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Enzymatic preparation of *cis* and *trans*-3-amino-4-hydroxytetrahydrofurans and *cis*-3-amino-4-hydroxypyrrolidines

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ABSTRACT

The lipase catalyzed resolution of *cis* and *trans*-3-amino-4-hydroxytetrahydrofurans and *cis*-3-amino-4-hydroxypyrrolidines have been studied. For all the heterocycles, the best enantioselectivity was obtained using *Candida antarctica* lipases A and B as catalysts in hydrolytic processes. The absolute configuration of the optically pure obtained heterocycles has been assigned.

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1. Introduction

The importance of optically active 3,4-disubstituted tetrahydrofurans and pyrrolidines is evident from their presence in a wide variety of bioactive natural products and drugs, and their successful applications as stereochemical control elements. In particular, the vicinal heterocyclic amino alcohols are used in organocatalysis,[1] as chiral auxiliaries,[2] and building blocks for the synthesis of complex molecules that exhibit interesting biological activities.[3]

As a part of our research on the enzymatic preparation of optically active functionalized heterocycles, we have reported the synthesis and enzymatic resolution of *trans*-3-amino-4-hydroxypyrrolidines.[4] Now, we are interested in extending this biocatalytic method to the synthesis of the *cis*-isomer derivatives, and also to the *cis*- and *trans*-3-amino-4-hydroxytetrahydrofuran analogs, since the presence of these moieties and their absolute (as well as relative) stereochemistry are important for the biological activity of the molecules that contain them.

Figure 1 shows three examples of these pharmacologically active molecules: the pyrrolidine derivative of guanine, **1** is a new class of bi-substrate inhibitor of human purine nucleoside,[3a] the quinolone derivative, **2** shows antibacterial activity[3f] and

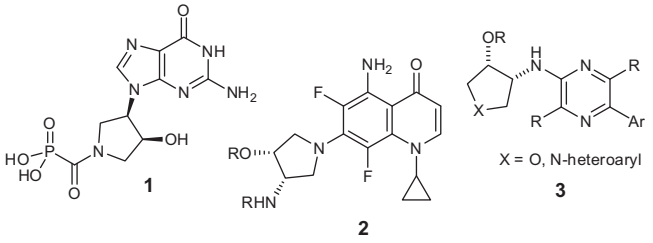

Figure 1.

1,4-pyrazine derivatives, **3** are claimed as corticotropin releasing factor, receptor antagonists.[3e]

Various synthetic approaches have been described for the synthesis of these structures.[5] Using biocatalytic methods, the *cis*-relative configuration can be obtained by the S_N2 displacement reaction of the bromo functionality in the 3,4-heterocyclic bromohydrins (±)-*trans*-**5** and (±)-*trans*-**12**, whose resolution has been recently reported.[6] Another synthetic approach for the preparation of the optically active 3-amino-4-hydroxypyrrolidines, is the enzymatic resolution of the 3-azido-4-hidroxypyrrolidines (±)-*cis*-**15** and also the *trans*-isomer, described by Kamal et al.[7]

The method described herein is a new and convenient biocatalytic approach for the preparation of all the isomers of these heterocyclic amino alcohols.

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2. Results and discussion

On the bases of our previous experience[4,6] we choose as substrates the N-Boc derivatives (±)-*cis*-**8**, (±)-*trans*-**8** and (±)-*cis*-**17a**, that have been synthesized in good yields by conventional methods, summarized in Scheme 1.

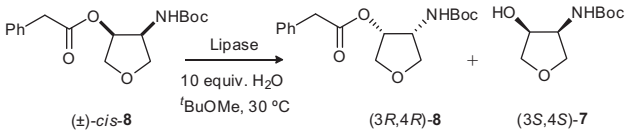
We have proven that the presence of a bulky substituent adjacent to the secondary carbon bearing the hydroxyl group could favour the enzymatic resolution of these compounds.[4] Also, the *tert*-butoxycarbonyl protecting group can be selectively removed in the presence of the benzyloxycarbonyl group in the case of the pyrrolidine ring. On the other hand, the presence of the phenylacetyl ester is very convenient for a good light-absorbing in UV-detector HPLC.

Initially, we studied the enzymatic hydrolysis of (±)-*cis*-3-*tert*-butoxycarbonylamino-4-phenylacetoxytetrahydrofuran, (±)-*cis*-**8**. The reactions were carried out at 30 °C in organic solvent (^{t}BuOMe) and using 10 equiv of water. Lipases from *Candida antarctica* (CAL-A and CAL-B), *Burkholderia cepacia* (PSL-IM) and *Thermomyces lanuginosus* (TL-IM) were tested. All the processes were carried out on a 15 mg scale (Scheme 2).

The lipases CAL-A and CAL-B catalysed the hydrolysis of substrate (±)-*cis*-**8**, (Table 1, entries 1 and 2) whereas, using TL IM or PSL-IM the unaltered starting material was recovered after three days of reaction. Both enzymes, CAL-A and CAL-B, showed very high enantioselectivity but different reaction rate: using CAL-B, a 50% of conversion was achieved after 10 h of reaction, whereas the process catalysed by CAL-A was slower, three days were necessary to achieve the same conversion than with CAL-B. In both processes, the product and the remaining substrate were obtained in enantiopure form. On the other hand, the two enzymes showed the same stereochemical preference. The assignation of absolute configurations of the products and the remaining substrate is described later.

Taking into account the results obtained in the resolution of the substrate (±)-*cis*-**8**, we carried out the enzymatic hydrolysis of the (±)-*trans*-3-*tert*-butoxycarbonylamino-4-hydroxytetrahydrofuran, (±)-*trans*-**8**, using the same lipases and reaction conditions. (Scheme 3, Table 1, entries 3 and 4).

The obtained results were very similar to those obtained in the hydrolysis of the *cis*-isomer: very high enantioselectivity and different reaction rates were observed depending on the biocatalyst used. The process catalysed by CAL-A was very slow, while in only three hours of reaction a 50% conversion was achieved using CAL-B as catalyst.



Scheme 2. Lipase catalysed hydrolysis of (±)-*cis*-**8**.

In view of the excellent results obtained in the resolution of the tetrahydrofuran derivatives, we tested the resolution of substrate (±)-*cis*-1-benzyloxycarbonyl-3-*tert*-butoxycarbonylamino-4-hydroxypyrrolydine, (±)-*cis*-**17a** under the same reaction conditions (Scheme 4). The CAL-A catalysed hydrolysis showed again a high enantioselectivity and a moderate reaction rate, both enantiomers were obtained in enantiopure forms, after 48 h of reaction (Table 1, entry 5). Surprisingly, the unaltered starting material was recovered after two days of reaction when CAL-B was used as biocatalyst.

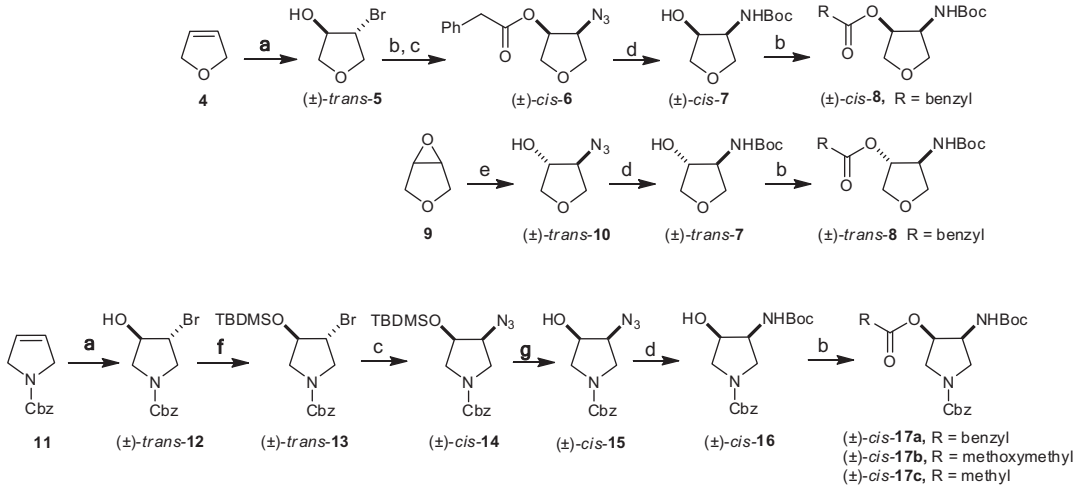
In order to improve the reaction rate, we tested the methoxymethyl derivative (±)-*cis*-**17b** as substrate in the hydrolytic process catalysed by lipases CAL-A or CAL-B, under the same reaction conditions (Scheme 4).

Effectively, the reaction rate was improved. In the case of CAL-A (Table 1, entry 7), only 23 h of reaction were necessary to achieved a 50% of conversion, but more surprising was the strong influence of the substrate structure in the process catalyzed by CAL-B since in this case a 50% of conversion can be achieved after 10 h (Table 1, entry 8). A very high enantioselectivity was observed for both enzymes and, again, the substrate and the product were obtained in their optically pure forms.

Finally, the enzymatic acylation processes of substrates (±)-*cis*-**7**, (±)-*trans*-**7** and (±)-*cis*-**16** ware examined. For both enzymes, very low or no conversions were observed when 2-phenyl acetate, ethyl acetate or methoxymethyl acetate were used as acylating agents. Also, using vinyl acetate, very low conversion was observed in the acylation of (±)-*cis*-**7** and (±)-*trans*-**7**. In the case of substrate (±)-*cis*-**16**, CAL-B showed a high enantioselectivity (E >200) and a moderate reaction rate, after 48 h of a 50% of conversion. These process are shown in Scheme 5, the results were very similar to those already described for (±)-*trans*-**16**.[4]

The assignment of the absolute configurations to the optically pure heterocycles obtained in the enzymatic processes was established as follows.

The optically pure products of the lipase catalysed hydrolysis (−)-*cis*-**7** and (−)-*trans*-**7** were treated with aq 3N HCl in methanol



Scheme 1. Synthesis of substrates: (a) NBS, H_2O, 4 h, rt.[6] (b) RCOCl, K_2CO_3, CH_3CN, 12 h, rt. (c) NaN_3, DMSO, 110 °C, 12 h. (d) PMe_3, Boc_2O, NaOH 1 M, THF, rt, 3 h. (e) NaN_3, H_2O/1,4-dioxane 1:4, 100 °C, 14 h. (f) TBDMSCl, imidazole, CH_2Cl_2, rt, 5 h. (g) CSA, anhydrous MeOH, rt, 3 h.

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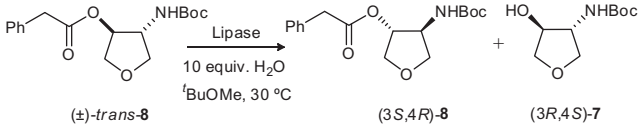
Table 1
Lipase catalysed hydrolysis, in ^{t}BuOMe at 30 °C, using 10 equiv of water

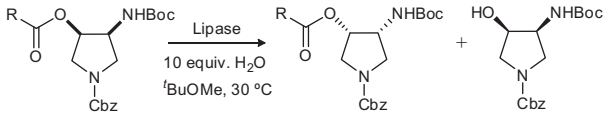
Entry	Lipase[a]	Substrate	t (h)	ee_s[b] (%)	Configuration	ee_p[a] (%)	Configuration	c[c] (%)	E[c]
1	CAL-A	(±)-*cis*-**8**	75	>99	(3*R*,4*R*)	>99	(3*S*,4*S*)	50	>200
2	CAL-B	(±)-*cis*-**8**	10	>99	(3*R*,4*R*)	>99	(3*S*,4*S*)	50	>200
3	CAL-A	(±)-*trans*-**8**	7 (days)	>99	(3*S*,4*R*)	>99	(3*R*,4*S*)	50	>200
4	CAL-B	(±)-*trans*-**8**	3	>99	(3*S*,4*R*)	>99	(3*R*,4*S*)	50	>200
5	CAL-A	(±)-*cis*-**17a**	48	>99	(3*R*,4*S*)	>99	(3*S*,4*R*)	50	>200
6	CAL-B	(±)-*cis*-**17a**	48	—	—	—	—	—	—
7	CAL-A	(±)-*cis*-**17b**	23	>99	(3*R*,4*S*)	>99	(3*S*,4*R*)	50	>200
8	CAL-B	(±)-*cis*-**17b**	10	>99	(3*R*,4*S*)	>99	(3*S*,4*R*)	50	>200

[a] Weight ratio enzyme/substrate 1:1.
[b] Determined by chiral HPLC.
[c] Conversion, $c = ee_s/(ee_s + ee_p)$, enantiomeric ratio, $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$.[8]



(±)-*trans*-**8** (3*S*,4*R*)-**8** (3*R*,4*S*)-**7**

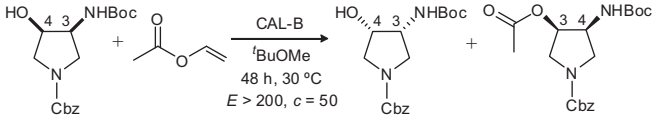
Scheme 3. Lipase catalysed hydrolysis of (±)-*trans*-**8**.



(±)-*cis*-**17a,b** (3*R*,4*S*)-**17a,b** (3*S*,4*R*)-**16**

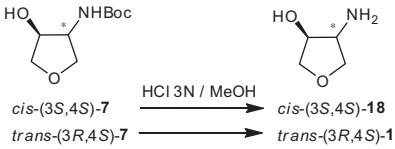
a, R = benzyl
b, R = metoxymethyl

Scheme 4. Lipase catalysed hydrolysis of (±)-*cis*-**17a**,**b**.



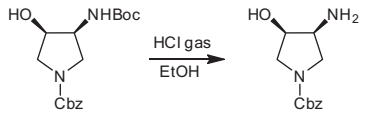
(±)-*cis*-**16** (3*R*,4*S*)-**16** (3*R*,4*S*)-**17c**

Scheme 5. CAL-B catalysed acetylation of (±)-*cis*-**16**.



cis-(3*S*,4*S*)-**7** *cis*-(3*S*,4*S*)-**18**
trans-(3*R*,4*S*)-**7** *trans*-(3*R*,4*S*)-**18**

Scheme 6. Deprotection of Boc group in aq 3N HCl.



cis-(3*S*,4*R*)-**16** *cis*-(3*S*,4*R*)-**19**

Scheme 7. Deprotection of Boc group in HCl saturated ethanol.

at room temperature to remove the Boc group and isolate (−)-*cis*-**18** and (−)-*trans*-**18**, whose specific rotation signs were in agreement with those reported for the (3*S*,4*S*) and (3*R*,4*S*)-enantiomers respectively[9] (Scheme 6).

In the case of the pyrrolidine derivatives, a better yield in the selective deprotection of (+)-*cis*-**16** was obtained when the reaction was accomplished by saturating the solution of (+)-*cis*-**16** in ethanol with HCl and allowing the mixture to stir at room temperature (Scheme 7).The specific rotation sign of the amino alcohol (+)-*cis*-**19** obtained, was opposite to that reported for the (3*R*,4*S*)-enantiomer.[3e]

It should be noted that, for all the substrates, the lipases preferentially catalyzed the hydrolysis of the expected enantiomer in accordance with the Kazlauskas' rule.[10]

3. Conclusion

This paper describes a convenient approach to the preparation of all the isomers of *cis* and *trans*-3-amino-4-hydroxytetrahydrofuran and *cis*-3-amino-4-hydroxypyrrolidine in their optically pure forms, via a CAL-A or CAL-B catalyzed hydrolysis. The pyrrolidine derivative (±)-*cis*-**16** that can also be resolved by a CAL-B catalysed acylation of the hydroxyl group. In general, the hydrolysis catalysed by CAL-B is faster than by CAL-A.

4. Experimental

General Remarks. 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, immobilized *Burkholderia cepacia* lipase (PS-SD, 23,000 U/g) is commercialized by Amano Pharmaceuticals and *Thermomyces lanuginosus* (TL-IM, 560 TBU/g) is commercialized by Novo Nordisk. Chemical reagents were commercialized by Aldrich, Merck, ACROS organics or Alfa Aesar. Solvents were distiled 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 $CDCl_3$ or MeOH-d_4, as solvent. The chemical shift values (δ) are given in ppm. For pyrrolidins, duplicity of some carbon signals (especially for those of the ring) and broad signals in ^{1}H NMR were observed due to the presence of different conformers around the N(1) (C=O) bond. 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 IA column (4.6 × 250 mm).

The starting materials (±)-*cis*-3-azido-4-phenylacetoxytetrahydrofuran, (±)-*cis*-**6**, and (±)-*cis*-1-benzyloxycarbonyl-3-azido-4-hydroxypyrrolidine, (±)-*cis*-**15**, were prepared following the methodology described in Ref. [6]

4.1. Synthesis of (±)-*trans*-3-azido-4-hydroxytetrahydrofuran, (±)-*trans*-10

To a solution of 3,4-epoxytetrahydrofuran **9** (1.0 g, 11.6 mmol) in a mixture of 1,4-dioxane/H_2O 4:1 (50 mL), sodium azide (2.2 g, 33.8 mmol) was added. The mixture was stirred at 100 °C, for 12 h, in a sealed tube. After this time, the resulting mixture was extracted with EtOAc (3 × 10 mL), the organic phase was dried over Na_2SO_4 and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 7:3) to afford the product (±)-*trans*-**10** as a colourless oil in a 80% yield. The spectroscopic data were consistent with those previously described.[5d] Better overall yield can be achieved using the crude residue in the next step of the synthesis without further purification.

4.2. General procedure for the synthesis of (±)-*cis*- and (±)-*trans*-3-*tert*-butoxycarbonylamino-4-hydroxytetrahydrofuran and (±)-*cis*-1-benzyloxycarbonyl-3-*tert*-butoxycarbonylamino-4-hydroxypyrrolidine, (±)-*cis*-7, (±)-*trans*-7 and (±)-*cis*-16

To a solution of (±)-*cis*-**6**, (±)-*trans*-**10** or (±)-*cis*-**15** (4 mmol) in THF (40 mL), NaOH 1M (8 mL), PMe_3 (12 mmol) and di-*tert*-butylpyrocarbonate (12 mmol) was added. The resulting mixture was stirred at room temperature for 3 h. After this time, the solvent was removed under reduced pressure to afford the corresponding crude residue that was purified by flash chromatography on silica gel (hexane/EtOAc 3:7).

4.2.1. (±)-*cis*-3-*tert*-Butoxycarbonylamino-4-hydroxytetrahydrofuran, (±)-*cis*-7

White solid, yield 85%; mp 148–154 °C. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 5.22 (br s, 1H), 4.32 (br s, 1H, H-4), 4.16 (br s, 1H, H-3), 4.07–3.96 (m, 2H, H-2 and H-5), 3.78 (dd, *J* 10.1, *J* 2.3 Hz, 1H, H-5), 3.53 (t, *J* 8.0 Hz, 1H, H-2), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 155.9 (CO), 80.0 (C), 74.4 (CH), 70.6 (CH_2), 70.4 (CH_2), 53.5 (CH), 28.3 (CH_3); IR (neat, NaCl): ν 3400–3200, 1690, cm^{-1}; HRMS-ESI$^+$ calcd for $[C_9H_{17}NO_4Na]^+$ (M+Na)$^+$ 226.1050 *m*/*z*, found 226.1030.

4.2.2. (±)-*trans*-3-*tert*-Butoxycarbonylamino-4-hydroxytetrahydrofuran, (±)-*trans*-7

White solid, yield 85%; mp 144–146 °C. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 4.79 (br s, 1H), 4.30 (m, 1H, H-4), 4.09 (m, 2H, H-2 and H-5), 3.97 (m, 1H, H-3), 3.71 (dd, *J* 9.9 Hz, *J* 2.9, 1H, H-5), 3.64 (dd, *J* 9.4 Hz, *J* 2.9, 1H, H-2), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 155.9 (CO), 80.3 (C), 77.3 (CH), 73.6 (CH_2), 71.0 (CH_2), 59.8 (CH), 28.3 (CH_3); IR (neat, NaCl): ν 3400–3200, 1689, cm^{-1}; HRMS-ESI$^+$ calcd for $[C_9H_{17}NO_4Na]^+$ (M+Na)$^+$ 226.1050 *m*/*z*, found 226.1061.

4.2.3. (±)-*cis*-1-Benzyloxycarbonyl-3-*tert*-butoxycarbonylamino-4-hydroxypyrrolidine, (±)-*cis*-16

Colourless oil, yield 87%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.35 (m, 5H), 5,26 (m, 1H), 5.12 (s, 2H), 4.41–4.05 (m, 2H, H-3 and H-4), 3.71 (m, 1H, H-2 or H-5), 3.54 (m, 2H, H-2 and H-5), 3.35–3.17 (m, 1H, H-2 or H-5); 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 155.6 (CO), 155.1 (CO), 136.5 (C), 128.5 (CH), 128.1 (CH), 127.9 (CH), 80.0 (C), 70.4 and 69.6 (CH), 67.1 (CH_2), 53.1 (CH_2), 52.6 (CH), 48.0 (CH_2), 28.3 (CH_3); IR (neat, NaCl): ν 3400–3200, 1689, cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{17}H_{24}N_2O_5Na]^+$ (M+Na)$^+$ 359.1583 *m*/*z*, found 359.1577.

4.3. General procedure for the acylation of (±)-*cis*-7, (±)-*trans*-7 and (±)-*cis*-16

To a solution of the corresponding *N*-*tert*-butoxycarbonylaminoalcohol (3.0 mmol) in acetonitrile (30 mL), the corresponding acyl chloride (4.6 mmol) and K_2CO_3 (623 mg, 4.5 mmol) were added. The mixture was stirred at room temperature for 12 h. Then, 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.

4.3.1. (±)-*cis*-3-*tert*-Butoxycarbonylamino-4-phenylacetoxytetrahydrofuran, (±)-*cis*-8

Flash chromatography eluent: hexane/EtOAc 6:4 Yellow oil, yield 80%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.42–7.27 (m, 5H), 5.25 (m, 1H, H-4), 4.45–4.43 (m, 2H, NH and, H-2 or H-5), 4.14–4.01 (m, 2H, H-2 and H-5), 3.82 (m, 1H, H-2 or H-5), 3.72 (s, 2H), 3.43 (m, 1H, H-3), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.5 (CO), 155.7 (CO), 133.4 (C), 129.1 (CH), 128.8 (CH), 127.4 (CH), 80.0 (C), 73.3 (CH), 72.6 (CH_2), 70.0 (CH_2), 52.1 (CH), 41.5 (CH_2), 28.3 (CH_3); IR (neat, NaCl): ν 3341, 1730, 1711 cm^{-1}, HRMS-ESI$^+$ calcd for $[C_{17}H_{23}NO_5Na]^+$ (M+Na)$^+$ 344.1468 *m*/*z*, found 344.1466.

4.3.2. (±)-*trans*-3-*tert*-Butoxycarbonylamino-4-phenylacetoxytetrahydrofuran, (±)-*trans*-8

Flash chromatography eluent: hexane/EtOAc 6:4 Yellow oil, yield 80%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.40–7.25 (m, 5H), 5.11 (m, 1H, H-4), 4.85 (br s, 1H, NH), 4.12–4.02 (m, 3H, H-2, H-3 and H-5), 3.74–3.68 (m, 2H, H-2 and H-5), 3.66 (s, 2H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.9 (CO), 155.9 (CO), 133.4 (C), 129.2 (CH), 128.8 (CH), 127.2 (CH), 79.0 (CH), 77.6 (C), 72.1 (CH_2), 71.9 (CH_2), 56.6 (CH), 41.10 (CH_2), 28.3 (CH_3); IR (neat, NaCl): ν 3341, 1730, 1711 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{17}H_{23}NO_5Na]^+$ (M+Na)$^+$ 344.1468 *m*/*z*, found 344.1478.

4.3.3. (±)-*cis*-1-Benzyloxycarbonyl-3-*tert*-butoxycarbonylamino--4-phenylacetoxypyrrolidine, (±)-*cis*-17a

Flash chromatography eluent: hexane/EtOAc 8:2 Yellow oil, yield 95%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.45–7.25 (m, 10H), 5.15 (s, 2H), 4.32 (m, 1H, H-4), 3.87–3.43 (m, 4H, H-3 and, H-2 or H-5), 3.36 (s, 2H), 3.2 (m, 1H, H-2 or H-5), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 175.8 (CO), 170.4 (CO), 154.7 (CO), 136.5 (C), 133.6 (C), 129.4 (CH), 129.1 (CH), 128.8 (CH), 128.6 (CH), 128.1 (CH), 127.5 (CH), 127.2 (CH), 80.0 (C), 73.1 and 72.9 (CH), 67.1 and 65.9 (CH_2), 50.9 and 50.6 (CH_2), 49.4 (CH_2), 48.1 (CH), 41.6 and 41.0 (CH_2), 28.3 and 27.0 (CH_3). IR (neat, NaCl): ν 3053, 1715, 1705 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{25}H_{30}N_2O_6Na]^+$ (M+Na)$^+$ 477.1996 *m*/*z*, found 477.1949.

4.3.4. (±)-*cis*-1-Benzyloxycarbonyl-3-*tert*-butoxycarbonylamino--4-(2-methoxyacetoxy)pyrrolidine, (±)-*cis*-17b

Yellow oil, yield 85%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.35 (s, 5H), 5.34 (br s, 1H, H-4), 5.13 (s, 2H), 4.82 (m, 1H, H-3), 4.44 (br s, 1H, NH) 4.08 (s, 2H), 3.89 (m, 1H, H-2 or H-5), 3.65 (m, 2H, H-2 and H-5), 3.44 (s, 3H), 3.19 (m, 1H, H-2 or H-5), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 169.9 (CO), 155.3 (CO), 155.1 (CO), 136.8 (C), 128.9 (CH), 128.5 (CH), 128.4 (CH), 80.7 (C), 73.8 and 73.1 (CH), 70.0 (CH_2), 67.5 (CH_2), 59.8 (CH_3), 51.7 (CH), 51.2 and 50.9 (CH_2), 48.4 (CH_2), 28.7 (CH_3); IR (neat, NaCl): ν 3345, 1730, 1710 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{25}H_{30}N_2O_6Na]^+$ (M+Na)$^+$ 431.1794 *m*/*z*, found 431.1789.

4.3.5. (±)-*cis*-1-Benzyloxycarbonyl-3-acetoxy-4-*tert*-butoxycarbonylaminopyrrolidine, (±)-*cis*-17c

Colourless oil, yield 80%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.52–7.31 (m, 5H), 5.23 (m, 1H, H-3), 5.13 (s, 2H), 4.75 (m, 1H, H-4), 4.41 (m, 1H, NH), 3.89 (m, 1H, H-2 or H-5), 3.62 (m, 2H, H-2 and H-5), 3.19 (m, 1H, H-2 or H-5), 2.13 (s, 3H), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.7 (CO), 154.93 (CO), 130.7 (C), 128.5 (CH), 128.1 (CH), 128.0 (CH), 80.3 (C), 77.3 (CH), 67.2 (CH_2), 50.9 (CH), 48.26 (CH_2), 28.3 (CH_3), 22.65 (CH_3); IR (neat, NaCl): ν 1744, 1719, 1706 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{19}H_{26}N_2O_6Na]^+$ $(M+Na)^+$ 401.1683 *m*/*z*, found 401.1660.

4.4. General procedure for the enzymatic hydrolysis

The reaction mixture, containing the corresponding hydroxyacylated-N-Boc-aminoalcohol (15 mg), the lipase (15 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 analyzed by TLC until the achievement of the required conversion (approximately). Then, the enzyme was removed by filtration and washed with ^{t}BuOMe. The crude residue was purified by flash chromatography on silica gel using a gradient hexane/EtOAc from 8:2 to 3:7.

4.4.1. (3*S*,4*S*)-3-*tert*-Butoxycarbonylamino-4-hydroxytetrahydrofuran (3*S*,4*S*)-7

White solid, yield 48%; $[\alpha]_D^{20}$ −3.6 (*c* 0.10, MeOH), ee >99%.

4.4.2. (3*R*,4*R*)-3-*tert*-Butoxycarbonylamino-4-phenylacetoxytetrahydrofuran, (3*R*,4*R*)-8

Yellow oil, yield 49%; $[\alpha]_D^{20}$ +16.3 (*c* 0.10, EtOH), ee >99%.

4.4.3. (3*R*,4*S*)-3-*tert*-Butoxycarbonylamino-4-hydroxytetrahydrofuran, (3*R*,4*S*)-7

White solid, 48%; $[\alpha]_D^{20}$ −4.2 (*c* 0.15, MeOH), ee >99%.

4.4.4. (3*S*,4*R*)-3-*tert*-Butoxycarbonylamino-4-phenylacetoxytetrahydrofuran, (3*S*,4*R*)-8

Yellow oil, yield 49%; $[\alpha]_D^{20}$ +9.0 (*c* 0.10, MeOH), ee >99%.

4.4.5. (3*S*,4*R*)-1-Benzyloxycarbonyl-3-*tert*-butoxycarbonylamino-4-hydroxypyrrolidine, (3*S*,4*R*)-16

Colourless oil, yield 47%; $[\alpha]_D^{20}$ +7.2 (*c* 0.07, MeOH), ee >99%.

4.4.6. (3*R*,4*S*)-1-Benzyloxycarbonyl-3-*tert*-butoxycarbonylamino-4-phenylacetoxypyrrolidine, (3*R*,4*S*)-17a

Yellow oil, yield 49%; $[\alpha]_D^{20}$ +35.0 (*c* 0.10, $CHCl_3$), ee >99%.

4.4.7. (3*R*,4*S*)-1-Benzyloxycarbonyl-3-*tert*-butoxycarbonylamino-4-(2-methoxyacetoxy)pyrrolidine, (3*R*,4*S*)-17b

Yellow oil, yield 49%; $[\alpha]_D^{20}$ +10.9 (*c* 0.10, MeOH), ee >99%.

4.5. Determination of the ee by HPLC analysis

Furan derivatives: previous to HPLC analysis, the hydroxyl group in *cis* or *trans*-**8**, was acylated to the phenyl acetyl ester; Chiralpak OJ-H, 30 °C, hexane/2-propanol (90:10), UV 210 nm, 0.8 mL min^{-1}, t_R 17.25 min (**3*R*,4*R***)-**8**, t_R 20.20 min (**3*S*,4*S***)-**8**, t_R 21.47 min (**3*S*,4*R***)-**8**, 29.43 min (**3*R*,4*S***)-**8**. Pyrrolidine derivatives: Chiralpak IA, 30 °C, hexane/2-propanol (75:25), UV 210 nm, 0.8 mL min^{-1}, t_R 11.12 min (**3*S*,4*R***)-**16**, t_R 14.79 min (**3*R*,4*S***)-**16**; t_R 8.32 min (**3*R*,4*S***)-**17a**; t_R 10.53 min (**3*S*,4*R***)-**17a**; t_R 8.58 min (**3*R*,4*S***)-**17b**; t_R 9.21 min (**3*S*,4*R***)-**17b**; t_R 28.88 min (**3*R*,4*S***)-**17c**; t_R 13.74 (**3*S*,4*R***)-**17c**.

4.6. General procedure for the enzymatic acylation

The reaction mixture containing the corresponding N-Boc-amino alcohol (15 mg), the lipase (15 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 until achievement of 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 using a gradient hexane/EtOAc from 8:2 to 3:7.

4.6.1. (3*R*,4*S*)-1-Benzyloxycarbonyl-3-acetoxy-4-*tert*-butoxycarbonylaminopyrrolidine. (3*R*,4*S*)-17c

Yellow oil, yield 18%; $[\alpha]_D^{20}$ −2.0 (*c* 0.07, MeOH), ee >99%.

4.7. Assignment of the absolute configuration of tetrahydrofuran derivatives

The CAL-B catalyzed hydrolysis of (±)-*cis*-**8**, (±)-*trans*-**8** and (±)-*cis*-**17b** were carried out on a 50 mg scale in order to obtain the optically pure 3-*tert*-butoxycarbonylamino-4-hydroxyheterocycles, that were deprotected to the corresponding amino alcohols.

4.7.1. Synthesis of (3*S*,4*S*)-3-amino-4-hydroxytetrahydrofuran, (3*S*,4*S*)-(−)-18

The optically pure product (−)-*cis*-**7** or (−)-*trans*-**7** (10 mg, 0.05 mmol) obtained from the CAL-B catalyzed hydrolysis, was dissolved in MeOH (1 mL) and treated with aq 3N HCl (20 μL). The mixture was stirred at room temperature for 24 h. Then, the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography on silica gel (dichloromethane/methanol 8:2). The specific rotation signs of the pure products were in accordance with that reported for the (3*S*,4*S*)-(−)-3-amino-4-hydroxytetrahydrofuran and the (3*R*,4*S*)-(−)-3-amino-4-hydroxytetrahydrofuran.[9]

4.7.2. (3*S*,4*S*)-(−)-18

Yield 50%. $[\alpha]_D^{25}$ −6.0 (*c* 0.01, MeOH), ee >99%.

4.7.3. (3*R*,4*S*)-(−)-18

Yield 50%. $[\alpha]_D^{25}$ −14.0 (*c* 0.01, MeOH), ee >99%.

4.8. Assignment of the absolute configuration of pyrrolidine derivative

4.8.1. Synthesis of (3*S*,4*R*)-1-Benzyloxycarbonyl-3-amino-4-hydroxypyrrolidine, (3*S*,4*R*)-19

To a solution of the optically pure product (+)-*cis*-**16** (20 mg, 0.059 mmol) obtained from the CAL-B catalyzed hydrolysis of (±)-*cis*-**17b** in ethanol (1.2 mL), HCl-saturated ethanol (10 mL) was added and the mixture was stirred at room temperature for 2 h. Then, the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography on silica gel (dichloromethane/methanol 8:2). The specific rotation signs of the pure products were in accordance with that reported for the (3*S*,4*R*)-(+)-1-benzyloxycarbonyl-3-amino-4-hydroxypyrrolidine.[3e]

4.8.2. (3*S*,4*R*)-(+)-19

Yield 80%. $[\alpha]_D^{25}$ +14.0 (*c* 0.03, $CHCl_3$), ee >99%.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.05.014. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Martín-Rapún, R.; Fan, X.; Sayalero, S.; Bahramnejad, M.; Cuevas, F.; Pericás, M. A. *Chem. Eur. J.* **2011**, *17*, 8780.
2. Ager, D. J.; Prakash, I.; Schaad, D. R. *Chem. Rev.* **1996**, *96*, 835.
3. Some representative examples: (a) Rejman, D.; Panova, N.; Klener, P.; Maswabi, B.; Pohl, R.; Rosenberg, I. *J. Med. Chem.* **2012**, *55*, 1612–1621; (b) Scatena, C. D.; Kumer, J. L.; Arbitrario, J. P.; Howlett, A. R.; Hawtin, R. E.; Fox, J. A.; Silverman, J. A. *Cancer Chemother. Pharmacol.* **2010**, *66*, 881; (c) Fliri, A. F. J.; Gallaschun, R. J.; O'Donnell, C. J.; Schwarz, J. B.; Segelstein, B. E. WO 2010150192 A1, 2010, CAN, 154:64617.; (d) Baldwin, J. J.; Claremon, D.A.; Tice, C. M.; Cacatian, S.; Dillar, L. W.; Ishchenko, A. V.; Yuan, J.; Xu, Z.; Mcgeehan, G.; Zhao, W.; Simpson, R. D.; Singh, S. B. WO 2007117559 A2 2007, CAN, 147:469236.; (e) Corbett, J. W.; Ennis, M. D.; Frank, K. E.; Fu, J.-M.; Hoffman, R. L.; Verhoest, P.R. WO 2004099201 A1, 2004, CAN,141:410966, ibid. WO 2003045924 A1, 2003, CAN, 139:6891.; (f) Okada, T.; Sato, H.; Tsuji, T.; Tsushima, T.; Nakai, H.; Yoshida, T.; Matsuura, S. *Chem. Pharm. Bull.* **1993**, *41*, 132; (g) Schenke, T.; Krebs, A.; Petersen, U. 1993, DE 4200415 A1, CAN, 119:271179.
4. Rodríguez-Rodríguez, J. A.; Quijada, F. J.; Brieva, R.; Rebolledo, F.; Gotor, V. *Tetrahedron* **2013**, *69*, 5407.
5. In addition to the methods described in Ref. [3], some representative examples of asymmetric synthesis: (a) Martin, M.; El Hellani, A.; Yang, J.; Collin, J.; Bezzenine-Lafollee, S. *J. Org. Chem.* **2011**, *76*, 9801; (b) Arai, K.; Salter, M. M.; Yamashita, Y.; Kobayashi, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 955; (c) Schaus, S. E.; Larroe, J. F.; Jacobsen, E. N. *J. Org. Chem.* **1997**, *62*, 4197; (d) Martínez, L. E.; Leighton, J. L.; Carsten, D. H.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1995**, *117*, 5897.
6. Villar-Barro, A.; Gotor, V.; Brieva, R. *Tetrahedron: Asymmetry* **2013**, *24*, 694.
7. (a) Kamal, A.; Shaik, A. A.; Sandbhor, M.; Malik, M. S.; Kaga, H. *Tetrahedron Lett.* **2004**, *45*, 8057; (b) Kamal, A.; Shaik, A. A.; Sandbhor, M.; Malik, M. S.; Azeeza, S. *Tetrahedron: Asymmetry* **2006**, *17*, 2876.
8. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.
9. Börner, A.; Holz, J.; Kagan, H. B. *Tetrahedron Lett.* **1993**, *34*, 5273.
10. Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656.