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Synthesis and Inhibitory Activity of Optically Active 2-Benzyl-3-mercaptopropanoic Acid against Carboxypeptidase A

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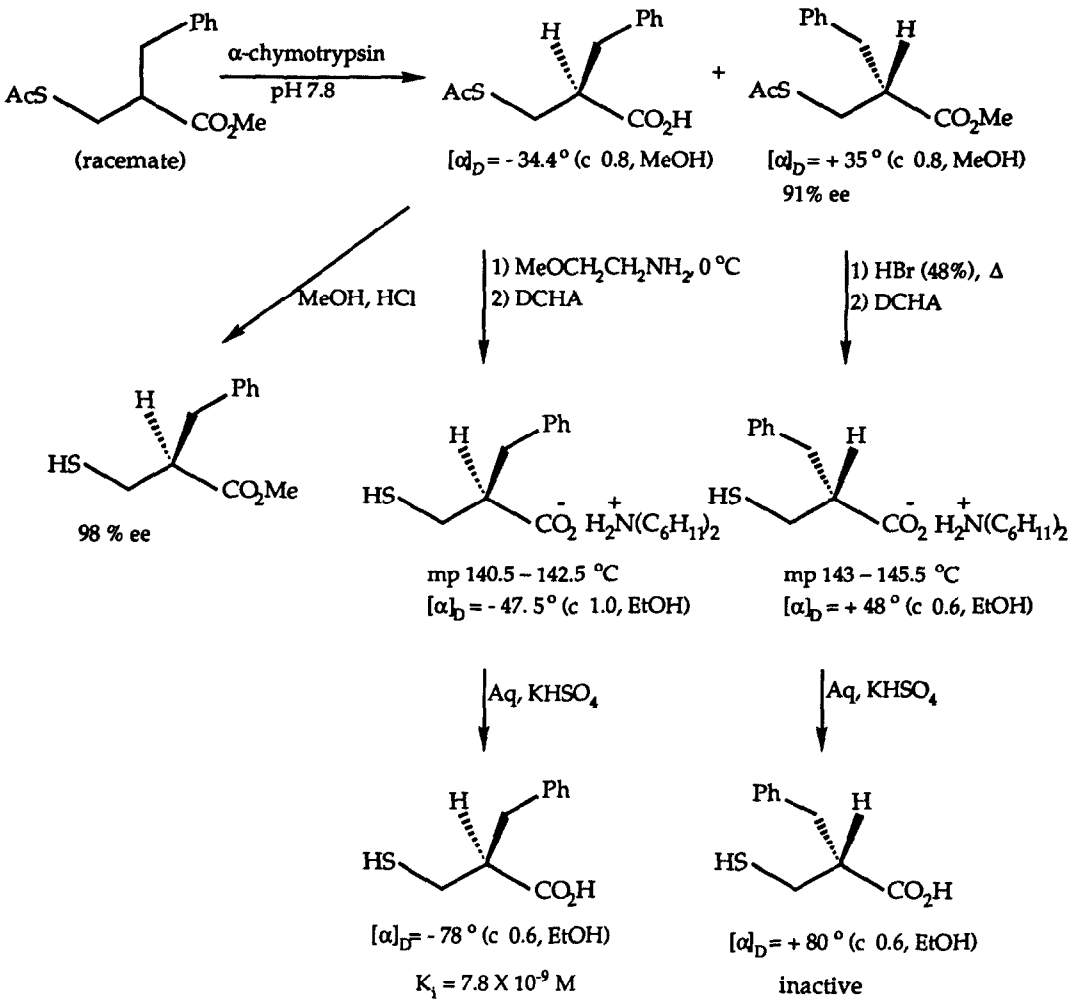
Abstract: Both enantiomers of 2-benzyl-3-mercaptopropanoic acid were synthesized starting with racemic 3-acetylthio-2-benzylpropanoic acid methyl ester using a kinetic resolution with α-chymotrypsin as a key step, and their inhibitory activities against carboxypeptidase A were determined to show that the *S*-isomer is much more potent ($K_i = 7.8 \times 10^{-9}$ M) than the racemic acid ($K_i = 1.1 \times 10^{-8}$ M).

Previously we have reported on the structural feature and the functional mode of the primary recognition pocket present at the S_1' subsite of carboxypeptidase A (CPA), which were put forward on the basis of K_i values obtained with a series of substrate analog inhibitors of the enzyme[1,2]. The inhibitors used at the time were, however, racemic form of 2-benzyl-3-mercaptopropanoic acids (BMPAs) having various substituents on the phenyl ring. This communication describes the syntheses of optically active BMPA and inhibitory activity of each enantiomer against the enzyme.

α-Chymotrypsin is an endopeptidase which hydrolyzes with L-stereospecificity the carboxyl side of the peptide bond of the amino acid residue having a hydrophobic side chain[3,4]. It also catalyzes the hydrolysis of simple esters of L-amino acids with a hydrophobic side chain[5] and structually related esters[6]. It was, therefore, envisioned that racemic esters of BMPA or its



Scheme 1

precusor may be resolved enzymatically using α-chymotrypsin. Indeed, when the methyl ester of racemic 3-acethylthio-2-benzylpropanoic acid[7] was treated with α-chymotrypsin for 12 hrs in the phosphate buffer solution of pH 7.8, a chemoselective and stereospecific hydrolysis of the ester moiety occurred, affording the corresponding acid with *S*-configuration in a high optical purity

(Scheme 1)[8]. The *R*-enantiomer remained intact. The unhydrolyzed methyl ester of the *R*-isomer was recovered by extraction with ether, and the *S*-acid was isolated from the aqueous layer after acidification with dilute hydrochloric acid. The acetyl group of the thioester of the resolved acid was then removed by the treatment with methoxyethylamine[9]. Treatment of the *R*-ester with hydrobromic acid resulted in hydrolysis of both ester functions. The mercapto acids thus obtained were purified as a dicyclohexylamine salt by recrystallizations, and the purified salts were distributed between ethyl acetate and 10% aqueous potassium bisulfate solution to generate optically active BMPA as an oil (Scheme 1).

The enantiomeric purity of the hydrolyzed 3-acetylthio-2-benzylpropanoic acid was determined as its methyl ester[10] to be 98% ee by the nmr method using $Eu(hfc)_3$, and the assignment of the *S*-absolute stereochemistry is based on the established L-stereospecificity of α-chymotrypsin[3-6, 11]. The stereochemical assignment was further supported by the optical rotation, $[\alpha]_D$ = - 34.4 ° (c 0.8, MeOH), of the product, which is in good agreement with reported values of $[\alpha]_D$ = - 36.4 ° (c 1.3, MeOH), and $[\alpha]_D$ = - 37.2 ° (c 0.62, EtOH) for the identical compound obtained by the chemical resolution using (-)-ephedrin[12] or (*R*)-IPAOL[13], respectively. The unhydrolyzed methyl ester of *R*-configuration had the ee value of 91%.

When inhibitory activities (K_i values) of both enantiomers against CPA were determined[14] using Hip-Phe as the substrate at pH 7.5, only the *S*-isomer was active, showing $K_i = 7.8 \times 10^{-9}$ M which compares favorably with $K_i = 1.1 \times 10^{-6}$ M of racemic BMPA[14]. On the other hand, no inhibitory effect was observed up to the concentration of 20 nM in the case of (*R*)-BMPA. This enantioselective inhibitory activity of the *S*-isomer is in accord with the L-stereospecificity of CPA, demonstrating that the enzyme has a distinctive propensity toward the *S*-isomer in binding.

In summary, we have synthesized the potent competitive inhibitor of CPA, 2-benzyl-3-mercaptopropanoic acid in an optically active form, and determined their inhibitory activity against CPA to find that the *S*-isomer is considerably more potent than the racemic form. The kinetic resolution of racemic 3-acetylthio-2-benzylpropanoic acid methyl ester using α-chymotrypsin constitutes a key step for the enantioselective synthesis of the BMPA.

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