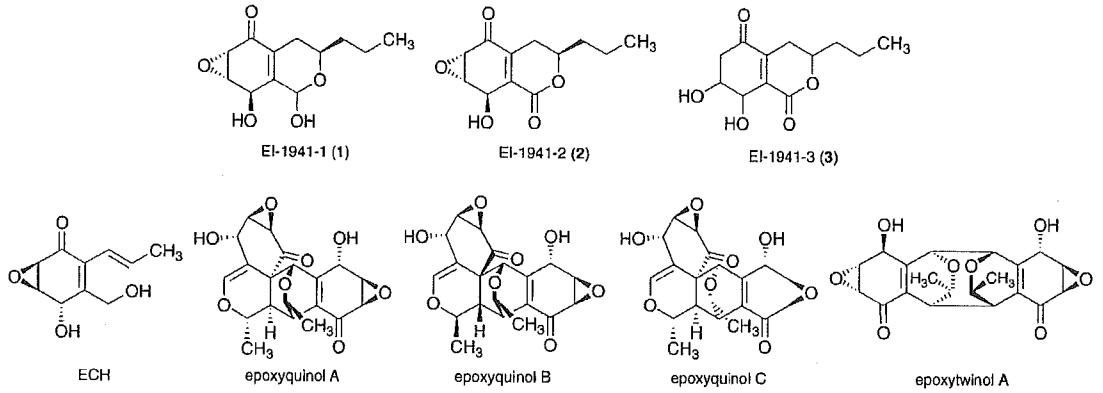


FIGURE 1. Natural products of epoxyquinol monomers and dimers.

bacteria, and moderate activity against *Proteus vulgaris*.[4] A more potent and effective ICE inhibitor would be desired, and EI-1941−1 and −2 would be suitable lead compounds for the study of the structure−activity relationship.

Structurally, EI-1941−1 and EI-1941−2 have an epoxyquinone core and a side chain. We have been interested in the synthesis and biology of epoxyquinone derivatives such as ECH ((2*R*,3*R*,4*S*)-2,3-epoxy-4-hydroxy-5-hydroxymethyl-6-(1*E*)-propenylcyclohex-5-en-1-one),[5] an inhibitor of Fas-mediated apoptosis, and its dimer, epoxyquinols A, B, and C, and epoxytwinol A, angiogenesis inhibitors.[6] At the time that we started this project, the relative and absolute stereochemistries of EI-1941−1, −2, and −3 were not known. As most of the epoxyquinol natural products have a trans relationship between the epoxide and the 4-hydroxy group on the cyclohexenone,[7] work on a synthetic route by which the two diastereomers (EI-1941−2 and epi-EI-1941−2) can be generated with high optical purity was undertaken in order to determine the relative stereochemistries. As for the absolute stereochemistry, with the structural similarity between EI-1941 and ECH, we tried to synthesize the (1*R*,5*S*,6*R*)-1,6-epoxy-5-hydroxycyclohexenone derivative as our first target.

When we had nearly finished the synthesis of the targeted isomer of EI-1941−2 and its epimer, the absolute and relative stereochemistries of EI-1941−1 and −2 were reported (see Figure 1),[8] whereas those of EI-1941−3 were not determined because of its low availability from the fermentation broth. Those determinations were made on the basis of the crystallographic analysis of the *p*-bromobenzoyl ester of EI-1941−2 and the chemical correlation between EI-1941−1 and −2. These results indicate that the compounds we have synthesized are opposite enantiomers of the natural EI-1941−2 and its epimer, which was communicated in a previous letter.[9]

As for the biosynthesis, we postulated the following path: Oxidation of alcohol **4** would afford aldehyde **5**, from which 6π-electrocyclization[10] proceeds to generate 2*H*-pyran **6**. Hydration and isomerization would afford EI-1941−1, the oxidation of which would provide EI-1941−2 (eq 1). Another possible path involves the oxidation of alcohol **4** to carboxylic acid **7**, from which 6π-electrocyclization proceeds to generate hydroxy-2*H*-pyran **8** (eq 2). Isomerization would afford EI-1941−2, the reduction of which would provide EI-1941−1. Similar 2*H*-pyran **10** is a key intermediate of our biomimetic total synthesis of epoxyquinols A, B, and C, and epoxytwinol A, which was generated by the oxidation of ECH followed by the 6π-electrocyclization.[6c,f,g]

(5) Kakeya, H.; Miyake, Y.; Shoji, M.; Kishida, S.; Hayashi, Y.; Kataoka, T.; Osada, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3743.

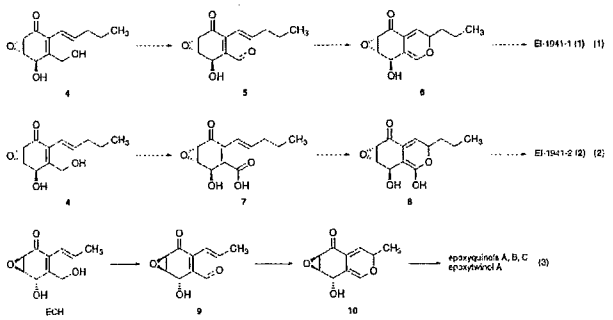
(6) Isolation: (a) Kakeya, H.; Onose, R.; Koshino, H.; Yoshida, A.; Kobayashi, K.; Kageyama, S.-I.; Osada, H. *J. Am. Chem. Soc.* **2002**, *124*, 3496. (b) Kakeya, H.; Onose, R.; Yoshida, A.; Koshino, H.; Osada, H. *J. Antibiot.* **2002**, *55*, 829. Total syntheses by our group, see: (c) Shoji, M.; Yamaguchi, J.; Kakeya, H.; Osada, H.; Hayashi, Y. *Angew. Chem., Int. Ed.* **2002**, *41*, 3192. (d) Shoji, M.; Kishida, S.; Takeda, M.; Kakeya, H.; Osada, H.; Hayashi, Y. *Tetrahedron Lett.* **2002**, *43*, 9155. (e) Shoji, M.; Kishida, S.; Kodera, Y.; Shiina, I.; Kakeya, H.; Osada, H.; Hayashi, Y. *Tetrahedron Lett.* **2003**, *44*, 7205. (f) Shoji, M.; Imai, H.; Shiina, I.; Kakeya, H.; Osada, H.; Hayashi, Y. *J. Org. Chem.* **2004**, *69*, 1548. (g) Shoji, M.; Imai, H.; Mukaida, M.; Sakai, K.; Kakeya, H.; Osada, H.; Hayashi, Y. *J. Org. Chem.* **2005**, *70*, 79. Other groups' total syntheses, see: (h) Li, C.; Bardhan, S.; Pace, E. A.; Liang, M.-C.; Gilmore, T. D.; Porco, J. A., Jr. *Org. Lett.* **2002**, *4*, 3267. (i) Mehta, G.; Islam, K.; *Tetrahedron Lett.* **2003**, *44*, 3569. (j) Mehta, G.; Islam, K. *Tetrahedron Lett.* **2004**, *45*, 3611. (k) Kuwahara, S.; Imada, S. *Tetrahedron Lett.* **2005**, *46*, 547. (l) Li, C.; Porco, J. A., Jr. *J. Org. Chem.* **2005**, *70*, 6053.

(7) (a) Epiepoformin: Nagasawa, H.; Suzuki, A.; Tamura, S. *Agric. Biol. Chem.* **1978**, *42*, 1303. (b) Epiepoxydon: Ichihara, A.; Kimura, R.; Oda, K.; Koichi, M.; Sakamura, S. *Tetrahedron Lett.* **1976**, 4741. Nagasawa, H.; Suzuki, A.; Tamura, S. *Agric. Biol. Chem.* **1978**, *42*, 1303. Sekiguchi, J.; Gaucher, G. M. *Biochem. J.* **1979**, *182*, 445. (c) Harveynone: Nagata, T.; Ando, Y.; Hirota, A. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 810. Kawazu, K.; Kobayashi, A.; Oe, K. JP 03041075, 1991; *Chem. Abstr.* **1991**, *115*, 181517. (d) Tricholomenyn A: Garlaschelli, L.; Magistrali, E.; Vidari, G.; Zuffardi, O. *Tetrahedron Lett.* **1995**, *36*, 5633. (e) Panepoxydon, Isopanepoxydon: Kis, Z.; Closse, A.; Sigg, H. P.; Hruban, L.; Snatzke, G. *Helv. Chim. Acta* **1970**, *53*, 1577. Erkel, G.; Anke, T.; Sterner, O. *Biochem. Biophys. Res. Commun.* **1996**, *226*, 214. (f) Cycloepoxydon: Gehrt, A.; Erkel, G.; Anke, T.; Sterner, O. *J. Antibiot.* **1998**, *51*, 455. Gehrt, A.; Erkel, G.; Anke, H.; Anke, T.; Sterner, O. *Nat. Prod. Lett.* **1997**, *9*, 259. (g) Terremutin: Miller, M. W. *Tetrahedron* **1968**, *24*, 4839. Read, G.; Ruiz, V. M. *J. Chem. Soc. C* **1970**, 1945. (h) Bromoxone: Higa, T.; Okuda, R. K.; Severns, R. M.; Scheuer, P. J.; He, C.-H.; Changfu, X.; Clardy, J. *Tetrahedron* **1987**, *43*, 1063. (i) Chaloxone: Fex, T.; Trofast, J.; Wickberg, B. *Acta Chem. Scand. B* **1981**, *35*, 91. Fex, T.; Wickberg, B. *Acta Chem. Scand. B* **1981**, *35*, 97. Grewe, R.; Kersten, S. *Chem. Ber.* **1967**, *100*, 2546. (j) Jesterone: Li, J. Y.; Strobel, G. A. *Phytochemistry* **2001**, *57*, 261. (k) Epoxyquinomycin C, D: Matsumoto, N.; Tuchida, T,; Umekita, M.; Kinoshita, N.; Iinuma, H.; Sawa, T.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1997**, *50*, 900. Matsumoto, N.; Tuchida, T.; Sawa, R.; Iinuma, H.; Nakamura, H.; Naganawa, H.; Sawa, T.; Takeuchi, T. *J. Antibiot.* **1997**, *50*, 912. For reviews, see: Marco-Contelles, J.; Molina, M. T.; Anjum, S. *Chem. Rev.* **2004**, *104*, 2857.

(8) Koizumi, F.; Takahashi, Y.; Ishiguro, H.; Tanaka, R.; Ohtaki, S.; Yoshida, M.; Nakanishi, S.; Ikeda, S. *Tetrahedron Lett.* **2004**, *45*, 7419.

(9) Shoji, M.; Uno, T.; Hayashi, Y. *Org. Lett.* **2004**, *6*, 4535.

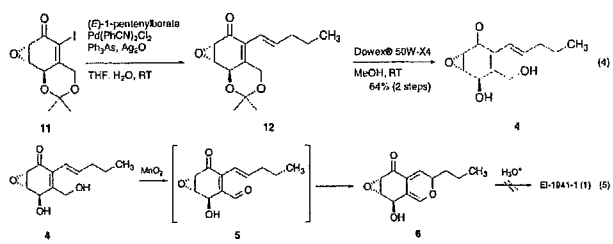
(10) Marvell, E. N. *Thermal Electrocyclic Reactions*; Academic Press: New York, 1980.



In this full paper we will describe the highly stereoselective, asymmetric total synthesis of the natural enantiomers of EI-1941−1, −2, and −3 in a full account, including an attempted total synthesis based on a biomimetic route with the theoretical calculation of 6π-electrocyclization of dienecarboxylic acid derivatives. We also describe the biological properties of EI-1941−1, −2, and −3 and their derivatives, including the finding of a more superior ICE inhibitor that is less cytotoxic than EI-1941−1 and −2.

Results and Discussion

Synthetic Study Based on Our Postulated Biosynthetic Pathway. As described in the Introduction, EI-1941−1 would be generated by the hydration and isomerization of 2*H*-pyran **6**, and we already found that the similar 2*H*-pyran **10**, generated by the oxidation of ECH, dimerized gradually under neat conditions or in a rather condensed solution (eq 3).[6c,f,g] We thought the vinyl ether moiety of **6** would react with H_2O before it dimerizes when 2*H*-pyran **6** was treated with acid in a dilute solution. Epoxycyclohexenol **4**, the starting material, was synthesized from the chiral iodocyclohexenone **11**,[6c,d,g] an enantiomer of the intermediate of our total synthesis of epoxyquinols, by the Suzuki coupling reaction with (*E*)-1-pentenylborate[11] and Ag_2O in the presence of a catalytic amount of $Pd(PhCN)_2Cl_2$ and Ph_3As,[12] followed by cleavage of the acetonide on acid treatment (eq 4). 2*H*-Pyran derivative **6** was isolated after the oxidation of alcohol **4** with MnO_2, followed by 6π-electrocyclization. Though 2*H*-pyran **6** was treated with several acids such as PPTS, $TsOH·H_2O$, and CF_3CO_2H in several dilute aqueous solvents, a complex mixture was obtained without isolation of the desired product (eq 5), which prompted us to examine the 6π-electrocyclization of a diene carboxylic acid derivative.

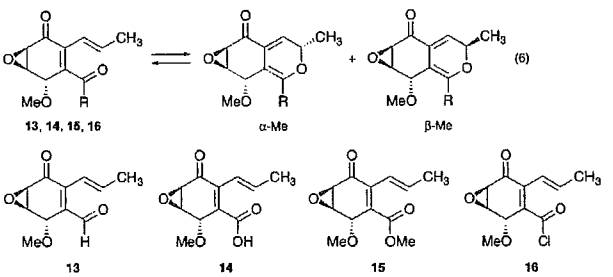


Despite the facile 6π-electrocyclization of dienal **5**, 6π-electrocyclization has generally been regarded as a difficult reaction.[10] According to the recent theoretical calculation of 2,4-pentadienal, a simple dienal, 6π-electrocyclization is an equilibrium that shifts to the starting material, and 2*H*-pyran is more energetically unstable than the parent aldehyde with a TS energy of 3.44 kcal/mol versus 21.52 kcal/mol.[13] In the case of ECH, however, an electron-withdrawing keto group on cyclohexane reduces the TS energy with the stabilization of the 2*H*-pyran intermediate **10** (eq 3).[6f] As 6π-oxa-electrocyclization has been investigated only for the diene-aldehydes without a systematic study of that for diene-carboxylic acids or esters, the theoretical calculations for the substrates having a propenyl side chain and functional groups such as aldehyde **13**, carboxylic acid **14**, methyl ester **15**, and acid chloride **16** were performed at the B3LYP/6-31G* level with the program package *TITAN* 1.0.5 including DFT engine Jaguar 3.5.042.2.[14] For all the transition-state searches, vibrational frequencies were computed after completion of the optimization from analytic second derivatives.

TABLE 1. Calculated TS Energy and Relative Energy between Diene Carbonyl Compound and α- and β-Methyl 2*H*-Pyran Derivatives

entry	starting material	TS energy (kcal mol^{-1})	relative energy[a] (kcal mol^{-1})	isomer[b]
1	**13**	17.74	−4.16	α
2	**13**	15.50	−4.18	β
3	**14**	25.34	+5.78	α
4	**14**	8.83	+6.26	β
5	**15**	25.65	+9.24	α
6	**15**	10.23	+8.66	β
7	**16**	22.50	+1. 73	α
8	**16**	9.46	+1. 58	β

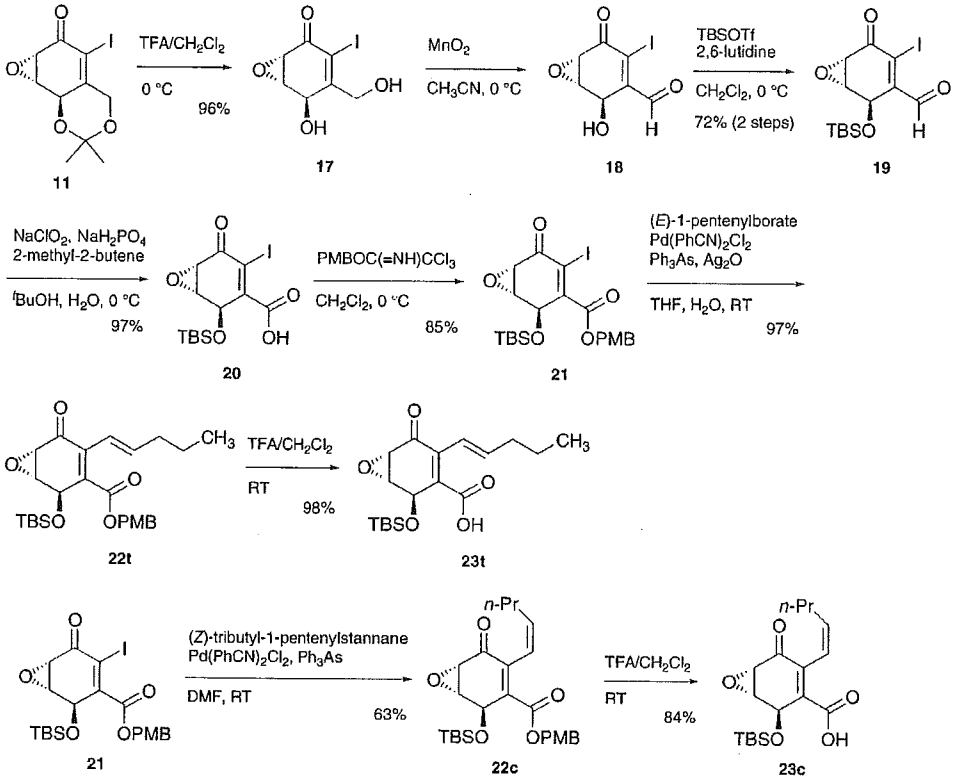
[a] The values are relative energies between the diene carbonyl compound and 2*H*-pyran in the 6π-electrocyclization. [b] α indicates the α-methyl isomer, whereas β indicates the β-methyl isomer.



There are two isomers for the 6π-oxa-electrocyclized products such as α- and β-methyl derivatives (eq 6). The transition-state energies for both α- and β-methyl-2*H*-pyran derivatives, and the relative energies between diene carbonyl derivatives and 2*H*-pyran derivatives, are calculated with the results summarized in Table 1. We have experimentally demonstrated the facile electrocyclization of diene aldehyde **13**, which is supported by the calculation showing that the 6π-electrocyclized product is more stable than the starting material with the low transition-state energies of 17.74 and 15.50 kcal/mol to α- and β-methyl 2*H*-pyran derivatives, respectively (en-

(11) Brown, H. C.; Gupta, S. K. *J. Am. Chem. Soc.* **1972**, *94*, 4370.
(12) Ruel, F. S.; Braun, M. P.; Johnson, C. R. *Org. Synth.* **1997**, *75*, 69.
(13) Rodriguez-Otero, J. *J. Org. Chem.* **1999**, *64*, 6842.
(14) *TITAN* 1.0.5. Schrodinger, Inc.: Irvine, CA, and Wavefunction, Inc.: Portland, OR, 2000. http://www.schrodinger.com; http://www.wavefun.com.

SCHEME 1. Synthesis of 23t and 23c



tries 1, 2). Diene carboxylic acid derivatives **14**, **15**, and **16** showed results different from those of diene aldehyde **13**. 6π-Electrocyclization might proceed, judging from the low transition-state energy to the β-methyl isomer, whereas that to the α-methyl isomer is too high for the 6π-electrocyclization to proceed at room temperature. As for the relative energies between the starting materials and the 2*H*-pyran derivatives, 2*H*-pyran derivatives are very unstable except for the acid chloride **16**, indicating that the equilibrium shifts mostly to the starting material, and the concentration of the 2*H*-pyran derivatives would be quite low. Even in the case of acid chloride **16**, though the 2*H*-pyran derivative is slightly more unstable (1.58 kcal/mol) than the starting material, the equilibrium also shifts to the starting material with the low concentration of 2*H*-pyran.

Synthesis of Carboxylic Acid Derivatives. With the calculation results in hand, we examined the 6π-oxa-electrocyclization of several derivatives. Before describing the results of 6π-oxa-electrocyclization, we will briefly mention the synthesis of carboxylic acid **23t** (see Scheme 1). Cleavage of the acetonide of the chiral iodocyclohexenone **11** with an acid treatment gave diol **17**. Selective oxidation of the primary alcohol with excess MnO_2 in CH_3CN gave aldehyde **18**; the secondary alcohol was protected by the use of TBSOTf and 2,6-lutidine, affording **19** in 72% yield over two steps. Oxidation of this aldehyde to the carboxylic acid was successfully performed under Kraus' conditions.[15] The carboxylic acid was protected as its *p*-methoxybenzyl ester **21** by the reaction with 4-methoxybenzyl trichloroacetimidate[16] in 85% yield. The introduction of a side chain by the Suzuki coupling reaction with (*E*)-1-pentenylborate and Ag_2O in the presence of a catalytic amount of $Pd(PhCN)_2Cl_2$ and Ph_3As afforded **22t** in 97% yield. Acid treatment then gave carboxylic acid **23t** in excellent yield. The isomer with the *Z* side chain, **23c**, was prepared in good yield by the Stille coupling reaction using (*Z*)-tributyl-1-pentenylstannane in the presence of a catalytic amount of $Pd(PhCN)_2Cl_2$ and Ph_3As, followed by the acid treatment.

With the starting materials in hand, we investigated the 6π-electrocyclization of dienecarboxylic acid. No reaction proceeded even at reflux in toluene, with the recovery of the starting materials in the cases of carboxylic acid **23t** and ester **22t**. When acid chloride generated from carboxylic acid **23t** with oxalyl chloride and a catalytic amount of DMF was gently heated to 60 °C in $CDCl_3$, a complex mixture was obtained. Only decomposition occurred when acid chloride was treated with $AlCl_3$ to generate the acylium ion.

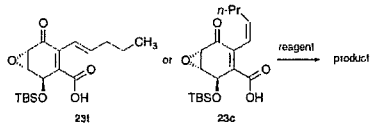
As all our trials using 6π-electrocyclization as a key step were in vain, we pursued another synthetic route using intramolecular carboxymetalation.

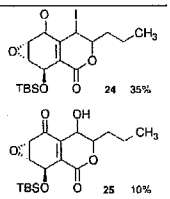
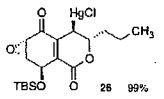
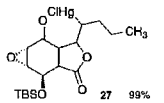
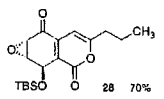
Intramolecular Carboxymetalation. Intramolecular addition of the carboxylic acid onto the alkene activated with iodine or metal salts was examined, though diastereoselectivity and alternate reaction modes such as 6-endo or 5-exo are possible problems with this approach (Table 2). In fact, iodolactonization proceeded

(15) (a) Kraus, G. A.; Taschner, M. J. *J. Org. Chem.* **1980**, *45*, 1175. (b) Bal, B. S.; Childers, W. E., Jr.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091.

(16) Nakajima, N.; Horita, K.; Abe, R.; Yonemitsu, O. *Tetrahedron Lett.* **1988**, *29*, 4139.

TABLE 2. Intramolecular Cyclization of 23t and 23c



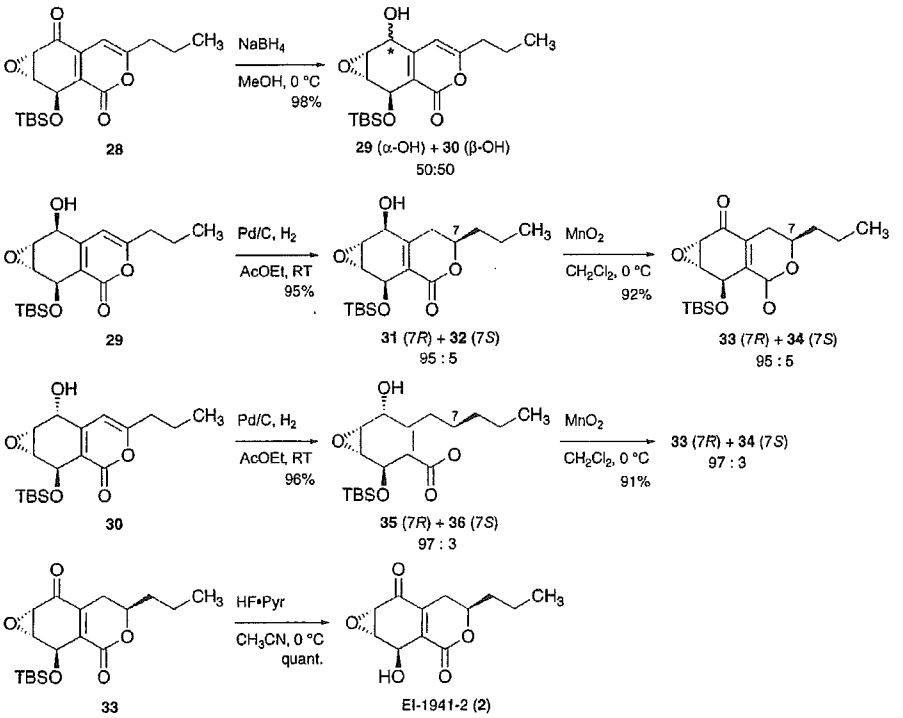
entry	reagent	SM[a]	products
1	NIS, $NaHCO_3$ THF/H_2O, RT, 1 h	**23t**	 **24** 35% **25** 10%
2	1) $Hg(OTf)_2$, MS4A, EtCN/MeCN -78 °C, 3 min 2) aq. NaCl	**23t**	 **26** 99%
3	1) $Hg(OTf)_2$, MS4A, EtCN/MeCN -78 °C, 3 min 2) aq. NaCl	**23c**	 **27** 99%
4	$Pd(PhCN)_2Cl_2$[b] *p*-benzoquinone THF, RT, 24 h	**23t**	 **28** 70%

[a] Starting material. [b] Ten mole percent was employed.

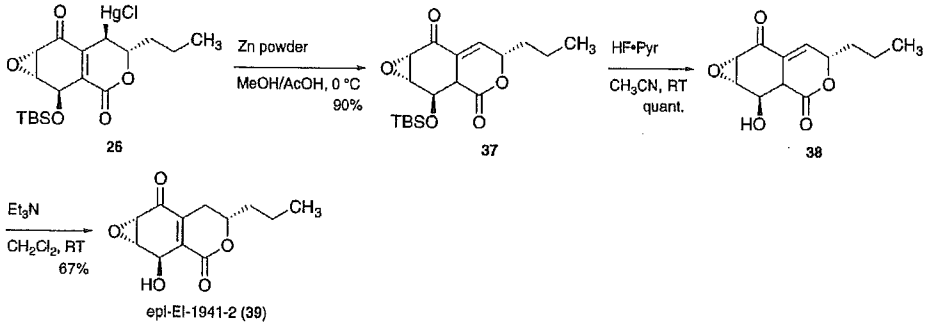
in the 6-endo mode with low yield (entry 1), whereas in the case of carboxymercuration using $Hg(OTf)_2$,[17] the 6-endo cyclized product was obtained in excellent yield as a single isomer, albeit with the incorrect side-chain relative stereochemistry at C7 in the reaction of *E* isomer **23t** (vide infra, entry 2). Undesired 5-exo cyclization was observed in that of the *Z* isomer **23c** (entry 3). Unlike these unsuccessful results, the 7,8-dihydro-6*H*-isochromen-1,5-dione structure **28** was formed when palladium(II) was used as a catalyst. That is, when **23t** was treated with *p*-benzoquinone and a catalytic amount of $Pd(PhCN)_2Cl_2$,[18] carboxypalladation proceeded, followed by the β-hydride elimination, affording **28** in 70% yield (entry 4).

The remaining steps are reduction of the double bond and deprotection (see Scheme 2). Hydrogenation of **28** under an H_2 atmosphere in the presence of Pd/C or $Pd(OH)_2$ did not proceed. As the keto group might be the cause of this reluctance to undergo hydrogenation, it was reduced with $NaBH_4$ in MeOH to afford alcohols **29** and **30** in 98% yield and equal amounts, which were separated by column chromatography. The relative stereochemistry is determined by the modified MTPA-ester method[19] of **29**. Hydrogenation of α-alcohol **29** proceeded smoothly and stereoselectively, affording an inseparable mixture of **31** and **32** in excellent yield (95%) and with high diastereoselectivity (95:5). It should be noted that the concentration is important for the diastereoselectivity. Whereas excellent diastereoselectivity (95:5) was obtained at 0.01 M, lower selectivity (87:13) was observed at a higher concentration (0.1 M).[9,20] The mixture of **31** and **32** was oxidized with MnO_2, affording ketones **33** and **34** in 92% yield (95:5), which were easily separated by thin-layer chromatography (TLC). Though hydrogenation of β-alcohol **30** proceeded slowly, the reduced products **35** and **36** were obtained in 96% yield with the desired isomer stereoselectivity (97:3). In this hydrogenation, the concentration is also crucial. Excellent diastereoselectiv-

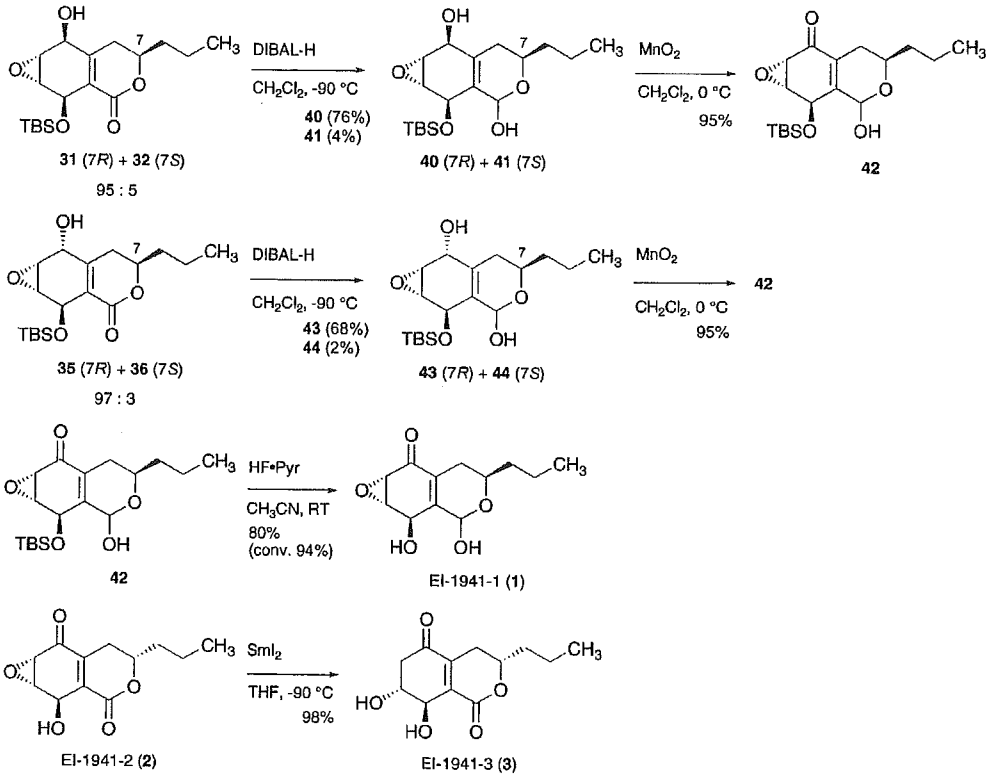
SCHEME 2. Synthesis of EI-1941−2 (2)



SCHEME 3. Synthesis of Epi-EI-1941−2 (39)



SCHEME 4. Synthesis of EI-1941−1 (1) and EI-1941−3 (3)



ity (97:3) is observed at low concentration (0.01 M) in contrast to the lower selectivity (83:17) at higher concentration (0.1 M).[9] Oxidation of alcohols **35** and **36** with MnO_2 gave **33** and **34** in 91% yield in a 97:3 ratio, and these were separated by TLC.

Removal of the TBS group of **33** afforded EI-1941−2 (**2**) quantitatively. Synthetic EI-1941−2 (**2**) exhibited properties identical to those of the natural product,[4b,8] including the optical rotation.

epi-EI-1941−2 was also prepared stereoselectively from carboxymercurated derivative **26**. Though conventional demercuration using Bu_3SnH in the presence of AIBN[21] did not work, affording **23t**, we found that the treatment of **26** with Zn powder in MeOH and AcOH[22] gave β,γ-unsaturated lactone **37**. After removal of the TBS group, treatment with a catalytic amount of amine isomerized the double bond to provide epi-EI-1941−2 (**39**) in 67% yield (Scheme 3).

Synthesis of EI-1941−1 and −3. EI-1941−1 was synthesized (see Scheme 4) from α-alcohol **31** and β-alcohol **35**, the intermediates of EI-1941−2, as follows: When α-alcohols **31** and **32** (**31**:**32** = 95:5) were treated with DIBAL-H in CH_2Cl_2 at low temperature (−90 °C),

(17) (a) Nishizawa, M.; Takenaka, H.; Nishida, H.; Hayashi, Y. *Tetrahedron Lett.* **1983**, *24*, 2581. (b) Nishizawa, M.; Morikuni, E.; Asoh, K.; Kan, Y.; Uenoyama, K.; Imagawa, H. *Synlett* **1995**, 169. (c) Imagawa, H.; Shigaraki, T.; Suzuki, T.; Takao, T.; Yamada, H.; Sugihara, T.; Nishizawa, M. *Chem. Pharm. Bull.* **1998**, *46*, 1341. (d) Nishizawa, M.; Kashima, T.; Sakakibara, M.; Wakabayashi, A.; Takahashi, K.; Takao, T.; Imagawa, H.; Sugihara, T. *Heterocycles* **2000**, *54*, 629.

(18) (a) Korte, D. E.; Hegedus, L. S.; Wirth, R. K. *J. Org. Chem.* **1977**, *42*, 1329. (b) Minami, T.; Nishimoto, A.; Hanaoka, M. *Tetrahedron Lett.* **1995**, *36*, 9505.

(19) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.

(20) The reason for the effect of the concentration on the diastereoselectivity is not clear.

(21) Whitesides, G. M.; San Filippo, J., Jr. *J. Am. Chem. Soc.* **1970**, *92*, 6611.

(22) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; John Wiley and Sons: New York, 1967; Vol. 1, p 1276.

TABLE 3. Summary of IC_{50} Values of Test Compounds on IL-1β Secretion from LPS-Stimulated THP-1 Cells and on Cell Viability of THP-1 Cells

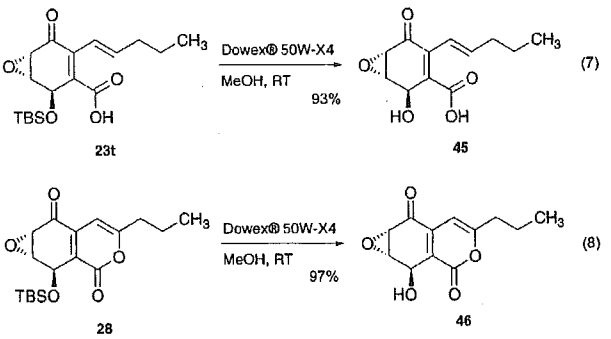
compound	IC_{50} values[a] on IL-1β secretion (μM)	IC_{50} values[b] on cell viability (μM)
EI-1941−1 (**1**)	56	>100
EI-1941−2 (**2**)	15	40
ent-**2**	10	68
EI-1941−3 (**3**)	>100	>100
45	>100	>100
ent-**45**	74	>100
46	14	20
ent-**46**	10	30

[a] Concentration of 50% inhibitory activity on LPS-stimulated IL-1β secretion. [b] Concentration of 50% inhibitory activity on cell viability.

lactone was reduced stereoselectively to lactols **40** and **41**, which were separated by TLC (76% **40**, 4% **41**). Oxidation of **40** with MnO_2 gave ketone **42** in excellent yield (95%). β-Alcohols **35** and **36** were also reduced with DIBAL-H to afford lactols **43** and **44**, which were separated by TLC (68% **43**, 2% **44**). Oxidation of **43** gave the same ketone **42** in 95% yield. Deprotection with HF·pyridine afforded EI-1941−1 (**1**) in good yield.

Reduction of the epoxide with SmI_2[23] at low temperature (−90 °C) cleanly converted EI-1941−2 into EI-1941−3 nearly quantitatively. Synthetic EI-1941−1, −2, and −3 exhibited properties identical to those of the natural products, including the optical rotation, which indicate that natural enantiomers were successfully synthesized. Comparison of the optical rotation of EI-1941−3 (synthetic **3**: $[\alpha]^{30}_D$ −88.7, natural **3**:[4b,8] $[\alpha]^{23}_D$ −87.5) determined its absolute stereochemistry.

Biological Evaluation. We evaluated the effects of EI-1941−1 (**1**), −2 (**2**), and −3 (**3**) and their derivatives on the extracellular release of IL-1β from THP-1 cells and cell viability in THP-1 cells, with the results summarized in Table 3 and Figure 2. The derivatives examined are *ent*-EI-1941−2 (*ent*-**2**), hydroxy carboxylic acid **45** and its enantiomer *ent*-**45**, and tetrahydro isocoumarin derivative **46** and its enantiomer *ent*-**46**. Hydroxy carboxylic acid **45** and tetrahydro isocoumarin derivative **46** were easily prepared from **23t** and **28**, respectively, by removal of the TBS group on acid treatment in MeOH, in good yields (eqs 7 and 8).



Compounds **1** and **2** inhibited IL-1β secretion in a dose-dependent manner; IC_{50} values of **1** and **2** in our assay system were 56 and 15 μM, respectively. IC_{50} values of **1** and **2** on cell viability were >100 and 40 μM, respectively. Compound **3**, which has been reported as an inactive compound in an in vitro system,[4] was inactive in THP-1 cells. These results indicate that an epoxide ring in **2** is essential for exhibiting the biological activities of **2**. Moreover, IC_{50} values of *ent*-**2** on IL-1β secretion and cell viability were 10 and 68 μM, respectively, which was a most effective derivative because the differences in IC_{50} values against IL-1β secretion and cell viability were greatly significant. The inhibition of IL-1β secretion by hydroxy carboxylic acids **45** and *ent*-**45** was weak (IC_{50} values of >100 and 74 μM, respectively), suggesting that a three-fused-ring system was important for the biological activity. Moreover, IC_{50} values of tetrahydro isocoumarin derivatives **46** and *ent*-**46** on IL-1β secretion were 14 and 10 μM, respectively. However, these two compounds **46** and *ent*-**46** also affected the cell viability at IC_{50} values of 20 and 30 μM, respectively. These results indicate that the enantiomer of EI-1941−2 (*ent*-**2**) is a more potent and effective ICE inhibitor than natural EI-1941−2 in a cultured cell system.

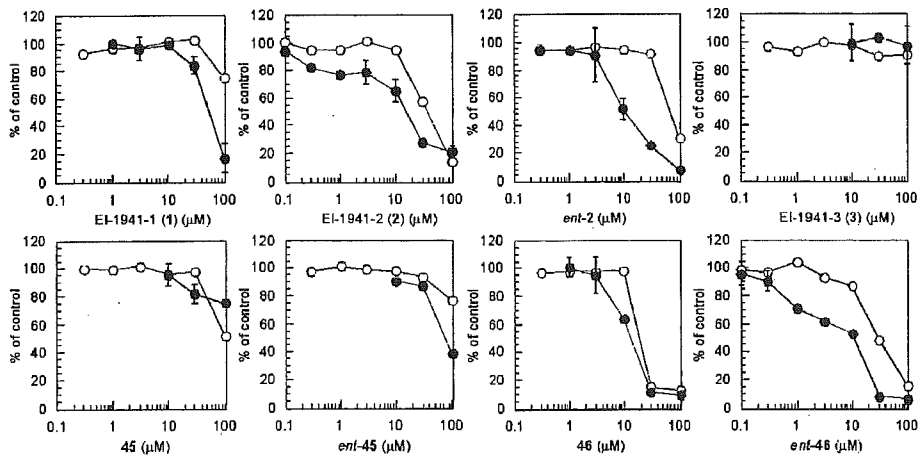


FIGURE 2. Effect of EI-1941−1 (**1**), −2 (**2**), and −3 (**3**) and their derivatives on IL-1β secretion from LPS-stimulated THP-1 cells and on cell viability of THP-1 cells. Symbols indicate IL-1β secretion (closed circle) and percentage of viable cells (open circle).

Conclusion

We have accomplished the first asymmetric total synthesis of EI-1941−1, −2, and −3, starting from the chiral epoxy iodoquinone **11**, a key intermediate in our total synthesis of epoxyquinols A and B. A key step is the intramolecular, metal-mediated carboxylation of an alkene via 6-endo cyclization, in which Pd(II) gave 2*H*-pyran-2-one via *β*-hydride elimination, affording EI-1941−2 after stereoselective hydrogenation. $Hg(OTf)_2$ afforded a carboxymercurated product of a side-chain relative stereochemistry opposite to that of the natural product, leading eventually to epi-EI-1941−2 with high diastereoselectivity. EI-1941−1 was synthesized stereoselectively from the intermediate of EI-1941−2, whereas EI-1941−3 was synthesized in one step from EI-1941−2. By using this asymmetric total synthesis, we determined the absolute stereochemistry of EI-1941−3. The structure−activity relationship of EI-1941−1, −2, and −3 and their synthetic derivatives revealed that an enantiomer of EI-1941−2 is a more potent ICE inhibitor than EI-1941−2, as it is less cytotoxic.

Experimental Section

(1*R*,5*S*,6*R*)-5-Hydroxy-4-hydroxymethyl-3-iodo-7-oxabicyclo[4.1.0]hept-3-ene-2-one (17). To a solution of acetonide **11** (100 mg, 0.311 mmol) in CH_2Cl_2 (3.1 mL) was added trifluoroacetic acid (3.1 mL) at 0 °C, and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced preaaure. The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:1) to afford diol **18** (84 mg, 96%) as a yellow oil: ^{1}H NMR (400 MHz, CD_3OD) δ 3.63 (1H, dd, J = 1.6, 3.6 Hz), 3.85 (1H, dd, J = 1.4, 3.6 Hz), 4.44 (2H, d, J = 3.2 Hz), 4.98 (1H, br-s); ^{13}C NMR (100 MHz, CD_3OD) δ 52.5, 57.4, 64.6, 69.6, 102.1, 163.2, 189.8; FT-IR (neat) ν 3392, 1682, 1591, 1273, 1228, 1080, 1051, 931, 856, 758, 525 cm^{-1}; HRMS (FAB) $[M + Na]^+$ calcd for $[C_7H_7IO_4 + Na]^+$ 304.9287, found 304.9301; $[\alpha]^{23}_D$ −31.7 (*c* 0.212, MeOH).

(1*S*,2*S*,6*R*)-2-(*tert*-Butyldimethylsiloxy)-4-iodo-5-oxo-7-oxabicyclo[4.1.0]hept-3-ene-3-carbaldehyde (19). To a solution of diol **17** (81 mg, 0.287 mmol) in CH_3CN (2.9 mL) was added MnO_2 (624 mg, 7.18 mmol) at 0 °C under an argon atmosphere, and the reaction mixture was stirred for 10 min at that temperature. The reaction mixture was filtered through a pad of Celite, and concentrated in vacuo. The residue was filtered through a pad of silica gel (AcOEt/hexane = 1:3) to afford aldehyde **18**, and the crude product was used for the next reaction without further purification. To a solution of aldehyde **18** (170 mg, 0.606 mmol) and TBSOTf (481 mg, 1.82 mmol) in CH_2Cl_2 (6.1 mL) was added 2,6-lutidine (0.23 mL, 1.94 mmol) at 0 °C, and the mixture was stirred for 1 h under an argon atmosphere. The reaction mixture was quenched with saturated aqueous NH_4Cl, and diluted with AcOEt. The organic phase was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:10) to afford siloxy aldehyde **19** (170 mg, 72%, 2 steps) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.08 (3H, s), 0.22 (3H, s), 0.82 (9H, s), 3.73 (2H, d, J = 1.2 Hz), 5.21 (1H, br-s), 9.79 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.8, 18.0, 25.6, 51.8, 56.2, 63.5, 117.9, 148.2, 190.6, 197.0; FT-IR (neat) ν 2954, 2929, 2858, 1705, 1689, 1581, 1471, 1340, 1255, 1167, 1049, 839, 781, 511 cm^{-1}; HRMS (FAB) $[M + H]^+$ calcd for $C_{13}H_{20}IO_4Si$ 395.0176, found 395.0178; $[\alpha]^{22}_D$ −3.2 (*c* 0.56, MeOH).

(23) Molander, G. A.; Hahn, G. *J. Org. Chem.* **1986**, *51*, 2596.

(1*S*,2*S*,6*R*)-2-(*tert*-Butyldimethylsiloxy)-4-iodo-5-oxo-7-oxabicyclo[4.1.0]hept-3-ene-3-carboxylic Acid (20). To a solution of siloxy aldehyde **19** (100 mg, 0.25 mmol), NaH_2PO_4 (40 mg, 0.25 mmol), and 2-methyl-2-butene (0.1 mL, 1.11 mmol) in *tert*-BuOH (1.8 mL) and H_2O (0.5 mL) was added $NaClO_2$ (78 mg, 0.86 mmol) at 0 °C, and the reaction mixture was stirred for 1 h under an argon atmosphere. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$ = 1:10) to afford carboxylic acid **20** (104 mg, 97%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.07 (3H, s), 0.14 (3H, s), 0.84 (9H, s), 3.62 (1H, br-*d*, J = 3.0 Hz), 3.69 (1H, br-s), 3.79 (1H, br-s), 5.18 (1H, br-s); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.6, −4.56, 18.0, 25.6, 51.0, 56.8, 66.7, 103.2, 152.9, 170.8, 189.1; FT-IR (neat) ν 3199, 2954, 2929, 2858, 1689, 1604, 1389, 1259, 839, 781, 756, 501 cm^{-1}; HRMS (FAB) $[M + Na]^+$ calcd for $[C_{13}H_{19}IO_5Si + Na]^+$ 432.9944, found 432.9938; $[\alpha]^{23}_D$ +26.1 (*c* 0.12, MeOH).

(1*S*,2*S*,6*R*)-2-(*tert*-Butyldimethylsiloxy)-4-iodo-5-oxo-7-oxabicyclo[4.1.0]hept-3-ene-3-carboxylic Acid 4-Methoxybenzyl Ester (21). To a solution of carboxylic acid **20** (50 mg, 0.12 mmol) in CH_2Cl_2 was added $PMBOC(=NH)CCl_3$ (102 mg, 0.24 mmol) at 0 °C, and the reaction mixture was stirred for 1 h under an argon atmosphere. The reaction mixture was quenched with pH 7.0 phosphate buffer, and diluted with AcOEt. The organic phase was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:3−1:10) to afford PMB ester **21** (55 mg, 85%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.01 (3H, s), 0.11 (3H, s), 0.83 (9H, s), 3.62 (1H, dd, J = 1.3, 3.7 Hz), 3.64 (1H, dd, J = 1.0, 3.7 Hz), 3.79 (3H, s), 5.04 (1H, m), 5.21 (2H, dd, J = 11.7, 18.4 Hz), 6.87 (2H, m), 7.35 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.1, −4.6, 17.9, 25.4, 50.9, 55,3, 57.0, 66.8, 68.2, 103.8, 114.0, 126.3, 131.0, 151.3, 160.1, 166.0, 188.6; FT-IR (neat) ν 2954, 2858, 1699, 1516, 1241, 1107, 1034, 841, 781 cm^{-1}; HRMS (FAB) $[M]^+$ calcd for $C_{21}H_{27}IO_6Si$ 530.0622, found 530.0637; $[\alpha]^{22}_D$ +19.9 (*c* 0.158, MeOH).

(1*S*,2*S*,6*R*)-2-(*tert*-Butyldimethylsiloxy)-5-oxo-4-pentyl-7-oxa-bicyclo[4.1.0]hept-3-ene-3-carboxylic Acid 4-Methoxybenzyl Ester (22t). To a solution of PMB ester **21** (48 mg, 0.09 mmol), (*E*)-1-pentenylborate (16 mg, 0.136 mmol), Ag_2O (33.6 mg, 0.145 mmol), and Ph_3As (2.8 mg, 0.009 mmol) in $THF.H_2O$ (8:1, 0.45 mL) was added $Pd(PhCN)_2Cl_2$ (1.7 mg, 0.0045 mmol) at room temperature in the dark, and the reaction mixture was stirred for 14 h under an argon atmosphere. The reaction mixture was quenched with saturated aqueous NH_4Cl, and stirred for 30 min at that temperature. The reaction mixture was filtered through a pad of Celite, and the organic materials were extracted with AcOEt. The organic phase was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:5~1:20) to afford dienone **22t** (42 mg, 97%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (3H, s), 0.09 (3H, s), 0.82 (9H, s), 0.84 (3H, t, J = 7.3 Hz), 1.32 (2H, sextet, J = 7.3 Hz), 1.90−2.02 (2H, m), 3.54 (1H, d, J = 4.0 Hz), 3.60 (1H, dd, J = 1.9, 4.0 Hz), 3.79 (3H, s), 5.10 (2H, d, J = 11.8 Hz), 5.20−5.23 (2H, m), 6.23 (1H, td, J = 6.6, 15.9 Hz), 6.36 (1H, d, J = 15.9 Hz), 6.86−6.88 (2H, m), 7.29−7.32 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.8, −4.7, 13.7, 17.9, 21.8, 25.5, 35.9, 53.6, 55.3, 56.0, 65.7, 67.2, 114.0, 121.7, 127.0, 130.7, 135.5, 136.2, 142.1, 160.0, 167.2, 195.7; FT-IR (neat) ν 2956, 2931, 2359, 1716, 1699, 1516, 1244, 1101, 1078, 839, 779 cm^{-1}; HRMS (FAB) $[M + H]^+$ calcd for $C_{26}H_{37}O_6Si$ 473.2359, found 473.2385; $[\alpha]^{23}_D$ −14.5 (*c* 0.078, MeOH).

(1*S*,2*S*,6*R*)-2-(*tert*-Butyldimethylsiloxy)-5-oxo-4-pent-1-enyl-7-oxabicyclo[4.1.0]hept-3-ene-3-carboxylic Acid (23t). To a solution of **22t** (32 mg, 0.068 mmol) in CH_2Cl_2 (0.7 mL) was added trifluoroacetic acid (0.07 mL) at room temper-

ature, and the reaction mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:1−1:3) to afford carboxylic acid **23t** (23 mg, 98%) as a colorless oil: ^{1}H NMR (400 MHz, CD_3OD) δ 0.14 (3H, s), 0.21 (3H, s), 0.90 (9H, s), 0.92 (3H, t, J = 7.3 Hz), 1.44 (2H, sextet, J = 7.3 Hz), 1.99−2.21 (2H, m), 3.53 (1H, d, J = 3.9 Hz), 3.72 (1H, dd, J = 3.9 Hz), 5.21 (1H, br-s), 6.32 (1H, td, J = 6.8 Hz, 16.0 Hz), 6.46 (1H, d, J = 16.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ −4.7, −4.7, 13.7, 17.9, 21.8, 25.5, 36.1, 53.6, 55.7, 65.6, 122.1, 134.5, 137.9, 143.4, 172.6, 196.3; FT-IR (neat) ν 3375, 2929, 2858, 1693, 1680, 1464, 1338, 1099, 781, 741 cm^{-1}; HRMS (FAB) [M + Na]$^+$ calcd for $[C_{18}H_{28}O_5Si + Na]^+$ 375.1604, found 375.1577; $[\alpha]^{22}_D$ −61.4 (*c* 0.11, MeOH).

(2*S*,3*S*,7*R*,8*S*,11*R*)-3-(*tert*-Butyldimethylsiloxy)-8-chloromercurio-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-4-en-5,10-dione (26). To a solution of carboxylic acid **23t** (21.0 mg, 0.06 mmol) in EtCN (2.1 mL) were added MS4A (6.3 mg, 30 wt %) and $Hg(OTf)_2$/MeCN (0.25 mL, 0.071 mmol) at −78 °C, and the reaction mixture was stirred for 3 min. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl (1:1). The organic materials were extracted with AcOEt, washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:3) to afford **26** (35.0 mg, 99%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.12 (3H, s), 0.24 (3H, s), 0.87 (9H, s), 0.96 (3H, t, J = 7.3 Hz), 1.49−1.70 (2H, m), 1.77−1.93 (2H, m), 2.82 (1H, br-*d*, J = 11.6 Hz), 3.70 (1H, br-*d*, J = 3.8 Hz), 3.75 (1H, dd, J = 1.9, 3.8 Hz), 4.55 (1H, ddd, J = 3.5, 7.9, 11.6 Hz), 5.43 (1H, br-s); ^{13}C NMR (150 MHz, $CDCl_3$) δ −4.9, −4.5, 13.7, 18.2, 25.6, 29.7, 38.8, 42.4, 53.1, 55.4, 61.9, 80.6, 136.5, 142.2, 164.2, 196.9; FT-IR (neat) ν 2958, 2929, 2858, 2360, 1714, 1680, 1252, 1090, 839, 781 cm^{-1}; HRMS (FAB) [M + H]$^+$ calcd for $C_{18}H_{28}ClHgO_5Si$ 589.1101, found 589.1074; $[\alpha]^{23}_D$ +48.8 (*c* 0.087, MeOH).

(2*R*,3*S*,11*S*)-3-(*tert*-Butyldimethylsiloxy)-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-4,7-dien-5,10-dione (28). To a solution of carboxylic acid **23t** (100.0 mg, 0.567 mmol) in THF (5.7 mL) was added *p*-benzoquinone (26.2 mg, 2.84 mmol) and $Pd(PhCN)_2Cl_2$ (12.4 mg, 0.0567 mmol) at room temperature, and the reaction mixture was stirred for 20 h. The reaction mixture was filtered through a pad of Celite, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:3−1:5) to afford **28** (70.0 mg, 70%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.15 (3H, s), 0.26 (3H, s), 0.85 (9H, s), 0.96 (3H, t, J = 7.5 Hz), 1.68 (2H, sextet, J = 7.5 Hz), 2.47 (2H, t, J = 7.5 Hz), 3.63 (1H, br-*d*, J = 3.9 Hz), 3.77 (1H, dd, J = 2.0, 3.9 Hz), 5.29 (1H, br-s), 6.28 (1H, br-s); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.9, −4.6, 13.5, 20.1, 25.7, 35.8, 52.6, 56.7, 61.9, 98.1, 125.4, 139.6, 139.6, 162.3, 166.9, 192.9; FT-IR (neat) ν 2956, 2929, 2856, 1732, 1705, 1641, 1577, 1464, 1257, 1086, 839, 781 cm^{-1}; HRMS (FAB) [M]$^+$ calcd for $C_{18}H_{26}O_5Si$ 350.1550, found 350.1579; $[\alpha]^{23}_D$ −14.1 (*c* 0.17, MeOH).

(2*S*,3*S*,10*R*,11*S*)-3-(*tert*-Butyldimethylsiloxy)-10-hydroxy-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-4,7-dien-5-one (29) and (2*S*,3*S*,10*S*,11*S*)-3-(*tert*-Butyldimethylsiloxy)-10-hydroxy-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-4,7-dien-5-one (30). To a solution of **28** (43.0 mg, 0.123 mmol) in MeOH (1.3 mL) was added $NaBH_4$ (14.0 mg, 0.368 mmol) at 0 °C, and the reaction mixture was stirred for 20 min at that temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl. The organic materials were extracted with AcOEt, washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$) to afford **29** and **30** (42.0 mg, 98%, 50:50 diastereoselectivity) as a colorless oil. **29**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.13 (3H, s), 0.25 (3H, s), 0.86 (9H, s), 0.96 (3H, t, J = 7.5 Hz), 3.43 (1H, m), 3.52 (1H, m), 4.55 (1H, br-*d*, J = 10.2 Hz), 5.23 (1H, d, J = 2.8 Hz), 5.94 (1H, br-s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.5, 17.9, 25.7, 29.6, 50.0, 51.3, 62.7, 67.3, 104.7, 117.2, 149.8, 162.5, 166.9; FT-IR (neat) ν 3419, 2927, 2856, 1726, 1651, 1585, 1464, 1254, 1080, 974, 839, 781 cm^{-1}; HRMS (FAB) [M]$^+$ calcd for $C_{18}H_{28}O_5Si$ 352.1706, found 352.1680; $[\alpha]^{23}_D$ +104.1 (*c* 0.08, MeOH). **30**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.12 (3H, s), 0.22 (3H, s), 0.85 (9H, s), 0.95 (3H, t, J = 7.4 Hz), 1.67 (2H, sextet, J = 7.4 Hz), 2.44 (2H, t, J = 7.4 Hz), 3.47 (1H, dd, J = 2.5, 4.3 Hz), 3.59 (1H, dd, J = 2.2, 4.3 Hz), 4.79 (1H, d, J = 8.8 Hz), 5.12 (1H, d, J = 2.2 Hz), 6.29 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.0, −4.6, 13.5, 18.1, 20.2, 25.8, 29.7, 35.8, 53.1, 54.4, 62.4, 66.1, 101.4, 117.2, 149.4, 162.3, 165.4; FT-IR (neat) ν 3410, 2929, 2856, 1722, 1645, 1574, 1464, 1252, 1117, 1065, 920, 839, 777 cm^{-1}; HRMS (FAB) [M]$^+$ calcd for $C_{18}H_{28}O_5Si$ 352.1706, found 352.1689; $[\alpha]^{23}_D$ +81.3 (*c* 0.21, MeOH).

(2*S*,3*S*,7*S*,10*R*,11*S*)-3-(*tert*-Butyldimethylsiloxy)-10-hydroxy-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-4-en-5-one (31) and (2*S*,3*S*,7*R*,10*R*,11*S*)-3-(*tert*-Butyldimethylsiloxy)-10-hydroxy-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-4-en-5-one (32). To a solution of **29** (10.0 mg, 0.0284 mmol) in AcOEt (2.8 mL) was added 10% Pd/C (3.0 mg, 0.0028 mmol) at room temperature, and the reaction mixture was stirred for 3 h under an H_2 atmosphere. The reaction mixture was filtered through a pad of Celite, and concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (AcOEt/hexane = 1:3) to afford an inseparable mixture of **31** and **32** (9.5 mg, 95%, 95:5 diastereoselectivity) as a colorless oil. **31**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.12 (3H, s), 0.22 (3H, s), 0.85 (9H, s), 0.91 (3H, t, J = 7.3 Hz), 1.37−1.58 (2H, m), 1.74−1.79 (1H, m), 2.29−2.34 (1H, m), 2.38 (1H, dd, J = 4.9, 18.0 Hz), 2.62 (1H, dd, J = 8.4, 18.0 Hz), 3.34−3.36 (1H, m), 3.43−3.45 (1H, m), 4.32 (1H, br-*d*, J = 8.7 Hz), 4.45−4.51 (1H, m), 5.03 (1H, d, J = 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.0, −4.7, 13.7, 17.9, 18.2, 25.7, 32.0, 36.4, 50.3, 51.8, 62.2, 67.5, 124.3, 148.6, 163.9; FT-IR (neat) ν 3423, 2958, 2931, 2858, 1716, 1254, 1084, 868, 839, 779 cm^{-1}; HRMS (FAB) [M + H]$^+$ calcd for $C_{18}H_{31}O_5Si$ 355.1941, found 355.1948.

(2*S*,3*S*,7*S*,11*R*)-3-(*tert*-Butyldimethylsiloxy)-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-4-en-5,10-dione (33). To a solution of **31** and **32** (10.0 mg, 0.0283 mmol) in CH_2Cl_2 (1.0 mL) was added MnO_2 (61.3 mg, 0.705 mmol) at 0 °C under an argon atmosphere, and the reaction mixture was stirred for 1 h at that temperature. The reaction mixture was filtered through a pad of Celite, and concentrated in vacuo. The residue was purified by preparative thin-layer chromatography (AcOEt/hexane = 1:5) to afford **33** (9.2 mg, 92%; 95:5 diastereoselectivity) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.14 (3H, s), 0.45 (3H, s), 0.85 (9H, s), 1.33−1.58 (3H, m), 1.81−1.72 (1H, m), 2.52 (1H, dd, J = 4.6, 18.1 Hz), 2.63 (1H, dd, J = 7.9, 18.1 Hz), 3.56 (1H, br-*d*, J = 3.9 Hz), 3.71 (1H, dd, J = 1.9, 3.9 Hz), 4.48−4.54 (1H,m), 5.16 (1H, br-s); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.8, −4.75, 13.7, 18.1, 18.2, 25.7, 29.7, 36.2, 52.3, 56.4, 61.9, 135.8, 139.5, 163.5, 194.4; FT-IR (neat) ν 2927, 2854, 1726, 1695, 1464, 1252, 1240, 1101, 1084, 839, 781 cm^{-1}; HRMS (FAB) [M + H]$^+$ calcd for $C_{18}H_{29}O_5Si$ 353.1784, found 353.1782; $[\alpha]^{22}_D$ −10.5 (*c* 0.12, MeOH).

(2*R*,3*S*,7*S*,11*R*)-3-Hydroxy-7-propyl-1,6-dioxatricyclo-[8.1.0.0^{4,9}]undec-4-en-5,10-dione (EI-1941−2 (2)). To a solution of **33** (16.6 mg, 0.0471 mmol) in CH_3CN (1.9 mL) was added HF·Pyr (0.5 mL) at 0 °C, and the reaction mixture was stirred for 4 h at that temperature. The reaction mixture was quenched with saturated aqueous $NaHCO_3$. The organic materials were extracted with AcOEt, washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (AcOEt/hexane = 1:1) to afford EI-1941−2 (**2**) (11.2 mg, quant.) as a colorless powder: ^{1}H NMR (400 MHz, CD_3CN) δ 0.93 (3H, t, J = 7.3 Hz), 1.35−1.51 (2H, m), 1.57−1.66 (1H, m), 1.69−1.78 (1H, m), 2.46 (1H, ddd, J = 1.0 9.7, 18.1 Hz), 2.54 (1H, ddd, J = 1.3, 4.6, 18.1 Hz), 3.56 (1H, dd, J = 1.0, 3.7 Hz), 3.85 (1H, dd,

J = 1.6, 3.7 Hz), 4.46−4.53 (1H, m), 4.97 (1H, br-s); ^{13}C NMR (100 MHz, CD_3CN) δ 12.5, 17.3, 25.3, 35.6, 51.8, 55.8, 60.4, 77.1, 135.1, 140.0, 163.7, 193.9; FT-IR (neat) ν 3448, 2960, 2873, 1716, 1695, 1417, 1244, 1124, 1099, 1041 cm^{-1}; HRMS (FAB) [M + H]$^+$ calcd for $C_{12}H_{15}O_5$ 239.0920, found 239.0932; $[\alpha]^{22}_D$ −299.9 (*c* 0.48, MeOH).

(2*S*,3*S*,7*R*,11*R*)-3-(*tert*-Butyldimethylsiloxy)-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-8-en-5,10-dione (37). To a solution of **26** (5.0 mg, 0.009 mmol) in MeOH (0.1 mL) were added zinc powder (2.8 mg, 0.045 mmol) and AcOH (0.002 mL) at 0 °C, and the reaction mixture was stirred for 10 min. The reaction mixture was quenched with saturated aqueous $NaHCO_3$. The reaction mixture was filtered through a pad of Celite, and the organic materials were extracted with AcOEt. The organic phase was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (AcOEt/hexane = 1:5) to afford **37** (2.7 mg, 90%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.07 (1H, s), 0.12 (1H, s), 0.78 (1H, s), 0.93 (3H, t, J = 7.2 Hz), 1.39−1.53 (2H, m), 1.66−1.76 (2H, m), 3.47 (1H, d, J = 4.1 Hz), 3.52 (1H, q, J = 3.0 Hz), 3.61 (1H, t, J = 4.1 Hz), 5.06 (1H, t, J = 3.0 Hz), 7.05 (1H, t, J = 3.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.1, −4.8, 14.0, 18.2, 25.9, 38.8, 40.7, 54.8, 56.6, 67.5, 79.5, 127.9, 135.1, 168.3, 190.7; FT-IR (neat) ν 2927, 2856, 1743, 1709, 1655, 1464, 1389, 1117, 839, 781 cm^{-1}; HRMS (FAB) [M + H]$^+$ calcd for $C_{18}H_{29}O_5Si$ 353.1784, found 353.1791; $[\alpha]^{23}_D$ −31.6 (*c* 0.113, MeOH).

(2*R*,3*S*,7*R*,11*R*)-3-Hydroxy-7-propyl-1,6-dioxatricyclo-[8.1.0.0^{4,9}]undec-8-en-5,10-dione (38). To a solution of **37** (3.5 mg, 0.01 mmol) in CH_3CN (0.2 mL) was added HF·Pyr (0.05 mL) at room temperature, and the reaction mixture was stirred for 6 h at that temperature. The reaction mixture was quenched with saturated aqueous $NaHCO_3$. The organic materials were extracted with AcOEt, washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (AcOEt/hexane = 1:1) to afford **38** (2.4 mg, quant.) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (3H, t, J = 7.4 Hz), 1.38−1.60 (2H, m), 1.67−1.79 (2H, m), 3.52−3.55 (2H, m), 3.79 (1H, dd, J = 3.8, 3.6 Hz), 5.14 (1H, dd, J = 3.1, 3.6 Hz), 7.13 (1H, t, J = 3.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.6, 18.1, 37.0, 41.1, 54.0, 55.3, 65.0, 79.6, 127.3, 135.9, 169.0, 189.7; FT-IR (neat) ν 2956, 2927, 2854, 1743, 1709, 1655, 1250, 1117, 1061, 839, 781 cm^{-1}; HRMS (FAB) [M + H]$^+$ calcd for $C_{12}H_{15}O_5$ 239.0920, found 239.0909; $[\alpha]^{32}_D$ +37.4(*c* 0.113, MeOH).

(2*R*,3*S*,7*R*,11*R*)-3,5-Dihydroxy-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-4-en-10-one (epi-EI-1941−2 (39)). To a solution of **38** (5.8 mg, 0.024 mmol) in CH_2Cl_2 (0.24 mL) was added Et_3N (1.7 μL, 0.012 mmol) at room temperature, and the reaction mixture was stirred for 2 h. The reaction mixture was quenched with buffer, and diluted with AcOEt. The organic phase was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (AcOEt/hexane = 1:1) to afford epi-EI-1941−2 (**39**) (3.5 mg, 67%) as a colorless powder: ^{1}H NMR (400 MHz, CD_3CN) δ 0.95 (3H, t, J = 7.3 Hz), 1.55−1.38 (2H, m), 1.77−1.62 (2H, m), 2.88 (1H, dd, J = 3.4, 18.1 Hz), 3.59 (1H, dd, J = 1.0, 3.6 Hz), 3.84 (1H, dd, J = 1.5, 3.6 Hz), 4.35−4.42 (1H, m), 5.11 (1H, br-s); ^{13}C NMR (100 MHz, CD_3CN) δ 14.1, 18.7, 26.3, 36.3, 53.3, 57.0, 61.3, 78.7, 136.5, 141.3, 165.5, 194.4; FT-IR (neat) ν 3438, 2958, 2871, 1716, 1697, 1417, 1124, 1113, 1246, 1041 cm^{-1}; HRMS (FAB) [M + H]$^+$ calcd for $C_{12}H_{15}O_5$ 239.0920, found 239.0922; $[\alpha]^{23}_D$ −29.5 (*c* 0.087, MeOH).

(2*R*,3*R*,7*R*,10*S*,11*R*)-3-(*tert*-Butyldimethylsiloxy)-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-4-en-5,10-diol (40). To a solution of **31** and **32** (9.0 mg, 0.0254 mmol) in CH_2Cl_2 (0.9 mL) was added a hexane solution of DIBAL-H (0.94 M, 0.09 mL, 0.0787 mmol) at −90 °C under an argon atmosphere, and the reaction mixture was stirred for 20 min at that temperature. The reaction mixture was quenched with saturated aqueous Rochelle salt. The organic materials were extracted with AcOEt, washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (AcOEt/hexane = 1:3) to afford **40** (6.9 mg, 80%, 95:5 diastereoselectivity) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.14 (3H, s), 0.17 (3H, s), 0.89 (9H, s), 0.91 (3H, t, J = 7.2 Hz), 1.32−1.60 (2H, m), 1.80 (1H, dd, J = 1.9, 15.5 Hz), 1.88 (1H, br-*d*, J = 11.1 Hz), 2.32 (1H, dd, J = 11.1, 17.4 Hz), 2.62 (1H, br-*d*, J = 5.3 Hz), 3.23−3.24 (1H, m), 3.38−3.39 (1H, m), 3.93 (1H, ddt, J = 4.3, 7.5, 11.1 Hz), 4.18 (1H, br-*d*, J = 9.9 Hz), 4.59 (1H, br-s), 5.37 (1H, d, J = 4.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.9, −4.5, 14.0, 18.0, 18.5, 25.8, 33.3, 37.2, 52.5, 52.7, 63.3, 65.8, 67.4, 88.8, 129.1, 131.6; FT-IR (neat) ν 3410, 2956, 2929, 2858, 2364, 2341, 1259, 1082, 1059, 1003, 974, 837, 779 cm^{-1}; HRMS (FAB) [M]$^+$ calcd for $C_{18}H_{32}O_5Si$ 356.2019, found 356.2004; $[\alpha]^{22}_D$ −7.13 (*c* 0.70, MeOH).

(2*R*,3*R*,7*R*,11*S*)-3-(*tert*-Butyldimethylsiloxy)-5-hydroxy-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-4-en-10-one (42). To a solution of **40** (10.5 mg, 0.0295 mmol) in CH_2Cl_2 (1.0 mL) was added MnO_2 (64.0 mg, 0.736 mmol) at 0 °C under an argon atmosphere, and the reaction mixture was stirred for 1 h at that temperature. The reaction mixture was filtered through a pad of Celite, and concentrated in vacuo. The residue was purified by preparative thin-layer chromatography (AcOEt/hexane = 1:5) to afford **42** (10.0 mg, 95%; 95:5 diastereoselectivity) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.17 (3H, s), 0.19 (3H, s), 0.89 (9H, s), 0.91 (3H, t, J = 7.3 Hz), 1.38−1.61 (2H, m), 2.06 (1H, dd, J = 10.8, 17.6 Hz), 2.18 (1H, br-*d*, J = 17.6 Hz), 2.80 (1H, br-s), 3.47 (1H, dd, J = 1.0, 3.6 Hz), 3.61 (1H, dd, J = 1.0, 3.6 Hz), 3.86−3.92 (1H, m), 4.82 (1H, br-s), 5.56 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.8, −4.3, 13.9, 18.0, 18.4, 25.6, 27.7, 37.1, 52.6, 57.6, 63.3, 66.3, 88.0, 129.8, 146.0, 193.4; FT-IR (neat) ν 3431, 2956, 2931, 2860, 1684, 1464, 1259, 1074, 866, 739 cm^{-1}; HRMS (FAB) [M + H]$^+$ calcd for $C_{18}H_{32}O_5Si$ 356.2019, found 356.1990; $[\alpha]^{21}_D$ −137.2 (*c* 0.593, MeOH).

(2*R*,3*R*,7*R*,11*S*)-3,5-Dihydroxy-7-propyl-1,6-dioxatricyclo[8.1.0.0^{4,9}]undec-4-en-10-one (EI-1941−1 (1)). To a solution of **42** (6.0 mg, 0.0169 mmol) in CH_3CN (0.5 mL) was added HF·Pyr (0.05 mL) at room temperature, and the reaction mixture was stirred for 4 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$. The organic materials were extracted with AcOEt, washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (AcOEt/hexane = 1:1) to afford EI-1941−1 (**1**) (3.2 mg, 94% based on conversion) as a brownish oil: ^{1}H NMR (400 MHz, CD_3CN) δ 0.92 (1H, t, J = 7.2 Hz), 1.35−1.54 (4H, m), 1.96 (1H, br-dd, J = 11.1, 17.7 Hz), 2.09 (1H, ddd, J = 1.8, 3.2, 17.7 Hz), 3.43 (1H, dd, J = 1.0, 3.7 Hz), 3.74 (1H, dd, J = 1.3, 3.7 Hz), 3.85 (1H, m), 4.59 (1H, br-s), 5.51 (1H, s); ^{13}C NMR (100 MHz, CD_3CN) δ 14.2, 19.2, 28.3, 37.9, 53.4, 57.7, 62.9, 66.3, 88.2, 129.9, 148.3, 194.9; FT-IR (neat) ν 3419, 2960, 2933, 2873, 1682, 1456, 1281, 1028, 874, 725 cm^{-1}; HRMS (FAB) [M + H]$^+$ calcd for $C_{12}H_{17}O_5$ 241.1076, found 241.1075; $[\alpha]^{20}_D$ −188.8 (*c* 0.16, MeOH).

(3*R*,7*R*,8*R*)-7,8-Dihydroxy-3-propylisochromene-1,5-dione (EI-1941−3 (3)). To a solution of EI-1941−2 (**2**) (4.6 mg, 0.0193 mmol) in THF (0.39 mL) was added a THF solution of SmI_2 (0.1 M, 0.58 mL, 3.0 mmol) at −90 ° C under an argon atmosphere, and the reaction mixture was stirred for 20 min at that temperature. The reaction mixture was quenched with pH 7.0 phosphate buffer, and diluted with AcOEt. The organic phase was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (AcOEt/hexane = 1:0) to afford EI-1941−3 (**3**) (4.5 mg, 98%) as a reddish oil: ^{1}H NMR (400 MHz, CD_3CN) δ 0.94 (3H, t, J = 7.3 Hz), 1.35−1.56 (1H, m), 1.60−1.69 (1H,

m), 1.71−1.80 (1H, m), 2.26 (1H, br-dd, J = 11.3, 18.2 Hz), 2.51 (1H, dd, J = 4.0, 16.7 Hz), 2.76 (1H, ddd, J = 1.4, 3.9, 18.2 Hz), 2.93 (1H, dd, J = 3.0, 16.7 Hz), 3.38 (1H, br-s), 3.76 (1H, br-s), 4.22 (1H, q, J = 3.2 Hz), 4.42−4.49 (1H, m); ^{13}C NMR (100 MHz, CD_3CN) δ 14.1, 18.9, 26.3, 37.4, 41.7, 66.7, 70.5, 79.0, 136.9, 143.6, 167.0, 197.7; FT-IR (neat) ν 3419, 2960, 2933, 2873, 1716, 1693, 1410, 1230, 1024 cm^{-1}; HRMS (FAB) $[M + H]^+$ calcd for $C_{12}H_{17}O_5$ 241.1076, found 241.1076; $[\alpha]^{30}_D$ −88.7 (*c* 0.28, MeOH).

(1*S*,2*S*,6*R*)-2-Hydroxy-5-oxo-4-pent-1-enyl-7-oxabicyclo-[4.1.0]hept-3-ene-3-carboxylic Acid (45). To a solution of **23t** (5.0 mg, 0.014 mmol) in MeOH (0.05 mL) was added Dowex 50W-X4 (10.0 mg), and the mixture was stirred at room temperature for 24 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by preparative thin-layer chromatography (MeOH/$CHCl_3$ = 1:10) to afford carboxylic acid **45** (2.3 mg, 93%) as a colorless oil: ^{1}H NMR (400 MHz, CD_3OD) δ 0.92 (3H, t, J = 7.3 Hz), 1.45 (2H, sextet, J = 7.3 Hz), 2.11 (2H, q, J = 6.9 Hz), 3.54 (1H, d, J = 3.9 Hz), 3.77 (1H, d, J = 3.9 Hz), 5.00 (1H, br-s), 6.33 (1H, dt, J = 6.9, 16.1 Hz), 6.45 (1H, d, J = 16.1 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 13.9, 23.1, 36.9, 55.0, 57.3, 65.9, 123.5, 134.1, 141.3, 143.0, 174.6, 197.2; FT-IR (neat) ν 3342, 2960, 2925, 2873, 2854, 2360, 1695, 1633, 1576, 1261, 1041, 970, 739 cm^{-1}; HRMS (FAB) $[M + H]^+$ calcd for $[C_{12}H_{14}O_5 + H]^+$ 239.0919, found 239.0934; $[\alpha]^{25}_D$ −73.8 (*c* 0.32, MeOH).

(2*R*,3*S*,11*S*)-3-Hydroxy-7-propyl-1,6-dioxatricyclo-[8.1.0.0^{4,9}]undec-4,7-dien-5,10-dione (46). To a solution of **28** (3.6 mg, 0.0074 mmol) in MeOH (0.05 mL) was added Dowex 50W-X4 (7.2 mg), and the mixture was stirred at room temperature for 24 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by preparative thin-layer chromatography (AcOEt/hexane = 1:5) to afford **46** (1.7 mg, 97%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (3H, t, J = 7.5 Hz), 1.67 (2H, sextet, J = 7.5 Hz), 2.50 (2H, t, J = 7.5 Hz), 3.66 (1H, d, J = 3.5 Hz), 3.94 (1H, dd, J = 1.4, 3.5 Hz), 5.28 (1H, br-s), 6.38 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.4, 20.2, 35.8, 52.8, 56.6, 61.4, 98.2, 125.7, 139.1, 163.4, 166.9, 191.4; FT-IR (neat) ν 3431, 2964, 2931, 2875, 1722, 1705, 1641, 1577, 1045, 852, 760 cm^{-1}; HRMS (FAB) $[M]^+$ calcd for $C_{12}H_{12}O_5$ 236.0685, found 236.0664; $[\alpha]^{24}_D$ −96.1 (*c* 0.43, MeOH).

Measurement of Interleukin-1β) Secretion. THP-1 cells were suspended in RPMI1640 medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum, and seeded on 48-well plates (5 × 10^4 cells/well). The cells were differentiated with 30 nM of pharbol-12-myristate-13-axetate (PMA) for 72 h. After the plate was rinsed with serum-free RPMI1640 medium to remove unadherent cells, adherent cells were stimulated with 100 μg/ml of lipopolysaccharide (LPS; Sigma) for 4 h in the presence of various concentrations of test compounds. The culture media were harvested, and mature IL-1β was measured by an ELISA method using an IL-1β assay kit (Amersham Biosciences, Tokyo, Japan).

Measurement of Cell Viability. THP-1 cells (2.5 × 10^4 cells/well) were differentiated with 30 nM PMA as described above. The differentiated cells were then treated with test compounds for 4 h. The cell number was evaluated by the subsequent color reaction. WST-8 solution 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt (Nacalai tesque, Kyoto, Japan), was added to the medium, and the cells were further incubated for 3 h at 37 °C. The absorbance (A_{450}) of each well was measured using a plate reader (Wallac 1420 multilabel counter; Amersham Biosciences). Cell viability (%) was calculated as (experimental absorbance − background absorbance)/(control absorbance − background absorbance) × 100.

Acknowledgment. We thank Dr. Fumito Koizumi at Kyowa Hakko Kogyo Co., Ltd., for the NMR charts of EI-1941−1, −2, and −3. This work was partially supported by a Grant-in-Aid for Scientific Research on Priority Areas 16073219 from The Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Supporting Information Available: Copies of ^{1}H and ^{13}C NMR and IR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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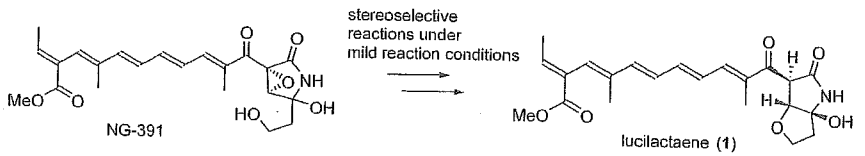
Natural Products Synthesis

Determination by Asymmetric Total Synthesis of the Absolute Configuration of Lucilactaene, a Cell-Cycle Inhibitor in p53-Transfected Cancer Cells



A biomimetic pathway to lucilactaene (**1**) from NG-391 has been developed which involves stereoselective reactions under very mild conditions. It was demonstrated that **1** racemizes rapidly, and the conditions under which racemization occurs were elucidated. Lucilactaene (**1**) isolated under neutral conditions is racemic, which suggests that either the natural product is racemized rapidly in the mycelia, or racemic **1** is biosynthesized.

Keywords: asymmetric synthesis · biosynthesis · lucilactaene · racemization · total synthesis

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Natural Products Synthesis

Determination by Asymmetric Total Synthesis of the Absolute Configuration of Lucilactaene, a Cell-Cycle Inhibitor in p53-Transfected Cancer Cells**

*Junichiro Yamaguchi, Hideaki Kakeya, Takao Uno, Mitsuru Shoji, Hiroyuki Osada, and Yujiro Hayashi**

The tumor-suppressor gene p53 is involved in important cellular events, such as cell-cycle control and apoptosis.[1] The p53 gene is lost or mutated in many types of human tumors. Small molecules that induce cell-cycle arrest or apoptosis in a p53-independent manner or allow mutant p53 to alter a conformationally active form of p53 may be good candidates for treating various types of cancers.[2] Recently we isolated lucilactaene (**1**), which arrests cell-cycle progression in the G1 phase at the nonpermissive temperature of 37 °C in Hl299/tsp53 cells, from *Fusarium* sp. RK97-94.[3] Lucilactaene (**1**) is a synthetically challenging molecule because of its rare hexahydro-3a-hydroxy-5-oxo-2*H*-furo[3,2-b]pyrrol-6-yl ring system and its substituted and conjugated *E*,*E*,*E*,*E* pentaene moiety, which is unstable to acid, base, and light.

Along with lucilactaene (**1**), we isolated a known neuronal-cell-protecting compound, NG-391 (**2**),[4] which possesses the same pentaene portion but a different γ-lactam moiety, and which is probably biosynthesized from the same intermediate as **1**.[3] These natural products **1** and **2** are close structural relatives of fusarins A and C,[5] nonmutagenic metabolites of *Fusarium moniliforme*. Though the biosynthetic pathways that lead to **1** and **2** remain unclear, the following is a plausible pathway based on the proposed biosynthetic route to fusarin C:[5] The fully elaborated polyketide reacts with homoserine aldehyde by an intramolecular Knoevenagel reaction to form the unmodified 1,5-dihydropyrrol-2-one **3**, a possible common key intermediate of **1** and **2**, after cleavage of the thioester (NADPH reduction) and condensation (Scheme 1). In the case of NG-391 (**2**), the remaining steps are epoxidation and oxidation to form the hemiaminal, either by ether hydroxylation α to the nitrogen atom or oxidation to an imine and addition of water (the

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

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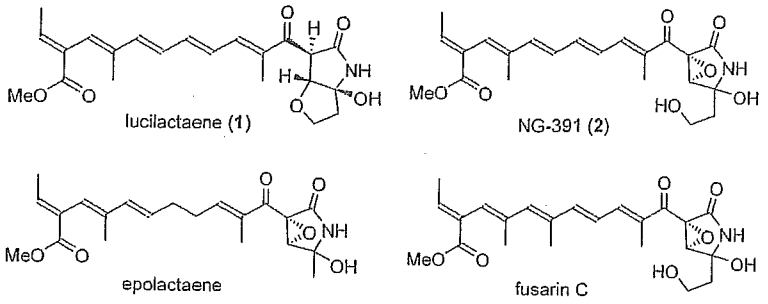
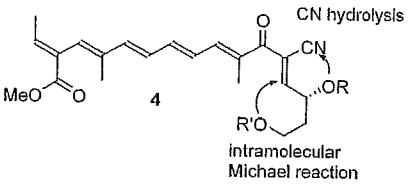


reactions might occur in the reverse order). In the case of lucilactaene (**1**), an intramolecular Michael reaction and oxidation to form the hemiaminal are the remaining reactions (again the order of reactions might be reversed). Another possibility for the biosynthesis of **1** is via **2** through intramolecular epoxide-ring opening by the primary hydroxy group, followed by reduction.

A similar side chain is also found in epolactaene, a neuritogenic compound isolated from the fungal strain *Penicillium* sp. BM-1689-P.[6] Clarification of the structure–activity relationships of lucilactaene (**1**), NG-391 (**2**), epolactaene, and their derivatives is highly desirable for elucidating their mechanism of action. We completed the first total syntheses of **2**[7] and epolactaene,[8] and developed a biologically more potent molecule, epolactaene tertiary butyl ester (ETB).[9] Recently, we revealed that both epolactaene and ETB bind to human Hsp60 and inhibit Hsp60 chaperon activity in vitro and in cultured cells,[9] whereas Kobayashi and co-workers reported that epolactaene is an inhibitor of mammalian topoisomerases α and β in vitro.[10]

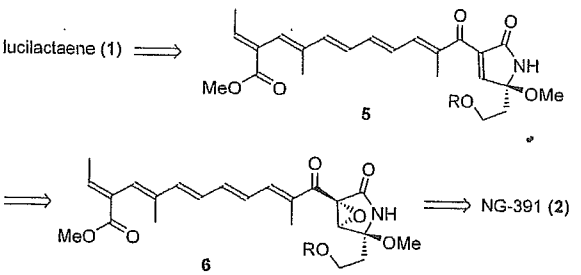
The absolute configuration of NG-391 (**2**) was determined by us by asymmetric total synthesis.[7] The optical rotation of lucilactaene (**1**) is zero in two different solvents (methanol and chloroform), which indicates the possibility that **1** is racemic. That **1** should be racemic appears strange when one considers its structural resemblance with **2**. Because of the interesting biological properties of **1**, its lability, and its rare structure, and because of the puzzle concerning its absolute configuration, we have investigated its asymmetric total synthesis by a biomimetic route.

On the basis of the proposed biosynthetic pathway, we planned to synthesize **1** from the key intermediate **4**, which corresponds to the key biosynthetic intermediate **3**. We had already synthesized **2** from **4**.[7] The remaining steps from **4** to lucilactaene (**1**) would be hydrolysis of the nitrile group, an intramolecular Michael reaction with the hydroxy group (R′ = H) as the nucleophile, and functional-group transformations (Scheme 2). However, all attempts, including changing of the order in which the reactions were carried out, were unsuccessful.

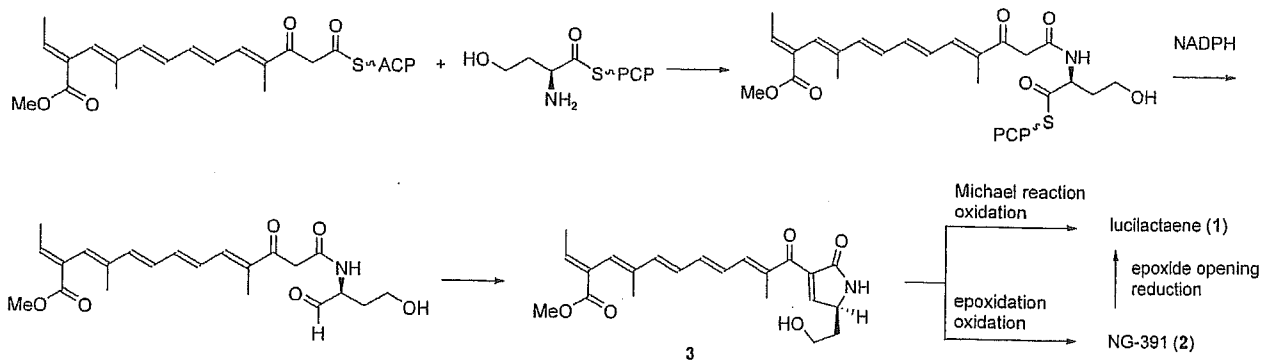


Scheme 2. Proposed synthetic approach to lucilactaene from **4**.

We therefore considered an approach to **1** from NG-391 (**2**)[7] (Scheme 3). The formation of methyl ether **6**, followed by reductive removal of the epoxide to give an alkene **5**, an intramolecular Michael reaction, and deprotection would afford lucilactaene (**1**). For this approach to be successful the reactions would have to proceed under mild conditions to avoid decomposition of the labile pentaene moiety. Methyl ether formation and the Michael reaction must proceed with



Scheme 3. Retrosynthetic analysis of lucilactaene.



Scheme 1. Proposed biosynthesis of lucilactaene (**1**) and NG-391 (**2**). ACP = acyl carrier protein, NADPH = nicotinamide adenine dinucleotide phosphate, PCP = peptidyl carrier protein.

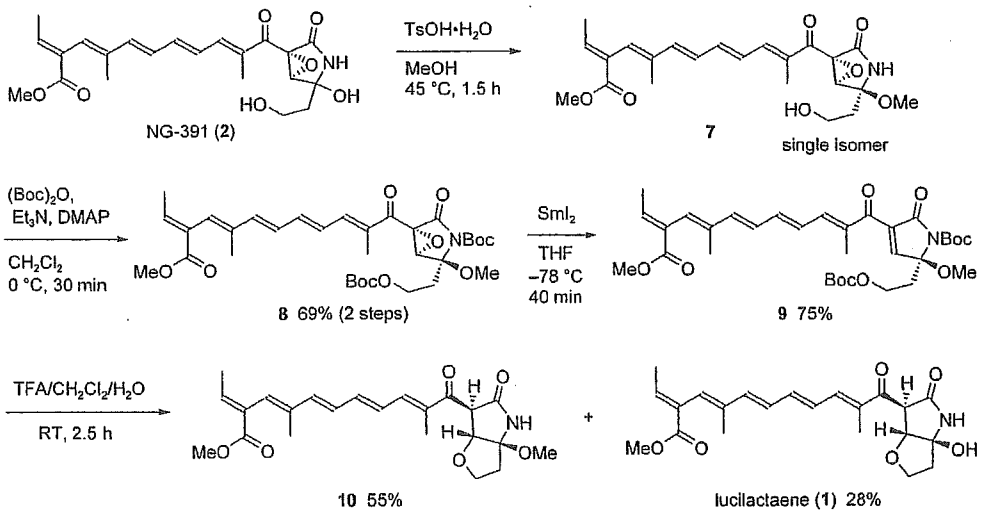
high diastereoselectivity for **1** to be generated with high enantiomeric excess.

The methyl ether was formed stereoselectively by the treatment of **2** with a catalytic amount of $TsOH{\cdot}H_2O$ in MeOH to afford β-methoxide **7** as a single isomer in which methanol had captured the acyliminium ion intermediate from the opposite face to that occupied by the epoxide (Scheme 4).[11] The next planned transformation was the reductive removal of the epoxide. Although SmI_2 is known to convert α,β-epoxyketones into α,β-unsaturated ketones,[12] in this case reductive demethoxylation is faster than epoxide removal. When epoxylactam **7** was treated with SmI_2, a 5-(2-hydroxyethyl)-2-pyrrolidone derivative was formed. After some experimentation, it was found that the protecting group on the nitrogen atom of the amide affects the reactivity of the compound towards reductive demethoxylation. Thus, **7** was treated with Boc_2O in the presence of triethylamine and a catalytic amount of DMAP to give the bis-Boc-protected derivative **8** in 69% yield over two steps. The reductive removal of the epoxide with SmI_2 (2 equiv) now proceeded efficiently at low temperature without affecting the methoxy group to afford **9** in 75% yield. The two Boc protecting groups were then removed by treatment with CF_3CO_2H (TFA) in CH_2Cl_2 at room temperature, whereupon a spontaneous Michael reaction and the conversion of the methyl ether into a hydroxy group gave lucilactaene (**1**) in 28% yield, along with lucilactaene methyl ether (**10**) in 55% yield. Synthetic **1** exhibited identical spectroscopic properties to those of the natural product (^1H NMR, ^{13}C NMR, IR).

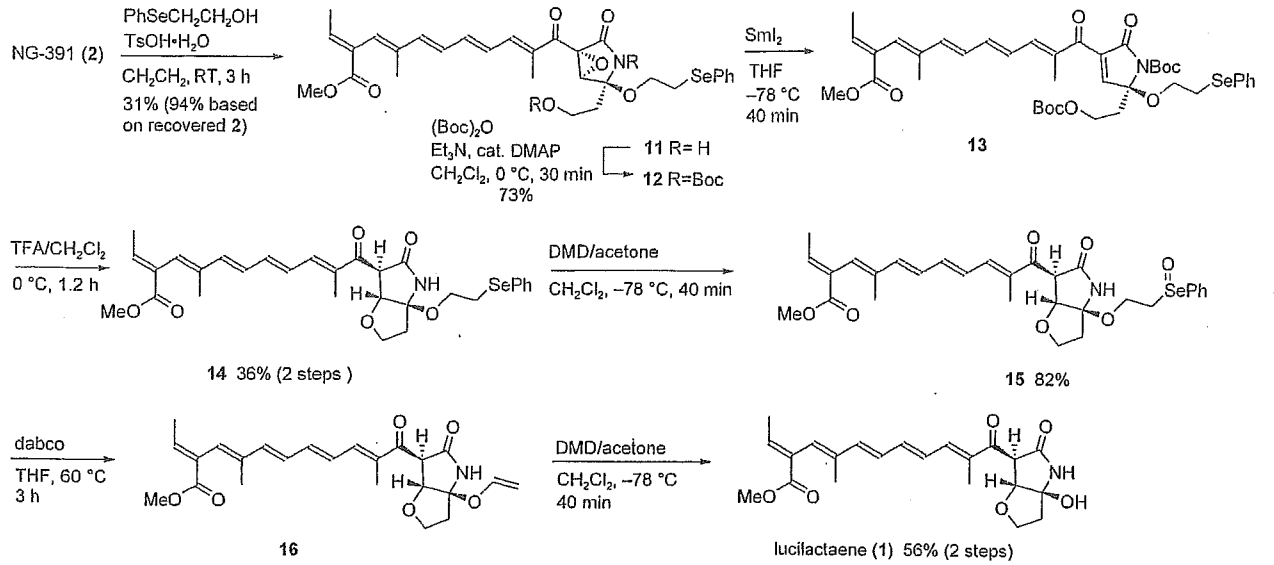
The optical rotation of this synthetic lucilactaene (**1**) was zero, identical to that of the isolated natural product and markedly different to that of **10** ($[\alpha]_D$ +36.6 (c = 0.17, MeOH)). Lucilactaene methyl ether (**10**) can also be converted into **1** in 60% yield by treatment with TFA in CH_2Cl_2; again the optical rotation of the product is zero. The large difference in optical rotation between **1** and its methyl ether **10** strongly suggests that the lucilactaene (**1**) formed is racemic. If racemization occurs during the synthesis, it must be during the final treatment with acid. To better understand the facile racemization of **1**, the synthesis of optically pure **1** was investigated.

To avoid possible racemization, the final cleavage of the hemiaminal protecting group should be conducted under neutral conditions. To this end we developed a novel deprotection method. When NG-391 (**2**) was treated with $PhSeCH_2CH_2OH$[13] in the presence of a catalytic amount of $TsOH{\cdot}H_2O$ in CH_2Cl_2 for 3 h at room temperature, phenylselenylethyl ether **11** was formed in 31% yield as a single isomer; **2** was recovered in 67% yield (Scheme 5). Although decomposition occurred upon longer treatment of **2** with acid, the repeated exposure of recovered **2** to acid led to its conversion into **11** in a high overall yield of 94%. Both the amide and the hydroxy groups were protected with Boc_2O to afford **12** in 73% yield. The sequence of steps involving reductive removal of the epoxide with SmI_2, removal of the Boc groups, and the Michael reaction proceeded as efficiently as for the methyl ether derivative **8** to afford the bicyclic compound **14** as a single isomer in 36% yield over two steps. The transformation of the 2-phenylselenylethoxy group into a hydroxy group could be carried out under mild reaction conditions in three novel steps: 1) The oxidation of the selenide to the selenoxide, which was isolated in good yield, proceeded smoothly at low temperature on treatment with dimethyldioxirane (DMD),[14] without affecting the pentaene moiety. 2) The elimination of benzeneselenenic acid occurred at 60°C in the presence of dabco to provide vinyl ether **16**.[15] 3) Final oxidative removal of the vinyl substituent was performed under neutral conditions by the use of DMD at low temperature (−78°C) to afford lucilactaene (**1**) in 56% yield from **15** *in optically pure form* ($[\alpha]_D$ +39.5 (c = 0.10, MeOH)).[16]

It is clear from the asymmetric total synthesis that isolated natural lucilactaene (**1**) is racemic. The facile racemization raises another question: There is the possibility that natural **1** is optically active but that racemization proceeds during the purification process. To investigate this possibility, it was necessary to isolate **1** under nonracemizing conditions. We



Scheme 4. Synthesis of lucilactaene. Boc = *tert*-butoxycarbonyl, DMAP = 4-dimethylaminopyridine, Ts = *p*-toluenesulfonyl.



Scheme 5. Synthesis of optically pure lucilactaene; dabco = 1,4-diazabicyclo[2.2.2]octane.

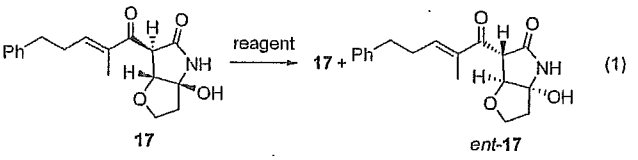
chose to use bicycle **17** as a model compound to establish such conditions.

Optically pure **17**, prepared by the same method as that used for **1**, was treated with various reagents, and after a certain period of time the optical purity of the recovered **17** was measured by HPLC analysis on a chiral phase; the results are summarized in Table 1. Under weakly acidic or basic conditions, for example, in the presence of PPTS in MeOH or NEt_3 in CH_2Cl_2, no racemization was observed. However, racemization occurred when **17** was treated with TFA/CH_2Cl_2 or K_2CO_3 in MeOH. It was also confirmed that no racemization occurred in the medium in which the fermentation was carried out. These results indicate that the purification of lucilactaene (**1**) should be performed under nearly neutral, mild reaction conditions. The racemization might occur via intermediates such as **18** or **19**, which arise from a reversible retro-Michael reaction, followed by acyliminium ion formation or keto–amide formation, though the order of the reactions might be different (Scheme 6).

As information about the racemization under a variety of conditions had been obtained, the production profile of lucilactaene (**1**) by *Fusarium* sp. RK97-94 was investigated further. All experiments were performed as rapidly as possible, with the temperature and pH value controlled carefully. The ethyl acetate extracts of the broth (supernatant) and the mycelia, which were obtained by centrifugation, were prepared under mild conditions at pH 7.0. The production profile of **1** in the broth is summarized in Table 2. The optical purity of **1** in the broth was very low (ca. 10% *ee*) throughout the fermentation. Moreover, the lucilactaene (**1**) in the mycelia was also nearly racemic (data not shown).

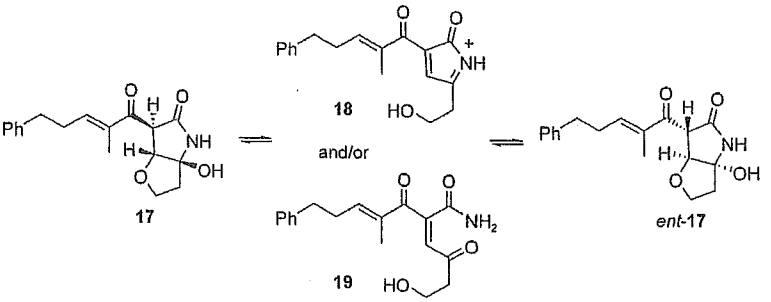
As shown in Scheme 1, lucilactaene (**1**) and NG-391 (**2**) may be biosynthesized from the same intermediate **3**. Epoxidation and oxidation to form the hemiaminal produce **2**; these two reactions proceed in this order, as **2** would otherwise be racemic. The absolute configura-

Table 1: Racemization of the lucilactaene model **17**.



Entry	Reagent	*T* [°C]	*t* [h]	*ee* [%][a]
1	none	23	24	100
2	$AcOH/CH_2Cl_2$ (1:20)	23	3	100
3	PPTS in MeOH (0.005 M)	23	3	100
4	DMD in acetone (0.07 M)	–78	0.5	100
5	Et_3N/CH_2Cl_2 (1:4)	23	3	100
6	TFA/CH_2Cl_2 (1:100)	0	0.1	98
7	TFA/CH_2Cl_2 (1:20)	0	0.25	57
8	$TsOH·H_2O$ in CH_2Cl_2 (0.013 M)	23	3	48
9	K_2CO_3 in MeOH (0.15 M)	23	3	2
10	TFA/CH_2Cl_2 (1:4)	0	2.5	0
11	culture medium[b]	28	48	100

[a] Optical purity was determined by HPLC analysis on a chiral phase (chirapak AD-H). [b] Culture medium: 2% glucose, 1% soluble starch, 0.3% meat extract, 2.5% yeast extract, 0.05% NaCl, 0.005% K_2HPO_4, 0.05% $CaCO_3$, and 0.05% $MgSO_4·H_2O$ adjusted to pH 7.2. PPTS = pyridinium *p*-toluenesulfonate.



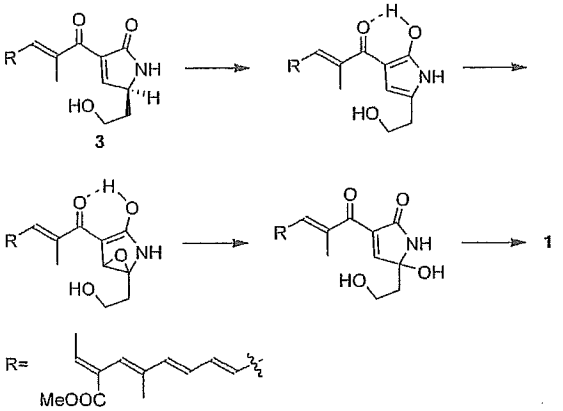
Scheme 6. Racemization of **17** via **18** and/or **19**.

Table 2: Production profile of **1** by *Fusarium sp.* RK97-94 in the broth.

Entry	*t* [h]	pH[a]	PCV[b] [%]	Production of **1** [$\mu g\,mL^{-1}$]	*ee* [%][c]
1	24	7.4	10	0	n.d.[d]
2	48	7.2	30	0.014	10.0
3	72	8.3	45	0.084	10.3
4	96	8.5	50	0.71	6.9

[a] pH value of the fermentation broth. [b] Packed-cell volume (*v*/*v*). [c] Optical purity was determined by HPLC analysis on a chiral phase (chiralcel OD-RH). [d] Not determined.

tion of the epoxide can also be explained reasonably as arising from the selective epoxidation from the opposite face to that with the hydroxyethyl substituent. In the synthesis of **1**, a Michael reaction and oxidation to form the hemiaminal are the remaining steps. Should the Michael reaction be the first step, then we suspect that racemization must occur during the subsequent oxidation step, because the internal Michael addition is a spontaneous reaction, as demonstrated for the synthetic conversion of **13** into **14** without racemization. That is, there is an acidic moiety in the enzyme responsible for this oxidation which causes racemization. If oxidation is the first step, a subsequent racemization process is conceivable (Scheme 7): Intermediate **3** may undergo tautomerism to a



Scheme 7. Possible racemization mechanism in the biosynthesis of lucilactaene.

2-hydroxypyrrole derivative, which could undergo epoxidation and isomerization. Racemization could occur via the achiral pyrrole tautomer. A subsequent Michael reaction would then afford racemic lucilactaene (**1**).

In summary, the labile natural product lucilactaene (**1**), which readily undergoes racemization, has been synthesized for the first time in optically pure form via a biomimetic pathway. The conditions under which racemization occurs were elucidated during this total synthesis. The careful isolation of lucilactaene (**1**) from both the broth and the mycelia under neutral, nonracemizing conditions demonstrated that the isolable natural product is in fact itself racemic. This total synthesis, which enabled verification of the absolute configuration of **1**, has several noteworthy features: All the reactions from NG-391 (**2**) are mild enough not to affect the labile *E*,*E*,*E*,*E*,*E* pentaene moiety; ether formation from **2** to **7** and **11** and the intramolecular Michael reaction from **9** to **10** or from **13** to **14** are both highly stereoselective; the reductive removal of the epoxide with SmI_2 without effecting demethoxylation, and the deprotection of a hemiaminal under neutral, oxidative conditions via vinyl ether **16** by using a newly developed phenylselenylethyl protecting group, are also useful transformations. Detailed biological studies on both enantiomers of lucilactaene are underway, the results of which will be reported in due course.

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Keywords: asymmetric synthesis · biosynthesis · lucilactaene · racemization · total synthesis

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[16] The optical purity was determined by HPLC analysis on a chiral phase (chiralcel OD-RH column, H_2O/CH_3CN (100:45), 1.0 mL min^{-1}; t_R ((−)-**1**): 27.7 min, t_R ((+)-**1**): 40.2 min).

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First Asymmetric Total Synthesis of Synerazol, an Antifungal Antibiotic, and Determination of Its Absolute Stereochemistry

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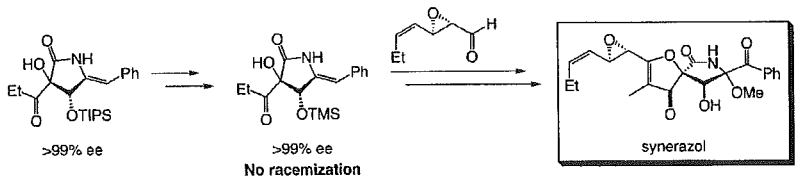

By synthesizing two possible diastereomers, the first asymmetric total synthesis of synerazol, an antifungal antibiotic, has been accomplished, allowing determination of its absolute stereochemistry. A more practical second generation route was also established. The key steps are racemization-free deprotection of a TIPS group and introduction of a methyl ether by DMD oxidation of the benzylidene moiety in a substrate having a small protecting group.

Introduction

Synerazol is an antifungal antibiotic isolated by Ando and co-workers in 1991 from the cultured broth of *Aspergillus fumigatus* SANK 10588.[1] Synerazol is active against *Candida albicans* and other fungi and shows marked synergistic activity with azole-type antifungal agents. Synerazol contains a highly oxygenated 1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione skeleton with benzoyl and epoxyalkene substituents. The core structure, a hetero-spirocyclic γ-lactam, is also found in the pseurotins[2] and azaspirene.[3] Pseurotin A, isolated from a culture broth of *Pseudeurotium ovalis* (strain S2269/F) in 1976 by Bloch et al.,[2a] was reported to inhibit chitin synthase by Sterner et al. in 1993[2c] and also found to induce cell differentiation of PC12 cells by Komagata et al. in 1996.[2d] Its structure, including the absolute stereochemistry, has been unambiguously determined by a single-crystal X-ray analysis of its 12,13-dibromo derivative.[2b] Azaspirene, possessing the same core structure, has been isolated from the fungus *Neosartorya* sp. by Kakeya and Osada et al. in 2002[3] and found to inhibit endothelial migration induced by vascular endothelial growth factor. Because of the unprecedented, densely functionalized core structure of the pseurotins and azaspirene, their total synthesis poses a significant challenge.[4] We have completed the first total synthesis of a member of this class of compounds, that of azaspirene, in 2002.[5] Through this asymmetric total synthesis the absolute stereochemistry of azaspirene was determined. Our group also accomplished the asymmetric total syntheses of pseurotin A and of 8-*O*-demethylpseurotin A in 2003.[6] Recently Tadano's group reported the total syntheses of pseurotin A, 8-*O*-demethylpseurotin A, and azaspirene from D-glucose.[7] Despite

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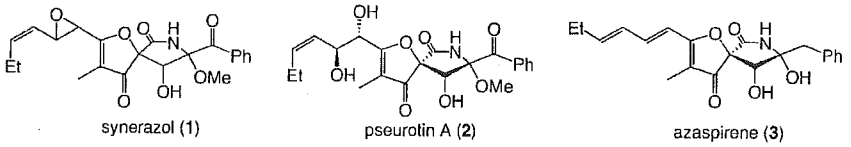


FIGURE 1. The structures of synerazol (**1**), pseurotin A (**2**), and azaspirene (**3**).

the similarity of their structures, the reported biological properties of synerazol, pseurotin A, and azaspirene are rather different as described above. Systematic comparison of the biological properties of these natural products and their derivatives is highly desirable, and a sufficient quantity of not only the natural products but also several derivatives is required for such biological study. As we had established a synthetic method for pseurotin A and azaspirene, we began to investigate the total synthesis of synerazol, the last member of this family of natural products remaining unsynthesized.The absolute stereochemistry of synerazol was unknown when we started this synthesis. We have determined this unambiguously by the first asymmetric total synthesis of synerazol as described in the first part of this paper[8] and in the second part a more practical synthesis of the natural isomer involving highly diastereoselective reactions. Recently Igarashi and co-workers have also determined the absolute stereochemistry using the modified Mosher's MTPA method on an alcohol derived from synerazol.[9]

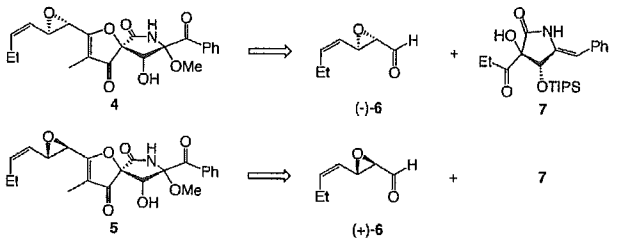
Results and Discussion

First Generation Synthesis of Synerazol. Retrosynthetic Analysis. While the absolute stereochemistry of synerazol was unknown, we postulated that the absolute stereochemistry of the 1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione ring system would likely be the same as pseurotin A[2b] and azaspirene.[5] As there was no information on the side-chain epoxyalkene, we decided to synthesize both possible diastereomers, **4** and **5**.

Using the methodology developed for the synthesis of pseurotin A and azaspirene, we planned to prepare the diastereoisomers **4** and **5** from ketone **7** containing the lactam moiety and epoxyaldehydes (−)-**6** and (+)-**6**, respectively, by aldol condensation followed by functional group transformations (Scheme 1).

Synthesis of the Epoxyaldehyde. The enantiomerically pure side-chain aldehyde has been prepared in a highly stereoselective manner (Scheme 2). The synthesis started from D-tartaric acid diethyl ester (−)-**8**, which was converted to epoxy diester (+)-**10** by Saito's procedure.[10] Careful optimization of the reaction conditions enabled the selective monoreduction of this diester to hydroxy ester (+)-**11** in reasonable yield (71%). Protection of the alcohol with TBSCl and imidazole, followed by reduction and oxidation, afforded aldehyde (+)-**14**. Wittig reaction provided the *Z*-olefin with excellent stereoselectivity (*Z*:*E* = >98:2). Deprotection and oxidation afforded side-chain aldehyde (−)-**6**, which was used immediately in the next reaction because it gradually decomposed. Starting from L-tartaric acid diethyl ester (+)-**8**, the enantiomer (+)-**6** was also prepared by the same route.

SCHEME 1. First Generation Retrosynthetic Analysis



With side-chain epoxyaldehydes (−)-**6** and (+)-**6** in hand, their reaction with ketone **7**, prepared by our established method,[5,6] was examined.

The lithium enolate of **7** reacted with side-chain epoxy aldehyde (−)-**6**, affording the aldol product **17** in 45% yield (diastereomer ratio = 2:1) with recovery of ketone **7** in 49% yield (Scheme 3). Thus, the yield based on the recovered starting material (BRSM) was 88%. Oxidation of aldol **17** with the Dess−Martin periodinane (DMP)[11] in CH_2Cl_2 proceeded smoothly, providing the 1,3-diketone, which owing to the mild acidity of silica gel was transformed into azaspiro compound **18** via cyclization and dehydration reactions during purification with thin-layer chromatography (TLC).

When **18** was treated with dimethyldioxirane (DMD)[12] at low temperature, selective oxidation of the benzylidene was achieved, affording diol **19** in 40% yield with 33% recovery of **18**. As overoxidation proceeded on prolonged reaction time or at higher temperature, quenching the reaction at an early stage and repeating the oxidation on recovered **18** are recommended. A subsequent DMP oxidation afforded benzoyl derivative **20** in good yield (95%).

In our previous total synthesis of pseurotin A,[6] successful transformation of a hydroxy group into the corresponding methyl ether was accomplished by treatment of **23** with AcCl in MeOH to afford pseurotin A in 25% yield (eq 1, Scheme 4). When *N*,*O*-acetal **25**, prepared by removal of the TIPS group of **20**, was treated with AcCl in MeOH in order to convert the hydroxy group into a methoxy group and form **4**, decomposition occurred because of the acid instability of the epoxyalkene moiety (eq 2, Scheme 4). Even in the presence of a milder acid

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(8) We have orally presented the first synthesis of synerazol and determination of its absolute stereochemistry at the following meeting: Hayashi, Y.; Shoji, M.; Mukaiyama, T.; Yamaguchi, S.; Gotoh, H.; Kakeya, H.; Osada, H. *Abstracts of Papers*, 84 Annual Meeting of Japan Chemical Society, Kobe, Japan, March 27, 2004; p 981.

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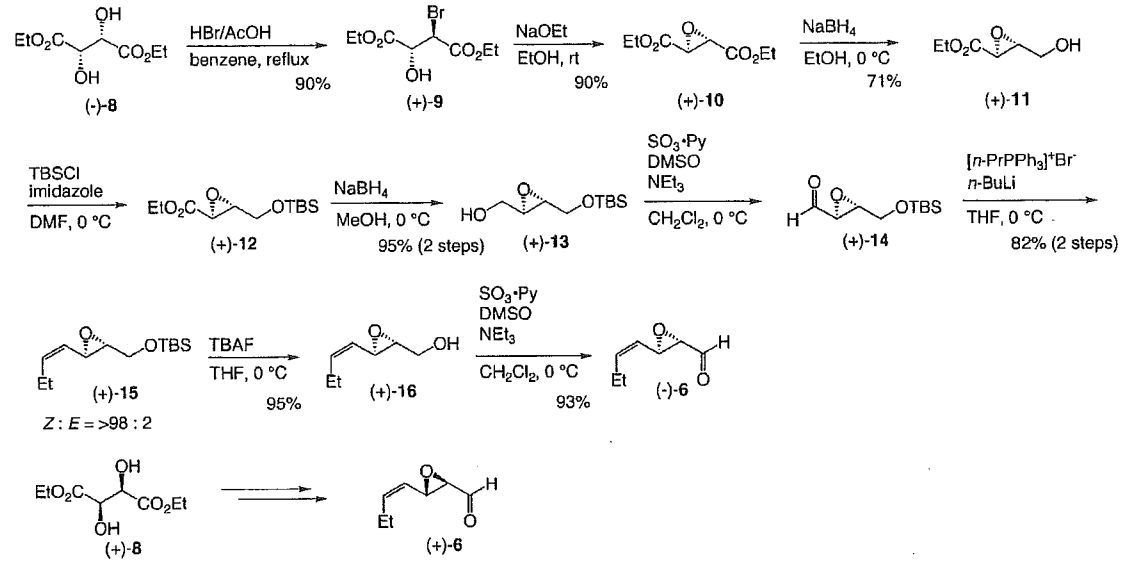
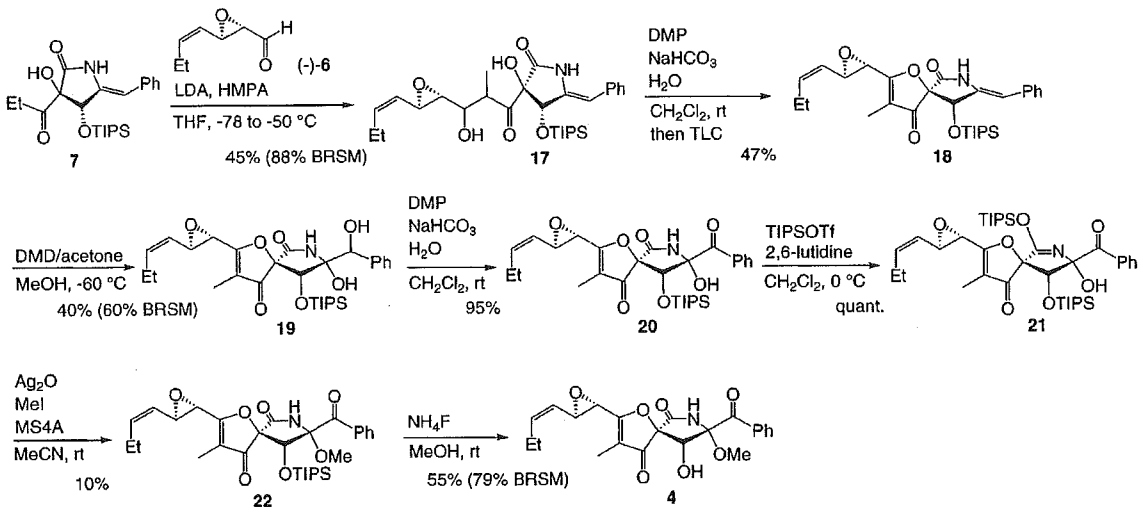
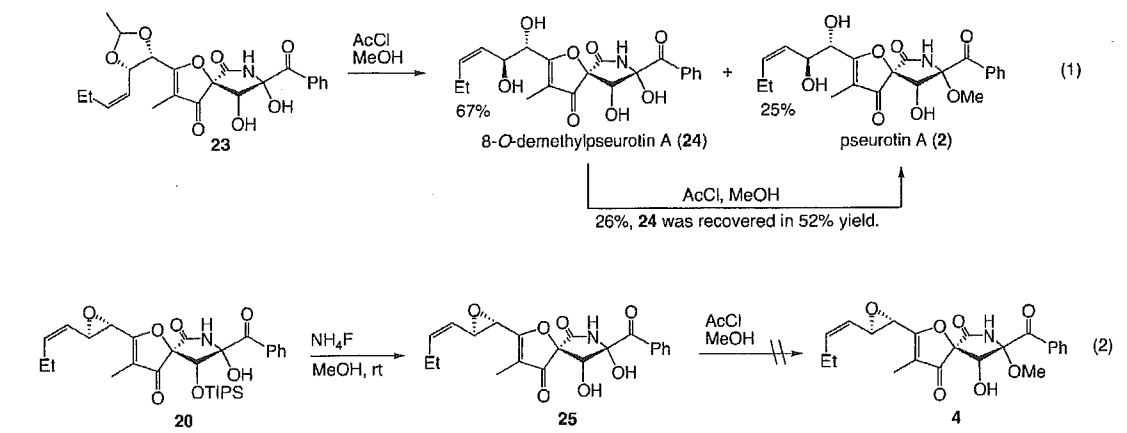
SCHEME 2. Synthetic Scheme of Epoxyaldehyde (−)-6 and (+)-6



SCHEME 3. Synthesis of 4



SCHEME 4



such as TsOH·H_2O or pyridinium *p*-toluenesulfonate (PPTS), decomposition also occurred without formation of **4**.

We next employed the Williamson ether synthesis. When **20** was treated with triisopropylsilyl trifluoromethanesulfonate (TIPSOTf) and 2,6-lutidine, *O*-sily-