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Chemoenzymatic Synthesis of the Polyketide Macrolactone 10-Deoxymethynolide

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Polyketides are a richly diverse class of natural products that display a wide variety of clinically important biological activities.[1] The enzymes that catalyze their formation, polyketide synthases (PKSs), are complex multienzyme systems that are characterized by a modular architecture that condense simple carboxylic acids such as malonyl-CoA and methylmalonyl-CoA.

The pikromycin (Pik) PKS system of *Streptomyces venezuelae* ATCC 15439 is notable among modular PKS systems in that it catalyzes the biosynthesis of both 12- and 14-membered macrolactones.[2] Upon thioesterase (TE)-catalyzed cyclization of the linear hexa- and heptaketide intermediates, 10-deoxymethynolide (**1**) and narbonolide (**2**) undergo post-PKS oxidation and glycosylation to methymycin and pikromycin, respectively (Figure 1). Our studies[3] and those of others[4] have focused on the ability of Pik monomodules to catalyze chain extension and processing of natural and unnatural substrates. To complement our work with Pik monomodules,[3] we initiated analysis of the Pik TE domain as an effective macrolactonization catalyst.

The ability of Pik TE to catalyze cyclization of both 12- and 14-membered ring macrolactones suggests that it has inherent substrate tolerance, which is consistent with structural information obtained by X-ray analysis.[5] Cane and co-workers have reported that relative to the 6-deoxyerythronolide B synthase (DEBS) TE, Pik TE has enhanced specific activity and broader substrate specificity.[6] These conclusions were based upon the use of simple diketide *N*-acetylcysteamine (SNAC) thioesters as substrates.

Recent work with TE domains from modular nonribosomal peptide synthetase (NRPS) and PKS systems has demonstrated their ability to accept natural and unnatural substrates for cyclization of linear polypeptide and polyketide chains.[7] For example, the TE domain isolated from the epothilone PKS system has been shown to catalyze macrocyclization of SNAC-*seco*-epothilone C obtained from epothilone C.[7e] The biochemical analysis of modular PKSs has been limited due in part to the challenge of obtaining suitable quantities of natural substrates and substrate analogues, particularly those involved in late stages of polyketide chain elongation. This challenge can be met by total synthesis to obtain natural substrates and substrate analogues in enantiomerically pure form.

We report here the synthesis of SNAC-*seco*-10-deoxymethynolide, a mimic of the natural hexaketide Pik TE chain elongation intermediate, and its enzymatic macrolactonization by Pik TE to complete the total synthesis of 10-deoxymethynolide. Further, we have extended the study of the substrate specificity of Pik TE through the use of unnatural hexaketide substrate analogues that were obtained by total synthesis.

While the target hexaketide may be obtained by degradation of 10-deoxymethynolide (**1**),[8] a more versatile total synthesis was developed based on the potential to generate analogues for substrate specificity studies. Synthesis of SNAC-*seco*-10-deoxymethynolide began with the opening of lactone **3**[9] to afford Weinreb amide **4**[10] (Scheme 1). The primary alcohol in Weinreb amide **4** was protected as PMB ether **5**, which was reduced to aldehyde **6** (the mirror image of **6** by different methodology has been reported[11]). Nozaki–Hiyama–Kishi coupling of aldehyde **6** with vinyl iodide **7**[12] provided allylic alcohol **8** as a 1:1 mixture of C-5 epimers, which underwent Dess–Martin oxidation to ketone **9**. Removal of the PMB ether afforded alcohol **10** in equilibrium with its hemiketal, which underwent SiO_2-catalyzed dehydration to a dihydropyran upon purification (see Supporting Information).

To avoid the problems associated with the instability of alcohol **10**, allylic alcohol **8** was protected as TBS ether **11**, followed by debenzylation to give alcohol **12** (Scheme 2). Alcohol **12** was

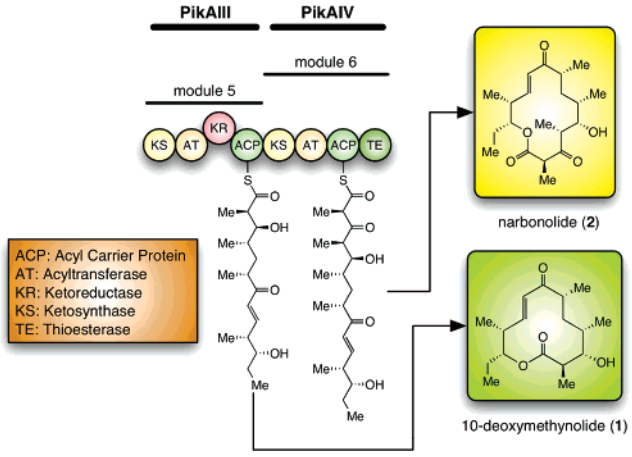
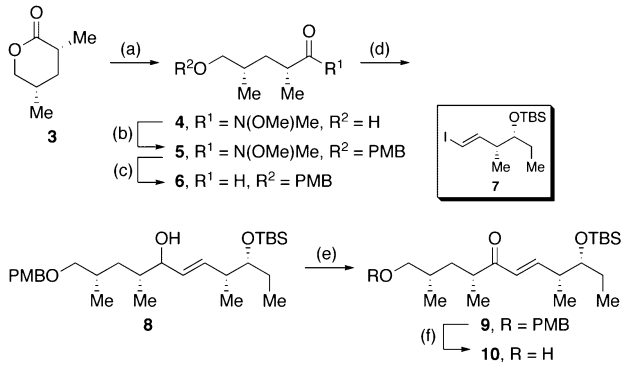


Figure 1. Final steps in the biosynthesis of 10-deoxymethynolide and narbonolide by the Pik PKS in *S. venezuelae*.

Scheme 1 [a]



[a] Key: (a) Me(OMe)NH•HCl, $AlMe_3$, 84%; (b) NaH, PMBCl, NaI, 93%; (c) DIBAL-H, 90%; (d) **7**, $CrCl_2$, $NiCl_2$, 70%; (e) Dess–Martin, 95%; (f) DDQ (see Supporting Information).

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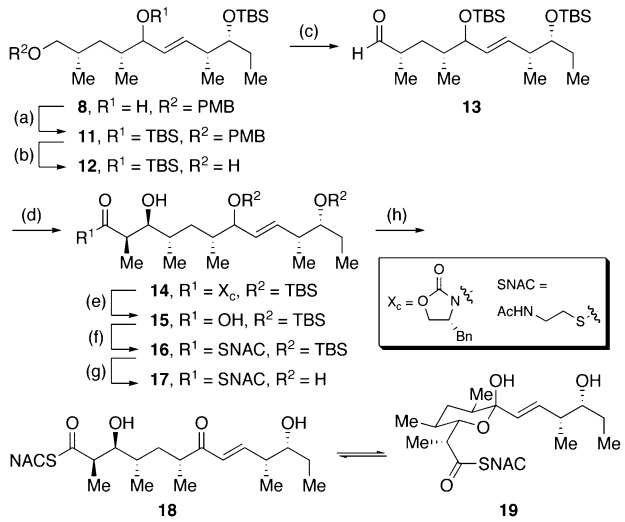
Scheme 2 [a]



[a] Key: (a) *i*-Pr_2NEt, TBSOTf, 97%; (b) DDQ, 87%; (c) TPAP, NMO, 81%; (d) *R*-4-benzyl-3-propionyl-2-oxazolidinone, Bu_2BOTf, *i*-Pr_2NEt, 75%; (e) LiOH, H_2O_2, 85%; (f) HSNAC, EDC, DMAP, 62%; (g) HF•pyr., pyr., 70%; (h) MnO_2, 35%.

oxidized to aldehyde **13**, which underwent a *syn*-aldol reaction to afford alcohol **14**. Removal of the chiral auxiliary gave acid **15**, and coupling with *N*-acetylcysteamine (HSNAC) afforded thioester **16** which was deprotected to triol **17**. For subsequent enzymatic reactions, the C-7 epimeric mixture of triol **17** was separated by reverse-phase HPLC. Chemoselective allylic oxidation of triol **17** with MnO_2 afforded SNAC-*seco*-10-deoxymethynolide **18** in 35% yield (based upon 60% conversion). Thioester **18** slowly decomposes at 23 °C, thus precluding longer reaction times to achieve 100% conversion. Upon the basis of ^{1}H NMR analysis, thioester **18** exists in equilibrium with hemiketal **19** as an approximately 4:5:1 ratio of **18:19**β**:19**α.

Incubation of SNAC-*seco*-10-deoxymethynolide (**18**, as a mixture with hemiketal **19**) with the purified Pik TE[6] (10 μM) at pH 7.0 afforded 10-deoxymethynolide (**1**) as the exclusive product as monitored by HPLC and mass spectrometry (Figure 2). Steady-state kinetic analysis was used to determine the kinetic parameters. The specificity constant k_{cat}/K_M for this reaction is 1.67 $\pm$ 0.027 mM^{-1} min^{-1}, which is approximately 4-fold greater than that obtained for the cyclization of SNAC-*seco*-epothilone C chain elongation intermediate with its cognate TE.[7e] As observed in the enzymatic cyclization of SNAC-*seco*-epothilone C,[7e] the low solubility of the substrate prevented determination of individual k_{cat} and K_M parameters, but the K_M is greater than 1 mM. In contrast to the reaction of SNAC-*seco*-epothilone C with its cognate TE,[7e] enzymatic hydrolysis of SNAC-*seco*-10-deoxymethynolide to *seco*-10-deoxymethynolide was not detected. The quantitative conversion and lack of observed partitioning to the *seco*-acid demonstrates the pronounced selectivity and efficiency of this biocatalytic process.

We also used non-native substrates to conduct a preliminary substrate specificity study of Pik TE. The reaction of the individual C-7 epimers of triol **17** with Pik TE at pH 7.0 was monitored, however, cyclization was not observed and the exclusive products were due to hydrolysis (see Supporting Information). These results show that Pik TE is highly optimized for its native substrate, as minor structural changes at C-7 are not tolerated. One potential explanation for this is the pH (7.0) at which these enzymatic reactions were conducted. Crystal structures of Pik TE show that the size of the substrate channel increases at higher pH,[5] suggesting that pH can influence the reactivity of Pik TE.[6] The effect of pH

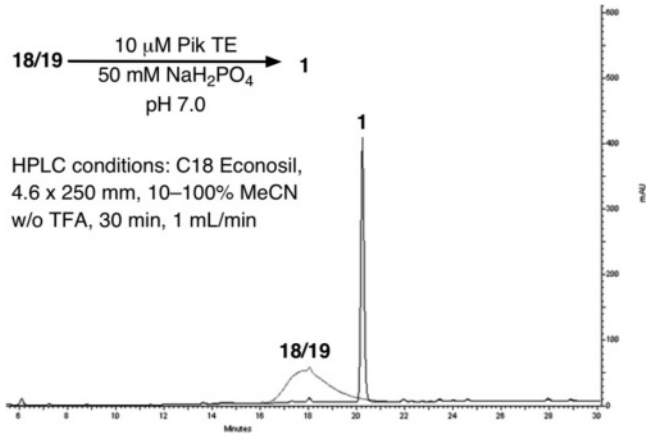


Figure 2. HPLC trace of SNAC-*seco*-10-deoxymethynolide (**18**, red) overlaid with trace following enzymatic reaction with Pik TE showing exclusive conversion to 10-deoxymethynolide (**1**, black).

on the reactivity of Pik TE with its native substrates will be the subject of future investigations.

As shown here, de novo synthesis of the natural pikromycin hexaketide chain elongation intermediate (SNAC-*seco*-10-deoxymethynolide) and its analysis as a substrate for Pik TE has revealed the exquisite selectivity and efficiency of this enzyme. Positional variation of functional groups and stereochemistry will enable investigation of the overall tolerance of Pik TE and suggest strategies for its optimization as a versatile biocatalyst toward diverse hydroxyl acyl-SNAC esters.

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Supporting Information Available: Experimental details and ^{1}H and ^{13}C NMR spectra for compounds **4**−**6**, **8**, **9**, and **11**−**18**, scheme for decomposition of compound **10**, enzymatic reaction conditions, and steady-state kinetic analysis details. This material is available free of charge via the Internet at http://pubs.acs.org.

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