Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 923–928

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Evaluation of Pseudopeptide Analogues of a Specific CXCR4 Inhibitor, T140: The Insertion of an (*E*)-Alkene Dipeptide Isostere into the βII′-Turn Moiety

Hirokazu Tamamura,[a,*] Kenichi Hiramatsu,[a] Kazuhide Miyamoto,[a] Akane Omagari,[a] Shinya Oishi,[a] Hideki Nakashima,[b] Naoki Yamamoto,[c] Yoshihiro Kuroda,[a] Terumichi Nakagawa,[a] Akira Otaka[a] and Nobutaka Fujii[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan*
[b]*St. Marianna University, School of Medicine, Miyamae-ku, Kawasaki 216-8511, Japan*
[c]*Tokyo Medical and Dental University, School of Medicine, Bunkyo-ku, Tokyo 113-8519, Japan*

Received 10 December 2001; accepted 14 January 2002

Abstract—A 14-residue peptide, T140, strongly inhibits the T-cell line-tropic HIV-1 (X4-HIV-1) infection, since this peptide functions as a specific antagonist against a chemokine receptor, CXCR4. T140 takes an antiparallel β-sheet structure with a type II′ β-turn. In the present paper, we have designed and synthesized several T140 analogues, in which an (*E*)-alkene dipeptide isostere was inserted into the type II′ β-turn moiety, as a bridging study to develop nonpeptidic CXCR4 inhibitors. It has been proven that the turn region of T140 can be replaced by the above surrogate with the maintenance of strong anti-HIV activity.

Introduction

Recently, a CXC-chemokine receptor, CXCR4, and a CC-chemokine receptor, CCR5, were proven to be major coreceptors for the entry of T-cell line-tropic HIV-1 (X4-HIV-1)[1] and macrophage-tropic HIV-1 (R5-HIV-1),[2] respectively. Since the identification of these coreceptors, several chemokine antagonists have been found by us[3] and others.[4] Currently, development of these chemokine antagonists is desired as the complement of 'highly active anti-retroviral therapy (HAART)' using multi-types of anti-HIV drugs, such as reverse transcriptase inhibitors and protease inhibitors.[5] We found that 14-residue peptides, T134 and T140, derived from our SAR studies on T22,[3a,6] are specific CXCR4 inhibitors that block the X4-HIV-1 entry.[7,8] These peptides have one disulfide bond and maintain an antiparallel β-sheet structure connected by a type II′ β-turn with D-Lys[8]-Pro[9] at the $(i+1)$ and $(i+2)$ positions (the amino acid sequences of T134 and T140 are shown in Table 1 and Fig. 1[7–9]). We wish to forward a project to develop nonpeptidic CXCR4 inhibitors based on T134 and T140. Initially, we attempted to restrict the secondary structure of these peptides. According to Wipf's study, D,L-type (or L,D-type) (*E*)-alkene dipeptide isosteres (EADIs) serve as the promoters of type II′ (or type II) β-turn structures in the crystal state.[10] Gellman[11] and Hoffmann[12] also reported (*E*)-alkene dipeptide mimetics that promote β- and βII-hairpin formation, respectively. Thus, in this study we have synthesized several T140 analogues, in which an EADI was inserted into the D-Lys[8]-Pro[9] sequence at the $(i+1)$ and $(i+2)$ positions of the turn to restrict the type II′ β-turn structure, and evaluated their anti-HIV activity in company with their conformational analysis.

Synthesis of T140 Analogues Containing (*E*)-Alkene Dipeptide Isosteres

An EADI, Fmoc-D-Lys(Cl-*Z*)-ψ[(*E*)-CH=CH]-L-Ala-OH **9**, was synthesized starting with N^{α}-Boc-N^{ε}-(Cl-Z)-D-lysine **1** as outlined in Scheme 1. Allylalcohol **4**, which was obtained as a major diastereoisomer by the Grignard reaction of aldehyde **3**, was purified by flash chromatography. γ-Acetoxy (*E*)-α,β-enoate **6**, which was given by the Wittig–Horner–Emmons reaction of

*Corresponding authors. Tel.: +81-75-753-4551; fax: +81-75-753-4570; e-mail: tamamura@pharm.kyoto-u.ac.jp; nfujii@pharm.kyoto-u.ac.jp

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00041-0

the aldehyde derived from **5**, was also purified by flash chromatography to eliminate the (*Z*)-isomer. Treatment of γ-mesyloxy (*E*)-α,β-enoate **7** with MeCu(CN)MgBr·BF_3 yielded the (D-Lys,Ala)-type EADI, Boc-D-Lys(Cl-Z)-ψ[(*E*)-CH=CH]-L-Ala-OBu^t **8**, in 95% yield via an anti-S_N2' mechanism, as previously reported by us.[13,14] Construction of protected peptidyl resins and introduction of the EADI **9** on the peptidyl resins were performed by the standard Fmoc-based solid-phase methodology. Treatment of the constructed peptidyl resins with 1 M trimethylsilyl bromide (TMSBr)–thioanisole/trifluoroacetic acid (TFA) in the presence of *m*-cresol and 1,2-ethanedithiol (EDT) resulted in cleavage of peptides from the resins and deprotection, followed by air oxidation (Fig. 1). The crude products were purified by preparative HPLC and gel-filtration to afford a white powder of the desired peptides. The integrity of peptides was determined by ion spray mass spectrometry analysis, and the purity was confirmed by analytical HPLC (data not shown).

Evaluation of anti-HIV activity and cytotoxicity

A strain of X4-HIV-1, HIV-1IIIB, was obtained from the culture supernatant of HIV-1 persistently infected MOLT-4/HIV-1IIIB cells. Anti-HIV activity was determined based on the protection against HIV-induced cytopathogenicity in MT-4 cells using the 3′-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (EC_{50}).[15] Cytotoxicity of the compounds was determined based on the reduction of the viability of mock-infected cells using the MTT method (CC_{50}).

Table 1. Anti-HIV activity, cytotoxicity and inhibitory activity against Ca^{2+} mobilization of T134, T140 and their analogues

Compd	X	Y	EC_{50} (nM)	CC_{50} (μM)	SI CC_{50}/EC_{50}	IC_{50} (nM)
T22			44	N.T.		N.T.
T134	Trp	OH	15	N.T.		30
T140	Nal	OH	<0.3	67	>220,000	6.9
T134-NH_2	Trp	NH_2	3.7	56	15,000	N.T.
T140-NH_2	Nal	NH_2	4.7	57	12,000	5.0
T141-NH_2	Cys(Bzl)	NH_2	67	54	810	N.T.
T142-NH_2	Cys(Ad)	NH_2	7.1	49	6900	N.T.
T(E)-134-OH	Trp	OH	<1.3	100	>78,000	27
T(E)-140-OH	Nal	OH	1.9	270	140,000	7.3
T(E)-134-NH_4	Trp	NH_2	2.9	58	20,000	N.T.
T(E)-140-NH_2	Nal	NH_2	1.9	58	31,000	10
T(E)-141-NH_2	Cys(Bzl)	NH_2	45	59	1300	36
T(E)-142-NH_2	Cys(Ad)	NH_2	17	57	3300	170
AZT			0.3	12	35,000	N.T.
ddC			33	48	1500	N.T.

T134, T140, T134-NH_2, T140-NH_2, T141-NH_2 and T142-NH_2 are peptides with a nomal imide bond in D-Lys[8]-Pro[9], whereas T(E)-134-OH, T(E)-140-OH, T(E)-134-NH_2, T(E)-140-NH_2, T(E)-141-NH_2 and T(E)-142-NH_2 are pseudopeptides with an EADI. The structures of these peptides containing X and Y are shown in Figure 1. EC_{50} values are based on the inhibition of HIV-induced cytopathogenicity in MT-4 cells. CC_{50} values are based on the reduction of the viability of mock-infected MT-4 cells. Selectivity index (SI) is shown as CC_{50}/EC_{50}. N.T., not tested. IC_{50} values are based on the inhibition of $[Ca^{2+}]$ mobilization induced by SDF-1 through CXCR4. 3′-Azido-3′-dideoxythymidine (AZT) and 2′,3′-dideoxycytidine (ddC) were tested for comparison. All data are the mean values for at least three experiments for EC_{50} and CC_{50} or two experiments for IC_{50}. Ad = *S*-1-adamantyl.

Evaluation of inhibitory activity against stromal cell-derived factor 1 (SDF-1)-induced $[Ca^{2+}]$ mobilization[16]

CXCR4-transfected CHO cell lines were loaded with Fura-2-AM for 1 to 2 h, and sequentially treated with T140 analogues, followed by the addition of recombinant human SDF-1α (PeproTech) after 3 min. $[Ca^{2+}]_i$ increases were measured by a spectrofluorometer by a modified procedure of the Fura-2 method.[16e] Inhibitory activity of T140 analogues was determined based on the inhibition of $[Ca^{2+}]$ mobilization induced by SDF-1α stimulation through CXCR4 (IC_{50}).

CD spectroscopy of T140 analogues

CD spectra of peptides in H_2O at 10 μM were recorded on a JASCO J-720 spectropolarimeter (Tokyo, Japan) as previously reported.[8]

^{1}H NMR spectroscopy of T(E)-140-OH

The NMR spectra of T(E)-140-OH in H_2O–D_2O (9:1) were recorded at 25 °C on a Varian UNITY 600 spectrometer at 600 MHz ^{1}H frequency. NMR experiments and assignments were achieved in the same manner as previously reported.[9]

Molecular dynamics calculations

Molecular dynamics calculations were made using the NMR refine program within the Insight II/Discover package (Molecular Simulation, Inc., CA, USA) on a Silicon Graphics Origin 2000 workstation. The dynamics calculations were carried out with distance constraints from NOEs and dihedral constraints from *J*-couplings. The 40 initial structures of the backbone of T(E)-140-OH were randomly generated using the NMR refine program. The direction of the two protons of the (*E*)-alkene in 20 initial structures was reversed from that of the two protons in the other 20 structures, since the backbone structure near the (*E*)-alkene in turn could not be determined by NMR. These initial structures

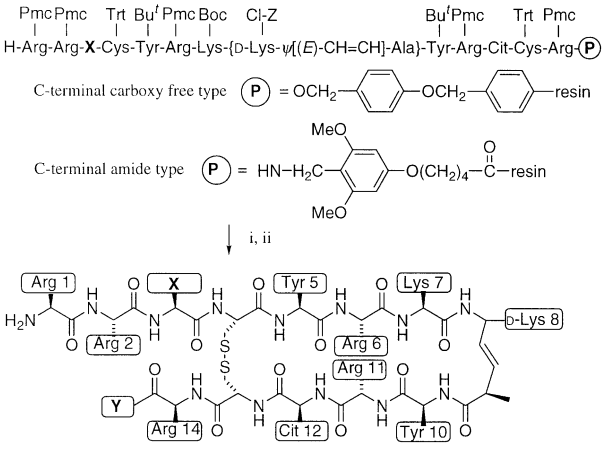
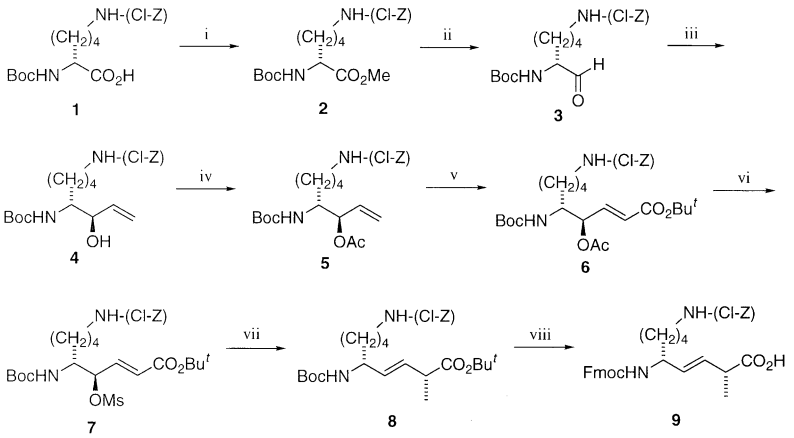


Figure 1. The synthesis of EADI-containing peptides: T(E)-134-OH, T(E)-140-OH, T(E)-134-NH_2, T(E)-140-NH_2, T(E)-141-NH_2 and T(E)-142-NH_2; (i) 1 M TMSBr–thioanisole/TFA, *m*-cresol, EDT; (ii) air oxidation; Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl; X and Y are shown in Table 1.



Scheme 1. (i) MeI, $KHCO_3$ (100%); (ii) DIBAL; (iii) vinylMgCl, LiCl, $ZnCl_2$ (40%, 2 steps); (iv) Ac_2O, pyridine, DMAP (50%); (v) O_3, Me_2S; $(EtO)_2P(O)CH_2COOBu^t$, LiCl, DIPEA (67%); (vi) Na_2CO_3, MeOH; MsCl, pyridine, DMAP (79%); (vii) MeCu(CN)MgBr·BF_3 (95%); (viii) TFA; Et_3N, MeCN–H_2O, Fmoc-Osu (17%); Cl-Z = 2-chloro-carbobenzoxy, Fmoc = 9-fluorenylmethyloxycarbonyl.

were subjected to the simulated annealing calculations in the same manner as previously reported.[17]

Results and Discussion

First, anti-HIV activity (EC_{50}) and cytotoxicity (CC_{50}) of T134, T140 and their analogues were evaluated by the MTT assay (Table 1). Since the cytotoxicity of T134 was previously evaluated ($CC_{50} > 50\,\mu M$),[8] the examination at high concentrations of T134 was omitted in this study. Our previous Ala-scanning study revealed that Pro^9 of T140 could be replaced by Ala without significant reduction of activity, indicating that the side-chain of Pro^9 is dispensable.[18] Thus, we adopted D-Lys-ψ[(*E*)-CH=CH]-L-Ala as a dipeptide isostere of the D-Lys^8-Pro^9 sequence (Fig. 1). T(E)-134-OH and T(E)-140-OH, which are T134 and T140 analogues containing an EADI D-Lys-ψ[(*E*)-CH=CH]-L-Ala, respectively, have strong anti-HIV activity, comparable to that of T134 and T140. Both analogues [T(E)-134-OH and T(E)-140-OH] have low cytotoxicity (CC_{50}s $> 100\,\mu M$) and very high SIs. Recently, we found that T140 is not completely stable and the C-terminal Arg can be easily hydrolyzed in the mouse and feline sera, and that T140-NH_2, which has a C-terminal carboxyl amide group, is stable in the serum.[19] Additionally, our previous study proved that an amino acid residue at the 3-position (Trp for T134, or Nal for T140) is important for anti-HIV activity (unpublished data). As such, we attempted to synthesize and evaluate several EADI-containing analogues, which possess Trp, Nal, Cys(Bzl) or Cys(Ad) at the 3 position with the C-terminal amide [Fig. 1 and Table 1, T(E)-134-NH_2, T(E)-140-NH_2, T(E)-141-NH_2 and T(E)-142-NH_2]. Their corresponding parent peptides, T134-NH_2, T140-NH_2, T141-NH_2 and T142-NH_2, which have a normal imide bond in the D-Lys^8-Pro^9 sequence, were also prepared for the comparative study (Table 1). T(E)-134-NH_2 and T(E)-140-NH_2 exhibited high anti-HIV activity, which is nearly equal to that of T(E)-134-OH and T(E)-140-OH, and higher than (or nearly equal to) that of T134-NH_2 and T140-NH_2. However, the cytotoxicity of T(E)-134-NH_2 and T(E)-140-NH_2 is relatively stronger than that of T(E)-134-OH and T(E)-140-OH. T141-NH_2, T142-NH_2, T(E)-141-NH_2 and T(E)-142-NH_2 showed modest anti-HIV activity. Next, inhibitory activity of novel representative compounds against [Ca^{2+}] mobilization induced by SDF-1α stimulation through CXCR4 (IC_{50}) was evaluated to confirm whether these compounds are targeted for CXCR4 (Table 1). All of the tested compounds inhibited [Ca^{2+}] mobilization ($IC_{50} < 170\,nM$), showing an apparent correlation between anti-HIV activity (EC_{50}) and inhibitory activity against [Ca^{2+}] mobilization (IC_{50}). As a result, the D-Lys^8-Pro^9 sequence at the ($i+1$) and ($i+2$) positions of the βII′-turn can be replaced by an EADI, D-Lys-ψ[(*E*)-CH=CH]-L-Ala, without significant reduction of anti-

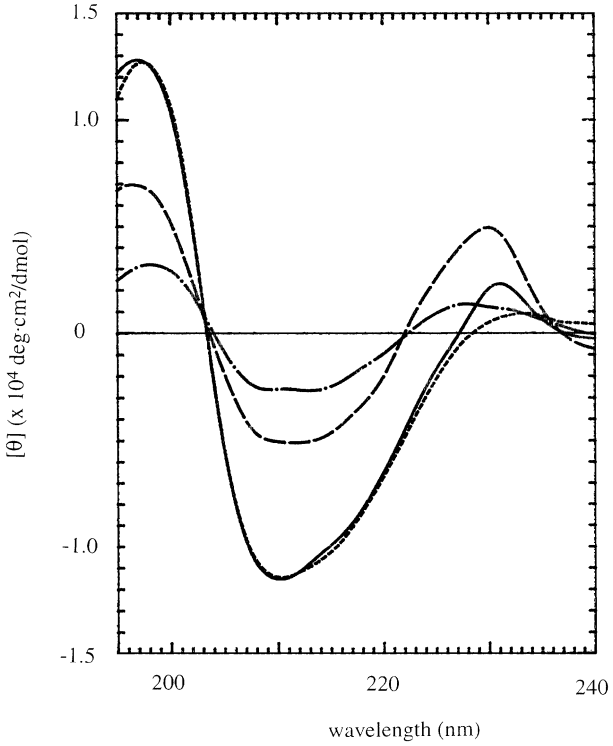


Figure 2. CD spectra of T140 (solid line), T134 (dotted line), T(E)-140-OH (dashed line) and T(E)-134-OH (center-dotted line).

HIV activity or CXCR4-inhibitory activity. T(E)-140-OH is the top analogue among all the tested EADI-containing surrogates in terms of both anti-HIV activity and CXCR4-inhibitory activity.

Second, the solution structures of the representative surrogates, T(E)-134-OH and T(E)-140-OH, were analyzed by CD spectroscopy to examine whether these pseudopeptides maintain the solution structures of the corresponding parent peptides, T134 and T140 (Fig. 2). T140 assumes an antiparallel β-sheet structure with a type II′ β-turn, which was determined by NMR and molecular dynamics calculations.[9] T134 is also thought to form a β-sheet structure similar to that of T140 according to CD spectra.[8] T(E)-134-OH and T(E)-140-OH showed CD patterns different from those of T134 and T140, respectively. Both a negative band near 210 nm and a positive band near 197 nm, which are characteristic bands of β-sheet structures, are markedly weaker in T(E)-134-OH and T(E)-140-OH than in T134 and T140, suggesting that the β-sheet structure in T(E)-134-OH and T(E)-140-OH is partially collapsed. Introduction of the EADI in the $(i+1)$ and $(i+2)$ positions of the βII′-turn region seems to impair the secondary structure composed of the β-sheet–βII′-turn structure.

Third, ^{1}H NMR analysis followed by molecular dynamics calculations was performed to disclose the detailed structure of T(E)-140-OH in solution. The backbones of 5 low-energy structures among 40 generated structures were superimposed, where the root mean square deviation value was 1.10 Å between Cys^4 and Cys^{13} (Fig. 3). The direction of the two protons of the (*E*)-alkene was almost similar in 40 generated structures. The direction of these two protons was nearly equal to that of the oxygen and the δ-carbon of the imide of D-Lys^8-Pro^9 at the $(i+1)$ and $(i+2)$ positions of the βII′-turn of T140. The three-dimensional structure of one of these structures is shown in Figure 4. The presence of β-sheet was seen from Cys^4 to Arg^6 and from Arg^{11} to Cys^{13}, whereas two strands were not parallel near Lys^7 and Tyr^{10}. In addition, judging from the distances between Lys^7 NH and Tyr^{10} CO and between Lys^7 CO and Tyr^{10} NH, hydrogen bonds could not be formed between these atoms. Furthermore, the superimposition was inferior, particularly in the D-Lys-ψ[(*E*)-CH=CH]-L-Ala region. Thus, the $Lys^7(i)$–D-$Lys^8(i+1)$-ψ[(*E*)-CH=CH]-L-$Ala^9(i+2)$-$Tyr^{10}(i+3)$ moiety did not assume a type II′ β-turn, which requires the formation of a hydrogen bond between the atoms of [Lys^7 CO, Tyr^{10} NH]. As a result, the complete antiparallel β-sheet structure connected by the type II′ β-turn, existing from Cys^4 to Cys^{13} of T140, was collapsed in T(E)-140-OH, although the simulated structure

Figure 3. The best-fit superposition of the backbone atoms of five low-energy structures. Green line: ribbon presentation of the backbones except for the (*E*)-alkene.

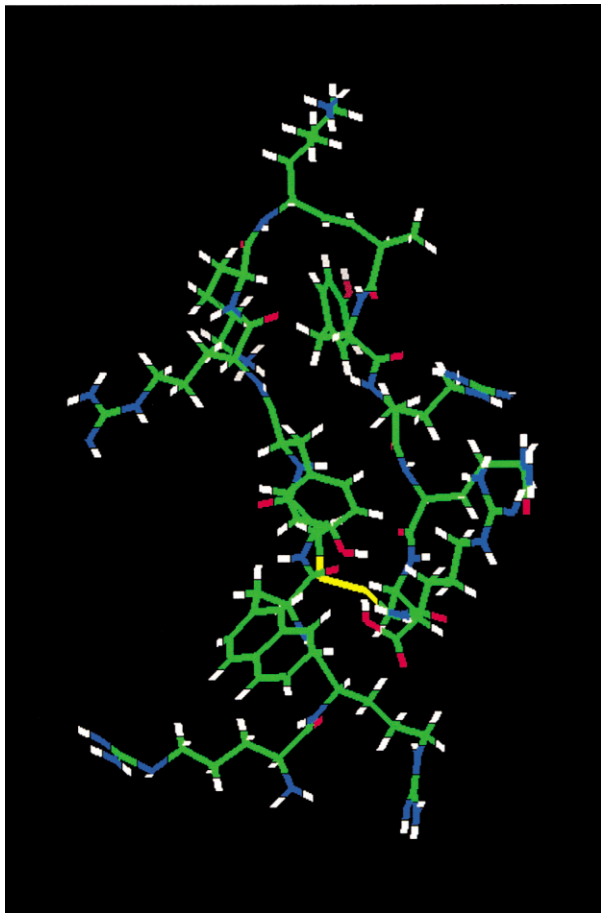


Figure 4. The three-dimensional structure of T(E)-140-OH refined by Insight II/Discover.

partly assumed the β-sheet structure. This result is compatible with that of CD spectroscopy. T(E)-140-OH frayed in its N- and C-terminal tails.

The difference in the solution structure from Arg^6 to Arg^{11} between T140 and T(E)-140-OH did not cause significant reduction in anti-HIV activity or CXCR4-inhibitory activity. According to our previous SAR study, Arg^2, Nal^3, Tyr^5 and Arg^{14} are indispensable residues, which form the intrinsic pharmacophore of anti-HIV activity and CXCR4-inhibitory activity.[18] Taken together, the third-dimensional structure from Arg^6 to Arg^{11} of T140 might not be significantly important for each activity. The region of Arg^2-Nal^3-Cys(S-)^4-Tyr^5 and Cys(S-)^{13}-Arg^{14}, which are linked by the disulfide bridge, is thought to be critical for the activity, although the backbones of Arg^2-Nal^3 and Arg^{14} were found to be flexible.

In conclusion, the pseudopeptide T(E)-140-OH, in which an EADI was inserted into the $(i+1)$–$(i+2)$ residue of the βII′ turn, exhibited strong anti-HIV activity and CXCR4-inhibitory activity comparable to those of the parent peptide T140, while its solution structure is different from that of T140, particularly in the turn region, which is not critical for each activity. In this case, partial nonpeptidylation was successful in terms of activity, although an EADI did not serve as a promoter of a βII′-turn structure. Our further study on nonpeptidylation of T140 will be followed to develop efficient CXCR4 inhibitors based on T140. In addition, we wish to investigate whether EADIs promote β- or βII-hairpin formation in other compounds in solution.

Acknowledgements

The authors wish to thank Drs. Hiromu Habashita, Yoshihiko Odagaki and Nobuyuki Hamanaka from Minase Research Institute, Ono Pharmaceutical Co., Ltd. for NMR analysis and technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan and the Japan Health Science Foundation. Computation times were provided by the Supercomputer Laboratory, Institute for Chemical Research, Kyoto University.

References and Notes

1. Feng, Y.; Broder, C. C.; Kennedy, P. E.; Berger, E. A. *Science* **1996**, *272*, 872.
2. (a) Deng, H.; Liu, R.; Ellmeier, W.; Choe, S.; Unutmaz, D.; Burkhart, M.; Marzio, P. D.; Marmon, S.; Sutton, R. E.; Hill, C. M.; Davis, C. B.; Peiper, S. C.; Schall, T. J.; Littman, D. R.; Landau, N. R. *Nature* **1996**, *381*, 661. (b) Dragic, T.; Litwin, V.; Allaway, G. P.; Martin, S. R.; Huang, Y.; Nagashima, K. A.; Cayanan, C.; Maddon, P. J.; Koup, R. A.; Moore, J. P.; Paxton, W. A. *Nature* **1996**, *381*, 667. (c) Alkhatib, G.; Combadiere, C.; Broder, C. C.; Feng, Y.; Kennedy, P. E.; Murphy, P. M.; Berger, E. A. *Science* **1996**, *272*, 1955. (d) Choe, H.; Farzan, M.; Sun, Y.; Sullivan, N.; Rollins, B.; Ponath, P. D.; Wu, L.; Mackay, C. R.; LaRosa, G.; Newman, W.; Gerard, N.; Gerard, C.; Sodroski, J. *Cell* **1996**, *85*, 1135. (e) Doranz, B. J.; Rucker, J.; Yi, Y.; Smyth, R. J.; Samson, M.; Peiper, S. C.; Parmentier, M.; Collman, R. G.; Doms, R. W. *Cell* **1996**, *85*, 1149.
3. (a) Murakami, T.; Nakajima, T.; Koyanagi, Y.; Tachibana, K.; Fujii, N.; Tamamura, H.; Yoshida, N.; Waki, M.; Matsumoto, A.; Yoshie, O.; Kishimoto, T.; Yamamoto, N.; Nagasawa, T. *J. Exp. Med.* **1997**, *186*, 1389. (b) Murakami, T.; Zhang, T.-Y.; Koyanagi, Y.; Tanaka, Y.; Kim, J.; Suzuki, Y.; Minoguchi, S.; Tamamura, H.; Waki, M.; Matsumoto, A.; Fujii, N.; Shida, H.; Hoxie, J.; Peiper, S. C.; Yamamoto, N. *J. Virol.* **1999**, *73*, 7489. (c) Xu, Y.; Tamamura, H.; Arakaki, R.; Nakashima, H.; Zhang, X.; Fujii, N.; Uchiyama, T.; Hattori, T. *AIDS Res. Hum. Retroviruses* **1999**, *15*, 419.
4. (a) Schols, D.; Struyf, S.; Van Damme, J.; Este, J. A.; Henson, G.; De Clercq, E. *J. Exp. Med.* **1997**, *186*, 1383. (b) Donzella, G. A.; Schols, D.; Lin, S. W.; Este, J. A.; Nagashima, K. A.; Maddon, P. J.; Allaway, G. P.; Sakmar, T. P.; Henson, G.; De Clercq, E.; Moore, J. P. *Nature Medicine* **1998**, *4*, 72. (c) Doranz, B. J.; Grovit-Ferbas, K.; Sharron, M. P.; Mao, S.-H.; Bidwell Goetz, M.; Daar, E. S.; Doms, R. W.; O'Brien, W. A. *J. Exp. Med.* **1997**, *186*, 1395. (d) Howard, O. M. Z.; Oppenheim, J. J.; Hollingshead, M. G.; Covey, J. M.; Bigelow, J.; McCormack, J. J.; Buckheit, R. W., Jr.; Clanton, D. J.; Turpin, J. A.; Rice, W. G. *J. Med. Chem.* **1998**, *41*, 2184. (e) Arenzana-Seisdedos, F.; Virelizier, J.-L.; Rousset, D.; Clark-Lewis, I.; Loetscher, P.; Moser, B.; Baggiolini, M. *Nature* **1996**, *383*, 400. (f) Simmons, G.; Clapham, P. R.; Picard, L.; Offord, R. E.; Rosenkilde, M. M.; Schwarts, T. W.; Buser, R.; Wells, T. N. C.; Proudfoot, A. E. I. *Science* **1997**, *276*, 276. (g) Baba, M.; Nishimura, O.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Iizawa, Y.; Shiraishi, M.; Aramaki, Y.; Okonogi, K.; Ogawa, Y.; Meguro, K.; Fujino, M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5698. (h) Maeda, K.; Yoshimura, K.; Shibayama, S.; Habashita, H.; Tada, H.; Sagawa, K.; Miyakawa, T.; Aoki, M.; Fukushima, D.; Mitsuya, H. *J. Biol. Chem.* **2001**, *276*, 35194.
5. Mitsuya, H.; Erickson, J. In *Textbook of AIDS Medicine*; Merigan, T. C., Bartlett, J. G., Bolognesi, D., Eds.; Williams & Wilkins: Baltimore, 1999; p 751.
6. Tamamura, H.; Kuroda, M.; Masuda, M.; Otaka, A.; Funakoshi, S.; Nakashima, H.; Yamamoto, N.; Waki, M.; Matsumoto, A.; Lancelin, J. M.; Kohda, D.; Tate, S.; Inagaki, F.; Fujii, N. *Biochim. Biophys. Acta* **1993**, *1163*, 209.
7. Tamamura, H.; Arakaki, R.; Funakoshi, H.; Imai, M.; Otaka, A.; Ibuka, T.; Nakashima, H.; Murakami, T.; Waki, M.; Matsumoto, A.; Yamamoto, N.; Fujii, N. *Bioorg. Med. Chem.* **1998**, *6*, 231.
8. Tamamura, H.; Xu, Y.; Hattori, T.; Zhang, X.; Arakaki, R.; Kanbara, K.; Omagari, A.; Otaka, A.; Ibuka, T.; Yamamoto, N.; Nakashima, H.; Fujii, N. *Biochem. Biophys. Res. Commun.* **1998**, *253*, 877.
9. (a) Tamamura, H.; Sugioka, M.; Odagaki, Y.; Omagari, A.; Kan, Y.; Oishi, S.; Nakashima, H.; Yamamoto, N.; Peiper, S. C.; Hamanaka, N.; Otaka, A.; Fujii, N. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 359. (b) Tamamura, H.; Sugioka, M.; Odagaki, Y.; Omagari, A.; Kan, Y.; Oishi, S.; Nakashima, H.; Yamamoto, N.; Peiper, S. C.; Hamanaka, N.; Otaka, A.; Fujii, N. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2409.
10. Wipf, P.; Henninger, T. C.; Geib, S. J. *J. Org. Chem.* **1998**, *63*, 6088.
11. Gardner, R. R.; Liang, G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280.
12. Schopfer, U.; Stahl, M.; Brandl, T.; Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1745.
13. (a) Ibuka, T.; Habashita, H.; Otaka, A.; Fujii, N.; Oguchi, Y.; Uyehara, T.; Yamamoto, Y. *J. Org. Chem.* **1991**, *56*, 4370. (b) Tamamura, H.; Yamashita, M.; Muramatsu, H.; Ohno, H.; Ibuka, T.; Otaka, A.; Fujii, N. *Chem. Commun.* **1997**, 2327.

14. Characterization data for the EADI **8**: colourless oil (found: C, 61.49; H, 7.86; N, 5.25. $C_{21}H_{41}ClN_2O_6$ requires: C, 61.76; H, 7.87; N, 5.34%); $[\alpha]_D^{26}$ −3.03 (*c* 0.330 in $CHCl_3$); Δε (CD spectroscopy) −2.83 (227 nm, isooctane); δ_H (300 MHz; $CDCl_3$) 1.20 (3H, d, $J=7.3$ Hz, Me), 1.43 (18H, s, Boc and Bu^t), 1.3–1.7 (6H, m, CH_2), 3.01 (1H, m, 2-H), 3.17 (2H, m, CH_2), 4.08 (1H, m, 5-H), 4.46 (1H, m, NH), 4.90 (1H, m, NH), 5.21 (2H, s, CH_2), 5.42 (1H, dd, $J=15.3$ and 6.3 Hz, CH=), 5.65 (1H, ddd, $J=15.5$, 7.6 and 1.0 Hz, CH=), 7.2–7.5 (4H, m, ArH); *m*/*z* (FAB-LRMS) 547 (MNa^+), 525 (MH^+), 425 (base peak), 369, 308, 184, 127, 125, 57. The EADI **9**: colourless crystals, mp 95–99 °C [from AcOEt–Et_2O (5:1)] (found: C, 66.76; H, 5.94; N, 4.66. $C_{33}H_{35}ClN_2O_6$ requires: C, 67.05; H, 5.97; N, 4.74%); $[\alpha]_D^{26}$ −0.005 (*c* 0.047 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.27 (3H, d, $J=7.3$ Hz, Me), 1.3–1.7 (6H, m, CH_2), 3.1–3.3 (3H, m, 2-H and CH_2), 4.1–4.3 (1H, m, 5-H), 4.42 (1H, m, NH), 4.8–4.9 (1H, m, NH), 5.20 (2H, s, CH_2), 5.4–5.8 (2H, m, CH=), 7.2–7.8 (12H, m, ArH).

15. Nakashima, H.; Masuda, M.; Murakami, T.; Koyanagi, Y.; Matsumoto, A.; Fujii, N.; Yamamoto, N. *Antimicrob. Agents Chemother*. **1992**, *36*, 1249.

16. (a) Nagasawa, T.; Kikutani, H.; Kishimoto, T. *Proc. Natl. Acad. Sci. U.S.A*. **1994**, *91*, 2305. (b) Bleul, C. C.; Farzan, M.; Choe, H.; Parolin, C.; Clark-Lewis, I.; Sodroski, J.; Springer, T. A. *Nature* **1996**, *382*, 829. (c) Oberlin, E.; Amara, A.; Bachelerie, F.; Bessia, C.; Virelizier, J.-L.; Arenzana-Seisdedos, F.; Schwartz, O.; Heard, J.-M.; Clark-Lewis, I.; Legler, D. F.; Loetscher, M.; Baggiolini, M.; Moser, B. *Nature* **1996**, *382*, 833. (d) Tashiro, K.; Tada, H.; Heilker, R.; Shirozu, M.; Nakano, T.; Honjo, T. *Science* **1993**, *261*, 600. (e) Grynkiewicz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem*. **1985**, *260*, 3440.

17. Miyamoto, K.; Nakagawa, T.; Kuroda, Y. *Biopolymers* **2001**, *59*, 380.

18. Tamamura, H.; Omagari, A.; Oishi, S.; Kanamoto, T.; Yamamoto, N.; Peiper, S. C.; Nakashima, H.; Otaka, A.; Fujii, N. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2633.

19. Tamamura, H.; Omagari, A.; Hiramatsu, K.; Gotoh, K.; Kanamoto, T.; Xu, Y.; Kodama, E.; Matsuoka, M.; Hattori, T.; Yamamoto, N.; Nakashima, H.; Otaka, A.; Fujii, N. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1897.