Practical synthesis of DOPA derivative for biosynthetic production of potent antitumor natural products, saframycins and ecteinascidin 743

Torikai, Kohei
Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, University of Shizuoka

Saruwatari, Takayoshi
Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, University of Shizuoka

Kitano, Tatsuya
Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, University of Shizuoka

Sano, Takuya
Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, University of Shizuoka

他

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Practical Synthesis of DOPA Derivative for Biosynthetic Production of Potent Antitumor Natural Products, Saframycins and Ecteinascidin 743

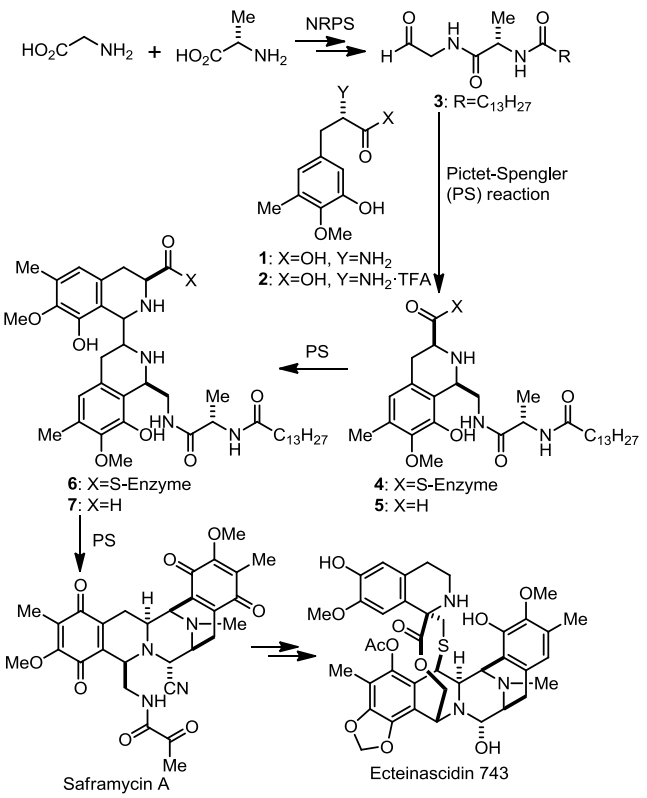
Kohei Torikai,[†,*] Takayoshi Saruwatari,[‡] Tatsuya Kitano,[‡] Takuya Sano, Atsuko Nakane, Hiroshi Noguchi, and Kenji Watanabe
Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan

(E-mail: torikai@chem.kyushu-univ.jp)

Abstract A practical synthetic route of DOPA derivative **2**, which should be useful for direct biosynthetic production of potent antitumor natural products, saframycins and ecteinascidin 743 was established. The developed strategy features i) easy-to-handle reactions without special care upon both dryness and inert atmopsphere, and ii) the facile HPLC-free purification of **2** via recrystallization enabling scalable synthesis of **2**.

Introduction Saframycins (SMs), produced by *Streptomyces* and various soil bacteria as well as marine vertebrates such as ascidians and sponges, are potent antitumor antibiotics [1]. In particular, a highly potent SM analog, ecteinascidin 743 [2] (ET-743), has recently been in use as an anticancer drug against soft-tissue sarcoma [3]. ETs share the central pentacyclic tetrahydroisoquinoline core with SMs, except for the oxidation state of their terminal rings and the additional ten-membered lactone bridge found in ET-743. Due to the short supply from natural resources, the production of ET-743 should depend on a semi-synthesis including 21 synthetic steps [4]. In order to facilitate the direct biosynthetic production of SMs including ETs, unremitting bioinfomatic analyses were carried out, and it was found that SMs are biosynthesized from L-alanine, glycine and two molecules of 3,4-dihydroxyphenylalanine (DOPA) derivative **1** [5] through dual Pictet-Spengler (PS) mechanism [6] (Scheme 1). Briefly, tetrahydroisoquinoline core is constructed by the following three steps; i) Schiff base is formed between DOPA derivative **1** and dipeptidic aldehyde **3**, generated from glycine and L-alanine by the aid of non-ribosomal polypeptide synthetases (NRPSs); ii) PS cyclization occurs to give **4**; and iii) enzymatic region is reductively eliminated to afford aldehyde **5**, which is involved in the same sequence to furnish SMs and ETs through intermediates **6** and **7**. Thus, to develop an engineered perpetual SM-producing system, we cloned necessary biosynthetic gene clusters for SMs and expressed them in model creatures, however, it was unsuccessful to detect the production of SMs even by mass spectrometric analysis [7]. We envisaged that one of the reasons would be insufficient amount of endogenous non-natural amino acid **1**, and if **2** were fed from outside the system, enough supply of SMs would be realized. Herein, we report practical and scalable synthesis of amino acid **2** to tolerate the feeding experiments.

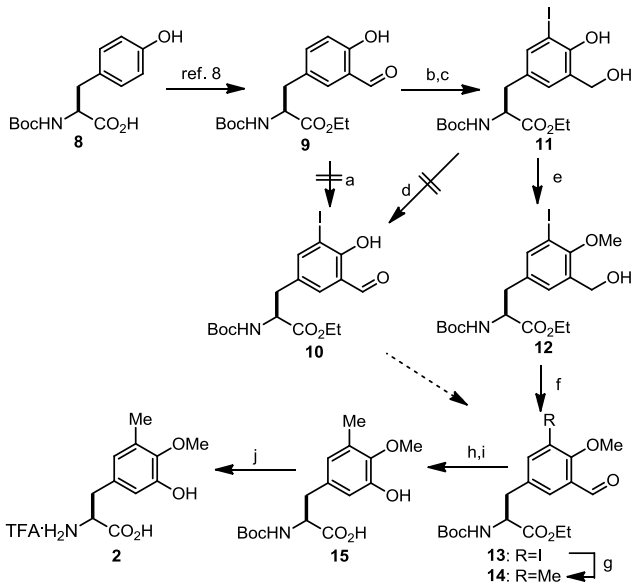
Results and Discussion Synthesis of DOPA derivative **2** commenced with *N*-*t*-butoxycabonyl (*N*-Boc) tyrosine **8** according to the Schmidt's report [8], one of the most expeditious and easy-to-handle methods to date [9] (Scheme 2). Aldehyde **9** was prepared by Reimer-Tiemann formylation [10a] and the subsequent esterification of *N*-Boc tyrosine **8** as reported previously [8,10b]. Although we attempted the



Scheme 1. Hypothetical Biosynthesis of Saframycin A and Ecteinascidin 743.

conversion of **9** into iodobenzene **10** by the action of I_2 and H_2O_2, the reaction could not be reproduced even in refluxing ethanol. Hence, we decided to set a robust and reproducible route for the two-steps-introduction of iodine as follows; (i) $NaBH_4$ reduction of aldehyde **9** into the corresponding alcohol [9a], and (ii) iodination of the resulting alcohol with I_2/H_2O_2 combination. As a result, probably due to the fact that electron-withdrawing formyl group was converted to electron-donating hydroxymethyl group, electrophilic iodination proceeded smoothly to afford **11** in good yield (69% for two steps).

Our next task was to oxidize alcohol **11** into Schmidt's intermediate **10** with MnO_2 under argon atmosphere, however, our endeavor was wasteful only to observe the decomposition of substrate **11**. We postulated that *o*-hydroxybenzaldehyde structure of **10** would be unstable even to mild heterogeneous oxidant under inert atmosphere, therefore, methylation of phenolic hydroxy group was first performed. After selective methylation of **11** using methyl iodide and potassium carbonate



Scheme 2. Reagents and Conditions: (a) I_2, 30% H_2O_2 aq, EtOH, rt, 115 h, no reaction; (b) $NaBH_4$, EtOH, 0 °C, 20 min, 78%; (c) I_2, H_2O_2, EtOH, rt, 1.5 h, 88%; (d) MnO_2, CH_2Cl_2, rt, 1 h, decomposed; (e) MeI, K_2CO_3, acetone, reflux, 15 h, 92%; (f) MnO_2, Na_2SO_4, CH_2Cl_2, rt, 25 min, 93%; (g) $Pd_2(dba)_3{\cdot}CHCl_3$, Me_4Sn, NMP, 70 °C, 71 h, 82%; (h) 4 M LiOH aq, THF, 0 °C, 3.5 h, then H_2O_2, rt, 41 h; (i) 4 M NH_3/MeOH, MeOH, rt, 3.5 h; (j) TFA, Me_2S, CH_2Cl_2, rt, 3.5 h, then recrystallization, 42% (over 3 steps).

(92%), the resultant *o*-methoxybenzylalcohol derivative **12** was subjected to MnO_2 oxidation. As expected, the reaction successfully furnished the desired aldehyde **13** in excellent yield with spot-to-spot manner (93%).

The final stage of this synthesis, that is, methylation of iodide **13** by Stille protocol using $Pd_2(dba)_3{\cdot}CHCl_3$ and Me_4Sn (**14**, 82%), followed by saponification, Dakin oxidation of **14** into DOPA derivative **15** and deprotection to give **2**, was all successful by the process of Schmidt's report. However, their reported route seemed not to be suitable for scale-up, for HPLC purification after the final step was inevitable.

To construct scalable and non-laborious HPLC-free system, we pursued clean conditions to afford **2**, as well as facile purification technique. After considerable experimentation, we found that (i) removal of Boc group with trifluoroacetic acid (TFA) in the presence of dimethylsulfide as cation scavenger to avoid side reactions, probably including Friedel-Crafts type *t*-butylation of electron-rich aromatic ring, and (ii) recrystallization of crude TFA salt from diethyl ether/methanol (10:1) furnished the desired product **2** with excellent purity [11].

Conclusion In conclusion, by improving the Schmidt's method, we have established a practical synthetic route of DOPA derivative **2**, which should be useful for biosynthetic production of saframycins and ecteinascidins, potent antitumor antibiotics. The developed route features higher reproducibility, more facile handling, and much better scalability than the original method to tolerate the feeding experiments. Moreover, this improved route without needs of setting dry, inert, and cold (< 0 °C) conditions should open the door to access amino acid **2** for researchers in biosynthetic community who are not so familiar with organic synthesis. Further studies toward totally biosynthetic production of saframycins and their analogs with potent biological activities, that are available only at low yields or produced by difficult-to-culture organisms, are now in progress in our laboratory.

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[11] Data of **2**: Mp. 233-236 °C; $[\alpha]_D^{28}$ -6.1 (*c* 0.13, H_2O); ^1H NMR (400 MHz, CD_3OD) δ 7.55 (1H, s), 7.34 (1H, s), 3.82 (4H, m), 3.14 (2H, m), 2.32 (3H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 173.2, 170.2, 158.7, 136.8, 134.4,

132.4, 130.9, 127.2, 61.9, 57.1, 37.3, 24.8, 16.2 ppm; IR (KBr) 3431, 2989, 2361, 2341, 1695, 1610, 1481, 1396, 1265, 1220, 1139, 1011, 897, 807 cm^{-1}

† Present address: Department of Chemistry, Graduate Faculty of Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan.

‡ These authors contributed equally to this work.