

Kinetic Resolution of Cyclic β -Amino Esters with Lipase B from *Candida antarctica* on Magnetic Nanoparticles

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Abstract

The biocatalytic kinetic resolution of three racemic cyclic β -amino esters ($\pm$)-ethyl *cis*-2-aminocyclopent-3-enecarboxylate ($\pm$)-**1a**, ($\pm$)-ethyl *cis*-2-aminocyclohex-3-enecarboxylate ($\pm$)-**1b**, and ($\pm$)-ethyl *trans*-6-aminocyclohex-3-enecarboxylate ($\pm$)-**1c** was investigated using lipase B from *Candida antarctica* immobilized on magnetic nanoparticles with three different acylating agents (**E**, **C**, **M**). Among the substrates examined, ($\pm$)-**1a** underwent a highly enantioselective *N*-acylation, displaying the characteristic profile of a classical kinetic resolution with conversions ~50% and excellent enantiomeric excess of the (*R*)-amide product ($ee_{(R)\text{-}2aE} > 99.9\%$). The reaction showed notable robustness across a wide substrate concentration range, highlighting its potential for larger-scale preparation of enantiomerically pure compounds. In contrast, substrates ($\pm$)-**1b** and ($\pm$)-**1c** were not resolved at preparative scale due to the low selectivity of the biocatalyst in kinetic resolution (for ($\pm$)-**1b**) or insufficient chromatographic separation of the corresponding racemic amide ($\pm$)-**2cE**). The three novel racemic amides [($\pm$)-**2a–cE**] and enantiopure (*R*)-**2aE** and (*S*)-**1a** were isolated and properly characterized.

Keywords

lipase B from *Candida antarctica*, kinetic resolution, acylation, racemic cyclic β -amino esters, isopropyl 2-ethoxyacetate, biocatalysis

1 Introduction

Cyclic β -amino esters constitute an important class of chiral building blocks widely applied in modern synthetic chemistry, serving as versatile precursors for the construction of heterocycles, peptides, and numerous other bioactive derivatives [1]. Owing to their rigidified frameworks, these compounds are frequently incorporated into bioactive molecules, enzyme inhibitors, and receptor ligands, where they enhance metabolic stability and modulate binding interactions [1]. They are found across a broad range of small- and large-molecule antifungal antibiotics (e.g., cispentacin, icofungipen, amipurimycin) and analgesics (e.g., tilidine) (Fig. 1) [1–5]. It was recently demonstrated that functionalized cyclic β -amino acid derivatives exhibited antiviral potential [6].

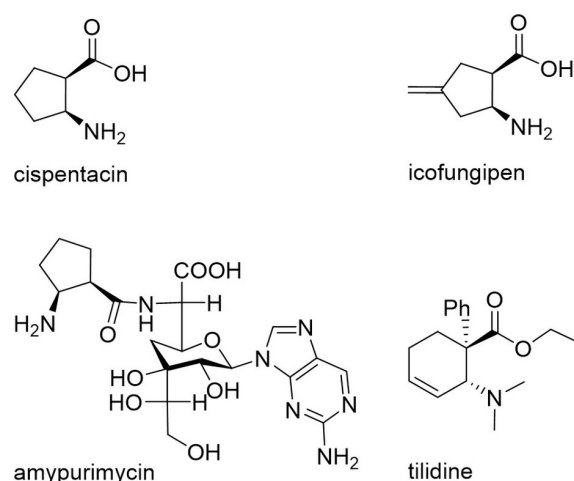


Fig. 1 Pharmacologically active β -amino acid derivatives

Biocatalysis has emerged as a highly efficient platform for constructing complex, biologically active molecules with exceptional selectivity. In contrast to non-stereoselective chemical routes that generate significant waste, enzymatic transformations offer inherently cleaner and more sustainable pathways. Advances in enzyme engineering and expanded catalytic repertoires now enable enzymes to operate with reagent-like reliability across diverse synthetic challenges, underscoring their growing importance in modern chemical synthesis [7–12].

The need for enantiomerically pure compounds is particularly pronounced in drug development, where stereochemistry often determines biological activity. More than half of all marketed drugs are chiral, and in many cases only one enantiomer exhibits the desired therapeutic effect [13]. The thalidomide tragedy further highlighted the critical importance of stereochemical control and rigorous enantiomeric purity in pharmaceutical synthesis [14, 15].

Lipases (EC 3.1.1.3) represent one of the most versatile enzyme families used in synthetic chemistry. They catalyze hydrolysis, esterification, transesterification, and aminolysis reactions, and their robustness in organic solvents makes them suitable for non-aqueous biotransformations [16, 17]. Many lipases show excellent enantiomer selectivity; as such, they have been used in e.g., synthesis of enantiopure amines using kinetic resolution and dynamic kinetic resolution [18, 19]. Among them, lipase B from *Candida antarctica* (CaLB) has emerged as a benchmark biocatalyst due to its broad substrate scope, operational stability, and pronounced enantioselectivity, since its first industrial application in the kinetic resolution (KR) of α -methylbenzylamine in the 1990s [20], CaLB has been widely employed in the preparation of enantiopure amines, alcohols, and amides.

Enzyme immobilization is widely used to enhance biocatalyst performance, as it can improve enzyme activity, stability, selectivity, and resistance toward inhibitors or organic solvents, while also enabling catalyst reuse [21, 22]. Although porous supports are commonly applied, they often suffer from diffusion limitations, enzyme desorption, mechanical brittleness, and even disintegration in certain organic media, as exemplified by the widely used Novozym 435, where CaLB is immobilized on macroporous Lewatit VP OC 1600 resin [23]. As an alternative, magnetic nanoparticles (MNPs) provide a non-porous, high-surface-area platform that minimizes mass-transfer barriers and prevents enzyme leaching, while their superparamagnetic properties allow simple magnetic separation

and efficient recyclability [24, 25]. These advantages make MNP-based immobilization a versatile tool across many areas of chemistry [26], while at the same time providing a highly suitable and efficient platform for biocatalytic applications, including the immobilization of CaLB for KR and dynamic kinetic resolution processes involving amines [27]. CaLB-MNP proved to be useful biocatalyst for KRs under continuous-flow conditions [28, 29].

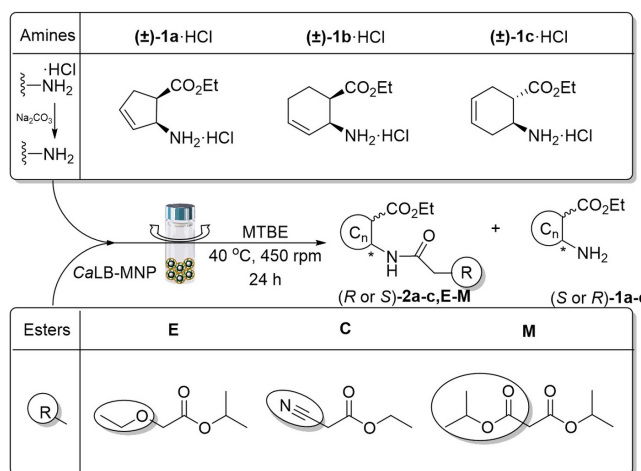
KR remains one of the most straightforward and widely applied biocatalytic strategies for obtaining enantiomerically enriched compounds. In CaLB-catalyzed KR of amines, the enzyme selectively acylates one enantiomer of the racemic mixture, yielding an optically enriched amide and the unreacted enantiopure amine [29]. The efficiency of the process strongly depends on the choice of acyl donor. Among the numerous acylating agents that have been studied previously, our research group investigated the reactivity of various acylating agents: ethyl acetate [30, 31], diisopropyl malonate **M** [32], isopropyl 2-cyanoacetate **C** [33], isopropyl 2-ethoxyacetate **E** [34–36]. In the case of activated esters, β -position electron-withdrawing groups- such as alkoxy, cyano, or halogen substituents- augment the partial positive character of the carbonyl carbon, resulting in increased acylation reactivity. The use of isopropyl esters rather than ethyl esters often enhances the overall selectivity of the biocatalytic transformation, as the bulkier isopropyl group diminishes competing non-enzymatic acylation pathways [33–36].

2 Results and discussion

The aim of this study was to achieve lipase-catalyzed kinetic resolution of three racemic cyclic β -amino esters – ethyl *cis*-2-aminocyclopent-3-enecarboxylate [(±)-**1a**], ethyl *cis*-2-aminocyclohex-3-enecarboxylate [(±)-**1b**], and ethyl *trans*-6-aminocyclohex-3-enecarboxylate [(±)-**1c**] – having relevance in peptide chemistry and drug design. To accomplish this, CaLB, immobilized on magnetic nanoparticles was applied in *N*-acylation reactions to obtain the corresponding enantiomerically enriched products. In this study, CaLB-MNP_{A10} from our previous work (almost fully immobilized: $Y_1 = 99\%$, after 60 min) [28] having a relatively high amount of CaLB (90 mg CaLB g_{MNP}⁻¹) was selected as biocatalyst.

2.1 Testing of various acylating agents in kinetic resolution

At the initial stage of our work, the major goal was to identify the most suitable acylating agent for the biocatalytic *N*-acylation reaction (Scheme 1).



Scheme 1 CaLB-catalyzed *N*-acylation of racemic amino esters (±)-1a–c·HCl with various isopropyl esters (**E**, **C**, **M**) as acylating agents. Reaction conditions: CaLB-MNP (10 mg), methyl *tert*-butyl ether (MTBE) (0.2 mL), (±)-1a–c (0.125 mmol), isopropyl 2-ethoxyacetate **E**, ethyl 2-cyanoacetate **C** or diisopropyl malonate **M** (1 equiv.), 40 °C, 450 rpm, 12 h.

Previous studies indicated that CaLB in soluble form was applicable for KR of (±)-1c [37], and other lipases could perform KR of (±)-1c with moderate selectivity [38]. Our research group has previously examined the performance of various acylating agents in lipase-catalyzed stereoselective biotransformations of chiral amines [30–36]. Based on these results, three acylating agents – isopropyl 2-ethoxyacetate **E**, ethyl 2-cyanoacetate **C**, and diisopropyl malonate **M** – were selected for preliminary evaluation with our substrates. Ethyl 2-cyanoacetate **C** and diisopropyl malonate **M** were readily available in our laboratory, whereas isopropyl 2-ethoxyacetate **E** had to be prepared synthetically, in two steps [34].

To enable the use of amines (±)-1a–c as substrates under the reaction conditions previously optimized [29] for the CaLB-MNP biocatalyst (40 °C, *tert*-butyl methyl ether (MTBE) as solvent), it was first necessary to liberate them from their salt forms (±)-1a–c·HCl (Scheme 1), following the protocol given in Section 4.3.

First, the three designed esters were tested as acylating agents in the CaLB-MNP-based kinetic resolution of the target cyclic amines ((±)-1a–c, Scheme 1). Preliminary activity tests revealed that isopropyl 2-ethoxyacetate **E** exhibited the highest activity. Diisopropyl malonate **M** was not an active acylating agent for our substrates (±)-1a–c, whereas ethyl 2-cyanoacetate **C** showed good activity towards the cyclic amine substrates (Figs. S1–S5). In the reaction of substrate (±)-1c with ethyl 2-cyanoacetate **C**, the mixture solidified during the process, preventing sampling; this phenomenon is presumably attributable to the sensitivity of the cyano group and the formation of side products. Consequently, isopropyl 2-ethoxyacetate **E**

was selected as the acylating agent for the subsequent lipase-catalyzed kinetic resolution experiments.

2.2 One-pot *N*-acylation of the racemic cyclic β-amino esters (±)-2a–cE as standards for gas chromatography

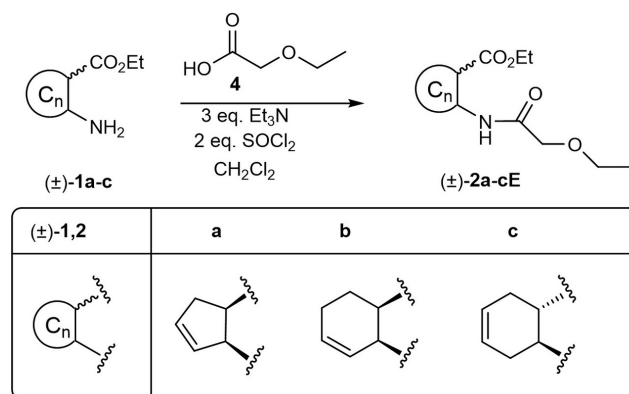
The appropriate racemic cyclic β-amino amides (±)-2a–cE – required as standards for gas chromatography (GC) – were prepared by chemical acylation carried out using a one-pot method adopted from the literature (Scheme 2) [32, 39]. Thus, the acyl chloride was not isolated in a separate step but – during the second stage of the one-pot process – the *in-situ* forming acid chloride performs the acylation. Consequently, the desired synthesis could be performed in short time yielding the racemic amides (±)-2a–cE after preparative thin-layer chromatography (TLC) in 36–71% yields.

2.3 *Candida antarctica* lipase B-catalyzed kinetic resolution of the cyclic β-amino esters (±)-1a–c with isopropyl 2-ethoxyacetate **E**

The biocatalytic *N*-acylation reactions were subsequently conducted under same conditions to those employed in the earlier experiments, using isopropyl 2-ethoxyacetate **E** as the acylating agent. To obtain more detailed information on the biocatalyzed reactions, samples were taken at 1, 2, 4, 8, and 24 h. Following appropriate sample work-up, these were analyzed by GC. By this stage, the racemic amides (±)-2a–cE had been prepared and were available as reference standards for the development and refinement of the chiral GC analytical method. For the three different substrates, three distinct outcomes and conclusions were obtained.

2.3.1 *Candida antarctica* lipase B-magnetic nanoparticle aided kinetic resolution of (±)-1a with isopropyl 2-ethoxyacetate **E**

For substrate (±)-1a the reaction proceeded with high enantiomer selectivity (Table 1). Since lipase B from *Candida antarctica* is known to exhibit (*R*)-selectivity,



Scheme 2 Synthesis of racemic cyclic β-amides (±)-2a–cE

Table 1 Results of the CaLB-catalyzed kinetic resolution of racemic cyclic β -amino ester ($\pm$)-**1a** with isopropyl 2-ethoxyacetate **E**

Reaction time [h]	c^a [%]	$ee_{(S)-1a}$ [%]	$ee_{(R)-2aE}$ [%]	E
1	16.8	17.0	99.5	>200
2	25.3	33.0	99.8	>200
4	37.5	59.6	99.8	>200
8	47.0	88.4	99.7	>200
24	50.3	99.9	99.1	>200

^a Reaction conditions: CaLB-MNP (10 mg), MTBE (0.2 mL), ($\pm$)-**1a** (0.125 mmol), isopropyl 2-ethoxyacetate **E** (1 equiv.), 40 °C, 350 rpm, 24 h.

the product formed can be assigned to the hypothetical (*R*)-amide. Because the enantiomers of the racemic amide ($\pm$)-**2aE** were separable on the given column (Hydrodex β -6TBDM, Figs. S6–S8), it could be concluded that the enantiomeric purity of the amide (*R*)-**2aE** remained quite high [$ee_{(R)-2aE} \geq 99.5\%$] up to 8 h. The kinetic resolution of ($\pm$)-**1a** catalyzed by CaLB-MNP displayed the characteristic behavior of a classical KR process (Table 1). The reaction proceeded rapidly up to 8 h reaching 47.0% conversion, followed by a markedly slower increase up to 50.3% at 24 h affording the amide (*R*)-**2aE** in slightly lower enantiomeric excess ($ee_{(R)-2aE} = 99.1\%$). This slight rise above the theoretical 50% limit can be attributed to excellent but non-exclusive enantiomer selectivity of the enzyme towards amine ($\pm$)-**1a**.

From this reaction the forming amide (*R*)-**2aE** and the residual amine (*S*)-**1a** could be isolated by preparative TLC (using an CH_2Cl_2 :EtOAc 1:1 as eluent) (Figs. S19 and S20). The isolation resulted in pure, brown oil products with 47.9% ((*R*)-**2aE**) and 44.0% ((*S*)-**1a**) yields.

2.3.2 *Candida antarctica* lipase B-magnetic nanoparticle aided kinetic resolution of ($\pm$)-**1b** with isopropyl 2-ethoxyacetate **E**

In case of screening the KR from amine ($\pm$)-**1b**, the racemic amide ($\pm$)-**2bE** could not be separated on either the chiral column Hydrodex β -6TBDM (Figs. S9–S11) or on Hydrodex β -6TBDAc (Figs. S15 and S16) and no direct conclusions could be drawn on the enantiopurity of the forming amide **2bE**. Since the enantiomers of amine ($\pm$)-**1b** – after derivatization to acetamide – could be distinguished, chromatographic data provided information on the selectivity of CaLB-MNP towards substrate ($\pm$)-**1b**. As the reaction progressed, both substrate peaks decreased almost equally, indicating that the CaLB-MNP exhibits low degree of enantiomer selectivity in this case.

2.3.3 *Candida antarctica* lipase B-magnetic nanoparticle aided kinetic resolution of ($\pm$)-**1c** with isopropyl 2-ethoxyacetate **E**

Similarly, for substrate ($\pm$)-**1c**, the synthesized racemic amide ($\pm$)-**2cE** could not be separated on either of the two chiral GC columns (Hydrodex β -6TBDM (Figs. S12–S14) or (Hydrodex β -6TBDAc, (Figs. S17 and S18)). However, in contrast to the observations made for ($\pm$)-**1b**, the chromatograms obtained for ($\pm$)-**1c** do allow conclusions to be drawn regarding enantiomer selectivity (E [40]) (Table 2).

Although the biocatalytic kinetic resolution of substrate ($\pm$)-**1c** could be successfully performed, isolation and detailed characterization of the corresponding product was not performed due to uncertainties in its analysis. Based on the chromatographic data, the elution-order-based assignment of the absolute configuration is not reliable. Although CaLB is known to exhibit (*R*)-selectivity with normal alcohol and amine substrates, the decreasing amine enantiomer peak corresponds to the opposite enantiomer. Moreover, the enantiomeric excess of the produced amide – presumably (*S*)-**2cE** – could not be determined due to insufficient enantiomer separation on either of our GC columns.

Thus, the isolation and absolute configuration determination of the product **2cE** will be investigated further in future works.

2.4 Substrate concentration screening in *Candida antarctica* lipase B-magnetic nanoparticle catalyzed kinetic resolution of ($\pm$)-**1a**

The acylation of ($\pm$)-**1a** with **E** was further examined by increasing the substrate concentration in a stepwise manner. Starting from the concentration applied in the initial experiments (0.125 mmol, 625 mM), the substrate load was progressively raised up to eightfold (1 mmol, 5 M). The results obtained after 24 h are summarized in Table 3.

Table 2 Results of the CaLB-catalyzed kinetic resolution of racemic cyclic β -amino ester ($\pm$)-**1c** with isopropyl 2-ethoxyacetate **E**

Reaction time [h]	c^a [%]	$ee_{(R)-1c}^b$ [%]	$ee_{(S)-2cE}^b$ [%]	E
1	4	4	99	156
2	8	8	98	131
4	16	18	98	114
8	20	25	98	122
24	40	63	96	100

^a Reaction conditions: CaLB-MNP (10 mg), MTBE (0.2 mL), ($\pm$)-**1c** (0.125 mmol), isopropyl 2-ethoxyacetate **E** (0.125 mmol, 1 equiv.), 40 °C, 350 rpm, 24 h.

^b Absolute configuration is hypothesized on basis of reversal of elution order of faster reaction enantiomer of ($\pm$)-**1c** on Hydrodex β -6TBDM chiral phase.

Table 3 Effect of substrate concentration [(±)-**1a**] on the CaLB-MNP catalyzed *N*-acylation with isopropyl 2-ethoxyacetate **E** after 24 h

[(±)- 1a] [M]	<i>c</i> ^a [%]	<i>ee</i> _{(S)-1a} [%]	<i>ee</i> _{(R)-2aE} [%]	<i>E</i>
0.63	50.3	>99.5	99.1	>200
0.78	49.7	>99.5	99.4	>200
0.94	49.9	>99.5	99.3	>200
1.10	50.1	>99.5	99.4	>200
1.25	50.3	>99.5	99.1	>200
1.88	50.0	>99.5	99.1	>200
2.50	49.6	>99.5	99.3	>200
5.00	50.3	>99.5	99.2	>200

^a Reaction conditions: CaLB-MNP (10 mg), MTBE (0.2 mL), (±)-**1a** (0.125–1 mmol), isopropyl 2-ethoxyacetate **E** (0.125–1 mmol, 1 equiv.), 40 °C, 350 rpm, 24 h.

Data indicates that the conversions remained within a narrow range – close to the theoretical limit – across all concentrations tested. These findings demonstrate that the reaction exhibits considerable robustness without apparent substrate or product inhibition up to multimolar concentration range, underscoring its suitability for scale-up.

3 Conclusion

Candida antarctica lipase B immobilized on magnetic nanoparticles (CaLB-MNP) proved to be an efficient biocatalyst for the kinetic resolution of racemic ethyl cis-2-aminocyclopent-3-enecarboxylate (±)-**1a** using isopropyl 2-ethoxyacetate **E** as acylating agent. The reaction displayed the characteristic features of a classical kinetic resolution, reaching ~50% conversion with excellent enantiomer selectivity (*E* > 200) leading to virtually enantiopure product (*ee*_{(R)-**2aE**} > 99%). The method showed excellent robustness across a wide substrate concentration range, underscoring its potential for large-scale preparation of enantiomerically pure compound.

In contrast, substrate (±)-**1b** could not be resolved with CaLB-MNP under the applied conditions due to the observed low enantiomer selectivity of CaLB-MNP and the lack of chromatographic separation of the corresponding racemic amides (±)-**2bE**.

Although significant enantiomer selectivity of CaLB-MNP was observable in KR of (±)-**1c** with ester **E** leaving the (*R*)-amine (*R*)-**1c** unreacted, assignation of the absolute configuration (*S*)-**2cE** for the reaction product remains ambiguous due to lack of GC enantiomer separation of the **2cE** enantiomers.

These findings suggest that while CaLB-MNP is highly effective for kinetic resolution of substrate (±)-**1a**, alternative amino-group-modifying enzymes may be required in future studies to achieve enantioselective transformation or resolution of substrates (±)-**1b** and (±)-**1c**.

4 Experimental section

4.1 Materials

Cyclic β-amino esters (±)-**1a–c** (Figs. S21, S22; S29, S30; S33, S34) prepared by known methods [41, 42] were obtained from Research Centre for Natural Sciences, Institute of Organic Chemistry, Budapest, Hungary in a solid crystalline state as HCl salt. Recombinant lipase B from *Candida antarctica* (CaLB, produced by microbial fermentation in *Pichia pastoris*, exhibiting a single band around 33 kDa on SDS gel EF; provided as a lyophilized powder, Lot-NO: MA-b-0002, activity: 59,900 TBU/g) was obtained from c-LEcta (Leipzig, Germany). Immobilization of the lyophilized CaLB on MNPs was performed as described earlier [28]. All other reagents and solvents were products of Sigma-Aldrich (Saint Louis, MO, USA), Merck (Darmstadt, Germany), or Alfa Aesar Europe (Karlsruhe, Germany).

4.2 Methods

Thin-layer chromatography was performed on precoated TLC ALUGRAM® Xtra SIL G/UV254 sheets from Macherey & Nagel (Düren, Germany), and the spots were visualized under UV light at a wavelength of 254 nm and with 5% phosphomolybdic acid solution followed by heating.

Infrared spectra of solid and liquid samples were recorded on a Bruker (Karlsruhe, Germany) ALPHA FT-IR spectrometer (in ATR mode). Wavenumbers (*ν*) of bands are listed in cm^{−1}.

The gas chromatographic analyses were performed with an Agilent (Santa Clara, CA, USA) 4890 gas chromatograph equipped with a flame ionization detector (FID) using H₂ 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*-butyldimethylsilyl)-β-cyclodextrine or Hydrodex β-6TBDAc column [25 m × 0.25 mm × 0.25 μm film with heptakis-(2,3-di-*O*-acetyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodextrin; Macherey & Nagel (Düren, Germany)]; using the temperature programs indicated in Tables S1 and S2. GC chromatograms are given in Figs. S1–S20 in the Supplement.

Conversion (*c*) and enantiomeric excess values (*ee*) were determined by GC measurements with base-line separations of the peaks for the enantiomers of racemic amines. The enantiomeric excess of the enantioenriched forms of amines (Eq. (1)) and amides (Eq. (2)) were calculated by Eq. (1):

$$ee_s [\%] = \frac{P_S - P_R}{P_S + P_R} \times 100, \quad (1)$$

where ee_S is the enantiomeric excess of the derivatized (*S*)-amine, P_R and P_S indicate the peak area of the corresponding enantiomer of the investigated amine:

$$ee_R [\%] = \frac{P_R - P_S}{P_R + P_S} \times 100, \quad (2)$$

where ee_R is the enantiomeric excess of the (*R*)-amide, P_R and P_S indicate the peak area of the corresponding enantiomer of the investigated amine.

The conversion of the amines (*c*) was calculated using the response factor *f* as follows (Eq. (3)):

$$c [\%] = \frac{P_R + P_S}{S_{SR} \times f + (P_R + P_S)} \times 100, \quad (3)$$

where S_{SR} indicates the peak area of the starting material. Enantiomeric ratio/selectivity (*E*) for KR from (±)-**1a** was calculated from the enantiomeric excess of the substrate (ee_S) and product (ee_p) fractions by using Eq. (4) [43]:

$$E = \frac{\ln[(1 - ee_S)/(1 + ee_S/ee_R)]}{\ln[(1 + ee_S)/(1 + ee_S/ee_R)]}, \quad (4)$$

Enantiomeric ratio/selectivity (*E*) for KR from (±)-**1c** was calculated from the conversion (*c*) and enantiomeric excess of the substrate fraction (ee_S) by using Eq. (5) [40]:

$$E_{c,ee(s)} = \frac{\ln[(1 - c) \times (1 - ee_S)]}{\ln[(1 - c) \times (1 + ee_S)]}, \quad (5)$$

In case of irreversible kinetic resolution, the enantiomeric composition of the product fraction (ee_p) can be calculated from the conversion (*c*) and enantiomeric excess of the substrate fraction (ee_S) [43] by using Eq. (6):

$$ee_S [\%] = \frac{c \times ee_p}{(1 - c)} \times 100, \quad (6)$$

Nuclear Magnetic Resonance (NMR) spectra were recorded using a Bruker (Karlsruhe, Germany) DRX-500 spectrometer, operating at frequencies of 500 or 300 MHz for ^1H and 126 or 75 MHz for ^{13}C , respectively. The NMR-signals were reported in parts per million (ppm) on the δ scale. NMR spectra are given in Figs. S21–S36 in the Supplement.

4.3 Liberation of cyclic β -amino ester salts (±)-1a-c**·HCl**
Na₂CO₃ solution (10 mL, 5 m/m%) was added to 150 mg of the corresponding amine salt of (±)-**1a-c**·HCl. The solution was extracted with ethyl acetate (EtOAc, 3 × 20 mL), and the organic phase was washed with brine (15 mL), and the mixture was dried over anhydrous Na₂SO₄. The ethyl acetate was removed by vacuum rotary evaporation, resulting in the free cyclic β -amino esters (±)-**1a-c**.

4.4 Synthesis of 2-ethoxyacetic acid **4**

2-Ethoxyacetic acid **4** was prepared using the published method [34] with minor modifications.

Sodium metal (5 g, 0.217 mol) was dissolved in ethanol (250 mL) at 60 °C, after which benzyltriethylammonium chloride (0.5 g, 0.018 mol) and sodium chloroacetate **3** (20 g, 0.172 mol) were added to the solution. The mixture was refluxed while stirring for 8 h, resulting in a white suspension. Then, the ethanol was removed by vacuum rotary evaporation. After adding distilled water (200 mL), the mixture was acidified to pH 1–2 by addition of 5M HCl. The resulting pale-yellow solution was extracted with ethyl acetate (3 × 100 mL), the combined organic phases were washed with brine (40 mL) and dried over anhydrous Na₂SO₄. After removing the solvent in rotary evaporator, the oily, brown residue was further purified with vacuum distillation, yielding a colorless oil.

Yield: 16.2 g (90%); bp = 107 °C (14 torr); n_D^{20} = 1.420; ^1H -NMR (500 MHz, CDCl₃) δ 8.91 (s, 1H), 4.14 (s, 2H), 3.64 (q, *J* = 7.0 Hz, 2H), 1.28 (t, *J* = 7.0 Hz, 3H); ^{13}C -NMR (126 MHz, CDCl₃) δ 175.43, 76.96, 67.42 (d, *J* = 7.8 Hz), 14.90.

4.5 Synthesis of isopropyl 2-ethoxyacetate **E**

Isopropyl 2-ethoxyacetate **E** was prepared using the published method [34] with minor modifications.

2-Ethoxyacetic acid **4** (14.0 g, 0.136 mol, 12.7 mL) and *p*-toluenesulfonic acid (0.46 g, 2.7 mmol) were dissolved in 2-propanol (250 mL). The reaction mixture was refluxed for 12 h, after which the solvent was distilled off slowly at atmospheric pressure (removing most of the formed water in the minimum BP azeotropic mixture). Then water-free 2-propanol (35 mL) was added and distilled off slowly (to remove the residual amount of water). The resulting crude ester **E** was purified by distillation at atmospheric pressure through a Vigreux column to yield isopropyl 2-ethoxyacetate **E** as a colorless oil.

Yield: 15.05 g (75%); bp = 161 °C (760 torr); n_D^{20} = 1.4031; ^1H -NMR (300 MHz, CDCl₃): 1.20 (3H, t, *J* = 6.41 Hz, CH₃); 1.25 (6H, d, *J* = 6.41 Hz, 2 × CH₃); 3.58 (2H, q, *J* = 6.41 Hz, CH₂); 4.01 (2H, s, CH₂); 5.08 (1H, m, CH); ^{13}C -NMR (75 MHz, CDCl₃) 170.12, 68.37, 68.29, 67.12, 21.81, 15.00.

4.6 One-pot acylation of racemic amines (±)-**1a-c** with 2-ethoxyacetic acid **4**

Triethylamine (140 μL , 1.005 mmol) and 2-ethoxyacetic acid (32 μL , 0.335 mmol) were added to a solution of the corresponding amine (0.335 mmol) in dry

dichloromethane (5 mL) under an argon atmosphere at room temperature. The mixture was stirred for 40 min, then cooled in an ice bath until $\sim 0^\circ\text{C}$. Then, 2 equivalents of thionyl chloride (49 μL , 0.670 mmol), dissolved in dichloromethane (2 mL), were added dropwise, while keeping the reaction mixture temperature below 5°C . After the addition was completed, the reaction was allowed to reach room temperature and proceeded for an additional 1 h and 30 min. The product was quenched with 10 mL of distilled water. The organic phase was separated and dried over anhydrous Na_2SO_4 . The residue was purified by preparative TLC using silica gel plates and CH_2Cl_2 :EtOAc, 1:1 as eluent.

($\pm$)-Ethyl *cis*-2-ethoxyacetamidocyclopent-3-enecarboxylate ($\pm$)-**2aE**: yield: 71%, yellowish-brown oil. ^1H -NMR (300 MHz, CDCl_3) δ 6.84 (d, $J = 9.2$ Hz, 2H), 5.99 (dq, $J = 5.9$, 2.1 Hz, 2H), 5.64 (dq, $J = 4.7$, 2.3 Hz, 2H), 5.43 (t, $J = 9.4$ Hz, 2H), 4.14 (q, $J = 7.1$ Hz, 4H), 4.02–3.81 (m, 4H), 3.73–3.35 (m, 6H), 2.96–2.79 (m, 2H), 2.59 (dd, $J = 17.2$, 8.8 Hz, 2H), 2.12 (s, 4H), 2.05 (d, $J = 5.8$ Hz, 1H), 1.38–1.16 (m, 17H), 1.27 (s, 16H), 0.88 (q, $J = 8.6$, 7.4 Hz, 4H); ^{13}C -NMR (75 MHz, CDCl_3) δ 172.95, 169.53, 133.71, 129.47, 77.21, 69.76, 67.20, 60.79, 55.18, 45.78, 34.80, 31.92, 31.43, 29.69, 22.69, 20.50, 14.95, 14.12. IR (film, ν) 3435, 2977.55, 1728.44, 1681.42, 1112.86.

($\pm$)-Ethyl *cis*-2-ethoxyacetamidocyclohex-3-enecarboxylate ($\pm$)-**2bE**: yield: 41%, yellowish-brown oil. ^1H -NMR (500 MHz, CDCl_3) δ 7.11 (d, $J = 9.8$ Hz, 1H), 5.87 (dtd, $J = 9.5$, 3.6, 1.8 Hz, 1H), 5.65 (ddt, $J = 9.9$, 4.0, 2.2 Hz, 1H), 4.98–4.89 (m, 1H), 4.24–4.07 (m, 2H), 3.96 (d, $J = 15.4$ Hz, 1H), 3.89 (d, $J = 15.3$ Hz, 1H), 3.57 (qd, $J = 7.0$, 2.6 Hz, 2H), 2.88 (dt, $J = 7.3$, 5.0 Hz, 1H), 2.20–2.02 (m, 1H), 2.00–1.92 (m, 2H), 1.27 (dt, $J = 9.7$, 7.1 Hz, 7H); ^{13}C -NMR (75 MHz, CDCl_3) δ 173.16, 169.29, 129.71, 126.87, 77.21, 69.91, 67.20, 60.63, 44.14, 43.16, 23.23, 22.02, 15.01, 14.13. IR (film, ν) 3352, 2977.30, 2905, 1728.76, 1663, 1112.27.

($\pm$)-Ethyl *trans*-6-ethoxyacetamidocyclohex-3-enecarboxylate ($\pm$)-**2cE**: yield: 36%, yellowish-brown oil. ^1H -NMR (500 MHz, CDCl_3) δ 7.00 (d, $J = 9.9$ Hz, 1H), 5.78 (dtd, $J = 9.6$, 3.6, 1.8 Hz, 1H), 5.56 (ddt, $J = 10.0$, 4.1, 2.2 Hz, 1H), 4.85 (dddt, $J = 11.0$, 5.7, 4.0, 2.0 Hz, 1H), 4.13–3.98 (m, 2H), 3.86 (d, $J = 15.3$ Hz, 1H), 3.79 (d, $J = 15.3$ Hz, 1H), 3.48 (qd, $J = 7.0$, 2.6 Hz, 2H), 2.79 (dt, $J = 7.5$, 5.0 Hz, 1H), 2.11–1.93 (m, 1H), 1.91–1.80 (m, 2H), 1.18 (dt, $J = 9.6$, 7.0 Hz, 6H); ^{13}C -NMR (126 MHz, CDCl_3) δ 173.14, 169.23, 129.69, 126.86, 77.28, 69.90, 67.18, 60.61, 44.08, 43.15, 23.22, 21.99, 15.00, 14.12. IR (film, ν) 3421, 2977, 1729.48, 1680.47, 1507.38, 1109.39.

4.7 Preparation of immobilized *Candida antarctica* lipase B-magnetic nanoparticle biocatalyst

The preparation of the MNP carriers, including the preparation of the magnetite core and its modifications with silica shell, and surface modification with trimethoxy aminopropyl silane were performed using previously published methods [28]. Immobilization of the lyophilized CaLB followed the previously published method with slight modifications [28].

Magnetic nanoparticles (40 mg) added to a mixture of phosphate buffer (1000 μL , sodium phosphate buffer, 100 mM, pH = 7.5) and a mixture of n-butanol:ethanol (7:3, 160 μL) were sonicated at 45 kHz for 10 min [EMAG AG (Mörfelden-Walldorf, Germany) Emmi 20HC ultrasonic bath]. Next, lyophilized CaLB (2.6 mg) was added to sodium phosphate buffer (1200 μL , strength, pH) and the resulting CaLB solution was mixed with the initial MNPs suspension and shaken at 450 rpm and room temperature for 2 h. After mixing, magnetic separation of the nanoparticles was performed by removal of the resulting supernatant, and the residual immobilized CaLB-MNP biocatalyst was left to dry at room temperature for 48 h.

4.8 Screening *Candida antarctica* lipase B-magnetic kinetic resolution of the cyclic β -amino esters ($\pm$)-**1a-c** with isopropyl 2-ethoxyacetate **E**

In a 4 mL screw-cap vial, MNP-CaLB (10 mg) was added to methyl *tert*-butyl ether (200 μL), and the forming suspension was sonicated for 5 min. The corresponding racemic amine ($\pm$)-**1a-c** (0.125 mmol) and isopropyl 2-ethoxyacetate **E** (0.125 mmol) were added to this suspension, and the reaction mixture was shaken at 350 rpm and 40°C for 24 h. The reaction mixture was monitored by taking samples at different intervals (1 h, 2 h, 4 h, 8 h, 24 h). Each sample was analyzed by chiral GC [5 μL of the reaction sample dissolved in 600 μL ethyl acetate was derivatized by the addition of acetic anhydride (10 μL) at 350 rpm and 40°C for 20 min, followed by quenching with 1 drop of distilled water and drying over anhydrous sodium sulfate].

4.9 Isolation of the forming amide (*R*)-**2aE** the residual amine (*S*)-**1a** and from kinetic resolution with ester **E** by *Candida antarctica* lipase B-magnetic nanoparticle

At room temperature, the immobilized MNP-CaLB was removed by magnetic separation from the reaction mixture. The remaining reaction mixture was purified by preparative TLC on silica gel using CH_2Cl_2 :EtOAc = 1:1 as eluent, yielding the amide (*R*)-**2aE**, and the amine (*S*)-**1a**.

(*R*)-Ethyl *cis*-2-(2-ethoxyacetamido)cyclopent-3-ene-1-carboxylate (*R*)-**2aE**: yield: 47.9%, brown oil, $[\alpha]_D^{27.5} = -79.5$ ($c = 1 \text{ mg mL}^{-1}$, CH_2Cl_2). $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ 6.80–6.74 (m, 1H), 5.97 (dq, $J = 6.1$, 2.2 Hz, 1H), 5.62 (dq, $J = 4.6$, 2.2 Hz, 1H), 5.41 (dddd, $J = 9.9$, 7.3, 2.6, 1.3 Hz, 1H), 4.12 (q, $J = 7.1$ Hz, 2H), 3.92 (d, $J = 15.3$ Hz, 1H), 3.84 (d, $J = 15.3$ Hz, 1H), 3.53 (qd, $J = 7.0$, 1.2 Hz, 2H), 3.40 (td, $J = 8.8$, 6.4 Hz, 1H), 2.86 (ddq, $J = 17.2$, 6.6, 2.4 Hz, 1H), 2.62–2.52 (m, 1H), 1.39–1.17 (m, 9H); $^{13}\text{C-NMR}$ (126 MHz, CDCl_3) δ 172.93, 169.28, 133.63, 129.55, 76.93, 69.86, 67.18, 60.75, 55.12, 45.81, 34.78, 29.69, 14.96, 14.14.

(*S*)-Ethyl *cis*-2-aminocyclopent-3-enecarboxylate (*S*)-**1a**: yield: 44.0 %, brown oil, $[\alpha]_D^{27.5} = +51.4$ ($c = 1 \text{ mg mL}^{-1}$, CH_2Cl_2). $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ 5.89–5.82 (m, 1H), 5.74 (dt, $J = 5.6$, 2.2 Hz, 1H), 4.21 (q, $J = 7.1$ Hz, 3H), 4.13 (q, $J = 7.2$ Hz, 7H), 3.22 (dt, $J = 8.8$, 7.7 Hz, 1H), 2.87 (ddq, $J = 17.1$, 7.4, 2.4 Hz, 1H), 2.46 (ddt, $J = 17.0$, 8.8, 2.3 Hz,

1H), 1.77 (s, 3H), 1.28 (dt, $J = 21.0$, 7.2 Hz, 15H); $^{13}\text{C-NMR}$ (126 MHz, CDCl_3) 173.20, 171.11, 132.96, 131.75, 77.22, 60.50, 60.37, 58.90, 48.29, 33.16, 21.02, 14.39, 14.18.

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