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The first aminoacylase-catalyzed enantioselective synthesis of aromatic β-amino acids

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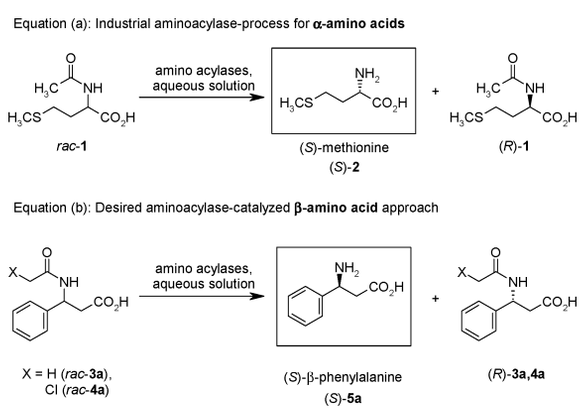
The first aminoacylase-catalyzed enantioselective synthesis of aromatic β-amino acids is reported. The presence of an *N*-chloroacetyl group as acyl group in the substrate as well as the use of porcine kidney acylase I as a suitable enzyme component are prerequisites for this resolution process whereby optically active β-amino acids are formed with high enantioselectivities of >98% ee.

The focus on the preparation of enantiomerically pure β-amino acids increased recently due to promising pharmaceutical applications thereof.[1,2] Popular synthetic accesses are still – in spite of disadvantages such as the need for a stoichiometric amount of a chiral auxiliary – the "classic" chemical resolution with chiral auxiliaries, and the diastereoselective addition of chiral lithiated amides to α,β-unsaturated esters.[2] Very recently, several attractive asymmetric metal-catalyzed syntheses for β-amino acids have been reported.[3] Biocatalysis provides an interesting alternative way, and in spite of the fact that "enzymes commonly used to resolve α-amino acids have narrow substrate tolerance of β-amino acids and often exhibit low selectivity in the process",[2c] several successful examples have already been reported. For example, lipase-catalyzed acylation of β-amino acid esters,[4] hydrolase-catalyzed hydrolysis of β-amino acid esters and *N*-acyl derivatives thereof,[5] as well as the PenG amidase-catalyzed deacetylation of *N*-phenylacetylated β-amino acids[6] have been proven to represent efficient routes.

An access to enantiomerically pure β-amino acids by means of an aminoacylase-catalyzed hydrolysis of *N*-acetylated β-amino acids according to Scheme 1, equation (b) could represent an interesting alternative to the existing biocatalytic approaches. Such a resolution concept with aminoacylases[7] [E.C. 3.5.1.14] would be very desirable due to their successful application in the industrial production of α-amino acids. For example, L-methionine L-**2** is produced *via* an aminoacylase-based resolution with an annual amount of several hundred tons at Degussa (Scheme 1, equation (a)).[8] Advantages are, *e.g.*, the easy access to the starting materials, the excellent enantioselectivity, and the simple isolation of the product. However, in spite of many successful examples for α-amino acids, this type of resolution turned out not to be suitable for the enantioselective synthesis of β-amino butyric acid.[7] In the following we report our preliminary results towards the use of aminoacylases for the enzymatic resolution of racemic *N*-acetyl-type substituted aromatic β-amino acids.



Scheme 1 General reaction concept.

At first, the preparation of optically active β-phenylalanine (3-phenyl-3-aminopropionic acid) (*S*)-**5a** according to Scheme 1, equation (b) was investigated as a model reaction since data of alternative routes are already available for this compound. The initial step was the selection of potentially useful substrates, and enzymes. To start with the choice of the substrate, *N*-acetyl- as well as *N*-chloroacetyl-substituted β-amino acids represent particularly desirable starting materials due to their simple preparation. In addition, both acyl groups are based on very cheap, industrially produced, and easily available raw materials. Furthermore, the separation of the product and remaining starting material from the carboxylic acid formed by *N*-acyl cleavage is easy and economical. As potentially suitable enzymes a broad variety of hydrolases were chosen. Besides several aminoacylases – in which we were most interested – also proteases, and lipases were included in the screening program. The examined biocatalyst library consists of 20 proteases/acylases and 10 lipases. The biocatalyst library was tested for the resolution according to Scheme 1, equation (b) by carrying out the biotransformation in parallel using a Beckman Biomek2000® as pipettor.[9]

Independent of the type of aminoacylase, an aminoacylase-mediated resolution could not be found using *N*-acetylated β-phenylalanine *rac*-**3a** as a starting material. In addition, other hydrolases are not suitable catalysts for *rac*-**3a**. However, changing the substrate to the *N*-chloroacetylated derivative *rac*-**4a** as a starting material we were pleased to find that porcine kidney acylase of type I (PKA I) has remarkable catalytic activity with the *N*-chloroacetylated β-amino acid. Other hydrolases also turned out to be useful, but gave lower catalytic activities.[10]

As a next step we carried out the reaction on a preparative scale † under standard reaction conditions for the PKA acylase, investigating its synthetic potential with respect to yield and enantioselectivity. For the model reaction using the racemate **4a** as a substrate, a good conversion of 43% with respect to

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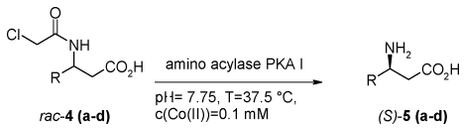
Table 1 Substrate range of the porcine kidney aminoacylase-based approach



rac-**4 (a-d)** *(S)*-**5 (a-d)**

product	R	time/h	conversion [%]	ee [%]
5a	Phenyl	24	43	
		48	49	>98
5b	4-Fluoro-phenyl	24	33	
		48	45	95
5c	4-Methoxy-phenyl	20	38	
		44	46	96
5d	2-Thienyl	24	46	
		45	~50	>99

the formation of the (*S*)-enantiomer was obtained after 24 h (Table 1, entry 1). Extending the reaction time to 48 h led to a nearly quantitative formation of the product **5a** (49%). As it is known that Co-ions enhance the specific activity of porcine kidney acylase, the reaction is carried out in the presence of 0.1 mM Co^{2+}.[7] It is noteworthy that the product (*S*)-**5a** is formed with a high enantioselectivity of >98% ee (entry 1).

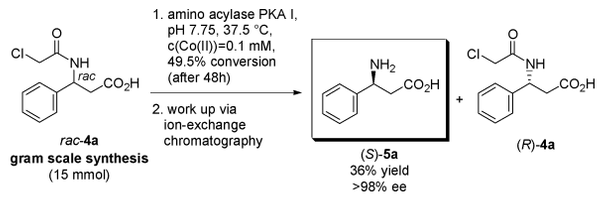
We further investigated the extension of this aminoacylase-catalyzed synthesis of optically active β-amino acids towards the use of other substrates (see Table 1). The conversions were determined directly from the reaction mixture *via* chiral HPLC after reaction times of 24 h and 44–48 h, respectively. Starting from the *p*-fluoro-substituted aromatic substrate *rac*-**4b**, the reaction proceeds with a conversion of 45% after 48 h (33% after 24 h), and with an enantioselectivity of 95% ee (entry 2). An enantioselectivity of 96% ee was obtained for the racemic *p*-methoxy-substituted aromatic substrate, *rac*-**4c** (entry 3) with a conversion of 46% after 44 h. Using a heteroaryl-based substrate of type **4d** gave a complete conversion of ~50% after 45 h. Even after 24 h, the conversion rate was already 46%. We were also pleased to find a high enantioselectivity of >99% ee for the formation of the β-amino acid product **5d** (entry 3).

Besides the highly enantioselective access and the good conversions, a further advantage is the easy work up. This has been demonstrated in a preparative experiment on a gram-scale. Comparable conversion rates were observed (41.9% after 24 h; 49.5% after 48 h and ultrafiltration). Downstream-processing involving ion-exchange chromatography for product isolation gave the β-amino acid (S)-**5a** in 36% yield, and with an enantioselectivity of >98% ee (Scheme 2).

In conclusion, this preliminary work has revealed the first aminoacylase-based access to aromatic-β-amino acids furnishing the products **5** in enantioselectivities of up to >99% ee. Further experiments addressing the issue of scopes and limitations of the reaction like the substrate spectrum and process optimisation are currently in progress. Among challenges for the future in order to match the requirements for a technically applicable process are the reduction of the (bio-)catalytic amount, and the large-scale availability of the biocatalyst, *e.g.* by developing suitable expression systems.

Notes and references

† Preparative scale experiments: An aqueous buffer solution (25 mL of phosphate buffer, 50 mM, pH 7.75), containing 0.25 mmol of the *rac*-*N*-chloroacetyl-β-amino acid and 0.0025 mmol of cobalt(II) chloride, was warmed up to 37 °C. Subsequently 60 mg of porcine kidney acylase I (source: Sigma, grade 2) were added, and the reaction mixture was stirred at 37.5 °C for 44–48 h. Reaction samples were taken at regular intervals, and analyzed with respect to conversion by means of HPLC chromatography. Subsequently, the reaction mixture was separated from the enzyme by ultrafiltration. The filtrate with the desired β-amino acid was analysed with respect to conversion and enantioselectivity. For the gram scale synthesis according to Scheme 2, the downstream processing after ultrafiltration consists of an ion exchange chromatography using the acidic ion exchanger "amberlite 252C". The final elution of the β-amino acid product (*S*)-**5a** was conducted with a 5% aqueous ammonia solution.



rac-**4a**
gram scale synthesis
(15 mmol)

(*S*)-**5a**
36% yield
>98% ee

(*R*)-**4a**

Scheme 2 Preparative synthesis and work up on gram scale.

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9 The enzyme screening was carried out as follows: In a GC-vial of 1 mL, 100 µl of *N*-acetylated- or *N*-chloroacetylated *rac*-β-phenylalanine, *rac*-**3a** and *rac*-**4a**, respectively (0.1 M solution in acetone and phosphate-buffer with pH 8.0) and 5 mg of the corresponding enzyme were dissolved in 0.9 ml of phosphate-buffer (50 mM, pH 8.0). The reaction was stirred for 24 h at 30 °C. Subsequently, the reactions were analyzed *via* HPLC (column: Nautilus, eluent: H_2O (80%), acetonitrile (20%), and 0.1% TFA, flow: 1 ml min^{-1}, 220 nm, injection: 900 µl Eluent + 100 µl reaction mixture).

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