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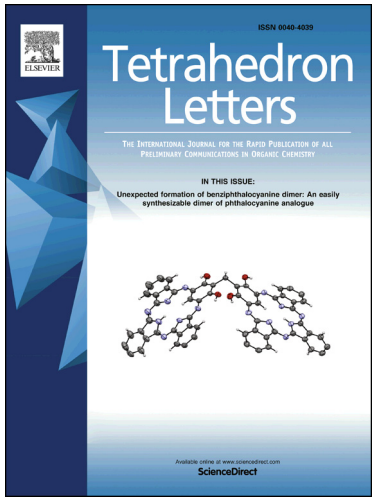

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Stereoselective Synthesis of Two Potential Metabolites of *Cis*-Metconazole

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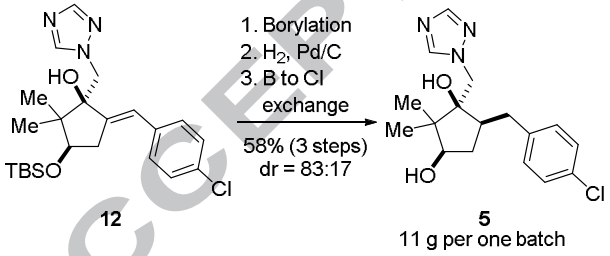
Abstract

The stereoselective synthesis of compounds **5** and **6**, potential hydroxylated metabolites of the agricultural fungicide *cis*-metconazole, is reported. In a key step of the initially surveyed synthetic route, hydrodechlorination of **12** was competitive with hydrogenation of the trisubstituted olefin. Application of a Miyaura borylation/hydrogenation/boron-to-halogen exchange reaction sequence solved the chemoselectivity issue.

Graphical abstract



Keywords: metconazole, metabolites, 1,2,4-triazole, Miyaura borylation

Introduction

Metconazole (*cis*-**1** and *trans*-**1**, Fig. 1A), belonging to the 1,2,4-triazole-containing fungicide family, is extensively used in agriculture to protect cereals, vegetables, and fruits against fungal infections.[1-6] In fungi, **1** inhibits lanosterol 14α-demethylase (CYP51A1), a cytochrome P450 enzyme engaged in the biosynthesis of ergosterol, a constituent of cell membranes which is essential for proper fungal growth.[7] Numerous 1,2,4-triazoles have found applications as drugs and agrochemicals, e.g. tebuconazole (**2**), fluconazole (**3**), and voriconazole (**4**) (Fig. 1B).

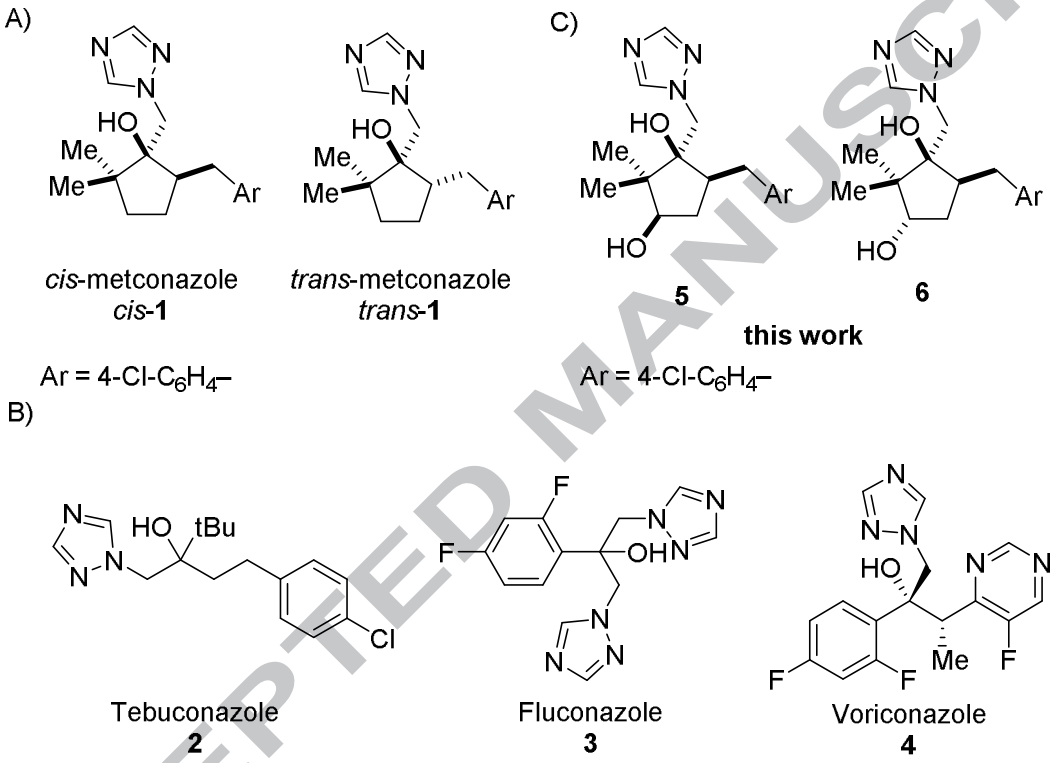


Figure 1. (A) *Cis*- and *trans*-metconazole; (B) Selected important triazole fungicides; (C) Potential metabolites of *cis*-metconazole.

Cytochrome P450 enzymes (CYPs) also play important roles in humans, including the synthesis of steroids and participation in the metabolism of xenobiotics. Similarly to other triazoles, **1** may inhibit CYPs, resulting in adverse effects such as hormonal and metabolic disorders.[8-11] Moreover, long-term exposure to fungicides may be connected with the development of drug resistance for pathogenic fungi.[12-15] Concerns regarding the impact of widespread agricultural applications of **1** on human health

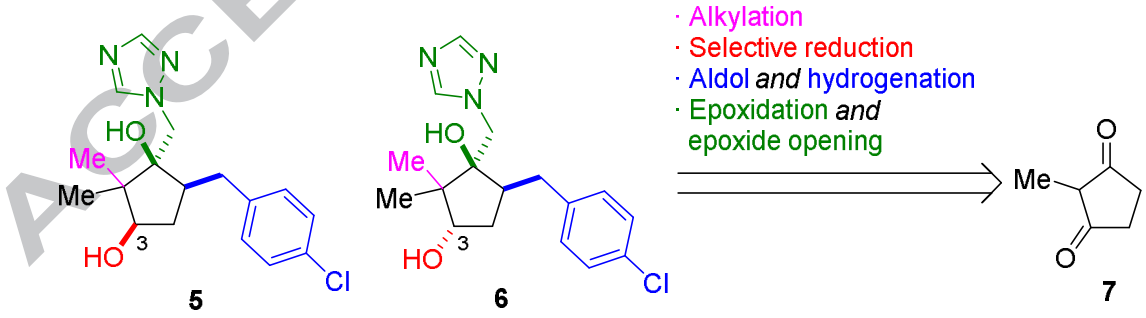
have arisen, and in order to address these questions, the biological activity of **1** as well as its metabolites must be studied more closely.[16]

Although, the synthesis of metabolites and metabolite-like derivatives of biologically active compounds have attracted significant attention,[17-21] the preparation of metconazole metabolites have not been reported; only the *in vitro* monooxygenation of **1** catalyzed by human CYP3A4, providing two hydroxylated products with undetermined structures, has been studied.[22] Among the twenty C-H bonds in **1**, one tertiary and two benzylic bonds are the most electronically prone toward monooxygenation, although other bonds may also be reactive with specific CYPs since electronic factors are not the only determinants of enzymatic oxidation selectivity.[23]

Herein, we disclose a concise synthesis of a pair of diastereomeric hydroxymetconazoles **5** and **6** (Fig. 1C), representing potential metabolites of *cis*-**1**, which is the more abundant metconazole isomer in the manufactured product.

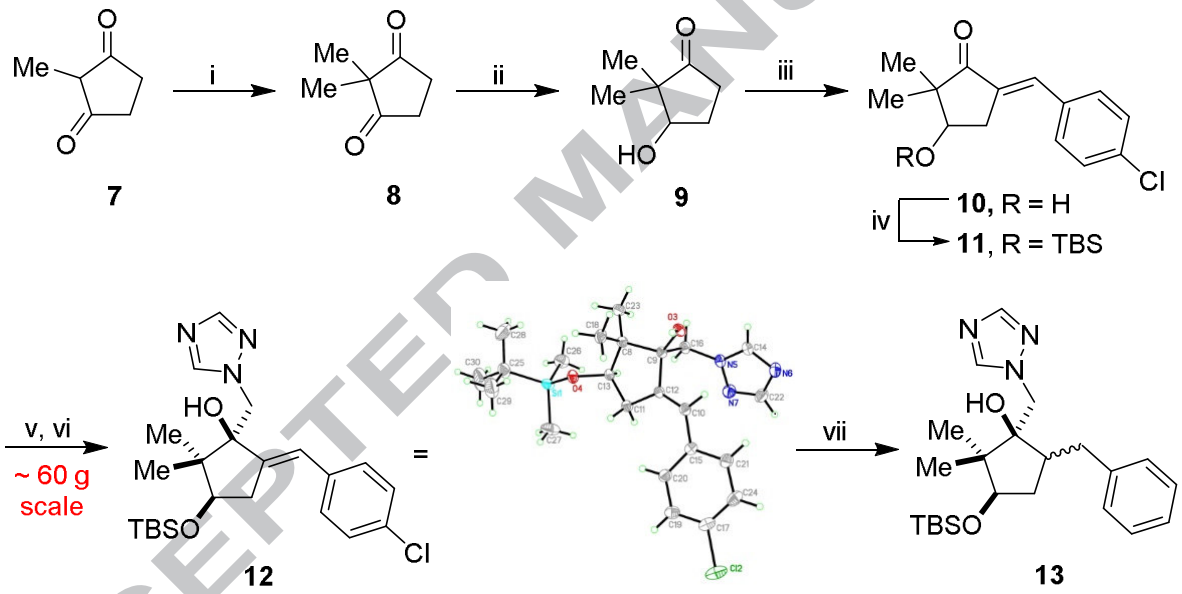
Results and Discussion

Our general strategy to access **5** and **6** is depicted in Scheme 1. We proposed that both compounds could be derived from commercially available **7** and the remaining structural motifs could be introduced using well-established chemistry. We also envisioned the relative stereochemistry between the cyclopentane ring substituents could be controlled by a bulky protecting group on C(3)-OH.



Scheme 1. Retrosynthetic analysis of compounds **5** and **6**.

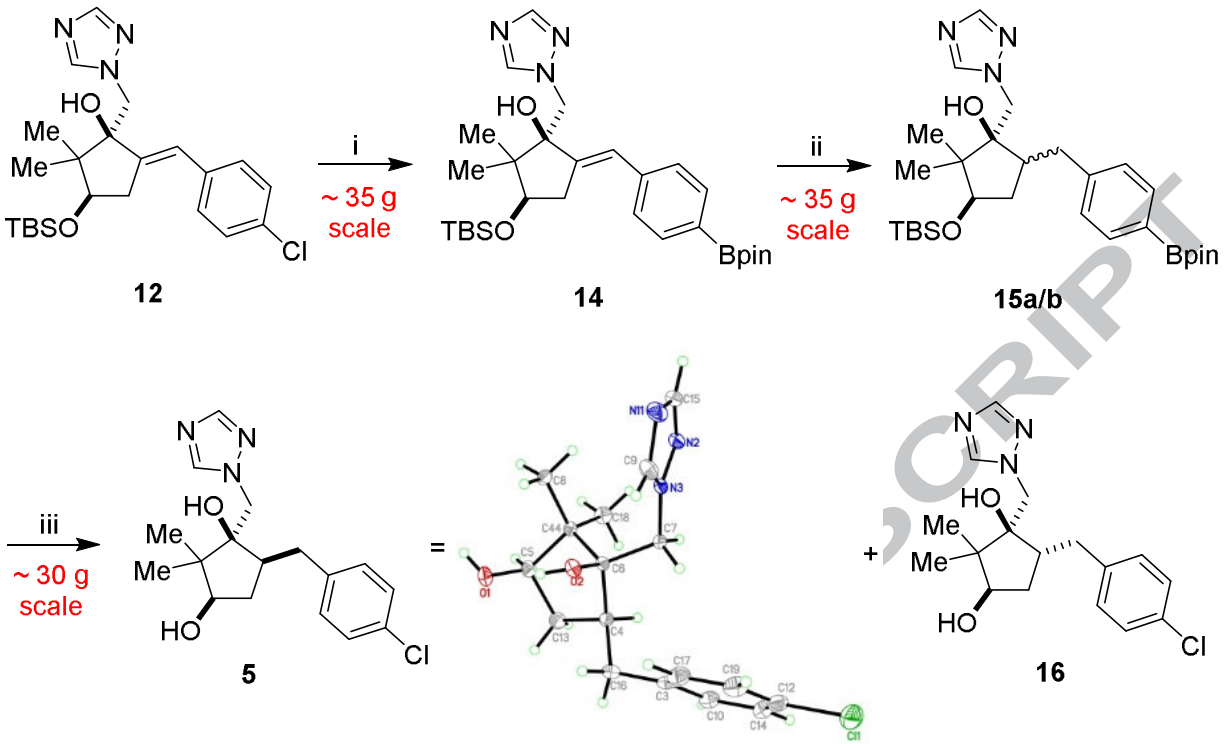
First, precursor **7** was alkylated with MeI to afford **8** in 36% yield.[24] Then, a single carbonyl group in **8** was selectively reduced using a substoichiometric amount of $NaBH_4$, affording alcohol **9** in 94% yield.[25] Cross-aldol condensation of alcohol **9** and 4-chlorobenzaldehyde followed by silylation of the hydroxyl group, furnished **11** in good overall yield. The Corey-Chaykovsky epoxidation of **11**, performed under Danishefsky conditions,[26] gave the corresponding epoxide which was directly subjected to the next step due to its instability. Treatment of the epoxide with 1,2,4-triazole/K_2CO_3 in DMF at 85 °C afforded **12** in 50% yield as a single diastereomer; the relative stereochemistry was confirmed by X-ray crystallography. The stereochemical outcome of the epoxidation reaction can be explained by shielding the top face of the cyclopentane ring by the TBS-protecting group, which forces attack of the sulfonium ylide on the C=O bond from the opposite side (see ESI, Fig. S1 for details).



Scheme 2. Synthesis and attempted hydrogenation of key intermediate **12**. Reagents and conditions: i. a) NaOH (1 eq), H_2O, rt, 15 min; b) MeI (1.5 eq), DMF, rt, overnight; c) $HCl_{(aq)}$, 1 h, reflux, 36% (3 steps) ii. $NaBH_4$ (0.27 eq), MeOH/H_2O (4:1), 0 °C -> rt, 30 min, 94%; iii. 4-chlorobenzaldehyde (1 eq), NaOH (1 eq), MeOH/H_2O (4:1), rt, 1 h, 89%; iv. TBSCl (1.5 eq), imidazole (3.0 eq), DMF, rt, overnight, 91%; v. trimethylsulfonium iodide (1.2 eq), KHMDS (1.1 eq), THF, 0 °C, then **11**, 0 °C, 1.5 h; vi. 1,2,4-triazole (1.5 eq), K_2CO_3, (1.5 eq), DMF, 60 °C, overnight, 50% (2 steps); vii. 10% Pd/C (16% w/w), HCO_2NH_4 (10 eq), EtOH, reflux, 1 h, 75%, dr = 88:12.

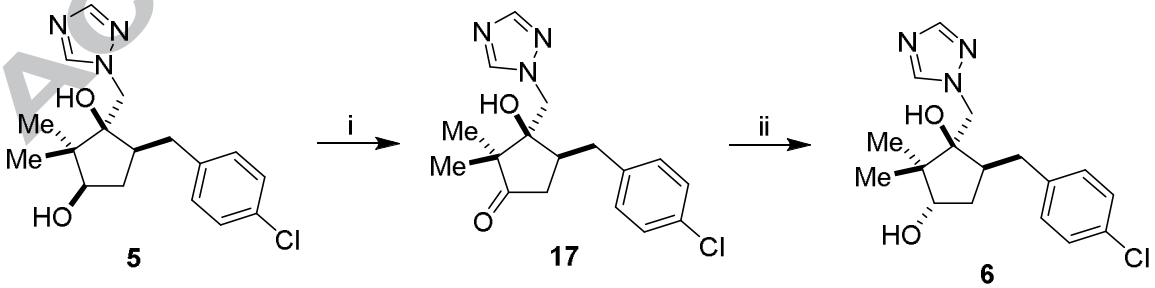
Hydrogenation of the trisubstituted alkene bond in **12** was challenging. Under typical conditions (Pd/C, 1 atm H_2, EtOH or EtOAc, rt; Pd/C, 1 atm H_2, EtOAc, 65 °C; Pd/C, 100 atm H_2, EtOH) compound **12** reacted sluggishly with low conversions, leading to dechlorinated **13** as the main product. Attempts to modify the catalyst activity by using Ph_2S[27,28] as a catalyst poison failed. With low Ph_2S loadings, no changes in the chemoselectivity were observed; however, higher loadings stopped the reaction completely. Remarkably, in a weakly basic environment with ammonium formate as the hydrogen source, **12** was reduced smoothly, providing **13** in 75% yield as a mixture of diastereomers. Cationic reduction of **12** was also attempted, however, this approach led to partial deprotection of the TBS-protecting group (Et_3SiH/TFA) or to complete decomposition of the starting material (Et_3SiH, TfOH).

To avoid chloroarene hydrodechlorination, we decided to exchange chlorine with the pinacolboronate group, which is considered to be stable in Pd-catalyzed hydrogenations.[29,30] We envisioned chlorine could be easily reintroduced after olefin reduction *via* a copper-mediated process.[31] Thus, Suzuki-Miyaura borylation of **12** furnished pinacolboronate **14** on a multigram scale in 79% yield.[32] Hydrogenation of **14** proceeded smoothly (Pd/C, EtOAc, 65 °C) without significant formation of the protodeboration product, affording **15a/b** in 93% yield with good diastereoselectivity (dr = 83:17) (see ESI, Fig. S2 for conformational analysis). Treatment of **15a/b** with excess of $CuCl_2$ in *i*PrOH/H_2O led to hydroxymetconazoles **5** and **16** in 79% yield (61% combined yield after separation by fractional crystallization). The relative stereochemistry of diastereomer **5** was confirmed by X-ray crystallography (Scheme 3).



Scheme 3. Synthesis of hydroxymetconazole **5**. Reagents and conditions: i. pin_2B_2 (1.05 eq), $Pd_2(dba)_3$ (2.5 mol%), XPhos (10 mol%), KOAc (3 eq), 1,4-dioxane, 110 °C, overnight, 79%; ii. 10% Pd/C (30% w/w), H_2 (balloon), EtOAc, 65 °C, 6 h, 93%, dr = 83:17; iii. $CuCl_2 \times H_2O$ (4.0 eq), *i*PrOH/H_2O (3:1), reflux, 48 h, 79%; 55% for **5** and 6% for **16** after fractional crystallization.

Finally, we applied a two-step sequence to convert **5** into epimeric **6**. In the first step, Swern oxidation of **5** furnished ketone **17** in 84% yield, which was stereoselectively reduced with $NaBH_4$ to provide **6** in 74% yield (Scheme 4).



Scheme 4. Synthesis of hydroxymetconazole **6**. Reagents and conditions: i. $(COCl)_2$ (5 eq), DMSO (5 eq), DCM, -78 °C, 30 min, then **5**, -78 °C, 90 min, then Et_3N (50 eq), rt, 30 min, 84%; ii. $NaBH_4$ (4 eq), MeOH/H_2O (15:1), 30 min, rt, 74%.

Conclusions

In summary, we have developed a stereoselective, multigram synthesis of hydroxymetconazole **5** and proposed a two-step sequence for the conversion of **5** to **6**. These compounds may find applications as chromatographic standards in the detection of metconazole metabolites. During the synthesis, we tackled the undesired chloroarene hydrodechlorination which was competitive with alkene bond hydrogenation. We proposed a three-step route involving Miyaura borylation/hydrogenation/boron-to-chlorine exchange reactions as a solution for this issue. Further applications of this method for the synthesis of other metconazole metabolites are ongoing in our laboratory.

Supplementary data

Experimental procedures, copies of ^{1}H, ^{13}C, 2D NMR spectra and detailed crystallographic data for compounds **6** and **13**. CIF files containing crystallographic data for compounds **6** and **13**.

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Notes

Authors declare no competing financial interest.

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Highlights:

- Synthesis of two possible metabolites of *cis*-metconazole
- Chloroarene was temporary converted into the corresponding pinacolboronate to prevent hydrodechlorination
- The developed method was applied to the multigram synthesis of **5**