Synthesis and Biological Evaluation of Peptide Mimics Derived from First Extracellular Loop of CCR5 toward HIV-1

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Peptide mimics derived from the first extracellular loop of CCR5 bearing non-peptide spacers in place of Ala-Ala-Ala sequence in the peptide moiety were synthesized, and the effects of these compounds on the inhibition against HIV-1 were examined. Compound 2b having *m*-aminophenoxyacetic acid derivative as a non-peptide spacer significantly inhibited HIV-1.

Key words HIV-1 inhibitor; first extracellular loop; CCR5 peptide mimic; chemokine receptor; non-peptide spacer

The chemokine receptor CCR5, a member of the seven-transmembrane G-protein coupled family of receptors,[1] has been identified as a primary co-receptor with CD4 by which macrophage tropic human immunodeficiency virus type-1 (HIV-1) strains infect their host cells.[2] Since the discovery of CCR5 as a co-receptor with CD4 for HIV-1 cell entry, there has been interest in discovering small molecule CCR5 antagonists[3—6] as well as CXCR4 antagonists[7] and HIV-1 protease inhibitors[8] as potential agents for the treatment of HIV-1 infection.

The entry of HIV-1 to cells involves the binding of the trimeric viral envelope glycoprotein gp120/gp41 to cell surface receptor CD4 and chemokine co-receptor CXCR4 or CCR5, which triggers conformational changes in the envelope proteins. Gp120 then dissociates from gp41, allowing for the fusion peptides to be inserted into the target membrane and the pre-hairpin configuration of the ectodomain to form.[9—11] Regulation of CCR5 number on cells is important in determining the infection rate by HIV-1. Therefore, CCR5 is a highly valued target for the treatment of HIV-1 infection.

It was demonstrated that the Gly-Gly-Gly sequence of the peptide moiety in lipopeptides could be replaced with a non-peptide spacer.[12] We designed compounds with non-peptide spacers such as a benzene ring in place of the Ala-Ala-Ala sequence of the peptide moiety of CCR5. As part of a program aimed at the development of new HIV-1 inhibitors,[13—18] we would like to report the design and synthesis of CCR5 peptide mimics derived from the first extracellular loop of CCR5 bearing non-peptide spacers in place of Ala-Ala-Ala sequence in the peptide moiety for prevention of HIV-1 infection based on a strategy of binding to gp120.

Results and Discussion

In the amino acid sequence of the *N*-terminal domain of CCR5, we chose the region of Tyr^{89}-Gly^{97} including alanine tripeptide. The peptide of natural type peptide **1** and its mimicking peptides **2a**—**c** consisting of unnatural-type amino acids derived from aminophenol as the spacer unit were prepared. First, the nona-peptide **1** was synthesized manually by a stepwise liquid-phase procedure. The synthesis of **2a**—**c** is shown in Chart 2. Aminophenoxyacetates **3a**—**c** as spacers were prepared from the corresponding nitrophenols and *tert*-butyl bromoacetate.[19,20] Coupling of compounds **3a**—**c** with 9-fluorenylmethoxycarbonyl (Fmoc)-Tyr(Bn) in the presence of 1-benzotriazoyloxy-tris-dimethylaminophosphonium hexafluorophosphate (BOP) and 1-hydroxy-1*H*-benzotriazole (HOBt) in *N*,*N*-dimethylformamide (DMF) gave compounds **4a**—**c** in 72%, 73% and 86% yield, respectively. The deprotection of *tert*-butyl group of **4a**—**c** with trifluoroacetic acid (TFA) afforded compounds **5a**, **5b**, and **5c** in 71%, quant. and 93% yield, respectively. Compounds **5a**—**c** were coupled with penta-peptide **6** in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl) and HOBt in DMF to give compounds **7a**—**c** in 78%, 81% and 95% yield, respectively. Finally, hydrogenolysis of compounds **7a**—**c** catalyzed by 20% $Pd(OH)_2$ in EtOH–AcOH (6 : 1) at 45 °C, followed by treatment with TFA–CH_2Cl_2–anisole–ethanedithiol (43 : 50 : 2 : 5), then 20% piper-

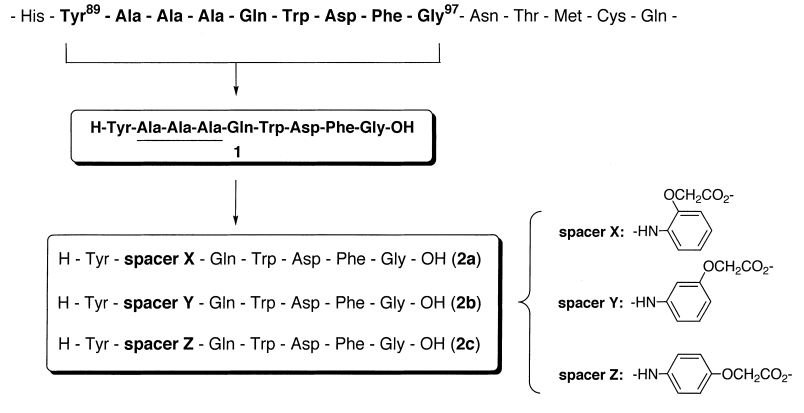

Chart 1. Amino Acid Sequence of First Extracellular Loop of CCR5

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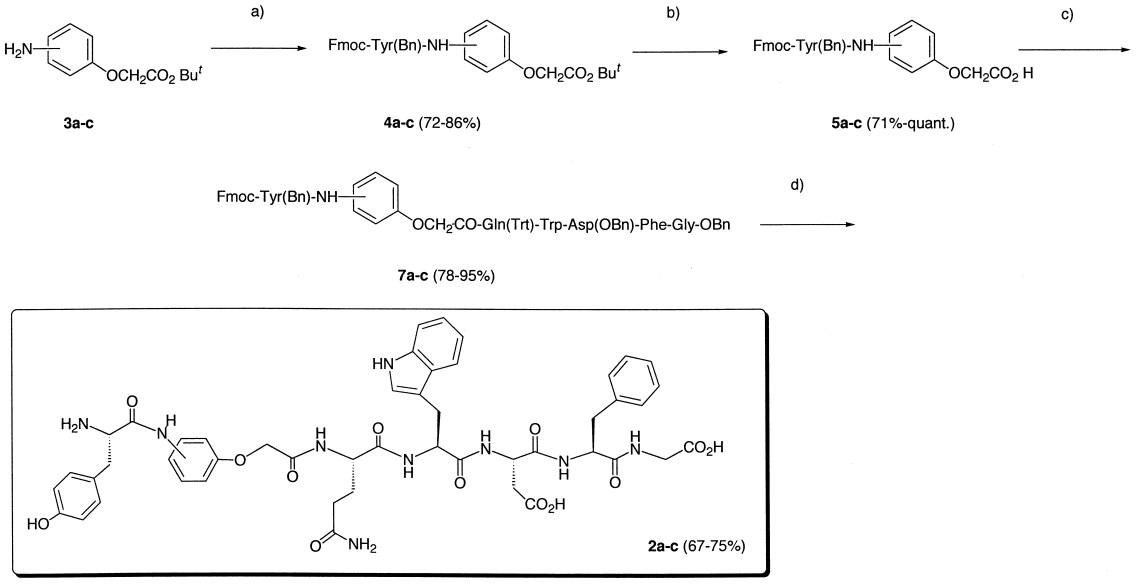

Reagents a) Fmoc-Tyr(Bn), BOP, HOBt, DMF; b) TFA, CH_2Cl_2; c) H-Gln(Trt)-Trp-Asp(OBn)-Phe-Gly-OBn (**6**), WSC HCl, HOBt, DMF; d) 1) 20% $Pd(OH)_2/C$, H_2, EtOH-AcOH (6:1), 2) TFA-CH_2Cl_2-anisole-ethanedithiol (43:50:2:5), 3) 20% piperidine, DMF

Chart 2. Synthesis of Peptide Mimics Derived from First Extracellular Loop of CCR5 toward HIV-1

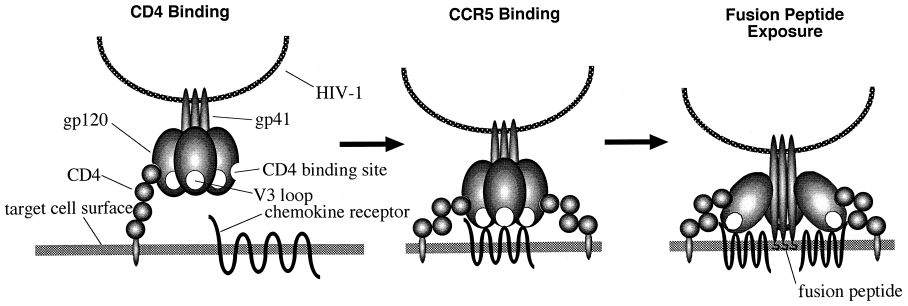

Fig. 1. Mechanism of HIV-1 Fusion with Target Cell Membrane

idine-DMF afforded target compounds **2a**—**c** in 75%, 67% and 67% yields, respectively.

Biological Activity

The anti-HIV-1 activities of **1** and **2a**—**c** are shown in Table 1. The anti-HIV-1 activity was tested by the syncytium-formation assay method using MT-4 cells according to our previously reported method.[8] Among the synthesized compounds, interestingly, **2b** as *m*-aminophenoxyacetic acid type showed the most potent inhibitory activity against HIV-1 and had cytotoxicity at 1000 μg/ml. This finding is in distinct contrast to the result that peptide **1** derived from the native sequence of CCR5 had little antiviral activity against HIV-1. However, **2a** had a moderate activity and **2c** was practically inactive against HIV-1. The reason compound **2b** showed higher inhibitory activity is unclear in this study, but there is the possibility that the configuration of aminophenoxyacetic acid derivatives as a non-peptide spacer affects the expression of antiviral activity against HIV-1.

In conclusion, it was demonstrated that the Ala-Ala-Ala sequence of the peptide moiety could be replaced with a non-peptide spacer. Synthesized peptide analogues are attractive candidates as new lead compounds for the development of chemokine receptor-directed anti-HIV-1 drugs.

Table 1. Anti-HIV Activities of Peptide Mimics (**2a**—**c**) Derived from First Extracellular Loop of CCR5[a)]

Compd.	Drug concentration (μg/ml)[b)]			Cytotoxicity (1000 μg/ml)
	1000	100	10	
1	+/−	−	−	−
2a	+	+/−	−	+/−
2b	n.d.[c)]	++	−	++
2c	+/−	−	−	−

a) C8166/GUN1WT (dual cell-tropic HIV strain) syncytium assay; *b*) inhibitory activity: −, 0%; +, <10%; ++, 10—50%; *c*) not determined.

Experimental

All melting points are uncorrected. Optical rotations were measured with a JASCO P-1030 polarimeter. IR spectra were recorded on a JASCO IR-810 spectrometer. ^{1}H-NMR spectra were recorded with a JEOL JNM-EX 270 (270 MHz) spectrometer. ^{1}H chemical shifts are given in ppm relative to Me_4Si (δ=0) in $CDCl_3$ or CD_3OD as internal standards at ambient temperature. Fast atom bombardment (FAB) mass spectra were obtained with a JEOL JMS SX-102 mass spectrometer in the positive ion mode using NBA or glycerol-thioglycerol matrix. Column chromatography was performed on silica gel 60 (70—230 mesh, Merck) and Sephadex LH-20 (Pharmacia). Precoated thin-layer chromatography (TLC) plates (silica gel 60-F_{254} (Merck)) were used to monitor the reaction and ascertain the purity of the reaction products. The spots were detected by spraying the plates with 5%

aqueous sulfuric acid and 10% 12-molybdo(VI) phosphoric acid *n*-hydrate in EtOH solution, and then heating.

L-Tyrosinyl-L-alanyl-L-alanyl-L-alanyl-L-glutaminyl-L-tryptophanyl-L-aspartyl-L-phenylalanyl-glycine (1) A solution of Fmoc-Tyr(Bn)-Ala-Ala-Ala-Gln(Trt)-Trp-Asp(Bn)-Phe-Gly-OBn (0.025 g, 0.014 mmol) in EtOH–AcOH (6 : 1) (2.0 ml) was hydrogenated over 20% $Pd(OH)_2$–C (0.030 g) for 20 h at 45 °C, then filtered and concentrated. The residue was treated with TFA–CH_2Cl_2–anisole–ethanedithiol (43 : 50 : 2 : 5) (1.5 ml) at room temperature for 20 h to give nona-peptide derivative which was dissolved in 20% piperidine-DMF (1.0 ml) for 18 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH–H_2O 60 : 60 : 10) and Sephadex LH-20 (CH_2Cl_2–MeOH–H_2O 12 : 8 : 1) to give amorphous **1** (0.014 g, 97%). Positive FAB-MS *m/z*: 1028 MH^+.

***tert*-Butyl [2-(N^{α}-9-Fluorenylmethoxycarbonyl-*O*-benzyl-L-tyrosinamido)phenoxy]acetate (4a)** BOP (2.44 g, 4.7 mmol) was added to a solution of **3a** (0.88 g, 3.9 mmol), Fmoc-L-Tyr(Bn) (1.93 g, 3.9 mmol) and HOBt (0.63 g, 4.7 mmol) in DMF (15 ml) at 0 °C and the mixture was stirred for 1 h at 0 °C and 15 h at room temperature under an Ar atmosphere. After removal of the solvent, the residue was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$, 10% aqueous citric acid and saturated aqueous NaCl, dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2–acetone 10 : 1) to give **4a** (0.92 g, 72%) as prisms, mp 65—67 °C, $[\alpha]_D$ −8.6° (c=1.2, $CHCl_3$). IR (KBr): 3318, 2977, 1725, 1607, 758, 741, 696 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (s, 9H, *tert*-Bu), 3.01—3.17 (m, 2H, Tyr; $CHCH_2$), 4.18—4.44 (m, 4H, Tyr; C<u>H</u>CH_2, Fmoc; $CHCH_2O$), 4.47 (s, 2H, OCH_2CO), 4.97 (s, 2H, Bn; CH_2), 6.81—7.77 (m, 16H, aromatic-H). Positive FAB-MS *m/z*: 699 MH^+.

4b (*m*-Form Compound) The reaction was carried out using **3b** (0.94 g, 4.2 mmol) and Fmoc-L-Tyr(Bn) (2.07 g, 4.2 mmol) in a manner similar to the preparation of **4a** to give **4b** (2.25 g, 83%) as colorless prisms, mp 106—107 °C, $[\alpha]_D$ −9.2° (c=2.1, $CHCl_3$). IR (KBr): 3299, 2977, 2930, 1752, 1671, 1611, 843, 739, 696 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (s, 9H, *tert*-Bu), 3.05—3.10 (m, 2H, Tyr; $CHCH_2$), 4.17—4.45 (m, 4H, Tyr; $CHCH_2$, Fmoc; $CHCH_2O$), 4.49 (s, 2H, OCH_2CO), 5.00 (s, 2H, Bn; CH_2), 6.64—7.76 (m, 16H, aromatic-H). Positive FAB-MS *m/z*: 699 MH^+.

4c (*p*-Form Compound) The reaction was carried out using **3c** (0.847 g, 3.8 mmol) and Fmoc-L-Tyr(Bn) (1.88 g, 3.8 mmol) in a manner similar to the preparation of **4a** to give **4c** (2.29 g, 86%) as colorless prisms, mp 154—156 °C, $[\alpha]_D$ −4.2° (c=2.2, $CHCl_3$). IR (KBr): 3306, 2978, 2938, 1752, 1694, 1663, 1611, 826, 741, 696 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (s, 9H, *tert*-Bu), 2.93—2.99 (m, 2H, Tyr; $CHCH_2$), 4.09—4.38 (m, 4H, Tyr; $CHCH_2$, Fmoc; $CHCH_2O$), 4.42 (s, 2H, OCH_2CO), 4.92 (s, 2H, Bn; CH_2), 6.76—7.69 (m, 16H, aromatic-H). Positive FAB-MS *m/z*: 699 MH^+.

[2-(N^{α}-9-Fluorenylmethoxycarbonyl-*O*-benzyl-L-tyrosinamido)phenoxy]acetic Acid (5a) A solution of **4a** (0.87 g, 1.24 mmol) and TFA–CH_2Cl_2 (1 : 3) (20 ml) was stirred for 7 h. After evaporation of the solvent, the residue was chromatographed on silica gel (CH_2Cl_2–MeOH 10 : 1) to give **5a** (0.57 g, 71%) as prisms, mp 150—153 °C, $[\alpha]_D$ −6.3° (c=1.2, AcOH). IR (KBr): 3295, 3063, 3036, 1694, 1607, 741, 696 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 2.93—3.23 (m, 2H, Tyr; $CHCH_2$), 4.15—4.37 (m, 4H, Tyr; $CHCH_2$, Fmoc; $CHCH_2O$), 4.58 (s, 2H, OCH_2CO), 4.95 (s, 2H, Bn; CH_2), 6.87—7.77 (m, 16H, aromatic-H). Positive FAB-MS *m/z*: 643 MH^+, 665 $(M+Na)^+$.

5b (*m*-Form Compound) The reaction was carried out using **4b** (1.42 g, 2.0 mmol) in a manner similar to the preparation of **5a** to give **5b** (1.30 g, quant) as greenish prisms, mp 182—183 °C, $[\alpha]_D$ +10.2° (c=0.71, AcOH). IR (KBr): 3299, 3063, 1694, 1659, 1613, 824, 802, 741, 696 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 2.91—3.14 (m, 2H, Tyr; $CHCH_2$), 4.14—4.50 (m, 4H, Tyr; $CHCH_2$, Fmoc; $CHCH_2O$), 4.63 (s, 2H, OCH_2CO), 4.97 (s, 2H, Bn; CH_2), 6.70—7.78 (m, 16H, aromatic-H). Positive FAB-MS *m/z*: 643 MH^+, 665 $(M+Na)^+$, 681 $(M+K)^+$.

5c (*p*-Form Compound) The reaction was carried out using **4c** (1.39 g, 2.0 mmol) in a manner similar to the preparation of **5a** to give **5c** (1.20 g, 93%) as prisms, mp 223—225 °C, $[\alpha]_D$ +21.6° (c=1.2, AcOH). IR (KBr): 3299, 3063, 3034, 1667, 1607, 808, 758, 741, 696 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 2.87—2.99 (m, 2H, Tyr; $CHCH_2$), 4.07—4.34 (m, 4H, Tyr; $CHCH_2$, Fmoc; $CHCH_2O$), 4.49 (s, 2H, OCH_2CO), 4.90 (s, 2H, Bn; CH_2), 6.77—7.68 (m, 16H, aromatic-H). Positive FAB-MS *m/z*: 643 MH^+, 665 $(M+Na)^+$, 681 $(M+K)^+$.

N^{ω}-Trityl-L-glutaminyl-L-tryptophanyl-L-aspartyl (β-benzyl ester)-L-phenylalanyl-glycine Benzyl Ester (6) Fmoc-Gln(Trt)-Trp-Asp(Bn)-Phe-Gly-OBn (0.96 g, 0.74 mmol) was dissolved in 20% piperidine-DMF (15 ml) for 2 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH 10 : 1) to give **6** (0.70 g, 88%) as powder. Positive FAB-MS *m/z*: 1074 MH^+.

[N^{α}-2-(N^{α}-9-Fluorenylmethoxycarbonyl-*O*-benzyl-L-tyrosinamido]phenoxy]acetyl-L-alanyl-L-alanyl-L-alanyl-N^{ω}-trityl-L-glutaminyl-L-tryptophanyl-L-aspartyl (β-benzyl ester)-L-phenylalanyl-glycine Benzyl Ester (7a) To a solution of **5a** (0.116 g, 0.18 mmol), peptide **6** (0.197 g, 0.18 mol) and HOBt (0.031 g, 0.20 mmol in DMF (2.0 ml) was added EDCl (0.038 g, 0.20 mmol) at 0 °C and the mixture was stirred for 15 h at room temperature. The reaction mixture was washed with saturated aqueous $NaHCO_3$, 10% aqueous citric acid and saturated aqueous brine, dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel (CH_2Cl_2–MeOH 10 : 1) to give **7a** (0.240 g, 78%) as prisms, mp 129—131 °C, $[\alpha]_D$ −49° (c=1.2, $CHCl_3$). IR (KBr): 3303, 3059, 3032, 2932, 1719, 1659, 1512, 745, 700 cm^{-1}. Positive FAB-MS *m/z*: 1699 MH^+.

7b (*m*-Form Compound) The reaction was carried out using **5b** (0.122 g, 0.19 mmol) and **6** (0.208 g, 0.19 mmol) in a manner similar to the preparation of **7a** to give **7b** (0.260 g, 81%) as prisms, mp 127—129 °C, $[\alpha]_D$ −26° (c=1.1, $CHCl_3$). IR (KBr): 3297, 3059, 3032, 2930, 1719, 1663, 743, 700 cm^{-1}. Positive FAB-MS *m/z*: 1699 MH^+.

7c (*p*-Form Compound) The reaction was carried out using **5c** (0.135 g, 0.21 mmol) and **6** (0.224 g, 0.21 mmol) in a manner similar to the preparation of **7a** to give **7c** (0.340 g, 95%) as prisms, mp 126—128 °C, $[\alpha]_D$ −42° (c=2.5, $CHCl_3$). IR (KBr): 3301, 3058, 2928, 1719, 1659, 741, 698 cm^{-1}. Positive FAB-MS *m/z*: 1699 MH^+.

[N^{α}-(2-L-Tyrosinamido]phenoxy]acetyl-L-alanyl-L-alanyl-L-alanyl-L-glutaminyl-L-tryptophanyl-L-aspartyl-L-phenylalanyl-glycine (2a) A solution of **7a** (0.049 g, 0.029 mmol) in EtOH–AcOH (6 : 1) (3.0 ml) was hydrogenated over 20% $Pd(OH)_2$–C (0.070 g) for 20 h at 45 °C, then filtered and concentrated. The residue was treated with TFA–CH_2Cl_2–anisole–ethanedithiol (43 : 50 : 2 : 5) (1.5 ml) at room temperature for 20 h to give Fmoc compound which was dissolved in 20% piperidine-DMF (1.5 ml) for 21 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH–H_2O 60 : 60 : 10) and Sephadex LH-20 (CH_2Cl_2–MeOH–H_2O 12 : 8 : 1) to give amorphous **2a** (0.021 g, 75%), after lyophilization from a H_2O suspension. Positive FAB-MS *m/z*: 964 MH^+.

2b (*m*-Form Compound) The reaction was carried out using **7b** (0.052 g, 0.031 mmol) in a manner similar to the preparation of **2a** to give amorphous **2b** (0.020 g, 67%). Positive FAB-MS *m/z*: 986 $(M+Na)^+$.

2c (*p*-Form Compound) The reaction was carried out using **7c** (0.053 g, 0.031 mmol) in a manner similar to the preparation of **2a** to give amorphous **2c** (0.020 g, 67%). Positive FAB-MS *m/z*: 964 MH^+, 986 $(M+Na)^+$.

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