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Total Synthesis of (+)-Sch 725680: Inhibitor of Mammalian A−, B−, and Y−Family DNA Polymerases

Toshifumi Takeuchi,[†] Yoshiyuki Mizushina,[‡] Satoshi Takaichi,[†] Natsuki Inoue,[§] Kouji Kuramochi,[∥] Satomi Shimura,[†] Yusuke Myobatake,[†] Yuri Katayama,[†] Kenji Takemoto,[†] Shogo Endo,[†] Shinji Kamisuki,[†] and Fumio Sugawara*,[†]

Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science (RIKADAI), Noda-shi, Chiba 278-8510, Japan, Laboratory of Food & Nutritional Sciences, Department of Nutritional Science, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan, Department of Radiation Biosciences, Faculty of Pharmaceutical Sciences, Tokyo University of Science (RIKADAI), Noda-shi, Chiba 278-8510, Japan, and Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Sakyo-ku, Kyoto 606-8522, Japan

sugawara@rs.noda.tus.ac.jp

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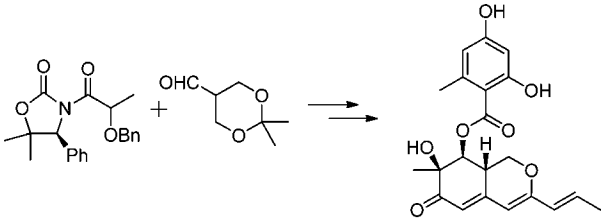
ABSTRACT



Sch 725680, **1**

The total synthesis of (+)-Sch 725680, a member of the hydrogenated azaphilone family, has been accomplished. The synthesis confirmed the absolute configuration and biological activities of the natural product. A key reaction to construct a hydrogenated azaphilone core skeleton is a Ti-mediated aldol reaction.

Sch 725680, **1**, was originally isolated by Yang et al. from a culture of an *Aspergillus* sp. and was shown to display growth inhibitory activity against *Saccharomyces cerevisiae* PM503 and *Candida albicans* C43 with MICs of 8 and 64 μg/mL, respectively (Figure 1).[1] Recently, Stierle et al. reisolated (+)-**1**, named berkazaphilone C, together with berkazaphilone B, **2**, from a culture of *Penicillium rubrum* as caspase 1 inhibitors with an IC_{100} of 25 μM.[2] They also reported that **1** selectively inhibits cell proliferation against the SR cell line in the National Cancer Institute (NCI) 60 cell line assay with a GI_{50} of 0.38 μM. In our natural product isolation program for the construction of a small molecule library,[3] we also independently reisolated (+)-**1** together with **2**, named pinophilin A, and pinophilin B, **3**, from a culture of *Penicillium pinophilum* Hedgcok as inhibitors of the mammalian A-, B-, and Y-family of DNA polymerases.[3g] Using the excitation chirality method, we also determined that the absolute configurations are 7*S*,8*S*,8a*S*.

Compounds **1**, **2**, and **3** belong to a class of natural products called hydrogenated azaphilones, a subclass of azaphilones, that share a highly oxygenated bicyclic core, a

[†] Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science.
[‡] Laboratory of Food & Nutritional Sciences, Department of Nutritional Science, Kobe-Gakuin University.
[§] Department of Radiation Biosciences, Faculty of Pharmaceutical Sciences, Tokyo University of Science.
[∥] Graduate School of Life and Environmental Sciences, Kyoto Prefectural University.

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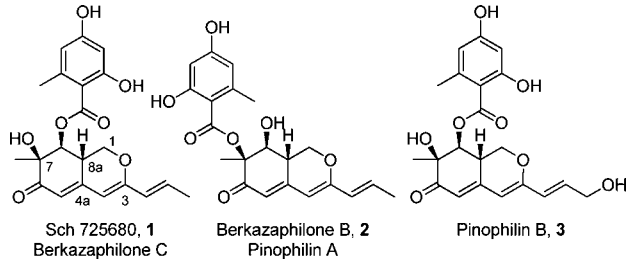
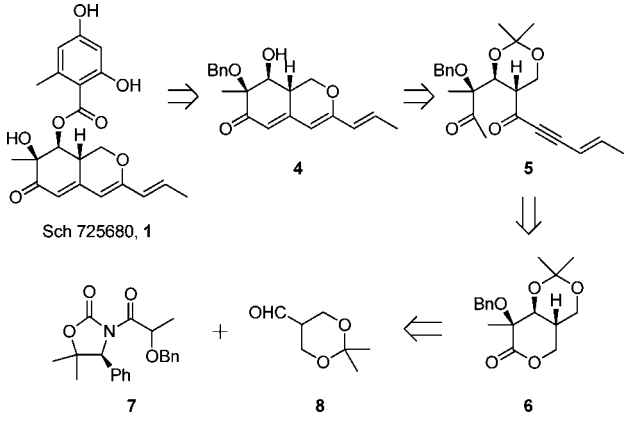


Figure 1. Structure of Sch 725680, Berkazaphilone B, and Pinophilin B.

chiral tertiary hydroxyl group, and a trienone moiety.[4] Although a number of synthetic studies of azaphilones, such as mitorubrinic acid,[5] have been reported, no synthetic study of hydrogenated azaphilones has been disclosed. A flexible and scalable synthetic route for structure–activity relationship studies is required for further biological studies of hydrogenated azaphilones. Herein, we report the first total synthesis of (+)-**1**, as well as confirmation of its absolute configuration and inhibitory activity against the mammalian A-, B-, and Y-family of DNA polymerases and the proliferation of human cancer cell lines.

Our retrosynthesis of (+)-Sch 725680 is depicted in Scheme 1. Sch 725680, **1**, should be derived from hydrogenated azaphilone core structure **4** by the attachment of an orsellinate fragment. The hydrogenated azaphilone core skeleton should be prepared from diketone **5** using intramolecular aldol cyclization and 1,6-oxy-Michael cyclization. Diketone **5** should be derived from lactone **6**, which should be stereoselectively obtained from readily available imide **7**[6] and aldehyde **8**[7] using the Ti-mediated aldol reaction developed by Kobayashi's group.[8]

Scheme 1. Retrosynthetic Analysis of Sch 725680



The Ti-mediated aldol reaction of imide **7** and aldehyde **8** (LDA, $ClTi(O\text{-}i\text{-}Pr)_3$, THF, 81%) gave *anti*-1,2-diol **9**,[9] followed by the deprotection of isopropylidene acetal and cleavage of the chiral auxiliary group ($Zn(NO_3)_2 \cdot 6H_2O$, MeCN, 89%) to afford lactone **10** as a diastereomeric mixture of the hydroxy methyl group (α/β = 4:1). Isopropylidene acetal protection of 1,3-diol **10** ($(MeO)_2CMe_2$, 10-camphorsulfonic acid (CSA), CH_2Cl_2) gave lactone **6α** and **6*β*** in yields of 70% and 18%, respectively. After both lactone **6α** and **6*β*** were converted to Weinreb amides (MeO(Me)NH·HCl, *i*-PrMgBr, THF), oxidation of alcohols (PCC, MS4A, CH_2Cl_2) gave corresponding aldehydes **11*β*** and **11*β*** in yields of 65% and 68% over two steps, respectively. The stereochemistry of the C8a formyl group in aldehyde **11*β*** was inverted under basic conditions (DBU, THF, 96%) to afford aldehyde **11α**, as shown in Scheme 2.

Freshly prepared dibromoalkene **12**[10] was treated with *n*-BuLi (THF, −78 °C to rt, 2 h in the dark) to give alkynyl lithium **13**; the resulting solution was immediately used in the next step (Scheme 3). The treatment of aldehyde **11α** with alkynyl lithium **13** followed by treatment with MeLi and oxidation of the resultant alcohol gave diketone **5**. Aldol cyclization[11] of diketone **5** (TBAF, THF, 0 °C, 2 h) gave β-hydroxy ketone **14** and α,β-unsaturated ketone **15**

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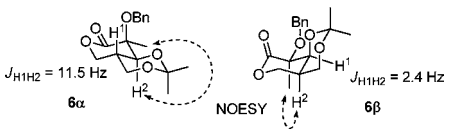
(9) The absolute configuration of secondary alcohol was determined as *S* by modified Mosher's method using (*S*)- and (*R*)-MTPA esters of **9**. The relative configuration of 1,2-diol was determined as the *anti* configuration by NMR studies of acetonide derivatives **6α** and **6*β***. See Supporting Information.



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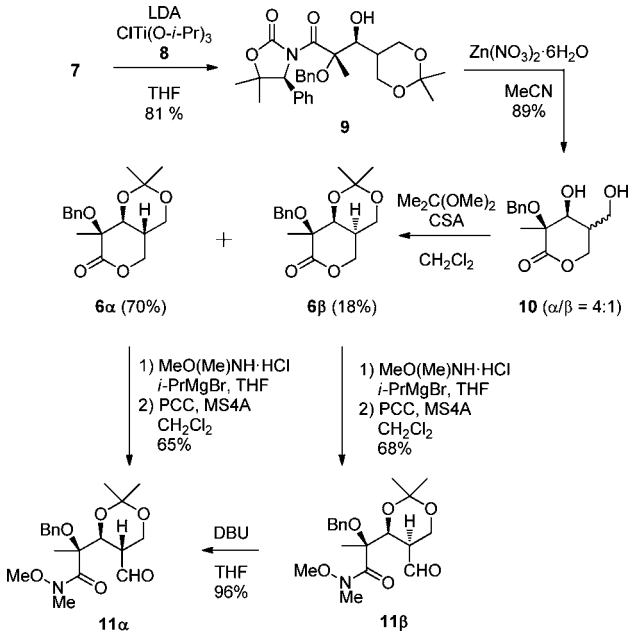
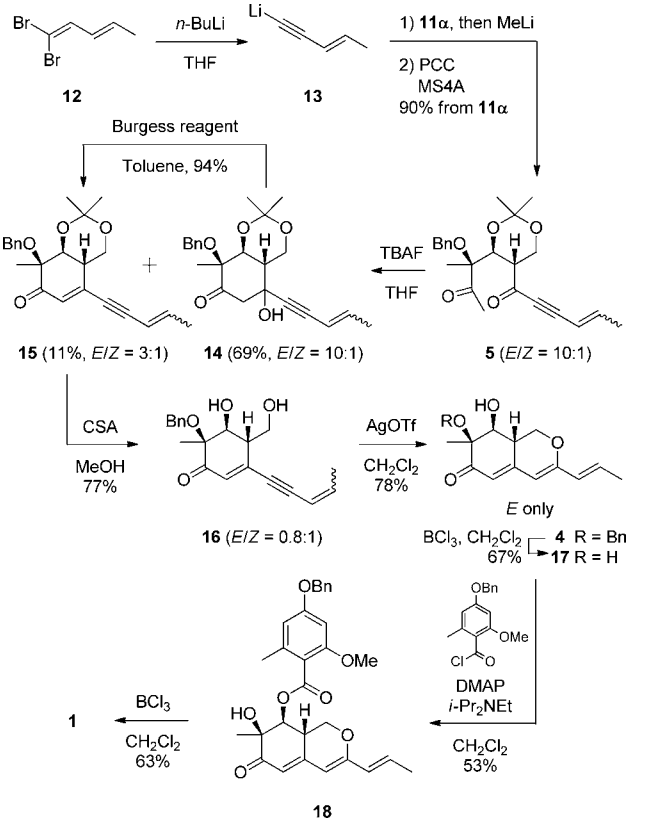
Scheme 2. Preparation of Aldehyde **11α**



at yields of 69% and 11% as ca. 10:1 and ca. 3:1 mixtures of *E*/*Z* isomers, respectively. Although isomerization of *E*-olefin to *Z*-olefin occurred,[12] we thought *Z*-olefin should be isomerized to *E*-olefin under 1,6-oxy-Michael cyclization conditions. Dehydration of alcohol **14** (Burgess reagent, toluene, 100 °C) gave enone **15** in 94% yield as a ca. 3:1 mixture of *E*/*Z* isomers. Isopropylidene acetal deprotection gave alcohol **16** (CSA, MeOH, 77% as a ca. 0.8:1 mixture of *E*/*Z* isomers) to cause further *E*/*Z*-isomerization. 1,6-Oxy-Michael cyclization of alcohol **16** was achieved using silver trifluoromethanesulfonate[13] in CH_2Cl_2 to give hydrogenated azaphilone core skeleton **4** with only an *E*-olefin configuration, as expected. After benzyl deprotection (BCl_3, CH_2Cl_2, 0 °C, 67%) and the introduction of protected orsellinate (4-benzyloxy-2-methoxy-6-methylbenzoyl chloride, DMAP, *i*-Pr_2NEt, CH_2Cl_2, 53%),[5d] both benzyl and methyl deprotection (BCl_3, CH_2Cl_2, 63%)[5d] gave hydrogenated azaphilone Sch 725680, **1**. The characterization data (^1H and ^{13}C NMR, IR, MS, UV, CD, inhibitory activity of mammalian DNA polymerases and of the proliferation of human cancer and normal cells[14]) for **1** ($[\alpha]_D^{22}$ +103 (*c* 0.1, MeOH)) was in agreement with those reported for **1**[1,2,3g] ($[\alpha]_D^{22}$ +103 (*c* 0.27, MeOH)[3g]).

Scheme 3. Completion of the Total Synthesis of (+)-Sch 725680



In summary, we have accomplished the first total synthesis of hydrogenated azaphilone Sch 725680, thereby, confirming its absolute configuration and biological activities. The natural product was obtained in a 13-step sequence with an overall yield of 6.0%, starting from known aldehyde **8**. Key steps to establish the hydrogenaged azaphilone core skeleton were a Ti-mediated aldol reaction with controlled introduction of the C7- and C8-stereochemistry and a silver-catalyzed 1,6-oxy-Michael cyclization.

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Supporting Information Available. Detailed experimental procedures, characterization data, ^1H and ^{13}C NMR spectra, and biological activities of both synthetic and natural Sch 725680. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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(14) Synthetic Sch 725680's ability to inhibit the activity of DNA polymerases and proliferation of five human cancer cell lines was investigated and compared to that of the natural substance. Synthetic Sch 725680 showed identical biological activity. See Supporting Information.