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RESEARCH ARTICLE

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Stereoselective synthesis of (−)-cytoxazone and its unnatural congener (+)-5-*epi*-cytoxazone

Izabel Luzia Miranda[1] | **Pedro Henrique Costa dos Santos**[1] | **Markus Kohlhoff**[2] | **Gislaine Aparecida Purgato**[3] | **Marisa Alves Nogueira Diaz**[3] | **Gaspar Diaz-Muñoz**[1]

[1]Department of Chemistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

[2]Chemistry of Bioactive Natural Products, René Rachou Institute, Oswaldo Cruz Foundation (Fiocruz), Belo Horizonte, Minas Gerais, Brazil

[3]Department of Biochemistry and Molecular Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

Correspondence
Gaspar Diaz-Muñoz, Department of Chemistry, Federal University of Minas Gerais, PO Box 31270-901, Belo Horizonte, Brazil.
Email: gaspardm@qui.ufmg.br

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Abstract

An interesting protocol for stereoselective synthesis of (−)-cytoxazone and its unnatural stereoisomer (+)-5-*epi*-cytoxazone from D-4-hydroxyphenylglycine in overall yields of 10% and 16%, respectively, is described. The stereoselective addition of cyanide to an *N*-Boc protected aminoaldehyde (*tert*-butyl ((*R*)-1-(4-methoxyphenyl)-2-oxoethyl)carbamate) (**5**) constitutes the key step in this approach, producing a mixture of cyanohydrins **6a** and **b** (1,2-*anti* and 1,2-*syn* *tert*-butyl (2-cyano-2-hydroxy-1-(4-methoxyphenyl)ethyl)carbamate) in 89% yield, with reasonable stereoselectivity (1.0:1.8) in favor of the *anti*-Felkin product (1,2-*syn*). A one-pot sequence of three successive steps from this mixture produced the oxazolidinone isomers **9a** and **b** ((4*R*,5*R*)- and (4*R*,5*S*)-4-(4-methoxyphenyl)-2-oxooxazolidine-5-carboxylate). Chromatographic column separation and reduction of the ester function of both precursors led to (−)-cytoxazone and (+)-5-*epi*-cytoxazone.

KEYWORDS

D-4-hydroxyphenylglycine, oxazolidinone, stereoisomer, stereoselective addition

1 | INTRODUCTION

Oxazolidinones constitute a class of natural and synthetic compounds with multiple biological activities. Naresh and coworkers[1] produced synthetic derivatives of oxazolidinones with good anticancer activity against lung A549 and prostate DU145 cancer cells and therapeutic potential for the treatment of schizophrenia.[1] The biological versatility of oxazolidinones is also made evident by their neuroleptic,[2] psychotropic,[3] and anti-allergic activities.[4]

Two oxazolidinones with interesting biological activities are (−)-cytoxazone and its unnatural congener (+)-*epi*-cytoxazone (Figure 1). (−)-Cytoxazone was isolated in 1998 by Kakeya and coworkers[5] from bacteria of the genus *Streptomyces*. A year later, Kakeya et al.[6] observed that (−)-cytoxazone had a modulating effect on cytokines by inhibiting the signaling pathway of T helper type 2 cells (involved in cell growth and differentiation) but not of T helper type 1 cells (responsible for hypersensitivity reactions). Research indicates that an imbalance in the production of cytokines can cause immunological disorders, such as allergies, progressive lymphoproliferation, and severe immunodeficiency,[6] as cytokines are directly linked to the production of antibodies. The promising mechanism of action of (−)-cytoxazone aroused the interest of researchers in investigating its pharmacological potential.

The absolute configuration (4*R*,5*R*) of (−)-cytoxazone was confirmed by nuclear magnetic resonance (NMR) spectroscopy, circular dichroism spectroscopy, and X-ray crystallography by Sakamoto et al.[7] and Seki and Mori[8] in the first total asymmetric syntheses.

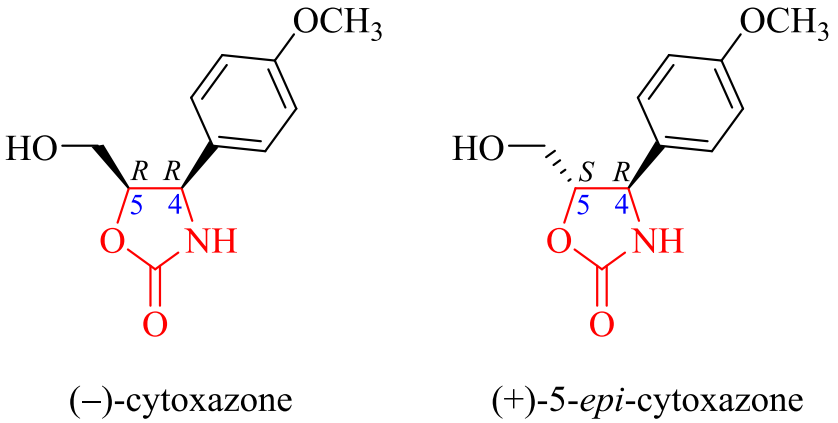


FIGURE 1 Chemical structure of (−)-cytoxazone and its unnatural epimer (+)-5-*epi*-cytoxazone

Of note, Kumar et al.[9] found (+)-5-*epi*-cytoxazone (Figure 1) to have higher antibacterial activity than (−)-cytoxazone against Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli*. The discoveries made by these groups encouraged the development of research aimed at the total synthesis of (−)-cytoxazone and congeners.[5,6,10]

1.1 | Total syntheses of (−)-cytoxazone and congeners: Background

The synthetic approaches for (−)-cytoxazone and congeners published so far start from simple substrates, such as cinnamic acid derivatives. Their β-amino acid and α-hydroxy functionalities are introduced via Sharpless enantioselective epoxidation, hydroxylation, and aminohydroxylation or by means of well-known (Evans oxazolidinone, *N*-*tert*-butanesulfinylimines) and less-known chirality inducers.[10] Figure 2 shows a compilation of some synthetic protocols as well as the key steps for the construction of the basic skeleton of (−)-cytoxazone and congeners.[7–9,11–19]

Sharpless asymmetric dihydroxylation (Figure 2, route **A**) using AD-mix-α in *t*-BuOH/H_2O was the key step in the control of stereogenic centers at C-4 and C-5 of (−)-cytoxazone, according to Sakamoto et al.[7] and Seki and Mori.[8]

Carter et al.[12] explored the 1,2-*syn* aldol reaction (Figure 2, route **D**), starting from chiral imide and an aldehyde and mediated by Bu_2BOTf, in the development of a new enantioselective synthesis of (−)-cytoxazone. Auxiliary removal and subsequent steps provided enantiomerically pure (−)-cytoxazone in 84% yield.

By applying a new approach based on the Petasis protocol,[20] Sugiyama and collaborators[13] developed a route for the synthesis of (−)-cytoxazone (Figure 2, route **E**).

Application of the highly enantioselective Henry (nitroaldol) reaction between an aldehyde and 1-methoxy-4-nitromethylbenzene using guanidine-thiourea as chiral catalyst provided nitro alcohol with high 1,2-*syn* selectivity (90:10) and 99% enantiomeric excess (Figure 2, route **H**). This adduct was the precursor to (−)-*epi*-cytoxazone in the study of Sohtome and coworkers.[16]

(−)-Cytoxazone was also synthesized by George and coworkers in 2007[17] (Figure 2, route **I**). Diastereoselective bromohydroxylation of α,β-unsaturated carboxamide in the presence of $NaIO_4$ and LiBr produced 1,2-*anti* bromohydrin. The stereochemistry of the intermediate allowed generating the correct stereocenters in (−)-cytoxazone.

Narina and coworkers[18] reported an elegant enantioselective synthetic route for (−)-cytoxazone (Figure 2, route **J**). Two key steps were required to introduce stereocenters: L-proline-catalyzed asymmetric α-aminooxylation and Rh-catalyzed diastereoselective oxidative C–H amination.

This literature survey revealed that the development of a large number of synthetic routes for (−)-cytoxazone and its congeners was motivated by the relevance of their bioactivities. From a synthetic point of view, the construction of the oxazolidinone carbon skeleton is challenging because, although it is a relatively simple molecule, it contains a five-membered ring with two consecutive stereogenic centers. The facts discussed above motivated us to develop this new study. Some specific aspects of the project stand out: the key step, considered of lower complexity than the routes reported in the literature, was the stereoselective addition of cyanide to *N*-Boc aldehyde **5**, obtained from the low-cost chiral substrate D-4-hydroxyphenylglycine. Another step, no less important, was the chromatographic separation of a mixture of oxazolidinone isomers **9a** and **b**, which corresponded to the advanced precursors of (−)-cytoxazone and (+)-5-*epi*-cytoxazone, respectively. Also noteworthy was the stereoselective synthesis of two stereoisomers from a single common intermediate.

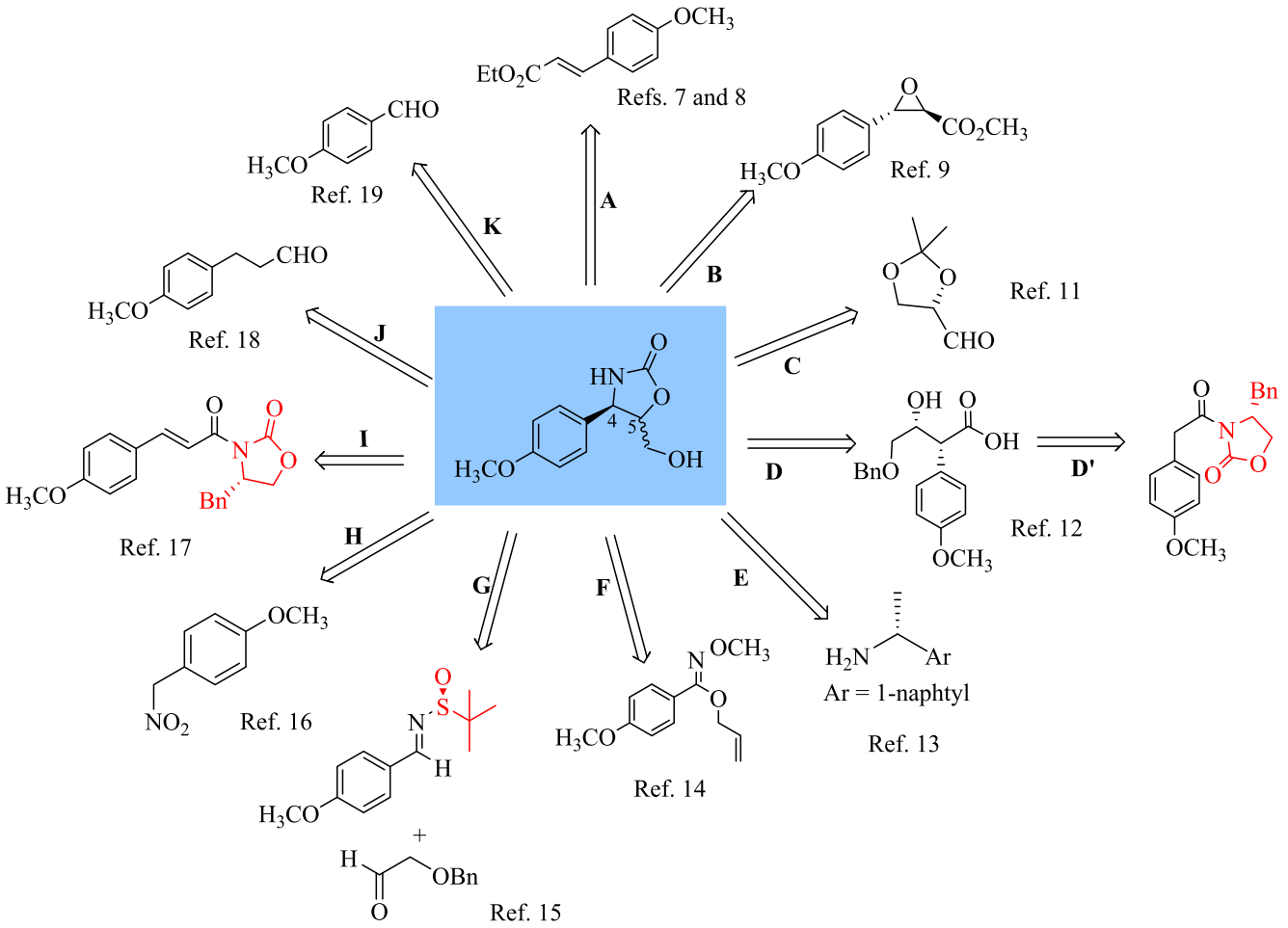


FIGURE 2 Substrates and key steps involved in some synthetic routes to (−)-cytoxazone and congeners. **A**, Sharpless asymmetric dihydroxylation; **B**, enzymatic kinetic resolution; **C**, stereoselective Grignard addition; **D**, Curtius rearrangement; **D′**, stereoselective aldol addition; **E**, Petasis coupling; **F**, 1,2-Wittig rearrangement; **G**, reductive cross-coupling; **H**, diastereoselective Henry reaction; **I**, stereoselective bromohydroxylation; **J**, stereoselective amination; **K**, Sharpless kinetic resolution

2 | MATERIALS AND METHODS

2.1 | General information

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer at 400 and 100 MHz, respectively. The liquid chromatography–tandem mass spectrometry (LC–MS/MS) analyses were performed in a Nexera UHPLC system (Shimadzu) hyphenated to a maXis ETD high-resolution ESI-QTOF mass spectrometer (Bruker). The infrared (IR) spectra were recorded on a Fourier-transform infrared (FTIR) spectrometer Varian 660 with a diamond attenuated total reflectance (ATR) accessory as a thin film. The melting points were measured in MQAPF-302 MICROCHEMICAL apparatus and uncorrected. $[\alpha]_D$ Values were measured using Anton Paar MCP 300 polarimeter equipped with a 589-nm wavelength sodium vapor lamp from the Oswaldo Cruz Foundation (Fiocruz) Natural Products Laboratory (610), Instituto René Rachou (IRR), Belo Horizonte, MG. The concentration of the solutions was denoted as *c* and was calculated as grams per milliliter (g/100 ml), where the solvent was indicated as (*c*, solvent). Purifications by column chromatography were performed on silica gel, using normal or flash chromatography, and neutral alumina. Tetrahydrofuran (THF) was distilled from sodium metal and benzophenone ketyl under nitrogen. *N*,*N*-Dimethylformamide (DMF) and dichloromethane were distilled from CaH_2. Acetonitrile was dried with anhydrous $MgSO_4$, distilled, and stored under 3-Å molecular sieve. Methanol and ethanol were dried using Mg^0 and I_2 (cat.) under reflux until Mg^0 total consumption, distilled, and stored under 3-Å molecular sieve. Thin-layer chromatography (TLC) visualization was achieved in chamber under ultraviolet (UV) light (λ 254 nm), and by spraying with $KMnO_4$ solution (1.00-g

$KMnO_4$, 6.66-g K_2CO_3, and 1.66-ml 5% KOH in 100-ml distilled water) and heating, and/or resublimated iodine. All of the chemicals were used as received unless otherwise stated. Analytical high-performance liquid chromatography (HPLC) experiments were performed on a Prominence LC-20AR Shimadzu using column CHIRALCEL OD-R (250 × 4.6 mm, 10 μm), mobile phase 100% acetonitrile, and UV–Vis detector. HPLC grade acetonitrile was degassed and filtered on a 45-μm Millipore membrane before use. Retention times Rt were in minutes.

2.2 | Synthesis

2.2.1 | (*R*)-2-((*tert*-Butoxycarbonyl)amino)-2-(4-methoxyphenyl)methyl acetate (**3**)

A mixture of D-4-hydroxyphenylglycine (2.0 g, 11.9 mmol), di-*tert*-butyldicarbonate (2.59 g, 11.9 mmol), dioxane (22 ml), water (11 ml), and aqueous Na_2CO_3 (26 ml, 0.5 mol ml^{-1}) was stirred for 5 min at 0°C under an argon atmosphere and for a further 24 h at room temperature. An aqueous solution of HCl (2 mol ml^{-1}) was added slowly to adjust the pH to 2–3, and the mixture was extracted with EtOAc (3 × 30 ml). The organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The crude *N*-Boc protected 4-hydroxy-D-phenylglycine thus obtained was used without further purification. To a 100-ml flask containing the residue were added anhydrous DMF (10 ml), anhydrous K_2CO_3 (1.99 g, 14.4 mmol), and CH_3I (3.6 ml, 57.6 mmol). After stirring for 8 h at room temperature, the mixture was neutralized with an aqueous solution of HCl (30 ml, 1 mol L^{-1}) and treated with a saturated solution of $NaHCO_3$ (30 ml). The aqueous phase was extracted with ethyl ether (3 × 30 ml). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/EtOAc 85:15), providing ester **3** (94% yield, two steps) as a yellowish solid, mp 65.5–66.6°C (literature,[9] 66–67°C). R_f 0.5 (hexane/EtOAc 70:30). $[\alpha]_D^{22} = -93.8°$ ($c = 1.3$, $CHCl_3$) (literature,[9] $[\alpha]_D^{25} = -95.3°$ [$c = 1.2$, $CHCl_3$]). IV (neat): $\bar{\nu}_{max}$ 3375, 2977, 2839, 1743, 1706, 1611, 1586, 1510, 1462, 1438, 1392, 1366, 1305, 1244, 1214, 1158, 1112, 1052, 1028, 914, 894, 852, 832, 797, 781, 763 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, δ) 7.27 (d, $J = 8.3$ Hz, 2H, Ar H), 6.86 (d, $J = 8.5$ Hz, 2H, Ar H), 5.50 (s, 1H, NH), 5.25 (s, 1H, CH), 3.78 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 1.42 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 172.11 (C1), 159.93 (C9), 155.06 (C6), 129.27 (C3), 128.63 (C4 and C4′), 114.54 (C5 and C5′), 80.30 (C10), 57.30 (C2), 55.51 (C7), 52.80 (C8), 28.55 (C11). HRMS (ESI, *m/z*): $[M + Na]^+$ calcd for $C_{15}H_{21}NO_5$, 318.1312; found, 318.1320.

2.2.2 | (*R*)-(2-Hydroxy-1-(4-methoxyphenyl) ethyl)*tert*-butyl carbamate (**4**)

To a 100-ml flask containing ester **3** (0.806 g, 2.73 mmol) in anhydrous ethanol (55 ml), under an argon atmosphere, were added $NaBH_4$ (0.413 g, 10.92 mmol) and LiCl (0.463 g, 42.44 mmol). The mixture was kept under stirring at room temperature for 18 h, an aqueous solution of HCl (30 ml, 1 mol ml^{-1}) and distilled water (30 ml) were added, and the mixture was extracted with ethyl ether (3 × 30 ml). The organic phase was washed with a saturated solution of $NaHCO_3$ (30 ml) and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Recrystallization of the crude residue in ethanol gave alcohol **4** in 92% yield as a white solid, mp 130.4–131.3°C (literature,[9] 130–131°C). R_f 0.4 (hexane/EtOAc 1:1). $[\alpha]_D^{23} = -40.1°$ ($c = 1.0$, $CHCl_3$) (literature,[9] $[\alpha]_D^{25} = -43.59°$ [$c = 1.09$, $CHCl_3$]). IV (neat): $\bar{\nu}_{max}$ 3373, 2983, 2937, 1682, 1613, 1585, 1514, 1462, 1390, 1366, 1350, 1319, 1303, 1284, 1266, 1245, 1167, 1111, 1090, 1052, 1030, 934, 910, 871, 837, 822, 782 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, δ) 7.21 (d, $J = 8.5$ Hz, 2H, Ar H), 6.87 (d, $J = 8.55$ Hz, 2H, Ar H), 5.27 (s, 1H, NH), 4.71 (s, 1H, CH), 3.78 (s, 5H, CH_2 and CH_3), 2.67 (s, 1H, OH), 1.42 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 159.24 (C8), 156.27 (C6), 131.86 (C3), 127.84 (C4 e C4′), 114.29 (C5 and C5′), 80.01 (C9), 66.86 (C1), 56.62 (C2), 55.39 (C7), 28.46 (C10). HRMS (ESI, *m/z*): $[M + Na]^+$ calcd for $C_{14}H_{21}NO_4$, 290.1357; found, 290.1367.

2.2.3 | (*R*)-(1-(4-Methoxyphenyl)-2-oxoethyl) *tert*-butyl carbamate (**5**)

Swern oxidation

To a 50-ml flask containing freshly distilled oxalyl chloride (0.16 ml, 1.96 mmol) in anhydrous CH_2Cl_2 (14 ml) under stirring at −78°C and an argon atmosphere was added anhydrous dimethyl sulfoxide (DMSO) (0.14 ml, 2.07 mmol) dropwise. After stirring for 30 min at the same temperature, a solution of alcohol **4** (0.35 g, 1.31 mmol) in CH_2Cl_2 (10 ml) was added slowly. The system was kept under stirring for 1 h, the temperature was raised to −35°C, and stirring was maintained for a further 1 h. Anhydrous diisopropylethylamine (0.5 ml, 2 mmol) was added slowly, and the mixture was stirred for 10 min at 0°C. Dichloromethane (5 ml) and a saturated solution of NH_4Cl (5 ml) were added, the phases were separated, and the aqueous layer was extracted with

CH_2Cl_2 (3 × 7 ml). The organic phases were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was rapidly filtered over silica gel (hexane/EtOAc 95:5), providing aldehyde **5** as a yellowish oil in 63% yield. The aldehyde was used immediately after synthesis.

Dess–Martin periodinane oxidation

To a 25-ml flask containing Dess–Martin periodinane (DMP) (0.31 g, 0.74 mmol) in anhydrous CH_2Cl_2 (3 ml) under an argon atmosphere were added alcohol solution **4** (0.10 g, 267.15 mmol) in CH_2Cl_2 (13 ml) and water (0.013 ml). The reaction mixture was kept under stirring at room temperature for 90 min. After the addition of dichloromethane (5 ml), rapid filtration through silica gel (hexane/EtOAc 95:5) provided aldehyde **5** as a yellowish oil in 71% yield.

IBX oxidation

To a 25-ml flask containing a solution of alcohol **4** (0.20 g, 0.75 mmol) in anhydrous CH_3CN under an argon atmosphere was added 2-iodoxybenzoic acid (IBX) (0.56 g, 2.25 mmol) in a single portion. The mixture was refluxed for 2 h, cooled to room temperature, and filtered through Celite. The filtrate was washed with a saturated solution of $NaHCO_3$ (5 ml) and brine (5 ml). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Then, the residue was rapidly filtered through silica gel (hexane/EtOAc 95:5), providing aldehyde **5** as a yellowish oil in 68% yield.

2.2.4 | *tert*-Butyl ((1*R*,2*R*)- and (1*R*,2*S*)-2-cyano-2-hydroxy-1-(4-methoxyphenyl)ethyl) carbamate (**6a** and **b**)

A solution containing NaCN (0.019 g, 0.4 mmol), anhydrous MeOH (0.2 ml), and acetic acid (0.02 ml, 0.43 mmol) in a 10-ml flask was stirred for 5 min at room temperature under an argon atmosphere. The temperature was lowered to 0°C, and a solution of aldehyde **5** (0.042 g, 0.16 mmol) in CH_2Cl_2 (0.5 ml) was added. The mixture was kept under stirring at room temperature for 6 h, brine (1 ml) and a saturated solution of $NaHCO_3$ (1 ml) were added, and stirring was maintained for 5 min. After phase separation, the aqueous layer was extracted with CH_2Cl_2 (3 × 5 ml). Organic phases were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified on a chromatographic column (hexane/EtOAc 80:20 → 70:30), providing a mixture of cyanohydrins **6a** and **b** in 89% yield as a yellowish solid (R_f 0.25 and 0.29, hexane/EtOAc 7:3). IV (neat): $\bar{\nu}_{max}$ 3379, 2982, 2932, 2837, 1666, 1613, 1509, 1364, 1302, 1244, 1158, 1083, 1021, 893, 782 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, δ) 7.32–7.28 (m, 4H, Ar H), 6.92–6.89 (m, 4H, Ar H), 5.74 (s, 1H, NH), 5.39 (s, 2H, NH), 4.91–4.85 (m, 2H, CH), 4.73 (s, 1H, CH), 4.59 (s, 1H, CH), 3.80 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 1.45 (s, 9H, CH_3), 1.43 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 160.04 (C9), 159.97 (C9), 157.42 (C7), 156.50 (C7), 128.60 (C5 and C5′), 128.17 (C5 and C5′), 127.57 (C4), 118.33 (C1), 117.93 (C1), 114.61 (C6 and C6′), 114.45 (C6 and C6′), 81.74 (C10), 81.24 (C10), 67.95 (C2), 65.54 (C2), 58.80 (C3), 55.35 (C8), 55.33 (C8), 28.25 (C11), 28.23 (C11). HRMS (ESI, *m/z*): $[M + Na]^+$ calcd for $C_{15}H_{20}N_2O_4$, 315.1321; found, 315.1313.

2.2.5 | (4*R*,5*R*)- and (4*R*,5*S*)-4-(4-Methoxyphenyl)-2-oxooxazolidine-5-methyl carboxylate (**9a** and **b**)

To a 10-ml flask containing a mixture of cyanohydrins **6a** and **b** (0.054 g, 0.184 mmol) was added an aqueous solution of HCl (3 ml, 15%) and refluxed for 1 h. The aqueous solution was evaporated under reduced pressure. The mixture of *N*-unprotected amino acids (**7a** and **b**) thus obtained was used in the next step without any purification. Ethyl ether (2 ml) and an aqueous solution of NaOH (0.086 g, 2.15 mmol, 5 ml) were added to a 10-ml flask containing **7a** and **b** (0.071 g, 0.28 mmol) under an argon atmosphere at 0°C and stirred for 10 min. Triphosgene (0.125 g, 0.42 mmol) was added, and the mixture was maintained at this temperature. Reaction progress was monitored by TLC. Upon completion of the reaction, the pH was adjusted to 2–3 by the addition of an aqueous solution of HCl (2 mol ml^{-1}), and the mixture was extracted with EtOAc (3 × 10 ml). The organic phases were combined, dried over Na_2SO_4, and concentrated under reduced pressure to provide a mixture of oxazolidinones **8a** and **b**, which was subjected to carboxyl esterification without further purification. To a 10-ml flask equipped with a reflux condenser under an argon atmosphere at 0°C were added a mixture of **8a** and **b** (0.082 g, 0.35 mmol), anhydrous MeOH (1 ml), and freshly distilled $SOCl_2$ (0.076 ml, 1.05 mmol). The mixture was refluxed until the reaction was complete. Reaction progress was monitored by TLC. After cooling to 0°C, the mixture was treated with a saturated solution of $NaHCO_3$ (3 ml) and extracted with EtOAc (3 × 10 ml). Organic phases were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc 75:25 → 70:30 → 60:40) to afford, as yellow solids, the diastereoisomeric oxazolidinones **9a** and **b** in three steps and yields of 20% and 32%, respectively.

Data for oxazolidinone ***9a***

Mp 104.2–105.1°C. R_f 0.36 (hexane/EtOAc 25:75). $[\alpha]_D^{22} = -82.9°$ ($c = 0.25$, CH_3OH) (literature,[21] $[\alpha]_D^{23} = -94.0°$ [$c = 1.0$, CH_3OH]). IV (neat): $\bar{\nu}_{max}$ 3375, 2954, 2841, 2035, 1741, 1612, 1516, 1251, 1182, 1025, 837 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, δ) 7.21 (d, $J = 8.7$ Hz, 2H, Ar H), 6.88 (d, $J = 8.7$ Hz, 2H, Ar H), 5.32 (s, 1H, NH), 5.26 (d, $J = 9.2$ Hz, 1H, CH), 5.17 (d, $J = 9.2$ Hz, 1H, CH), 3.80 (s, 3H, CH_3), 3.33 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 166.75 (C6), 160.38 (C11), 157.73 (C1), 128.17 (C9 and C9′), 126.96 (C8), 114.14 (C10 and C10′), 78.17 (C5), 57.89 (C4), 55.34 (C12), 52.10 (C7). HRMS (ESI, *m/z*): $[M + Na]^+$ calcd for $C_{12}H_{13}NO_5$, 274.0686; found, 274.0689.

Data for oxazolidinone ***9b***

Mp 91.8–92.6°C. R_f 0.56 (hexane/EtOAc 25:75). $[\alpha]_D^{22} = +83.4°$ ($c = 0.33$, CH_3OH) (literature,[21] $[\alpha]_D^{23} = +90°$ [$c = 1.0$, CH_3OH]). IV (neat): $\bar{\nu}_{max}$ 3245, 3142, 2962, 2925, 2840, 1722, 1614, 1586, 1512, 1414, 1304, 1249, 1229, 1172, 1093, 1021, 954, 830 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, δ) 7.29 (d, $J = 8.6$ Hz, 2H, Ar H), 6.93 (d, $J = 8.6$ Hz, 2H, Ar H), 5.48 (s, 1H, NH), 4.92 (d, $J = 5.2$ Hz, 1H, CH), 4.75 (d, $J = 5.2$ Hz, 1H, CH), 3.86 (s, 3H, CH_3), 3.82 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 168.99 (C6), 160.55 (C11), 157.43 (C1), 130.86 (C8), 127.52 (C9 and C9′), 114.95 (C10 and C10′), 80.77 (C5), 58.89 (C4), 55.62 (C12), 53.27 (C7). HRMS (ESI, *m/z*): $[M + Na]^+$ calcd for $C_{12}H_{13}NO_5$, 274.0686; found, 274.0687.

2.2.6 | (−)-Cytoxazone (**1**)

To a 10-ml flask containing $NaBH_4$ (0.003 g, 0.1 mmol) and LiCl (0.004 g, 0.1 mmol) in anhydrous MeOH (0.5 ml) under an argon atmosphere at 0°C was added a solution of oxazolidinone ester **9a** (0.010 g, 0.025 mmol) in anhydrous MeOH (0.5 ml). The temperature was raised to room temperature, and the mixture stirred for 2 h until total consumption of the substrate, as monitored by TLC. Water (1 ml) was added, and the mixture extracted with EtOAc (3 × 3 ml). The organic phases were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc 25:75), yielding 90% (86.6% de) of **1** as a white solid, mp 120.5–121.3°C. R_f 0.30 (hexane/EtOAc 25:75). $[\alpha]_D^{25} = -63.16°$ ($c = 0.1$, CH_3OH) (literature,[21] $[\alpha]_D^{25} = -65.7°$ [$c = 0.38$, CH_3OH]). IV (neat): $\bar{\nu}_{max}$ 3441, 3238, 2960, 2928, 2841, 1709, 1611, 1513, 1401, 1249, 1174, 1047, 1026, 992 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, δ) 7.21 (d, $J = 8.7$ Hz, 2H, Ar H), 6.91 (d, $J = 8.7$ Hz, 2H, Ar H), 5.18 (s, 1H, NH), 5.01 (d, $J = 8.4$ Hz, 1H, CH), 4.90 (ddd, $J = 8.4$, 7.6 and 4.0 Hz, 1H, CH), 3.82 (s, 3H, CH_3), 3.48–3.42 (m, 1H, CH_b), 3.30–3.23 (m, 1H, CH_a). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 160.21 (C1), 148.12 (C10), 127.89 (C8 and C8′), 127.24 (C7), 114.45 (C9 e C9′), 80.27 (C5), 62.07 (C6), 57.30 (C4), 55.38 (C11). HPLC column CHIRALCEL OD-R (250 × 4.6 mm, 10 μm): 100% acetonitrile, detector: 234 nm, flow rate: 0.5 ml min^{-1}, (4*R*,5*S*) = 6.58 min, (4*R*,5*R*) = 7.78 min. HRMS (ESI, *m/z*): $[M + Na]^+$ calcd for $C_{11}H_{13}NO_4$, 246.0737; found, 246.0735.

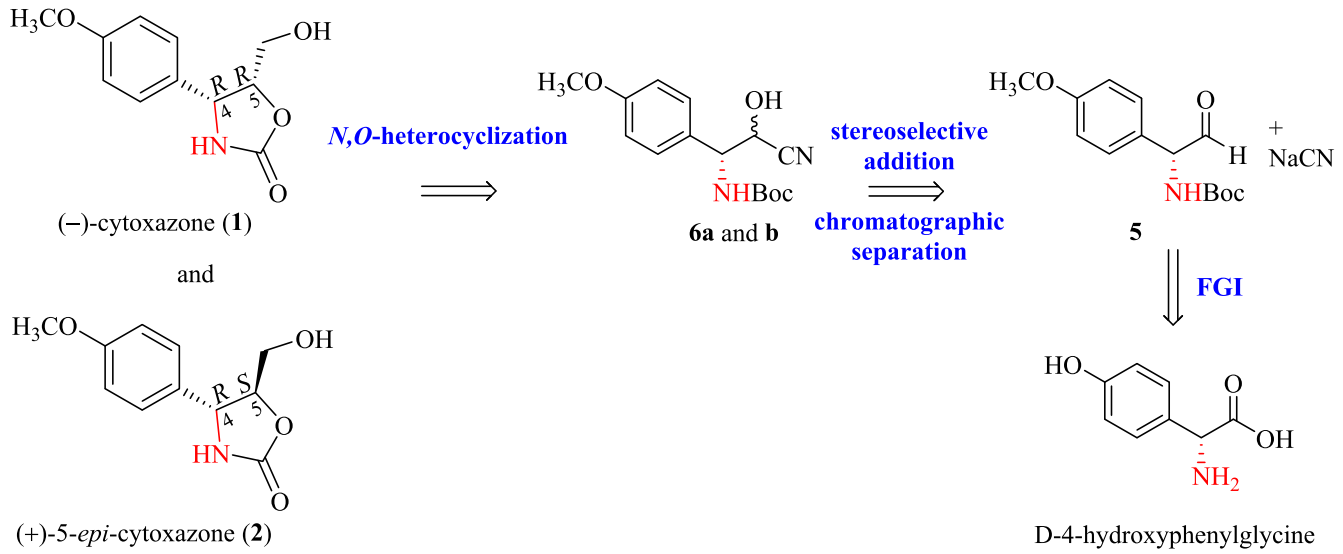
2.2.7 | (+)-5-epi-Cytoxazone (**2**)

(+)-5-*epi*-Cytoxazone was prepared in a similar manner to **1** but from oxazolidinone ester **9b**. The residue was purified by silica gel column chromatography (hexane/EtOAc 25:75) yielding 92% (97.4% de) of **2** as a white solid, mp 161.3–162.3°C. R_f 0.38 (hexane/EtOAc 25:75). $[\alpha]_D^{25} = +21.3°$ ($c = 0.15$, CH_3OH) (literature,[21] $[\alpha]_D^{25} = +32.0°$ [$c = 0.4$, CH_3OH]). IV (neat): $\bar{\nu}_{max}$ 3328, 2935, 1733, 1692, 1610, 1514, 1425, 1384, 1299, 1243, 1176, 1028, 831 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6, δ) 7.33 (d, $J = 8.7$ Hz, 2H, Ar H), 6.95 (d, $J = 8.7$ Hz, 2H, Ar H), 4.78 (d, $J = 6.4$ Hz, 1H, CH), 4.35 (t, $J = 6.0$ Hz, 1H, OH), 4.29–4.21 (m, 1H, CH), 3.86–3.80 (m, 1H, CH_b), 3.79 (s, 3H, CH_3), 3.75–3.66 (m, 1H, CH_a). ^{13}C NMR (100 MHz, acetone-d_6, δ) 160.71 (C1), 159.13 (C10), 133.98 (C7), 128.49 (C8 and C8′), 115.13 (C9 and C9′), 85.69 (C5), 62.52 (C6), 57.76 (C4), 55.68 (C11). HPLC column CHIRALCEL OD-R (250 × 4.6 mm, 10 μm): 100% acetonitrile, detector: 234 nm, flow rate: 0.5 ml min^{-1}, (4*R*,5*S*) = 6.57 min, (4*R*,5*R*) = 7.79 min. HRMS (ESI, *m/z*): $[M + Na]^+$ calcd for $C_{11}H_{13}NO_4$, 246.0737; found, 246.0730.

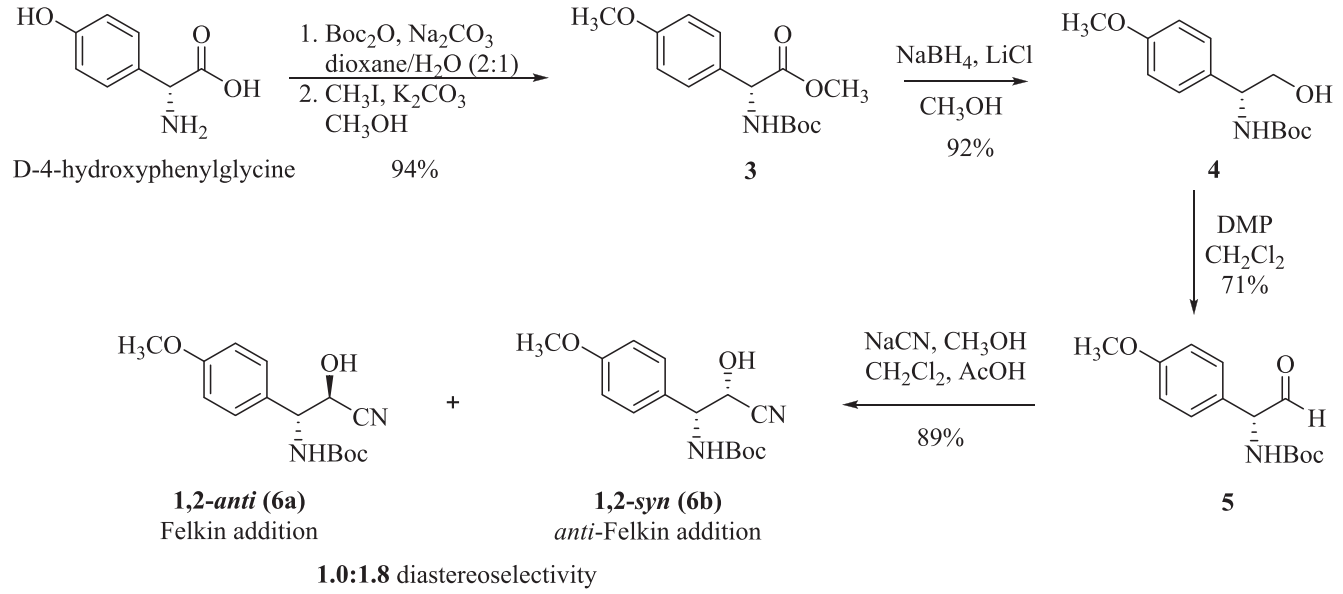
3 | RESULTS AND DISCUSSION

3.1 | Retrosynthetic analysis of (−)-cytoxazone (1) and (+)-5-*epi*-cytoxazone (2)

The retrosynthetic analysis of (−)-cytoxazone (**1**) and (+)-5-*epi*-cytoxazone (**2**) is presented in Scheme 1. The formation of the oxazolidinone rings must be achieved through an *N*,*O*-heterocyclization, mediated by triphosgene, of the respective amino acid precursors, derived from acid hydrolysis of cyanohydrins **6a** and **b**. Preparation of **6a** and **b** and separation of the diastereoisomers should be possible through a stereoselective addition of cyanide to aldehyde **5**, which in turn can be produced from commercially available D-4-hydroxyphenylglycine.



SCHEME 1 Retrosynthetic analysis of (−)-cytoxazone (**1**) and (+)-5-*epi*-cytoxazone (**2**) from D-4-hydroxyphenylglycine



SCHEME 2 Synthesis of cyanohydrins **6a** and **b**

3.2 | Synthesis of cyanohydrins 6a and b

The synthesis of **1** and **2** started with the preparation of *N*-Boc amino ester **3**. *N*-Boc protection and methoxylation with concomitant esterification from D-4-hydroxyphenylglycine produced *N*-Boc ester **3** in 94% yield (two steps) (Scheme 2). Reduction of the ester function of **3** using $LiBH_4$ (generated in situ from $NaBH_4$ and LiCl), followed by DMP oxidation from the resulting alcohol **4**, provided *N*-Boc aldehyde **5** in 65% yield (two steps). Three oxidizing agents were tested in this last step, Swern reagent, IBX, and DMP, giving yields of 63%, 68%, and 71%, respectively. The aldehyde should be used immediately after preparation to avoid or minimize racemization. Attempts to purify aldehyde **5** by silica gel chromatography resulted in partial racemization. Thus, as the diastereoselectivity of the reaction with cyanide depends on the diastereoisomeric purity of the aldehyde, **5** was used in its crude form in the current study.

The synthesis of cyanohydrins **6a** and **b**, in 89% overall yield and stereoselectivity (1.0:1.8) toward the *anti*-Felkin (1,2-*syn*) addition product (**6b**), was performed by reacting HCN (generated in situ by treatment of a methanolic solution of NaCN with acetic acid) with *N*-Boc aldehyde **5** in

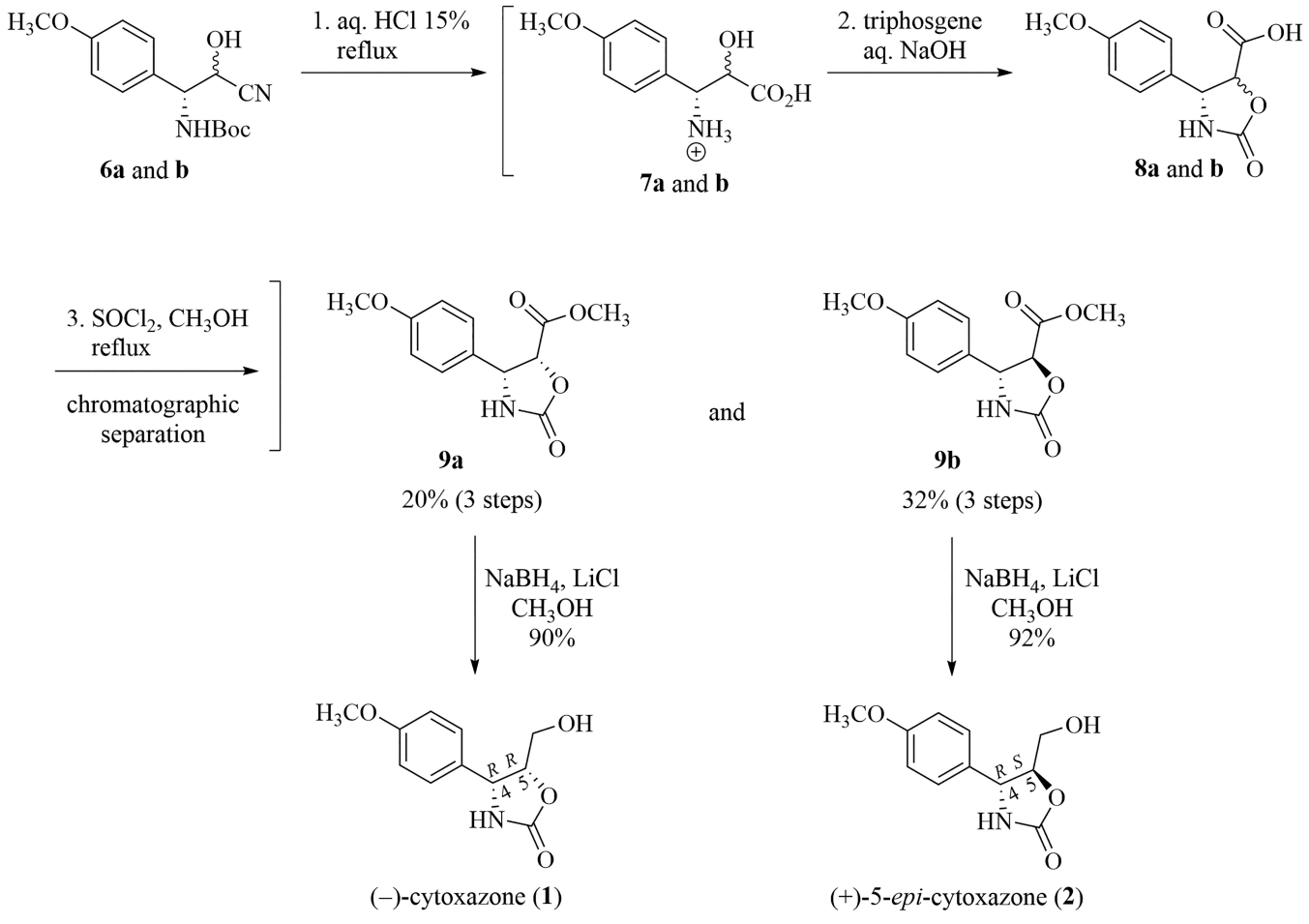
CH_2Cl_2.[22] An acidic medium (HCN) in the nucleophilic addition to the carbonyl of the aldehyde is necessary, because, under these conditions, the carbonyl oxygen is protonated, generating a carboxonium ion that facilitates the reaction; that is, in an acidic environment, the thermodynamic balance of the reaction between HCN and aldehyde is shifted toward the products.[23]

The stereoisomeric ratio of cyanohydrins **6a** to **b** (1.0:1.8) was determined by analyzing the ^{1}H NMR spectrum of the mixture. However, this initial evaluation did not allow identifying which stereoisomer was formed preferentially; this was only possible in later steps.

3.3 | Synthesis of (−)-cytoxazone and (+)-5-*epi*-cytoxazone from cyanohydrins 6a and b

Because of the difficulties in separating cyanohydrins **6a** and **b**, after several attempts, we decided to proceed with the synthesis using the diastereoisomeric mixture (Scheme 3). Acid hydrolysis of the nitrile group in the presence of a 15% HCl aqueous solution under reflux led to the removal of the protective *N*-Boc group and gave a mixture of carboxylic acids (**7a** and **b**). The very polar crude product was subjected to the next step without previous purification. *N*,*O*-heterocyclization of **7a** and **b** was performed by using triphosgene in basic medium to provide a mixture of the respective oxazolidinone acid derivatives **8a** and **b**. All attempts to purify this mixture resulted in product decomposition.

The crude mixture of **8a** and **b** was subjected to esterification of the carboxyl function by treatment with $SOCl_2$ and MeOH under reflux, resulting in a mixture of the respective oxazolidinone esters **9a** and **b**. Purification of **9a** and **b** by column chromatography allowed the separation of diastereoisomers, obtained in three steps from cyanohydrins **6a** and **b** in yields of 20% and 32%, respectively.



SCHEME 3 Synthesis of (−)-cytoxazone (**1**) and (+)-5-*epi*-cytoxazone (**2**) from cyanohydrins **6a** and **b**

By separating the oxazolidinone isomers **9a** and **b**, we were able to determine, through their masses, that the addition of cyanide to the carbonyl of aldehyde **5** favored the formation of 1,2-*syn* cyanohydrin (**6b**) at a ratio of 1.0:1.8 (Scheme 2).

The stereochemistry of the major cyanohydrin (**6b**) can be rationalized by the Felkin–Ahn transition state model (Figure 3).[24] It suggests a balance between conformers **A** and **B**.[25] Thus, it was expected that the use of a bulky *N*-protective group (Boc) would hinder the formation of the N–H bond, favoring the formation of conformer **B**. However, this was not observed in practice, as *N*-Boc favored the five-membered cyclic conformer **A**, resulting preferentially in *anti*-Felkin **6b** (1,2-*syn*).

Finally, reduction of the respective ester functions of **9a** and **b** produced (−)-cytoxazone (**1**) and (+)-5-*epi*-cytoxazone (**2**) in yields of 90% and 92%, respectively.

Spectroscopic data of **1** and **2** were in agreement with those reported in the literature,[21,26–28] as were their specific rotation values $[\alpha]_D^{24} = -63.16°$ ($c = 0.1$, CH_3OH) and $[\alpha]_D^{24} = +21.3°$ ($c = 0.15$, CH_3OH).[21,26–28]

The relative stereochemistry of **1** and **2** (*cis* and *trans*, respectively) was unequivocally established by

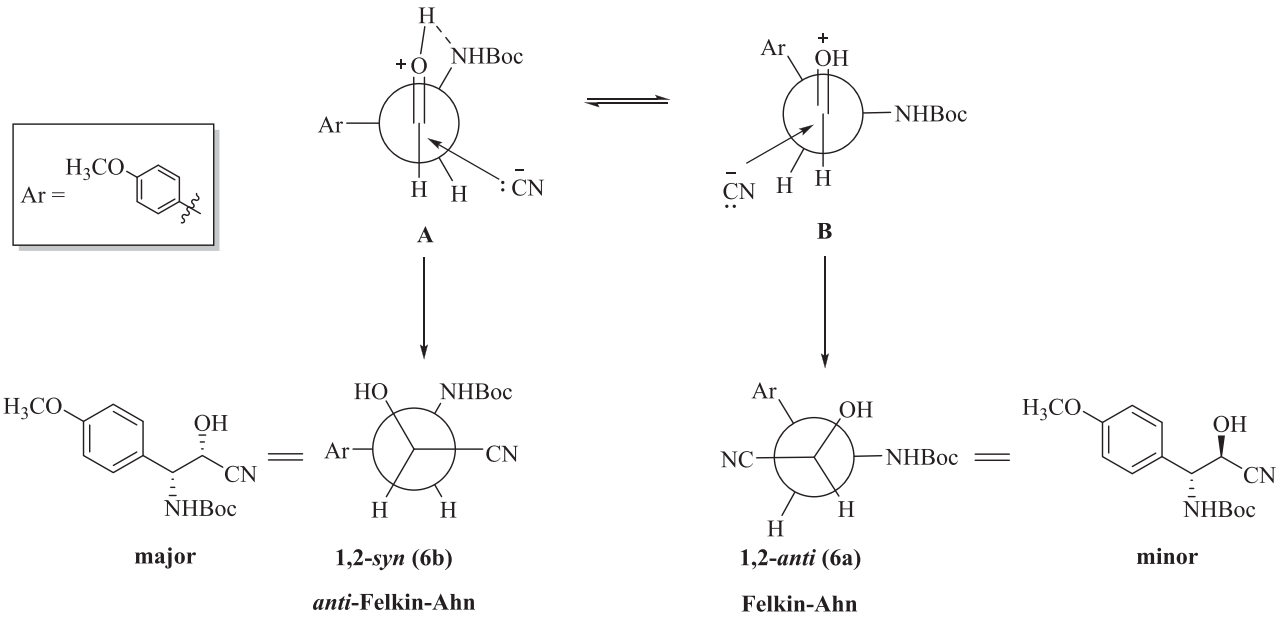


FIGURE 3 Felkin–Ahn transition state model

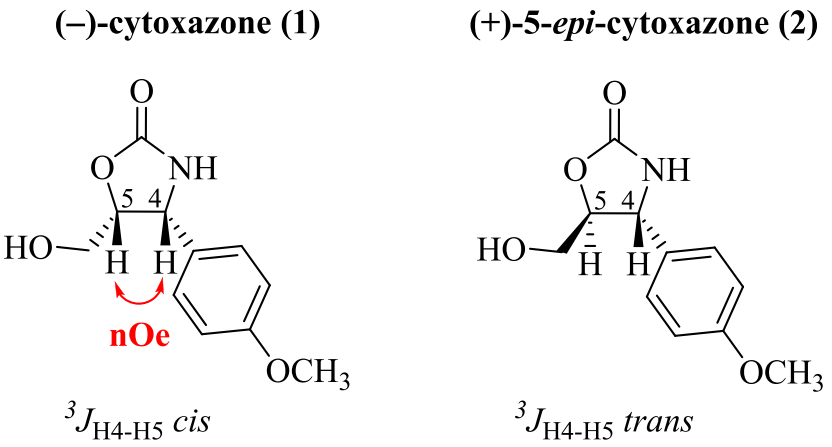


FIGURE 4 Analysis of the coupling constant and determination of the relative stereochemistry of (−)-cytoxazone (**1**) and (+)-5-*epi*-cytoxazone (**2**)

analyzing their ^{1}H NMR spectra. The coupling constant ($^{3}J_{H4–H5} = 8.4$ Hz) indicates that these hydrogens are on the same side of the heterocyclic ring in *cis*-oxazolidinone **1** (Figure 4).[29,30] The coupling constant ($^{3}J_{H4–H5} = 6.4$ Hz), on the other hand, indicates that the hydrogens are on opposite faces of the ring in *trans*-oxazolidinone **2**.[29,30]

The nuclear Overhauser effect spectroscopy (NOESY) contour map of (−)-cytoxazone (**1**) (see Supporting Information, Appendix S11) showed a correlation signal between hydrogens 4 and 5, corroborating the *cis* configuration of the oxazolidinone ring.

4 | CONCLUSION

In summary, the versatility of our method enabled the stereoselective synthesis of the oxazolidinones, (−)-cytoxazone (**1**) and (+)-5-*epi*-cytoxazone (**2**) in overall yields of 10% and 16%, respectively, from a single common chiral intermediate, *N*-Boc amino aldehyde **5**, derived from D-4-hydroxyphenylglycine. Insertion of the second oxazolidinone stereocenter was controlled by adding cyanide to the aldehyde, generating both 1,2-*anti* and 1,2-*syn* diastereoisomeric cyanohydrins (**6a** and **b**), the precursors of **1** and **2**, respectively. Independent synthesis of target molecules was made possible by silica gel chromatographic separation of the advanced oxazolidinone diastereoisomers **9a** and **b**. The method is very advantageous, as it allows the synthesis of two oxazolidinones via a single protocol.

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DATA AVAILABILITY STATEMENT

I declare to make my data available.

ORCID

Marisa Alves Nogueira Diaz https://orcid.org/0000-0002-3370-4149

Gaspar Diaz-Muñoz https://orcid.org/0000-0002-7515-0420

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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