

# Enzymatic Basis of “Hybridity” in Thiomarinol Biosynthesis\*\*

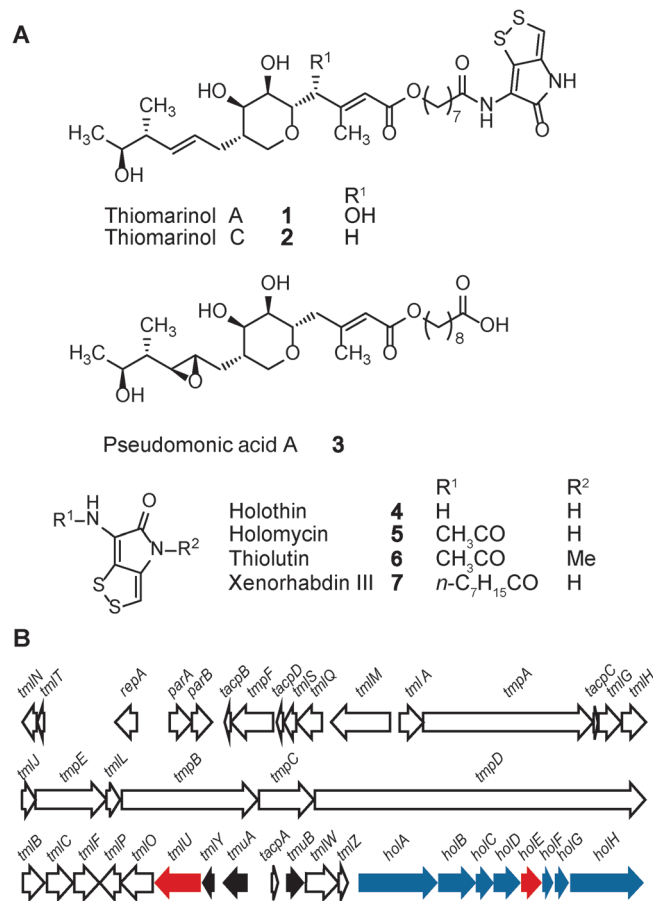
Zachary D. Dunn, Walter J. Wever, Nicoleta J. Economou, Albert A. Bowers, and Bo Li\*

**Abstract:** Thiomarinol is a naturally occurring double-headed antibiotic that is highly potent against methicillin-resistant *Staphylococcus aureus*. Its structure comprises two antimicrobial subcomponents, pseudomonic acid analogue and holothin, linked by an amide bond. TmlU was thought to be the sole enzyme responsible for this amide-bond formation. In contrast to this idea, we show that TmlU acts as a CoA ligase that activates pseudomonic acid as a thioester that is processed by the acetyltransferase HolE to catalyze the amidation. TmlU prefers complex acyl acids as substrates, whereas HolE is relatively promiscuous, accepting a range of acyl-CoA and amine substrates. Our results provide detailed biochemical information on thiomarinol biosynthesis, and evolutionary insight regarding how the pseudomonic acid and holothin pathways converge to generate this potent hybrid antibiotic. This work also demonstrates the potential of TmlU/HolE enzymes as engineering tools to generate new “hybrid” molecules.

The rapid rise of antibiotic resistance creates an urgent need for new antibiotics. One strategy to meet the diminishing returns on traditional antibiotics is to covalently link combinations of existing antibiotics to produce novel hybrids. These hybrid antibiotics exhibit enhanced bioactivity and pharmacology compared to the parent compounds. This strategy has a synergistic effect because it improves activity against drug-resistant bacteria, expands the spectrum of the individual compounds, and reduces the potential for new resistance.<sup>[1]</sup> A drawback of synthetic hybrid antibiotics is that the partner compound or the covalent linker may hinder target binding. Furthermore, the effective concentration of both species may be reduced if each compound targets disparate cellular sites.<sup>[2]</sup> Naturally occurring hybrid antibiotics, such as the marine

natural product thiomarinols (**1** and **2**) from *Pseudoalteromonas* spp. SANK73390,<sup>[3]</sup> have already been honed by nature for selectivity and biological activity. Thus, these natural hybrids can provide valuable insight into useful therapeutic combinations and linker strategies.

Thiomarinol combines the monic acid warhead of the FDA-approved agent mupirocin (pseudomonic acids, e.g. **3**, Bactroban—GlaxoSmithKline)<sup>[4]</sup> and the compact holothin (**4**) core of the dithiopyrrolones (DTPs), such as holomycin (**5**)<sup>[5]</sup> and thiolutin (**6**; Figure 1A).<sup>[6]</sup> These fragments, which in thiomarinol are linked by a fatty acyl amide bridge, exhibit broadly different antibiotic activities: mupirocin exhibits high specificity for the bacterial isoleucyl-tRNA synthetase and subsequent inhibition of protein synthesis,<sup>[7]</sup> whereas the



**Figure 1.** A) Structures of thiomarinols, pseudomonic acids, and dithiopyrrolones. B) Gene cluster for thiomarinol. Open arrows indicate ORFs with homology to the mupirocin pathway; blue ORFs are homologous to DTP biosynthetic genes; black ORFs are unique to the thiomarinol pathway; red ORFs, TmlU and HolE, the targets of this study, have counterparts in the mupirocin and holomycin pathway, respectively. They are represented as red arrows instead of open and blue arrows for clarity.

[\*] Z. D. Dunn, Prof. B. Li  
Department of Chemistry  
University of North Carolina at Chapel Hill  
Carolina Center for Genome Sciences  
Chapel Hill, NC, 27599 (USA)  
E-mail: boli@email.unc.edu

W. J. Wever, Dr. N. J. Economou, Prof. A. A. Bowers  
Division of Chemical Biology and Medicinal Chemistry  
Eshelman School of Pharmacy  
University of North Carolina at Chapel Hill  
Chapel Hill, NC, 27599 (USA)

[\*\*] We acknowledge Dr. Wilfred van der Donk and Dr. Christopher T. Walsh for helpful discussions. We thank Dr. Gary Pielak, Andrew Chan, Kevin Santa Maria, and Dr. Christopher Neumann for careful reading of the manuscript. Financial support was provided by the University of North Carolina at Chapel Hill and NIH grant 5R00GM099904-03 (B.L.).

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201411667>.

proposed holomycin mechanism of action involves inhibition of bacterial transcription.<sup>[8]</sup> Importantly, despite these differences, the hybrid is more potent than its constituents, with enhanced activity against many drug-resistant pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA).<sup>[3a]</sup>

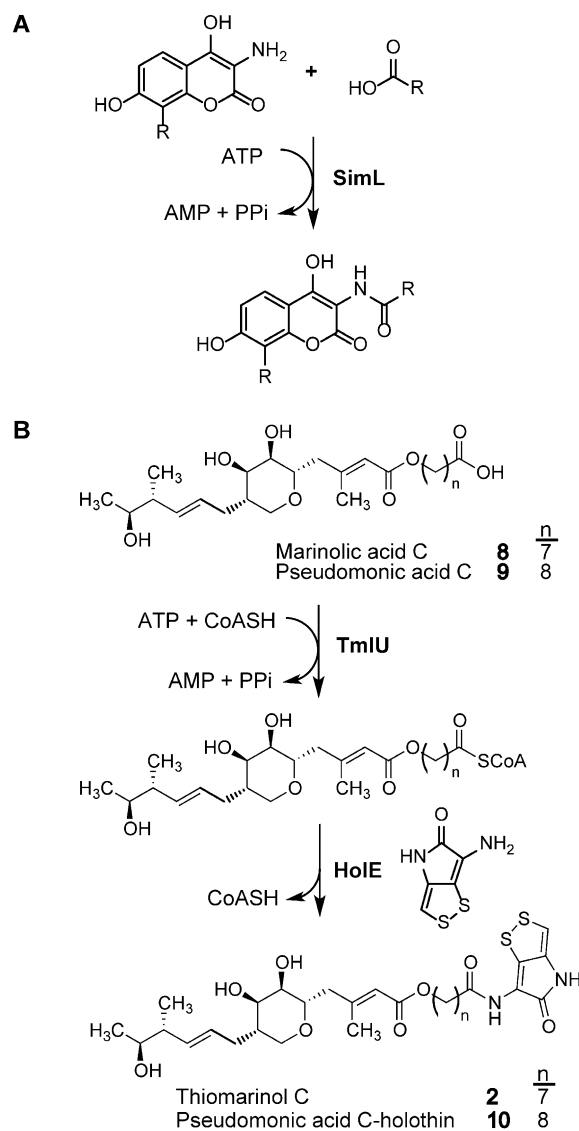
Thiomarinol's hybrid structure generates at least two thought-provoking questions. How did nature come to couple these two distinct moieties? What are the mechanistic benefits of combining these seemingly unrelated antibiotic motifs? Here, we begin to answer the first question by detailed characterization (and functional reassignment) of the *tmlU* and *holE* gene products.

The thiomarinol gene cluster was discovered by whole genome sequencing of *Pseudoalteromonas* spp. SANK73390.<sup>[9]</sup> The genes are present on a 97 kb plasmid comprising a polyketide synthase (PKS) portion and a non-ribosomal peptide synthetase (NRPS) portion, which are responsible for synthesizing the marinolic acid and holothin segments, respectively (Figure 1 B). Subsequent genetic deletions by Simpson and co-workers identified TmlU as a key enzyme responsible for coupling the holothin and marinolic acid moieties.<sup>[9,10]</sup> TmlU was initially assigned as an amide ligase on the basis of its significant sequence identity with the amide ligases NovL (20.7%), CouL (21.1%), CloL (19.7%), and SimL (18.5%), which catalyze amide bond formation in the biosynthesis of the aminocoumarin antibiotics novobiocin, coumermycin, chlorobiocin, and simocyclinone, respectively (Scheme 1 A, see Figure S1 in the Supporting Information).<sup>[11]</sup>

We tested whether TmlU could similarly act as a stand-alone amide ligase by generating recombinant TmlU in *E. coli*. The proposed amine donor for TmlU-mediated amide coupling, holothin, could be readily accessed by total synthesis in five steps with 13% overall yield.<sup>[13]</sup> The C<sub>7</sub> fatty acyl monic acid, marinolic acid (**8**), was not readily available. The C<sub>8</sub> fatty acyl monic acid, pseudomonic acid A (PAA), which is commercially available (Sigma), was used instead. The epoxy group in PAA was reduced in three steps to give pseudomonic acid C (PAC, **9**) with a C-10,11 *trans*-olefin, similar to marinolic acid.

Despite investigating a large number of conditions, TmlU failed to yield the anticipated product with the substrates holothin and either PAA or PAC. This observation led us to reexamine the assignment of TmlU. A Phyre2 homology model (Figure S2) indicated close structural similarity to the SrfA-C termination module of the nonribosomal peptide synthetase responsible for surfactin biosynthesis.<sup>[14]</sup> Moreover, we found that TmlU has 14% sequence identity to MupU, a putative acyl-CoA ligase in the mupirocin biosynthetic pathway (Figures S1 and S3). As a result, we wondered whether TmlU might activate marinolic acid by linking to CoA or an acyl carrier protein, which could then be transferred onto the acceptor holothin through tandem action of another enzyme from the cluster, and speculated that the putative acyltransferase HolE might play this auxiliary role.

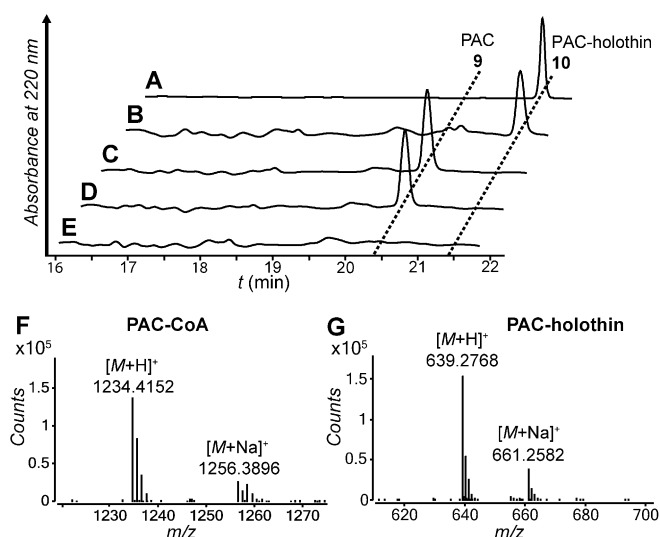
HolE is a homologue of HlmA, which is present in the holomycin pathway from *Streptomyces clavuligerus*, catalyz-



**Scheme 1.** A) Reported mechanism of amide formation in the biosynthesis of simocyclinone catalyzed by SimL.<sup>[12]</sup> B) Our proposed mechanism of thiomarinol formation catalyzed by TmlU/HolE. ATP = adenosine triphosphate, AMP = adenosine monophosphate, PPi = inorganic pyrophosphate, CoASH = coenzyme A.

ing the terminal acylation of holothin with acetyl-CoA.<sup>[15]</sup> It was hypothesized that HolE was solely responsible for the “background” acylation observed in isolates from *Pseudoalteromonas* spp. SANK73390, which gives rise to a series of short-chain xenorhabdin-like molecules.<sup>[16]</sup>

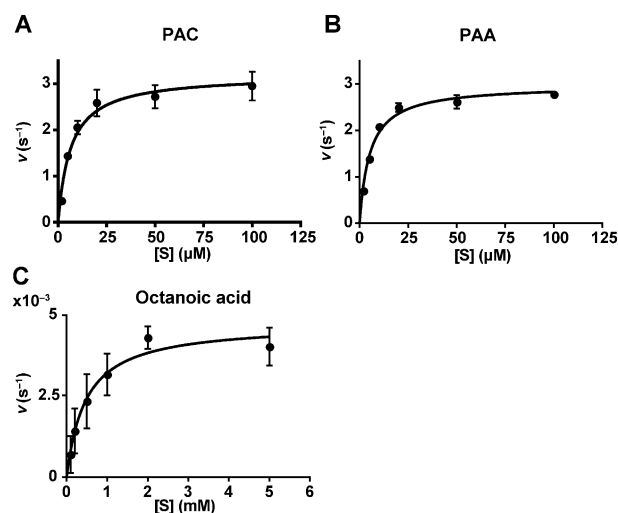
HolE was expressed in *E. coli* and purified to homogeneity. For *in vitro* reconstitution of enzyme activities, we treated PAC and holothin with 1  $\mu$ M purified TmlU and 1  $\mu$ M HolE in the presence of CoASH, MgCl<sub>2</sub>, and ATP. We detected a significant peak for the pseudomonic acid C-holothin (PAC-holothin, **10**) conjugate, which displayed the same molecular weight and retention time as a synthetic standard (Figure 2 A, B, and G; see the Supporting Information for synthesis). Omitting CoA abolished PAC-holothin production, which suggests that CoA is necessary for efficient conversion (Figure 2 C). Assays carried out in the absence of



**Figure 2.** Enzymatic production of PAC-holothin, a thiomarinol analogue, in vitro by TmlU and HolE, in the presence of 1 mM ATP, 2 mM  $MgCl_2$ , and 1 mM CoASH at pH 7.5. A) Synthetic PAC-holothin standard, B) in vitro reconstitution of TmlU and HolE activity generating PAC-holothin, C) control lacking CoA, D) control lacking TmlU, E) control lacking HolE, F) mass spectrum of PAC-CoA generated by TmlU (calculated  $[M+H]^+$ , 1234.4155), G) mass spectrum of PAC-holothin generated enzymatically by TmlU and HolE (calculated  $[M+H]^+$ , 639.2768).

TmlU or ATP failed to yield the expected product (Figure 2D and Figure S6). Furthermore, when HolE was omitted, the substrate PAC was consumed, but the final PAC-holothin product was not observed (Figure 2E). Instead, a pseudomonic acid C CoA (PAC-CoA) adduct accumulated (Figure 2F and Figures S7 and S8). These results demonstrate that TmlU is an acyl-CoA ligase and that HolE catalyzes the subsequent acyl-transfer step required for thiomarinol biosynthesis. With this understanding, we assessed the kinetics and promiscuity of this two-step enzymatic process.

Kinetic parameters for TmlU were measured using saturating concentrations of the cosubstrates CoA and ATP with PAC as a substrate. The formation of the PAC-CoA product was measured by a coupled assay with saturating concentrations of HolE and 3-aminocoumarin. 3-Aminocoumarin was used instead of holothin, because we found it to be well accepted by HolE and more stable than holothin. Under these conditions, TmlU displays a  $K_M$  value of  $(6 \pm 1) \mu M$  for PAC and a  $k_{cat}$  value of  $(3.2 \pm 0.1) s^{-1}$  (Figure 3A). These parameters are consistent with those reported for other acyl-CoA ligases, such as 4-chlorobenzoate-CoA ligase.<sup>[17]</sup> Interestingly, the use of PAA as substrate yielded similar values to PAC (Figure 3B), thus suggesting that the presence of the epoxy group does not affect the TmlU activity. Thus, the lack of the epoxide moiety in thiomarinols is likely due to the absence of epoxide-forming enzymes in thiomarinol biosynthesis rather than the substrate selectivity of TmlU or HolE. We additionally investigated the substrate scope against a number of other carboxylic acids. Under high enzyme concentration, TmlU was capable of activating acetic, octanoic, 2,4-dodecadienoic, and 2,4-decadienoic acids, albeit to



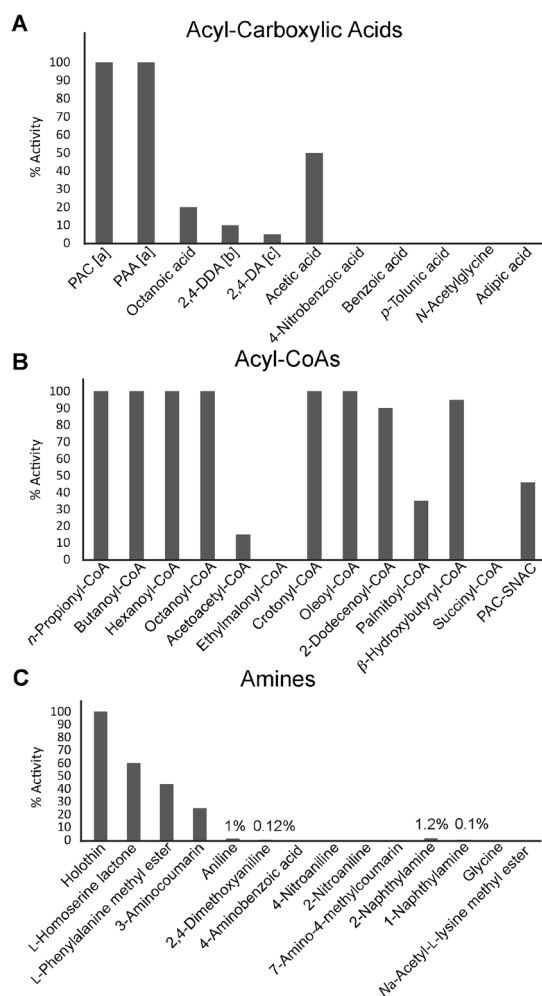
**Figure 3.** Kinetic measurement of TmlU with different substrates.

A) PAC,  $K_M = 6 \pm 1 \mu M$  and  $k_{cat} = 3.2 \pm 0.1 s^{-1}$ . B) PAA,  $K_M = 5.2 \pm 0.5 \mu M$  and  $k_{cat} = 3.0 \pm 0.1 s^{-1}$ . C) Octanoic acid,  $K_M = 0.5 \pm 0.1 mM$  and  $k_{cat} = (5.0 \pm 0.3) \times 10^{-3} s^{-1}$ .

a lesser extent (Figure 4A). In particular, we measured the  $k_{cat}/K_M$  value of TmlU for octanoic acid and found it to be 50000 fold less than those for PAC or PAA (Figure 3C). Overall, TmlU appears selective for long and relatively complex fatty acyl carboxylates.

To assess the kinetics of HolE, the substrate PAC-CoA was generated using TmlU under the conditions of full conversion. HolE and holothin were subsequently added and the conversion of PAC-CoA into PAC-holothin was observed. For a fixed concentration of PAC-CoA ( $100 \mu M$ ), holothin displayed inhibitory activity against HolE at concentrations above  $20 \mu M$  (Figure S5). This prevented determination of the  $k_{cat}/K_M$  value. Efforts instead turned to assessing its promiscuity at a fixed concentration of holothin and in the presence of several different, commercially available acyl-CoA substrates. HolE accepted linear CoA substrates of different lengths, including propionyl-, hexanoyl-, octanoyl-, oleoyl-, and dodecanoyl-CoA, readily converting all into the corresponding acyl-holothin adducts (Figure 4B and Figure S12). This finding is consistent with our observation regarding the substrate tolerance of HlmA, the acetyltransferase in the holomycin biosynthetic pathway.<sup>[15]</sup> The promiscuity of HolE with respect to fatty acyl CoA derivatives suggests that it is likely responsible for the formation of the xenorhabdins (7), which were seen as pathway by-products by Simpson et al.<sup>[10]</sup>

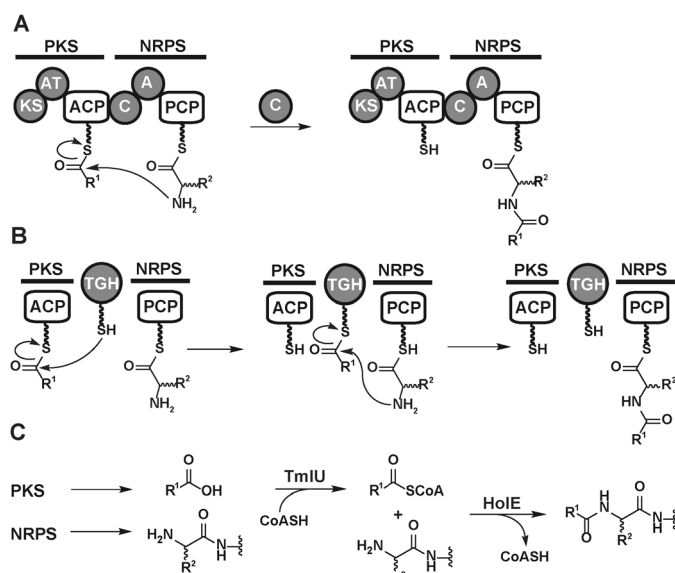
Given the potential for new and useful hybrid antibiotics from this pathway, we explored the promiscuity of the HolE/TmlU pair by supplying the reaction with various amine donors and measuring the conversion to PAC-amine products over a fixed time (Figure 4C and Figure S10). The HolE/TmlU pair could readily attach PAC to a variety of primary amines including 3-aminocoumarins, but was less effective with a series of aryl amines. Overall, an adjacent substrate carbonyl group appears useful or important for recognition of the amine donor. This promiscuity stands in contrast to the related enzyme systems, SimL and CouL from simocyclinone



**Figure 4.** Substrate promiscuity of TmlU and HolE. A) Incorporation of carboxylic acids into thiomarinol by 5  $\mu$ M TmlU in the presence of saturating concentrations of HolE and holothin. Activity was measured by the formation of the acyl-holothin products at  $\lambda = 360$  nm and normalized to activity with PAC as substrate. [a] 1  $\mu$ M TmlU was used in this assay. [b] 2,4-DDA: (2E,4E)-2,4-dodecadienoic acid. [c] 2,4-DA: (2E,4E)-2,4-decadienoic acid. B) Activity of HolE with acyl-CoA derivatives as substrates to generate acyl-holothin products, measured as described in (A). SNAC: N-acetyl cysteamine thioester. C) Activity of HolE with different amines as substrates. Cosubstrate PAC-CoA was generated by TmlU. Activity was measured by integration of ion intensities in mass spectra.

and coumermycin biosynthesis, respectively. Both SimL and CouL reportedly show strong preference for native aminocoumarin derived substrates.<sup>[11b,12,18]</sup> The biological activities of PAC-aminocoumarin and other analogues generated in this study will be the subject of future studies.

TmlU/HolE points to an intriguing convergent strategy for creating PKS/NRPS hybrid molecules. While many known hybrid linkages are installed on the PKS/NRPS assembly line (Figure 5A,B),<sup>[19]</sup> TmlU/HolE makes use of a fully mature PKS product, which is reactivated by a stand-alone acyl-CoA ligase (TmlU) and transferred to a fully mature NRPS product by a stand-alone acyltransferase (HolE; Figure 5C). This strategy does not require direct participation of assembly proteins or attachment to a carrier protein and is highly





mechanism of action: the crystal structure of Ile-tRNA synthetase bound pseudomonic acid A shows the carboxylate group jutting from the active site, uninvolved in the key inhibitory binding event.<sup>[23]</sup> Although the cyclic disulfide in holomycin was shown to be important for the antimicrobial action,<sup>[24]</sup> additional mechanistic studies are needed to reveal the role that holothin could play in the Ile-tRNA synthetase steric space. The combination of the two molecules is a potentially fortuitous evolutionary event, not easily predicted by a modern structure-based approach. We anticipate that our characterization of TmlU and HolE will aid efforts to gain insight into the evolution and confluence of biosynthetic pathways.

**Keywords:** antibiotics · biosynthesis · dithiopyrrolone · enzymes · evolution

**How to cite:** *Angew. Chem. Int. Ed.* **2015**, *54*, 5137–5141  
*Angew. Chem.* **2015**, *127*, 5226–5230

- [1] a) C. K. Chu, T. J. Bardos, *J. Med. Chem.* **1977**, *20*, 312–314; b) D. D. Long, D. G. Marquess, *Future Med. Chem.* **2009**, *1*, 1037–1050; c) T. Karoli, S. K. Mamidyalu, J. Zuegg, S. R. Fry, E. H. L. Tee, T. A. Bradford, P. K. Madala, J. X. Huang, S. Ramu, M. S. Butler, M. A. Cooper, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2428–2433.
- [2] a) V. Pokrovskaya, T. Baasov, *Expert Opin. Drug Discovery* **2010**, *5*, 883–902; b) S. Shapiro, *J. Antibiot.* **2013**, *66*, 371–386.
- [3] a) H. Shiozawa, T. Kagasaki, T. Kinoshita, H. Haruyama, H. Domon, Y. Utsui, K. Kodama, S. Takahashi, *J. Antibiot.* **1993**, *46*, 1834–1842; b) H. Shiozawa, T. Kagasaki, A. Torikata, N. Tanaka, K. Fujimoto, T. Hata, Y. Furukawa, S. Takahashi, *J. Antibiot.* **1995**, *48*, 907–909; c) H. Shiozawa, A. Shimada, S. Takahashi, *J. Antibiot.* **1997**, *50*, 449–452.
- [4] a) A. T. Fuller, G. Mellows, M. Woolford, G. T. Banks, K. D. Barrow, E. B. Chain, *Nature* **1971**, *234*, 416–417; b) E. B. Chain, G. Mellows, *J. Chem. Soc. Perkin Trans. 1* **1977**, 318–322; c) E. B. Chain, G. Mellows, *J. Chem. Soc. Perkin Trans. 1* **1977**, 294–309; d) J. P. Clayton, P. J. O'Hanlon, N. H. Rogers, T. J. King, *J. Chem. Soc. Perkin Trans. 1* **1982**, 2827–2833.
- [5] L. Ettlinger, E. Gaumann, R. Hutter, W. Kellerschierlein, F. Kradolfer, L. Neipp, V. Prelog, H. Zahner, *Helv. Chim. Acta* **1959**, *42*, 563–569.
- [6] W. D. Celmer, I. A. Solomons, *J. Am. Chem. Soc.* **1955**, *77*, 2861–2865.
- [7] a) J. Hughes, G. Mellows, *Biochem. J.* **1978**, *176*, 305–318; b) C. M. Thomas, J. Hothersall, C. L. Willis, T. J. Simpson, *Nat. Rev. Microbiol.* **2010**, *8*, 281–289.
- [8] a) B. Oliva, A. O'Neill, J. M. Wilson, P. J. O'Hanlon, I. Chopra, *Antimicrob. Agents Chemother.* **2001**, *45*, 532–539; b) B. Li, W. J. Wever, C. T. Walsh, A. A. Bowers, *Nat. Prod. Rep.* **2014**, *31*, 905–923.
- [9] D. Fukuda, A. S. Haines, Z. S. Song, A. C. Murphy, J. Hothersall, E. R. Stephens, R. Gurney, R. J. Cox, J. Crosby, C. L. Willis, T. J. Simpson, C. M. Thomas, *Plos ONE* **2011**, *6*, e18031.
- [10] A. C. Murphy, D. Fukuda, Z. S. Song, J. Hothersall, R. J. Cox, C. L. Willis, C. M. Thomas, T. J. Simpson, *Angew. Chem. Int. Ed.* **2011**, *50*, 3271–3274; *Angew. Chem.* **2011**, *123*, 3329–3332.
- [11] a) E. Schmutz, M. Steffensky, J. Schmidt, A. Porzel, S. M. Li, L. Heide, *Eur. J. Biochem.* **2003**, *270*, 4413–4419; b) C. L. Free-1 Meyers, M. Oberthur, L. Heide, D. Kahne, C. T. Walsh, *Biochemistry* **2004**, *43*, 15022–15036; c) M. Steffensky, S. M. Li, L. Heide, *J. Biol. Chem.* **2000**, *275*, 21754–21760.
- [12] M. Pacholec, C. L. F. Meyers, M. Oberthur, D. Kahne, C. T. Walsh, *Biochemistry* **2005**, *44*, 4949–4956.
- [13] T. Hjelmgaard, M. Givskov, J. Nielsen, *Org. Biomol. Chem.* **2007**, *5*, 344–348.
- [14] a) A. Tanovic, S. A. Samel, L. O. Essen, M. A. Marahiel, *Science* **2008**, *321*, 659–663; b) A. M. Gulick, *ACS Chem. Biol.* **2009**, *4*, 811–827.
- [15] B. Li, C. T. Walsh, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 19731–19735.
- [16] A. C. Murphy, S. S. Gao, L. C. Han, S. Carobene, D. Fukuda, Z. S. Song, J. Hothersall, R. J. Cox, J. Crosby, M. P. Crump, C. M. Thomas, C. L. Willis, T. J. Simpson, *Chem. Sci.* **2014**, *5*, 397–402.
- [17] R. Wu, J. Cao, X. Lu, A. S. Reger, A. M. Gulick, D. Dunaway-Mariano, *Biochemistry* **2008**, *47*, 8026–8039.
- [18] a) U. Galm, M. A. Dessoy, J. Schmidt, L. A. Wessjohann, L. Heide, *Chem. Biol.* **2004**, *11*, 173–183; b) U. Galm, S. Heller, S. Shapiro, M. Page, S. M. Li, L. Heide, *Antimicrob. Agents Chemother.* **2004**, *48*, 1307–1312.
- [19] a) L. Du, C. Sanchez, B. Shen, *Metab. Eng.* **2001**, *3*, 78–95; b) A. C. Mercer, M. D. Burkart, *Nat. Prod. Rep.* **2007**, *24*, 750–773; c) C. M. Rath, J. B. Scaglione, J. D. Kittendorf, D. H. Sherman in *Comprehensive Natural Products II: Chemistry and Biology*, Vol. 1 (Eds.: L. Mander, H.-w. Liu), Elsevier, Amsterdam, **2010**, pp. 453–492; d) D. Boettger, C. Hertweck, *ChemBioChem* **2013**, *14*, 28–42.
- [20] a) C. Fischer, L. Rodriguez, E. P. Patallo, F. Lipata, A. F. Brana, C. Mendez, J. A. Salas, J. Rohr, *J. Nat. Prod.* **2002**, *65*, 1685–1689; b) M. Yang, M. R. Proctor, D. N. Bolam, J. C. Errey, R. A. Field, H. J. Gilbert, B. G. Davis, *J. Am. Chem. Soc.* **2005**, *127*, 9336–9337; c) M. Perez, F. Lombo, I. Baig, A. F. Brana, J. Rohr, J. A. Salas, C. Mendez, *Appl. Environ. Microbiol.* **2006**, *72*, 6644–6652; d) G. J. Williams, C. Zhang, J. S. Thorson, *Nat. Chem. Biol.* **2007**, *3*, 657–662; e) R. W. Gantt, R. D. Goff, G. J. Williams, J. S. Thorson, *Angew. Chem. Int. Ed.* **2008**, *47*, 8889–8892; *Angew. Chem.* **2008**, *120*, 9021–9024; f) R. W. Gantt, P. Peltier-Pain, W. J. Cournoyer, J. S. Thorson, *Nat. Chem. Biol.* **2011**, *7*, 685–691; g) M. D. Shepherd, T. Liu, C. Mendez, J. A. Salas, J. Rohr, *Appl. Environ. Microbiol.* **2011**, *77*, 435–441; h) G. Wang, P. Pahari, M. K. Kharel, J. Chen, H. Zhu, S. G. Van Lanen, J. Rohr, *Angew. Chem. Int. Ed.* **2012**, *51*, 10638–10642; *Angew. Chem.* **2012**, *124*, 10790–10794.
- [21] P. D. Fortin, C. T. Walsh, N. A. Magarvey, *Nature* **2007**, *448*, 824–827.
- [22] S. S. Gao, J. Hothersall, J. Wu, A. C. Murphy, Z. Song, E. R. Stephens, C. M. Thomas, M. P. Crump, R. J. Cox, T. J. Simpson, C. L. Willis, *J. Am. Chem. Soc.* **2014**, *136*, 5501–5507.
- [23] L. F. Silvan, J. M. Wang, T. A. Steitz, *Science* **1999**, *285*, 1074–1077.
- [24] a) B. Li, C. T. Walsh, *Biochemistry* **2011**, *50*, 4615–4622; b) B. Li, R. R. Forseth, A. A. Bowers, F. C. Schroeder, C. T. Walsh, *ChemBioChem* **2012**, *13*, 2521–2526.

Received: December 4, 2014

Published online: February 26, 2015