

Biomimetic Asymmetric Synthesis of (*R*)-GTRI-02 and (3*S*,4*R*)-3,4-Dihydroxy-3,4-dihydronaphthalen-1(2*H*)-ones

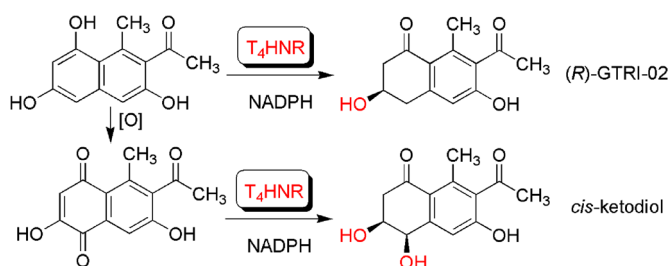
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ABSTRACT



The NADPH-dependent tetrahydroxynaphthalene reductase (T₄HNR) from *Magnaporthe grisea* was used for the biomimetic synthesis of (*R*)-GTRI-02 by stereoselective reduction of 1-(3,6,8-trihydroxy-1-methylnaphthalen-2-yl)ethanone. This also led to the isolation of a (3*S*,4*R*)-*cis*-ketodiol formed by T₄HNR-catalyzed reduction of the corresponding hydroxynaphthoquinone. Flaviolin and lawsone also reduced to corresponding *cis*-ketodials in good yields.

Naphthol reductases belong to the large family of short-chain dehydrogenases/reductases (SDR) and show a unique ability to catalyze asymmetric NADPH-dependent reduction of polyhydroxynaphthalenes.¹ Although the use of naphthol reductases as biocatalysts in synthesis looks promising, so far their application has remained limited to the reduction of only a few physiological substrates.^{1–4} Tetrahydroxynaphthalene reductase (T₄HNR) from *Magnaporthe grisea* has been used by us and others to catalyze the reduction of 1,3,6,8-tetrahydroxynaphthalene (T₄HN, **1**) to (*R*)-scytalone (**2**) in 33% yield (Scheme 1, A).^{1,4,5} T₄HNR is

one of the two naphthol reductases involved in the biosynthesis of dihydroxynaphthalene melanin.^{2–4} For naphtholic substrates to be reduced by T₄HNR, the 1,3-dihydroxy substitution pattern represents the essential structural motif.¹ Herein, we report the chemoenzymatic synthesis of the natural product GTRI-02 (**3**) using T₄HNR as well as the unexpected recognition of hydroxynaphthoquinones as substrates which are reduced by T₄HNR to *cis*-ketodials.

The putatively polyketidic GTRI-02 (**3**), isolated from soil actinomycetes *Micromonospora* sp.,⁶ has been shown to possess antioxidant properties. More recently, it has also been extracted from *Streptomyces* strain GW4184 and *Streptomyces* sp. ANK313.^{7,8} We proposed that **3** is biosynthesized via an enzymatic reduction.

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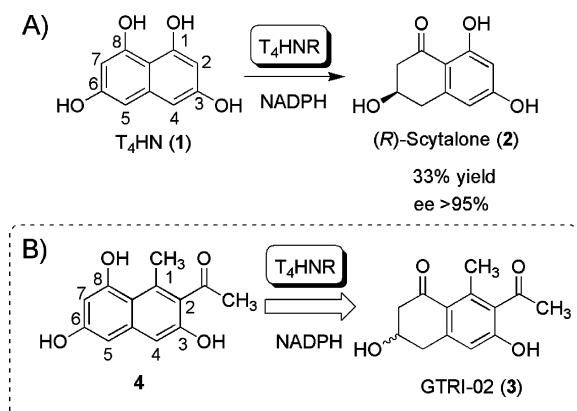
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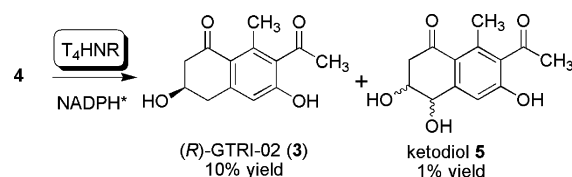
Scheme 1. (A) T₄HNR-Catalyzed Reduction of its Physiological Substrate T₄HN (1). (B) Retrosynthetic Proposal for the Biomimetic Enzymatic Synthesis of GTRI-02 (3)



The biomimetic retrosynthetic analysis of **3** guided us to the acetylated trihydroxynaphthalene **4** as the required substrate (Scheme 1, B). Compared to T₄HN (**1**), naphthol **4** contains an additional acetyl group, in principle a potentially better substrate unit to be reduced by oxidoreductases.

Compound **4** was synthesized in four straightforward steps starting from 3,5-dimethoxyphenylacetic acid in an overall yield of 25% (see the Supporting Information). Naphthol **4** was then reduced with T₄HNR. NADPH was used as a cofactor and regenerated using glucose and glucose dehydrogenase (GDH) in all enzyme-catalyzed reactions. The transformation proceeded as proposed and resulted in the formation of GTRI-02 (**3**) as the major product after 24 h (17% conversion), supporting the argument for the putative involvement of a similar naphthol reductase in the biosynthesis of **3**.⁹ Unexpectedly, ketodiol **5** was obtained as a side product in the same transformation (Scheme 2).

Scheme 2. T₄HNR-Catalyzed Reduction of **4**



* NADPH was regenerated using glucose/glucose dehydrogenase.

GTRI-02 (**3**) was isolated and purified by preparative HPLC (RP-18) to provide pure material in 10% isolated yield. The absolute configuration was determined as (*R*) by using circular dichroism (CD). Additionally, the

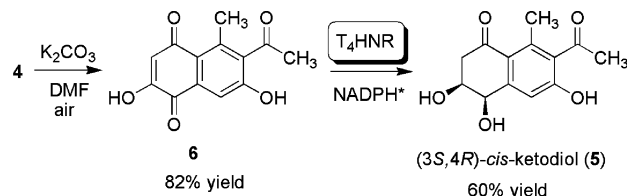
(9) 2-Acetyl-1,3,6,8-tetrahydroxynaphthalene (a) and 6-hydroxymusizins (b) were synthesized and tested with T₄HNR; however, the transformations did not result in any reduction. (a) Wheeler, M. H.; Abramczyk, D.; Puchhaber, L. S.; Naruse, M.; Ebizuka, Y.; Fujii, I.; Szaniszlo, P. J. *Eukaryotic Cell* **2008**, *7*, 1699–1711. (b) Harris, T. H.; Witte, P. J. *J. Am. Chem. Soc.* **1975**, *97*, 3270–3271.

bis-*p*-bromobenzoate derivative of **3** was prepared, and CD spectroscopy, according to the method of Harada and Nakanishi (exciton coupling), was used to unambiguously verify the assignment.¹⁰ Since both the natural substance⁶ and the enzymatically synthesized product show negative specific rotation, the absolute configuration of the former must also be (*R*).

The unexpected side product of the enzymatic reduction, ketodiol **5**, was isolated in 1% yield by column chromatography. Further analysis of the T₄HNR-catalyzed reduction reaction of **4** led to the detection of the presence of 2-hydroxy-1,4-naphthoquinone **6**, possibly formed by nonenzymatic aerobic oxidation. Although the transformation was performed under N₂, incomplete removal of oxygen from the buffer solution might account for the oxidation of compound **4**. We assumed that 2-hydroxy-1,4-naphthoquinone **6** could have been reduced to diol **5** by T₄HNR, using 2 equiv of NADPH.

In order to prove this assumption, **6** was prepared in 82% isolated yield by the oxidation of compound **4** using K₂CO₃ in DMF.¹¹ Hydroxynaphthoquinone **6** was then employed as a substrate and was reduced by T₄HNR to exclusively give the above-mentioned vicinal diol **5** with 80% conversion (60% yield, dr_{cis/trans} ≥ 99:1) (Scheme 3). The relative configuration of **5** was elucidated by single-crystal X-ray analysis of the 4-biphenylboronic ester derivative. The absolute (3*S*,4*R*)-configuration was determined by vibrational circular dichroism (VCD) and quantum chemical calculations (Gaussian 09¹²) (see the Supporting Information).

Scheme 3. T₄HNR-Catalyzed Reduction of Hydroxynaphthoquinone **6**



T₄HNR-catalyzed reduction of 2-hydroxy-1,4-naphthoquinone **6** to *cis*-ketodiol **5** also hints at the possible

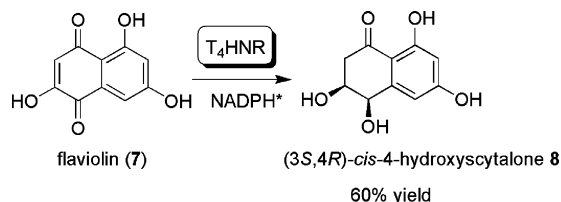
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involvement of T₄HNR or related enzymes in the putative biosynthetic reduction of flaviolin (**7**) to 4-hydroxyscytalone (**8**).¹³ To support our argument, **7** was synthesized in 80% yield by the oxidation of T₄HN (**1**)¹¹ and was used as a substrate for T₄HNR (Scheme 4).

Scheme 4. T₄HNR-Catalyzed Reduction of Flaviolin (**7**)

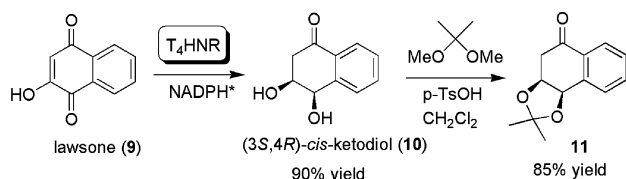


The transformation was performed using glucose/GDH for cofactor regeneration and resulted in the formation of *cis*-4-hydroxyscytalone (**8**) with quantitative conversion and high diastereoselectivity (*dr*_{*cis/trans*} = 99:1) (determined by ¹H NMR spectroscopy). A lower isolated yield of 60% was obtained due to decomposition of **8**.

Relative to transformations performed in the presence of air, much better yields were obtained when the transformation was performed under N₂ and after degassing. *cis*-4-Hydroxyscytalone (**8**) has been isolated previously from various sources and has also been proposed to be formed during melanin biosynthesis of *Wangiella dermatitidis* along with other naphthalene-based metabolites.^{9a,13–15}

To stress hydroxynaphthoquinones as general substrates for T₄HNR, another natural product, lawsone (**9**), was used as a substrate (Scheme 5). This transformation resulted in quantitative reduction, and the *cis*-ketodiols **10** was isolated in 90% yield.

Scheme 5. Reduction of Lawsone (**9**) Catalyzed by T₄HNR



The same transformation was performed in up to a 100-mg scale with no significant change in the yield of ketodiols **10**. The configuration of **10** was determined by VCD of its acetonide **11** and assigned as (3*S*,4*R*) (Figure 1).

For *cis*-4-hydroxyscytalone (**8**), the absolute configuration (3*S*,4*R*) was assigned by comparison of the CD spectra of **8** and **10** (see the Supporting Information).

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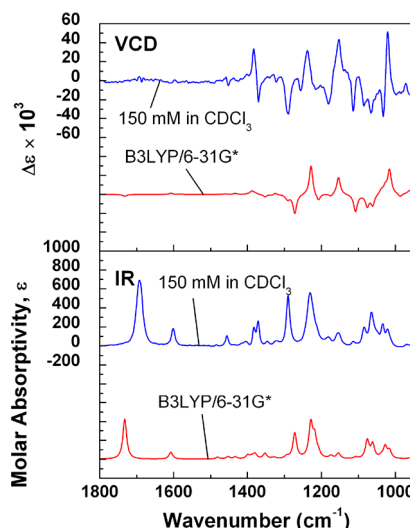


Figure 1. Experimental IR and VCD spectra of the acetonide **11** (150 mM in CDCl₃) and the theoretical IR and VCD spectra calculated¹² (B3LYP/6-31G* level) for the (3*S*,4*R*)-configured acetonide **11**. Good agreement in frequencies and sign between the calculated and observed spectra allows for assignment of the absolute configuration.

Although several studies^{13b} have proposed that *cis*-4-hydroxyscytalone (**8**) is formed by the reduction of flaviolin (**7**), the absolute configuration of **8** and the enzymatic reduction of flaviolin by T₄HNR have now been established for the first time.

Acceptance by T₄HNR of different hydroxynaphthoquinones as substrates and their reduction to homochiral *cis*-ketodiols hint at further biosynthetic considerations: we propose that in the biosynthesis of 3-hydroxy-3,4-dihydronaphthalen-1(2*H*)-ones (and the corresponding anthracenones), e.g., GTRI-02 (**3**), T₄HNR-like enzymes might be involved. Characterization of these enzymes with a probably expanded substrate range will broaden the

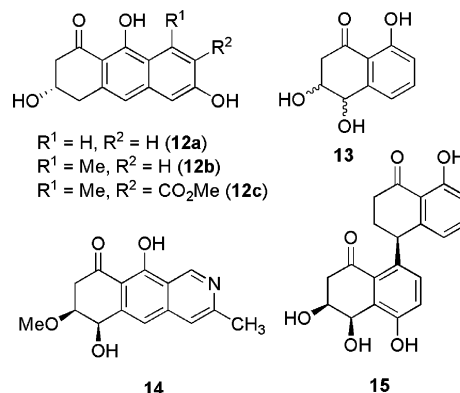


Figure 2. Natural products containing 3-hydroxy-3,4-dihydroanthracen-1(2*H*)-one (**12a–c**) and 3,4-dihydroxy-3,4-dihydronaphthalen-1(2*H*)-one substructures (**13–15**).

scope of asymmetric “phenol”-reducing methods. Moreover, the biosynthesis of 3,4-dihydroxy-3,4-dihydronaphthalen-1(2*H*)-ones (and the corresponding anthracenones) might proceed through T₄HNR-like transformations, as shown above for the reduction of hydroxy-1,4-naphthoquinones **6**, **7**, and **9**.

Several natural products with similar substructures show interesting biological activities. For example, **12a** isolated from *Dendrobium* sp.¹⁶ and **12b** and **12c** isolated from the roots of *Lomatophyllum*¹⁷ possess the 3-hydroxy-3,4-dihydroanthracen-1(2*H*)-one substructure (Figure 2).

cis-Ketodiols contain a 3,4-dihydroxy-1-tetralone substructure which is also a part of several natural products. Such an example is 3,4,8-trihydroxy-1-tetralone (**13**) which has been isolated from several fungal species and shows antifungal activity.^{18–20} Chrysanthone A (**14**)²¹ and

cladosporol D (**15**)²² are further examples of natural products with a vicinal *cis*-diol containing a similar substructure.²³

In summary, T₄HNR was successfully applied for the chemoselective reduction of naphtholic substrates; no reduction of the acetyl side chain present in compounds **4** and **6** was observed. We have shown that the enzymatic reduction of aromatic hydroxy groups and, especially, 2-hydroxy-1,4-naphthoquinones can be used for the asymmetric synthesis of related compounds.²³ Moreover, the discussed biosynthetic considerations will help to identify related enzymes.

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Supporting Information Available. Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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The authors declare no competing financial interest.

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