Synthesis of Peptides Mimicking Chemokine Receptor CCR5 and Their Inhibitory Effects against HIV-1 Infection

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Peptides mimicking chemokine receptor CCR5 were synthesized and their anti-HIV-1 activities evaluated. Prepared compounds, especially a sulfated derivatives, showed significant anti-HIV-1 activities. Furthermore, a hybrid molecule linked to an *N*-carbomethoxycarbonyl-prolyl-phenylalanine (CPF) moiety had a greater effect.

Key words mimicking peptide; anti-HIV-1 activity; CPF; sulfation; chemokine receptor; hybrid molecule

CD4 is the primary cellular receptor for human immunodeficiency virus type 1 (HIV-1), but CD4 alone is not sufficient to allow the entry of HIV-1 into cells. In 1996, the cellular coreceptors that HIV-1 requires in conjunction with CD4 were identified as members of the chemokine receptor family of seven-transmembrane G protein-coupled receptors.[1] This discovery of distinct chemokine receptors explains the differences in cell tropism between viral strains. Thus CXCR4 supports entry of T cell (T)-tropic HIV-1 strains, whereas CCR5 supports macrophage (M)-tropic HIV-1 strains. Recently, these chemokine receptors have received much attention as attractive targets for new antiviral therapies.[2] The mechanism of entry of HIV-1 into cells is thought to be: first, gp120-CD4 conjugation induces conformational changes in the gp120 subunit, including exposure of the V3 loop, and then conjugation with the coreceptor occurs.[3] It is known that these coreceptors are rich in tyrosines and acidic amino acids at their *N*-terminal region, and this contributes to the ability of HIV-1 to fuse with and enter into target cells.[4] A strongly positive region of the V3 loop has been shown to be important for the association with CCR5. Consequently, it can be considered that some of these positively charged residues may directly and complementarily interact with a sequence of highly negatively charged acidic amino acids. It is known that *N*-carbomethoxycarbonyl-prolyl-phenylalanine (CPF)[5] mimics CD4 and binds selectively to gp120. We previously reported the synthesis of the hybrid compounds linked to the CPF moiety as HIV protease inhibitors[6] and showed that these compounds bind selectively to infected cells, probably based on an interaction between CPF and gp120. Therefore we focused on the synthesis of peptides mimicking CCR5 with a CPF moiety. As a part of a program aimed at the development of new HIV-1 inhibitors, we would like to report the design and synthesis of peptides mimicking CCR5 for prevention of HIV-1 infection based on a strategy of binding to gp120.

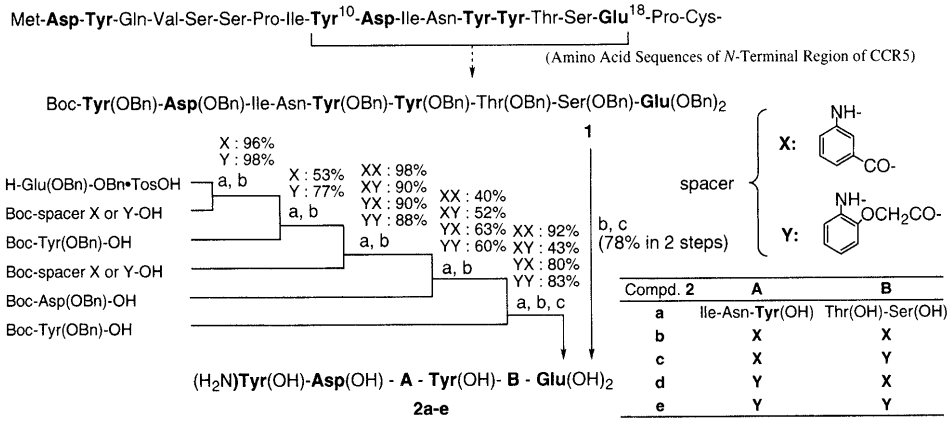
Results and Discussion

In the amino acid sequences of the *N*-terminal domain of CCR5, we first chose the region of Tyr^{10}-Glu^{18} including tyrosines, glutamic acid, and aspartic acid as a target peptide moiety. The peptide of natural type **2a** from **1** and its mimicking peptides **2b**—**e** consisting of acidic amino acids as well as unnatural-type amino acids as the spacer unit were prepared (Chart 1).[7] Resistance to the action of protease was expected from the latter compounds.

Compounds **2a**—**e** had no expected anti-HIV-1 activities[8] against M-tropic strains of HIV-1; on the other hand, these compounds showed significant anti-HIV-1 activities against T-tropic strains. Thus the percentage of HIV-1 antigen-positive cells with **2a**—**e** was 23.3—28.3%, whereas that with CPF was 27.7%. Furthermore, the anti-HIV-1 activities of **2a**—**e** were increased by the addition of CPF as an additive (11.0—26.7%).

To enhance the selectivity of binding to HIV-1 and hence the anti-HIV-1 activity, a hybrid compound **3**[9] of **2a** and CPF was designed and prepared (Chart 2). The synthesis of the hybrid molecule was straightforward and the construction of the covalent linkage between the CPF moiety and the peptide moiety was carried out using spacers derived from *o*-aminophenol. However, compound **3** prepared in this way exhibited the same level of anti-HIV-1 activity as that of **2a**.

Recently, it was suggested that the hydroxyl groups of ty-



Compd. 2	A	B
a	Ile-Asn-**Tyr**(OH)	Thr(OH)-Ser(OH)
b	X	X
c	X	Y
d	Y	X
e	Y	Y

Reagents : a) WSC•HCl, HOBt, NMM, DMF; b) TFA-CH_2Cl_2; c) $Pd(OH)_2$/C, H_2, MeOH

Chart 1

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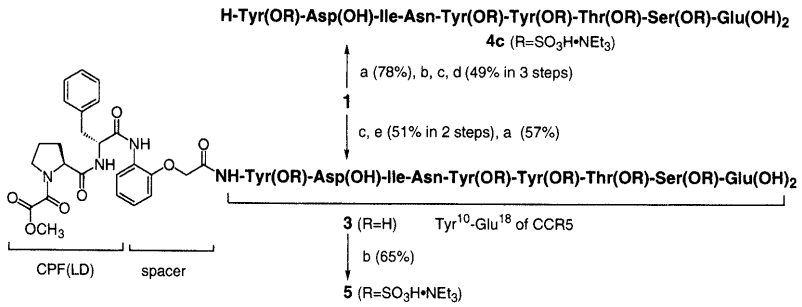

Reagents : a) $Pd(OH)_2/C$, H_2, MeOH; b) SO_3-pyridine, then NEt_3; c) 4*N* HCl, 1,4-dioxane; d) NEt_3; e) CPF-spacer, WSC•HCl, HOBt, NMM, DMF

Chart 2

Table 1. The Anti-HIV-1 Activities of **4a**—**c** and **5**[a]

Compd.	$-SO_3X$	Drug concentration (μg/ml)			
		1000[b]	200	50	0
4a	H	232	258	225	219
4b	Na	207	236	255	251
4c	NEt_3	57	138	245	n.t.[c]
5	NEt_3	2	40	223	n.t.

a) C8166/GUN1WT syncytium assay. *b*) No significant cytotoxicity of the compounds at 1000 μg/ml was observed. *c*) Not tested.

rosines in the *N*-terminal region of CCR5 are modified in the form of sulfates.[10] For the purpose of achieving increased anti-HIV-1 activity, some sulfated analogues were prepared. The sulfates[11] **4a**—**c** and **5** were derived from **1** and **3**, respectively, and these transformations are also shown in Chart 2.[12] The results of biological assay for sulfated compounds **4a**—**c** and **5** are listed in Table 1. As can been seen from the table, in spite of the low activity of **4a** and **b**, compounds **4c** and **5**, especially **5**, showed significantly higher anti-HIV-1 activity.

Conclusions

In this study, it was confirmed that enhancement of anti-HIV-1 activities with mimicking peptides was achieved by the addition of CPF. Additionally, the sulfation of the parent peptide, and more effectively, the conjugation of the sulfated peptide with CPF, caused an increase in anti-HIV-1 activity. These results suggest that highly negatively charged sulfated groups interact strongly with a positively charged region of the V3 loop. The observations in this study will be helpful for a better understanding of the mechanism of HIV-1 infection, and hence development of new types of drugs against AIDS.

References and Notes

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7) **2a**: FAB-MS *m/z* 1167 $(M+1)^+$, **2b**: FAB-MS *m/z* 827 $(M+1)^+$, **2c**: FAB-MS *m/z* 857 $(M+1)^+$, **2d**: FAB-MS *m/z* 857 $(M+1)^+$, **2e**: FAB-MS *m/z* 887 $(M+1)^+$.
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9) **3**: FAB-MS *m/z* 1669 $(M+Na)^+$.
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12) **4c**: FAB-MS *m/z* 1567 M^+, **5**: FAB-MS *m/z* 1849 $(M-3SO_3H+2Na-2H)^+$.