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Enzymatic resolution of five membered heterocyclic bromohydrins

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ABSTRACT

The lipase catalyzed resolution of *trans*-3,4-tetrahydrofuran and pyrrolidine bromohydrins by acylation or hydrolysis of their acylated derivatives has been studied. For both heterocycles, the best enantioselectivity was obtained using *Candida antarctica* lipase B as the catalyst in the hydrolytic processes. The enantiomerically pure bromohydrins are useful intermediates for the preparation of 3,4-fuctionalized *cis*-heterocycles.

1. Introduction

Optically active 3,4-disubstituted tetrahydrofurans and pyrrolidines are common structural subunits found in many natural compounds and synthetic derivatives that exhibit interesting biological activities. For example, enantiomerically pure tetrahydrofurans can serve as monosaccharide mimetic structures while some *cis*-3,4-pyrrolidine derivatives have been described as inhibitors of the natural enzyme renin.[1] Their presence as substituents in quinoline antibacterial derivatives,[2] or thiopenems,[3] is crucial for their biological activity. Moreover, non-racemic vicinal heterocyclic diols, diamines, or aminoalcohols, occupy a central role in catalytic asymmetry synthesis as chiral auxiliaries or ligands.

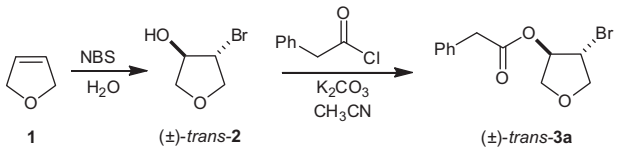
On the other hand, easily accessible vicinal *trans*-bromohydrins, are common intermediates in numerous synthetic routes for the preparation of pharmaceuticals, agrochemicals, and other fine chemicals. The S_N2 displacement reaction of the bromo functionality in 3,4-disubstituted tetrahydrofurans and pyrrolidines yields heterocycles with a *cis*-relative configuration.

As part of our research on the enzymatic preparation of optically active functionalized heterocycles,[4] we are interested in extending biocatalytic methods to the resolution of bromohydrin heterocyclic structures. We have studied the enzymatic resolution of *trans*-3-bromo-4-hydroxytetrahydrofuran and its analogue pyrrolidine derivative.

We have also carried out the S_N2 displacement reaction of the bromo functionality with sodium azide in order to obtain the enantiomerically pure *cis*-hydroxyazide derivatives. The comparison of the specific rotation of these compounds to those described in the literature[5] led us to determine the absolute configuration of the enantiomerically pure bromohydrins obtained.

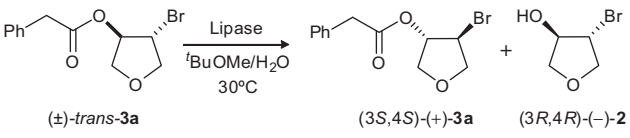
2. Results and discussion

Initially, we studied the enzymatic hydrolysis of (±)-*trans*-3-bromo-4-(2-phenylacetoxy)tetrahydrofuran, (±)-*trans*-**3a**, which was prepared in high yield by treatment of 2,5-dihydrofuran **1** with *N*-bromosuccinimide and subsequent acylation of the bromohydrin obtained with 2-phenylacetylchloride (Scheme 1).



Scheme 1. Synthesis of bromohydrins (±)-*trans*-**2** and (±)-*trans*-**3a**.

We started testing the enzymatic hydrolysis of (±)-*trans*-**3a** (Scheme 2) using two different reaction conditions: with 10 equiv of water in organic solvent (^{t}BuOMe) or in a 1:2 ratio of water/organic solvent. The obtained results are summarized in Table 1.



Scheme 2. Enzymatic hydrolysis of (±)-*trans*-**3a** (the configuration shown was obtained using CAL-B).

Five of the lipases tested catalyzed the hydrolysis of substrate (±)-*trans*-**3a**: lipases A and B from *Candida antarctica* (CAL-A), *Pseudomonas fluorescens* (AK), *Burkholderia cepacia* (PS-SD), and

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Table 1
Lipase catalyzed hydrolysis of (±)-*trans*-**3a**, in ^{t}BuOMe at 30 °C

Entry	Lipase	H_2O	*t* (h)	ee_s[a] (%) *trans*-**3a**	Configuration	ee_p[a] (%) *trans*-**2**	Configuration	*c*[b] (%)	*E*[b]
1	CAL-A	10 equiv	22	59	(3*R*,4*R*)	27	(3*S*,4*S*)	69	3
2	CAL-B	10 equiv	16	>99	(3*S*,4*S*)	>99	(3*R*,4*R*)	50	>200
3	AK	10 equiv	22	33	(3*S*,4*S*)	61	(3*R*,4*R*)	35	6
4	CAL-A	^{t}BuOMe/H_2O 2:1	17	86	(3*R*,4*R*)	31	(3*S*,4*S*)	73	5
5	CAL-B	^{t}BuOMe/H_2O 2:1	17	97	(3*S*,4*S*)	83	(3*R*,4*R*)	54	45
6	TL-IM	^{t}BuOMe/H_2O 2:1	22	16	(3*S*,4*S*)	37	(3*R*,4*R*)	30	3
7	PS-SD	^{t}BuOMe/H_2O 2:1	8 (day)	4	(3*R*,4*R*)	21	(3*S*,4*S*)	16	2

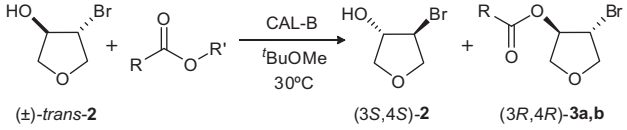
[a] Determined by chiral HPLC.
[b] Conversion, $c = ee_s/(ee_s + ee_p)$, enantiomeric ratio, $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)((1+ee_s)]$.[6]

Thermomyces lanuginosus (TL IM). Using the latter two enzymes, the product was only detected using a proportion of solvent/H_2O (2:1), but low enantioselectivity and conversion were achieved (Table 1, entries 6 and 7).

In general, better results were obtained using a small amount of water (10 equiv), particularly in the case of the hydrolysis catalyzed by CAL-B (Table 1, entry 2). Using this enzyme, 50% conversion was achieved after 16 h of reaction and both the product and the remaining substrate were obtained in enantiopure form.

A different stereochemical preference of some lipases was observed. Lipases CAL-B, AK, and TL-IM catalyzed the hydrolysis of the (3*R*,4*R*)-configured ester while CAL-A and PS-D catalyzed the hydrolysis of the (3*S*,4*S*)-configured ester. The assignation of the absolute configurations of the products and the remaining substrate is described later.

We also examined the acylation process of substrate (±)-*trans*-**2**, using CAL-B as catalysts, but a lower reaction rate and enantioselectivity were achieved in these processes (Scheme 3). The results obtained are summarized in Table 2.



Scheme 3. CAL-B catalyzed acylation of (±)-*trans*-**2**.

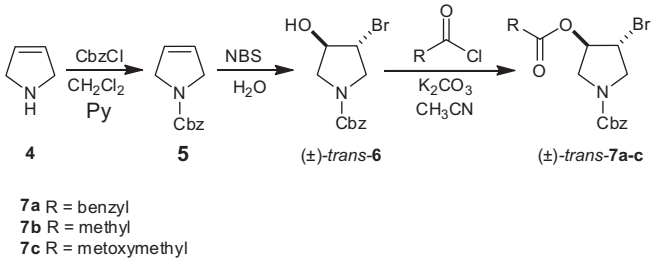
Table 2
CAL-B catalyzed acylation of (±)-*trans*-**2** in ^{t}BuOMe, using 5 equiv of the acylating agent

Entry	Product	R	R'	*t* (h)	ee_s[a] (%)	ee_p[a] (%)	*c*[b] (%)	*E*[b]
1	**3a**	Benzyl	ethyl	48	38	56	40	5
2	**3b**	Methyl	vinyl	48	14	42	25	3

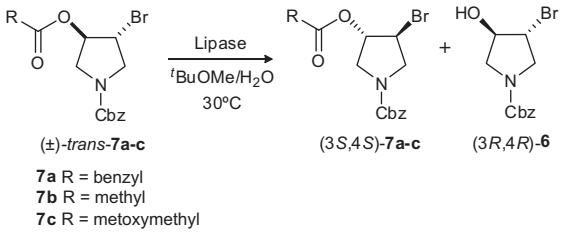
[a] Determined by chiral HPLC.
[b] Conversion, $c = ee_s/(ee_s + ee_p)$, enantiomeric ratio, $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)((1+ee_s)]$.[6]

Taking into account the results obtained in the resolution of the tetrahydrofuran bromohydrin (±)-*trans*-**3a**, we studied the enzymatic hydrolysis of (±)-*trans*-1-benzyloxycarbonyl-3-bromo-4-(2-phenylacetoxy)pyrrolidine, (±)-*trans*-**7a**, which was prepared using the same methodology as for the tetrahydrofuran derivative (Scheme 4).

The CAL-B catalyzed hydrolysis of (±)-*trans*-**7a** was slower than for substrate (±)-*trans*-**3a**: under the same reaction conditions, only 22% conversion was achieved after 12 days and the enantioselectivity was also lower, *E* = 23, (Scheme 5, Table 3, entry 1). We then attempted to improve these results by testing different lipases and analyzing the influence of different reaction parameters.



Scheme 4. Synthesis of bromohydrins (±)-*trans*-**6** and (±)-*trans*-**7a–c**.



Scheme 5. Lipase catalyzed hydrolysis of (±)-*trans*-**7a–c** (the configuration shown was obtained using CAL-B).

Under the same reaction conditions, lipase CAL-A catalyzed the process in a moderate yield but with low enantioselectivity (Table 3, entry 2). The lipases AK and TL IM also catalyzed the hydrolysis but the product was only detected when using solvent/H_2O in a ratio of 2:1 and low enantioselectivity and conversion were achieved (Table 3, entries 4 and 5).

Dioxane, toluene, acetonitrile, and THF were also examined as solvents; the rate and enantioselectivity were not improved in comparison with the results obtained in ^{t}BuOMe.

As in the case of the tetrahydrofuran derivative, a different stereochemical preference of some lipases was observed. Lipases CAL-B, AK, and TL-IM catalyzed the hydrolysis of the (3*R*,4*R*)-configured ester while CAL-A catalyzed the hydrolysis of the (3*S*,4*S*)-configured ester.

Since the results obtained for substrate (±)-*trans*-**7a** were not satisfactory, we prepared two new derivatives (±)-*trans*-**7b** and (±)-*trans*-**7c** and carried out the hydrolysis processes catalyzed by CAL-B under the best conditions found for derivative (±)-*trans*-**7a** (Table 3, entries 6 and 7). For both substrates, the rate and enantioselectivity of the process were higher in comparison with those of substrate (±)-*trans*-**7a**, particularly for the methoxyacetylated derivative (±)-*trans*-**7c**. In this process, 50% conversion was achieved after 9 h of reaction and both the product and the remaining substrate were obtained in enantiopure form.

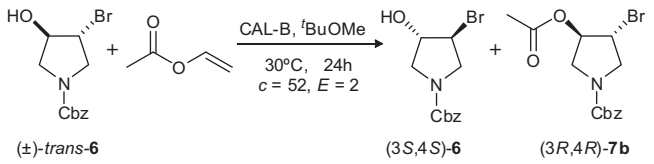
Finally, we examined the enzymatic acylation of the bromohydrin (±)-*trans*-**6**. When vinyl acetate was used as the acylating

Table 3
Lipase catalyzed hydrolysis of (±)-*trans*-**7a–c**, in ^{t}BuOMe at 30 °C

Entry	Substrate	Lipase	H_2O	*t* (h)	ee_s[a] (%) *trans*-**7a–c**	Configuration	ee_p[a] (%) *trans*-**6**	Configuration	*c*[b] (%)	*E*[b]
1	(±)-*trans*-**7a**	CAL-B	10 equiv	12 (days)	25	(3*S*,4*S*)	89	(3*R*,4*R*)	22	23
2	(±)-*trans*-**7a**	CAL-A	10 equiv	24	5	(3*R*,4*R*)	12	(3*S*,4*S*)	31	1
3	(±)-*trans*-**7a**	CAL-B	^{t}BuOMe /H_2O 2:1	7 (days)	17	(3*S*,4*S*)	88	(3*R*,4*R*)	16	18
4	(±)-*trans*-**7a**	AK	^{t}BuOMe /H_2O 2:1	7 (days)	1	(3*S*,4*S*)	15	(3*R*,4*R*)	8	1
5	(±)-*trans*-**7a**	TL-IM	^{t}BuOMe /H_2O 2:1	7 (days)	6	(3*S*,4*S*)	66	(3*R*,4*R*)	8	5
6	(±)-*trans*-**7b**	CAL-B	10 equiv	48	76	(3*S*,4*S*)	91	(3*R*,4*R*)	46	50
7	(±)-*trans*-**7c**	CAL-B	10 equiv	9	>99	(3*S*,4*S*)	>99	(3*R*,4*R*)	50	>200

[a] Determined by chiral HPLC.
[b] Conversion, $c = ee_s/(ee_s + ee_p)$, enantiomeric ratio, $E = \ln[(1 - c)(1 - ee_s)]/\ln[(1 - c)((1 + ee_s)]$.[6]

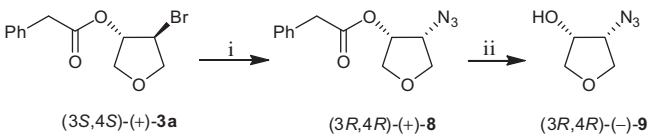
agent, very low enantioselectivity was observed for all of the catalysts tested. Scheme 6 shows the best results, obtained in the process catalyzed by CAL-B.



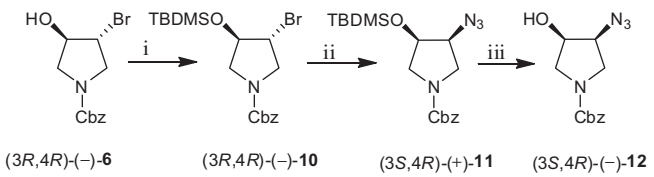
Scheme 6. CAL-B catalyzed acylation of (±)-*trans*-**6**.

Using less activated acylating agents such as ethyl 2-phenylacetate, only lipase CAL-A catalyzed the process, but low conversion and enantioselectivity were observed.

In order to determine the absolute configuration of the bromohydrins obtained we carried out an S_N2 displacement reaction of the bromo functionality with sodium azide and then subsequent hydrolysis of the ester to yield the corresponding enantiomerically pure *cis*-hydroxyazides (Schemes 7 and 8). In the case of the tetrahydofuran bromohydrins, we used the remaining enantiomerically pure substrate (+)-**3a** obtained in the lipase-catalyzed kinetic resolution. The treatment of this compound with sodium azide in DMSO yielded the corresponding *cis*-azido ester (+)-**8**, with an enantiomeric excess higher than 99%. The hydrolysis of (+)-**8** yielded the azido alcohol (−)-**9**, whose specific rotation sign was compared with that reported for the (3*R*,4*R*)-enantiomer;[5b] the



Scheme 7. Reagents and conditions: (i) NaN_3, DMSO, 110 °C, 7 h; (ii) K_2CO_3, MeOH/H_2O, rt, 2 h.



Scheme 8. Reagents and conditions: (i) TBDMSCl, imidazole, CH_2Cl_2, rt, 24 h; (ii) NaN_3, DMSO, 110 °C, 7 h; (iii) CSA, anhydrous MeOH, rt, overnight.

configuration of the remaining substrate in the enzymatic process was therefore established as (3*S*,4*S*)-(+)-**3a**.

In the case of the pyrrolidine bromohydrins, we started with the enantiomerically pure product (−)-**6** obtained via the biocatalytic process. In order to avoid possible racemization, the hydroxyl group was protected as a ^{t}butyldimethylsilylated derivative (−)-**10**, and then treated with sodium azide in DMSO to yield the corresponding *cis*-azido derivative (+)-**11**, with an enantiomeric excess higher than 99%. The deprotection of (+)-**11** yielded the azido alcohol (−)-**12**. The comparison of the specific rotation of this compound to that described in the literature[5a] led us to determine the (3*S*,4*R*)-absolute configuration for the azido alcohol (−)-**12**; the product of the enzymatic hydrolysis was therefore established as the (3*R*,4*R*)-enantiomer (Scheme 8).

It should be noted that for both substrates, CAL-B preferentially catalyzed the hydrolysis of the (3*R*,4*R*) enantiomer, as expected by Kazlauskas' rule.[7]

3. Conclusions

Herein we have described an easy method for the resolution of *trans*-3-bromo-4-hydroxytetrahydrofuran and the analogous pyrrolidine derivatives via a CAL-B catalyzed hydrolytic process. These compounds could be useful intermediates in the preparation of enantiomerically pure 3,4-disubstituted heterocycles.

The best substrates for the resolution are the phenylacetylated derivative of the bromohydrin, in the case of the tretrahydrofuran ring, and the methoxyacetylated derivative in the case of the pyrrolidine. Good yields and high enantioselectivities can be achieved by the appropriate selection of the reaction parameters. The two enantiomers of both substrates can be obtained in good yield and in enantiomerically pure forms. On the other hand, enzymatic hydrolysis of an ester derivative is preferable to acylation of the hydroxyl group of the bromohydrins.

4. Experimental

4.1. General

Enzymatic reactions were carried out in a Gallenkamp incubatory orbital shaker. Immobilized *Candida antarctica* lipase B, CAL-B (Novozym 435, 7300 PLU/g), was a gift from Novo Nordisk co., immobilized CAL-A (lipase NZL-101, 6.2 U/g) is commercialized by Codexis, and immobilized *Burkholderia cepacia* lipase (PS-SD, 23000 U/g) is commercialized by Amano Pharmaceuticals. *Pseudomonas fluorescens* (AK, 22100 U/g)), and *Thermomyces lanuginosus* (TL IM, 560 TBU/g) are commercialized by Novo Nordisk. Chemical reagents were commercialized by Aldrich, Fluka, Lancaster or Prolabo. Solvents were distilled over an appropriate desiccant under nitrogen. Flash chromatography was performed using Merck silica gel 60 (230–400 mesh). Optical rotations were measured using a Perkin–Elmer 343 polarimeter and are quoted in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR, ^{13}C NMR, and DEPT spectra were recorded in a Bruker AC-300, Bruker AC-300 DPX, or Bruker NAV-400 spectrometer using $CDCl_3$ as solvent. The chemical shift values (δ) are given in ppm. APCI$^+$ and ESI$^+$ using a Hewlett–Packard 1100

chromatograph mass detector or EI$^+$ with a Hewlett–Packard 5973 mass spectrometer were used to record mass spectra (MS). IR spectra were recorded in a UNICAM Mattson 3000 FT. The enantiomeric excesses were determined by chiral HPLC analysis on a Hewlett–Packard 1100, LC liquid chromatograph, using a CHIRALPAK OJ-H column (4.5 × 250 mm) and CHIRALPAK IC column (4.0 × 250 mm).

4.2. General procedure for the preparation of bromohydrins

2,5-Dihydrofuran (175 mg, 2.50 mmol) or 1-benzyloxycarbonyl-3-pyrroline (500 mg, 2.46 mmol) was added to a stirred solution of *N*-bromosuccinimide (NBS) (650 mg, 3.7 mmol) in water and cooled on an ice bath. The resulting mixture was stirred 4 h at rt. After this time, 15 mL of ethyl ether was added and the resulting mixture was washed with brine separated and dried over Na_2SO_4. The solvent was removed under reduced pressure to give a yellow oil as a crude residue in a 70–80% yield. The crude product obtained was used for the chemical acylation without further purification. When the pure bromohydrin was required, the crude was purified by flash chromatography on silica gel (hexane/EtOAc 6:4).

4.3. General procedure for the acylation of bromohydrins

To a solution of the crude bromohydrin (3 mmol) in acetonitrile (30 mL), the corresponding acyl chloride (4.6 mmol) and K_2CO_3 (623 mg, 3 mmol) were added. The mixture was stirred at room temperature for 12 h. Next, the solvent was removed under reduced pressure and ethyl acetate (25 mL) was added to the crude residue; the mixture was washed with water (3 × 15 mL) and the organic phase was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 8:2) to afford the corresponding ester.

4.3.1. (±)-*trans*-3-Bromo-4-(2-phenylacetoxy)-tetrahydrofuran (±)-*trans*-3a

Yellow oil, yield 80%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.49–7.18 (m, 5H), 5.40 (d, 1H, J_{HH} 6.6), 4.30 (m, 3H), 4.09 (m, 1H), 3.87 (m, 1H), 3.66 (s, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.9 (CO), 133.6 (C), 129.6 (CH), 129.1 (CH), 127.8 (CH), 81,0 (CH), 75.0 (CH_2), 71.7 (CH_2), 48.4 (CH), 41.5 (CH_2); IR (neat, NaCl): ν 1739 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{12}H_{13}BrO_3Na]^+$ (M+Na)$^+$ 306.9940 *m*/*z*, found: 306.9913.

4.3.2. (±)-*trans*-3-Acetoxy-4-bromotetrahydrofuran (±)-*trans*-3b

Yellow oil yield 82%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 5.29–5.19 (m, 1H), 4.28–4.15 (m, 3H), 3.97 (m, 1H), 3.74 (m, 1H), 1.98 (s, 3H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 169.7 (CO), 81,2 (CH), 74.4 (CH_2), 71.3 (CH_2), 48.2 (CH), 20.7 (CH_3); IR (neat, NaCl): ν 1742 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_6H_9BrO_3Na]^+$ (M+Na)$^+$ 230.9627 *m*/*z*, found: 232.9618.

4.3.3. (±)-*trans*-1-Benzyloxycarbonyl-3-bromo-4-(2-phenyl acetoxy)pyrrolidine (±)-*trans*-7a

Yellow oil yield 53%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.52–7.19 (m, 10H), 5.35 (d, 1H, $^3J_{HH}$ 4.3), 5.19 (s, 2H), 4.2 (br s, 1H), 4.11–4.00 (m, 1H), 3.94 (m, 1H), 3.75–3.53 (m, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.7 (CO), 155.1 (CO), 137.8 (C), 133.5 (C), 129.5 (CH), 129.2 (CH), 129.0 (CH), 128.6 (CH), 128.4 (CH), 127.88 (CH), 78.9 (CH), 67.7 (CH_2), 52.85 (CH_2), 51.53 (CH_2), 49.9 (CH), 41.5 (CH_2); IR (neat, NaCl): ν 1740, 1704 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{20}H_{20}BrNO_4Na]^+$ (M+Na)$^+$ 440.0468 *m*/*z*, found 440.0457.

4.3.4. (±)-*trans*-1-Benzyloxycarbonyl-3-acetoxy-4-bromo pyrrolidine (±)-*trans*-7b

Yellow oil, yield 50%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.39 (br s, 5H), 5.33 (m, 1H), 5.18 (br s, 2H), 4.41–4.24 (m, 1H), 4.12–3.86 (m, 3H), 3.71–3.5 (m, 1H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 169.6 (CO), 154.7 (CO), 136.4 (C), 128.5 (CH), 128.2 (CH), 128.0 (CH), 128.6 (CH), 78.3 (CH), 67.3 (CH_2), 53.4 (CH_2), 59.56 (CH_2), 46.9 (CH), 20.9 (CH_3); IR (neat, NaCl): ν 1744, 1704 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{14}H_{16}BrNO_4Na]^+$ (M+Na)$^+$ 364.0155 *m*/*z*, found: 364.0169.

4.3.5. (±)-*trans*-1-Benzyloxycarbonyl-3-bromo-4-(2-methoxy acetyl)pyrrolidine (±)-*trans*-7c

Yellow oil, yield 65%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.52–7.27 (m, 5H), 5.42 (m, 1H), 5.17 (br s, 2H), 4.38–4.24 (m, 1H), 4.21–3.84 (m, 5H), 3.62 (m, 1H), 3.44 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 168.1 (CO), 154.6 (CO), 136.3 (C), 128.5 (C), 128.2 (CH), 128.0 (CH), 78.5 (CH), 69.5 (CH_2), 67.3 (CH_2), 59.5 (CH_3), 53.3 (CH_2), 49.4 (CH_2), 46.3 (CH); IR (neat, NaCl): ν 1758, 1704 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{15}H_{18}BrNO_5Na]^+$ (M+Na)$^+$ 394.0261 *m*/*z*, found: 394.0258.

4.4. General procedure for the enzymatic hydrolysis

The reaction mixture, which contained the corresponding acylated bromohydrin (20 mg), the lipase (20 mg), and H_2O (10 equiv) in ^{t}BuOMe (2.5 mL), was shaken at 30 °C and 250 rpm in an orbital shaker. The progress of the reaction was monitored by TLC (hexane/EtOAc 8:2) until required conversion was achieved. Next, the enzyme was removed by filtration and washed with ^{t}BuOMe. The crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 8:2).

4.4.1. (3*S*,4*S*)-3-Bromo-4-hydroxytetrahydrofuran (3*R*,4*R*)-2

Yellow oil, yield 48%; $[\alpha]_D^{25} = -27.6$ (*c* 0.08, CH_2Cl_2), ee >99%.

4.4.2. (3*S*,4*S*)-3-Bromo-4-(2-phenylacetoxy)tetrahydrofuran (3*S*,4*S*)-3a

Yellow oil, yield 49%; $[\alpha]_D^{25} = +73.1$ (*c* 0.1, CH_2Cl_2), ee >99%.

4.4.3. (3*R*,4*R*)-1-Benzyloxycarbonyl-3-bromo-4-hydroxypyrrolidine (3*R*,4*R*)-6

Yellow oil, yield 47%; $[\alpha]_D^{20} = -5.0$ (*c* 0.1, $CHCl_3$), ee >99%.

4.4.4. (3*S*,4*S*)-1-Benzyloxycarbonyl-3-bromo-4-(2-methoxyacetyl)pyrrolidine (3*S*,4*S*)-*trans*-7c

Yellow oil, yield 49%; $[\alpha]_D^{20} = -10.0$ (*c* 0.05, $CHCl_3$), ee >99%.

4.5. Determination of the enantiomeric excess by HPLC analysis

Chiralpak OJ-H, 30 °C, hexane/2-propanol (95:5), UV 210 nm, 0.8 mL min^{-1}, t_R 26.96 min (3*S*,4*S*)-**3a**, t_R 23.76 min (3*R*,4*R*)-**3a**. Chiralpak IC; hexane/2-propanol (90:10) UV 210 nm, 0.8 mL min^{-1}, t_R 15.77 min (3*R*,4*R*)-**6**, t_R 13.5 min (3*S*,4*S*)-**6;** t_R 56.5 min (3*R*,4*R*)-**7c**; t_R 47.38 min (3*S*,4*S*)-**7c**.

4.6. General procedure for the enzymatic acylation

The reaction mixture containing the corresponding bromohydrin (50 mg), the lipase (100 mg), and the acylating agent (5 equiv) in ^{t}BuOMe (1 mL) was shaken at 30 °C and 250 rpm in an orbital shaker. The progress of the reaction was monitored by TLC (hexane/EtOAc 8:2) until the required conversion. The enzyme was then removed by filtration and washed with ^{t}BuOMe. The solvent was evaporated under reduced pressure and the crude residue

was purified by flash chromatography on silica gel (hexane/EtOAc 8:2).

4.7. Assignment of the absolute configuration of tetrahydrofuran derivatives

4.7.1. Synthesis of (3*R*,4*R*)-(+)-3-azido-4-phenylacetoxytetrahydrofuran, (3*R*,4*R*)-(+)-8

To a solution of the enantiomerically pure substrate (+)-*trans*-**3a** (30 mg, 0.1 mmol), obtained from the CAL-B catalyzed hydrolysis, in DMSO (2 mL), sodium azide (33 mg, 0.5 mmol) was added under a nitrogen atmosphere at room temperature. The mixture was stirred at 110 °C for 7 h. Next, the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 7:3) to afford the product as a yellow oil in 52% yield. $[\alpha]_D^{25} = +1.4$ (*c* 0.08, CH_2Cl_2), ee >99%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.40–7.28 (m, 5H), 5,20 (m, 1H) 4.11 (m, 1H), 4.08–3.96 (m, 2H), 3.90–3.72 (m, 2H), 3.67 (s, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 171.1 (CO), 133.2 (C), 129.3 (C), 128.7 (CH), 127.3 (CH), 73.7 (CH), 70.8 (CH_2), 70.1 (CH_2), 60.7 (CH), 40.9 (CH_2; IR (neat, NaCl): ν 2355.5, 1738.05 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{12}H_{13}N_3O_3Na]^+$ (M+Na)$^+$ 270.0855 *m*/*z*, found 270.0849.

4.7.2. Synthesis of (3*R*,4*R*)-3-azido-4-hydroxytetrahydrofuran, (3*R*,4*R*)-(−)-9

A solution of (+)-*cis*-**8** (8 mg, 0.03 mmol) and K_2CO_3 in methanol/water 2:1 (1.5 mL) was stirred at room temperature for 6 h. Next, ethyl acetate (10 mL) was added, the resulting mixture was washed with water (3 × 15 mL) and the organic phase was dried over Na_2SO_4. The solvent was removed under reduced pressure to afford the product as a colorless oil in 75% yield. The crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 7:3). The specific rotation sign of the pure product was in accordance with that reported for the (3*R*,4*R*)-(−)-3-azido-4-hydroxytetrahydrofuran.[5b]

4.8. Assignment of the absolute configuration of pyrrolidine derivatives

4.8.1. Synthesis of (3*R*,4*R*)-(−)-1-benzyloxycarbonyl-3-bromo-4-(*tert*-butyldimethylsilyloxy)pyrrolidine (3*R*,4*R*)-(−)-10

To a solution of enantiomerically pure product (−)-*trans*-**6** (25 mg, 0.083 mmol), obtained from the CAL-B catalyzed hydrolysis, in CH_2Cl_2 (8.8 mL), imidazole (7.48 mg, 0.11 mmol), and *t*-butyldimethylsilyl chloride, TBDMSCl (15 mg, 0.1 mmol), was added under a nitrogen atmosphere at room temperature. The mixture was then stirred for 24 h. The crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 75:35) to afford the product as a colorless oil in 88% yield. $[\alpha]_D^{25} = -3.0$ (*c* 0.08, $CHCl_3$), ee >99%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.38 (m, 5H), 5.18 (s, 2H), 4.41 (m, 1H), 4.18–4.01 (m, 2H), 3.97–3.72 (m, 2H), 3.40 (m, 1H), 0.89 (s, 9H), 0.15 (s, 6H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 155.4 (CO), 137.1 (C), 128.9 (CH), 128.4 (CH), 128.3 (CH), 77.6 (CH), 67.4 (CH_2), 53.4 (CH), 52.7 (CH_2), 50.7 (CH_2), 26.02 (CH_3), 18.3 (C), −4.3 (CH_3); IR (neat, NaCl): ν 1708.8 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{18}H_{28}BrNO_3SiNa]^+$ (M+Na)$^+$ 436.0914 *m*/*z*, found: 436.0899.

4.8.2. Synthesis of (3*S*,4*R*)-(+)-(-1-benzyloxycarbonyl-3-azido-4-(*tert*-butyldimethylsilyloxy)pyrrolidine (3*S*,4*R*)-(+)-11

To a solution of (−)-*trans*-**10** (30 mg, 0.1 mmol) in DMSO (3 mL), sodium azide (23.4 mg, 0.36 mmol) was added under a nitrogen atmosphere at room temperature. The mixture was then stirred at 110 °C for 12 h. Next, the solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 8:2) to afford the product as a colorless oil in 57% yield. $[\alpha]_D^{25} = +35.0$ (*c* 0.1, $CHCl_3$), ee >99%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.38 (br s, 5H), 5.16 (s, 2H), 4.43 (m, 1H), 4.05–3.70 (m, 1H), 3.50 (m, 4H), 0.94 (s, 9H), −0.29 (s, 6H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 154.8 (CO), 136.6 (C), 128.5 (CH), 128.1 (CH), 128.0 (CH), 72.5 (CH), 67.1 (CH_2), 61.4 (CH), 50.4 (CH_2), 47.63 (CH_2), 25.29 (CH_3), 18.1 (C), −5.0 (CH_3); IR (neat, NaCl): ν 2104.3, 1704.5 cm^{-1}. HRMS-ESI$^+$ calcd for $[C_{18}H_{29}N_4O_3Si]^+$ (M+1)$^+$ 377.2003 *m*/*z*, found: 377.1996.

4.8.3. Synthesis of (3*S*,4*R*)-(−)-(-1-benzyloxycarbonyl-3-azido-4-hydroxypyrrolidine (3*S*,4*R*)-(−)-12

To a solution of (+)-*cis*-**11** (10 mg, 0.03 mmol), in dry methanol (300 μL), (−)-Camphor sulfonic acid, CSA, (35 mg, 0.15 mmol) was added. The mixture was stirred overnight at room temperature. Then, the solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 6:4) to afford the product as a colorless oil in 71% yield. The specific rotation sign of the pure product was in accordance with that reported for the (3*S*,4*R*)-(−)-(-1-benzyloxycarbonyl-3-azido-4-hydroxypyrrolidine[5a]

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