

Linear Aglycones Are the Substrates for Glycosyltransferase DesVII in Methymycin Biosynthesis: Analysis and Implications

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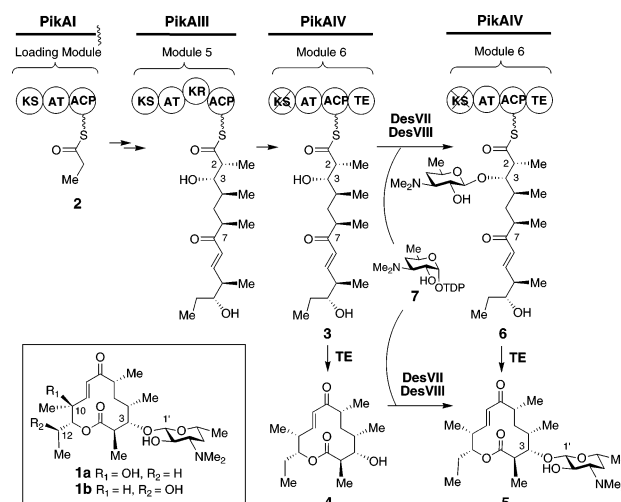
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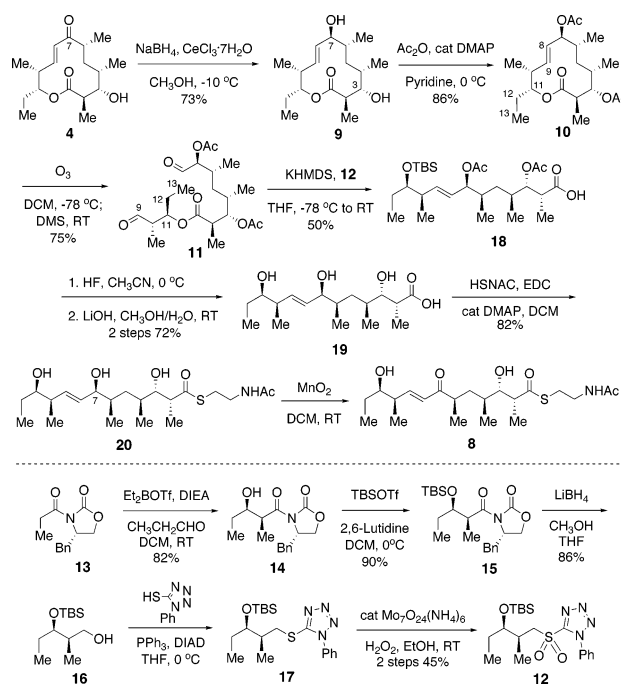
Macrolides are an important class of antibiotics used to treat infections caused by Gram-positive pathogens. This group of compounds consists of two essential structural components: a polyketide aglycone and the appended sugars. The aglycone is biosynthesized by the sequential condensation of acyl thioesters catalyzed by polyketide synthases (PKS).¹ The PKS genes (*pikAI* to *pikAIV*) in the *pik* gene cluster from *Streptomyces venezuelae*, which produces methymycin (**1a**) and neomethymycin (**1b**), represent a well characterized system.² The polyketide chain is made starting from **2** by PikAI and passed on to the subsequent modules in an assembly line fashion (Scheme 1). The mature polyketide chain (**3**) is released in the cyclic form (**4**) from PKS by the action of a thioesterase (TE) domain located at the C-terminus of the last module (module 6). This 12-membered ring macrolactone (**4**) is the precursor of methymycin (**1a**) and neomethymycin (**1b**).³

Advances in our understanding of polyketide biosynthesis have enabled the recombination of the PKS genes to construct a diverse array of new ring systems.^{1c,e,4} Without the sugar components, however, these new compounds are usually biologically inactive. It is generally believed that glycosylation by the action of dedicated glycosyltransferases occurs after the cyclic aglycone (such as **4**) is released from the PKS. However, the failure of an *in vitro* demonstration of macrolide glycosyltransferase activity in the presence of a necessary TDP-sugar donor and aglycone acceptor raised questions about the glycosylation step. The situation was clarified somewhat by the recent discovery of an auxiliary protein (DesVIII), which activates the glycosyltransferase (DesVII) responsible for the attachment of TDP-desosamine (**7**) to **4**.⁵ This unusual property of DesVII may account for the fact that activity has not been previously verified by conventional assays.⁶ However, whether the acyl carrier protein (ACP)-bound linear polyketide chain (**3**) can also be processed by the corresponding glycosyltransferase in addition to the cyclic aglycone has never been explored. Reported herein are the results of our investigation indicating that the linear polyketide chain **8** (an analogue of **3**) can also serve as a chemically competent substrate for the macrolide glycosyltransferase DesVII.

As depicted in Scheme 2, the synthesis of the linear substrate **8** from **4**, which was produced by *S. venezuelae* mutant KdesI-80,⁷ was initiated by reduction of the C-7 keto group (**4** → **9**), followed by protection of the C-3 and C-7 hydroxyl groups (**9** → **10**), and oxidative cleavage of the double bond between C-8 and C-9 of **10** to give **11**.⁸ The sulfone derivative of the six-carbon (C₉–C₁₃) fragment **12** was prepared using **13** as the chiral building block, which was reacted with propionaldehyde to yield the syn aldol product **14**. Subsequent reactions involving silyl protection (**14** → **15**), reduction with LiBH₄ (**15** → **16**), Mitsunobu reaction with 1-phenyl-1*H*-tetrazole-5-thiol (**16** → **17**), and oxidation using a molybdate catalyst⁹ led to the sulfone **12**. This fragment (**12**) was



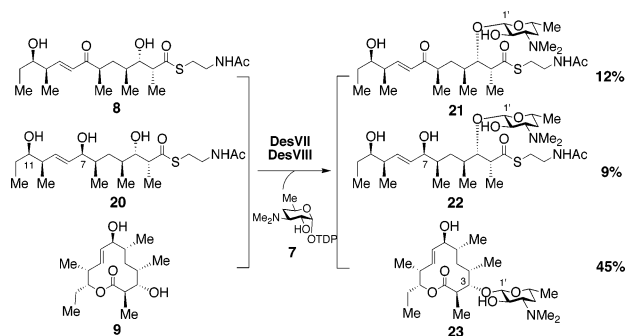
Scheme 2



then coupled to **11** under the Julia-Kocieniński olefination conditions.^{9,10} Interestingly, the anticipated diene compound was produced only in a trace amount. Instead, the hydrolyzed acid **18** was obtained in 50% yield.¹¹ Consecutive deprotection of **18** gave **19** in 72% yield. Due to the difficulty of preparing the ACP-bound linear polyketide chain (**3**), the intended linear substrate was synthesized

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Scheme 3



as the *N*-acetylcysteamine (NAC) thioester via esterification of **19** with *N*-acetylcysteamine in 82% yield. Selective oxidation of **20** afforded the desired product **8**.¹²

Incubation of **8** with TDP-D-desosamine (**7**) in the presence of DesVII and DesVIII resulted in the formation of a more polar compound.¹³ This compound was purified by HPLC, and the high-resolution CI-MS mass (calcd for $C_{29}H_{53}N_2O_7S$ [$M + H$]⁺ 573.3573, found 573.3566) is consistent with the anticipated glycosylated product **21** (Scheme 3). The 7-OH compound **20** was also tested for chemical competence as a substrate for DesVII/DesVIII. Two new compounds were isolated from this incubation mixture. The major product is singly glycosylated as indicated by the high-resolution CI-MS mass (calcd for $C_{29}H_{53}N_2O_7S$ [$M + H$]⁺ 575.3730, found 575.3729). The predicted structure of this product is **22**. The minor and less polar product has a molecular mass corresponding to the dehydrated **22** (CI-MS calcd for $C_{29}H_{53}N_2O_6S$ [$M + H$]⁺ 557.3624, found 557.3618). The dehydration is unlikely enzyme-catalyzed but an artifact of the incubation (pH 8) or isolation conditions. HPLC analysis of the incubation mixtures enabled the estimation of a 12% yield for **21** from **8** and a 9% yield for **22** and the dehydrated product from **20**. The low yields of the products precluded a thorough characterization of the structures, but a single product formation in all cases suggests that these compounds are glycosylated at the 3-OH group.¹⁴

For comparison, glycosylation of the cyclic aglycone **4** and the C-7 reduced form **9** were also examined under the same conditions. Again, a single glycosylated product was isolated in each case. NMR analysis confirmed the structure of the product resulting from **9** to be **23** (obtained in 45% yield), with the correct MS mass (calcd for $C_{25}H_{46}NO_6$ [$M + H$]⁺ 456.3325, found 456.3327). As expected, compound **5** was obtained from the incubation with **4** in nearly quantitative yield (CI-MS calcd for $C_{25}H_{44}NO_6$ [$M + H$]⁺ 454.3169, found 454.3173).¹⁵ Clearly, the cyclic forms are much better substrates: the yield of glycosylation is 5-fold higher for **9** versus **20** and at least 8-fold more for **4** versus **8**. The analysis also shows that DesVII prefers the C-7 keto group over the C-7 OH group (**8** versus **20**, and **4** versus **9**).

In summary, this study demonstrated for the first time that a macrolide glycosyltransferase can also recognize and process the linear precursor of its macrolactone substrate with reduced but measurable activity. The reaction is regioselective because only one singly glycosylated product is generated in each case despite the presence of multiple potential glycosylation sites. This finding is significant for three reasons. First, a similar capability is possible for other macrolide glycosyltransferases, and the glycosylation of the linear polyketide may be a minor glycosylation pathway during the biosynthesis of macrolide antibiotics. Second, the substrate flexibility of macrolide glycosyltransferases is apparently extended

to linear aglycones as well as cyclic analogues. This property expands the opportunities for glycodiversification of secondary metabolites. More importantly, this study implicates that the substitution patterns nearby the glycosylation site, and not the ring conformation, are the major recognition determinants for glycosyltransferases.¹⁶ This finding sets the stage for a future investigation to establish the minimal recognition elements for this class of glycosyltransferases. However, the fact that **8** and **4** are better substrates (than **20** and **9**, respectively) indicates that the specificity of DesVII is also sensitive to the substituents distant from the glycosylation site. It has been shown that **8** can be cyclized to **4** by *pik*-TE in vitro.¹² It remains to be tested if the thioesterase is also capable of cyclizing the glycosylated linear polyketides.

Acknowledgment. This work was supported by the National Institutes of Health Grant GM54346.

Supporting Information Available: The conditions for the enzymatic glycosylations as well as characterization of glycosylated products (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (11) Such a transformation could occur by a β -fragmentation facilitated by the oxyanion generated after the addition of **12** to **11**.
- (12) A more efficient synthesis of **8** based on direct hydrolysis to open the macrolactone ring of **4** was recently reported [(a) Aldrich, C. C.; Beck, B. J.; Fecik, R. A.; Sherman, D. H. *J. Am. Chem. Soc.* **2005**, *127*, 8441–8452. (b) Aldrich, C. C.; Venkatraman, L.; Sherman, D. H.; Fecik, R. A. *J. Am. Chem. Soc.* **2005**, *127*, 8910–8911]. However, the hydrolysis was slow, and two C-2 stereoisomers were generated during hydrolytic ring opening, which had to be separated by HPLC.
- (13) See Supporting Information for details.
- (14) However, glycosylation at 11-OH remains a possibility.
- (15) Since neither **9** nor **23** possess a chromophore, an HPLC instrument equipped with the universal Corona charged aerosol detector (CAD) was used to show the conversion of **9** to **23** as the only product.
- (16) This is in agreement with our previous observation of aglycone specificity for DesVII (see ref 6d).

JA058433V