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PAPAIN-CATALYZED SYNTHESIS OF PEPTIDE ISOSTERES

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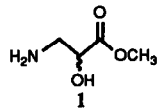
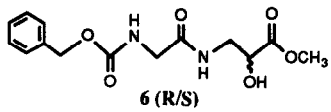
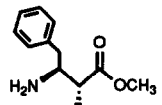
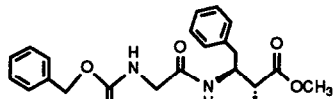
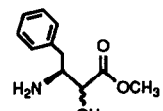
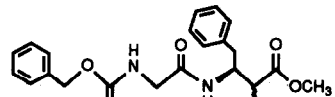
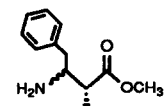
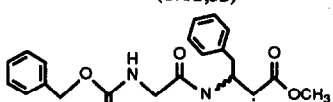
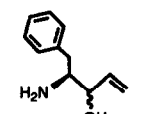
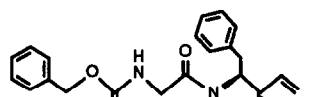
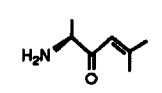
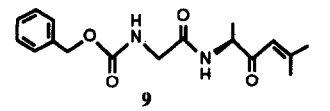
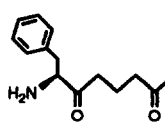
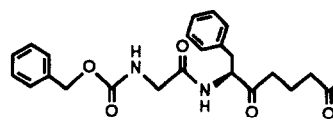
Abstract: The thiol protease papain catalyses the transfer of the Cbz-Gly moiety from the donor substrate Cbz-Gly-OMe to the amino group of various α-hydroxy- and α-keto-amines, providing a new route to peptide isosteres.

Isosteres of natural peptides, especially of dipeptides, are valuable building blocks for the synthesis of mechanism-based protease inhibitors[1] and proteolytically stable peptides.[2] As more and more peptide isosteres are being discovered as potent protease inhibitors, considerable effort has been directed towards the development of efficient and practical procedures for the synthesis of this class of compounds on large scales for therapeutic evaluation.[3]

This initial study investigates the ability of proteases to catalyze the formation of peptide bonds[4] involving the amino group of hydroxy-amines and keto-amines. The thiol protease papain was chosen as catalyst due to its efficient catalysis of the acyl transfer to amine nucleophiles.[5] Synthetic reactions[6] were carried out in a kinetically controlled approach. The acyl donor Cbz-Gly-OMe which meets the primary binding requirement of papain[7] was chosen as a donor substrate in this study. The reactions were performed in 50% methanol in order to provide high concentrations of both the acyl donor and the acyl acceptor in the reaction mixture.

The α-hydroxy- and α-keto-amines prepared[8] as well as the yield and enantioselectivity in papain-catalyzed reactions are given in Table 1. The conversion of hydroxy-amine **1** demonstrates the general applicability of papain to the synthesis of peptide isosteres. Interestingly, the methyl ester moiety of both the amine substrate and the reaction product are not attacked by papain; in contrast, the acyl donor ester is a substrate for the enzyme. This observation suggests that the α-hydroxycarboxyl moiety is bound to the active site with low affinity and/or in a nonproductive manner. The synthesis yield is increased by introduction of a benzyl group at the β-carbon of the amine nucleophile (diasteriomers of **2** in Table 1), probably due to improved binding to the hydrophobic P_1'-subsite of papain. As demonstrated in Table 1, the acyl enzyme does not significantly discriminate between any diastereomer of **2**. This finding is in

Table 1. Synthesis of peptide isosteres and enantioselectivity in papain-catalyzed reactions using Cbz-Gly-OMe as acylating agent..

amine nucleophile	reaction product	enantioselectivity (R/S)[9]	yield (%)
 1	 6 (R/S)	not determined	25
 2 (2R,3S)	 7 (2R,3S)	-	37
 2 (2R/S,3S)	 7 (2R/S,3S)	0.8	47
 2 (2R,3R/S)	 7 (2R,3R/S)	0.9	48
 3	 8 (3R,S)	0.6	45
 4	 9	-	63
 5	 10	-	23

contrast to the expressed enantioselectivity of acyl papains towards D/L-amino acid amides.[5] The observation indicates that the nucleophiles are bound to the acyl enzyme in a distorted manner compared to analogous natural amino acid derivatives. Low enantioselectivity is also observed when the racemic compound **3** is used as acyl acceptor. The keto-amines **4** and **5** show a similar substrate behaviour as the hydroxy-amines discussed above.

Conclusions

It was demonstrated that the amino group of hydroxy-amines as well as of keto-amines can be acylated in a papain-catalyzed reaction to prepare peptide isosters. No reaction takes place at the hydroxy function of the hydroxy-amines, and remarkably, papain does not discriminate between the four diastereomeres of Phe-bestatin type isostere **2**. Thus, a variety of stereochemically different peptide isosteres is accessible using this method with enantiomerically pure α-hydroxy-β-amino esters, α-hydroxy-amines or α-keto-amines as nucleophiles. Work is in progress to examine the scope of protease-catalyzed acylation of unnatural amino acids and peptides.

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4. for recent reviews on enzyme-catalyzed peptide synthesis see : Schellenberger, V. and Jakubke, H.-D. *Angew. Chem.* **1991**, *103*, 1440; Wong, C.-H. and Wang, K.T. *Experientia* **1991**, *47*, 1123.
5. Schuster, M., Aaviksaar, A. and Jakubke, H.-D. *Biochim. Biophys. Acta* **1992**, *1121*, 207.
6. In a typical experiment 0.1 mmol of acyl donor and 0.1 mmol of peptide isostere were dissolved in 100 µl methanol. After addition of 90 µl 1.5 M carbonate buffer (pH 9, containing 5 mM dithioeritritol) the pH was carefully adjusted to 8.5-9.0 using 6 M KOH. The reaction was started by addition of 5 mg papain (crude powder, SIGMA, lot 123F-0456). The reaction was followed by RP-HPLC (Pep-S column, Pharmacia; acetonitrile/0.1% TFA mixtures as eluents) and/or TLC. When the reaction was completed, the reaction mixture was extracted with ethyl acetate and washed with 0.1 M HCl, water, sat. sodium bicarbonate (except in case of **10**) and water. After removal of the solvent the product was separated by chromatography on Kieselgel using ethyl acetate/hexane mixtures as eluent and characterized by ^{1}H NMR, HRMS and Elemental Analysis:

6 (R/S): ^{1}H NMR (CD_3OD, 500MHz) δ 3.45 (dd, 1H, *J* = 6.5, 13.5Hz), 3.55 (dd, 1H, *J* = 5.0, 13.5Hz), 3.71 (s, 3H), 3.76 (s, 2H), 4.17-4.28 (m, 1H), 5.10 (s, 2H), 7.25-7.37 (m, 5H); calc. 54.17% C, 5.85% H, 9.03% N, found 53.76% C, 5.88% H, 8.50% N; HRMS ($M+H^+$): calc. 311.1243, found 311.1246.

7 (2R,3S): ^{1}H NMR ($CDCl_3$, 300MHz) δ 2.91 (d, 2H, *J* = 7.78Hz), 3.69 (s, 3H), 3.71 (dd, 1H, *J* = 5.5, 16.5Hz), 3.83 (dd, 1H, *J* = 5.8, 16.5Hz), 4.07-4.15 (m, 1H), 4.52-4.64 (m, 1H), 5.12 (s, 2H), 5.60-5.70 (m, 1H), 6.69 (d, 1H, *J* = 9.0Hz), 7.20-7.39 (m, 10H); calc. 62.99% C, 6.04% H, 7.00% N, found 62.91% C, 6.03% H, 7.11% N; HRMS ($M+H^+$): calc.533.0689, found 533.0694.

7 (2S,3S):. ^{1}H NMR ($CDCl_3$, 300MHz) δ 2.78 (d, 2H, *J* = 7.0Hz), 3.57 (s, 3H), 3.72 (dd, 1H, *J* = 5.3, 16.6Hz), 3.83 (dd, 1H, *J* = 5.7, 16.7Hz), 4.27-4.34 (m, 1H), 4.55-4.69 (m, 1H), 5.10 (s, 2H), 5.55-5.65 (m, 1H), 6.73 (d, 1H, *J* = 8.2Hz), 7.06-7.34 (m, 10H); calc. 62.99% C, 6.04% H, 7.00% N, found 63.32% C, 5.78% H, 7.11% N; HRMS ($M+Cs^+$): calc. 533.0689, found 533.0673.

8 (3R)[9]: ^{1}H NMR ($CDCl_3$, 300MHz), δ 2.83 (dd, 1H, *J* = 7.3, 13.4Hz), 2.94 (dd, 1H,*J* = 7.1, 13.3Hz), 3.73-3.82 (m, 2H), 4.10-4.22 (m, 2H), 5.10 (s, 2H), 5.13 (d, 1H, *J* = 9.9Hz), 5.23 (d, 1H, *J* = 17.1Hz), 5.55-5.64 (m, 1H), 5.73-5.88(m, 1H), 6.52 (d, 1H, *J* = 7.9Hz), 7.18-7.27 (m, 5H), 7.31-7.38 (m, 5H); calc. 68.46% C, 6.57% H, 7.60% N, found 68.47% C, 6.56% H, 7.59% N; HRMS ($M+Cs^+$): calc. 501.0790, found 501.0790.

8 (3S)[9]: ^{1}H NMR ($CDCl_3$, 300MHz), δ 2.62-2.92 (m, 2H), 3.53-3.80 (m, 3H), 4.20-4.30 (m, 2H), 5.06 (s, 2H), 5.23 (d, 1H, *J* = 10.6Hz), 5.34 (d, 1H, *J* = 17.2Hz), 5.70-5.81 (m, 1H), 5.81-5.95 (m, 1H), 6.61 (d, 1H, *J* = 8.5Hz), 7.09-7.26 (m, 5H), 7.27-7.83 (m, 5H); calc. 68.46% C, 6.57% H, 7.60% N, found 68.30% C, 6.65% H, 7.60% N; HRMS ($M+Cs^+$): calc. 501.0790, found 501.0795.

9:^{1}H NMR ($CDCl_3$, 300MHz) δ 1.34 (d, 3H, *J* = 7.1Hz), 1.95 (s, 3H), 2.20 (s, 3H), 3.85-3.95 (m, 2H), 4.50-4.62 (m, 1H), 5.13 (s, 2H), 5.45-5.55 (m, 1H), 6.08 (s, 1H), 6.88-6.97 (d, 1H, *J* = 5.54Hz).7.28-7.31 (m, 5H); calc. 64.13% C, 6.97% H, 8.80% N, found 64.11% C, 7.20% H, 9.01% N; MS ($M+Cs^+$): calc. 451.0634, found 451.0630.

10: ^{1}H NMR ($CDCl_3$, 300MHz) δ 1.75-1.90 (m, 2H), 2.22-2.55 (m, 4H), 2.96 (dd, 1H, *J* = 7.1, 14.2Hz), 3.05 (dd, 1H, *J* = 6.8, 14.2Hz), 3.75-3.89 (m, 2H), 4.76-4.85 (m, 1H), 5.11 (s, 2H), 5.43-5.53 (m, 1H), 6.75 (d, 1H, *J* = 7.0Hz), 7.08-7.40 (m, 10H); HRMS ($M+Cs^+$): calc. 559.0845, found 559.0855.

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8. Compound **1** was obtained by esterification of commercially available isoserine (Aldrich).The synthesis of the diastereomeres of **2** has been described (Ocain, T.D., Rich, D.H. *J. Med. Chem.* **1988**, *31*, 2193; Yuan, W., Zhong, Z., Wong, C.-H., Haeggström, J.Z., Wetterholm, A., Samuelsson, B. *BioMed. Chem. Lett.* **1991**, *1*, 551). The preparation of **3 (3R)**, **3 (3S)**, **4** and **5** will be published elsewhere.
9. The assignment of the stereochemistry of the diastereomeres of **8** is tentative. It is based on their relative mobilities in TLC compared to those of **7 (2R,3S)** and **7 (2S,3S)**, respectively.

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