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Synthesis of a new class of cathepsin B inhibitors exploiting a unique reaction cascade

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Abstract

Chiral 5-substituted 3-pyrrolin-2-ones bearing L-Ile-L-Pro-OH or L-Phe-NHCH$_2$Ph were successfully synthesized by utilizing a characteristic reaction cascade based on alkaline hydrolysis of the corresponding diethyl α-hydroxy-α-(β-propiolamide)malonates. Among the synthesized chiral pyrrolinones, compound 5*S*-**9** proved to be the most potent inhibitor against cathepsin B. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: alkynes; cyclization; enzyme inhibitor; lactams; X-ray crystal structures.

Cathepsins B, H, L, S, and K are mammalian lysosomal cysteine proteases that belong to the papain superfamily. These cathepsins most probably play an important role in the regulation of the amount of specific enzymes and hormones. Katunuma and colleagues have discussed the participation of cathepsin B in antigen processing and the important role of cathepsin L in bone collagenolytic activity in bone resorption.[1] Our recent focus of research has been the development of new specific inhibitors against cathepsin B.[2] There have been several epoxysuccinic acid derivatives bearing the L-isoleucyl-L-proline (L-Ile-L-Pro-OH) moiety as the irreversible inhibitors to cathepsin B;[3] many kinds of peptidyl aldehydic reversible and other irreversible inhibitors to various cathepsins.[4] However, novel characteristic lead compounds are still extensively required in the research field of specific enzyme inhibitors against several selected cathepsins. We have focused on the functionalized chiral 3-pyrrolin-2-one derivatives as the new active center moiety of various SH-enzyme inhibitors on the basis of earlier research on tenuazonic acid and *N*-ethylmaleimide, and to develop an efficient synthetic methodology for them.[5] We describe here the synthesis of a new class of cathepsin B inhibitors, 3-pyrrolin-2-one L-Ile-L-Pro-OH derivatives, which can be obtained by a unique reaction cascade exploiting the particular trigger moiety, diethyl α-alkynylmalonate. This diethyl α-alkynylmalonate trigger system could be useful for the construction of

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a conjugated allenyl ester system under mild basic conditions in an aqueous solution, as it has been efficiently utilized to inhibit SH-enzymes,[2a] oxazole ring formation via 5-*endo*-mode cyclization,[2a] and Myers-type cycloaromatization of some diethyl α-enediynyl-α-methoxymalonates.[6]

Based on the background described above and on a series of studies of the cyclization reactions of the conjugated allenyl esters and ketones,[2,7] we anticipated a novel reaction cascade for the conversion of diethyl α-hydroxy-α-(β-propiolamide)malonates **A** into 3-pyrrolin-2-one derivatives **E**, as shown in Fig. 1. Namely, alkaline hydrolysis of **A**, followed by decarboxylation, will initiate the desirable reaction cascade to generate cumulenolates **B** and then hydroxy allenyl esters **C**. Cyclization in the resultant *Z*-olefinic ketones **D**, obtained by stereospecific protonation at the sterically less hindered side of the enol moiety of **C**, will afford 5-substituted 3-pyrrolin-2-ones **E**.

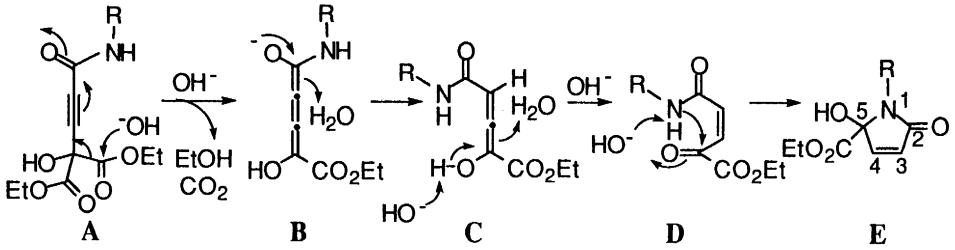
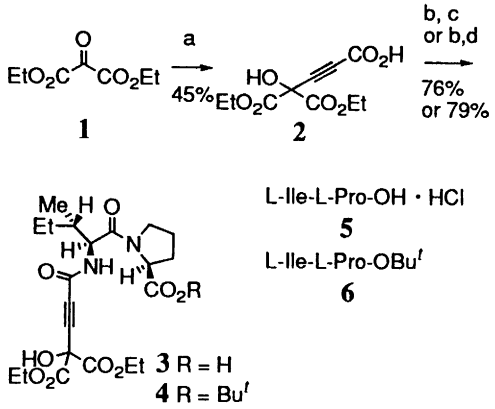


Fig. 1. Possible reaction cascade from diethyl α-alkynylmalonates **A** into 3-pyrrolin-2-ones **E**

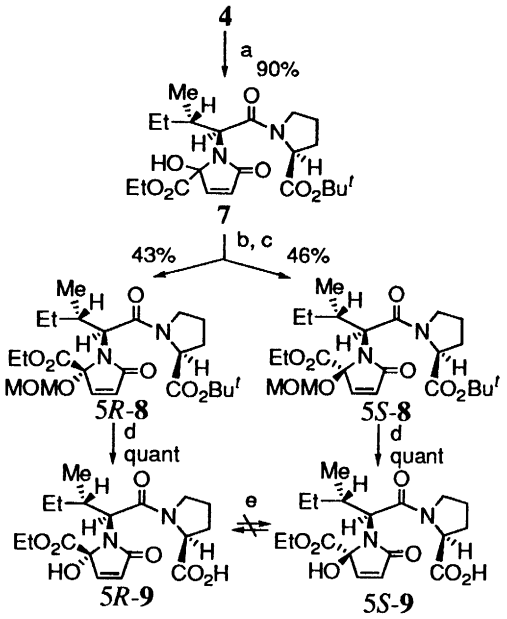
Thus, the diethyl α-hydroxy-α-(β-propiolamide)malonates **3** or **4** were readily synthesized by a reaction of diethyl ketomalonate **1** with 3-lithiopropiolic acid lithium salt followed by dehydrative condensation [isobutyl chloroformate (IBCF) and *N*-methylmorpholine (NMM)] of the resultant **2** with L-Ile-L-Pro-OH·HCl **5** or L-Ile-L-Pro-OBu^t **6** obtained by conventional dipeptide synthetic methodology (Scheme 1). Compound **4** was submitted to alkaline hydrolysis with 1N KOH in EtOH at 0°C for 30 min to give 3-pyrrolin-2-one **7** as an inseparable diastereomeric mixture in 90% yield. After treatment of **7** with methoxymethyl chloride (MOMCl) in the presence of the Hünig base, the corresponding MOM-diastereomers, 5*R*-**8** and 5*S*-**8**, could be separated by silica gel column chromatography with hexane:AcOEt (1:1) in 43% yield [5*R*-**8**: mp 107–108°C, $[\alpha]_D^{25}$ −91.4 (*c* 1.03, $CHCl_3$)] and 46% yield [5*S*-**8**: mp 110.5–111.5°C, $[\alpha]_D^{24}$ −106.7 (*c* 0.83, $CHCl_3$)] as colorless needles from CH_2Cl_2–hexane, respectively (Scheme 2). The absolute configuration of C5 of 5*S*-**8** was determined by its X-ray crystallographic analysis (Fig. 2),[8] and, therefore, another diastereomer is 5*R*-**8**. Removal of MOM and the *t*-Bu groups of 5*R*-**8** and 5*S*-**8** employing CF_3CO_2H proceeded smoothly to give 5*R*-**9** [colorless amorphous powder, $[\alpha]_D^{23}$ −144.3 (*c* 1.44, $CHCl_3$)] and 5*S*-**9** [colorless amorphous powder, $[\alpha]_D^{23}$ −97.6 (*c* 0.54, $CHCl_3$)] in a quantitative yield, respectively.

Similar alkaline hydrolysis of **3** furnished 3-pyrrolin-2-one **10** as a diastereomeric mixture in 86% yield. Methoxymethylation of the mixture **10**, followed by chromatographic separation of the resultant C5–MOM derivatives on a silica gel column with hexane:AcOEt (1:3), gave the corresponding bis MOM derivatives, 5*R*-**11** [colorless oil, $[\alpha]_D^{22}$ –144.2 (*c* 2.84, $CHCl_3$)] in 43% yield and 5*S*-**11** [colorless oil, $[\alpha]_D^{23}$ −88.0 (*c* 1.68, $CHCl_3$)] in 27% yield. On the other hand, treatment of **10** with triethylsilyl (TES) chloride in the presence of dimethylaminopyridine (DMAP) exclusively yielded the TES derivative 5*R*-**12** [colorless needles, mp 43–44°C, $[\alpha]_D^{24}$ –173.5 (*c* 0.49, $CHCl_3$)] in 55% yield. Its 5*S* diastereomer might decompose through the silylation reaction. The stereochemistry of newly formed chiral C5 in the compounds 5*R*-**11**, 5*S*-**11** and 5*R*-**12** was determined by chemical conversion of 5*R*-**11** and 5*R*-**12** to the known compound 5*R*-**9**, as shown in Scheme 3.

Subsequently, we have tentatively prepared 3-pyrrolin-2-one derivatives bearing the L-Phe-$NHCH_2Ph$ group. A precursor **13** [colorless oil, $[\alpha]_D^{25}$ +6.4 (*c* 0.52, $CHCl_3$)], obtained from the conventional



Scheme 1. (a) Li–≡–CO_2Li/THF/−78°C→rt; (b) IBCF/NMM/THF/−16–−18°C; (c) **5**/NMM/THF–DMF/−18°C→rt; (d) **6**/THF/−16°C→rt



Scheme 2. (a) 1N KOH/EtOH/0°C; (b) MOMCl/DIEA/CH_2Cl_2/rt; (c) silica gel column/hexane:AcOEt (1:1); (d) CF_3CO_2H/0°C; (e) acetate buffer (pH 5.5)/37°C

dehydrative condensation reaction of **2** with L-Phe-$NHCH_2Ph$ **14**, was allowed to react with 1N KOH in EtOH at 0°C for 40 min. The desirable cascade reaction proceeded in a highly diastereoselective manner to afford 5*R*-**15** [colorless amorphous powder, $[\alpha]_D^{23}$ +5.1 (*c* 0.93, $CHCl_3$)] in 72% yield as the major product with the minor 5*S* diastereomer in a 9.1:0.9 ratio (^{1}H NMR analysis). The absolute configuration of chiral C5 in 5*R*-**15** was established by X-ray analysis (Fig. 2)[8] of its crystalline MOM derivative 5*R*-**16** [mp 115–117°C (CH_2Cl_2–hexane), $[\alpha]_D^{23}$ +19.5 (*c* 1.31, $CHCl_3$)], which was converted to 5*R*-**15** by treatment with 4N HCl in dioxane, as shown in Scheme 4.

Finally, we examined the inhibition of several 3-pyrrolin-2-one derivatives, namely 5*R*-**8**, 5*S*-**8**, 5*R*-**9**, 5*S*-**9**, 5*R*-**11**, 5*S*-**11**, 5*R*-**12** and 5*R*-**15**, against enzymatic hydrolysis of *Z*-L-Phe-L-Arg-MCA (MCA: methylcoumarylamino group) with human liver cathepsin B and rat liver cathepsin L in sodium acetate buffer (pH 5.5) under the Barrett assay procedure.[9] Compounds 5*R*-**8** and 5*S*-**8** did not inhibit enzymatic

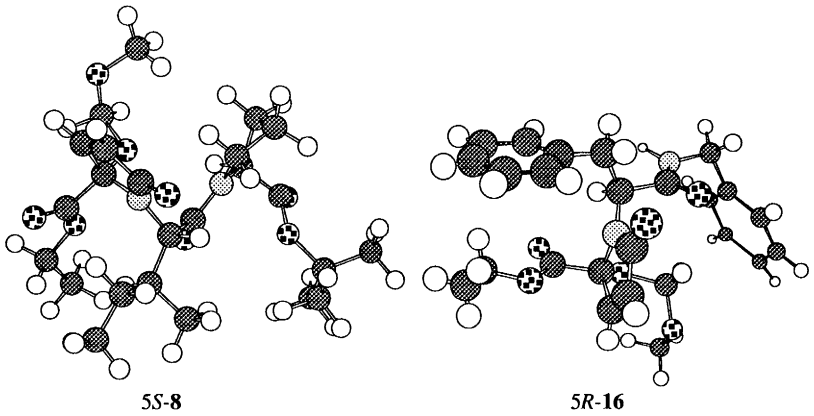
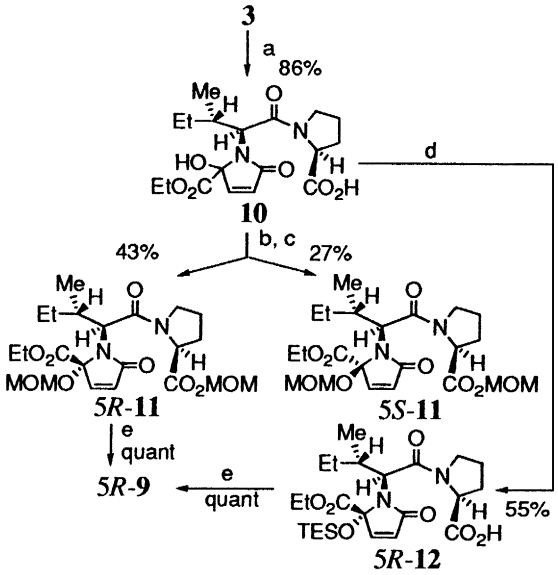
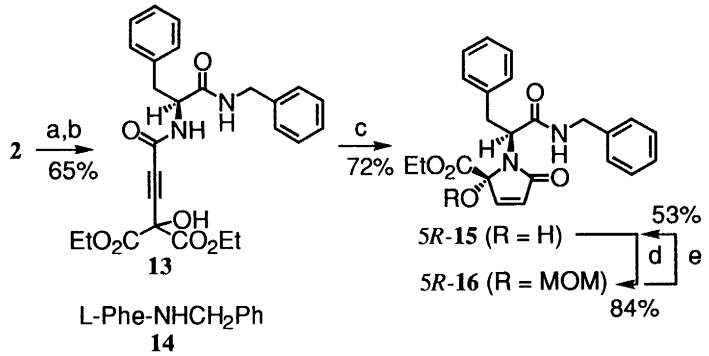


Fig. 2. Computer-generated drawing of 5*S*-**8** and 5*R*-**16** derived from the X-ray coordinates



Scheme 3. (a) 1N KOH/EtOH/0°C; (b) MOMCl/DIEA/CH_2Cl_2/0°C→rt; (c) silica gel column/hexane:AcOEt (1:3); (d) TESCl/DMAP/CH_2Cl_2/rt; (e) CF_3CO_2H/0°C



Scheme 4. (a) IBCF/NMM/THF/−18°C; (b) **14**/THF/−18°C→rt; (c) 1N KOH/EtOH/0°C; (d) MOMCl/DIEA/CH_2Cl_2/0°C→rt; (e) 4N HCl/dioxane/0°C

hydrolysis with both cathepsins B and L, even at their high concentration orders, 10^{-5} M and 10^{-6} M. Compounds 5*R*-**11**, 5*S*-**11**, and 5*R*-**12** exhibited weak inhibitory activities against cathepsin B (5*R*-**11**: 50.3% at 10^{-5} M, 5*S*-**11**: 40.7% at 10^{-5} M, 5*R*-**12**: 62.1% at 10^{-6} M) and cathepsin L (5*R*-**11**: 25.6% at 10^{-5} M, 5*S*-**11**: 50.5% at 10^{-5} M, 5*R*-**12**: 37.5% at 10^{-5} M), respectively. Other selected enzyme-inhibitory assay data are listed in Table 1. Among the 3-pyrrolin-2-one derivatives, compounds 5*R*-**9** and 5*S*-**9** bearing the 5-hydroxy and 5-ethoxycarbonyl groups exhibited selective inhibitory activities against cathepsin B in comparison with those against cathepsin L. Specifically, 5*S*-**9** proved to be the most potent inhibitor against cathepsin B. Because a maleimide derivative **17** did not exhibit any inhibitory activity against cathepsins B and L, the chiral 3-pyrrolin-2-one moiety, having the functional group(s) (e.g., OH, CO_2Et, etc.) at the C5 position, must be essential for the molecular recognition to the active center[10] in the cathepsin B. Interestingly, a 3-pyrrolin-2-one derivative 5*R*-**15** with L-Phe-$NHCH_2Ph$ exhibited higher inhibition values against cathepsin L than those against cathepsin B, as shown in Table 1.

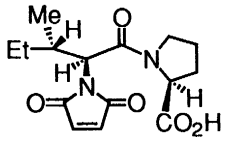


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Table 1
Inhibition with 3-pyrrolin-2-ones against cathepsins B and L

compound	cathepsin B		cathepsin L	
	conc (M)	inhibition (%)	conc (M)	inhibition (%)
5*R* - **9**	1×10^{-7}	20.4	1×10^{-7}	nd [a]
	1×10^{-6}	79.7	1×10^{-6}	0.0
	1×10^{-5}	95.8	1×10^{-5}	0.3
5*S* - **9**	1×10^{-7}	90.7	1×10^{-7}	nd [a]
	1×10^{-6}	98.7	1×10^{-6}	-5.0
	1×10^{-5}	99.8	1×10^{-5}	-0.3
5*R* - **15**	1×10^{-7}	17.8	1×10^{-7}	58.1
	1×10^{-6}	68.2	1×10^{-6}	91.5
	1×10^{-5}	96.5	1×10^{-5}	99.0

a) nd = not determined

Acknowledgements

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8. The crystallographic data of compounds 5*S*-**8** and 5*R*-**16** are as follows. For 5*S*-**8**: $C_{24}H_{38}N_2O_8$, *FW*=482.57, orthorhombic, space group $P2_12_12_1$ (#19), *a*=11.262(3) Å, *b*=21.019(4) Å, *c*=11.268(4) Å, *Z*=4, D_{calc}=1.202 g/cm^3, *V*=2667.4(10) Å^3, *R*=0.051; for 5*R*-**16**: $C_{25}H_{28}N_2O_6$, *FW*=452.51, orthorhombic, space group $P2_12_12$ (#18), *a*=15.413(6) Å, *b*=24.062(8) Å, *c*=6.650(4) Å, *Z*=4, D_{calc}=1.218 g/cm^3, *V*=2466(2) Å^3, *R*=0.041.
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