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A Rapid and Highly Diastereoselective Synthesis of Enantiomerically Pure (4R,5S)- and (4S,5S)- isocytoxazone

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Abstract: A three step protocol for the highly diastereoselective (>98%) synthesis of both (4R,5S)- and (4S,5S)-iso-cytoxazone from (D)- or (L)-tyrosine is reported. The diastereoselection was confirmed by X-ray crystallography. This synthesis is currently the highest yielding approach towards these enantiomerically pure biologically active oxazolidinones.

Key words: Diastereoselective, Cytoxazone, Tyrosine, Oxazolidinone, Oxidation.

During the course of screening for chemical immunomodulators from microbial metabolites, Osada found that an actinomycete strain (RK95-31) produced cytoxazone 1, an oxazolidinone that interferes with cytokine IL-4, IL-10 and IgG production. Several groups have synthesized (−)-cytoxazone 1 and (+)-epi-cytoxazone 2, and Šunic has described the racemic syntheses of all of the stereoisomers and cogeners of iso-cytoxazone 3; enantiomerically pure samples were isolated by preparative HPLC. A theoretical study on the absolute configurations of 1 and 3 has been carried out by Berova, as they have extensive X-ray crystallographic studies.

Rozwadowska and Tomczak have recently reported the synthesis of (4S,5S)-(-)-iso-cytoxazone 3, but the synthesis required seven synthetic steps, the last of which afforded a mixture of regioisomers in an overall yield of 8.1%. Prompted by these studies after reporting several routes to 1,3 amino diols we herein report our efforts in this area, utilizing a rapid highly diastereoselective three-step process from Boc-protected D- or L-tyrosine.

![Figure 1 The cytoxazones](image)

Our original strategy towards (4S,5S)-iso-cytoxazone was similar to that of Rozwadowska, using diazotization as a key reaction step (Scheme 1). Hydrogenation of the nitro group in the formate-protected amino diol 4 to give the corresponding amino compound 5 proved highly successful (99% yield). The subsequent diazotization reaction, however, was extremely poor, giving at best 15% yield in our hands (the reaction has previously been reported to give yields of up to 35%). Further manipulation of product 6 afforded the 4-hydroxyphenyl-1,3-dioxide 7. Methylation, after some experimentation, was achieved with caesium carbonate and dimethyl sulfate, producing the 4-methoxyformate 8, but separation from by-products brought through from the initial diazotization reaction proved difficult (Scheme 2). Before this approach was abandoned, an interesting reaction reported by Quin and Macdiarmid was attempted, whereby direct conversion from an amine group to a methoxy group is possible using iso-amyl nitrite in methanol (Scheme 3). Unfortunately, when using our substrate, this led to a complex mixture of products, including loss of the formate protecting group.

![Scheme 1 Retrosynthetic route towards (4S,5S)-iso-isocytoxazone](image)

![Scheme 2 Initial synthesis of the p-methoxy formate 8.](image)

**Reagents and Conditions**: i: H₂/Pd, ethanol, r.t. 24 h, 99%; ii: a) NaNO₂, H₂SO₄, b) pH 6, Δ, 15%; iii, 2,2-DMP, acetone, CSA, r.t., 4 h, 72%; iv: Cs₂CO₃, Me₃SO₄, CH₂Cl₂, 48 h, 72%.

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Reagents and Conditions: i: a) H₂SO₄, MeOH, (CH₃)₂CHCH₂CH₂ONO, 0 °C, 3 h; b) Δ, 1 h.

Scheme 3 Attempted direct incorporation of the methoxy group using Quin and Macdiarmid’s method.

We next reasoned that (4S,5S)-iso-cytoxazone 3 could be prepared from commercially available Boc-(D)-tyrosine (Scheme 4).

Scheme 4 A revised retrosynthetic route towards (4S,5S)-iso-cytoxazone from Boc-D-tyrosine

We initially aimed to prepare the enantiomer of 3 (4R,5R)-iso-cytoxazone ent-3 from the cheaper Boc-(L)-tyrosine (Schemes 5, 6 and 7). Methylation of the acid and phenol components within Boc-(L)-tyrosine with potassium hydroxide and iodomethane afforded 10, the required precursor to 11.

Scheme 5 Formation of Boc-protected, dimethylated (L)-tyrosine 10.

Reagents and Conditions: i: Mel (2.2 eq.), KOH (2.2 eq.), DMF, 0 °C-r.t. 3.5 h, 74 %.

Following the work of Ohfune, the benzylic position of 10 was oxidized with potassium persulfate (K₂S₂O₈) and catalytic copper sulfate to form the oxazolidinone 11 in a highly diastereoselective manner (diastereomeric ratio ≥ 98 % R at C3) (Scheme 6).

Scheme 6 Ohfune’s cyclic carbamate formation.

The authors suggest that this high selectivity in cyclic carbamate formation arises because the reaction proceeds via the more stable conformer of benzyl cation intermediate 10b. The conformer 10a is more strained than 10b due to steric interaction between the ester group and the ortho hydrogen atom. Intramolecular trapping of the cation by a carbonyl oxygen and subsequent release of the tert-butyl cation, which is believed to be more stable than the benzylic cation of 10, is thought to be a driving force for the reaction. This was supported by Ohfune’s observation that only poor yields were obtained from compounds containing other amino protecting groups, such as the Cbz group. Confirmation of the stereoselectivity was achieved from the X-ray crystal structure of compound 11, as shown in Figure 2.

Figure 2 X-ray crystal structure of 11

Although this reaction is highly stereoselective, some problems were encountered during the synthesis. Yields tended to vary on scale-up of the reaction. Initially, the reaction on 4 mmol of substrate afforded 50% of the desired product (a good yield given the reported yield of 55%), but on increasing the scale to 26 mmol a drop in yield to 40% was observed. Optimum conditions were found when carrying the reaction out on a 16 mmol scale - a 52 % yield of product was obtained. Attempts to drive the reaction to completion proved fruitless. Generally, increased reaction times and temperatures decreased the overall yield due to generation of higher levels of the side product 4-methoxy benzaldehyde (a product of over-oxidation). Milder reaction conditions resulted in no product formation.

In order to afford the desired (4R,5R)-iso-cytoxazone ent-3 the carbamate 11 was then reduced; this was initially achieved with lithium aluminium hydride, but sodium borohydride provided a superior yield (91 % compared to 77 %), probably due to the ease of work-up associated with the sodium borohydride reactions, (Scheme 7). (4S,5S)-Iso-cytoxazone 3 was prepared by repeating this optimized sequence using (D)-tyrosine as the starting material, in an overall yield of 33%.
Reagents and Conditions: i: NaBH₄, EtOH, 0 °C to r.t. 45 min, 91%.

Scheme 1 Formation of (4R,5R)-iso-cytaxzone ent-3.

In conclusion we have reported here the highly diasteroselective synthesis of (4R,5R)-iso-cytaxzone ent-3 and (4S,5S)-iso-cytaxzone 3 in just three synthetic steps from (D)- or (L)- Boc-tyrosine, and confirmation of the stereoselection by X-ray crystallography. This is the shortest and most high yielding approach (35% overall yield compared to 8.1% overall yield reported previously) currently known for this class of biologically active oxazolidinones.

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References
(9) O-Methyl-N-tert-butoxycarbonyl-L-tyrosine methyl ester 10: A solution of N-tert-butoxycarbonyl-L-tyrosine (8.00 g, 28.5 mmol) in dimethylformamide (80 ml) was cooled using an ice bath, treated with freshly ground potassium hydroxide (1.72 g, 31.3 mmol), and a cooled solution of iodomethane (1.95 ml, 31.3 mmol) in dimethylformamide (20 ml) added dropwise over 5 min. The mixture was stirred at room temperature for 30 min, cooled using an ice bath, and additional potassium hydroxide (1.72 g, 31.3 mmol) and a cooled solution of iodomethane (1.95 ml, 31.3 mmol) in dimethylformamide (20 ml) added. The mixture was stirred for 3 h, poured onto ice (150 ml), and extracted with ethyl acetate (3 x 75 ml). The organic layers were washed with water (3 x 50 ml), brine (2 x 50 ml) and dried (MgSO₄). The solvent was removed under reduced pressure to afford a colourless oil. Crystallization was achieved from ethyl acetate/light petroleum, to give 10 as colourless crystals (6.5 g, 74%); m.p. 52-53 °C; [α]D +58.9 (c 1.2, CHCl₃), lit.
(10) Crystal data for H₂C₂H₄NO₂: M = 251.23, monoclinic, α = 9.0384(10) Å, β = 60.2297(10) Å, γ = 28.6973(9) °, V = 1562.01(12) Å3, space group P2₁/a, Z = 2, μ = 0.109 mm⁻¹, ρcalcd = 1.386 Mg cm⁻³, 5035 data (1542 unique, R = 0.0157) collected on an Apex II diffractometer at 150K. Solved by direct methods and refined by full-matrix least-squares on F². R(F > 2σ(F)) = 0.0298 and wR(F) (all data) = 0.0745. Goodness of fit on F² = 1.079. CCDC No. 804966.
(11) (4R,5S)-iso-Cytaxzone ent-3: Methyl (4R,5S)-5-[4-methoxyphenyl]-1,3-oxazolidin-2-one 4-carboxylate 11 (2.20 g, 8.8 mmol) was dissolved in ethanol (25 ml) and the solution cooled using an ice bath. A solution of NaBH₄ (0.70 g, 19.3 mmol) in ethanol (8 ml) was added dropwise with stirring. After the addition was complete the ice bath was removed and the mixture stirred for 45 min. The mixture was cooled to 0 °C and conc. HCl (1.5 ml) added, followed by water (15 ml). The ethanol was removed under reduced pressure and the remaining aqueous solution extracted with ethyl acetate (3 x 50 ml). The combined organic solutions were dried (MgSO₄) and concentrated under reduced pressure to give a dark yellow oil. Column chromatography, eluting with ethyl acetate/light petroleum (1:10-1:1), afforded a colourless solid, which was recrystallized from ethyl acetate/light petroleum to give 11 as a colourless crystalline solid (2.10g, 52%); m.p. 94-96 °C; [α]D +83.5 (c 1.15, CHCl₃), νmax (film) /cm⁻¹ 3316, 2956, 2362, 2337, 1762, 1613, 1515, 1382, 1250, 1224, 1026, 834, 763; δH (400 MHz; CDCl₃) δH (313), 3.83 (3 H, s), 4.51 (1 H, q, J 5.7 Hz), 5.00 (1 H, d, J 6.7 Hz), 6.82 (2 H, d, J 8.7 Hz), 7.03 (2 H, d, J 8.7 Hz); δC (100 MHz; CDCl₃) 28.3, 37.6, 52.7, 54.7, 55.3, 79.9, 114.1, 128.1, 130.3, 151.5, 158.8, 172.4; m/z 309.1578; C₆H₆NO₂ requires 309.1576.
(13) Synthetic Methods: (4R,5S)-5-[4-methoxynaphthalen-1-yl]-3-oxazolidin-2-one 4-carboxylate 11 (2.20 g, 8.8 mmol) was dissolved in ethanol (25 ml) and the solution cooled using an ice bath. A solution of NaBH₄ (0.70 g, 19.3 mmol) in ethanol (8 ml) was added dropwise with stirring. After the addition was complete the ice bath was removed and the mixture stirred for 45 min. The mixture was cooled to 0 °C and conc. HCl (1.5 ml) added, followed by water (15 ml). The ethanol was removed under reduced pressure and the remaining aqueous solution extracted with ethyl acetate (3 x 50 ml). The combined organic solutions were dried (MgSO₄) and the solvents removed to afford an off-
white solid, which was recrystallized from ethyl acetate/light petroleum to give (4R,5R)-iso-cytoxzone ent-3 as a colourless crystalline solid (1.75 g, 90%); m.p. 140-142 °C; [α]D20 +74.8 (c 1.08, CH3COCH3, lit.); [α]D20 +70 c 0.4, MeOH); ʋmax (nujol) /cm-1 3239, 1725, 1614, 1514, 1459, 1376, 1251, 1174, 1062, 1016, 828; δH (250 MHz; acetone-d6) 3.71-3.87 (3 H, m), 3.86 (3 H, s), 5.35 (1 H, d, J 5.3 Hz), 7.01 (2 H, d, J 8.6 Hz), 7.41 (2 H, d, J 8.6 Hz); δC (100 MHz; CDCl3) 56.0, 63.1, 64.2, 80.4, 115.4, 128.7, 133.2, 159.6, 161.3; m/z 223.0842; C11H13NO4 (M+) requires 223.0845.

(14) See the supplementary material for the experimental data.
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