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Lipase-catalyzed kinetic resolution of 7-, 8- and 12-membered alicyclic β-amino esters and *N*-hydroxymethyl-β-lactam enantiomers

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Abstract—Enzymatic kinetic resolutions of methyl *cis*-2-aminocycloheptane-, 2-aminocyclooctane- and 2-aminocyclododecanecarboxylates with *Candida antarctica* lipase A in diisopropyl ether (E >200) and of the corresponding *N*-hydroxymethyl-β-lactams with *Pseudomonas cepacia* lipase in dry acetone (E from 27 to >200) has been performed with 2,2,2-trifluoroethyl butanoate as the best acyl donor, with both enantiomers being obtained. *trans*-13-Hydroxymethyl-13-azabicyclo[10.2.0]tetradecan-14-one was resolved with vinyl butanoate and *Candida antarctica* lipase B (E=26) in acetone.

1. Introduction

Recently, the preparation and importance of optically active β-amino acids and β-lactams as versatile building blocks for synthetic chemistry has been reviewed.[1–6] 1,2-Dipolar cycloaddition of chlorosulfonyl isocyanate to cycloalkenes is a method widely used for the preparation of racemic cycloalkane-fused β-lactams in a stereoselective manner.[7–9] The hydrolysis or the alcoholysis of the prepared β-lactams in concentrated (18–22%) hydrogen chloride in water or in an alcohol provides easy access to the corresponding β-amino acids or esters, respectively.[5,10–14] The interest in β-amino acids is mainly due to their ability to serve as starting substances for heterocycles, pharmacons, natural products and chiral auxiliaries. Most recently, interest has focused on the bioactivity of β-peptides which are stable against peptidase degradation and often capable of forming stable tertiary structures.[2,5,15] Alicyclic β-amino acids comprise of an interesting class among the β-amino acids. Some of them, such as cispentacin [(−)-(1*R*,2*S*)-2-aminocyclopentanecarboxylic acid, FR 109615], are biologically active natural products.[16,17]

For the preparation of enantiopure compounds, enzymatic kinetic resolution is one of the most efficient methods. In particular, lipase catalysis is increasingly replacing the classical diastereomer separation of racemates. Our studies on lipase catalysis puts us amongst the pioneers in resolving alicyclic β-amino esters using enzymes. For instance, various monocyclic (rings containing 5 or 6 carbon atoms) and bicyclic (bicyclo[2.2.1]heptane or heptene) β-amino esters have been resolved in organic solvents, using lipase-catalyzed *N*-acylation with activated esters at the stereogenic (2*R*) center.[18] As another possibility, *N*-hydroxymethyl-6-azabicyclo[3.2.0]heptan-7-one and *exo*-3-azatricyclo[4.2.1.0^{2,5}]nonan-4-one that can act as precursors for β-amino acids (hydrolysis of the β-lactam ring in concentrated HCl) or β-lactams (removal of the *N*-hydroxymethyl group with NH_4OH/MeOH) were prepared with paraformaldehyde by sonication and subsequent highly enantioselective lipase-catalyzed *O*-acylation at the primary hydroxymethyl function was reported.[10] The *N*-hydroxymethyl-β-lactam method was later successfully exploited for the resolution of *N*-hydroxymethyl-9-azabicyclo[6.2.0]dec-4-en-10-one, in addition to compounds with smaller fused rings.[11–13] As a third resolution method, the enantioselective hydrolysis of

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6-azabicyclo[3.2.0]hept-3-en-7-one in water in the presence of a whole-cell preparation (*Rhodococcus equi*)[19] or isolated enzymes (lactamases)[20] and the lipase catalysed hydrolysis of β-lactams (*cis*-fused with alicycles, ring size 5–8 carbon atoms) by 1 equivalent of water with diisopropyl ether allows the direct ring opening of the lactam ring.[14]

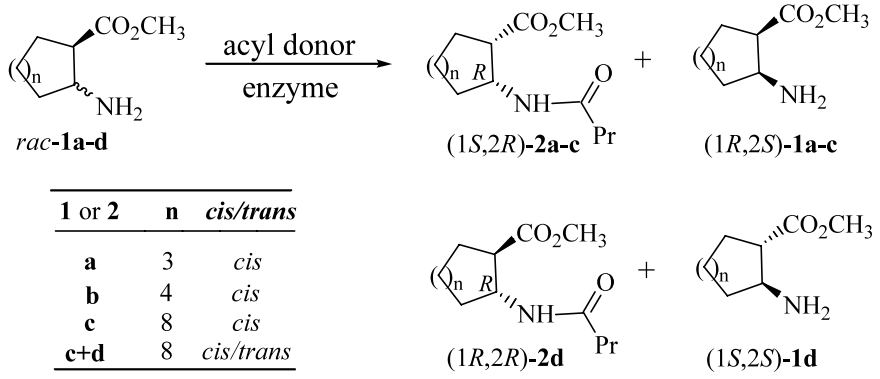
Seven-membered and larger rings are present in many natural products. This gave us a reason to study how well the β-amino ester[18] and *N*-hydroxymethyl-β-lactam[10] methods are suitable for the resolution of compounds containing larger alicyclic rings. Accordingly, the lipase-catalyzed asymmetric *N*-acylation of 7-, 8- and 12-membered alicyclic β-amino acid esters, *rac*-**1a–c** and *rac*-(**1c**+**1d**) (Scheme 1), and the *O*-acylation of the corresponding *N*-hydroxymethyl-β-lactams, *rac*-**3a–d** (Scheme 2), have now been studied. None of the substrates have been enzymatically resolved before by these methods, and **1c**, **1d**, **3c** and **3d**, including the corresponding free acids and β-lactams are new compounds. Gram-scale resolutions were performed under optimized conditions. One beneficial feature of the present enzymatic acylation methods is that the water activity[21] can be expected to stay constant throughout the reaction.

2. Results and discussion

2.1. Enantioselective *N*-acylation of β-amino esters

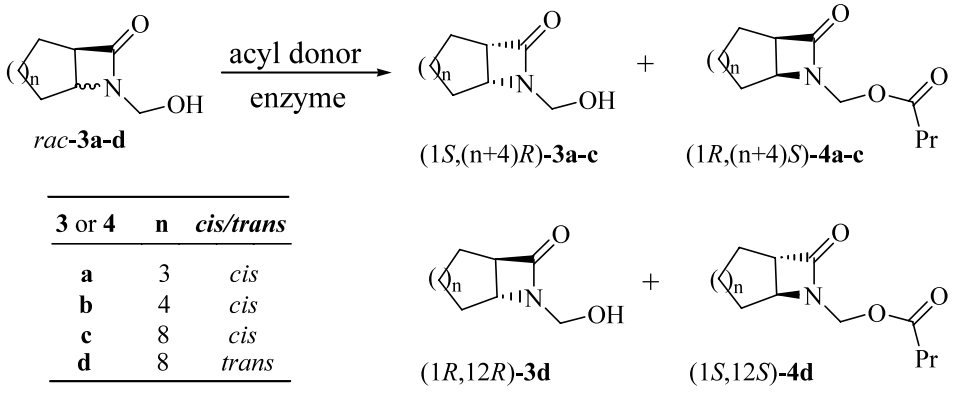
Extensive lipase screening for the asymmetric acylation of 5- and 6-membered alicyclic *cis*- and *trans*-β-amino esters has previously revealed that two lipases, lipase PS (lipase from *Pseudomonas cepacia*) and CAL-A (lipase A from *Candida antarctica*), display complementary behavior where CAL-A proved to be more applicable for the *cis*-isomers.[18] Later, it became clear that CAL-A is exceptional amongst lipases by catalyzing the *N*- and *O*-acylations of various sterically hindered substances in a highly enantioselective manner.[22–26] The enzyme works well even for the *N*-acylation of the secondary amino groups in proline and pipecolic acid esters.[27,28] Previous solvent screening involved simple ethers, such as diisopropyl and *tert*-butyl methyl ethers, which were the most favorable solvents for the enzymatic acylation of alicyclic β-amino esters.[18]

Encouraged by these findings, we subjected *rac*-**1a–c** (*cis* isomers) and a mixture of *rac*-**1c** and *rac*-**1d** (*cis*:*trans* 1:2) to acylation with a CAL-A preparation in the presence of 2,2,2-trifluoroethyl butanoate in



1 or 2	n	*cis/trans*
a	3	*cis*
b	4	*cis*
c	8	*cis*
c+d	8	*cis/trans*

Scheme 1.



3 or 4	n	*cis/trans*
a	3	*cis*
b	4	*cis*
c	8	*cis*
d	8	*trans*

Scheme 2.

Table 1. Enantioselectivity of the CAL-A preparation for the acylation of *rac*-**1a–c** (0.1 M) and *rac*-[**1c**+**1d**, (1:2)] (0.1 M) with 2,2,2-trifluoroethyl butanoate (0.2 M) in diisopropyl ether (2 ml) at room temperature

Entry	Compounds	Enzyme content (mg/ml)	Time (h)	Conversion (%)	*E*
1	**1a**	10	1	28	>200
2	**1a**	20	1	48	>200
3	**1a**	25	1	49	>200
4	**1a**	50	1	48	>200
5	**1a**	75	1	46	>200
6	**1b**	5	1	24	130±7
7	**1b**	10	1	33	170±7
8	**1b**	20	1	34	>200
9	**1b**	40	1	18	>200
10	**1b**	75	1	17	>200
11	**1c**	50	24	50[a]	>200
12	**1c**+**1d**	50	48	–	–
13	**1c**+**1d**	50	24	50/8[a]	116±13/7±1

[a] Temperature 47°C.

diisopropyl ether (Scheme 1). Excellent enantioselectivity in the terms of *E* values is obvious for the present *cis*-amino esters (Table 1, entries 1–11 and 13), while the *trans*-isomer **1d** in the *cis*/*trans*-mixture (entry 13) reacts with negligible enantioselectivity. The reactivity (reflected by the conversion reached after a certain time) of the *cis*-isomers decreased considerably with increasing size of the alicyclic ring. Thus, conversions of 48% for **1a** (entry 2) and 34% for **1b** (entry 8) were reached after 1 h when 20 mg/ml of the enzyme preparation was used, whereas there was no reaction in the case of the mixture of **1c** and **1d** as substrate (entry 12) after 2 days at room temperature, even though the enzyme content was high (50 mg/ml of the enzyme preparation). In the case of the mixture of **1c** and **1d**, the replacement of diisopropyl ether with acetone, acetonitrile, toluene or diethyl ether as solvent did not improve the reactivity. When lipase PS, lipase AK (lipase from *Pseudomonas fluorescens*), CAL-B (lipase B from *Candida antarctica*) or PPL (porcine pancreatic lipase) was used in place of CAL-A in diisopropyl ether, negligible reactivity (conversion 0–7% after 1 day) with practically no enantioselectivity was observed. When 2,2,2-trifluoroethyl chloroacetate was used with lipase PS in diisopropyl ether, a chemical reaction strongly disturbed the enzymatic acylation. For the *cis*-substrate **1c** (entry 11) and the *cis*/*trans*-mixture of **1c** and **1d** (entry 13), raising the temperature (47°C) was necessary for acylation to proceed.

Successful gram-scale resolutions of **1a–c** with CAL-A in diisopropyl ether were performed as described in Section 5. For substrates **1a** and **1b**, 10 or 20 mg/ml of the enzyme preparation was taken as optimal enzyme contents. For the acylation of **1b**, high enzyme contents tended to halt the reactivity (entries 9 and 10). Aggregation of the enzyme preparation under the given reaction conditions was taken as an explanation. As shown later, (1*S*,2*R*)-**1a–c** and (1*R*,2*R*)-**1d** are the more reactive enantiomers in the racemates.

2.2. Enantioselective *O*-acylation of *N*-hydroxymethyl-β-lactams

For a second synthetic approach to the enantiomers of alicyclic β-amino acids, *N*-hydroxymethyl-β-lactams *rac*-**3a–d** were subjected to lipase-catalyzed acylation in dry acetone (Scheme 2). As concerns enzymatic enantioselectivity and suppression of the enzymatic hydrolysis (caused by the water adsorbed on the seemingly dry enzyme preparation) of new ester product **4** back to **3**, acetone has previously been shown to be the most appropriate solvent for β-lactam substrates.[10] Vinyl esters were expected to be excellent as achiral acyl donors because the vinyl alcohols produced (another product of enzymatic acylation) were unstable and thus decomposed with the formation of acetaldehyde. Accordingly, the reverse enzymatic reactions of (1*R*,(*n*+4)*S*)-**4a–c** ($n=3$, 4 and 8) and (1*S*,12*S*)-**4d** with the released vinyl alcohol and the consequent racemization were avoided.

As expected on the basis of the earlier studies,[10–13] lipases PS (Table 2, entries 1–6) and AK (entry 7) smoothly catalyzed the formation of (1*R*,7*S*)-**4a** with vinyl esters in dry acetone with the combination of lipase PS and vinyl butanoate (entry 5) being the most enantioselective. For appropriate reactivity, 50 mg/ml of the enzyme preparation was chosen for further studies. In the cases of substrates *rac*-**3b–d**, the acylation reactions with vinyl butanoate tended to stop at an early stage (entries 9, 11 and 15). It is well known that aldehydes form hemiacetals under both acidic (with this reaction continuing to form an acetal) and basic conditions. The formation of hemiacetal esters in appreciable yields was previously observed when sterically hindered secondary alcohols were treated with lipase PS or CAL-B and vinyl acetate in organic solvents.[29] However, side-products were not detected by the GC method with the present primary alcohols. Any drop in e.e. for the separated substrates **3** due to the decomposition of free hemiacetals (if present) during the work-up when comparing the e.e. with the resolved mixture was not

Table 2. Enzymatic acylation of *rac*-**3a**–**d** with acyl donors (0.2 M) in acetone (2 ml) at room temperature

Entry	Compounds	Acyl donor	Enzyme content (mg/ml)	Time (h)	Conversion (%)	*E*
1	**3a** (0.1 M)	$MeCO_2CH{=}CH_2$	Lipase PS (50)	1	48	50±10
2	**3a** (0.1 M)	$PrCO_2CH{=}CH_2$	Lipase PS (10)	1	17	>200
3	**3a** (0.1 M)	$PrCO_2CH{=}CH_2$	Lipase PS (30)	1	36	>200
4	**3a** (0.1 M)	$PrCO_2CH{=}CH_2$	Lipase PS (40)	1	40	>200
5	**3a** (0.1 M)	$PrCO_2CH{=}CH_2$	Lipase PS (50)	1	45	>200
6	**3a** (0.1 M)	$PrCO_2CH{=}CH_2$	Lipase PS (75)	1	48	>200
7	**3a** (0.1 M)	$PrCO_2CH{=}CH_2$	Lipase AK (50)	1	46	82±9
8	**3b** (0.1 M)	$PrCO_2CH_2CF_3$	Lipase PS (50)	2	32	27±1
9	**3b** (0.1 M)	$PrCO_2CH{=}CH_2$	Lipase PS (50)	2	30[b]	–
10	**3c** (0.05 M)	$PrCO_2CH_2CF_3$	Lipase PS (50)	3	48	83±10
11	**3c** (0.05 M)	$PrCO_2CH{=}CH_2$[a]	Lipase PS (25)	3	30[b]	–
12	**3c** (0.05 M)	$PrCO_2CH{=}CH_2$	CAL-B (2.5)	1	62	3±1
13	**3d** (0.1 M)	$PrCO_2CH_2CF_3$	Lipase PS (50)	2	39	5±1
14	**3d** (0.1 M)	$PrCO_2CH_2CF_3$[a]	Lipase PS-C (50)	2	49	10±2
15	**3d** (0.1 M)	$PrCO_2CH{=}CH_2$	Lipase PS (50)	2	23[b]	–
16	**3d** (0.05 M)	$PrCO_2CH{=}CH_2$	PPL (25)	2	11	18±2
17	**3d** (0.05 M)	$PrCO_2CH{=}CH_2$	CAL-B (2.5)	2	54	26±2
18	**3d** (0.05 M)	$PrCO_2(CH_2)_3CH_3$	CAL-B (2.5)	2	40	15±5

[a] 0.4 M.
[b] Reaction ceases.

detected. However the present observations do not exclude the possibility of the inactivation of lipase PS by unstable hemiacetal formation. It is worth emphasizing that vinyl esters are commonly accepted by lipase PS.[30] Another possibility is that the enzymatic reaction between the produced (1*R*,(*n*+4)*S*)-**4** and the less reactive primary alcohol (1*S*,(*n*+1)*R*)-**3** becomes increasingly important in the reaction mixture at longer reaction times: If true, a drop in e.e. is expected for both resolution products. As the e.e. values in the resolution mixture remained practically unchanged for hours after the reaction had stopped and because the change of the vinyl ester to 2,2,2-trifluoroethyl butanoate as acyl donor (entries 8, 10 and 13) allowed us to complete the resolutions, the possibility was not studied further. The same reactions with CAL-B and PPL proceeded without difficulty, but with low selectivity (entries 12, 16 and 17).

Gram-scale resolutions with lipase PS, vinyl butanoate (*rac*-**3a**) and 2,2,2-trifluoroethyl butanoate (*rac*-**3b** and **3c**) in acetone were performed as described in Section 5. The results are shown in Table 3. In accordance with the relatively low enantioselectivity of lipase PS for *rac*-**3b** and *rac*-**3c** (Table 2, entries 8 and 10) the resolution products (1*S*,8*R*)-**3b**, (1*R*,8*S*)-**4b**, (1*S*,12*R*)-**3c** and (1*R*,12*S*)-**4c** were not enantiopure at 50% conversion. For the resolution of the *trans*-β-lactam analogue **3d**, CAL-B-catalyzed acylation with vinyl butanoate in acetone was the most appropriate (Table 2, entry 17).

3. Absolute configuration

The less reactive enantiomers of **1a** and **1b** were obtained from the gram-scale resolutions by CAL-A as

Table 3. Acylation results for *rac*-**1** with CAL-A (10–50 mg/ml of the preparation) in diisopropyl ether and for *rac*-**3** with lipase PS (50 mg/ml of the preparation) in dry acetone, using 2,2,2-trifluoroethyl butanoate (vinyl butanoate in the case of *rac*-**3a**) at room temperature

Substrate	Time (h)	Conversion (%)	$Ee^{1\text{ or }3}$ (%) (configuration)	$Ee^{2\text{ or }4}$ (%) (configuration)
rac-**1a**	1.8	50	98 (1*R*,2*S*)	97 (1*S*,2*R*)
rac-**1b**	4	50	99 (1*R*,2*S*)	95 (1*S*,2*R*)
rac-**1c**	24[a]	49	96 (1*R*,2*S*)	99 (1*S*,2*R*)
rac-**3a**	2	49	93 (1*S*,7*R*)	97 (1*R*,7*S*)
rac-**3b**	24	52	90 (1*S*,8*R*)	82 (1*R*,8*S*)
rac-**3c**	10	50	90 (1*S*,12*R*)	–[c]
rac-**3d**[b]	2.5	50	85 (1*R*,12*R*)	–[c]
rac-**5**[d]	3	51	96 (1*S*,8*R*)	92 (1*R*,8*S*)

[a] Temperature 47°C.
[b] CAL-B as enzyme.
[c] Determination of e.e. was not possible in the resolution mixture.
[d] Acylation of *N*-hydroxymethyl-9-azabicyclo[6.2.0]dec-4-en-10-one **5** with vinyl butanoate and lipase PS in diisopropyl ether, temperature –15°C; Ref. 13.

shown in Section 5. In order to confirm the absolute configurations, the amino esters were subjected to hydrolysis in 18% HCl, followed by treatment of the acid hydrochlorides obtained with a basic Amberlite IRA 904 resin. The specific rotations, $[\alpha]_D^{25}=-4.6$ (*c* 0.5, H_2O) for the free acid produced from ester **1a** and $[\alpha]_D^{25}=+11.0$ (*c* 0.5, H_2O) for that produced from **1b**, are in accordance with the literature values,[14] $[\alpha]_D^{25}=-7.2$ (*c* 0.5, H_2O) and +17.8 (*c* 0.4, H_2O) for the (1*R*,2*S*) acids. As a consequence of the slight differences between our $[\alpha]_D^{25}$ values and the literature values, the enantiopurity was confirmed by transforming the free acids back to the amino esters and determining the e.e. = 96% for the reproduced **1a** and **1b** (as compared with e.e. = 98 and 99% for the resolved products) by the GC method. Accordingly, the *N*-acylations by CAL-A had proceeded at the stereogenic (2*R*) center, as expected on the basis of previous work.[18] There is every reason to presume that the same enantiopreference will hold for the CAL-A-catalyzed *N*-acylation of **1c**. It is therefore concluded that the more reactive enantiomer of *rac*-**1a**–**c** has the (1*S*,2*R*) absolute configuration and for the same reasoning the (1*R*,2*R*) enantiomer reacts in the case of *rac*-**1d**.

Opposite enantiodiscrimination has previously been reported for the acylation of alicyclic β-amino esters and for the acylation of the corresponding *N*-hydroxymethyl-β-lactams with lipases.[10,18] This is also true for the present work, where the separated unreacted enantiomers of *rac*-**3b** and *rac*-**3d** were first transformed to the amino esters **1b** and **1d** by treatment with MeOH/HCl and NH_3, after which the positions of the peaks in the GC chromatograms were compared with those for the CAL-A-catalyzed acylations of the amino esters. This result justifies the conclusion concerning the absolute configurations of (1*S*,8*R*) for the unreacted enantiomer of *rac*-**3b** and (1*R*,12*R*) for *rac*-**3d**. In general, the (1*R*,(*n*+4)*S*) enantiomers (*n* = 3, 4 and 8) are more reactive for the lipase-catalyzed acylation of racemic *cis* isomers **3a**–**c**.

4. Conclusions

The present work extends the usability of known enzymatic kinetic resolution methods (method 1 and 2) for the preparation of medium- (8-membered) and large-ring (12-membered) alicyclic β-amino acid derivatives in addition to normal-ring (5- to 7-membered) analogues.[10,18] In method 1, the *N*-acylation of alicyclic β-amino esters *rac*-**1a**–**c** (*cis*-isomers) with 2,2,2-trifluoroethyl butanoate has been studied (Scheme 1). The reactions in diisopropyl ether proceeded with excellent enantioselectivity (usually *E*>200, Table 1) in the presence of CAL-A, allowing the enantioseparation as a one-pot acylation at 50% conversion (Table 3). The alicyclic ring size did not exert any effect on the enantioselectivity and an amide product **2** with a (1*S*,2*R*) configuration. In accordance with the previous results,[18] the method works poorly (*E* = 7) for the resolution of *trans* isomer **1d**. In method 2 (Scheme 2), the corresponding *N*-hydroxymethyl-β-lactams *rac*-**3a**–**c** have been enantioselectively *O*-acylated with vinyl and 2,2,2-trifluoroethyl butanoates in dry acetone with lipase PS acting as the catalyst. The enantioselectivity of lipase PS is highly dependent on the molecular size of the substrate alcohol (*E* between >200 and 27, Table 2) and the results in Table 3 clearly indicate that Method 1 is by far the more favorable enzymatic kinetic resolution method for the preparation of normal-, medium- and large-ring alicyclic β-amino esters (or β-amino acids) with the *cis* configuration.

The β-lactam method with CAL-B acting as the catalyst and vinyl butanoate as the acyl donor, is usable for the gram-scale resolution of the *trans*-β-amino ester precursor **3d**.

5. Experimental

5.1. Materials and methods

Chlorosulfonyl isocyanate, cycloalkenes, propylene oxide, vinyl and methyl butanoates and 2,2,2-trifluoroethanol were products of Aldrich or Fluka. All solvents were of the highest analytical grade and were dried over molecular sieves (3 Å) before use. *Pseudomonas cepacia* lipase (lipase PS) was from Amano Europe, while lipases A (CAL-A, Chirazyme L5, lyo.) and B (CAL-B, Chirazyme L2, cf. C2, lyo) from *Candida antarctica* were products of Roche. Before use, lipases PS and CAL-A were adsorbed on celite by dissolving the enzyme (5 g) and sucrose (3 g) in a tris–HCl buffer (250 ml, 20 mM, pH 7.8), followed by the addition of celite (17 g).[31] The mixture was dried by allowing the water to evaporate. The final lipase content in the enzyme preparation was 20% (w/w). Most of the enzymatic reactions were performed at room temperature (23–24°C). 2,2,2-Trifluoroethyl butanoate was prepared from butyric acid by using thionyl chloride and 2,2,2-trifluoroethanol.

In a typical small-scale experiment, one of the substrates *rac*-**1a**–**c** and (**1c**+**1d**) (0.2 mmol) in diisopropyl ether (2 ml), or one of the substrates *rac*-**3a**–**d** (0.1–0.2 mmol) in dry acetone (2 ml), was added to a lipase preparation (20–150 mg, corresponding to 4–30 mg of a lipase) or to a commercial lipase (5–100 mg), followed by the addition of an achiral acyl donor (2 equivalents to the substrate). The progress of the reactions and the e.e. values were followed by taking samples (0.1 ml) at intervals and analyzing them by gas chromatography. For good baseline separation, the unreacted amino (substrates **1**) and hydroxy (substrates **3**) groups in the sample were derivatized with acetic anhydride in the presence of pyridine containing 1% 4-*N*,*N*-dimethylaminopyridine (DMAP) before injection. The GC was equipped with a Chrompack CP-Chirasil-DEX CB or Chrompack CP-Chirasil-L-Valine column. HPLC equipped with a Sumichiral OA-4900 (46 mm×0.25 m, $\lambda = 210$ nm) column was used to obtain the e.e.[3d] value. Conversions were calculated from the e.e. values $c = ee_S/(ee_S+ee_P)$ except in the cases of **3c** and **3d** as substrates, where hexadecane served as an internal standard in the GC method. Preparative chromatographic separations were performed by column chromatography on Merck Kieselgel 60 (0.063–0.200 μm). TLC was carried out with Merck Kieselgel $60F_{254}$ sheets. Spots were visualized with 5% ethanolic phosphomolybdic acid solution and heating.

Elemental analyses were carried out with a Perkin–Elmer 2400 CHNS instrument. The ^{1}H and ^{13}C NMR spectra were recorded on a Brucker 400 spectrometer operating at 400 and 100 MHz, respectively. ^{1}H and ^{13}C spectra were referenced internally to TMS as internal standard. MS spectra were taken on a VG 7070E mass spectrometer. Melting points were determined by the hot plate method and are uncorrected. Optical rotations were determined with a JASCO Model DIP-360 digital polarimeter. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2/g. The determination of E was based on the equation $E=\ln[1-ee_S)/(1-ee_S/ee_P)]/\ln[(1-ee_S)/(1+ee_S/ee_P)]$ with $c=ee_S/(ee_S+ee_P)$, as derived from the original equations of Chen et al.,[32] using linear regression, with E as the slope of the line $\ln[(1-c)(1-ee_S)]$ vs. $\ln[(1-c)(1+ee_S)]$.

5.2. Preparation of β-lactams and β-amino acids

Alicyclic β-lactams were prepared by the cycloaddition of chlorosulfonyl isocyanate to the corresponding cycloalkenes and thereafter transformed to β-amino acids.[5,7–14] For the synthesis of 13-azabicyclo[10.2.0]-tetradecan-14-one as a new compound, a mixture of *cis*- and *trans*-cyclododecene (*cis*:*trans*=2:1) gave a mixture of isomers (*cis*:*trans*=45%:55%) which were separated by column chromatography on silica gel with diethyl ether:ethyl acetate (4:1). The products were recrystallized from ethyl acetate and hydrolyzed to the corresponding β-amino acids. The other β-lactams and β-amino acids were described earlier.[13,14]

5.2.1. *cis*-13-Azabicyclo[10.2.0]tetradecan-14-one. Yield: 60%. Mp: 147–148°C. Anal. calcd for $C_{13}H_{23}NO$: C, 74.59; H, 11.07; N, 6.69. Found: C, 74.25; H, 10.94; N, 6.66%. HRMS M$^+$ found (M$^+$ calcd): 209.17822 (209.17796); MS: m/z (relative intensity)=210 (2), 209 (1), 208 (1) [M]$^+$: 168 (1), 167 (8), 166 (57); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.26–1.84 (20H, m, 10×C$\underline{H}_2$), 3.11–3.16 (1H, m, C$\underline{H}$CO), 3.65–3.69 (1H, m, C$\underline{H}$NH), 5.87 (1H, bs, N$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 22.44, 23.03, 23.41, 25.11, 28.83, 27.92, 28.05, 28.32, 28.42, 29.42, 53.43, 54.41, 171.89.

5.2.2. *cis*-2-Aminocyclododecanecarboxylic acid. Yield: 72%. Mp: 239–241°C. Anal. calcd for $C_{13}H_{25}NO_2$: C, 68.68; H, 11.08; N, 6.16. Found: C, 68.47; H, 10.95; N, 6.14%. HRMS M$^+$ found (M$^+$ calcd): 227.18857 (227.18853); MS: m/z (relative intensity)=228 (1), 227 (2), 226 (1), [M]$^+$: 211 (1), 210 (3), 198 (1), 192 (1), 185 (1), 184 (5), 182 (1), 180 (1), 170 (2), 169 (1), 168 (5), 167 (1), 166 (2);); ^{1}H NMR (D_2O in the presence of KOH) δ (ppm): 1.34–1.80 (20H, m, 10×C$\underline{H}_2$), 2.62–2.64 (1H, m, C$\underline{H}$CO), 3.45–3.47 (1H, m, C$\underline{H}$NH). ^{13}C NMR (D_2O in the presence of KOH) δ (ppm): 20.44, 21.86, 21.95, 22.21, 23.04 (2 overlapping signals), 24.54, 25.55 (2 overlapping signals), 30.85, 48.17, 51.91, 183.95.

5.2.3. *trans*-13-Azabicyclo[10.2.0]tetradecan-14-one. Yield: 63%. Mp: 126–127°C. Anal. calcd for $C_{13}H_{23}NO$: C, 74.59; H, 11.07; N, 6.69. Found: C, 74.13; H, 10.97; N, 6.65%. HRMS M$^+$ found (M$^+$ calcd): 209.17814 (209.17796); MS: m/z (relative intensity)=210 (2), 209 (1), 208 (1) [M]$^+$:181 (1), 180 (1), 169 (1), 168 (1), 167 (8), 166 (63), 164 (1); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.16–1.97 (20H, m, 10×C$\underline{H}_2$), 2.84–2.88 (1H, m, C$\underline{H}$CO), 3.42–3.46 (1H, m, C$\underline{H}$NH), 5.97 (1H, bs, N$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 23.23, 23.32, 24.27, 25.08, 25.27, 25.75, 27.83, 28.06, 28.43, 35.07, 54.32, 55.22, 172.55.

5.2.4. *trans*-2-Aminocyclododecanecarboxylic acid. Yield: 74%. Mp: 261–262°C. Anal. calcd for $C_{13}H_{25}NO_2$: C, 68.68; H, 11.08; N, 6.16. Found: C, 68.93; H, 10.92; N, 6.08%. HRMS M$^+$ found (M$^+$ calcd): 227.18887 (227.18853); MS: m/z (relative intensity)=227 (2), [M]$^+$: 210 (3), 198 (1), 185 (1), 184 (4), 183 (1), 170 (2), 169 (1), 168 (4), 167 (1), 166 (2); ^{1}H NMR (D_2O in the presence of KOH) δ (ppm): 1.28–1.75 (20H, m, 10×C$\underline{H}_2$), 2.66–2.70 (1H, m, C$\underline{H}$CO), 3.25–3.29 (1H, m, C$\underline{H}$NH). ^{13}C NMR (D_2O in the presence of KOH) δ (ppm): 19.68, 22.90 (2 overlapping signals), 23.23, 23.63, 23.72, 24.70, 24.95, 26.88, 29.75, 50.07, 51.67, 183.33.

5.3. Preparation of methyl β-aminocycloalkanecarboxylates 1a–c

Distilled thionyl chloride (1.1 ml, 14 mmol) was added dropwise to a solution of a racemic 2-aminocycloalkanecarboxylic acid (12.7 mmol) in methanol (40 ml) at −5°C. The reaction mixture was first stirred at −5°C (0.5 h), then at room temperature (1 h) and then finally refluxed (2 h). The solvent was evaporated off and the resulting semisolid product was crystallized from diethyl ether. NH_3 gas was bubbled into a solution of a β-amino ester hydrochloride (12 mmol) in chloroform (20 ml) for 0.5 h. The reaction mixture was extracted with distilled water (3×10 ml) and the organic layer dried over sodium sulfate and concentrated in vacuo.

5.3.1. Methyl *cis*-2-aminocycloheptanecarboxylate 1a. Yield: 93%. Anal. calcd for $C_9H_{17}NO_2$: C, 63.13; H, 10.01; N, 8.18. Found: C, 63.06; H, 10.04; N, 8.15%. HRMS M$^+$ found (M$^+$ calcd): 171.12600 (171.12593); MS: m/z (relative intensity)=171 (3), [M]$^+$: 156 (3), 142 (3), 140 (2), 128 (4), 114 (13), 97 (5); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.38–1.90 (10H, m, 5×C$\underline{H}_2$), 2.65–2.69 (1H, m, C$\underline{H}$CO), 3.39–3.46 (1H, m, C$\underline{H}$NH), 3.69 (3H, m, COOC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ (ppm): 23.59, 24.52, 26.82, 28.53, 36.06, 50.47, 51.44, 51.82, 175.89

5.3.2. Methyl *cis*-2-aminocyclooctanecarboxylate 1b. Yield: 85%. Anal. calcd for $C_{10}H_{19}NO_2$: C, 64.83; H, 10.34; N, 7.56. Found: C, 64.63; H, 10.30; N, 7.55%. HRMS M$^+$ found (M$^+$ calculated for): 185.14169 (185.14158); MS: m/z (relative intensity)=186 (21), 185 (7), 184 (26); [M]$^+$ 169 (4), 168 (5), 155 (5), 154 (35), 153 (10), 138 (4), 137 (39), 136 (5), 126 (19), 125 (18), 112(13), 111 (7), 110 (7), 109 (24); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.33–1.87 (12H, m, 6×C$\underline{H}_2$), 2.76–2.79 (1H, m, C$\underline{H}$CO), 3.31–3.35 (1H, m, C$\underline{H}$NH), 3.69 (3H, m, COOC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ (ppm): 23.21, 23.65, 25.63, 26.59, 28.00, 33.86, 46.66, 51.37, 51.55, 176.45.

5.3.3. Methyl *cis*-2-aminocyclododecanecarboxylate 1c. Yield: 60%. Mp: 54–57°C. Anal. calcd for $C_{14}H_{27}NO_2$: C, 69.66; H, 11.27; N, 5.80. Found: C, 69.85; H, 11.30; N, 5.80%. HRMS M^+ found (M^+ calcd): 241.20430 (241.20418); MS: m/z (relative intensity) = 242 (1), 241 (2), 240 (1); $[M]^+$: 224 (4), 211 (1), 210 (7), 199 (1), 198 (5), 192 (3), 184 (2), 170 (3), 169 (1), 168 (6). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.23–1.72 (20H, m, 10×C$\underline{H}_2$), 2.60–2.62 (1H, m, C$\underline{H}$CO), 3.10–3.12 (1H, m, C$\underline{H}$NH), 3.70 (3H, s, COOC$\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ (ppm): 20.99, 22.11, 22.14, 22.45, 23.53, 23.69, 24.47, 25.61, 25.68, 31.25, 46.04, 47.32, 52.00, 176.69.

5.4. Gram-scale resolution of 2-aminocycloalkanecarboxylates

5.4.1. Methyl *cis*-2-aminocycloheptanecarboxylate *rac*-1a. *rac*-**1a** (0.85 g, 5.0 mmol) and 2,2,2-trifluoroethyl butanoate (1.5 ml, 10.0 mmol) in diisopropyl ether (50 ml) were added to the CAL-A preparation (1.0 g). The mixture was stirred for 1.8 h at room temperature. The reaction was stopped by filtering off the enzyme at 50% conversion, with 98% e.e. for (1*R*,2*S*)-**1a** and 97% e.e. for (1*S*,2*R*)-**2a**. The temperature was lowered to 0°C and gaseous hydrogen chloride bubbled through the solution for 30 min. After evaporation, the residue was dissolved in diethyl ether (20 ml) after which (1*R*,2*S*)-**1a** was allowed to precipitate as a white hydrochloride salt (0.40 g, 1.9 mmol, mp 146–148°C; $[\alpha]_D^{22} = +12.2$ (*c* 1, H_2O); e.e. = 99%). Anal. calcd for $C_9H_{18}ClNO_2$: C, 52.05; H, 8.74; N, 6.74. Found: C, 51.98; H, 8.75; N, 6.73%. ^{1}H NMR (D_2O) δ (ppm): 1.30–1.96 (10H, m, 5×C$\underline{H}_2$), 3.02–3.05 (1H, m, C$\underline{H}$CO), 3.53–5.57 (1H, m, C$\underline{H}$NH), 3.65 (3H, s, COOC$\underline{H}_3$); ^{13}C NMR (D_2O) δ (ppm): 23.95, 25.44, 26.95, 27.00, 30.77, 45.77, 53.40, 53.47, 176.13.

(1*S*,2*R*)-**2a** was obtained as a yellow oil after column chromatography involving elution with dichloromethane:ethyl acetate (1:1) (0.51 g, 2.1 mmol, $[\alpha]_D^{22} = +11.9$ (*c* 1, $CHCl_3$); e.e. = 98%). Anal. calcd for $C_{13}H_{23}NO_3$: C, 64.70; H, 9.61; N, 5.80. Found: C, 64.52; H, 9.59; N, 5.81%. HRMS M^+ found (M^+ calcd): 241.16793 (241.16779); MS: m/z (relative intensity) = 242 (4), 241 (16), $[M]^+$: 210 (10), 198 (3), 182 (4), 171 (10), 170 (59), 154 (19), 140 (8), 139 (10), 138 (10), 123 (13), 122 (12), 114 (34), 112 (14), 97 (14), 95 (30). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.93 (3H, t, $J = 7$ Hz, $CH_2CH_2C\underline{H}_3$), 1.41–1.99 (12H, m, 6×CH_2), 2.11 (2H, t, $J = 7$ Hz, $C\underline{H}_2CH_2CH_3$), 2.89–2.93 (1H, m, C$\underline{H}$CO), 3.70 (3H, s, COOC$\underline{H}_3$), 4.23–4.30 (1H, m, C$\underline{H}$NH), 6.17 (1H, bs, N$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 13.59, 19.15, 24.45, 25.04, 26.92, 27.17, 32.47, 38.83, 47.45, 50.43, 51.48, 171.63, 175.07.

5.4.2. Methyl *cis*-2-aminocyclooctanecarboxylate *rac*-1b. *rac*-**1b** (0.37 g, 2.0 mmol) and 2,2,2-trifluoroethyl butanoate (0.60 ml, 5.0 mmol) in diisopropyl ether (20 ml) were added to the CAL-A preparation (0.20 g). The mixture was stirred for 4 h at room temperature. The reaction was stopped by filtering off the enzyme at 50% conversion, with 99% e.e. for (1*R*,2*S*)-**1b** and 95% e.e. for (1*S*,2*R*)-**2b**. The work-up followed the above procedure. (1*R*,2*S*)-Methyl 2-aminocyclooctanecarboxylate hydrochloride (0.21 g, 0.94 mmol, mp 143–145°C; $[\alpha]_D^{22} = +33.4$ (*c* 1, H_2O); e.e. = 99%) was obtained as white crystals. Anal. calcd for $C_{10}H_{20}ClNO_2$: C, 54.17; H, 9.09; N, 6.32. Found: C, 54.00; H, 9.12; N, 6.31%. ^{1}H NMR (D_2O) δ (ppm): 1.53–1.95 (12H, m, 6×C$\underline{H}_2$), 3.12–3.16 (1H, m, C$\underline{H}$CO), 3.77 (3H, s, COOC$\underline{H}_3$), 3.79–3.84 (1H, m, C$\underline{H}$NH); ^{13}C NMR (D_2O) δ (ppm): 23.89, 25.30, 26.08, 26.83, 27.19, 29.37, 43.48, 52.19, 53.07, 175.21.

(1*S*,2*R*)-**2b** (0.25 g, 0.98 mmol, $[\alpha]_D^{22} = +41.7$ (*c* 1, $CHCl_3$); e.e. = 95%) was obtained as a yellow oil after column chromatography. Anal. calcd for $C_{14}H_{25}NO_3$: C, 65.85; H, 9.87; N, 5.49. Found: C, 66.00; H, 9.89; N, 5.50%. HRMS M^+ found (M^+ calcd): 255.18401 (255.18344); MS: m/z (relative intensity) = 256 (3), 255 (15), $[M]^+$: 224 (7), 212 (2), 195(4), 185 (10), 184 (54), 182 (4), 169 (3), 168 (13), 156 (3), 154 (5), 153 (4), 152 (4), 142 (6), 140 (3), 137 (9), 136 (11), 128 (3), 127 (3), 126 (10), 125 (5), 124 (3), 115 (4), 114 (39), 113 (14), 112 (7), 110 (3). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.94 (3H, t, $J = 7$ Hz, $CH_2CH_2C\underline{H}_3$), 1.50–2.01 (12H, m, 6×C$\underline{H}_2$), 1.67 (2H, m, $CH_2C\underline{H}_2CH_3$), 2.22 (2H, t, $J = 7$ Hz, $C\underline{H}_2CH_2CH_3$), 2.84–2.89 (1H, m, C$\underline{H}$CO), 3.69 (3H, s, COOC$\underline{H}_3$), 4.43–4.49 (1H, m, C$\underline{H}$NH), 6.40 (1H, bs, N$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 13.46, 19.40, 24.88, 25.11, 25.79, 26.39, 30.93, 35.80, 37.85, 46.33, 49.16, 51.75, 173.47, 174.95.

5.4.3. Methyl *cis*-2-aminocyclododecanecarboxylate *rac*-1c. *rac*-**1c** (0.17 g, 0.70 mmol) and 2,2,2-trifluoroethyl butanoate (0.27 g, 0.25 ml, 1.6 mmol) in diisopropyl ether (8 ml) were added to the CAL-A preparation (0.4 g). The mixture was stirred for 24 h at 47°C. The reaction was stopped by filtering off the enzyme at 49% conversion, with 96% e.e. for (1*R*,2*S*)-**1c** and 99% e.e. for (1*S*,2*R*)-**2c**. The work-up followed the above procedure. (1*R*,2*S*)-Methyl 2-aminocyclododecanecarboxylate hydrochloride (0.097 g, 0.35 mmol, mp 174–175°C; $[\alpha]_D^{21} = +33.4$ (*c* 1, MeOH); e.e. = 97%) was obtained as white crystals. Anal. calcd for $C_{14}H_{28}ClNO_2$: C, 60.52; H, 10.16; N, 5.04. Found: C, 60.39; H, 10.14; N, 5.04%. ^{1}H NMR (D_2O) δ (ppm): 1.32–1.88 (20H, m, 10×C$\underline{H}_2$), 2.89–2.93 (1H, m, C$\underline{H}$CO), 3.65–3.68 (1H, m, C$\underline{H}$NH), 3.75 (3H, s, COOC$\underline{H}_3$); ^{13}C NMR (D_2O) δ (ppm): 21. 10, 21.26, 21.55, 21.99, 22.67, 23.13, 23.68, 24.19, 25.48, 25.95, 47.68, 50.65, 53.10, 176.22.

(1*S*,2*R*)-**2c** (0.093 g, 0.30 mmol, $[\alpha]_D^{22} = +28.6$ (*c* 1, $CHCl_3$); e.e. = 99%) was obtained as a yellow oil after column chromatography. Anal. calcd for $C_{18}H_{33}NO_3$: C, 69.41; H, 10.68; N, 4.50. Found: C, 69.54; H, 10.73; N, 5.49%. HRMS M^+ found (M^+ calcd): 311.24634 (311.24604); MS: m/z (relative intensity) = 312 (2), 311 (7); $[M]^+$: 281(1), 280 (3), 279 (1), 268 (2), 251 (1), 242 (1), 241 (5), 240 (20), 238 (4), 225 (2), 224 (6), 212 (1), 211 (1), 210 (6), 198 (4), 196 (2), 182 (3), 180 (1), 170 (3), 169 (2), 168 (7), 164 (1). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.96 (3H, t, $J = 7$ Hz, $CH_2CH_2C\underline{H}_3$), 1.34–0.74 (22H, m, 10×C$\underline{H}_2$, $CH_2C\underline{H}_2CH_3$), 2.22 (2H, t, $J = 7$ Hz, $C\underline{H}_2CH_2CH_3$), 2.59–2.61 (1H, m, C$\underline{H}$CO), 3.69 (3H, s, COOC$\underline{H}_3$), 4.36–4.39 (1H, m, C$\underline{H}$NH), 6.57 (1H, bs, N$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 13.54, 18.17, 21.73, 23.06, 23.28, 23.42, 25.08, 25.22, 26.58, 30.28, 35.81, 38.45, 46.50, 46.92, 51.66, 173.18, 174.74.

5.5. Preparation of *N*-hydroxymethyl-β-lactams 3a–d

A β-lactam (30 mmol) was dissolved in tetrahydrofuran (60 ml). Paraformaldehyde (1.08 g, 36 mmol), K_2CO_3 (0.42 g, 3 mmol) and distilled water (3 ml) were added. The solution was sonicated for 8 h. The solvent was evaporated off and the residue dissolved in diethyl ether (50 ml). The solution was dried over sodium sulfate for 12 h. The solvent was evaporated off and recrystallization from diisopropyl ether afforded colorless crystals of **3a**–**d**.

5.5.1. *cis*-8-Hydroxymethyl-8-azabicyclo[5.2.0]nonan-9-one 3a. Yield: 75%. Mp: 73–75°C. Anal. calcd for $C_9H_{15}NO_2$: C, 63.88; H, 8.93; N, 8.28. Found: C, 64.09; H, 8.89; N, 8.26%. HRMS M^+ found (M^+ calcd): 169.11102 (169.11028); MS: *m*/*z* (relative intensity) = 171 (1), 170 (4), 169 (2), $[M]^+$: 152 (6), 151 (5), 124 (1), 97 (7), 96 (96) 95 (13); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.20–2.09 (10H, m, 5×C<u>H</u>$_2$), 3.28–3.39 (1H, m, C<u>H</u>CO), 3.50 (1H, bs, OH), 3.86–3.96 (1H, m, C<u>H</u>NH), 4.35–4.48 (1H, m, C<u>H</u>$_2$OH), 4.68–4.83 (1H, m, C<u>H</u>$_2$OH); ^{13}C NMR ($CDCl_3$) δ (ppm): 24.59, 24.97, 27.90, 29.53, 31.35, 54.25, 55.71, 62.35, 170.63.

5.5.2. *cis*-9-Hydroxymethyl-9-azabicyclo[6.2.0]decan-10-one 3b. Yield: 80%. Mp: 67–69°C. Anal. calcd for $C_{10}H_{17}NO_2$: C, 65.54; H, 9.35; N, 7.64. Found: C, 65.23; H, 9.32; N, 7.66%. HRMS M^+ found (M^+ calcd): 183.12595 (183.12593); MS: *m*/*z* (relative intensity) = 185 (1), 184 (5), 183 (4), $[M]^+$: 166 (8), 138 (2), 124 (2), 110 (65); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.94–2.08 (12H, m, 6×C<u>H</u>$_2$), 3.02–3.13 (1H, m, C<u>H</u>CO), 3.55 (1H, bs, OH), 3.70–3.81 (1H, m, C<u>H</u>NH), 4.39–4.51 (1H, m, C<u>H</u>$_2$OH), 4.65–4.81 (1H, m, C<u>H</u>$_2$OH); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.60, 25.60, 25.94, 25.96, 27.32, 29.01, 53.62, 56.42, 62.72, 171.00.

5.5.3. *cis*-13-Hydroxymethyl-13-azabicyclo[10.2.0]tetradecan-14-one 3c. Yield: 77%. Mp: 126–128°C. Anal. calcd for $C_{14}H_{25}NO_2$: C, 70.25; H, 10.53; N, 5.85. Found: C, 70.04; H, 10.55; N, 5.86%. HRMS M^+ found (M^+ calcd): 239.18904 (239.18853); MS: *m*/*z* (relative intensity) = 240 (1), 239 (1), $[M]^+$: 222 (5), 210 (2), 208 (2), 194 (3), 180 (2), 168 (3), 167 (25), 166 (74); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.29–1.79 (20H, m, 10×C<u>H</u>$_2$), 3.09–3.14 (1H, m, C<u>H</u>CO), 3.78–3.84 (1H, m, C<u>H</u>NH), 3.85 (1H, lb, OH), 4.49 (1H, d, J = 11 Hz, C<u>H</u>$_2$OH), 4.77 (2H, d, J = 11 Hz, C<u>H</u>$_2$OH); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.83, 22.73, 23.07, 24.57, 24.63, 26.01, 26.23, 27.50, 27.60, 27.96, 54.00, 56.39, 67.34, 170.96.

5.5.4. *trans*-13-Hydroxymethyl-13-azabicyclo[10.2.0]-tetradecan-14-one 3d. Yield: 83%. Mp: 92–93°C. Anal. calcd for $C_{14}H_{25}NO_2$: C, 70.25; H, 10.53; N, 5.85. Found: C, 70.28; H, 10.51; N, 5.86%. MS M^+ found (M^+ calcd): 239.18809 (239.18853); MS: *m*/*z* (relative intensity) = 240 (1), $[M]^+$: 222 (4), 210 (2), 194 (2), 180 (2), 168 (4), 167 (25), 166 (78); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.12–2.05 (20H, m, 10×C<u>H</u>$_2$), 2.84–2.89 (1H, m, C<u>H</u>CO), 3.56–3.60 (1H, m, C<u>H</u>NH), 4.10 (1H, lb, OH), 4.48 (1H, d, J = 11 Hz, C<u>H</u>$_2$OH), 4.81 (1H, d, J = 11 Hz, C<u>H</u>$_2$OH); ^{13}C NMR ($CDCl_3$) δ (ppm): 23.27, 23.34, 24.35, 24.63, 25.33, 25.66, 27.81, 27.93, 28.04, 32.62, 54.32, 57.02, 63.46, 171.97.

5.6. Gram scale resolution of *N*-hydroxymethyl-β-lactams *rac*-3a–d

5.6.1. *cis*-8-Hydroxymethyl-8-azabicyclo[5.2.0]nonan-9-one 3a. After *rac*-**3a** (0.80 g, 4.7 mmol) was dissolved in dry acetone (47 ml), lipase PS preparation (2.4 g) and vinyl butanoate (2.16 g, 18.9 mmol, 2.40 ml) were added. The mixture was stirred for 2 h at room temperature. The enzyme was filtered off at 49% conversion, with 93% e.e. for the unreacted **3a** and 97% e.e. for the produced **4a**. The acetone was evaporated off and the residue chromatographed on silica, with elution with dichloromethane:acetone (4:1), affording (1*S*,7*R*)-**3a** (0.41 g, 2.4 mmol; $[\alpha]_D^{22} = -26.2$ (*c* 1, $CHCl_3$); e.e. = 97%) and (1*R*,7*S*)-**4a** (0.59 g, 2.4 mmol, $[\alpha]_D^{22} = -52.3$ (*c* 1, $CHCl_3$); e.e. = 97%), both as colorless oils.

(1*R*,7*S*)-**4a**: Anal. calcd for $C_{13}H_{21}NO_3$: C, 65.25; H, 8.84; N, 5.85. Found: C, 65.06; H, 8.86; N, 5.84%. HRMS M^+ found (M^+ calcd): 239.152680 (239.15214); MS: *m*/*z* (relative intensity) = 239 (2); $[M]^+$: 169 (1), 168 (3), 153 (4), 152 (35), 151 (7), 144 (10), 124 (4), 101 (10), 97 (8), 96 (100). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.95 (3H, t, J = 8 Hz, $CH_2CH_2C\underline{H}_3$), 1.33–2.04 (10H, m, 5×C<u>H</u>$_2$), 1.61–1.70 (2H, m, $CH_2C\underline{H}_2CH_3$) 2.31 (2H, t, J = 8 Hz, C<u>H</u>$_2CH_2CH_3$), 3.31–3.36 (1H, m, C<u>H</u>CO), 3.81–3.86 (1H, m, C<u>H</u>NH), 5.06 (1H, d, J = 6 Hz, NC<u>H</u>$_2$O), 5.18 (1H, d, J = 6 Hz, NC<u>H</u>$_2$O); ^{13}C NMR ($CDCl_3$) δ (ppm): 13.62, 18.22, 24.60, 25.03, 27.95, 29.42, 31.39, 35.89, 54.90, 57.35, 62.71, 170.51, 173.62.

5.6.2. *cis*-9-Hydroxymethyl-9-azabicyclo[6.2.0]decan-10-one 3b. After *rac*-**3b** (0.36 g, 2.0 mmol) was dissolved in dry acetone (20 ml), lipase PS preparation (1.0 g) and 2,2,2-trifluoroethyl butanoate (1.35 g, 7.9 mmol, 1.20 ml) were added and the mixture stirred for 24 h at room temperature. The enzyme was filtered off at 52% conversion, with 90% e.e. for the unreacted **3b** and 82% e.e. for the produced **4b**. The work-up carried out as previously described, afforded (1*S*,8*R*)-**3b** (0.17 g, 0.93 mmol, $[\alpha]_D^{22} = -38.8$ (*c* 1, $CHCl_3$); e.e. = 91%) and (1*R*,8*S*)-**4b** (0.20 g, 0.79 mmol, $[\alpha]_D^{22} = -39.9$ (*c* 1, $CHCl_3$); e.e. = 82%) as colorless oils.

(1*R*,8*S*)-**4b**: Anal. calcd for $C_{14}H_{23}NO_3$: C, 66.37; H, 9.15; N, 5.53. Found: C, 66.54; H, 9.17; N, 5.52%. HRMS M^+ found (M^+ calcd): 253.166840 (253.16779); MS: *m*/*z* (relative intensity) = 254 (1), 253 (5); $[M]^+$: 182 (4), 183 (1), 167 (3), 166 (31), 138 (4), 124 (2), 111 (7), 110 (75), 109 (7). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.95 (3H, t, J = 8 Hz, $CH_2CH_2C\underline{H}_3$), 1.25–2.12 (14H, m, 7×C<u>H</u>$_2$), 2.31 (2H, t, J = 8 Hz, C<u>H</u>$_2CH_2CH_3$), 3.05–3.10 (1H, m, C<u>H</u>CO), 3.66–3.71 (1H, m, C<u>H</u>NH), 5.08 (1H, d, J = 6 Hz, NC<u>H</u>$_2$O), 5.15 (1H, d, J = 6 Hz, NC<u>H</u>$_2$O); ^{13}C NMR ($CDCl_3$) δ (ppm): 13.57, 18.20, 21.57, 25.62, 25.82, 25.97, 27.35, 28.94, 35.87, 54.21, 58.02, 62.08, 170.86, 173.52.

5.6.3. *cis*-13-Hydroxymethyl-13-azabicyclo[10.2.0]tetradecan-14-one 3c. After *rac*-**3c** (0.30 g, 1.3 mmol) was dissolved in dry acetone (25 ml), lipase PS preparation (1.25 g), 2,2,2-trifluoroethyl butanoate (0.85 g, 5.0 mmol, 0.75 ml) and hexadecane (56 mg) were added and the mixture stirred for 10 h at room temperature. The enzyme was filtered off at 50% conversion, with 90% e.e. for the unreacted **3c**; for **4c** e.e. determination was not possible in the resolution mixture. The work-up as previously described, afforded (1*S*,12*R*)-**3c** (0.15 g, 0.63 mmol, mp 128–130°C; $[\alpha]_D^{22}=-30.2$ (*c* 1, $CHCl_3$); e.e. = 93%) and (1*R*,12*S*)-**4c** (0.17 g, 0.55 mmol, mp 72–74°C; $[\alpha]_D^{22}=-24.5$ (*c* 1, $CHCl_3$); e.e. = 94%), both as white crystals. The value of the e.e.[4c] was obtained by the GC method using NH_2OH/MeOH followed by MeOH/HCl treatments and preparing the acetamide of the amino ester.

(1*R*,12*S*)-**4c**: Anal. calcd for $C_{18}H_{31}NO_3$: C, 69.86; H, 10.10; N, 4.53. Found: C, 69.99; H, 10.12; N, 4.54%. HRMS M^+ found (M^+ calcd): 309.23026 (309.23039); MS: *m*/*z* (relative intensity) = 309 (1); $[M]^+$: 279 (1), 266 (1), 239 (2), 238 (6), 224 (6), 223(6), 222 (40), 221(4), 220 (5), 211 (1), 210 (3), 209 (2), 208 (4), 204 (3), 197 (1), 196 (3), 195 (3) 194 (10), 193 (6), 192 (5), 180 (5), 178 (5), 168 (8), 167 (13), 166 (100), 165 (7), 164 (6). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.95 (3H, t, $J=7$ Hz, $CH_2CH_2C\underline{H}_3$), 1.26–1.76 (22H, m, $10\times C\underline{H}_2$, $CH_2C\underline{H}_2CH_3$), 2.30 (2H, t, $J=7$ Hz, $C\underline{H}_2CH_2CH_3$), 3.13–3.16 (1H, m, $C\underline{H}CO$), 3.67–3.70 (1H, m, $C\underline{H}NH$), 5.07 (1H, d, $J=6$ Hz, $NC\underline{H}_2O$), 5.14 (1H, d, $J=6$ Hz, $NC\underline{H}_2O$); ^{13}C NMR ($CDCl_3$) δ (ppm): 13.56, 18.23, 21.79, 22.66, 22.75, 23.10, 24.51, 25.87, 27.40, 27.57, 29.33, 31.90, 35.90, 54.25, 57.18, 62.30, 170.87, 173.48.

5.6.4. *trans*-13-Hydroxymethyl-13-azabicyclo[10.2.0]-tetradecan-14-one 3d. After *rac*-**3d** (0.30 g, 1.3 mmol) was dissolved in dry acetone (25 ml), CAL-B (63 mg), vinyl butanoate (0.57 g, 5.0 mmol, 0.64 ml) and hexadecane (56 mg) were added and the mixture stirred for 2.5 h at room temperature. The enzyme was filtered off at 50% conversion, with 85% e.e. for the unreacted **3d**; for **4d** e.e. determination was not possible in the resolution mixture. The work-up as above afforded (1*R*,12*R*)-**3d** (0.12 g, 0.50 mmol, mp 93–94°C; $[\alpha]_D^{22}=-146.6$ (*c* 1, $CHCl_3$); e.e. = 91%) as white crystals and (1*S*,12*S*)-**4d** (0.19 g, 0.61 mmol, $[\alpha]_D^{22}=54.4$ (*c* 1, $CHCl_3$); e.e. = 91%) as a colorless oil. The value of e.e.[4d] was obtained by the GC method using NH_2OH/MeOH followed by MeOH/HCl treatments and preparing the acetamide of the amino ester.

(1*S*,12*S*)-**4d**: Anal. calcd for $C_{18}H_{31}NO_3$: C, 69.86; H, 10.10; N, 4.53. Found: C, 70.00; H, 10.14; N, 4.54%. HRMS M^+ found (M^+ calcd): 309.23056 (309.23039); MS: *m*/*z* (relative intensity) = 309 (1); $[M]^+$: 281 (1), 264 (1), 239 (2), 238 (4), 224 (2), 223(8), 222 (41), 221(1), 220 (1), 211 (1), 210 (1), 209 (1), 208 (1), 204 (1), 196 (1), 195 (2) 194 (8), 193 (1), 192 (1), 180 (2), 169 (2), 168 (2), 167 (14), 166 (100), 165 (2). ^{1}H NMR ($CDCl_3$) δ (ppm): 0.95 (3H, t, $J=7$ Hz, $CH_2CH_2C\underline{H}_3$), 1.21–1.68 (22H, m, $10\times C\underline{H}_2$, $CH_2C\underline{H}_2CH_3$), 2.31 (2H, t, $J=7$ Hz, $C\underline{H}_2CH_2CH_3$), 2.88–2.91 (1H, m, $C\underline{H}CO$), 3.46–3.48 (1H, m, $C\underline{H}NH$), 5.09 (1H, d, $J=6$ Hz, $NC\underline{H}_2O$), 5.16 (1H, d, $J=6$ Hz, $NC\underline{H}_2O$); ^{13}C NMR ($CDCl_3$) δ (ppm):13.53, 18.24, 22.68, 22.97, 23.07, 23.92, 25.04, 25.22, 27.35, 29.30, 31.88, 32.08, 35.96, 54.65, 58.40, 62.64, 171.27, 173.39.

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