Understanding a Molecular Pharmaceutical Factory
Synthesis of Proposed Intermediates for Polyketide Synthase LovB

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Introduction

- LovB is an enzyme from the fungus *Aspergillus terreus*[1], belonging to the polyketide synthases (PKSs): a family of multi-domain, complex enzymes occurring naturally in living organisms[2]
- PKSs produce polyketides, complex organic compounds with potent bioactivities from which many pharmaceuticals are derived
- The cholesterol lowering prescription drug lovastatin is a polyketide formed from its precursor dihydromonacolin L (DHML), which is formed by LovB and other cofactors (see figure 1)
- Lovastatin inhibits HMG-CoA reductase, a critical enzyme involved in the cholesterol biosynthesis in humans[1]
- Lovastatin and its derivatives, called statins (simvastatin, atorvastatin), account for 5.4% (11 Billion USB) of all drug sales in the US in 2002[1]
- Statins may be used to prevent heart diseases, reduce the risk of cardiovascular disease, Alzheimer's and multiple sclerosis[1]
- **LovB and other PKSs resemble eukaryotic fatty acid synthases, (see figure 2) but the exact assembly of polyketides is unresolved**
- **This resemblance leads to proposed intermediates for the assembly of lovastatin by LovB**

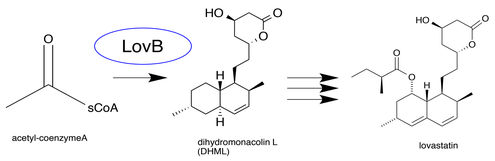
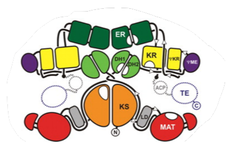


Figure 1: Biosynthesis of lovastatin

Figure 2: Illustration of Fatty acid synthase showing its similarity to LovB[3]



Purpose

- To synthesize the sequence of proposed intermediates: the hexaketide, heptaketide and octaketide
  - The hexaketide is synthesized from glutamic acid using a key stereospecific Diels-Alder reaction
  - The hepta- and octaketides are made using degradation chemistry from DHML isolated from genetically modified *Aspergillus* (previous work)
- To determine whether the proposed intermediates are true intermediates
  - The ketides are labelled with deuterium to track their incorporation by LovB into DHML
- To investigate the assembly of Lovastatin and discover more about LovB and other polyketide synthases

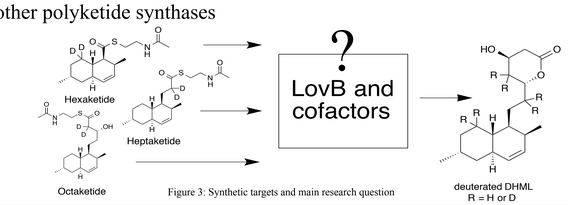


Figure 3: Synthetic targets and main research question

Synthesis

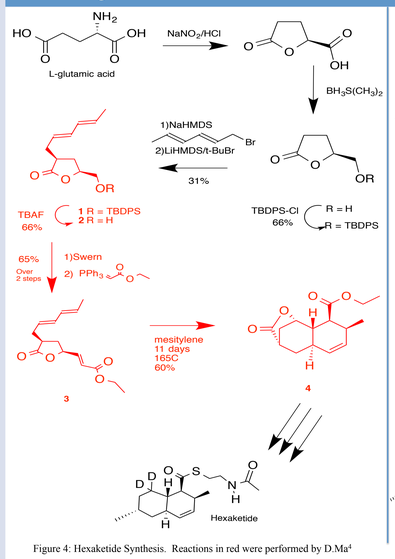


Figure 4: Hexaketide Synthesis. Reactions in red were performed by D.Ma[4]

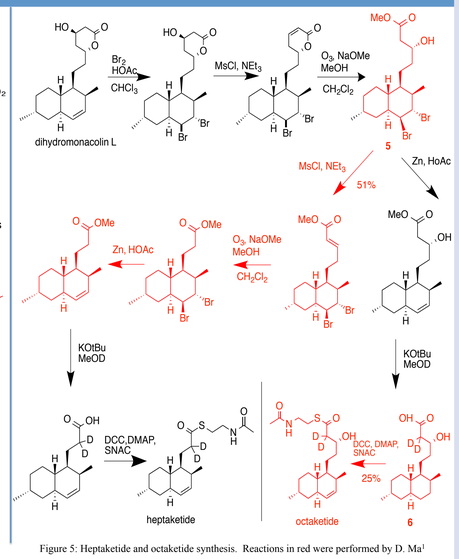


Figure 5: Heptaketide and octaketide synthesis. Reactions in red were performed by D. Ma[1]

Hexaketide

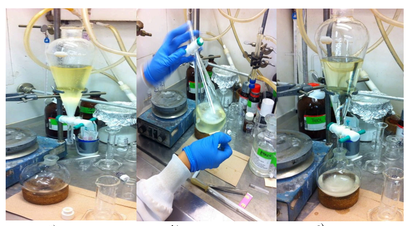
- TBAF was added dropwise to a cool stirred solution of **1** at 0℃ in dry THF
- The reaction mixture was warmed to room temperature for over 3 hours before diluting with Et_2O
- The mixture was washed with a saturated solution of ammonium chloride (see figure 4)
- The aqueous layers were combined and back extracted with Et_2O
- The organic fractions were combined, washed with brine, dried with $MgSO_4$, filtered and the solvent was removed
- To get the product **3,** DMSO was added to a cooled solution of CO_2Cl_2 at -78℃ in dry DCM for over 25 mins (see figure 5)
- After 20 mins the alcohol **2** in dry THF was added over 5 mins and left to stir for 20 min
- A solution of DIPEA was added over 5 mins and left to stir for 10 mins before warming to -5℃
- The mixture was added to a cool stirred solution of triphenyl phosphorane at 0℃ (see figure 6) in dry THF for over 1 hour and allowed to stir and warm to room temperature for 20 hours in darkness
- The solvent was evaporated and the remaining mixture dissolved in EtOAc, washed 2X with HCl.
- Aqueous layer was back extracted with EtOAc and the organic layer combined, washed with brine, dried with $MgSO_4$, filtered and solvents removed
- To get the product **4)**, product **3** was dissolved in mesitylene, BHT was added and the mixture was refluxed under argon for 11 days at 165℃ (see figure 7)

Heptaketide

- **5** was dissolved and cooled on ice
- NEt3was added, then MsCl slowly
- Ice was melted, mixture stirred for 3 days
- The reaction was quenched with HCl and the organic layer was washed with water, sat'd sodum bicarbonate, water and brine
- Dried over Na_2SO_4, filtered and solvent removed

Octaketide

- **6** was dissolved in dry DCM directly in a vial
- It was cooled to 0℃ in an ice-water bath
- DMAP, SNAC and DCC were added respectively
- 4The reaction was left for 30 mins on ice and overnight at room temperature
- A white precipitate formed and solvent removed, the residue was applied directly to a column



a) b) c)

Figure 4: a) acid wash b) shaking c)washing with brine to pre-dry organic phase

Figure 5: Swern Oxidation: CO_2Cl_2, DMSO and DCM at -78℃

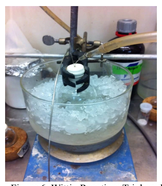


Figure 6: Wittig Reaction: Triphenyl phosphorane at 0℃

Figure 7: Reaction under argon at 165℃,

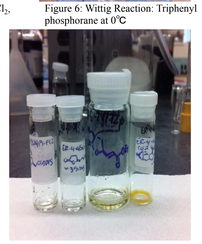
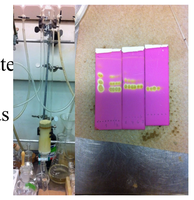


Figure 8 From left to right, the products **1, 2, 3, 4**

Purification, Characterization

- Purification of the products was achieved through column chromatography and Thin Layer Chromatography (TLC), using hexanes and ethyl acetate or pentane and diethyl ether as eluents
- Nuclear Magnetic Resonance (NMR) Spectroscopy was used to check the structure and purity of the products
- Mass Spectrometry was also used to characterize the products



a) b)

Figure 10: a) A column used for purification b) TLC plates

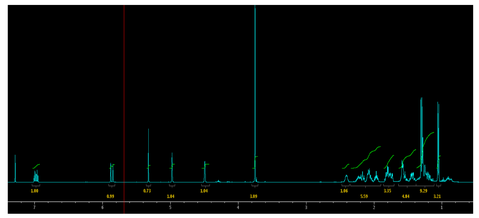


Figure 9: Proton NMR Spectrum of the product with starting material **5**

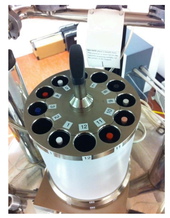


Figure 11: NMR sample tubes ready to be scanned

Discussion

- Once the proposed intermediates are synthesized, collaborators at UCLA will be using LovB enzyme assays to check if the intermediates are true intermediates
- We are also studying the domains as stand-alone proteins, to study interactions between the ACP and other domains
- The aim of further research is to understand polyketide synthases and how they assemble polyketides
- **Once PKSs are better understood, can we manipulate synthases to produce analogues of lovastatin or improved pharmaceutical drugs?**

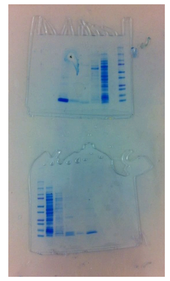


Figure 12: Protein gels after expression

Acknowledgements

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