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Chemoenzymatic Synthesis of Spinosyn A

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Abstract

Following the biosynthesis of polyketide backbones by polyketide synthases (PKSs), post-PKS modifications result in a significantly elevated level of structural complexity that renders the chemical synthesis of these natural products challenging. We report herein a total synthesis of the widely used polyketide insecticide spinosyn A by exploiting the prowess of both chemical and enzymatic methods. As more polyketide biosynthetic pathways are characterized, this chemoenzymatic approach is expected to become readily adaptable to streamlining the synthesis of other complex polyketides with more involved post-PKS modifications.

Keywords

polyketide macrolide; polyketide biosynthesis; post-polyketide synthase (PKS) modifications; chemoenzymatic synthesis; spinosyns

Natural products are rich sources of lead compounds for the development of new pharmaceuticals. Yet, their structural complexity renders their chemical synthesis challenging. There is a pressing need to develop efficient strategies for the synthesis of natural product-based or -like libraries for the drug discovery program.[1,2]

Macrolide polyketides are an important class of natural products having widespread clinical applications. Due to their complex structures, this class of secondary metabolites have often been targets for synthetic chemists to showcase newly developed methods. Over the years, chemical approaches to prepare macrolides have improved significantly to the point where Krische *et al.* reported a 14-step synthesis of deoxyerythronolide B in 2013.[3] Such remarkable innovations have greatly advanced the synthesis of linear polyketides and macrolactone backbones, which are biosynthetically assembled through the catalysis of

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polyketide synthases (PKSs).[4] However, many polyketides possess additional structural sophistication due to various post-PKS modifications of the initially formed linear and monocyclic structures.[5] Syntheses of polyketides whose structures are derived from a series of complicated post-PKS modifications are much more demanding.

Spinosyn A (**1**), a commercially important, polyketide-derived insecticide isolated from *Saccharopolyspora spinosa*,[6] consists of four fused rings, seventeen stereogenic centers, and multiple functional groups, including two unusual carbohydrate moieties, all of which are built through post-PKS modifications. Total syntheses of this complex framework have been reported by the Evans, Paquette, and Roush groups.[7-10] A Diels-Alder reaction (Evans, Roush) or an oxy-Cope rearrangement (Paquette) was employed to construct the octahydro-*as*-indacene core in these syntheses. Since, in all cases, the stereochemical control was directed by the conformation of the reactant, a major challenge of these early syntheses was the development of effective routes to assemble the stereocontrolling template motifs. To streamline the synthesis of more complex polyketides, We envisioned that the challenge of devising strategies for the stereo- and regiochemical control may better be met by using the corresponding biosynthetic enzymes.[11,12]

The entire biosynthetic pathway of spinosyn A has been elucidated as shown in Scheme 1.[13-19] Post-PKS modifications of the aglycone core commence following release of macrolactone **11** from the PKS acyl carrier protein. The monocyclic intermediate **11** is first primed via the SpnJ-catalyzed dehydrogenation at C-15 (**11**→**12**),[15] which facilitates the subsequent 1,4-dehydration catalyzed by SpnM.[19] The resulting intermediate **13** is susceptible to a transannular [4+2]-cycloaddition reaction (**13** → **14**) that can be accelerated by SpnF.[19] This cyclization step to form the tricyclic hexahydro-1*H*-indene intermediate **14** has attracted much attention, because SpnF may operate as a Diels-Alderase.[19-21]

The C-C bond formation between C-2 and C-14, which is catalyzed by SpnL, occurs after the rhamnosylation of **14** via the action of SpnG.[17,18,22] This affords the tetracyclic core in **16**, and the attached rhamnose moiety is subsequently permethylated in the presence of the SAM-dependent methyltransferases SpnH, SpnI, and SpnK to produce pseudoaglycone **17**.[18] SpnP completes the biosynthesis of spinosyn A by catalyzing the final coupling of **17** with forosamine.[13,23] The two sugar appendages (rhamnose and forosamine) are both biosynthesized from TDP-D-glucose (**2**) through pathways that have been fully established (see Scheme 1A).[14,16,24]

Considering the wealth of information available in the chemical literature concerning the biosynthesis of polyketides, we envisioned that a chemoenzymatic synthesis, which exploits advantages offered by both chemical and biological approaches, would provide a practical alternative to conventional chemical synthesis for the assembly of complex polyketide structures. Herein, we report the first chemoenzymatic synthesis of spinosyn A encompassing chemical preparation of the spinosyn polyketide backbone and subsequent application of post-PKS modification enzymes to complete the final construction. Our results demonstrate that a “one pot, multi-step” chemoenzymatic synthesis is a viable and effective approach to prepare complex polyketide natural products.

The first phase of our synthesis is the preparation of macrolactone **11**, which is biosynthesized via the actions of five polyketide synthases SpnA–E. Although **11** has never been isolated, its structure was deduced according to the established principles of linear polyketide chain assembly by type I PKSs[4] as well as the hydroxyl group stereochemistry prediction based on sequence analysis of the ketoreductase domains.[25] The retro-synthetic analysis for the preparation of **11** is depicted in Scheme 2. The linear polyketide chain **18** is assembled from three fragments via two C-C bond coupling reactions: a Julia-Kocieński olefination between sulfone **19** (fragment A) and aldehyde **20** (fragment B) to form the Δ^{12}-(*E*)-olefin, and a palladium-catalyzed Stille coupling using stannyl dienoate **21** (fragment C) to generate the C-5/C-6 linkage. Subsequent cyclization of **18** by Yamaguchi macrolactonization completes the synthesis of **11** following global deprotection.

Synthesis of fragment A (**19**) was initiated by applying Soai's asymmetric ethylation to Weinreb amide **22**[26] using (−)-DBNP as the chiral catalyst.[27] Mosher ester analysis of product **23** confirmed the (*S*)-configuration at C-21. Aldehyde **24**, prepared by *O*-silylation of **23** followed by DIBAL-H reduction, was utilized in an asymmetric aldol reaction with propionate **25**, carrying a Crimmins' chiral auxiliary.[28] The anticipated *syn*-aldol adduct **26** was obtained in good yield with high diastereoselectivity (>19:1). The auxiliary group of **26** was reductively cleaved after *O*-silyl protection and the resulting hydroxyl group was oxidized using TPAP to afford aldehyde **27**. Incorporation of a homoallyl extension was achieved by Brown's asymmetric allylation methodology.[29,30] The desired homoallylic alcohol **28** was produced as a single isomer at C-15.[31,32] The terminal olefin of **28** was transformed to a primary alcohol by Lemieux-Johnson oxidation under buffered conditions (pH 7) followed by $NaBH_4$ reduction. Introduction of a 1*H*-phenyltetrazolyl-5-thioether moiety onto **29** to give **30** was accomplished under Mitsunobu conditions. Conversion of **30** to sulfone **31** was found problematic as common oxidants gave either a partially oxidized sulfoxide or no product at all. The desired sulfone was eventually obtained in 91% yield after overnight incubation at 4 °C with H_2O_2 in the presence of an ammonium molybdate catalyst.[33] Although cleavage of the TES group at C-21 was observed, it could be re-introduced quantitatively to complete the synthesis of fragment A (**19**).

Preparation of fragment B (**20**) started with regioselective epoxide opening of PMB-protected (*S*)-glycidol (**32**) using lithiated 1,3-dithiane to give **33**. After *O*-silyl protection followed by hydrolysis of the 1,3-dithiane moiety, another application of Brown's allylation methodology afforded **35** with the desired stereochemistry at C-9 (d.r. > 20:1). Incorporation of the terminal vinyl iodide group began with TBS-protection of **35** followed by oxidative cleavage of the terminal olefin by Jin's procedure.[34] The resulting aldehyde **36** was subjected to Takai iodoolefination[35] providing **37** with >9:1 (*E*):(*Z*) stereoselectivity. Subsequent oxidative removal of the PMB ether followed by Dess-Martin oxidation completed the synthesis of fragment B (**20**).

Preparation of fragment C (**21**) was straightforward as shown in Scheme 4A. Regioselective addition of tributytin radical to propargyl alcohol (**38**) afforded (*E*)-vinylstannane **39**. Oxidation of the hydroxyl group followed by Horner-Wadsworth-Emmons olefination of the resulting aldehyde furnished dienoate **21** in excellent overall yield. With all fragments in

hand, a Julia-Kocieński olefination connecting **19** and **20** was achieved using KHMDS at –78 °C to give the desired product **40** with (*E*)-selectivity in 82% yield (Scheme 4B). Then, a palladium-catalyzed Stille coupling between **40** and fragment C (**21**) effectively generated the linear ketide **18**. The final steps to complete the synthesis of **11** involved chemoselective deprotection of the TES group at C-21, hydrolysis of the ethyl ester moiety to afford *seco*-acid **41**, and lactonization under Yamaguchi conditions to give macrolactone **42**.[36] The finishing global deprotection of **42** proved to be more challenging encountering partial deprotection or decomposition under various conditions. We were pleased to find that treatment of **42** with HF•pyridine in ethanol at 4 °C for ca. 4 days eventually led to successful production of **11** in satisfactory yield (64%).

In the second phase of our synthesis, we opted to take advantage of our knowledge regarding spinosyn biosynthesis and the availability of all post-PKS tailoring enzymes, and apply a series of enzymatic transformations in one-pot to convert macrolactone **11** to 17-pseudoaglycone (**17**). As shown in Figure 1A, the genes responsible for post-PKS modifications in the spinosyn pathway are translationally controlled by at least four operons (operons I–IV).[13] In our previous study of *O*-methylation of the rhamnose moiety in spinosyn A, it was found that prudent coordination of the three rhamnose methyltransferases *in vitro* could generate distinct methylation patterns of rhamnose.[18] In fact, accumulation of mono- and di-methylated products could be avoided when 10 μM SpnI, 5 μM SpnK, and 1 μM SpnH were used to permethylate rhamnose. These results suggested that the *in vivo* control of rhamnose methylation is likely achieved via differential expression of these methyltransferase genes, *spnH*, *spnI* and *spnK*, which are located on three different operons (III, II, and I, respectively, see Figure 1A).[18]

Hence, proper adjustment of the relative concentrations of enzymes catalyzing the tailoring reactions of spinosyn A biosynthesis may also be crucial to minimizing the possible accumulation of dead-end intermediates in a one-pot conversion of **11** to **17**. While the actual mechanism for *in vivo* metabolic flux control is likely to be more complicated, we proceeded on the assumption that the expression levels of the genes (i.e., the concentration of the encoded enzymes) from the same operon would be similar. Thus, guided by results from the rhamnose permethylation work, the concentrations of enzymes from operon I (SpnM, SpnL, SpnK, and SpnJ) were set at 5 μM, those from operon II (SpnI) at 10 μM, and those from operon III (SpnH, SpnG) at 3 μM.[32]

SpnF catalyzes the [4+2] cycloaddition of **13** to yield **14** (Scheme 1).[19] The cyclization could also occur in the absence of SpnF, albeit at a reduced rate. Since **13** is susceptible to Michael addition by cellular nucleophiles and/or radicals, the physiological function of SpnF may be to prevent the formation of byproducts from such off-path reactions by accelerating the cycloaddition step. Since SpnF is the sole gene encoded in operon IV, the proper concentration of SpnF used in the incubation must be determined separately. Accordingly, a model system was devised in which the product profiles and yields of a series of incubations containing **12** and TDP-L-rhamnose with SpnM, SpnG, SpnL and varied concentrations of SpnF (0 to 20 μM) were analyzed. Our results showed that addition of 20 μM of SpnF clearly suppressed the formation of minor byproducts and elevated the yield of the

tetracyclic octahydro-*as*-indacene product (Figure S1 and S2).[32] Hence, the concentration of SpnF in the *in vitro* experiment was set at 20 μM.

Having the concentrations of all enzymes required for post-PKS modifications adjusted, the one-pot reaction was conducted by incubation of 1 mM **11**, excess SAM, and TDP-L-rhamnose (**5**) with the aforementioned enzymes in 50 mM Tris•HCl buffer (pH 8) at 30 °C. As shown in Figure 1B, transformation of **11** to product **17** was achieved with an overall conversion yield estimated to be 19.6% (average yield per step = 81.6%) based on HPLC analysis.[32]

To complete the synthesis of spionsyn A (**1**), the attachment of forosamine at C-17 of **17** was attempted using SpnP, which is the glycosyltransferase assigned for this transformation.[13] Specifically, SpnP was incubated with all enzymes involved in TDP-D-forosamine biosynthesis (SpnO, SpnN, SpnQ, SpnR, and SpnS),[14,16] TDP-4-keto-6-deoxyglucose (**3**), and **17** in one-pot. Unfortunately, production of **1** was not observed. Further sequence alignment and crystal structural analysis suggested that SpnP belongs to a group of glycosyltransferases requiring an auxiliary protein for activation.[23] However, no putative auxiliary protein gene could be found in the spinosyn biosynthetic gene cluster. Thus, the failure of SpnP to forosamylate **17** might be due to the absence of the cognate auxiliary protein to reconstitute its activity *in vitro*. Consequently, chemical glycosylation was adopted instead, and treatment of **17** and D-forosamine with BF_3•OEt_2 successfully led to spinosyn A (**1**).[32]

In summary, a chemoenzymatic strategy was effectively applied in our synthesis of spinosyn A. Construction of monocyclic precursor **11** was achieved chemically by assembling three synthesized fragments in a linear form followed by a controlled macrolactonization. The more challenging post-PKS modifications converting **11** to **17** were accomplished using a total of eight enzymes all in one-pot. While the overall enzymatic transformations were highly effective, it was rather unfortunate that direct transformation of **11** to spinosyn A (**1**) was hampered by missing the putative auxiliary protein for SpnP. This prevented the exploitation of the full capacity of the spinosyn biosynthetic machinery. Nevertheless, the feasibility of a chemoenzymatic synthesis of a complex polyketide-derived natural product has been demonstrated. When compared to the reported chemical syntheses of spinosyn A, our chemoenzymatic approach is not without its shortcomings. In particular, an in-depth understanding of the biosynthetic pathway is an essential prerequisite with its own set of challenges. Thus, it may be premature at the present time to claim a general applicability of the chemoenzymatic approach for the synthesis of complex natural products. However, continuing innovation in the fields of synthetic chemistry and natural product biosynthesis indicate that the current technical impediments to chemoenzymatic synthesis will be overcome before long. This approach is thus expected to become more readily adaptable in the future, and offer a valuable alternative for streamlining the synthesis of polyketides with more involved post-PKS modifications as well as the preparation of modified polyketide targets for mechanistic and pharmaceutical research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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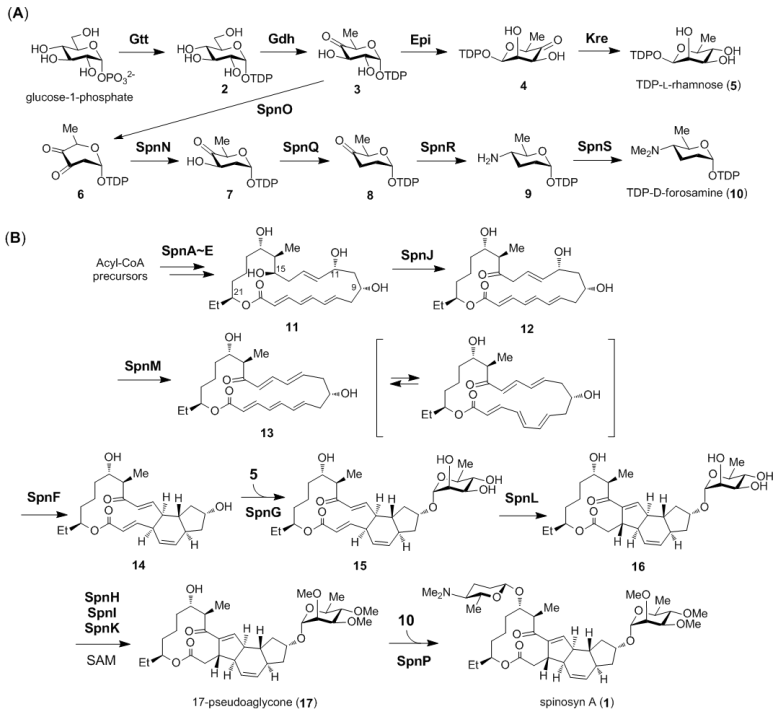
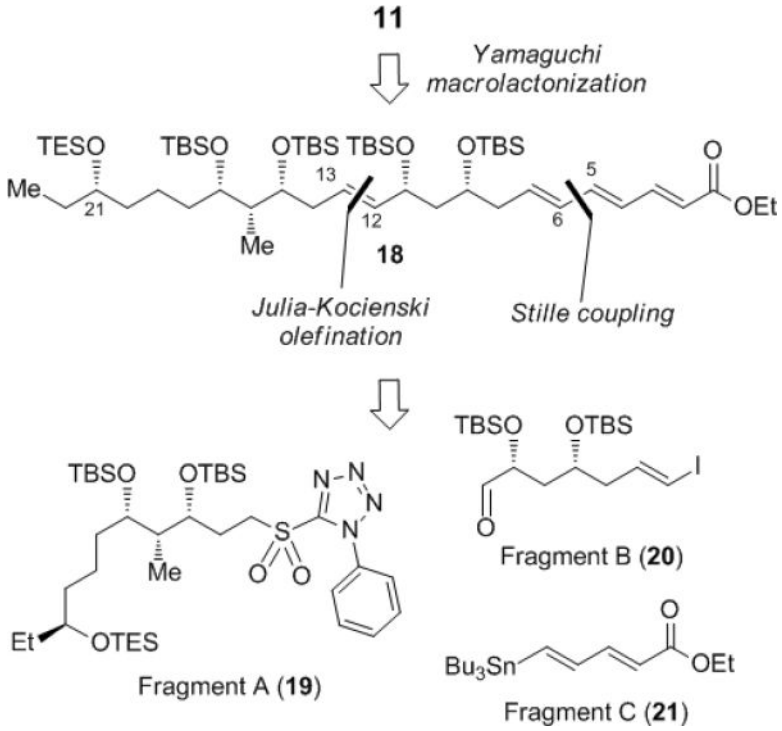
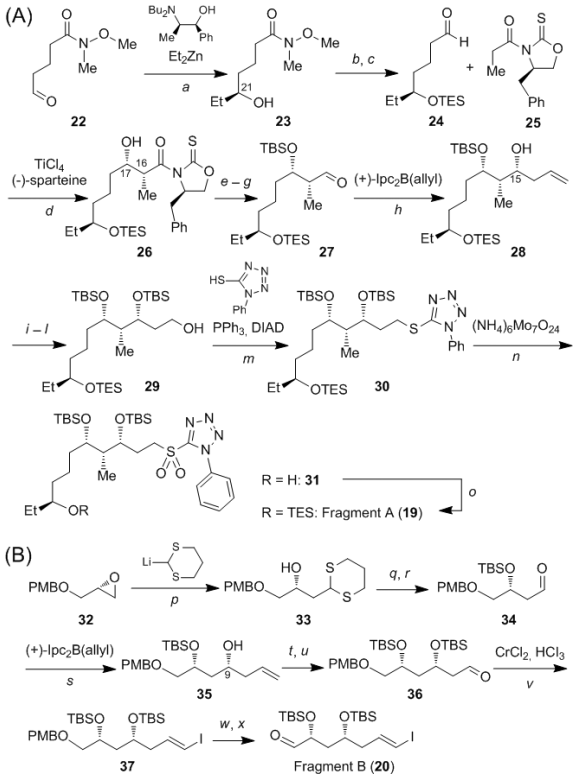


Scheme 1.

Established biosynthetic pathway for spinosyn A (**1**).

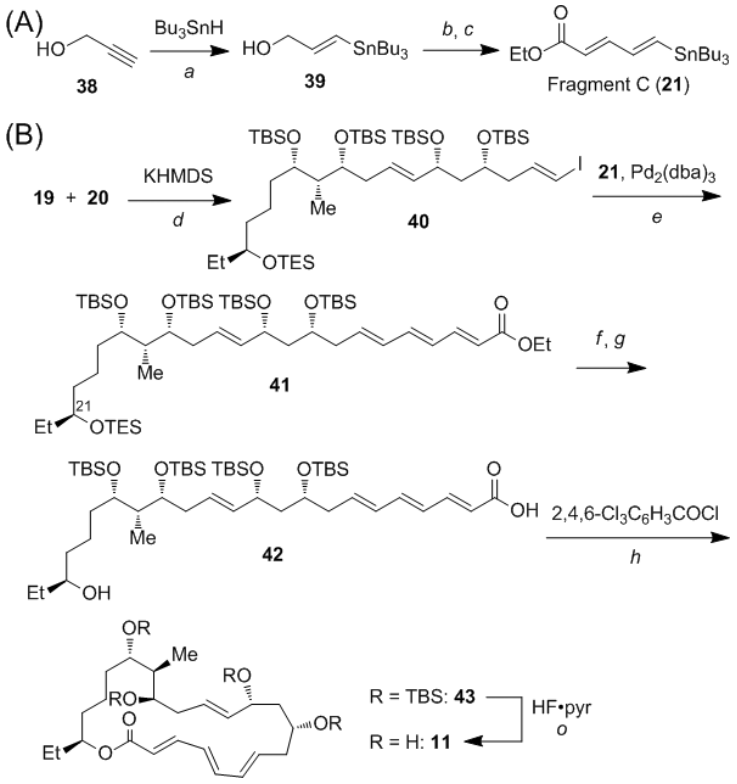


Scheme 2.
Retrosynthetic analysis.



Scheme 3.

Synthesis of (A) fragment A (**19**) and (B) fragment B (**20**). Reagents and conditions: a) (−)-DBNP, Et_2Zn, hexanes, 0 °C, 66%, 92% ee; b) TESOTf, 2,6-lutidine, CH_2Cl_2, −78 °C; c) *i*-Bu_2AlH, toluene, −78 °C, 2 steps 78%; d) $TiCl_4$, (−)-sparteine, CH_2Cl_2, 0 °C, 84%; e) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C; f) $LiBH_4$, Et_2O, CH_3OH, 0 °C, 2 steps 75%; g) TPAP, NMO, 4Å MS, CH_2Cl_2, rt; h) (+)-Ipc_2B(allyl), Et_2O, −78 °C, 2 steps 60%; i) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 87%; j) OsO_4, NMO, THF, acetone, pH 7 buffer, rt; k) $NaIO_4$, THF, pH 7 buffer, rt; l) $NaBH_4$, EtOH, rt, 3 steps 67%; m) PTSH, PPh_3, DIAD, THF, rt, 86%; n) $(NH_4)_6Mo_7O_{24}$, H_2O_2, EtOH, H_2O, 4 °C; o) TESOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 2 steps 91%; p) 1,3-dithiane, *n*-BuLi, THF, 0 °C, 94%; q) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 95%; r) MeI, $CaCO_3$, CH_3CN, H_2O, reflux, quantitative; s) (+)-Ipc_2B(allyl), THF, −78 °C; t) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 2 steps 67%; u) OsO_4, $NaIO_4$, 2,6-lutidine, dioxane, H_2O, rt, 83%; v) $CrCl_2$, HCI_3, dioxane, THF, rt, 87%; w) DDQ, aq CH_2Cl_2, rt; x) Dess-Martin periodinane, CH_2Cl_2, rt, 2 steps 83%.



Scheme 4.

(A) Synthesis of fragment C (**21**) and (B) completion of preparation of **11**. Reagents and conditions: a) Bu_3SnH, AIBN, benzene, reflux, 50%; b) MnO_2, acetone, rt, 85%; c) $EtO_2CCH_2P(O)(OEt)_2$, NaH, THF, 0 °C to rt, 74%; d) KHMDS, THF, −78 °C, 82%; e) **21**, $Pd_2(dba)_3$, Ph_3As, DMF, rt, 70%; f) PPTS, EtOH, 0 °C; g) LiOH, THF, CH_3OH, H_2O, reflux, 2 steps 62%; h) 2,4,6-$Cl_3C_6H_3COCl$, Et_3N, THF; DMAP, toluene, rt, 75%; o) HF•pyr, EtOH, 4 °C, 4 days, 64%.

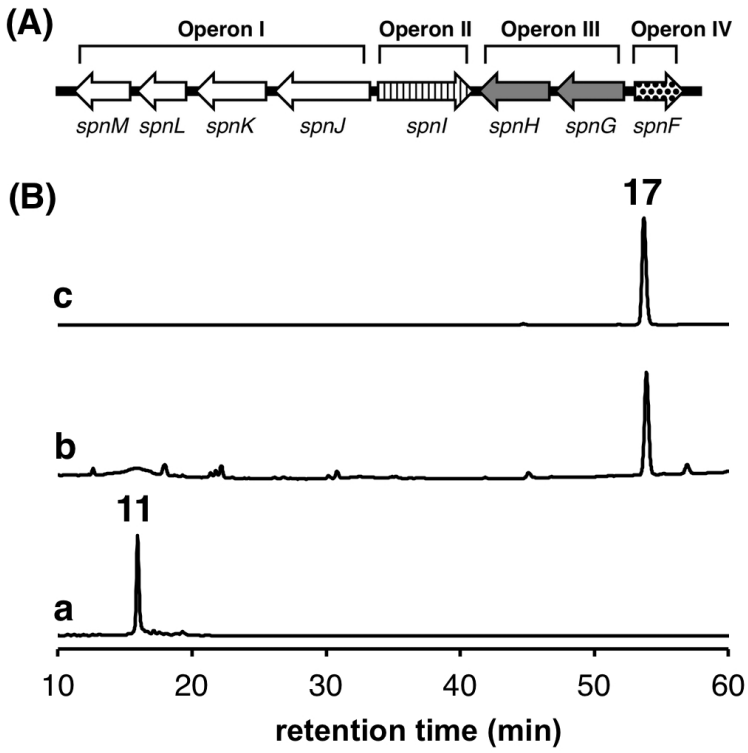


Figure 1.

(A) The organization of putative operons encoding enzymes responsible for the post-PKS modifications in the spinosyn biosynthetic gene cluster. (B) HPLC analysis of the enzymatic tandem reactions to convert **11** to **17**; **11** alone (**a**), enzyme reaction mixture (**b**), and **17**