclo(-D-Nal-Gly-D-Tyr-L-Arg-L-Arg-)	C	11	730.3761
8o	cyclo(-L-Nal-Gly-D-Tyr-D-Arg-D-Arg-)	C	13	730.3774
8p	cyclo(-D-Nal-Gly-L-Tyr-L-Arg-L-Arg-)	C	12	730.3797

[a] FAB-MS (HR), *m/z* calcd for $C_{36}H_{48}N_{11}O_6$ 730.3789.

Table S2. Structure, chemical yield and IS-mass of linear peptides.

	compound	yield (%)	IS-MS (reconstructed)[a]
13a	Ac-D-Tyr-D-Arg-L-Arg-D-Nal-Gly-NH_2	59	789.5
13b	Ac-Gly-D-Tyr-D-Arg-L-Arg-D-Nal-NH_2	61	789.0
13c	Ac-D-Nal-Gly-D-Tyr-D-Arg-L-Arg-NH_2	41	789.0
13d	Ac-L-Arg-D-Nal-Gly-D-Tyr-D-Arg-NH_2	61	789.5
13e	Ac-D-Arg-L-Arg-D-Nal-Gly-D-Tyr-NH_2	48	789.0
14a	Ac-D-Tyr-D-Arg-L-Arg-L-Nal-Gly-NH_2	40	789.0
14b	Ac-Gly-D-Tyr-D-Arg-L-Arg-L-Nal-NH_2	10	789.0
14c	Ac-L-Nal-Gly-D-Tyr-D-Arg-L-Arg-NH_2	47	789.0
14d	Ac-L-Arg-L-Nal-Gly-D-Tyr-D-Arg-NH_2	14	789.0
14e	Ac-D-Arg-L-Arg-L-Nal-Gly-D-Tyr-NH_2	23	789.0
15a	Ac-D-Tyr-L-Arg-L-Arg-L-Nal-Gly-NH_2	24	789.5
15b	Ac-Gly-D-Tyr-L-Arg-L-Arg-L-Nal-NH_2	17	789.5
15c	Ac-L-Nal-Gly-D-Tyr-L-Arg-L-Arg-NH_2	50	789.0
15d	Ac-L-Arg-L-Nal-Gly-D-Tyr-L-Arg-NH_2	36	789.0
15e	Ac-L-Arg-L-Arg-L-Nal-Gly-D-Tyr-NH_2	33	789.5

[a] IS-MS (reconstructed), *m/z* calcd for $C_{38}H_{52}N_{12}O_7$ 788.90.

Table S3. Optical rotation of peptides.

compound	α_D	temp.	*c*
1a	- 13.5	26.9	0.591
2a	- 21.6	28.9	0.185
3a	- 18.3	28.9	0.493
4a	+ 10.4	25.5	0.386
5a	+ 3.27	27.4	0.611
6a	- 19.6	28.6	0.459
7a	- 58.2	24.8	0.481
8a	- 19.9	27.6	0.201
9a	+ 52.2	28.3	0.134
10a	- 11.3	26.1	0.265
11a	- 66.2	26.9	0.468
12a	- 47.1	27.6	0.361
1b	- 7.37	26.8	0.407
2b	- 6.34	27.3	0.315
3b	- 49.9	26.2	0.661
4b	- 58.5	24.1	0.650
5b	- 52.6	28.1	0.666
6b	- 42.9	28.5	0.396
7b	- 15.4	27.5	0.650
8b	+ 14.7	25.5	0.612
9b	- 34.5	27.0	0.290
10b	- 37.1	27.8	0.485
11b	- 18.2	25.9	0.549
12b	- 32.6	26.7	0.368

Table S3. Optical rotation of peptides. (continued)

compound	α_D	temp.	*c*
1c	- 12.8	27.1	0.470
2c	+ 2.77	29.2	0.360
3c	+ 0.00	28.1	0.201
4c	- 16.4	26.8	0.365
5c	+ 7.40	28.3	0.405
6c	+ 16.0	28.1	0.188
7c	+ 52.6	27.2	0.304
8c	+ 9.34	28.1	0.214
9c	+ 32.6	28.5	0.092
10c	+ 13.4	26.5	0.224
11c	+ 44.3	27.2	0.271
12c	+ 18.9	28.2	0.265
1d	+ 9.54	27.1	0.524
2d	+ 37.0	27.8	0.513
3d	+ 73.1	28.3	0.383
4d	+ 54.6	27.2	0.513
5d	+ 62.3	28.2	0.610
6d	+ 50.7	28.3	0.691
7d	+ 7.40	27.7	0.810
8d	- 5.22	25.7	0.957
9d	+ 33.5	27.1	0.388
10d	+ 32.2	27.7	0.900
11d	+ 25.3	26.4	0.831
12d	+ 49.5	26.4	0.465

Table S3. Optical rotation of peptides. (continued)

compound	α_D	temp.	*c*
8e	+ 21.7	30.3	0.046
8f	- 48.3	23.8	0.269
8g	- 27.5	22.4	0.545
8h	+ 41.7	20.5	0.216
8i	- 53.1	30.3	0.113
8j	+ 32.1	22.5	0.280
8k	- 67.4	30.3	0.089
8l	+ 47.1	21.8	0.255
8m	+ 51.9	18.5	0.231
8n	- 32.5	30.3	0.154
8o	+ 32.9	30.2	0.152
8p	- 54.8	30.3	0.073
13a	- 1.45	22.6	0.688
13b	- 8.00	22.6	0.375
13c	+ 2.84	22.8	0.352
13d	- 4.29	22.8	0.699
13e	- 9.75	23.2	0.615
14a	- 2.56	27.3	0.781
14b	+ 10.5	27.4	0.190
14c	+ 9.99	27.4	0.400
14d	+ 9.61	27.9	0.208
14e	+ 1.84	28.0	0.541
15a	- 23.2	23.5	0.388
15b	+ 15.3	23.5	0.327
15c	+ 32.2	23.2	0.373
15d	+ 14.7	23.1	0.408
15e	+ 39.6	24.4	0.303

Table S4. Anti-HIV antivities of cyclic peptides in the "conformation-based" library.

compound	IC_{50}(μM)[a]	EC_{50}(μM)[b]	compound	IC_{50}(μM)[a]	EC_{50}(μM)[b]
1a	> 10	>80	**1c**	> 10	>80
2a	> 10	>80	**2c**	> 10	>80
3a	> 10	>80	**3c**	1.0 - 10	53
4a	> 10	>80	**4c**	> 10	41
5a	> 10	>80	**5c**	> 10	>80
6a	> 10	>80	**6c**	1.0 - 10	>80
7a	0.1 – 1.0	6.2	**7c**	> 10	63
8a	0.1 – 1.0	7.2	**8c**	> 10	66
9a	> 10	>80	**9c**	> 10	>80
10a	1.0 - 10	40	**10c**	> 10	39
11a	1.0 - 10	60	**11c**	> 10	>80
12a	> 10	>80	**12c**	1.0 - 10	38
1b	1.0 - 10	9.6	**1d**	> 10	61
2b	1.0 - 10	>80	**2d**	1.0 - 10	15
3b	1.0 - 10	25	**3d**	> 10	34
4b	1.0 - 10	55	**4d**	> 10	61
5b	> 10	>80	**5d**	1.0 - 10	59
6b	> 10	>80	**6d**	> 10	>80
7b	> 10	37	**7d**	1.0 - 10	46
8b	> 10	12	**8d**	0.016	0.28
9b	> 10	>80	**9d**	> 10	>80
10b	> 10	>80	**10d**	0.1 – 1.0	>80
11b	> 10	>80	**11d**	> 10	>80
12b	1.0 - 10	>80	**12d**	1.0 - 10	58

[a] IC_{50} values for the cyclic pentapeptides are based on inhibition of [^{125}I]SDF-1 binding to CXCR4 transfectants. [b] EC_{50} values are based on the inhibition of HIV-induced cytopathogenicity in MT-4 cells.

Table S5. Anti-HIV activities of linear peptides.

	$EC_{50}(\mu M)$[a]
8d	0.28
13a	>190
13b	>190
13c	180
13d	78
13e	96
8g	0.11
14a	>190
14b	>190
14c	>190
14d	>190
14e	19
8k	0.038
15a	>23
15b	>23
15c	>23
15d	>23
15e	>23

[a] EC_{50} values are based on the inhibition of HIV-induced cytopathogenicity in MT-4 cells.

Figure S1. ^{1}H-NMR spectrum of *cyclo*(-L-Nal-Gly-D-Tyr-L-Arg-L-Arg-)

8k

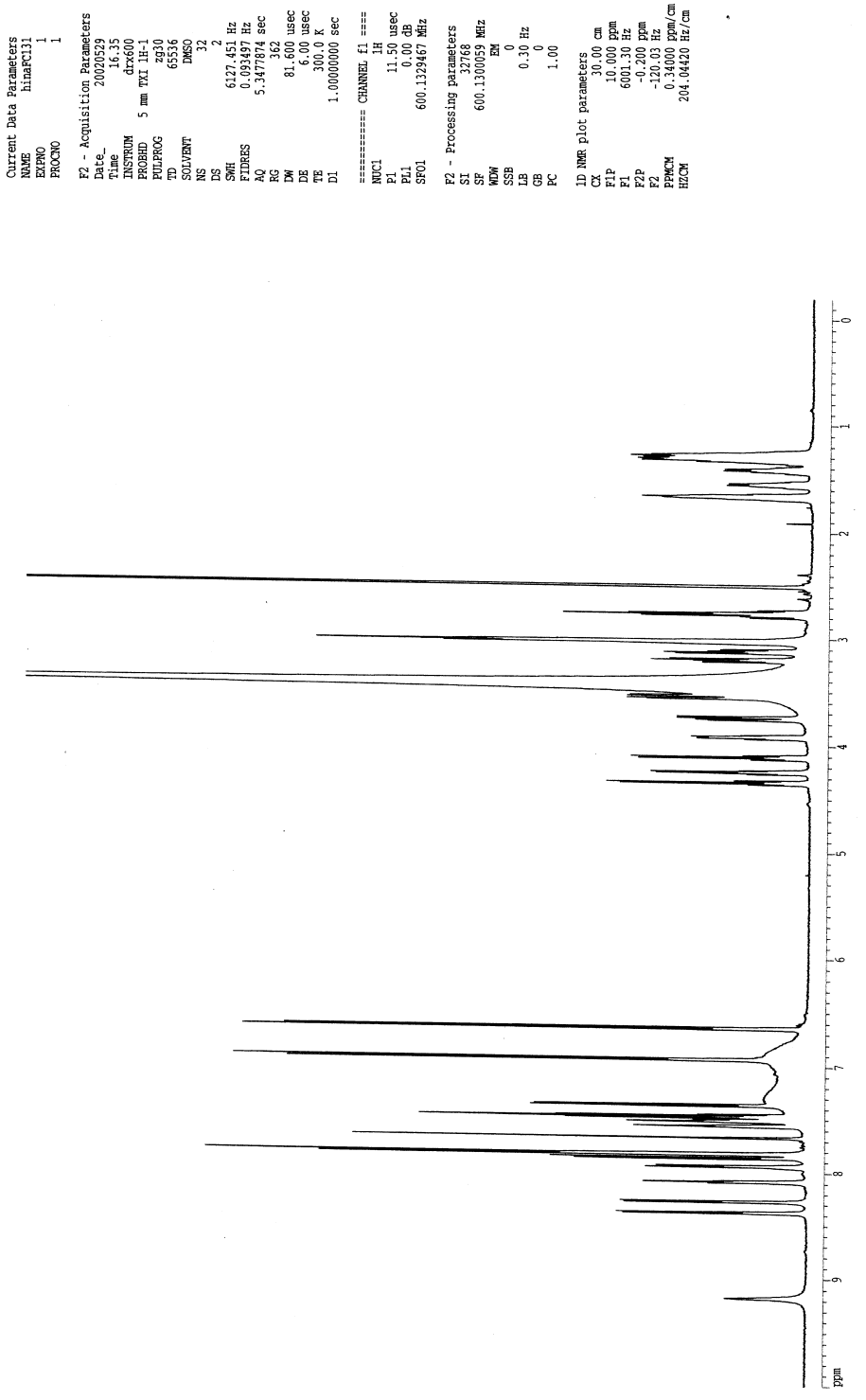

Figure S2. HPLC data of *cyclo*(-L-Nal-Gly-D-Tyr-L-Arg-L-Arg-) **8k**; linear gradient of solvent B in solvent A, 15 to 30% over 30 min

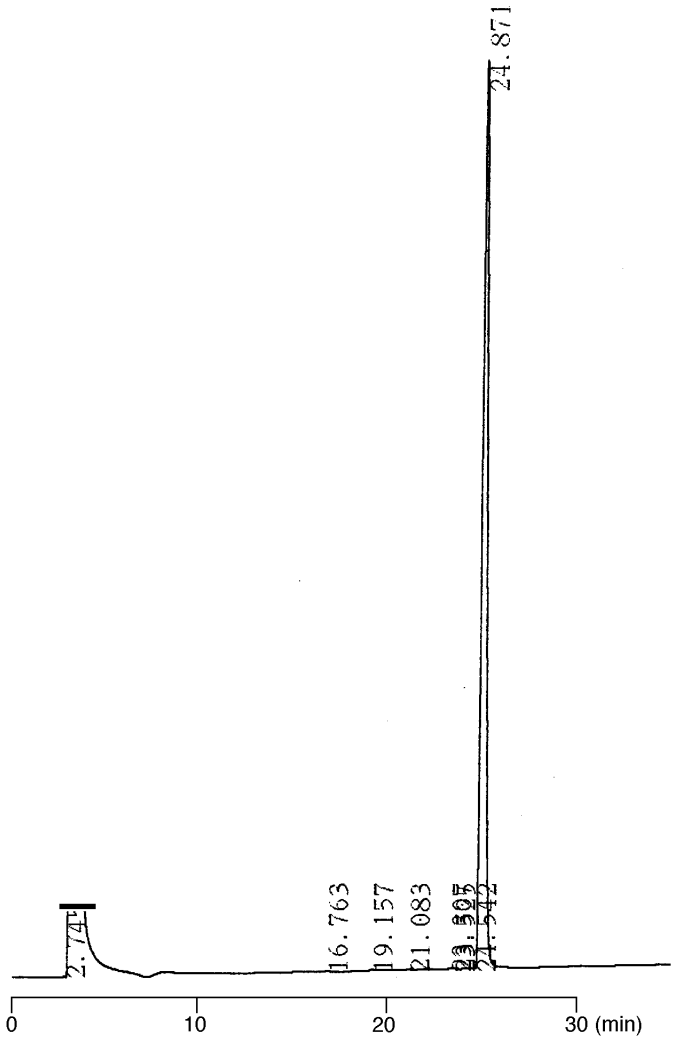

Table S6. Observed ^{1}H-NMR chemical shifts, J values and temperature coefficients of **8k**.

residue	chemical shift (ppm)						$^{3}J(H^{N},H^{\alpha})$	$^{3}J(H^{\alpha},H^{\beta})$	$-\Delta\delta/\Delta T$[a] (ppb/K)
	H^{N}	H^{α}	H^{β}	H^{γ}	H^{δ}	H^{ε}			
Nal[1]	8.37	4.34	3.12 3.20				7.33	7.27	5.3
Gly[2]	8.08	3.55 3.75					4.76		2.7
D-Tyr[3]	7.93	4.24	2.74 2.78				6.67	6.18	2.5
Arg[4]	8.27	3.92	1.41 1.66	1.33	3.03	7.50	6.74	- -	6.5
Arg[5]	7.82	4.11	1.55 1.66	1.27	3.03	7.55	-[b]	-	2.3

[a] Temperature dependence of amide proton chemical shifts. [b] The ^{3}J value was not determined due to the broad peak.

Table S7. Upper-limit distance restraints for the structure calculation of **8k**.[a]

atom1		atom2		distance (Å)	atom1		atom2		distance (Å)
Nal^{1}	H^{N}	Arg^{5}	H^{N}	3.5	Arg^{4}	H^{N}	Arg^{4}	H^{α}	5.0
Nal^{1}	H^{N}	Arg^{5}	H^{α}	3.5	Arg^{4}	H^{N}	Arg^{4}	$H^{\beta}*$	4.5
Nal^{1}	H^{N}	Arg^{5}	$H^{\beta}*$	4.5	Arg^{4}	H^{N}	Arg^{4}	$H^{\gamma}*$	4.5
Nal^{1}	H^{N}	Nal^{1}	H^{α}	3.5	Arg^{4}	H^{N}	Arg^{5}	H^{N}	3.5
Nal^{1}	H^{N}	Nal^{1}	$H^{\beta}*$	6.0	Arg^{4}	H^{α}	Arg^{4}	$H^{\beta}*$	6.0
Nal^{1}	H^{N}	Gly^{2}	H^{N}	5.0	Arg^{4}	H^{α}	Arg^{4}	$H^{\gamma}*$	6.0
Nal^{1}	H^{α}	Nal^{1}	$H^{\beta}*$	4.5	Arg^{4}	$H^{\beta1}$	Arg^{4}	$H^{\beta2}$	2.7
Nal^{1}	$H^{\phi1}$[b]	Nal^{1}	$H^{\beta}*$	4.5	Arg^{4}	$H^{\beta}*$	Arg^{4}	$H^{\gamma}*$	5.5
Gly^{2}	H^{N}	Tyr^{3}	H^{N}	3.5	Arg^{4}	$H^{\beta}*$	Arg^{4}	$H^{\delta}*$	7.0
Gly^{2}	H^{N}	Gly^{2}	$H^{\alpha1}$	3.5	Arg^{5}	H^{N}	Arg^{4}	H^{α}	5.0
Gly^{2}	H^{N}	Gly^{2}	$H^{\alpha2}$	3.5	Arg^{5}	H^{N}	Arg^{4}	$H^{\beta}*$	6.0
Gly^{2}	H^{N}	Nal^{1}	H^{α}	3.5	Arg^{5}	H^{N}	Arg^{5}	H^{α}	3.5
Gly^{2}	$H^{\alpha1}$	Gly^{2}	$H^{\alpha2}$	2.7	Arg^{5}	H^{N}	Arg^{4}	$H^{\beta}*$	6.0
Tyr^{3}	H^{N}	Tyr^{3}	H^{α}	5.0	Arg^{5}	H^{N}	Arg^{5}	H^{α}	3.5
Tyr^{3}	H^{N}	Tyr^{3}	$H^{\beta}*$	4.5	Arg^{5}	H^{N}	Arg^{5}	$H^{\beta}*$	6.0
Tyr^{3}	H^{N}	Gly^{2}	$H^{\alpha1}$	3.5	Arg^{5}	H^{α}	Arg^{5}	$H^{\beta}*$	6.0
Tyr^{3}	H^{N}	Gly^{2}	$H^{\alpha2}$	5.0	Arg^{5}	H^{α}	Arg^{5}	$H^{\gamma}*$	6.0
Tyr^{3}	H^{α}	Tyr^{3}	$H^{\beta}*$	3.7	Arg^{5}	$H^{\beta1}$	Arg^{5}	$H^{\beta2}$	2.7
Tyr^{3}	H^{α}	Tyr^{3}	$H^{\delta}*$	7.0	Arg^{5}	$H^{\beta}*$	Arg^{5}	$H^{\gamma}*$	5.5
Tyr^{3}	$H^{\beta}*$	Tyr^{3}	$H^{\delta}*$	6.5	Arg^{5}	$H^{\beta}*$	Arg^{5}	$H^{\delta}*$	7.0
Arg^{4}	H^{N}	Tyr^{3}	H^{α}	2.7					

[a] Asterisks indicate the defined pseudoatoms. [b] $H^{\phi1}$ means the proton at the 1-position of naphthalene.