Developing Novel Antibiotics to Kill Vancomycin-Resistant Enterococcus

This report features the work of Syue-Yi Lyu, Tsung-Lin Li and their co-workers published in J. Am. Chem. Soc. ***136****, 10989 (2014).*

Lipoglycopeptide antibiotic teicoplanin A2-2 **1** (Tei)/A40926 **2** carries a unique long aliphatic acyl side chain attached to glucosamine at the central residue of the Tei/A40926 pseudoaglycone scaffold **3** (Fig. 1). The gene products *orf11** and *dbv8* were characterized to be *N*-acyltransferases (NAT) responsible for *N*-acylation in the biosynthesis of Tei/A40926.[1] This modification endows the lipoglycopeptide antibiotic with an ability to exterminate multidrug-resistant Gram-(+) pathogens, e.g. methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococcus (VRE).[2,3] New applications of the enzyme have since been implied but without elaboration and detail.[4] It is thus of great interest to understand how two bulky substrates, Tei/A40926 pseudoaglycone **3** and decanoyl-CoA **4**, are recruited and how the acyl-transfer reaction is executed. To address these questions, Tsung-Lin Li and his co-workers at Academia Sinica pursued the complicated structures of NAT at various stages of reaction using BL13B1 and BL13C1 at the TLS and BL12B2 at SPring-8. In parallel with the structural determination, the authors discovered two unusual acyl-transfer reactions, providing a new chemoenzymatic strategy to obtain products in new classes that would be extremely difficult to obtain by other means, and that offer improved and complementary profiles for antimicrobial drug discovery efforts.

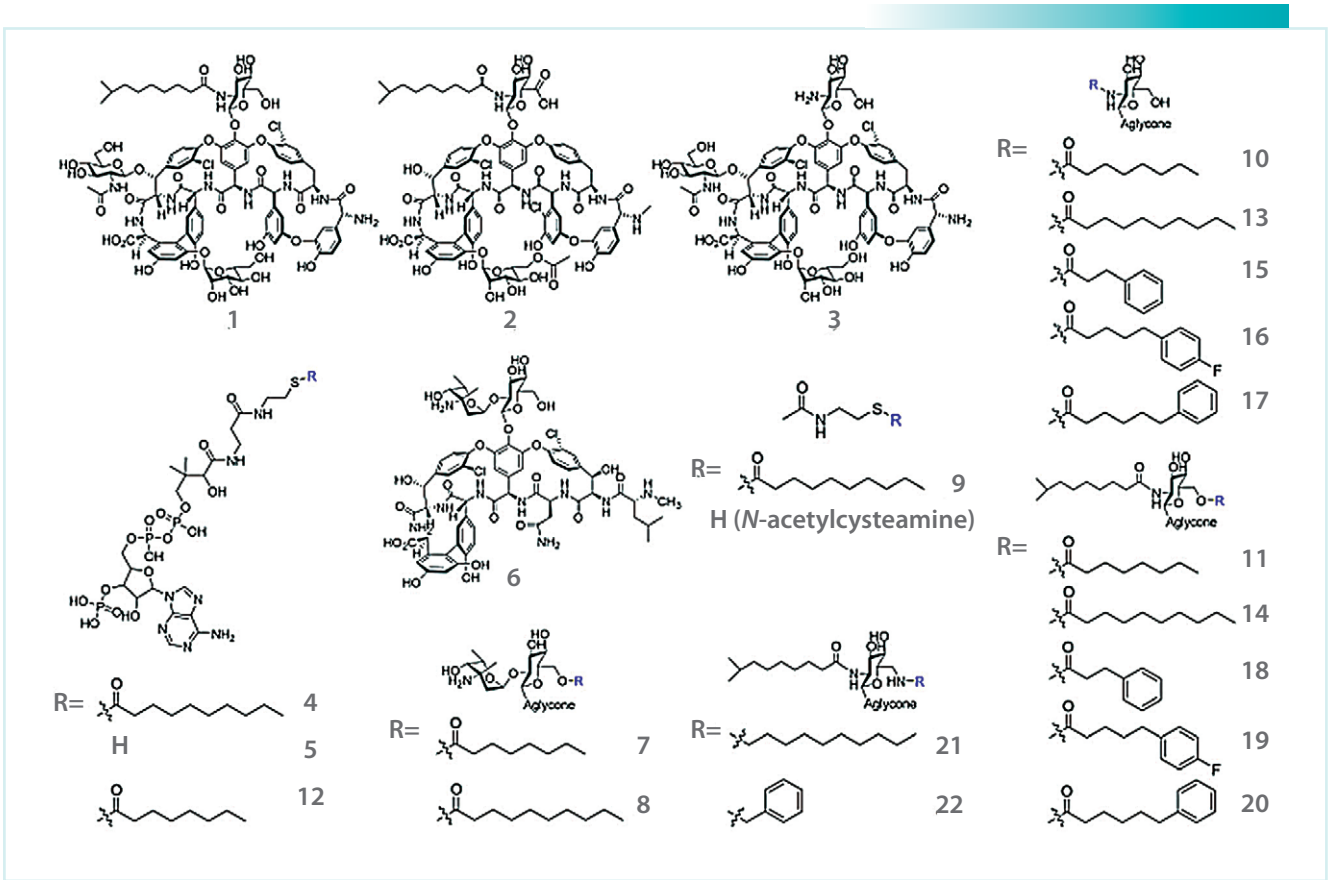

Fig. 1: Chemical structures of glycopeptides, acyl-*N*-acetyl cysteamine, and CoA derivatives.

The authors first presented eight high-resolution X-ray crystallographic unary, binary and ternary complexes to decipher the molecular basis for the functionality of NAT. Both Orf11* and Dbv8 fold in a dumbbell-like architecture, with two sizable subdomains—an unusual all-helix N-domain and a GNAT-like C-domain. Superimposition of the ligand-free and ligand-bound structures revealed that the enzyme undergoes a multistage conformational change upon binding of acyl-CoA, thus allowing the uploading of Tei pseudoaglycone to enable the acyl-transfer reaction to occur in the occlusion between the N- and C-halves of the protein.

The authors further demonstrated that Orf11*/Dbv8 is an adaptable enzyme that can generate numerous new glycopeptide analogues. The acyl moiety of acyl-CoA can be bulky or lengthy, allowing much diversity in new derivatives that can be formed upon its transfer. Interestingly, vancomycin **6** and synthetic acyl-*N*-acetyl cysteamine **9** were not expected to be able to serve as surrogates for an acyl acceptor and donor, respectively.

It has been well documented that the acyl transfer can proceed in two ways, a direct transfer and an acyl-enzyme mediated transfer as seen in histone AT. Three reaction states—pre-acylation, tetrahedral intermediate and post-acylation—were spotted in three individual ternary structures (Fig. 2), indicating that the acyl transfer of Orf11*/Dbv8 follows the direct transfer mechanism. In brief, the H196 acts as a general base deprotonating the C2 NH^{3+} of glucosamine at 4Hpg, which then attacks the thioester carbonyl carbon of acyl-CoA. The resulting tetrahedral transition structure is stabilized by the main-chain amide of V197, in which an oxyanion hole likely resides. Collapse of the transition structure results in *N*-acylated Tei pseudoaglycone. The departure of CoASH might be facilitated by S236 through protonating the sulfur anion to sulfhydryl (Fig. 2). This mechanistic notion was supported by mutagenic and biochemical assays, as relative activities of mutants H196A and H196A/S236A plunged significantly (5% and 0%, respectively, relative to WT).

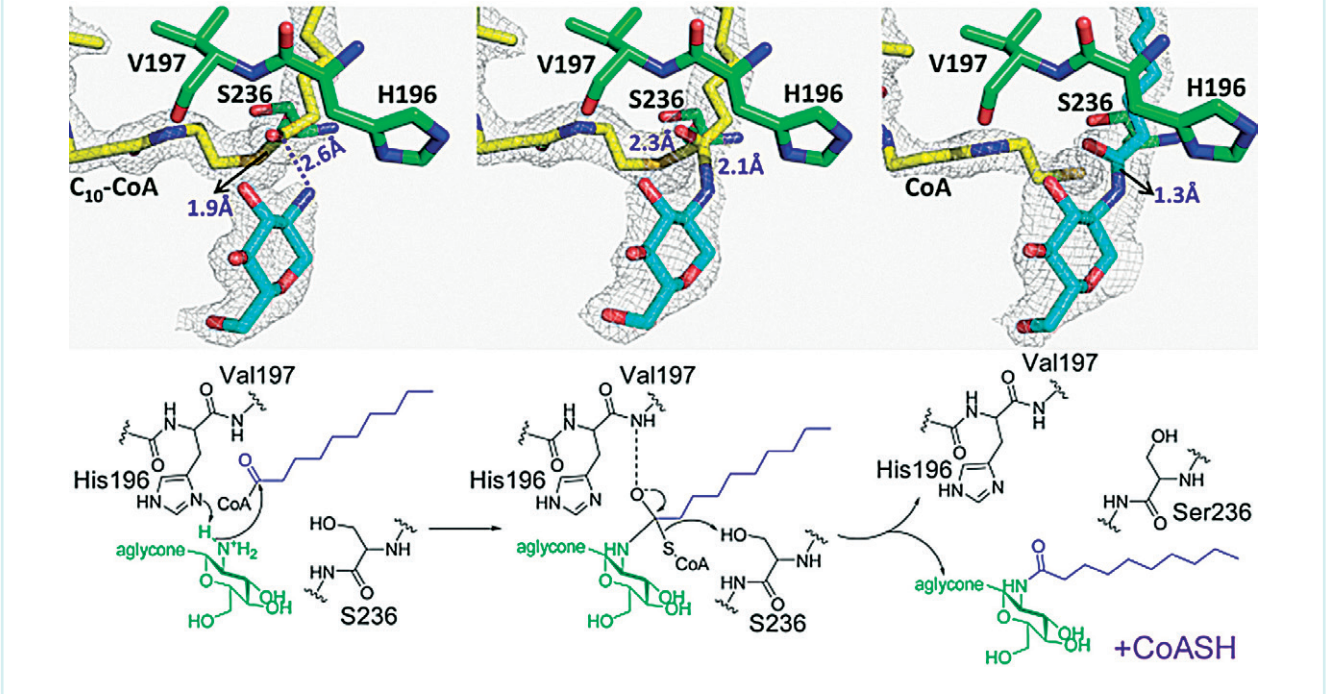

Fig. 2: Reaction intermediates and proposed enzymatic mechanisms.

Most strikingly, a double-acylation reaction and an acyl-substitution reaction were discovered, whereby Tei **1** becomes converted to C_8-Tei **10** and C_8, C_{10}-Tei **11** in an enzyme reaction containing Tei **1** and octanoyl-CoA **12**. The authors reasoned that the *2N,6O*-diacyl glucosamine moiety subject to intramolecular restraints under the active site constraints experiences an equatorial-axial interconversion via a twist-boat state. In a 1,4-diaxial fashion (a boat conformation) the C6 acyl and the C2 secondary amine are within a bond-length distance allowing a C6⟶C2 acyl migration to form a germinal diacyl intermediate. Given that the migrated acyl moiety likely remains inside a lipid tunnel, the solvent-exposed C2-*N*-acyl group is more accessible to water attack, thus resulting in formation of the acyl-substituted Tei. Two additional crystal structures were solved to verify this working model. The electron densities for both the C2-*N*-acyl and aglycone moieties were too faint to be modeled, likely due to large molecular oscillation, whereas the electron density of the glucosamine moiety was well defined, with the C6 OH clearly pointing to the acyl-CoA carbonyl carbon in one structure and with formation of a C6-*O*-acyl bond in the other, supporting the first two stages in the working model (Fig. 3).

The authors proceeded to examine the reaction conditions in a bid to control the reaction. One set of conditions (pH 7.0, 1 molar equiv of acyl-NAC/Tei) gives ratio 4:1 for acyl-substituted Tei versus diacyl Tei; the ratio is reversed (1:4) in another set of conditions (pH 9.0, 3 molar equiv of acyl-NAC/Tei, 30% DMSO). Overall, the conversion is a mild and effective one-step reaction, of which the final yield is large because the minor product is recyclable in each condition. The authors reasoned that the reaction mechanism is a synergy of stoichiometric and solvational effects. This work affords an expedient way to generate new Tei analogues without problematic deacylation and reacylation processes.[4,5] This finding also provides a one-pot solution to convert Tei mixtures of natural isolates to a single uniform compound, which

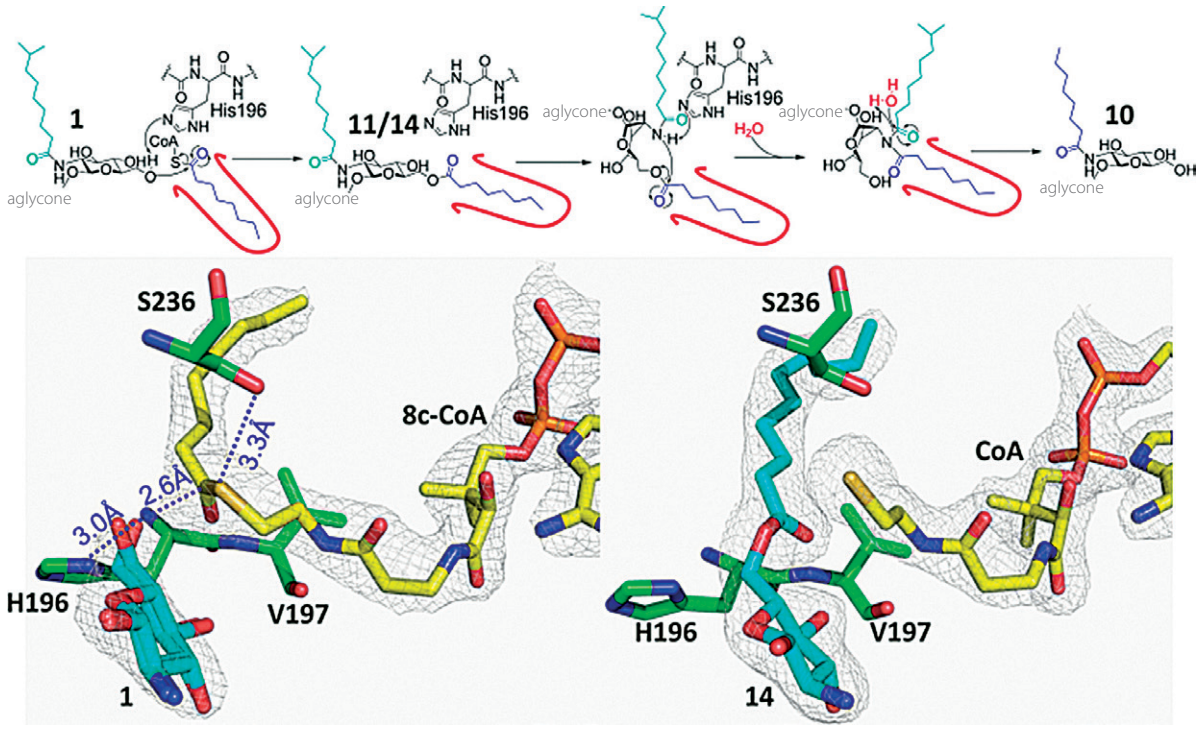

Fig. 3: Proposed mechanism of Orf11*-mediated 1,4-acyl-migration reaction. The lipid tunnel in Orf11* is schematized in red. Bottom panel: structural views for the first two acyl-migration reaction states.

should facilitate the development of approvable drugs (teicoplanin in mixtures is one reason that it is not approved by the USA FDA).

Having added new chemical functionality with the modified Tei analogues, the authors sought to examine whether they had similarly expanded new biological functionality; accordingly, the authors determined the minimum inhibition concentrations of several compounds against collections of major types (VanABC) of VRE. The 2*N*,6*O*-diacyl analogues (**11**, **18**, **19**, **20**) showed significantly enhanced bactericidal activities against all nine tested strains (both sensitive and resistant strains) when compared with mono *N*-acyl-substituted Tei, vancomycin and teicoplanin. On investigating the bactericidal variations among these diacyl analogues, the authors found that straight-chain octanoyl analogue **11** slightly outperformed phenylpropionyl analogue **18**, which, however, is compensated on increasing the chain length (analogues **19** and **20**). VanC VRE is more sensitive to analogue **19**, wherein the fluorine substituent at the *para* position of the phenyl ring might exert some electronic influence; both straight-chain analogue **11** and phenylhexanoyl analogue **20** are more effective against VanA VRE.

The development of new antibiotics with enhanced or broadened antimicrobial efficacy remains a pressing and challenging goal. Elucidation of the key long-aliphatic *N*-acyltransferase complexes in the biosynthesis of the clinically important antibiotic teicoplanin/A40926 not only resolved the difficult problems but also expanded the ordinary task of the enzyme. The authors expect that the broad-substrate acyl-transfer, diacyl- transfer and acyl-substitution reactions identified will offer a valuable way to make more effective and synthetically challenging biochemicals to meet strong synthetic and medicinal demands.

References

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