NITRILE OXIDES IN MEDICINAL CHEMISTRY. 5.[*] LIPASE PS-CATALYZED RESOLUTION OF A SET OF HETEROCYCLIC DERIVATIVES.

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Abstract: Lipase from *Pseudomonas cepacia* (lipase PS) catalyzed the hydrolysis of a series of butyrates of racemic primary alcohols carrying a Δ^2-isoxazoline or an isoxazolidin-3-one nucleus. Within this set of compounds, the enantiopreference of the catalyst can be accounted for by a rule recently proposed for lipase PS-catalyzed resolution of secondary alcohols. Nevertheless much work needs to be done in order to become predictive of the degree of enantioselectivity and of the relative reaction rates.

INTRODUCTION

The use of lipases, esterases and proteases in the resolution of racemic alcohols, acids and esters[1] is particularly appealing in medicinal chemistry as well in agrochemistry due to the fact that individual enantiomers are usually provided with different biological activities and/or selectivities.[2] As a consequence a racemate may act as a mixture of drugs. Presently, there is an increasing awareness of the drug regulatory authorities about the consequences of stereochemistry on biological activity. While there are no common rules yet, the guidelines of various countries state that enantiomers may be considered as impurities and, consequently, safety and efficacy data need to be produced for each stereoisomer.

In the past we applied the chemoenzymatic methodology to the synthesis of the enantiomers of broxaterol,[3] a β_2-adrenergic stimulant presently under clinical investigation for the treatment of bronchial asthma,[4] and to the resolution of iopanoic acid which is currently employed as an X-ray contrast medium for the diagnosis of bile stones.[5]

As a part of a programme devoted to the study of the relationship between structure and activity of cholinergic ligands related to natural muscarine **1**, we tackled the synthesis of the enantiomers of **2**, a potent muscarinic-receptor agonist[6] and of **3**, a valuable antimuscarinic agent[7] (Figure 1).

Quite interestingly, derivative **2**, which is more potent than muscarine, showed a reversal in the enantioselectivity and a very low potency ratio among the enantiomers (eudismic ratio, ER). The ER values

Figure 1

(2S,4R,5S)-1

2: R=Me
3: R=Ph
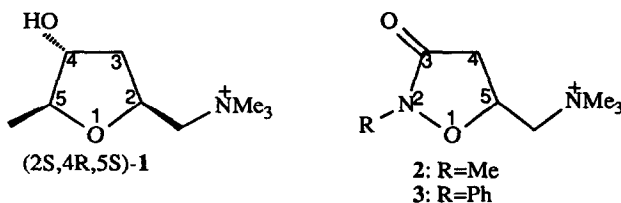
of **2** spanned the range 2.5-10.4 in a number of functional test preparations[8] at variance with muscarine[9] and all the major chiral muscarinic agonists, where the eudismic ratios are higher than 100. Similar results were recently obtained on the structurally related antagonist **3**.[7] Such an anomaly has been rationalized on the basis of molecular mechanics calculations and X-ray analysis data.[10]

The key step of the synthesis of the enantiomers of **2** and **3** was a lipase PS-catalyzed kinetic resolution of precursors **4** and **6** respectively (Scheme I), followed by standard chemical transformations.[11] The attempted resolution of **8** failed due to the lack of selectivity of the catalyst for the substrate.[11] It is worth
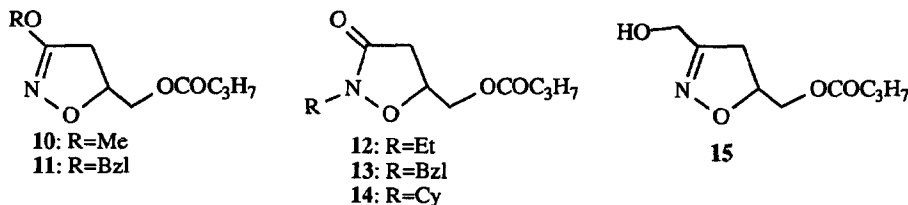
Scheme I
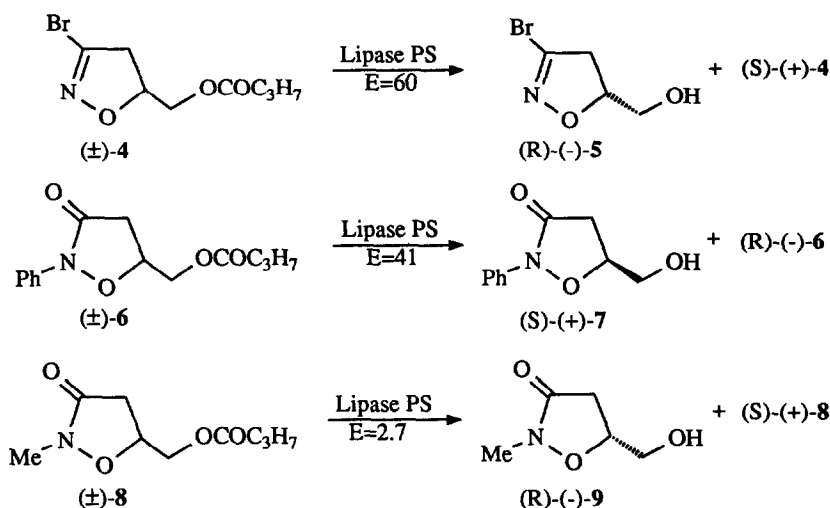
pointing out the sharp change in the lipase PS enantiopreference on passing from **4** (E=60 for R) to **6** (E=41 for S); substrate **8** lies in between being poorly discriminated by the enzyme (E=2.7).

In view of further applications of this strategy to the synthesis of biologically active compounds and to challenge the active-site models proposed to rationalize the results,[12-14] we thought it interesting to study the lipase PS-catalyzed hydrolysis of a series of esters structurally related to derivatives **4** and **6**.

This paper deals with the synthesis and the lipase PS-catalyzed hydrolysis of butyrates **10-15** (Figure 2).

Figure 2

10: R=Me
11: R=Bzl

12: R=Et
13: R=Bzl
14: R=Cy

15

RESULTS AN DISCUSSION

As shown in Scheme II, substrates **10** and **11** were obtained by refluxing a methanol solution of isoxazoline **5** and potassium hydroxide or by reacting at room temperature a DMSO mixture of **5** and lithium benzylate. Intermediates **16** and **17** were then transformed into **10** and **11** by a standard reaction. On the other hand, N-alkylation of Δ^2-isoxazoline **4** was achieved at room temperature with a dichloromethane solution of ethyl- or cyclohexyltriflate or at reflux with a DMF solution of benzyl bromide. In all three cases the

Scheme II

16: R=Me
17: R=Bzl

12
13
14

18
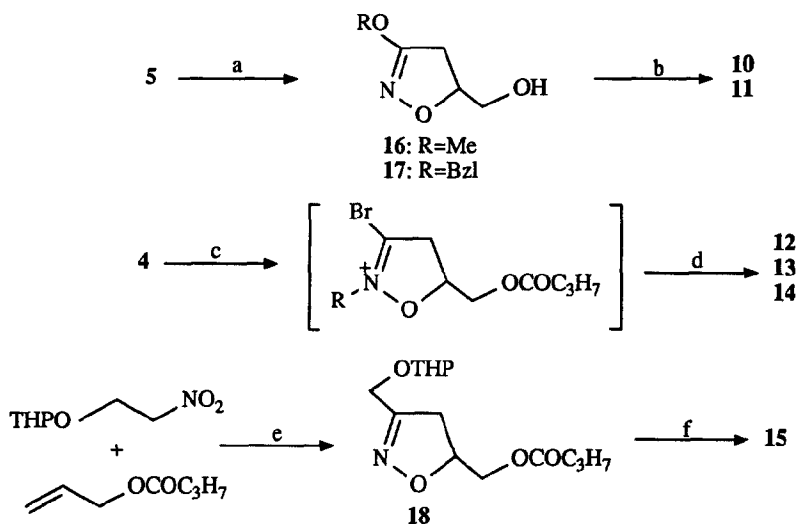
a:MeOH/KOH or PhCH$_2$OLi/DMSO; b:C$_3$H$_7$COCl/Py; c:CF$_3$SO$_3$R/CH$_2$Cl$_2$ or PhCH$_2$Br/DMF; d:NaHCO$_3$/H$_2$O; e:PhNCO-NEt$_3$/C$_6$H$_6$; f:IR-120/MeOH.

intermediate isoxazolinium salts were not isolated but directly transformed into final derivatives **12-14** by treatment with an aqueous solution of sodium bicarbonate. Isoxazoline **15** was prepared via 1,3-dipolar cycloaddition of 2-nitroethanol tetrahydropyranyl ether with allyl alcohol butyrate following the Mukaiyama procedure.[15] Intermediate **18** was transformed into **15** by treatment with a strongly acidic resin.

Butyrates **10-15** were submitted to hydrolysis in a phosphate buffer solution (pH 7) under the catalysis of lipase PS. The results of such a reaction are collected in Table I. The degree of conversion and the enantiomeric excess (e.e.) were evaluated by chiral HPLC which gave base-line separation for the enantiomers of the produced alcohols and/or residual butyrates (see Experimental Section). The absolute configuration of the resulting alcohols had previously been assigned i.e. **5, 7, 9** and **17** or was attributed by chemical correlation with (R)-(-)-**5** or (S)-(+)-**4**.

Table I. Lipase PS-catalyzed hydrolysis of butyrates **4, 6, 8, 10-15**.

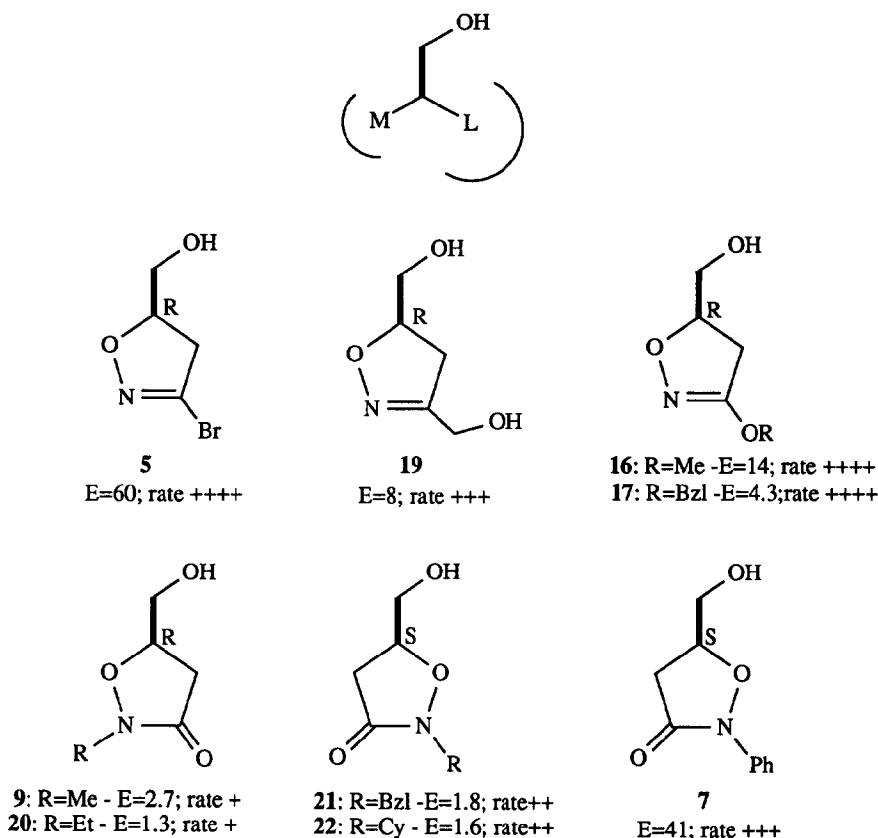| Substrate	E^a	degree of conv.(%)	configuration of produced alcohol (e.e. %)	configuration of residual ester (e.e. %)
4	60	39	R(94)	
		55		S(>99)
6	41	41	S(91)	
		55		R(98)
8	2.7	40	R(36)	
		51		S(38)
10	14	51	R(73)	S(75)
11	4.3	66	R(36)	S(71)
12	1.3	37	R(10)	S(6)
13	1.8	45	S(21)	R(18)
14	1.6	57	S(16)	R(21)
15	8	22	R(73)	S(21)

^{a}The E values were calculated according to the equations reported in Ref. 1.

Inspection of the data collected in Table I reveals a strong dependence of the enzyme enantiopreference on the shape of the heterocycle carrying the alcohol moiety. Within the set of Δ^2-isoxazoline derivatives **4, 10, 11, 15**, lipase PS recognized invariably the (R)-enantiomer even though the degree of the observed enantioselectivity was strongly influenced by the substituent of the heterocyclic nucleus. As a matter of fact the remarkable enantiomeric ratio obtained with derivative **4** (E=60) was drastically reduced in the other three cases (E=14-4.3). Worth mentioning are the results obtained with N-substituted isoxazolidin-3-one derivatives since an increase in the bulkiness of the N-substituent determined a change of the lipase PS-enantiopreference from the (R)- to the (S)-enantiomer. Of particular relevance is the enantioselectivity obtained with the N-phenyl derivative **6** (E=41).

Recent papers deal with lipases active-site models, designed to account for the experimental results and to predict the enantiopreference and/or the degree of enantioselectivity of enzymes for new substrates.[12-14] In our opinion the most general and easily applicable model is the one recently proposed by Kazlauskas et al. to predict the enantiopreference of some lipases and yeast-catalyzed hydrolyses of esters derived from secondary

alcohols.[13,14,16,17] Here we have applied such a model to a series of primary alcohols. In the upper part of Figure 3 is dipicted the shape of the enantiomer of a primary alcohol which, according to the above-mentioned rule, should fit at best the catalytic site of the enzyme. The rule is based on the relative

Figure 3

5
E=60; rate ++++

19
E=8; rate +++

16: R=Me -E=14; rate ++++
17: R=Bzl -E=4.3;rate ++++

9: R=Me - E=2.7; rate +
20: R=Et - E=1.3; rate +

21: R=Bzl -E=1.8; rate++
22: R=Cy - E=1.6; rate++

7
E=41; rate +++

bulkiness of the substituents around the stereogenic center which will be accommodated in lipophilic pockets of different sizes. In sequence we report the configuration of the alcohol experimentally obtained from the lipase PS-catalyzed hydrolysis of butyrates **4, 6, 8, 10-15** which have been taken into account in this paper. The degree of enantioselectivity is expressed by the value of the enantiomeric ratio (E). Qualitative reaction rates are also reported. The results depicted in Figure 3 are qualitatively predicted by the model proposed by Kazlauskas[13,14] since the left part of the heterocyclic ring of derivatives **5, 16, 17, 19,** and perhaps **9** and **20** is sterically less demanding than the corresponding right part. Nevertheless the enzyme enantioselectivity is not related to the sole bulkiness of the 3-substituent since derivative **17** should give rise to a higher selectivity

than **5**. In our opinion the degree of enantioselectivity is also somehow dependent on the lipophilicity and/or the electronic characteristics of the substituent. Similar conclusions can be deduced by comparison of the results obtained with isoxazolidin-3-ones **9, 20, 21, 22, 7** where a stepwise increase in the size of the N-substituent changes the enantiopreference from R to S. In any case, the huge difference in the E value on passing from **7** to **21** and **22** cannot be accounted for by a difference in the bulkiness of the substituent. Such a remarkable change in enantioselectivity provoked by a phenyl ring has precedents in the literature[13,18] and could be attributed to a specific interaction, i.e. π-π interaction, of such a group with a juxtaposed substituent in the polypeptide chain of the enzyme active-site.

EXPERIMENTAL SECTION

Materials and Methods. Lipase from *Pseudomonas cepacia* (lipase PS) was purchased from Amano. Porcine pancreatic lipase (P.P.L.) was bought from Sigma. Organic solvents were reagent grade. ^{1}H NMR spectra were recorded at 200 MHz in CDCl$_3$ solution (unless otherwise stated); chemical shifts (δ) are expressed in ppm and coupling constants (J) in hertz. HPLC analyses were performed on Chiralcel OB or OD columns (4.6x250 mm); experimental conditions are listed in Table II. Rotary power determinations were carried out with a Perkin Elmer 241 Polarimeter coupled with a Haake N3-B thermostat. Liquids were characterized by the oven temperature for bulb to bulb distillations. TLC analyses were carried out on commercial silica gel GF$_{254}$ plates; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution. Microanalyses of new compounds agree with theoretical value ±0.4%.

Synthesis of butyrates (±)-10 and (±)-11. Intermediates (±)-**16**[19] and (±)-**17**[20] were prepared according to the previously reported procedures.

(RS)-3-Methoxy-5-(hydroxymethyl)-Δ^2-isoxazoline [(±)-**16**]: ^{1}H NMR 2.33 (bs, 1, OH); 2.94 (dd,1, H-4; J=8.7 and 16.4); 2.97 (dd, 1, H-4'; J=9.6 and 16.4); 3.57-3.90 (m, 2, CH$_2$OH); 3.84 (s, 3, CH$_3$); 4.72(m, 1, H-5).

(RS)-3-Benzyloxy-5-(hydroxymethyl)-Δ^2-isoxazoline [(±)-**17**]: 2.40 (bs, 1, OH); 3.08 (m, 2, H-4 and H-4'); 3.78 (m, 2, CH$_2$OH); 4.81 (m, 1, H-5); 5.20 (s, 2, CH$_2$O); 7.43 (m, 5, arom.).

To a mixture of (±)-**16** (or **17**) (5 mmol) and pyridine (0.8 mL, 10 mmol) in anhydrous dichloromethane (20 mL) was added dropwise a dichloromethane solution (5 mL) of butyryl chloride (2.08 mL, 20 mmol) at 0°C. The disappearance of the starting material was monitored by TLC (eluent: ethyl acetate). The mixture was washed with a 20% solution of CuSO$_4$ (3x5 mL) and the organic phase was dried and concentrated. The residue was purified by silica gel column chromatography (eluent: cyclohexane/ethyl acetate 3:1).

(RS)-3-Methoxy-5-(hydroxymethyl)-Δ^2-isoxazoline butyrate [(±)-**10**]: 83% yield; bp 110-115°C/0.5 mmHg; R$_f$ 0.27 (cyclohexane/ethyl acetate 3:1); ^{1}H NMR: 0.93 (t, 3, CH$_2$CH$_3$; J=7.6); 1.64 (m, 2, CH$_2$CH$_3$); 2.32 (t, 2, OCOCH$_2$; J=7.5); 2.76 (dd, 1, H-4; J=7.6 and 16.5); 3.02 (dd, 1, H-4'; J=10.2 and 16.5); 3.84 (s, 3, OCH$_3$); 4.18 (m, 2, CH$_2$O); 4.83 (m, 1, H-5). *Anal.* Calcd for C$_9$H$_{15}$NO$_4$: C, 53.72; H, 7.51; N, 6.96. Found: C, 54.02; H, 7.77; N, 6.59.

(RS)-3-Benzyloxy-5-(hydroxymethyl)-Δ^2-isoxazoline butyrate [(±)-**11**]: 89% yield; colorless viscous oil which decomposes on distillation at 0.5 mmHg; R$_f$ 0.39 (cyclohexane/ethyl acetate 3:1); ^{1}H NMR: 0.95 (t, 3,

CH$_2$CH_3; J=7.5); 1.66 (m, 2, CH_2CH$_3$); 2.33 (t, 2, OCOCH$_2$; J=7.4); 2.83 (dd, 1, H-4; J=7.6 and 16.4); 3.13 (dd, 1, H-4'; J=10.3 and 16.4); 4.23 (m, 2, CH$_2$O); 4.87 (m, 1, H-5); 5.15 (s, 2, OCH_2Ph); 7.39 (m, 5, arom.). *Anal.* Calcd for C$_{15}$H$_{19}$NO$_4$: C, 64.97; H, 6.91; N, 5.05. Found: C, 64.59; H, 7.07; N, 5.39.

Synthesis of butyrates (±)-12 and (±)-14.
A. To a cold (-30°C) and stirred solution of cyclohexanol (1.5 g, 15 mmol) and dry pyridine (1.21 mL, 15 mmol) in anhydrous dichloromethane (15 mL) was added trifluoromethanesulfonic anhydride (3 mL, 18 mmol) dropwise in 15 min under nitrogen. The disappearance of the starting material was checked by TLC (eluent: petroleum ether/ethyl acetate 3:2). After treatment with 3% HCl (pH 5), the organic phase was dried and concentrated under vacuum at room temperature. A dichloromethane solution (15 mL) of (±)-4[11](0.863 g, 3.45 mmol) was added to the crude cyclohexyl trifluoromethanesulfonate (3.2 g, 13.8 mmol) and the mixture was stirred at room temperature overnight; it was treated with an aqueous solution of sodium bicarbonate and the organic layer was collected. The aqueous phase was extracted with dichloromethane (3x10 mL). After the usual workup, the residue of the organic extracts was column chromatographed (eluent: cyclohexane/ethyl acetate 1:1) to yield 0.538 g (58%) of the desired ester.
(RS)-2-Cyclohexyl-5-(hydroxymethyl)isoxazolidin-3-one butyrate [(±)-14]: bp 190-195°C/0.5 mmHg; R$_f$ 0.38 (cyclohexane/ethyl acetate 1:1); ^{1}H NMR: 0.96 (t, 3, CH$_2$CH_3; J=7.4); 1.05-1.42 (m, 3); 1.47-1.89 (m, 9); 2.34 (t, 2, OCOCH$_2$; J=7.4); 2.63 (dd, 1 ,H-4; J=7.2 and 16.4); 2.86 (dd, 1, H-4'; J=8.5 and 16.4); 3.97 (m, 1, NCH); 4.21 (m, 2, CH$_2$O); 4.67 (m, 1, H-5). *Anal.* Calcd for C$_{14}$H$_{23}$NO$_4$: C, 62.43; H, 8.61; N, 5.20. Found: C, 62.18; H, 8.40; N, 5.49.
Butyrate (±)-12 was similarly obtained from (±)-4 by using a fourfold excess of commercial ethyl trifluoromethanesulfonate (64% yield). The reaction was carried out at reflux in 1,2-dichloroethane.
(RS)-2-Ethyl-5-(hydroxymethyl)isoxazolidin-3-one butyrate [(±)-12]: bp 150-155°C/0.5 mmHg; R$_f$ 0.26 (cyclohexane/ethyl acetate 1:1); ^{1}H NMR : 0.95 (t, 3, CH$_2$CH_3; J=7.4); 1.20 (t, 3, NCH$_2$CH_3; J=7.2); 1.66 (m, 2, CH_2CH$_3$); 2.34 (t, 2, OCOCH$_2$; J=7.4); 2.62 (dd, 1, H-4; J=7.7 and 16.5); 2.87 (dd, 1, H-4'; J=8.6 and 16.5); 3.58 (q, 2, NCH_2CH$_3$; J=7.2); 4.24 (m, 2, CH$_2$O); 4.69 (m, 1, H-5). *Anal.* Calcd for C$_{10}$H$_{17}$NO$_4$: C, 55.80; H, 7.96; N, 6.51. Found: C, 56.12; H, 7.70; N, 6.37.

Synthesis of butyrate (±)-13. A solution of (±)-4 (1 g, 4 mmol) and benzyl bromide (2.38 mL, 20 mmol) in anhydrous DMF (40 mL) was stirred and heated overnight at 120°C under nitrogen. After cooling at room temperature, the mixture was treated with a slurry of sodium bicarbonate (20 min), then diluted with water (20 mL) and repeatedly extracted with dichloromethane. The pooled organic phases were dried and concentrated; residual DMF was distilled off (60°C/1 mmHg) and the residue was purified by column chromatography (eluent: cyclohexane/ethyl acetate 1:1) to give 0.355 g (32%) of the desired product.
(RS)-2-Benzyl-5-(hydroxymethyl)isoxazolidin-3-one butyrate [(±)-13]: bp 175-180°C/0.5 mmHg; R$_f$ 0.35 (cyclohexane/ethyl acetate 1:1); ^{1}H NMR: 0.93 (t, 3, CH$_2$CH_3; J=7.5); 1.62 (m, 2, CH_2CH$_3$); 2.28 (t, 2, OCOCH$_2$; J=7.5); 2.67 (dd, 1, H-4; J=7.6 and 16.6); 2.91 (dd, 1, H-4'; J=8.5 and 16.6); 4.18 (m, 2, CH$_2$O); 4.65 and 4.73 (d, 2, NCH_2Ph; J=15.6); 4.70 (m, 1, H-5); 7.32 (m, 5, arom.). *Anal.* Calcd for C$_{15}$H$_{19}$NO$_4$: C, 64.97; H, 6.91; N, 5.05. Found: C, 64.72; H, 6.58; N, 5.32.

Synthesis of butyrate (±)-15.

A. To a stirred solution of 2(2-nitroethoxy)tetrahydropyran[21] (3.5 g, 20 mmol), allyl butyrate[22] (3.84 g, 30 mmol) and triethylamine (0.5 mL) in dry benzene (50 mL) was added dropwise (30 min) phenyl isocyanate (5 mL, 46 mmol). The mixture was stirred overnight at room temperature, then refluxed for 3h. After cooling at room temperature and filtration by suction, the residue of the filtrate was purified by silica gel column chromatography (eluent: cyclohexane/ethyl acetate 3:1) to yield 2.25 g (39.5%) of the expected cycloadduct.

(RS)-3-(Tetrahydropyranyloxy)methyl-5-(butyryloxy)methyl-Δ^2-isoxazoline [(±)-18]: bp 170-175°C/0.5 mmHg; R_f 0.23 (cyclohexane/ethyl acetate 3:1); ^{1}H NMR 0.95 (t, 3, CH_2CH_3; J=7.5); 1.47-1.85 (m, 8); 2.33 (t, 2, OCOCH$_2$; J=7.5); 2.88 (dd, 1, H-4; J=7.5 and 17.4); 3.17 (dd, 1, H-4'; J=10.8 and 17.4 Hz); 3.52 (m, 1); 3.84 (m, 1); 4.17 (m, 2, 5-CH$_2$O); 4.30 and 4.43 (d, 2, CH_2OTHP; J=12.8); 4.64 (m, 1); 4.85 (m, 1, H-5).

B. A methanol solution (40 mL) of (±)-18 (1.8 g, 6.3 mmol) was mixed with 0.5 g of Amberlite IR-120 and stirred at 40°C overnight. The acidic resin was removed by filtration and the solvent was evaporated at reduced pressure. The residue distilled at 180-185°/0.5 mmHg as a colorless oil (1 g, 79% yield).

(RS)-3-(Hydroxymethyl)-5-(butyryloxymethyl)-Δ^2-isoxazoline [(±)-15]: R_f 0.20 (eluent: cyclohexane/ethyl acetate 1:1); ^{1}H NMR 0.97 (t, 3, CH$_2$CH_3; J=7.4); 1.67 (m, 2, CH_2CH$_3$); 2.35 (t, 2, OCOCH$_2$; J=7.4); 2.40 (bs, 1, OH); 2.88 (dd, 1, H-4; J=7.2 and 17.4); 3.19 (dd, 1, H-4'; J=10.8 and 17.4); 4.19 (m, 2, CH$_2$OCO); 4.44 (s, 2, CH_2OH); 4.89 (m, 1, H-5). *Anal.* Calcd for C$_9$H$_{15}$NO$_4$: C, 53.72; H, 7.51; N, 6.96. Found: C, 54.01; H, 7.70; N, 6.83.

Standard procedure for the preparation of alcohols (±)-19-(±)-22. The title primary alcohols were conveniently prepared by P.P.L.-catalyzed hydrolysis of butyrates (±)-15, (±)-12, (±)-13 and (±)-14 respectively. The following protocol is representative. A solution of the appropriate ester (2 mmol) in acetone (8 mL) and 0.1M potassium phosphate buffer, pH 7 (80 mL) was hydrolyzed in the presence of P.P.L. (0.5 g). Hydrolysis was complete in about 6h. The reaction mixture was lyophilized., taken up with acetone/methanol (10 mL, 3:1) and filtered. After evaporation of volatiles, the residue was submitted to silica gel column chromatography (eluent: ethyl acetate/methanol 9:1). R_f values were measured with ethyl acetate as the mobile phase.

(RS)-3,5-Dihydroxymethyl-Δ^2-isoxazoline [(±)-19]: 77% yield; R_f 0.12; ^{1}H NMR (CDCl$_3$/acetone d$_6$ 1:1): 2.40 (bs, 2, OH); 2.88 (dd, 1, H-4; J=8.0 and 17.3); 2.93 (dd, 1, H-4'; J=10.5 and 17.3); 3.48 and 3.57 (dd, 2, 5-CH_2OH; J=4.7, 3.6 and 12.1); 4.24 (s, 2, 3-CH_2OH); 4.52 (m, 1, H-5). *Anal.* Calcd for C$_5$H$_9$NO$_3$: C, 45.80; H, 6.92; N, 10.68. Found: C, 45.62; H, 6.80; N, 10.39.

(RS)-2-Ethyl-5-(hydroxymethyl)isoxazolidin-3-one [(±)-20][11]: 82% yield; bp 180-185°C/0.5 mmHg.

(RS)-2-Benzyl-5-(hydroxymethyl)isoxazolidin-3-one [(±)-21]: 69% yield; R_f 0.30; ^{1}H NMR 1.93 (bs, 1, OH); 2.80 (dd, 1, H-4; J=7.9 and 16.5); 2.83 (dd, 1, H-4'; J=8.4 and 16.5); 3.60 and 3.69 (dd, 2, CH_2OH; J=5.5, 3.1 and 12.5); 4.56 (m, 1, H-5); 4.68 and 4.71 (d, 2, NCH$_2$; J=15.5); 7.34 (m, 5, arom.). *Anal.* Calcd for C$_{11}$H$_{13}$NO$_3$: C, 63.76; H, 6.32; N, 6.76. Found: C, 63.51; H, 6.47; N, 6.95.

(RS)-2-Cyclohexyl-5-(hydroxymethyl)isoxazolidin-3-one [(±)-22]: 87% yield; bp 210-215°C/1 mmHg; R_f 0.24; ^{1}H NMR 1.05-1.26 (m, 3); 1.30-1.88 (m, 7); 2.28 (bs, 1, OH); 2.70 (dd, 1, H-4; J=8.0 and 16.2); 2.77 (dd, 1, H-4'; J=8.0 and 16.2); 3.73 (m, 2, CH_2OH); 3.96 (m, 1, NCH); 4.57 (m, 1, H-5). *Anal.* Calcd for C$_{10}$H$_{17}$NO$_3$: C, 60.28; H, 8.60; N, 7.03. Found: C, 60.22; H, 8.73; N, 7.09.

Standard procedure for lipase PS-catalyzed hydrolyses of (±)-10-(±)-15. Lipase PS-catalyzed hydrolyses of the title butyrates were carried out according to the methodology previously reported for substrates (±)-4, (±)-6 and (±)-8.[11] The following procedure is representative. A 100-mL Erlenmeyer flask was charged with (±)-10 (0.603 g, 3 mmol), lipase PS (3 mg), 0.1 M potassium phosphate buffer, pH 7 (60 mL) and acetone (3 mL). The mixture was stirred at room temperature for 3h (51% conversion), then lyophilized. The residue was taken up with acetone/methanol (15 mL, 4:1). After evaporation of the solvents, the residue was purified by silica gel column chromatography (eluent: cyclohexane/ethyl acetate 3:2) to afford (R)-(-)-16 (0.160 g, 1.22 mmol) and (S)-(+)-10 (0.231 g, 1.15 mmol).

(R)-(-)-16: $[\alpha]_{20}^D$-63.96 (c0.980, CHCl$_3$); e.e.% = 73.

(S)-(+)-10: $[\alpha]_{20}^D$+39.28 (c0.804, CHCl$_3$); e.e.% = 75.

The same protocol was applied to the lipase PS-catalyzed hydrolyses of butyrates (±)-11-(±)15. The data, collected in Table I, were obtained by chiral HPLC under the experimental conditions detailed in Table II.

Table II. Chiral HPLC data.

Compound	Column (Chiralcel)	λ (nm)	flow rate (mL/min)	mobile phase (n.hexane/EtOH)	t_R(min)
(R)-(-)-10	OB	210	0.5	97:3	65.4
(S)-(+)-10	OB	210	0.5	97:3	69.9
(R)-(-)-11	OD	254	0.3	60:40	19.5
(S)-(+)-11	OD	254	0.3	60:40	21.0
(S)-(+)-12	OB	220	1.0	96:4	24.9
(R)-(-)-12	OB	220	1.0	96:4	29.0
(R)-(-)-13	OB	220	1.0	90:10	24.3
(S)-(+)-13	OB	220	1.0	90:10	24.3
(R)-(-)-14	OD	254	1.5	97:3	15.7
(S)-(+)-14	OD	254	1.5	97:3	17.9
(R)-(-)-15	OD	220	0.5	95:5	45.5
(S)-(+)-15	OD	220	0.5	95:5	49.3
(R)-(-)-16	OB	210	1.0	96:4	35.8
(S)-(+)-16	OB	210	1.0	96:4	40.7
(R)-(-)-17	OD	254	0.3	60:40	16.5
(S)-(+)-17	OD	254	0.3	60:40	16.5
(R)-(-)-19	OD	220	1.0	80:20	33.6
(S)-(+)-19	OD	220	1.0	80:20	33.6
(S)-(+)-20	OB	220	1.0	96:4	35.1
(R)-(-)-20	OB	220	1.0	96:4	52.0
(R)-(-)-21	OB	220	1.0	90:10	15.5
(S)-(+)-21	OB	220	1.0	90:10	17.9
(R)-(-)-22	OD	254	1.5	97:3	22.3
(S)-(+)-22	OD	254	1.5	97:3	22.3

Absolute configurations to (R)-(-)-17 and (S)-(+)-17 were previously assigned.[20] Absolute configurations (R)-(-)-16, (S)-(+)-12, (S)-(+)-13 and (S)-(+)-14 were attributed by chemical correlations with (R)-(-)-5[11] and (S)-(+)-4[11] respectively. Absolute configurations (S)-(+)-15 and (R)-(-)-19 were assigned by comparing their sign of rotary power with that of the above-reported 3-substituted-Δ^2-isoxazolines.

(R)-(-)-17: $[\alpha]_{20}^D$-25.75 (c0.750, CHCl$_3$); e.e.% = 36.

(S)-(+)-11: $[\alpha]_{20}^D$+29.25 (c0.810, CHCl$_3$); e.e.% = 71.

(R)-(-)-**19**: $[\alpha]_{20}{}^D$-42.94 (c0.815, CHCl$_3$); e.e.% = 73.

(S)-(+)-**15**: $[\alpha]_{20}{}^D$+19.72 (c0.710, CHCl$_3$); e.e.% = 21.

(S)-(+)-**22**: $[\alpha]_{20}{}^D$+2.98 (c0.852, MeOH); e.e.% = 16.

(R)-(-)-**14**: $[\alpha]_{20}{}^D$-5.09 (c0.820, CHCl$_3$); e.e.% = 21.

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