Elucidating bottromycin biosynthesis

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Bottromycins were originally discovered as antibacterial peptides of unknown biosynthetic origin that are effective against major Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Vancomycin-resistant Enterococci* (VRE)[1]. More importantly, they provide a completely new scaffold with a molecular target at the A-site of the prokaryotic 50S ribosome[2], not tackled by any other antibiotic used in the clinic. In 2012 it was reported that bottromycins belong to the fast-growing natural product superfamily of ribosomally synthesized and post-translationally modified peptides (RiPPs)[3-6]. We set out to establish an *in vitro* route to bottromycin derivatives using the biosynthetic enzymes rather than total synthesis. In the process, we gained detailed insights into several biosynthetic steps, including heterocyclization of a cysteine to thiazoline, macroamidine formation and macroamidine-dependent proteolytic processing of a biosynthetic intermediate.[7-9] Recently, we discovered and characterized a novel amino acid epimerase that is responsible for the posttranslational conversion of L-Asp to D-Asp in bottromycin biosynthesis. In addition, we have characterized the P450 enzyme BotCYP that is responsible for an oxidative decarboxylation reaction to yield the bottromycin core scaffold (without methylation). The amalgamation of these insights has enabled a facile, two-pot route to the bottromycin core scaffold with near-quantitative yields and will allow the rapid generation of bottromycin analogues for compound development.

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