Letter

A Highly Promiscuous #-Ketoacyl-ACP Synthase (KAS) III-like Protein is Involved in Pactamycin Biosynthesis

Mostafa E. Abugrain, Corey J. Brumsted, Andrew R. Osborn, Benjamin Philmus, and Taifo Mahmud

ACS Chem. Biol., **Just Accepted Manuscript** • DOI: 10.1021/acschembio.6b01043 • Publication Date (Web): 06 Jan 2017

ACS Publications

ACS Chemical Biology is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

A Highly Promiscuous ß-Ketoacyl-ACP Synthase (KAS) III-like Protein is Involved in Pactamycin Biosynthesis

Mostafa E. Abugrain,[†,¶] Corey J. Brumsted,[‡,¶] Andrew R. Osborn,[†] Benjamin Philmus,[†] and Taifo Mahmud[†,‡,*]

[†]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, Oregon 97333, United States

[‡]Department of Chemistry, Oregon State University, Corvallis, Oregon 97333, United States

*To whom correspondence should be addressed

[¶]These authors contributed equally

ABSTRACT

β-Ketoacyl-acyl carrier protein (β-Ketoacyl-ACP) synthase (KAS) III catalyzes the first step in fatty acid biosynthesis, involving a Claisen condensation of the acetyl-CoA starter unit with the first extender unit, malonyl-ACP, to form acetoacetyl-ACP. KAS III-like proteins have also been reported to catalyze acyltransferase reactions using coenzyme A esters or discrete ACP-bound substrates. Here, we report the in vivo and in vitro characterizations of a KAS III-like protein (PtmR), which directly transfers a 6-methylsalicylyl moiety from an iterative type I polyketide synthase to an aminocyclopentitol unit in pactamycin biosynthesis. PtmR is highly promiscuous, recognizing a wide array of *S*-acyl-*N*-acetylcysteamines as substrates to produce a suite of pactamycin derivatives with diverse alkyl and aromatic features. The results suggest that KAS III-like proteins may be used as versatile tools for modifications of complex natural products.

β-Ketoacyl-acyl carrier protein synthase (KAS) enzymes play a central role in type I (modular) and type II (dissociable) fatty acid synthases (FASs) and polyketide synthases (PKSs), as well as in chalcone synthases.[1] In the type II dissociable FASs, three types of KAS enzymes are known: KAS I (FabB) and KAS II (FabF) are responsible for the elongation steps, whereas KAS III (FabH) catalyzes the initiation step, involving a Claisen condensation of the acetyl-CoA starter unit with the first extender unit, malonyl-ACP, to form acetoacetyl-ACP (Figure S1a).[1] In *Streptomyces*, KAS III can also recruit alternative starter units, such as isobutyryl-CoA and methylbutyryl-CoA, to form branched chain fatty acids. Less efficiently, it can also catalyze acyl-CoA:ACP transacylase (ACAT) reactions.[2]

Over the past decade, a number of KAS III-like enzymes have been reported to have unusual catalytic functions. The *cloN2* gene of the clorobiocin cluster in *Streptomyces roseochromogenes* var. *oscitans* has been found through a gene inactivation study to be responsible for the transfer of the pyrrolylcarbonyl unit to the sugar moiety of clorobiocin.[3] The KAS III-like protein CerJ uses malonyl-CoA analogs to form sugar esters in cervimycin biosynthesis (Figure S1b),[4] whereas ChlB6 from the chlorothricin pathway transfers a 3-chloro-6-methoxy-2-methylbenzoyl moiety from a discrete ACP, ChlB2, to a sugar moiety of chlorothricin (Figure S1c).[5] The 3-chloro-6-methoxy-2-methylbenzoyl unit is derived from a 6-methylsalicylyl (6MSA) unit, a product of an iterative type I PKS (ChlB1). Incorporation of this PKS product to the sugar moiety of chlorothricin requires multiple intermediary acyl transfer reactions involving two discrete KAS III-like proteins (ChlB3 and ChlB6) and a discrete ACP (ChlB2) (Figure S1c).[5]

Genes encoding proteins homologous to ChlB6 have been found in a number of natural products biosynthetic gene clusters, such as *aviN* of the avilamycin A cluster in *Streptomyces viridochromogenes* Tü57,[6] *evrI* of the evernimicin cluster in *Micromonospora carbonacea* var. *africana* ATCC 39149,[7] *calO4* of the calicheamicin cluster in *Micromonospora echinospora* subsp. *calichensis*,[8] *pokM2* of the polyketomycin cluster in *Streptomyces diastatochromogenes* Tü6028,[9] *tiaF* of the tiacumicin cluster in *Dactylosporangium aurantiacum* subsp. *hamdenensis* NRRL 18085,[10] and *esmD1* of the esmeraldin pathway in *Streptomyces antibiotics* Tü 2706.[11] Except esmeraldin, all of these natural products contain one or more sugar moieties decorated by 6MSA or orsellinic acid derivatives. However, in contrast to the chlorothricin pathway, they lack the genes that code for the second KAS III and the discrete ACP in their clusters.

A gene encoding a protein homologous to KAS III was also found in the pactamycin cluster in *Streptomyces pactum* (Figure S2).[12] This gene (*ptmR*) may be involved in the attachment of 6MSA, which is synthesized by the iterative type I PKS PtmQ,[12] to the aminocyclopentitol core unit in pactamycin biosynthesis. Also present in the cluster are genes that code for a KAS I protein (PtmK), a discrete ACP (PtmI), and a putative hydrolase/acyltransferase (PtmO). The roles of these genes in pactamycin biosynthesis are currently unknown, but their possible involvement in the attachment of 6MSA cannot be ruled out.

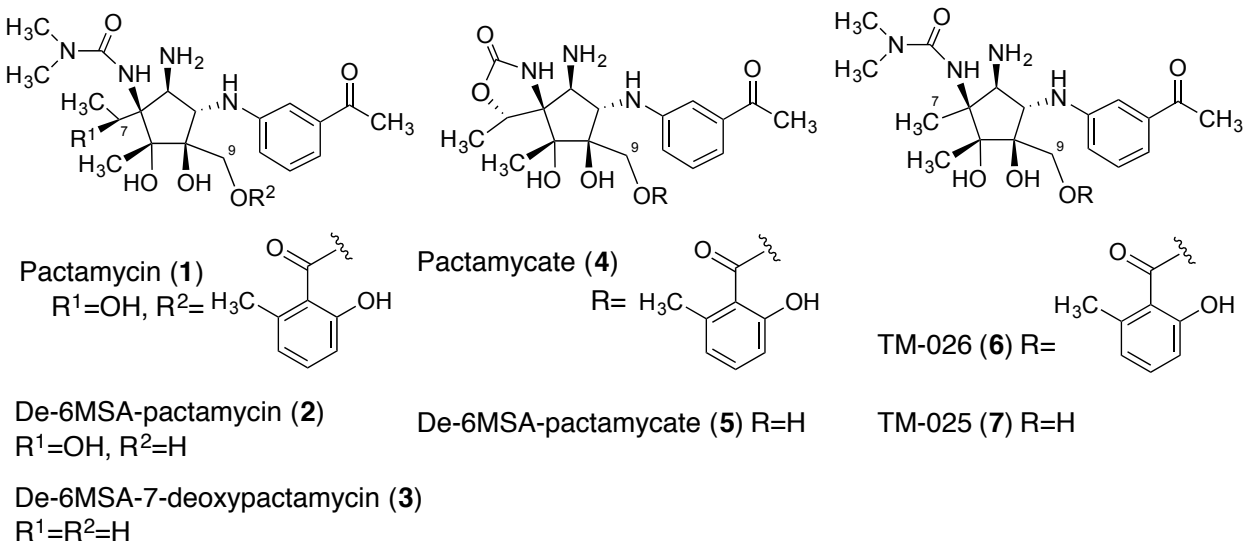


Figure 1. Chemical structures of pactamycin analogs.

RESULTS AND DISCUSSION

To investigate the involvement of *ptmI*, *ptmK*, *ptmO*, and *ptmR* in the transfer of the 6MSA moiety, we generated mutant strains of *S. pactum* by either in-frame deletion or gene disruption with *aac(3)IV* (Figures S3 and S4), and characterized their products by ESI-MS and HPLC. Inactivation of *ptmI, ptmK,* or *ptmO* completely abrogated the production of pactamycin and its analogs (Figure S5),[12] suggesting that these genes are involved in the early steps of the pathway, not in the attachment of 6MSA, which occurs later in the pathway.[12, 13] On the other hand, the ΔptmR mutant produced de-6MSA-pactamycin (**2**) and its degradation product, de-6MSA-pactamycate (**5**) (Figures 1, S6a and S7), consistent with the absence of 6MSA-transferase activity in this mutant. Furthermore, we inactivated *ptmR* in a mutant strain of *S. pactum*, ΔptmH, which produces 7-deoxy-7-demethylpactamycin (TM-026, **6**).[14] The double gene

knockout mutant, ΔptmH/ΔptmR, produces de-6MSA-7-deoxy-7-demethylpactamycin (TM-025, **7**) (Figures S6g and S8), confirming the function of PtmR as a 6MSA-transferase. These results were further corroborated by a gene complementation experiment, in which an integrative plasmid harboring intact *ptmR* was introduced into ΔptmH/ΔptmR. As expected, the resulting conjugants were able to produce TM-026 (Figure S6f).

To characterize the catalytic function of PtmR in vitro, we cloned the gene in the expression vector pET-20b(+) and heterologously expressed it in *Escherichia coli* BL21(DE3) pLysS to give a 39-kDa C-terminal His_6-tagged protein (Figure S9). Initial enzymatic experiments were carried out using cell-free extracts of *E. coli* containing PtmR, with de-6MSA-pactamycin (**2**), de-6MSA-7-deoxypactamycin (**3**), de-6MSA-pactamycate (**5**), or TM-025 (**7**) as substrates. The synthetically prepared *N*-acetylcysteamine (NAC) thioester of 6MSA, which mimics an ACP-bound substrate, was used as a model acyl donor substrate. ESI-MS and HPLC analyses of the reaction products revealed the conversion of the de-6MSA analogs to their corresponding 6MSA esters (Figures S10 and S11). Similar experiments using purified PtmR protein and de-6MSA-pactamycin also gave pactamycin, and its degradation product, pactamycate (Figure 2), which unambiguously confirm the 6MSA-transferase activity of PtmR. Incubations of the enzyme and TM-025 with 6MSA free acid as substrate did not give any products, indicating PtmR only recognizes an activated substrate (data not shown). Moreover, addition of 6MSA to ΔptmQ and ΔptmQ/ΔptmH cultures did not give pactamycin or TM-026 (Figure S12), suggesting that free 6MSA is not involved in the pathway.

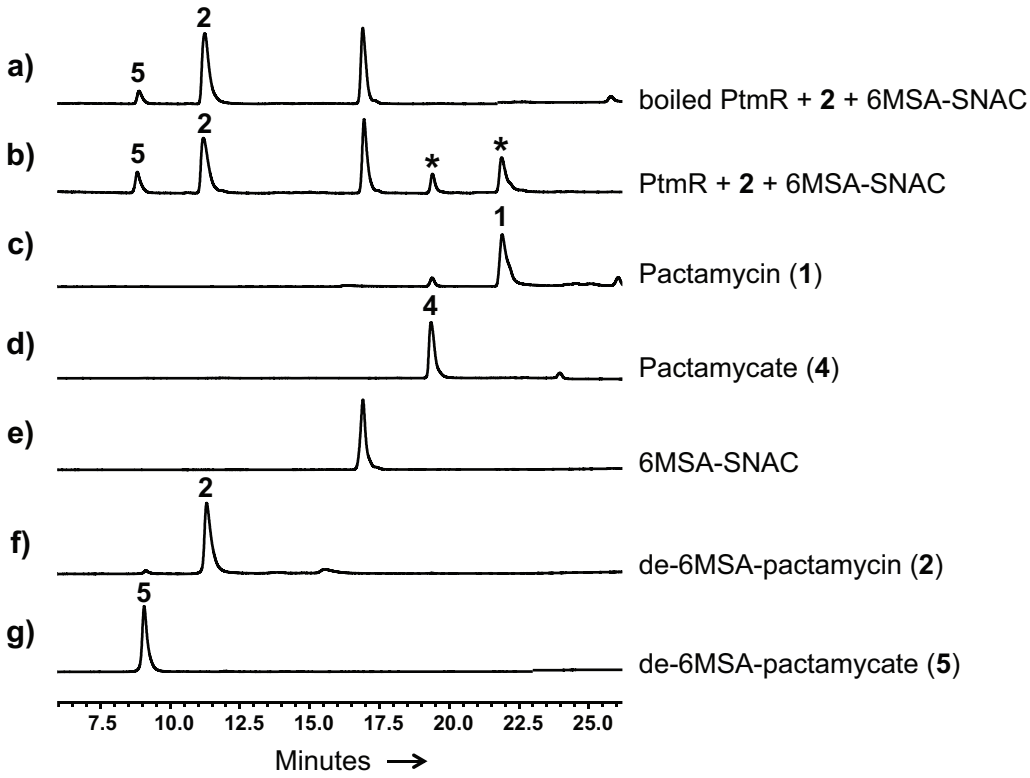


Figure 2. HPLC analyses of PtmR reactions using de-6MSA-pactamycin (**2**) and 6MSA-SNAC as substrates. Stars indicate the products of PtmR. Compounds **1** and **2** can non-enzymatically convert to **4** and **5**, respectively.

However, in saphenamycin biosynthesis, it is proposed that 6MSA is first activated by a 6MSA adenylase (EsmD2) to its AMP derivative and loaded onto a carrier protein (EsmD3) before being transferred to saphenic acid by EsmD1, a ChlB6 homologue.[11] In fact, a gene encoding a putative AMP-forming acyl-CoA synthetase is present in the pactamycin cluster. The gene product (PtmS) has been proposed to catalyze the activation of 3-aminobenzoic acid (3ABA), the precursor of the 3-aminoacetophenone moiety of pactamycin.[12, 15] However, its role in the pactamycin pathway has yet to be experimentally established.

To investigate the possible involvement of PtmS in the 6MSA transfer, we inactivated the gene in *S. pactum* by in frame-deletion and analyzed the products by ESI-MS. Similar to the ΔptmI, ΔptmK, and ΔptmO mutants, ΔptmS was not able to produce pactamycin or its analogs (Figure S13), suggesting that it is involved in an early step of the pathway. However, the results cannot rule out the possibility of PtmS playing a dual role in the pathway – that is activating both 3ABA and 6MSA. This may also be the case with the discrete ACP PtmI. To test this possibility, we carried out co-culture experiments using ΔptmQ/ΔptmH + ΔptmI and ΔptmQ/ΔptmH + ΔptmS. Co-cultures of ΔptmQ/ΔptmH + ΔptmJ (which lacks the glycosyltransferase activity)[12] were used as a positive control. It is expected that the ΔptmQ/ΔptmH mutant product (TM-025) will be taken up by ΔptmJ and converted to TM-026. A similar phenomenon should occur in the ΔptmQ/ΔptmH + ΔptmI and ΔptmQ/ΔptmH + ΔptmS cultures. However, if PtmI and/or PtmS are involved in the 6MSA attachment, no conversion from TM-025 to TM-026 should be observed. As expected, the ΔptmQ/ΔptmH + ΔptmJ cultures produced TM-026 as a major metabolite (Figure S14). The same result was also observed in the cultures of ΔptmQ/ΔptmH + ΔptmI and ΔptmQ/ΔptmH + ΔptmS, indicating that both PtmI and PtmS do not play a role in 6MSA attachment. Altogether, the results show that PtmR is responsible for a direct transfer of the 6MSA moiety from the iterative type I PKS PtmQ to the aminocyclopentitol unit in pactamycin biosynthesis (Figure 3).

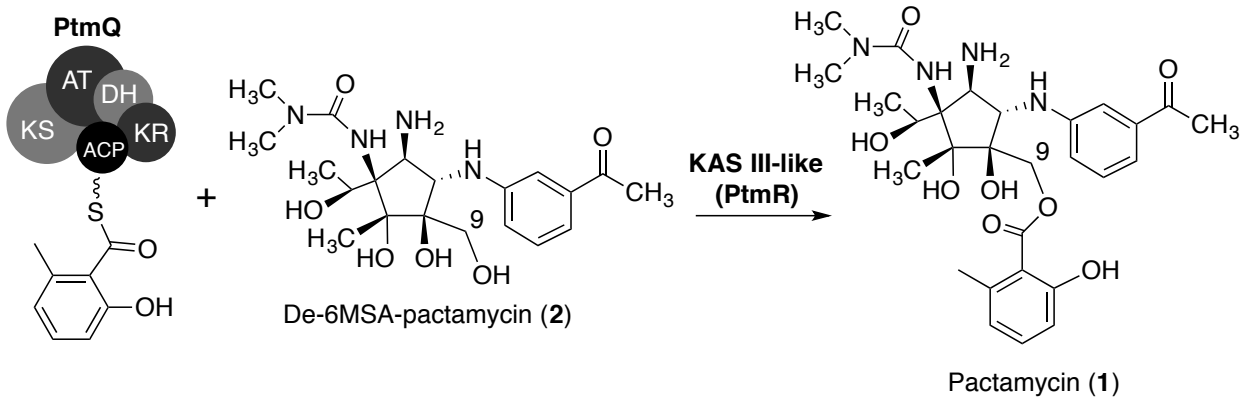
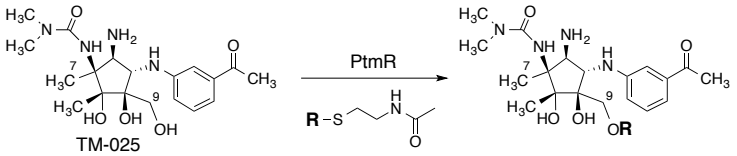
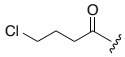
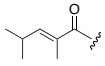
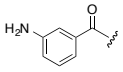
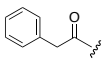
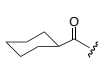
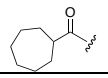


Figure 3. Proposed catalytic activity of PtmR.

The fact that PtmR does not only process de-6MSA-pactamycin but also de-6MSA-7-deoxypactamycin, de-6MSA-pactamycate, and TM-025 indicates that it has relaxed substrate specificity. To explore if PtmR can also transfer acyl groups other than 6MSA, we synthesized an additional 13 NAC thioesters with a variety of alkyl and aromatic features ranging from acetyl-SNAC to 3-aminobenzoyl-SNAC to cycloheptanecarbonyl-SNAC (Table 1), and incubated them with TM-025 and a cell-free extract of *E. coli* containing PtmR. ESI-MS analysis of the enzymatic reactions revealed the ability of PtmR to use all 13 NAC thioesters, resulting in a suite of new pactamycin analogs with diverse functionalities (Table 1 and Figures S10, S15–S17). Parallel experiments with benzoyl-CoA, acetyl-CoA, propionyl-CoA, and butyryl-CoA did not give any products (Figure S18), indicating that PtmR does not recognize CoA esters as substrates. Scaled-up enzymatic reactions using 4-chlorobutyryl-SNAC furnished TM-107, whose complete chemical structure was determined by 1D and 2D NMR spectroscopic data (Figures S48–S52).

Table 1. Conversion of TM-025 to various pactamycin analogs by PtmR.



R	Product	HR ESI-MS *(m/z)*		Mol. Formula	Relative Conversion[a]
		Calcd	Observed		
	TM-026	529.2657 $[M+H]^+$	529.2664 $[M+H]^+$	$C_{27}H_{36}N_4O_7$	100%
	TM-104	437.2395 $[M+Na]^+$	437.2396 $M+Na]^+$	$C_{21}H_{32}N_4O_6$	71%
	TM-105	473.2371 $[M+Na]^+$	473.2383 $[M+Na]^+$	$C_{22}H_{34}N_4O_6$	87%
	TM-106	465.2708 $[M+H]^+$	465.2718 $[M+H]^+$	$C_{23}H_{36}N_4O_6$	91%
	TM-107	499.2318 $[M+H]^+$	499.2327 $[M+H]^+$	$C_{23}H_{35}ClN_4O_6$	100%
	TM-108	465.2708 $[M+H]^+$	465.2718 $[M+H]^+$	$C_{23}H_{36}N_4O_6$	83%
	TM-109	479.2864 $[M+H]^+$	479.2874 $[M+H]^+$	$C_{24}H_{38}N_4O_6$	61%
	TM-110	505.3021 $[M+H]^+$	505.3025 $[M+H]^+$	$C_{26}H_{40}N_4O_6$	44%
	TM-111	479.2864 $[M+H]^+$	479.2872 $[M+H]^+$	$C_{24}H_{38}N_4O_6$	20%
	TM-112	499.2551 $[M+H]^+$	499.2558 $[M+H]^+$	$C_{26}H_{34}N_4O_6$	43%
	TM-113	514.2660 $[M+H]^+$	514.2690 $[M+H]^+$	$C_{26}H_{35}N_5O_6$	17%
	TM-114	513.2708 $[M+H]^+$	513.2738 $[M+H]^+$	$C_{27}H_{36}N_4O_6$	95%
	TM-115	505.3021 $[M+H]^+$	505.3022 $[M+H]^+$	$C_{26}H_{40}N_4O_6$	57%
	TM-116	519.3177 $[M+H]^+$	519.3182 $[M+H]^+$	$C_{27}H_{42}N_4O_6$	87%

[a]Based on relative intensity of (+)-ESI-MS substrate (*m/z* 395) and observed product peak

The ability of PtmR to utilize a broad range of substrates is somewhat surprising, as so far KAS III-like proteins, e.g., CloN2, ChlB6, CalO4, AviN, and EvrI, have only been associated with the transfer of an aryl or pyrrolylcarbonyl group to a sugar moiety. A phylogenetic analysis

of PtmR and homologous proteins showed that PtmR falls within a clade of KAS III-like enzymes, but it forms a separate sub-clade together with a number of hypothetical proteins from *Streptomyces niveiscabiei* NRRL B-24457, *Streptomyces acidiscabies* 84-104, and *Streptomyces* sp. DSM 15324 (Figure S19). Genome mining studies revealed that these genes are part of gene clusters that resemble the pactamycin cluster of *S. pactum*. Similar to *ptmR*, they are also located next to a gene encoding an iterative type I PKS (6MSA synthase). Therefore, we predict that the encoding proteins are also responsible for the attachment of 6MSA or its analogs to the corresponding natural products.

In conclusion, the present work showed that a highly promiscuous KAS III-like enzyme is responsible for the attachment of 6MSA directly from an iterative type I PKS to an aminocyclopentitol unit in pactamycin biosynthesis. The results also serve as a starting point for the development of KAS III-like proteins as versatile tools for creation of new libraries of complex natural products.

METHODS

Construction of ΔptmR and ΔptmH/ΔptmR mutant strains. The target genes were inactivated using a gene in-frame deletion strategy (Figure S3). Two ~1 kb PCR fragments upstream (HindIII/EcoRI) and downstream (EcoRI/XbaI) of the *ptmR* gene were fused and cloned into the HindIII/XbaI site of pBluescript II SK(–) vector to generate pTMM056 (Table S2). The fused PCR fragment was excised and cloned into the HindIII/XbaI site of pTMN002 to

generate pTMM057. The plasmid was then introduced into the wild-type and the ΔptmH mutant strains of *S. pactum* ATCC 27456 by conjugation using *E. coli* ET12567/pUZ8002 as a donor strain. Apramycin resistant strains representing single crossover mutants were obtained and grown on BTT [glucose (1%), yeast extract (0.1%), beef extract (0.1%), casein hydrolysate (0.2%), agar (1.5%), pH 7.4] agar plates containing apramycin (50 μg mL^{-1}). Subsequently, apramycin sensitive colonies were counter-selected by replica plating on BTT agar with and without apramycin (50 μg mL^{-1}). The resulting double-crossover candidate strains were confirmed by PCR amplification with *ptmR*-F1 and *ptmR*-R2 primers (Table S3) flanking the respective targeted gene (Figure S3).

Complementation of ΔptmH/ΔptmR mutant. For complementation of the ΔptmH/ΔptmR mutant, the *ptmR* gene was amplified by PCR using the primers PtmR-pET-F and PtmR-C-R (Table S3). The PCR products were digested with BglII and EcoRI and ligated into the BamH/EcoRI sites of the cloning vector pBluescript II SK(–) to generate pTMM058. DNA sequencing confirmed the correct sequence of the construct. The resulting plasmid was digested with NdeI/EcoRI and the DNA fragment was ligated into the integration vector pTMW50, predigested with the same restriction enzymes, to generate pTMM059. The pTMM059 plasmid was then transferred into *E. coli* ET12567/pUZ8002, which was subsequently used to transform ΔptmH strain by conjugation.[16] Selection of the exconjugants (ΔptmH/ΔptmR mutants) was performed on BTT agar containing apramycin (50 μg mL^{-1}) and ampicillin (100 μg mL^{-1}).

Analysis of ΔptmR, ΔptmH/ΔptmR, and ΔptmH/ΔptmR+pTMM059 metabolic profiles. The ΔptmR, ΔptmH/ΔptmR, and ΔptmH/ΔptmR+pTMM059 strains were grown on BTT agar at 30 °C for 3 days. Single colonies were used to inoculate the BTT seed cultures [medium for ΔptmH/ΔptmR+pTMM059 was supplemented with apramycin (10 μg mL^{-1}) and ampicillin (20 μg mL^{-1})] and incubated at 30 °C for 2 days. Production cultures were prepared in modified Bennett's medium (50 mL)[12] and inoculated with seed cultures [10% (v/v)]. The production cultures were incubated at 30 °C for 5 days under vigorous shaking (200 rpm). The mycelia were centrifuged and the supernatants were extracted twice with equal volumes of EtOAc followed by extraction with n-BuOH. The organic solvent from each extraction was evaporated *in vacuo* and the residues dissolved in MeOH and analyzed by reversed-phase HPLC and/or ESI-MS. Analysis of the metabolites of the mutants and those complemented with *ptmR* was carried out by reversed-phase HPLC with a C-18 column (Supelcosil LC-18-DB 15 cm x 4.6 cm, 5 μm) using H_2O [95% (v/v)] and CH_3CN [5% (v/v)] containing TFA [0.1% (v/v)] as mobile phase at 1 mL min^{-1} flow rate.

Construction of ΔptmI, ΔptmO, and ΔptmS mutant strains. The target genes were inactivated using a gene in-frame deletion strategy (Figure S3a). For details, see the Supporting Information.

Construction of *ptmK::aac(3)IV* mutant. The *ptmK* gene (1.7 kb) was inactivated using a gene disruption strategy (Figure S3b). For details, see the Supporting Information.

Feeding experiments with 6-MSA to ΔptmQ and ΔptmH/ΔptmQ mutants. The ΔptmQ and ΔptmH/ΔptmQ mutants were streaked on BTT agar [glucose (1% (w/v)), yeast extract (0.1% (w/v)), beef extract (0.1% (w/v)), casein hydrolysate (0.2% (w/v)), agar (1.5% (w/v)), pH 7.3] and incubated at 30 °C for 3 days. Spores of the ΔptmQ and ΔptmH/ΔptmQ mutants were individually grown in two Erlenmeyer flasks (125 mL) containing seed medium [glucose (1% (w/v)), yeast extract (0.1% (w/v)), beef extract (0.1% (w/v)), casein hydrolysate (0.2% (w/v)), pH 7.3] (50 mL) for 3 days at 30°C and 200 rpm. Each of these seed cultures (10 mL) was used to inoculate 4 Erlenmeyer flasks (250 mL) containing modified Bennett's medium (100 mL). After incubation for 18 h under the same conditions, the cultures were grouped into two groups; the first group was supplemented with 6-MSA (5 mM, 250 μL) and the second group was used as control. The feeding was repeated every 12 h for 2 days. All experiments were done in triplicate. After five days of incubation, the cultures were centrifuged. The metabolites of each group were extracted with ethyl acetate (2 x 100 mL). The organic solvent was evaporated using rotary evaporator and the products were analyzed by MS.

Co-culture experiments. For details on co-culture experiments with ΔptmJ, ΔptmI, ΔptmS, and ΔptmH/ΔptmQ mutants, see the Supporting Information.

Production of PtmR. The ptmR gene was amplified using primer pairs PtmR-pET-F and PtmR-pET-R (Table S3). High Fidelity Taq DNA Polymerase (Invitrogen) was used for PCR, and the resulting 1.0-kb PCR product was cloned into the pET-20b(+) vector (Novagen) to generate expression vector pTMM060, which was introduced into *E. coli* BL21(DE3) pLysS (Invitrogen).

For protein production, the bacteria were grown in LB medium supplemented with ampicillin (100 μg mL^{-1}) and chloramphenicol (25 μg mL^{-1}) at 30 °C with shaking at 250 rpm until an OD_{600} of 0.8–1 was reached. The culture was shaken at 16 °C for 1 h. Protein expression was induced by addition of IPTG (0.5 mM) with further cultivation for 36 h. The cells were harvested by centrifugation and resuspended in sodium phosphate buffer (40 mM, pH 7.5) containing NaCl (300 mM) and imidazole (10 mM), and then disrupted by sonication. After centrifugation of the sample, the supernatant was directly loaded onto a Ni-NTA spin column (Qiagen). The recombinant PtmR was eluted using sodium phosphate buffer (40 mM, pH 7.5) containing NaCl (300 mM) and imidazole (250 mM). The PtmR-containing fractions were dialyzed (3 times, 5 h each) in 1 L solution containing sodium phosphate buffer (20 mM, pH 7), $MgCl_2$ (10 mM), glycerol (10% (w/v)) and dithiothreitol (0.5 mM) at 4 °C.

Acyltransferase assay. The acyltransferase reaction was typically carried out in 50 μL mixtures containing TM-25 or de-6MSA-pactamycin (1 mM), NAC thioester (2 mM), purified PtmR or a cell free extract of *E. coli* containing PtmR (47 μL), and $MgCl_2$ (10 mM) in sodium phosphate buffer (50 mM, pH 7.5). Boiled protein or cell free extract of empty pET-20b(+) was used as negative control. The reaction was incubated at 30 °C. After 4 h, the reaction was quenched by the addition of one volume MeOH, centrifuged at 14,000 rpm for 15 min, and the supernatants were analysed by reversed-phase HPLC and/or ESI-MS.

Scaled-up enzymatic reaction and isolation of TM-107. Scaled-up enzymatic reactions (100 μL x 100, 10 mL total) were carried out using the cell free extract of *E. coli* containing PtmR in

sodium phosphate buffer (40 mM, pH 7.5, contains 10 mM $MgCl_2$ and 10% (w/v) glycerol), TM-025 (1 mM), and 4-chlorobutyryl-SNAC (2 mM). The mixtures were incubated at 30 °C for 3 h. The reactions were then quenched by addition of one volume of MeOH and centrifuged at 14,000 rpm for 15 min. The supernatants were pooled and subjected to rotary evaporator to remove MeOH, and the aqueous mixture was extracted twice with two volumes of EtOAc. The EtOAc extract was dried in vacuo and the residue was dissolved in MeOH (400 μL) and subjected to reverse-phase HPLC (YMC-Pack ODS-A, 250 x 10 mm I.D., 5 μm), solvent gradient: CH_3CN-H_2O (5:95) to CH_3CN (100%) containing TFA [0.1% (v/v)] over 60 min, flow rate 4.7 mL min^{-1}, detection at 254 nm) to give TM-107 (1.2 mg).

TM-107: a white powder, $[\alpha]_D$ = +37° (*c* 0.267, MeOH, 20 °C); UV (MeOH) λ_{max} 240 nm (ε 1.6 x 10^3), 264 nm (ε 4.65 x 10^2), and 354 (ε 24.4); IR (KBr, MeOH) ν 3359, 2919, 2856, 1679, 1536, 1483, 1206, and 1138 cm^{-1}. ^{1}H NMR (700 MHz, CD_3OD): Table S5; ^{13}C NMR (175 MHz, CD_3OD): Table S5.

Chemical synthesis of NAC thioesters. For details, see the Supporting Information.

Associate Content

Supporting Information

The Supporting Information is available free of charge on the ACS Publication Website at DOI:…

Supporting methods, figures and tables (PDF).

Author Information

Corresponding Authors

***Address:** 203 Pharmacy Building, Oregon State University, Corvallis, Oregon 97331, United States. Phone: (541) 737-9679. Fax: (541) 737-3999. E-mail: Taifo.mahmud@oregonstate.edu.

Notes

The authors declare no competing financial interest.

Acknowledgements

The authors thank P. Proteau for a critical reading of this manuscript, N. Roongsawang, E. Wan-Knight, and K. W. Wen for technical assistance, and A. deBarber for HR-ESI-MS analysis. The project described was supported by the Oregon State University College of Pharmacy Faculty Development Funds.

References

(1) Dawe, J. H., Porter, C. T., Thornton, J. M., and Tabor, A. B. (2003) A template search reveals mechanistic similarities and differences in beta-ketoacyl synthases (KAS) and related enzymes, *Proteins 52*, 427-435.

(2) Han, L., Lobo, S., and Reynolds, K. A. (1998) Characterization of beta-ketoacyl-acyl carrier protein synthase III from *Streptomyces glaucescens* and its role in initiation of fatty acid

biosynthesis, *J. Bacteriol. 180*, 4481-4486.

(3) Xu, H., Kahlich, R., Kammerer, B., Heide, L., and Li, S. M. (2003) CloN2, a novel acyltransferase involved in the attachment of the pyrrole-2-carboxyl moiety to the deoxysugar of clorobiocin, *Microbiology 149*, 2183-2191.

(4) Bretschneider, T., Zocher, G., Unger, M., Scherlach, K., Stehle, T., and Hertweck, C. (2012) A ketosynthase homolog uses malonyl units to form esters in cervimycin biosynthesis, *Nat. Chem. Biol. 8*, 154-161.

(5) He, Q. L., Jia, X. Y., Tang, M. C., Tian, Z. H., Tang, G. L., and Liu, W. (2009) Dissection of two acyl-transfer reactions centered on acyl-S-carrier protein intermediates for incorporating 5-chloro-6-methyl-*O*-methylsalicyclic acid into chlorothricin, *ChemBioChem 10*, 813-819.

(6) Weitnauer, G., Muhlenweg, A., Trefzer, A., Hoffmeister, D., Sussmuth, R. D., Jung, G., Welzel, K., Vente, A., Girreser, U., and Bechthold, A. (2001) Biosynthesis of the orthosomycin antibiotic avilamycin A: deductions from the molecular analysis of the avi biosynthetic gene cluster of *Streptomyces viridochromogenes* Tu57 and production of new antibiotics, *Chem. Biol. 8*, 569-581.

(7) Hosted, T. J., Wang, T. X., Alexander, D. C., and Horan, A. C. (2001) Characterization of the biosynthetic gene cluster for the oligosaccharide antibiotic, Evernimicin, in *Micromonospora carbonacea* var. *africana* ATCC39149, *J. Ind. Microbiol. Biotechnol. 27*, 386-392.

(8) Ahlert, J., Shepard, E., Lomovskaya, N., Zazopoulos, E., Staffa, A., Bachmann, B. O., Huang, K., Fonstein, L., Czisny, A., Whitwam, R. E., Farnet, C. M., and Thorson, J. S. (2002) The calicheamicin gene cluster and its iterative type I enediyne PKS, *Science 297*, 1173-1176.

(9) Daum, M., Peintner, I., Linnenbrink, A., Frerich, A., Weber, M., Paululat, T., and Bechthold, A. (2009) Organisation of the biosynthetic gene cluster and tailoring enzymes in the biosynthesis of the tetracyclic quinone glycoside antibiotic polyketomycin, *ChemBioChem 10*, 1073-1083.

(10) Xiao, Y., Li, S., Niu, S., Ma, L., Zhang, G., Zhang, H., Zhang, G., Ju, J., and Zhang, C. (2011) Characterization of tiacumicin B biosynthetic gene cluster affording diversified tiacumicin analogues and revealing a tailoring dihalogenase, *J. Am. Chem. Soc. 133*, 1092-1105.

(11) Rui, Z., Ye, M., Wang, S., Fujikawa, K., Akerele, B., Aung, M., Floss, H. G., Zhang, W., and Yu, T. W. (2012) Insights into a divergent phenazine biosynthetic pathway governed by a plasmid-born esmeraldin gene cluster, *Chem. Biol. 19*, 1116-1125.

(12) Ito, T., Roongsawang, N., Shirasaka, N., Lu, W., Flatt, P. M., Kasanah, N., Miranda, C., and Mahmud, T. (2009) Deciphering pactamycin biosynthesis and engineered production of new pactamycin analogues, *ChemBioChem 10*, 2253-2265.

(13) Abugrain, M. E., Lu, W., Li, Y., Serrill, J. D., Brumsted, C. J., Osborn, A. R., Alani, A., Ishmael, J. E., Kelly, J. X., and Mahmud, T. (2016) Interrogating the tailoring steps of pactamycin biosynthesis and accessing new pactamycin analogues, *ChemBioChem 17*, 1585-1588.

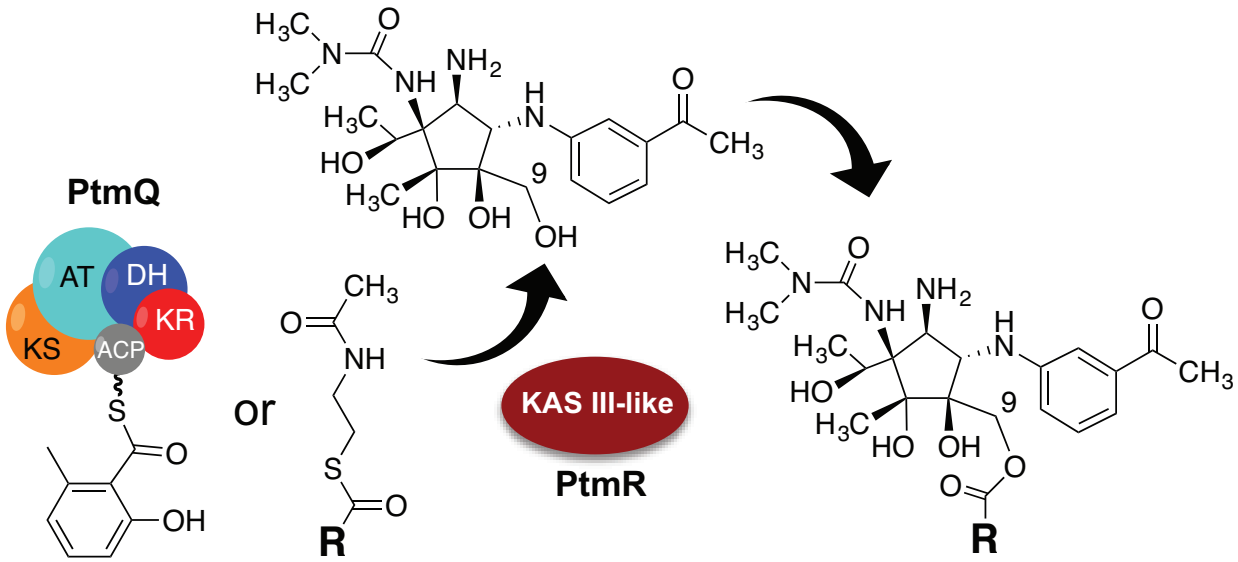
(14) Lu, W., Roongsawang, N., and Mahmud, T. (2011) Biosynthetic studies and genetic engineering of pactamycin analogs with improved selectivity toward malarial parasites, *Chem. Biol. 18*, 425-431.

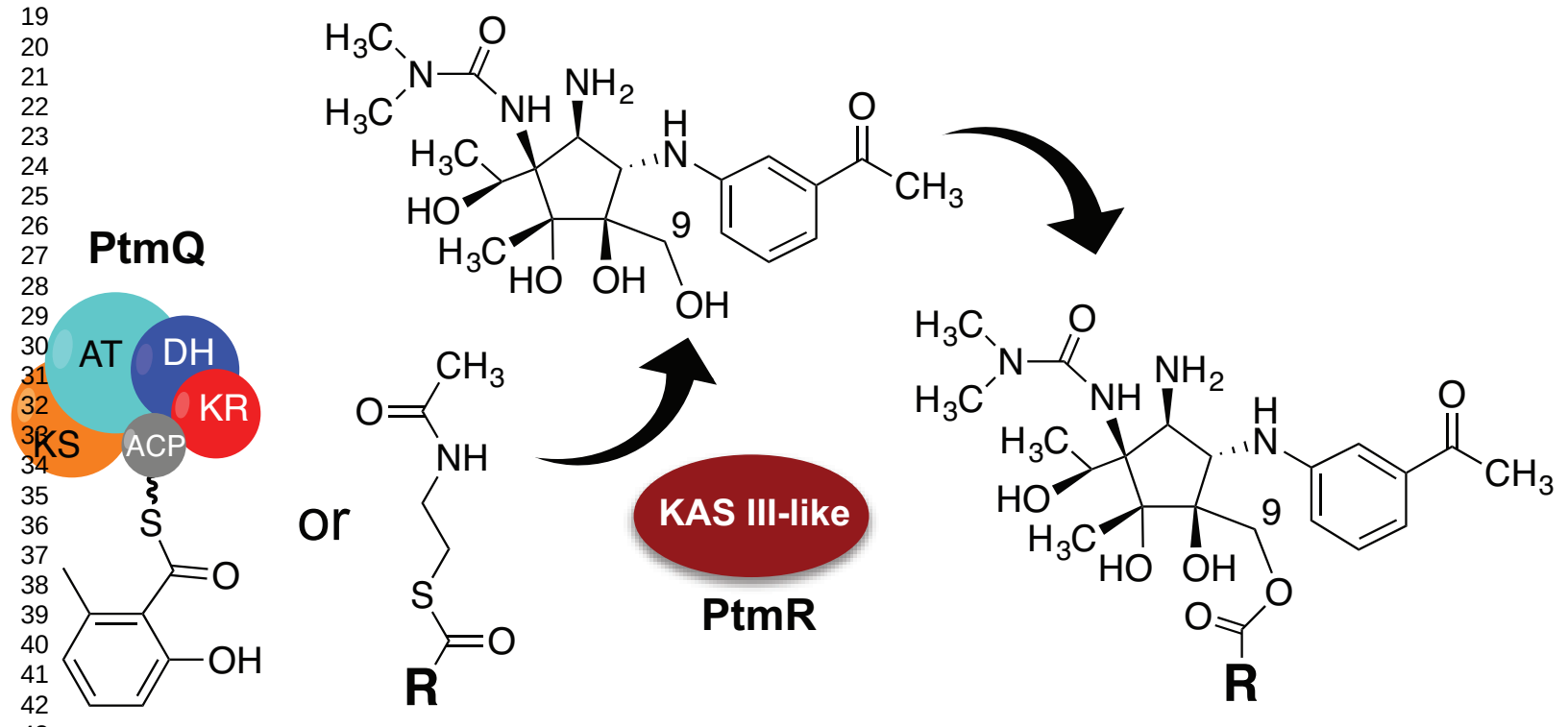
(15) Kudo, F., Kasama, Y., Hirayama, T., and Eguchi, T. (2007) Cloning of the pactamycin biosynthetic gene cluster and characterization of a crucial glycosyltransferase prior to a unique

cyclopentane ring formation, *J. Antibiot. 60*, 492-503.

(16) Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F., and Hopwood, D. A. (2000) "Practical *Streptomyces* Genetics", *The John Innes Foundation Norwich*, England.

Graphic for TOC




PtmQ
AT
DH
KR
KS
ACP
S
O
OH
or
CH3
O
NH
S
O
R
KAS III-like
PtmR
H3C
N
O
H3C
NH
NH2
H3C
HO
H3C
HO
OH
OH
9
H
N
O
CH3
R