**Synthesis and Evaluation of Retro-inverso-modified HTLV-1 Protease Inhibitor**

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*The effects of additional substituents covering the prime-site of retro-inverso (RI)-modified HTLV-1 protease inhibitors containing a hydroxyethylamine (HEA) isoster were clarified. Stereo-selective construction of the most potent isoster backbone was achieved by the Evans-aldol reaction. Addition of N-acetylated D-amino acid corresponding to the P2 site gave an RI-modified inhibitor showing superior inhibitory activity to the previous inhibitor. Inhibitory activities of the newly synthesized inhibitors suggest that partially modified RI inhibitors would interact with HTLV-1 protease in the same manner as the parent HEA inhibitor.*

**Keywords:** Fmoc-based SPPS, HTLV-1 protease, hydroxyethylamine isoster, Inhibitor, Retro-inverso peptide

**Introduction**

Human T-cell leukemia virus type 1 (HTLV-1) is a retrovirus which cause adult T-cell leukemia (ATL) and related diseases. The key enzyme in the processing of virus proteins in the HTLV-1 is an aspartic protease called HTLV-1 protease. Thus, HTLV-1 protease inhibitor is considered to be an attractive agent for effective treatment of ATL.

We have previously reported the structure–activity relationship of HTLV-1 protease inhibitors containing a transition-state mimic, HEA dipeptide isoster (Fig. 1) [1,2]. The results clearly showed that the configurations at the hydroxyl- and side chain-bearing asymmetric centers of the mimic have marked effects on inhibitory activity. Based on these studies, we found that retro-inverso (RI) modification of the inhibitor containing the transition-state mimic can retain inhibitory activity. In this study, we examined whether the additional D-amino acids or substituents covering the prime site would have effects on inhibitory activity.



*Fig. 1. The structure of previously reported inhibitors.*

**Results and Discussion**

We previously found that the most potent inhibitor has *syn*-configuration at the HEA part. Thus, we selected diastereo-selective aldol reaction using Evans auxiliary to construct the *syn*-configuration. Starting from **4** and **5**, we synthesized 12 inhibitors via Fmoc-based solid phase peptide synthesis (Scheme 1) [3].



*Scheme 1. Synthetic route for RI-modified inhibitors.*

The inhibitory activities of newly synthesized inhibitors against HTLV-1 protease are summarized in Table 1. While inhibitors having an *N*-terminal aromatic ring substituents showed moderate inhibitory activity, saturated alkyl group showed lower inhibitory activity. Although *N*-terminal imino or amino group without the acyl group showed no inhibitory activity, acetylated inhibitor showed clear activity. Based on these results, further studies on structure-activity relationships are now underway.

*Table 1. IC50 values of RI-modified inhibitors.*



**References**

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