Chemoenzymatic Synthesis of All Four Cytoxazone Stereoisomers

Zdenko Hameršak,[a] Edina Ljubović,[a] Mladen Merćep,[b] Milan Mesić,[b] Vitomir Šunjić*[a]

[a] Ruđer Bošković Institute, P.O.B. 180, HR-10002, Zagreb, Croatia

[b] PLIVA d.d., Research Institute, Prilaz baruna Filipovića 25, HR-10000, Zagreb, Croatia
Fax +385(1)4680195; E-mail: sunjic@rudjer.irb.hr

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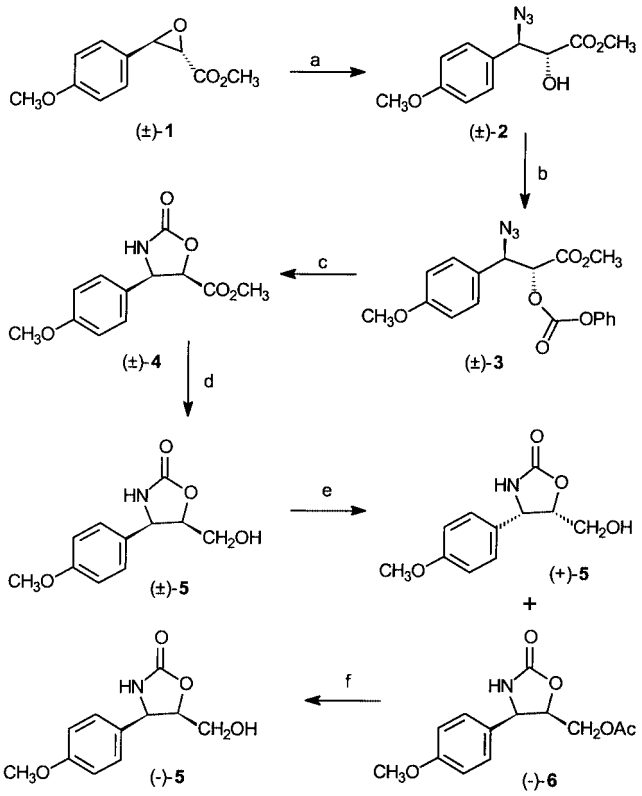
Abstract: Racemic cytoxazone (±-**5**) was synthesized starting from easily available glycidic ester (±)-**1** by nucleophilic epoxide ring opening, followed by 2-oxazolidinone ring construction and calcium chloride/sodium borohydride reduction of the intermediary ester (±)-**4**. Kinetic resolution of (±)-**5** performed by acetylation with vinyl acetate catalyzed by *Penicillium camemberti* lipase (PcamL) afforded, on hydrolysis of acetate (–)-**6**, cytoxazone (–)-**5** in 33% overall yield and 88.2% enantiomeric excess (ee), and its enantiomer (+)-**5** (38% yield, 89.3% ee). Base-catalyzed epimerization of intermediary (±)-**4** to (±)-**7**, and reduction and kinetic resolution with *Candida antarctica* lipase (CAL) led to *epi*-cytoxazones (–)-**8** and (+)-**8**.

Key words: oxazolidinones, epoxide ring opening, lipases, kinetic resolution

(–)-Cytoxazone (–)-**5**, (4*R*,5*R*)-5-(hydroxymethyl)-4-(4-methoxyphenyl)-1,3-oxazolidin-2-one), a novel cytokine modulator produced by *Streptomyces sp*. was isolated from the fermentation broth in a low yield,[1] and its absolute configuration has been determined by X-ray crystallographic analysis and CD-spectroscopy.[2] Cytoxazone and its analogues have become a new subject of synthetic studies because of their immunostimulating activity.[1] As yet, three syntheses of cytoxazone stereoisomers have been published. Two of them, reported by Nakata's group[3] and by Mori's group[4], employed Sharpless asymmetric dihydroxylation of *p*-methoxy cinnamic derivative, while the third route involved imino-1,2-Wittig rearrangement of hydroximate and conventional optical resolution of racemates in the final step.[5] 4-*epi*-Cytoxazone has been prepared by asymmetric dihydroxylation of *p*-methoxy cinnamic ester followed by cyclic iminocarbonate rearrangement.[6]

Here, we describe the chemoenzymatic synthesis of cytoxazone and its stereoisomers starting from readily available glycidic ester (±)-**1**,[7] Scheme 1.

Epoxide ring opening in (±)-**1** with sodium azide proceeded, according to a well-known mechanism,[8] with complete regio and stereoselectivity affording azido alcohol (±)-**2**. Construction of the 2-oxazolidinone ring was completed on conversion of (±)-**2** to phenyl carbonate (±)-**3** followed by simultaneous reduction and cyclization. Reduction of the ester group in *cis*-oxazolidinone (±)-**4** was



Scheme 1 Synthesis of (–)- and (+)-cytoxazone; *Reagents and conditions*: a) aq NaN_3, dioxane, 50 °C, 3 h, 56%; b) $ClCO_2Ph$, CH_2Cl_2, –5 °C, 1 h, 100%; c) Ph_3P, aq THF, 50 °C, 90 min, 88%; d) $NaBH_4$, $CaCl_2$, absolute EtOH, r.t., 20 min, 79%; e) PcamL, vinyl acetate, 30 °C; f) KOH, MeOH, r.t., 1 h

accomplished with calcium chloride/sodium borohydride in absolute ethanol; in the absence of calcium chloride, slow reaction and poor selectivity was observed. Correlation of ^1H and ^{13}C NMR spectra of (±)-**5** with literature data for (–)-cytoxazone[3] confirmed its structure and *cis*-configuration.

Kinetic resolution of (±)-**5** was attempted by a number of commercial lipases: *Penicillium camambertii* (PcamL), *Candida antarctica* (CAL) immobilized in Sol-Gel-AK, *Candida antarctica A*, *Penicillium roqueforti*, *Humicola lanuginosa*, *Pseudomonas fluorescens*, *Pseudomonas cepacia*, *Porcine pancreas*, *Candida cylindracea*, *Aspergilus niger*, *Mucor miehei*, *Mucor iavanicus*, *Candida lipolitica*, *Rhizopus niveus* and *Rhisopus delemar*. Most of them exhibited low activity and very low enantioselectiv-

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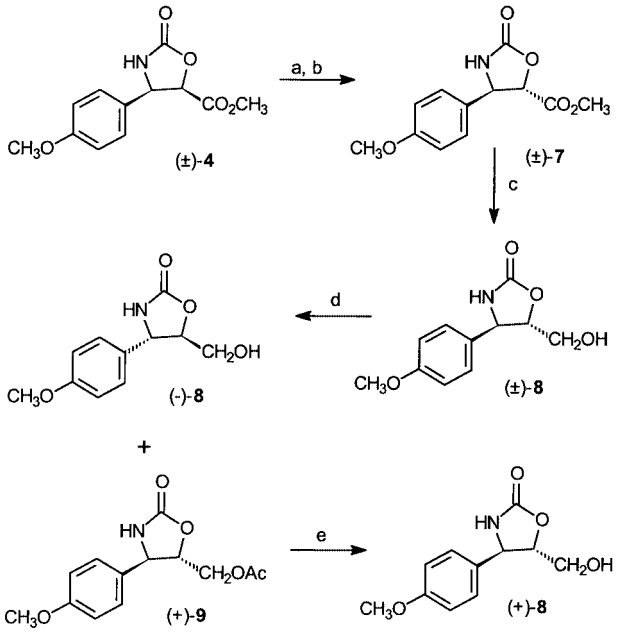
ity (E values < 5); PcamL was selected for the preparative enzymatic resolution (E value = 39).[9] At 50.7% conversion, the ee of alcohol (+)-**5** was 89.3%, and the ee of acetate (–)-**6** was 88.2%. Upon their separation by silica gel column chromatography and subsequent hydrolysis of (–)-**6**, both alcohols were crystallized to obtain (–)-cytoxazone (–)-**5** with 99.0% ee, and (+)-cytoxazone, (+)-**5** with 95.2% ee.

(±)-*epi*-Cytoxazone (±)-**8** was obtained from common intermediate, oxazolidinone (±)-**4**, which was epimerized at C(5) using potassium hydroxide in ethanol followed by esterification with methyl iodide (Scheme 2). Reduction of (±)-**7** with calcium chloride/sodium borohydride afforded (±)-**8**, whose structure was confirmed by correlation of ^{1}H and ^{13}C NMR data with those reported for (–)-*epi*-cytoxazone.[3]



Scheme 2 Synthesis of (–)- and (+)-*epi*-cytoxazone; a) KOH, EtOH, reflux, 1 h; b) MeI, K_2CO_3, DMF, r.t., 16 h, 46%; c) $NaBH_4$, $CaCl_2$, absolute EtOH, r.t., 20 min, 82%; d) CAL in SOL-Gel-AK, vinyl acetate, 30 °C; e) KOH, MeOH, r.t., 1 h

Interestingly, *epi*-racemate (±)-**8** proved a better substrate for microbial lipases than (±)-**5**. *Penicillium camambertii*, *Candida antarctica* immobilized in Sol-Gel-AK, *Candida antarctica A*, *Penicillium roqueforti*, *Humicola lanuginosa*, *Pseudomonas fluorescens* and *Pseudomonas cepacia* immobilized on ceramics exhibited high activity, but most of them showed relatively low enantioselectivity (E values 2–5). With CAL in Sol-Gel-AK, the best selectivity was obtained (E value = 37), so this lipase was selected for the preparative resolution; at 49.1% conversion, ee of alcohol (–)-**8** was 84.2%, and ee of acetate (+)-**9** was 87.3%, which was hydrolyzed to (+)-**8** with 79.1% ee.

In conclusion, all four cytoxazone stereoisomers have been prepared by the chemoenzymatic route starting from easily available glycidic ester (±)-**1**. This method is a valuable alternative to the earlier reported synthetic approaches to these chiral compounds.

^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 FT spectrometer; chemical shifts (ppm) are reported downfield from TMS. IR spectra were recorded on Perkin–Elmer 297 spectrometer. High resolution mass spectrometry was performed on an Extrel-FTMS 2001 DD Instrument. Optical rotations were determined on an AA-10 polarimeter. HPLC analyses were performed on a Hewlett Packard instrument Series 1050 with UV detector (λ = 235 nm) and Hewlett Packard integrator 3396A. Conversions were monitored by HPLC chromatography using Nucleosil C-18 7 μm column (250 × 4.6 mm); flow rate 0.8 mL/min; using gradient program from 10% to 100% MeOH over 20 min. Enzymatic kinetic resolutions were monitored using Nucleosil C-18 7 μm column (250 × 4.6 mm) under the following conditions: MeOH–H_2O (1:1) as eluent, flow rate 0.8 mL/min. Determination of the enantiomeric purity was performed using Chiralcel AS column (250 × 4.6 mm) with Chiralcel AS precolumn (50 × 4.6 mm) in *n*-hexane–*i*-PrOH (1:1) for (±)-**5**, and (7:4.5) for (±)-**8**, flow rate 1.0 mL/min, method accuracy ±0.2%. Enzymatic acylations were performed in a thermostated shaker (Tehtnica Co.) at 220 rpm. Lipases from the following microorganisms (purchased from Amano Co.) were tested: *Penicillium camambertii*, *Candida antarctica* immobilized in Sol-Gel-AK, *Candida antarctica A*, *Penicillium roqueforti*, *Humicola lanuginosa*, *Pseudomonas fluorescens*, *Pseudomonas cepacia*, *Pseudomonas cepacia* immobilized on ceramics, *Porcine pancreas*, *Candida cylindracea*, *Aspergilus niger*, *Mucor miehei*, *Mucor iavanicus*, *Candida lipolitica*, *Rhizopus niveus* and *Rhisopus delemar*.

Methyl 3-azido-2-hydroxy-3-(4-methoxyphenyl)propanoate [(±)-2]

To a solution of NaN_3 (2.90 g, 52 mmol) in H_2O (30 mL), a solution of epoxide (±)-**1** (6.60 g, 31 mmol) in dioxane (80 mL) was added. The reaction mixture was stirred at 50 °C for 3 hours. EtOAc (80 mL) was added, the solution was washed with H_2O (3 × 50 mL), and dried. Upon evaporation of the solvent, crude product was purified by column chromatography on silica gel (EtOAc–hexane, 2:8) yielding 4.29 g (56%) of oily azide (±)-**2**, which was 98% pure by HPLC.

IR (neat): 3500, 2960, 2100, 1745, 1615, 1515, 1250 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 3.03 (d, 1 H, *J* = 6.5 Hz), 3.72 (s, 3 H), 3.80 (s, 3 H), 4.50 (dd, 1 H, *J* = 6.5, 4.0 Hz), 4.83 (d, 1 H, *J* = 4.0 Hz), 6.90 (d, 2 H, *J* = 8.5 Hz), 7.27 (d, 2 H, *J* = 8.5 Hz).

^{13}C NMR ($CDCl_3$): δ = 52.6, 55.1, 66.4, 73.5, 113.9, 126.0, 129.0, 159.8, 171.7.

HRMS: *m/z* calcd. for $C_{11}H_{13}N_3O_4$ [M$^+$]: 252.0978. Found: 252.0891.

Methyl-3-azido-3-(4-methoxyphenyl)-2-[(phenoxycarbonyl)-oxy]propanoate [(±)-3]

To a solution of azide **(**±)-**2** (3.80 g, 16 mmol) and pyridine (1.4 mL, 17 mmol) in anhyd CH_2Cl_2 (60 mL), a solution of phenylchloroformate (2.2 mL, 2.75 g, 17 mmol) in CH_2Cl_2 (5.0 mL) was added at -5 °C over 10 min. After stirring at –5 °C for 1 h, the reaction mixture was poured into H_2O. The organic layer was washed with 1% H_3PO_4, then with 3% $NaHCO_3$, and dried. Upon evaporation of the solvent, 6.10 g (quantitative) of oily (±)-**3** was obtained; HPLC analysis revealed 97% purity.

IR (neat): 2955, 2100, 1760, 1610, 1510, 1245 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 3.75 (s, 3 H), 3.83 (s, 3 H), 5.05 (d, 1 H, *J* = 5.0 Hz), 5.35 (d, 1 H, *J* = 5.0 Hz), 6.82–7.46 (m, 9 H).

^{13}C NMR ($CDCl_3$): δ = 52.4, 54.7, 63.7, 76.9, 113.7, 113.9, 120.4, 124.9, 125.8, 128.6, 129.0, 150.4, 152.2, 159.7, 166.6, 166.8.

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Methyl 4-(4-Methoxyphenyl)-2-oxo-1,3-oxazolidine-5-carboxylate [(±)-4]

Azido-ester (±)-**3** (6.0 g, 16 mmol) and Ph_3P (12.6 g, 48 mmol) were dissolved in THF (160 mL) and H_2O (3.3 mL). The reaction mixture was heated at 50 °C for 1.5 h. Evolution of N_2 was observed during the first 30 min of the reaction. The solvent was evaporated, the solid residue was dissolved in EtOAc (150 mL), washed with 5% NaCl (3 × 50 mL), and dried. Crude product was crystallized from *i*-Pr_2O to obtain 3.55 g (88%) of oxazolidinone (±)-**4**, which was 98% pure by HPLC.

Mp 160–161 °C.

IR (KBr): 3240, 2945, 1745, 1720, 1510, 1460, 1215, 1090 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 3.39 (s, 3 H), 3.80 (s, 3 H), 5.19 (d, 1 H, *J* = 9.0 Hz), 5.26 (d, 1 H, *J* = 9.0 Hz), 6.21 (br s, 1 H), 6.87 (d, 2 H, *J* = 8.5 Hz), 7.21 (d, 2 H, *J* = 8.5 Hz).

^{13}C NMR ($CDCl_3$): δ = 51.9, 55.2, 57.8, 78.0, 113.9, 128.0, 128.5, 158.1, 160.1, 166.7.

HRMS: *m/z* calcd. for $C_{12}H_{13}NO_5$ [M^+]: 252.0866. Found: 252.0811.

5-(Hydroxymethyl)-4-(4-methoxyphenyl)-1,3-oxazolidin-2-one [(±)-5]

Oxazolidinone (±)-**4** (50 mg, 0.22 mmol) was dissolved in absolute EtOH (4 mL), $CaCl_2$ (50 mg, 0.45 mmol) and $NaBH_4$ (40 mg, 1.0 mmol) was added, and reaction mixture was stirred for 20 min at r.t. The excess of reducing agent was destroyed by the addition of sat. NH_4Cl (0.5 mL), EtOH was evaporated; the residue was dissolved in EtOAc (30 mL), washed with 5% NaCl (2 × 20 mL), and dried. Crude product was crystallized from *t*-butyl methyl ether (MTBE)–MeOH (95:5) to give 35 mg (79%) of (±)-**5**, which was >99% pure by HPLC.

Mp 143–144 °C.

IR (KBr): 3350, 3240, 2940, 1720, 1215, 1025, 965 cm^{-1}.

^1H NMR (acetone-d_6): δ = 3.15–3.26 (m, 3 H), 3.80 (s, 3 H), 4.82 (ddd, 1 H), 5.03 (d, 1 H, *J* = 8.0 Hz), 6.94 (d, 2 H, *J* = 8.5 Hz), 6.96 (br s, 1 H), 7.24 (d, 2 H, *J* = 8.5 Hz).

^{13}C NMR (acetone-d_6): δ = 54.9, 57.2, 61.9, 80.8, 113.9, 128.3, 129.6, 158.8, 159.9.

Methyl 4-(4-Methoxyphenyl)-2-oxo-1,3-oxazolidine-5-carboxylate [(±)-7]

To a solution of (±)-**4** (1.30 g, 5.1 mmol) in EtOH (6 mL), KOH (320 mg, 5.7 mmol) was added. The resulting mixture was kept at reflux for 1 h, then cooled to r.t., acidified with 10% HCl, and extracted with EtOAc (3 × 30 mL). Combined extracts were washed with H_2O (30 mL), dried (Na_2SO_4), and evaporated in vacuo. The residue was dissolved in DMF (15 mL), K_2CO_3 (700 mg, 5.1 mmol) and MeI (980 mg, 7.0 mmol) were added. The reaction mixture was stirred overnight at r.t., then H_2O (30 mL) and EtOAc (60 mL) were added. The organic layer was successively washed with H_2O (5 × 30 mL), dried, and evaporated. Crude product was purified by silica gel chromatography (MTBE–hexane, 4:1). Evaporation of the fractions with (±)-**7** afforded 530 mg (46%) of pure product (>99% by HPLC).

Mp 123–124 °C.

IR (KBr): 3270, 2960, 1760, 1710, 1515, 1255, 1205, 1090, 920, 835 cm^{-1}.

^1H NMR ($CDCl_3$): δ = 3.81 (s, 3 H), 3.86 (s, 3 H), 4.72 (d, 1 H, *J* = 5.2 Hz), 4.93 (d, 1 H, *J* = 5.2 Hz), 6.60 (br s, 1 H), 6.92 (d, 2 H, *J* = 8.1 Hz), 7.28 (d, 2 H, *J* = 8.1 Hz).

^{13}C NMR ($CDCl_3$): δ = 52.9, 55.2, 58.6, 80.3, 114.4, 127.1, 130.6, 157.8, 159.9, 168.7.

HRMS: *m/z* calcd. for $C_{12}H_{13}NO_5$ [M^+]: 252.0866. Found: 252.0792.

5-*epi*-5-(Hydroxymethyl)-4-(4-methoxyphenyl)-1,3-oxazolidin-2-one [(±)-8]

5-*epi*-Cytoxazone [(±)-**8**] was obtained in 82% yield, as described for (±)-**5**.

Mp 158–159 °C.

IR (KBr): 3290, 2920, 1720, 1510, 1240, 1025, 830 cm^{-1}.

^1H NMR (CD_3OD): δ = 3.39 (br s, 1 H), 3.77 (dd, 1 H, *J* = 12.0, 3.0 Hz), 3.88 (s, 3 H), 3.90 (dd, 1 H, *J* = 12.0, 3.0 Hz), 4.35–4.45 (m, 1 H), 4.83 (d, 1 H, *J* = 6.5 Hz), 7.05 (d, 2 H, *J* = 8.5 Hz), 7.39 (d, 2 H, *J* = 8.5 Hz).

^{13}C NMR (CD_3OD): δ = 55.9, 58.7, 62.6, 87.0, 115.5, 128.7, 133.7, 161.0, 161.5.

Preparative Kinetic Resolution of [(±)-5] Catalyzed by Lipase from *Penicillium camembertii*

To the solution of (±)-**5** (200 mg) in vinyl acetate (140 mL), lipase from PcamL (4.0 g) was added. The reaction was performed in a thermostated shaker at 30 °C and 220 rpm. After 9 days, at 50.7% conversion, the reaction mixture was filtered. The filtrate was evaporated and products were separated on a silica gel column (EtOAc–CH_2Cl_2, 3:1). Combined fractions yielded pure (+)-**5** (81 mg, 89.3% ee) and (–)-**6** (92 mg, 88.2% ee). Acetate (–)-**6** was hydrolysed with KOH/MeOH (1.1 mol equiv of KOH) at r.t. Upon crystallization from MTBE, (–)-**5** was obtained in 99.0% ee, and (+)-**5** in 95.2% ee.

(–)-**5**

Mp 122–123 °C (Lit.[3] mp 122–123 °C).

$[\alpha]_D^{25}$ –74.0 (*c* 0.5, MeOH) [Lit.[3] $[\alpha]_D$ –75.7 (*c* 1.00, MeOH)].

(+)-**5**

$[\alpha]_D^{25}$ +70.1 (*c* 0.5, MeOH).

(–)-**6**

$[\alpha]_D^{25}$ –45.2 (*c* 1.01, MeOH).

Preparative Kinetic Resolution of [(±)-8] Catalyzed by Lipase from *Candida antarctica* Immobilised in Sol-Gel-AK

To a solution of (±)-**8** (157 mg) in vinyl acetate (220 mL), lipase from CAL in SOL-Gel-AK (455 mg) was added. Using the same conditions and isolation procedure as described for (±)-**5**, pure alcohol (–)-**8** (68 mg, 84.2% ee), and acetyl derivative (+)-**9** (90 mg, 87.3% ee) were obtained. Acetyl derivative (+)-**9** was hydrolyzed with KOH–MeOH (1.1 equiv) at r.t. affording 58 mg of (+)-**8** (79.1% ee). Upon crystallization from MTBE, both enantiomers were obtained with >96% ee.

(–)-**8**

Mp 160–161 °C (Lit.[3] mp 161.5–162.5 °C).

$[\alpha]_D^{25}$ –29.7 (*c* 1.32, MeOH) [Lit.[3] $[\alpha]_D$ –30.4 (*c* 1.01, MeOH)].

(+)-**8**

Mp 159–160 °C.

$[\alpha]_D^{25}$ +28.8 (*c* 0.59, MeOH).

(+)-**9**

$[\alpha]_D^{25}$ +42.7 (*c* 1.68, MeOH).

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