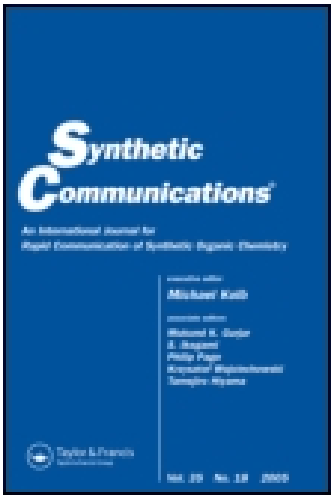

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Synthesis of (-)-Cytoxazone and (+)-epi-Cytoxazone: The Chiral Pool Approach

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Abstract: Immunomodulator (−)-cytoxazone and its epimer (+)-*epi*-cytoxazone were synthesized starting from (D)-(−)-4-hydroxyphenylglycine. Homologation of the amino acid was achieved via the corresponding aldehyde, by a cyanohydrin reaction. The racemization of highly sensible amido aldehyde was efficiently suppressed when the oxidation of the parent aminoalcohol was performed by a modified Dess-Martin procedure.

Keywords: Amino alcohols, cyanohydrins, cytoxazone, oxazolines

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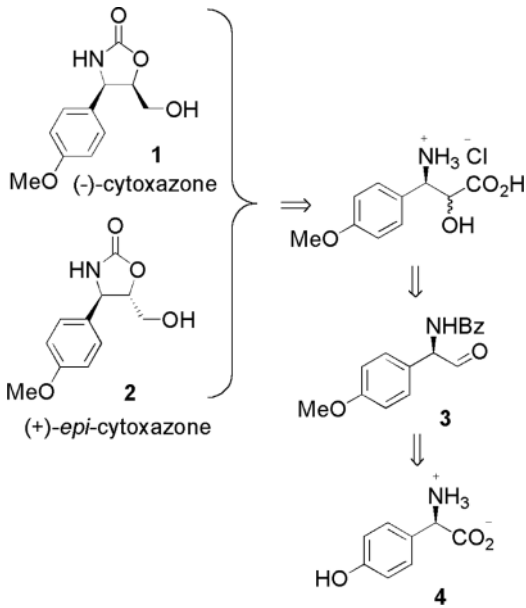
INTRODUCTION

Owing to its strong, yet selective, immunosuppressive activity, cytoxazone **1**—a secondary metabolite of *Streptomyces sp.*—has attracted attention of synthetic chemists. Since its isolation in 1998,[1,2] several syntheses of the compound,[3–12] as well as of its epimer *epi*-cytoxazone **2**,[9–11] have been reported. These approaches involved asymmetric synthesis,[3–12];) as well as the separation of the racemic compound.[5,8] No attempt has been made, however, to prepare the optically pure **1** starting from an amino acid, using a chiral pool approach, which is the subject of this study.

RESULTS AND DISCUSSION

We considered using (D)-(−)-4-hydroxyphenylglycine **4**—a commercially available, nonexpensive amino acid—as a starting compound for the synthesis of both (−)-cytoxazone **1** and (+)-*epi*-cytoxazone **2** (Scheme 1). Amino acids are routinely used as chiral starting material for syntheses of optically pure compounds,[13] and their homologation into amino alcohols, via the corresponding amino aldehydes,[14] is a well-known process. Addition of vinyl organo metalics,[15] acetylide anion,[16] as well as cyanohydrin reaction,[17] were usually used for this purpose (when one-carbon



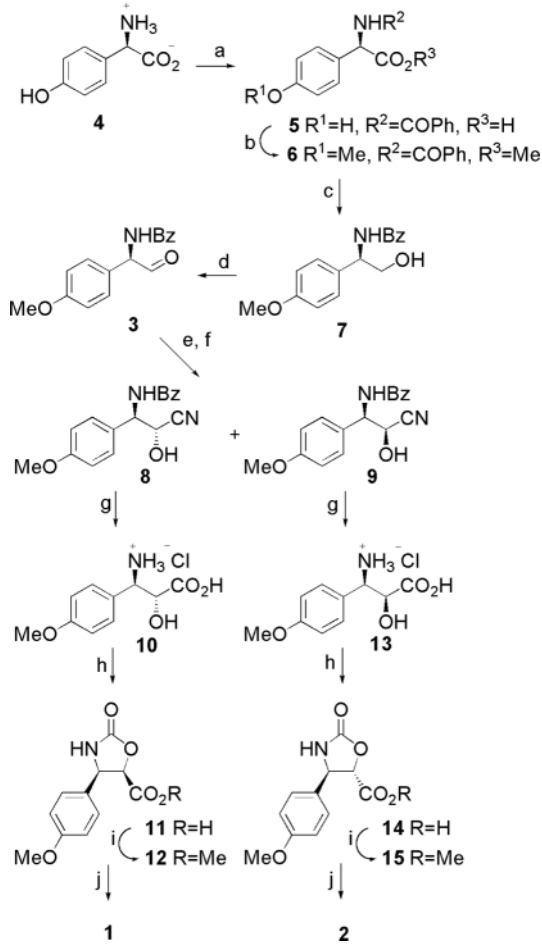
Scheme 1.

homologation is required, the first two methods are used in combination with subsequent oxidative fragmentation). However, the aminoaldehyde-type intermediates can easily epimerize and require a careful choice of protective group on the nitrogen atom; most often, these include carbamates, sulfonamides, and alkyl substituted amine derivatives. We intended to perform the homologation using a cyanohydrin reaction. Rather than using its more sophisticated variants, such as trimethylsilyl cyanide,[18] tributylstannyl cyanide,[19] with or without added Lewis acids, or diethylaluminum cyanide (Nagata's reagent),[20] we opted for performing the cyanohydrin reaction under operationally simple conditions, with cheap and environmentally least-harmful sodium cyanide. The diastereoselectivity of such a reaction is reagent controlled and would not be expected to be high; therefore, the homologated intermediate should be amenable to configurational inversion at the newly created stereocenter. Previously, it was shown that this transformation can be conveniently effected via oxazoline intermediates. In order to minimize protective group manipulations, this approach would require the use of amido aldehyde **3** as a homologation intermediate. However, due to their extreme proclivity towards epimerization, these species have not found synthetic application. Thus, the feasibility of the approach was to be tested experimentally.

The course of synthesis is displayed in Scheme 2. (D)-(−)-4-Hydroxyphenylglycine was converted into the corresponding amido alcohol **7** via a three-step procedure (71% overall).[1] Given the high propensity of α-amido aldehydes toward racemization, we performed a short study on the oxidation of **7** under a variety of conditions. The optical purity of the aldehyde **3** was determined by its reduction with sodium borohydride back to **7**, and the comparison of the optical rotation value with the original sample. Swern oxidation on a closely related system was reported to give amido aldehyde with a high level of optical purity.[15,10] In our hands, however, the enantiomeric excess of the amido aldehyde **3** was 83%. Initial experiments with TEMPO mediated oxidation were quite disappointing, as the product completely racemized.[21] After careful optimization (time of the reaction; solvent; concentration; stoichiometry), we were able to obtain the product with 85% ee, which was still unsatisfactory. Dess-Martin oxidation in THF afforded the aldehyde with 67% ee; in acetonitrile the optical purity improved to 75%.[2] Recently, the mechanism of the Dess-Martin oxidation was studied in more detail and it was found that the water accelerated oxidation with freshly prepared periodinane can afford amido aldehydes with excellent levels of optical purity.[22] Indeed, this protocol

[1]It should be noted that (D)-(−)-*N*-benzoyl-4-hydroxyphenylglycine **5** easily epimerizes: when the benzoylation was performed in refluxing dichloromethane using triethylamine as a base, the racemic *N,O*-dibenzoylated product was obtained.

[2]Dichloromethane could not be used as a solvent due to the insolubility of the starting amidoalcohol **7**.



Scheme 2. a) BzCl, NaOH, H_2O, 0°C-rt, 2.5 h (88%); b) Me_2SO_4, K_2CO_3, acetone, reflux, 3 h (89%); c) $NaBH_4$, MeOH, rt, 3 h (91%); d) DMP, CH_2Cl_2, H_2O (cat.), 12 min (94%); e) KCN, CH_2Cl_2, MeOH, AcOH (92%, **8:9** = 1:1.5); f) separation by column chromatography; g) 15% HCl, 110°C. 3 h h) triphosgene, NaOH, H_2O, 0°C, 1 h; i) CH_2N_2, THF (65% from **8**, 71% from **9**); j) $NaBH_4$, THF, H_2O, 0°C, 2 h (83%).

allowed us to obtain the amido aldehyde **3** in 94% yield, with 98% ee. Cyanohydrin reaction of **3** proved also to be potentially epimerizing step (as with the amido aldehyde, the optical purity of the cyanohydrins **8** and **9** was determined by their reduction with sodium borohydride to the starting amidoalcohol). Thus, when the cyanohydrin reaction with optically pure **3** was performed under basic conditions according to described procedures,[23] partial racemization occured. Neutral conditions proved more suitable for this

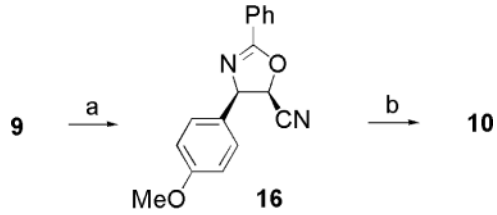
transformation:[22] with in situ generated methanolic hydrogen cyanide the reaction was very fast and afforded essentially optically pure cyanohydrins **8** and **9** in 92% yield. The product was obtained as a diastereomeric mixture in a ratio **8**:**9** = 1:1.5–2.0 and the isomers were separated by flash chromatography.

The *anti*-isomer **8** was hydrolyzed into α-hydroxy-β-amino acid **10**, which was converted into (−)-cytoxazone **1** by a previously reported three-step procedure (54% from **8**).[7] The physical data of the obtained product were in agreement with those of the natural compound; in addition to the optical rotation value, its optical purity was confirmed by HPLC analysis on a chiral column.[5] By the same sequence of reactions the *syn*-isomer **9** was converted into enantiopure (+)-*epi*-cytoxazone **2**. However, this compound could also be used for (−)-cytoxazone synthesis: treatment of **9** with triphenylphosphine/DEAD afforded oxazoline **16** with the inversion of configuration at the α-carbon atom (73%; Scheme 3). Acidic hydrolysis of **16**, followed by the aforementioned protocol, furnished (−)-cytoxazone identical in all respects to the natural sample (56% from **16**). Conversely, *anti*-cyanohydrin **8** could be converted into (+)-*epi*-cytoxazone, by an analogous set of reactions (43% overall yield).

To summarize, optically pure (−)-cytoxazone **1** and (+)-*epi*-cytoxazone **2** were synthesized from (D)-(−)-4-hydroxyphenylglycine **4**. It is shown that the homologation of amino acids can be effected without epimerization via amido aldehydes, under carefully controlled conditions, where *N*-benzoyl group serves both as a protective group and the internal nucleophile in the configurational inversion step.

EXPERIMENTAL

All chromatographic separations were performed on silica, 10–18, 60A, ICN Biomedicals. Standard techniques were used for the purification of reagents and solvents. NMR spectra were recorded on a Varian Gemini 200, ^{1}H NMR at 200 MHz, ^{13}C NMR at 50 MHz. Chemical shifts are expressed in ppm using tetramethylsilane as internal standard, coupling constants (*J*) are



Scheme 3. a) Ph_3P, DEAD, C_6H_6, rt, 30 min (73%); b) 15% HCl, 110°C, 1.5 h.

in Hz. IR spectra were recorded on a Perkin-Elmer 457 grating FT instrument, and are expressed in cm^{-1}. Mass spectra were obtained on a Finnigan ITDS 700 instrument. Microanalyses were performed at the Vario EL III instrument CHNOS Elementar Analyzer, Elemantar Analysensysteme GmbH, Hanau-Germany. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotation measurements were performed on Perkin Elmer 141 MC polarimeter. HPLC analyses were performed on the Hewlett Packard 1100 Agilent Technologies instrument, with HP 1100 UV-VIS detector. Measurements were performed at 235 nm wavelength, at 40°C, at a flow rate of 1 mL/min. Chiracel OD column (200 × 4.6 mm) was used with a solvent mixture: *n*-hexane/*iso*-propanol = 80/20 as a mobile phase for the determination of cytoxazone, and *n*-hexane/ethanol = 85/15 for *epi*-cytoxazone.

(*R*)-*N*-Benzoyl-4-hydroxyphenylglycine (5). To a cold (0°C), vigorously stirred solution of (D)-(−)-4-hydroxyphenylglycine **4** (9.00 g; 53.8 mmol) in aqueous NaOH (156 mL of 2 M solution) was added benzoyl chloride (15.6 mL; 134 mmol), drop-wise, over 30 min. After the addition was complete, the reaction mixture was stirred for 2.5 h at room temperature (rt), followed by the addition of aqueous NaOH (30 mL of 4 M solution) and another 30 min stirring at rt, after which time no starting compound could be detected in the reaction mixture (the course of the reaction was monitored by TLC, eluent: toluene/ethyl acetate = 7/3, with 2 drops of formic acid). The reaction mixture was filtered with suction and the product was precipitated from the cold filtrate by the addition of concentrated HCl (ca. 18 mL) until pH 1-2. The precipitate was separated on a Buchner funnel, washed with water until pH 4 (5 × 10 mL), dried in a vacuum dessicator over P_2O_5. The amorphous solid was first recrystallized from toluene and then from water, to give 12.8 g (88%) of the title compound **5** as white crystals.

Physical data for **5**: m.p. 223°C; $[\alpha]_D$ − 137.9° (c 1.0, THF, 18°C); Anal. calcd. for $C_{15}H_{13}NO_4$: C 66.41, H 4.83, N 5.16; found: C 66.72, H 4.87, N 4.88; IR_{KBr}: 3412, 3358, 1738, 1614, 1567, 1539, 1518, 1491, 1378, 1360, 1270, 1209; ^{1}H NMR (DMSO) δ: 5.46 (d, J = 7.2, 1H); 6.77 (d, J = 8.4, 2H); 7.30 (d, J = 8.4, 2H); 7.41–7.58 (m, 3H); 7.94 (d, J = 1.2, 2H); 8.89 (d, J = 7.2, N**H**); 9.52 (bs, COO**H**); ^{13}C NMR (DMSO) δ: 56.67; 115.38; 127.38; 127.98; 128.44; 129.70; 131.69; 134.08; 157.48; 166.56; 172.66.

Methyl (*R*)-*N*-benzoyl-4-methoxyphenylglycinate (6). To a solution of (*R*)-*N*-benzoyl-4-hydroxyphenylglycine **5** (12.00 g; 44 mmol) in acetone (420 mL) was added anhydrous K_2CO_3 (18.30 g; 133 mmol) and dimethyl sulphate (13.95 g; 10.52 mL; 111 mmol). The resulting suspension was stirred and heated to reflux for 3 h (the course of the reaction was monitored by TLC, eluent: toluene/ethyl acetate = 7/3). The mixture was cooledto rt, filtered with suction and the filtrate was concentrated under reduced pressure to give amorphous, ochre-colored crude ester **6** (12.80 g).

The solid was crystallized from ethyl acetate with the addition of activated carbon. The second crystallization from ethyl acetate/petroleum ether afforded 11.80 g (89%) of the title compound **6**, as colorless needles.

Physical data for **6**: m.p. 144°C; $[\alpha]_D$ − 123° (c 1.0, $CHCl_3$, 21°C); Anal. calcd. for $C_{17}H_{17}NO_4$: C 68.21, H 5.72, N 4.68; found: C 68.08, H 5.95, N 4.52; IR_{KBr}: 3314, 1741, 1638, 1516, 1320, 1277, 1249, 1186, 1027; ^1H NMR ($CDCl_3$) δ: 3.76 (s, 3H); 3.78 (s, 3H); 5.71(d, J 6.8, 1H); 6.86–6.93 (m, 2H); 7.18 (d, $J = 6.8$, 1H); 7.26–7.51 (m, 5H); 7.79–7.84 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 52.79; 55.24; 56.19; 114.36; 127.10; 128.54; 128.58; 131.80; 133.59; 159.75; 171.73.

(*R*)-*N*-Benzoyl-4-methoxyphenylglycinol (7). Sodium borohydride (5.42 g; 143 mmol) was added in small portions to a solution of Methyl (*R*)-N-benzoyl-4-methoxyphenylglycinate **6** (7.16 g; 24 mmol) in dry methanol (400 mL). After the addition was complete (ca 30 min) the reaction mixture was stirred at rt for 3 h, when the reaction was complete (the course of the reaction was monitored by TLC, eluent toluene/ethyl acetate = 6 : 4). The reaction mixture was concentrated under reduced pressure to a volume of ca 60 mL, cooled to 0°C, carefully acidified with 2M HCl (ca 18 mL) to a pH 2 and extracted with 4 × 150 mL of ethyl acetate. The combined organic extract was washed successively with 10% aqueous NaOH (3 × 50 mL) until pH 9, then with 2 M HCl, water, and brine until pH 7. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give the crude product (6.7 g). Purification by dry-flash chromatography (gradient elution with toluene/ethyl acetate = 7/3-1/1) afforded 5.90 g (91%) of the title compound **7** as colorless crystals.

Physical data for **7**: m.p. 154°C; $[\alpha]_D$ + 16.7° (*c* 1.0, AcOEt, 20°C); Anal. calcd. for $C_{16}H_{17}NO_3$: C 70.83, H 6.32, N 5.16; found: C 71.09, H 6.30, N 5.34; IR_{KBr}: 3431, 3331, 2923, 1634, 1532, 1491, 1300, 1248, 1180, 1033; ^1H NMR (DMSO) δ: 3.39–3.69 (m, 2H); 3.72 (s, 3H); 4.91 (t, $J = 5.7$, 1H); 5.03 (dd, $J_1 = 5.7$, $J_2 = 8.0$, 1H); 6.88 (d, $J = 8.8$, 2H); 7.32 (d, $J = 8.8$, 2H); 7.42–7.50 (m, 3H); 7.90 (d, $J = 8.0$, 2H); 8.65 (d, $J = 8.0$, 1H); ^{13}C NMR (DMSO) δ: 55.24; 55.56; 64.79; 113.78; 127.64; 128.38; 128.44; 131.37; 133.65; 134.99; 158.48; 166.34.

(*R*)-*N*-Benzoyl-4-methoxyphenylglycinal (3). Freshly prepared DMP[24] (2.62 g; 619 mmol) was added with stirring to a suspension of (*R*)-*N*-benzoyl-4-methoxyphenylglycinol **7** (0.80 g; 295 mmol) in dichloromethane saturated with water (16 mL). After 2 min the reaction mixture became clear, and after 12 min the reaction was complete (TLC: toluene/ethyl acetate = 3/2). The reaction was quenched by the addition of diethyl ether (12 mL) and the solution of $Na_2S_2O_3$ (8.05 g; 32 mmol; dissolved in 10 mL of the saturated aqueous solution of $NaHCO_3$), and the mixture was stirred at rt until it cleared up completely, with the separation of layers. The organic layer was separated, the aqueous layer was extracted with diethyl ether (3 × 20 mL), and the combined extract was washed successively with

saturated aqueous $Na_2S_2O_3$ (2 × 10 mL), $NaHCO_3$ (2 × 5 mL), water and brine. Drying over anhydrous $MgSO_4$, followed by solvent evaporation under reduced pressure gave 0.75 g (94%) of (*R*)-*N*-benzoyl-4-methoxyphenylglycinal **3**, as pale-yellow crystals, which was used in the next step without further purification.

Physical data for **3**: $IR_{film\ in\ CHCl3}$: 3318, 2927, 1713, 1620, 1516, 1320, 1249, 1184, 1027, 829; ^1H NMR ($CDCl_3$) δ: 3.80 (s, 3H); 5.71 (d, $J = 5.8$, 1H); 6.94 (d, $J = 8.8$, 2H); 7.30 (d, $J = 8.8$, 2H); 7.38–7.56 (m, 4H); 7.87 (d, $J = 8.0$, 2H); 9.62 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 55.29; 63.24; 114.87; 124.97; 127.14; 128.62; 129.51; 131.93; 133.49; 160.06; 166.72; 194.96.

The optical purity of (*R*)-*N*-benzoyl-4-methoxyphenylglycinal **3** was determined by its' reduction into (*R*)-*N*-benzoyl-4-methoxyphenylglycinol **7**, and the comparison of the optical rotation value with the original sample: Sodium borohydride (20 mg; 0.529 mmol) was added to the solution of (*R*)-*N*-benzoyl-4-methoxyphenylglycinal **3** (30 mg; 0.112 mmol) in ethanol (3 mL) and THF (1 mL). The mixture was stirred at rt for 30 min, after which time the reaction was complete. The reaction mixture was carefully acidified to pH ~4 by the addition of 1.5 M HCl and extracted with ethyl acetate (3 × 10 mL). The combined organic extract was washed with saturated aqueous $NaHCO_3$ (2 × 5 mL), water and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The solid residue was purifies by dry-flash chromatography (gradient elution with toluene/ethyl acetate = 7/3-1/1) afforded 25 mg (83%) of (*R*)-*N*-benzoyl-4-methoxyphenylglycinol **7** as colorless crystals. m.p. 154°C; $[\alpha]_D + 16.5$ (*c* 1.0, AcOEt, 24°C) ⇒ 98.2% ee.

(2*R*,3*R*)-2-Hydroxy-3-(4-methoxyphenyl)-3-*N*-benzoylaminopropionitrile (8) and **(2*S*,3*R*)-2-Hydroxy-3-(4-methoxyphenyl)-3-*N*-benzoylaminopropionitrile (9)** **(*CAUTION:* Hydrogen cyanide is highly toxic! All operations with hydrogen cyanide should be performed in a well ventilated hood).** Acetic acid (0.43 g; 0.41 mL; 7.167 mmol) was added drop-wise to a suspension of potassium cyanide (0.43 g; 6.603 mmol) in dry methanol (3.52 mL), with stirring. After 5 min stirring at rt, the clear methanolic solution of HCN was added drop-wise (with a syringe) to a cold (0°C) suspension of (*R*)-*N*-benzoyl-4-methoxyphenylglycinal **3** (0.71 g; 2.636 mmol) in dichloromethane (8.8 mL), with vigorous stirring, under an argon atmosphere. After 10 min TLC indicated the complete conversion of the starting material (eluent: toluene/ethyl acetate = 7/3). The reaxtion mixture was quenched by the addition of the mixture of water (18 mL), brine (14.4 mL) and saturated aqueous $NaHCO_3$ (3.6 mL) and the stirring was continued for 5 more min. The organic layer was separated and the aqueous layer was extracted with ether (3 × 15 mL). The combined organic extract was washed with a mixture of brine and saturated aqueous $NaHCO_3$ in a 4 : 1 ratio (10 mL) and water, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Purification by dry-flash chromatography (eluent: toluene/ethyl acetate = 4/1)

afforded 0.71 g (92%) of the mixture of diastereoisomeric cyanohydrins **8** (*anti*): **9** (*syn*) = 1 : 1.5–1 : 2, as a colorless foam. The isomers were separated by flash-chromatography (eluent: chloroform/methanol = 50/1).

Physical data for the mixture of **8** and **9**: m.p. 45°C; $IR_{film\ in\ CHCl3}$: 3327, 3068, 2053, 1898, 1642, 1613, 1579, 1515, 1488, 1464, 1306, 1251, 1182, 1081, 1029, 832; NMR spectra of pure compounds **8** and **9**: (2*R*,3*R*)-2-Hydroxy-3-(4-methoxyphenyl)-3-*N*-benzoylaminopropionitrile (**8**): ^{1}H NMR ($CDCl_3$): 3.82 (s, 3H); 4.74 (br s, 1H); 5.32 (dd, $J_1 = 2.2$, $J_2 = 2.5$, 1H); 6.32 (br s, 1H); 6.85 (d, $J = 5.1$, NH); 6.98 (d, $J = 8.7$, 2H); 7.26–7.59 (m, 5H); 7.78 (d, $J = 6.9$, 2H); ^{13}C NMR ($CDCl_3$) δ: 55.33; 59.12; 67.73; 114.85; 117.89; 127.31; 128.25; 128.81; 132.58; 160.28; 169.94. (2*S*,3*R*)-2-Hydroxy-3-(4-methoxyphenyl)-3-*N*-benzoylaminopropionitrile (**9**): ^{1}H NMR ($CDCl_3$): ^{1}H NMR ($CDCl_3$) δ: 3.77 (s, 3H); 4.84 (br s, 1H); 5.37 (dd, $J_1 = 5.4$, $J_2 = 6.2$, 1H); 5.50 (d, $J = 5.4$, 1H); 6.89 (d, $J = 8.8$, 2H); 7.27–7.47 (m, 5H); 7.48 (d, $J = 7.0$, 1H) 7.75 (d, $J = 6.8$, 2H); ^{13}C NMR ($CDCl_3$) δ: 55.26; 56.65; 64.82; 114.41; 118.49; 127.25; 127.72; 128.67; 132.20; 133.19; 159.89; 168.60.

The optical purity of the cyanohydrins **8** and **9** was determined by their reduction with sodium borohydride to (*R*)-*N*-benzoyl-4-methoxyphenylglycinol **7** and the comparison of the optical rotation data to those of the original sample, as described for (*R*)-*N*-benzoyl-4-methoxyphenylglycinal **3**. The obtained sample had m.p. 154°C and the optical rotation value $[\alpha]_D + 16.3$ (*c* 1.0, AcOEt, 24°C). Similarly, to verify that cyanohydrins **8** and **9** do not racemize on silica-gel, the purified samples of the separated cyanohydrins **8** and **9** were also submitted to the reduction. The obtained alcohol had the physical properties identical to the reference compound **7**.

(2*R*,3*R*)-3-Amino-3-(4-methoxyphenyl)-2-hydroxypropanoic acid hydrochloride (10). A mixture of (2*R*,3*R*)-2-Hydroxy-3-(4-methoxyphenyl)-3-*N*-benzoylaminopropionitrile **8** (72 mg; 0.243 mmol) and 15% HCl (7.5 mL; 34 mmol) was heated to reflux with stirring. After 3 h TLC (eluent: toluene/ethyl acetate = 3/2, with 3 drops of formic acid) indicated the full consumption of the starting material. The reaction mixture was concentrated to dryness under reduced pressure, dry ethanol (8 mL) was added to the viscous residue, and the mixture was evaporated to dryness. This procedure was repeated twice, and then three times by substituting acetone for ethanol. Ether (20 mL) was added, heated to the boiling point and the etheral extract was decanted. This operation was repeated one more time, with the same amount of ether, when benzoic acid was completely removed. Drying under vacuum (0.1 mmHg) afforded 62 mg of the title compound **10** as the amorphous, colorless powder, which was used in the next step without further purification.

(4*R*,5*R*)-4-(4-Methoxyphenyl)-1,3-Oxazolidine-2-one-5-carboxylic acid (11) (*CAUTION:* Phosgene is highly toxic! All operations with trichloromethyl chloroformate should be performed in a well ventilated

hood). (2*R*,3*R*)-3-Amino-3-(4-methoxyphenyl)-2-hydroxypropanoic acid hydrochloride **10** (62 mg; 0.243 mmol) was added to a cold (0°C) solution of NaOH (75 mg; 1.875 mmol) in water (4 mL) and the mixture was stirred for 10 min in an ice bath. Trichloromethyl chloroformate (45 μL; 73.8 mg; 0.373 mmol) was added drop-wise and the mixture was stirred for 90 min at that temperature; pH of the reaction mixture should be 9-10. The progress of the reaction was monitored by TLC (eluent: toluene/ethyl acetate = 3/2, with 3 drops of formic acid). As TLC indicated the presence of starting material (about 20%), additional NaOH (25 mg) and trichloromethyl chloroformate (15 μL) were added and stirring was continued for 1 h.The reaction was quenched by the addition of 1.5 M HCl until pH 2 (with stirring and cooling). The reaction mixture was extracted with ethyl acetate (3 × 15 mL), the combined organic extract was washed with water (2 × 5 mL), brine and dried over anhydrous $MgSO_4$. Evaporation of solvent under reduced pressure afforded 45 mg of the title compound **11** as beige-colored, amorphous powder, which was used in the next step without further purification.

Methyl (4*R*,5*R*)-4-(4-methoxyphenyl)-1,3-oxazolidine-2-one-5-carboxylate (12) (*CAUTION:* Diazomethane is explosive!). Nitrosomethylurea (80 mg) was added in small portions to a cold (0°C) solution of KOH (200 mg) in water (0.2 mL) and ether (4.8 mL). The yellow etheral solution was decanted, dried over anhydrous Na_2SO_4 and added drop-wise to a cold solution of (4*R*,5*R*)-4-(4-methoxyphenyl)-1,3-oxazolidine-2-one-5-carboxylic acid **11** (45 mg, from the previous reaction) in THF (2.5 mL), with stirring, while the progress of the reaction was monitored by TLC (eluent: toluene/ethyl acetate = 4/1). Evaporation of solvent under reduced pressure, followed by purification by dry-flash chromatography (eluent: toluene/ethyl acetate = 4/1) afforded 39 mg (65% from cyanohydrin **8**) of the title compound **12**, as colorless, star-shaped crystals.

Physical data for **12**: m.p. 106°C; recrystallization from ethyl acetate/*n*-hexane gives colorless crystals m.p. 110–110.5°C; $[\alpha]_D$ − 94.0 (*c* 1.0, MeOH, 23°C); Anal. calcd. for $C_{12}H_{13}NO_5$: C 57.37, H 5.22, N 5.58; found: C 57.10, H 5.31, N 5.54; IR_{KBr}: 3465, 3271, 3012, 1757, 1738, 1517, 1458, 1280, 1217, 1029, 935; ^1H NMR ($CDCl_3$) δ: 3.31 (s, 3H); 3.80 (s,3H); 5.18 (d, $J = 9.3$; 1H); 5.26 (d, $J = 9.3$, 1H); 6.09 (br s, 1H); 6,88 (d, $J = 8.8$, 2H); 7.21 (d, $J = 8.8$, 2H); ^{13}C NMR($CDCl_3$) δ: 52.00; 55.26; 57.88; 78.13; 114.05; 126.99; 128.14; 158.24; 160.28; 166.85.

(4*R*,5*R*)-4-(4-Methoxyphenyl)-5-Hydroxymethyl-1,3-oxazolidine-2-one (cytoxazone, 1). Sodium borohydride (36.4 mg; 0.963 mmol) was added in one portion to a cold (0°C) solution of methyl (4*R*,5*R*)-4-(4-methoxyphenyl)-1,3-oxazolidine-2-one-5-carboxylate **12** (29.1 mg; 0.116 mmol) in THF (1.9 mL) and water (0.2 mL), and the reaction mixture was stirred for 1 h while monitoring the progress of the reaction by TLC (eluent: toluene/ethyl acetate = 7/3). Additional sodium borohydride (10 mg; 0.264 mmol) was added and stirring was continued for one more hour, when no starting

compound could be detected in the reaction mixture. 1 M HCl was added until pH 6-7 and the mixture was extracted with ethyl acetate (3 × 10 mL). The combined extract was washed with 5% aqueous NaOH (2 × 4 mL), water and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The solid residue was purified by dry-flash chromatography (eluent: toluene/ethyl acetate = 45/55) to give 22 mg (83%) of (−)-cytoxazone **1** as shiny, colorless crystals, m.p. 120–121°C (lit.[2] m.p. 118–121°C); Anal. calcd. for $C_{11}H_{13}NO_4$: C 59.20, H 5.87, N 6.27; found: C 58.90, H 5.76, N 6.27; $[\alpha]_D$ − 65.7 (*c* 0.38, MeOH, 25°C); (lit.[2] $[\alpha]_D$ − 75.7 (*c* 1.0, MeOH, 26°C)); Spectral data identical to those reported in the literature.[2] HPLC analysis on Chiracel OD column showed the compound to be >98% ee.

(4*R*,5*R*)-2-Phenyl-4-(4-methoxyphenyl)-1,3-oxazoline-5-carbonitrile (16; isomerization of *syn*-cyanohydrin 9). Diethyl azodicarboxylate (394 mg; 352 μL; 2.263 mmol) was added over 4 min to a solution of (2*S*,3*R*)-2-Hydroxy-3-(4-methoxyphenyl)-3-*N*-benzoylaminopropionitrile **9** (100 mg; 0.338 mmol), triphenylphosphine (594 mg; 2.263 mmol) and *p*-nitrobenzoic acid (101.7 mg; 0.608 mmol), with stirring, at rt, under an argon atmosphere. The course of the reaction was monitored by TLC (eluent: toluene/ethyl acetate = 9/1). After 3 h the mixture was filtered through a sintered glass funnel, diluted with dichloromethane (50 mL), washed with saturated aqueous $NaHCO_3$ (2 × 5 mL), water and brine. The organic extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The pale-yellow viscous residue was purified by dry-flash chromatography (eluent: toluene/ethyl acetate = 39/1) to give 68 mg (73%) of the title compound **16**, which crystallizes from ethyl acetate/*n*-hexane as shiny needles.

Physical data for **16**: m.p. 115°C; $[\alpha]_D$ + 3.1 (*c* 0.38, EtOAc, 23°C); Anal. calcd. for $C_{17}H_{14}N_2O_2$: C 73.37, H 5.07, N 10.07; found: C 73.01, H 5.08, N 10.06; IR_{KBr}: 2965, 2930, 1667, 1581, 1513, 1456, 1327, 1271, 1249, 1176, 1059, 1028, 787; ^1H NMR ($CDCl_3$) δ: 3.81 (s, 3H); 5.52 (d, *J* = 10.0, 1H); 5.66 (d, *J* = 10.0, 1H); 6.94 (d, *J* = 8.8, 2H); 7.27 (d, *J* = 8.8, 2H); 7.43–7.61 (m, 3H); 8.04 (d, *J* = 6.5, 2H); ^{13}C NMR ($CDCl_3$) δ: 55.20; 71.59; 72.75; 114.24; 114.70; 125.77; 128.28; 128.61; 128.64; 128.70; 132.46; 160.04; 163.37.

Hydrolysis of **16** into (2*R*,3*R*)-3-Amino-3-(4-methoxyphenyl)-2-hydroxypropanoic acid hydrochloride **10** was carried out according to the procedure described above for the conversion of **8** to **10**. Conversion of **10** into cytoxazone was accomplished by a three-step procedure, as described above. The cytoxazone thus obtained was identical in all respect to the sample obtained from **8**.

Methyl (4*R*,5*S*)-4-(4-methoxyphenyl)-1,3-oxazolidine-2-one-5-carboxylate (15). This compound was prepared from **9** by a three-step procedure, as described for the preparation of methyl (4*R*,5*R*)-4-(4-

methoxyphenyl)-1,3-oxazolidine-2-one-5-carboxylate **12** from **8**. Starting from 84 mg of **9**, 51 mg (71% over three steps) of **15** was obtained, as colorless crystals.

Physical data for **15**: m.p. 91–92°C; $[\alpha]_D + 90.0$ (*c* 1.0, MeOH, 20°C); Anal. calcd. for $C_{12}H_{13}NO_5$: C 57.37, H 5.22, N 5.58; found: C 57.51, H 4.99, N 5.54; IR_{KBr}: 3230, 2966, 1784, 1729, 1613, 1513, 1251, 1192, 1069, 938; ^1H NMR ($CDCl_3$) δ: 3.82 (s, 3H); 3.87 (s, 3H); 4.75 (d, $J = 5.3$, 1H); 4.93 (d, $J = 5.3$, 1H); 5.76 (br s, 1H); 6.94 (d, $J = 8.8$, 2H); 7.29 (d, $J = 8.8$, 2H); ^{13}C NMR ($CDCl_3$) δ: 53.02; 55.33; 58.70; 80.46; 114.61; 127.25; 130.73; 157.80; 160.17; 168.83.

(4*R*,5*S*)-*epi*-Cytoxazone 2. According to the procedure described for cytoxazone **1**. Starting from 38.1 mg of Methyl (4*R*,5*S*)-4-(4-methoxyphenyl)-1,3-oxazolidine-2-one-5-carboxylate **15**, 36 mg (93%) of (4*R*,5*S*)-*epi*-cytoxazone **2** was obtained in form of colorless crystals, m.p. 161–162°C, (lit.[11] m.p. 161.5–162.5°C); $[\alpha]_D + 32.0$ (*c* 0.4, MeOH, 25°C); (lit.[11] for the enantiomeric, (4*S*,5*R*)-*epi*-cytoxazone: $[\alpha]_D - 30.4$ (*c* 1.0, MeOH, 20°C)); Spectral data identical to those previously reported.[11] Determination of optical purity by HPLC analysis on Chiracel OD column showed the compound to be >96% ee.

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