Nitrile Oxides in Medicinal Chemistry. 4.[†,‡] Chemoenzymatic Synthesis of Chiral Heterocyclic Derivatives

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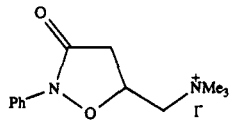
The two enantiomers of 3-bromo-5-(hydroxymethyl)-Δ^2-isoxazoline (1) and 2-phenyl-5-(hydroxymethyl)-isoxazolidin-3-one (9) have been prepared in enantiomeric excess higher than 90% by hydrolysis of the corresponding butyrates under the catalysis of lipase PS, which was the most selective catalyst of the enzymes tested. The pairs of enantiomers of 1 and 9 were transformed into the chiral forms of the potent muscarinic ligands 3 and 5. The results obtained with the homogeneous set of esters 6, 7, 10a–d evidence a strong dependence of reaction rate and enantioselectivity of the lipase PS-catalyzed transformations upon both the size of the acyl moiety and the shape of the group carrying the alcoholic part of the ester. In the series of esters 10a–d, the best results were obtained with butyrate 10b. Quite interestingly, on passing from the butyrate of 1 to that of 9, the value of the enantiomeric ratio remained remarkably high but the enantiopreference switched from *R* to *S*. In between lies the butyrate of 2-methyl-5-(hydroxymethyl)isoxazolidin-3-one [(±)-6] which was barely recognized by lipase PS and yielded alcohol (*R*)-(−)-2 in a modest enantiomeric excess.

Introduction

The isoxazoline nucleus constitutes the basic skeleton of derivatives provided with a variety of biological activities.[1,2] Some years ago we started a program based on the use of nitrile oxide chemistry as a tool for the synthesis of biologically active compounds.[3–9] In such a context, bromonitrile oxide turned out to be the most versatile nitrile oxide.[6–9] As shown in Scheme I, its 1,3-dipolar cycloaddition to allyl alcohol represents the key step in the reaction sequence to (±)-3, a potent muscarinic-receptor agonist.[7] The same strategy applied to (*S*)-(+)-isopropylidene-3-butene-1,2-diol yielded the two enantiomers of 3 in high enantiomeric excess (Scheme I).[8] The major drawback of this methodology is the rather laborious manipulation necessary to transform cycloadducts 4a and 4b into key intermediates (*S*)-(+)-1 and (*R*)-(−)-1. Furthermore, the relative availability of these intermediates is strictly dependent upon the stereoselectivity of the cycloaddition step (4a/4b = 76/24).[9]

As part of a program devoted to the application of bioconversion processes to asymmetric synthesis of chiral compounds endowed with biological activity,[10–12] we now describe a practical and straightforward approach to the enantiomers of 3 via enzyme-catalyzed resolution of suitable precursors. This paper reports the results of a study on the enzymatic resolutions of intermediates (±)-1 and (±)-2, which provided the synthesis of (*R*)-(−)-3 and (*S*)-(+)-3 in very high optical purity through a simplified procedure. The same methodology was also applied to the preparation of the enantiomers of 5, designed as a potent antimuscarinic agent.



(±)-5

Table I. Enzyme-Catalyzed Transformations of Substrates (±)-1, (±)-6, and (±)-7

substrate	enzyme	E^a	deg of convn (%)	confign of alcohol (ee, %)	confign of ester (ee, %)
(±)-1[b]	lipase PS	14	31		*R* (81)
(±)-1[c]	lipase PS	16	54	*S* (84)	
(±)-6	lipase PS	2.7	40	*R* (36)	
			51		*S* (38)
(±)-6	C.E. NP	13	40	*R* (77)	
			55		*S* (83)
(±)-7	C.E. NP	26	37	*R* (88)	
			55		*S* (94)
(±)-7	lipase PS	60	39	*R* (94)	
			55		*S* (>99)
(±)-7	PPL	2.3	31	*R* (33)	
			61		*S* (41)
(±)-7	CCL	2.1	33	*R* (32)	
			57		*S* (33)

[a] The values of *E* were calculated according to the equations reported in ref 13. [b] The acylating agent is trifluoroethyl butyrate. [c] The acylating agent is vinyl acetate.

Results and Discussion

As the first approach to the synthesis of the two enantiomers of 3, we chose to investigate the enzyme-catalyzed

[†] Dedicated to the memory of professor Davide Pitrè.

[‡] Part 3: De Amici, M.; De Micheli, C.; Grana, E.; Lucchelli, A.; Zonta, F. *Il Farmaco* **1990**, *45*, 859–866.

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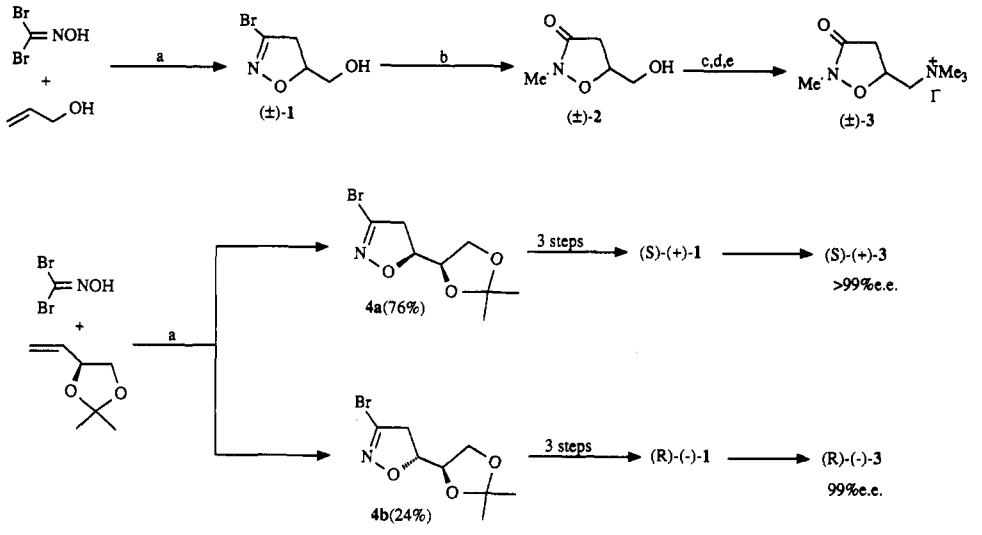
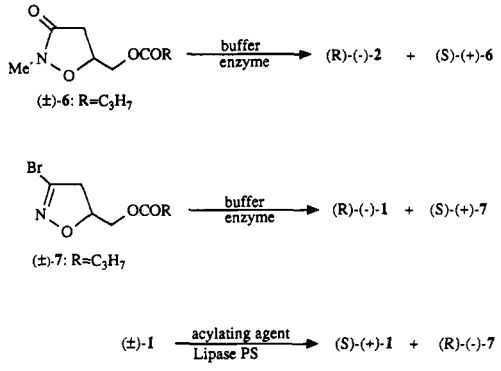
Scheme I[a]



[a] Key: (a) $NaHCO_3$/EtOAc; (b) $Me_3O^+BF_4^-$; (c) Tf_2O/Py; (d) $NHMe_2$/MeOH; (e) MeI.

Scheme II



hydrolyses of (±)-6 (Scheme II), whose alcohol (±)-2 is the most advanced intermediate in the reaction sequence. All the enzymes tested evidenced little activity and modest enantioselectivity for (±)-6 which precluded any synthetic application; Table I exemplifies the results obtained with lipase PS and carboxylesterase NP. Therefore, we discontinued the research on (±)-6 and turned our attention to (±)-1, the immediate precursor of (±)-2. Butyrate (±)-7 was hydrolyzed in a phosphate buffer solution (pH 7) with catalysis by the enzymes listed in Table I. The degree of conversion and the enantiomeric excess (ee) were evaluated by chiral HPLC which gave base-line separation for both the enantiomers of the produced alcohol and residual substrate (see Experimental Section). The absolute configuration of the resulting alcohol had previously been assigned.[9]

Among the enzymes tested, porcine pancreatic lipase (PPL) and *Candida cylindracea* lipase (CCL) showed a negligible propensity to discriminate between the enantiomers of (±)-7 (E = 2.3 and 2.1) whereas carboxylesterase NP- and especially lipase PS-catalyzed hydrolysis proceeded with remarkable degrees of enantioselectivity (Table I) to yield alcohol (*R*)-(−)-1. For preparative purposes, we selected lipase PS, the most specific catalyst. The hydrolysis of (±)-7 was stopped at 39% conversion and the optically enriched alcohol [(*R*)-(−)-1, 94% ee] was recovered. The residual ester was subjected to a second enzyme-catalyzed hydrolysis to yield, at 55% overall conversion, (*S*)-(+)-7 as a single enantiomer (ee >99%). (*S*)-(+)-7 was then quantitatively transformed into (*S*)-(+)-1 by chemical hydrolysis under standard conditions. The pair of enantiomeric alcohols was transformed into the final derivatives (*S*)-(+)-3 and (*R*)-(−)-3 through the reaction sequence previously reported.[8] Their specific rotations matched those reported for these compounds.[8] The present route, based on the lipase PS kinetic resolution of (±)-7, constitutes, therefore, a profitable alternative to the sequence based on the asymmetric induction caused by a chiral dipolarophile.[8] Resolution of (±)-1 was also attempted by lipase PS-catalyzed transesterification with trifluoroethyl butyrate or vinyl acetate as acylating agents in an organic solvent (toluene). The enantioselectivities (E = 14 and 16) were, in both cases, lower than those obtained by the more conventional hydrolysis of butyrate (±)-7.

The easy access to large quantities of the enantiomers of 1 induced us to make use of such intermediates for the preparation of the enantiomers of 5, designed as a new antimuscarinic agent.

Since the synthesis of *N*-methylisoxazolidin-3-one 3 has been efficiently accomplished by methylation of 3-bromo-5-(hydroxymethyl)-Δ^2-isoxazoline (1) followed by treatment with a base,[7] in our first approach to derivative 5 we explored the possibility of adding electrophiles to different 3-bromoisoxazolines (see Experimental Section). Among the tested electrophiles, trimethyloxonium tetrafluoroborate was the most efficient alkylating agent with all the substrates and produced *N*-methylisoxazolidin-3-ones in 82–88% yield; alkyl triflates were also very effective in some instances. On the contrary, phenyl trifluoromethanesulfonate turned out to be completely unreactive with all the substrates, even in refluxing 1,2-dichloroethane. The inability of such an excellent electrophile as phenyl triflate to arylate 3-bromoisoxazolines forced us to develop a new strategy for the synthesis of derivative 5. It has been reported that *N*-benzylisoxazolidin-3-ones can be prepared by cyclization of functionalized *N*-benzylhydroxamic acids.[14] Following the same strategy, chlorosuccinic acid monobenzyl ester was transformed into isoxazolidin-3-one (±)-9 through the intermediacy of hydroxamic acid (±)-8 (Scheme III). Intermediate 9 was transformed into the final trimethylammonium derivative (±)-5 through the reaction sequence reported in the same scheme.

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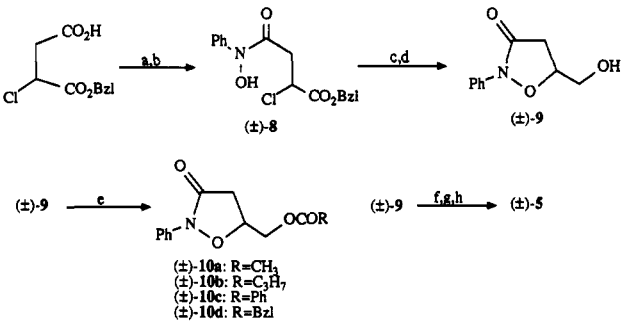
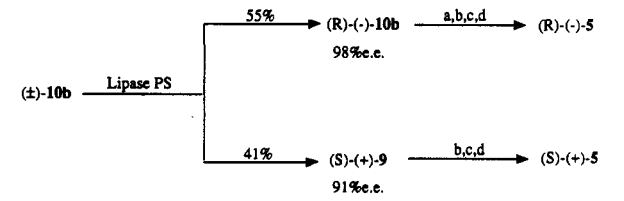
Scheme III[a]



[a] Key: (a) $SOCl_2$; (b) PhNHOH; (c) $NaHCO_3$–H_2O; (d) $LiBH_4$; (e) RCOCl–Py; (f) Tf_2O–Py; (g) $NHMe_2$; (h) MeI.

Scheme IV[a]



[a] Key: (a) PPL-catalyzed hydrolysis; (b) Tf_2O–Py; (c) $NHMe_2$–MeOH; (d) MeI–acetone.

Table II. Lipase PS- and Carboxylesterase NP (C.E. NP)-Catalyzed Hydrolysis of Esters 10a–d

substrate	enzyme	E	convn (%)	confign of alcohol 9 (ee, %)	confign of residual ester (ee, %)
10a	lipase PS	18	32	*S* (85)	
			54		*R* (86)
	C.E. NP	23	40	*R* (86)	
			58		*S* (97)
10b	lipase PS	41	41	*S* (91)	
			55		*R* (98)
	C.E. NP	8	40	*R* (68)	
			53		*S* (70)
10c	C.E. NP	8	40	*R* (68)	
			57		*S* (75)
10d	lipase PS	2.1	21	*S* (32)	
	C.E. NP	16	22	*R* (85)	
			56		*S* (89)

Subsequently, the synthesis of the two enantiomers of **5** was attempted by enzymatic hydrolysis of esters (±)-**10a–d**. In analogy, Watanabe et al.[15,16] prepared the active form of β-blockers through the asymmetric hydrolysis of (±)-5-[(acyloxy)methyl]-3-alkyl-2-oxazolidinones. Derivatives **10a–d** were prepared (Scheme III) and submitted to hydrolysis catalyzed by either lipase PS or carboxylesterase NP (Table II). The degrees of conversion and the ee for both the alcohol and residual ester were determined by chiral HPLC. The *R* configuration for the levorotatory ammonium salt (−)-**5** was preliminarily inferred from analogy with the structurally related (*R*)-(−)-**3** and was subsequently secured by single-crystal X-ray diffraction analysis (Figure 1). The series of data collected in Table II shows that the most favorable way for synthesis of the enantiomers of **9** was lipase PS-catalyzed hydrolysis of (±)-**10b**. For preparative purposes, (±)-**10b** was submitted to this catalyzed hydrolysis in a buffer medium at pH 7 and the reaction was interrupted at 41% conversion

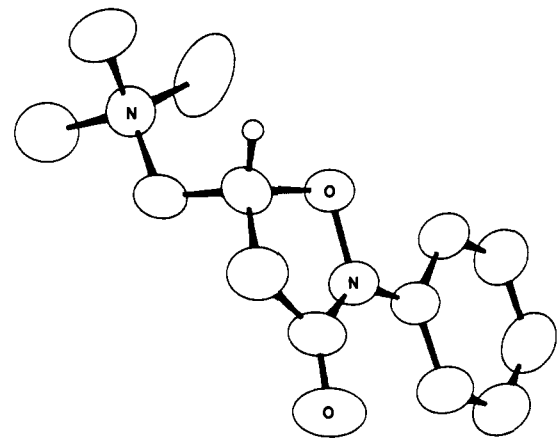


Figure 1. X-ray crystal structure of (−)-**5**. Hydrogens have been omitted except at the stereogenic center.

Table III. Relative Rates of the Lipase PS and Carboxylesterase NP (C.E. NP) Hydrolyses of Esters 6, 7, 10a–d

substrate	lipase PS rel rate	E	confign of alcohol	C.E. NP rel rate	E	confign of alcohol
(±)-**6**	1	2.7	*R*	35	13	*R*
(±)-**7**	100	60	*R*	100	26	*R*
(±)-**10a**	8	18	*S*	100	23	*R*
(±)-**10b**	20	41	*S*	60	8	*R*
(±)-**10c**				15	8	*R*
(±)-**10d**	0.2	2.1	*S*	75	16	*R*

(Scheme IV). Alcohol (*S*)-(+)-**9** (91% ee) was separated from the remaining ester which was hydrolyzed up to 55% overall conversion. The ee value of the recovered ester (*R*)-(−)-**10b** was 98%. Since this ester is particularly labile in alkaline conditions, it was hydrolyzed to (*R*)-(−)-**9** in a buffer solution at pH 7 in the presence of the aspecific porcine pancreatic lipase (PPL). Intermediates (−)-**9** and (+)-**9** were then transformed into the final derivatives (*R*)-(−)-**5** and (*S*)-(+)-**5** (Scheme IV), without any loss of optical purity, according to the protocol previously applied to **3**.[7,8]

In Table III the major features of the hydrolyses catalyzed by lipase PS and carboxylesterase NP (C.E. NP) are collected. It is worth pointing out that, among this set of data, carboxylesterase NP-catalyzed hydrolyses are modestly influenced by structural variations in both the alcohol and ester moieties and always produce the (*R*)-alcohol. On the contrary, the hydrolyses catalyzed by lipase PS are influenced either by a variation in the size of the acyl group, i.e., from **10a** to **10d**, or by a change in the shape of the heterocycle that carries the alcohol moiety, i.e., the three butyrates (±)-**6**, (±)-**7**, and (±)-**10b**. The major characteristic of these reactions is the sharp change in the enzyme enantiopreference on passing from (±)-**7** (E = 60 for *R*) to (±)-**10b** (E = 41 for *S*); butyrate (±)-**6** lies in between being poorly discriminated by lipase PS (E = 2.7) (Table III). In any case, the overall results could hardly be predicted by the current active-site models, developed either for secondary alcohols[17,18] or for α-hydroxycarboxylic esters.[19]

To conclude, the synthesis of the enantiomers of the potent muscarinic ligands **3** and **5** has been efficiently

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accomplished by lipase PS-catalyzed hydrolysis of the butyrates of suitable precursors. The present results represent a further example of the usefulness and versatility of the chemoenzymatic approach to the preparation of the chiral form of biologically active compounds.

Experimental Section

Materials and Methods. Carboxylesterase NP was donated by International Bio-Synthetics (I.B.I.S., The Netherlands). Lipase PS (from *Pseudomonas cepacia*) was purchased from Amano; porcine pancreatic lipase (PPL) and *C. cylindracea* lipase (CCL) were bought from Sigma. Organic solvents were reagent grade. ^{1}H NMR spectra were recorded in $CDCl_3$. GLC analyses were carried out on a 5-m HP1 capillary silica gel column coated with methylsilicone gum. Chiral HPLC analyses were performed on a Chiralcel OB column (4.6 × 250 mm) at a flow rate of 0.5 mL/min. The composition of the mobile phase is specified in the appropriate paragraph. TLC were carried out on commercial silica gel GF_{254} plates. Melting points are uncorrected. Liquids were characterized by the oven temperature for bulb to bulb distillations.

X-ray Analysis. A crystal of (−)-**5** was mounted on a X-ray diffractometer equipped with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) and using the $\omega - 2\theta$ scan technique. Enantiomer (−)-**5**, $C_{13}H_{19}IN_2O_2$, crystallizes in the orthorombic space group $P2_12_12_1$ with a = 7.652 (1) Å, b = 12.261 (2) Å, c = 32.557 (3) Å, Z = 8, and d_{calc} = 1.575 g/cm^3. In order to obtain the absolute configuration of this compound, two whole octants of hkl were collected up to θ = 26.0°. A total of 5985 independent reflections were obtained over the ranges: $-9 \le h \le 9$, $0 \le k \le 15$, $0 \le l \le 40$ of which 4691 with $I > \sigma(I)$ were used in the refinement. The structure was solved by standard heavy atom Patterson methods followed by weighted Fourier synthesis. Refinement was by full-matrix least-squares techniques, minimizing the quantity $\Sigma w(|F_0| - k|F_c|)^2$, with weight $w = 1/\sigma^2(I)$. Non-hydrogen atoms were refined anisotropically. Scattering factors including anomalous dispersion terms were taken from International *Tables for X-ray Crystallography* (Vol. IV, Tables 2.2B and 2.3.1). Both structures (−)-**5** and (+)-**5** were refined. The (−)-structure converged to R = 0.0457 and R_w = 0.0450, while the (+)-structure converged to R = 0.0488 and R_w = 0.0487. This means that the (−)-structure is the correct one with a level of confidence >0.999.[20] Bond lengths and angles are in the normal range. The two independent molecules of the crystal cell possess different conformations. In particular, their heterocyclic ring has an ^5E and E_5 conformation respectively.

General Procedure for the Alkylation of 3-Bromo-5-substituted-Δ^2-isoxazolines. A. A solution of 2.5 mmol of 3-bromo-5-substituted-Δ^2-isoxazoline[9] in nitromethane (10 mL) was treated with 3–5 mmol of trimethyloxonium tetrafluoroborate and stirred overnight at room temperature. To the reaction mixture was added an aqueous slurry of potassium carbonate, and the resulting suspension was stirred until evolution of gas ceased (30 min). After evaporation of the solvent the residue was column chromatographed to give the products in 82–88% yield.

B. A solution of a Δ^2-isoxazoline (2.5 mmol) and an excess alkyl trifluoromethanesulfonate (3–5 mmol) in dichloromethane (20 mL) was stirred at room temperature until disappearance of the starting material (24–48 h). The reaction mixture was reacted with wet potassium carbonate and worked up according to the above-reported procedure. Phenyl trifluoromethanesulfonate[21] failed to react with all the substrates even at reflux in 1,2-dichloroethane. R_f values of the N-alkyl derivatives were measured using either *n*-hexane/ethyl acetate (3:2) (method a) or pure ethyl acetate (method b) as the mobile phase.

2-Methyl-5-phenylisoxazolidin-3-one: bp 150–155 °C/0.8 mmHg; R_f(a) 0.38; ^{1}H NMR δ 2.81 (dd, 1, H-4′; J = 9.6 and 16.7 Hz), 3.12 (dd, 1, H-4; J = 8.0 and 16.7 Hz), 3.23 (s, 3, NMe), 5.48 (dd, 1, H-5; J = 8.0 and 9.6 Hz), 7.42 (m, 5, arom).

2-Ethyl-5-phenylisoxazolidin-3-one: bp 145–150 °C/0.4 mmHg; R_f(a) 0.48; ^{1}H NMR δ 1.27 (t, 3, CH_2CH_3; J = 7.2 Hz), 2.85 (dd, 1, H-4′; J = 8.4 and 17.0 Hz), 3.09 (dd, 1, H-4; J = 9.6 and 17.0 Hz), 3.68 (q, 2, NCH_2; J = 7.2 Hz), 5.43 (dd, 1, H-5, J = 8.4 and 9.6 Hz), 7.42 (m, 5, arom).

2-Methyl-5-*n*-butylisoxazolidin-3-one: bp 75–80 °C/0.5 mmHg; R_f(a) 0.43; ^{1}H NMR δ 0.95 (t, 3, CH_2CH_3; J = 6.3 Hz), 1.13–2.05 (m, 6, $(CH_2)_3$), 2.43 (dd, 1, H-4′; J = 11.0 and 16.2 Hz), 2.79 (dd, 1, H-4; J = 7.8 and 16.2 Hz), 3.17 (s, 3, NMe), 4.15–4.80 (m, 1, H-5).

2-Ethyl-5-*n*-butylisoxazolidin-3-one: bp 85–90 °C/0.5 mmHg; R_f(a) 0.50; ^{1}H NMR δ 0.70–1.9 (m, 12, NCH_2CH_3 and $(CH_2)_3CH_3$), 2.43 (dd, 1, H-4′; J = 9.4 and 16.6 Hz), 2.78 (dd, 1, H-4; J = 7.4 and 16.6 Hz), 3.57 (q, 2, NCH_2; J = 7.0 Hz), 3.95–4.75 (m, 1, H-5).

2-Methyl-5-(hydroxymethyl)isoxazolidin-3-one (2):[7] bp 180–185 °C/1 mmHg; R_f(b) 0.19.

2-Ethyl-5-(hydroxymethyl)isoxazolidin-3-one: bp 185–190 °C/0.8 mmHg; R_f(b) 0.27; ^{1}H NMR δ 1.22 (t, 3, Me; J = 7.2 Hz), 2.48 (bs, 1, OH), 2.80 (m, 2, H-4 and H-4′), 3.62 (q, 2, NCH_2; J = 7.2 Hz), 3.80 (m, 2, CH_2O), 4.63 (m, 1, H-5).

Synthesis of (±)-2-Phenyl-5-(hydroxymethyl)isoxazolidin-3-one [(±)-9]. A. A mixture of 1-benzyl-2-chlorosuccinic acid[14] (4.85 g, 20 mmol) and thionyl chloride (2.25 mL, 27.6 mmol) was heated at 70 °C for 2 h under inert atmosphere. Excess $SOCl_2$ was removed under reduced pressure, and the resulting oil was not characterized but directly used in the following step.

B. A solution of the crude acid chloride (4.80 g, 18.4 mmol) in diethyl ether (30 mL) was cooled at 0 °C and stirred under nitrogen during the dropwise addition of a solution of freshly prepared *N*-phenylhydroxylamine[22] (4.01 g, 36.8 mmol) in diethyl ether (20 mL). At the end of the addition, the slurry was stirred for additional 15 min and then diluted with diethyl ether (80 mL). The clear solution resulting from a filtration under vacuum was washed with 1 N HCl (2 × 10 mL) and the crude 3-(benzyloxycarbonyl)-3-chloro-*N*-phenylpropiono-*N*-hydroxamic acid [(±)-**8**] was used without any further purification.

C. The ethereal solution of hydroxamic acid (±)-**8** was shaken with a saturated aqueous solution of sodium bicarbonate (100 mL) and stirred overnight at room temperature. The organic layer was separated, and the aqueous phase was extracted "in continuum" with diethyl ether. The pooled organic extracts were dried and concentrated. The residue was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate (3:1)) to give 2.85 g (48% overall yield) of benzyl 2-phenyl-3-oxo-5-isoxazolidinecarboxylate as a thick yellow oil which solidified when taken up with diisopropyl ether; mp 66–67 °C; R_f 0.55 (benzene/diethyl ether (4:1)); ^{1}H NMR δ 3.13 (dd, 1, H-4′; J = 6.0 and 17.6 Hz), 3.45 (dd, 1, H-4; J = 8.4 and 17.6 Hz), 5.12 (dd, 1, H-5; J = 6.0 and 8.4 Hz), 5.28 (s, 2, CH_2Ar), 7.10–7.95 (m, 10, arom).

D. To a solution of benzyl (±)-2-phenyl-3-oxo-5-isoxazolidinecarboxylate (2.5 g, 8.42 mmol) and lithium chloride (0.714 g, 16.84 mmol) in THF/methanol (4:3) was added sodium borohydride (0.637 g, 16.84 mmol) portionwise under stirring. After the addition was completed, the reaction mixture was stirred for an additional 10 min. The reaction was quenched by the addition of 1 N HCl (pH 4.5), the solvents were removed at reduced pressure, and the resulting slurry was flash-chromatographed (eluent: ethyl acetate). Alcohol (±)-**9** (0.991 g, 61%) was collected as a viscous colorless oil. An attempted distillation under vacuum failed due to its lability to heating: R_f 0.61 (eluent: ethyl acetate); ^{1}H NMR δ 1.58 (s, 1, OH), 2.97 (dd, 1, H-4′; J = 8.2 and 16.5), 3.06 (dd, 1, H-4; J = 8.4 and 16.5), 3.89 (m, 2, CH_2O), 4.82 (m, 1, H-5), 7.10–7.80 (m, 5, arom).

(±)-2-Phenyl-5-[(dimethylamino)methyl]isoxazolidin-3-one Methiodide Salt [(±)-5]. A. To a cold (−25 °C) and stirred solution of (±)-**9** (0.425 g, 2.20 mmol) and pyridine (178 μL, 2.20 mmol) in anhydrous dichloromethane (15 mL) was added trifluoromethanesulfonic anhydride (444 μL, 2.64 mmol) dropwise in 10 min under nitrogen. The disappearance of the starting material was checked by TLC (eluent: ethyl acetate). The mixture was washed with cold 1 N HCl (2 × 5 mL) and, after the usual workup, a methanolic solution (10 mL) of the crude triflate (R_f 0.66 cyclohexane/ethyl acetate (2:3)) was immediately reacted

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with excess dimethylamine at −15 °C. The solution was stirred for 3 h, the solvent was removed at reduced pressure, and the residue was treated with water (10 mL) and extracted with dichloromethane (3 × 5 mL). The product was purified by column chromatography (eluent: dichloromethane/methanol 9:1, R_f 0.53) to yield 0.334 g (69%) of a dark yellow oil.

(±)-2-Phenyl-5-[(dimethylamino)methyl]isoxazolidin-3-one: bp 160–165 °C/0.8 mmHg; ^{1}H NMR δ 2.38 (s, 6, NMe_2), 2.62–3.05 (m, 4, H-4, H-4′ and CH_2N), 4.86 (m, 1, H-5), 7.10–8.05 (m, 5, arom).

B. A solution of the tertiary base (0.25 g, 1.14 mmol) in acetone/ether (1:3) was treated with excess methyl iodide. The resulting ammonium salt separated quantitatively from the solution. **(±)-5** crystallized as colorless needles from absolute ethanol: mp 210–222 °C dec; ^{1}H NMR (D_2O) δ 2.95 (dd, 1, H-6′; J = 8.4 and 17.0), 3.24 (s, 9, NMe_3), 3.30 (dd, 1, H-6; J = 8.3 and 17.0), 3.70 (d, 1, H-4′; J = 14.6), 4.00 (dd, 1, H-4; J = 9.7 and 14.6), 5.47 (m, 1, H-5); 7.28–7.60 (m, 5, arom).

Standard Procedure for the Synthesis of Esters (±)-6, (±)-7, and (±)-10a–d. To a solution of (±)-1 (2 or 9) (10 mmol) and pyridine (1.6 mL, 20 mmol) in anhydrous dichloromethane (30 mL) was added dropwise a dichloromethane solution (5 mL) of the appropriate acyl chloride (15–20 mmol) at 0 °C. The disappearance of the starting material was monitored by TLC (eluent: ethyl acetate). The mixture was washed with a 20% aqueous solution of $CuSO_4$ (3 × 10 mL), and the organic phase was dried and concentrated. The residue was purified by column chromatography (eluent: cyclohexane/ethyl acetate (3:1)) to give the desired esters in 79–92% yield.

(±)-2-Methyl-5-(hydroxymethyl)isoxazolidin-3-one butyrate [(±)-6]: bp 135-140 °C/0.6 mmHg; ^{1}H NMR δ 0.96 (t, 3, CH_2CH_3; J = 7.0 Hz), 1.68 (m, 2, CH_2CH_3), 2.35 (t, 2, $OCOCH_2$; J = 7.1 Hz), 2.77 (m, 2, H-4 and H-4′), 3.15 (s, 3, NMe), 4.27 (m, 2, CH_2O), 4.71 (m, 1, H-5).

(±)-3-Bromo-5-(hydroxymethyl)-Δ²-isoxazoline butyrate [(±)-7]: bp 125–130 °C/0.3 mmHg; ^{1}H NMR δ 0.96 (t, 3, CH_2CH_3; J = 7.0 Hz), 1.70 (m, 2, CH_2CH_3), 2.35 (t, 2, $OCOCH_2$; J = 7.0 Hz), 3.06 (dd, 1, H-4′; J = 7.6 and 16.6 Hz), 3.32 (dd, 1, H-4; J = 9.2 and 16.6 Hz), 4.25 (m, 2, CH_2O), 4.90 (m, 1, H-5).

(±)-2-Phenyl-5-(hydroxymethyl)isoxazolidin-3-one acetate [(±)-10a]: 160–164 °C/0.7 mmHg; ^{1}H NMR δ 2.08 (s, 3, CH_3), 2.90 (dd, 1, H-4′; J = 8.0 and 18.2 Hz), 3.09 (dd, 1, H-4; J = 8.6 and 18.2 Hz), 4.38 (m, 2, CH_2O), 4.90 (m, 1, H-5), 7.10–7.90 (m, 5, arom).

(±)-2-Phenyl-5-(hydroxymethyl)isoxazolidin-3-one butyrate [(±)-10b]: bp 160–165 °C/0.5 mmHg; ^{1}H NMR δ 0.96 (t, 3, CH_2CH_3; J = 7.1 Hz), 1.67 (m, 2, CH_2CH_3), 2.37 (t, 2, $OCOCH_2$; J = 7.0 Hz), 2.89 (dd, 1, H-4; J = 8.6 and 17.0 Hz), 3.09 (dd, 1, H-4′; J = 8.0 and 17.0 Hz), 4.38 (m, 1, H-5), 4,88 (m, 1, H-5), 7.05–7.90 (m, 5, arom).

(±)-2-Phenyl-5-(hydroxymethyl)isoxazolidin-3-one benzoate [(±)-10c]: ^{1}H NMR δ 3.01 (dd, 1, H-4′; J = 8.0 and 17.0 Hz), 3.25 (dd, 1, H-4; J = 7.8 and 17.0 Hz), 4.60 (m, 2, CH_2O), 5.04 (m, 1, H-5), 7.10–8.05 (m, 10, arom).

(±)-2-Phenyl-5-(hydroxymethyl)isoxazolidin-3-one phenylacetate [(±)-10d]: ^{1}H NMR δ 2.84 (dd, 1, H-4′; J = 7.8 and 16.8 Hz), 3.05 (dd, 1, H-4; J = 8.0 and 16.8 Hz), 3.67 (s, 2, $OCOCH_2$), 4.39 (m, 2, CH_2O), 4.83 (m, 1, H-5), 7.0–7.85 (m, 10, arom).

Standard Procedure for the Lipase PS-Catalyzed Hydrolyses of (±)-6, (±)-7, and (±)-10a,b,d. The following procedure is representative. A 500-mL Erlenmeyer flask was charged with (±)-7 (2 g, 8 mmol), lipase PS (8 mg), 0.1 M potassium phosphate buffer, pH 7 (240 mL), and acetone (40 mL). The mixture was stirred at room temperature for 65 min (39% conversion) and then extracted with ethyl acetate (5 × 70 mL). After the usual workup, the organic extracts were column chromatographed (eluent: n-hexane/ethyl acetate (7:3)) to yield (*R*)-(−)-1 (0.55 g, 3.05 mmol): bp 160–165 °C/1 mmHg; $[\alpha]^{20}_D$ −130.25 (*c* 1.01, $CHCl_3$) [lit.[8] $[\alpha]^{20}_D$ −138.81 (*c* 1.054, $CHCl_3$)].

The unreacted butyrate (1.12 g) was resubmitted to enzymatic hydrolysis until 55% total conversion. The mixture was worked up as reported above, and the residue was chromatographed to yield (*S*)-(+)-7 (0.84 g, 3.36 mmol) as a colorless oil: $[\alpha]^{20}_D$ +98.79 (*c* 0.992, $CHCl_3$).

Chiral HPLC: wavelength, 215 nm; eluent, n-hexane/n-propanol (4.5:1). Retention times: (*R*)-(−)-1, 23.5 min; (*S*)-(+)-1, 27.9 min; (*S*)-(+)-7, 34.1 min; (*R*)-(−)-7, 43.7 min.

The same protocol was applied to the lipase PS-catalyzed hydrolyses of **(±)-6**, **(±)-10a**, **(±)-10b**, and **(±)-10d** and to the hydrolyses catalyzed by carboxylesterase NP, PPL, and CCL. The results are reported in Tables I and III.

Chiral HPLC of compounds **2** and **6**: wavelength, 220 nm; eluent, n-hexane/n-propanol (7:3). Retention times: (*R*)- and (*S*)-**2**, 12.4 min; (*R*)-(−)-**6**, 21.8 min; (*S*)-(+)-**6**, 24.8 min.

Chiral HPLC of compounds **9** and **10a–d**: wavelength, 254 nm; eluent, n-hexane/ethanol (9:1). Retention times: (*S*)-(+)-**9**, 31.2 min; (*R*)-(−)-**9**, 38.1 min; (*S*)-(+)-**10a**, 66.1 min; (*R*)-(−)-**10a**, 94.8 min; (*S*)-(+)-**10b**, 57.5 min; (*R*)-(−)-**10b**, 60.7 min; (*S*)-(+)-**10c**, 146.9 min; (*R*)-(−)-**10c**, 156.3 min; (*S*)-(+)-**10d**, 143.6 min; (*R*)-(−)-**10d**, 152.7 min.

(*S*)-(+)-**9**: $[\alpha]^{20}_D$ +12.33 (*c* 1.01, MeOH).

(*R*)-(−)-**10b**: $[\alpha]^{20}_D$ −19.19 (*c* 0.964, MeOH).

Lipase PS-Catalyzed Transesterifications of (±)-1. A 0.500-g (2.78-mmol) portion of (±)-1 in 100 mL of toluene was reacted under stirring with 2 equiv of trifluoroethyl butyrate (or vinyl acetate) in the presence of 0.5 g of lipase PS and 5 g of molecular sieves (3 Å). The progress of the reaction was monitored by chiral HPLC, and the results are reported in Table I.

Synthesis of (*S*)-(+)-3 and (*R*)-(−)-3. A 0.84-g (3.36-mmol) portion of (*S*)-(+)-7 was dissolved in methanol (15 mL) and treated with a 20% aqueous solution of potassium carbonate (7 mL). The mixture was stirred at room temperature until disappearance of the starting material (3 h), and then methanol was evaporated at reduced pressure and the aqueous phase was thoroughly extracted with dichloromethane. (*S*)-(+)-1 was obtained in 87% yield.

(*S*)-(+)-1: $[\alpha]^{20}_D$ +140.87 (*c* 0.91, $CHCl_3$) [lit.[8] $[\alpha]^{20}_D$ + 141.44 (*c* 0.934, $CHCl_3$)].

(*S*)-(+)-1 and (*R*)-(−)-1 were transformed into final derivatives (*S*)-(+)-3 and (*R*)-(−)-3 through the reaction sequence previously described.[8]

(*S*)-(+)-3: $[\alpha]^{20}_D$ +34.28 (*c* 0.726, MeOH) [lit.[8] $[\alpha]^{20}_D$ +34.75 (*c* 0.315, MeOH)].

(*R*)-(−)-3: $[\alpha]^{20}_D$ −32.77 (*c* 0.637, MeOH) [lit.[8] $[\alpha]^{20}_D$ −34.18 (*c* 0.32, MeOH)].

Synthesis of (*R*)-(−)-5 and (*S*)-(+)-5. A solution of (*R*)-(−)-**10b** (0.580 g, 2,31 mmol) in acetone (10 mL) and 0.1 M potassium phosphate buffer, pH 7 (100 mL) was hydrolyzed in the presence of PPL (0.5 g). The substrate was completely hydrolyzed in about 5 h. The mixture was thoroughly extracted with ethyl acetate (4 × 25 mL), and (*R*)-(−)-**9** was collected as a colorless oil (0.365 g, 87% yield): $[\alpha]^{20}_D$ −12.50 (*c* 1.02, MeOH).

Intermediates (*R*)-(−)-**9** and (*S*)-(+)-**9** were transformed into the final derivatives (*R*)-(−)-**5** and (*S*)-(+)-**5** through the reaction sequence described in a previous section for the racemic form.

(*R*)-(−)-2-Phenyl-5-[(dimethylamino)methyl]isoxazolidin-3-one: pale yellow oil; bp 160–165 °C/0.8 mmHg; $[\alpha]^{20}_D$ −7.74 (*c* 0.994, $CHCl_3$).

(*R*)-(−)-5: colorless needles from absolute methanol; mp 212–220 °C dec; $[\alpha]^{20}_D$ −12.19 (*c* 0.746, MeOH).

(*S*)-(+)-2-Phenyl-5-[(dimethylamino)methyl]isoxazolidin-3-one: $[\alpha]^{20}_D$ +7.96 (*c* 1.03, MeOH).

(*S*)-(+)-5: $[\alpha]^{20}_D$ +11.63 (*c* 0.748, MeOH).

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Supplementary Material Available: Tables of X-ray final atomic positional coordinates, atomic thermal parameters, bond distances, and bond angles of compound (−)-**5** and analytical data of the new compounds as well as the ^{1}H NMR spectra of (+)-**9** and (±)-**5** (14 pages). Ordering information is given on any current masthead page.