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Development of Specific CXCR4 Inhibitors Possessing High Selectivity Indexes as Well as Complete Stability in Serum Based on an Anti-HIV Peptide T140

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Abstract—We previously reported a truncated polyphemusin peptide analogue, T140, which efficiently inhibits infection of target cells by T-cell line-tropic strains of HIV-1 (X4-HIV-1) through its specific binding to a chemokine receptor, CXCR4. We have found that T140 is not stable in feline serum due to the cleavage of the C-terminal Arg,[14] indispensable for anti-HIV activity. On the other hand, a C-terminally amidated analogue of T140, TZ14004, has been found to be completely stable in incubation in the serum for 2 days. The C-terminal amide is thought to be needed for stability in serum. However, TZ14004 does not have fairly strong anti-HIV activity, but has relatively strong cytotoxicity, probably due to an increase by +1 charge from total +7 charges of T140. In our previous study, the number of total +6 charges seemed to be a suitable balance between activity and cytotoxicity. In this study, we have conducted a double-L-citrulline (Cit)-scanning study on TZ14004 based on the C-terminally amidated form in due consideration of the total net charges in the whole molecule to find novel effective CXCR4 inhibitors, TN14003 ([Cit[6]]-T140 with the C-terminal amide) and TC14012 ([Cit[6], D-Cit[8]]-T140 with the C-terminal amide), which possess high selectivity indexes (SIs) and complete stability in feline serum.

Introduction

Recently, the function of a subset of chemokine receptors as coreceptors for the entry of HIV-1 was clarified: A CXC-chemokine receptor, CXCR4, and a CC-chemokine receptor, CCR5, are 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. We[3] and others[4] previously developed several compounds targeting chemokine receptors. Furthermore, we found a specific CXCR4 inhibitor, T140,[5] which is a 14-residue peptide possessing a disulfide bridge and taking an antiparallel β-sheet structure with a type II′ β-turn involving D-Lys[8]-Pro[9] at the (i+1) and (i+2) site (Fig. 1).[6] T140 possesses the highest level of anti-HIV activity and antagonism of the entry by X4-HIV-1 among all antagonists of CXCR4 that have been reported to date. T140 is a typically amphiphilic peptide containing basic residues (5 Arg, 1 Lys and 1 D-Lys residues) and hydrophobic residues, exhibiting total +7 positive charges. The electrostatic and/or hydrophobic interaction of such peptides with membranes might be related to its cytotoxicity.[7,8] In the course of development of T140, we found that there is an apparent correlation between the number of the net positive charges and anti-HIV activity or cytotoxicity, and that reduction of total positive charges resulted in even less cytotoxicity whereas extreme reduction caused a significant decrease in anti-HIV activity. The strategy of reduction of total positive charges by substitution for basic residues (Arg and Lys) with nonbasic polar amino acids, such as L-citrulline (Cit), was useful for developing effective

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T140	H-Arg-Arg-Nal-Cys-Tyr-Arg-Lys-DLys-Pro-Tyr-Arg-Cit-Cys-Arg-OH
TZ14004	H-Arg-Arg-Nal-Cys-Tyr-Arg-Lys-DLys-Pro-Tyr-Arg-Cit-Cys-Arg-NH_2
TC14003	H-Arg-Arg-Nal-Cys-Tyr-Cit-Lys-DLys-Pro-Tyr-Arg-Cit-Cys-Arg-OH
TN14003	H-Arg-Arg-Nal-Cys-Tyr-Cit-Lys-DLys-Pro-Tyr-Arg-Cit-Cys-Arg-NH_2
TC14005	H-Arg-Arg-Nal-Cys-Tyr-Arg-Lys-DCit-Pro-Tyr-Arg-Cit-Cys-Arg-OH
TN14005	H-Arg-Arg-Nal-Cys-Tyr-Arg-Lys-DCit-Pro-Tyr-Arg-Cit-Cys-Arg-NH_2
TC14011	H-Arg-Arg-Nal-Cys-Tyr-Cit-Lys-DCit-Pro-Tyr-Arg-Cit-Cys-Arg-OH
TC14012	H-Arg-Arg-Nal-Cys-Tyr-Cit-Lys-DCit-Pro-Tyr-Arg-Cit-Cys-Arg-NH_2
TC14013	H-Arg-Arg-Nal-Cys-Tyr-Cit-Lys-DLys-Pro-Tyr-Cit-Cit-Cys-Arg-OH
TC14014	H-Arg-Arg-Nal-Cys-Tyr-Cit-Lys-DLys-Pro-Tyr-Cit-Cit-Cys-Arg-NH_2
TC14015	H-Cit-Arg-Nal-Cys-Tyr-Cit-Lys-DLys-Pro-Tyr-Arg-Cit-Cys-Arg-OH
TC14016	H-Cit-Arg-Nal-Cys-Tyr-Cit-Lys-DLys-Pro-Tyr-Arg-Cit-Cys-Arg-NH_2
TC14017	H-Cit-Arg-Nal-Cys-Tyr-Arg-Lys-DCit-Pro-Tyr-Arg-Cit-Cys-Arg-OH
TC14018	H-Cit-Arg-Nal-Cys-Tyr-Arg-Lys-DCit-Pro-Tyr-Arg-Cit-Cys-Arg-NH_2
TC14019	H-Arg-Arg-Nal-Cys-Tyr-Arg-Lys-DCit-Pro-Tyr-Cit-Cit-Cys-Arg-OH
TC14020	H-Arg-Arg-Nal-Cys-Tyr-Arg-Lys-DCit-Pro-Tyr-Cit-Cit-Cys-Arg-NH_2
TC14021	H-Cit-Arg-Nal-Cys-Tyr-Arg-Lys-DLys-Pro-Tyr-Cit-Cit-Cys-Arg-OH
TC14022	H-Cit-Arg-Nal-Cys-Tyr-Arg-Lys-DLys-Pro-Tyr-Cit-Cit-Cys-Arg-NH_2

Figure 1. Amino acid sequences of T140 and its analogues. A disulfide linkage is shown by a solid line. The substituted amino acids are underlined. Nal = L-3-(2-naphthyl)alanine, Cit = L-citrulline.

analogues possessing high activity and low cytotoxicity. Cit is a neutral amino acid analogue possessing an Arg-like isosteric structure without charges. Therefore, through Cit-scanning of the Arg and Lys residues in T140, we previously developed more effective anti-HIV peptides with less cytotoxicity, TC14003 ([Cit^6]-T140) and TC14005 ([D-Cit^8]-T140) (both total net charges: +6).[9] In this study, we have initially investigated the behaviour of T140 analogues in feline serum to find their instability. Subsequently, modification of T140 analogues and substitution for Arg/Lys with Cit have allowed us to discover practically useful anti-HIV peptides with high selectivity indexes (SIs) [50% cytotoxic concentration (CC_{50})/50% effective concentration (EC_{50})] and complete stability in serum.

Investigation on Behaviour of T140 Analogues in Feline Serum

Test compounds (100 nmol) were dissolved in feline serum (100 μL)/H_2O (100 μL), and incubated at 37 °C. At intervals (0, 1, 2, 5, 24 h), an aliquot (8 μL) was sampled and examined by analytical HPLC with an isocratic mode of 16% (v/v) CH_3CN/H_2O containing 0.1% (v/v) trifluoroacetic acid. HPLC peaks of the starting compounds and the generated products were identified by the ion spray mass spectrometry (IS-MS) analysis and the HPLC analysis for co-injection with the corresponding authentic samples. The amounts of the starting compounds and the generated products were quantified from the peak areas.

Synthesis of T140 Analogues

The protected peptidyl resins were constructed by 9-fluorenylmethyloxycarbonyl (Fmoc)-based solid-phase methodology on 4-(2′,4′-dimethoxyphenylaminomethyl)-phenoxy resin[10] (for C-terminally amidated type) or *p*-benzyloxy benzyl alcohol resin[11] (for C-terminally carboxy-free type), followed by treatment with 1 M trimethylsilyl bromide–thioanisole/trifluoroacetic acid in the presence of *m*-cresol and 1,2-ethanedithiol at 4 °C for 2 h. After air-oxidization in the NH_4OAc aqueous solution at pH 7.8, the crude products were purified by preparative HPLC and gel-filtration to afford a white powder following lyophilization.[9] The integrity of peptides was determined by IS-MS, and the purity was confirmed by analytical HPLC (data not shown).

Virus

The HIV-1_{IIIB} strain was obtained from the culture supernatant of X4-HIV-1 persistently infected MOLT-4/HIV-1_{IIIB} cells. Luciferase reporter viruses were prepared in 293T cells[12] by co-transfection of pNL4-3-Luc-E^-R^- vector and Env-expressing plasmids using lipofectamine as described by the manufacturer (GibcoBRL, Grand Island, NY, USA).[13] Pseudotyped viruses in supernatants of transfected 293T cells were quantified by p24 ELISA (ABOTT, Abott Park, IL, USA) to normalize virus stocks for infection.

Evaluation of Anti-HIV Activity and Cytotoxicity

In this study, anti-HIV activity of the analogues was determined by the 3′-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[14] and the inhibition assay on an HIV infection model mediated by CXCR4.[2a,15] In the MTT assay, anti-HIV activity was based on the protection against HIV-induced cytopathogenicity in MT-4 cells.[14] Cytotoxicity of the compounds was based on the reduction of the viability of mock-infected cells using the MTT method (test I). Cytotoxicity was also determined by the trypan blue exclusion staining method in human peripheral blood mononuclear cells (PBMC) (test II).[16] 3′-Azido-2′, 3′-dideoxythymidine (AZT)[17] and 2′,3′-dideoxycytidine (ddC)[18] were tested as controls. In the inhibition assay

on an HIV infection model mediated by CXCR4, pseudotyped viruses were used for the infection of U87.CD4.CXCR4 cells.[2a] All data are the mean values of at least three experiments.

Results and Discussion

First, to investigate the stability of T140 under physiological conditions, this peptide was incubated in feline serum at 37 °C, and its behaviour was monitored by the analytical HPLC profile. As shown in Figure 2, the peak area of T140 was decreased and another peak appeared with the passage of time. The newly generated product was identified as a C-terminally deleted derivative of T140, des-Arg^{14}-T140 (lacking C-terminal Arg^{14}), by the IS-MS analysis and the HPLC analysis for co-injection with the authentic sample. T140 was proven to be unstable due to the cleavage of Arg^{14} from the C-terminal end. Since this type of degradation reaction involving one substrate and one enzyme is theoretically performed by first-order kinetics, the half-life of T140 in serum is estimated by linearization of plots of a logarithm of the concentration of T140 versus time: $t_{1/2}$ = 9.6 h. Further degradation was not detected after the cleavage of Arg^{14} in serum, suggesting that the generated 13-residue peptide, des-Arg^{14}-T140, is stable probably due to the disulfide bridge between Cys^4 and Cys^{13}. According to our previous SAR study, Arg^{14} is an indispensable residue of T140 for strong anti-HIV activity.[9] In fact, the anti-HIV activity of des-Arg^{14}-T140 is very weak (EC_{50} = 2.5 μM, unpublished data). Therefore, we investigated the stability of a C-terminal amidated derivative of T140 (TZ14004). TZ14004 was proven to be completely stable even in 2 days' incubation with feline serum (Fig. 2). Therefore, T140 analogues are thought to require the derivation by C-terminal

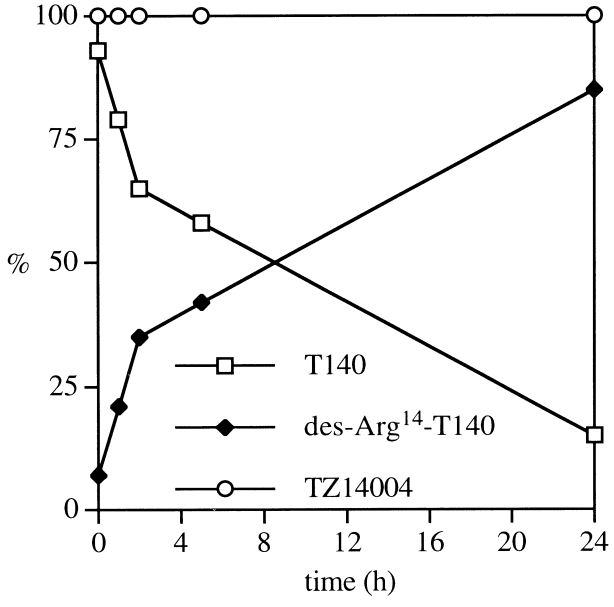

Figure 2. Behaviours of T140 and TZ14004 in feline serum. HPLC peak areas (%) of the starting material (T140) and the degraded product (des-Arg^{14}-T140) were shown with the passage of time (h). The peak area of TZ14004 was not changed for 2 days.

amidation, whereas TZ14004 does not have fairly strong anti-HIV activity but relatively strong cytotoxicity [EC_{50} = 72 nM, CC_{50} = 10 μM (test I) in Table 1]. This is probably due to an increase in total positive charges (+7 to +8). The cytotoxicity of T140 was previously evaluated as CC_{50} > 40 μM,[5] although the estimation of T140 at high concentrations was omitted in this study (test I). According to our previous study, total +6 charges are favorable for the expression of high activity and low cytotoxicity, as TC14003 and TC14005 (Fig. 1), which are the most effective compounds among all the T140 analogues found hitherto, have +6 charges.[9] Modification by the C-terminal amidation of TC14003 and TC14005 also causes an increase by +1 charge (+6 to +7). Thus, we conducted double-Cit-substitution scanning for 2 Arg or Lys residues in T140 analogues based on total +6 charges as well as the C-terminally amidated form to attempt to find more effective compounds, which are completely stable in serum and possess high anti-HIV activity and low cytotoxicity.

We initially prepared C-terminally amidated analogues of TC14003 and TC14005, TN14003 and TN14005 (Fig. 1), respectively. In our previous Cit-scanning study, Arg^6 or D-Lys^8 in T140 was substituted with Cit or D-Cit, respectively, with an apparent increase in SI, and Arg^1 or Arg^{11} was also able to be replaced by Cit with the maintenance of high SI.[9] Therefore, we synthesized several analogues, where two basic residues among Arg^1, Arg^6, D-Lys^8 and Arg^{11} were substituted by 2 Cit (D-Cit), in both types of C-terminally carboxy-free and amidated forms for a comparative study. TN14003 showed high anti-HIV activity (EC_{50} = 0.6 nM), which is superior to that of T140 (EC_{50} = 3.3 nM) or TC14003 (EC_{50} = 2.8 nM) whereas TN14003 showed low cytotoxicity [CC_{50} (test I) > 100 μM, CC_{50} (test II) = 410 μM], which is remarkably weaker than that of T140 [CC_{50} (test II) = 59 μM], but slightly stronger than that of TC14003 [CC_{50} (test II) = 510 μM]. Thus, TN14003 has very high SI [SI (test II) = 680,000]. TN14005 also showed high activity (EC_{50} = 4.6 nM), which is nearly equal to that of T140 or TC14005 (EC_{50} = 4.0 nM), but relatively lower than that of TN14003. On the other hand, TN14005 exhibited modest cytotoxicity [CC_{50} (test I) > 100 μM, CC_{50} (test II) = 98 μM], which is weaker than that of T140, but nearly equal to that of TC14005 [CC_{50} (test II) = 110 μM]. In these analogues, the C-terminal amidation did not cause any remarkable changes in activity or cytotoxicity although the number of net positive charges was changed (+7 to +6). TC14011 ([Cit^6, D-Cit^8]-T140, 2 Cit-substitution for Arg^6 and D-Lys^8 in T140) exhibited very high anti-HIV activity (EC_{50} = 0.5 nM), low cytotoxicity [CC_{50} (test I) > 100 μM, CC_{50} (test II) > 800 μM] and very high SI [SI (test II) > 1,600,000]. This is thought to be reasonable since Arg^6 or D-Lys^8 was substituted by Cit or D-Cit with an apparent increase in SI, as seen in TC14003 or TC14005, respectively. Its amidated analogue, TC14012 ([Cit^6, D-Cit^8]-T140 with the C-terminal amide), also exhibited excellent activity profile [EC_{50} = 0.4 nM, CC_{50} (test I) > 100 μM, CC_{50} (test II) > 800 μM, SI (test II) > 2,000,000]. TC14013

Table 1. Anti-HIV activity and cytotoxicity of T140 and its analogues

Compound	Charges	SI		CC_{50} (μM)		EC_{50} (nM)	IC_{50} (nM)
		CC_{50} (test I)/$EC_{50}\times10^3$	CC_{50} (test II)/$EC_{50}\times10^3$	(test I)	(test II)		
T140	7	≫0.3	18	≫1	59	3.3	10.2±1.17
TZ14004	8	0.14	N.T.[a]	10	N.T.	72	N.T.
TC14003	6	>29	180	>80	510	2.8	8.01±0.94
TN14003	7	>170	680	>100	410	0.6	12.4±0.26
TC14005	6	>20	28	>80	110	4.0	22.8±1.02
TN14005	7	>22	21	>100	98	4.6	23.5±1.22
TC14011	5	>200	>1600	>100	>800	0.5	50.6±3.27
TC14012	6	>250	>2000	>100	>800	0.4	19.3±0.97
TC14013	5	>11	>90	>100	>800	8.9	N.T.
TC14014	6	>15	>59	>100	>400	6.8	N.T.
TC14015	5	>2.7	>22	>100	>800	37	N.T.
TC14016	6	>15	53	>100	350	6.6	N.T.
TC14017	5	>2.8	>22	>100	>800	36	N.T.
TC14018	6	>83	420	>100	500	1.2	N.T.
TC14019	5	>13	>100	>100	>800	7.8	N.T.
TC14020	6	>37	250	>100	680	2.7	N.T.
TC14021	5	>1.5	>12	>100	>800	68	N.T.
TC14022	6	>2.5	12	>100	470	40	N.T.
AZT		1.5	N.T.	12	N.T.	7.9	N.T.
ddC		0.16	N.T.	14	N.T.	88	N.T.

Charges: the number of total positive charges of each peptide. EC_{50} values are the concentrations for 50% protection of HIV-induced cytopathogenicity in MT-4 cells on the MTT assay. CC_{50} values (test I) are based on the reduction of the viability of mock-infected MT-4 cells on the MTT assay. CC_{50} values (test II) are determined by the trypan blue exclusion staining method in PBMC. SI is shown as CC_{50}/EC_{50}. IC_{50} values are determined by the inhibition assay on an HIV (the 89.6 strain) infection model mediated by CXCR4.
[a]N.T., not tested.

([Cit^{6,11}]-T140, 2 Cit-substitution for Arg^{6,11} in T140), and its amidated analogue, TC14014 ([Cit^{6,11}]-T140 with the C-terminal amide), exhibited modest anti-HIV activity (EC_{50}s = 8.9 and 6.8 nM, respectively) and low cytotoxicity (CC_{50} (test I) > 100 μM for both, CC_{50} (test II) > 800 μM for TC14013, > 400 μM for TC14014). This is probably due to the Cit-substitution for Arg^{11}, which caused a slight decrease in anti-HIV activity in the case of the Cit-substitution for Arg^{11} in T140 (TC14006, EC_{50} = 15 nM).[9] The activity of TC14015 ([Cit^{1,6}]-T140, 2 Cit-substitution for Arg^{1,6} in T140, EC_{50} = 37 nM) is 10-fold weaker than that of T140 whereas its amidated analogue, TC14016 ([Cit^{1,6}]-T140 with the C-terminal amide), exhibited relatively high anti-HIV activity (EC_{50} = 6.6 nM). A similar phenomenon was seen in the activity of TC14017 ([Cit^{1}, D-Cit^{8}]-T140, 2 Cit-substitution for Arg^{1}, D-Lys^{8} in T140) and its amidated analogue TC14018 ([Cit^{1}, D-Cit^{8}]-T140 with the C-terminal amide) (TC14017, EC_{50} = 36 nM; TC14018, EC_{50} = 1.2 nM). It is suggested that at least +3 charges are required in the N- and C-terminal regions (positions 1–3 and 14) for high anti-HIV activity: TC14016 and TC14018 have +3 charges based on 1 N-α amino group and 2 γ-guanidine groups of Arg^{2,14} whereas TC14015 and TC14017 have +2 charges based on the 1 N-α amino group, 2 γ-guanidine groups of Arg^{2,14} and the C-terminal carboxy group. According to our previous study, Arg^{2}, Nal^{3}, Tyr^{5} and Arg^{14} are the critical residues, which are spatially close to each other and located in the region near the N- or C-terminus.[6] It is suggested that some amount of charges is required in this pharmacophore region. In any case, the anti-HIV activity of these four analogues is lower than that of TC14011 or TC14012, partially due to the Cit-substitution for Arg^{1}, which caused a slight decrease in anti-HIV activity in the case of the Cit-substitution for Arg^{1} in T140 (TC14001, EC_{50} = 15 nM).[9] TC14019 ([D-Cit^{8}, Cit^{11}]-T140, 2 Cit-substitution for D-Lys^{8} and Arg^{11} in T140) did not exhibit stronger anti-HIV activity than its amidated analogue, TC14020 ([D-Cit^{8}, Cit^{11}]-T140 with the C-terminal amide) (TC14019, EC_{50} = 7.8 nM; TC14020, EC_{50} = 2.7 nM). Either activity is lower than that of TC14011 or TC14012, due to the Cit-substitution for Arg^{11} involving a similar phenomenon as seen in the activity of TC14013 and TC14014. TC14021 ([Cit^{1,11}]-T140, 2 Cit-substitution for Arg^{1,11} in T140) and its amidated analogue, TC14022 ([Cit^{1,11}]-T140 with the C-terminal amide), exhibited low anti-HIV activity (EC_{50}s = 68 and 40 nM, respectively), due to the double-Cit-substitution for Arg^{1,11}. In all the 2 Cit-substituted analogues, C-terminally amidated compounds have stronger anti-HIV activity than the corresponding C-terminally carboxy-free compounds, suggesting that +6 is more suitable than +5 as net charges for high activity. However, in terms of cytotoxicity, the C-terminally amidated compounds (TC14016, TC14018, TC14020 and TC14022) are more cytotoxic than the corresponding C-terminally carboxy-free compounds (TC14015, TC14017, TC14019 and TC14021), probably due to an increase in total positive charges (+5 to +6), whereas TC14012 and TC14014 did not show significant cytotoxicity up to 800 and 400 μM, respectively. We confirmed that the C-terminally amidated analogues such as TN14003 and TC14012 are completely stable even in 24 h incubation with feline or mouse serum (data not shown).

Next, we wish to confirm whether novel compounds with high SIs specifically inhibit the X4-HIV-1 entry mediated by CXCR4. The effect of these peptides on the entry of X4-, R5- and dual-tropic HIV-1 into cells was examined as follows: HIV-1 entry events were

quantitatively evaluated using pseudotyped viruses with different envelopes by a single round replication.[2a] Envelope genes used were derived from HXB2 (X4), SF162 (R5) and 89.6 (dual-tropic) viruses.[19] U87 cells expressing CD4 and CXCR4 or CCR5 were employed as the model of target cells. T140, TC14003, TN14003, TC14005, TN14005, TC14011 and TC14012 were selected as representatives and conducted in this assay. All these peptides (1 μM) inhibited by more than 95% the infection of the CXCR4-expressing cells by the HXB2 (X4) or 89.6 (dual-tropic) strain whereas these peptides (1 μM) did not inhibit at all the infection of the CCR5-expressing cells by the SF162 (R5) or 89.6 (dual-tropic) strain (data not shown). This result suggests that these peptides specifically inhibit the X4-(or dual-tropic) HIV-1 infection mediated by CXCR4. IC_{50} values of these peptides on the entry of the 89.6 strain into the CXCR4-expressing cells are 8–24 nM, except for TC14011 ($IC_{50} = 50.6$ nM). We cannot explain the reason for the relatively low inhibitory activity of TC14011. The IC_{50} values of the other peptides on the HIV-1 entry are mostly equivalent to their EC_{50} values on the MTT assay, although all the IC_{50} values are relatively larger than the corresponding EC_{50} values, due to the difference in the assay systems.

Finally, we wish to examine whether novel compounds with high SIs maintain the solution structures of T140, TC14003 and TC14005. T140 takes an antiparallel β-sheet structure with a type II′ β-turn, which was determined by NMR and molecular dynamic calculations.[6]

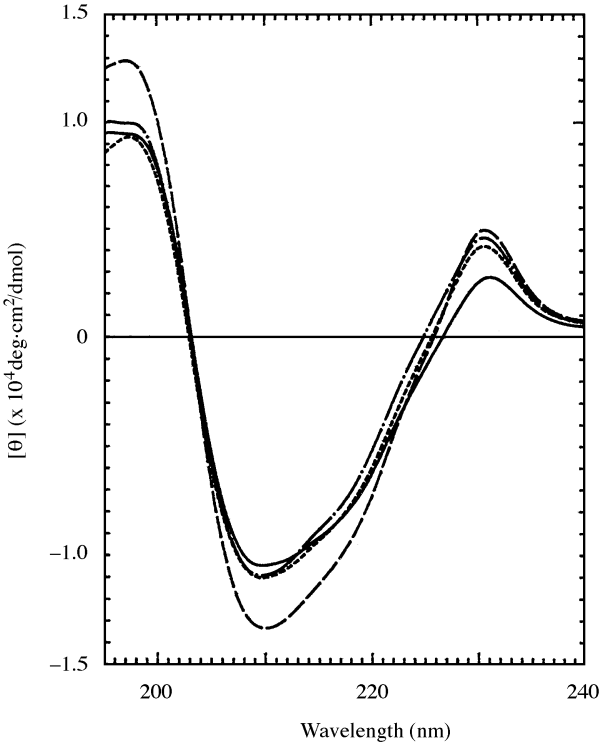

Figure 3. CD spectra of TN14003, TN14005, TC14011 and TC14012. Dashed line: TN14003, center-dotted line: TN14005, solid line: TC14011, dotted line: TC14012. These peptides were dissolved in H_2O at concentration of 10 μM. CD spectra were recorded on a JASCO J-720 spectropolarimeter (Tokyo, Japan) using 1 cm cells at 1 nm intervals, with five scans averaged for each.

CD spectroscopic analysis revealed that TC14003 and TC14005 take β-sheet structures similar to that of T140.[9] In this study, the conformation of the representative analogues (TN14003, TN14005, TC14011 and TC14012) was analyzed by CD. All the analogues were found to form β-sheet structures as T140 (Fig. 3). These analogues exhibited a strong negative band near 210 nm and a strong positive band near 197 nm, relevant to β-sheet structures.

In conclusion, we have found that T140 is not stable in serum due to the cleavage of Arg^{14}, and that the C-terminal amidation of T140 is capable of resisting from the degradation. The C-terminal amidation and the double-Cit-scanning of T140 have led to development of the novel effective CXCR4 inhibitors, TN14003 ([Cit^6]-T140 with the C-terminal amide) and TC14012 ([Cit^6, D-Cit^8]-T140 with the C-terminal amide), which possess high SIs (680,000 and >2,000,000, respectively) and complete stability in serum, without significant change in the secondary structure. The Cit-substitution for a reduction of total cationic charges in the molecule is thought to be useful for developing effective anti-HIV peptides with low cytotoxicity (high SIs). These results will give rise to the rational design and synthesis of a new type of anti-HIV agents against CXCR4. Since involvement of CXCR4 in the pancreatic cancer progression and breast cancer metastasis has been currently reported, small molecule antagonists against CXCR4, such as TN14003 and TC14012, might be attractive lead compounds even for cancer chemotherapy.[20]

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