Unprecedented biological cyclopropanation in the biosynthesis of FR-900848†

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We were able to show the predominant incorporation of a single enantiomer and intact incorporation of multiply labelled synthetic diketide precursors (14 and 16), which established the intermediacy of cyclopropanated diketide and led to our proposal for the unprecedented biological cyclopropanation, *via* PKS (polyketide synthase) having a novel cyclopropanase domain, in the biosynthesis of FR-900848 (1).

FR-900848 (**1**) is a polyketide-nucleoside produced by *Streptoverticillium fervens* HP-891 and shows potent activity against phytopathogenic fungi.[1] Its structure consists of a cyclopropanated fatty acid containing one isolated and four contiguous cyclopropanes,[2] and 5″-amino-5″-deoxy-5′,6′-dihydrouridine (Fig. 1). The closely related compound U-106305 (**3**), an inhibitor of the cholesteryl ester transfer protein (CETP), was isolated from *Streptomyces* sp. UC 11136 by Kuo *et al.*[3] and was found to have the same absolute stereochemistry for cyclopropane rings.[4] To elucidate the mechanism for enzymatic construction of polycyclopropanes, we have started on a biosynthetic study of FR-900848 (**1**) and determined its biosynthetic building units by a series of feeding experiments with isotopically labelled precursors.[5a,b] These results established that the backbone of **1** was constructed *via* a polyketide pathway and was coupled with an aminonucleoside unit derived from dihydrouridine. In addition, methylenes of the most characteristic cyclopropane moieties in **1** were derived from L-methionine.

The introduction of the highly unusual contiguous cyclopropane moieties is the most remarkable step in the biosynthesis of FR-900848 (**1**). For the intriguing cyclopropanations, two biosynthetic pathways were proposed as depicted in Scheme 1. Kuo *et al.* proposed that in the biosynthesis of U-106305 the octaenoic acid **6**, which is constructed *via* the polyketide pathway, is cyclopropanated by methyl transfer from *S*-adenosylmethionine (SAM), followed by ring closure (Scheme 1, path a).[3] Alternatively, based on the well-known cyclopropanation of the α,β-unsaturated carbonyl system with sulfur-ylide,[6] Barrett *et al.* proposed a biosynthetic pathway in which cyclopropanation occurs in the growing the polyketide chain (Scheme 1, path b).[4] Herein we describe the incorporation of plausible cyclopropanated diketide precursors into **1**, and we further propose an unprecedented biological cyclopropanation involved in the biosynthesis of FR-900848.

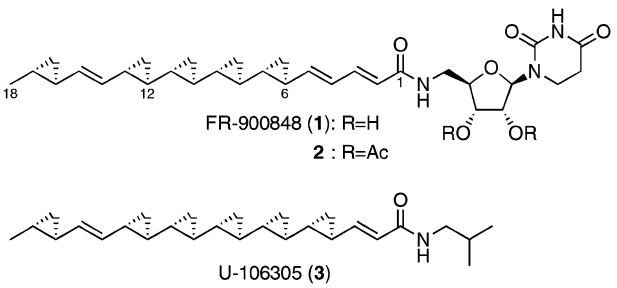
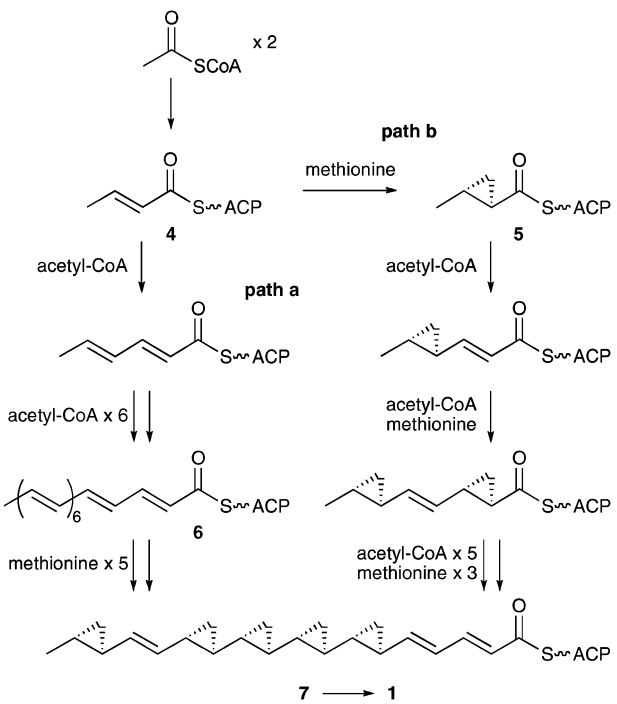


Fig. 1

To examine the mechanism of the step-wise introduction of the cyclopropane system (Scheme 1, path b), we planned a feeding experiment with several cyclopropanated diketide precursors labelled with deuterium. Incorporation of advanced intermediates as thioester-activated forms is a well



Scheme 1 Two proposed biosynthetic pathways to the polycyclopropane unit of **1**.

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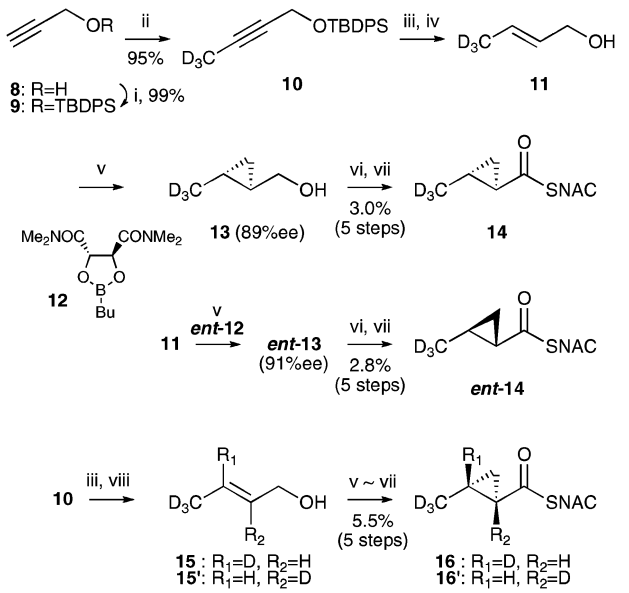


Scheme 2 *Reagents and conditions*: (i) TBDPSCl, imidazole, DMF, 25 °C; (ii) BuLi, CD_3OTs, THF, 25 °C; (iii) HF–pyridine, THF, 25 °C; (iv) $LiAlH_4$, NaOMe, THF, −10 °C to reflux; (v) CH_2I_2, Et_2Zn, CH_2Cl_2, 0 °C; (vi) $RuCl_3$, $NaIO_4$, CCl_4–H_2O–CH_3CN, 25 °C; (vii) *N*-acetylcysteamine, DCC, Ph_3P, DMAP, CH_2Cl_2, 0 °C; (viii) $LiAlH_4$, NaOMe, THF, −10 °C to reflux, then D_2O.

established strategy in biosynthetic studies of polyketides.[7a] Synthesis of the plausible precursor **14** and its enantiomer (*ent*-**14**) is shown in Scheme 2. The lithium acetylide prepared from the corresponding silyl ether **9** was reacted with C^2H_3OTs to afford **10** in 95% yield (>99 atom% ^2H). After deprotection of the silyl ether **10**, hydroalumination of the resulting 2-butyn-1-ol with $LiAlH_4$–NaOMe yielded [4,4,4-$^2H_3$]-2-buten-1-ol **11**. Cyclopropanation of **11** using Et_2Zn–CH_2I_2 in the presence of D-tartramide-derived dioxaborolane (**12**) according to the Charette protocol[8] gave **13**. The optical purity of **13** was determined to be 89% ee by ^1H-NMR analysis of the corresponding (*R*)-MTPA ester. Oxidation of the alcohol **13** with $RuCl_3$–$NaIO_4$ followed by condensation with *N*-acetyl cysteamine using DCC, Ph_3P, and DMAP afforded the thioester **14**. Charette cyclopropanation with the enantiomeric dioxaborolane afforded *ent*-**13** (91% ee) which was converted to *ent*-**14** in the same procedure. To confirm the intact incorporation, the multiply deuterium labelled diketide precursor **16** was synthesized. Hydroalumination of [4,4,4-$^2H_3$]-2-butynl-1-ol followed by quenching with ^2H_2O provided a mixture of [3,4,4,4-$^2H_4$]-2-buten-1-ol (**15**) and [2,4,4,4-$^2H_4$]-2-buten-1-ol (**15′**). The labelled 2-buten-1-ols (**15** and **15′**) were converted to **16** and **16′** as a 65 : 35 mixture[9] according to the same procedure as that to obtain **14**.

Separate administrations of synthetic analogues **14** and *ent*-**14** to *S. fervens* HP-891 followed by the work-up procedure described previously afforded diacetate **2** which was subjected to NMR analysis.[5a] The ^2H-NMR spectrum of **2** derived from the feeding experiment with **14** having the same absolute stereochemistry as that of FR-900848, showed the signal at 1.02 ppm corresponding to 18-Me (Fig. 2, b). In the ^2H-NMR spectrum of **2** provided by the administration of *ent*-**14**, the 18-C^2H_3 signal with very low intensity was

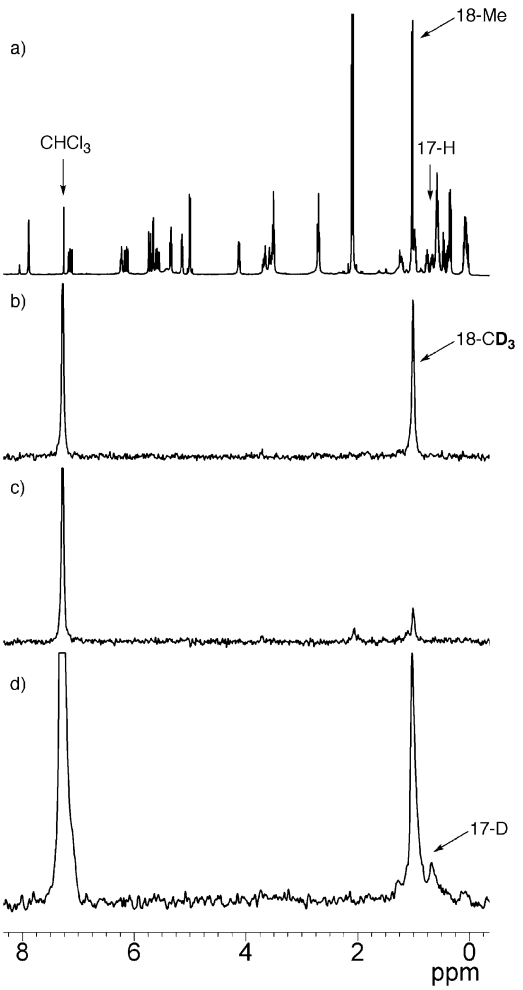
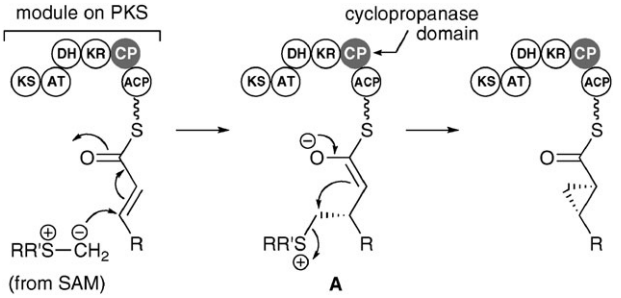


Fig. 2 (a) ^1H NMR spectrum of **2**; (b, c, d) ^2H NMR spectra of **2** derived from the feeding experiments with **14**, *ent*-**14**, and **16** (**16′**), respectively.

observed (Fig. 2, c). Incorporations of **14** and *ent*-**14** into **2** were 0.11% and 0.01%, respectively, as determined with reference to the natural abundance solvent ($CHCl_3$) peaks. Considering the optical purity of *ent*-**14**, the signal is likely derived from a small amount of **14**. To obtain further support for these experimental results, the incorporation of multiply deuterium labelled **16** and **16′** was employed. The ^2H-NMR spectrum of **2** from the feeding experiment with the mixture of **16** and **16′** showed signals of 18-C^2H_3, which overlapped with 16-^2H, and 17-^2H at 1.02 and 0.67 ppm, respectively (Fig. 2, d), in the integral ratio of 6.8 : 1 which is nearly identical to the corresponding ratio, 5.2 : 1 [(H4 + H3) : (H2)] in **16** and **16′**. These results strongly indicated the specific and intact incorporation of the precursors **14** and **16** into **1** without degradation in a metabolic pathway.

Since we have already established that the backbone of **1** is biosynthesized *via* a polyketide pathway, probably by putative type-I PKS,[5a] predominant incorporation of the chiral diketide precursor **14** strongly indicated that cyclopropanation occurred at the stage of α,β-unsaturated thioester bound to PKS. In the biosynthesis of cyclopropanated natural products, such as bacterial lipids, mycolic acids[10] and sterols,[11] the



Scheme 3 Proposed cyclopropanation with PKS having a cyclopropanase domain.

cyclopropane moiety is introduced to the isolated olefin with SAM and a corresponding enzyme. However, it is less likely that the electrophile SAM directly methylates the electron deficient olefin **4** to give the cyclopropanated diketide **5**. On the other hand, the alternative cyclopropanation of the α,β-unsaturated carbonyl system with sulfur-ylide (Scheme 3) fits well with our experimental results. Considering the involvement of type-I PKS, which consists of common domains,[7a,b] we propose an additional domain, cyclopropanase (CP), which catalyzes the conjugate addition of the SAM-derived ylide to the α,β-unsaturated thioester, followed by ring closure of the resulting enolate intermediate (Scheme 3, **A**) thus affording the cyclopropane ring. This domain could be regarded as a derivative of the methylation domain which is found in the bacterial type-I PKS.[12a,b] In the putative FR-900848 PKS, the 1st and 3rd–6th modules could have CP domains responsible for cyclopropanation in the corresponding position.

In conclusion, our experimental results on intact incorporation of multiply labelled biosynthetic precursor analogues established the intermediacy of the cyclopropanated diketide and suggested the unprecedented biological cyclopropanation with PKS, having novel a cyclopropanase domain, in the biosynthesis of FR-900848 (**1**). Based on the pathway proposed in this paper, we are currently identifying the biosynthetic gene cluster.

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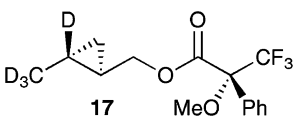

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