Review article

Resolution of α-methylserine derivatives via lipase mediated acetylation

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Abstract: *(±)-N-Benzoyl-α-methylserine ethyl ester and (±)-4-hydroxymethyl-4-methyl-2-phenyl-1,3-oxazol-5(4H)-one were synthesized and used as substrates for enzyme catalyzed kinetic resolution in organic solvents. Several lipases active for O-acetylation of both compounds were found. The most active enzymes were screened for influence of solvent, acetyl donor type, its concentration, biocatalyst quantity and reaction temperature on resolution rate and enantioselectivity ratio E. For acetylation of (±)-N-benzoyl-α-methylserine ethyl ester with isopropenyl acetate in the presence of Novozym®435 the highest enantioselectivity E = 35 was found, yielding at 54% conversion (S)-N-Benzoyl-α-methylserine ethyl ester with 94% ee and (R)-O-acetyl-N-benzoyl-α-methylserine ethyl ester with 82% ee.*

Keywords: *α-methylserine, racemate resolution, lipase, acetylation, organic solvents.*

Introduction

In nature there are twenty protein amino acids, present in every living organism. Although the number of combinations they can produce is almost infinite, nowadays chemists, biologists and medicine experts are interested in less abundant non-standard or non-protein amino acids. They are not coded in the DNA, but are produced either as intermediates or final products of metabolic pathways, or appear in proteins as an effect of post-translational modifications. Their low abundance in living organisms suggests, that they may play an important role in specific intracellular processes, leading to the assumption of their important function in bioactive compounds. Furthermore, enantiopure non-protein amino acids can be used as chiral building blocks in organic synthesis.

The α-substituted-α-amino acids known as "chimeric amino acids" contain two side chains coming from different standard amino acids. Apart from a few examples, it is not yet known how such amino acids are recognized and utilized in living cells. For example (S)-α-methylserine is a side product of one of metabolic pathways – the one carbon pool by folate pathway in *Pseudomonas* sp. [1], was found in a natural antibiotic (-)-amicetin [2] from *Streptomyces plicatus*,

as well as in (+)-conagenin [3], a secondary metabolite of *Streptomyces roseosporus* (Figure 1).

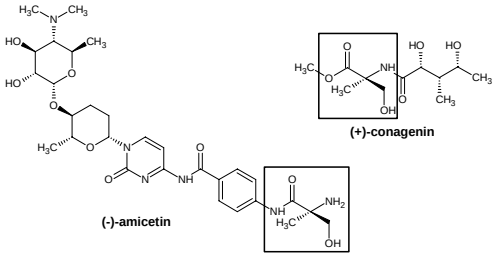


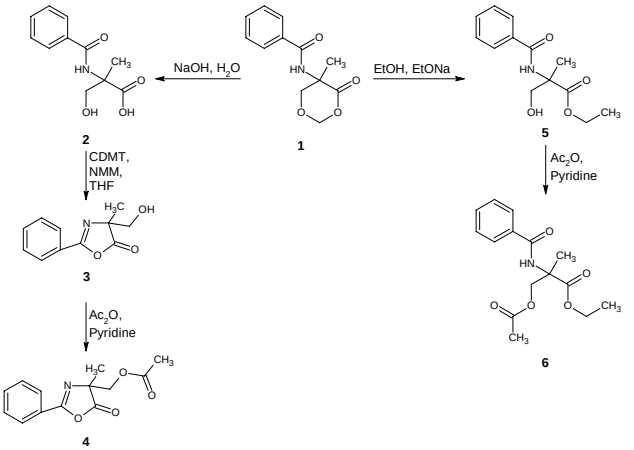
Figure 1. Bacterial metabolites containing (*S*)-α-methylserine

α-Methylserine is used for chemical synthesis of polypeptides, as well as their modification, changing the molecule flexibility [4]; its O-phosphate esters show neurological activity [5]; specific pentapeptides containing α-methylserine have a strong analgesic effect [6]; and it is useful as a chiral building block in organic synthesis [7,8]. α-Methylserine due to the presence of three functionalities, hydroxyl, carboxyl and amino group, is a good starting material for asymmetric synthesis of more complex compounds. The fact, that it is present in living organisms in very low amounts calls for application of chemical synthesis for preparation of this amino acid. Chiral synthesis is costly and of low efficiency, whereas racemate preparations are cheap and effective processes. Currently the price of enantiopure α-methylserine is rather high (ca. 600 €/g [9]), in spite of numerous asymmetric syntheses reported in the literature [10]. This led to a concept of inventing an effective technology for both α-methylserine enantiomers preparation by biocatalytic approach. Resolution of (±)-N-benzoyl-α-methylserine via lipase-mediated *O*-acetylation was attempted by Olczyk et al. [11], however at conditions they used two subsequent enzymatic enrichments and crystallization were necessary to attain high enantiomeric excess because of relatively low enantioselectivity ($E < 10$). This led to the idea of substrate structure modification in order to improve enantioselectivity of enzymatic resolution. Herein we report the synthesis of two novel (±)-α-methylserine derivatives and their application as substrates in enzyme catalyzed *O*-acetylation.

Results and Discussion

(±)-5-Benzoylamino-5-methyl-4-oxo-1,3-dioxane (**1**) obtained from (±)-alanine according to Kamiński and Leplawy [12] was used as starting material for the preparation of (±)-*N*-benzoyl-α-methylserine ethyl ester (**5**) and (±)-4-(hydroxymethyl)-4-methyl-2-phenyl-1,3-oxazol-5(4*H*)-one (**3**) (Scheme 1). Ethanolysis of oxodioxane **1** catalyzed by sodium ethoxide led to ester **5** in 90% yield. Alkaline hydrolysis of **1** to (±)-*N*-benzoyl-α-methylserine (**2**), followed by activation with 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) [13] in the presence of N-methylmorpholine (NMM) gave oxazolone **3** in 40% overall yield.

Derivatives **3** and **5** were acetylated with acetic anhydride in pyridine yielding corresponding (±)-acetates **4** and **6** in 52% and 55% yield, respectively.



Scheme 1. Synthesis of (±)-α-methylserine derivatives

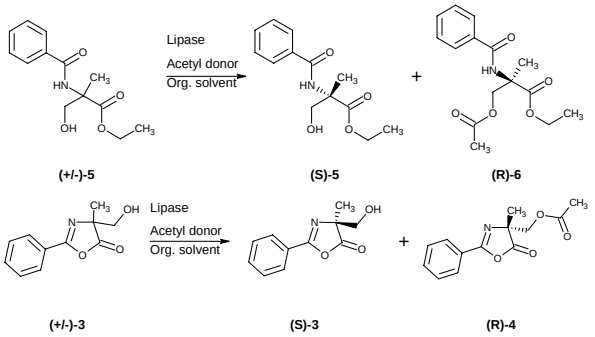
Having in hands racemic compounds **3** and **5** as well as their *O*-acetyl derivatives **4** and **6**, HPLC analysis method was elaborated using Chiralcel®OD column, and hexane/isopropanol (95:5) as mobile phase (0.5 mL/min). Under these conditions excellent enantiomers separation was achieved with signals at: 27.2 and 28.3 min. for oxazolone **(±)-3**, 11.9 and 15.5 min. for acetylated oxazolone **(±)-4**. Enantiomers of ester **(±)-5** appeared at 39.7 (*S*) and 43.3 min. (*R*), while those of acetylated ester **(±)-6** at 25.5 (*R*) and 32.2 min. (*S*). Therefore, measurements of conversion and enantiomeric excesses of substrate and product for evaluation of enantioselectivity were feasible.

In preliminary screening for active enzymes, ester **(±)-5** and oxazolone **(±)-3** were tested as substrates for O-acetylation with vinyl acetate (VA) in hexane/THF mixtures (Scheme 2). Hexane/THF ratio was found to be dependent on substrate solubility and was established experimentally in order to obtain homogeneous solution.

Thirteen commercial enzyme preparations (twelve lipases and one acylase) together with two non-commercial lipases were used as biocatalysts (see Table 1).

For ester **(±)-5** nine lipases were active, but only two showed moderate enantioselectivity (entry 3, 4), while oxazolone **(±)-3** was acetylated by eight lipases, three of them showing moderate enantioselectivity (entry 6-8). For all enzymes the same enantiopreference was observed - substrate of longer retention time was acetylated faster, yielding acetylated product of shorter retention time. It should also be noticed that in most cases acetylation of oxazolone **(±)-3** was significantly faster than that of the ester **(±)-5**. Inspection of molecular models revealed that substrate **(±)-3** is less bulky**,** with easy access to hydroxyl group,

while molecule **(±)-5** is larger and strongly hindered at quaternary carbon, what probably makes the formation of O-acylation transition state more difficult.



Scheme 2. Kinetic resolution of α-methylserine derivatives **(±)-3** and **(±)-5**

Table 1. Biocatalysts activity in acetylation of **(±)-5** and **(±)-3** with vinyl acetate

Entry	Enzyme	Ester **(±)-5**[a]				Oxazolone **(±)-3**[b]			
		c [%]	**ee$_s$** [%]	**ee$_p$** [%]	**E**	**c** [%]	**ee$_s$** [%]	**ee$_p$** [%]	**E**
1	*M. racemosus* lipase	inactive				inactive			
2	*M. circinelloides* lipase	12	3	23	2	38	11	56	4
3	Lipozyme® RM IM	29	31	76	**10**	>95	-	-	~1
4	Lipozyme® TL IM	44	49	61	**7**	>95	-	-	~1
5	Novozym® 435	91	53	5	2	>95	-	-	~1
6	PPL	inactive				69	55	51	**5**
7	Lipase AK	5	0	3	~1	89	99	16	**5**
8	Lipase PS	46	2	2	1	70	85	51	**8**
9	Lipase M	inactive				inactive			
10	Lipase G	inactive				inactive			
11	*R. oryzae* lipase	inactive				inactive			
12	CCL	22	6	21	2	13	18	31	2
13	Lipase A	26	2	5	1	inactive			
14	Lipase AY	34	3	7	1	inactive			
15	Acylase	inactive				inactive			

c-conversion, ee$_p$/ee$_s$-enantiomeric excess of product/substrate, E-enantioselectivity

[a] Standard conditions: 0.25 mmol **(±)-5**, 1 mmol VA, 100 mg enzyme, 3 mL hexane/THF 7:3, 30°C, 48 h

[b] 0.25 mmol **(±)-3**, 1 mmol VA, 100 mg enzyme, 4.2 mL hexane/THF 1:1, 30°C, 48 h

[c] Enantioselectivity calculated from ee$_s$ and ee$_p$ according to [14]

For effective kinetic resolution, when both enantiomers of high ee are required, enantioselectivity E > 20 is necessary. For further experiments to improve E value we decided to use ester **(±)-5** as substrate and the most enantioselective catalysts – Lipozyme® RM IM and Lipozyme® TL IM.

In addition, Novozym® 435 as the most active lipase was also included despite its low enantioselectivity in preliminary screening. It is known that such process parameters as kind of solvent and/or acetyl donor, reactant to catalyst ratio, concentration or quantity of enzyme, strongly affect the reaction rate and enantioselectivity [15].

Influence of acetyl donor

Two enol esters – vinyl (VA) and isopropenyl acetate (IPA), and ethyl acetate were tested as acetyl donors. Enol esters were used in limited amount (molar ratio 0.5:1 with respect to substrate **(±)-5**), while ethyl acetate acting simultaneously as acetyl donor and solvent was used in large excess. The initial concentrations of enol esters were eight times lower in comparison to previous screening.

Table 2. Influence of acetyl donor on ester **(±)-5** resolution

Enzyme	Vinyl acetate [a]		Isopropenyl acetate [a]		Ethyl acetate [b]	
	c [%]	E	c [%]	E	c [%]	E
Lipozyme® RM IM	No reaction		No reaction		No reaction	
Lipozyme® TL IM	No reaction		No reaction		25	1
Novozym® 435	9	3	8	5	37	**16**

[a] Standard conditions: 0.25 mmol **(±)-5**, 0.125 mmol acetyl donor, 50 mg enzyme, 3 mL hexane/THF 7:3, 30°C, 24 h

[b] 3 mL ethyl acetate as acetyl donor and solvent

At these conditions, after 24 h Lipozyme® RM IM and Lipozyme® TL IM were found to be inactive, while for Novozym® 435 the conversion in a range 8-9% and enantioselectivity E = 3 and E = 5 for VA and IPA, respectively was observed (see Table 2).

Ethyl acetate was not accepted as acetyl donor by Lipozyme® RM IM, however was active in the presence of Lipozyme® TL IM in a non-enantioselective manner (E = 1). For Novozym® 435 all acetyl donors were active, and suprisingly for ethyl acetate was observed a significantly improved enantioselectivity (E = 16) in comparison to enol esters. Therefore, resolution of **(±)-5** in ethyl acetate by Novozym® 435 was carried out with the monitoring of reaction progress in time. We found that after 19 h a 31% conversion was reached and product **6** with ee 79% and substrate **5** with ee 36% were obtained. Then, enantiomeric excesses of substrate and product gradually decreased due to reaction reversibility. After 11 days, an equlibrium state was reached at ca. 45% conversion and ee of product and substrate lower than 10%.

Influence of solvent

To study the effect of solvent on the reaction rate and enantioselectivity, acetone and methyl *tert*-butyl ether (MTBE) were selected and compared to hexane/THF 7:3 mixture. The choice of solvent was based on the substrate

solubility. Vinyl acetate in 2:1 molar ratio to substrate was used in these experiments.

Table 3. Influence of solvent on ester **(±)-5** resolution

Enzyme	Acetone		MTBE		Hexane/THF 7:3	
	c [%]	E	c [%]	E	c [%]	E
Lipozyme® RM IM	2	1	9	7	29[a]	**10**
Lipozyme® TL IM	7	1	27	**8**	30	3
Novozym® 435	5	**7**	40	2	84	3

Standard conditions: 0.25 mmol (±)-**5**, 0.5 mmol VA, 50 mg enzyme, 3 mL solvent, 30°C, 24 h
[a] Reaction time 48 h

In acetone the lowest reaction rate for Lipozyme® TL IM and Novozym® 435 were found, while in MTBE Lipozyme® RM IM showed the lowest activity. In hexane/THF (7:3) similar conversions were observed for both Lipozymes, while Novozym® 435 showed the highest activity (see Table 3). However, the highest enantioselectivity for each enzyme was observed in different solvents: for Novozym® 435 in acetone (E = 7), for Lipozyme® TL IM in MTBE (E = 8) and for Lipozyme® RM IM in hexane/THF (7:3) mixture (E = 10). These results confirm the importance of enzyme-solvent 'compatibility' for effective kinetic resolution.

Choice of the most enantioselective enzyme – acetyl donor – solvent combination

In the next series of experiments we performed acetylation of **(±)-5** with monitoring of conversion and enantioselectivity in time. For Lipozyme® RM IM in hexane/THF (E = 10) and Lipozyme® TL IM in MTBE (E = 8) vinyl acetate was used as acetyl donor. Novozym® 435 in hexane/THF was included into this experiment, however was used in 2.5 times lower amount than Lipozymes, because of very fast reaction. Moreover, for Novozym® 435 we expected to improve enantioselectivity by using isopropenyl acetate instead of vinyl acetate and that was found to be the case (see Table 4).

Table 4. Parameters of ester **(±)-5** resolution monitored in time

Time [h]	Lipozyme® RM IM VA, hexane/THF 7:3		Lipozyme® TL IM VA, MTBE		Novozym® 435[a] IPA, hexane/THF 7:3	
	c [%]	E	c [%]	E	c [%]	E
2	8	8	9	7	24	26
4	18	11	18	7	45	26
6	25	13	24	7	54	27
8	30	13	29	7	59	34
26	56	15	55	9	79	8

Standard conditions: 1.25 mmol **(±)-5**, 2.5 mmol acetyl donor, 250 mg enzyme, 15 mL solvent, 30°C; [a] 100 mg enzyme

In this series of experiments the same activity for Lipozyme® RM IM and Lipozyme® TL IM was observed, but the latter as less enantioselective (E < 10) was excluded from further optimization. Therefore Lipozyme® RM IM with vinyl acetate and Novozym® 435 with isopropenyl acetate as acetyl donor were chosen for further study.

Influence of acetyl donor concentration and biocatalyst quantity

For Lipozyme® RM IM and Novozym® 435, experiments with monitoring in time were carried out in order to evaluate the influence of acetyl donor concentration and biocatalyst quantity on conversion and enantioselectivity. Reactions were performed in hexane/THF 7:3 mixture.

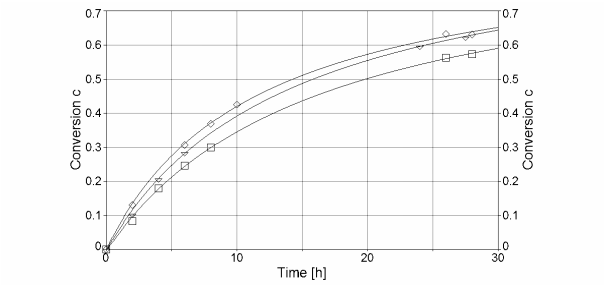


Figure 2. Conversion *vs* time for Lipozyme® RM IM/VA. Reaction conditions: 30°C, [**(±)-5**] = 0.083 mmol/mL; (◊) 250 mg enzyme, [VA] = 0.167 mmol/mL, $t_{½}$ = 14.3 h; (∇) 250 mg enzyme, [VA] = 0.333 mmol/mL $t_{½}$ = 15.8 h; (□) 125 mg enzyme, [VA] = 0.167 mmol/mL, $t_{½}$ = 19.8 h

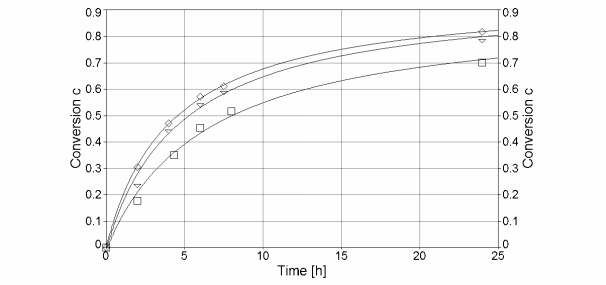


Figure 3. Conversion *vs* time for Novozym® 435/IPA. Reaction conditions: 30°C, [**(±)-5**] = 0.083 mmol/mL; (◊) 100 mg enzyme, [IPA] = 0.083 mmol/mL, $t_{½}$ = 4.6 h; (∇) 100 mg enzyme, [IPA] = 0.167 mmol/mL, $t_{½}$ = 5.3 h; (□) 50 mg enzyme, [IPA] = 0.167 mmol/mL, $t_{½}$ = 8.0 h

Both lipases exhibit typical Michaelis-Menten kinetics, however the initial reaction rate was more than twice higher for Novozym® 435, despite the fact its quantity was 2.5 times lower than that of Lipozyme® RM IM (Figure 2, 3). Estimated time of 50% conversion ($t_{½}$) for acetylation with vinyl acetate by Lipozyme® RM IM was 14.3 h, while for acetylation with isopropenyl acetate by

Novozym® 435, was 4.6 h. Two-fold increase of acetyl donor concentration resulted in slight decrease of reaction rate, with elongation of $t_{½}$ to 15.8 h for Lipozyme® RM IM, and to 5.3 h for Novozym® 435. More pronounced decrease of reaction rate was observed, when enzymes were used in two-fold lower quantity. Under these conditions for Lipozyme® RM IM $t_{½}$ increased to 19.8 h, and for Novozym® 435 to 8 h.

On plotting enantioselectivity E *vs* conversion, different behavior for each enzyme was found (see Figure 4, 5). For Lipozyme® RM IM trend of slow increase of enantioselectivity was observed up to ca. 57-60% conversion, then a slight drop occurred. The highest E values were found at higher acetyl donor concentration and larger enzyme quantity.

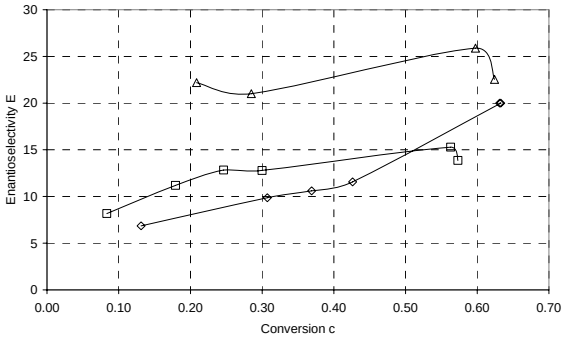


Figure 4. Enantioselectivity *vs* conversion for Lipozyme® RM IM/VA. Reaction conditions: 30°C, [**(±)-5**] = 0.083 mmol/mL; (◊) 250 mg enzyme, [VA] = 0.167 mmol/mL; (Δ) 250 mg enzyme, [VA] = 0.333 mmol/mL; (□) 125 mg enzyme, [VA] = 0.167 mmol/mL

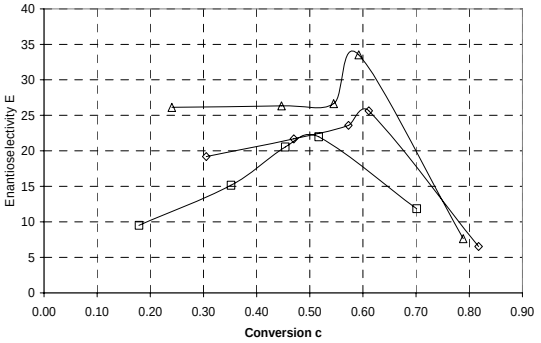


Figure 5. Enantioselectivity *vs* conversion for Novozym® 435/IPA. Reaction conditions: 30°C, [**(±)-5**] = 0.083 mM/mL ; (◊) 100 mg enzyme, [IPA] = 0.083 mM/mL; (Δ) 100 mg enzyme, [IPA] = 0.167 mM/mL; (□) 50 mg enzyme, [IPA] = 0.167 mM/mL

For Novozym® 435 a rapid drop of enantioselectivity was observed at conversion around 60% indicating, that the 'wrong' enantiomer of substrate started to react. Thus, it was necessary to terminate the resolution at conversion below 50% in order to obtain product of a high ee, and around 60% if substrate of high ee is required. It was established, that Novozym® 435 shows higher enantioselectivity (E = 34) than Lipozyme® RM IM (E = 26) as well as higher activity under conditions tested.

Influence of temperature

For evaluating the temperature effect on ester **(±)-5** resolution by Novozym® 435 with isopropenyl acetate, reactions monitored at 5°C and 30°C were compared (Figure 6).

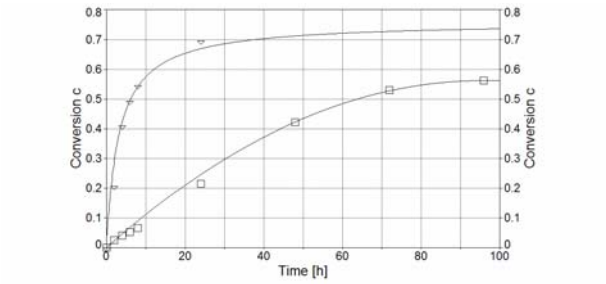


Figure 6. Effect of temperature on conversion rate for Novozym® 435/IPA. Reaction conditions: [**(±)-5**] = 0.25 mmol/mL , 50 mg enzyme, [IPA] = 0.5 mmol/mL; ∇ at 30°C, $t_{½}$ = 6.2 h ; □ at 5°C, $t_{½}$ = 63.9 h

It was found that lowering of the reaction temperature to 5°C resulted in over 10-fold elongation of time of 50% conversion (from 6.2 h to 63.6 h) and did not improve enantioselectivity (E = 35 at 30°C, E = 37 at 5°C).

Assignment of absolute configuration of preferred enantiomer

Acetylated ester **6** (59% ee), isolated from lipase catalyzed reaction was hydrolyzed at room temperature with sodium hydroxide in water/methanol solution yielding (+)-*N*-benzoyl-α-methylserine of specific rotation $[\alpha]_D^{19,5}$ = 3.5° (0.7, MeOH). For (*S*)-*N*-benzoyl-α-methylserine specific rotation $[\alpha]_D$ = -12.8° (1.5, MeOH) was reported [16]. Hence, absolute configuration of faster acetylated enantiomer was assigned as *R*.

Experimental

Materials

Chemicals and solvents of reagent grade were purchased from Fluka, Sigma-Aldrich or Chempur and were used without additional purification. Whole-cells preparations of *Mucor racemosus* and *Mucor circinelloides* were kindly donated by Prof. Tadeusz Antczak from Institute of Technical Biochemistry, Technical University of Łódź. Immobilized lipases Novozym 435® (*Candida antarctica B*), Lipozyme RM® IM (*Rhizomucor miehei*), Lipozyme TL® IM (*Thermomyces*

lanuginosus) were obtained from Novozymes, lipase AK (*Pseudomonas fluorescens*), PS (*Burkholderia cepacia*), M (*Mucor javanicus*), G (*Penicillium camemberti*), A (*Aspergillus niger*), AY (*Candida rugosa*) and acylase (*Aspergillus sp.*) were purchased from Amano, lipase *Candida cylindracea* and porcine pancreas lipase (PPL) from Sigma and *Rhizopus oryzae* lipase were obtained from Fluka.

Methods

Synthesis

(±)-5-Benzoylamino-5-methyl-4-oxo-1,3-dioxane (**1**) was synthesized in three steps from (±)-alanine according to [12]; m.p. 152-154°C; lit. m.p. 153-154°C.

(±)-*N*-benzoyl-α-methylserine ethyl ester (5). Oxodioxane **1** (7.006 g, 30 mmol) was dissolved in 20 mL 0.1 M EtONa in EtOH at r.t. After 3 h TLC control (hexane/THF 3:2) indicated disappearance of substrate **1**. Ethanol was evaporated, the residue was diluted with sat. $NaHSO_4$ (50 mL) and extracted with AcOEt (4×50 mL). Extract was successively washed with sat. $NaHSO_4$ (1×100 mL), sat. $NaHCO_3$ (4×100 mL) and quickly dried over anh. $MgSO_4$. After solvent evaporation, ester **5 (**6.582 g, 90%) was obtained as an yellow oil.

Purity >99% by HPLC.

^{1}H-NMR; δ [ppm]: 1.25 (t, J = 7 Hz, 3H); 1.59 (s, 3H); 3.83 (d, J = 12 Hz, 1H); 4.16 (d, J = 12 Hz, 1H); 4.22 (q, J = 7 Hz, 2H); 7.19 (s, 1H); 7.35-7.46 (m, 3H); 7.72-7.76 (m, 2H).

(±)-*O*-Acetyl-*N*-benzoyl-α-methylserine ethyl ester (6). To the ester **5 (**340 mg, 1.35 mmol) dissolved in 3 ml of pyridine Ac_2O (152 mg, 1.5 mmol) was added at r.t. After 3 days TLC control (hexane/THF 3:2) indicated disappearance of substrate **5**. Mixture was diluted with water (10 mL) and extracted with $CHCl_3$ (4×10 mL). Organic layer was successively washed with sat. $NaHSO_4$ (4×10 mL), sat. $NaHCO_3$ (3×10 mL) and was quickly dried over anh. $MgSO_4$. After evaporation, crude **6** (200 mg, 52%) was obtained as an yellow oil. Analytical sample was purified by column chromatography and obtained as yellow oil. Purity >99% by HPLC.

^{1}H-NMR; δ [ppm]: 1.30 (t, J = 7 Hz, 3H); 1.73 (s, 3H); 2.02 (s, 3H); 4.29 (dq, J = 7 Hz, 2H); 4.51 (d, J = 11Hz, 1H); 4.84 (d, J = 11Hz, 1H); 7.22 (s, 1H); 7.26-7.52 (m, 3H); 7.79-7.82 (m, 2H).

^{13}C-NMR; δ [ppm]: 13.94, 19.86, 20.55, 59.86, 62.12, 65.64, 126.86, 128.45, 131.55, 134.24, 166.51, 170.51, 172.14

(±)-*N*-benzoyl-α-methylserine (2) Oxodioxane **1** (3.932 g, 16.7 mmol) was dissolved in 1.5 M aq. NaOH (13.3 mL) with stirring at r.t. Termination of the reaction was marked with the solution turning orange and translucent. The mixture without stirring separated into two layers and crude N-benzoyl-α-methylserine crystallized spontaneously. Precipitate was separated by filtration and mother liquors were extracted with AcOEt (5×10 mL). Combined extract was evaporated to give residual product. Crystals of the product were dried in

a dessicator over P_2O_5 for 2 days yielding (±)-*N*-benzoyl-α-methylserine (2.608 g, 70%); m.p. 151-152°C, lit. [12] m.p. 154-155°C.

(±)-4-(Hydroxymethyl)-4-methyl-2-phenyl-1,3-oxazol-5(4*H*)-one (3). To CDMT (1.750 g, 10 mmol) suspended in THF (20 mL) and cooled to 0°C, NMM (1.16 mL, 10.6 mmol) was added while stirring and mixture was kept at 0°C for 30 min. Then substrate **2** (2.234 g, 10 mmol) was added and the mixture was kept at 0°C. After 2 hours TLC control (THF/MeOH 85:15) indicated disappearance of substrate. Then the mixture was stirred at r.t. with TLC monitoring until disappearance of triazinyl ester (ca. 48 h). The precipitate was filtered off, and washed with THF (30 mL). The filtrate was evaporated, yielding 3.694 g of yellowish, viscous oil containing some crystals. The crude product was dissolved in the mixture of AcOEt (100 mL) and water (10 mL), and the solution was successively washed with 1M citric acid (3×10 mL) 1M $NaHCO_3$ (3×10 mL) and water (1×10 mL). Organic layer was quickly dried over anh. $MgSO_4$ and evaporated, yielding **3** (1.698 g, 57%) as white, crystalline powder. M.p. 113-115°C; lit. [13] m.p. 125-127°C. Purity >99% by HPLC.

^{1}H-NMR; δ [ppm]: 1.52 (s, 3H), 3.93 (d, J = 11.3 Hz, 1H), 4.00 ppm (d, J = 11.3 Hz, 1H), 7.48-7.62 (m, 3H), 8.00-8.04 ppm (m, 2H)

(±)-4-(Acetoxymethyl)-4-methyl-2-phenyl-1,3-oxazol-5(4*H*)-one (4). To oxazolone **3** (205 mg, 0.92 mmol) dissolved in pyridine (0.5 mL), Ac_2O (112 mg, 1.1 mmol) was added at r.t. After 24 h additional Ac_2O (45 mg, 0.4 mmol) was added. After 3 days TLC monitoring (THF/MeOH 85:15) indicated disappearance of substrate **3**. The mixture was diluted with water (10 mL) and extracted with $CHCl_3$ (4×10 mL). Extract was successively washed with sat. $NaHSO_4$ (1×10 mL), sat. $NaHCO_3$ (4×10 mL) and water (1×10 mL). After drying over anh. $MgSO_4$ and solvent evaporation product **4** (126 mg, 55% yield) was obtained as oil, which crystallized after 24 h at 4°C forming yellow crystals. M.p. 50-52°C; purity >99% by HPLC.

^{1}H NMR; δ [ppm]: 1.58 (s, 3H), 2.00 (s, 3H), 4.32 (d, J = 11 Hz, 1H), 4.50 (d, J = 11 Hz, 1H), 7.30-7.64 (m, 3H), 8.04-8.09 ppm (m, 2H)

Analysis

Enzymatic reactions were performed in glass vials using thermostated Biosan Environmental Shaker-Incubator ES-20.

TLC was performed on Kieselgel 60 plates (Merck, art. No. 5715) with I_2 staining. For column chromatography Kieselgel 60 (Merck) was used with hexane/THF as eluent.

HPLC analyses were performed on ASI 100 Dionex apparatus with UVD 340S detector using analytical Chiralcel OD column (250×4.60 mm) and hexane/isopropanol 95:5 mobile phase (0.5 mL/min.) at 25ºC. UV detection at 225 nm for **5** and **6**, and at 250 nm for **3** and **4** was applied.

^{1}H- and ^{13}C-NMR spectra were recorded on Bruker DPX 200 Avance (250 MHz for proton, 62.5 MHz for carbon) in $CDCl_3$ with TMS as internal standard.

Optical rotations were measured at 20°C in a 1 dm cell using Rudolph Research polarimeter model Autopol IV at 589 nm wavelength.

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