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An effective approach to the enantiomers of alicyclic β-aminonitriles by using lipase catalysis

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Abstract—Lipase-catalyzed N-acylations of racemic *cis*- and *trans*-2-aminocyclopentane- (and cyclohexane-) carbonitriles with 2,2,2-trifluoethyl butanoate in *tert*-butyl methyl ether (TBME) and in room-temperature ionic liquids (RTILs) were studied. The racemates were effectively resolved ($E > 200$) on a preparative scale by lipase PS-C II (lipase from *Burkholderia cepacia*) in TBME, resulting in two enantiomers in their enantiopure forms at 50% conversion. The reactions in RTILs with Novozym 435 (*Candida antarctica* lipase B) were slow and proceeded with low enantioselectivity.

1. Introduction

Alicyclic $\beta^{2,3}$-amino acids are structural units of oligopeptides, intermediates in the preparation of pharmaceutically important 1,3-heterocycles, and building blocks of potential pharmacons.[1] They include, for instance, cispentacin [FR 109615, (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid] and its derivatives, which exhibit antifungal activity against *Candida* strains.[2,3]

Nitriles are known to be highly valuable intermediates in synthetic chemistry. Both acid- and base-induced hydrolysis of a nitrile gives a carboxylic acid, while metal hydrides reduce nitriles to give primary amines. Accordingly, β-aminonitriles are valuable intermediates in the preparation of β-amino acids and the corresponding diamines. Besides chemical transformations, nitrile-converting enzymes are used to hydrolyze nitriles in the formation of carboxylic acids and amides.[4] Thus, enantioselective hydrolysis of the nitrile group by nitrilases has provided an attractive approach for the production of alicyclic $\beta^{2,3}$-amino acids from the corresponding racemic β-aminonitriles.[5–7] Interestingly, the enantiomers of the same alicyclic $\beta^{2,3}$-amino acids have previously been resolved as amino esters for lipase-catalyzed asymmetric N-acylation, as N-hydroxymethylated β-lactam intermediates for lipase-catalyzed asymmetric O-acylation, and as amino acids for direct lipase-catalyzed hydrolysis of the β-lactam ring.[8–11] These examples indicate a wide area of economical application of the readily availability lipases compared to enzymes with more targeted substrate specificities.

Herein, we report the application of the lipase-induced asymmetric acylation of β-aminonitriles *rac*-**1**–**4** in *tert*-butyl methyl ether (TBME) and in room-temperature ionic liquids (RTILs) for the preparation of enantiopure counterparts (Schemes 1 and 2). To the best of our knowledge, the asymmetric N-acylation of 3-amino-4-(1*H*-3-indolyl)butanenitrile is the only reported lipase-catalyzed kinetic resolution of β-aminonitriles so far.[12] On the other hand, the enzymatic acylation of α-aminonitriles in the presence of lipases has already been described.[13] Although nitriles are not carbonyl compounds, we found it interesting to compare the enzymatic enantioselectivities for the acylation of alicyclic β-aminonitriles herein with those observed earlier for the corresponding β-amino esters. We expected that the absolute configurations of the lipase-catalyzed resolution products **1**–**8** would be the same as in the case of the corresponding β-amino esters.[8] In order to verify this expectation, (1*R*,2*S*)-**2** was reduced with $LiAlH_4$ to the (1*S*,2*S*)-diamine, and (1*S*,2*S*)-**4** to the (1*S*,2*R*)-diamine, with known specific rotations (Scheme 3).[14,15]

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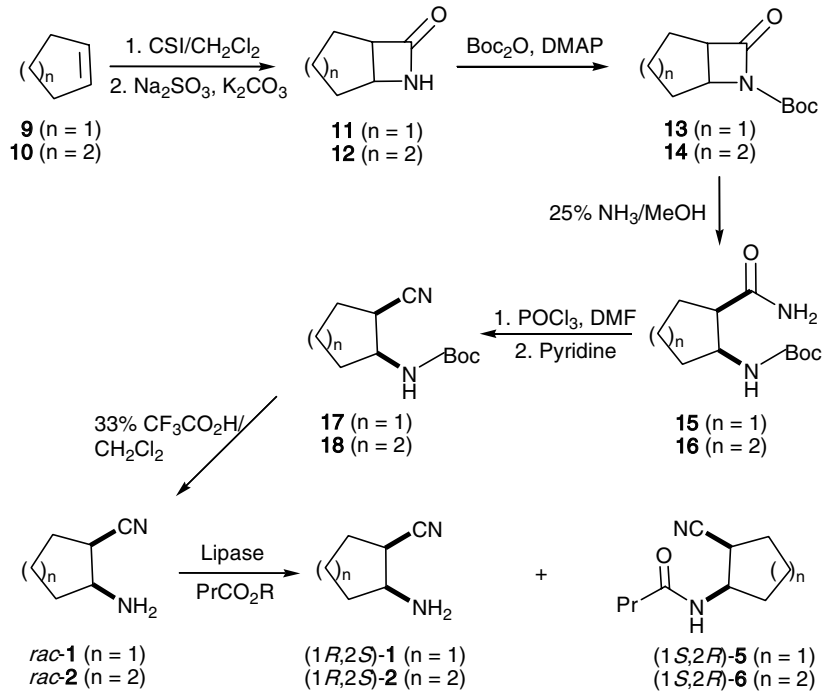
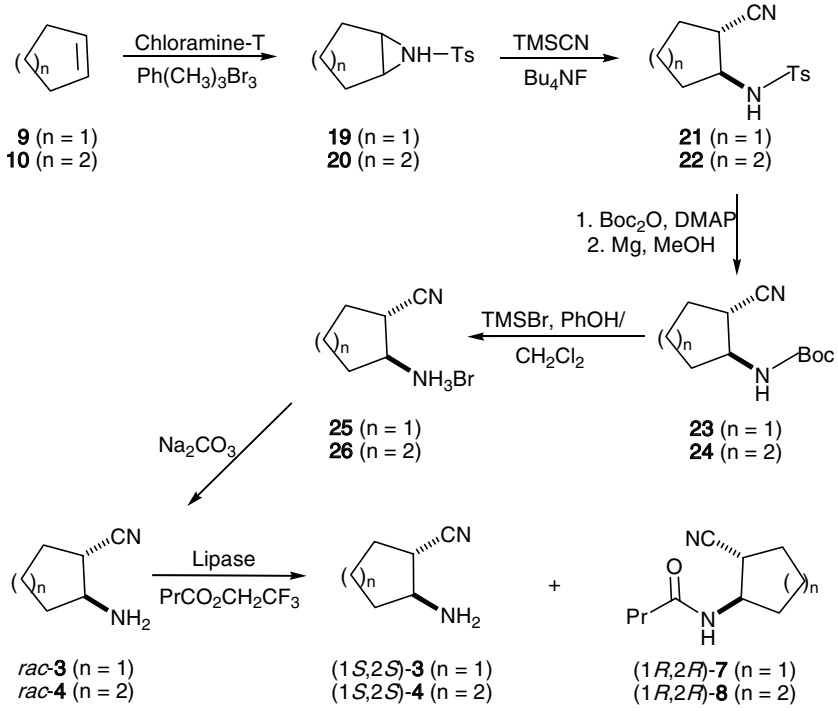


Scheme 1. Transformation of cyclopentene and cyclohexene into the enantiomers of *cis*-β-aminonitriles **1** and **2**.

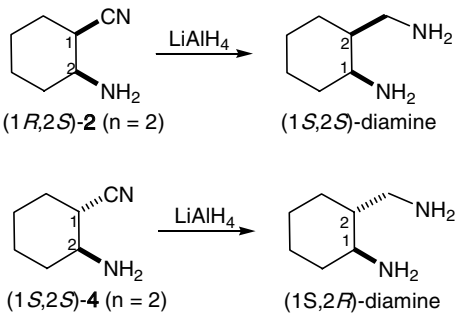


Scheme 2. Transformation of cyclopentene and cyclohexene into the enantiomers of *trans*-β-aminonitriles **3** and **4**.

2. Results and discussion

2.1. Preparation of racemic substrates *rac*-1–4

Substrates *rac*-**1** and *rac*-**2** (the racemic *cis* isomers) were prepared by following the protocol shown in Scheme 1. In this protocol, the alicyclic β-lactams **11** and **12** were readily available by the cycloaddition of chlorosulfonyl isocyanate to cycloalkenes.[9–11] *N*-Boc protection of the β-lactam, followed by ring opening through ammonolysis, gave the Boc-protected amides **15** and **16**, as previously described.[16] Dehydration of the amide with phosphorus oxychloride,[17] followed by *N*-Boc deprotection with 2,2,2-trifluoroacetic acid, furnished the racemic substrates. A three-step synthesis of *rac*-**1** and *rac*-**2** has previously been reported, starting from adiponitrile and heptanedinitrile, respectively, where the *cis* product had to be separated from the *trans* by-product.[7]



Scheme 3. Reduction of β-aminonitrile enantiomers to the diamine enantiomers.

Substrates *rac*-**3** and *rac*-**4** (the racemic *trans*-isomers) were prepared by following the protocol shown in Scheme 2. Catalytic aziridination of the cycloalkenes with chloramine-T gave the *N*-Ts-aziridines **19** and **20,** and subsequent opening of the aziridine ring with trimethylsilyl cyanide in the presence of tetrabutylammonium fluoride proceeded by a known method.[5,6] In the next step, the tosyl group in **21** and **22** was changed to Boc in two steps. The use of the protective groups Ts and Boc was due to the previous observation that the ring opening of *N*-Boc-aziridine was incomplete.[6] Moreover, the removal of Ts with the present procedure was clean.[18] Deprotection of **23** and **24** with bromotrimethylsilane and phenol, and neutralization of the resulting hydrobromides **25** and **26** with sodium carbonate, furnished the racemic substrates as described in the Experimental.

2.2. Kinetic resolution of *rac*-1–4 in TBME

In our earlier studies on the enantioselective N-acylation of alicyclic β-amino esters in ether (Et_2O, $^iPr_2O$ and TBME) solutions, lipase PS from *Burkholderia cepacia* (earlier lipase from *Pseudomonas cepacia*) was the most enantioselective lipase, in the case of the racemic trans-isomers, as was *Candida antarctica* lipase A (CAL-A) in the case of the racemic *cis*-isomers.[8] As an exception to this rule, lipase PS also exhibited excellent enantioselectivity for the acylation of cispentacin ethyl ester. Herein, these lipases, together with some others, were screened for the acylation of *rac*-**1** and *rac*-**2** with 2,2,2-trifluoroethyl butanoate in TBME. The enantioselectivities observed in terms of the enantiomeric ratios *E* and conversions after a certain time are reported in Tables 1 and 2. The lipase PS preparations (PS on Celite and PS-C II on ceramic particles) gave excellent enantioselectivities for both *cis*-β-aminonitriles (entries 1 and 2, Table 1; entries 2 and 3, Table 2), while CAL-A resulted in a fast reaction without enantioselectivity. It was impossible to increase the low enantioselectivity of CAL-A by changing the solvent from TBME to either toluene, acetonitrile, *tert*-amyl alcohol or ethyl butanoate, as indicated by the *E* values constantly being between 2 and 4. Accordingly, there is a clear difference in the enantioselective behavior of the two lipases toward nitrile substrates as compared with the previous ester substrates with a *cis*-configuration (entries 3 and 7 as compared with entries 1, 2, and 4–6, Table 3). Good (entry 5, Table 2) to excellent (entry 4, Table 1) enantioselectivities were observed in the case of *C. antarctica* lipase B (CAL-B) as catalyst. For acylation in neat ethyl butanoate, the lipases totally lost their enantioselectivity (Table 2).

Table 1. Lipase screening for the enantioselective acylation of *rac*-**1** (0.05 M) with $PrCO_2CH_2CF_3$ (0.1 M) in TBME in the presence of the enzyme preparation (50 mg/mL) at room temperature

Entry	Lipase	Time (h)	Conversion (%)	*E*
1	Lipase PS	0.5	50	≫200
2	Lipase PS-C II	2	51	>200
3	CAL-A	0.5	83	2 ± 1
4	CAL-B	1	50	>200

On the basis of the lipase screening, the lipase PS-C II-catalyzed acylation of *rac*-**2** with 2,2,2-trifluoroethyl butanoate in TBME was further optimized with respect to the amount of the enzyme. The reason for this was the observation that, although aminonitrile **1** reacted rapidly to 50% conversion in the presence of 50 mg/mL of the enzyme, the reaction in the case of *rac*-**2** tended to slow down at close to 50% conversion (Table 4). Thus, its took 6 h to reach 49% conversion, whereas the first 46% was reached within only 2 h (entries 3 and 4). In order to obtain both enantiomers in highly enantiopure form in a reasonable time, the gram-scale resolution of *rac*-**2** was performed at 70 mg/mL of lipase PS-C II (entries 5 and 6). Chromatographic purification finally furnished (1*R*,2*S*)-**1** (ee = 98%) and (1*S*,2*R*)-**5** (ee = 98%) from *rac*-**1**, and (1*R*,2*S*)-**2** (ee >99%) and (1*S*,2*R*)-**6** (ee = 96%) from *rac*-**2**, as shown in Section 4.

Table 2. Lipase screening for the enantioselective acylation of *rac*-**2** with $PrCO_2R$ in TBME at room temperature

Entry	Lipase	R = Et[a]			R = CH_2CF_3[b]		
		Time (h)	Conversion (%)	*E*	Time (h)	Conversion (%)	*E*
1	Lipase AK	40	33	1.3 ± 0.1	6	12	30 ± 0.3
2	Lipase PS	40	49	3.2 ± 0.2	4	43	>200
3	Lipase PS-C II	—	—	—	4	48	>200
4	CAL-A	—	—	—	0.5	83	2 ± 1
5	CAL-B	40	62	9 ± 1	6	48	79 ± 5
6	PPL	40	29	1.1 ± 0.01	—	—	—
7	CRL	40	39	1.2 ± 0.1	—	—	—

[a] Reaction of *rac*-**2** (0.1 M) in neat ethyl butanoate in the presence of the enzyme preparation (100 mg/mL).
[b] Reaction of *rac*-**2** (0.05 M) with the acyl donor (0.1 M) in TBME in the presence of the enzyme preparation (50 mg/mL).

Table 3. Enantioselectivity for the lipase-catalyzed N-acylation of ethyl *cis*-2-aminocyclopentyl- **1a** and *cis*-2-aminocyclohexyl- **2a** 1-carboxylates and that of *rac*-**1** and *rac*-**2**

Entry	Ref.	Substrate	Acyl donor	Solvent	Lipase PS *E*	CAL-A *E*
1	8	**1a** (CO_2Et)	$AcOCH_2CF_3$	Et_2O	$\gg 100$[a]	31
2	8	**1a** (CO_2Et)	$AcOCH_2CF_3$	TBME	>100[a]	37
3	—	**1** (CN)	$PrCO_2CH_2CF_3$	TBME	>200[a]/>200[b]	2
4	8	**2a** (CO_2Et)	$AcOCH_2CF_3$	Et_2O	6	51
5	8	**2a** (CO_2Et)	$PrCO_2CH_2CF_3$	Et_2O	2	$\gg 100$
6	8	**2a** (CO_2Et)	$AcOCH_2CF_3$	TBME	7	57
7	—	**2** (CN)	$PrCO_2CH_2CF_3$	TBME	>200[a]/>200[b]	2

In **1a** and **2a** CO_2Et is in the place of CN of compounds **1** and **2**.
[a] Lipase PS preparation on Celite.
[b] Lipase PS-C II.

Table 4. Effect of the amount of the lipase PS-C II on the enantioselective acylation of *rac*-**2** (0.05 M) with $PrCO_2CH_2CF_3$ (0.1 M) in TBME at room temperature

Entry	Enzyme (mg/mL)	Time (h)	Conversion (%)	$ee^{(1R,2S)\text{-}2}$ (%)	$ee^{(1S,2R)\text{-}6}$ (%)	*E*
1	25	2	42	70	99	>200
2	25	8	50	96	97	>200
3	50	2	46	83	98	>200
4	50	6	49	92	97	>200
5	70	2	49	93	98	>200
6	70	4	50	97	96	>200

Excellent enantioselectivities ($E \gg 200$) for the acylations of *rac*-**3** and *rac*-**4** (racemic *trans* isomers) with 2,2,2-trifluoroethyl butanoate were observed in the presence of lipase PS-C II (Table 5). Accordingly, the *trans*-aminonitriles behaved as expected on the basis of the previous results for the corresponding β-aminocarboxylates as substrates.[8] Due to the low solubility of *rac*-**3** in particular, *tert*-amyl alcohol was added to the TBME. However, the presence of *tert*-amyl alcohol somewhat lowered the reactivity as compared with the reaction in neat TBME, as shown for *rac*-**4** (entries 3 and 4). For this reason, and because of the somewhat better solubility of *rac*-**4**, its gram-scale kinetic resolution was performed in TBME at 45 °C, as described in Section 4, which allowed the preparations of (1*S*,2*S*)-**4** (ee = 99%) and (1*R*,2*R*)-**8** (ee >99%) at 50% conversion. *rac*-**3** was resolved in TBME/*tert*-amyl alcohol (9:1) at room temperature, yielding (1*S*,2*S*)-**3** (ee = 98%) and (1*R*,2*R*)-**7** (ee >99%).

2.3. Asymmetric acylation of *rac*-2 with CAL-B in TBME and in RTILs

RTILs serve as non-toxic, non-volatile, and non-flammable alternatives to classical organic solvents in various applications, including their use as biocatalytic reaction media. The results for lipase-catalyzed reactions in RTILs have been promising with respect to enhanced enzymatic activities and stabilities and to enzymatic regio- and enantioselectivities. CAL-B (Novozyme 435) is perhaps the most commonly used enzyme in RTILs. Most of these studies relate to the transformations of secondary alcohols.[19,20] Amines have served as substrates less commonly.[21]

The acylation of *rac*-**2** with 2,2,2-trifluoroethyl butanoate in TBME in the presence of CAL-B gave good (entry 5, Table 2), but not excellent enantioselectivity, as observed in the case of *rac*-**1** (entry 4, Table 1). It was also clear that an enzyme content of 30 mg/mL or higher had no effect on the reactivity of *rac*-**2** (entries 3–5, Table 6), with identical progression curves being obtained (not shown here). As RTILs have been described, which have several attractive properties, with enantioselectivity enhancement for biocatalysis in particular, we decided to study the CAL-B-catalyzed acylation of *rac*-**2** in RTILs. The RTILs were selected according to our previous work and are described in Scheme 4.[22] The results in Table 6 did not meet fully with our expectations (entries 6–12). The reactions in all cases proceeded slowly as compared with the reaction in TBME. The reactivity was best in hydrophobic EMIM·NTf_2 (entry 9), while

Table 5. Acylation of *rac*-**3** (0.05 M) and *rac*-**4** (0.05 M) with $PrCO_2CH_2CF_3$ (0.1 M) in TBME in the presence of lipase PS-C II (50 mg/mL)

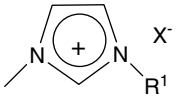
Entry	Substrate	Solvent	Temperature (°C)	Conversion (%)	*E*
1	*rac*-**3**	TBME/*tert*-amyl alcohol (8/2)	23	49	$\gg 200$
2	*rac*-**3**	TBME/*tert*-amyl alcohol (9/1)	23	50	$\gg 200$
3	*rac*-**4**	TBME/*tert*-amyl alcohol (9/1)	23	17	$\gg 200$
4	*rac*-**4**	TBME	23	30	$\gg 200$
5	*rac*-**4**	TBME	65	32	$\gg 200$

Reaction time 24 h.

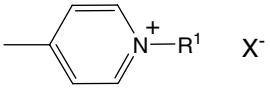
Table 6. Solvent screening for the CAL-B-catalyzed acylation of *rac*-**2** (0.05 M) with $PrCO_2CH_2CF_3$ (0.1 M) at room temperature

Entry	Solvent	CAL-B (mg/mL)	Time (h)	Conversion (%)	*E*
1	TBME	10	6	35	57 ± 2
2	TBME	20	6	45	63 ± 2
3	TBME	30	6	48	74 ± 10
4	TBME	50	6	48	79 ± 5
5	TBME	70	6	48	73 ± 4
6	BMIM·PF_6	50	96	11	14 ± 3
7	BMPyr·BF_4	50	96	32	24 ± 3
8	EMIM·BF_4	50	96	9	12 ± 1
9	EMIM·NTf_2	50	96	46	22 ± 1
10	EMIM·TfO	50	96	23	51 ± 6
11	EMIM·TfO	75	96	26	71 ± 4
12	EMIM·TfO/TBME[a]	75	96	46	89 ± 4

[a] A mixture of 1:1; two-phase medium.



[EMIM][BF_4]: R^1 = Et, X = BF_4
[BMIM][PF_6]: R^1 = Bu, X = PF_6
[EMIM][NTf_2]: R^1 = Et, X = $(CF_3SO_2)_2N$
[EMIM][TfO]: R^1 = Et, X = CF_3SO_3



[BMPyr][BF_4]: R^1 = Bu, X = BF_4

Scheme 4. Imidazolium- and pyridinium-based ionic liquids.

the enantioselectivity reached the same level as in TBME only in the case of hydrophilic EMIM·TfO (entry 11). In accordance with what we have seen before,[23] the higher reactivity and enantioselectivity of *rac*-**2** are evident in a solvent mixture of EMIM·TfO and TBME (entry 12).

3. Conclusions

We have shown that alicyclic *cis*- and *trans*-aminonitriles *rac*-**1**–**4** can be effectively resolved on a preparative scale by lipase PS-C II, on acylation with 2,2,2-trifluoroethyl butanoate in TBME. Thus, both enantiomers are simultaneously present at 50% conversion, one as the unreacted aminonitrile **1**–**4** and the other as the produced N-butanoylated counterpart **5**–**8** (Schemes 1 and 2). For both *cis*- and *trans*-isomers, the enantiomer in which the amino group is at the (*R*)-stereocenter reacts. This is in accordance with the previously observed enantiopreference in the N-acylation of the corresponding *cis*- and *trans*-amino esters.[8] In disagreement with the previous results, however, the highly enantioselective (E >200) N-acylation of *rac*-**2** proceeded quickly in TBME. The reactions in RTILs with CAL-B catalysis were less successful.

We have also demonstrated that reduction by $LiAlH_4$ can produce *cis*-(1*S*,2*S*)-1-amino-2-aminomethylcyclohexane from (1*R*,2*S*)-**2**, and *trans*-(1*S*,2*R*)-1-amino-2-aminomethylcyclohexane from (1*S*,2*S*)-**4** (Scheme 3). In this process, however, the enantiomeric excess tended to drop somewhat. These reductions were used for the determination of absolute configurations, and need more optimization in order to be used for preparative purposes.

4. Experimental

4.1. Materials and methods

2,2,2-Trifluoroethyl butanoate was prepared from 2,2,2-trifluoroethanol and butanoyl chloride. Organic solvents were of the highest analytical grade. BMPyr·BF_4 and EMIM·TfO were products of Fluka. The other RTILs were prepared by slightly modifying methods described in the literature.[19,23,24] *C. antarctica* lipase A (lipase SP 526) and B (Novozym 435) were purchased from Roche and Novozyme, respectively. Lipases PS and PS-C II from *B. cepacia* (former *P. cepacia*) and lipase AK from *Pseudomonas fluorescens* were product of Amano Europe, England. Before use, CAL-A and lipases PS and AK were adsorbed on Celite (4.0 g) by dissolving the enzyme and sucrose (0.24 g) in Tris–HCl buffer (pH 7.9), and thereafter left to dry at room temperature, with the final lipase content in the enzyme preparation being 20% (w/w).[25] Porcine pancreatic lipase (PPL) and *Candida rugosa* lipase (CRL) were purchased from Sigma.

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ and D_2O at ambient temperature on a Bruker DRX400 spectrometer. Chemical shifts are given in δ (parts per million) relative to TMS as the internal standard; multiplicities were recorded as s (singlet), br s (broad

singlet), d (doublet), t (triplet), m (multiplet), or om (overlapping multiplet). Optical rotations were measured with a Perkin Elmer 341 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

In a typical small-scale experiment, one or more of racemates **1**–**4** (0.05 M) was dissolved in TBME (or some other organic solvent) or in one of the RTILs (1 mL), followed by addition of the enzyme preparation (10–75 mg/mL). The addition of 2,2,2-trifluoroethyl butanoate (0.1 M) initiated the reaction. For the reactions in ethyl butanoate, the addition of the enzyme initiated the reaction. The reaction mixture was shaken at room temperature (23 °C) if not otherwise stated. The progress of the reactions and the ee values were calculated by taking samples (0.05 mL) at intervals, filtering off the enzyme, and analyzing them by gas chromatography on a Chrompack CP-Chirasil-DEX CB column (25 m). In the case of RTILs, the products were extracted into TBME before the GC analysis. For good baseline separation, the unreacted amino group in the sample was acylated with acetic anhydride in the presence of pyridine containing 1% 4,4-dimethylaminopyridine. The determination of E was based on the equation $E = \ln[(1 - ee_s)/(1 + ee_s/ee_p)]/\ln[(1 + ee_s)/(1 + ee_s/ee_p)]$, where $c = ee_s/(ee_s + ee_p)$, with the use of linear regression, E being the slope of the line $\ln[(1 - c)(1 - ee_s)]$ versus $\ln[(1 - c)(1 + ee_s)]$ the subscripts referring to the less reactive substrate (s) and to the formed product (p).[26]

4.2. Preparation of *cis*-2-aminocyclopentanecarbonitrile, *rac*-1

The preparation of **15** has already been described (Scheme 1).[16] Compound **15** (3.60 g; 16 mmol) was added to a solution of the freshly formed Vilsmeier reagent (*N*,*N*-dimethylformamide and $POCl_3$; 1.47 mL (19 mmol) and 1.60 mL (18 mmol), respectively) in acetonitrile (20 mL) under an argon atmosphere at 0 °C, followed by the addition of pyridine in order to cleave the intermediate.[17] Compound **17** was obtained as white crystals (2.05 g; 60%), mp: 86–88 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (9H, s, *t*-Bu), 1.55–2.10 (6H, om, $3 \times CH_2$), 3.21 (1H, m, H-1), 4.12 (1H, m, $J = 7.28$ Hz, H-2), 4.84 (1H, s, N*H*) ppm; Anal. Calcd for $C_{11}H_{18}N_2O_2$: C, 62.83; H, 8.63; N, 13.32. Found: C, 62.40; H, 8.52; N, 13.01.

Boc-deprotection of **17** involved standard procedures. Compound **17** (1.26 g, 6 mmol) was dissolved in dichloromethane (60 mL), which contained 33% trifluoroacetic acid. The reaction mixture was stirred at room temperature for 49 h before evaporation, leading to the trifluoroacetic salt as an oily residue (4.9 g). The residue (1.0 g) was dissolved in 0.5 M aqueous sodium carbonate (70 mL) and this mixture extracted three times with dichloromethane. After drying of the organic phases and evaporation, the free aminonitrile was purified by column chromatography, using dichloromethane/methanol (19:1) as eluent resulting in *rac*-**1** (0.089 g, 60%) as white crystals, mp: 59–61 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90–2.35 (6H, om, $3 \times CH_2$), 1.63 (2H, br s, NH_2), 2.92 (1H, m, H-1), 3.59 (1H, m, H-2) ppm, ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.8 (C4), 29.2 (C5), 33.6 (C3), 37.1 (C2), 54.9 (C1), 121.0 (CN) ppm. Anal. Calcd for $C_6H_{10}N_2$: C, 65.42; H, 9.15; N, 25.43. Found: C, 65.23; H, 9.05; N, 25.12.

4.3. Preparation of *cis*-2-aminocyclohexanecarbonitrile, *rac*-2

The preparation of *rac*-**2** was performed in the same way as that of *rac*-**1**. Compound **12** in the synthetic path is well characterized.[10] The characterization of other intermediates is given as below.

4.3.1. 7-*tert*-Butoxycarbonyl-7-azabicyclo[4,2,0]hexan-8-one, 14. White crystals (yield 65%), mp: 69–72 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.52 (9H, m, *t*-Bu), 1.56–2.06 (8H, om, $4 \times CH_2$), 3.24 (1H, m, $J = 3.52$ Hz, H-1), 4.10 (1H, m, $J = 2.94$ Hz, H-2) ppm. Anal. Calcd for $C_{12}H_{19}NO_3$: C, 63.98; H, 8.50; N, 6.22. Found: C, 63.54; H, 8.42; N, 6.14.

4.3.2. *cis*-2-*tert*-Butoxycarbonylaminocyclohexanecarboxamide, 16. White crystals (yield 70%), mp: 163–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (9H, s, *t*-Bu), 1.56–2.04 (8H, om, $4 \times CH_2$), 2.92 (1H, m, $J = 7.23$ Hz, H-1), 4.14 (1H, m, $J = 7.76$ Hz, H-2), 5.02 (1H, d, $J = 6.72$ Hz, NH), 5.32 (1H, s, NH_2), 5.70 (1H, s, NH_2) ppm. Anal. Calcd for $C_{12}H_{22}N_2O_3$: C, 59.48; H, 9.15; N, 11.56. Found: C, 59.15; H, 9.02; N, 11.37.

4.3.3. *cis*-2-*tert*-Butoxycarbonylaminocyclohexanecarbonitrile, 18. White crystals (yield 56%), mp: 105–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (9H, s, *t*-Bu), 1.36–2.03 (8H, m, $4 \times CH_2$), 3.33 (1H, br s, H-1), 3.58–3.63 (1H, m, $J = 4.00$ Hz, H-2), 4.75 (1H, d, $J = 5.47$ Hz, N*H*) ppm. Anal. Calcd for $C_{12}H_{20}N_2O_2$: C, 64.26; H, 8.99; N, 12.49. Found: C, 63.91; H, 8.72; N, 12.28.

4.3.4. *cis*-2-Aminocyclohexanecarbonitrile, *rac*-2. White crystals (yield 62%), mp: 74–75.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25–2.05 (8H, om, $4 \times CH_2$), 1.61 (2H, br s, NH_2), 2.86 (1H, m, H-1), 2.97 (1H, m, H-2), ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.3 (C4), 24.6 (C6), 28.1 (C5), 33.0 (C3), 38.0 (C2), 51.0 (C1), 120.9 (CN) ppm. Anal. Calcd for $C_7H_{12}N_2$: C, 67.70; H, 9.74; N, 22.56. Found: C, 67.45; H, 9.65; N, 22.25.

4.4. Preparation of *trans*-2-aminocyclopentanecarbonitrile, *rac*-3

The preparation of *rac*-**3** was performed in the same way as that of *rac*-**4** below, the synthetic path to intermediate **23** following the known procedure (Scheme 2).[5,6]

4.4.1. *trans*-2-Aminocyclopentanecarbonitrile hydrobromide, 25. Light-yellowish crystals (yield 67%), mp: 212–214 °C; ^{1}H NMR (400 MHz, D_2O): δ 1.30–2.25 (6H, om, $3 \times CH_2$), 1.45 (2H, br s, NH_2), 2.92 (1H, m, H-1), 3.55 (1H, m, H-2) ppm. Anal. Calcd for $C_6H_{10}N_2 \cdot HBr$: C, 37.72; H, 5.80; N, 14.66. Found: C, 37.49; H, 5.76; N, 14.41.

4.4.2. *trans*-2-Aminocyclopentanecarbonitrile, *rac*-3. White crystals (yield 63%), mp: 72–74 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30–2.30 (6H, om, $3\times CH_2$), 1.70 (2H, br s, NH_2), 2.42 (1H, m, H-1), 3.54 (1H, m, H-2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.0 (C4), 29.8 (C5), 35.1 (C3), 39.2 (C2), 59.2 (C1), 122.8 (CN) ppm. Anal. Calcd for $C_6H_{10}N_2$: C, 65.42; H, 9.15; N, 25.43. Found: C, 65.06; H, 9.07; N, 25.12.

4.5. Preparation of *trans*-2-aminocyclohexanecarbonitrile, *rac*-4

The preparation of intermediate **24** has been described before (Scheme 2).[5,6] Boc-deprotection in **24** was performed by adding **24** (0.4 g, 1.8 mmol) in dry dichloromethane (4 mL) to a solution of bromotrimethylsilane (0.41 g, 2.7 mmol) and phenol (0.01 g, 0.12 mmol) in dry dichloromethane (5 mL) under an argon atmosphere at room temperature. After stirring for 2.5 h the white crystals produced were filtered off and washed with diethyl ether, resulting in **26** (0.23 g, 66%), mp: 248–250 °C; ^{1}H NMR (400 MHz, D_2O): δ 1.20–2.30 (8H, om, $4\times CH_2$), 1.45 (2H, br s, NH_2), 2.89 (1H, m, H-1), 3.48 (1H, m, H-2) ppm. Anal. Calcd for $C_7H_{12}N_2\cdot$HBr: C, 40.99; H, 6.39; N, 13.66. Found: C, 40.62; H, 6.29; N, 13.32.

Hydrobromide **26** (0.17 g, 0.83 mmol) was neutralized with sodium carbonate (0.5 M, 5 mL) and the aqueous phase extracted with 3×50 mL dichloromethane. The combined organic phase was dried and evaporated. The residue was purified by column chromatography, using dichloromethane/methanol (19:1) as eluent yielding *rac*-**4** (0.26 g, 83%) as a yellowish oil; ^{1}H NMR (400 MHz, CDCl3): δ 1.10–2.20 (8H, om, $4\times CH_2$), 1,65 (2H, br s, NH_2), 2.46 (1H, m, H-1), 3.47 (1H, m, H-2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.0 (C4), 24.9 (C6), 29.4 (C5), 34.4 (C3), 38.8 (C2), 52.9 (C1), 122.0 (CN) ppm. Anal. Calcd for $C_7H_{12}N_2\cdot$HBr: C, 67.70; H, 9.74; N, 22.56. Found: C, 67.43; H, 9.62; N, 22.26.

4.6. Preparative-scale resolution of *rac*-1

rac-**1** (0.5 g, 4.54 mmol) was dissolved in TBME (80 mL) and lipase PS-C II (4.50 g, 50 mg/mL) was added. The reaction was started by the addition of 2,2,2-trifluoroethyl butanoate (1.38 mL, 9.14 mmol). The reaction mixture was shaken at room temperature. The reaction was stopped after 5 h at 50% conversion ($ee^{(1R,2S)\text{-}\mathbf{1}} = 98\%$, $ee^{(1S,2R)\text{-}\mathbf{5}} > 99\%$) by filtering off the enzyme. After evaporation, the resolution products were separated by column chromatography, using CH_2Cl_2/MeOH (19:1), yielding (1*R*,2*S*)-**1** {0.182 g, 1.65 mmol; white crystals, mp: 59–61 °C, ee = 98%, $[\alpha]_D^{20} = -5.9$ (*c* 1.0, MeOH); the ^{1}H and ^{13}C NMR and elemental analysis data were identical with those for *rac*-**1**} and (1*S*,2*R*)-**5** {0.280 g, 1.55 mmol; light-yellow oil, ee = 98%, $[\alpha]_D^{20} = +127.5$ (*c* 0.53, MeOH)}. Spectral data for (1*S*,2*R*)-**5**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97 (3H, t, $J = 7.39$ Hz, CH_3), 1.55–2.25 (6H, om, $3\times CH_2$), 1.69 (2H, m, $CH_3CH_2CH_2$), 2.21 (2H, t, $J = 7.13$ Hz, $CH_2CH_2CH_3$), 3.29 (1H, m, H-1), 4.39 (1H, m, H-2), 5.98 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (CH_3), 19.7 ($CH_2CH_2CH_3$), 22.2 (C4), 29.4 (C5), 30.8 (C3), 35.0 ($CH_2CH_2CH_3$), 47.1 (C2), 52.3 (C1), 121.0 (CN), 174.1 (NHCO) ppm. Anal. Calcd for $C_{10}H_{16}N_2O$: C, 66.64; H, 8.95; N, 15.54. Found: C, 66.36; H, 9.21; N, 15.10.

4.7. Preparative-scale resolution of *rac*-2

rac-**2** (0.4 g, 3.23 mmol) was dissolved in TBME (65 mL) and lipase PS-C II (4.52 g, 70 mg/mL) added. The reaction was started by the addition of 2,2,2-trifluoroethyl butanoate (0.97 mL, 6.43 mmol). The reaction mixture was shaken at room temperature. The reaction was then stopped after 2.25 h at 50% conversion [$ee^{(1R,2S)\text{-}\mathbf{2}} = 95\%$, $ee^{(1S,2R)\text{-}\mathbf{6}} = 95\%$], with the following work-up as above, yielding (1*R*,2*S*)-**2** {0.136 g, 1.1 mmol; white crystals, mp: 74–76 °C, ee >99%, $[\alpha]_D^{20} = +13.2$ (*c* 0.5, MeOH); the ^{1}H and ^{13}C NMR and elemental analysis data were identical to those of the racemic *rac*-**2**} and (1*S*,2*R*)-**6** {0.126 g, 0.65 mmol; white crystals, mp: 87–89 °C, ee = 96%, $[\alpha]_D^{20} = +98.7$ (*c* 0.5, MeOH)}. Spectral data for (1*S*,2*R*)-**6**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, $J = 7.38$ Hz, CH_3), 1.30–2.10 (8H, om, $4\times CH_2$), 1.66 (2H, m, $CH_3CH_2CH_2$), 2.18 (2H, t, $J = 7.46$ Hz, $CH_2CH_2CH_3$), 2.74 (1H, m, H-1), 4.24 (1H, m, H-2), 7.46 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (CH_3), 19.8 ($CH_2CH_2CH_3$), 21.9 (C4), 25.2 (C6), 28.0 (C5), 29.2 (C3), 34.5 ($CH_2CH_2CH_3$), 39.1 (C2), 48.8 (C1), 120.7 (CN), 172.0 (NHCO) ppm. Anal. Calcd for $C_{11}H_{18}N_2O$: C, 68.01; H, 9.34; N, 14.42. Found: C, 67.64; H, 9.87; N, 14.37.

4.7.1. Absolute configuration of (1*R*,2*S*)-2. The separated unreacted **2** (50 mg, 0.403 mmol) was reduced with lithium–aluminum hydride in the formation of 1-amino-2-aminomethylcyclohexane (30 mg, 0.234 mmol; a yellow oil, ee = 92%) with the following spectral data: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20–2.00 (9H, om, H-2-6), 1.58 (2H, s, CH_2NH_2), 2.80 (2H, br s, CH_2NH_2), 3.40 (1H, m, H-1), 5.10 (2H, br s, $CHNH_2$) ppm. Anal. Calcd for $C_7H_{16}N_2$: C, 65.57; H, 12.58; N, 21.85. Found: C, 65.64; H, 12.87; N, 22.07.

The obtained $[\alpha]_D^{20} = +20.5$ (*c* 1.0, EtOH) corresponds to the literature value[15] $[\alpha]_D^{20} = +21.8$ (*c* 2.18, EtOH) for (1*S*,2*S*)-*cis*-(+)-1-amino-2-aminomethylcyclohexane. This indicates a (1*R*,2*S*)-absolute configuration for the unreacted **2** (Scheme 3).

4.8. Preparative-scale resolution of *rac*-3

rac-**3** (0.255 g, 2.32 mmol) was dissolved in a mixture of TBME and *tert*-amyl alcohol (9:1), after which lipase PS-C II (2.26 g, 50 mg/mL) was added. The reaction was started by the addition of 2,2,2-trifluoroethyl butanoate (0.68 mL, 4.50 mmol). The mixture was shaken at room temperature. The reaction was stopped after 9.5 h at 50% conversion ($ee^{(1S,2S)\text{-}\mathbf{3}} = 98\%$, $ee^{(1R,2R)\text{-}\mathbf{7}}$ >99%), with the following work-up as above, yielding (1*S*,2*S*)-**3** {0.085 g, 0.77 mmol; colorless crystals, mp: 72–74 °C, ee = 98%, $[\alpha]_D^{20} = +54.1$ (*c* 0.5, MeOH); the

^{1}H and ^{13}C NMR and elemental analysis data were identical with those for racemic **3**} and (1*R*,2*R*)-**7** {0.105 g, 0.58 mmol; white crystals, mp: 73–74 °C, ee >99%, $[\alpha]_D^{20} = -44.2$ (*c* 0.5, MeOH)}. Spectral data for (1*R*,2*R*)-**7**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (3H, t, $J = 7.36$ Hz, CH_3), 1.55–2.25 (6H, om, $3 \times CH_2$), 1.69 (2H, m, $CH_3CH_2CH_2$), 2.16 (2H, t, $J = 7.30$ Hz, $CH_2CH_2CH_3$), 2.87 (1H, m, H-1), 4.35 (1H, m, H-2), 5.94 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (CH_3), 19.7 ($CH_2CH_2CH_3$), 23.5 (C4), 29.9 (C5), 32.1 (C3), 35.3 ($CH_2CH_2CH_3$), 39.1 (C2), 56.2 (C1), 122.1 (CN), 173.8 (NHCO) ppm. Anal. Calcd for $C_{10}H_{16}N_2O$: C, 66.64; H, 8.95; N, 15.54. Found: C, 66.39; H, 9.30; N, 15.53.

4.9. Preparative-scale resolution of *rac*-4

rac-**4** (0.22 g, 1.77 mmol) was dissolved in TBME (39 mL) and lipase PS-C II (1.95 g, 50 mg/mL) was added. The reaction was started by the addition of 2,2,2-trifluoroethyl butanoate (0.59 mL, 3.91 mmol). The mixture was shaken at 45 °C. The reaction was stopped after 206 h at 50% conversion [$ee^{(1S,2S)\text{-}4} = 99\%$, $ee^{(1R,2R)\text{-}8} > 99\%$], with the following work-up as above, yielding (1*S*,2*S*)-**4** {0.101 g, 0.81 mmol; a slowly crystallizing yellowish oil, ee = 98% $[\alpha]_D^{20} = +34.6$ (*c* 0.5, MeOH); the ^{1}H and ^{13}C NMR and elemental analysis data were identical with those for racemic **4**}; and (1*R*,2*R*)-**8** (0.110 g, 0.57 mmol), white crystals, mp: 76–78 °C, ee >99% $[\alpha]_D^{20} = -6.7$ (*c* 0.5, MeOH). Spectral data for (1*R*,2*R*)-**8**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97 (3H, t, $J = 7.38$ Hz, CH_3), 1.20–2.25 (8H, om, $4 \times CH_2$), 1.69 (2H, m, $CH_3CH_2CH_2$), 2.19 (2H, t, $J = 7.42$ Hz, $CH_2CH_2CH_3$), 2.66 (1H, m, H-1), 3.99 (1H, m, H-2), 5.84 (1H, s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (CH_3), 19.9 ($CH_2CH_2CH_3$), 24.5 (C4), 29.4 (C6), 32.3 (C5), 35.0 (C3), 39.4 ($CH_2CH_2CH_3$), 49.2 (C2), 50.2 (C1), 121.2 (CN), 173.4 (NHCO) ppm. Anal. Calcd for $C_{11}H_{18}N_2O$: C, 68.01; H, 9.34; N, 14.42. Found: C, 67.83; H, 9.14; N, 13.98.

4.9.1. Absolute configuration of (1*S*,2*S*)-4. The separated unreacted **4** (30 mg, 0.242 mmol) was reduced with lithium–aluminum hydride in the formation of 1-amino-2-aminomethylcyclohexane (19 mg, 0.148 mmol) as a light-yellowish oil, ee = 90%, $[\alpha]_D^{20} = +21.1$ (*c* 0.5, EtOH) with the following spectral data: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.05–2.20 (9H, om, H-2-6), 1.50 (2H, s, CH_2NH_2), 2.45 (1H, m, H-1), 2.90 (2H, m, CH_2NH_2), 5.04 (2H, br s, $CHNH_2$) ppm. Anal. Calcd for $C_7H_{16}N_2$: C, 65.57; H, 12.58; N, 21.85. Found: C, 65.72; H, 12.27; N, 21.97.

The obtained $[\alpha]_D^{20} = +21.1$ (*c* 0.5, EtOH) corresponds to the literature value[14] $[\alpha]_D^{20} = +22.8$ (*c* 0.724, EtOH) for (1*S*,2*R*)-*trans*-(+)-1-amino-2-aminomethylcyclohexane. This indicates a (1*S*,2*S*)-absolute configuration for the unreacted **4** (Scheme 3).

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