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α-Chymotrypsin Catalyzed Enantioselective Hydrolysis of Alkenyl-α-Amino Acid Esters

Bettina Schricker, Klaus Thirring and Heinz Berner

Sandoz Forschungsinstitut, Brunnerstraße 59, A-1235 Wien, Austria

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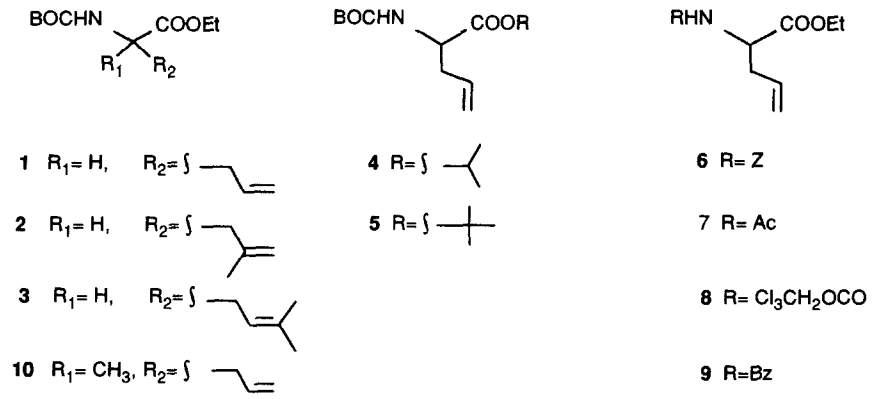
Abstract. *- Preparative scale hydrolyses (0.1-0.3*M*) were carried out on a series of alkenyl-α-aminoacid esters catalyzed by different hydrolytic enzymes. Within the scope of investigated substrates (**1-10**) and enzymes (PLE, chymopapain, papain, chymotrypsin, trypsin) α-chymotrypsin was shown to be the most effective. It showed broad substrate specificity, high stereoselectivity (ee 86-96%) and gave high chemical yields (up to 99%).*

Introduction. - There is increasing interest in stereocontrolled synthesis of lipopeptides, due to the range of biological activities associated with this class of compounds.[1,2] In the course of our own studies on lipopeptides containing nonproteinogenic amino acids, *N*-protected (*S*)-alkenyl-glycine-derivatives proved to be valuable starting compounds for syntheses, and we now became interested in preparative scale production of these compounds.[3] There are several known methods for the stereoselective alkylation of glycine. In most cases, bis-lactim-ethers[4], imidazolidinones[5], oxazolidinones[6] or oxazinones[7] are used as chiral intermediates. However, considering the time and costs involved, none of these methods can compete with an efficient separation of readily available enantiomers.

Here we report the results of a stereoselective enzymatic hydrolysis[8] of a series of structurally related racemic *N*-protected α-alkenylglycine esters.

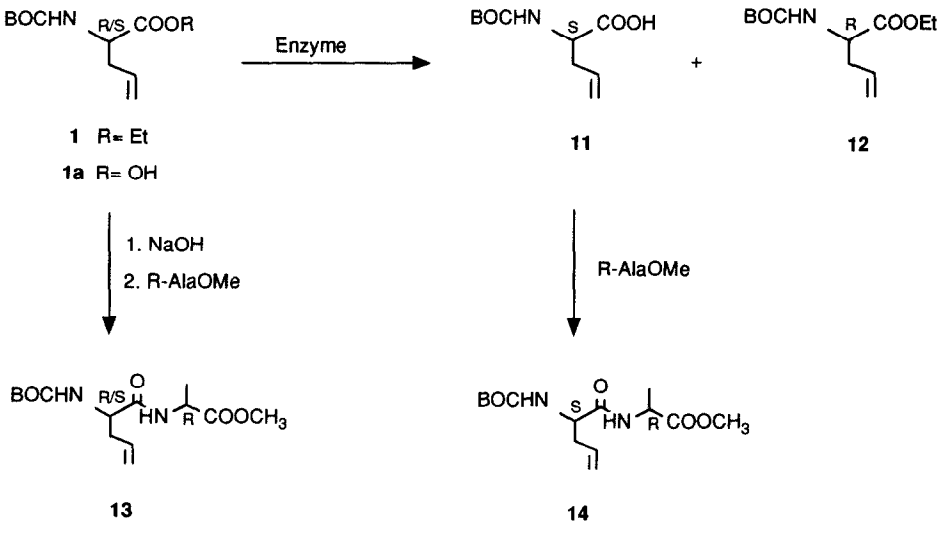
Methods and Results. - All substrates were synthesized according to published methods, **1**-**9**[9,10], **10**[11,12] (**Scheme 1**). The preparative scale hydrolyses (0.1-0.3M) were carried out in a mixture of phosphate buffer (0.1M,pH 8), ethanol (0-10%) and the appropriate enzyme (75-100 units/mmol substrate) at 37 °C. In a typical procedure, 50g (0.19 mol) of **1** were suspended in 3 L 0.1M phosphate buffer (pH 8) and treated with 250mg of α-chymotrypsin (75 units/mg, Fluka 27270) with gentle stirring at 37 °C. The pH was kept constant within the range of 7.5-8 by continous addition of 1N NaOH. After 24 h, when 171 ml (ca. 1 equiv.) of 1N NaOH had been consumed, the unreacted (*R*)-ester **12** (**Scheme 2**) was recovered by extracting the reaction mixture with ethyl acetate. Subsequently, the aqueous solution was acidified (pH 2.5) with 1N HCl and continously extracted with ethyl acetate. After the usual workup 21.37g (95.8%) of (*S*)-configurated acid **11** were obtained.

Scheme 1. Substrates for enzymatic hydrolysis



The enantiomeric purity of the deprotected (*S*)-acid **11** and of the dipeptide **14** was determined by HPLC[13]- and/or NMR-analyses.[14] The dipeptide **14** as well as the diastereomeric mixture **13** could be easily obtained by coupling racemic **1a** or the (*S*)-acid **11** with (*R*)-alanine methyl ester by using the mixed anhydride method.[15] The absolute configuration of deprotected **11** was determined by comparison of its optical rotation with an authentic sample of (*S*)-allylglycine.[16]

Scheme 2. Enzymatic reaction of substrate 1



By using **1** as a substrate, different enzymes were tested for their effect on chemical and stereochemical yields. The data in **Table 1** show that α-chymotrypsin and papain were the most efficient .

Table 1. Reaction of substrate 1 with different enzymes

Enzyme		Yield %	e.e.%
PLE	(E.C.3.1.1.1.)	64	14
Chymopapain	(E.C.3.4.22.6.)	94	93
Papain	(E.C.3.4.22.2.)	88	96
α-Chymotrypsin	(E.C.3.4.21.1.)	95	94
Trypsin	(E.C.3.4.21.2.)	72	94

Table 2. Reaction of different substrates with α-chymotrypsin

Substrate	Reaction time	Yield %	e.e. %
2	24h	99	91 [a]
3	24h	79	96 [a]
4	3d	39	91 [a]
5	4d	21	93 [a]
6	3d	41	86 [b]
7	24h	71	90 [b]
8	48h	86	88 [a]
9	24h	96	96 [b]
10	3d	0	0

[a] Enantiomeric purity determined by HPLC analysis of the deprotected acid

[b] Enantiomeric purity determined by HPLC or NMR analysis of the corresponding dipeptides **14**

Chymotrypsin was finally chosen since, during workup of the reaction mixtures, the use of papain gave rise to stable emulsions which could be handled only with difficulty. In order to elucidate the scope and limitations of this enzyme-catalyzed hydrolysis, substrates **2-10** were subjected to the treatment with α-chymotrypsin under the above mentioned conditions. The results are summarized in **Table 2.**

Discussion. - All of the investigated compounds but **10** proved to be satisfactory substrates for the α-chymotrypsin catalyzed hydrolysis, giving high chemical yields and high enantiomeric excess. Bulky substituents in the domain of the aminogroup (**6** - **9**) or at the α-C-atom (**3**) did not exert any appreciable effect, either on stereospecifity or on chemical yield. Only α-disubstituted compounds such as **10** are not accepted as a substrate. The reaction rates slowed down only for the more hydrophobic esters (**4** , **5**) and the Z-protected compound **6**, whose low water solubility is probably responsible. There is no reason to argue substrate specificity of these compounds since their enantiomeric excess is very high.

An application of this enzymatic reaction in lipopeptide synthesis will be described in a forthcoming publication.[3]

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