Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 2741–2751

TETRAHEDRON: *ASYMMETRY*

A chemoenzymatic approach to the synthesis of the stereoisomers of a β-adrenergic receptor antagonist

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Received 11 May 2000; accepted 16 June 2000

Abstract

The four stereoisomers of Δ^2-isoxazoline **2**, a β-adrenergic receptor antagonist structurally related to Falintolol **1**, were prepared by an enzyme-catalyzed kinetic resolution of the unsaturated secondary alcohol (±)-**7** followed by its cycloaddition to pyruvonitrile oxide. Through this strategy, diastereomeric amino-alcohols (+)-**2a**/(–)-**2b** and (–)-**2a**/(+)-**2b** were obtained in 99 and 92% enantiomeric excess, respectively. The absolute configuration to the target compounds was assigned via chemical correlation to the enantiomers of epoxides **4a** and **4b**, whose stereochemistry had been previously established.

1. Introduction

The use of the cycloaddition approach in the synthesis of biologically active heterocycles has characterized our research in the last decade.[1–5] Recently, we applied the cycloaddition of nitrile oxides to the preparation of a set of Δ^2-isoxazoline derivatives,[6] designed as semirigid analogues of the β-blocking agent Falintolol **1** (Fig. 1).[7,8] The 3-isopropenyl stereoisomers *anti*-(±)-**2a** and *syn*-(±)-**2b** (Fig. 1) were revealed as the most interesting among the investigated compounds. In particular, (±)-**2a** behaved as a non-selective β-adrenergic receptor antagonist, and displayed a binding affinity to β_1- and β_2-adrenergic receptors very close to that reported for the model compound **1**.[6] Since our goal was a deeper investigation of the pharmacological profile of derivatives **2**, we planned the synthesis of the two enantiomeric pairs (+)-**2a**/(–)-**2a** and (+)-**2b**/(–)-**2b**.

Any speculation on the relationship between the structure and biological properties of chiral isomers requires their availability in very high enantiomeric purity. To attain such a goal, we tackled the synthesis of target compounds by means of biocatalytic methods, which represent an

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0957-4166/00/$ - see front matter
PII: S0957-4166(00)00241-X

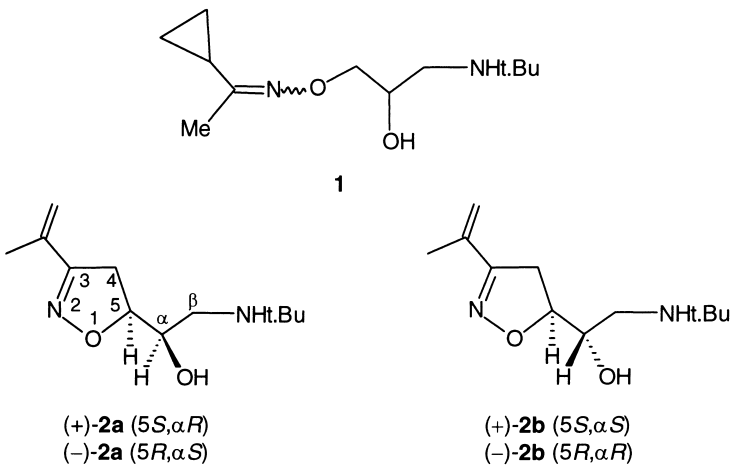
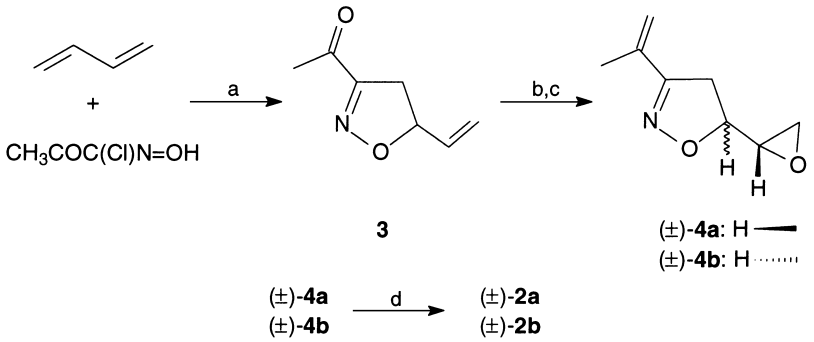


Figure 1.

increasingly valuable alternative to common synthetic approaches, i.e. asymmetric synthesis and resolution procedures.[9–11] In the past, we used enzyme-catalyzed reactions as a key step in the preparation of enantiomerically pure compounds provided with a variety of biological activities.[12–16] As an extension of our efforts along this research line, we now report the application of the chemoenzymatic strategy to the synthesis of the four stereoisomers of **2**. In addition, our approach allowed the assignment of the absolute configuration to the enantiomers (+)-**2a**/(–)-**2a** and (+)-**2b**/(–)-**2b**.

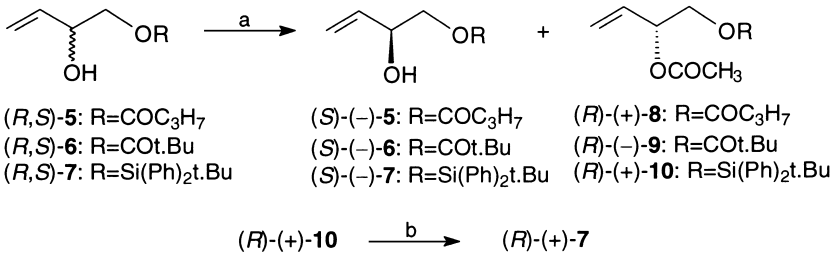
2. Results and discussion

The sequence used to prepare stereoisomers (±)-**2a** and (±)-**2b** is reported in Scheme 1.[6] The cycloaddition of pyruvonitrile oxide to butadiene yielded 3-acetyl-5-vinyl-Δ^2-isoxazoline **3**, which, after epoxidation and Wittig methylenation, afforded a 1:1 mixture of stereoisomeric epoxides *anti*-(±)-**4a** and *syn*-(±)-**4b**, whose relative configuration was assigned.[6] The desired amino alcohols (±)-**2a** and (±)-**2b** were then obtained by reacting (±)-**4a** [(±)-**4b**] with excess *tert*-butylamine.



Scheme 1. (a) AcOEt/$NaHCO_3$; (b) MCPBA/CH_2Cl_2; (c) $Ph_3P{=}CH_2$; (d) tBuNH$_2$/MeOH

Based on these results, we extended the above strategy to the preparation of the four stereoisomers of **2** in enantiomerically pure form. For such a purpose, we studied the kinetic resolution of derivatives related to (*RS*)-3-butene-1,2-diol through the enzyme-catalyzed transesterification of their secondary alcohol. The transacetylation of 2-hydroxy-but-3-enyl butyrate (±)-**5** (Scheme 2) under the catalysis of different lipases[17] gave a moderate enantioselection, as reflected by the low values of the enantiomeric ratio (E) reported in Table 1. Accordingly, residual monoester was obtained as a single enantiomer only by increasing the degree of conversion (≥70%). An inversion of the enantiopreference was detected on passing from *Candida antarctica* lipase B [CALB, E = 18 for (*R*)-(+)-**5**] to *Pseudomonas fluorescens* lipase [lipase AK, E = 14 for (*S*)-(–)-**5**]. The degree of conversion and the enantiomeric excess (e.e.) of reagent and product were both evaluated by chiral GLC analysis.[17] The absolute configuration of residual monoester (*S*)-(–)-**5** [(*R*)-(+)-**5**] and produced diester (*R*)-(+)-**8** [(*S*)-(–)-**8**] was assigned through their conversion into the enantiomers of 3-butene-1,2-diol, whose absolute configurations are known from the literature.[17,18]



Scheme 2. (a) Enzyme/vinyl acetate; (b) $NaOH/H_2O$–MeOH

Table 1
Lipase-catalyzed transacetylation of substrates (±)-**5**, (±)-**6** and (±)-**7**

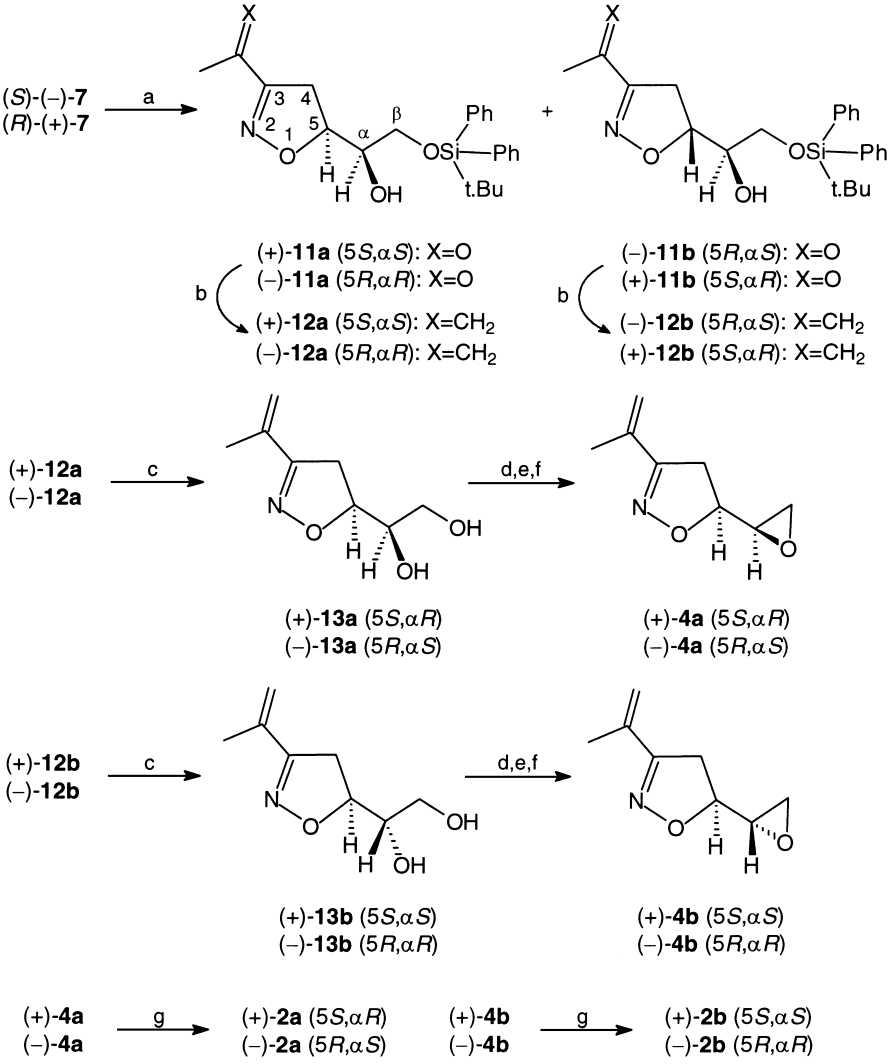
Substrate	Enzyme	E[a]	Degree of conv.(%)	Residual substrate (e.e.%)[b]	Produced ester (e.e.%)[b]
(±)-**5**	CALB	18	70	(*S*)-(–)-**5** (≥99)	(*R*)-(+)-**8** (n.d.)
(±)-**5**	Lipase AK	14	76	(*R*)-(+)-**5** (≥99)	(*S*)-(–)-**8** (n.d.)
(±)-**6**	Lipase PS	>100	52	(*S*)-(–)-**6** (≥99)	(*R*)-(–)-**9** (92)
(±)-**7**	CALB	>100	52	(*S*)-(–)-**7** (≥99)	(*R*)-(+)-**10** (92)

[a]The E values were calculated according to the equations reported in Ref.9. [b]See the Experimental Section for the details of determination of e.e.

Since our goal was the synthesis of both enantiomers in very high enantiomeric excess, we investigated the biotransformation of derivatives (±)-**6** and (±)-**7**, which are characterized by the presence of a sterically hindered ester or silyl ether, respectively. As shown in Table 1, the results on the transacetylation of substrates (±)-**6** and (±)-**7** revealed that the structural modifications taken into account significantly improved the enantioselectivity. Among the tested enzymes, Lipase PS was the most selective catalyst of the transacetylation of derivative (±)-**6** (E > 100),

whereas CALB efficiently catalyzed the acetyl transfer on substrate (±)-**7** (E > 100). Therefore, both the enzyme/substrate couples were suited for their utilization on a preparative scale. However, we chose monoester (±)-**7**, owing to the higher rate of transacetylation in the presence of CALB and a better efficiency of the chiral HPLC evaluation of the enantiomeric purity.

As shown in Table 1, the CALB-catalyzed transesterification of substrate (±)-**7** with vinyl acetate allowed the preparation of either residual substrate (*S*)-(–)-**7** and produced ester (*R*)-(+)-**10** in gram amounts with a very high enantiomeric purity (e.e. higher than 99% and equal to 92%, respectively). The product of the enzyme-catalyzed transesterification (*R*)-(+)-**10** was submitted to an alkaline hydrolysis to yield secondary alcohol (*R*)-(+)-**7**. The two enantiomers (*S*)-(–)-**7** and (*R*)-(+)-**7** were separately submitted to 1,3-dipolar cycloaddition with pyruvonitrile oxide. As reported in Scheme 3, the pericyclic reaction gave rise to equimolar amounts of stereoisomeric 3-acetyl-Δ^2-isoxazolines (+)-**11a**/(–)-**11b** and (–)-**11a**/(+)-**11b**, which were separated



Scheme 3. (a) $CH_3COC(Cl)=NOH/AcOEt/NaHCO_3$; (b) $Ph_3P=CH_2$; (c) TBAF/THF; (d) $MeC(OMe)_3$/*p*TosOH; (e) Me_3SiCl/CH_2Cl_2; (f) K_2CO_3/MeOH; (g) *t*$BuNH_2$/MeOH

by column chromatography. Treatment of isomers (+)-**11a**, (–)-**11a**, (+)-**11b** and (–)-**11b** with triphenylmethylene phosphorane produced the corresponding 3-isopropenyl derivatives (+)-**12a**, (–)-**12a**, (+)-**12b** and (–)-**12b**. Cleavage of the silyl ether was accomplished by reacting (+)-**12a** [(–)-**12a**] and (+)-**12b** [(–)-**12b**] with a THF solution of tetrabutyl ammonium fluoride, and the resulting diols (+)-**13a** [(–)-**13a**] and (+)-**13b** [(–)-**13b**] were submitted to Sharpless' 'one pot' procedure,[19] which smoothly afforded epoxides (+)-**4a** [(–)-**4a**] and (+)-**4b** [(–)-**4b**] with retention of configuration at Cα. Since the configuration to diastereomeric *anti–syn* epoxides **4a** and **4b** was previously attributed,[6] the sequence of steps depicted in Scheme 3 allowed the assignment of absolute configurations to cycloadducts (5*S*,α*S*)-(+)-**11a** and (5*R*,α*S*)-(–)-**11b**.

Treatment of (+)-**4a** [(–)-**4a**] and (+)-**4b** [(–)-**4b**] with an excess of *tert*-butylamine gave the desired aminoalcohols (5*S*,α*R*)-(+)-**2a** [(–)-**2a**] and (5*S*,α*S*)-(+)-**2b** [(–)-**2b**], which were transformed into the corresponding 1:1 oxalates. An HPLC analysis of both diastereomeric pairs (+)-**2a**/(–)-**2b** and (–)-**2a**/(+)-**2b** on a chiral stationary phase (Chirobiotec T) demonstrated that the sequence of reactions applied did not alter the value of enantiomeric purity gained in the enzymatic kinetic resolution.

In summary, the use of enzymes allowed a convenient synthesis of new chiral heterocyclic derivatives of interest to medicinal chemistry. The preparation of the four stereoisomers of a β-adrenoceptor antagonist was achieved in high enantiomeric purity through an efficient CALB-catalyzed transesterification coupled to a pericyclic reaction involving a nitrile oxide. The evaluation of the pharmacological profile of target compounds will be reported in due course.

3. Experimental

Lipases from *Pseudomonas cepacia* (lipases PS) and *Pseudomonas fluorescens* (lipase AK) were purchased from Amano Pharmaceutical Co. Lipase B from *Candida antarctica* (CALB) was bought from Novo Nordisk in immobilized form with the commercial name of NOVOZYM 435. Organic solvents were reagent grade. Racemic 3-butene-1,2-diol was kindly donated by Bayer AG, Leverkusen. ^{1}H NMR spectra were recorded at 200 MHz in $CDCl_3$ solutions; chemical shifts (δ) are expressed in ppm and coupling constants (J) in hertz. Chiral GLC analyses were conducted on a gas chromatograph equipped with a Chrompack CP-Cyclodextrin-2,3,6-M-19 column (50 m, 0.25 mm ID). Chiral HPLC analyses were performed on a Chiralcel OD column (4.6×250 mm) and on a Chirobiotic T (4.6×250 mm). The experimental conditions of chromatographic analyses are specified in the appropriate paragraph. Melting points were determined on a Mod. B 540 Büchi apparatus and are uncorrected. Liquid compounds were characterized by the oven temperature for bulb to bulb distillations. Rotary power determinations were carried out with a Perkin–Elmer 241 polarimeter coupled with a Haake N3-B thermostat. TLC analyses were performed on commercial silica gel 60 F254 aluminum sheets: spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution. Microanalyses (C, H, N) of new compounds agreed with the theoretical value ±0.4%.

3.1. Synthesis of (±)-6 and (±)-7

A. To a stirred solution of (±)-3-butene-1,2-diol (2 g, 22.72 mmol) and triethylamine (3.80 mL, 27.26 mmol) in dichloromethane (100 mL) was added dropwise a solution of pivaloyl chloride (3.08 mL, 25 mmol) in dichloromethane (30 mL) at 0°C. The reaction mixture was stirred at room

temperature for 3 h, then acidified to pH 3 with 3N HCl. The organic layer was separated and the aqueous phase was further extracted with dichloromethane (2×70 mL). The pooled organic extracts were dried over anhydrous sodium sulfate and the solvent was removed at reduced pressure. A silica gel column chromatography of the residue (eluant: 10% ethyl acetate/petroleum ether) gave 3.05 g (78% yield) of the desired monoester.

(±)-2-Hydroxy-but-3-enyl pivalate **6**: colorless oil, bp 155–160°C/20 mmHg, R_f 0.23 (eluant: 15% ethyl acetate/cyclohexane); ^{1}H NMR: 1.20 (s, 9), 2.43 (bs, 1, OH), 4.07 (dd, 1, CHOCO, J = 6.8 and 11.2), 4.10 (dd, 1, CHOCO, J = 4.0 and 11.2), 4.36 (m, 1, C*H*OH), 5.22 (d, 1, C*H*=CHCHOH, J = 10.4), 5.36 (d, 1, C*H*=CHCHOH, J = 17.5), 5.85 (ddd, 1, CH_2=C*H*, J = 5.6, 10.4 and 17.5).

B. To a stirred solution of (±)-3-butene-1,2-diol (4.0 g, 45.45 mmol), triethylamine (12.68 mL, 91 mmol) and 4-dimethylaminopyridine (0.556 g, 4.55 mmol) in anhydrous THF (300 mL) was added dropwise under nitrogen a solution of *tert*-butyldiphenylchlorosilane (12.85 mL, 50 mmol) in anhydrous THF (100 mL). The reaction mixture was stirred overnight at room temperature, the precipitate was filtered off and the volatiles were evaporated at reduced pressure. The residue was submitted to a silica gel column chromatography (eluant: 5% ethyl acetate/petroleum ether) to afford 13.04 g (88% yield) of the expected silyl ether.

(±)-1-(*tert*-Butyldiphenylsilyloxy)-2-hydroxy-3-butene **7**: viscous colorless oil, bp 190–195°C/0.3 mmHg, R_f 0.44 (eluant: 5% ethyl acetate/cyclohexane); ^{1}H NMR: 1.08 (s, 9), 2.73 (bs, 1, OH), 3.58 (dd, 1, CHOSi, J = 7.4 and 9.9), 3.68 (dd, 1, CHOSi, J = 3.8 and 9.9), 4.27 (m, 1, C*H*OH), 5.16 (d, 1, C*H*=CHCHOH, J = 10.5), 5.32 (d, 1, C*H*=CHCHOH, J = 17.3), 5.79 (ddd, 1, CH_2=C*H*, J = 5.6, 10.5 and 17.3), 7.43 (m, 6, arom.), 7.67 (m, 4, arom.).

*3.2. Lipase-catalyzed preparative transacetylation of substrates (±)-**6** and (±)-**7***

A. A solution of 1.72 g (10 mmol) of (±)-**6** in petroleum ether (150 mL) was treated with vinyl acetate (7.5 mL) in the presence of lipase PS (1.5 g). The reaction mixture was stirred (200 rpm) at room temperature and stopped at 52% conversion (about 60 h). The degree of conversion was determined by GLC analysis, carried out on a 25 m HP1 capillary silica gel column coated with methylsilicone gum under the following conditions: oven temperature from 85°C (initial time 5 min) to 130°C (final time 10 min). H_2 was used as the carrier gas at a heating rate of 1.2°C/min. The enzyme was filtered off and the solvent evaporated at reduced pressure. The residue was submitted to silica gel column chromatography (eluant: 10% ethyl acetate/petroleum ether) to afford 0.757 g (4.4 mmol) of (*S*)-(–)-**6** and 1.017 g (4.75 mmol) of (*R*)-(–)-**9**.

(*S*)-2-Hydroxy-but-3-enyl pivalate (–)-**6**: $[\alpha]_D^{20} = -5.3$ (*c* 1.040, $CHCl_3$), e.e. > 99%. The e.e. was determined by chiral GLC analysis on a Chrompack CP-Cyclodextrin column, after conversion into the corresponding (*S*)-(+)-**9**, following the temperature program utilized with the achiral stationary phase. Relative retention times (min): (*S*)-(+)-**9**, 30.15; (*R*)-(–)-**9**, 30.55.

(*R*)-2-Acetyloxy-but-3-enyl pivalate (–)-**9**: colorless oil, bp 165–170°C/20 mmHg, R_f 0.56 (eluant: 15% ethyl acetate/cylohexane). $[\alpha]_D^{20} = -0.5$ (*c* 1.0, $CHCl_3$); $[\alpha]_{436}^{20} = -2.7$ (*c* 1.0, $CHCl_3$); $[\alpha]_{365}^{20} = -5.5$ (*c* 1.0, $CHCl_3$), e.e. = 92%, determined by chiral GLC analysis (see above). ^{1}H NMR: 1.18 (s, 9), 2.08 (s, 3, CH_3CO), 4.13 (dd, 1, C*H*OCO*t*Bu, J = 6.7 and 11.6), 4.20 (dd, 1, C*H*OCO*t*Bu, J = 4.3 and 11.6), 5.28 (d, 1, C*H*=CHCHOCO, J = 10.8), 5.35 (d, 1, C*H*=CHCHOCO, J = 17.3), 5.51 (m, 1, C*H*OCOCH$_3$), 5.80 (ddd, 1, CH_2=C*H*, J = 5.8, 10.8 and 17.3).

The assignment of absolute configurations to (*S*)-(–)-**6** and (*R*)-(–)-**9** was confirmed by their conversion, through an alkaline hydrolysis, into (*S*)-(–)- and (*R*)-(+)-3-butene-1,2-diol, $[\alpha]_D^{20} = -9.5$ (*c* 1.0, $CHCl_3$) and $[\alpha]_D^{20} = +8.8$ (*c* 1.0, $CHCl_3$), respectively.

B. A solution of 12.08 g (37 mmol) of (±)-**7** in petroleum ether (500 mL) was reacted with vinyl acetate (27.75 mL) in the presence of CALB (7 g). The reaction mixture was stirred (200 rpm) at room temperature for about 18 h (52% conversion). The degree of conversion was determined by achiral GLC analysis (see above): oven temperature from 200°C (initial time 5 min) to 230°C (final time 10 min); heating rate, 2°C/min. The enzyme was filtered off and the solvent evaporated at reduced pressure. The residue was submitted to silica gel column chromatography (eluant: 3% ethyl acetate/petroleum ether) to afford 5.24 g (16.07 mmol) of (*S*)-(–)-**7** and 6.73 g (18.28 mmol) of (*R*)-(+)-**10**.

(*S*)-1-(*tert*-Butyldiphenylsilyloxy)-2-hydroxy-3-butene (–)-**7**: $[\alpha]_D^{20} = -5.0$ (*c* 1.040, $CHCl_3$), e.e. > 99%. The HPLC analysis was carried out on a Chiralcel OD column: eluant 0.2% 2-propanol/petroleum ether, flow rate 1 mL/min, λ 254 nm. Relative retention times (min): (*R*)-(+)-**7**, 29.25; (*S*)-(–)-**7**, 40.02. To corroborate the assignment of absolute configuration, a sample (200 mg) of (*S*)-(–)-**7** was treated with a 1 M solution of tetrabutylammonium fluoride in THF and converted into known (*S*)-(–)-3-butene-1,2-diol.

(*R*)-2-Acetyloxy-1-(*tert*-butyldiphenylsilyloxy)-3-butene (+)-**10**: viscous colorless oil, bp 205–210°C/0.3 mmHg, R_f 0.64 (eluant: 5% ethyl acetate/cyclohexane); $[\alpha]_D^{20} = +10.8$ (*c* 1.010, $CHCl_3$); e.e. = 92% [from HPLC analysis, after conversion into the corresponding (*R*)-(+)-**7**]. ^{1}H NMR: 1.05 (s, 9), 2.06 (s, 3, CH_3CO), 3.73 (m, 2, CH_2OSi), 5.22 (d, 1, C*H*=CHCHOCO, J = 10.6), 5.29 (d, 1, C*H*=CHCHOCO, J = 17.3), 5.41 (m, 1, CHOCO), 5.81 (ddd, 1, CH_2=C*H*, J = 6.3, 10.6 and 17.3), 7.43 (m, 6, arom.), 7.67 (m, 4, arom.).

3.3. Synthesis of aminoalcohols (+)-**2a**/(–)-**2a** and (+)-**2b**/(–)-**2b**

A. To an ethyl acetate solution (200 mL) of pyruvohydroximoyl chloride[20] (2.67 g, 22 mmol) and (*S*)-(–)-**7** (4.78 g, 14.67 mmol) was added solid sodium bicarbonate (9.25 g, 0.11 mol). The mixture was stirred at room temperature until disappearance of the starting dipolarophile (about 18 h). The slurry was then poured into water, the organic layer separated and the aqueous phase was further extracted with ethyl acetate (2×50 mL). The pooled organic extracts were dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. A silica gel column chromatography of the residue (eluant: 10% ethyl acetate/petroleum ether) gave 2.38 g of the *anti*-isomer (+)-**11a** and 2.03 g of the *syn*-isomer (–)-**11b** (73% overall yield).

(+)-**11a** (5*S*,α*S*): thick colorless oil, R_f 0.27 (eluant: 20% ethyl acetate/*n*-hexane); $[\alpha]_D^{20} = +66.4$ (*c* 0.990, $CHCl_3$); ^{1}H NMR: 1.07 (s, 9), 2.48 (s, 3, CH_3CO), 2.50 (bs, 1, OH), 3.11 (dd, 1, H-4′, J = 11.3 and 17.6), 3.14 (dd, 1, H-4, J = 7.9 and 17.6), 3.73 (m, 2, CH_2OSi), 3.82 (m, 1, C*H*OH), 4.85 (ddd, 1, H-5, J = 5.7, 7.9 and 11.3), 7.41 (d, 6, arom.), 7.66 (d, 4, arom.). Anal. calcd for $C_{23}H_{29}NO_4Si$: C, 67.12; H, 7.10; N, 3.40. Found: C, 67.55; H, 6.90; N, 3.27.

(–)-**11b** (5*R*,α*S*): thick colorless oil; R_f 0.33 (eluant: 20% ethyl acetate/*n*-hexane); $[\alpha]_D^{20} = -108.1$ (*c* 0.990, $CHCl_3$); ^{1}H NMR: 1.07 (s, 9), 2.49 (s, 3, CH_3CO), 2.65 (bs, 1, OH), 3.13 (m, 2, H-4), 3.68–3.80 (m, 3), 4.89 (ddd, 1, H-5, J = 2.4, 9.8 and 9.8), 7.39 (d, 6, arom.), 7.65 (d, 4, arom.). Anal. calcd for $C_{23}H_{29}NO_4Si$: C, 67.12; H, 7.10; N, 3.40. Found: C, 66.70; H, 6.75; N, 3.71.

B. To a stirred solution of 6.40 g (17.39 mmol) of (*R*)-(+)-**10** in MeOH (120 mL) 40 mL of 2N NaOH was added. The reaction mixture was stirred for 2 h at room temperature, then methanol was evaporated under vacuum and the residual aqueous phase was extracted with ethyl acetate (3×30 mL). After the usual work-up, crude (*R*)-(+)-**7** was purified by distillation (5.025 g, 89% yield).

(*R*)-1-(*tert*-Butyldiphenylsilyloxy)-2-hydroxy-3-butene (+)-**7**: $[\alpha]_D^{20} = +4.4$ (*c* 1.020, $CHCl_3$), e.e. = 92% [HPLC analysis on a Chiralcel OD column, following the conditions reported above for (–)-**7**].

The cycloaddition reaction to (*R*)-(+)-**7**, performed following the above described procedure, allowed the isolation of stereoisomers (–)-**11a** and (+)-**11b**.

(–)-**11a** (5*R*,α*R*): thick colorless oil; $[\alpha]_D^{20} = -61.2$ (*c* 0.995, $CHCl_3$). Anal. calcd for $C_{23}H_{29}NO_4Si$: C, 67.12; H, 7.10; N, 3.40. Found: C, 66.82; H, 7.51; N, 3.19.

(+)-**11b** (5*S*,α*R*): thick colorless oil; $[\alpha]_D^{20} = +98.9$ (*c* 1.015, $CHCl_3$). Anal. calcd for $C_{23}H_{29}NO_4Si$: C, 67.12; H, 7.10; N, 3.40. Found: C, 66.91; H, 6.87; N, 3.65.

C. To an ice-cooled stirred suspension of potassium *tert*-butoxide (1.40 g, 12.48 mmol) in anhydrous toluene (100 mL) was added portionwise methyltriphenylphosphonium bromide (4.78 g, 13.37 mmol). After heating at reflux for 1 h, the suspension was cooled at room temperature. A solution of (+)-**11a** (2.20 g, 5.35 mmol) in toluene (5 mL) was then added dropwise. The mixture was stirred at room temperature for about 3 h, until disappearance of the starting material; the progress of the reaction was monitored by TLC (eluant: 20% ethyl acetate/petroleum ether). Acetone (10 mL) and water (50 mL) were then added, the organic phase was separated and the aqueous phase was extracted with ether (3×30 mL). After the usual work-up, the residue was submitted to column chromatography (eluant: 5% ethyl acetate/petroleum ether) affording 1.16 g (53% yield) of the desired *anti* 3-isopropenyl-Δ^2-isoxazoline (+)-**12a**.

(+)-**12a** (5*S*,α*S*): thick colorless oil; R_f 0.30 (eluant: 15% ethyl acetate/*n*-hexane); $[\alpha]_D^{20} = +73.3$ (*c* 0.992, $CHCl_3$); ^{1}H NMR: 1.08 (s, 9), 2.03 (s, 3, C*H*$_3$-C=CH$_2$), 2.48 (d, 1, OH, J = 3.5), 3.10 (dd, 1, H-4′, J = 10.8 and 16.5), 3.18 (dd, 1, H-4, J = 7.6 and 16.5), 3.70–3.90 (m, 3), 4.72 (ddd, 1, H-5, J = 5.0, 7.6 and 10.8), 5.21 (bs, 1, CH$_3$-C=C*H*), 5.33 (bs, 1, CH$_3$-C=C*H*), 7.39 (d, 6, arom.), 7.67 (d, 4, arom.). Anal. calcd for $C_{24}H_{31}NO_3Si$: C, 70.38; H, 7.63; N, 3.42. Found: C, 70.06; H, 7.98; N, 3.12.

The same procedure carried out on 3-acetyl-Δ^2-isoxazolines (–)-**11a**, (–)-**11b** and (+)-**11b** gave the corresponding 3-isopropenyl derivatives (–)-**12a**, (–)-**12b** and (+)-**12b** in comparable yields.

(–)-**12a** (5*R*,α*R*): thick colorless oil; $[\alpha]_D^{20} = -67.1$ (*c* 1.01, $CHCl_3$). Anal. calcd for $C_{24}H_{31}NO_3Si$: C, 70.38; H, 7.63; N, 3.42. Found: C, 69.95; H, 8.04; N, 3.17.

(–)-**12b** (5*R*,α*S*): thick colorless oil; R_f 0.26 (eluant: 15% ethyl acetate/*n*-hexane); $[\alpha]_D^{20} = -91.1$ (*c* 1.0, $CHCl_3$); ^{1}H NMR: 1.07 (s, 9), 2.04 (s, 3, C*H*$_3$-C=CH$_2$), 2.32 (d, 1, OH, J = 5.7), 3.12 (m, 2, H-4), 3.63–3.82 (m, 3), 4.78 (ddd, 1, H-5, J = 3.4, 9.6 and 9.6), 5.18 (bs, 1, CH$_3$-C=C*H*), 5.33 (bs, 1, CH$_3$-C=C*H*), 7.39 (d, 6, arom.), 7.65 (d, 4, arom.). Anal. calcd for $C_{24}H_{31}NO_3Si$: C, 70.38; H, 7.63; N, 3.42. Found: C, 70.45; H, 8.01; N, 3.15.

(+)-**12b** (5*S*,α*R*): thick colorless oil; $[\alpha]_D^{20} = +82.2$ (*c* 0.990, $CHCl_3$). Anal. calcd for $C_{24}H_{31}NO_3Si$: C, 70.38; H, 7.63; N, 3.42. Found: C, 70.70; H, 7.91; N, 3.32.

D. To a stirred solution of (+)-**12a** (1.08 g, 2.64 mmol) in THF (50 mL) 3.5 mL of a 1 M solution of tetrabutylammonium fluoride in THF was added. After stirring for 1 h at room temperature, the reaction mixture was concentrated and directly submitted to a silica gel column chromatography (eluant: 30% petroleum ether/ethyl acetate) to give 0.384 g (85% yield) of the desired diol (+)-**13a**.

(+)-**13a** (5*S*,α*R*): mp 88.5–90.5°C (colorless leaflets from diisopropyl ether); R_f 0.22 (eluant: 50% ethyl acetate/cyclohexane); $[\alpha]_D^{20} = +172.1$ (*c* 1.050, $CHCl_3$); ^{1}H NMR: 2.04 (s, 3, CH$_3$), 2.11 (bs, 2, OH), 3.16 (dd, 1, H-4′, J = 11.0 and 16.4), 3.19 (dd, 1, H-4, J = 8.5 and 16.4), 3.71 (dd, 1, J = 6.1 and 11.5), 3.73–3.92 (m, 2), 4.63 (ddd, 1, H-5, J = 4.7, 8.5 and 11.0), 5.26 (bs, 1, CH$_3$-C=C*H*), 5.36 (bs, 1, CH$_3$-C=C*H*). Anal. calcd for $C_8H_{13}NO_3$: C, 56.13; H, 7.65; N, 8.18. Found: C, 56.27; H, 7.90; N, 8.02.

The same procedure carried out on 3-isopropenyl-Δ^2-isoxazolines (–)-**12a**, (–)-**12b** and (+)-**12b** gave the corresponding diols (–)-**13a**, (–)-**13b** and (+)-**13b** in comparable yields.

(–)-**13a** (5*R*,α*S*): mp 88–90.5°C (colorless leaflets from diisopropyl ether); $[\alpha]_D^{20} = -157.1$ (*c* 1.025, $CHCl_3$). Anal. calcd for $C_8H_{13}NO_3$: C, 56.13; H, 7.65; N, 8.18. Found: C, 56.38; H, 7.73; N, 8.41.

(–)-**13b** (5*R*,α*R*): mp 86–88°C (colorless leaflets from diisopropyl ether); R_f 0.14 (eluant: 50% ethyl acetate/*n*-hexane); $[\alpha]_D^{20} = -232.4$ (*c* 0.998, $CHCl_3$); ^{1}H NMR: 2.04 (s, 3, CH_3), 2.12 (bs, 1, OH), 2.48 (d, 1, OH, J = 6.2), 3.16 (m, 2, H-4), 3.60–3.85 (m, 3), 4.73 (ddd, 1, H-5, J = 4.5, 9.6 and 9.6), 5.24 (bs, 1, CH_3-C=C*H*), 5.36 (bs, 1, CH_3-C=C*H*). Anal. calcd for $C_8H_{13}NO_3$: C, 56.13; H, 7.65; N, 8.18. Found: C, 55.91; H, 7.97; N, 7.88.

(+)-**13b** (5*S*,α*S*): mp 85–88°C (colorless leaflets from diisopropyl ether); $[\alpha]_D^{20} = +211.0$ (*c* 1.0, $CHCl_3$). Anal. calcd for $C_8H_{13}NO_3$: C, 56.13; H, 7.65; N, 8.18. Found: C, 56.37; H, 7.79; N, 8.39.

E. To a solution of 0.350 g (2.05 mmol) of (+)-**13a** in dichloromethane (5 mL) were added toluene-4-sulfonic acid monohydrate (5 mg) and trimethyl orthoacetate (310 μL, 2.46 mmol). After stirring at room temperature for 0.5 h, the volatiles were evaporated at reduced pressure and the residue was dissolved in dichloromethane (5 mL). Trimethylchlorosilane (365 μL, 2.88 mmol) was then added and the reaction mixture was stirred for 4 h at room temperature. After removal of the solvent, the residue was taken up with MeOH (10 mL) and treated with potassium carbonate (0.570 g). The suspension was vigorously stirred for 1 h, then 20 mL of a saturated aqueous solution of ammonium chloride was added. The reaction mixture was extracted with dichloromethane (3×10 mL) and, after the usual work-up, the residue was purified by column chromatography (eluant: 20% ethyl acetate/petroleum ether), affording 0.244 g (78% yield) of the desired epoxide (+)-**4a**.

(+)-**4a** (5*S*,α*R*): colorless liquid, bp 80–85°C/1.5 mmHg, R_f 0.51 (eluant: 30% ethyl acetate/cyclohexane); $[\alpha]_D^{20} = +167.5$ (*c* 1.040, $CHCl_3$). The ^{1}H NMR spectrum was identical to that reported for (±)-**4a**.[6] Anal. calcd for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14. Found: C, 62.44; H, 7.03; N, 9.37.

Epoxides (–)-**4a**, (–)-**4b** and (+)-**4b** were prepared from diols (–)-**13a**, (–)-**13b** and (+)-**13b** in comparable yields through the same sequence of steps.

(–)-**4a** (5*R*,α*S*): colorless liquid, bp 80–85°C/1.5 mmHg; $[\alpha]_D^{20} = -155.3$ (*c* 1.150, $CHCl_3$). Anal. calcd for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14. Found: C, 62.59; H, 7.50; N, 8.92.

(–)-**4b** (5*R*,α*R*): colorless liquid, bp 80–85°C/1.5 mmHg (crystalline on standing, mp 51.5–54.5°C), R_f 0.46 (eluant: 30% ethyl acetate/cyclohexane); $[\alpha]_D^{20} = -211.7$ (*c* 0.990, $CHCl_3$). The ^{1}H NMR spectrum was identical to that reported for (±)-**4b**.[6] Anal. calcd for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14. Found: C, 63.01; H, 7.05; N, 9.16.

(+)-**4b** (5*S*,α*S*): colorless liquid, bp 80–85°C/1.5 mmHg; $[\alpha]_D^{20} = +194.9$ (*c* 0.995, $CHCl_3$). Anal. calcd for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14. Found: C, 62.60; H, 7.41; N, 9.35.

F. A stirred solution of (+)-**4a** (230 mg, 1.50 mmol) and *tert*-butylamine (950 mL, 9 mmol) in methanol (10 mL) was refluxed until TLC (eluant: 20% ethyl acetate/petroleum ether) evidenced the disappearance of the starting material. The solvent and excess reagent were removed under vacuum, the oily residue was dissolved in 3N HCl (15 mL) and washed with ethyl ether (2×10 mL). The aqueous layer was alkalinized with solid sodium carbonate and extracted with ethyl acetate (3×10 mL). After the usual work-up, the residue of the pooled organic extracts (278 mg, 82% yield) was dissolved in anhydrous ethyl ether and treated with a threefold excess of anhydrous oxalic acid. The corresponding oxalate, which precipitated immediately, was recovered by suction filtration.

(+)-**2a** (5*S*,α*R*)×$C_2H_2O_4$: colorless prisms (from absolute ethanol), mp 191–194°C, dec.; $[\alpha]_D^{20} = +159.6$ (*c* 1.0, MeOH), e.e. > 99%, determined by chiral HPLC analysis on a Chirobiotic T

column [eluant: MeOH/AcOH (0.05%)/TEA (0.05%), injection vol.: 2 μL, flow rate: 0.6 mL/min, λ 254 nm]. Relative retention times (min): (–)-**2a**, 24.52; (+)-**2a**, 26.22. The ^{1}H NMR spectrum (recorded on the corresponding free base) was identical to that reported for (±)-**2a**.[6] Anal. calcd for $C_{14}H_{24}N_2O_6$: C, 53.15; H, 7.65; N, 8.86. Found: C, 52.92; H, 7.59; N, 8.89.

Isomeric aminoalcohols (–)-**2a**, (–)-**2b** and (+)-**2b** were similarly obtained in comparable yields from epoxides (–)-**4a**, (–)-**4b** and (+)-**4b**, respectively.

(–)-**2a** (5*R*,α*S*)×$C_2H_2O_4$: colorless prisms (from absolute ethanol), mp 191–193°C, dec.; $[\alpha]_D^{20}$ = –147.9 (*c* 1.0, MeOH), e.e. = 92% [HPLC analysis on a Chirobiotic T column, following the conditions reported above for (+)-**2a**]. Anal. calcd for $C_{14}H_{24}N_2O_6$: C, 53.15; H, 7.65; N, 8.86. Found: C, 53.37; H, 7.45; N, 8.74.

(–)-**2b** (5*R*,α*R*)×$C_2H_2O_4$: colorless prisms (from absolute ethanol), mp 164.5–168°C, dec.; $[\alpha]_D^{20}$ = –167.9 (*c* 0.990, MeOH); e.e. > 99%, determined by chiral HPLC analysis on a Chirobiotic T column [eluant: MeOH/AcOH (0.25%)/TEA (0.25%), injection vol.: 2 μL, flow rate: 0.6 mL/min, λ 254 nm]. Relative retention times (min): (+)-**2b**, 13.30; (–)-**2b**, 14.31. The ^{1}H NMR spectrum (recorded on the corresponding free base) was identical to that reported for (±)-**2b**.[6] Anal. calcd for $C_{14}H_{24}N_2O_6$: C, 53.15; H, 7.65; N, 8.86. Found: C, 53.10; H, 7.76; N, 8.84.

(+)-**2b** (5*S*,α*S*)×$C_2H_2O_4$: colorless prisms (from absolute ethanol), mp 164–167.5°C, dec.; $[\alpha]_D^{20}$ = +154.3 (*c* 1.010, MeOH); e.e. = 92% [HPLC analysis on a Chirobiotic T column, following the conditions reported above for (–)-**2b**]. Anal. calcd for $C_{14}H_{24}N_2O_6$: C, 53.15; H, 7.65; N, 8.86. Found: C, 52.97; H, 7.60; N, 8.71.

Acknowledgements

This research was financially supported by grants from the National Council of Research (CNR, Target Project on Biotechnology) and from the Ministero dell'Università Scientifica e Tecnologica (MURST, Rome).

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