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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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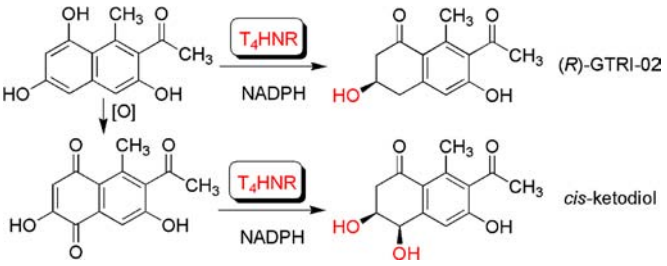
ABSTRACT



The NADPH-dependent tetrahydroxynaphthalene reductase (T_4HNR) 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 T_4HNR-catalyzed reduction of the corresponding hydroxynaphthoquinone. Flaviolin and lawsone also reduced to corresponding *cis*-ketodiols 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 (T_4HNR) from *Magnaporthe grisea* has been used by us and others to catalyze the reduction of 1,3,6,8-tetrahydroxynaphthalene (T_4HN, **1**) to (*R*)-scytalone (**2**) in 33% yield (Scheme 1, A).[1,4,5] T_4HNR is one of the two naphthol reductases involved in the biosynthesis of dihydroxynaphthalene melanin.[2−4] For naphtholic substrates to be reduced by T_4HNR, 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 T_4HNR as well as the unexpected recognition of hydroxynaphthoquinones as substrates which are reduced by T_4HNR to *cis*-ketodiols.

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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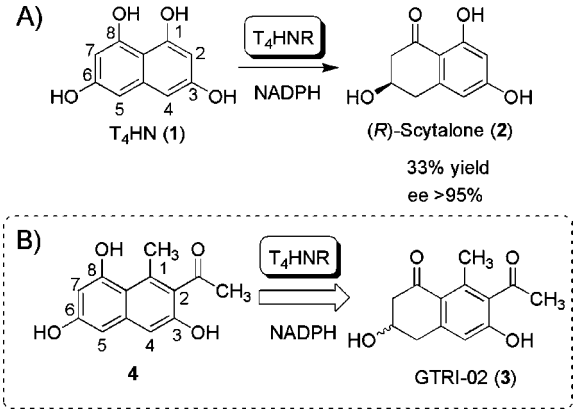
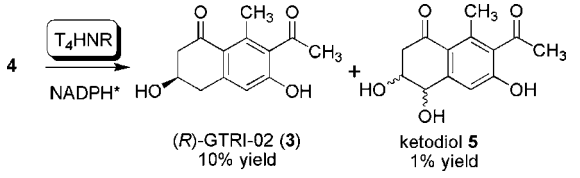
Scheme 1. (A) T_4HNR-Catalyzed Reduction of its Physiological Substrate T_4HN (**1**). (B) Retrosynthetic Proposal for the Biomimetic Enzymatic Synthesis of GTRI-02 (**3**)



The biomimetic retrosynthetic analysis of **3** guided us to the acetylated trihydroxynaphthalene **4** as the required substrate (Scheme 1, B). Compared to T_4HN (**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 T_4HNR. 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. T_4HNR-Catalyzed Reduction of **4**



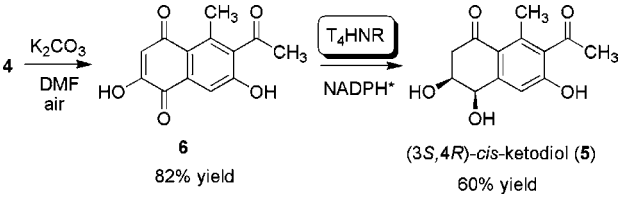
* NADPH was regenerated using glucose/glucose dehydrogenase.

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 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 substance[6] 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 T_4HNR-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 N_2, 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 T_4HNR, 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 K_2CO_3 in DMF.[11] Hydroxynaphthoquinone **6** was then employed as a substrate and was reduced by T_4HNR to exclusively give the above-mentioned vicinal diol **5** with 80% conversion (60% yield, $dr_{cis/trans} \geq 99:1$) (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 09[12]) (see the Supporting Information).

Scheme 3. T_4HNR-Catalyzed Reduction of Hydroxynaphthoquinone **6**



T_4HNR-catalyzed reduction of 2-hydroxy-1,4-naphthoquinone **6** to *cis*-ketodiol **5** also hints at the possible

(9) 2-Acetyl-1,3,6,8-tetrahydroxynaphthalene (a) and 6-hydroxymusizin (b) were synthesized and tested with T_4HNR; 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.; Wittek, P. J. *J. Am. Chem. Soc.* **1975**, *97*, 3270–3271.

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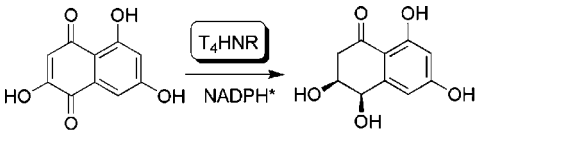
involvement of T_4HNR or related enzymes in the putative biosynthetic reduction of flaviolin (**7**) to 4-hydroxyscytalone (**8**).[13] To support our argument, **7** was synthesized in 80% yield by the oxidation of T_4HN (**1**)[11] and was used as a substrate for T_4HNR (Scheme 4).

Scheme 4. T_4HNR-Catalyzed Reduction of Flaviolin (**7**)

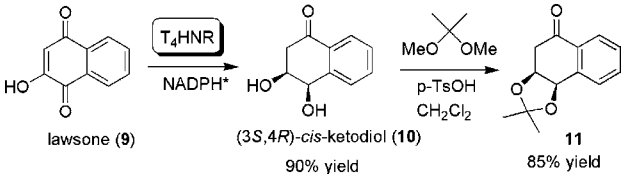


The transformation was performed using glucose/GDH for cofactor regeneration and resulted in the formation of *cis*-4-hydroxyscytalone (**8**) with quantitative conversion and high diastereoselectivity ($dr_{cis/trans}$ = 99:1) (determined by ^{1}H NMR spectroscopy). A lower isolated yield of 60% was 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 N_2 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 T_4HNR, another natural product, lawsone (**9**), was used as a substrate (Scheme 5). This transformation resulted in quantitative reduction, and the *cis*-ketodiol **10** was isolated in 90% yield.

Scheme 5. Reduction of Lawsone (**9**) Catalyzed by T_4HNR



The same transformation was performed in up to a 100-mg scale with no significant change in the yield of ketodiol **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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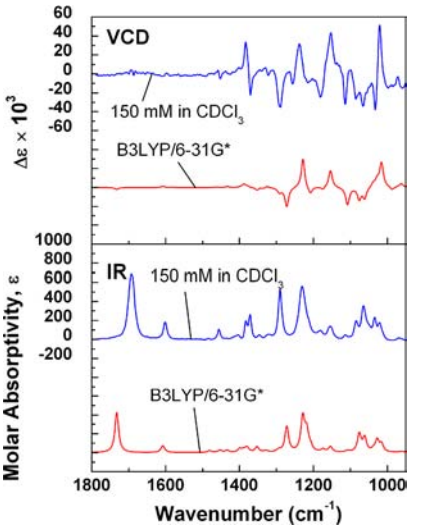

Figure 1. Experimental IR and VCD spectra of the acetonide **11** (150 mM in $CDCl_3$) and the theoretical IR and VCD spectra calculated[12] (B3LYP/6-31G* level) for the (3*S*,4*R*)-configured acetonide **11**. Good agreement in frequencies and sign between the calculated and observed spectra allows 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 T_4HNR have now been established for the first time.

Acceptance by T_4HNR 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**), T_4HNR-like enzymes might be involved. Characterization of these enzymes with a probably expanded substrate range will broaden the

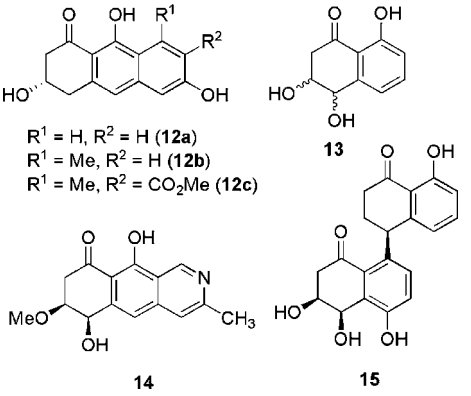

Figure 2. Natural products containing 3-hydroxy-3,4-dihydroanthracen-1(2*H*)-one (**12a**−**c**) and 3,4-dihydroxy-3,4-dihydronaphthalen-1(2*H*)-one substructures (**13**−**15**).

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 T_4HNR-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, T_4HNR 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.