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# 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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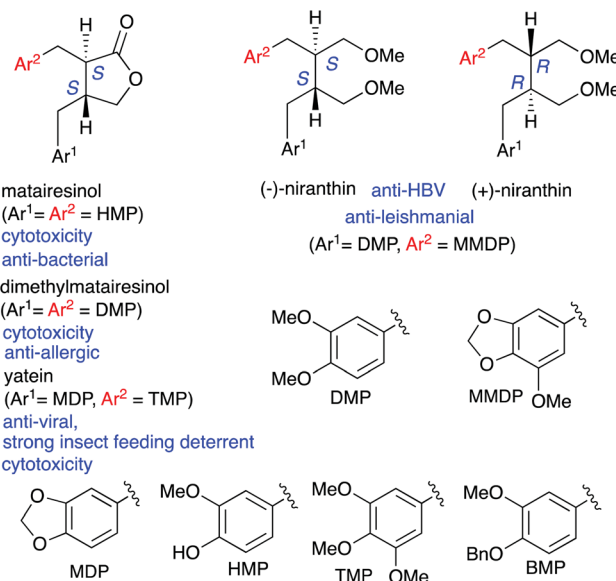
The asymmetric total synthesis of four lignans, dimethylmatairesinol, matairesinol, (–)-niranthin, and (+)-niranthin has been achieved using reductive ring-opening of cyclopropanes. Moreover, we performed bioassays of the synthesized (+)- and (–)-niranthins using hepatitis B and influenza viruses, which 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.<sup>1–5</sup> For example, matairesinol,<sup>2</sup> dimethylmatairesinol,<sup>3</sup> yatein,<sup>4</sup> and niranthin<sup>5</sup> are found in nature and exhibit *e.g.*, cytotoxicity,<sup>2b,d,3b,4b</sup> anti-bacterial,<sup>2c</sup> anti-allergic,<sup>3c</sup> anti-viral,<sup>4d,5b,e</sup> anti-leishmanial,<sup>5d</sup> and strong insect-feeding-deterrent activity.<sup>4c</sup> 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),<sup>5b,e</sup> 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.<sup>6</sup> During our recent studies on the transformation of cyclopropanes,<sup>7</sup> we have reported a reductive ring-opening of enantioenriched donor–acceptor (D–A) cyclopropanes and its application to an asymmetric total synthesis of yatein.<sup>7i</sup> 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,<sup>7i</sup> we attempted to synthesize the enantio-enriched bicyclic lactones **4a** and **4b**.

Initially, the cyclopropanation of enal **1** with dimethyl  $\alpha$ -bromomalonate **2** using the Hayashi–Jørgensen catalyst afforded the desired optically active cyclopropylaldehydes **3a** and **3b** in good to high yield with high ee.<sup>7c,e,h–j,8,9</sup> The reduction of the



Scheme 1 Some examples of bioactive dibenzyl lignans.

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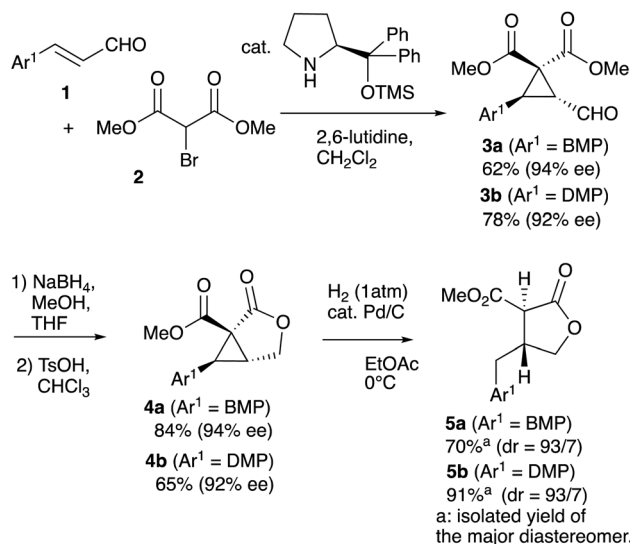
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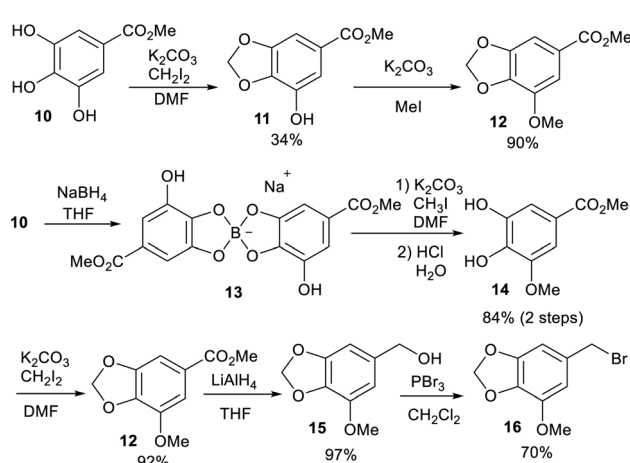
Scheme 2 Enantioselective synthesis of key intermediates 5a and 5b.

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.<sup>7i</sup>

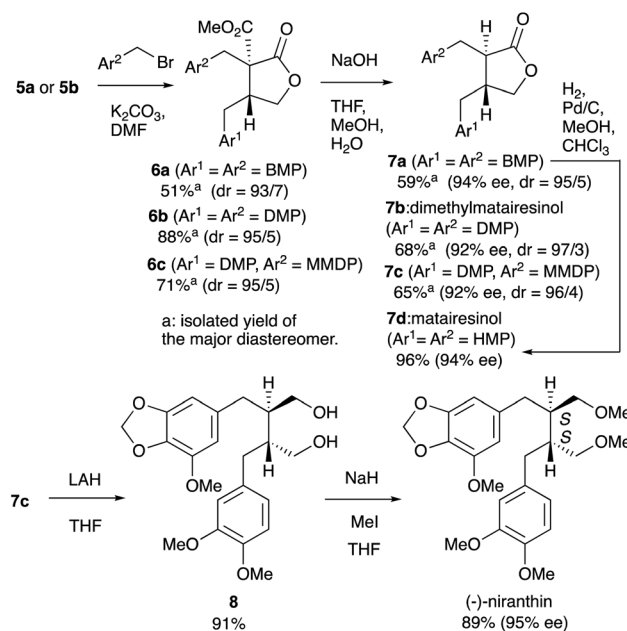
For the  $\alpha$ -benzylation of **5a** and **5b** to afford **6a-c**, the corresponding substituted benzylhalides were necessary. 3-Methoxy-4-benzyloxybenzylbromide and 3,4-dimethoxybenzylbromide were easily prepared by known methods (for details, see the ESI<sup>†</sup>); however, the preparation 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**.<sup>10</sup> Consequently, we successfully synthesized **12** using a cyclic boron-ester system.<sup>11</sup> 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  $\text{K}_2\text{CO}_3$  in DMF, and successfully attacked the benzylhalides on the less-hindered side to afford  $\alpha$ -benzyl lactones **6a-c** with excellent dr values (Scheme 4).<sup>7i,12</sup> The *trans*- $\alpha,\beta$ -disubstituted lactones **7a-c** were obtained *via* the hydrolysis of the  $\alpha$ -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.<sup>7i,12</sup> 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.<sup>2e,3d</sup> 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.<sup>13</sup> Spectral data of (–)-niranthin was also consistent with reported data.<sup>5b,e,6</sup> 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).<sup>14</sup>

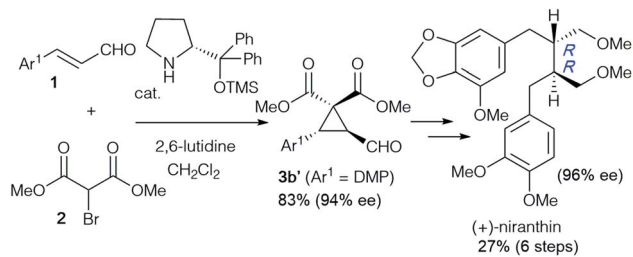


Scheme 3 Preparation of substituted benzylbromide 16.



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.<sup>5b,e</sup> 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

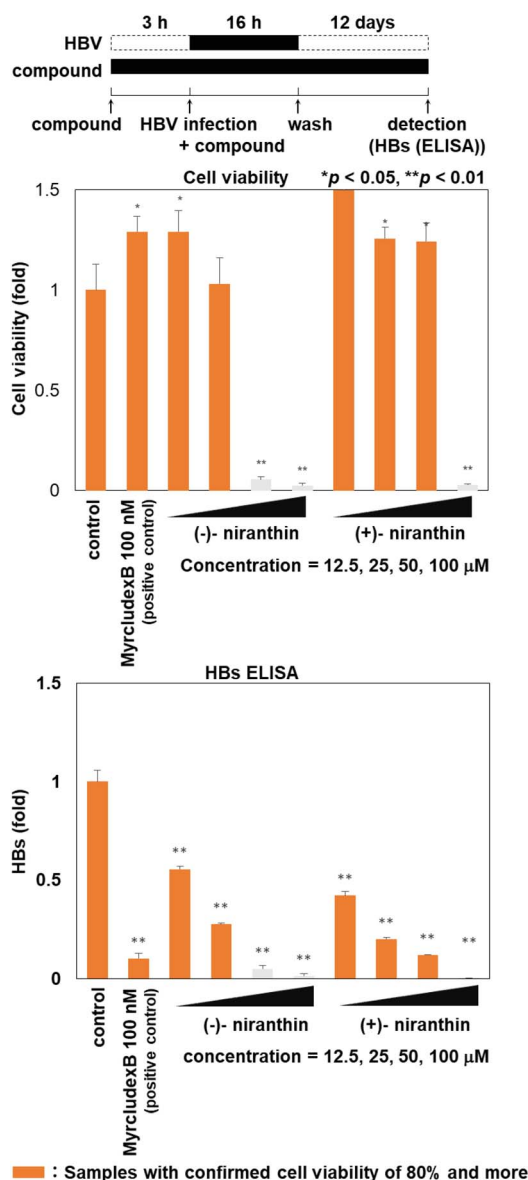


Fig. 1 HBV-infection assay using (-) and (+)-niranthin.

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  $\mu\text{M}$  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-

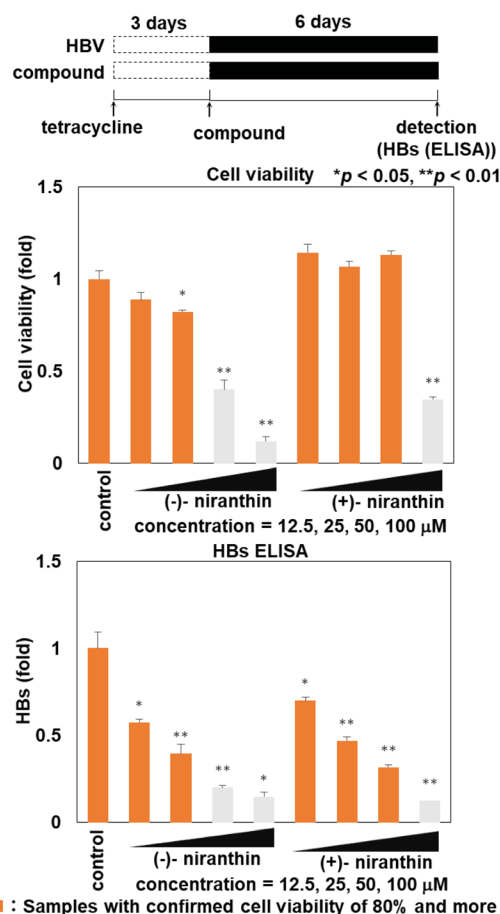


Fig. 2 HBV-replication assay using (-) and (+)-niranthin.



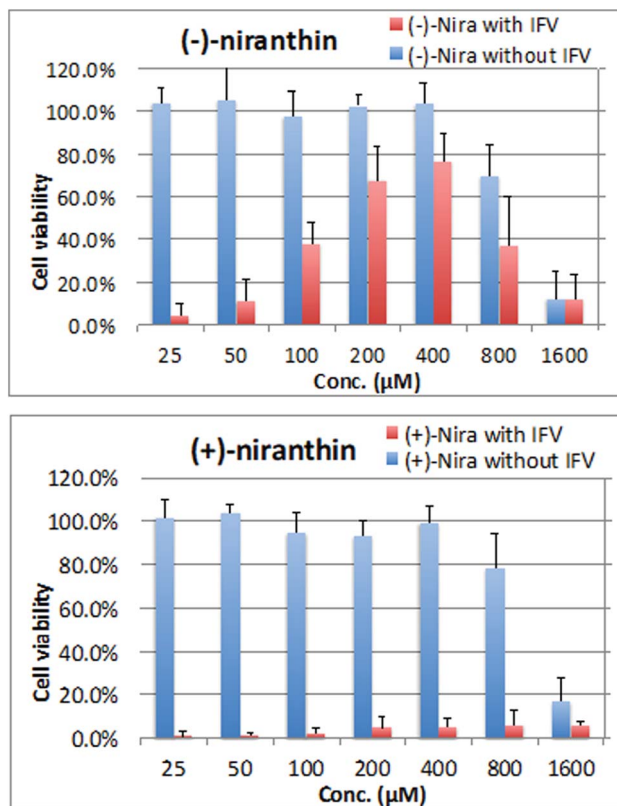
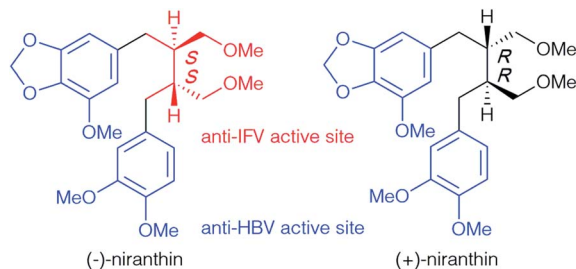


Fig. 3 Growth-inhibition assay of IFV using (–)- and (+)-niranthin.



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

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## Notes and references

- For reviews, see: (a) R. S. Ward, *Nat. Prod. Rep.*, 1999, **16**, 75; (b) M. Saleem, H. J. Kim, M. S. Ali and Y. S. Lee, *Nat. Prod. Rep.*, 2005, **22**, 696; (c) J.-Y. Pan, S.-L. Chen, M.-H. Yang, J. Wu, J. Shinkkonen and K. Zou, *Nat. Prod. Rep.*, 2009, **26**, 1251; (d) R. G. Reynolds, H. Q. A. Nguyen, J. C. T. Reddel and R. J. Thomson, *Nat. Prod. Rep.*, 2022, DOI: 10.1039/D1NP00057H, and other recent references cited therein.
- (a) T. H. Esterfield and J. Bee, *J. Chem. Soc., Trans.*, 1910, **97**, 1028; (b) T. Hirano, M. Gotoh and K. Oka, *Life Sci.*, 1994, **55**, 1061; (c) Y. Kumarasamy, L. Nahar, P. J. Cox, L. N. Dinan, C. A. Ferguson, D. A. Finnie, M. Jaspars and S. D. Sarker, *Pharm. Biol.*, 2003, **41**, 203; (d) S. Su, X. Cheng and M. Wink, *J. Pharm. Pharmacol.*, 2015, **67**, 1316, for a reference of synthesis of matairesinol, see: (e) P. C. Eklund, F. J. Sundell, A. I. Smeds and R. E. Sjöholm, *Org. Biomol. Chem.*, 2004, **2**, 2229.
- (a) D. Takaoka, N. Takamatsu, Y. Saheki, K. Kono, C. Nakaoka and M. Hiroi, *Nippon Kagaku Kaishi*, 1975, **12**, 2192; (b) S.-T. Chang, D. S.-Y. Wang, C.-L. Wu, S.-G. Shiah, Y.-H. Kuo and C.-J. Chang, *Phytochemistry*, 2000, **55**, 227; (c) H. Tanabe, R. Fukutomi, K. Yasui, A. Kaneko, S. Imai,



- T. Nakayama and M. Isemura, *J. Health Sci.*, 2011, **57**, 184, for a recent reference of synthesis of dimatairesinol, see: (d) S. Yamauchi, A. Nishimoto, H. Nishiwaki, K. Nishi and T. Sugahara, *Bioorg. Med. Chem. Lett.*, 2020, **30**, 127191.
- 4 (a) P. B. McDoniel and J. R. Cole, *J. Pharm. Sci.*, 1972, **61**, 1992; (b) M. Novelo, J. G. Cruz, L. Hernández, R. Pereda-Miranda, H. Chai, W. Mar and J. M. Pezzuto, *J. Nat. Prod.*, 1993, **56**, 1728; (c) J. Harmatha and J. Nawrot, *Entomol. Exp. Appl.*, 2002, **104**, 51; (d) Y.-C. Kuo, Y.-H. Kuo, Y.-L. Lin and W.-J. Tsai, *Antiviral Res.*, 2006, **70**, 112.
- 5 For the reference regarding the isolation of natural (–)-niranthin, see: (a) A. S. R. Anjaneyulu, K. J. Rao, L. R. Row and C. Subrahmanyam, *Tetrahedron*, 1973, **29**, 1291, for references regarding bioassays using isolated natural (–)-niranthin, see: (b) R.-L. Huang, Y.-L. Huang, J.-C. Ou, C.-C. Chen, F.-L. Hsu and C. Chang, *Phytother. Res.*, 2003, **17**, 449; (c) C. A. L. Kassuya, A. Silvestre, O. Menezes-de-Lima, D. M. Marotta, V. L. G. Rehder and J. B. Calixto, *Eur. J. Pharmacol.*, 2006, **546**, 182, (anti-inflammatory); (d) S. Chowdhury, T. Mukherjee, R. Mukhopadhyay, B. Mukherjee, S. Sengupta, S. Chattopadhyay, P. Jaisankar, S. Roy and H. K. Majumder, *EMBO Mol. Med.*, 2012, **4**, 1126; (e) S. Liu, W. Wei, K. Shi, X. Cao, M. Zhou and Z. Liu, *J. Ethnopharmacol.*, 2014, **155**, 1061.
- 6 For a reference regarding the total synthesis of (±)-niranthin, see: G. E. Schneiders and R. Stevenson, *Org. Prep. Proced. Int.*, 1982, **14**, 1.
- 7 For selected references in last decade, see: (a) E. Yoshida, K. Nishida, K. Toriyabe, R. Taguchi, J. Motoyoshiya and Y. Nishii, *Chem. Lett.*, 2010, **39**, 194; (b) D. Sakuma, J. Ito, R. Sakai, R. Taguchi and Y. Nishii, *Chem. Lett.*, 2014, **43**, 610; (c) J. Ito, D. Sakuma and Y. Nishii, *Chem. Lett.*, 2015, **44**, 297; (d) D. Sakuma, Y. Yamada, K. Sasazawa and Y. Nishii, *Chem. Lett.*, 2015, **44**, 818; (e) S. Takada, K. Iwata, T. Yubune and Y. Nishii, *Tetrahedron Lett.*, 2016, **57**, 2422; (f) S. Takada, T. Saito, K. Iwata and Y. Nishii, *Asian J. Org. Chem.*, 2016, **5**, 1225; (g) K. Sasazawa, S. Takada, T. Yubune, N. Takaki, R. Ota and Y. Nishii, *Chem. Lett.*, 2017, **46**, 524; (h) S. Takada, N. Takaki, K. Yamada and Y. Nishii, *Org. Biomol. Chem.*, 2017, **15**, 2443; (i) Y. Sone, Y. Kimura, R. Ota, T. Mochizuki, J. Ito and Y. Nishii, *Eur. J. Org. Chem.*, 2017, 2842; (j) Y. Kimura, Y. Sone, T. Saito, T. Mochizuki and Y. Nishii, *Asian J. Org. Chem.*, 2017, **6**, 977; (k) T. Saito, Y. Shimizu, Y. Araki, Y. Ohgami, Y. Kitazawa and Y. Nishii, *Eur. J. Org. Chem.*, and other references cited therein, DOI: 10.1002/ejoc.202101213.
- 8 For a reference regarding the asymmetric cyclopropanation using a Hayashi-Jørgensen catalyst, see: H. Xie, L. Zu, H. Li, J. Wang and W. Wang, *J. Am. Chem. Soc.*, 2007, **129**, 10886.
- 9 For references regarding the original Hayashi-Jørgensen catalyst, see: (a) M. Marigo, T. C. Wabnitz, D. Fielenbach and K. A. Jørgensen, *Angew. Chem., Int. Ed.*, 2005, **44**, 794; (b) Y. Hayashi, H. Gotoh, T. Hayashi and M. Shoji, *Angew. Chem., Int. Ed.*, 2005, **44**, 4212; (c) J. Franzén, M. Marigo, D. Fielenbach, T. C. Wabnitz, A. Kjærsgaard and K. A. Jørgensen, *J. Am. Chem. Soc.*, 2005, **127**, 18296, for a recent review of total synthesis and patents using Hayashi-Jørgensen catalyst, see; (d) G. J. Reyes-Rodríguez, N. M. Rezayee, A. Vidal-Albalat and K. A. Jørgensen, *Chem. Rev.*, 2019, **119**, 4221.
- 10 (a) C. Song, P. Zhao, Z. Hu, S. Shi, Y. Cui and J. Chang, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 2297; (b) S. Takaoka, N. Takaoka, Y. Minoshima, J.-M. Huang, M. Kubo, K. Harada, H. Hioki and Y. Fukuyama, *Tetrahedron*, 2009, **65**, 8354.
- 11 (a) K. Cormier, R. D. Curry, M. P. Betsch, J. A. Goguen, C. M. Vogels, A. Decken, S. Turcotte and S. A. Westcott, *J. Heterocycl. Chem.*, 2016, **53**, 1807; (b) G. R. Pettit and S. B. Singh, *Can. J. Chem.*, 1987, **65**, 2390.
- 12 (a) L. Ferrié, D. Bouyssi and G. Balme, *Org. Lett.*, 2005, **7**, 3143; (b) K.-i. Yamada, T. Konishi, M. Nakano, S. Fujii, R. Cadou, Y. Yamamoto and K. Tomioka, *J. Org. Chem.*, 2012, **77**, 5775.
- 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.

