


 Cite this: *RSC Adv.*, 2022, 12, 4635

 Received 24th January 2022
 Accepted 31st January 2022

DOI: 10.1039/d2ra00499b

rsc.li/rsc-advances

Asymmetric total synthesis of four bioactive lignans using donor–acceptor cyclopropanes and bioassay of (–)- and (+)-niranthin against hepatitis B and influenza viruses†

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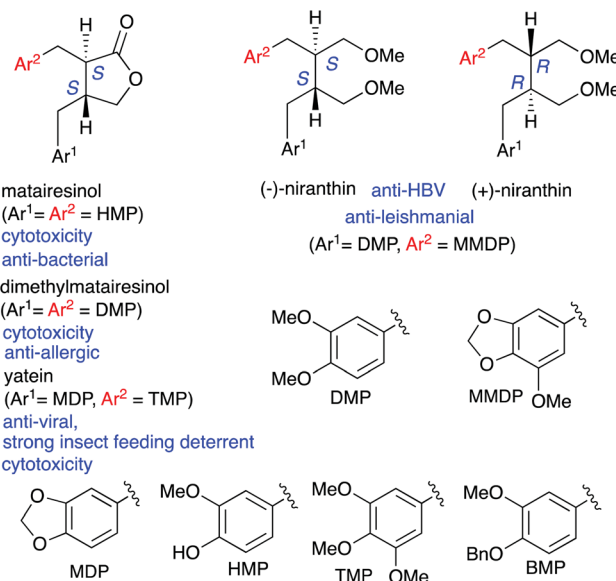
The asymmetric total synthesis of four lignans, dimethylmatairesinol, matairesinol, (–)-niranthin, and (+)-niranthin has been achieved using reductive ring-opening of cyclopropanes. Moreover, we performed bioassays of the synthesized (+)- and (–)-niranthins using hepatitis B and influenza viruses, which revealed the relationship between the enantiomeric structure and the anti-viral activity of niranthin.

Lignans are attracting considerable attention due to their widespread distribution in plants and their varied bioactivity.^{1–5} For example, matairesinol,² dimethylmatairesinol,³ yatein,⁴ and niranthin⁵ are found in nature and exhibit *e.g.*, cytotoxicity,^{2b,d,3b,4b} anti-bacterial,^{2c} anti-allergic,^{3c} anti-viral,^{4d,5b,e} anti-leishmanial,^{5d} and strong insect-feeding-deterrent activity.^{4c} Among these compounds, anti-viral compounds have received significant attention owing to the worldwide pandemic of coronavirus disease 2019 (COVID-19). Although niranthin exhibits anti-viral activity toward the hepatitis B virus (HBV),^{5b,e} the enantiomeric SAR (structure–activity relationship) for the anti-viral activity of niranthin has not been revealed so far. To examine the SAR for a pair of enantiomers, an independent asymmetric synthesis of both enantiomers is necessary. However, the alternative synthesis of (–)- or (+)-niranthin has not been reported.⁶ During our recent studies on the transformation of cyclopropanes,⁷ we have reported a reductive ring-opening of enantioenriched donor–acceptor (D–A) cyclopropanes and its application to an asymmetric total synthesis of yatein.⁷ⁱ As a further extension of this synthetic method, we disclose here the asymmetric total synthesis of

(–)-dimethylmatairesinol, (–)-matairesinol, (+)-niranthin, and (–)-niranthin. Moreover, the results of bioassays using (+)-niranthin and (–)-niranthin against HVB and influenza virus (IFV) are described (Scheme 1).

Scheme 2 outlines the enantioselective synthesis of optically active lactones **5a** and **5b**. Following our previous report,⁷ⁱ we attempted to synthesize the enantio-enriched bicyclic lactones **4a** and **4b**.

Initially, the cyclopropanation of enal **1** with dimethyl α -bromomalonate **2** using the Hayashi–Jørgensen catalyst afforded the desired optically active cyclopropylaldehydes **3a** and **3b** in good to high yield with high ee.^{7c,e,h–j,8,9} The reduction of the



Scheme 1 Some examples of bioactive dibenzyl lignans.

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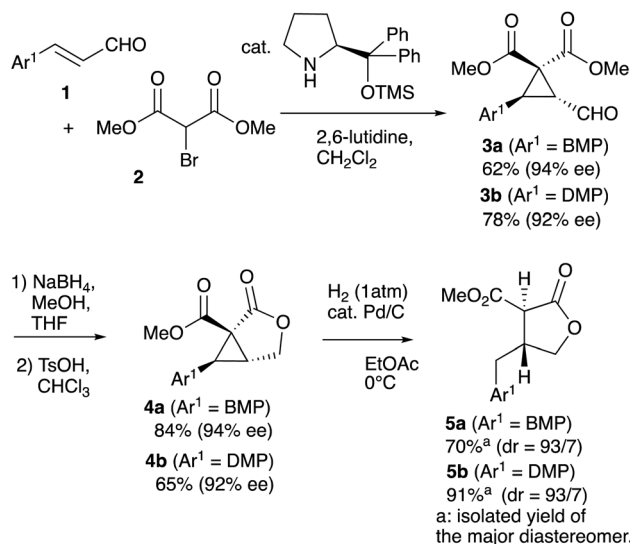
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† Electronic supplementary information (ESI) available. See DOI: 10.1039/d2ra00499b





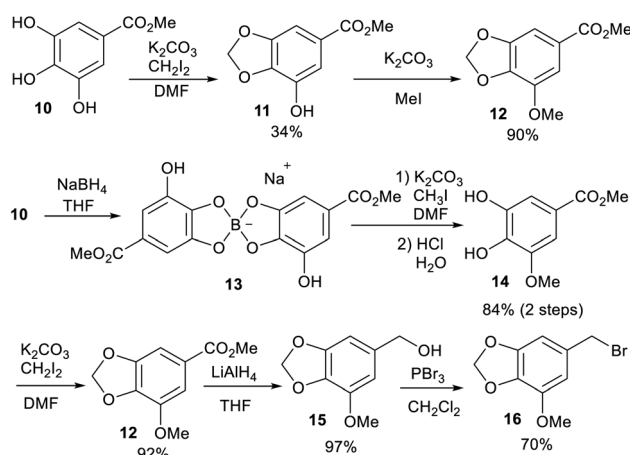
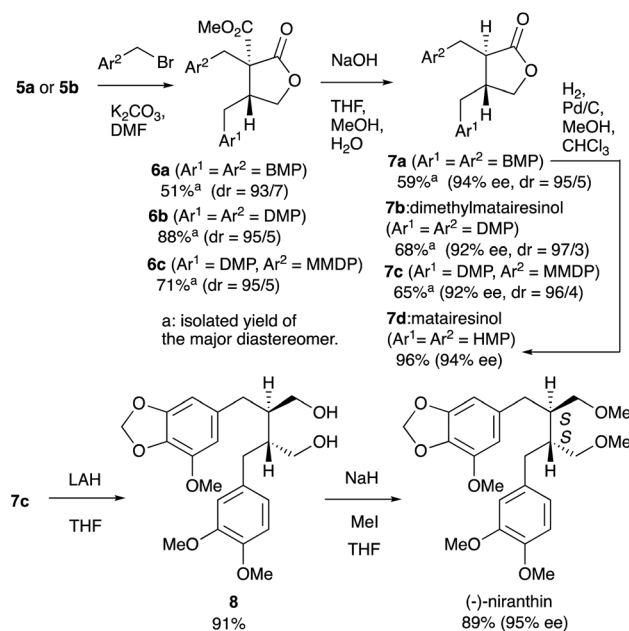
Scheme 2 Enantioselective synthesis of key intermediates 5a and 5b.

aldehydes to alcohols and subsequent lactonization with *p*-TsOH afforded lactones **4a** and **4b** in high yield with high ee. The optical purity of lactones **4a** and **4b** were determined using HPLC analyses on a chiral column, and the ee values of the enantioselective cyclopropanations were estimated based on these HPLC analyses. Next, treatment of bicyclic lactones **4a** and **4b** with hydrogen in the presence of a catalytic amount of Pd-C in AcOEt at 0 °C resulted in a regioselective reductive ring-opening to furnish benzyloxylactones **5a** and **5b** in good to high yield with high dr and high ee. In the hydrolysis step, debenylation of the benzyloxyaryl group did not occur under these mild conditions, *i.e.*, in AcOEt at 0 °C.⁷ⁱ

For the α -benzylation of **5a** and **5b** to afford **6a–c**, the corresponding substituted benzylhalides were necessary. 3-Methoxy-4-benzyloxybenzylbromide and 3,4-dimethoxybenzylbromide were easily prepared by known methods (for details, see the ESI[†]); however, the preparation of 3,4-methylenedioxy-5-

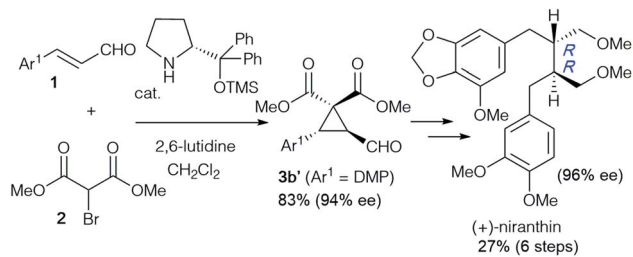
methoxybenzylbromide (**16**) required a modified procedure that involves the regioselective protection of the hydroxy group at the 3-position of 3,4,5-trihydroxybenzene (Scheme 3). The methylenedioxylation of gallic acid (**10**) during the first step resulted in a low yield of **11**.¹⁰ Consequently, we successfully synthesized **12** using a cyclic boron-ester system.¹¹ Arylmethylbromide **16** was derived from ester **12** in two steps in good to high yield.

Next, enolates were generated from lactones **5a** and **5b** using K_2CO_3 in DMF, and successfully attacked the benzylhalides on the less-hindered side to afford α -benzyl lactones **6a–c** with excellent dr values (Scheme 4).^{7i,12} The *trans*- α,β -disubstituted lactones **7a–c** were obtained *via* the hydrolysis of the α -methoxycarbonyllactones **6a–c** followed by decarboxylation. The transformation from the enol form to the keto form gave the thermodynamically favored *trans* products (**7a–c**) with excellent dr values.^{7i,12} Finally, the debenylation of **7a** using a catalytic amount of Pd-C in methanol under a hydrogen atmosphere afforded matairesinol (**7d**) in 96% yield. Thus, the total syntheses of dimethylmatairesinol (**7b**) and matairesinol (**7d**) were achieved, and spectral data of these natural products were consistent with reported data.^{2e,3d} The absolute configuration of these compounds were determined using the known data of optical rotation values. The reduction of lactone **7c** using LAH afforded diol **8** in 91% yield (Scheme 4). Subsequent dimethylation of the resulting diol **8** using NaH and MeI furnished (–)-niranthin in 89% yield with 95% ee.¹³ Spectral data of (–)-niranthin was also consistent with reported data.^{5b,e,6} Following the total synthesis of (–)-niranthin, we also achieved the total synthesis of (+)-niranthin *via* an alternative enantioselective cyclopropanation using a different enantiomeric Hayashi-Jørgensen catalyst derived from *D*-proline instead of *L*-proline (Scheme 5).¹⁴

Scheme 3 Preparation of substituted benzylbromide **16**.

Scheme 4 Alternative asymmetric total synthesis of dimethylmatairesinol, matairesinol, and (–)-niranthin.





Scheme 5 Alternative asymmetric total synthesis of (+)-niranthin.

(-)-Niranthin has been reported to exhibit anti-HBV activity.^{5b,e} Aiming to shed light on the relationship between its enantiomeric structure and activity, we performed a bioassay on the synthesized (-) and (+)-niranthin against not only HBV, but

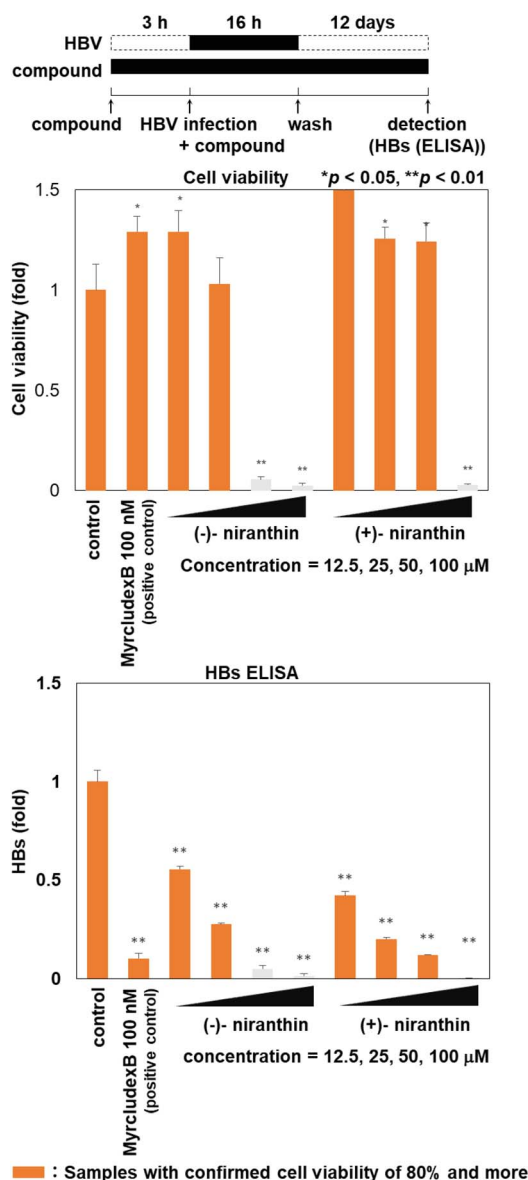


Fig. 1 HBV-infection assay using (-) and (+)-niranthin.

also the influenza virus (IFV). The anti-HBV activity results are summarized in Fig. 1 and 2, while the anti-IFV activity is summarized in Fig. 3 (for details, see the ESI†).

Based on the assays using HBV-infected HepG2-hNTCP-C4 cells and HBV-replicating Hep38.7-tet cells, the amount of HBs antigen decreased in a concentration-dependent manner without apparent cytotoxicity. The 50% inhibition concentration (IC_{50}) in the HBV-infected cells was calculated to be $14.3 \pm 0.994 \mu\text{M}$ for (-)-niranthin and $9.11 \pm 0.998 \mu\text{M}$ for (+)-niranthin (Fig. 1), while the IC_{50} in the HBV-replicating cells was calculated to be $16.2 \pm 0.992 \mu\text{M}$ for (-)-niranthin and $24.2 \pm 0.993 \mu\text{M}$ for (+)-niranthin (Fig. 2). These results show that (-)-niranthin and (+)-niranthin exhibit anti-HBV activity, and that there is no remarkable difference between the anti-HBV activity of both enantiomers. In contrast, based on the bioassay of (-) and (+)-niranthins against IFV using MDCK cells, cytotoxicity of (-)-niranthin appears at $>400 \mu\text{M}$ judging that cell viability without IFV is less than 80%, and (-)-niranthin inhibited IFV-infection to cells in a concentration-dependent manner on the concentration range of non-cytotoxicity, and exhibits anti-IFV activity at 200–400 μM judging that cell viability with IFV is over 50% (Fig. 3). However, (+)-niranthin does not exhibit anti-IFV activity, and similarly to (-)-niranthin, cytotoxicity appears at $>400 \mu\text{M}$. Thus, the anti-

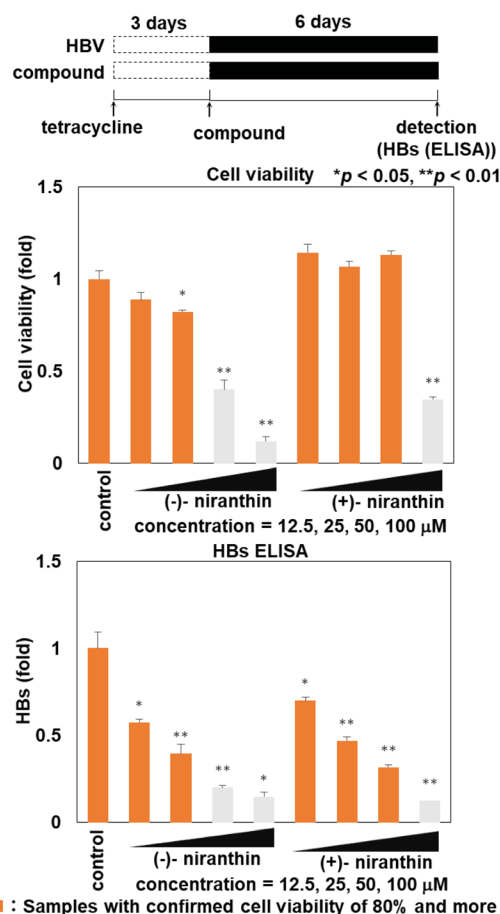


Fig. 2 HBV-replication assay using (-) and (+)-niranthin.



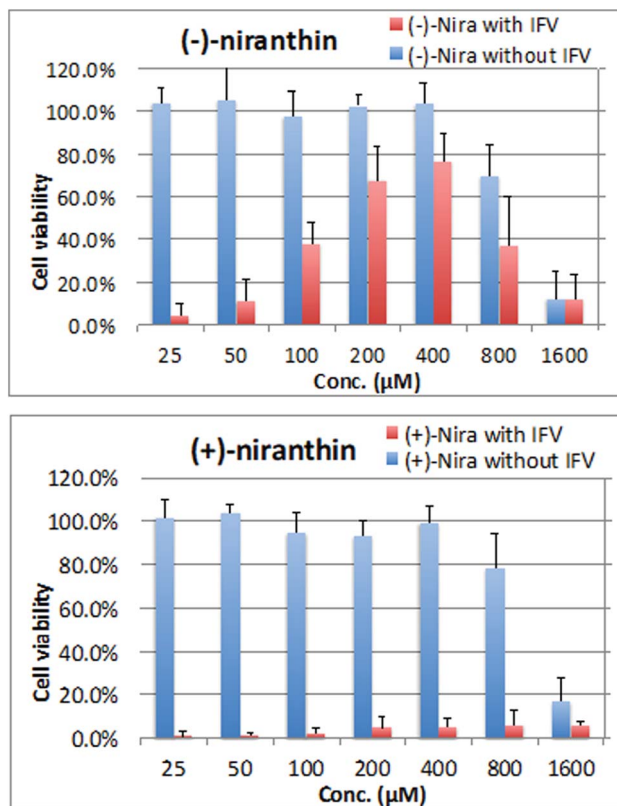
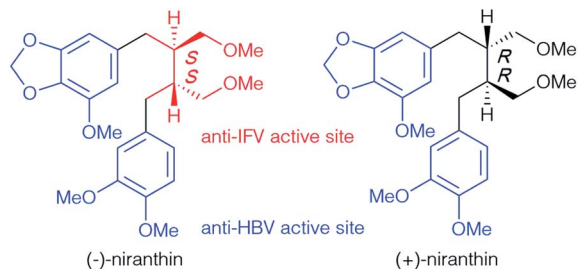


Fig. 3 Growth-inhibition assay of IFV using (–)- and (+)-niranthin.



Scheme 6 A speculation for the bioactive site of niranthin against HBV and IFV.

IFV activity between (–)- and (+)-niranthins is clearly different. Our findings suggest that the enantiomeric site in niranthin endows (–)-niranthin with more potent anti-IFV activity than (+)-niranthin. We speculated that the anti-HBV active site of niranthin might be a part of the molecular structure such as aromatic groups which are far from chiral centers. In contrast, anti-IFV active site of niranthin might be closer to the chiral centers (Scheme 6).

Conclusions

We achieved the asymmetric total syntheses of four bioactive lignans: matairesinol, dimethylmatairesinol, (–)-niranthin, and (+)-niranthin. Key reactions include the Pd-catalyzed reductive ring-opening reaction of enantioenriched cyclopropanes under

a hydrogen atmosphere and a highly stereoselective decarboxylation. Thus, we have achieved the first alternative total synthesis of (–)-niranthin and (+)-niranthin. Using the synthesized niranthin enantiomers, we investigated the relationship between the enantiomer structure and its anti-viral activity against the hepatitis B virus (HBV) and the influenza virus (IFV). The results indicate that although the anti-HBV activity does not differ significantly between these two enantiomers, the anti-IFV activity of (–)-niranthin is more potent than that of (+)-niranthin. This result may be interpreted in terms of a different recognition of the enantiomeric structure of a bioactive compound among different virus species.

Author contributions

R. Ota: investigation for the synthesis of lignans. D. Karasawa: investigation for the synthesis of lignans. M. Oshima: investigation for bioassay of niranthin using HBV. K. Watashi: investigation for bioassay and writing original draft of bioassay using HBV. N. Shimasaki: investigation for bioassay and writing original draft of bioassay using IFV. Y. Nishii: methodology, investigation and writing – original draft.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This research was partially supported by Grants-in-Aid for Scientific Research on Basic Areas (A) '20H00288' and (C) '18K05120' from the JSPS. We thank Prof. Mutsumi Kimura (Shinshu University) for the HRMS measurements.

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- 13 During the isolation process, a recrystallization of (–)-niranthin increased the ee value from 92% ee to 95% ee.
- 14 Similar to (–)-niranthin, a recrystallization of (+)-niranthin increased the ee value from 94% ee to 96% ee.

