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12/08/17

FK228 (Figure 1) and its analogues have been molecules of biological and chemical interest due to their histone deacetylase (HDAC) inhibition that allow this class of molecules to have the potential for chemotherapeutic properties.[1,2] Solution-phase synthetic routes for such molecules have been well studied;[3-7] however, the effect of structure of the analogues on their potency and effectiveness as chemotherapeutic molecules can still be explored further. All depsipeptides in question contain cysteine, are cyclic, and many occur naturally, such as FK228.[8] FK228 is the only natural HDAC inhibitor to make it to trials as an anticancer drug,[5] as it is deadly against a variety of tumor cells. FK228 has the ability to focus on the epigenetic silencing mechanism of HDACs, in which histone deacetylase keeps DNA bound to its histone, causing the suppression of genes necessary for apoptosis and the propagation of cancer cells.[9,10] In this and similar molecules, it has been shown that (*S*,*E*)-3-hydroxy-7-mercapto-4-heptenoate (Hmh, shown in blue) is significant in preserving HDAC inhibition[2] by interacting with the zinc binding pocket of zinc-dependent HDACs.[9] Once in the cancerous cell, the disulfide bond of the Hmh breaks to form two thiols; the long chain ending in thiol on the Hmh can then interact with the zinc in the active site of HDAC.[2] This deactivates the HDAC and allows transcription of DNA to resume, and programmed cell death of the cancerous cell can occur.[11] Further research into how and if changing the structure of the FK228 analogues would allow better binding to the active site, and if the molecule be tuned so HDAC inhibitor binds to specific HDACs. The goal of this synthesis is not only to synthesize an analog (**2**) of the FK228 through the solid phase,[12] but to eventually test the impact of the analog's structure on its biological activity, such as how these amino acid residues affect its ability as an HDAC inhibitor and potential anticancer drug.

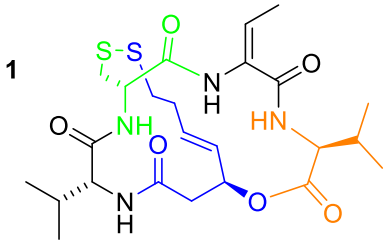


Figure 1: FK228 is a HDAC inhibitor. Once in the cell, disulfide bond on the Hmh (blue) forms thiols, fitting into HDAC's binding pocket to deactivate the enzyme.

Previous synthetic strategies of FK228 have all used solution-phase synthesis, and have encountered low yields, mostly due to problems when macrocyclizing. These problems included sensitivity of eliminations or steric hindrance during macrolactamization or macrolactonization.[13-16,4,5,9] This route was unique and novel in that it utilized solid-phase synthesis (SPS), and because it that took advantage of a latent thioester[3] and native chemical ligation (NCL)[17] as key mechanistic steps of chemoselective macrocyclization. Solid-phase synthesis of isosteres of FK228 have been completed before, but using a different linker and a different isostere of Hmh that was less biologically active.[15] Solid-phase synthesis allows a high degree of control over structure and the production of many analogues,[15] connecting the depsipeptide to a solid resin. SPS can also be a less laborious

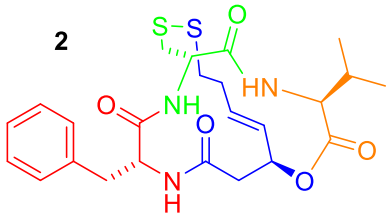
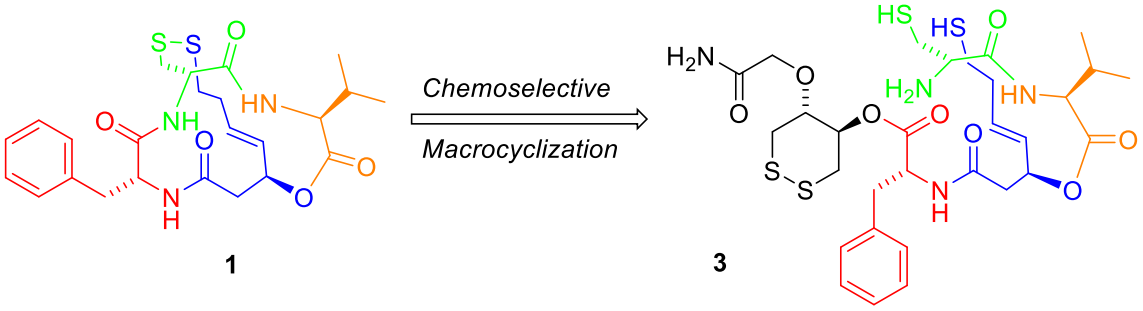


Figure 2: The final structure of the depsipeptide sythesized as an FK228 analog, containing Hmh (blue), valine (orange), cysteine (green), and phenylalaine (red). These colors of amino acid residues to are consistent throughout figures.

process due to all reactions taking place in one vessel, with purification only needed once the molecule is cleaved from the resin.

Latent thioesters have been shown to be solid-phase linkers in the past,[18] allowing macrocyclization and an easy way to handle transitions from solid to solution phase. This latent thioester was present in the linker (black on **3**) had the reactivity of a typical thioester, but after it was activated by MES-Na, allowing native chemical ligation to cyclize by bonding phenylalanine and cysteine, only after all constituent amino acid residues have been added in the desired configuration. After that, an oxidative cyclization occurred to get the disulfide bond and the second large ring to yield the bicyclic depsipeptide. The overall process of macrocyclization is shown retrosynthetically in Scheme 1.

Scheme 1: Retrosynthesis showing chemeoselective macrocyclization FK228 analouge (**2**)



1 **3**

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