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Resolution of Δ^2-Isoxazoline-5-carboxylates by a Protease from *Aspergillus Oryzae* Providing Masked Synthons for Enantiopure β-Aminoalcohols and Related Structures [1]

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Summary. A series of racemic Δ^2-isoxazolinecarboxylates have been synthesized and subjected to enzymatic hydrolysis by a protease from *Aspergillus oryzae* in a two-phase system. Out of these compounds only isoxazoline-5-carboxylates unsubstituted at C-4 were hydrolyzed. Thus, from 3-ethoxycarbonyl-, 3-methyl-, and 3-phenyl-Δ^2-isoxazoline-5-carboxylates the corresponding (*R*)-configurated carboxylic acids are obtained. In contrast, an additional methyl group at C-5 changes the steric course of the hydrolysis to give predominantly the (*S*)-acid. The enantioselectivities obtained are in the range of $E = 5$–35.

Keywords. *Aspergillus oryzae*; Chemoenzymatic synthesis; Enantioselective hydrolysis; Δ^2-Isoxazoline-carboxylates; Protease.

Racematspaltung von Δ^2-Isoxazolin-5-carboxylaten mittels einer Protease von *Aspergillus oryzae* zur Darstellung maskierter Synthons für enantiomerenreine β-Aminoalkohole und verwandte Strukturen

Zusammenfassung. Zahlreiche Δ^2-Isoxazolincarbonsäureester wurden in racemischer Form dargestellt und einer enzymatischen Hydrolyse mittels einer Protease aus *Aspergillus oryzae* in einem Zwei-Phasen-System unterworfen. Lediglich an C-4 unsubstituierte Isoxazolin-5-carbonsäureester wurden hydrolysiert. Aus 3-Ethoxycarbonyl-, 3-Methyl- und 3-Phenyl-Δ^2-isoxazolin-5-carbonsäureestern wurden die entsprechenden (*R*)-konfigurierten Carbonsäuren erhalten, während eine zusätzliche Methylgruppe an C-5 eine bevorzugte Bildung der (*S*)-Säure bewirkte. Die erzielten Enantiomerenüberschüsse liegen im Bereich von $E = 5$–35.

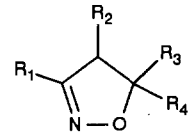
Introduction

Δ^2-Isoxazolines are an interesting class of heterocycles that have been widely used as stable equivalents of β-hydroxyketones [2, 3] and β-aminoalcohols [4, 5]. These compounds are easily available in excellent yields by 1,3-dipolar cycloadditions [6]. Several attempts have been made to synthesize enantiomerically pure isoxazolines using enantiopure dipolarophiles, but the level of chiral induction often was quite low [7]. Our recent finding that (*RS*)-diethyl 5-methyl-4,5-dihydroisoxazole-3,5-

dicarboxylate (**1i**) can be hydrolyzed regio- and enantioselectively by a protease from *Aspergillus oryzae* [8] prompted us to study the action of that enzyme on a series of Δ^2-isoxazolinecarboxylates.

Results and Discussion

Racemic isoxazolines **1** were prepared by 1,3-dipolar cycloaddition reaction from the corresponding nitrile oxides generated in situ by standard procedures [9–11]



(*RS*)-**1**

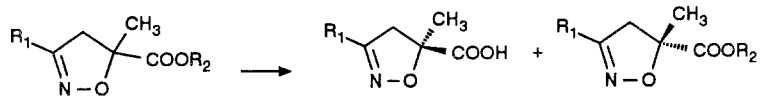
	R_1	R_2	R_3	R_4
a	COOEt	-$(CH_2)_3$-		H
b	COOEt	-$(CH_2)_4$-		H
c	COOEt	H	n-C_4H_9	H
d	COOEt	H	n-C_6H_{13}	H
e	C_6H_5	CH_3	H	COOEt
f	C_6H_5	COOEt	H	CH_3
g	CH_3	C_6H_5	H	COOEt
h	CH_3	COOEt	H	C_6H_5

	R_1	R_2	R_3	R_4
i	COOEt	H	CH_3	COOEt
j	COOEt	H	H	COOEt
k	CH_3	H	H	COOEt
l	CH_3	H	CH_3	COOEt
m	C_6H_5	H	H	COOEt
n	C_6H_5	H	H	COOMe
o	C_6H_5	H	CH_3	COOEt
p	C_6H_5	H	CH_3	COOMe

and were subsequently subjected to enzymatic hydrolysis in a two-phase system as previously described [8]. Out of all the compounds tested only Δ^2-isoxazoline-5-carboxylates **1e**, **1g** and **1i**–**1p** were cleaved under the conditions used (Scheme 1), whereas the 3- and 4-carboxylates remained unaffected. In case of a possibility for discrimination between two ester groups (**1i** and **1j**) the reaction is regioselective and only the carboxylate at C-5 is cleaved in the enzyme-catalyzed reaction. The course of the reaction was monitored by using an automatic burette, where by addition of aqueous sodium hydroxide *pH* 7.0 was kept constant, connected with a recorder. In each case the overall reaction rate slowed down at about 50% conversion. If the hydrolysis was stopped at this point, good to medium enantioselectivities were obtained. For preparative purposes, where enantiopure compounds are needed, the enantiomeric purity can be raised by a modification in the degree of conversion, a repetition of the resolution using enantiomerically enriched material and, in most cases, by recrystallisation of either crystalline derivatives or intermediates of the further pathway of the synthetic sequence.

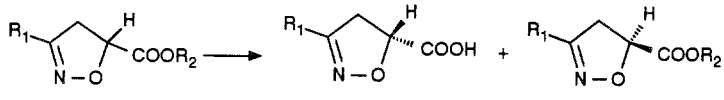
It has to be mentioned that, contrary to the results given above for substances **1a**–**1d**, (±)-(4*RS*, 5*RS*)-methyl 5-(4-methoxyphenyl)-4-methyl-Δ^2-isoxazoline-3-carboxylate can be hydrolyzed enantioselectively using this protease from *Aspergillus oryzae*. Thus, a chemo-enzymatic synthetic access to antibiotics of the nikkomycin-type [13] has been made possible [21].

For the determination of the absolute configuration, two independent reaction sequences were chosen (Scheme 2). Either ester **3n** was reduced with sodium borohydride and then converted into iodide **6** via tosylate **5**. Reduction with



(*RS*)-1 (*S*)-2 (*R*)-3

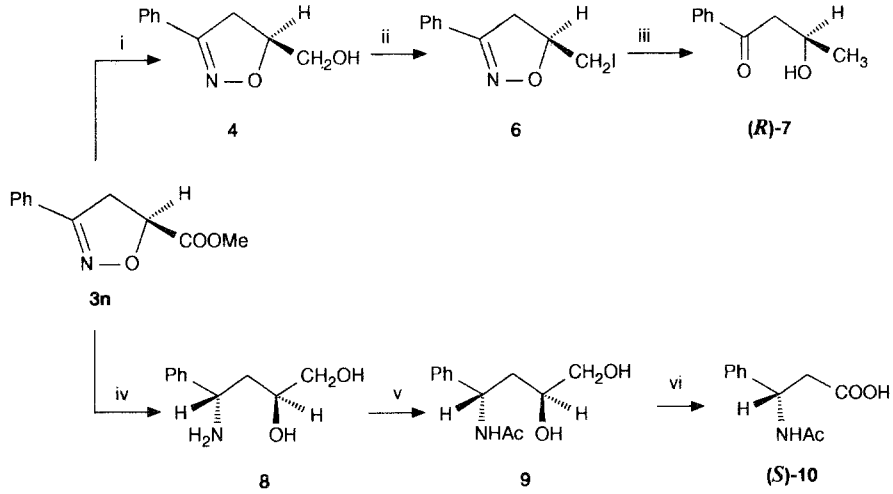
1 - 3	R_1	R_2	% conversion (h reaction time)	e.e.(%)	$[\alpha]_D^{20}$	e.e.(%)	$[\alpha]_D^{20}$	E
i	COOEt	Et	55 (2)	77	+81°	97	-187°	35
l	CH_3	Et	40 (5)	54	+70°	36	-82°	5
o	C_6H_5	Et	43 (17)	75	+109°	51	-108°	11
p	C_6H_5	Me	57 (5)	60	+87°	80	-143°	10



(*RS*)-1 (*R*)-2 (*S*)-3

1 - 3	R_1	R_2	% conversion (h reaction time)	e.e.(%)	$[\alpha]_D^{20}$	e.e.(%)	$[\alpha]_D^{20}$	E
j	COOEt	Et	100 (2)	0	0	0	0	1
k	CH_3	Et	40 (5)	64	-117	57	+92	8
m	C_6H_5	Et	48 (15)	66	-116	95	+156	17
n	C_6H_5	Me	57 (4)	64	-113	> 97	+197	17

Scheme 1. Enzymatic Hydrolysis using Protease of *Aspergillus oryzae*



Reaction conditions: i) $NaBH_4$/EtOH ii) a)TosCl/Pyridine b) NaI/DMAP
iii) Raney-Nickel/H_2 iv) $LiAlH_4$ v) 4-$NO_2(C_6H_4)$OAc vi) $NaIO_4$

Scheme 2. Assignment of absolute configuration

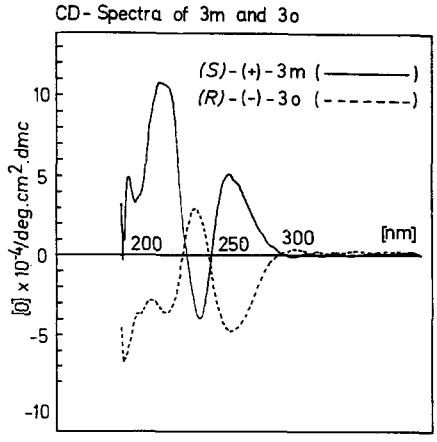


Fig. 1. CD-Spectra of (*R*)-**3o** and (*S*)-**3m**

Raney-nickel gave (*R*)-alcohol **7** [14]. Alternatively **3n** was reduced with *LAH*; as the ester group is reduced first, chiral induction gives rise to the predominant formation of the *S*-enantiomer in the β-position to the first chiral center. Subsequent acetylation and periodate cleavage gave β-aminoacid **10** [15]. In addition, the CD spectra of (*R*)-**3o** and (*S*)-**3m** were recorded [16] which clearly demonstrate that these compounds are of opposite configuration (Fig. 1).

Experimental Part

Melting points were obtained on a Büchi-Tottoli apparatus and are uncorrected. Column chromatograpy (CC) was performed on silica gel 60, 230–400 mesh, ASTM, Merck, Darmstadt, and TLC on aluminum sheets, silica gel 60 F_{254}, Merck, Darmstadt. Optical rotations were determined on a Jasco DIP 370 polarimeter, ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker MSL 300 instrument (*TMS* as internal standard, δ-values in ppm, $CDCl_3$ as solvent unless otherwise indicated), IR spectra were determined as films on a Beckman IR-33 infrared spectrophotometer. The elemental analyses were performed at the Institute of Organic Chemistry, University of Graz. The enantiomeric excesses were determined by ^{1}H-NMR spectroscopy using Eu(*hfc*)$_3$ as shift reagent, or by GC of the menthyl esters.

General Procedures for the Synthesis of Isoxazolines 1

Method A: To a vigorously stirred solution of 10.0 g (0.066 mol) of ethyl chlorooximidoacetate [17] and 49 g of dipolarophile in 170 ml of ether was added dropwise over a 5-h period triethylamine (10.0 ml, 0.072 mol) in 100 ml of ether. Triethylamine hydrochloride was filtered off, the organic phase washed with water, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography, recrystallisation or vacuum distillation. During workup, extreme caution has to be applied, as during this reaction always a dimer of the nitril oxide is formed [18]. This compound causes severe skin irritations and allergies.

Method B: To a suspension of 4.41 g of N-chlorosuccinimide (*NCS*, 33 mmol) in 30 ml of dichloromethane and 0.2 ml of pyridine was added 4 g of benzaldoxime (33 mmol) under stirring. When the solution was clear, (after about 10 minutes), the dipolarophile (41 mmol) was added at once and the internal temperature raised to 40–50 °C. Then a solution of 34.7 mmol of triethylamine in 5 ml of dichloromethane was dropped to the reaction mixture within 30 min. After stirring for further 20 min at room temperature the reaction mixture was washed with water 2 times with 25 ml each, dried over

sodium sulphate and the solvent evaporated in vacuo. The crude products were purified by column chromatography or recrystallisation.

Method C: 4.50 g of nitroethane (60 mmol) and 14.3 g of phenylisocyanate (120 mmol) were dissolved in 30 ml dipolarophile and cooled to 10 °C in an ice bath. After addition of 10 drops of triethylamine, the solution was stirred for one hour at 10 °C, then warmed up to 60 °C for one hour. After dilution with 50 ml of benzene diphenylurea was filtered off, benzene removed in vacuo and the crude products were purified by distillation in vacuo or by column chromatography.

(±)-Ethyl cis-3a,5,6,6a-Tetrahydro-4H-cyclopenta[d]isoxazole-3-carboxylate (**1a**) [19]

Method A. Yield 10 g (83.3%) after CC (toluene/ethyl acetate 10/1) and subsequent bulb-to-bulb distillation.

(±)-Ethyl cis-3a,4,5,6,7,7a-Hexahydrocyclohexa[d]isoxazole-3-carboxylate (**1b**) [19]

Method A. Yield 10.6 g (81.3%) after CC (toluene/ethyl acetate 10/1) and subsequent bulb-to-bulb distillation.

(±)-Ethyl 5-Butylisoxazole-3-carboxylate (**1c**) [19]

Method A. Yield 13.25 g (92.4%) after bulb-to-bulb distillation.

(±)-Ethyl 5-Hexylisoxazole-3-carboxylate (**1d**) [19]

Method A. Yield 14.8 g (99%) after bulb-to-bulb distillation.

(±)-Ethyl 3-Phenyl-2-isoxazoline-4-methyl-5-carboxylate (**1e**) *and (±)-Ethyl 3-Phenyl-2-isoxazolin-5-methyl-4-carboxylate* (**1f**)

Method B. The crude mixture of isomers **1e** and **1f** (9.2 g, 80%) was separated by CC (toluene/ethyl acetate 20/1. **1e**: ^{1}H-NMR: 1.30 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.40 (d, J = 7.2 Hz, 3H, 4-CH_3), 3.95 (m, 1H, 4-H), 4.23 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 4.75 (d, J = 4.2 Hz, 1H, 5-H), 7.41 and 7.66 (m, 5H, aromatic H). ^{13}C-NMR: 14.33 (OCH_2CH_3), 18.28 (4-CH_3), 47.09 (C-4), 62.02 (OCH_2CH_3), 85.38 (C-5), 127.5–130.5 (C_6H_5), 160.51 (C-3), 170.54 (5-COO). $C_{13}H_{15}NO_3$ (233.27): calcd. C 66.94, H 6.48, N 6.00; found C 66.66, H 6.31, N 5.96. **1f**: ^{1}H-NMR: 1.16 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.45 (d, J = 6.3 Hz, 3H, 5-CH_3), 4.07 (d, J = 6.3 Hz, 1H, 4-H), 4.14 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 5.07 (m, 1H, 5-H), 7.38 and 7.67 (m, 5H, aromatic H). ^{13}C-NMR ($CDCl_3$): 14.14 (OCH_2CH_3), 20.90 (5-CH_3), 60.48 (C-4), 62.10 (OCH_2CH_3), 127.04–130.29 (C_6H_5), 154.07 (C-3), 169.57 (4-COO). $C_{13}H_{15}NO_3$ (233.27): calcd. C 66.94, H 6.48, N 6.00; found C 67.31, H 6.40, N 6.09.

(±)-Ethyl 3-Methyl-4-phenyl-2-isoxazoline-5-carboxylate (**1g**) *and (±)-Ethyl 3-Methyl-5-phenyl-2-isoxazoline-4-carboxylate* (**1h**)

Method C. The isomers were separated by CC (toluene/ethyl acetate 10/1). **1g** (5.6 g, 40.2%): ^{1}H-NMR: 1.29 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.83 (s, 3H, 3-CH_3), 4.24 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 4.46 (d, J = 5.72 Hz, 1H, 4-H), 4.83 (d, J = 5.72 Hz, 1H, 5-H), 7.17 and 7.31 (m, 5H, aromatic H). ^{13}C-NMR ($CDCl_3$): 11.41 (3-CH_3), 14.27 (OCH_2CH_3), 61.84 (C-4), 62.05 (OCH_2CH_3), 85.39 (C-5), 127.76–129.55 (C_6H_5), 157.18 (C-3), 170.11 (5-COO). $C_{13}H_{15}NO_3$ (233.27): calcd. C 66.94, H 6.48, N 6.00; found C 66.71, H 6.60, N 5.77. **1h** (3 g, 21.6%): ^{1}H-NMR: 1.31 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 2.07 (s, 3H, 3-CH_3), 3.93 (d, J = 8.6 Hz, 1H, 4-H), 4.26 (m, 2H, OCH_2CH_3), 5.85 (d, J = 8.6 Hz, 1H, 5-H), 7.34 (m, 5H, aromatic H). ^{13}C-NMR: 12.63 (3-CH_3), 14.34 (OCH_2CH_3), 62.26 (OCH_2CH_3), 64.59 (C-4), 85.07 (C-5),

125.93–139.76 (C_6H_5), 151.94 (C-3), 168.94 (4-COO). $C_{13}H_{15}NO_3$ (233.27): calcd. C 66.94, H 6.48, N 6.00; found C 66.55, H 6.20, N 6.20.

(±)-Diethyl 4,5-Dihydroisoxazole-3,5-dicarboxylate (**1j**)

Method A. Yield 92.2%, b.p.$_{0.2}$ 90–92 °C. ^{1}H-NMR: 1.17 and 1.23 (2 × t, J = 7.2 Hz, 2 × 3H, OCH_2CH_3), 3.33 and 3.39 (2 × d, 2 × 1H, 4-CH_2), 4.12 and 4.21 (2 × q, J = 7.2, 2 × 2H, 2 × OCH_2CH_3), 5.09 (q, J = 9.3 Hz, 1H, 5-H). ^{13}C-NMR: 14.00 and 14.13 (2 × OCH_2CH_3), 37.55 (4-CH_2), 62.11 and 62.21 (2 × OCH_2CH_3), 79.87 (C-5), 151.10 (C-3), 159.82 (3-COO), 168.86 (5-COO). $C_9H_{13}NO_5$ (215.21): calcd. C 50.23, H 6.09, N 6.51; found C 49.55, H 5.66, N 6.51.

(±)-Ethyl 3-Methyl-4,5-dihydroisoxazole-5-carboxylate (**1k**)

Method C. Yield 3.44 g (73%) after distillation (b.p.$_{14}$ = 130°) and subsequent CC (cyclohexane/ethyl acetate 1/1). ^{1}H-NMR: 1.18 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.89 (s, 3H, 3-CH_3), 3.10 and 3.14 (2 × d, 2 × 1H, 4-CH_2), 4.10 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 4.83 (q, J = 9.3 Hz, 1H, 5-H). ^{13}C-NMR: 12.43 (3-CH_3), 13.93 (OCH_2CH_3), 42.30 (C-4), 61.55 (OCH_2CH_3), 77.01 (C-5), 154.71 (C-3), 170.31 (5-COO). $C_7H_{11}NO_3$ (157.17): calcd. C 53.50, H 7.06, N 8.91; found C 53.72, H 6.91, N 8.70.

(±)-Ethyl 3,5-Dimethyl-4,5-dihydroisoxazole-5-carboxylate (**1l**)

Method C. Yield 8.48 g (82.7%), b.p.$_{11}$ 122–123 °C. IR (film): 1987, 1734 (C = O), 1434, 1300, 1185, 1113, 1024. ^{1}H-NMR: 1.20 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.51 (s, 3H, 5-CH_3), 1.90 (s, 3H, 3-CH_3), 2.73 and 3.36 (2 × d, 2 × 1H, J = 17.0 Hz, AB-system, 4-CH_2), 4.13 (q, J = 7.2 Hz, 2H, OCH_2CH_3). ^{13}C-NMR: 13.08 (3-CH_3), 14.13 (OCH_2CH_3), 23.49 (5-CH_3), 48.33 (C-4), 61.88 (OCH_2CH_3), 85.19 (C-5), 155.28 (C-3), 172.29 (5-COO). $C_8H_{13}NO_3$ (171.20): calcd. C 56.13, H 7.65, N 8.18; found C 55.86, H 7.39, N 8.08.

(±)-Ethyl 3-Phenyl-4,5-dihydroisoxazole-5-carboxylate (**1m**)

Method B. Yield 6.24 g (84.8%), b.p.$_{0.2}$ 150 °C. IR (film): 3651, 3462, 3065, 2986, 1741, 1602, 1570, 1447, 1370, 1356, 1206, 1164, 1032, 896, 760, 693, 672. ^{1}H-NMR: δ = 1.25 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 3.58 (d, 2H, 4-CH_2), 4.17 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 5.10 (q, J = 9.3 Hz, 1H, 5-H), 7.34 and 7.60 (m, 5H, aromatic H). ^{13}C-NMR: 14.08 (OCH_2CH_3), 38.83 (C-4), 61.91 (OCH_2CH_3), 78.13 (C-5), 127.0–130.4 (–C_6H_5), 156.04 (C-3), 170.17 (5-COO). $C_{12}H_{13}NO_3$ (219.24): calcd. C 65.74, H5.98, N 6.39; found C 65.30, H 5.78, N 6.47.

(±)-Methyl 3-Phenyl-4,5-dihydroisoxazole-5-carboxylate (**1n**) [20]

Method B. Yield 5.4 g (80%), mp. 72–73 °C (methanol).

(±)-Ethyl 3-Phenyl-5-methyl-4,5-dihydroisoxazole-5-carboxylate (**1o**)

Method B. Yield 7.8 g (89.0%). Colorless liquid, b.p.$_{0.15}$ 140 °C. IR (film): 3651, 3460, 3030, 2985, 2939, 1737, 1446, 1360, 1303, 1190, 1108, 1021, 912, 761, 693. ^{1}H-NMR: δ = 1.28 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.68 (s, 3H, 5-CH_3), 3.19 and 3.86 (2d, J = 17.1 Hz, 2H, AB-system, 4-CH_2), 4.19 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 7.37 and 7.63 (m, 5H, aromatic H). ^{13}C-NMR: 14.13 (OCH_2CH_3), 23.67 (5-CH_3), 44.60 (C-4), 62.05 (OCH_2CH_3), 86.25 (C-5) 156.30 (C-3), 172.07 (5-COO). $C_{13}H_{15}NO_3$ (233.27): calcd. C 66.94, H 6.48, N 6.01; found C 67.19, H 5.96, N 5.80.

(±)-Methyl 3-Phenyl-5-methyl-4,5-dihydroisoxazole-5-carboxylate (**1p**)

Method B. Yield 6.75 g (65.9%), m.p. 58–58.5 °C (methanol). IR (Nujol): 2929 cm^{-1}, 1750 (C=O), 1459, 1378, 1282, 1198, 761, 691. $C_{12}H_{13}NO_3$ (219.24): calcd. C 65.74, H 5.98, N 6.39; found C 66.01, H 5.82, N 6.23.

Enzymatic Hydrolysis of the Isoxazolinecarboxylates

Protease (2.0 g, from *Aspergillus oryzae*, Sigma Type XXIII) was suspended in a two phase system prepared from 200 ml of phosphate buffer (0.1 *M*, *pH* 7) and 150 ml of toluene. The suspension was stirred vigorously and the *pH* was kept at 7 with 1 *N* sodium hydroxide by means of an autoburette. Isoxazoline (3.0 g) was added, and consumption of alkali was followed. When the consumption of 1 *N* sodium hydroxide showed the conversion desired, the mixture was extracted with toluene, the combined organic phases were dried over Na_2SO_4, concentrated in vacuo and the crude ester purified by the procedure described above for the racemic derivatives. The aqueous solution was brought to *pH* 2–3 with hydrochloric acid (10%), saturated with NaCl, extracted with ethyl acetate (3 × 70 ml) and the combined organic phases were dried (Na_2SO_4) and evaporated to yield the corresponding acid. Data for the compounds are given in Schemes 1 and 2.

(S)-(+)-4,5-Dihydro-3-phenyl-5-isoxazolemethanol (**4**)

1 g (4.83 mmol) of methyl (*S*)-(+)-3-phenyl-2-isoxazolin-5-carboxylate **3n** (e.e. = 85%) was added to a solution of 0.75 g of $NaBH_4$ in 30 ml of ethanol at room temperature. After stirring overnight the reaction was quenched by careful addition of diluted hydrochloric acid. The mixture was extracted three times with CH_2Cl_2, the combined extracts were dried over Na_2SO_4, CH_2Cl_2 removed in vacuo and the crude product was recrystallized from $CHCl_3$/petrol ether to yield 0.8 g (93.1%), m.p. 78–79 °C, $[\alpha]_D^{20} = +106°$ (*c* 0.57, $CHCl_3$). IR (Nujol): 3547, 2900, 1104, 1052, 917, 903, 814, 766. ^{1}H-NMR: $\delta = 3.33$ (2 × dd, 2H, CH_2OH), 3.69 and 3.87 (2 × dd, 2H, 4-CH_2), 4.87 (m, 1H, 5-H), 7.40 and 7.66 (m, 5H, aromatic H). ^{13}C-NMR: 36.61 (C-4), 63.91 (CH_2OH), 81.51 (C-5), 125.73–130.39 (aromatic C), 157.28 (C=N). $C_{10}H_{11}NO_2$ (177.21): calcd. C 67.78, H 6.26, N 7.90; found C 67.70, H 6.17, N 7.77.

(S)-(+)-4,5-Dihydro-3-phenyl-5-isoxazolymethyl (4-methylbenzene)sulfonate (**5**)

To a solution of 0.8 g (4.49 mmol) of (*S*)-(+)-4,5-dihydro-3-phenyl-5-isoxazolmethanol **4** and 1.2 g (6.3 mmol) of *p*-toluenesulfonyl chloride in 50 ml of dichloromethane were added 1 ml of pyridine and 60 mg of 4-dimethylaminopyridine. After stirring overnight, the solvent was evaporated in vacuo and the crude product was dissolved in 200 ml of ether and filtered. The filtrate was evaporated and the residue was recrystallized from ether to yield 1.43 g (95.7%), m.p. 103–104 °C, $[\alpha]_D^{20} = +138°$ (*c* = 0.45, acetone). IR (Nujol): 2900, 1595, 1365, 1191, 985, 829, 690, 668. ^{1}H-NMR: $\delta = 2.45$ (s, 3H, Ar–CH_3), 3.25 and 3.45 (2 × dd, 2H, 4-CH_2), 4.10 and 4.19 (2 × dd, 2H, –CH_2–O–Tos), 4.95 (m, 1H, 5-H), 7.27–7.81 (m, 10H, aromatic H). ^{13}C-NMR: 21.88 (CH_2–O–Tos), 37.61 (4-CH_2), 69.50 (C-5), 127–132 and 145.43 (aromatic C), 156.49 (C=N). $C_{17}H_{16}NSO_4$ (331.39): calcd. C 61.62, H 4.87, N 4.23; found C 61.50, H 5.05, N 4.02.

(S)-(+)-4,5-Dihydro-5-iodomethyl-3-phenylisoxazole (**6**)

A solution of 1.24 g (3.73 mmol) of tosylate **5**, NaI (2.17 g, 14.5 mmol), and $NaHCO_3$ (180 mg) in 150 ml of 2-butanone was refluxed for 15 h. After removal of the solvents the residue was partitioned between ether (100 ml) and 5% sodium thiosulphate solution (100 ml). The organic phase was washed with H_2O (100 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was recrystallized from ether/petrol ether, yield 1.04 g (97.6%), m.p. 75–76 °C, $[\alpha]_D^{20} = +18.0°$ (*c* = 0.91, $CHCl_3$). IR (Nujol): 2900, 1174, 900, 754, 688. ^{1}H-NMR: 3.25 (m, 2H, 4-CH_2), 3.49 (m, 2H, –CH_2I),

4.94 (m, 1H, 5-H), 7.43 (m, 3H, aromatic H), 7.68 (m, 2H, aromatic H). ^{13}C-NMR: 7.61 (CH_2I), 41.33 (4-CH_2), 80.73 (C-5), 125–130 (aromatic C), 156.04 (C=N). $C_{10}H_{10}NOI$ (286.09): calcd. C 41.83, H 3.51, N 4.88; found C 41.77, H 3.65, N 4.67.

(R)-(−)-3-Hydroxy-1-phenyl-1-butanone (**7**) [14]

To a solution of iodide **6** (0.35 g, 1.22 mmol), boric acid (0.2 g), and methanol/water (15:1) (30 ml) was added excess Raney nickel. The mixture was stirred vigorously in a hydrogen atmosphere at room temperature for 15 h. After filtration of the catalyst, the solution was diluted with 50 ml of water and extracted with CH_2Cl_2 (three times). The combined organic phases were dried over Na_2SO_4, evaporated under reduced pressure and the product was purified by CC (CH_2Cl_2). Yield 70 mg (34.9%), $[\alpha]_D^{20} = -55.5°$ ($c = 2$, $CHCl_3$) (lit. [12] −59.7°, e.e. = 90%).

(2S,4S)-(−)-4-Amino-4-phenyl-1,2-butanediol (**8**)

0.5 g (2.4 mmol) of methyl (*S*)-(+)-3-phenyl-2-isoxazoline-5-carboxylate **3n** (e.e. = 95%) in 25 ml of dry ether was added to the suspension of 0.4 g (10.5 mmol) of $LiAlH_4$ in 25 ml of ether at 0 °C in 5 min. After 22 h reflux to the stirred mixture were added slowly 0.4 ml of water, 0.4 ml 20% NaOH and 1.5 ml water and the product was continuously extracted with $CHCl_3$. After removal of the solvent and recrystallisation from $CHCl_3$ 0.4 g product with about 4:1 (*R,S*:*R,R*) isomer ratio were obtained (97%), m.p. 102–102.5 °C ($CHCl_3$), $[\alpha]_D^{20} = -23°$ ($c = 0.3$, $CHCl_3$). IR (Nujol): 3352, 3310, 2925, 1608, 1110, 1075, 1064, 1022, 990, 980, 868, 755. ^{1}H-NMR (*DMSO-d*$_6$): 1.5–1.8 (m, 2H, 3-CH_2), 3.35 (m, 2H, 1-CH_2), 3.65 (m, 1H, 4-CH), 4.05 (m, 1H, 2-CH), 7.2–7.5 (m, 5H, aromatic H). ^{13}C-NMR (*DMSO-d*$_6$) *R,S*-isomer: 44.00 (3-C), 52.28 (4-C), 66.57 (1-C), 69.23 (2-C), 126–128 and 148.29 (aromatic C). ^{13}C-NMR (*DMSO-d*$_6$) for *R,R*-isomer: 43.00 (3-C), 54.20 (4-C), 66.57 (1-C), 71.20 (2-C), 126–128 and 147.99 (aromatic C). $C_{10}H_{15}NO_2$ (180.34): calcd. C 66.60, H 8.38, N 7.77; found C 65.79, H 8.12, N 7.51.

(2S,4S)-(−)-4-Acetamido-4-phenyl-1,2-butandiol (**9**)

A solution of 0.4 g (2.2 mmol) of (2*S*,4*S*)-(−)-4-amino-4-phenyl-1,2-butandiol **8** and 0.4 g (2.2 mmol) of *p*-nitrophenyl acetate in 5 ml of *DMF* was stirred overnight at room temperature. After removal of the solvent under reduced pressure the residue was purified by CC (CH_3Cl/MeOH = 9:1) and recrystallized from MeOH–$CHCl_3$ to yield 0.49 g (98.6%), m.p. 141–142 °C, $[\alpha]_D^{20} = -84°$ ($c = 7$, MeOH). IR (Nujol): 3322, 2900, 1632, 1554, 1038, 1020, 958, 758, 694. ^{1}H-NMR (*DMSO-d*$_6$): $\delta = 1.60$–1.89 (m, 2H, 3-CH_2), 1.94 (s, 3H, $COCH_3$), 3.40 (m, 2H, 1-CH_2), 3.60 (m, 1H, 4-CH), 5.11 (m, 1H, 2-CH), 7.2–7.45 (m, 5H, aromatic H), 8.4 (d, 1H, NH). ^{13}C-NMR (*DMSO-d*$_6$) *R,S*-isomer: 23.11 (CH_3), 41.31 (3-CH_3), 49.89 (4-CH), 66.61 (1-CH_2), 68.64 (2-CHOH), 126–128.6 (aromatic C), 145.09 (aromatic C), 169.45 (C=O). ^{13}C-NMR (*DMSO-d*$_6$) *R,R-isomer*: 23.11 (CH_3), 41.00 (3-CH_3) 50.30 (4-CH), 66.25 (1-CH_2), 68.84 (2-CHOH), 126–128.6 (aromatic C), 144.09 (aromatic C), 168.95 (C=O). $C_{12}H_{17}NO_3$ (223.28): calcd. C 64.56, H 7.68, N 6.27; found C 64.32, H 7.62, N 6.11.

S(−)-3-Phenyl-3-acetamidopropanoic acid (**10**) [15]

A solution of 0.48 g (2.14 mmol) of (2*S*,4*S*)-(−)-4-acetamido-4-phenyl-1,2-butanediol **9** and 0.51 g of $NaIO_4$ (2.38 mmol) in 20 ml of H_2O was stirred at room temperature for 30 min., saturated with NaCl and extracted with CH_2Cl_2 (3 × 60 ml). The combined organic phases were evaporated and the residue was dissolved in 50 ml ethanol and combined with a solution of 1.43 g (8.5 mmol) of $AgNO_3$ in 10 ml of water. Then 10 ml of 1*N* KOH was added to the mixture. After stirring at room temperature for 3 h, the suspension was filtered, the filtrate was neutralized with dilute HCl and evaporated under reduced pressure. The product was extracted with methanol and recrystallized from water to yield 0.2 g (45%), m.p. 162–164 °C, $[\alpha]_D^{20} = -50.7°$ ($c = 1.17$, EtOH). ^{1}H-NMR (*DMSO-d*$_6$): $\delta = 1.85$ (s, 3H,

$-CH_3$), 2.70 (2 × d, 2H, 2-CH_2), 5.20 (q, 1H, β-CH), 7.30 (m, 5H, aromatic H), 8.45 (d, 1H, NH). ^{13}C-NMR (*DMSO-d_6*): 27.94 (CH_3), 46.22 (C-3), 54.80 (C-2), 131–133 and 147.88 (aromatic C), 173.76 (CH_3CO), 176.97 (COO). $C_{11}H_{13}NO_3$ (207.23): calcd. C 63.76, H 6.32, N 6.76; found C 62.32, H 6.54, N 6.65.

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