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Synthesis of (–)-Cytoxazone, a Novel Cytokine Modulator Isolated from *Streptomyces* sp.

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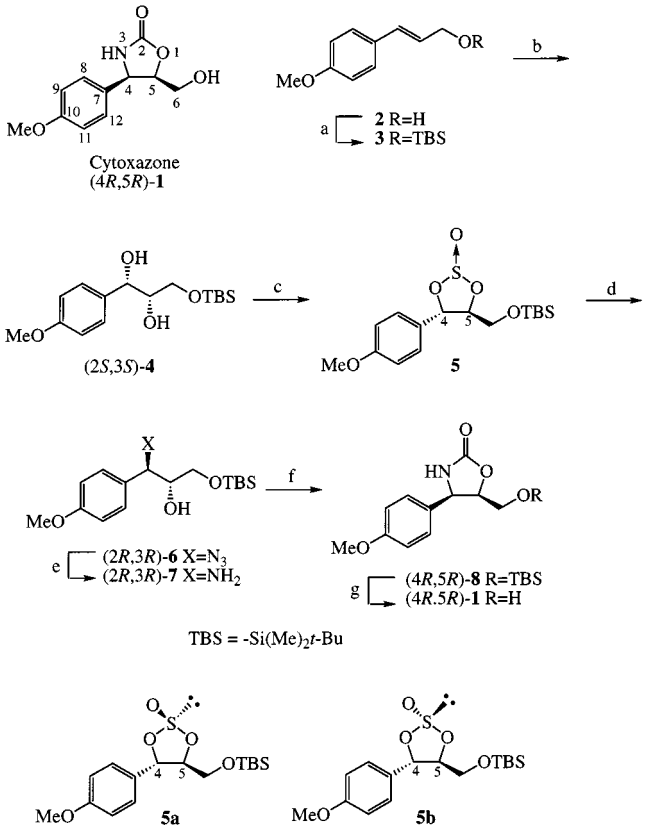
Cytoxazone [(4*R*,5*R*)-(–)-5-hydroxymethyl-4-(4-methoxyphenyl)-2-oxazolidinone, **1**], a new immunosuppressant, was synthesized by starting from *p*-methoxycinnamyl alcohol (**2**) employing the Sharpless asymmetric dihydroxylation as the key reaction in 26% overall yield (7 steps).

In 1998 Osada and co-workers isolated 140 mg of a novel cytokine modulator from 18 L of the culture broth of *Streptomyces* RK95−31 isolated from a soil sample in Hiroshima Prefecture.[1] That new immunosuppressant was named cytoxazone and its structure **1** was elucidated on the basis of its NMR, CD and X-ray analysis.[1][2] Cytoxazone inhibits the cytokine production via the signaling pathway of Th2 cells, but not Th1 cells. We envisaged that **1** with a 2-oxazolidinone ring might readily be synthesized from *p*-methoxycinnamyl alcohol (**2**) by employing the Sharpless asymmetric dihydroxylation as the key reaction.[3]

Scheme 1 summarizes our synthesis of cytoxazone (**1**). The commercially available **2** was converted into the corresponding *tert*-butyldimethylsilyl (TBS) ether **3**. This silyloxy olefin **3** was dihydroxylated by treatment with an osmium tetroxide based oxidant in the presence of 1,4-bis(9-*O*-dihydroquinidine)phthalazine [$(DHQD)_2PHAL$] under the so-called AD-mix-α® conditions[3] to give (2*S*,3*S*)-**4** (99.6% *ee*) in 99% yield. Conversion of such a 1,2-glycol as **4** to an azido alcohol **6** was reported to be feasible via a cyclic sulfate[4] or a sulfite like **5**.[5−7] In the present case, the cyclic sulfite **5** is thought to be reactive enough to give **6**, because C-4 of **5** is the activated benzylic position to which the nucleophilic attack of the azide anion is expected. Accordingly, **4** was treated with thionyl chloride and triethylamine to afford **5**, which was a mixture of two diastereomers **5a** and **5b** as revealed by its ^{1}H-NMR spectrum. Cleavage of the cyclic sulfite **5** was executed with lithium azide in hot *N*,*N*-dimethylformamide (DMF).[5][6] The resulting azide **6** was fortunately a single stereoisomer as evidenced by its ^{1}H-NMR spectrum (see Experimental Section). This was presumably caused by rapid S_N2-type ring cleavage at C-4 with the azide anion. The azide **6** was then hydrogenated with ammonium formate and palladium/charcoal to give **7**.

Construction of the 2-oxazolidinone ring was achieved as usual[8] by treatment of **7** with diethyl carbonate in the presence of potassium carbonate to furnish **8**. Removal of the TBS protective group of **8** with tetrabutylammonium fluoride (TBAF) yielded (4*R*,5*R*)-(−)-cytoxazone (**1**), whose spectral properties are identical with those of the natural product.[1][2] The overall yield of **1** was 26% based on **2** (7 steps).



Scheme 1. Synthesis of (−)-cytoxazone (**1**): reagents: (a) TBSCl, imidazole, DMF (97%); (b) AD mix-α® [$(DHQD)_2PHAL$, $K_2O_sO_2(OH)_4$, $K_3Fe(CN)_6$, K_2CO_3], $MeSO_2NH_2$, *t*BuOH/H_2O (99%); (c) $SOCl_2$, Et_3N, CH_2Cl_2 (88%); (d) LiN_3, DMF, 100°C, then H_2O at 0°C (61%); (e) HCO_2NH_4, Pd/C, MeOH, 50°C (87%); (f) $CO(OEt)_2$, K_2CO_3 (66%); (g) TBAF, THF (89%)

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Experimental Section

General: M.p.: Yanaco MP-S3; uncorrected values. − IR: Jasco A-102 and Perkin−Elmer 1640. − ^{1}H NMR: Jeol JNM-LA300 (300 MHz) and Jeol JNM-LA500 (500 MHz) ($CHCl_3$ at δ = 7.26 or CD_3OD at δ = 3.30 or DMSO at δ = 2.49 as an internal standard). − ^{13}C NMR: Jeol JNM-LA500 (125 MHz) (DMSO at δ = 39.75 as an internal standard). − Optical rotation: Jasco DIP-1000. − MS: Jeol JMS-SX102A. − CD: Jasco J-725 spectropolarimater. − Column chromatography: Merck Kieselgel 60 Art. 1.07734. − TLC: 0.25-mm Merck silica gel plates (60F−254).

(*E*)-1-(*tert*-Butyldimethylsilyloxy)-3-(4-methoxyphenyl)-2-propene (3): To a stirred solution of *p*-methoxycinnamyl alcohol (**2**; 2.49 g, 15.2 mmol) in dry DMF (25 mL), imidazole (1.55 g, 22.7 mmol) and TBSCl (2.75 g, 18.2 mmol) were added portionwise. This mixture was stirred at room temperature for 12 h and then quenched with MeOH. It was poured into water and extracted with diethyl ether. The organic phase was washed with water, a satd. sodium hydrogen carbonate solution and brine, dried with $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel (40 g; hexane/ethyl acetate, 300:1) to give 4.12 g (97%) of **3** as a white crystalline solid; m.p. 32−34°C. − IR (KBr): $\tilde{\nu}_{max}$ = 2955 cm^{-1} (m), 1605 (m), 1510 (s), 1240 (s), 1120 (m), 840 (s), 775 (s). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.11 [s, 6 H, $Si(Me)_2$], 0.94 [s, 9 H, $SiC(Me)_3$], 3.81 (s, 3 H, OMe), 4.33 (d, *J* = 5.3 Hz, 2 H, 1-H), 6.14 (dt, *J* = 16, 5.3 Hz, 1 H, 2-H), 6.52 (d, *J* = 16 Hz, 1 H, 3-H), 6.85 (d, *J* = 8.8 Hz, 2 H, 6,8-H), 7.31 (d, *J* = 8.8 Hz, 2 H, 5,9-H). − $C_{16}H_{26}O_2Si$ (278.5): calcd. C 69.01, H 9.41; found C 69.14, H 9.62.

(2*S*,3*S*)-1-(*tert*-Butyldimethylsilyloxy)-3-(4-methoxyphenyl)-2,3-propanediol (4): To a mixture of water (15 mL) and 2-methyl-2-propanol (10 mL) was added AD mix-α® (2.51 g) and methanesulfonamide (171 mg, 1.80 mmol) at room temperature. The mixture was stirred for 10 min, and then cooled to 0°C. A solution of **3** (500 mg, 1.80 mmol) in 2-methyl-2-propanol (5 mL) was added and the mixture was stirred for 7 h at 0°C. The reaction was quenched by the addition of sodium sulfite heptahydrate (2.40 g, 9.52 mmol) at 0°C. Then the mixture was warmed to room temperature, stirred for 30 min, and extracted with ethyl acetate. The organic phase was washed with aqueous 2 M KOH, dried with $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel (6 g; hexane/ethyl acetate, 8:1) to give 554 mg (99%) of **4** as a colorless oil; n_D^{25} = 1.5012. − $[\alpha]_D^{19}$ = +10.7 (*c* = 1.00, $CHCl_3$). − IR (film): $\tilde{\nu}_{max}$ = 3420 cm^{-1} (s, O−H), 2955 (m), 1615 (m), 1515 (s), 1250 (s, Si−Me), 1120 (m), 1040 (m), 835 (s), 780 (s). − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.06 [s, 6 H, $Si(Me)_2$], 0.91 [s, 9 H, $SiC(Me)_3$], 2.71 (d, *J* = 6.0 Hz, 1 H, 2-OH), 3.11 (d, *J* = 1.8 Hz, 1 H, 3-OH), 3.52 (dd, *J* = 10.3, 4.9 Hz, 1 H, 1-H), 3.61 (dd, *J* = 10.3, 3.4 Hz, 1 H, 1-H), 3.65−3.70 (m, 1 H, 2-H), 3.81 (s, 3 H, OMe), 4.65 (dd, *J* = 6.0, 1.8 Hz, 1 H, 3-H), 6.89 (d, *J* = 8.3 Hz, 2 H, 6,8-H), 7.29 (d, *J* = 8.3 Hz, 2 H, 5,9-H). − $C_{16}H_{28}O_4Si$ (312.5): calcd. C 61.50, H 9.03; found C 61.23, H 9.18. − HPLC analysis [column: Chiralcel-OD®, 4.6 mm × 25 cm; solvent: hexane/*i*PrOH, 60:1; flow rate: 0.5 mL/min; detection: 254 nm], t_R = 49.4 min [(2*R*,3*R*)-**4**, 0.20%], 56.3 [(2*S*,3*S*)-**4**, 99.8%]. The enantiomeric purity of (2*S*,3*S*)-**4** was 99.6% *ee*.

(4*S*,5*S*)-5-(*tert*-Butyldimethylsilyloxymethyl)-4-(4-methoxyphenyl)-1,3,2-dioxathiolane 2-Oxide (5): To a solution of **4** (220 mg, 0.70 mmol) and triethylamine (0.43 mL, 3.07 mmol) in CH_2Cl_2 (3 mL) at 0°C was added a solution of thionyl chloride (0.06 mL) in CH_2Cl_2 (0.5 mL). Stirring was continued for 10 min at 0°C. The mixture was diluted with cold diethyl ether, washed with cold water and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (3 g, hexane/ethyl acetate, 150:1) to give 223 mg (88%) of **5** as a pale yellow oil, which was used immediately in the next reaction. − IR (film): $\tilde{\nu}_{max}$ = 2955 cm^{-1} (s), 1615 (m), 1515 (m), 1255 (s), 1210 (m), 1145 (m), 1035 (w), 950 (m), 835 (s). − **5a:** ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.09 [s, 6 H, $Si(Me)_2$], 0.88 [s, 9 H, $SiC(Me)_3$], 3.83 (s, 3 H, OMe), 3.84−4.04 (m, 2 H, 6-H), 4.39 (dt, *J* = 8.1, 5.2 Hz, 1 H, 5-H), 5.77 (d, *J* = 8.1 Hz, 1 H, 5-H), 6.95 (d, *J* = 9.0 Hz, 2 H, 9,11-H), 7.42 (d, *J* = 9.0 Hz, 2 H, 8,12-H). − **5b**: δ = 0.09 [s, 6 H, $Si(Me)_2$], 0.88 [s, 9 H, $SiC(Me)_3$], 3.83 (s, 3 H, OMe), 3.84−4.04 (m, 2 H, 6-H), 4.75 (dt, *J* = 9.1, 6.3 Hz, 1 H, 4-H), 5.35 (d, *J* = 9.1 Hz, 1 H, 5-H), 6.94 (d, *J* = 9.0 Hz, 2 H, 9,11-H), 7.32 (d, *J* = 9.0 Hz, 2 H, 8,12-H).

(2*R*,3*R*)-3-Azido-1-(*tert*-butyldimethylsilyloxy)-3-(4-methoxyphenyl)-2-propanol (6): A mixture of cyclic sulfite **5** (220 mg, 0.61 mmol) and LiN_3 (54 mg, 1.1 mmol) in dry DMF (3 mL) was stirred under Ar for 2 h at 100°C. After this, it was ice-cooled and quenched with water, and extracted with ethyl acetate. The organic phase was washed with water and brine, dried with $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel (15 g; hexane/ethyl acetate, 20:1) to give 127 mg (61%) of **6** as a colorless oil; n_D^{25} = 1.5041. − $[\alpha]_D^{18}$ = −88.1 (*c* = 0.99, $CHCl_3$). − IR (film): $\tilde{\nu}_{max}$ = 3470 cm^{-1} (m, O−H), 2955 (w), 2105 (s, azido), 1610 (s), 1515 (s), 1250 (s), 1115 (m), 835 (s). − ^{1}H NMR (300 MHz, CD_3OD): δ = 0.05 (s, 3 H, Si−Me), 0.06 (s, 3 H, Si−Me), 0.92 [s, 9 H, $SiC(Me)_3$], 3. 53 (dd, *J* = 10.5, 5.7 Hz, 1 H, 1-H), 3. 66 (dd, *J* = 10.5, 4.8 Hz, 1 H, 1-H), 3.75−3.85 (m, 1 H, 2-H), 3.80 (s, 3 H, OMe), 4.55 (d, *J* = 6.4 Hz, 1 H, 3-H), 6.93 (d, *J* = 8.8 Hz, 2 H, 6,8-H), 7.31 (d, *J* = 8.8 Hz, 2 H, 5,9-H). − $C_{16}H_{27}N_3O_3Si$ (337.5): calcd. C 56.94, H 8.06, N 12.45; found C 57.11, H 8.20, N 12.53. − A regioisomer (N_3 at C-2) of **6** was also obtained (25 mg, 12%), whose structure was assigned by its ^{1}H-NMR analysis. − ^{1}H NMR (300 MHz, $CDCl_3$): δ = −0.29 (s, 3 H, Si−Me), −0.02 (s, 3 H, Si−Me), 0.80 [s, 9 H, $SiC(Me)_3$], 3. 61 (ddd, *J* = 7.5, 5.0, 4.2 Hz, 1 H, 2-H), 3.70−3.85 (m, 2 H, 1-H), 3.82 (s, 3 H, OMe), 4.59 (d, *J* = 7.5 Hz, 1 H, 3-H), 6.90 (d, *J* = 8.8 Hz, 2 H, 6,8-H), 7.25 (d, *J* = 8.8 Hz, 2 H, 5,9-H).

(2*R*,3*R*)-3-Amino-1-(*tert*-butyldimethylsilyloxy)-3-(4-methoxyphenyl)-2-propanol (7): To a stirred solution of **6** (337 mg, 1.00 mmol) and 10% Pd/C (70 mg) in MeOH (6 mL) was added a solution of ammonium formate (252 mg, 4.00 mmol) in MeOH (3 mL). After having been stirred at 50°C for 4 h, the mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (5 g; $CHCl_3$/MeOH, 50:1) to give 272 mg (87%) of **7** as a white solid, which was used without further purification. − IR (film): $\tilde{\nu}_{max}$ = 3385 cm^{-1} (m, O−H), 2930 (w), 1615 (m), 1515 (m), 1255 (s), 1120 (m), 1095 (m), 835 (s). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.04 [s, 6 H, $Si(Me)_2$], 0.89 [s, 9 H, $SiC(Me)_3$], 1.71 (br., 2 H, NH_2), 3.53 (dd, *J* = 10.2, 4.6 Hz, 1 H, 1-H), 3.59 (dd, *J* = 10.2, 5.9 Hz, 1 H, 1-H), 3.76 (ddd, *J* = 5.9, 5.4, 4.6 Hz, 1 H, 2-H), 3.81 (s, 3 H, OMe), 4.07 (d, *J* = 5.4 Hz, 1 H, 3-H), 6.88 (d, *J* = 8.8 Hz, 2 H, 6,8-H), 7.28 (d, *J* = 8.8 Hz, 2 H, 5,9-H).

(4*R*,5*R*)-5-(*tert*-Butyldimethylsilyloxymethyl)-4-(4-methoxyphenyl)-2-oxazolidinone (8): A mixture of **7** (160 mg, 0.51 mmol), diethyl carbonate (0.14 mL, 1.20 mmol), and anhydrous potassium carbonate (1.1 mg, 8.5 μmol) was heated at 120°C for 2 h with stirring. The mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (2 g; hexane/ethyl acetate, 5:1) to give 115 mg (66%) of **8** as a white solid. The solid **8** was recrystallized from hexane/ethyl acetate (10:1) to give colorless needles; m.p. 71−73°C.

− $[\alpha]_D^{19}$ = −24.5 (*c* = 0.98, $CHCl_3$). − IR (KBr): $\tilde{\nu}_{max}$ = 3280 cm^{-1} (m, N−H), 2955 (w), 1745 (s, C=O), 1615 (m), 1515 (s), 1250 (s), 1105 (m), 835 (s). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = −0.14 (s, 3 H, Si−Me), −0.08 (s, 3 H, Si−Me), 0.80 [s, 9 H, $SiC(Me)_3$], 3.26 (dd, *J* = 11.0, 5.9 Hz, 1 H, 6-H), 3.46 (dd, *J* = 11.0, 5.9 Hz, 1 H, 6-H), 3.81 (s, 3 H, OMe), 4.80 (ddd, *J* = 8.3, 5.9, 5.9 Hz, 1 H, 5-H), 4.92 (d, *J* = 8.3 Hz, 1 H, 4-H), 5.86 (s, 1 H, N−H), 6.88 (d, *J* = 8.8 Hz, 2 H, 9,11-H), 7.17 (d, *J* = 8.8 Hz, 2 H, 8,12-H). − $C_{17}H_{27}NO_4Si$ (337.5): calcd. C 60.50, H 8.06, N 4.15; found C 60.33, H 8.15, N 4.01.

Cytoxazone [(4*R*,5*R*)-5-(Hydroxymethyl)-4-(4-methoxyphenyl)-2-oxazolidinone, 1]: To a solution of **8** (84 mg, 0.25 mmol) in THF (1 mL), TBAF (1.00 M in THF; 0.30 mL, 0.30 mmol) was added dropwise at room temperature. Stirring was continued for 10 min. After this, it was quenched with water and extracted with ethyl acetate. The organic phase was washed with brine, dried with $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel (1 g, $CHCl_3$/MeOH, 70:1) to give 49 mg (89%) of **1** as a white crystalline solid. The crystalline **1** was recrystallized from MeOH/H_2O (3:7) to give colorless prisms; m.p. 117−119°C [ref.[1] 118−121°C (from MeOH/H_2O, 3:7)]. − $[\alpha]_D^{19}$ = −70.9 (*c* = 0.098 MeOH) {ref.[1] $[\alpha]_D^{23}$ = −71 (*c* = 0.1 MeOH)}. − CD (*c* = 0.098, MeOH): Δε (λ) = +0.25 (226 nm). − IR (KBr): $\tilde{\nu}_{max}$ = 3480 cm^{-1} (m, O−H), 3255 (m, N−H), 2955 (m), 1715 (s, C=O) 1615 (w), 1515 (m), 1405 (m), 1255 (s, C−O), 1050 (s), 995 (m). − ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 2.94−2.98 (m, 2 H, 6-H), 3.74 (s, 3 H, O−Me), 4.68 (ddd, *J* = 8.3, 7.8, 4.4 Hz, 1 H, 5-H), 4.80 (t, *J* = 5.2 Hz, O−H), 4.89 (d, *J* = 8.3 Hz, 4-H), 6.92 (d, *J* = 8.3 Hz, 2 H, 9,11-H), 7.14 (d, *J* = 8.3 Hz, 2 H, 8,12-H), 8.04 (s, 1 H, N−H). − ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 55.1 (O−Me), 56.2 (4-C), 61.0 (6-C), 80.0 (5-C), 113.7 (9,11-C), 128.0 (8,12-C), 129.3 (7-C), 158.7 (2-C), 159.0 (10-C). − HRFAB MS ($C_{11}H_{13}NO_4$): calcd. 224.0923; found 224.0928. − $C_{11}H_{13}NO_4$ (223.2): calcd. C 59.19, H 5.87, N 6.27; found C 58.98, H 5.90, N 6.18.

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