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L-Methionine related L-amino acids by acylase cleavage of their corresponding *N*-acetyl-DL-derivatives †,‡

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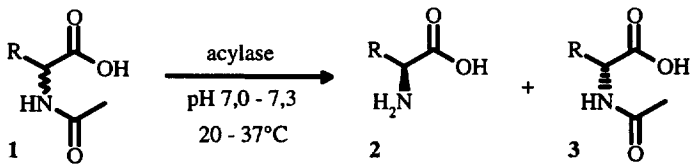
Abstract: Acylase I from *Aspergillus oryzae* is an even more useful enzyme than suggested so far. Besides standard amino acids such as L-Met, L-Val and L-Phe, a number of additional sulfur- and selenium-containing amino acids can be obtained at useful reaction rates and in very high enantiomeric purity by kinetic resolution of the respective *N*-acetyl-DL-amino acids. © 1997 Elsevier Science Ltd

Enantiomerically pure α-amino acids are increasingly important products with extended applications in food and feed stuffs, pharmaceutical or agrochemical industries.[1] In particular, non-proteinogenic amino acids have attracted considerable research interest due to the special effects which can be achieved by incorporating them into biologically active compounds or by preparing derivatives for applications in asymmetric synthesis.[2] Many general procedures are suitable for the preparation of both proteinogenic and non-proteinogenic amino acids.[3] Thus, *N*-acetyl-DL-amino acids **1** are cleaved enantioselectively into L-amino acids **2** and *N*-acetyl-D(L)-amino acids **3** by acylase (E.C. 3.5.1.14) from porcine kidney or especially *Aspergillus oryzae* (Scheme 1). Based on this reaction, Degussa operates a production process for L-Met **2a** and L-Val **2k** using an enzyme membrane reactor.[4] However, acylase accepts many different *N*-acyl amino acids as substrates.[5] Therefore, we wanted to produce a variety of non-proteinogenic amino acids using acylase from *Aspergillus oryzae*.



1-3a: R = $H_3CSC_2H_4$ **1-3b**: R = $H_5C_2SC_2H_4$ **1-3c**: R = $H_3CSeC_2H_4$ **1-3d**: R = H_3CSCH_2
1-3e: R = $H_3CS_2C_2H_4$ **1-3f**: R = $HSCH_2$ **1-3g**: R = H_3C **1-3h**: R = H_5C_2
1-3i: R = H_7C_3 **1-3j**: R = H_9C_4 **1-3k**: R = $(H_3C)_2CH$ **1-3l**: R = $H_3CS(O)_2C_2H_4$

Scheme 1.

† Dedicated to Prof. Dr Heribert Offermanns (Degussa AG) on the occasion of his 60th birthday.
‡ Amino acid transformations, no. 14. For no. 13, see: U. Eichhorn, A. S. Bommarius, K. Drauz, H.-D. Jakubke, *J. Pept. Sci.* **1997**, *3*, 245.
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Derivatives of L-methionine-sulfone **2l** have been developed as thrombin inhibitors.[6] Therefore, we prepared *N*-Ac-DL-Met-sulfone **1l** by oxidation of inexpensive DL-Met with 30% hydrogen peroxide (yd. 72%), followed by acetylation with acetic anhydride (yd. 67%). Incubation with acylase gave enantiomerically pure (e.e. >99.6%, $[\alpha]_D^{20}$=12.6, c=2 in water) **2l** in 35% yield.[7]

The copper complex of L-dithiomethionine **2e** has been described as suitable for the treatment of cancer.[8] Also, it was already known in principle that **2e** could be obtained from **1e** by acylase reaction.[9] We obtained **1e** as a yellowish oil by demethylation of DL-Met with sodium in ammonia and methylthiolation using methanethiosulfonic acid *S*-methylester (yd. 83%), followed by acetylation with acetic anhydride.[8,10] After reaction of **1e** with acylase (c=0.6 M, pH 7.0, 2 d, room temperature), analytically pure **2e** was isolated in 22% yield[7] (e.e. >99.6%; $[\alpha]_D^{20}$=39.7, c=1 in 6 N HCl, obtained for **2e** prepared from L-methionine).

α-^{13}C-L-Met (^{13}C-**2a**) was needed for a research project related to the molecular origin of Alzheimer's disease.[11] ^{13}C-**1a** was obtained by Strecker synthesis starting from methylmercaptopropionic aldehyde, ammonia and K^{13}CN, hydrolysis of the intermediate hydantoin and subsequent acetylation. Acylase cleavage and ion exchange purification gave ^{13}C-**2a** (17.0 g, yd. 42.5%[7]) in 99.8% e.e. and 98.5% isotopic purity. The rate of recovery for the highly expensive ^{13}C-compounds ^{13}C-**2a** and ^{13}C-**1a** reached 99.4%, clearly demonstrating the suitability of the acylase reaction system for substrate/product combinations with need of near-total recovery.

Enantiomerically pure amino alcohols obtained by reduction of the corresponding amino acids[12] are very important intermediates for the preparation of pharmaceutically active compounds or chiral auxiliaries for asymmetric synthesis.[13] However, our model substrate *N*-acetyl-L-methioninol failed to react with acylase. This is in accordance with previous results that acylase accepts only free *N*-acyl-α-aminocarboxylic acids and does not tolerate derivatization towards the amide, ester or, as shown now, towards the alcohol.

S-Methyl-L-cysteine **2d** has become highly important as building block of HIV-protease inhibitors.[14] L-Selenomethionine **2c** was described as suitable for treatment of Alzheimer's disease and Parkinson syndrome.[15] We have performed kinetic measurements for these and several other interesting amino acids to determine the reactivity of the corresponding *N*-acetyl-DL-amino acids **1b–j** in comparison to our model substrate *N*-acetyl-DL-methionine **1a** (Table 1). Clearly, acylase from *Aspergillus oryzae* converts all the investigated substrates **1a–j** at good reaction rates. **1a** showed average reactivity in our investigations while carbon analogs such as *N*-acetyl-DL-norleucine **1j** or more hydrophobic S-containing acetyl amino acids such as *N*-acetyl-DL-ethionine **1b** or *N*-acetyl-DL-dithiomethionine **1e** were better substrates. Preferably, the amino acid sidechain should be 4 to 5 atoms long, regardless of whether it contains only carbon atoms or sulfur or selenium in addition (exception: *N*-acetyl-DL-cysteine **1f**). Acylase converts *N*-acetyl-DL-amino acids with S- or Se-containing sidechains at similar rates (relative activities≤factor 2 refering to **1a**) as their carbon analogs. The reversibility of the acylase reaction is not a major problem because the equilibrium rests strongly on the side of the desired products **2** in all cases. The L-enantiomers of *N*-acetyl-DL-α-amino butyric acid **1h** and *N*-acetyl-DL-norleucine **1j** react almost quantitatively[7] while *N*-acetyl-DL-cysteine **1f** gives the lowest conversion.

In summary, we have shown that acylase is even more useful than previously known for the preparation of enantiomerically pure L-amino acids.

Experimental

Acylase from *Aspergillus oryzae* was bought from Amano, methanethiosulfonic acid *S*-methyl ester from Fluka. DL-Amino acids were prepared by Strecker synthesis following known procedures. Acetylations were done at pH 7.5–9 and 15–30°C with 1.1–1.3 equivalents of acetic anhydride over 2–4 h. *N*-Acetyl-DL-amino acids **1** were extracted with MTBE, crystallized by evaporation, filtered off, washed with water and dried *in vacuo*. Isolated yields: **1b**: 96.0%; **1c**: 84.1%; **1d**: 97.4%; **1j**: 71.0%. Kinetic investigations were performed at 37°C, pH 7, 0.3 M of **1**, [E] of 30 g/l and monitored by polarimetry. Equilibrium conversions at the end of each reaction were determined by HPLC (Jasco

Table 1. Cleavage of *N*-acetyl-DL-amino acids **1** with acylase

Edukt	R	$[\alpha]_{equ}$ (°/mol)	equilibrium conversion (%)	$\Delta\alpha/\Delta t$ (m°/mol·h)	r_0 (mol/l·h)	relative activity (%)	e.e. of **2** (%)
1a	$H_3CSC_2H_4$	-0.513	40.8	169.3	0.0404	100.0	> 99.6
1b	$H_5C_2SC_2H_4$	-0.856	48.1	299.3	0.0503	124.6	> 99.6
1c	$H_3CSeC_2H_4$	-0.863	37.7	333.0	0.0436	108.1	> 99.6
1d	H_3CSCH_2	-0.084	41.0	18.2	0.0266	65.9	> 99.6
1e	$H_3CS_2C_2H_4$	0.123	49.9	54.3	0.0659	163.0	> 99.6
1f	$HSCH_2$	-0.078	32.9	52.4	0.0648	160.3	> 99.6
1g	H_3C	1.027	41.6	210.7	0.0256	63.4	> 99.6
1h	H_5C_2	0.693	ca. 50	156.5	0.0333	82.3	> 99.6
1i	H_7C_3	0.605	43.8	187.5	0.0401	99.4	> 99.6
1j	H_9C_4	0.084	ca. 50	29.8	0.0532	132.0	> 99.6

(**1k** and **1l** have not been measured kinetically.)

800; eluent 30% acetonitrile, 70% 0.1 M phosphate, pH 5.2) by comparison with calibration curves. Initial conversions were calculated from initial slopes of $\alpha-t$-curves ($\Delta\alpha-\Delta t$). Combination with substrate concentrations gave initial reaction rates r_0. To isolate the amino acids **2**, reacted solutions were passed through a strongly acidic ion exchange column (Amberlite® 252 C), the amino acids eluted with 5% ammonia, evaporated to dryness and dried *in vacuo*. E.e. values were determined by GC on Chirasil-L-Val (**2a**, **2f–i**) or by HPLC on Crownpak(+) (**2b–e**, **2j**, **2l**), absolute configurations by comparison with authentic material.

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