Peptide bond mimicry by (*E*)-alkene and (*Z*)-fluoroalkene peptide isosteres: synthesis and bioevaluation of α-helical anti-HIV peptide analogues†

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The α-helix structures of the anti-HIV fusion inhibitory peptides are stabilized by the amino acid sequence and by intrachain hydrogen bonds. The study of peptide analogues using (*E*)-alkene and (*Z*)-fluoroalkene dipeptide isosteres demonstrated the substantial, yet position-dependent, contribution of hydrogen bonds to the α-helix stability and anti-HIV bioactivity.

Introduction

The α-helix represents one of the largest classes of secondary structure elements found in protein and peptide structures.[1] The cylindrical structures are stabilized by intrachain hydrogen bond (H-bond) networks which are formed between the C=O of residue *i* and the amide N–H of the *i*+4 residue to generate 13-membered pseudocyclic structures. The functional and/or interactive surface(s) of the α-helix are revealed by the arrangement of the distribution residues in the linear sequence upon folding.

In order to stabilize the α-helix structure of bioactive peptides, there are two possible approaches: (1) bridging side-chains by covalent or non-covalent bond(s) or (2) mimicking intrachain H-bond(s).[2] Recently, we reported a novel design concept of fusion inhibitory peptides active against HIV-1 by utilizing an X-EE-XX-KK motif (X: original residue; E: glutamic acid; K: lysine).[3,4] This motif contributes to the stabilization of the bioactive α-helix conformation by forming two potential salt bridges between Glu and Lys side-chains without altering the location of residues that form the interactive surface with the viral protein gp41.[4c] The peptides, named SC35EK and T-20EK, exhibit highly potent anti-HIV activity by inhibition of the rearrangement of HIV-1 gp41 that facilitates fusion between the host cellular and viral membranes. In addition, a structure–activity relationship study identified a novel amphiphilic peptide, SC29EK, with a minimal sequence for anti-HIV activity.[5] In light of its high potency of SC29EK, it was of interest to estimate the effect of intrachain H-bond(s) on α-helix stabilization in the presence of the X-EE-XX-KK motifs. Accordingly, efforts herein have been undertaken to comparatively evaluate the anti-HIV activity and biophysical properties of SC29EK analogues containing peptide bond mimetics.

(*E*)-Alkene dipeptide isostere (EADI) **1** and (*Z*)-fluoroalkene dipeptide isostere (FADI) **2** of Lys-Lys were chosen as planar peptide bond surrogates for positional scanning of each Lys-Lys dipeptide in four repeat motifs (Fig. 1).[6] Two potential H-bonds may be missing when replacing the peptide bond in Lys-Lys with the olefin congeners: (1) between the C=O of the first Lys (*i*) and the N–H of the downward Glu (*i*+4), (2) between the N–H of the second Lys (*i*+1) and the C=O of the upward Glu (*i*−3) (Fig. 1). In the case of FADI substitution, the presence of the first H-bond was expected, because of the potential ability of a fluorine atom to act as a H-bond acceptor.[7]

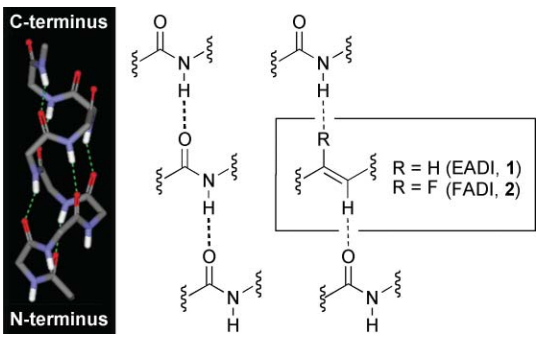

Fig. 1 Structures of (*E*)-alkene and (*Z*)-fluoroalkene dipeptide isosteres and the potential mimicry of H-bonds stabilizing the α-helix structure.

Results and discussion

Lys-Lys EADI[8] and FADI[9] were prepared by the established procedures shown in Schemes 1 and 2, respectively. Briefly, allyl alcohol **3**[10] derived from a protected amino acid was converted into Ns-amide **4**. Aziridination of **4** by the Mitsunobu reaction followed by C-1 elongation afforded the β-aziridinyl-α,β-unsaturated ester **6**. Organocopper-mediated alkylation of **6** provided an α-alkyl adduct **7** regio- and stereoselectively. Subsequent functional group manipulations generated the expected Fmoc-protected EADI **10**.

FADI synthesis began with mono-TBS-protected 1,5-pentanediol **11**. Rh-catalyzed Reformatsky–Honda reaction[11] of the corresponding aldehyde gave α,α-difluoro-β-amino ester **12**. The simultaneous hydrogenolysis and Boc protection followed by C-2 elongation using the Horner–Wadsworth–Emmons reaction produced a key γ,γ-difluoro-α,β-enoyl sultam **14**. One-pot reduction/asymmetric alkylation *via* transmetalation with allyl bromide formed the FADI scaffold **15**. Selective

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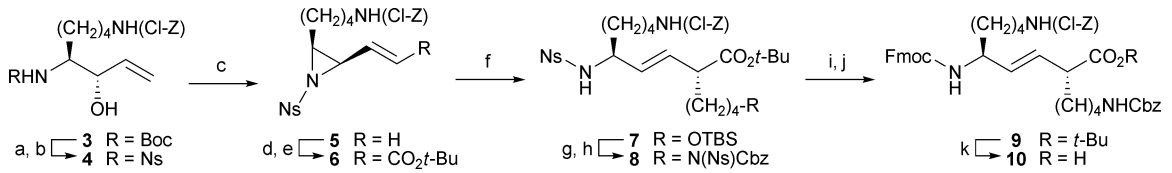
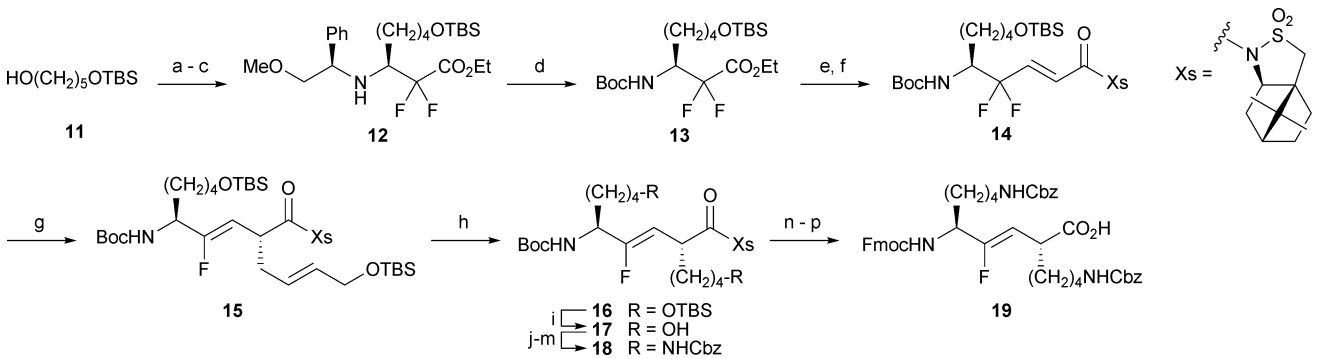


Scheme 1 Synthesis of the Lys-Lys-type alkene dipeptide isostere. *Reagents and conditions*: (a) 4 N HCl/dioxane; (b) NsCl, 2,4,6-collidine, $CHCl_3$, 65% (2 steps); (c) DIAD, PPh_3, THF/toluene, 0 °C, 84%; (d) O_3, AcOEt, −78 °C, then Me_2S; (e) $(EtO)_2P(O)CH_2CO_2t$-Bu, LiCl, DIEA, CH_3CN, 0 °C, 46% (2 steps); (f) $TBSO(CH_2)_4I$, *t*-BuLi, CuCN, LiCl, *n*-pentane/Et_2O/THF, −78 °C, 60%; (g) H_2SiF_6 aq., CH_3CN/CH_3OH, 0 °C; (h) CbzNHNs, PPh_3, DIAD, THF/toluene, 81% (2 steps); (i) PhSH, K_2CO_3, DMF; (j) Fmoc-OSu, Et_3N, DMF, 84% (2 steps); (k) 4 N HCl/dioxane, 96%.



Scheme 2 Synthesis of the Lys-Lys-type fluoroalkene dipeptide isostere. *Reagents and conditions*: (a) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C; (b) (*R*)-2-methoxy-1-phenylethylamine, 3 Å MS, THF, 0 °C; (c) $BrCF_2CO_2Et$, $RhCl(PPh_3)_3$, Et_2Zn, 0 °C, 43% (3 steps); (d) $Pd(OH)_2$, H_2, Boc_2O, EtOH, 87%; (e) DIBAL-H, CH_2Cl_2/toluene −78 °C; (f) $(EtO)_2P(O)CH_2COXs$, LiCl, DIEA, CH_3CN, 0 °C, 87% (2 steps); (g) $Me_2CuLi{\cdot}LiI$, THF/Et_2O, −78 °C, then HMPA, then Ph_3SnCl, −78 °C to −40 °C, then $BrCH_2$–(*E*)-CH=CH–CH_2OTBS, −40 °C, 78%; (h) 4.5% Pd/C(en), EtOH, H_2; (i) aq. H_2SiF_6, CH_3CN/CH_3OH, 78% (2 steps); (j) TsCl, Et_3N, CH_2Cl_2; (k) NaN_3, DMF; (l) PPh_3, THF/H_2O; (m) Cbz-OSu, Et_3N, DMF, 65% (4 steps); (n) 1 N LiOH, 50% H_2O_2, THF/H_2O; (o) TFA, CH_2Cl_2; (p) Fmoc-OSu, Et_3N, MeCN/DMF/H_2O, 64% (3 steps).

hydrogenation in the presence of Pd/C(en)[12] and step-wise modifications afforded the Fmoc-protected FADI **19**. The resulting isosteres **10** and **19** were incorporated into the KK dipeptide of SC29EK sequence by standard Fmoc-based solid-phase peptide synthesis.

Anti-HIV activities of the isostere-containing peptides **20E–23E** and **20F–23F** were examined using the MAGI assay (Table 1). Substitutions of the first and second N-terminal Lys-Lys dipeptides with EADI (**20E** and **21E**) resulted in the loss of the anti-HIV activity (EC_{50} >10 μM). In contrast, the FADI congeners exhibited weak or moderate anti-HIV activities (**20F**: EC_{50} = 5.2 μM; **21F**: EC_{50} = 599 nM). Both EADI and FADI analogues with substitution at the third Lys-Lys showed slightly lower anti-HIV potency than wild-type C29[5] without the α-helix inducible XEEXXKK motifs (**22E**: EC_{50} = 865 nM; **22F**: EC_{50} = 663 nM). The best peptide analogues were obtained by replacement of the C-terminal Lys-Lys with the isosteres (**23E**: EC_{50} = 43 nM; **23F**: EC_{50} = 37 nM); however, the potency was lower than the original SC29EK peptide (EC_{50} = 2.2 nM). Similar bioactivities of peptide **20E–23E** and **20F–23F** were also observed against the other HIV-1 strains (Table 2). These observations suggest that all the peptide bonds within the Lys-Lys and the related H-bonding are essential for the potent anti-HIV activity of SC29EK.

The α-helix properties of these peptides were determined by circular dichroism (CD) analysis (Fig. 2a,b). The stable α-helix

Table 1 Sequences and anti-HIV activities of C29 and its derivatives and T_m values of the mixture with N36

	EADI analogues **E**		FADI analogues **F**	
Sequence[a]	EC_{50} (nM)[b]	T_m (°C)[c]	EC_{50} (nM)[b]	T_m (°C)[c]
WMEWDREINNYTSLIHSLIEESQNQQEKN C29	308 ± 144	51.7	—	—
WEEWDKKIEEYTKKIEELIKKSEEQQKKN SC29EK	2.2 ± 0.2	67.4	—	—
WEEWDKKIEEYTKKIEELIKKSEEQQKKN **20E/20F**	>10000	43.9	5220 ± 202	44.1
WEEWDKKIEEYTKKIEELIKKSEEQQKKN **21E/21F**	>10000	40.1	599 ± 96	49.5
WEEWDKKIEEYTKKIEELIKKSEEQQKKN **22E/22F**	865 ± 317	62.2	663 ± 242	60.9
WEEWDKKIEEYTKKIEELIKKSEEQQKKN **23E/23F**	43 ± 7	64.1	37 ± 6	64.8

[a] The underlined KK dipeptide indicates the position of the dipeptide isostere. [b] EC_{50} was determined as the concentration that blocked HIV-1 (NL4–3 strain) replication by 50%. [c] T_m values were defined by the midpoint of the thermal unfolding transition state as determined from $[\theta]_{222}$ readings.

Table 2 Anti-HIV activities of C29 and its derivatives against three HIV-1 strains

	EC_{50} (nM)[a]		
Peptides	NL4–3	IIIB	Ba-L
C29	308 ± 144	396 ± 83	42 ± 8
SC29EK	2.2 ± 0.2	6.5 ± 0.9	1.9 ± 0.2
20E	>10000	>10000	>10000
20F	5220 ± 202	>10000	5580 ± 1920
21E	>10000	>10000	>10000
21F	599 ± 96	3010 ± 554	600 ± 302
22E	865 ± 317	5110 ± 2,750	2630 ± 386
22F	663 ± 242	2200 ± 712	527 ± 95
23E	37 ± 6	153 ± 27	33 ± 2
23F	43 ± 7	237 ± 16	51 ± 7

[a] EC_{50} is the concentration that blocks HIV-1 replication by 50%.

structure of SC29EK was disrupted by a single substitution of the second or third Lys-Lys peptide bond with the isosteres in **21E/21F** and **22E/22F**. This suggests that the contribution of the H-bonds to the stability of the α-helix is likely to be superior to the multiple introductions of the X-EE-XX-KK motifs at these positions. Conversely, the effects of N- and C-terminal substitution were less significant as observed in **20E/20F** and **23E/23F**. This may be rationalized by the fact that these peptide bonds of SC29EK are positioned at the edge of the helix and that C-terminal Lys-Lys is involved in only upward H-bonding through the donor N–H moiety. CD spectra of SC29EK analogues in the presence of an interactive counterpart N36 indicated the formation of stable six-helix coiled-coil structures (Fig. 2c,d).[13] This observation supports the concept that SC29EK analogues exert their anti-HIV activity by inhibiting the folding process of the HIV-1 envelop protein gp41.

Binding affinity of SC29EK analogues to a viral protein was determined by the thermal stability of the six-helix complexes formed between SC29EK and N36 peptides. The melting temperature (T_m), representing 50% disruption of the six-helix bundle, was comparatively evaluated by monitoring the change in the circular dichroism signal at 222 nm as a function of increasing temperature (Table 1). The complexes involving peptides **20E/20F** and **21E/21F** showed significantly lower thermal stability, which correlates with the observed absent or low anti-HIV activities of these peptides. In contrast, potent analogues **23E/23F** form stable complexes with N36 with T_m values comparable to the value measured for SC29EK (**23E**: $T_m = 64.1$ °C; **23F**: $T_m = 64.8$ °C). The N-terminal tryptophan-rich domain (WRD) of inhibitory peptides such as C34 is essential for binding to the cavity formed by the N36 coiled-coil.[11] H-Bonds linked by the first and second Lys-Lys peptide bonds in SC29EK would reinforce the arrangement of these tryptophans. Interestingly, less potent anti-HIV activity of peptide **22E/22F** was observed compared with C29, whereas the complexes with N36 showed higher thermal stability. This result suggests that the loss of crucial H-bonds could reduce the anti-HIV activity, even though the X-EE-XX-KK motifs apparently aid the conformational stability of the six-helix bundle.

In terms of the mimicking ability of the two-peptide-bond isosteres, FADI peptides **20F–23F** exhibited slightly more potent anti-HIV activity and formed more stable complexes with N36 (except for **22F**). Although a fluoroalkene with a large dipole moment imperfectly reproduces the H-bonds needed for α-helix stabilization, FADI is an appropriate peptide bond surrogate to investigate structural requirements in bioactive peptides.

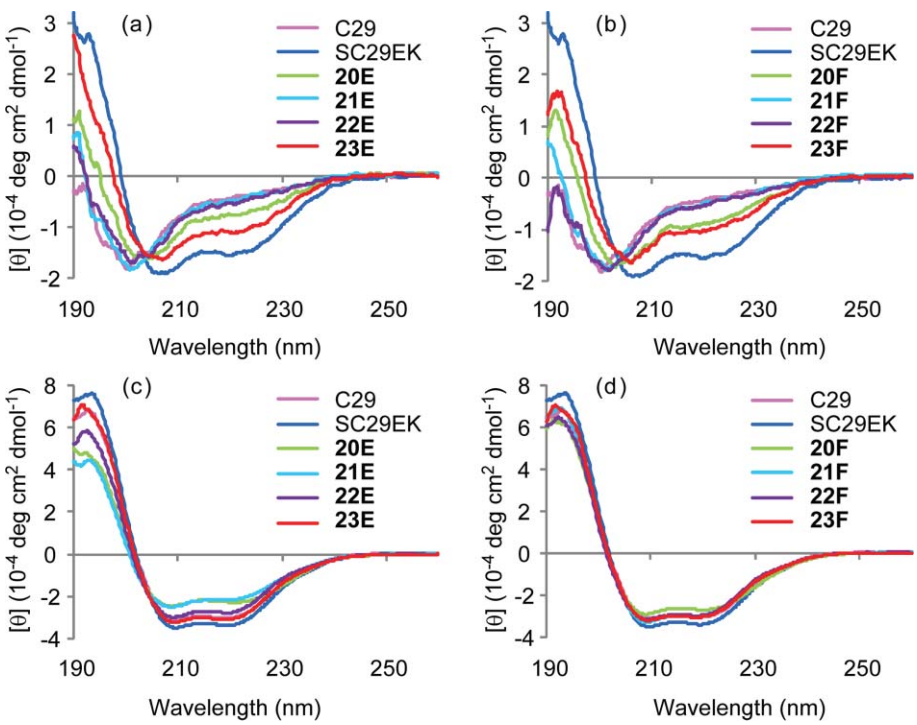


Fig. 2 CD spectra of EADI- and FADI-containing SC29EK analogues in the absence (a,b) and presence (c,d) of N36.

Conclusions

The effects of H-bonds on the stability of the α-helix of an HIV-1 fusion inhibitor were investigated by positional-scanning of the Lys-Lys dipeptides using EADI and FADI. As demonstrated by CD analysis of the SC29EK analogues, H-bonds in the middle of the sequence contribute significantly to the stabilization of the α-helix. In contrast, the effect of H-bonds on the anti-HIV activity of the peptides depends on the distance from the crucial interactive domain. As such, we have shown that EADI and FADI can be used for conformational evaluation of bioactive and/or functional α-helical peptides.

Experimental section

Synthesis

***tert*-Butyl (2*R*,5*S*,3*E*)-2-[4-(*tert*-butyldimethylsiloxy)butyl]-9-[*N*-(2-chlorobenzyloxycarbonyl)amino]-5-[*N*-(2-nitrophenylsulfonyl)amino]non-3-enoate (7).** To a stirred solution of TBSO-$(CH_2)_4$I (236 mg, 0.75 mmol) in dry Et_2O (0.5 cm^3), was added dropwise 1.59 M *t*-BuLi in Et_2O solution (1.0 cm^3, 1.58 mmol) under Ar at −78 °C. After being stirred at this temperature for 30 min, the mixture was stirred at 0 °C for 30 min. To a stirred solution of CuCN (61 mg, 0.61 mmol) and LiCl (52 mg, 1.23 mmol) in dry THF (0.8 cm^3), was added dropwise the above 0.5 M TBSO$(CH_2)_4$Li in THF solution (1.2 cm^3) under Ar at −78 °C, and the mixture was stirred at 0 °C for 10 min. A solution of aziridinyl enoate **6** (91 mg, 0.15 mmol) in dry THF (1.0 cm^3) was added dropwise to the above mixture at −78 °C with stirring, and the stirring was continued for 1.5 h followed by quenching with saturated NH_4Cl/28% NH_4OH solution (1/1, 2.0 cm^3). The mixture was washed with H_2O and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (3:1) gave the title compound **7** (72 mg, 60%) as a colorless oil; $[\alpha]^{23}_D$ −73.2 (*c* 0.87 in $CHCl_3$); ν_{max}/cm^{-1} 3349 (NHCO), 1725 (CO); δ_H (500 MHz; $CDCl_3$) 0.04 (6H, s), 0.89 (9H, s), 1.08–1.64 (21H, m), 2.64 (1H, dt, *J* 8.0 and 6.3), 3.07–3.21 (2H, m), 3.55 (2H, t, *J* 6.3), 3.86–3.96 (1H, m), 4.92 (1H, br s), 5.21 (2H, s), 5.26 (1H, dd, *J* 15.5 and 7.5), 5.39 (1H, d, *J* 8.0), 5.40 (1H, dd, *J* 15.5 and 8.0), 7.22–7.30 (2H, m), 7.37 (1H, dd, *J* 5.7 and 2.3), 7.42 (1H, dd, *J* 5.7 and 2.3), 7.65–7.74 (2H, m), 7.83 (1H, dd, *J* 6.9 and 2.3), 8.09 (1H, dd, *J* 6.9 and 2.3); δ_C (100 MHz; $CDCl_3$) −5.3 (2C), 18.3, 22.5, 23.3, 25.9, 28.0 (3C), 29.3 (3C), 32.3, 32.6, 35.4, 40.6, 49.3, 56.7, 62.8, 63.9, 80.6, 125.3, 126.9, 129.3, 129.5, 129.8, 130.9 (2C), 131.2, 132.8, 133.3, 133.5, 134.3, 135.1, 147.8, 156.2, 172.8; *m/z* (FAB) 782.3246 ([M + H]$^+$, $C_{37}H_{57}ClN_3O_9SSi$ requires 782.3273).

(2*R*,5*S*,3*E*)-2-{4-[*N*-(*tert*-Butoxycarbonyl)amino]butyl}-9-[*N*-(2-chlorobenzyloxycarbonyl)amino]-5-[*N*-(9-fluorenylmethoxycarbonyl)amino]non-3-enoic acid (10). To the Fmoc-protected amine **9** (435 mg, 0.52 mmol) was added 4 N HCl/dioxane (5.0 cm^3) at 0 °C, and the mixture was stirred for 20 h at room temperature. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (1:1) gave the title compound **10** (391 mg, 96%) as a semisolid; $[\alpha]^{22}_D$ −18.0 (*c* 0.87 in $CHCl_3$); ν_{max}/cm^{-1} 3324 (NHCO), 1703 (CO); δ_H (500 MHz; $CDCl_3$) 1.09–1.81 (12H, m), 2.85–3.01 (1H, m), 3.03–3.21 (4H, m), 4.01–4.21 (2H, m), 4.30–4.54 (2H, m), 4.81–5.26 (6H, m), 5.30–5.81 (3H, m), 7.19–7.41 (13H, m), 7.52–7.59 (2H, m), 7.74 (2H, d, *J* 7.5); δ_C (125 MHz; $CDCl_3$) 22.5, 24.0, 29.2, 29.3, 31.5, 34.3, 40.6 (2C), 47.1, 48.4, 52.3, 63.7, 66.4 (2C), 119.8 (2C), 124.9 (2C), 126.7 (2C), 126.9 (2C), 127.5 (2C), 127.7, 127.9, 128.3, 129.2 (2C), 129.3 (2C), 129.5, 133.3, 134.2, 136.5, 141.1, 143.7 (2C), 143.8 (2C), 155.8, 156.3, 156.4, 178.0; *m/z* (FAB) 782.3201 ([M + H]$^+$, $C_{44}H_{49}ClN_3O_8$ requires 782.3208).

(2*R*,5*S*,3*Z*)-5-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[(*E*)-4-(*tert*-butyldimethylsiloxy)but-2-enyl]-9-(*tert*-butyldimethylsiloxy)-4-fluoronon-3-enoyl (*S*)-sultam (15). To a suspension of CuI (180 mg, 0.94 mmol) in THF (4.8 cm^3) at −78 °C under argon was added dropwise a solution of MeLi·LiBr complex in Et_2O (1.5 M, 1.3 cm^3, 1.89 mmol), and the mixture was stirred for 10 min at 0 °C. To the solution of the above organocopper reagent at −78 °C was added dropwise a solution of the *N*-enoyl sultam **14** (150 mg, 0.24 mmol) in THF (4.8 cm^3). The mixture was stirred for 30 min at −78 °C and HMPA (0.66 cm^3, 3.78 mmol) was added dropwise to the mixture. After stirring for 30 min at −78 °C, a solution of triphenyltin chloride (182 mg, 0.47 mmol) in THF (3.0 cm^3) was added dropwise, and the mixture was then stirred for 30 min at −40 °C. (*E*)-(4-Bromobut-2-enyloxy)(*tert*-butyl)dimethylsilane (501 mg, 1.89 mmol) in THF (3.0 cm^3) was added dropwise and the mixture was stirred for 20 h at −40 °C. The reaction was quenched at −40 °C by addition of a saturated NH_4Cl/28% NH_4OH solution (1/1, 6.0 cm^3) and the mixture was stirred at room temperature for additional 30 min. The mixture was extracted with Et_2O and the extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (5:1) gave the title compound **15** (148 mg, 78% yield) as a colorless oil; $[\alpha]^{25}_D$ −47.1 (*c* 1.00 in $CHCl_3$); ν_{max}/cm^{-1} 3317 (NHCO), 1693 (CO); δ_H (500 MHz; $CDCl_3$) 0.03 (6H, s), 0.04 (6H, s), 0.88 (9H, s), 0.89 (9H, s), 0.96 (3H, s), 1.15 (3H, s), 1.24–1.64 (17H, m), 1.83–1.91 (3H, m), 2.02–2.05 (2H, m), 2.33–2.37 (1H, m), 2.51–2.55 (1H, m), 3.41 (1H, d, *J* 13.7), 3.49 (1H, d, *J* 13.7), 3.58 (2H, t, *J* 6.3), 3.86 (1H, t, *J* 6.3), 4.06 (2H, d, *J* 3.4), 4.12–4.21 (2H, m), 4.60–4.72 (1H, m), 4.97 (1H, dd, *J* 36.7 and 8.6), 5.58 (2H, m); δ_C (125 MHz; $CDCl_3$) −5.3 (4C), 18.2, 18.3, 19.8, 20.7, 21.9, 25.9 (6C), 26.4, 28.3 (3C), 32.2, 32.3, 32.8, 36.9, 38.3, 41.0, 44.6, 47.6, 48.2, 51.6 (d, *J* 27.6), 53.0, 62.8, 63.5, 65.1, 79.4, 103.3 (d, *J* 12.0), 125.9, 132.7, 154.8, 158.6 (d, *J* 261.5) 172.2; δ_F (470 MHz; $CDCl_3$) −119.1—119.8 (m); *m/z* (FAB) 801.4732 ([M + H]$^+$, $C_{40}H_{74}FN_2O_7SSi_2$ requires 801.4739).

(2*R*,5*S*,3*Z*)-2-{4-[*N*-(Benzyloxycarbonyl)amino]butyl}-9-[*N*-(benzyloxycarbonyl)amino]-5-[*N*-(9-fluorenylmethoxycarbonyl)-amino]-4-fluoronon-3-enoic acid (19). To a solution of the sultam **18** (376 mg, 0.34 mmol) and aqueous 50% H_2O_2 (0.12 cm^3, 1.75 mmol) in THF/H_2O (5/1, 6.0 cm^3) at 0 °C was added aqueous 1 N LiOH (0.67 cm^3, 0.67 mmol), and the mixture was stirred at room temperature for 2 h. After being diluted with EtOAc (20 cm^3), the mixture was washed with 0.1 N HCl and dried over $MgSO_4$. Concentration under reduced pressure gave the corresponding acid, which was used in the next reaction without purification. To a solution of the above acid in CH_2Cl_2 (15 cm^3) at 0 °C was added TFA (4.0 cm^3), and the mixture was stirred at room temperature for 0.5 h. Concentration under reduced pressure gave an oily residue, which was dissolved in MeCN/DMF/H_2O

(10/9/1, 20 cm^3). Fmoc-OSu (159 mg, 0.472 mmol) and Et_3N (0.094 cm^3, 0.675 mmol) were added to the mixture at 0 °C, and the mixture was stirred at room temperature for 12 h. After being diluted with EtOAc (70 cm^3), the reaction mixture was washed with 1 N HCl and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (1:1) gave the title compound **19** (267.3 mg, 65% yield) as a semisolid; $[\alpha]^{24}_D$ −19.6 (*c* 1.13 in DMSO); ν_{max}/cm^{-1} 3333 (OH), 1693 (CO); δ_H (500 MHz; DMSO-d_6) 1.04–1.70 (12H, m), 2.89–3.02 (4H, m), 3.21 (1H, dt, *J* 9.7 and 7.5), 3.98–4.10 (1H, m), 4.22 (1H, t, *J* 6.9), 4.30 (2H, d, *J* 6.9), 4.85 (1H, dd, *J* 37.2 and 9.7), 4.99 (4H, s), 7.19–7.44 (16H, m), 7.65–7.74 (3H, m), 7.89 (2H, d, *J* 7.5), 12.35 (1H, br s); δ_C (125 MHz; DMSO-d_6) 22.6, 23.7, 28.9, 29.0, 30.9, 31.9, 40.0, 40.1, 40.3, 46.6, 51.3 (d, *J* 31.2), 65.0, 65.1, 65.4, 104.0 (d, *J* 12.0), 120.0 (2C), 125.1 (2C), 127.0 (2C), 127.6 (2C), 127.6 (4C), 127.7 (2C), 128.3 (4C), 137.2 (2C), 140.7 (2C), 143.7, 143.8, 155.6, 156.0 (2C), 159.4 (d, *J* 257.9), 174.4; δ_F (470 MHz; DMSO-d_6) −117.9–−118.5 (m); *m/z* (FAB) 766.3512 ($[M + H]^+$, $C_{44}H_{49}FN_3O_8$ requires 766.3504).

General procedure for preparation of peptide by Fmoc-SPPS

The protected peptide chains were constructed on the Novasyn® TGR resin (0.26 mmol g^{-1}, 96 mg, 0.025 mmol). *t*-Bu ester for Asp and Glu; 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for Arg; *t*-Bu for Thr, Tyr and Ser; Boc for Lys; and Trt for Gln, Asn and His were employed for side-chain protection. Fmoc-amino acids (0.075 mmol) were coupled by using *N*,*N*′-diisopropylcarbodiimide (DIC; 0.012 cm^3, 0.075 mmol) and *N*-hydroxybenzotriazole monohydrate ($HOBt\cdot H_2O$, 11.5 mg, 0.075 mmol) in DMF for 2 h. Coupling of dipeptide isosteres (EADI **10**: 49 mg, 0.063 mmol; FADI **19**, 48 mg, 0.063 mmol) was carried out with DIC and $HOBt\cdot H_2O$ for 12 h. The peptide resins were treated with 1 M TMSBr-thioanisole/TFA in the presence of *m*-cresol and 1,2-ethanedithiol as scavengers. The reaction mixture was precipitated with diethyl ether. The resulting powder was collected by centrifugation and then washed three times with diethyl ether. The crude product was purified by preparative HPLC to afford the expected peptides as a colorless powder. The purity of each compound was assessed analytical RP-HPLC prior to the CD analysis and biological testing (>98%).

Anti-HIV-1 activity

Anti-HIV-1 activity was determined by the multinuclear activation of a galactosidase indicator (MAGI) assay as described previously.[14] Briefly, the MAGI cells (10^4 cells $well^{-1}$) were seeded in flat-bottom 96-well microtitre plates. The following day, the cells were inoculated with HIV-1 (60 MAGI units/well, yielding 60 blue cells after 48 h incubation) and cultured in the presence of various concentrations of peptide inhibitors in fresh medium. After 48 h incubation, all the blue cells stained with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) in each well were counted. The activity of inhibitors was determined as the concentration that blocked HIV-1 replication by 50% (50% effective concentration [EC_{50}]).

Measurement of CD spectra

Peptides were incubated at 37 °C for 30 min (the final concentrations of peptides were 10 μM in 5 mM HEPES buffer, pH 7.2). CD spectra were acquired on a Jasco spectropolarimeter (Model J-710, Jasco Inc., Tokyo, Japan) at 25 °C as the average of 8 scans. Thermal unfolding at intervals of 0.5 °C was performed after a 0.25-min equilibration at the desired temperature and an integration time of 1.0 s. The mid point of the thermal unfolding transition (melting temperature, T_m) of each complex was determined from the maximum of the first derivative, with respect to the reciprocal of the temperature, of the $[\theta]_{222}$ values.

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