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Divergent Synthesis of Nerolidol-Type Sesquiterpenoids Produced by Soil Bacterium from an Identical Starting Material via Diepoxide Precursors: Stereochemical Revision and Absolute Configuration of a THF Natural Product

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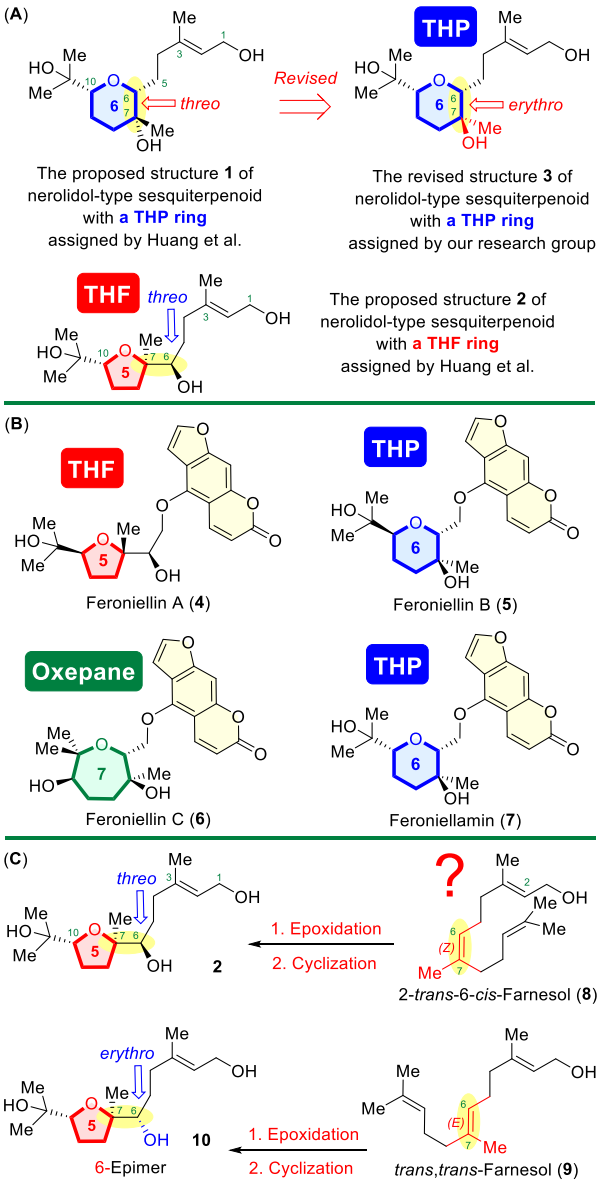
A divergent asymmetric synthesis of two nerolidol-type sesquiterpenoids with a five- or a six-membered ether ring was established from an identical commercially available *trans*,*trans*-farnesyl acetate through the cyclization of diepoxide precursors under simple acidic or neutral conditions, respectively. In addition, the relative configuration of a nerolidol-type sesquiterpenoid with a tetrahydrofuran ring was revised. Its absolute configuration was determined by its asymmetric synthesis and a modified Mosher's analysis. Furthermore, the cytotoxicity and nitric oxide production inhibitory activity of the synthesized compounds were assessed.

Keywords: Divergent synthesis, Stereochemical revision, Absolute configuration

In 2016, Huang et al. isolated two new nerolidol-type sesquiterpenoids from the fermentation broth of the forest soil bacterium *Streptomyces scopuliridis*.[1] The chemical structures of nerolidol-type sesquiterpenoids include a five-membered ether (tetrahydrofuran, THF) ring or a six-membered ether (tetrahydropyran, THP) ring which attaches an alkyl substituent including a trisubstituted olefin and a 1-hydroxy-1-methylethyl group. Their biological activities had yet to be discovered. Although their relative configurations have been assigned (as shown in the proposed structures **1** and **2** with the *threo* configuration between the vicinal oxygens at C6 and C7), their absolute configurations have yet to be determined (Scheme 1A). Our research group synthesized a 7-epimer of the proposed structure **1** (namely **3**) in 2019 using our original cyclization in H_2O from a diepoxide under neutral conditions, and the correct structure of nerolidol-type sesquiterpenoid with a THP ring has been revised to **3** with an *erythro* configuration, and its absolute configuration has been assigned by its asymmetric synthesis.[2] However, no one has reported the synthesis and biological activity of the nerolidol-type sesquiterpenoid with a THF ring.

The Cane-Celmer-Westley hypothesis[3] proposed the epoxide-opening cascade biogenesis for ionophoric polyether antibiotics and epoxide-opening cascades have been used to quickly construct polyether skeletons.[4] Previously, authors have synthesized a marine natural polyether teurilene, isolated from the red alga *Laurencia obtusa*, from squalene tetraepoxide via three 5-*exo*-selective cascade cyclizations triggered by Brønsted acid-catalyzed terminal epoxide hydrolysis.[5] Then, in the epoxide-opening cascade cyclization of polyepoxide, we described a method for switching the cyclization mode from THF to THP formation.[2] The THP formation proceeded via the epoxonium ion

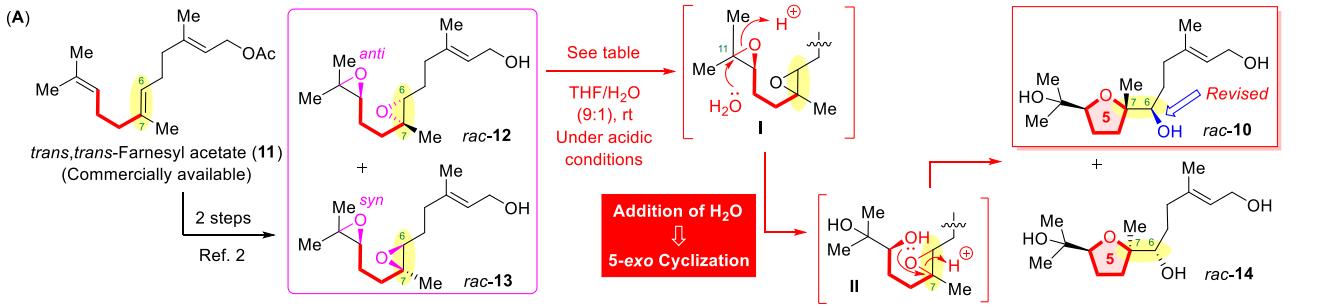


Scheme 1. (**A**) Chemical structures of nerolidol-type sesquiterpenoids derived from the culture broth of *Streptomyces* sp., (**B**) chemical structures of feroniellins derived from a fruit tree in Thailand, and (**C**) hypothetical biogenesis of the nerolidol-type sesquiterpenoid with a THF ring.

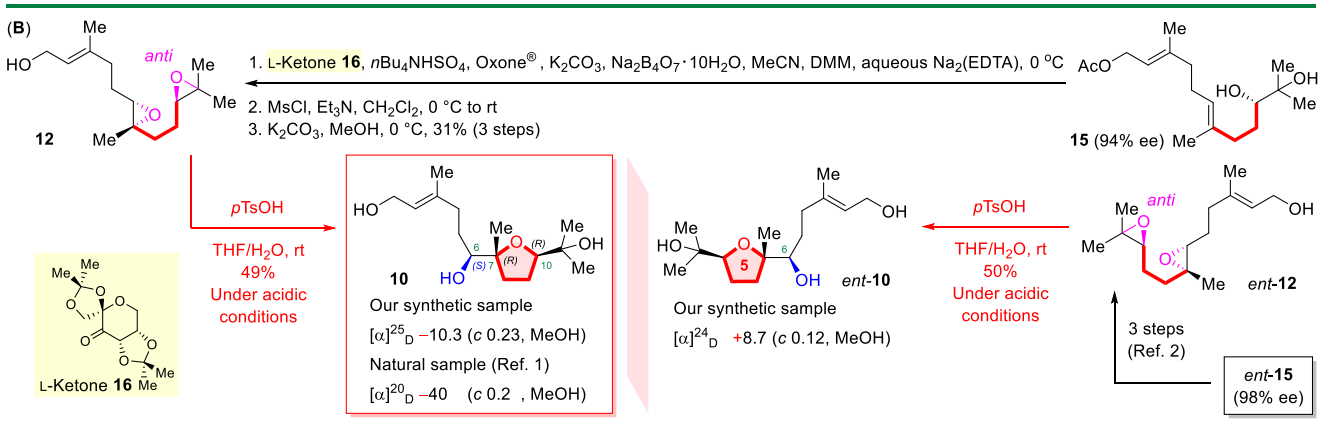
intermediate by simply heating in neutral water. Furthermore, we developed a "ring-size-divergent" synthetic strategy that allowed us to construct the five-, six-, and seven-membered ether rings from identical diepoxide precursors under simple acidic or neutral conditions, and demonstrated the application of the synthetic strategy to the divergent syntheses of feroniellins derived from a fruit tree in Thailand (Scheme 1B).[6] The authors present a concise and comprehensive synthesis of nerolidol-type sesquiterpenoids using the "ring-size-divergent" strategy, which resulted in revision of the proposed structure **2**, and the determination of its absolute configuration. Furthermore, the cytotoxicity and the nitric oxide (NO) production inhibitory activity of the synthesized compounds were also evaluated.

As shown in Scheme 1C, if the proposed THF ring of the nerolidol-type sesquiterpenoid structure **2** is generated by regio- and stereoselective epoxidation of the two alkenes followed by an oxacyclization like the THF formation in feroniellin A (**4**) (Scheme 1B) from a diepoxide,[6] the proposed structure **2** would be biosynthesized from 2-*trans*-6-*cis*-farnesol (**8**) because of the *threo* configuration between the vicinal oxygens at C6 and C7 in **2**. However, we consider that this case is very rare in nature, and the THF-type sesquiterpenoid could be derived from *trans,trans*-farnesol (**9**), which is ubiquitous in nature.[7] As a result, we determined to synthesize a 6-epimer of the proposed structure **2** (namely **10**) with an *erythro* configuration derived from **9**.

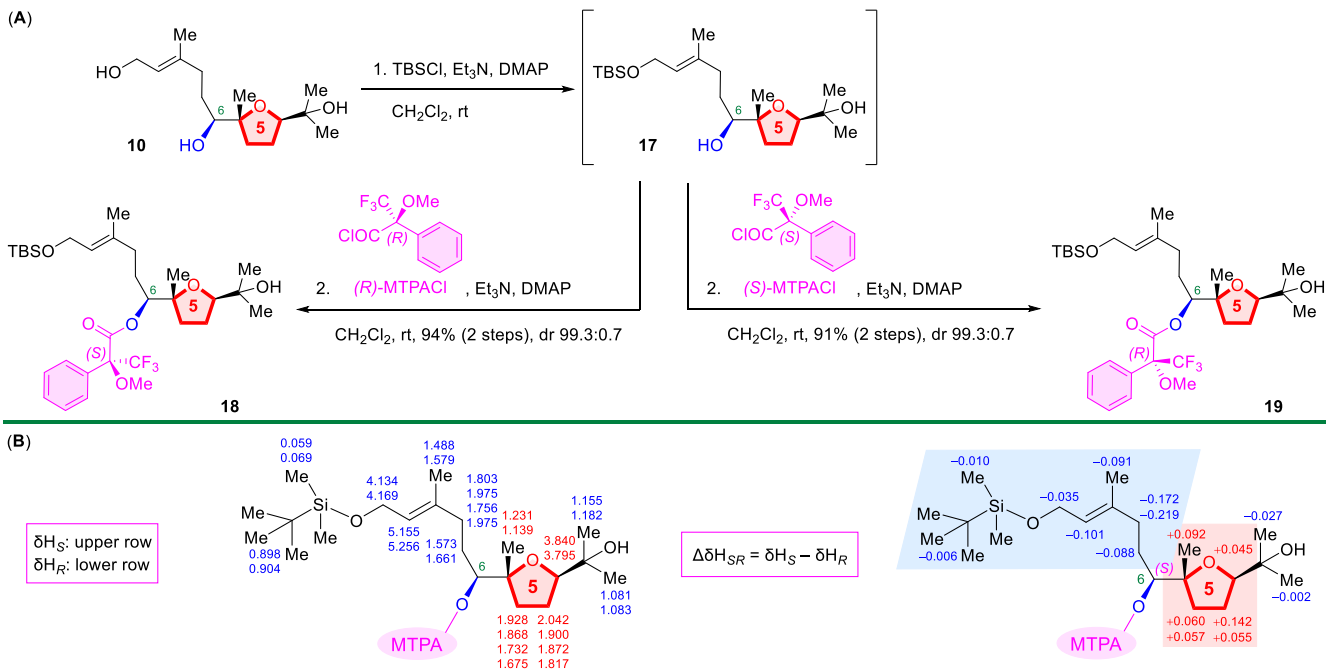
From commercially available *trans,trans*-farnesyl acetate (**11**), we developed the racemic synthesis of the nerolidol-type sesquiterpenoid with a THF ring (Scheme 2A). Our previous synthetic method yielded the cyclization precursor, which was an inseparable mixture of *anti*- and *syn*-diepoxides *rac*-**12** and *rac*-**13**, respectively, from **11**.[2] Various Brønsted acids were used to investigate the cyclization of *rac*-**12** and *rac*-**13** in acidic aqueous media in this study. Our proposed structure *rac*-**10** with an *erythro* configuration of nerolidol-type sesquiterpenoid with a THF ring was isolated in 12% yield after cyclization of *rac*-**12** and *rac*-**13** with a protic acid H_2SO_4, as well as its diastereomer *rac*-**14** (13%) (Scheme 2A, Entry 1).[8a,9] The reaction mechanism commenced with protic acid-catalyzed hydrolysis at the more substituted C11 of the more water-accessible terminal epoxide in **I**. The mechanism including the next kinetically favored 5-*exo* cyclization could explain the stereospecific construction of THF products, as expected.[2,5,6] The spectral data of synthetic *rac*-**10** were identical to those reported for the natural sample.[1,8b] As a result, the structure **2** of the proposed nerolidol-type sesquiterpenoid with a THF ring must be revised to *rac*-**10**. Among various Brønsted acids (H_2SO_4, TfOH, TFA, and *p*TsOH), concentrations, and reaction times investigated, it



Entry	Catalyst	Concentration (M)	Time (h)	Yields (%) *rac*-**10**	Yields (%) *rac*-**14**	Entry	Catalyst	Concentration (M)	Time (h)	Yields (%) *rac*-**10**	Yields (%) *rac*-**14**
1	H_2SO_4	0.072	3	12	13	4	*p*TsOH	0.072	3	16	16
2	TfOH	0.072	3	10	10	5	TFA	0.036	6	21	21
3	TFA	0.072	6	17	17	6	*p*TsOH	0.036	2.5	23	24



Scheme 2. (**A**) Racemic synthesis of nerolidol-type sesquiterpenoid with a THF ring derived from soil bacterium, (**B**) its asymmetric synthesis, and the determination of its absolute configuration.



Scheme 3. (**A**) Syntheses of MTPA esters **18** and **19** of nerolidol-type sesquiterpenoid **10** with a THF ring, and (**B**) its modified Mosher's analysis.

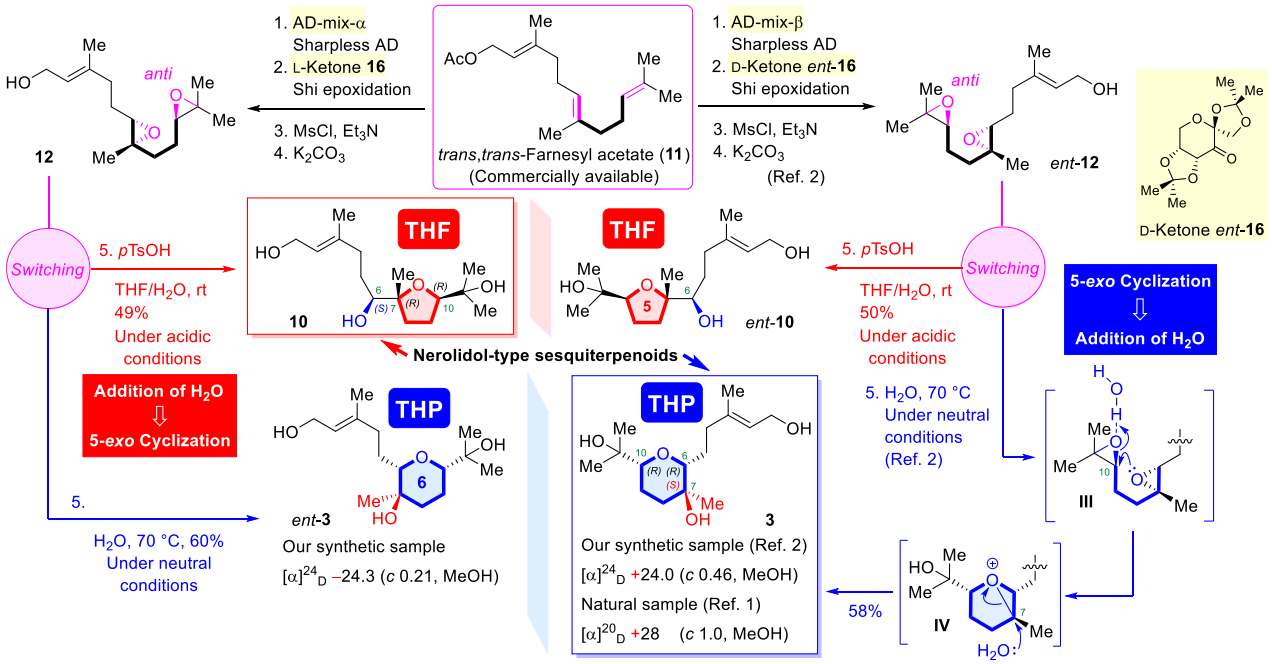
was discovered that the Entry 6 (catalyst: *p*TsOH, concentration: 0.036 M, and reaction time: 2.5 h) promoted the cyclization with a higher yield of *rac*-**10** (23% based on the diastereomeric mixture of *rac*-**12** and *rac*-**13**) than the other conditions (Scheme 2A, Entries 1–5).[9]

To determine the absolute configuration of nerolidol-type sesquiterpenoid having a THF ring, a divergent asymmetric total synthesis was performed (Scheme 2B). The asymmetric synthesis began with the optically active known diol **15** (94% ee), which was prepared via Sharpless asymmetric dihydroxylation (Sharpless AD) of commercially available *trans*,*trans*-farnesyl acetate.[10] According to our previous report,[2] after the stereoselective epoxidation of the trisubstituted olefin in **15** using Shi's L-ketone **16**,[11] the mesylation of a secondary hydroxy group followed by the formation of the terminal epoxide and the deprotection of an acetyl group, yielded the cyclization precursor **12**. The cyclization of **12** with Brønsted acid *p*TsOH yielded nerolidol-type sesquiterpenoid **10** with a THF ring and an optical rotation of $[\alpha]^{25}_{D}$ –10.3 (*c* 0.23, MeOH) that was identical in sign to that of the natural product, $[\alpha]^{20}_{D}$ –40 (*c* 0.2, MeOH).[1] We also synthesized its enantiomer *ent*-**10** from the known optically active *ent*-**15** (98% ee)[10] via *ent*-**12** using the four synthetic steps previously reported by authors,[2] including the epoxidation reaction using Shi's D-ketone *ent*-**16**.[11] The optical rotation of *ent*-**10**, $[\alpha]^{24}_{D}$ +8.7 (*c* 0.12, MeOH), was opposite in sign to that of the natural sample.

The absolute configuration of C6 stereochemistry of **10** was confirmed using MTPA esterifications of synthetic nerolidol-type sesquiterpenoid **10** with a THF ring.[8c,12] Following TBS protection of a primary hydroxy group in **10**, the esterification of **17** with (*R*)- and (*S*)-MTPACl using Et_3N and DMAP yielded MTPA esters **18** and **19** in high yields, respectively (94% and 91% in 2 steps) (Scheme 3A).[8a] Scheme 3B summarizes Mosher's analysis of **18** and **19**. The absolute configuration at C6 stereochemistry in **10** is (*S*)-configuration, according to the analytical result. As a result, the authors propose that the previously unknown absolute configuration of nerolidol-type sesquiterpenoid with a THF ring was determined to be (6*S*, 7*R*, 10*R*).

As summarized in Scheme 4, a divergent synthesis of two nerolidol-type sesquiterpenoids with a five- or a six-membered ether ring was established from commercially available *trans*,*trans*-farnesyl acetate (**11**) through the cyclization of diepoxide precursors under simple acidic or neutral conditions, respectively. Authors synthesized natural product **10** with a THF ring from **11** via diepoxide **12** through the five steps including Sharpless AD of **11** using AD-mix-α,[10] Shi epoxidation using L-ketone **16**,[11] and the cyclization of **12** by the use of Brønsted acid, and also synthesized its enantiomer *ent*-**10** via the same synthetic method of reactions as that to **10** except for using AD-mix-β and D-ketone. We previously investigated the cyclization of *ent*-**12** in neutral water and obtained the desired THP-type sesquiterpenoid **3**.[2] Its absolute configuration was determined to be (6*R*, 7*S*, 10*R*). The THP ring was produced stereospecifically via a kinetically favored *exo*-selective S_N2 attack of the internal epoxide oxygen to the less substituted C10 of the H_2O-activated terminal epoxide in **III**, followed by a ring-opening process by H_2O at C7 in epoxonium ion **IV**. In this work, the *exo*-selective epoxide-opening cascade of **12** in H_2O was then performed, yielding the THP product *ent*-**3** in 60% yield. The optical rotation of synthetic *ent*-**3** was $[\alpha]^{24}_{D}$ –24.3 (*c* 0.21, MeOH). As a result, we were able to confirm that *ent*-**3** has the opposite optical rotation.

Although the biological activity of the natural nerolidol-type sesquiterpenoids with a THF ring had not been reported, it is reported that many sesquiterpenoids exhibit strong cytotoxic activity[13] and NO production inhibitory activity.[14] Therefore, THF ringed compounds, nerolidol-type



Scheme 4. Summary for the divergent asymmetric synthesis of nerolidol-type sesquiterpenoids.

sesquiterpenoid **10**, its enantiomer *ent*-**10**, TBS ether **17**, and MTPA esters **18** and **19** were tested for cytotoxicity against a murine leukemia cell line (P388).[8d] Compound **17** had the highest cytotoxicity with an IC_{50} value of 23 μM while IC_{50} values of compounds **10**, *ent*-**10**, **18**, and **19** were more than 50 μM. These findings suggested that protecting the primary hydroxy group with a TBS group increased the cytotoxicity of natural product **10**. The cytotoxicity of compound **17** was reduced by MTPA esterification of a secondary hydroxy group. Furthermore, the anti-inflammatory activity of the synthesized compounds was assessed by looking at their ability to inhibit NO production in lipopolysaccharide (LPS)-induced murine macrophage cell line (RAW264).[8d] However, at concentrations above 80% cell viability, none of the compounds had an inhibitory effect on NO production though quercetin (a positive control) had NO inhibitory activity without cytotoxicity.

Under simple acidic or neutral conditions, the divergent asymmetric synthesis of two nerolidol-type sesquiterpenoids with the five- or six-membered ether rings, respectively, was achieved from an identical commercially available *trans*,*trans*-farnesyl acetate via diepoxide precursors. In addition, the relative configuration of a THF ringed nerolidol-type sesquiterpenoid was revised to *rac*-**10**. The asymmetric synthesis and modified Mosher's analysis were carried out to determine its absolute configuration to be (6*S*, 7*R*, 10*R*). Furthermore, the TBS ether of a natural nerolidol-type sesquiterpenoid had the highest cytotoxicity. Application of this synthetic strategy to other natural products is currently being researched.

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Supporting Information is available on http://dx.doi.org/10.1246/cl.******.

References and Notes

1 L. Li, R. Liu, L. Han, Y. Jiang, J. Liu, Y. Li, C. Yuan, X. Huang, *Magn. Reson. Chem.* **2016**, *54*, 606.
2 K. Nishikawa, K. Morita, S. Hashimoto, A. Hoshino, T. Ikeuchi, M. Kumagai, Y. Morimoto, *Angew. Chem., Int. Ed.* **2019**, *58*, 10168.
3 a) J. W. Westley in Antibiotics IV. Biosynthesis (Ed.: J. W. Corcoran), Springer-Verlag, New York, **1981**, pp. 41–73. b) D. E. Cane, W. D. Celmer, J. W. Westley, *J. Am. Chem. Soc.* **1983**, *105*, 3594.
4 I. Vilotijevic, T. F. Jamison, *Angew. Chem., Int. Ed.* **2009**, *48*, 5250.
5 a) Y. Morimoto, E. Takeuchi, H. Kambara, T. Kodama, Y. Tachi, K. Nishikawa, *Org. Lett.* **2013**, *15*, 2966. b) T. Kodama, S. Aoki, T. Matsuo, Y. Tachi, K. Nishikawa, Y. Morimoto, *Chem. Lett.* **2014**, *43*, 1662.
6 K. Nishikawa, T. Niwa, K. Nishikibe, M. Kumagai, Y. Morimoto, *Chem. Eur. J.* **2021**, *27*, 11045.
7 a) O. E. Edwards, J. L. Douglas, B. Mootoo, *Can. J. Chem.* **1970**, *48*, 2517. b) L. H. Zalkow, J. T. Baxter, R. J. McClure, Jr., M. M. Gordon, *J. Nat. Prod.* **1980**, *43*, 598.
8 a) The relative configurations of compounds *rac*-**10**, *rac*-**14**, **18**, and **19** were determined by the NOESY spectra; see the Supporting Information (SI). b) For the comparison of ^{1}H-NMR and ^{13}C-NMR spectra between synthetic *rac*-**10** and the natural nerolidol-type sesquiterpenoid with a THF ring; see the SI. c) For modified Mosher's analyses of **10**, the MTPA esterification of two hydroxy groups in **10** was carried out. Unfortunately, the opposite signs of the $\Delta\delta H_{SR}$ values were mixed; see the SI. d) For the detailed data of cytotoxicities (P388) of the synthesized compounds (Figure S1), their NO production inhibitory activities, and their cell viabilities (Figure S2); see the SI.

9 In these reactions, the starting diepoxides have completely been consumed. Many by-products including the THP products were observed as the minor products in its TLC analysis. Their separations and structural determinations are very difficult, but their examinations are ongoing.
10 G. Vidari, A. Dapiaggi, G. Zanoni, L. Garlaschelli, *Tetrahedron Lett.* **1993**, *34*, 6485.
11 a) Z.-X. Wang, Y. Tu, M. Frohn, J.-R. Zhang, Y. Shi, *J. Am. Chem. Soc.* **1997**, *119*, 11224. For a review, see: b) O. A. Wong, Y. Shi, *Chem. Rev.* **2008**, *108*, 3958.
12 M. Kodama, S. Yoshio, T. Tabata, Y. Deguchi, Y. Sekiya, Y. Fukuyama, *Tetrahedron Lett.* **1997**, *38*, 4627.
13 For a review, see: O.-F. Chen, Z.-P. Liu, F.-P. Wang, *Mini-Rev. Med Chem.* **2011**, *11*, 1153.
14 For a review, see: J. Ge, Z. Liu, Z. Zhong, L. Wang, X. Zhuo, J. Li, X. Jiang, X.-Y. Ye, T. Xie, R. Bai, *Bioorg. Chem.* **2022**, *124*, 105817

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Title(required)	Divergent Synthesis of Nerolidol-Type Sesquiterpenoids Produced by Soil Bacterium from an Identical Starting Material via Diepoxide Precursors: Stereochemical Revision and Absolute Configuration of a THF Natural Product
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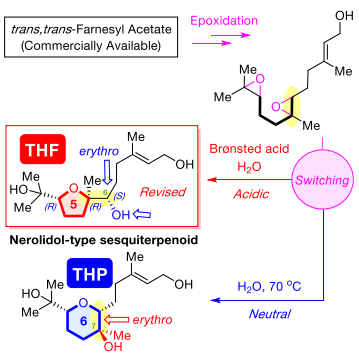

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