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Evaluation of retro-inverso modifications of HTLV-1 protease inhibitors containing a hydroxyethylamine isoster

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ABSTRACT

Effects of retro-inverso (RI) modifications of HTLV-1 protease inhibitors containing a hydroxyethylamine isoster backbone were clarified. Construction of the isoster backbone was achieved by a stereoselective aldol reaction. Four diastereomers with different configurations at the isoster hydroxyl site and the scissile site substituent were synthesized. Inhibitory activities of the new inhibitors suggest that partially modified RI inhibitors would interact with HTLV-1 protease in the same manner as the parent hydroxyethylamine inhibitor.

1. Introduction

Retro-inverso (RI) modifications involve a reversion of the peptide sequence accompanied by the replacement of each L-amino acid with the corresponding D-amino acid[1] (Fig. 1). Fully or partially RI modified biologically active peptides have been found to retain the properties or biological activity of the parent peptides, since a similar topography of side chain orientations is retained by the RI modification.[2] The modification primarily reverses the direction of amide bonds. The introduction of D-amino acids enhances the resistance of fully or partially RI modified peptides to enzymatic degradation.[3] Few applications of the RI modification to protease inhibitors, however, have been reported, although similar or improved interactions of RI modified peptides with proteins or receptors have been reported.[4] In this paper, we report the RI modification of a HTLV-1 protease inhibitor containing the substrate sequence which would improve its resistance to enzymatic degradation without significantly affecting its inhibitory activity.

Human T-cell leukemia virus type 1 (HTLV-1) is a retro virus etiologically associated with adult T-cell leukemia and a number of other chronic diseases.[5] HTLV-1 protease is an aspartic protease, and crucial for processing of the virus' proteins. Thus, HTLV-1 protease is a suitable target for the development of inhibitors for therapeutic use. In previous papers,[6] we reported the synthesis and structure–activity relationship (SAR) of HTLV-1 protease inhibitors containing a hydroxyethylamine dipeptide isoster.[7] We found the configurations at the hydroxyl- and side chain-bearing asymmetric centers to have remarkable effects on the inhibitory activity. Evaluations of substrate specificity at the site of cleavage by this protease also revealed unique preferences for Pro at the P_1' position and for Ile at the P_2 position.[8]

2. Results and discussion

Based on our previous studies on HTLV-1 protease inhibitors, effects of RI modifications of substrate-based inhibitors containing a hydroxyethylamine isoster backbone were examined in the present study (Fig. 2). Type-I RI inhibitor **2** based on the total RI modification of parent inhibitor **1** and type-II RI inhibitor **3** based on a partial RI modification were designed and synthesized. In the present RI modifications, Ile at the P_2 position was replaced with (D)-Ile to retain the side chain orientation after the modification, but the chirality at the β-position of the side chain is not retained. The relative relationship between the isoster hydroxyl and scissile site substituent in the type-II inhibitor **3** is the same as that in the parent inhibitor **1**, in which the side chain substituent is supposed to interact with the protease S_1 pocket.

Type-I RI inhibitor **2** was synthesized on a solid support using an acid-stable succinate ester linker according to our previous report[6] (Scheme 1). (*R*,*S*)-Aminoalkylepoxide **4** derived from (1*R*,2*S*)-1-arylsulfonamide-2-indanyl ester[9] was reacted with H-(D)-Ile-OAllyl and the resulting hydroxyethylamine product was anchored to H-Gly-MBHA resin[10] through a succinic acid linker

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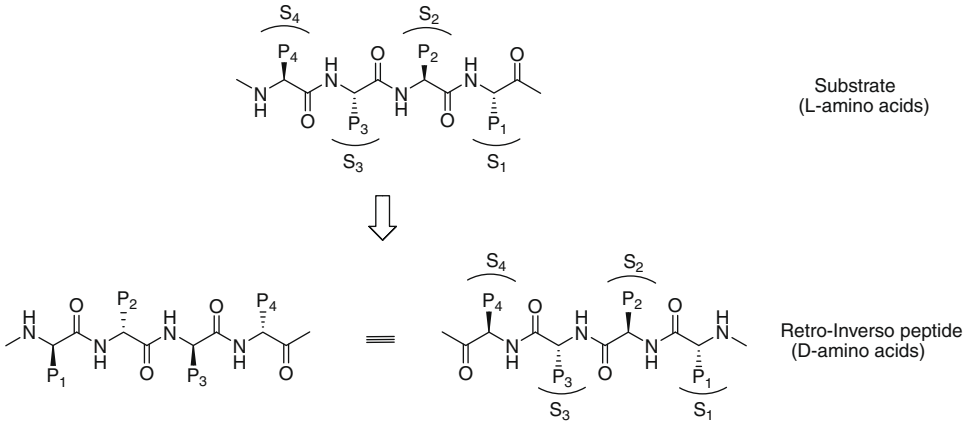


Figure 1. Retro-inverso modification.

to give **6**. After cleavage of the C-terminal allyl group of **6** with $[(C_6H_5)_3P]_4$ Pd(0), H-(D)-Val-OAllyl was coupled using DIPCDI/HOBt as coupling reagents. An aliquot of the product was treated with HF to give a product having a major peak on analytical HPLC, which suggests that no significant racemization occurred during the coupling. Further elongation in the C-terminal direction with H-(D)-Pro-NH_2 was conducted in a similar manner to give resin **7**, and an aliquot of **7** was similarly examined as above. The product gave a single major peak when the coupling was conducted with HATU/HOAt,[11] whereas two major peaks were observed when DIPCDI/HOBt were used as the coupling reagents.[12] After cleavage of the Boc group of the resin **7** with 50% TFA/CH_2Cl_2, Boc-(D)-Pro-OH was coupled with DIPCDI/HOBt. The Boc group of the product was similarly removed by TFA, and the resulting resin was treated with acetic anhydride. The acetylated peptide was then cleaved from the resin by treatment with HF at 4 °C. The succinic acid linker of the crude product was removed by treatment with $AcONH_4$ at pH 9 for 20 h, and the crude product was purified by preparative HPLC to give **2**. The truncated analog **9** was similarly synthesized from the intermediate **7**.

Type-II RI inhibitors based on a partial RI modification were synthesized by a conventional solution-phase procedure. Construction of the isoster backbone containing a hydroxyl group and scissile site substituent was achieved with a stereoselective aldol reaction,[9] which made it possible to selectively prepare all four diastereomers containing different configurations at the isoster hydroxyl site and scissile site substituent (Scheme 2). Boc-(D)-Pro-ol **10** was oxidized with SO_3·Py, and the resulting aldehyde was reacted with methoxymethyltriphenylphosphonium chloride at 50 °C for 3 h. The product **11** was treated with pyridinium *p*-toluenesulfonate (PPTS) to afford the homologated aldehyde **12**. A known ester containing a chiral auxiliary, (1*S*,2*R*)-1-arylsulfonamido-2-indanyl 4-methylvalerate **13**,[6] was then reacted with the aldehyde **12**. While the aldol condensation was conducted according to our previous protocol,[6] a mixture of diastereomers was obtained in the present reaction. To confirm the configuration, each diastereomer, separated by column chromatography, was hydrolyzed and the resulting β-hydoxycarboxlic acid was converted to the oxazolidinone **22** or **23** through a Curtius rearrangement. From the coupling constant of the vicinal protons obtained in a homonuclear decoupling experiment, it was supposed that the polar ester **14** has the *syn* configuration and the less polar ester **15** has the anti configuration. NOE spectroscopy of oxazolidinones supported this estimation.

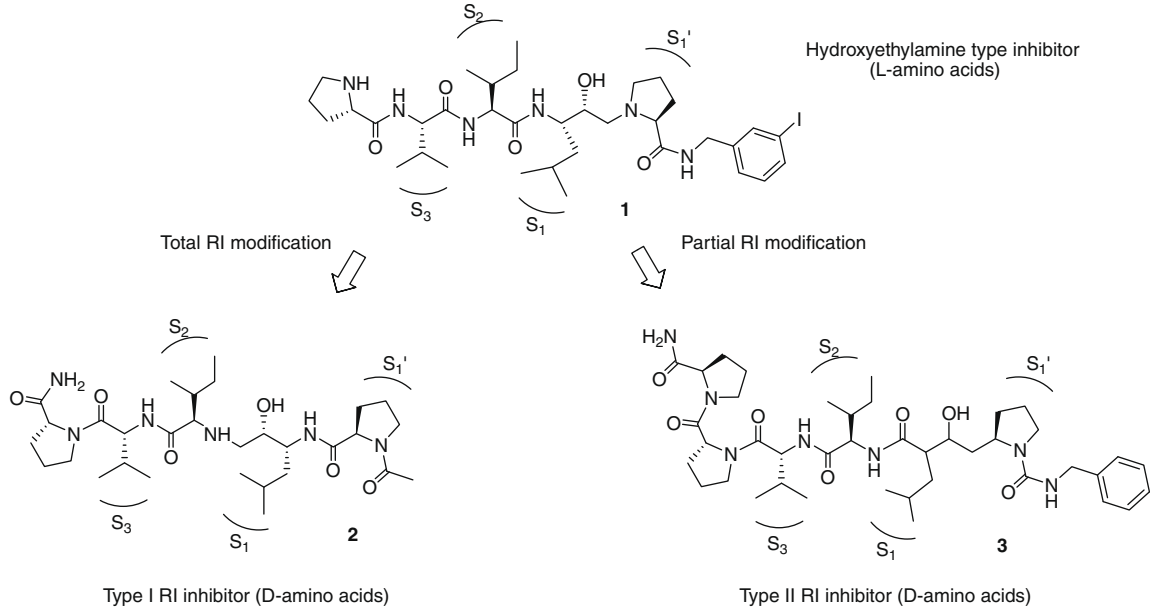
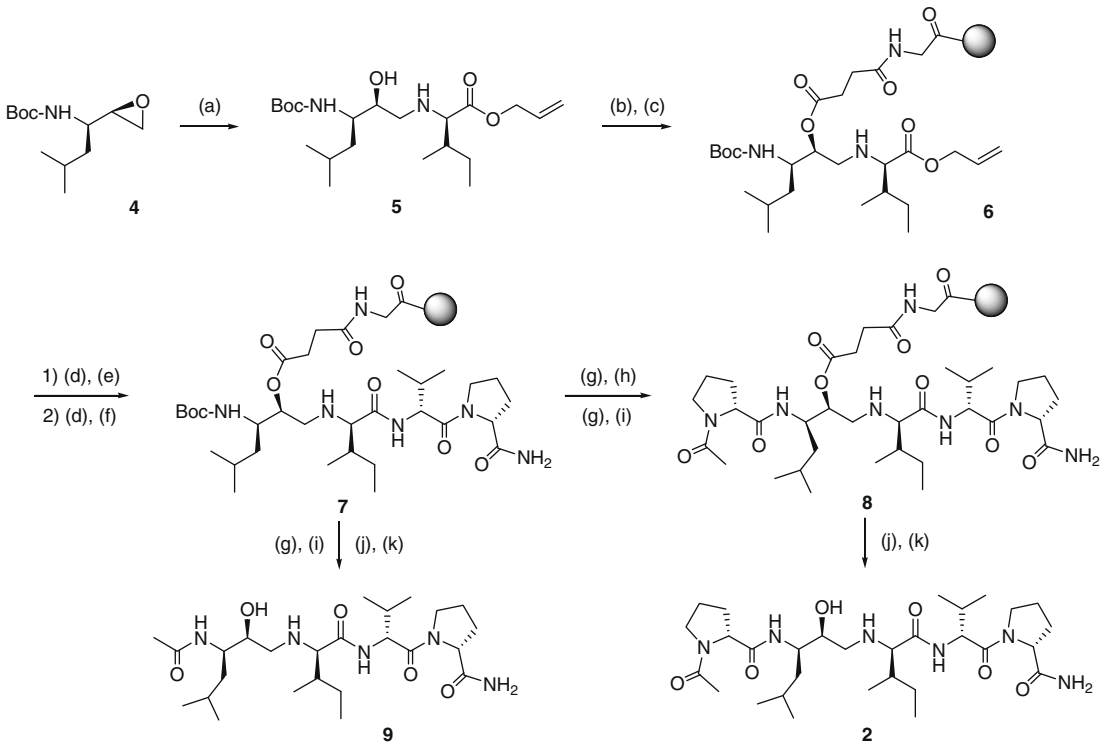
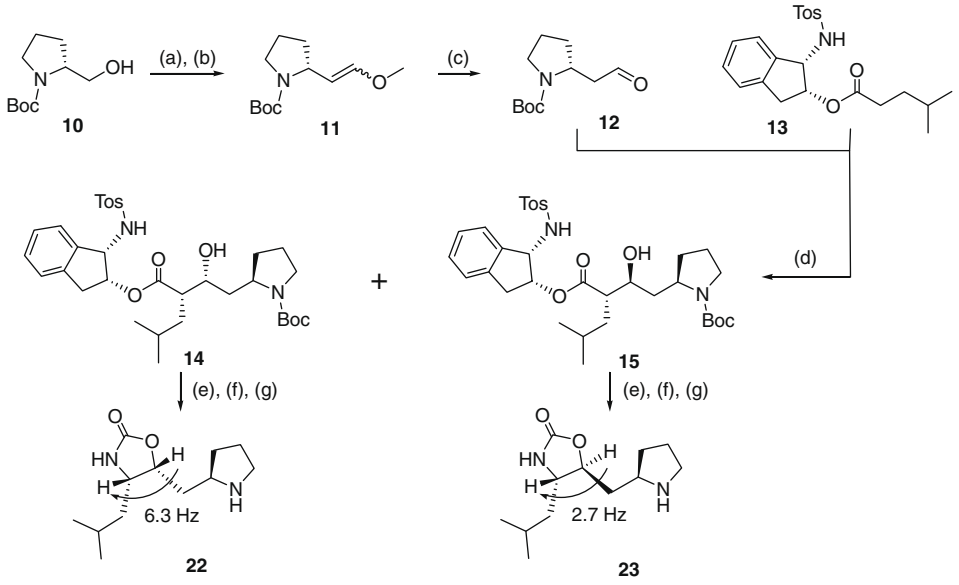


Figure 2. Retro-inverso modification of HTLV-1 protease inhibitors containing a hydroxyethylamine isoster.



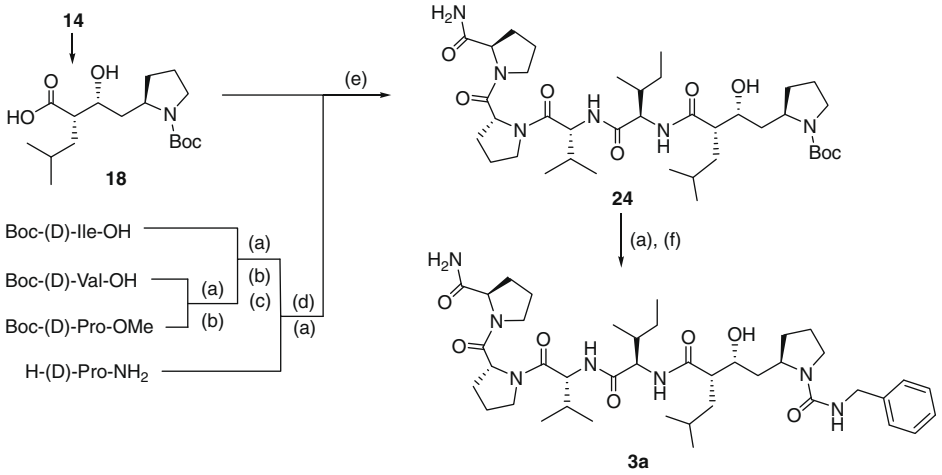
Scheme 1. Synthesis of type-I RI inhibitors. Reagents and conditions: (a) H-(D)-Ile-OAllyl in 2-propanol, 60 °C, 4 h and 25 °C, 18 h; (b) succinic anhydride/DMAP, 25 °C, 16 h; (c) H-Gly-MBHA resin/DIPCDI/HOBt/DIEA, 25 °C, 18 h; (d) $Pd[P(Ph)_3]_4$, 25 °C, 2 h; (e) H-(D)-Val-OAllyl/DIPCDI/HOBt, 25 °C, 18 h; (f) H-(D)-Pro-NH_2/coupling reagent, 25 °C, 2–18 h; (g) TFA, 25 °C, 15 min; (h) Boc-(D)-Pro-OH/DIPCDI/HOBt, 25 °C, 1.5 h; (i) Ac_2O/DIEA, 25 °C, 20 min; (j) HF, 4 °C, 1 h; (k) aq $AcONH_4$, pH 8.5–9, 25 °C, 20 h.



Scheme 2. Construction of the isoster backbone. Reagents and conditions: (a) SO_3·Py/DMSO, Et_3N, 25 °C, 0.5 h; (b) $Cl^-P^+Ph_3CH_2OMe$/*t*BuOK, 50 °C, 3 h; (c) PPTS, 25 °C, 4 h; (d)1 M $TiCl_4/CH_2Cl_2$, DIEA, −78 °C, 2 h; (e) 30%H_2O_2, LiOH, 25 °C, 24 h; (f) DPPA/Et_3N, 80 °C, 0.5 h; (g) TFA, 25 °C, 15 min.

The isoster backbone thus prepared was then introduced into the RI modified substrate sequence (Scheme 3). First, the β-hydoxycarboxlic acid **18** was obtained by the hydrolysis of polar ester **14** with LiOH. **18** was then coupled with a tetrapeptide synthesized by a conventional solution-phase method using WSCDI/HOBt as coupling reagents.[13] The Boc group of the resulting product **24** was removed with TFA and the product was reacted with benzylisocyanate to afford the Diastereomer **3a** of a Type-II RI inhibitor. The diastereomer 3b was similarly synthesized from β-hydoxycarboxlic acid **15** prepared from the chiral ester **13**. Diastereomers **3c** and **3d** of Type-II RI inhibitors were similarly synthesized from (1*R*,2*S*)-1-arylsulfonamido-2-indanyl 4-methylvalerates. Each diastereomer showed a single major peak at different elution times on analytical HPLC.

The inhibitory activities of Type-I and -II RI inhibitors thus obtained were examined using a synthetic dodecapeptide as a substrate according to our previous procedure.[6] Cleavage of the substrate by a mutant HTLV-1 protease[14] in the presence of the



Scheme 3. Synthesis of type II RI inhibitor **3a**. Reagents and conditions: (a) TFA, 25 °C, 0.5–2 h; (b) BOP/DIEA, 25 °C, 3 h-2 days; (c) 1 M NaOH, 25 °C, 2 days; (d) DPPA/DIEA, 25 °C, 2 days; (e) WSCDI/HOBt, 25 °C, 16 h; (f) benzylisocyanate/DIEA, 25 °C, 1.5 h.

RI inhibitor was monitored by analytical HPLC. The rate of cleavage was estimated from the decrease in the substrate during the incubation. The inhibitory activity of each RI inhibitor was evaluated using the corresponding IC_{50} value obtained from the sigmoidal dose–response curve[15] (Table 1).

No inhibitory activity was detected for type-I RI inhibitors **2** and **9** even at 10 mM. In contrast, all diastereomers of type-II RI inhibitors showed inhibitory activity with IC_{50} values in the order of μM. These results indicate that the relative position of the isostere hydroxyl group for the isobutyl group directing the S_1 site is critical for the RI modification of a hydroxyethylamine backbone. Among type-II RI inhibitors, **3a**, with an R-configuration for the hydroxyl group and S-configuration for the scissile site isobutyl substituent, showed the most effective inhibitory activity (IC_{50} = 160 μM). The diastereomers **3b** and **3d** having the reversed configuration of **3a** at the isoster hydroxyl group showed decreased inhibitory activities; one quarter or less the activity of **3a**. Reversal of the isobutyl substituent had a relatively small effect on the inhibitory activity. These structure–activity relationships of type-II RI inhibitors are consistent with those of parent hydroxylethylamine inhibitors,

Table 1
IC_{50} values of RI inhibitors

	Compound	IC_{50}
Type I; **2**	H_2N-Pro(D)-Val(D)-Ile(D)	>10 mM
9	H_2N-Pro(D)-Val(D)-Ile(D)	>10 mM
Type II; **3a**	H_2N-Pro(D)-Pro(D)-Val(D)-Ile(D)	160 μM
3b	H_2N-Pro(D)-Pro(D)-Val(D)-Ile(D)	550 μM
3c	H_2N-Pro(D)-Pro(D)-Val(D)-Ile(D)	350 μM
3d	H_2N-Pro(D)-Pro(D)-Val(D)-Ile(D)	1500 μM

which suggest that type-II RI inhibitors would interact with HTLV-1 protease in the same manner as hydroxylethylamine inhibitors. The parent inhibitor **1** (IC_{50} = 5.8 μM),[6b] however, is still relatively potent compared with a type-II RI modified inhibitor **3a**. To improve the inhibitory activities of the RI modified inhibitors, SAR studies on side chain characteristics including (D)-alloisoleucine are now underway.

In conclusion, retro-inverso (RI) modification of HTLV-1 protease inhibitors containing a hydroxyethylamine isoster backbone was effective without significantly influencing the inhibitory activity. Structure–activity relationships regarding the isoster hydroxyl group and scissile site substituent suggest that the RI modified inhibitor would retain a similar topography to the parent inhibitor.

3. Experimental

3.1. General

All solvents were of reagent grade. THF was distilled from sodium and benzophenone ketyl. CH_2Cl_2 was distilled from CaH_2. All commercial reagents were of the highest purity available. Amino acid derivatives were purchased from Peptide Institute, Inc. (Osaka, Minoh, Japan) or Watanabe Chemical, Inc. (Hiroshima, Japan). Analytical TLC was performed on silica gel (60 F-254, Plates 0.25 mm). Column chromatography was carried out on Wakogel 60 (particle size, 63–200 μm) or Wakogel FC-40 (particle size, 20–40 μm). Analytical and preparative HPLCs were performed using a HITACHI ELITE LaChrom system (OD, 220 nm). ^{1}H NMR (300 MHz or 400 MHz) spectra were recorded on a Bruker AM-300 or Bruker-DMX 400. Chemical shifts are expressed in ppm relative to TMS (0 ppm) or $CHCl_3$ (7.28 ppm). High-resolution mass spectra (HRMS) were obtained on a JMS-HX-110A (FAB) or a Bruker Autoflex-II (MALDI-TOF). Optical rotations were recorded on a HORIBA SEPA-300 polarimeter at the sodium D line.

3.2. Boc-NHCH($CH_2CH(CH_3)_2$)CH(OH)CH_2-(D)-Ile-OAllyl (Boc-HEA-(D)-Ile-OAllyl) (5)

To a solution of Boc-aminoalkylepoxide (**4**)[6a] (40 mg, 170 μmol) in 2-propanol (0.5 mL) was added H-(D)-Ile-OAllyl (320 mg, 1.7 mmol). The mixture was stirred at 60 °C for 4 h, and then at 25 °C for 18 h. The mixture was concentrated in vacuo and $CHCl_3$ was added to the residue. The organic layer was washed successively with 5% aq citric acid and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by flash chromatography (hexane/AcOEt = 3:1) to give **5** (35 mg, 51%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.88–0.94 (12H, m), 1.17–1.23 (1H, m), 1.25 (1H, s), 1.30–1.34 (1H, dd, J = 5.7, 4.5 Hz), 1.35–1.40 (1H, dd, J = 10.0, 4.0 Hz), 1.43 (9H, s), 1.48–1.55 (1H, m), 1.67–1.77 (2H, m), 2.38–2.43 (1H, br m), 2.82 (1H, br d, J = 10.8 Hz), 3.08 (1H, br d, J = 5.9 Hz), 3.45 (1H, br m), 3.64 (1H, br m), 4.62 (2H, d, J = 5.8 Hz), 4.76 (1H, br d, J = 9.4 Hz), 5.25 (1H, dd, J = 10.4, 1.4 Hz), 5.32–5.36 (1H, dd, J = 15.8, 1.4 Hz), 5.87–5.97 (1H, m).

3.3. Boc-HEA ($COCH_2CH_2CO$-Gly-MBHA)-(D)-Ile-OAllyl (6)

To a solution of **5** (59 mg, 150 μmol) in $CHCl_3$ (1 mL) were added succinic anhydride (19 mg, 190 μmol) and DMAP (5.3 mg, 44 μmol), and the mixture was stirred at 25 °C for 16 h. The mixture was washed successively with 5% aq citric acid and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by flash chromatography (hexane/AcOEt = 3:1) to give Boc-HEA ($COCH_2CH_2$-COOH)-(D)-Ile-OAllyl (44 mg, 60%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.89–0.98 (12H, m), 1.24–1.29 (2H, m), 1.30–1.37 (1H, m), 1.44 (9H, s), 1.64–1.78 (2H, m), 2.03 (1H, br s), 2.37 (1H, br s), 2.67 (4H, m), 2.87–2.96 (1H, m), 3.17 (1H, br d, J = 14.6 Hz), 3.51 (1H, br m), 3.65 (1H, br m), 4.65 (2H, m), 5.27 (1H, d, J = 10.6 Hz), 5.30–5.40 (1H, m), 5.85–5.98 (1H, m).

To 150 mg of *p*-methylbenzhydrylamine (MBHA) resin (0.66 mmol/g resin) swelled in DMF (1.5 mL) were added Fmoc-Gly-OH (72 mg, 0.24 mmol), HOBt (37 mg, 0.24 mmol), DIPCDI (39 μL, 0.24 mmol), and DIEA (42 μL, 0.24 mmol). The mixture was agitated for 90 min at 25 °C. After the resin was washed with DMF, piperidine (20%) in DMF was added to the resin. After agitating for 20 min at 25 °C, the N^{α}-deprotected resin was washed with DMF. To this resin were added the above carboxylic acid (78 mg, 0.16 mmol), HOBt (24 mg, 0.16 mmol), DIPCDI (25 μL, 0.16 mmol), and DIEA (27 μL, 0.16 mmol). The mixture was agitated for 18 h at 25 °C, and washed with DMF. Acetic anhydride (46 μL, 0.49 mmol) and DIEA (84 μL, 0.49 mmol) were added to the resin. After agitating for 20 min at 25 °C, the product resin **6** was washed with DMF and MeOH.

3.4. Ac-(D)-Pro-HEA($COCH_2CH_2CO$-Gly-MBHA)-(D)-Ile-(D)-Val-(D)-Pro-NH_2 (8)

To the resin **6** (65 mg) in $CHCl_3$/AcOH/NEM (37:2:1, 2 mL) was added $Pd[PPh_3]_4$ (230 mg, 0.20 mmol), and the mixture was agitated under darkness and an Ar atmosphere for 2 h. The resin was washed with $CHCl_3$, THF, and DMF, and then H-(D)-Val-OAllyl (25 mg, 0.15 mmol), HOBt (23 mg, 0.15 mmol), DIPCDI (24 μL, 0.15 mmol), and DIEA (26 μL, 0.15 mmol) were added. The mixture was agitated for 18 h at 25 °C, washed with DMF and MeOH, and dried. The resin was similarly treated with $Pd[PPh_3]_4$ (230 mg, 0.20 mmol) as above, washed, and reacted with H-(D)-Pro-NH_2 (17 mg, 0.15 mmol), HOBt (23 mg, 0.15 mmol), DIPCDI (24 μL, 0.15 mmol), and DIEA (26 μL, 0.15 mmol) for 18 h at 25 °C. The product resin was treated with 50% TFA in CH_2Cl_2 for 15 min at 25 °C, and washed with CH_2Cl_2, DMF, and 5% DIEA in DMF. After the resin was washed with DMF, Boc-(D)-Pro-OH (27 mg, 0.13 mmol), HOBt (19 mg, 0.13 mmol), DIPCDI (20 μL, 0.13 mmol), and DIEA (22 μL, 0.13 mmol) were added. The mixture was agitated for 1.5 h at 25 °C, and washed with DMF. The resin was similarly treated with 50% TFA in CH_2Cl_2 as above, washed, and reacted with acetic anhydride (24 μL, 0.25 mmol), and DIEA (44 μL, 0.25 mmol). After agitating for 20 min at 25 °C, the product resin was washed with DMF and MeOH, and dried to yield 73 mg of **8**.

3.5. Ac-(D)-Pro-HEA-(D)-Ile-(D)-Val-(D)-Pro-NH_2 (2)

The resin **8** (58 mg) was treated with an anhydrous HF (5.0 mL) in the presence of anisole (50 mL) at 4 °C for 60 min. After evaporation of HF, ether (10 mL) was added to the reaction mixture. The resulting precipitate was washed with ether (10 mL) and dissolved in 0.1% aqueous TFA. The solution was freeze-dried to yield 12 mg of the type I RI inhibitor precursor as a white amorphous powder. HRFAB MS, m/z 751.4723 for $[M+H]^+$ (calcd 751.4718 for $C_{36}H_{62}N_8O_9$).

The precursor product (11 mg) was dissolved in 0.1 aq TFA (1.0 mL) and the pH of the solution was adjusted to 9.0. The mixture was stirred at 25 °C for 20 h, and then the pH was adjusted to 5.0 with 1 M AcOH. The solution was freeze-dried and the crude product was purified by preparative HPLC [YMC Pro C18 column (10 × 250 mm), CH_3CN (15–45%/60 min) in 0.1% aq TFA, 2.5 ml/min] to yield 2.1 mg (overall 7.3%) of **2** as a white powder. HRFAB MS, m/z 595.4186 for $[M+H]^+$ (calcd 595.4183 for $C_{30}H_{54}N_6O_6$).

3.6. Ac-HEA-(D)-Ile-(D)-Val-(D)-Pro-NH_2 (9); truncated analog of type I RI inhibitor

To Boc-HEA($COCH_2CH_2CO$-Gly-MBHA)-(D)-Ile-(D)-Val-OH, prepared as above starting from 62 mg of MBHA resin (0.66 mmol/g re-

sin), in DMF (0.5 ml) were added H-(D)-Pro-NH_2 (47 mg, 0.41 mmol), HOAt (8.4 mg, 62 μmol), HATU (78 mg, 0.21 mmol), and DIEA (18 μL, 0.21 mmol). The mixture was agitated at 25 °C for 2 h. The Boc group of the product resin was removed and the resulting amino group was acetylated as above to yield 76 mg of dried Ac-HEA($COCH_2CH_2CO$-Gly-MBHA)-(D)-Ile-(D)-Val-(D)-Pro-NH_2. HF treatment and removal of the linker at pH 8.5 were also achieved as described for **2**. The crude product was purified by preparative HPLC to yield 1.7 mg (overall 15%) of **9** as a white powder. HRFAB MS, *m/z* 498.637 for $[M+H]^+$ (calcd 498.370 for $C_{25}H_{47}N_5O_5$).

3.7. (1*S*,2*R*)-*N*-[2,3-dihydro-2-((2*S*,3*R*)-2-isobutyl-3-hydroxy-4-(*N*-Boc-(*R*)-pyrrolidin-2-yl)-1-oxobutoxy)indene-1-yl]-4-methylbenzene sulfonamide (14) and diasteromers (15, 16, and 17)

To a stirred solution of Boc-(D)-Pro-ol **10** (1.0 g, 5.0 mmol) in DMSO/AcOEt (5 mL/10 mL) were added Et_3N (2.1 mL, 15 mmol) and SO_3·Py (2.4 g, 15 mmol). The mixture was stirred at 25 °C for 30 min and poured into ice-water. The aqueous phase was extracted with AcOEt. The organic phase was washed with H_2O, dried over Na_2SO_4, and rotary evaporated. The crude product was used without further purification. To a suspension of methoxymethyltriphenylphosphine chloride (5.1 g, 15 mmol) in benzene was added *t*-BuOK (1.9 g, 17 mmol). The mixture was stirred at 50 °C for 30 min, and then crude aldehyde obtained above was added to the mixture. The whole solution was further stirred at 50 °C for 3 h and then quenched with saturated aqueous NH_4Cl. The organic phase was separated, and the remaining aqueous phase was extracted with AcOEt. The combined organic phase was washed with H_2O, dried over $MgSO_4$, and rotary evaporated. The crude product was partially purified by column chromatography (hexane/AcOEt = 2:1) to yield 0.70 g (61%) of **11** (*E*/*Z* mixture) as an oil.

To a stirred solution of **11** (0.66 g, 2.9 mmol) in acetone (30 mL) was added pyridinium *p*-toluenesulfonate (0.73 g, 2.9 mmol). The mixture was stirred at 25 °C for 4 h, and then poured into saturated aqueous $NaHCO_3$. The aqueous phase was extracted with AcOEt. The organic phase was washed with H_2O, dried over $MgSO_4$, and rotary evaporated. The crude product of **12** was used in the next reaction without further purification. To a solution of (1*S*,2*R*)-1-arylsulfonamido-2-indanyl 4-methylvalerate **13**[6a] (0.60 g, 1.5 mmol) in CH_2Cl_2 (10 mL) was added a 1 M solution of $TiCl_4$ (1.8 mL) in CH_2Cl_2 at 4 °C. The mixture was stirred at 25 °C for 15 min. To this solution was added DIEA (1.0 mL, 6.0 mmol) and the mixture was stirred for 2 h at 25 °C. The resulting solution was added to a stirred solution of aldehyde **12** (2.9 mmol) and a 1 M solution of $TiCl_4$ (3.5 mL) in CH_2Cl_2 (10 mL) at −78 °C. The mixture was stirred at −78 °C for 2 h and then quenched by the addition of aqueous ammonium chloride. The organic layer was washed with H_2O, dried over $MgSO_4$, and rotary evaporated. The crude product was purified by silica gel column chromatography (hexane/AcOEt = 5:1). Each diastereomixture was further purified by silica gel column chromatography (hexane/AcOEt = 40:1 to 20:1) to yield **14** (polar compound, 13%) and **15** (less polar compound, 20%) each as an amorphous powder.

Compound (**14**); ^1H NMR ($CDCl_3$) δ: 0.79 (3H, d, *J* = 6.3 Hz), 0.85 (3H, d, *J* = 6.3 Hz), 1.43 (9H, s), 1.1–1.6 (6H, m), 1.79 (2H, m), 2.43 (3H, s), 2.62 (1H, m), 2.85 (1H, d, *J* = 17.1 Hz), 3.05 (1H, dd, *J* = 17.1, 4.5 Hz), 3.20–3.36 (2H, m), 3.66 (1H, m), 4.02 (1H, m), 4.86 (1H, dd, *J* = 9.6, 4.5 Hz), 5.26 (1H, dd, *J* = 4.5, 4.5 Hz), 5.63 (1H, d, *J* = 2.7 Hz), 6.36 (1H, d, *J* = 9.6 Hz), 7.83 (2H, d, *J* = 8.4 Hz); HRFAB MS, *m/z* 615.3089 for $[M+H]^+$ (calcd 615.3104 for $C_{33}H_{47}O_7N_2S$).

(1*S*,2*R*)-*N*-[2,3-dihydro-2-((2*S*,3*S*)-2-isobutyl-3-hydroxy-4-(*N*-Boc-(*R*)-pyrrolidin-2-yl)-1-oxobutoxy)indene-1-yl]-4-methylbenzene sulfonamide (**15**); ^1H NMR ($CDCl_3$) δ: 0.74 (3H, d, *J* = 6.6 Hz), 0.83 (3H, d, *J* = 6.6 Hz), 1.43 (9H, s), 1.20–2.12 (8H, m), 2.38 (1H, m), 2.43 (3H, s), 2.85 (1H, d, *J* = 17.1 Hz), 3.06 (1H, dd, *J* = 17.1, 4.5 Hz), 3.28 (2H, m), 3.53 (1H, m), 4.26 (1H, m), 4.87 (1H, dd, *J* = 9.6, 4.5 Hz), 5.48 (1H, dd, *J* = 4.5, 4.5 Hz), 5.77 (1H, d, *J* = 3.6 Hz), 6.99 (1H, d, *J* = 9.6 Hz), 7.12–7.27 (4H, m), 7.30 (2H, d, *J* = 8.4 Hz), 7.85 (2H, d, *J* = 8.4 Hz); HRFAB MS, *m/z* 615.3115 for $[M+H]^+$ (calcd 615.3104 for $C_{33}H_{47}O_7N_2S$).

The diastereomers ((1*R*,2*S*)-*N*-[2,3-dihydro-2-((2*R*,3*R*)-2-isobutyl-3-hydroxy-4-(*N*-Boc-(*R*)-pyrrolidin-2-yl)-1-oxobutoxy)indene-1-yl]-4-methylbenzene sulfonamide **16** and (1*R*,2*S*)-*N*-[2,3-dihydro-2-((2*R*,3*S*)-2-isobutyl-3-hydroxy-4-(*N*-Boc-(*R*)-pyrrolidin-2-yl)-1-oxobutoxy)indene-1-yl]-4-methylbenzene sulfonamide **17**) were similarly prepared from (1*R*, 2*S*)-1-arylsulfonamido-2-indanyl 4-methylvalerate (**16**); ^1H NMR ($CDCl_3$) δ: 0.74 (d, *J* = 6.6 Hz, 2H), 0.83 (d, *J* = 6.6 Hz, 3H), 0.98–1.68 (m, 13H), 1.77–2.07 (m, 3H), 3.22–3.38 (m, 2H), 3.53 (m, 1H), 4.26 (m, 1H), 4.87 (dd, *J* = 9.2, 4.8 Hz, 1H), 5.48 (dd, *J* = 4.5, 4.5 Hz, 1H), 5.76 (d, *J* = 3.3 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 7.22–7.27 (m, 3H), 7.30 (d, *J* = 8.1 Hz, 2H), 7.85 (d, *J* = 8.1 Hz, 2H). (**17**) ^1H NMR ($CDCl_3$) δ: 0.79 (3H, d, *J* = 6.3 Hz), 0.85 (3H, d, *J* = 6.3 Hz), 1.08–1.56 (14H, m), 1.82 (3H, m), 2.43 (3H, s), 2.62 (1H, m), 2.85 (1H, d, *J* = 17.1 Hz), 3.05 (1H, dd, *J* = 17.1, 3.9 Hz), 3.20–3.36 (2H, m), 3.67 (1H, m), 4.02 (1H, m), 4.86 (1H, dd, *J* = 6.6, 4.5 Hz), 5.26 (1H, dd, *J* = 4.5, 4.5 Hz), 5.63 (1H, d, *J* = 2.7 Hz), 6.36 (1H, d, *J* = 9.6 Hz), 7.21–7.44 (6H, m), 7.83 (1H, d, *J* = 8.4 Hz).

3.8. (2*S*,3*R*)-2-Isobutyl-3-hydroxy-4-(*N*-Boc-(*R*)-pyrrolidine-2-yl)-butyric acid (18) and diasteromers (19, 20, and 21)

To a stirred solution of **14** (110 mg, 0.18 mmol) in THF/H_2O (3:1, 8 mL) were added LiOH·H_2O (30 mg, 0.72 mmol) and 30% aqueous H_2O_2 (100 μL). The mixture was stirred at 25 °C for 24 h, and then quenched with the addition of aqueous Na_2SO_3 and saturated aqueous $NaHCO_3$. The organic solvent was evaporated in vacuo. The resulting aqueous solution was diluted with H_2O, acidified with 1 M HCl, and extracted with AcOEt. The organic layer was washed with H_2O, dried over $MgSO_4$ and rotary evaporated to yield 32 mg (54%) of **18** as an amorphous powder. $[\alpha]_D^{25}$ −16.1 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.91 (3H, d, *J* = 6.3 Hz), 0.93 (3H, d, *J* = 6.6 Hz), 1.46 (9H, s), 1.02–2.07 (9H, m), 2.62 (1H, m), 3.32 (2H, m), 3.75 (1H, m), 4.16 (1H, m), 6.34 (1H, br s); HRFAB MS, *m/z* 330.2284 for $[M+H]^+$ (calcd 330.2280 for $C_{17}H_{32}O_5N$).

Hydrolysis of **15** (180 mg, 0.29 mmol) was similarly carried out as above to yield 92 mg (95%) of (2*S*,3*S*)-2-isobutyl-3-hydroxy-4-(*N*-Boc-(*R*)-pyrrolidine-2-yl)-butyric acid **19** as an amorphous powder: $[\alpha]_D^{25}$ −2.8 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.92 (3H, d, *J* = 6.6 Hz), 0.94 (3H, d, *J* = 6.6 Hz), 1.32–2.08 (18H, m), 2.42 (1H, m), 3.33 (2H, dd, *J* = 7.5, 5.4 Hz), 3.62 (1H, d, *J* = 9.6 Hz), 4.15 (1H, m), 6.59 (1H, br s); HRFAB MS, *m/z* 330.2284 for $[M+H]^+$ (calcd 330.2280 for $C_{17}H_{32}O_5N$).

Two additional diastereomers **20** and **21** were similarly prepared from the ester **16** and **17**. (2*R*,3*R*)-2-Isobutyl-3-hydroxy-4-(*N*-Boc-(*R*)-pyrrolidine-2-yl)-butyric acid **20** from **16** (210 mg, 0.34 mmol): yield 116 mg (quantitative), $[\alpha]_D^{25}$ 12.38 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.91 (3H, d, *J* = 6.6 Hz), 0.93 (3H, d, *J* = 6.6 Hz), 1.35–2.08 (18H, m), 2.47 (1H, m), 3.28–3.38 (2H, m), 3.63 (1H, m), 4.17 (1H, m), 6.56 (1H, br s); MALDI TOF-MS, *m/z* 352.330 for $[M+Na]^+$ (calcd 352.210 for $C_{17}H_{31}O_5NNa$). (2*R*,3*S*)-2-Isobutyl-3-hydroxy-4-(*N*-Boc-(*R*)-pyrrolidine-2-yl)-butyric acid **21** from **17** (280 mg, 0.46 mmol): yield 146 mg (97%), $[\alpha]_D^{25}$ 10.93 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.91 (3H, d, *J* = 6.6 Hz), 0.93 (3H, d, *J* = 6.6 Hz), 1.13–2.10 (18H, m), 2.64 (1H, ddd, *J* = 8.4, 4.8, 4.5 Hz), 3.26–3.38 (2H, m), 3.77 (1H, m), 4.17 (1H, m), 6.62 (1H, br s); MALDI TOF-MS, *m/z* 352.322 for $[M+Na]^+$ (calcd 352.210 for $C_{17}H_{31}O_5NNa$).

3.9. (4*S*,5*R*)-4-Isobutyl-5-(((*R*)-pyrrolidin-2-yl)methyl)oxazolidin-2-one (22)

To a stirred solution of **18** (16 mg, 4.8 μmol) in toluene (2.0 mL) were added Et_3N (10 μL, 7.3 μmol) and DPPA (10 μL, 7.3 μmol). The mixture was stirred at 80 °C for 30 min. After cooling, the mixture was rotary evaporated. The crude product was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to yield 14 mg (88%) of (4*S*,5*R*)-4-isobutyl-5-((*N*-Boc-(*R*)-pyrrolidin-2-yl)methyl)-oxazolidin-2-one. ^{1}H NMR ($CDCl_3$) δ: 0.90 (3H, d, *J* = 6.6 Hz), 0.96 (3H, d, *J* = 6.6 Hz), 1.21–1.27 (1H, m), 1.49 (9H, s), 1.37–2.27 (8H, m), 3.23–3.56 (2H, m), 3.83 (1H, ddd, *J* = 11.1, 7.5, 3.3 Hz), 3.93 (1H, br), 4.76 (1H, br), 6.50(1H, br). Nuclear overhauser and exchange spectroscopy of the ^{1}H NMR showed the NOE between vicinal protons of the oxazolidin ring (Hs of 3.83 ppm (NH–C*H*) and 4.76 ppm (O–C*H*)) clearly.

The product was dissolved in TFA (2 mL), and the mixture was stirred at 25 °C for 15 min and rotary evaporated. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 50:1) to yield **22** (quantitative). ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, d, *J* = 6.6 Hz), 0.94 (3H, d, *J* = 6.6 Hz), 1.18 (1H, m), 1.42 (1H, m), 1.58 (1H, m), 1.81 (1H, m), 1.92–2.46 (5H, m), 3.35 (2H, br s), 3.79 (1H, m), 4.02 (1H, m), 4.88 (1H, dt, *J* = 6.3, 7.5 Hz), 6.75 (1H, s).

(4*S*,5*S*)-4-Isobutyl-5-(((*R*)-pyrrolidin-2-yl)methyl)oxazolidin-2-one **23** was similary prepared from **19**. ^{1}H NMR ($CDCl_3$) δ: 0.90 (3H, d, *J* = 6.6 Hz), 0.92 (2H, m), 1.27–1.53 (2H, m), 1.62 (1H, m), 1.81 (1H, m), 1.90–2.42 (5H, m), 3.37 (2H, br s), 3.65 (1H, m), 3.79 (1H, br s), 4.37 (1H, dt, *J* = 2.7, 7.5 Hz), 6.67 (1H, s). Nuclear overhauser and exchange spectroscopy of ^{1}H NMR of (4*S*,5*S*)-4-isobutyl-5-((*N*-Boc-(*R*)-pyrrolidin-2-yl)methyl)oxazolidin-2-one was measured, but the NOE between vicinal protons of the oxazolidin ring (Hs of 3.50 ppm (NH–C*H*) and 4.20 ppm (O–C*H*)) was very weak.

3.10. Boc-(D)-Ile-(D)-Val-(D)-Pro-OMe

Boc-(D)-Pro-OMe (2.3 g, 10 mmol) was treated with TFA at 25 °C for 30 min, and excess TFA was rotary evaporated. The residue was dissolved in CH_2Cl_2 (30 mL) and neutralized with iPr_2NEt. To the solution were added Boc-(D)-Val-OH (2.6 g, 12 mmol) and BOP (5.3 g, 12 mmol). The mixture was stirred at 25 °C for 3 h and rotary evaporated. The residue was dissolved in AcOEt and the organic phase was washed with 10% citric acid, sat.Na_2CO_3, and H_2O, dried over $MgSO_4$, and rotary evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 50:1 to 20:1) to yield 2.4 g (74%) of Boc-(D)-Val-(D)-Pro-OMe as colorless oil. The product (0.54 g, 1.6 mmol) was then treated with TFA (5 mL) as above for 2 h. The product was dissolved in CH_2Cl_2 (20 mL) and neutralized with iPr_2NEt. To the solution were added Boc-(D)-Ile-OH (0.38 g, 1.6 mmol) and BOP (0.79 g, 1.8 mmol). The mixture was stirred at 25 °C for 2 days and rotary evaporated. The residue was dissolved in AcOEt and the organic layer was washed with 10% citric acid, sat.Na_2CO_3, H_2O, dried over $MgSO_4$, and rotary evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 50:1 to 30:1) to yield 0.60 g (84%) of Boc-(D)-Ile-(D)-Val-(D)-Pro-OMe as a white amorphous solid. ^{1}H NMR ($CDCl_3$) δ: 0.80–1.68 (23H, m), 1.72–2.30 (6H, m), 3.72 (3H, s), 3.62–3.88 (2H, m), 3.96 (1H, m), 4.50 (1H, dd, *J* = 8.7, 4.8 Hz), 4.61 (1H, dd, *J* = 8.7, 6.6 Hz), 4.97 (1H, d, *J* = 8.7 Hz), 6.52 (1H, d, *J* = 8.7 Hz).

3.11. Boc-(D)-Ile-(D)-Val-(D)-Pro-(D)-Pro-NH_2

To a solution of Boc-(D)-Ile-(D)-Val-(D)-Pro-OMe (0.75 g, 1.7 mmol) in MeOH (15 mL) was added 1 M NaOH (2.0 mL) and the mixture was stirred at 25 °C for 2 days. The organic solvent was rotary evaporated and the residual aqueous solution was diluted with H_2O, and washed with AcOEt. The aqueous solution was acidified with 1 N HCl and extracted with AcOEt. The organic layer was washed with H_2O, dried over $MgSO_4$, and rotary evaporated to yield 0.71 g (98%) of Boc-(D)-Ile-(D)-Val-(D)-Pro-OH. ^{1}H NMR ($CDCl_3$) δ: 0.83–1.60 (23H, m), 1.72–2.38 (6H, m), 3.67 (1H, m), 3.85 (1H, m), 4.01 (1H, m), 4.53 (1H, dd, *J* = 7.5, 5.7 Hz), 4.60 (1H, dd, *J* = 8.7, 7.5 Hz), 5.13 (1H, d, *J* = 8.7 Hz), 6.98 (1H, d, *J* = 8.7 Hz).

Boc-(D)-Pro-NH_2 (0.21 g, 1.0 mmol) was treated with TFA as above. The product was dissolved with CH_2Cl_2 (5 mL) and neutralized with iPr_2NEt. To the solution were added Boc-(D)-Ile-(D)-Val-(D)-Pro-OH (0.39 g, 0.92 mmol), DPPA (0.22 mL, 1.2 mmol) and iPr_2NEt (0.19 mL, 1.1 mmol), and the mixture was stirred at 25 °C for 2 days. The organic layer was washed with 10% citric acid, sat. Na_2CO_3, and H_2O, dried over $MgSO_4$, and rotary evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 50:1) to yield 0.40 g (56%) of Boc-(D)-Ile-(D)-Val-(D)-Pro-(D)-Pro-NH_2. ^{1}H NMR ($CDCl_3$) δ: 0.80–1.60 (23H, m, 1.60–2.66 (8H, m), 3.38–4.67 (8H, m), 5.08–5.50 (1H, m), 6.60–6.85 (1H, m), 7.62 (1H, br s), 8.13–8.78 (1H, m).

3.12. Type II RI inhibitor (3a) and diasteromers (3b, 3c, and 3d)

To a stirred solution of **18** (16 mg, 0.05 mmol) in THF (3 mL) were added WSCDI HCl (12 mg, 0.06 mmol) and HOBt (11 mg, 0.06 mmol), and the mixture was stirred at 25 °C for 1 h. H-(D)-Ile-(D)-Val-(D)-Pro-(D)-Pro-NH_2, prepared from Boc-(D)-Ile-(D)-Val-(D)-Pro-(D)-Pro-NH_2 (150 mg, 0.30 mmol) and TFA (1 mL), was dissolved in DMF, neutralized with iPr_2NEt, and added to the above solution of the activated ester of **18**. The mixture was stirred at 25 °C for 16 h, and diluted with AcOEt. The organic phase was washed with 10% citric acid, sat.d Na_2CO_3, and H_2O, dried over $MgSO_4$, and rotary evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 30:1) to yield 32 mg (89%) of **24**. 28 mg (0.04 mmol) of **24** was then treated with TFA as before, and the residue was dissolved with CH_2Cl_2 (3 mL) and neutralized with iPr_2NEt. To the solution were added benzylisocyanate (7.0 μL, 0.06 mmol) and iPr_2NEt (7.0 μL, 0.04 mmol), and the mixture was stirred at 25 °C for 1.5 h. The solvent was evaporated in vacuo and the residue was dissolved in AcOEt. The organic layer was washed with 1 M HCl, sat.Na_2CO_3, and H_2O, and dried over $MgSO_4$. The organic solvent was rotary evaporated, and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 30:1) to yield 25 mg (86%) of partially purified **3a**. The product was further purified by preparative HPLC [YMC Pro C18 column (10 × 250 mm), CH_3CN (40–80%/60 min) in 0.1% aq TFA, 2.5 mL/min] to yield homogeneous **3a** as a white amorphous powder (overall 43%): HPLC, 12.08 min [Cosmosil 5C18-ARII column (4.6 × 150 mm), 1.0 mL/min, CH_3CN (30–60%, 30 min)], ^{1}H NMR ($CDCl_3$) δ: 0.77–1.08 (18H, m), 1.08–2.65 (22H, m), 3.15–4.68 (14H, m), 6.32–7.00 (2H, m), 7.23–7.38 (5H, m). MALDI TOF MAS, *m*/*z* 790.297 for $[M+Na]^+$ (calcd 790.485 for $C_{41}H_{65}O_7N_7Na$).

Diasteromers **3b**, **3c**, and **3d** were similarly prepared from **19**, **20**, and **21**, respectively. Compound **3b**; HPLC, 15.22 min [Cosmosil 5C18-ARII column (4.6 × 150 mm), 1.0 mL/min, CH_3CN (30–60%, 30 min)], ^{1}H NMR ($CDCl_3$) δ: ^{1}H NMR ($CDCl_3$) δ: 0.82–1.17 (18H, m), 1.34–2.63 (22H, m), 3.15–3.80 (7H, m), 3.98 (1H, m), 4.18–4.65 (6H, m), 6.88–7.18 (2H, m), 7.23–7.38 (5H, m). MALDI TOF MAS, *m*/*z* 790.893 for $[M+Na]^+$ (calcd 790.485 for $C_{41}H_{65}O_7N_7Na$). **3c**; HPLC, 15.83 min [Cosmosil 5C18-ARII column (4.6 × 150 mm), 1.0 mL/min, CH_3CN (30–60%, 30 min)], ^{1}H NMR ($CDCl_3$) δ: 0.75–1.14 (19H, m), 1.22–2.63 (21H, m), 3.14–3.33 (2H, m), 3.45–3.60 (2H, m), 3.62–3.80 (2H, m), 4.07 (1H, m), 4.18–4.65 (7H, m), 6.44 (1H, m), 7.23–7.39 (5H, m), 7.56 (1H, m). MALDI TOF MAS, *m*/*z* 790.301 for $[M+Na]^+$ (calcd 790.485 for $C_{41}H_{65}O_7N_7Na$). Compound **3d**; HPLC, 13.59 min [Cosmosil 5C18-

ARII column (4.6 × 150 mm), 1.0 mL/min, CH_3CN (30–60%, 30 min)], ^1H NMR ($CDCl_3$) δ: 0.78–1.23 (19H, m), 1.23–2.68 (21H, m), 3.15–4.92 (14H, m), 6.60–7.03 (2H, m), 7.18–7.38 (5H, m). MALDI TOF MAS, *m*/*z* 790.316 for $[M+Na]^+$ (calcd 790.485 for $C_{41}H_{65}O_7N_7Na$).

3.13. Measurement of inhibitory activity

Enzyme assays were carried out using the synthetic HTLV-1 protease mutant[14] at an enzyme concentration of 5.6 nM. The reaction mixture (0.5 M sodium acetate buffer, pH 5.6, containing 10% glycerol, 10 mM DTT and 4 M NaCl) was analyzed on a Cosmosil 5C18-ARII column (4.6 × 150 mm), employing a linear gradient of CH_3CN (10–40%, 30 min) in aq 0.1% TFA. Each IC_{50} value was obtained from a sigmoidal dose–response curve[15] obtained from the decrease of the substrate in the reaction mixture. Each experiment was repeated three times.

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Supplementary data

Supplementary data (DL analyses of type I RI inhibitors, a typical sigmoidal dose-response curve, and HPLC chromatogram for compounds **3a**–**d**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.02.019.

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