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Efficient synthesis of (*R*)-ochratoxin alpha, the key precursor to the mycotoxin ochratoxin A

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ABSTRACT

Two new routes for the synthesis of enantiomerically pure ochratoxin alpha ((3*R*)-OTα) are presented, which is the key intermediate for the synthesis of ochratoxin A (OTA) by coupling reaction with the amino acid L-phenylalanine. The key step of both routes is the one pot directed *ortho*-metalation/alkylation/lactonization of unprotected and suitably functionalized aromatic carboxylic acids, using lithium tetramethylpiperidide (LTMP) and (*R*)-propylene oxide.

Introduction

Ochratoxin A (OTA) **1** is a metabolite of some species of the fungal genera *Aspergillus* and *Penicillium* and is known as a toxic contaminant in cereals, coffee, wine, beer, milk, and seasonings.[1] OTA **1** specifically is supposed to be the cause of the so-called 'Balkan endemic nephropathy',[2] a disease resulting in failure of kidney function and kidney cancer. Since then the European Union and several other countries established limits for OTA **1** in some foods (cereals 5 μg/kg, cereal products, 3 μg/kg, and raisins 10 μg/kg).[3] The control of these limits requires the availability of pure OTA **1** as a reference standard.

Ochratoxin α (OTα) **2** is also found as a metabolite derived from OTA **1** in mammals,[4] and, thus also in meat and milk.[5]

Racemic syntheses of (*R*/*S*)-OTα have been published, beginning with Steyn and Holzapfel[6] in 1967 (10% yield in nine steps), followed by Roberts and Woollven[7] (0.62% yield in eight steps), Kraus et al.,[8] (20% yield in four steps), Snieckus et al.[9] (6% yield in five steps), Gabriele et al.[10] (23% yield in five steps), and finally Cramer et al.[11] (8% yield in five steps). All racemic syntheses lead to the mixture of (*R*/*S*)-OTα and require enantiomeric separation in this step or posterior separation of the two OTA diastereoisomers formed by coupling of (*R*/*S*)-OTα with L-phenylalanine. The diastereoisomeric separation usually is done on a small scale by high performance liquid chromatography (HPLC) or preparative thin layer chromatography (TLC prep). This additional step leads to a loss of at least 50% of the product due to the presence of the non-natural diastereomer of OTA in equal proportion to the diastereomer of interest, beside the additional costs of the separation process. If enantiomerically pure OTα **2** is needed, an additional step of hydrolysis of isolated (2*S*,3*R*)-OTA **1** is required to obtain (3*R*)-ochratoxin α **2**.

Only one synthetic route to enantiomerically pure (3*R*)-OTα **2** was published by Donner and Gill,[12] starting from (*R*)-propylene oxide **5** with an overall yield of 10% in nine steps. The latter authors used the same synthetic approach developed earlier[13] for the synthesis of the similar benzoisochromane skeleton present in (*R*)-mellein employing Diels–Alder cycloaddition reactions. Other synthesis for (*R*)-mellein presented by Kobayashi and coworkers[14] uses the approach of *ortho*-lithiation of amide protected anisic acid and also alkylation with (*R*)-propylene oxide, thus pointing out that this reagent is the simplest way to introduce the appropriate stereochemistry in such isocoumarin systems.

The goal of the present work was to synthesize enantiomerically pure OTα **2** in a more efficient way than previously reported. OTα **2** obtained according to the present work is useful in itself as a reference substance (primary standard) in analytical chemistry and toxicological research, and also as the final intermediate for obtaining mycotoxin OTA **1** and/or its isomers and also the isotopically labeled analogs[15] for use in analytical chemistry food contamination analysis or in toxicological studies.[16] The synthesis of OTA **1** starting from the enantiomerically pure OTα **2** involves only a coupling reaction with the amino acid L-phenylalanine.

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Results and discussion

The present Letter describes two new synthetic routes for obtaining enantiomerically pure (3*R*)-ochratoxin α **2**, based on inserting the proper stereochemistry from the chiral reagent (*R*)-propylene oxide **5**, nevertheless using different approaches from the previous enantiomeric synthesis and also different from all other racemic syntheses already published. Both routes are based on the directed *ortho*-metalation of unprotected aromatic carboxylic acids containing the appropriate substituent groups for the synthesis of OTα **2** in few steps. The retrosynthesis of **2** is presented in Scheme 1.

The routes described here allow options in choosing aromatic precursors conveniently substituted as starting materials, according to their availability and/or costs, besides using simpler reagents and fewer reaction steps, thus increasing the overall efficiency. Furthermore the routes do not require the step of aromatic chlorination, which is central in most of the previously reported syntheses.

Synthetic route A starts from the commercially available reagent 5-chloro-2-methoxybenzoic acid **3**, and synthetic route B starts from the suitably substituted aromatic precursor 5-chloro-2-methoxybenzene-1,3-dicarboxylic acid **4** which in turn can be easily prepared by simple reactions[17] using options of reagents of better availability or convenience as starting materials. The direct metalation of unprotected aromatic acids was employed as the alternative to the tertiary or secondary benzamide systems, and avoided protecting and deprotecting steps allowing one pot alkylation-cyclization reactions. *ortho*-Metalation studies of disubstituted aromatic rings have demonstrated *ortho* orientation to the carboxylic acid group in *o*-anisic acid[18] when using LTMP or *s*-BuLi/TMEDA, whereas the use of *n*-BuLi/*t*-BuOK changed the orientation toward the *ortho* position of the methoxy group. Also the studies with 3-chlorobenzoic acid[19] showed that the hindered lithium dialkylamide LTMP was effective to promote the *ortho*-metalation at the position mutually adjacent, while LDA was not suitable. Therefore, for the aromatic rings tri-substituted **3** and tetra-substituted isophthalic acid **4**, LTMP was the first choice for the metalation of the mutually *ortho*-position for carboxylic acid and chlorine groups.

The synthetic route A is illustrated in Scheme 2. The unprotected 5-chloro-*o*-anisic acid **3** is submitted to directed *ortho*-metalation reaction with LTMP in position 6 followed by alkylation with (*R*)-propylene oxide **5** to produce lactone **6** by spontaneous cyclization after acidic quench, in a one pot reaction and 53% yield, which turns this approach attractive.

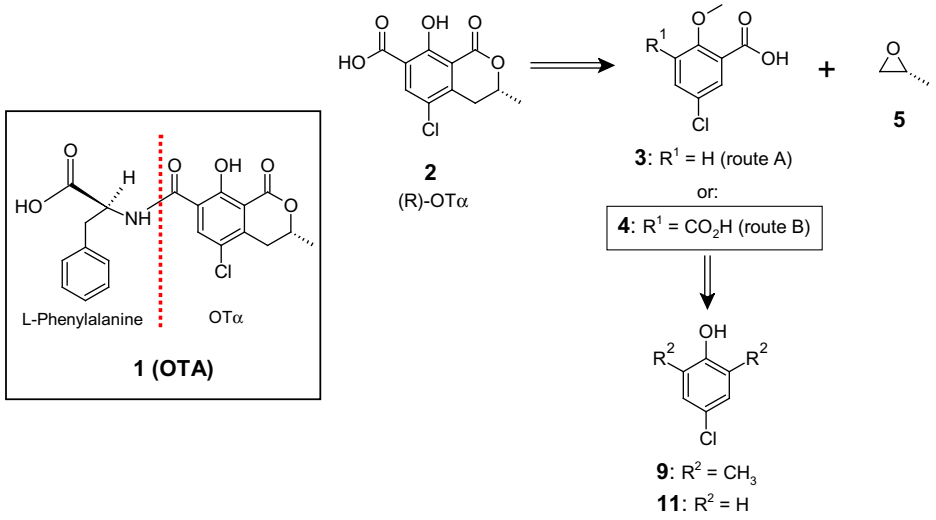
Treatment of **6** with BCl_3 in CH_2Cl_2 led smoothly to phenol[20] **7** in 93% yield. The formylation of **7** with dichloromethyl methyl ether and $TiCl_4$ to produce aldehyde[21] **8** was only achieved when a large excess of the reagents (10 equiv) was used and even then in only 48% yield, indicating that this aromatic system is not enough activated toward this reaction. The oxidation of aldehyde function in **8** to carboxylic acid was performed with hydrogen peroxide[22] in the presence of a catalytic amount of $AgNO_3$, to afford the final (*R*)-ochratoxin α **2** in 65% yield. Therefore, this synthetic route A led to the target compound (*R*)-OTα **2** in an overall yield of 15.4% over four steps.

The unsatisfactory performance in functionalization at the *ortho*-phenol position stimulated the search for alternatives, and the best situation would be to find an intermediate with the suitably functionalization already present, prior to formation of the chiral lactone system, to minimize the loss of the chiral reagent **5**. This highly functionalized aromatic intermediate was envisioned as the isophthalic system 5-chloro-2-methoxybenzene-1,3-dicarboxylic acid **4**, which can be easily prepared from simple reactions[17] and reagents as shown in Scheme 3.

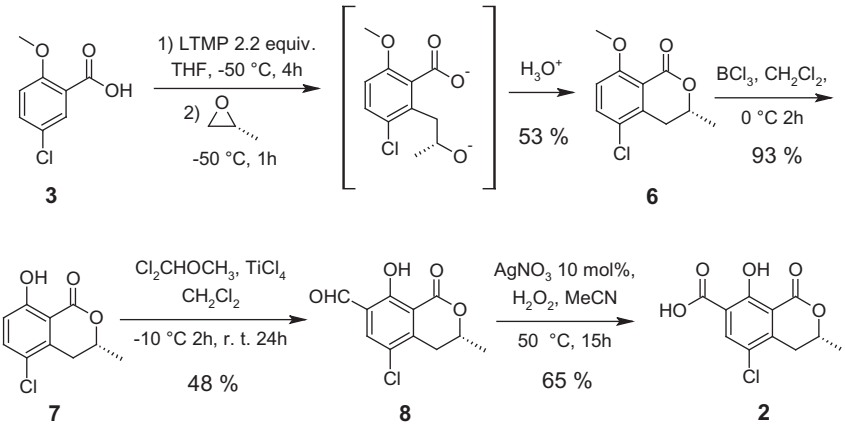
Starting from 4-chloro-2,6-dimethylphenol **9**, the aromatic intermediate **4** was obtained easily[17c,d] by protecting the phenol group of **9** with dimethyl sulfate (DMS) followed by $KMnO_4$ oxidation of the methyl groups, leading to intermediate **4** in a 51% yield over two steps. Starting from an even simpler and cheaper reagent, 4-chlorophenol **11**,[17a,b] the aromatic intermediate **4** was obtained in 50% yield over three steps, beginning with the reaction of **11** with formaldehyde in aqueous basic media to give 4-chloro-2,6-bis(hydroxymethyl)phenol **12** in 77%, which was directly submitted to the phenolic methyl protection reaction with DMS in aqueous basic media to give 4-chloro-2,6-bis(hydroxymethyl)anisole **13** in 85% yield. Then, the hydroxymethyl groups were oxidized with $KMnO_4$ in aqueous basic media to the isophthalic acid system in 75% yield.

With the tetra-substituted aromatic intermediate in hand, the directed *ortho*-metalation of unprotected dicarboxylic acid **4** was carried out as showed in Scheme 4.[23]

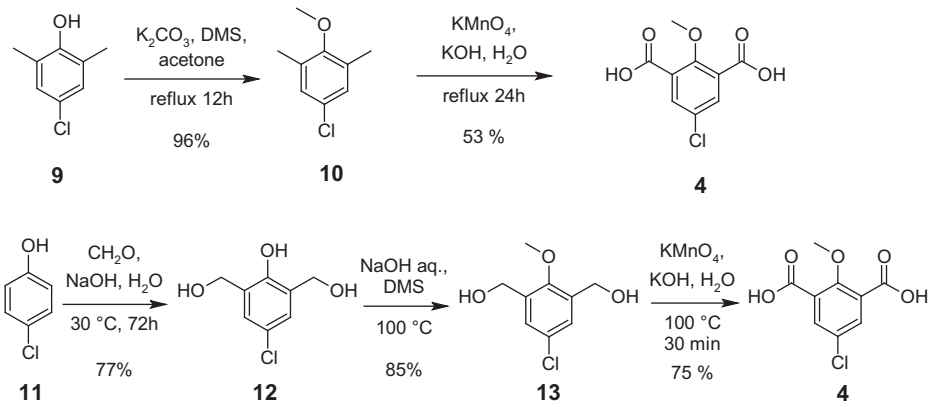
As could be expected, the reaction yield was low, partially due to the predictable low solubility of the trianion intermediate, and hence an excess of the solvent THF had to be used to carry out this reaction. A large excess of the base LTMP should also be avoided due to the presence of 2 equivalent positions on the aromatic ring,



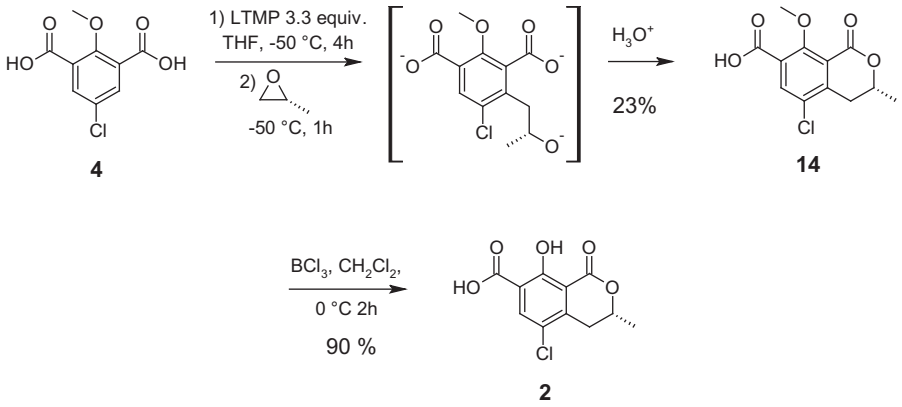
Scheme 1. Retrosynthesis of ochratoxin α **2**.



Scheme 2. Synthetic route A to ochratoxin α **2**.



Scheme 3. Preparation of intermediate **4**.



Scheme 4. Synthetic route B to ochratoxin α **2**.

which could lead to an aromatic dimetalation. Although this reaction resulted in a low yield of 23% and still has to be submitted to optimization studies, it was considered of great value in the synthesis of OTα due to the possibility of obtaining directly the phenolic methyl protected OTα **14** in only one reaction from the readily available aromatic intermediate **4**, which can be quickly prepared and easily stored for a long time. Furthermore, phenolic methyl protected OTα **14** can be submitted to the coupling reaction with L-phenylalanine to produce the phenolic methyl protected OTA for other research purposes, and can then be unprotected to give OTA **1**. The methyl group of **14** was cleanly removed with BCl_3 in CH_2Cl_2 to provide the final OTα **2** in 90% yield.

In conclusion, we have accomplished two rapid synthetic routes to the enantiomerically pure synthesis of (*R*)-ochratoxin α **2** based on inserting the proper stereochemistry from the commercially available (*R*)-propylene oxide **5**. Synthetic route A provided OTα **2** in 15.4% yield over four steps starting from commercially available 5-chloro-*o*-anisic acid **3**, while the synthetic route B provided OTα **2** in 20.7% yield over only two steps starting from the readily available intermediate 5-chloro-2-methoxybenzene-1,3-dicarboxylic

acid **4**. Due to their novelty, for both routes a Brazilian patent has been filed.[24] These syntheses of enantiomerically pure (*R*)-ochratoxin α **2** constitute also the formal total synthesis of enantiomerically pure ochratoxin A **1** by means of known coupling reactions[6,9,11,15] of OTα **2** with the amino acid L-phenylalanine and also of the isotopically labeled analogs of OTA by coupling OTα **2** with isotopically labeled reagents [^{13}C]-, [^{15}N]- or [^{2}H]-L-phenylalanine. In addition to these analogs derived from OTα **2**, both synthetic routes presented here can easily be adapted for the preparation of non-chlorinated analogs (*R*)-ochratoxin β and ochratoxin B (OTB) starting from non-chlorinated analogic reagents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.11.123.

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23. Procedure for synthesis of (3*R*)-5-chloro-8-methoxy-3-methyl-1-oxo-3,4-dihydro-1*H*-isochromene-7-carboxylic acid **14**. Lithium 2,2,6,6-tetramethylpiperidide (LTMP) was generated by adding tetramethylpiperidine (5.6 mL, 33 mmol) to a solution of *n*-butyl lithium 2.5 mol/L (13.4 mL, 33.5 mmol) in 300 mL of THF at −20 °C under argon atmosphere with agitation, and was maintained at this temperature for 15 min. Then, the mixture was cooled to −50 °C, and a solution of 5-chloro-2-methoxybenzene-1,3-dicarboxylic acid (**4**) (2.31 g, 10 mmol) in 20 mL of THF was added dropwise, and the mixture was stirred at this temperature for 4 h. (*R*)-Propylene oxide (**5**) was added in one portion (2.4 mL, 34.3 mmol) and the temperature was allowed to rise slowly to room temperature, and thereafter, HCl (4 mol/L) was added until a pH below 1 was obtained. The mixture was extracted with ethyl acetate (4 × 50 mL), the combined organic phases were extracted with aqueous sodium hydroxide (4 × 50 mL, 10%), the latter of which was extracted with dichloromethane to clean it from organic by-products. The aqueous alkaline solution was acidified with HCl (4 mol/L) until a pH below 1 and extracted again with ethyl acetate, which was washed with water and saturated NaCl solution, and dried over Na_2SO_4. After filtration and evaporation of the solvents, the crude material was purified by crystallization from acetone or by dry column vacuum chromatography (DCVC) using mixtures of ethyl acetate and methanol with polarity gradient elution, providing 0.623 g of product **14** as a white solid (23%). LC–MS, ESI positive mode (DP = 50 V): *m*/*z* = 293.1 (82) $[M+Na]^+$, 271.1 (100) $[M+H]^+$. LC–MS/MS $[M+H]^+$ (ESI positive mode, CE = 21 V): *m*/*z* = 253.1 (100), 223.1 (20), 195.1 (2).
Rapid derivatization for further confirmation by GC–MS: preparation of methyl (3*R*)-5-chloro-8-methoxy-3-methyl-1-oxo-3,4-dihydro-1*H*-isochromene-7-carboxylate (methyl ester of **14**). About 2 mg of the carboxylic acid **14** was dissolved in a vial with 1 mL of ether and 0.5 mL of methanol, followed by addition of an ether solution (2 mol/L) of trimethylsilyldiazomethane ($(CH_3)_3SiCHN_2$) until persistent yellow color. The vial was shaken for 5 min and the GC-MS spectrum of the derivative product was obtained: GC-MS (70 eV, IE): *m*/*z* = 284 $[M]^+$ (12) (cluster of Cl containing $[M]^+$ and $[M+2]^+$), 266 (35), 253 (40), 239 (58), 208 (57), 180 (67), 103 (70), 75 (100).
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