Tetrahedron: *Asymmetry* 19 (2008) 1684–1688

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Highly enantioselective transformations on 3,4-dihydroxytetrahydrofurans catalyzed by lipases

Verónica Recuero, Rosario Brieva, Vicente Gotor *

Departamento de Química Orgánica e Inorgánica and Instituto Universitario de Biotecnología de Asturias, Universidad de Oviedo, 33006 Oviedo, Asturias, Spain

ARTICLE INFO

Article history:
Received 22 May 2008
Accepted 28 May 2008
Available online 2 July 2008

ABSTRACT

Enzymatic acylations and alkoxycarbonylations of *cis*- and *trans*-3,4-dihydroxytetrahydrofuran and hydrolysis of their diacetylated and dialkoxycarbonylated derivatives have been studied. High enantioselectivity is obtained using *Pseudomonas cepacia* lipase as a catalyst in the hydrolysis of the *trans*-diacetyl derivative, while for the desymmetrization of the *cis*-3,4-dihydroxytetrahydrofuran the best results are obtained in the acylation process catalyzed by *Candida antarctica* lipase B.

1. Introduction

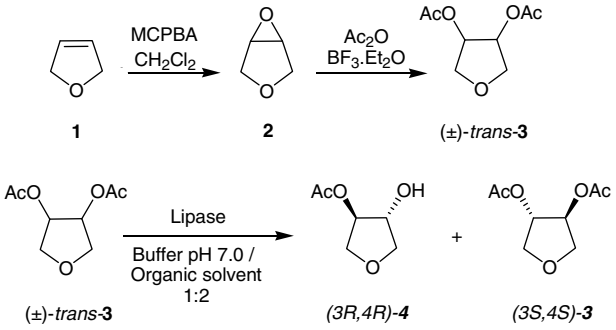
Optically active vicinal diols are versatile chemical intermediates for the production of pharmaceuticals, agrochemicals, ferroelectric liquid crystals, flavors, and fragrances. In particular, chiral oxygenated tetrahydrofuran derivatives are useful synthons in enantioselective synthesis[1] and are often found in physiologically active compounds.[2] Typically, enantiomerically enriched 3,4-dihydroxytetrahydrofuran derivatives are synthesized by hydride reduction of tartaric acid to give a tetralol, followed by subsequent cyclization.[3] Other procedures include the asymmetric opening of a *meso*-epoxide with alcohols in the presence of acid catalysts. The desymmetrization of a *meso*-diol by carbamoylation catalyzed with a chiral Cu(II) has recently been described.[4]

Biocatalytic methods are also employed for the preparation of enantiomerically pure *trans*-3,4-dihydroxytetrahydrofuran. Microbial epoxide hydrolases have been successfully employed for the asymmetric ring opening of the meso-epoxide.[5] The enzymatic resolution of the *trans*-isomer has been carried out by hydrolysis of the diacetyl derivative in the presence of lipase from *Pseudomonas* sp., but the enantioselectivity of the process was very low ($E = 4.4$).[6] Better results were obtained when the mono alkyloxy derivative was employed as a substrate, for example, in the resolution of the *cis*-3-benzyloxy-4-hydroxytetrahydrofuran[6] or *trans*-3-alkenyloxy-4-hydroxytetrahydrofuran.[7]

As part of our research on the enzymatic preparation of optically active polyhydroxyheterocycles, we are interested in the enzymatic resolution of *trans*-3,4-dihydroxytetrahydrofuran and in the desymmetrization of the *cis*-isomer, which, to the best of our knowledge, has not been studied.

2. Results and discussion

First, we studied the enzymatic hydrolysis of the *trans*-diacetyl derivative (±)-**3**. This substrate has been prepared in almost quantitative yield by treatment of 2,5-dihydrofuran **1** with *m*-chloroperbenzoic acid (*m*-CPBA) in CH_2Cl_2[8] and subsequent opening of the resulting epoxide **2** with acetic anhydride and $BF_3{\cdot}Et_2O$ (Scheme 1). According to the results reported by Seemayer and Schneider,[6] the enantioselectivity of the enzymatic hydrolysis catalyzed by *Pseudomonas cepacia* lipase (PSL-C) in an aqueous media showed a very low enantioselectivity. Next, we carried out the process in a mixture 1:2 of phosphate buffer 1 M and 1,4-dioxane. We tested several commercially available lipases [PSL-C, *Candida antarctica* lipases A and B (CAL-A and CAL-B), *Candida rugosa* (CRL), *Aspegillus melleus*, porcine pancreatic (PPL)]. Only the lipase PSL-C catalyzed the process and, surprisingly, under these conditions the enantioselectivity of the process was very high (E >200). The reaction rate was moderated, after one day of reaction, 51% conversion was achieved. Similar results were obtained when acetonitrile or THF was used as reaction solvent (Table 1).



Scheme 1. Synthesis and enzymatic hydrolysis of (±)-*trans*-**3**.

* Corresponding author. Tel./fax: +34 985 103 448.
E-mail address: vgs@fq.uniovi.es (V. Gotor).

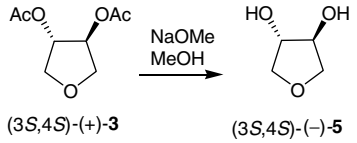
0957-4166/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2008.05.027

Table 1
PSL-C catalyzed hydrolysis of (±)-*trans*-**3** at 30 °C in phosphate buffer 1 M pH 7.0/organic solvent 1:2

Entry	Organic solvent	*t* (h)	*c*[a] (%)	ee_s[b] (%)	ee_p[b] (%)	*E*[c]
1	1,4-Dioxane	31	51	>99	95	>200
2	Acetonitrile	24	51	99	95	>200
3	THF	24	51	>99	94	>200

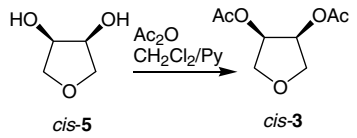
[a] Conversion, $c = ee_s/(ee_s + ee_p)$.
[b] Determined by chiral GC.
[c] Enantiomeric ratio, $E = \ln[(1 - c)(1 - ee_s)]/\ln[(1 - c)(1 + ee_s)]$.[9]

The absolute configurations of the product and the remaining substrate were assigned as follows. Hydrolysis of the remaining substrate (+)-*trans*-**3** using NaOMe in MeOH afforded the dihydroxy derivative (−)-*trans*-**5**, (Scheme 2) whose specific rotation sign was in agreement with that reported for (3*S*,4*S*)-(−)-**5**.[3a]



Scheme 2. Hydrolysis of (+)-**3**.

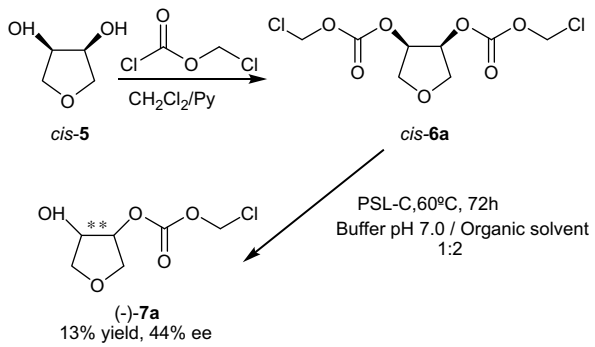
In view of these successful results, similar biocatalytic conditions were applied to the hydrolysis of the derivative *cis*-**3**, which was prepared by acetylation of the commercially available *cis*-**5** (Scheme 3).



Scheme 3. Synthesis of *cis*-**3**.

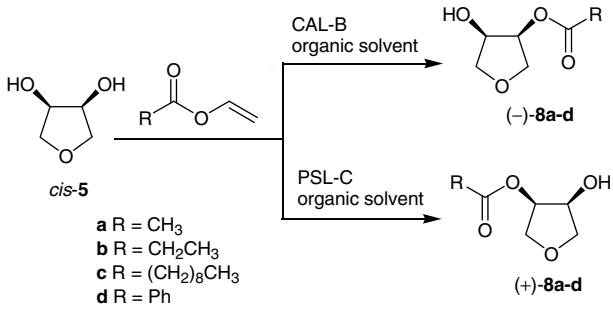
As a desymmetrization process, a maximum yield of 100% can be attained in the enzymatic hydrolysis of the *meso*-derivative. The PSL-C catalyzed hydrolysis of *cis*-**3** in a 1:2 mixture of phosphate buffer 1 M and 1,4-dioxane but, unfortunately, showed a very low selectivity. After 1 h of reaction, a mixture of the mono- and dihydroxylated products was detected and, after 14 h of reaction only the *meso*-diol *cis*-**5** was detected. No reaction was detected when CAL-B was used as catalyst of the reaction.

Taking into account that the low selectivity of the reaction catalyzed by PSL-C could be due to intramolecular acyl migrations under the hydrolytic conditions, we tested the dicarbonate *cis*-**6a** as substrate for the enzymatic hydrolysis (Scheme 4). When the reaction was carried out at 30 °C only a 7% of the monohydrolyzed product *cis*-**7a** was isolated after 29 h of reaction. In order to improve the yield of the reaction the same process was carried out at 60 °C, but after a period of three days only 13% of the monocarbonate (−)-**7a** was isolated and its enantiomeric excess was low. As in the case of the substrate *cis*-**3**, no product was detected when CAL-B was used as catalyst.



Scheme 4. Synthesis and enzymatic hydrolysis of *cis*-**6a**.

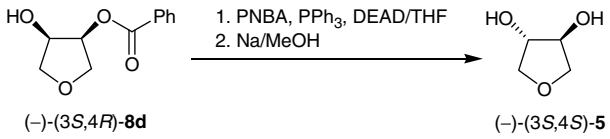
In light of these results, we decide to test the *meso*-diol *cis*-**5** as a substrate for the enzymatic acylation. First, we carried out the process at 30 °C, using 5 equiv of different acylating agents and ^{t}BuOMe as solvent (Scheme 5).



Scheme 5. Enzymatic acylation of *cis*-**5**.

Two of the enzymes tested catalyzed the reaction: PSL-C and CAL-B. It is noteworthy that depending on the biocatalyst employed, an opposite stereochemical preference in the asymmetric acylation was observed. PSL-C catalyzed the formation of derivatives (3*R*,4*S*)-(+)-**8**, while CAL-B catalyzed the formation of derivatives (3*S*,4*R*)-(−)-**8**. Unfortunately, the PSL-C showed low enantioselectivity in these processes.

The absolute configuration of these compounds were assigned as follows (Scheme 6): Compound (3*S*,4*R*)-(−)-**8d** was converted by a Mitsunobu inversion in (3*S*,4*S*)-(−)-*trans*-**5**, whose specific rotation was compared with the one established.[3a]



Scheme 6. Assignation of the absolute configuration of (−)-**8d**.

Table 2 summarized the results obtained in the lipase-catalyzed acylations of *cis*-**5**. In all cases, the reactions were finished when no further progress of the process was observed by HPLC analysis.

Table 2
Lipase-catalyzed acylation of *cis*-**5** in ^{t}BuOMe

Entry	Lipase	Acylating agent[a]	*T* (°C)	*t* (h)	*c*[b] (%)	ee_p[c] (%)
1	PSL-C	Vinyl acetate	30	4	—[d]	—[d]
2	PSL-C	Vinyl propionate	30	24	—	—
3	PSL-C	Vinyl decanoate	30	48	22	24
4	PSL-C	Vinyl benzoate	30	48	65	25
5	PSL-C	Vinyl benzoate	60	48	69	30
6	PSL-C	Ethyl acetate	30	30	—	—
7	CAL-B	Vinyl acetate	30	4	—[d]	—[d]
8	CAL-B	Vinyl propionate	30	24	—	—
9	CAL-B	Vinyl decanoate	30	24	29	32
10	CAL-B	Vinyl benzoate	30	24	68	74

[a] 5 equiv.
[b] Conversion determined by HPLC.
[c] Determined by chiral HPLC.
[d] A mixture of *cis*-**3**, (±)-**8a**, and the remaining *cis*-**5** was obtained.

Among the processes catalyzed by PSL-C only with the monoacylated vinyl decanoate (+)-**8c** and vinyl benzoate (−)-**8d** were obtained as products of respective reactions (Table 2, entries 3 and 4). However, the enantioselectivity and conversion of the reaction were low in both cases. When we carried out the process with vinyl benzoate at 60 °C (entry 5) the results were only slightly improved. The same reaction carried out with vinyl acetate afforded low conversion and a mixture of mono- and diacylated products. Using other acylating agents such as vinyl propionate or ethyl acetate, no product was detected after 24 h of reaction.

The preliminary results obtained in acylations catalyzed by CAL-B are summarized in entries 7–10 of Table 2. The best results were obtained when vinyl benzoate was used as an acylating agent (entry 10). After 24 h of reaction, only the monoacylated product (−)-**8d** was obtained with 68% of conversion and 74% enantiomeric excess.

In view of these moderate but promising results, we decided to study the influence of different reaction parameters in the asymmetric acylation of *cis*-**5** catalyzed by CAL-B, using vinyl benzoate as an acylating agent (Table 3).

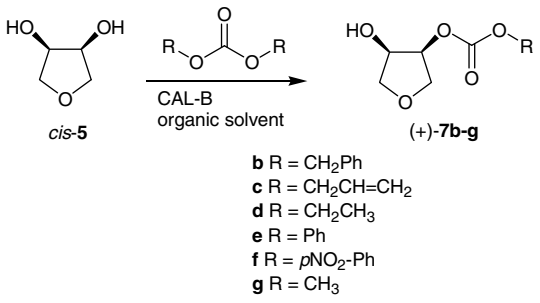
First, we studied the influence of the organic solvent at 30 °C, using 5 equiv of vinyl acetate. The results obtained are summarized in entries 1, 2, 9, 10, 15, and 19. The faster process was carried out in diethyl ether (entry 15), after 17 h of reaction an 80% of the monoacylated product was achieved with an 82% enantiomeric excess. Similar conversions were achieved in 1,4-dioxane (81%, entry 2) and THF (79%, entry 10) after longer reaction times. Higher enantiomeric excess was obtained in THF (83% ee, entry 10). The effect of the temperature was also analyzed in three of the solvents. Apparently, an increase in the temperature enhanced the initial reaction rate, but did not improve the final conversion of the process. For example, when the reaction was carried out at 40 °C in 1,4-dioxane, 82% conversion was obtained after 24 h (entry 3), but after 48 h of reaction, the conversion was only slightly higher (85%, entry 4). A small increase in the enantiomeric excess of the product was observed at 40 °C, but this tendency was not maintained at 50 °C (entry 5); both conversion and enantioselectivity were lower than at 40 °C. We also studied the process at lower temperature using diethyl ether as solvent (entry 16). Under these conditions the reaction was slower and less enantioselective than at 30 °C.

The influence of the concentration of the acylating agent was also examined in 1,4-dioxane, THF, and diethyl ether, using 2 or 10 equiv of the acylating agent. The results obtained are summarized in entries 7, 8, 13, 14, 17, and 18 of Table 3. Even though an increment of the initial reaction rate was observed using 10 equiv of the acylating agent, in any case an improvement in the final conversion of the process was observed, and the best enantioselectivities were always observed in processes carried out using 5 equiv of acylating agent.

In order to verify the possible racemization of the monoacylated product in the reaction media, the product (−)-**8d** obtained in one of the enzymatic processes was dissolved in 1,4-dioxane and shaken at 30 °C for seven days. Effectively, after this time HPLC analysis of the recovered product showed a racemization of 18%. This slow racemization, probably due to intramolecular acyl migrations, can be easily avoided by a convenient protection of the free hydroxyl group.

Another option to avoid the racemization of the product is the formation of a monocarbonate through an enzymatic alkoxycarbonylation process. With this in mind, we tested several carbonates in the lipase-catalyzed desymmetrization of substrate *cis*-**5**. (Scheme 7). First, we tested the reactions in 1,4-dioxane, at 30 °C and using 5 equiv of the corresponding carbonate as an alkoxycarbonylation agent. Entries 1–4 and 7–9 of Table 4 summarize the most significant results obtained in the alkoxycarbonylation processes.

As in the case of the enzymatic acylation, PSL-C showed very low selectivity: the reaction with dibenzyl carbonate afforded a mixture of mono- and dialcoxycarbonylated products. Better results were obtained using CAL-B as a catalyst. The best enantio-



Scheme 7. Enzymatic alkoxycarbonylation of *cis*-**5**.

Table 3
CAL-B catalyzed acylation of *cis*-**5** in organic solvents using vinyl benzoate as an acylating agent

Entry	Acylating agent (equiv)	Solvent	*T* (°C)	*t* (h)	*c*[a] (%)	ee_p[b] (%)
1	5	^{t}BuOMe	30	24	68	74
2	5	1,4-Dioxane	30	64	81	78
3	5	1,4-Dioxane	40	24	82	80
4	5	1,4-Dioxane	40	48	85	80
5	5	1,4-Dioxane	50	24	78	77
7	2	1,4-Dioxane	30	48	68	73
8	10	1,4-Dioxane	30	19	70	75
9	5	Acetonitrile	30	72	75	73
10	5	THF	30	96	79	83
11	5	THF	40	48	75	70
12	5	THF	50	48	62	46
13	2	THF	30	72	74	72
14	10	THF	30	24	72	70
15	5	Et_2O	30	17	80	82
16	5	Et_2O	15	27	78	80
17	2	Et_2O	30	24	70	71
18	10	Et_2O	30	24	75	73
19	5	Toluene	30	72	41	13

[a] Conversion determined by HPLC.
[b] Determined by chiral HPLC.

Table 4
Lipase-catalyzed alkoxycarbonylation of *cis*-**5** in 1,4-dioxane at 30 °C

Entry	Carbonate[a] R	Enzyme	Solvent	*T* (°C)	*t* (h)	*c*[b] (%)	ee$_p$[c] (%)
1	Benzyl	PSL-C	1,4-Dioxane	30	24	—[d]	—
2	Benzyl	CAL-B	1,4-Dioxane	30	24	—[d]	—
3	Allyl	CAL-B	1,4-Dioxane	30	24	52	38
4	Ethyl	CAL-B	1,4-Dioxane	30	15	30	78
5	Ethyl	CAL-B	1,4-Dioxane	40	24	55	31
6	Ethyl	CAL-B	Ether	30	24	22	16
7	Phenyl	CAL-B	1,4-Dioxane	30	72	—	—
8	*p*-Nitrophenyl	CAL-B	1,4-Dioxane	30	48	—	—
9	Methyl	CAL-B	1,4-Dioxane	30	72	—	—

[a] 5 equiv.
[b] Conversion determined by HPLC.
[c] Determined by chiral HPLC.
[d] A mixture of *cis*-**6b**, (±)-**7b**, and the remaining *cis*-**5** was obtained.

selectivity was observed when the diethyl carbonate was used as the alkoxycarbonylation agent. In this case, the product of the enzymatic process showed 78% enantiomeric excess but only 30% conversion was observed after 24 h of reaction (entry 4). In order to improve these results, we carried out the reaction at 40 °C (entry 5). Under these conditions, the conversion increased to 55% after 24 h of reaction, but the enantioselectivity was lower than in the process carried out at 30 °C. We also tested this enzymatic process in ether (entry 6), because in this media the acylation is faster than in the other solvents tested; however, for the alkoxycarbonylation process the reaction in ether is slower and less enantioselective than in 1,4-dioxane.

The absolute configuration of compound (3*S*,4*R*)-(−)-**7d** was assigned as in the case of (3*S*,4*R*)-(−)-**8d** by a Mitsunobu inversion and hydrolysis of the resulting product to afford (3*S*,4*S*)-(−)-*trans*-**5**, whose specific rotation was compared with the one established.[3a]

3. Conclusions

In conclusion, we have reported an easy methodology for the resolution of *trans*-3,4-dihydroxytetrahydrofuran via a PSL-C catalyzed hydrolysis of its diacetyl derivative. The high enantioselectivity obtained in this process is particularly noteworthy. On the other hand, the enzymatic acylation is preferable to alkoxycarbonylation for the desymmetrization of the *cis*-3,4-dihydroxytetrahydrofuran; the best results are obtained in the process catalyzed by *C. antarctica* lipase B using vinyl benzoate as an acylating agent, a conversion higher than 80% and a enantiomeric excess of 82% can be obtained by the appropriate selection of the reaction parameters.

4. Experimental

4.1. General

Enzymatic reactions were carried out in a Gallenkamp incubatory orbital shaker. Immobilized *C. antarctica* lipase B, CAL-B Novozym 435 (7300 PLU/g), was a gift from Novo Nordisk co. and immobilized *P. cepacia* lipase (PSL-C, 783 U/g) is from by Amano Pharmaceuticals.

Chemical reagents were purchased 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 241 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. Positive electrospray ionization (ESI$^+$) was used to record mass spectra on Hewlett–Packard 110 LC/MSD Series spectrometers. The enantiomeric excesses were determined by chiral HPLC analysis on a Hewlett–Packard 1100, LC liquid chromatograph, using a CHIRALPCK IA column (4.5 × 250 mm) or by GC analyses on a Hewlett Packard 6890 Series II chromatograph equipped with a Restek RtβDEXse (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2) column. For all the analyses, the injector temperature was 225 °C and the FID temperature was 250 °C.

4.2. Synthesis of (±)-*trans*-3,4-diacetoxytetrahydrofuran (±)-*trans*-3

To a solution of **2** (0.5 g, 5.1 mmol) in acetic acid (10 mL), acetic anhydride was added (2 mL, 21 mmol). Then, boron trifluoride etherate (635 μL, 5 mmol) was slowly added, and the mixture was stirred at room temperature for 24 h. An aqueous saturated solution of $NaHCO_3$ (10 mL) was added, and the mixture extracted with EtOAc (20 mL). The organic phase was washed with saturated aqueous Na_2CO_3 solution (20 mL) followed by saturated aqueous NaCl solution (20 mL), and then 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 1:1) to afford product **(±)-*trans*-4** as a white solid (0.75 g, 78%). Mp 34–36 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.04 (s, 6H), 3.72–3.76 (m, 2H), 4.06 (dd, 2H, J = 4.38 Hz, J = 10.53 Hz), 5.11–5.14 (m, 2H); ^{13}C NMR ($CDCl_3$, 100.5 MHz): δ 21.38 ($2CH_3$), 72.48 ($2CH_2$), 77.28 (2CH), 170.55 (2CO); IR (KBr, cm^{-1}) 1021.1, 1072.8, 1228.7, 1373.2, 1436.0, 1467.3, 1740.0, 2877.2, 2990.8 cm^{-1}; MS (ESI$^+$ m/z): 189 [(M+H)$^+$, 100].

4.2.1. Analysis by chiral GC of (±)-*trans* 3

Determination of the ee by GC analysis: RtβDEXse, 70 °C (5 min), 3 °C/min, 200 °C (10 min). t_R (3*R*,4*R*) 29.8 min; t_R (3*S*,4*S*) 30.3 min.

4.3. General procedure for the enzymatic hydrolysis of (±)-*trans*-3

The reaction mixture containing the substrate (100 g, 0.16 mmol), 1 mL of 1 M phosphate buffer (pH 7.0), the lipase (100 mg) and the corresponding organic solvent (2 mL) was shaken at 30 °C and 250 rpm in an orbital shaker. The progress of the reaction was monitored by TLC (hexane/EtOAc 1:1) until the achievement of the required conversion. Then, the enzyme was removed by filtration and washed with the same solvent that has been used in the reaction, 3 × 5 mL. The solvent was removed under reduced pressure, and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 1:1) to afford the monoacetylated product (3*R*,4*R*)-**4** and the remaining substrate (3*S*,4*S*)-**3**.

4.3.1. (+)-(3*S*,4*S*)-3,4-Diacetoxytetrahydrofuran, (+)-(3*S*,4*S*)-3. Determination of the ee by GC analysis

RtβDEXse, 70 °C (5 min), 3 °C/min, 200 °C (10 min). t_R (3*S*,4*S*) 30.0 min, ee >99%.

4.3.2. (−)-(3*R*,4*R*)-3-Acetoxy-4-hydroxytetrahydrofuran, (−)-(3*R*,4*R*)-4

White solid. Mp 37–38 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.22 (s, 3H), 3.56 (br s, OH), 3.79–3.90 (m, 2H), 4.01–4.09 (m, 2H), 4.24–4.32 (m, 2H); ^{13}C NMR ($CDCl_3$, 100.5 MHz): δ 21.31 (CH_3), 72.73 (CH_2), 72.98 (CH_2), 77.35 (CH), 81.92 (CH), 170.28 (CO); IR (KBr, cm^{-1}) 3122, 1743; MS (ESI$^+$ *m*/*z*): 147 [(M+H)$^+$, 100].

4.3.3. Determination of the ee by GC analysis

In order to determinate its enantiomeric excess, (−)-(3*R*,4*R*)-**4** was converted into the diacetyl derivative (−)-(3*R*,4*R*)-**3** and analyzed by GC. RtβDEXse, 70 °C (5 min), 3 °C/min, 200 °C (10 min). t_R (3*R*,4*R*) 29.8 min, ee >99%.

4.4. General procedure for the enzymatic acylation

The reaction mixture containing the corresponding substrate (0.15 mmol), the corresponding acylating agent (0.75 mmol), the lipase (100 mg) and the corresponding organic solvent (4 mL) was shaken at 30 °C and 250 rpm in an orbital shaker. The progress of the reaction was monitored by TLC (hexane/EtOAc 7:3) until the achievement of the required conversion. Then, the enzyme was removed by filtration and washed with $CHCl_3$ (3 × 5 mL). The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 7:3), to afford the corresponding monoacylated derivative.

4.4.1. (−)-(3*S*,4*R*)-3-Benzoyloxy-4-hydroxytetrahydrofuran, (−)-(3*S*,4*R*)-8d

White solid. Mp 48–49 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.42 (br s, OH), 3.78–3.98 (m, 1H), 4.00–4.20, (m, 3H), 4.57–4.59 (m, 1H), 5.36–5.41 (m, 1H), 7.43–7.59 (m, 3H), 8.05–8.08 (d, 2H, *J* 7.17 Hz), ^{13}C NMR ($CDCl_3$, 100.5 MHz): δ 70.93 (CH_2), 71.47 (CH_2), 72.72 (CH), 74.57 (CH), 128.86 (2CH), 129.61 (2CH), 130.08 (2CH), 133.86 (C), 166.67 (CO); IR (KBr, cm^{-1}) 1120.9, 1274.5, 1451.5, 1710.0, 3428.4; MS (ESI$^+$ *m*/*z*): 209 [(M+H)$^+$, 100].

4.4.2. Determination of the ee by HPLC analysis

Chiralpack IA, 20 °C, hexane/2-propanol (80:20), UV 210 nm, 0.5 mL min^{-1}, t_R 13.43 min (minor); t_R 15.23 min (major). $[\alpha]_D^{25} = -12$ (*c* 0.03, $HCCl_3$), ee = 83%.

4.5. General procedure for the enzymatic alkoxycarbonylations of *cis*-3,4-dihydroxytetrahydrofuran

The lipase (100 mg) and the corresponding carbonate (5 equiv) were added to a solution of *cis*-**5** (100 mg, 1 equiv) in 1,4-dioxane (4 mL). The mixture was shaken at the selected temperature and 250 r.p.m. in a rotatory shaker. The progress of the reaction was monitored by TLC. Once the reaction was finished, the enzyme was removed by filtration, washed with 1,4-dioxane, and the solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel to afford the corresponding monoalkoxycarbonyl derivatives *cis*-**7b–g**.

4.5.1. (−)-(3*S*,4*R*)-3-Ethoxycarbonyloxy-4-hydroxytetrehydrofuran, (−)-(3*S*,4*R*)-7d

White solid. Mp 39–41 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.42 (t, 3H), 2.29 (c, 2H), 3.57 (br s, OH), 3.77–3.87 (m, 2H), 4.01–4.13 (m, 2H), 4.19–4.31 (m, 2H). ^{13}C NMR ($CDCl_3$, 100.5 MHz): δ 11.52 (CH_3), 28.11 (CH_2), 72. 91 (CH_2), 73.06 (CH_2), 77.15 (CH), 82.88 (CH), 172.98 (CO); IR (KBr, cm^{-1}) 1138.5, 1422.1, 1744.8; MS (ESI$^+$ *m*/*z*): 199 [(M+Na)$^+$, 40].

4.5.2. Determination of the ee by HPLC analysis

Chiralpack IA, 20 °C, hexane/2-propanol (80:20), UV 210 nm, 0.5 mL min^{-1}, t_R 15,69 min (minor); t_R 17.48 min (major). $[\alpha]_D^{25} = -4.6$ (*c* 0.25, $HCCl_3$), ee = 78%.

Acknowledgments

This work was supported by the Principado de Asturias (project PC06-019). We express our appreciation to Novo Nordisk Co. for the generous gift of the lipase CAL-B.

References

1. See for example: Aghmiz, M. A.; Aghmiz, A.; Masdeu-Bulto, Y.; Claver, C.; Castillon, S. *J. Org. Chem.* **2004**, *69*, 7502–7510.
2. See for example: (a) Wang, W.; Jin, H.; Fuselli, N.; Mansour, T. S. *Bioorg. Med. Chem. Lett.* **1997**, 2567–2572; (b) Tatani, K.; Shuto, S.; Ueno, Y.; Matsuda, A. *Tetrahedron Lett.* **1998**, *39*, 5065–5068; (c) Hwang, D. J.; Kim, S. N.; Choi, J. H.; Lee, Y. S. *Bioorg. Med. Chem. Lett.* **2001**, 1429–1437.
3. (a) Dulphy, H.; Gras, J.-L.; Lejon, T. *Tetrahedron* **1996**, *52*, 8517–8524; (b) Skarżewski, J.; Gupta, A. *Tetrahedron: Asymmetry* **1997**, *8*, 1861–1867.
4. Matsumoto, K.; Mitsuda, M.; Ushijima, N.; Demizu, Y.; Onomura, O.; Matsumura, Y. *Tetrahedron Lett.* **2006**, *47*, 8453–8456.
5. (a) Zhao, L.; Han, B.; Huang, Z.; Miller, M.; Huang, H.; Malashock, D. S.; Zhu, Z.; Milan, A.; Robertson, D. E.; Weiner, D. P.; Burk, M. J. *J. Am. Chem. Soc.* **2004**, *126*, 11156–11157; (b) Botes, A. L.; Lotter, J. WO 2005100578 A2, CAN 143:438591.
6. Seemayer, R.; Schneider, M. P. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2359–2360.
7. Bayston, D. J.; Casson, S.; Lovell, J. M.; Scott, R. M. U.S. Patent 6,140,516, CAN 133:335151.
8. Xue, F.; Seto, C. T. *Bioorg. Med. Chem.* **2006**, *14*, 8467–8487.
9. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.