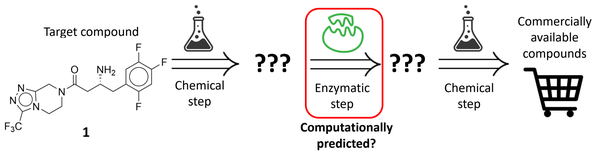
Multi-step chemo-enzymatic synthesis planning for process chemistry

Karthik Sankar, Willow Carretero Chavez, and Klavs F. Jensen

Massachusetts Institute of Technology

MLPDS

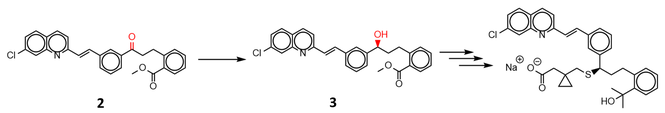
Goal: Create synthesis plans that combine chemical and biocatalytic steps



Why chemo-enzymatic synthesis?

- Enzymes operate under relatively mild aqueous, room-temperature conditions
  - Reduced need for organic solvents
  - Reduced need for reaction heating/cooling/pressurization
- Can replace expensive and toxic precious metal catalysts
- Enzymes are highly enantio-, regio-, and chemo-selective
  - Reduced need for protection/deprotection steps
  - Enables otherwise difficult/inaccessible synthesis routes

Industrial-scale, stereoselective, chemo-enzymatic case study: Montelukast sodium, 20 tons per annum



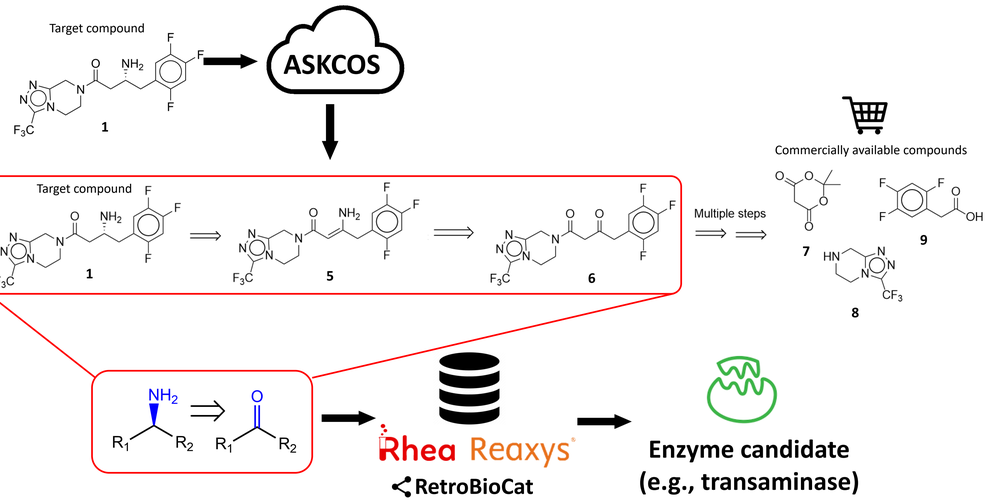
Reaction parameter	Biocatalytic Process	(S)-DIP-Cl Process
Catalytic/Stoichiometric	Catalytic	1.8 eq DIP-Cl
Temperature	45°C	-25°C
Solvent Use (L solvent/ kg product)	6	30-50
Waste Generated	Biodegradable enzyme, cofactor	Non-biodegradable borate salts, other inorganics, 3.6 eq pinene

- Reaction: Ketone to chiral alcohol, introducing lone stereocenter in product
- Organic approach: Chiral reagent (*(S)*-DIP-Cl) at -25°C
- Improved biocatalytic approach: Selective ketoreductase (KRED, refined via directed evolution) at 45°C

Approach: Identify chemical step(s) that can also be catalyzed by enzymes

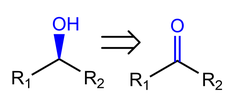
Step 1: Use ASKCOS Tree Builder to predict possible *purely chemical* synthesis plans for the target compound

Step 2: Identify candidates for replacement with biocatalytic step(s) using database of enzymatic retrosynthetic templates

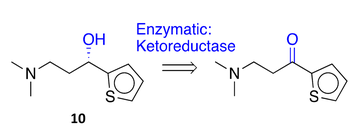


One step evaluation: Substrate scope is diverse within a given enzyme class

Core transformation:

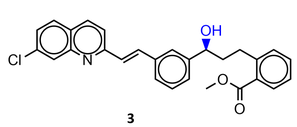


Example proposed reaction:

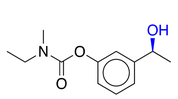


Tool can successfully recover *enantioselective* reduction of a ketone to a chiral alcohol by a ketoreductase (KRED) with a diverse scope of substrates. All example products have biocatalytic routes available for them in extant literature.

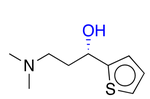
Example products:


3

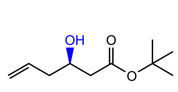
Montelukast sodium intermediate


11

Rivastigmine intermediate


12

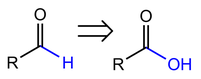
Duloxetine intermediate


13

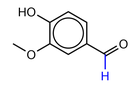
Statin sidechain intermediate

One step evaluation: different enzyme classes appropriately captured

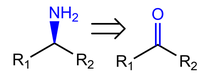
Carboxylic acid reduction



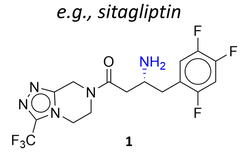
e.g., vanillin


14

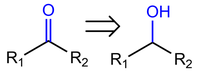
Transamination



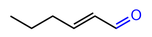
e.g., sitagliptin


1

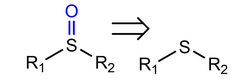
Alcohol oxidation



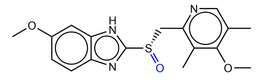
e.g., (E)-2-hexenal


15

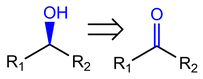
Sulfide oxidation



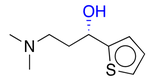
e.g., esomeprazole


16

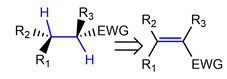
Ketone reduction



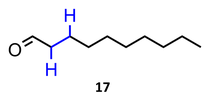
e.g., duloxetine intermediate


12

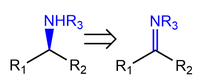
C=C Ene reduction



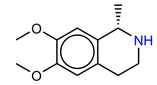
e.g., decanal


17

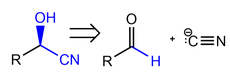
Imine reduction



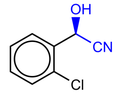
e.g., (S)-salsolidine


18

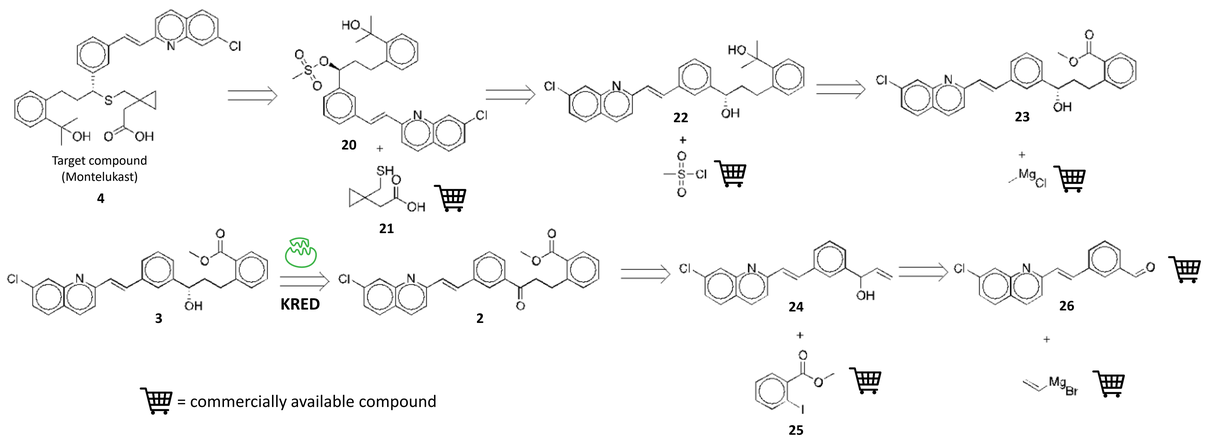
Hydroxynitrile lyase



e.g., clopidogrel intermediate


19

Application to multi-step planning of a complex anti-inflammatory drug



Process Mass Intensity (PMI) for prioritization of biocatalytic replacements

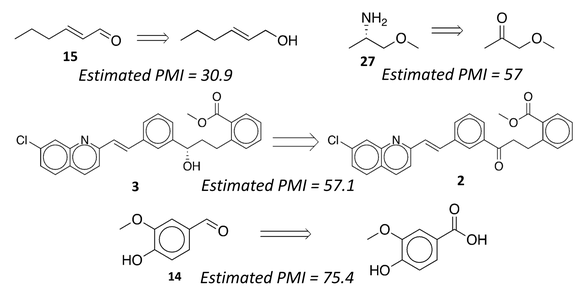
In order to prioritize wasteful chemical reactions that should be replaced with their biocatalytic equivalents, the Process Mass Intensity (PMI) of each reaction is calculated as follows:

$$PMI = \frac{\sum Mass\ of\ materials}{Mass\ of\ isolated\ product}$$

(*Mass of materials* includes mass of process solvents, chemical reagents, isolated product, and any single use process chemicals utilized in process execution)

PMI estimates are currently performed by the ACS PMI Predictor (https://www.acs.org/). Future versions of the tool will use an in-house machine learning-based predictor.

Example PMI analyses of biocatalysis candidates:



References and Acknowledgments

Bollikonda, S. et al. *J. Org. Chem*. **80**, 3891–3901 (2015).
Borovika, A. et al. *ChemRxiv* (2019).
Sherer, C. E. et al. Driving Aspirational Process Mass Intensity Using Simple Structure-Based Prediction. Org. Process Res. Dev. 26(5), 1405-1410 (2022).
Chen, X., Liu, Z.-Q., Lin, C.-P. & Zheng, Y.-G. *Bioorganic Chemistry* **65**, 82–89 (2016).
de Almeida, T. P. et al. *Advanced Synthesis & Catalysis* **361**, 2668–2672 (2019).
Glieder, A. et al. *Angewandte Chemie International Edition* **42**(39), 4815–4818 (2003).
Leipold, F., Hussain, S., Ghislieri, D. & Turner, N. J. *ChemCatChem* **5**, 3505–3508 (2013).
Papadopoulou, A., Peters, C., Borchert, S., Steiner, K., & Buller, R. *Org. Process Res. Dev.* **26**, 2102-2110 (2022).
Park, J., Lee, H.-S., Oh, J., Joo, J. C. & Yeon, Y. J. *Biochemical Engineering Journal* **161**, 107683 (2020).
Turner, N. J. & Humphreys, L. *Biocatalysis in Organic Synthesis: The Retrosynthesis Approach*. (RSC, 2018).
Wu, S., Snajdrova, R., Moore, J. C., Baldenius, K. & Bornscheuer, U. T. *Angewandte Chemie International Edition* **60**, 88–119 (2021).
Xu, N. et al. Org. Process Res. Dev. 24, 1124-1130 (2020).
Yan, P.-C. et al. Org. Process Res. Dev. 17, 307–312 (2013).
Ye, F. et al. RSC Advances 11, 4805–4809 (2021).
Yun, Y.-M. S. and H. J Microbiol Biotechnol 21, 1049–1052 (2011).

We would like to thank:

- Colin Lam
- Deeptak Verma
- Ania Fryszkowksa
- MIT Undergraduate Research Opportunities Program Office