Communication

Identification of the Formycin A Biosynthetic Gene Cluster from Streptomyces kaniharaensis Illustrates the Interplay between Biological Pyrazolopyrimidine Formation and de novo Purine Biosynthesis

Shao-An Wang, Yeonjin Ko, Jia Zeng, Yujie Geng, Daan Ren, Yasushi Ogasawara, Seema Irani, Yan Jessie Zhang, and Hung-wen Liu

J. Am. Chem. Soc., **Just Accepted Manuscript** • DOI: 10.1021/jacs.9b00241 • Publication Date (Web): 03 Apr 2019

Identification of the Formycin A Biosynthetic Gene Cluster from *Streptomyces kaniharaensis* Illustrates the Interplay between Biological Pyrazolopyrimidine Formation and *de novo* Purine Biosynthesis

Shao-An Wang,[†,‡] Yeonjin Ko,[†,‡] Jia Zeng,[±] Yujie Geng,[¶] Daan Ren,[†] Yasushi Ogasawara,[¶,§] Seema Irani,[±] Yan Zhang,[±] Hung-wen Liu*[,¶,†]

[†]Department of Chemistry, [±]Department of Molecular Biosciences, and [¶]Division of Chemical Biology and Medicinal Chemistry, College of Pharmacy, The University of Texas at Austin, Austin, TX, 78712

Supporting Information Placeholder

ABSTRACT: Formycin A is a potent purine nucleoside antibiotic with a *C*-glycosidic linkage between the ribosyl moiety and the pyrazolopyrimidine base. Herein, a cosmid is identified from the *Streptomyces kaniharaensis* genome library that contains the *for* gene cluster responsible for the biosynthesis of formycin. Subsequent gene deletion experiments and *in vitro* characterization of the *forBCH* gene products established their catalytic functions in formycin biosynthesis. Results also demonstrated that PurH from *de novo* purine biosynthesis plays a key role in pyrazolopyrimidine formation during biosynthesis of formycin A. The participation of PurH in both pathways represents a good example of how primary and secondary metabolism are interlinked.

Formycin A (**1**), also known as 8-aza-9-deazaadenosine, is a purine nucleoside antibiotic[1] isolated from *Nocardia interforma*,[2] *Streptomyces kaniharaensis* SF-557[3] and *Streptomyces* sp. MA406-A-1.[4] It is an isomer of adenosine (**2**); however, it is a *C*-nucleoside with a pyrazolopyrimidine instead of an imidazolopyrimidine nucleobase making it distinctly different from typical nucleosides.[5] Formycin A is a potent inhibitor of adenosine-utilizing enzymes such as bacterial purine nucleoside phosphorylase[6] and the adenosine kinase involved in the purine salvage pathway in *Mycobacterium tuberculosis*.[7] It is cytotoxic to *Leishmania* species due to the incorporation of the phosphorylated form of formycin A into RNA.[8] Formycin A also exhibits antiviral activity against influenza virus A1[9] and human immunodeficiency virus type 1.[10] Although the biological functions of formycin A have been well documented, little is known about how it is assembled in nature.[4,11]

To investigate the biosynthesis of formycin A, the genome of *S. kaniharaensis* was sequenced.[12] Preliminary analysis revealed the presence of two sets of *purA*, *purB*, *purC*, and *purH* genes. The *purA*, *purB*, *purC* and *purH* gene products are enzymes responsible for the conversion of carboxyaminoimidazole ribonucleotide (CAIR, **3a**) to adenosine 5'-phosphate (**9a**, see Figure 1) during the biosynthesis of purine nucleosides.[13] The discovery of two sets of *pur* homologous genes in the genome of *S. kaniharaensis* suggested that the second set of *pur*-like genes (i.e., the *for*-genes) (see Table S4) are likely involved in the biosynthesis of formycin A, since the pyrazolopyrimidine moiety of formycin A may be formed in a similar manner as the imidazolopyrimidine group in adenosine. This hypothesis was supported by the *in vitro* demonstration that the *purA*-like (*forA*) and *purB*-like (*forB*) gene products can catalyze the conversion of formycin B 5'-phosphate to formycin A 5'-phosphate (**7b**→**8b**→**9b**, Figure 1).[12] In the present work, the full *for* gene cluster for formycin A biosynthesis is identified from *S. kaniharaensis*. Moreover, investigation of the encoded enzymes indicates that the cluster alone is insufficient for the biosynthesis of formycin A and requires involvement of PurH from *de novo* purine biosynthesis.

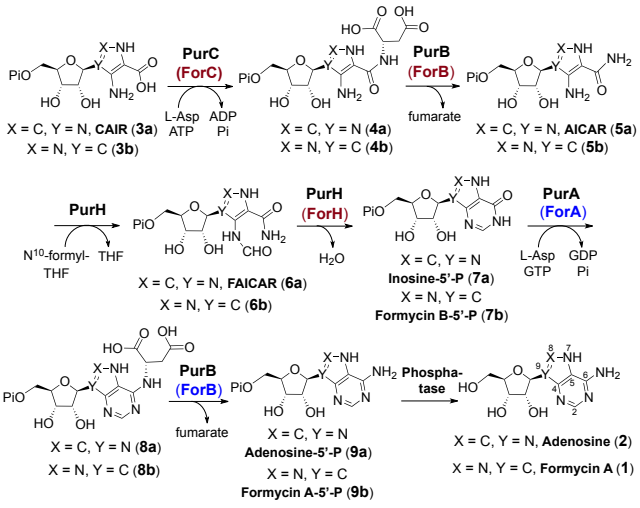

Figure 1. Later steps of the biosynthetic pathway for adenosine (**2**) and the proposed pathway for formycin A (**1**) formation.

To identify the formycin A gene cluster, a cosmid library from the genome of *S. kaniharaensis* was constructed and those cosmids harboring the *pur*-like genes were screened by PCR amplification with primers based on the sequences of the second set of *purA*-like, *purC*-like, and *purH*-like genes (i.e., *forA*, *forC* and *forH*). This effort led to the identification of a single cosmid, designated K24C, harboring the

forABCH genes. This cluster spans approximately 34 kbp with 29 open reading frames (ORFs) and is flanked by genes likely responsible for the biosynthesis of the adenosine deaminase inhibitor coformycin (**10**, see Figure 2 and Table S4). Co-localization and overlap of the formycin and coformycin clusters is not unexpected. The two species are known to be produced together,[3] and purine nucleoside antibiotics are in general produced synergistically with an adenosine deaminase inhibitor[14] to inhibit their deactivation.[15] To study the role of the *for* cluster in formycin A biosynthesis, the *forC*, *forH*, *forU*, *forF*, *forT* and *forL* genes of *S. kaniharaensis* SF-557 were selected for in-frame deletion. Except for the Δ*forC* and Δ*forL* mutants, formycin A production from all other gene deletion mutants was either abolished or significantly suppressed (Figure S1). These results are consistent with the *for* cluster being responsible for formycin A biosynthesis.

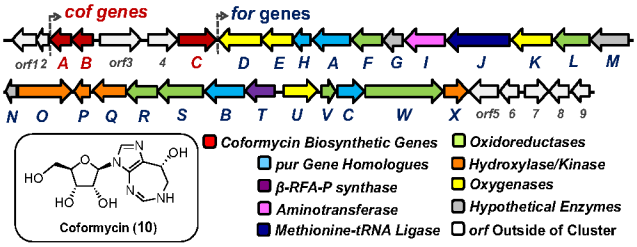

Figure 2. Structure of the formycin and coformycin biosynthetic gene cluster from cosmid K24C generated from S. kaniharaensis. ForT is homologous to β-ribofuranosyl-aminobenzene 5′-phosphate (β-RFA-P) synthase, which catalyzes the coupling between *p*-aminobenzoic acid and PRPP.[16]

Previous experiments showed that ForA and ForB could catalyze the conversion of formycin B 5'-phosphate (**7b**) to formycin A 5'-phosphate (**9b**), whereas the *de novo* purine biosynthetic enzymes PurA and PurB are unable to do so.[12] ForC is expected to operate together with ForB to catalyze the conversion of **3b** to **5b** in a similar fashion to the activity of PurC/PurB.[19] To examine this hypothesis, the putative substrate (**3b**) and product (**5b**) were synthesized from **11** and **12**, which had been prepared in an early work[12] (Figure 3).

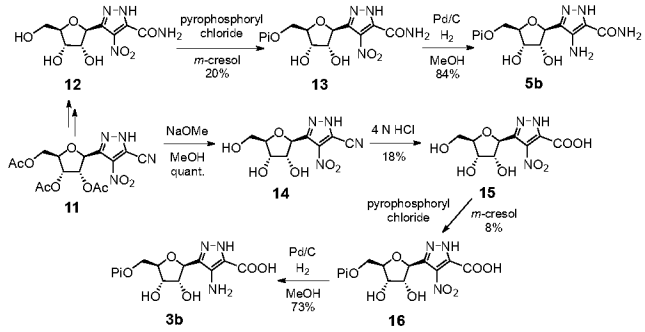

Figure 3. Synthesis of **3b** and **5b** standards. (see Supporting Information for details).

Incubation of **3b** with ForC along with L-aspartate and ATP led to the production of ADP and complete consumption of **3b** after 4 h (Figure 4, trace B). The expected product **4b** was not observable likely on account of its prolonged retention time on Dionex anion exchange HPLC. In contrast, incubation of **3b** with a ForC/ForB mixture (1:1 molar ratio) under the same conditions resulted in a new product peak eluting with a retention time of 7.8 min (Figure 4, trace C). This new product coeluted with a standard of **5b** prepared as shown in Figure 4 and had a molecular weight identical to that of **5b** (calculated for $C_9H_{14}O_8N_4P^-$ $[M-H]^-$: 337.0555 *m/z*, found: 337.0531 *m/z*). These results indicated that ForC indeed catalyzes amidation of **3b** with L-aspartate and ForB catalyzes the subsequent elimination of fumarate to produce **5b**. Interestingly, since the ForC/ForB reactions are analogous to those catalyzed by PurC/PurB during *de novo* purine biosynthesis, their functions can be substituted with PurC and PurB (Figure S3).

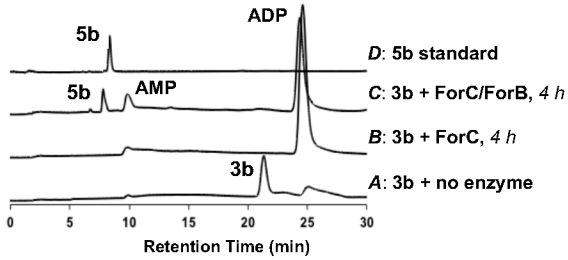

Figure 4. HPLC traces showing the reactions of ForC and ForB. Trace A: the reaction mixture of **3b**, L-aspartate, and ATP before addition of enzymes. Trace B: trace A mixture incubated with ForC for 4 h. Trace C: trace A mixture incubated with both ForC and ForB for 4 h. AMP was likely generated via non-enzymatic hydrolysis. Trace D: synthetic standard of **5b**.

Mirroring the PurH reaction (**5a** to **7a**), the amide product **5b** should then be processed by ForH via formylation followed by cyclization to yield **7b**.[20] However, the *forH* gene from *S. kaniharaensis* encodes a much shorter enzyme (196 aa) than PurH (529 aa in *E. coli* K12 and 502 aa in *S. kaniharaensis*). It is homologous only to the IMP cyclohydrolase domain of PurH. Due to the absence of other formyltransferase genes in the *for* cluster, it was hypothesized that the *de novo* purine biosynthetic enzyme PurH may serve a dual role to formylate not only its natural substrate **5a** but also **5b**.

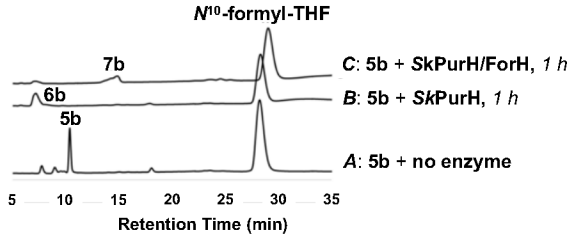

Figure 5. HPLC traces showing the reactions catalyzed by *Sk*PurH and ForH. Trace A: the reaction mixture of **5b** and N^{10}-formyl-THF before the addition of enzymes. Trace B: trace A mixture incubated with *Sk*PurH for 1 h. Trace C: trace A mixture incubated with both *Sk*PurH and ForH for 1 h.

As predicted, incubation of **5b**, N^{10}-formyltetrahydrofolate (N^{10}-formyl-THF) and ForH did not yield any detectable product (Figure S4). In contrast, incubation of **5b** with *S. kaniharaensis* PurH (*Sk*PurH) resulted in formylation of the pyrazolic amide to **6b** (see Figure 1) without subsequent cyclization to **7b** (Figure 5, trace B). The standard of **6b** was enzymatically prepared from **5b** using *E. coli* PurH as the catalyst (see Supporting Information S2.4). The fact that *Sk*PurH can convert **5b** to **6b** but not **6b** to **7b** demonstrates that the

enzyme is promiscuous with respect to its formylase activity but not its cyclase activity. PurH from *E. coli* K12 (*Ec*PurH), displays the same selectivity toward **6a** and **6b**, as **6a** could be processed to **7a**, while **6b** could not be converted to **7b** (Figure S5). However, when *Sk*PurH and ForH were incubated together with **5b**, complete conversion of **5b** to **7b** was observed based on both HPLC (Figure 5, trace C) and mass analyses. These results support the hypothesis that ForH functions as a cyclohydrolase catalyzing the cyclization of **6b** to **7b** during pyrazolopyrimidine formation. Interestingly, unlike PurH which only catalyzes cyclization of **6a**, ForH demonstrates cyclase activity towards both **6a** and **6b** (Figure 6) with a 1.7-2 fold greater preference for the latter based on substrate competition assays (Figure S6B).

The fact that ForH can catalyze the cyclization of both **6a** and **6b** prompted us to study the structural basis of the observed substrate promiscuity. The crystal structure of ForH from *S. kaniharaensis* was solved by molecular replacement using the cyclohydrolase domain of *Gallus gallus* PurH (*Gg*PurH, residues 4–199 from structure 1M9N in the protein data bank) as the search model.[21] The structure of ForH alone (2.4 Å resolution, Table S5), is characteristic of a classic Rossman fold containing a central stretch of four β-strands surrounded by α-helices (Figures S7 and S8).

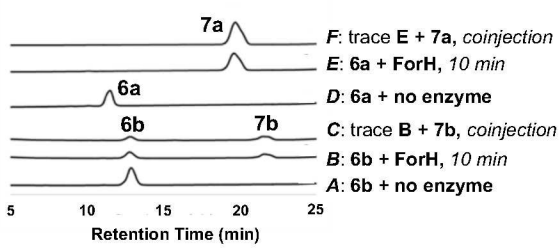

Figure 6. ForH reaction with **6a** and **6b**. Trace A: reaction mixture containing **6b** before addition of enzyme. Trace B: trace A mixture incubated with ForH for 10 min. Trace C: trace B mixture co-injected with **7b** standard. Trace D: reaction mixture containing **6a** before addition of enzyme. Trace E: trace D mixture incubated with ForH for 10 min. Trace F: trace E mixture coinjected with **7a**.

The overall fold of ForH and the cyclohydrolase domain of *Gg*PurH (38% sequence identity) are highly similar with a RMSD of 1.39 Å for C_α residues (Figure S9). Although the thin-plate morphology of the crystals made the effective incorporation of ligand in the active site challenging, the high conservation of the active site residues between PurH and ForH suggests a similar binding mode of nucleotide ligand (XMP, xanthosine-5'-monophosphate) between the homologues (Figure S10). The putative active site of ForH was identified by overlaying the ForH structure with that of *Gg*PurH bound with XMP (Figure 7A). The residues interacting with XMP in PurH are almost all conserved in both identity and backbone position in ForH (Figures 7A, S9, S10). However, there is structural variation between the two binding pockets adjacent to where the ribose moiety of **6a**/**6b** is predicted to bind (Figures 7B and 7C). Specifically, Ser11 in PurH is in close proximity to the nucleotide ring for potential hydrogen bond formation. However, this residue is replaced by Ala5 in ForH. Furthermore, substitution of Ser35 in PurH by Thr29 in ForH also increases the hydrophobicity of the binding pocket (Figure 7D). The more hydrophobic nature of this region in ForH may account of the reduced substrate specificity of ForH versus PurH.

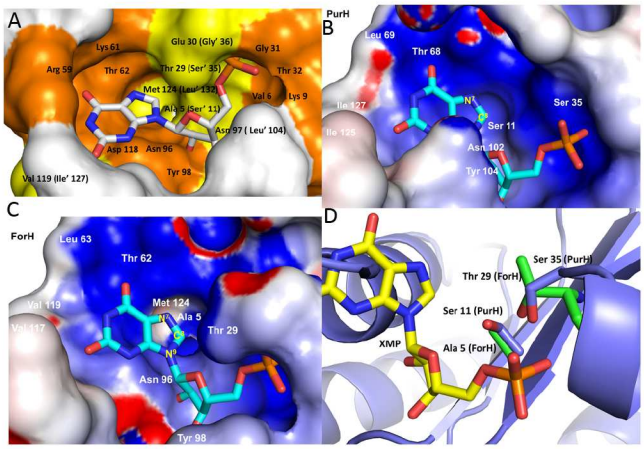

Figure 7. Structural comparison of ForH to the cyclohydrolase domain of *Gg*PurH. (A) Surface representation of the active site of ForH with conserved residues shown in orange and non-conserved residues shown in yellow. Residues are numbered according to the ForH sequence, and residues not conserved with PurH are denoted with a prime in parentheses. XMP (sticks) was modeled in the ForH active site via overlay with a previously solved PurH•XMP complex structure (pdb code:1M9N). (B-C) Electrostatic surface representation of the active site of PurH (B) and ForH (C) calculated using APBS, scaled from -10 (red for negative charge) to 10 (blue for positive charge) with white representing neutral. A surface loop (residues 62-67 (PurH) and 56-61 (ForH)) has been removed for visualization of the active site. (D) Superposition of PurH with XMP (sticks) and ForH active site.

In summary, the gene cluster responsible for the biosynthesis of formycin A in *S. kaniharaensis* has been identified. This represents only the second reported identification of a gene cluster for C-nucleoside biosynthesis.[22] Interestingly, the *for* gene cluster appears to be incomplete and lacks a complete formylase-cyclohydrolase. In fact, ForH only exhibits cyclohydrolase activity with reduced substrate specificity. However, its counterpart from *de novo* purine biosynthesis, PurH, is able to compensate for the missing formylase activity but not the cyclohydrolase activity. The involvement of PurH in *de novo* purine biosynthesis as well as its participation in the construction of pyrazolopyrimidine nucleosides illustrates just how deeply primary and secondary metabolic pathways are intertwined. While this work establishes the later steps in the biosynthesis of formycin A, the early steps involving formation of the pyrazole ring and the C-glycosidic linkage remain open to speculation based on gene sequence information and the pathway intermediates identified herein. Of particular note is a recent report by Matsuda *et al.*[18] that suggests ForJ, ForK and ForL could constitute a hydrazine forming system for introduction of the pyrazole ring (see Scheme 1 in Supporting Information). Consequently, this work lays the foundation for the future investigation of formycin A biosynthesis as a structurally unique *C*-nucleoside. Unraveling the hidden workings underlying the assembly of C-nucleoside antibiotics, an area that remains underexplored, could also facilitate metagenomic mining efforts and offer new avenues for engineering pathways to produce next-generation antibiotics with improved biomedical properties.

ASSOCIATED CONTENT

Supporting Information

Atomic coordinates and structure factors for the reported crystal structure in this work have been deposited to the Protein Data Bank (PDB) under accession number 6NKO. The Supporting Information is available free of charge on the ACS Publications website at DOI: xxxxx

Experimental details including synthesis of compounds, genomic analysis, gene deletion, complementation, and expression, protein isolation, and analytic methodologies (PDF).

AUTHOR INFORMATION

Corresponding Author

* h.w.liu@mail.utexas.edu.

ORCID

Hung-wen Liu: 0000-0001-8953-4794

Present Addresses

§Laboratory of Applied Biochemistry, Graduate School of Engineering, Hokkaido University, Sapporo, Hokkaido 060-8628, Japan.

Author Contributions

‡These authors contributed equally.

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENT

This work was supported by grants from the National Institutes of Health (GM035906 to H.-w.L. and GM104896 to Y.J.Z.) and the Welch Foundation (F-1511 to H.-w.L. and F1778 to Y.J.Z.).

ABBREVIATIONS

SAICAR, succino-AICAR; $NADP^+$, nicotinamide adenine dinucleotide phosphate; ATP, adenosine 5'-triphosphate; ADP, adenosine 5'-diphosphate; AMP, adenosine 5'-monophosphate; IMP, inosine 5'-monophsophate; *E. coli*, *Escherichia coli*; XMP, xanthosine 5'-monophosphate; N^{10}-formyl-THF, N^{10}-formyltetrahydrofolate.

REFERENCES

(1)(a) Ishizuka, M.; Takeuchi, T.; Nitta, K.; Koyama, G.; Hori, M.; Umezawa, H. Antitumor Activities of Formycin and Labilomycin. *J. Antibiot., Ser. A.* **1964**, *17*, 124-126. (b) Ishizuka, M.; Sawa, T.; Hori, S.; Takayama, H.; Takeuchi, T.; Umezawa, H. Biological Studies on Formycin and Formycin B. *J. Antibiot.* **1968**, *21*, 5-12

(2) Hori, M.; Ito, E.; Takita, T.; Koyama, G.; Takeuchi, T.; Umezawa H. A New Antibiotic, Formycin. *J. Antibiotics. Ser. A.* **1964**, *17*, 96-99.

(3) R. J. Suhadolnik "Nucleoside Antibiotics," Wiley-Interscience, New York, N. Y., 1970

(4) Ochi, K.; Iwamoto, S.; Hayase, E.; Yashima, S.; Okami, Y. Biosyntheis of Formycin: Role of Certain Amino Acids in Formycin Biosynthesis. *J. Antibiot.* **1974**, *27*, 909-916.

(5) Koyama, G.; Maeda, K.; Umezawa, H.; Iitaka, Y. The Structural Studies of Formycin and Formycin B. *Tetrahedron Lett.* **1966**, *7*, 597-602.

(6)(a) Bzowska, A.; Kulikowska, E.; Shugar, D. Formycins A and B and Some Analogues: Selective Inhibitors of Bacterial (*Escherichia coli*) Purine Nucleoside Phosphorylase. *Biochim. Biophys. Act.* **1992**, *1120*, 239-247. (b) Kierdaszuk, B.; Modrak-Wójcik, A.; Wierzchowski, J.; Shugar, D. Formycin A and its *N*-methyl Analogues, Specific Inhibitors of *E. coli* Purine Nucleoside Phosphorylase (PNP): Induced Tautomeric Shifts on Binding to Enzyme, and Enzyme→ligand Fluorescence Resonance Energy Transfer. *Biochim. Biophys. Act.* **2000**, *1476*, 109-128.

(7) Long, M. C.; Parker, W. B. Structure–activity Relationship for Nucleoside Analogs as Inhibitors or Substrates of Adenosine Kinase from *Mycobacterium tuberculosis*. I. Modifications to the Adenine Moiety. *Biochem. Pharmacol.* **2006**, *71*, 1671-1682.

(8)(a) Carson, D. A.; Chang, K.-P. Phosphorylation and Anti-leishmanial Activity of Formycin B. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 1377-1383. (b) Nelson, D. J.; Lafon, S. W.; Jones, T. E.; Spector, T.; Berens, R. L.; Marr, J. J. The Metabolism of Formycin B in *Leishmania donovani*. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 349-354. (c) Rainey, P.; Santi, D. V. Metabolism and Mechanism of Action of Formycin B in *Leishmania*. *Proc. Natl. Acad. Sci. U. S. A.* **1983**, *80*, 288-292.

(9) Takeuchi, T.; Iwanaga, J.; Aoyagi, T.; Umezawa, H. Antiviral Effect of Formycin and Formycin B. *J. Antibiot., Ser. A* **1966**, *19*, 286-287.

(10) Dapp, M. J.; Bonnac, L.; Patterson, S. E.; Mansky, L. M. Discovery of Novel Ribonucleoside Analogs with Activity against Human Immunodeficiency Virus Type 1. *J. Virol.* **2014**, *88*, 354-363.

(11)(a) Kunimoto, T.; Sawa, T.; Wakashiro, T.; Hori, M.; Umezawa, H. Biosynthesis of The Formycin Family. *J. Antibiot.* **1971**, *24*, 253-258. (b) Ochi, K.; Yashima, S.; Eguchi, Y. Biosynthesis of Formycin: Formation of Formycin from Formycin B. *J. Antibiot.* **1975**, *28*, 965-973. (c) Ochi, K.; Kikuchi, S.; Yashima, S.; Eguchi, Y. Biosynthesis of Formycin: Incorporation and Distribution of Labeled Compounds into Formycin. *J. Antibiot.* **1976**, *29*, 638-645. (d) Ochi, K.; Yashima, S.; Eguchi, Y. Matsushita, K. Biosynthesis of Formycin: Incorporation and Distribution of ^{13}C-, ^{14}C-, and ^{15}N-Labeled Compounds into Formycin. *J. Biol. Chem.* **1979**, *254*, 8819-8824. (e) Buchanan, J. G.; Hamblin, M. R. Sood, G. R.; Wightman, R. H. The Biosynthesis of Pyrazofurin and Formycin. *J. Chem. Soc. Chem. Commun.* **1980**, 917-918.

(12) Ko, Y.; Wang, S.-A.; Ogasawara, Y.; Ruszczycky, M. W.; Liu, H.-w. Identification and Characterization of Enzymes Catalyzing Pyrazolopyrimidine Formation in the Biosynthesis of Formycin A. *Org. Lett.* **2017**, *19*, 1426-1429.

(13)(a) Zalkin, H.; Dixon, J. E. *De Novo* Purine Nucleotide Biosynthesis. *Prog. Nucleic Acid Res. Mol. Biol.* **1992**, *42*, 259–287. (b) Zhang, Y.; Morar, M.; Ealick, S. E. Structural Biology of the Purine Biosynthetic Pathway. *Cell. Mol. Life Sci.* **2008**, *65*, 3699–3724.

(14)(a) Hanvey, J. C.; Hardman, J. K.; Suhadolnik, R. J.; Baker, D. C. Evidence for the Conversion of Adenosine to 2′-Deoxycoformycin by *Streptomyces antibioticus*. *Biochemistry*, **1984**, *23*, 904-907. (b) Hanvey, J. C.; Hawkins, E. S.; Tunac, J. B.; Dechter, J. J.; Baker, D. C.; Suhadolnik, R. J. Biosynthesis of 2′-Deoxycoformycin: Evidence for Ring Expansion of the Adenine Moiety of Adenosine to a Tetrahydroimidazo[4,5-*d*][1,3]diazepine System. *Biochemistry*, **1987**, *26*, 5636-5641. (c) Hanley, J. C.; Hawkins, E. S.; Baker, D. C.; Suhadolnik, R. J. 8-Ketodeoxycoformycin and 8-Ketocoformycin as Intermediates in the Biosynthesis of 2′-Deoxycoformycin and Coformycin. *Biochemistry*, **1988**, *27*, 5790-5795. (d) Wu, P.; Wan, D.; Xu, G.; Wang, G.; Ma, H.; Wang, T.; Gao, Y.; Qi, J.; Chen, X.; Zhu, J.; Li, Y.-Q.; Deng, Z.; Chen, W. An Unusual Protector-Protégé Strategy for the Biosynthesis of Purine Nucleoside Antibiotics. *Cell Chem. Biol.* **2017**, *24*, 171-181. (e) Xia, Y.; Luo, F.; Shang, Y.; Chen, P.; Lu, Y.; Wang, C. Fungal Cordycepin Biosynthesis Is Coupled with the Production of the Safeguard Molecule Pentostatin. *Cell Chem. Biol.* **2017**, *24*, 1479-1489. (f) Gao, Y.; Xu, G.; Wu, P.; Liu, J.; Cai, Y.-s.; Deng, Z.; Chen, W. Biosynthesis of 2′-Chloropentostatin and 2′-Amino-2′-Deoxyadenosine Highlights a Single Gene Cluster Responsible for Two Independent Pathways in *Actinomadura* sp. Strain ATCC 39365. *Appl. Environ. Microbiol.* **2017**, *83*, e00078-17.

(15)(a) Sawa, T.; Fukagawa, Y.; Homma, I.; Takeuchi, T.; Umezawa, H. Mode of Inhibition of Coformycin on Adenosine Deaminase. *J. Antibiot., Ser. A* **1967**, *20*, 227-231. (b) Woo, P. W. K.; Dion, H. W.; Lange, S. M.; Dahl, L. F.; Durham, L. J. A Novel Adenosine and Ara-A Deaminase Inhibitor, (*R*)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol. *J. Heterocycl. Chem.* **1974**, *11*, 641-643. (c) Kline, P. C.; Schramm, V. L. Electrostatic Potential Surfaces of the Transition State for AMP Deaminase and for (*R*)-Coformycin, a Transition State Inhibitor. *J. Biol. Chem.* **1994**, *269*, 22385-22390. (d) Marrone, T. J.; Straatsma, T. P.; Briggs, J. M.; Wilson, D. K.; Quiocho, F. A.; McCammon, J. A. Theoretical Study of Inhibition of Adenosine Deaminase by (*8R*)-Coformycin and (*8R*)-Deoxycoformycin. *J. Med. Chem.* **1996**, *39*, 277-284. (e) Castro, C.; Britt, B. M. Evidence for a Low Temperature Transition State Binding Preference in Bovine Adenosine Deaminase. *Biophys. Chem.* **1998**, *70*, 87-92. (f) Castro, C.; Britt, B. M. Binding Thermodynamics of the Transition State Analogue Coformycin and of the Ground State Analogue 1-Deazaadenosine to Bovine Adenosine Deaminase. *J. Enzyme Inhibition* **2001**, *16*, 217-232.

(16)(a) Dumitru, R. V.; Ragsdale, S. W. Mechanism of 4-(β-D-Ribofuranosyl)aminobenzene 5′-Phosphate Synthase, a Key Enzyme in the Methanopterin Biosynthetic Pathway. *J. Biol. Chem.* **2004**, *279*, 39389–39395. (b) White, R. H. The Conversion of a Phenol to an Aniline Occurs in the Biochemical Formation of the 1-(4-Aminophenyl)-1-deoxy-D-ribitol Moiety in Methanopterin. *Biochemistry* **2011**, *50*, 6041–6052.

(17) Aravind, L.; de Souza, R. F.; Iyer, L. M. Predicted Class-I Aminoacyl tRNA Synthetase-like Proteins in Non-ribosomal Peptide Synthesis. *Biol. Direct* **2010**, *5*, 48.

(18) Matsuda, K.; Tomita, T.; Shin-ya, K.; Wakimoto, T.; Kuzuyama, T.; Nishiyama, M. Discovery of Unprecedented Hydrazine-Forming Machinery in Bacteria. *J. Am. Chem. Soc.* **2018**, *140*, 9083-9086.

(19) Meyer, E.; Leonard, N. J.; Bhat, B.; Stubbe, J.; Smith, J. M. Purification and Characterization of the *purE*, *purK*, and *purC* Gene Products: Identification of a Previously Unrecognized Energy Requirement in the Purine Biosynthetic Pathway. *Biochemistry*, **1992**, *31*, 5022-5032.

(20) Rayl E. A.; Moroson, B. A.; Beardsley, G. P. The Human *purH* Gene Product, 5-Aminoimidazole-4-carboxamide Ribonucleotide Formyltransferase/IMP Cyclohydrolase. Cloning, Sequencing, Expression, Purification, Kinetic Analysis, and Domain Mapping. *J. Biol. Chem.* **1996**, *271*, 2225–2233.

(21) Wolan, D. W.; Greasley, S.E.; Beardsley, G. P.; Wilson, I. A. Structural Insights into the Avian AICAR Transformylase Mechanism. *Biochemistry*, **2002**, *41*, 15505-15513.

(22) Palmu, K.; Rosenqvist, P.; Thapa, K.; Ilina, Y.; Siitonen, V.; Baral, B.; Mäkinen, J.; Belogurov, G.; Virta, P.; Niemi, J.; Metsä-Ketelä, M. Discovery of the Showdomycin Gene Cluster from *Streptomyces showdoensis* ATCC 15227 Yields Insight into the Biosynthetic Logic of C-Nucleoside Antibiotics. *ACS Chem. Biol.* **2017**, *12*, 1472-1477.

Graphic entry for the Table of Contents (TOC)