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Chemo-enzymatic Total Syntheses of Jorunnamycin A, Saframycin A, and *N*-Fmoc Saframycin Y3

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Supporting Information

ABSTRACT: The antitumor tetrahydroisoquinoline (THIQ) alkaloids share a common pentacyclic scaffold that is biosynthesized by nonribosomal peptide synthetases involving unique enzymatic Pictet−Spengler cyclizations. Herein we report concise and divergent chemo-enzymatic total syntheses of THIQ alkaloids by merging precise chemical synthesis with *in vitro* engineered biosynthesis. A recombinant enzyme SfmC responsible for the biosynthesis of saframycin A was adapted for the assembly of these natural products and their derivatives, by optimizing designer substrates compatible with SfmC through chemical synthesis. The appropriately functionalized pentacyclic skeleton were efficiently synthesized by streamlining the linkage between SfmC-catalyzed multistep enzymatic conversions and chemical manipulations of the intermediates to install aminonitrile and *N*-methyl groups. This approach allowed rapid access to the elaborated pentacyclic skeleton in a single day starting from two simple synthetic substrates without isolation of the intermediates. Further functional group manipulations allowed operationally simple and expeditious syntheses of jorunnamycin A, saframycin A, and *N*-Fmoc saframycin Y3 that could be versatile and common precursors for the artificial production of other antitumor THIQ alkaloids and their variants.

With the exception of the semisynthetic approach, the two synthetic disciplines of chemical synthesis and biological synthesis have been developed essentially independently in an effort to gain efficient access to structurally complex and biologically intriguing natural products.[1] The two disciplines offer complementary strengths and weaknesses, yet very few attempts have been made to explore the interface of chemical and *in vitro* biological synthesis to achieve the total synthesis of natural products and their analogs.[2,3] Herein we report the chemo-enzymatic divergent total syntheses of saframycin A (**1**),[4] *N*-protected derivative of saframycin Y3 (**2**),[5] and jorunnamycin A (**3**)[6] by streamlining the integration of (i) chemical synthesis of designer substrates, (ii) an enzyme-catalyzed cascade synthesis working in conjunction with chemical manipulations to install an aminonitrile and a *N*-methyl substituent to forge a pentacyclic core scaffold, and (iii) subsequent two- or three-step chemical transformations to gain rapid access to the natural products (**1**−**3**). These natural products hold a promise as versatile intermediates for the production of tetrahydroisoquinoline (THIQ) antitumor antibiotics and their variants.

The THIQ alkaloids represented by saframycins (**1**, **2**), jorunnamycins (**3**), renieramycins (**4**), and safracins (**5**) constitute important families of antitumor antibiotics (Figure 1).[7] These alkaloids share a densely functionalized common pentacyclic scaffold capable of DNA alkylation through generation of an electrophilic iminium species exploiting either aminonitrile or hemiaminal group at C21. Ecteinascidin-743 (**6**) bearing an additional THIQ system with the formation of a macrolactone exhibits exceptionally potent antitumor activities and is clinically approved for the chemotherapy of ovarian neoplasms and sarcoma.[8] This anticancer agent **6**

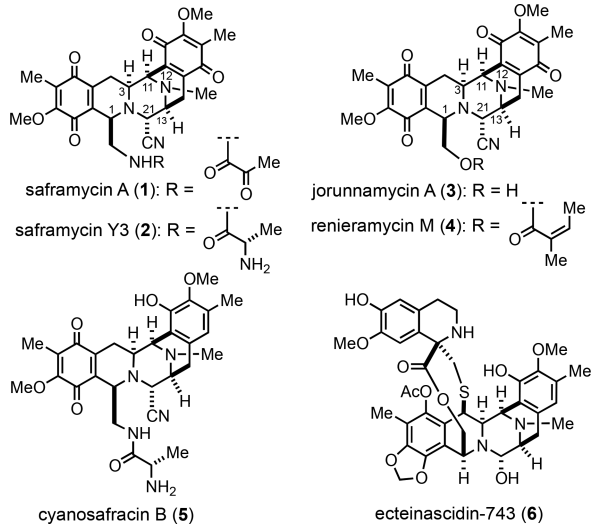

Figure 1. Structures of tetrahydroisoquinoline (THIQ) alkaloids.

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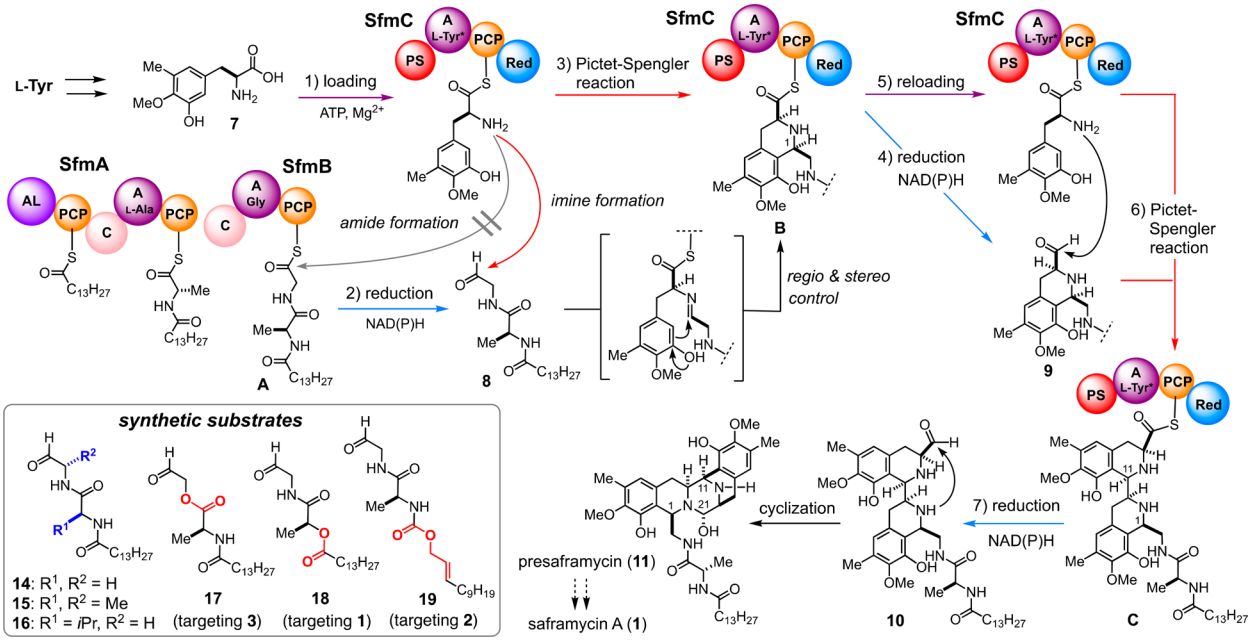


Figure 2. Proposed NRPS biosynthetic assembly line leading to saframycin A (**1**) and design of synthetic substrates (**7**, **8**, and **14**–**19**).

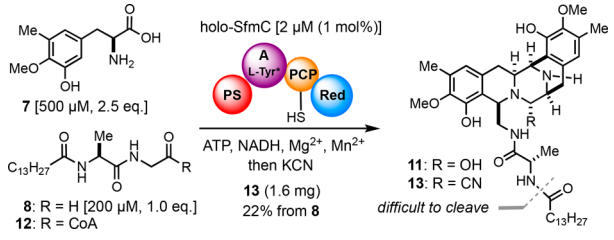
contains the common pentacyclic scaffold shared with other THIQ alkaloids, and its semisynthetic production in 21 chemical steps from fermentation-derived cyanosafracin B (**5**) has been developed to provide **6** for clinical applications.[9] The promising therapeutic potentials of THIQ alkaloids coupled with growing demand for artificial production of these complex structures have made the common THIQ scaffold an important target molecule for both chemical synthesis and engineered biosynthesis.[10,11]

The pentacyclic scaffold of saframycin A (**1**), a representative member of the THIQ alkaloids, is biosynthesized from L-alanine, glycine, and two molecules of the functionalized tyrosine derivative **7** (Figure 2). Nonribosomal peptide synthetases (NRPSs) composed of three modules (SfmA, SfmB, and SfmC) are responsible for the assembly of the pentacyclic scaffold.[12] Unlike the canonical NRPS system catalyzing the condensation of amino acid components,[13] the biosynthetic machinery of saframycins has two distinctive features.[14] First, SfmA contains an acyl-CoA ligase (AL) domain for loading a fatty acid, whereas such a long acyl chain is absent from the THIQ alkaloids. According to a collinearity rule, two modules (SfmA and SfmB) catalyze formation of *N*-acylated alanylglycinyl thioester **A** present on the peptidyl carrier protein (PCP) in SfmB. The second feature is the unique catalytic function of SfmC exploiting the Pictet–Spengler (PS) and reduction (Red) domains. Instead of amide bond formation being catalyzed by the canonical condensation (C) domain, the Red domain could catalyze reduction of the thioester substrate **A** on the upstream module (SfmB), liberating peptidyl aldehyde **8**. The Pictet–Spengler (PS) domain could mediate imine formation between the resultant **8** and the tyrosine derivative **7** loaded on the PCP in SfmC. Subsequent regio- and stereocontrolled cyclization would afford the THIQ intermediate **B** with formation of a sp^3-stereogenic center at C1. Then, SfmC composed of the four domains (PS, A, PCP, and Red) could allow iterative assembly of **7**. Thioester reduction to generate aldehyde **9** followed by reloading of **7** and subsequent Pictet–Spengler reaction could incorporate an additional stereocenter at C11 to produce intermediate **C** bearing two THIQ substructures. Further thioester reduction would furnish an aldehyde **10**, and spontaneous intramolecular cyclization would forge the pentacyclic scaffold **11** having a hemiaminal moiety at C21.

The chain length of the fatty acid group was shown to be critical for efficient enzymatic conversions. A series of experiments revealed cryptic roles played by the long fatty acyl chain and illustrated the elegant interplay of the four domains of SfmC. Tyrosine derivative **7** as well as peptidyl aldehydes such as **8** bearing the fatty acyl group are reported to be commonly involved in the biosynthesis of saframycin Mx1,[12b] quinocarcin,[15] SF-1739/naphthyridinomycins,[15,16] and ecteinscidins.[17] Consequently, the related NRPS biosynthetic machinery can likely be adopted to very similar and potentially universal strategies for the production of THIQ alkaloids.

The engineered biosynthesis of functionalized tyrosine derivative **7** has not been achieved yet, to our knowledge, while the biosynthetic enzymes responsible for the conversion of L-tyrosine into **7** have been suggested.[17,18] To secure a sufficient amount of **7**, a catalytic asymmetric synthetic route was developed.[19] We previously investigated the SfmC-catalyzed assembly of the tetracyclic scaffold (Scheme 1). Employment of *N*-myristoylated peptidyl aldehyde **8**, assumed to be liberated from the upstream module SfmB, markedly

Scheme 1. SfmC-Catalyzed Conversion of Synthetic Substrates



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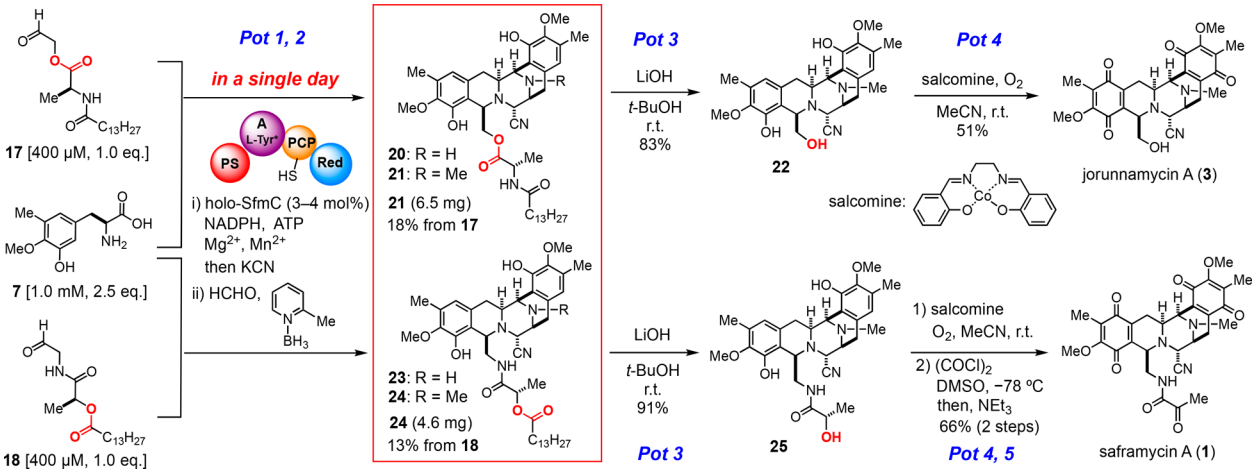
Scheme 2. Chemo-enzymatic Total Syntheses of Jorunnamycin A (3) and Saframycin A (1)



accelerated (greater than 10-fold) the formation of **11**, in comparison to the corresponding CoA thioester **12**. Upon incubation of **8** and tyrosine derivative **7** with recombinant holo-SfmC in the presence of ATP, Mg^{2+}, Mn^{2+}, and NAD(P)H, we observed formation of the pentacyclic **11**. One-pot treatment of the resulting crude reaction mixture with KCN and subsequent purification afforded aminonitrile **13** in up to 22% overall yield based on **8**.[14a] *N*-Myristoylated peptidyl aldehyde **8** is highly compatible with SfmC as a plausible biosynthetic substrate for the PS-domain, and thus we synthesized several peptidyl aldehydes (**14**–**16**) with various substitutions (Figure 2). Minor modifications of the peptidyl moiety of **8**, however, substantially decreased SfmC-catalyzed formation of the corresponding pentacyclic scaffold (SI-Figure S4–S6).

In addition to issues with the substrate tolerance, a major obstacle for postenzymatic chemical conversions could be the removal of the peptidyl side chain to access saframycins (**1**, **2**) and their congeners. While Tang and co-workers recently revealed that a membrane-bound peptidase SfmE, hydrolyzes the fatty acyl chain,[20] we adopted a convenient chemical approach employing artificial substrates compatible to SfmC. Taking the relatively strict substrate specificity into account, we minimized structural modification and thereby initially designed the substrates, **17** and **18** bearing an ester linkage in place of the amide bond in **8**, with maintenance of an almost identical chain length to that of **8** (Figure 2).

The SfmC-catalyzed conversion of **7** and **17** proceeded to generate the corresponding pentacyclic products based on LC-MS analysis (Scheme 2, SI-Figure S7). Chemical conversion of the hemiaminal group through one-pot treatment with KCN produced aminonitrile **20**. The use of NADPH in place of NADH provided a higher yield. However, despite the promising LC-MS profiles indicating preferential formation of the pentacyclic scaffolds (SI-Figure S7), the isolated yields of **20** were less than 10%. We also struggled to reproducibly attain optimum yield of **20**. Even for the reported system employing the plausible biosynthetic intermediate **8** of SfmC,[14a] we also faced difficulties to consistently reproduce the production of **13** in the range of 20% isolated yield (Scheme 1, SI-Figures S2 and S3).

In efforts to improve the enzymatic conversion, we optimized purification methods of the labile SfmC and also conducted direct *in vivo* preparation of phosphopantetheinylated SfmC (holo-SfmC, SI-Figure S1). While these improvements considerably shorten the preparation time for the holo-SfmC, a substantial decrease of **20** was occasionally observed upon handling and purification of the crude reaction mixture. We thus assumed that the secondary amino group of **20** could not only impair isolation efficiency but also entail unexpected side reactions or oxidative degradations.[21] According to this working hypothesis, we next performed *N*-methylation of the crude mixture containing secondary amine **20** by treatment with excess amounts of formaldehyde and 2-picoline borane.[22] Subsequent reductive amination to install the *N*-methyl group proceeded cleanly, and more importantly, the corresponding tertiary amine **21** was efficiently obtained in 18% overall yield based on **17** in a reproducible manner (Scheme 2). This *in situ* *N*-methylation provided significant improvements in the chemo-enzymatic synthesis,[23] which enabled facile access to pentacyclic scaffold **21** incorporating the three stereocenters (C1, C11, and C21) with accurate control of the chemo-, regio- and stereoselectivities of the nine-step conversions. Because we can complete the overall manipulations (**7** + **17** → **21**) within a single day, this chemo-enzymatic approach, which does not require the isolation of any intermediates, greatly reduces the time and labor involved in assembling the highly elaborated pentacyclic THIQ scaffold.

Having established the sequential process, we secured sufficient amount of **21** as the appropriately functionalized intermediates to achieve chemical conversions into **3** (Scheme 2). Hydrolysis of the ester linkage of **21** smoothly proceeded to furnish **22** in 83% yield. Oxidation of the phenols in the presence of a catalytic amount of salcomine gave rise to **3** (2.9 mg).[24] We thus developed the chemo-enzymatic synthetic process of jorunnamycin A (**3**) in just four-pot starting from the tyrosine derivative **7**. While elegant total syntheses of **3** by Zhu and Chen have been reported,[6b,c] our approach described herein could provide an alternative synthetic route to **3**.

This approach employing a designer peptidyl aldehyde can be readily applied to saframycin A (**1**) (Figure 2 and Scheme 2). The change of peptidyl aldehyde substrate **8** into **18** was demonstrated to be well tolerated to SfmC, and 4.6 mg of **24** was obtained in 13% yield based on **18** (SI-Figure S8). Hydrolysis of **24** (12.2 mg) gave secondary alcohol **25** in 91% yield. Salcomine-mediated oxidation of phenols followed by Swern oxidation of the alcohol allowed chemo-enzymatic total synthesis of **1** (5.0 mg).

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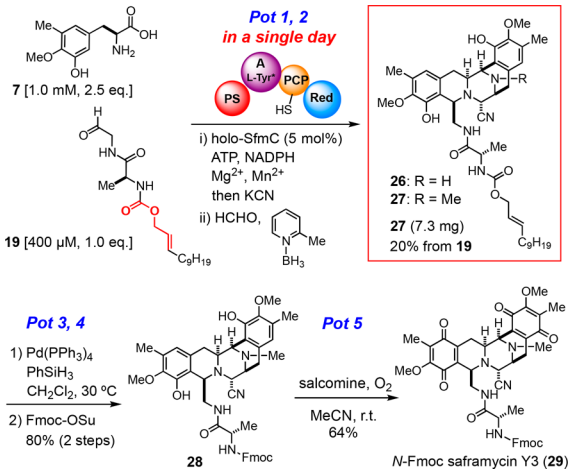
To expand the scope of this approach, we further designed substrate **19** having an allyl carbamate linkage to access saframycin Y3 (**3**) bearing a *N*-terminal alanine unit (Figure 2 and Scheme 3). SfmC-catalyzed skeleton forming reaction

Scheme 3. Chemo-enzymatic Synthesis of *N*-Fmoc Saframycin Y3 (29)



employing **19** with **7** also proceeded efficiently to furnish the corresponding **27** in 20% overall yield based on **19** (SI-Figure S9). Notably, 7.3 mg of **27** was successfully obtained in a day. Treatment of **27** (12.8 mg) with $Pd(PPh_3)_4$ in CH_2Cl_2 employing $PhSiH_3$ as a hydride source allowed rapid transformation to liberate the corresponding primary amine,[25] which was isolated after protection with Fmoc group to give **28** in 80% yield (2 steps). Salcomine-mediated oxidation of the phenols provided 6.9 mg of *N*-Fmoc saframycin Y3 (**29**) in 64% yield. Although removal of the *N*-Fmoc group in **29** to liberate saframycin Y3 (**2**) was feasible based on ESI-MS analysis, it was quite difficult to isolate **2** having both the primary amine and the bisquinone groups in a practical yield. Eventually, we thus report the synthesis of the *N*-Fmoc protected derivative of **2**.

In conclusion, the integration of precise chemical synthesis with *in vitro* engineered biosynthesis allowed us to achieve the chemo-enzymatic total syntheses of jorunnamycin A (**3**), saframycin A (**1**), and *N*-Fmoc saframycin Y3 (**29**). This approach exploiting the enzyme-catalyzed skeleton constructing conversions streamlines assembly of the simple synthetic components in conjunction with chemical installations of aminonitrile and *N*-methyl groups. In comparison to multistep chemical syntheses that usually necessitate isolation of the intermediates at each step, the key processes (**7** + **17**/**18**/**19** → **21**/**24**/**27**) minimizes the need to separate the intermediates, thus allowing labor-saving synthesis of the elaborated scaffolds only in a single day. The judicious choice of the designer substrates **17**−**19** for SfmC allowed divergent access to the natural products **1** and **3** as well as *N*-protected derivative **29** of **2**, which could be a versatile platform for the collective synthesis of saframycins, jorunnamycins, renieramycins, and their congeners through chemical manipulation of the hydroxyl/amino groups.[26]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b07161.

Detailed experimental procedures, spectroscopic data, LC-MS chromatograms, and copies of NMR spectra (PDF)

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Notes

The authors declare the following competing financial interest(s): We have patent applications on the development of the chemo-enzymatic synthetic process to generate tetrahydroisoquinoline scaffolds.

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