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Short-Step and Scalable Synthesis of (±)-Cytoxazone

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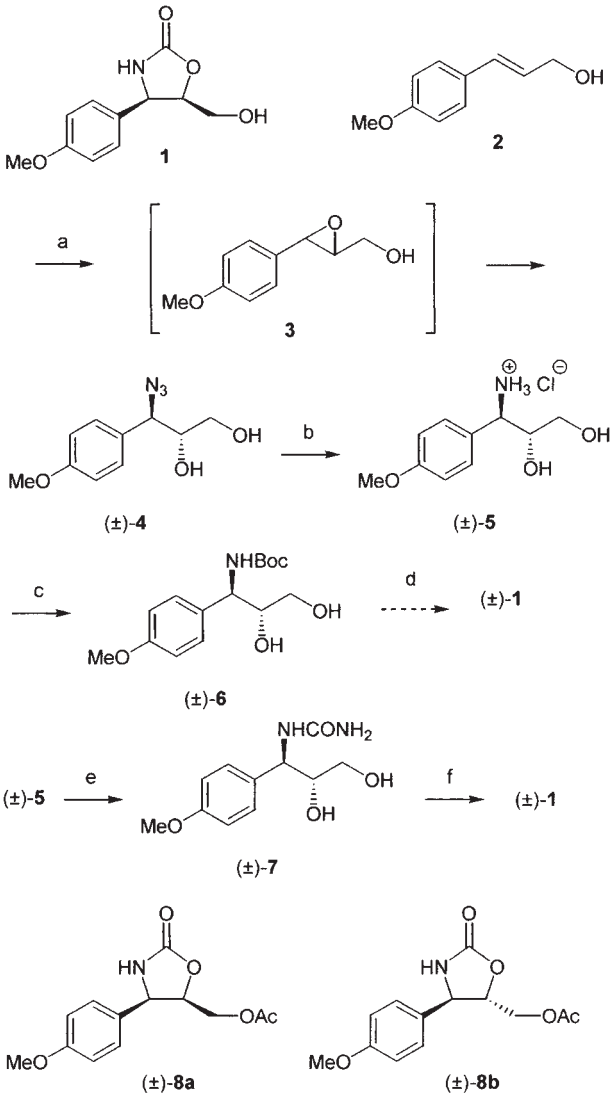
A five-step and scalable synthesis of racemic cytoxazone, a novel cytokine modulator, was accomplished in a total yield of 51% from *p*-methoxycinnamyl alcohol without any protective groups. The keystep was the new one-pot azidohydroxylation procedure by the combined use of NaN_3–H_2O_2–CH_3CN. The epoxidation of an olefin by means of an *in situ*-formed iminohydroperoxide worked well, accompanied by the concomitant regioselective ring opening reaction of the resulting highly reactive epoxide with an azide ion.

Key words: cytoxazone; oxazolidinone; azidohydroxylation; nitrosation; deaminocyclization

Cytoxazone (**1**) is a novel cytokine modulator[1,2] of microbial origin. Chemical syntheses of the natural enantiomer[3,4] as well as the stereoisomers[5–13] have so far been reported. The fact that there was no difference between the stereoisomers in their bioactivity[10] prompted us to develop a short-step, scalable route to its racemic form for more advanced biological studies.

Our synthesis is straightforward as described in Scheme 1. Initial attempts for epoxidation of commercially available *p*-methoxycinnamyl alcohol (**2**) were problematic, as the resulting epoxide (**3**) was very unstable and highly susceptible to attack by any nucleophilic substance. For example, *m*-CPBA oxidation resulted in an attack on concomitantly formed *m*-CBA together with the progress of epoxidation. We then tried epoxidation in the presence of an azide, expecting simultaneous nucleophilic ring-opening of the epoxide.

For this purpose, a combination of acetonitrile as the solvent and aqueous H_2O_2 served very well to provide the *in situ* epoxidizing reagent, $CH_3C(=NH)OOH$ (an iminohydroperoxide, Payne oxidation).[14] Under the conditions whereby substrate **2** was mixed with 30% H_2O_2, acetonitrile and NaN_3, azidoalcohol **4** was obtained in a 73% yield together with the recovered **2** (5%). This two-step, one-pot conversion was reproducible at the one-gram scale of the substrate under careful control of the reaction temperature to below 30 °C throughout the epoxidation process. Intermediate epoxide **3** was highly reactive toward the nucleophilic ring opening reaction, and we could do without any further addition of a Lewis acid such as $LiClO_4$.[15] Indeed, such addition did not affect either the yield of desired compound **4** or the formation of a triol originating from the action of water on the epoxide intermediate.



Scheme 1. Reagents: a) NaN_3, aq. H_2O_2, CH_3CN, 73%; b) 1) Ph_3P, aq. THF; 2) aq. HCl; c) Boc_2O, Et_3N, MS 3A, THF, quant.; d) Ref. 6; e) KCNO (1.5 eq), aq. HCl (0.2 eq); f) $NaNO_2$ (1.2 eq), aq. HCl (4.0 eq), 70% from **4**.

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The conversion of azidoalcohol to oxazolidinone was also straightforward. Reduction of the azide to amine with Ph_3P in aqueous THF gave amino alcohol (±)-**5** as a free base. After acidification with aqueous HCl, precipitated Ph_3PO could be simply removed by filtration, and we could avoid the previously reported extractive workup[16] with an organic solvent. Treatment of amino alcohol **5** with Boc_2O under anhydrous reaction conditions provided *N*-Boc aminodiol (±)-**6**,[6] a known precursor of cytoxazone, in a quantitative yield. An attempt at direct *in situ* transformation to desired **1** in the presence of either DBU or DMAP,[17] however, was unsuccessful.

At this stage, we turned our attention to an *N*-carbamyl derivative, another possible intermediate, whose preparation and cyclization can be performed in aqueous media. Obtained amine salt (±)-**5** was treated with 1.5 eq. of KCNO and an additional 0.2 eq. of aqueous HCl to give crude *N*-carbamylamino alcohol (±)-**7**. After separating from the inorganic materials by solvent extraction, $NaNO_2$ (1.2 eq.) was added, and the nitrosation–deaminocyclization took place.[18,19] The yield of (±)-**1** from **4** was 70% in three steps. The desired five-membered oxazolidinone ring formation to **1** proceeded smoothly. The only characterizable by-product was *trans*-isomer **8b**, after converting to the corresponding acetate. This was probably due to inversion at the secondary alcohol during the Ph_3P-mediated reduction,[20] although the amount was less than 0.2%. Fortunately, the undesired mode of cyclization to yield a six-membered ring did not occur, and this regioselective reaction enabled us to avoid any introduction of protecting groups on the primary hydroxy group in this synthesis.

In conclusion, a five-step (51% total yield) and scalable (up to one gram) synthesis of racemic cytoxazone was accomplished from *p*-methoxycinnamyl alcohol without the need for any protective groups. The first two-step azidohydroxylation was accomplished with a one-pot treatment. Moreover, all steps could be performed in aqueous media, and it was not necessary to take care with the ambient moisture conditions throughout the synthesis.

Experimental

All melting point (mp) data are uncorrected. IR spectra were measured as films for oil and as KBr disks for solids with a Jasco FT/IR-410 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with a Jeol JNM GX-270 or GX-400 spectrometer. Silica gel 60 (spherical, 100–210 μm) from Kanto Chemical Co. was used for column chromatography. Preparative TLC was performed with E. Merck silica gel 60 F_{256} plates (0.5 mm thickness, No. 5744).

(2R,3R*)-3-Azido-3-(4-methoxyphenyl)propane-1,2-diol (**4**).* NaN_3 (1.976 g, 30.4 mmol) was dissolved in water (30 ml), and to that a solution of **2** (1.004 g, 6.1 mmol) in acetonitrile (10 ml) was added while vigorously stirring, before a white precipitate appeared. The mixture was then cooled in an ice–water bath, and aqueous H_2O_2 (30% solution, 12.5 ml, 0.16 mol) was added dropwise. After completing the addition, the ice–water bath was replaced with a water bath, and the reaction temperature was allowed to rise to room temperature. The water bath was removed when the internal reaction temperature had reached the ambient temperature. The reaction was exothermic, and the internal temperature was carefully maintained at below 30 °C with occasional external cooling. During the progress of the reaction, effervescence of O_2 gas was observed. The mixture turned to a yellow homogeneous solution within 30 min, and the effervescence ceased. After stirring overnight at room temperature, the mixture was saturated with NaCl and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate and concentrated *in vacuo*. The residual brown viscous oil (1.294 g) was charged into a silica gel column (15 g). Elution with hexane–EtOAc (3:2) afforded recovered **2** (51 mg, 5%) and (±)-**4** (1.010 g, 73%) as an oil. IR ν_{max} cm^{-1}: 3356, 3005, 2935, 2839, 2102, 1612, 1585, 1514, 1464, 1032, 827; NMR δ_H (270 MHz, $CDCl_3$): 1.87 (1H, t, $J = 6.1$ Hz), 2.03 (1H, d, $J = 4.1$ Hz), 3.67–3.80 (3H, m), 3.76 (3H, s), 4.51 (1H, d, $J = 7.3$ Hz), 6.88 (2H, d, $J = 8.7$ Hz), 7.21 (2H, d, $J = 8.7$ Hz); NMR δ_C (100 MHz, $CDCl_3$): 55.3, 63.0, 66.5, 73.9, 114.4, 127.7, 129.0, 159.8. *Anal.* Found: C, 53.71; H, 5.82; N, 18.34%. Calcd. for $C_{10}H_{13}N_3O_3$: C, 53.80; H, 5.87; N, 18.82%.

(2R,3R*)-3-Amino-3-(4-methoxyphenyl)propane-1,2-diol HCl salt (**5**).* A mixture of azidoalcohol **4** (1.506 g, 6.75 mmol), Ph_3P (3.529 g, 13.5 mmol) and H_2O (1.3 ml, 72.2 mmol) in THF (24 ml) was stirred and heated overnight at 50 °C. The mixture was concentrated *in vacuo*, and the resulting residue was dissolved in aq. HCl (2 M, 10 ml), and Ph_3PO was precipitated. The solid was finely dispersed and filtered off. The combined filtrate and washings were concentrated *in vacuo* again to give amine salt (±)-**5** (2.276 g, quantitative). NMR δ_H (270 MHz, D_2O): 3.41 (1H, dd, $J = 6.4$, 11.7 Hz), 3.52 (1H, dd, $J = 5.4$, 11.7 Hz), 3.90 (3H, s), 4.18 (1H, ddd, $J = 4.0$, 5.4, 6.4 Hz), 4.55 (1H, d, $J = 4.0$ Hz), 7.11 (2H, d, $J = 8.9$ Hz), 7.45 (2H, d, $J = 8.9$ Hz). This salt was employed for the next step without further purification.

(2R,3R*)-3-tert-Butoxycarbonylamino-3-(4-methoxyphenyl)propane-1,2-diol (**6**).* Crude aminoalcohol HCl salt **5** (141.3 mg, 0.46 mmol was expected) was mixed with THF (2 ml) and Et_3N (0.127 ml, 0.91 mmol) in the presence of 3A molecular sieves (714 mg), and the solid material of the amine salt was finely dispersed ultrasonically under Ar. Di-*tert*-butyl dicarbonate (Boc_2O, 0.126 ml, 0.55 mmol) was then added, and the mixture

was stirred at room temperature. A conventional workup afforded (±)-**6** (143.2 mg, quantitative from **4**), whose purity was good enough as judged from its TLC analysis. This compound was recrystallized from a mixture of hexane (0.5 ml) and ethyl acetate (1.0 ml) to afford colorless fine needles (91.4 mg, 67%), mp 151.4–151.5 °C. NMR δ_H (270 MHz, $CDCl_3$): 1.41 (9H, s), 2.37 (1H, d, $J = 6.9$ Hz), 3.05 (1H, s), 3.65–3.75 (2H, m), 3.75–3.85 (1H, m), 3.79 (3H, s), 4.61 (1H, t, $J = 7.7$ Hz), 5.07 (1H, m), 6.89 (2H, d, $J = 8.6$ Hz), 7.24 (2H, d, $J = 8.6$ Hz). *Anal.* Found: C, 60.61; H, 7.74; N, 4.61%. Calcd. for $C_{15}H_{23}NO_5$: C, 60.59; H, 7.80; N, 4.71%. Its NMR spectrum was identical with that reported previously.[6] Any attempts at *in situ* cyclization by either applying refluxing temperature, or adding DBU (1.5 eq.) or DMAP (1.0 eq.) had no further effect on the desired reaction.

(2R,3R*)-3-Carbamylamino-3-(4-methoxyphenyl)propane-1,2-diol (**7**).* Crude aminoalcohol HCl salt **5** (2.276 g; 6.75 mmol was expected) was dissolved in aq. HCl (2 M, 0.7 ml) and H_2O (3.4 ml), before KCNO (822 mg, 10.1 mmol) was added portionwise at room temperature. The mixture was stirred overnight and concentrated *in vacuo*. The residual solid was extracted with EtOH, and the extract was concentrated *in vacuo* again to give carbamylamino alcohol (±)-**7** (1.996 g, quantitative) as a viscous oil. NMR δ_H (270 MHz, CD_3OD): 3.20–3.40 (2H, m), 3.67 (3H, s), 3.67–3.72 (1H, m), 4.64 (1H, d, $J = 5.2$ Hz), 6.77 (2H, d, $J = 8.6$ Hz), 7.17 (2H, d, $J = 8.6$ Hz). This was employed for the next step without further purification.

(4R,5R*)-5-Hydroxymethyl-4-(4-methoxyphenyl)-2-oxazolidinone (**1**).* Carbamylaminoalcohol **7** was dissolved in aq. HCl (3 M, 9.0 ml), the reaction vessel being briefly evacuated under ultrasonic vibration and then purged with Ar. To this mixture $NaNO_2$ (465.6 mg, 6.75 mmol) was added. Effervescence of N_2 gas was observed and a white solid precipitated. The mixture was stirred at room temperature for 2 hr, before $NaNO_2$ (95.5 mg, 1.38 mmol) was added and the reaction continued for a further 20 min. After being neutralized by adding aq. NaOH (1 M), the mixture was concentrated *in vacuo*. The residual solid was extracted with THF, and the extract was concentrated *in vacuo* again. The resulting solid residue was dissolved in THF (2 ml). After standing in a refrigerator, a solid (405.4 mg) of (±)-**1** was precipitated and then recovered by decantation. The supernatant was concentrated *in vacuo*, the resulting residue (1.20 g) being loaded into a silica gel column (120 g). Elution with THF–EtOAc (1:5) afforded partially purified (±)-**1** (1.13 g). The combined material was dissolved in THF (9.0 ml) and hexane (3 ml) while heating. The solution was left to stand in a refrigerator, and the precipitated crystals were collected by filtration. Racemic cytoxazone (±)-**1** (794.2 mg, 53% from **4**) was obtained as colorless prisms, mp 143.9–144.1 °C [lit.[7] mp 143–144 °C]. IR ν_{max} cm^{-1}: 3371, 3265, 2960, 1734, 1616, 1520, 1429, 1311, 1254, 1223, 1036, 980, 850, 814; NMR δ_H (400 MHz, DMSO-d_6): 2.90–3.00 (2H, m), 3.74 (3H, s), 4.69 (1H, ddd, $J = 4.4$, 7.3, 8.3 Hz), 4.82 (1H, t, $J = 5.1$ Hz), 4.90 (1H, d, $J = 8.3$ Hz), 6.92 (2H, d, $J = 8.6$ Hz), 7.14 (2H, d, $J = 8.6$ Hz), 8.06 (1H, s); NMR δ_C (100 MHz, DMSO-d_6): 55.01, 56.18, 61.03, 80.01, 113.56, 127.90, 129.14, 158.61, 158.84. *Anal.* Found: C, 59.02; H, 5.91; N, 6.26%. Calcd. for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87; N, 6.27%. The spectral data were identical with those reported previously.[1,2,7]

The mother liquor from the final recrystallization was concentrated *in vacuo*, and the residue was dissolved in Ac_2O (5.8 ml) and pyridine (5.8 ml). The mixture was stirred overnight at room temperature. After a conventional workup, the resulting oily residue (605.9 mg) was loaded into a silica gel column (30 g). Elution with hexane–EtOAc (2:3) afforded (±)-**8a**. The residue was recrystallized from a mixture of hexane (1.5 m) and EtOAc (1.4 ml) to afford colorless fine needles (277.4 mg). The mother liquor was further purified by preparative thin-layer chromatography [developed twice with hexane–EtOAc (2:3)] to give 34.3 mg of (±)-**8a**, mp 114.5–114.7 °C. IR ν_{max} cm^{-1}: 3452, 3263, 1776, 1734, 1612, 1514, 1250, 1068, 1047; NMR δ_H (270 MHz, $CDCl_3$): 1.98 (3H, s), 3.70–3.90 (2H, m), 3.80 (3H, s), 4.97 (2H, m), 5.37 (1H, s), 6.89 (2H, d, $J = 8.6$ Hz), 7.18 (2H, d, $J = 8.6$ Hz); NMR δ_C (100 MHz, $CDCl_3$): 20.64, 55.34, 57.35, 63.04, 77.50, 114.35, 126.96, 127.80, 158.70, 160.04, 170.17. *Anal.* Found: C, 58.67; H, 5.70; N, 5.17%. Calcd. for $C_{13}H_{15}NO_5$: C, 58.86; H, 5.70; N, 5.28%.

Epimeric (±)-**8b** was isolated in the course of preparative TLC separation (1.8 mg, 0.2% from **4**). NMR δ_H (270 MHz, $CDCl_3$): 2.05 (3H, s), 3.75 (3H, s), 4.22 (1H, dd, $J = 5.1$, 12.3 Hz), 4.28 (1H, dd, $J = 3.7$, 12.3 Hz), 4.43–4.47 (1H, m), 4.57 (1H, d, $J = 6.8$ Hz), 5.25 (1H, s), 6.87 (2H, d, $J = 8.5$ Hz), 7.20 (2H, d, $J = 8.5$ Hz).

The combined amount of (±)-**8a** was 311.7 mg, and this was converted into (±)-**1** in a quantitative manner by treating with triethylamine in refluxing methanol. The total yield of (±)-**1** then reached 70% from (±)-**4**.

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References

1) Kakeya, H., Morishita, M., Kobinata, K., Osono, M., Ishizuka, M., and Osada, H., Isolation and biological activity of a novel cytokine modulator, cytoxazone. *J. Antibioics*, **51**, 1126–1128 (1998).

2) Kakeya, H., Morishita, M., Koshino, H., Morita, T., Kobayashi, K., and Osada, H., Cytoxazone: A novel cytokine modulator containing a 2-oxazolidinone ring produced by *Streptomyces* sp. *J. Org. Chem.*, **64**, 1052–1053 (1999).
3) Sakamoto, Y., Shiraishi, A., Seonhee, J., and Nakata, T., Stereoselective syntheses of cytoxazone, a novel cytokine modulator, and its stereoisomers. *Tetrahedron Lett.*, **40**, 4203–4206 (1999).
4) Seki, M., and Mori, K., Synthesis of (−)-cytoxazone, a novel modulator isolated from *Streptomyces* sp. *Eur. J. Org. Chem.*, 2965–2967 (1999).
5) Park, J. N., Ko, S. Y., and Koh, H. Y., Synthetic application of the cyclic iminocarbonate rearrangement: enantioselective syntheses of chloamphenicol and 4-epi-cytoxazone. *Tetrahedron Lett.*, **41**, 5553–5556 (2000).
6) Madhan, A., Kumar, A. R., and Rao, B. V., Stereoselective synthesis of (−)-cytoxazone. *Tetrahedron: Asymmetry*, **12**, 2009–2011 (2001).
7) Hamersak, Z., Ljubovic, E., Mercep, M., Mesic, M., and Sunjic, V., Chemoenzymatic synthesis of all four cytoxazone stereoisomers. *Synthesis*, 1989–1992 (2001).
8) Carda, M., González, F., Sánchez, R., and Marco, J. A., Stereoselective synthesis of (−)-cytoxazone. *Tetrahedron: Asymmetry*, **13**, 1005–1010 (2002).
9) Madhusudhan, G., Reddy, G. O., Ramanatham, J., and Dubey, P. K., Stereoselective synthesis of *anti*-2-oxazolidinones by Ph_3P–CCl_4–Et_3N mediated S_N2 cyclization of *N*-Boc-*β*-amino alcohols. *Tetrahedron Lett.*, **44**, 6323–6325 (2003).
10) Carter, P. H., LaPorte, J. R., Scherle, P. A., and Decicco, C. P., A new synthesis of cytoxazone and its diastereomers provides key initial SAR information. *Bioorg. Med. Chem. Lett.*, **13**, 1237–1239 (2003).
11) Miyata, O., Koizumi, T., Asai, H., Iba, R., and Naito, T., Imino 1,2-Wittig rearrangement of hydroximates and its application to synthesis of cytoxazone. *Tetrahedron*, **60**, 3893–3914 (2004).
12) Milicevic, S., Matovic, R., and Saicic, R. N., Stereoselective synthesis of (−)-cytoxazone and (+)-epi-cytoxazone. *Tetrahedron Lett.*, **45**, 955–957 (2004).
13) Davies, S. G., Hughes, D. G., Nicholson, R. L., Smith, A. D., and Wright, A. J., Asymmetric synthesis of (4*R*,5*R*)-cytoxazone and (4*R*,5*S*)-epi-cytoxazone. *Org. Biomol. Chem.*, **2**, 1549–1553 (2004).
14) Payne, G. B., Deming, P. H., and Williams, P. H., Reactions of hydrogen peroxide. VII. Alkali-catalyzed epoxidation and oxidation using a nitrile as co-reactant. *J. Org. Chem.*, **26**, 659–663 (1961).
15) Chini, M., Crotti, P., and Macchia, F., Efficient metal salt catalyzed azidolysis of epoxides with sodium azide in acetonitrile. *Tetrahedron Lett.*, **31**, 5641–5644 (1990).
16) Knouzi, N., Vaultier, M., and Carrie, R., Reduction d'azides par la triphenylphosphine en presence d'eau: une methode generale et chimioselective d'acces aux amines primaires. *Bull. Soc. Chim. Fr.*, 815–819 (1985).
17) Knölker, H.-J., and Braxmeier, T., Isocyanates Part 5. Synthesis of chiral oxazolidin-2-ones and imidazolidin-2-ones *via* DMAP-catalyzed isocyanation of amines with di-*tert*-butyl dicarbonate. *Tetrahedron Lett.*, **39**, 9407–9410 (1998).
18) Miyahara, M., Nakadate, M., and Kamiya, S., Cyclization of (2-hydroxyethyl)urea derivatives to give 3-nitroso-2-oxazolidinones under usual nitrosation conditions. *Chem. Pharm. Bull.*, **33**, 497–502 (1985).
19) Suzuki, M., Yamazaki, T., Ohta, H., Shima, K., Ohi, K., Nishiyama, S., and Sugai, T., *N*-Carbamylamino alcohols as the precursors of oxazolidinones *via* nitrosation-deamination reaction. *Synlett*, 189–192 (2000).
20) Legters, J., Thijs, L., and Zwanenburg, B., A convenient synthesis of optically Active 1H-aziridine-2-carboxylic acids (esters). *Tetrahedron Lett.*, **30**, 4881–4884 (1989).