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Timing of the $\Delta_{10,12}$-$\Delta_{11,13}$ Double Bond Migration During Ansamitocin Biosynthesis in *Actinosynnema pretiosum*

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The plant-derived maytansinoids[1] and their microbial counterparts, the ansamitocins **1**−**3** (Figure 1),[2] are extraordinarily potent antitumor agents *in vitro* and in tumor-bearing animals, blocking the assembly of tubulin into functional microtubules.[3] Their biosynthesis in *Actinosynnema pretiosum* involves the assembly of a polyketide by chain extension of the starter unit 3-amino-5-hydroxybenzoic acid (AHBA, **4**)[4] by three molecules each of malonyl-CoA and 2-methylmalonyl-CoA and one molecule of 2-methoxymalonyl-acyl carrier protein (ACP) on a type I modular polyketide synthase (*asm* PKS) (Scheme 1).[5] Its product proansamitocin is then converted by six tailoring steps into the bioactive end product, ansamitocin *P*-3 **2** (AP-3).[6]

In ansamitocin the position of the diene moiety at C-11 and C-13 is shifted by one carbon from the position where normal PKS processing would place these double bonds, obliterating the stereocenter at C-14. It has been proposed that migration of the double bonds already occurs during processing on the PKS.[6]

Recently, we determined the cryptic configuration of the stereocenter at C-14 by synthesizing the two enantiomers of the intermediate diketide **II** as their *N*-acetylcysteamine (SNAC) thioesters and demonstrating that only the (*R*)-enantiomer complements an AHBA-nonproducing mutant *A. pretiosum* HGF073 to restore ansamitocin formation.[7] Surprisingly, AP-3 production could not be restored by supplementation with a racemic mixture of the triketide **5**. We now report the synthesis and feeding of the respective SNAC esters of the enantiopure triketide (*R*)-**5** and of the two isomeric tetraketides (*R*)-**6** and **7** to gain insight into the biosynthetic logic of diene migration.

N-Acetylcysteamine ester **5** was synthesized by Evans alkylation[8] of the protected benzyl iodide **8**[7] (Scheme 2). After auxiliary removal, aldehyde **10** was directly converted into the SNAC ester (*R*)-**5** by a Wittig olefination using ylide **11**[9,10] followed by deprotection. Aldehyde **10** also served to prepare tetraketide **6**. Wittig olefination using ylide **12** resulted in aldehyde **13** after a short reduction/oxidation sequence. This aldehyde was subjected to a second olefination protocol using the new phosphonate **14**, which already bears the methoxy group in the right place as well as the SNAC moiety. Deprotection finally yielded the target tetraketide (*R*)-**6**.

The isomeric tetraketide **7** was prepared from known vinyl iodide **16**[11] which was subjected to a Heck reaction with alkene **18** (Scheme 3). This butene derivative was prepared according to Trost's palladium-catalyzed kinetic resolution by ring opening of vinyl oxirane **15** in the presence of ligand **17**.[12] The Heck reaction yielded the desired diene **19** which was further converted into the target SNAC ester **7** by standard functional group transformations.

Compounds (*R*)-**5** (15 μmol) and (*R*)-**6** and **7** (21 μmol) were then each fed to cultures of *A. pretiosum* mutant HGF073 (Figure 2).[13] AP-3 was detected in cultures supplemented with the (*R*)-triketide SNAC **5** and with tetraketide SNAC **7**. ESI-MS spectral comparison of the biosynthetic samples with authentic AP-3 showed them to be identical, with a parent ion at *m/z* 635 $(M+H)^+$ and

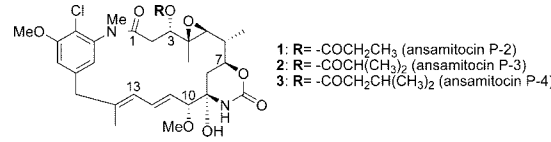
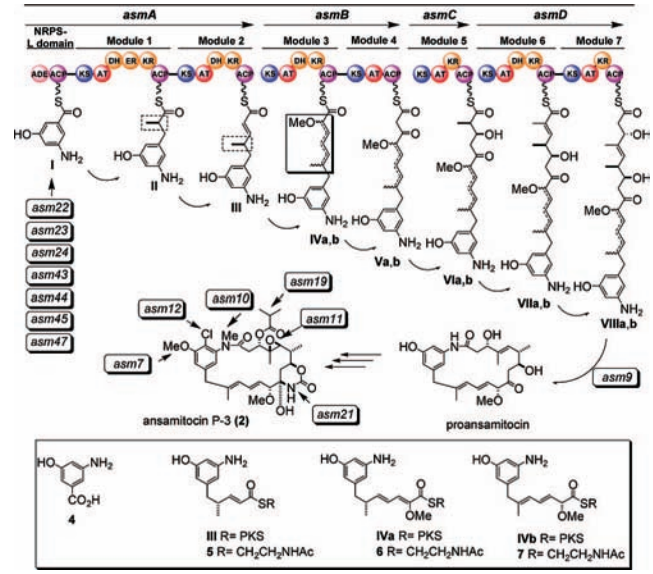


Figure 1

Scheme 1. Biosynthesis of AP-3



collision-induced fragmentation giving daughter ion spectra with a base peak at *m/z* 547 (from *m/z* 635) due to loss of the ester function. Based on quantitation by UV absorption, the relative amount of AP-3 formed from tetraketide **7** was 1.15% of that produced upon complementation with the same molar amount of AHBA. In contrast AP-3 formation was substantially lower after supplementation with triketide **5** so that MS signal intensities were used to quantify its formation.[13] Thus, incorporation of triketide **5** is very low and contrary to our recent preliminary studies.[7] We could only detect AP-3 by using improved MS-technology (ultra performance LC coupled ESI-MS). No AP-3 was detectible in the extract of the fermentation of HGF073 with tetraketide SNAC **6**, while formation of a new metabolite was observed, identified as tetraketide derivative **20**.[13]

The results with tetraketides **6** and **7** lead us to conclude that the unusually positioned diene system is generated during processing of the polyketide on module 3 and that only a rearranged diene, **IVb**, but not **IVa**, can be passed on to the KS4 domain for further processing. We propose that the final diene system is installed during the dehydration step on the DH of module 3 by a vinylogous *syn*-elimination (Scheme 4). However, sequence alignment of 856 bacterial PKS dehydratases showed that the *asm* DH3 seems to be a functional domain capable of catalyzing α,β-*syn*-dehydration (motifs: PWLADH, DXXXXXQ).[13−15] In the absence of a crystal

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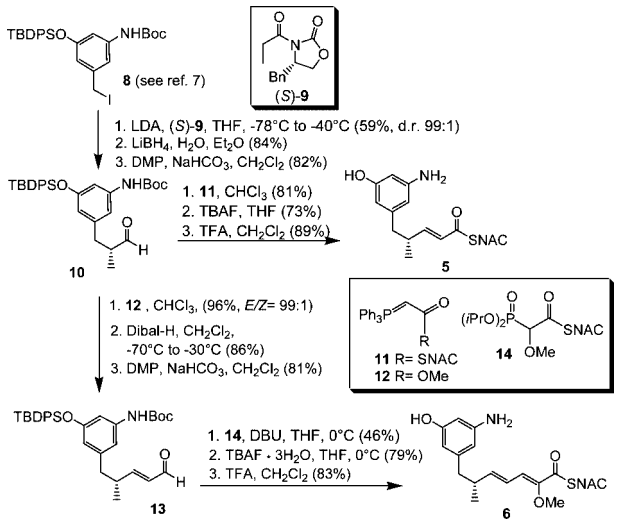
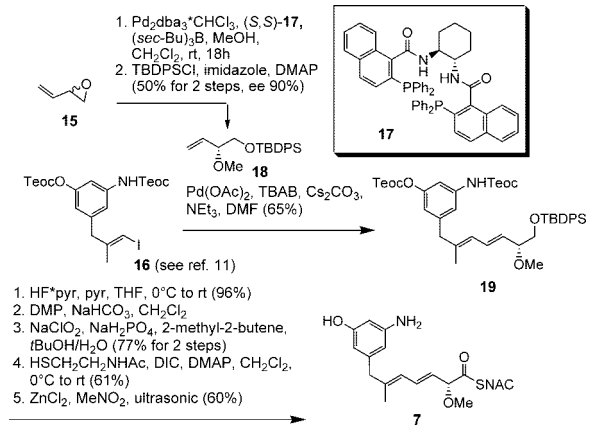
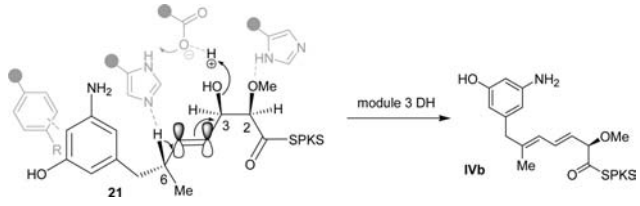
Scheme 2. Synthesis of Triketide **5** and Tetraketide **6**



Scheme 3. Synthesis of Tetraketide **7**



Scheme 4. Proposed Mechanism for Diene Formation via Vinylogous *syn*-Dehydration (DH Amino Acid Residues Shown in Gray).



Formation of AP-3 **2** after feeding to blocked mutant (HGF073) of *Actinosynnema pretiosum*

7: 1.15% relative to feeding of AHBA **4**
5: 0.20% relative to feeding of AHBA **4**
6: no AP-3 **2**, formation of derivative **20**

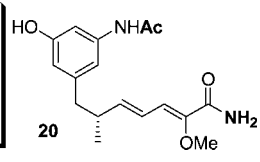


Figure 2. Feeding experiments with triketide **5** and tetraketides **6** and **7**.

structure of a PKS DH domain and due to the relatively low level of conservation between DHs, the reasons for its apparent ability to additionally or alternatively catalyze a vinylogous dehydration can only be speculated upon. The configuration at C-2[16] and C-6 of the presumed intermediate **21** has been determined by our feeding studies. The cryptic stereochemistry of the alcohol at C-3 is deduced from the KR3 sequence (motifs: LDD, AXXXN = B-type KR).[13,19] Thus restricted by the three stereocenters, vinylogous dehydration and formation of the final diene system can only occur when the tetraketide intermediate is fixed in conformation **21**. This could be achieved by coordination of the aromatic moiety via F/Y residues and additionally by H-bonding of the catalytic histidine residue with the oxygen of the methoxy group, thereby preventing a possible α,β-*syn*-dehydration.[15,20] Another histidine residue, possibly situated in the second subunit of the presumably dimeric DH domain,[15] would be required to carry out deprotonation at C-6. Since an *A. pretiosum* mutant incorporating malonyl-CoA instead of 2-methoxymalonyl-ACP was able to produce 10-desmethoxy-AP-3 in low yields,[21] the methoxy substituent is no prerequisite for vinylogous elimination but may increase its efficiency. Similar to tetraketide **6**, any products of an α,β-dehydration would not be further processed by the downstream enzymes. The low yield of 10-desmethoxy-AP-3 formation is probably due to poor transfer of the abnormal substrate analogue to module 4. The poor incorporation of SNAC derivatives **5** and **7** (relative to AHBA), due to inefficient loading of such polyketides onto the cognate PKS modules, appears to be a rather general phenomenon.[7]

In conclusion, our results lead us to conclude that the unusually positioned Δ_{11},Δ_{13} diene system in AP-3 is installed during processing of the nascent polyketide on module 3 of the asmPKS, with KS4 acting as a gatekeeper which accepts only the rearranged **IVb**, but not the unrearranged tetraketide **IVa** for further processing.

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Supporting Information Available: Synthesis and spectra of **5**, **6**, and **7**; protocols of feeding experiments; and their analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kupchan, S. M.; Komoda, Y.; Court, W. A.; Thomas, G. J.; Smith, R. M.; Karim, A.; Gilmore, C. J.; Haltiwanger, R. C.; Bryan, R. F. *J. Am. Chem. Soc.* **1972**, *94*, 1354–1356.
(2) Higashide, E.; Asai, M.; Ootsu, K.; Tanida, S.; Kozai, Y.; Hasegawa, T.; Kishi, T.; Sugino, Y.; Yoneda, M. *Nature* **1977**, *270*, 721–722.
(3) Review Cassady, J. M.; Chan, K. K.; Floss, H. G.; Leistner, E. *Chem. Pharm. Bull.* **2004**, *52*, 1–26.
(4) Hatano, K.; Akiyama, S.-L; Asai, M.; Rickards, R. W. *J. Antibiot.* **1982**, *35*, 1415–1417.
(5) Yu, T.-W.; Bai, L.; Clade, D.; Hoffmann, D.; Toelzer, S.; Trinh, K. Q.; Xu, J.; Moss, S. J.; Leistner, E.; Floss, H. G. *Proc. Nat. Acad. Sci. U.S.A.* **2002**, *99*, 7968–7973.
(6) Spiteller, P.; Bai, L.; Shang, G.; Carroll, B. J.; Yu, T.-W.; Floss, H. G. *J. Am. Chem. Soc.* **2003**, *125*, 14236–14237.
(7) Kubota, T.; Brünjes, M.; Frenzel, T.; Xu, J.; Kirschning, A.; Floss, H. G. *ChemBioChem* **2006**, *7*, 1221–1225.
(8) (a) Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737–1739. (b) Andrus, M. B.; Meredith, E. L.; Somar Sekhar, B. B. V. *Org. Lett.* **2001**, *3*, 259–262.
(9) Gilbert, I. H.; Ginty, M.; O'Neill, J. A.; Simpson, T. J.; Staunton, J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1587–1590.
(10) Formation of other diastereomers was not detected (^{1}H/^{13}C NMR) after purification by flash column chromatography.
(11) Frenzel, T.; Brünjes, M.; Quitschalle, M.; Kirschning, A. *Org. Lett.* **2006**, *8*, 135–138.
(12) Trost, B. M.; McEachern, E. J.; Toste, F. D. *J. Am. Chem. Soc.* **1998**, *120*, 12702–12703.
(13) See Supporting Information.
(14) Wu, J.; Zaleski, T. J.; Valenzano, C.; Khosla, C.; Cane, D. E. *J. Am. Chem. Soc.* **2005**, *127*, 17393–17404.
(15) Smith, S.; Tsai, S.-C. *Nat. Prod. Rep.* **2007**, *24*, 1041–1072.
(16) The configuration at this center is opposite from that predicted based on the origin of the precursor, methoxymalonyl-ACP, from 1,3-bisphospho-*D*-glycerate[17] and the known[18] inversion of configuration in KS-catalyzed Claisen condensations. Therefore, an epimerization must take place at this center at some stage in the biosynthesis prior to loading onto KS4.
(17) Dorrestein, P. C.; Van Lanen, S. G.; Li, W.; Zhao, C.; Deng, Z.; Shen, B.; Kelleher, N. L. *J. Am. Chem. Soc.* **2006**, *128*, 10386–10387.
(18) Weissman, K. J.; Timoney, M.; Bycroft, M.; Grice, P.; Hanefeld, U.; Staunton, J.; Leadlay, P. F. *Biochemistry* **1997**, *36*, 13849–18855.
(19) (a) Caffrey, P. *Chem. Biol.* **2003**, *10*, 1060–1062. (b) Reid, R.; Piagentini, M.; Rodriguez, E.; Ashley, G.; Viswanathan, N.; Carney, J.; Santi, D. V.; Hutchinson, R.; McDaniel, R. *Biochemistry* **2003**, *42*, 72–79.
(20) Castonguay, R.; Valenzo, C. R.; Chen, A. Y.; Keatinge-Clay, A.; Khosla, C.; Cane, D. E. *J. Am. Chem. Soc.* **2008**, *130*, 11598–11599.
(21) Carrol, B. J.; Moss, S. J.; Bai, L.; Kato, Y.; Toelzer, S.; Yu, T.-W.; Floss, H. G. *J. Am. Chem. Soc.* **2002**, *124*, 4176–4177.

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