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Design and Synthesis of Novel Protease Inhibitors. Tripeptide α',β'-Epoxyketones as Nanomolar Inactivators of the Proteasome.

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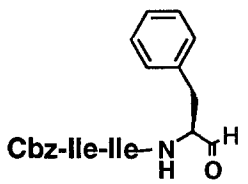
*Abstract: Tripeptide α',β'-epoxyketones were prepared stereospecifically starting from Boc-[S]-phenylalanine. Diastereomer **5b** inhibited the chymotrypsin-like activity of porcine endothelial cell derived proteasome at low nanomolar concentrations.*

The proteasome is a high molecular weight (ca. 700 kD) cytoplasmic enzyme complex composed of at least 14 different subunits and possessing five separate hydrolytic activities one of which was recently classified as a threonine protease.[1] In addition to its "housekeeping function" in cell protein degradation, the proteasome has been implicated in a variety of disease states ranging from immune diseases (inflammation, asthma)[2] to cancer.[3]

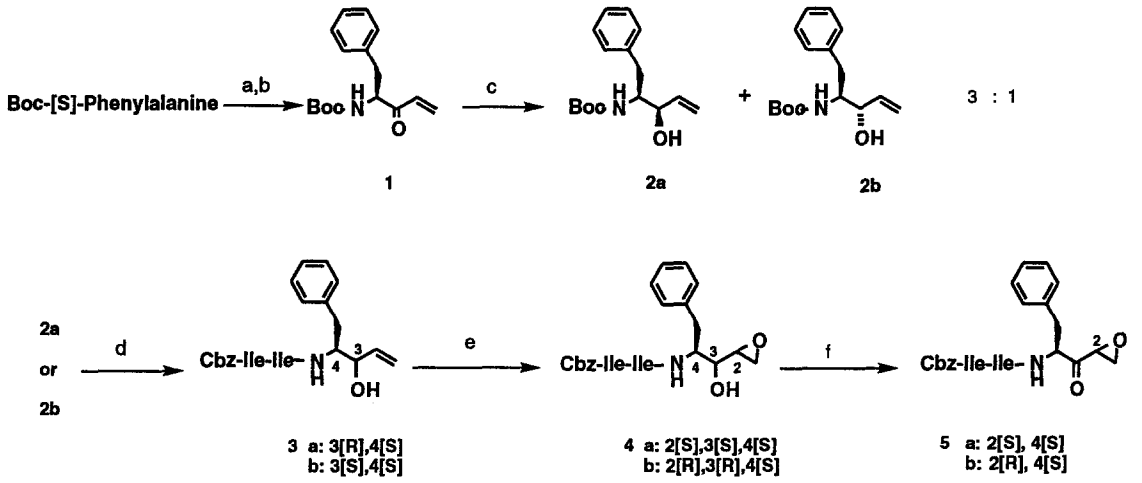
One approach to develop inhibitors for such proteases is to utilize part of the peptide sequence of a known substrate and attach a reactive moiety which is capable of inactivating the enzyme when bound to the active site.[4] A large number of such substrate-derived functionalities have been used as potential serine- and thiolprotease inhibitors. Among the more frequently utilized ones are aldehydes[5], chloromethyl ketones,[6] trifluoromethyl ketones,[7] α-keto-acids, -esters, and -amides.[8] Most of these compounds inhibit the enzyme either by mimicking the tetrahedral nature of the transitionstate of the enzymatic reaction (hydrated trifluoromethyl ketones), or by irreversibly alkylating a nucleophilic residue, usually a serine-, cysteine- or histidine-sidechain in the active site (chloromethyl ketones).

Here we report the synthesis of tripeptide α',β'-epoxyketones, a novel class of substrate-derived inhibitors of serine-type proteases. We reasoned that this class of compounds might be able to act either as a transitionstate mimetic due to their activated ketone functionality, or as a covalent irreversible inhibitor via alkylation of the enzyme with the reactive epoxide function. Based on BW2428, one of our most potent (IC_{50}= 200nM) proteasome inhibitor leads[9], we sought to introduce the α',β'-epoxyketone functionality into the peptide sequence Ile-Ile-Phe.



BW2428

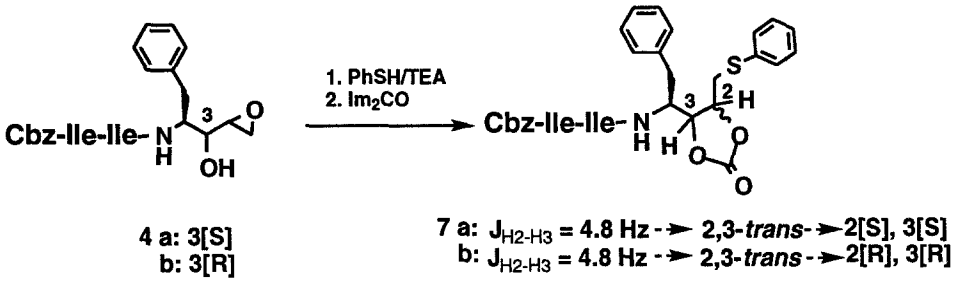
The synthesis of the targets, **5a** and **5b**, proceeded as follows (Scheme 1).[10,11] Boc-[S]-phenylalanine was converted to the Weinreb amide[12] and treated with vinylmagnesium bromide to afford vinylketone **1** in good yield. Reduction with sodiumborohydride / cerium chloride[13] gave a 3:1 mixture of diastereomers **2a** and **2b** which were separated on silica gel. The absolute stereochemistry at carbon C-3 was established as follows: treatment of **2a** and **2b** respectively with 1M HCl/dioxane, followed by cyclization with carbonyl diimidazole gave the two oxazolidinones **6a** and **6b**, which were analyzed by spectroscopic means and assigned as indicated.[14]



Scheme 1 Reagents and conditions: a. MeNHOMe x HCl, EDCI, NMM, HOBt, DMF, 0°, 12h, 80% b. vinylMgBr, THF, rt, 2h, 78% c. $NaBH_4$, $CeCl_3x7H_2O$, MeOH, 0°, 0.2h, 94%, then separation on silica gel (EtOAc:Hexane 1:3) d. *i.* TFA / CH_2Cl_2, 0°, 3h *ii.* Cbz-Ile-Ile-OSu / NMM, EtOAc,0°, 2h, 50% (2 steps) e. 6 equiv. mCPBA, $CHCl_3$, 0°, 8h, 69% f. DMSO / Ac_2O 5:1, 25°, 12-36h, 80%

Deprotection of the two isomers **2a** and **2b** with trifluoroacetic acid, followed by coupling with the N-hydroxysuccinimide ester[15] of Cbz-isoleucyl-isoleucine afforded the vinyl alcohols **3a** (50%, two steps) and **3b** (53%, two steps). The epoxidation of **3a** and **3b** with m-CPBA was effected in chloroform using 6 equivalents of the oxidant to give **4a** (67%) and **4b** (69%) respectively. In both cases, we only observed a single diastereomer from the epoxidation reaction. Oxidation of the epoxyalcohols **4a** and **4b** with DMSO / acetic anhydride[16] gave the desired epoxyketones **5a** and **5b** in 78% and 80% yields. The stereochemical assignment at the epoxide carbon C-2 was carried out as outlined in Scheme 2. Opening of the epoxides **4a** and **4b** with thiophenol[17], followed by treatment of the resulting diols with carbonyl diimidazole gave the cyclic carbonates **7a** and **7b**. ^1H NMR analysis showed a coupling constant J_{H2-H3} of 4.8 Hz for both isomers. MM2 calculations for the two possible ring configurations indicate a dihedral angle of 122° for the *trans* isomer and 2° for the *cis* isomer. This clear difference permits the assignment for **7a,b** as the *trans* isomers with a reasonable degree of certainty (a dihedral angle of

near 0° for the *cis* configuration would give rise to a significantly larger coupling constant)[18] and thus establishes the C-2 configuration of **7a** as [S] and of **7b** as [R].[19]



Scheme 2

Initial biological studies[20] showed that compound **5b** inactivates 50% of the proteasome activity at an inhibitor concentration of 5 nanomolar, while diastereomer **5a** was found to be at least 50-fold less potent. Preliminary kinetic results indicate, that the highly active isomer **5b** is a covalent, irreversible inhibitor of the proteasome. Further kinetic studies are in progress and will be reported in due course.

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10 all compounds gave NMR, and high- or low resolution MS data consistent with the proposed structures as well as satisfactory combustion analyses.

11. Abbreviations are: EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, NMM (N-methyl morpholine, DCC (dicyclohexyl carbodiimide), TFA (trifluoroacetic acid), mCPBA (m-chloroperbenzoic acid), HOBT (N-hydroxybenzotriazole), Cbz (benzyloxycarbonyl), Ile (L-isoleucine), Boc (tert.-butoxycarbonyl).

Spectral Data for selected compounds are as follows: ^{1}H NMR(DMSO-d6): **2a** 1.22(9H,s), 2.58(1H,d), 3.50(1H,m), 3.86(1H,dd), 5.03(2H,dd), 5.20(1H,d), 5.85(1H,dddd), 6.59(1H,d), 7.2(5H,m). **2b**1.22(9H,s), 2.52(1H,d), 2.78(1H,dd), 3.62(1H,m), 3.92(1H,dd), 5.00(1H,d), 5.05(1H,d), 5.20(1H,d), 5.82(1H,dddd), 6.42(1H,d), 7.2(5H,m). **3a** 0.7-0.9(12H,m) 1.10(2H,m), 1.40(2H,m), 1.70(2H,m), 2.70(1H,dd), 2.95(1H,dd), 3.95(3H,m), 4.22(1H,t), 5.08(2H,s), 5.12(1H,d), 5.28(1H,d), 5.96(1H,dddd), 7.2-7.4(10H,m), 7.45(1H,d), 7.75(1H,d), 7.82(1H,d). **3b** 0.6-0.8(12H,m), 1.00(2H,m), 1.30(2H,m), 1.65(2H,m), 2.50(1H,dd), 2.83(1H,dd), 3.90(1H,t), 3.99(2H,m), 4.18(1H,t), 5.00(2H,s), 5.02(1H,d), 5.18(2H,dd), 5.83(1H,dddd), 7.1-7.4(11H,m), 7.65(2H,m). **4a** 0.65-0.85(12H,s), 1.0(1H,m), 1.18(1H,m), 1.35(1H,m), 1.45(1H,m), 1.65(1H,m), 1.78(1H,m), 2.64(2H,m), 2.76(1H,dd), 2.95(1H,m), 3.01(1H,dd), 3.13(1H,dd), 3.97(1H,t), 4.08(1H,dd), 4.18(1H,t), 5.08(2H,s), 7.2-7.4(10H,m), 7.45(1H,d), 7.71(1H,d), 7.83(1H,d). **4b** 0.6-0.8(12H,m), 1.05(2H,m), 1.35(2H,m), 1.65(2H,m), 2.45(1H,dd), 2.62(2H,m), 2.82(2H,m), 3.05(1H,m), 3.85(1H,t), 4.04(1H,m), 4.18(1H,t), 4.99(2H),s), 5.38(1H,d), 7.1-7.4(11H,m), 7.72(1H,d), 7.80(1H,d). **5a** 0.80(12H,m), 1.15(2H,m), 1.35(2H,m), 1.65(2H,m), 2.50(1H,dd), 2.80(2H,m), 3.05(1H,dd), 3.66(1H,m,α'-H), 3.93(1H,t), 4.20(1H,t), 4.65(1H,m), 5.00(2H,s), 7.2--7.4(10H,m), 8.15(1H,d), 8.55(1H,d). **5b** 0.78(12H,m), 1.05(2H,m), 1.35(2H,m), 1.65(2H,m), 2.79(2H,m), 2.98(2H,m), 3.62(1H,m,α'-H), 3.85(1H,t), 4.18(1H,t), 4.58(1H,m), 5.00(2H,s), 7.2-7.4(10H,m), 7.68(1H,d), 8.44(1H,d).

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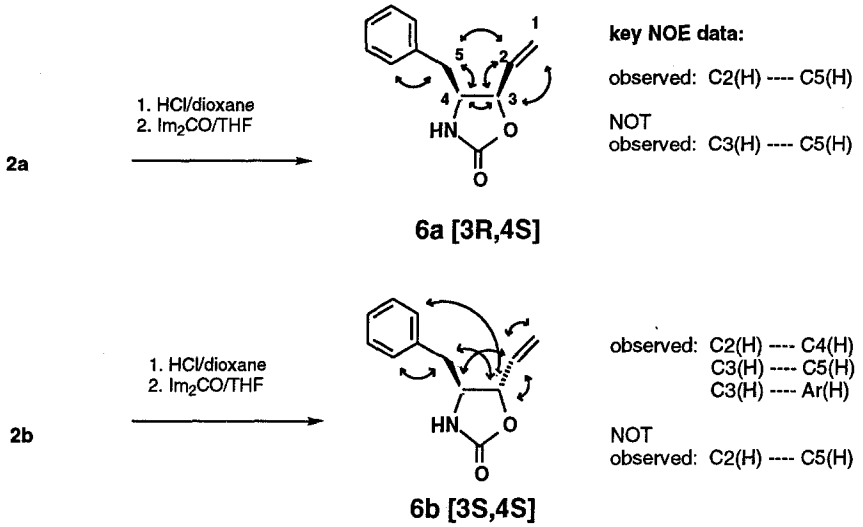

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19. We are currently attempting to obtain X-ray quality crystals of **4** and/or **7** to further substantiate the C-2 stereochemical assignment.
20. Fluorescence-based assay (Z-IIW-AMC substrate), the proteasome was purified from pig aorta endothelial cells. The enzyme was pre-incubated with the inhibitor for 1 hour before the reaction was initiated by addition of the substrate.

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