IX Biological Methods of Control

Objectives

By the end of this section you will:

1) appreciate that enzymes can be very useful in natural product synthesis;
2) understand the principles of kinetic resolution and desymmetrisation using lipases;
3) understand the useful properties of enzymes as well as some of their drawbacks.

Recommended Reading: C.-H. Wong, G. M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Tetrahedron Organic Chemistry Series Vol 12, Pergamon, Oxford, 1994.

IX.A Enzymes in Synthesis

Nature operates in a chiral environment; highly selective (chemo-, regio- and stereo-) transformations are routine. Enzymes - proteins that catalyse reactions *in vivo* - are Nature's catalysts for most of these transformations. Synthetic chemists have not surprisingly isolated and purified many enzymes from a range of organisms, and used them in the laboratory on both natural *and* unnatural substrates, often to great effect.

Advantages and useful properties of enzymes:

1. Reactions employing enzymes usually proceed under very mild conditions (often physiological).
2. They have low environmental impact ('green chemistry' is becoming increasingly important).
3. They can be extremely selective. As enantiomerically pure, chiral catalysts, they often impart exceptional levels of enantio- and diastereoselectivity on both natural *and* unnatural substrates.
4. They can be exceptionally efficient catalysts even with unnatural substrates - very few synthetic catalysts compare.
5. They can achieve transformations not possible using conventional chemical reagents (see methods of remote oxidation later).
6. A wide variety are now commercially available (although some are very expensive).

Disadvantages and some solutions.

i) Substrate specificity

Enzymes can exhibit extremely high substrate specificities, which can be a problem if the enzyme doesn't accept your substrate. However, by screening a wide range of enzymes it is sometimes possible to identify an enzyme that will accept the substrate.

ii) Enzyme stability

Enzymes usually operate in an aqueous environment; they therefore tend to be more unstable in the organic solvents which are often required to solubilise the reacting substrate. The stability of enzymes in organic solvents depends on the hydrophilicity of the enzyme - hydrophilic enzymes tend to be less stable in organic solvents. However careful choice of solvent can help minimise these problems: immiscible, non polar solvents often give the best results. Increased enzyme stability can also be achieved by immobilising the enzyme on a support. This has the added advantage of providing a simple purification procedure - the enzyme can be filtered off and recovered at the end of the reaction.

iii) Additives

Some enzymes require co-factors to operate. While co-factor recycling is possible it is not always easy.

Water is also often required for enzymes to maintain catalytic activity, which can be a problem if the substrate is water-sensitive. However such problems can be minimised by restricting the amount of water added: it is sometimes possible to use very small amounts of water and still maintain enzyme activity.

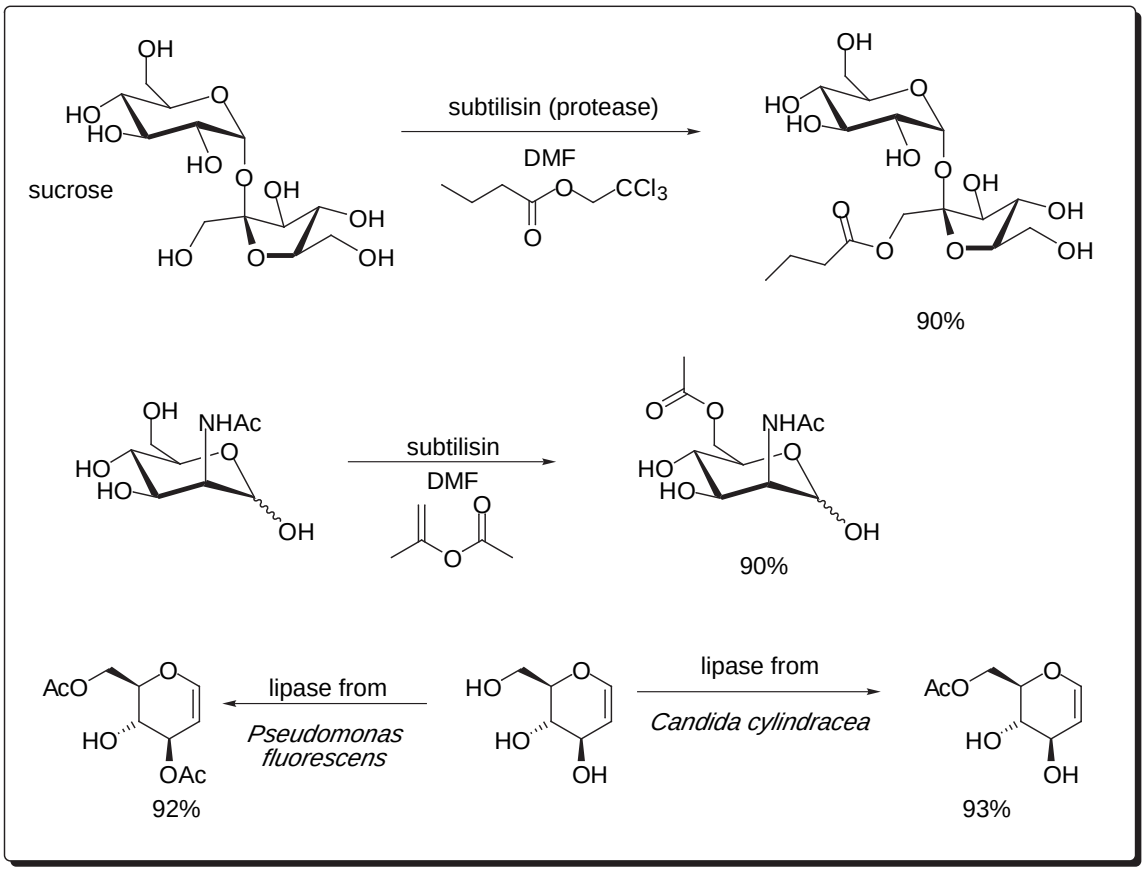
iv) Cost - some enzymes are very very expensive.

IX.B Hydrolytic Enzymes - Lipases, Esterases, Proteases and Amidases

- These enzymes catalyse the hydrolysis of ester and amide bonds.
- Depending on the precise reaction conditions, they can also be used in a reverse sense to catalyse the formation of ester and amide bonds.
- This is the most commonly used class of enzymes in synthesis:
  - they require no co-factor (so no need for co-factor recycling);
  - reactions are simple to carry out;
  - many 1000s of these enzymes are known and a large number are now commercially available and relatively inexpensive.

IX.B.1 Regioselective Protection of Alcohols

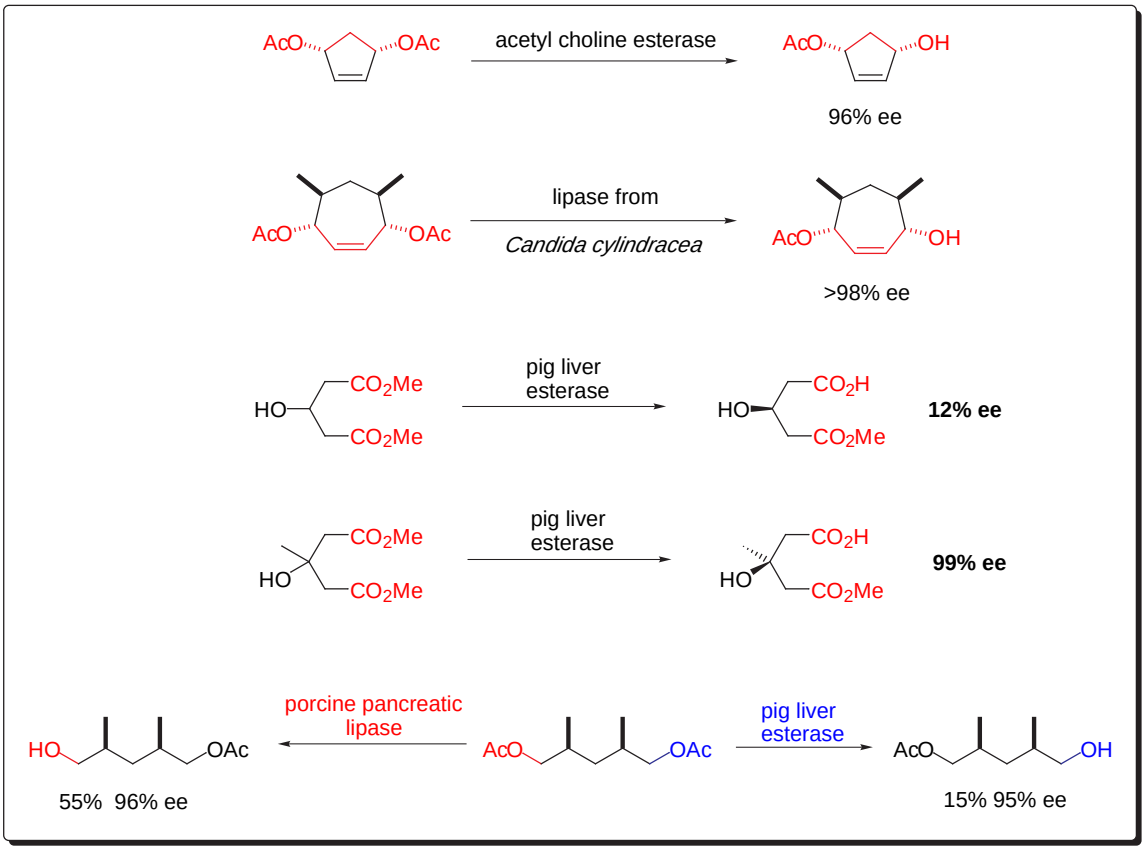
We have seen how protecting group strategies are very important for the synthesis of molecules that possess multiple reactive functional groups. Enzymes have been used for highly regioselective protection of alcohol functionality (as esters) in carbohydrates.



IX.B.2 Desymmetrisation of M*eso* Systems

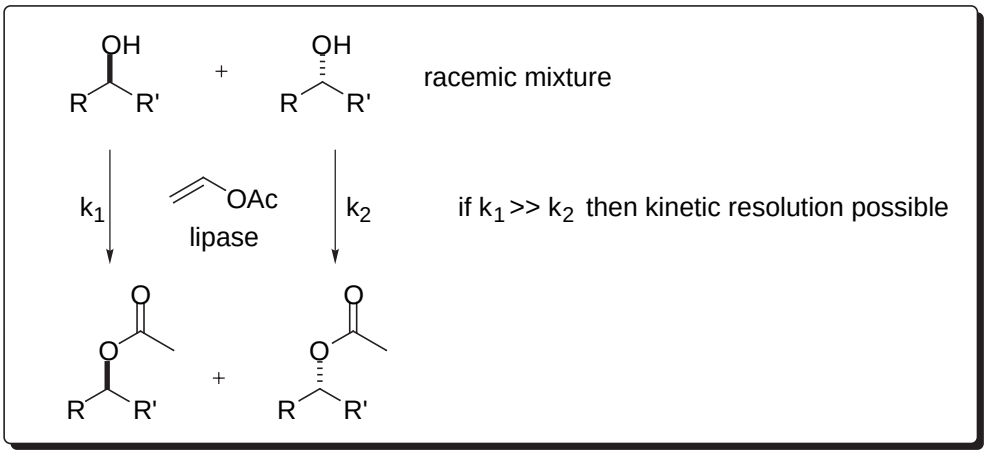
- a very useful method for accessing chiral compounds
- complete conversion possible (see kinetic resolution below) although lower yields are often obtained when hydrolysis of the remaining acetate becomes competitive.

Examples



IX.B.3 Kinetic Resolution of Racemic Alcohols and Esters

- This provides a very useful method for accessing enantiomerically enriched molecules from a readily prepared racemic starting material.
- Start with a racemate. Providing one enantiomer reacts faster than the other, then there is the potential for a kinetic resolution. The greater the difference in rates, the more efficient will be the resolution.
- The maximum yield is 50%, which can be a disadvantage.
- Unreacted starting material has to be separated from the desired product (usually not a major problem).



Problems with enzymatic kinetic resolutions of alcohols:

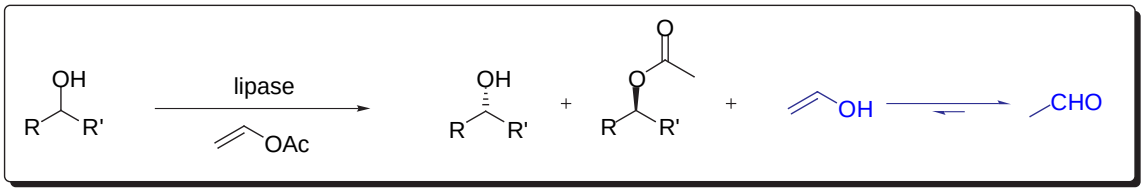
- The reaction is reversible. This is a problem in a kinetic resolution. The major product in the forward reaction is also going to be the faster reacting of the two enantiomeric products in the reverse reaction. Hence there will be an erosion in the enantioselectivity as the reverse reaction proceeds.

- Product inhibition caused by the release of an alcohol during the transesterification reaction can reduce the efficiency of the process.

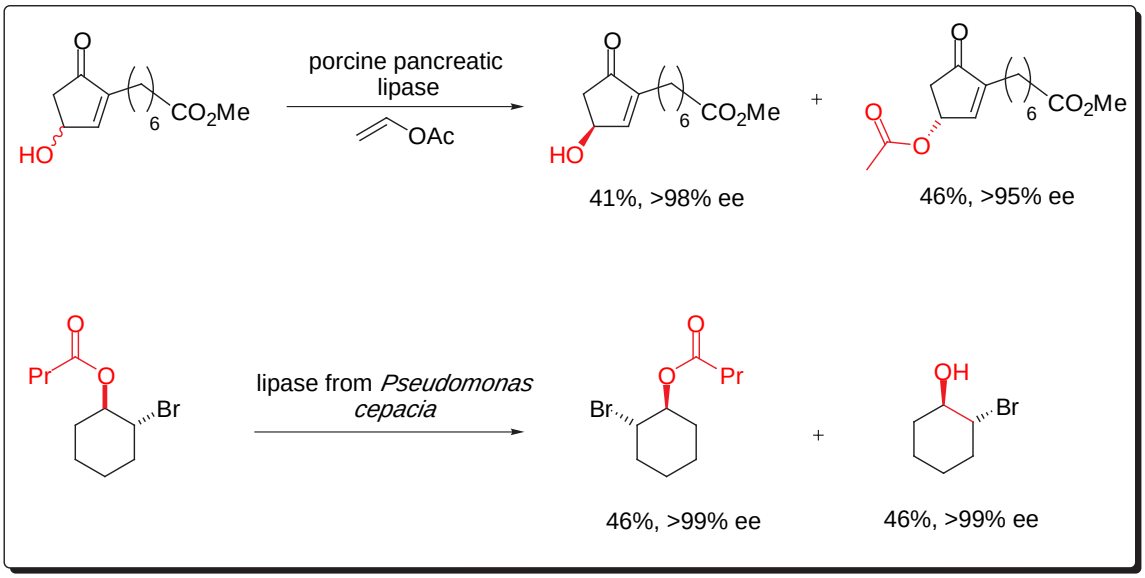
Solution:

- make the desired reaction irreversible and remove the alcohol transesterification product. Both of these can be achieved by using activated esters especially enol esters (*e.g.* vinyl acetate):

1. The increased reactivity of the ester facilitates the forward reaction;
2. The equilibrium is pushed over to the right if the ester is used in excess - vinyl acetate is cheap and volatile so it can be used as the solvent.
3. The product is an enol and therefore rapidly tautomerises to the aldehyde, thereby removing the product alcohol and rendering the reaction irreversible.

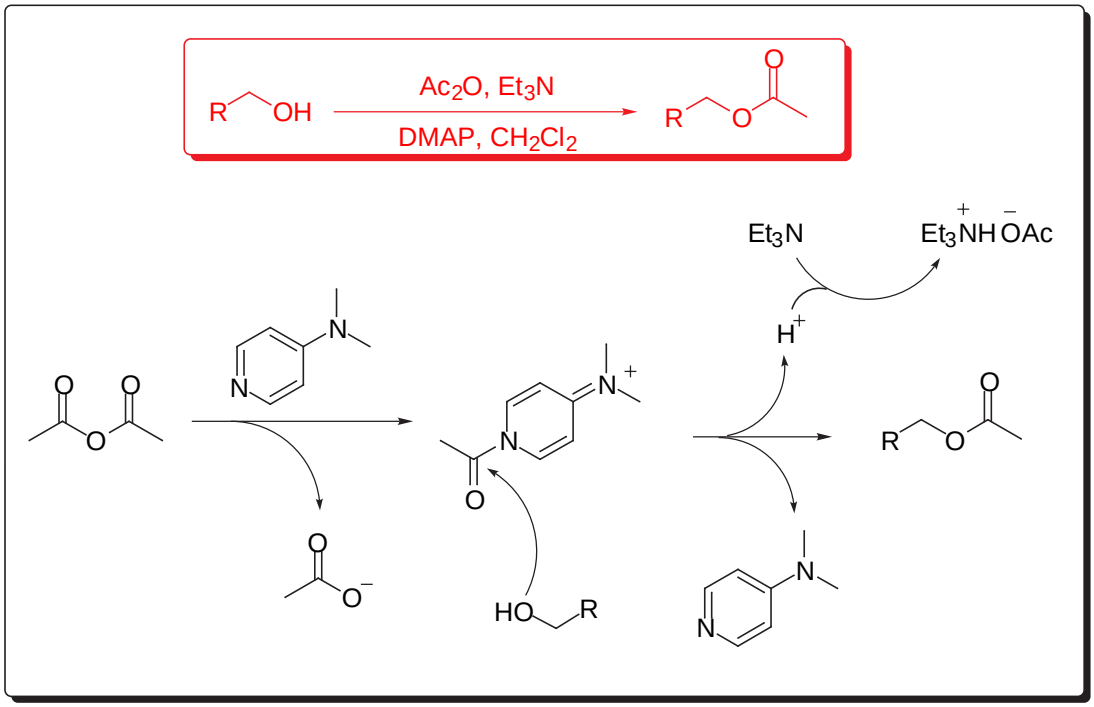


Examples:



IX.C Anything Nature can do We can do better? A Biomimetic Artificial Enzyme - Catalytic Enantioselective Acylation

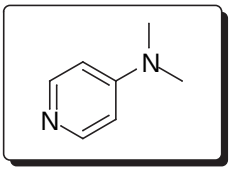
Standard method for acylating alcohols:



- DMAP (4-dimethylaminopyridine) acts as a **nucleophilic catalyst** and increases the rate of acylation by greater than 10^5.
- Reaction is very general and exhibits very broad substrate specificity.
- rapid and high yielding.
- The active acylating reagent is the acyl pyridinium species.

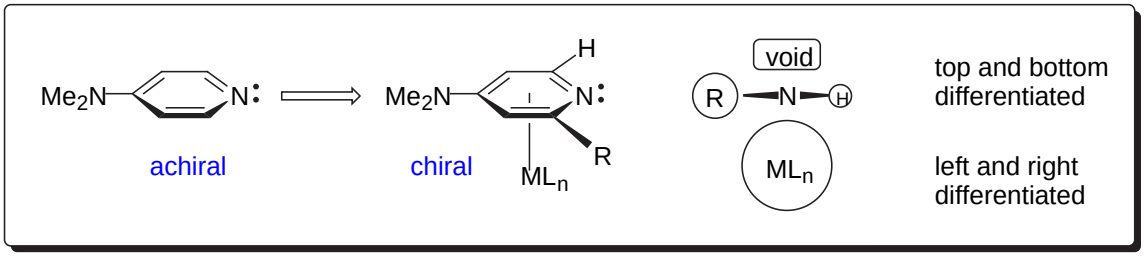
Can we design a chiral version of this nucleophilic catalyst?

starting point - DMAP



This molecule contains two mirror planes and therefore is achiral; however, if we can eliminate these two elements of symmetry, then we can generate a chiral molecule.

strategy: use π-complexation to destroy the symmetry elements:

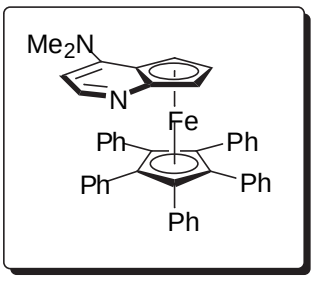


Convince yourself that π-complexation and introduction of a 2-substituent breaks the mirror planes and generates a chiral molecule. Draw the enantiomer.

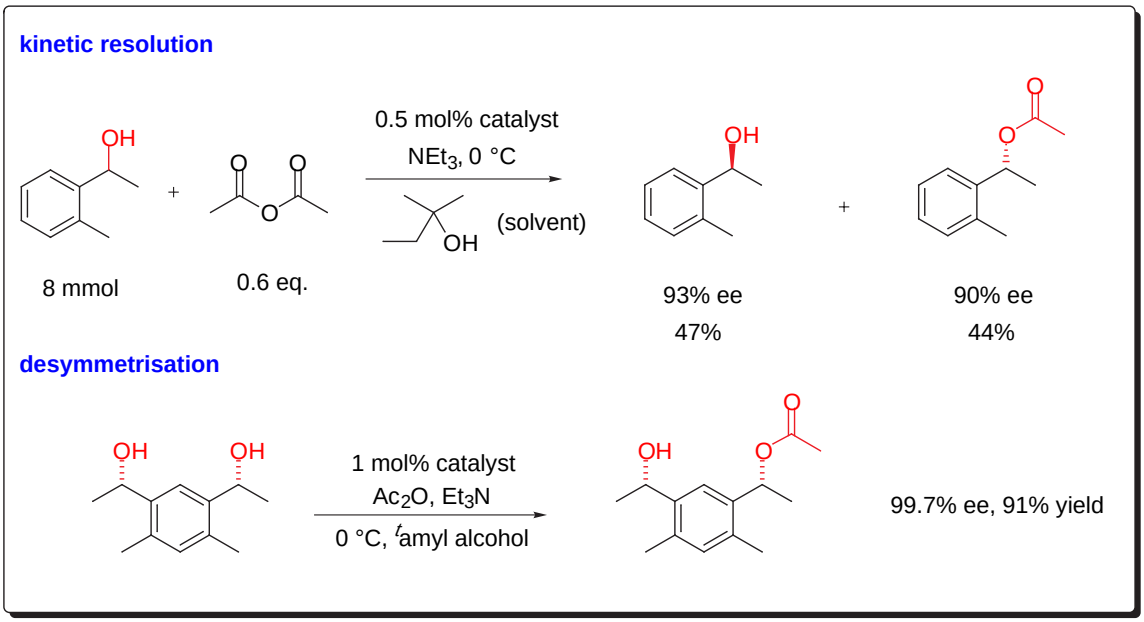
Other desirable properties of a nucleophilic catalyst include:

- electron rich;
- a tunable, steric environment;
- robust, easy to prepare, manipulate and recycle.

final product:



Does it work?

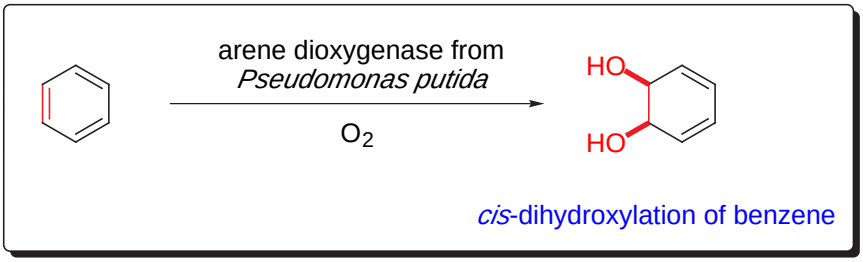


For an excellent review of this chemistry: G. C. Fu, *Acc. Chem. Res.*, 2000, **33**, 412-420.

IX.D Enzymatic Oxidation

Oxidation is as widespread a reaction in Nature as it is in the laboratory. Not surprisingly there are thousands of enzymes capable of carrying out most types of oxidation, and in cases where new stereogenic centres are created, these reactions are invariably highly enantioselective. The high specificities of enzymes can be an advantage where issues of chemo- and regioselectivity preclude the use of 'chemical reagents'. Some examples will serve to illustrate the utility of enzymes in oxidation reactions.

IX.D.1 Oxidation of Benzene.

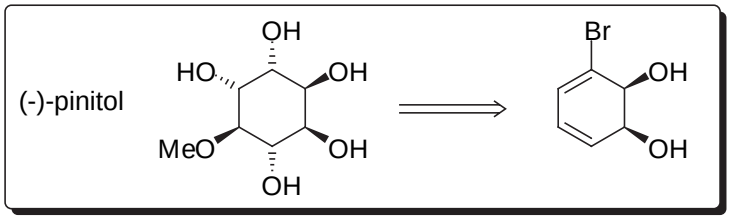


cis-dihydroxylation of benzene

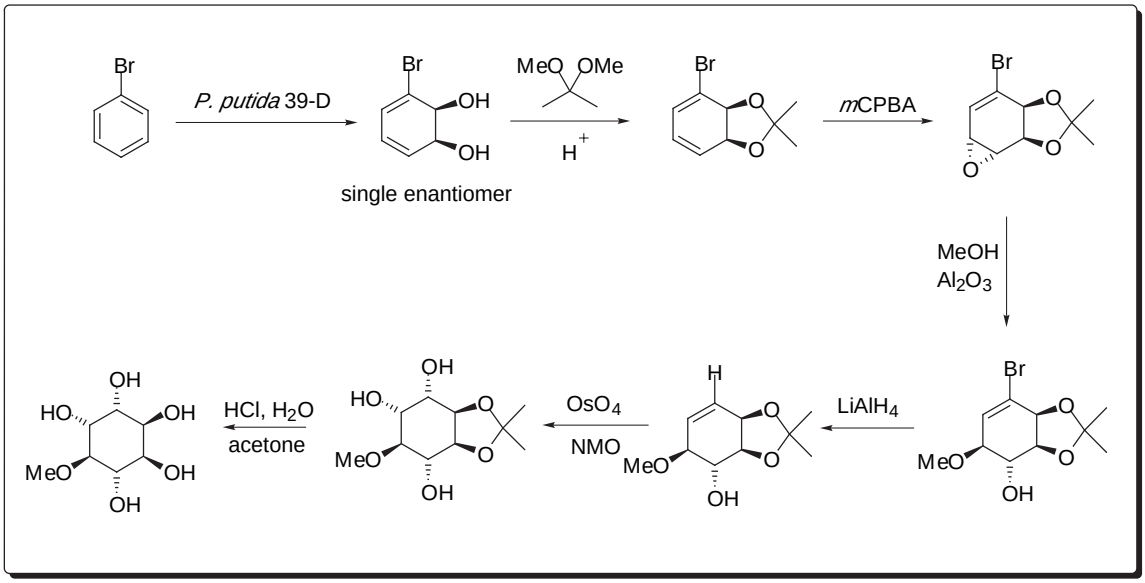
It is impossible to perform this transformation using standard chemical dihydroxylation (OsO_4).

How might you go about preparing the product by chemical means? Hint: start with methoxy benzene and a Birch reduction.

Use in synthesis. A route to (–)-pinitol



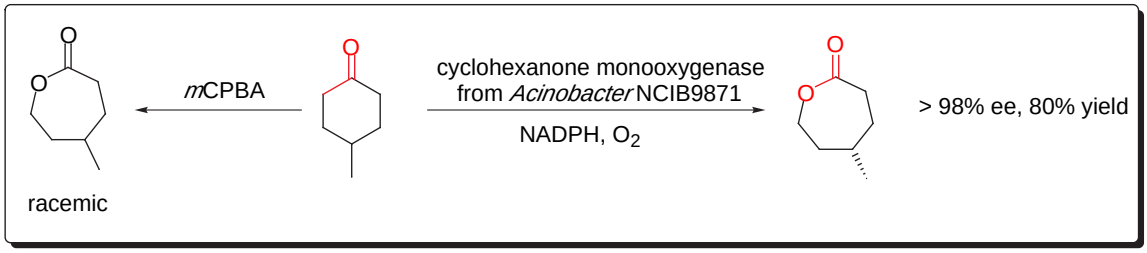
Forward Synthesis:



Some questions to consider in an analysis of this synthesis:

- The bromine substituent plays a number of important roles. What are they?
- The diol product from the *P. putida* oxidation is almost flat. Formation of the isopropylidene acetal changes the shape of the molecule. How does this control the stereoselectivity of subsequent reactions?
- Account for the reioselectivity of the epoxidation.
- Account for the regio- and stereoselectivity of the epoxide ring opening.
- How might you access the opposite enantiomer which itself is a natural product?

IX.D.2 Enantioselective Baeyer-Villiger Oxidation

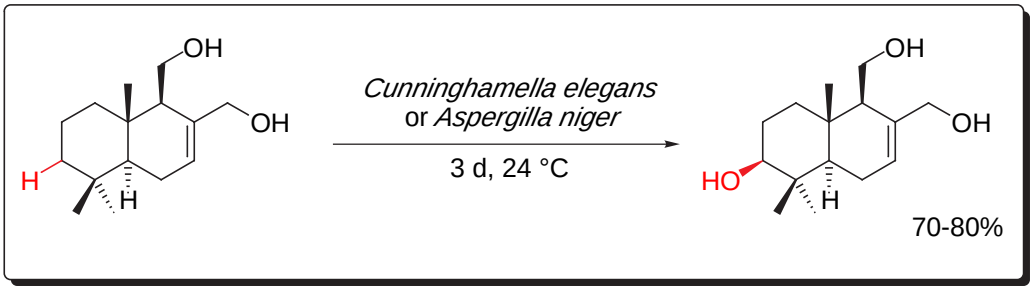


- This enzyme requires a cofactor (NADPH) for activity;
- Olefins are NOT epoxidised - this can be a competing process using chemical methods (chemoselectivity);
- Oxidation is highly enantioselective and generates a synthetically useful product;
- there are currently no efficient and general chemical methods for enantioselective Baeyer-Villiger oxidations.
- *you should be able to draw the mechanism of the Baeyer-Villiger oxidation using mCPBA.*

IX.D.3 Remote Oxidation

This is a very challenging problem largely unsolved in organic chemistry. However Nature carries out these tranformations routinely (definitely winning this battle!).

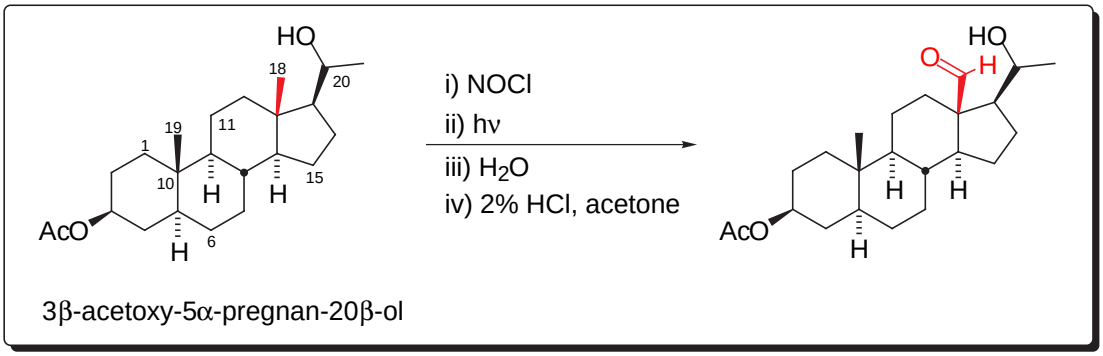
Example:



- This transformation uses the whole organism (a bacterium); there is no need to isolate and purify the active enzyme.
- To date it would be impossible to carry out this transformation using chemical reagents.

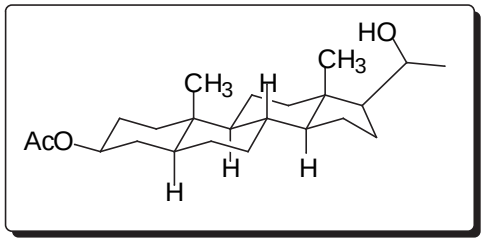
IX.D.3.i Chemical Approaches to Remote Oxidation

One of the most successful approaches to remote oxidation (although still very substrate-specific) was developed by Barton specifically for oxidising remote positions in the steroid skeleton. It involves a radical reaction process.

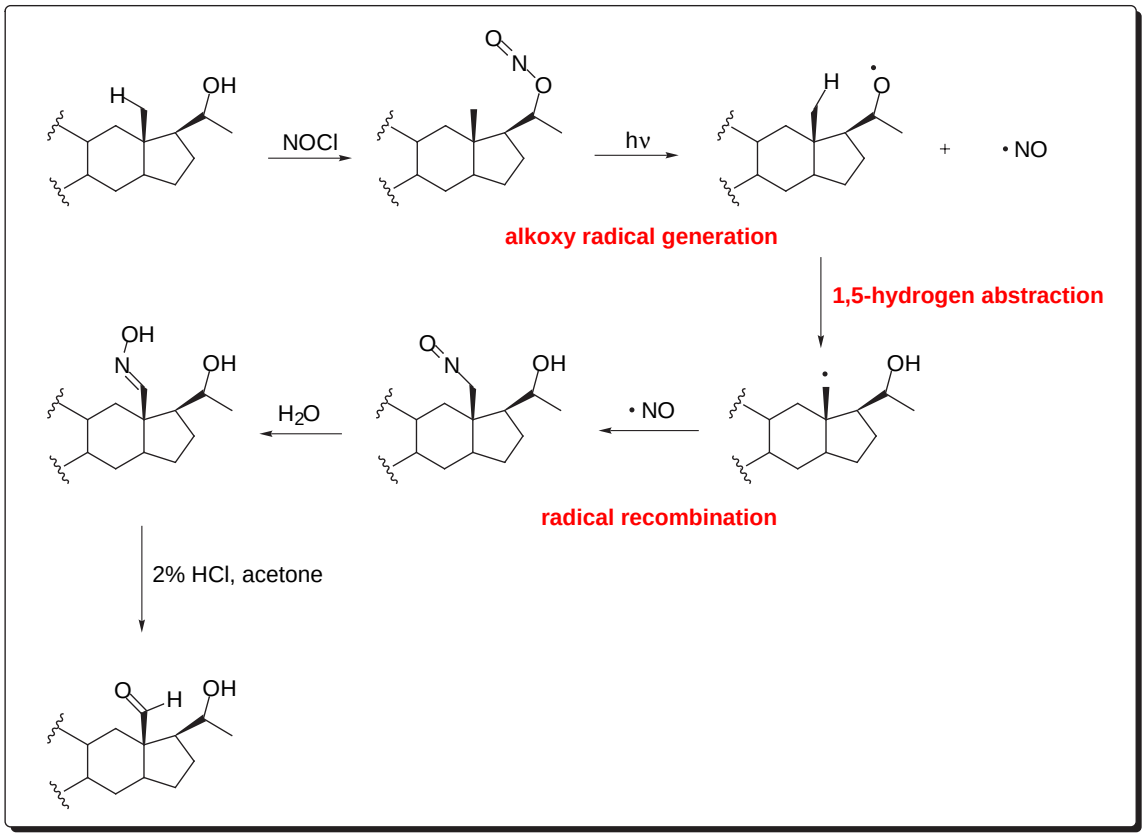


3β-acetoxy-5α-pregnan-20β-ol

The alcohol functionality at C(20) is crucial for enabling this reaction. The structure of the steroid is also very important - note in particular the close proximity of the OH and axial methyl group at C(18):

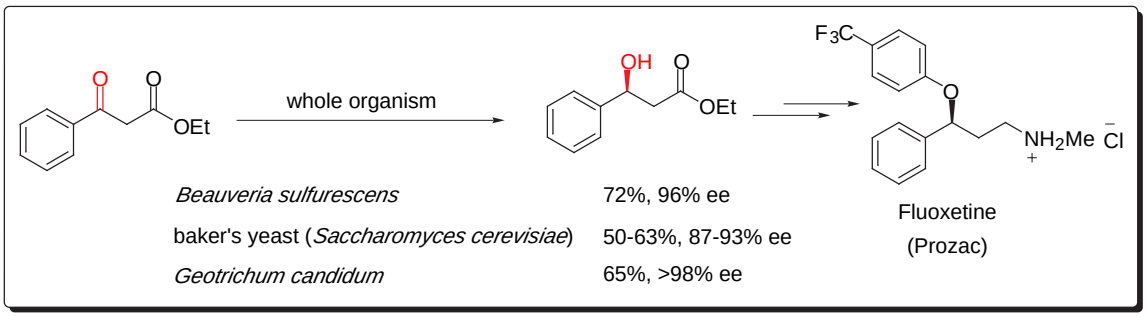


Mechanism



IX.E Reduction Reactions

Example:



- β-Ketoesters are particularly good substrates for a yeast reduction.
- The active enzyme is an alcohol dehydrogenase

- The reaction usually does not require the use of purified enzyme; instead, the whole organsim is used. However, this can lead to problems if the substrate is accepted by two enzymes (an organism will obviously contain thousands of enzymes) which impart opposing facial selectivities in the reduction. If both enzymes react at similar rates there will be an erosion in the enantioselectivity of the reaction; in these cases it is better to use the appropriate purified enzyme.

Q? How might you prepare prozac (both enantiomers) from the enantiomerically enriched β-hydroxyester?

Summary

There are many advantages to using enzymes and related systems in bond-forming processes and as a result biological methods are finding increasing utility in organic synthesis. To date their major application has been mainly in the desymmetrisation of *meso* diols / esters (lipases) and kinetic resolution of racemic alcohols / esters (lipases), and in the reduction of certain ketones (*e.g.* baker's yeast). However with the application of modern purification techniques and an increased understanding of co-factor recycling, a much wider variety of enzymes can now be routinely used in the laboratory. One area which has benefitted greatly from enzymatic reactions is the synthesis of oligosaccharides. We have seen earlier that the multifunctionality of monosaccharides necessitates elaborate and carefully designed protecting group strategies to ensure that only one alcohol is released at a time to permit regioselective glycosylation. Stereoselective glycosylation also relies heavily on the protecting groups around the sugar (especially at C(2)). Nature uses glycosyl transferases (amongst other enzymes) to effect highly chemo-, regio- *and* stereoselective glycosylation using *free* sugars. Synthetic chemists now routinely use these enzymes to carry out glycosylation reactions thereby circumventing the use of protecting groups altogether; it appears that the only limitation to this approach to oligosaccharide synthesis is the availability of the appropriate enzyme for carrying out the desired glycosylation.