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Diisopropyl Malonate as Acylating Agent in Kinetic Resolution of Chiral Amines with Lipase B from *Candida antarctica*

József Szemes[1], Ágnes Malta-Lakó[1], Regina Eszter Tóth[1], László Poppe[1,2,3*]

[1] Department of Organic Chemistry and Technology, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Műegyetem rkp. 3, H-1111 Budapest, Hungary
[2] Enzymology and Applied Biocatalysis Research Center, Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Arany János Str. 11, RO-400028 Cluj-Napoca, Romania
[3] SynBiocat Ltd., Szilasliget u. 3, H-1172 Budapest, Hungary
[*] Corresponding author, e-mail: poppe.laszlo@vbk.bme.hu

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Abstract

Activity of diisopropyl malonate (**2**) as a novel acylating agent was investigated in kinetic resolution (KR) of various racemic amines [(±)-**1a-d**] catalyzed by lipase B from *Candida antarctica*. Diisopropyl malonate (**2**) proved to be effective acylating agent with four racemic amines [(±)-2-aminoheptane, (±)-1-methoxy-2-propylamine, (±)-1-phenylethylamine and (±)-4-phenylbutan-2-amine; (±)-**1a-d**, respectively] selected for this study. The lipase-catalyzed acylation of the amines (±)-**1a-d** with **2** proceeded with good conversions (44.9–52.1%) and provided the expected (*R*)-amides [(*R*)-**3a-d**] in moderate to excellent yields (51–98%) with high enantiomeric excess ($ee_{(R)\text{-}\mathbf{3a\text{-}d}}$ 92.0–99.9%) after 4 h reaction time under mild reaction conditions in batch mode. The best conversion (50%) combined with high enantiomeric purity ($ee_{(R)\text{-}\mathbf{2d}}$ > 99%*ee*) was achieved in the KR from racemic 2-aminoheptane (±)-**1a**. The four novel (*R*)-amides [(*R*)-**3a-d**] were isolated and properly characterized.

Keywords

lipase from *Candida antarctica*, kinetic resolution, acylation, chiral amine, diisopropyl malonate, biocatalysis

1 Introduction

Nowadays, biocatalysis is an often-used technology for enantioselective synthesis, since biocatalysts may be more selective, efficient, easy-to-handle, economical and environmentally friendly compared to traditional chemical catalysts [1, 2]. Biocatalytic processes are used in many areas of industry (food, detergent, cosmetics, pharmaceuticals, biodiesel production) [3, 4].

The group of lipases (Enzyme Commision number – EC 3.1.1.3) is one of the most popular and often used enzyme family in asymmetric biotransformations and organic syntheses. Lipases primarily catalyze the degradation of triglycerides to fatty acids and glycerol, but they can also catalyze acylation, esterification, transesterification, aminolysis in organic solvents as well [5, 6]. Lipases are usually thermotolerant, do not require any cofactors and relatively stable in organic solvents. Besides the advantageous catalytic properties of lipases (high activity, wide substrate specificity, high chemo-, regio- and enantioselectivity), their properly immobilized forms can be easily recovered and reused.

Lipases also catalyze the kinetic resolution (KR) of racemic alcohols [7–9], or amines and their derivatives [10, 11]. It was found that lipase B from *Candida antarctica* (CaLB) is suitable for dynamic kinetic resolution (DKR) of primary amines [12, 13]. Chiral amines and their derivates [14, 15] are considered important building blocks in organic synthesis aiming a range of drugs, fine chemicals, and agrochemicals.

There are various acylating agents that can be advantageously used in lipase-catalyzed *N*-acylations. These include non-activated esters of acetic acid (e.g., ethyl acetate [16, 17], isopropyl acetate [18], *n*-butyl acetate), or the so-called activated esters (e.g., alkyl alkoxyacetates or alkyl cyanoacetates). The reactivity of activated esters as acylating agents is based on the presence of an electron withdrawing group (e.g., alkoxy, cyano, or halogen) at the

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β-position which enhances the partially positive character of the carbon of the ester function. For example, the activity in the lipase-catalyzed acylation with ethyl 2-methoxyacetate was more than one hundred times better than with butyl acetate [19]. This could be explained by the high electronegativity of the methoxy oxygen enhancing the electrophilicity of the carbonyl carbon during the action of lipase [20]. Due to its excellent productivity, efficiency, and selectivity, ethyl 2-methoxyacetate has been widely used by BASF since 1993 in the lipase-catalyzed kinetic resolution of various racemic amines [21].

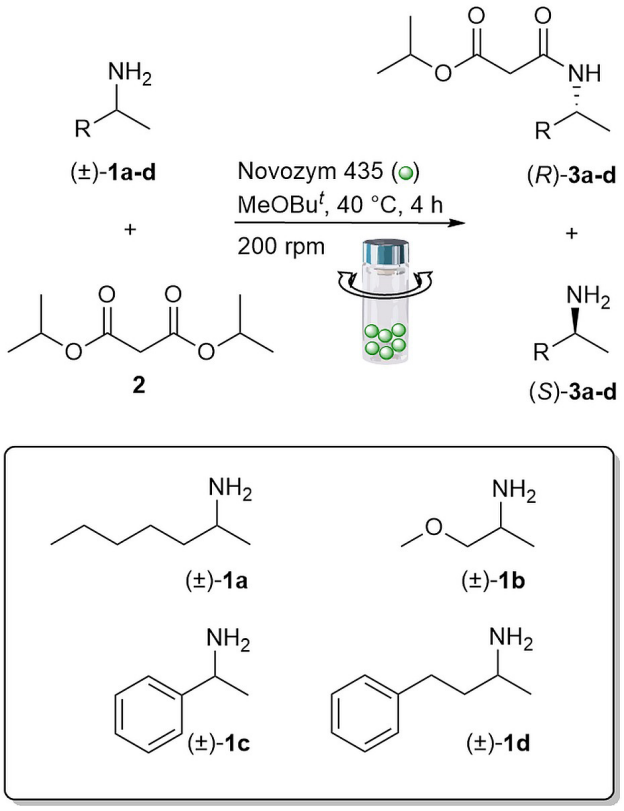
Our research group investigated the reactivity of various activated isopropyl esters such as 2-ethoxy-, 2-propoxy- and 2-butoxyacetates. The processes applying isopropyl 2-ethoxyacetate, and isopropyl 2-propoxyacetate as acylating agent in CaLB-catalyzed KRs could exceed the reactivity as well as the selectivity of the process with ethyl 2-methoxyacetate [22, 23]. Presumably, introduction of isopropyl as a leaving group into acylating agents diminished the non-selective chemical acylation as side-reaction with the ethyl esters, thereby increasing the apparent selectivity of the enzymatic process. In addition to the alkyl alkoxyacetates [22, 23], alkyl 2-cyanoacetates proved to be effective acylating agents with CaLB as well [24].

Garcia and his colleagues have demonstrated that immobilized CaLB (Novozym 435) catalyzes the aminolysis of β-ketoesters (ethyl 3-oxobutyrate and 3-oxo-3-phenylpropionate) with various racemic amines at room temperature in dioxane [25]. The corresponding optically active β-ketoamides were obtained in moderately high enantiomeric excess and yield. Diethyl malonate has been shown to be an efficient acyl donor in lipase-catalyzed resolution of aromatic amines [26]. Subsequently, a robust and efficient solvent-free method was developed for the kinetic resolution of racemic 1-phenylethane-1-amine with diethyl malonate catalyzed by immobilized CaLB [27].

Since our studies have demonstrated that changing ethyl esters to isopropyl esters [22–24] is advantageous due to diminishing the minor amount of chemical catalysis impairing the selectivity of the enzymatic process with ethyl esters, we investigated in this study the diisopropyl malonate as an acylating agent in the CaLB-catalyzed kinetic resolution of amines.

2 Results and discussion

Our aim was to study the *N*-acylating ability of diisopropyl malonate (**3**) with four different chiral aliphatic and aromatic primary amines (±)-**1a-d** (Scheme 1).



Scheme 1 CaLB-catalyzed kinetic resolution of racemic amines (±)-**1a-d** using diisopropyl malonate (**2**) as the acylating agent

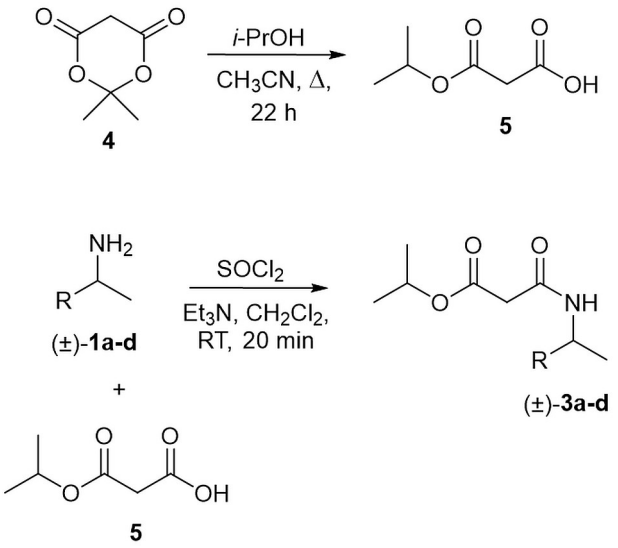
Before investigation of the CaLB-catalyzed enzymatic kinetic resolutions, the racemic amides (±)-**2a-d** were also synthesized as standards for enantiomer selective GC analysis to monitor these reactions.

2.1 Chemical synthesis of racemic amides (±)-3a-d

First, the corresponding racemic amides (±)-**3a-d** were prepared as standards for chiral GC analysis of the enzymic acylation. The racemic amides (±)-**3a-d** were synthetized smoothly starting from Meldrum's acid (**4**) and the racemic amines (±)-**1a-d** (Scheme 2). First, the 3-isopropoxy-3-oxopropanoic acid (**5**) as acylation agent was prepared from Meldrum's acid (**4**) by refluxing with isopropanol in acetonitrile for 22 h (Scheme 2).

The next step was the one-pot synthesis [28] of the racemic amides (±)-**3a-d** (Scheme 2).

Generally, the reaction was carried out by adding to the solution of racemic amines (±)-**1a-d** in dry dichloromethane (DCM) one equivalent of 3-isopropoxy-3-oxopropanoic acid (**5**) and three equivalents of Et_3N. After stirring the ice-cooled mixture for 5 min, one equivalent of $SOCl_2$ was added dropwise at a rate which kept the temperature of the reaction mixture below 15 °C. After $SOCl_2$ addition and a further 5 min stirring at 15–20 °C,



Scheme 2 Preparation of the racemic amides (±)-**3a-d** starting from Meldrum's acid (**4**) and the racemic amines (±)-**1a-d**

TLC analysis indicated a significant amount of the formed racemic amide (±)-**3a-d**. The highest proportion of product was present at a reaction time of 20 min, beyond which decomposition was observed. Although this one-step *N*-acylation provided lower yields (10–21%) than the usual two-step process, this method proved to be sufficient for quick preparation of the desired racemic amides (±)-**3a-d** being necessary as standards for chiral GC analysis.

2.2 CaLB-catalyzed kinetic resolution of chiral amines (±)-1a-d with diisopropyl-malonate (2)

After having the analytic methods enabling determination of the enantiomeric compositions of the KR processes in our hand, reaction conditions were optimized with KR of racemic 1-phenylethane-1-amine (±)-**3c**. The same reaction conditions were tried as described earlier for the acylations with isopropyl cyanoacetate [24]. First, the reaction was carried out without any solvent at 40 °C for 4 h, then the reactions using *tert*-amyl alcohol and methyl *tert*-butyl ether (MTBE) as solvents were tried. This short screen revealed the reaction in MTBE as the most efficient, since in the other two cases almost no product formation and low yields could be achieved. Thus, MTBE was used as solvent in the further experiments (Scheme 1). After purification, the formed isopropyl (*R*)-3-oxo-3-[(1-phenylethyl) amino]propanoate (*R*)-**3c** was obtained in yield of 49% (based on racemate) with excellent enantiomeric excess ($ee_{(R)\text{-}3c}$ = 99.9%). Thus, the other three amides (*R*)-**3a,b,d** were synthetized by applying the same reaction conditions.

The reactions were sampled in every hour and the conversion and enantiomeric composition of the products were determined by GC on a chiral column after derivatization of the residual amines (*S*)-**1a-d** in the KR mixtures to their acetamides by Ac_2O-treatment. As shown on Fig. 1 A), the progress of the conversion in the KRs depended on the nature of the starting amine (±)-**1a-d**. The *N*-acylation of the racemic 1-methoxy-2-propylamine (±)-**1b** catalyzed by Novozym 435 form of CaLB with diisopropyl malonate was the most rapid, while the enzymatic acylation of the bulkier amines (±)-**1a,c,d** was slower. However, proper conversions (≥45%) could be achieved in all KRs after 4 h (Table 1).

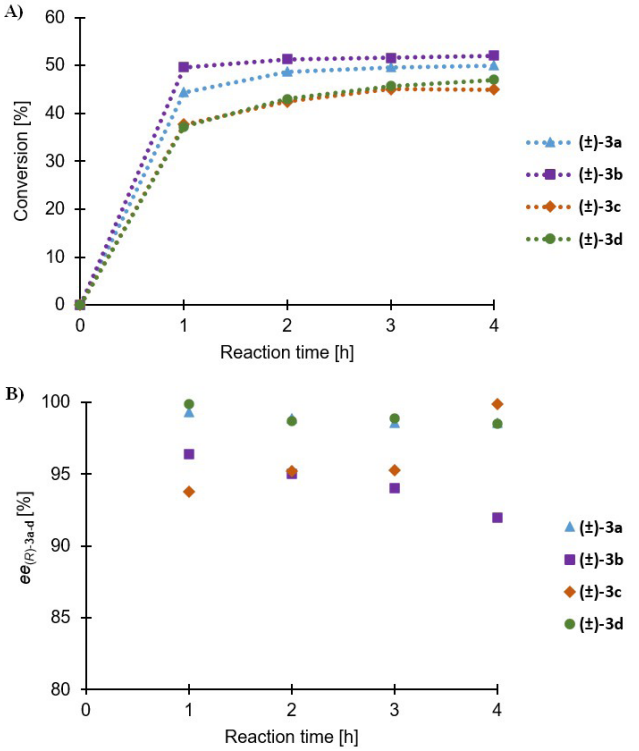


Fig. 1 CaLB catalyzed KR of (±)-**1a-d** with diisopropyl malonate (**2**) [A) Conversion-reaction time plot of KRs of (±)-**1a-d**; B) Enantiomeric excess ($ee_{(R)\text{-}2a\text{-}d}$)-reaction time plot]

Table 1 Results of the CaLB-catalyzed kinetic resolution of racemic amines (±)-**1a-d** amines with diisopropyl malonate (**2**)

Amine[a]	c^a [%]	$Y_{(R)\text{-}3a\text{-}d}{}^b$ [%]	$ee_{(R)\text{-}3a\text{-}d}$ [%]	$ee_{(S)\text{-}1a\text{-}d}$ [%]	E [-]
(±)-**1a**	50.0	31	98.5	98.7	»200
(±)-**1b**	52.1	25	92.0	99.9	>100
(±)-**1c**	45.0	49	99.9	81.5	»200
(±)-**1d**	47.0	30	98.5	87.5	>200

[a] Reaction conditions: Novozym 435 (20 mg), MTBE (0.2 mL), (±)-**1a-d** (0.5 mmol), diisopropyl malonate (**2**) (1 equiv.), 40 °C, 200 rpm, 4 h.

[b] Isolated yields of the (*R*)-amides (*R*)-**3a-d** are based on the amount of the starting racemic amine (±)-**1a-d**.

The reaction with racemic heptane-2-amine (±)-**1a** stopped at 50.0% conversion due to the high enantiomer selectivity of the process (E »200), but with 1-methoxy-2-propylamine (±)-**1b** the somewhat lower degree of enantiomer selectivity (E > 100) enabled to exceed the 50% conversion and resulted in decreased enantiomeric excess of the product ($ee_{(R)\text{-}\mathbf{3a\text{-}d}}$ = 92.0%). The lowest conversion in 4 h (c = 45.0%) could be achieved with 1-phenylethane-1-amine (±)-**1c**. By increasing the reaction time, the value of the conversion could presumably also be increased.

The known (*R*)-selectivity of lipase B from *Candida antartica* in KRs of amines was confirmed in our case by GC analysis. Expectedly, when the degree of the (*R*)-selectivity of the kinetic resolutions is not as high as for 1-phenylethane-1-amine (±)-**3c** (E »200) a slight decrease of the enantiomeric excess of the products at conversions close to 50% could be observed (Fig. 1 B)).

Although the *N*-acylation reactions proceeded with high conversion according to the CG analysis, the isolated yields were only moderate (except for (*R*)-**3c**). The reason of these lower yield could stem from the small-scale work up including preparative TLC and difficulties during the removal of the more polar amides [(*R*)-**3a,b,d**] from the chromatographic support.

Overall–based on the above reported results–diisopropyl malonate proved to be an excellent acylating agent in the CaLB-catalyzed kinetic resolutions of the four investigated racemic amines (±)-**1a-d** of various properties.

3 Conclusions

In summary, this study extended the armory of useful acylating agents for the lipase-catalyzed kinetic resolution of chiral amines with diisopropyl malonate (**2**) which proved to be an efficient activated acylating agent in KRs of four racemic amines (±)-**1a-d**. The four new amides (*R*)-**3a-d** have been characterized spectrally and by their specific optical rotation as well. The reactivity of the acyl moiety of the forming amides (*R*)-**3a-d** opens room for further synthetic applications of the novel process based on using of diisopropyl malonate (**2**) as acylating agent.

4 Experimental section

4.1 Materials

CaLB N435 (Novozym® 435, lipase B from *Candida antarctica*, recombinant, expressed in *Aspergillus niger*, adsorbed on acrylic resin) was obtained from Sigma–Aldrich (Saint Louis MO, USA). All other reagents and solvents were the products of Sigma–Aldrich (Saint Louis MO, USA), Merck (Darmstadt, Germany), or Alfa Aesar Europe (Karlsruhe, Germany).

4.2 Methods

TLC was carried out using Kieselgel 60 F254 (Merck) sheets. Spots were visualized under UV light (Vilber Lourmat VL-6.LC, 254 nm) or after treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates.

The NMR spectra were recorded in $CDCl_3$ on a Bruker Avance DRX300- or 500 spectrometers operating at 300 or 500 MHz for ^1H and 75 or 126 MHz for ^{13}C, and signals are given in ppm on the δ scale.

Infrared spectra were recorded on a Bruker ALPHA FT-IR spectrometer in ATR mode and wavenumbers of bands are listed in cm^{-1}.

Optical rotation was measured on Perkin–Elmer 241 polarimeter at the D-line of sodium. The polarimeter was calibrated with measurements of both enantiomers of menthol.

The gas chromatographic (GC) analyses were performed with an Agilent 4890 gas chromatograph equipped with flame ionization detector (FID) using H_2 carrier gas (injector: 250 °C, detector: 250 °C, head pressure: 12 psi, split ratio: 50:1) and a Hydrodex β-6TBDM column [25 m × 0.25 mm × 0.25 μm film with heptakis-(2,3-di-*O*-methyl-6-*O*-*t*-butyldimethyl-silyl)-β-cyclodextrine; Macherey & Nagel (Düren, Germany)] using the temperature programs indicated in Table S1 (ESI; Supplement). GC chromatograms and NMR spectra are given as Fig. S1–Fig. S31 in Supplement.

Conversion (c) and enantiomeric excess (ee) values were determined by GC. Conversion was calculated using Eq. (1):

$$c = ee_S \times \left(ee_S + ee_P\right)^{-1}, \tag{1}$$

where ee_S is the ee of the substrate and ee_P is the ee of the product). Enantiomeric ratio/selectivity (E) was calculated from the enantiomeric excess (ee) of the substrate (ee_S) and product (ee_P) using Eq. (2) [29]:

$$E = \frac{\ln\left[\left(1 - ee_S\right)/\left(1 + ee_S/ee_P\right)\right]}{\ln\left[\left(1 + ee_S\right)/\left(1 + ee_S/ee_P\right)\right]}. \tag{2}$$

Due to sensitivity of the E value above 100 to small deviations of experimental errors, E values calculated in the range of 100–200 were given as >100, those in the range of 200–500 as >200 and above 500 as »200.

4.3 Synthesis of 3-isopropoxy-3-oxopropanoic acid (5)

Meldrum's acid (**4**, 30.8 mmol) was dissolved in acetonitrile (16 mL), then the isopropanol (30.8 mmol, 1 equiv.) was added to the solution. The reaction mixture was refluxed and stirred for 22 h. Progress of the reaction was monitored by thin layer chromatography (TLC). After 20 min reaction time, the reaction mixture was concentrated on a rotary evaporator and the crude residue appearing as a yellow oil was applied in the further reaction as such.

Yield: 90%. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (d, J = 6.3 Hz, 6H, 2 × CH_3), 3.46 (s, 2H, CH_2), 5.10 (m, 1H, OCH), 10.90 (bs, OH). ^{13}C-NMR ($CDCl_3$) δ: 21.6 (CH_3), 41.1 (CH_2), 69.9 (CHO), 166.6 (COO), 171.7 (COO).

4.4 One-pot acylation of racemic amines (±)-1a-d with 3-isopropoxy-3-oxopropanoic acid (5)

General method: 3-Isopropoxy-3-oxopropanoic acid (**5**, 0.685 mmol), the corresponding amine ((±)-**1a-d**, 0.685 mmol) and triethylamine (2.055 mmol, 3 equiv.) were added to dry dichloromethane (6 mL) and the mixture was ice-cooled for 5 min. Then one equivalent of thionyl chloride (0.685 mmol, 1 equiv.) was added dropwise at a rate which kept the temperature of the reaction mixture below 15 °C. After $SOCl_2$ addition, the reaction was monitored by TLC. After 20 min stirring at room temperature, the reaction was quenched with distilled water (5 mL) and diluted with dichloromethane (10 mL). The organic phase was washed twice with 1M HCl (2 × 5 mL), once with 10% Na_2CO_3 (5 mL) and twice with brine (2 × 5 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by preparative TLC using silica gel plates and DCM:MeOH, 20:1 as eluent.

Racemic isopropyl 3-(heptan-2-ylamino)-3-oxopropanoate (±)-**3a**: White crystals. Yield: 10%. R_f = 0.70 (DCM:MeOH, 20:1). Melting point: 53.6 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.88 (d, J = 8.2 Hz, 1H, NH), 5.03 (h, J = 6.3 Hz, ^{1}H, CH-O), 3.97 (dq, J = 8.2, 6.3 Hz, 1H, CH-N), 3.24 (s, 2H, CO-CH_2-CO), 1.47–1.37 (m, 2H, 1 × CH_2), 1.35–1.20 (m, 12H, 3 × CH_2 + 2 × CH_3), 1.13 (d, J = 6.6 Hz, 3H, CH_3), 0.86 (t, J = 6.7 Hz, 3H, CH_3). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 169.3, 164.3, 69.3, 45.4, 41.6, 36.7, 31.7, 25.6, 22.5, 21.7, 20.8, 14.0. IR (cm^{-1}): 3291, 3084, 2955, 2923, 2873, 2854, 1736, 1639, 1551, 1467, 1453, 1373, 1261, 1161, 1144, 1105, 741, 724.

Racemic isopropyl 3-[(1-methoxypropan-2-yl) amino]-3-oxopropanoate (±)-**3b**: Yellow oil. Yield: 15%. R_f = 0.53 (DCM:MeOH, 20:1); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.13 (s, 1H, NH), 5.04 (h, J = 6.3 Hz, 1H, CH-O), 4.16 (m, 1H, CH-N), 3.35 (s, 3H, OCH_3), 3.40–3.31 (m, 2H, O-CH_2), 3.25 (s, 2H, CO-CH_2-CO), 1.24 (d, J = 6.3 Hz, 3H, 1 × CH_3), 1.18 (d, J = 6.8 Hz, 6H, 2 × CH_3); ^{13}C-NMR (126 MHz, $CDCl_3$) δ 168.8, 164.6, 77.2, 69.2, 59.1, 45.0, 41.8, 21.7, 17.5; IR (cm^{-1}): 3293, 3077, 2979, 2930, 2879, 2832, 2816, 1735, 1648,1543, 1452, 1374, 1258, 1145, 1199, 1103, 971.

Racemic isopropyl 3-oxo-3-[(1-phenylethyl)amino] propanoate (±)-**3c**: Yellow oil. Yield: 13%. R_f = 0.73 (DCM:MeOH, 20:1); ^{1}H-NMR (500 MHz,$CDCl_3$) δ 7.47 (d, J = 6.5 Hz, 1H, NH), 7.37–7.28 (m, 4H, 4 × ArH), 7.32–7.21 (m, 1H, 1 × ArH), 5.14 (m, 1H, CH-N), 5.04 (h, J = 6.3 Hz, 1H, CH-O), 3.33–3.18 (m, 2H, CO-CH_2-CO), 1.50 (d, J = 6.9 Hz, 3H, 1 × CH_3), 1.25 (dd, J = 6.9, 5.3 Hz, 6H, 2 × CH_3); ^{13}C-NMR (126 MHz, $CDCl_3$) δ 169.2, 164.2, 143.1, 128.7, 127.4, 126.4, 69.4, 48.9, 41.4, 22.1, 21.7; IR (cm^{-1}): 3291, 3066, 3032, 2978, 2934 2875, 1734, 1645 1542, 1495, 1450, 1374, 1270, 1180, 1146, 1104, 977, 950, 761, 699.

Racemic isopropyl 3-oxo-3-[(4-phenylbutan-2-yl) amino]propanoate (±)-**3d**: Yellow oil. Yield: 21%. R_f = 0.64 (DCM:MeOH, 20:1); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.30–7.23 (m, 1H, NH), 7.20–7.13 (m, 4H, 4 × ArH), 6.98 (m, 1H, 1 × ArH), 5.04 (h, J = 6.3 Hz, 1H, CH-O), 4.06 (dq, J = 8.2, 6.6 Hz, 1H, CH-N), 3.24 (s, 2H, CO-CH_2-CO), 2.64 (td, J = 7.3, 2.1 Hz, 2H, CH_2-Ph), 1.84–1.72 (m, 2H, CH_2), 1.26 (d, J = 6.3 Hz, 6H, 2 × CH_3), 1.19 (d, J = 6.6 Hz, 3H, 1 × CH_3); ^{13}C-NMR (126 MHz, $CDCl_3$) δ 169.3, 164.4, 141.7, 128.4, 128.3, 125.9, 69.4, 45.3, 41.5, 38.5, 32.5, 21.7, 20.9; IR (cm^{-1}): 3292, 3084, 3064, 3026, 2978, 2934, 2861, 1734, 1644, 1547, 1495, 1453, 1374, 1272, 1181, 1145, 1105, 952, 747, 699.

4.5 CaLB-catalyzed kinetic resolution of the racemic amines (±)-1a-d with diisopropyl malonate (2)

General method: Into a 4 mL screw-cap vial were added immobilized CaLB enzyme (20.0 mg, Novozym 435), methyl *tert*-butyl ether (200 µL), the corresponding amine ((±)-**1a-d**, 0.5 mmol, 1 equiv.) and diisopropyl malonate (**2**, 0.5 mmol). The reaction mixture was shaken (200 rpm) for 4 h at 40 °C and monitored by taking samples after different reaction times (1 h, 2 h, 3 h, 4 h). After 4 h, the reaction mixture was worked up.

The enzyme was filtered through a glass filter and washed with methyl *tert*-butyl ether (2 × 0.5 mL). After evaporation of the solvent the residue was purified by preparative TLC using silica gel plates and DCM:MeOH 20:1 as eluent. After evaporation of the solvent in vacuum, the corresponding (*R*)-**3a-d** amides were obtained as light-yellow crystals/oil.

Isopropyl (R)-3-(heptan-2-ylamino)-3-oxopropanoate (*R*)-**3a**: Light yellow crystals. Yield: 31%. Melting point: 49.5 °C. $[\alpha]_D^{27.5}$ = −2.4 (*c* = 0.43, CH_2Cl_2). ^{1}H-NMR (500 MHz, $CDCl_3$) *δ* 6.93–6.87 (m, 1H, NH), 5.04 (h, *J* = 6.3 Hz, 1H, CH-O), 3.98 (dq, *J* = 8.4, 6.5 Hz, 1H, CH-N), 3.24 (s, 2H, CO-CH_2-CO), 1.47–1.37 (m, 2H, 1 × CH_2), 1.36–1.21 (m, 12H, 3 × CH_2 + 2 × CH_3), 1.14 (d, *J* = 6.6 Hz, 3H, CH_3), 0.87 (t, *J* = 6.7 Hz, 3H, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$) *δ* 169.5, 164.4, 69.4, 45.6, 41.8, 36.9, 31.9, 25.8, 22.7, 21.9, 21.0, 14.2. IR (cm^{-1}): 3297, 3084, 2964, 2925, 2873, 2857, 1742, 1640, 1552, 1469, 1455, 1374, 1263, 1159, 1148, 1106, 741, 725.

Isopropyl (R)-3-[(1-methoxypropan-2-ylamino]-3-oxopropanoate (*R*)-**3b**: Light yellow oil. Yield: 25%. $[\alpha]_D^{27.5}$ = −1.4 (*c* = 0.44, CH_2Cl_2). ^{1}H-NMR (500 MHz, $CDCl_3$) *δ* 7.14 (s, 1H, NH), 5.04 (h, *J* = 6.3 Hz, 1H, CH-O), 4.16 (m, 1H, CH-N), 3.40–3.31 (m, 2H, OCH_2), 3.35 (s, 3H, OCH_3), 3.25 (s, 2H, CO-CH_2-CO), 1.24 (d, *J* = 6.3 Hz, 3H, 2 × CH_3), 1.18 (m, 3H, CH_3); ^{13}C-NMR (126 MHz, $CDCl_3$) *δ* 168.8, 164.6, 77.2, 69.3, 59.1, 45.0, 41.8, 21.7, 17.5; IR (cm^{-1}): 3292, 3078, 2980, 2935, 2880, 2832, 2817, 1735, 1648,1543, 1453, 1374, 1253, 1145, 1179, 1104, 967.

Isopropyl (R)-3-oxo-3-[(1-phenylethyl)amino]propanoate (*R*)-**3c**: Light yellow crystal. Yield: 49%. Melting point: 61 °C. $[\alpha]_D^{27.5}$ = −2.8 (*c* = 0.43, CH_2Cl_2). ^{1}H-NMR (500 MHz, $CDCl_3$) *δ* 7.46 (s, 1H, NH), 7.37–7.29 (m, 4H, 4 × ArH), 7.29–7.21 (m, 1H, 1 × ArH), 5.14 (m, 1H, CH-N), 5.04 (h, *J* = 6.3 Hz, 1H, CH-O), 3.33–3.18 (m, 2H, CO-CH_2-CO), 1.50 (d, *J* = 6.9 Hz, 3H, 1 × CH_3), 1.25 (dd, *J* = 6.9, 5.3 Hz, 6H, 2 × CH_3); ^{13}C-NMR (126 MHz, $CDCl_3$) *δ* 169.2, 164.2, 143.1, 128.7, 127.3, 126.1, 69.4, 48.9, 41.4, 22.1, 21.7; IR (cm^{-1}): 3262, 3088, 2984, 2971, 2931, 2874, 1744, 1648, 1570, 1494, 1451, 1377, 1280, 1195, 1172, 1144, 1097, 759, 663.

Isopropyl (R)-3-oxo-3-[(4-phenylbutan-2-yl)amino] propanoate (*R*)-**3d**: Light yellow crystals. Yield: 30%. Melting point: 68 °C. $[\alpha]_D^{27.5}$ = −2.6 (*c* = 0.43, CH_2Cl_2). ^{1}H-NMR (500 MHz, $CDCl_3$) *δ* 7.26 (t, *J* = 7.5 Hz, 2H, 2 × ArH), 7.19–7.13 (m, 3H, 3 × ArH), 6.97 (d, *J* = 8.2 Hz, 1H, 1 × ArH), 5.04 (h, *J* = 6.2 Hz, 1H, CH-O), 4.06 (dq, *J* = 8.3, 6.5 Hz, 1H, CH-N), 3.23 (s, 2H, CO-CH_2-CO), 2.64 (td, *J* = 7.4, 2.5 Hz, 2H, CH_2-Ph), 1.82–1.73 (m, 2H, CH_2), 1.25 (d, *J* = 6.3 Hz, 6H, 2 × CH_3), 1.18 (d, *J* = 6.6 Hz, 3H, 1 × CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) *δ* 169.4, 164.6, 141.9, 128.6, 128.5, 126.0, 69.5, 45.4, 41.7, 38.7, 32.6, 21.9, 21.1; IR (cm^{-1}): 3292, 3085, 3064, 3029, 2980, 2936, 2859, 1732, 1642, 1555, 1493, 1454, 1376, 1272, 1187, 1146, 1108, 956, 752, 700.

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