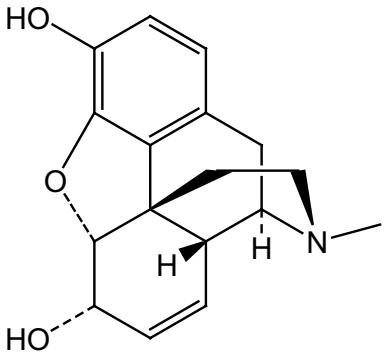
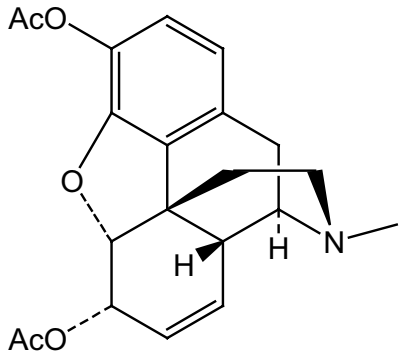
Why is asymmetric synthesis important?

Nature yields an enormous variety of chiral compounds among its natural products *e.g*.



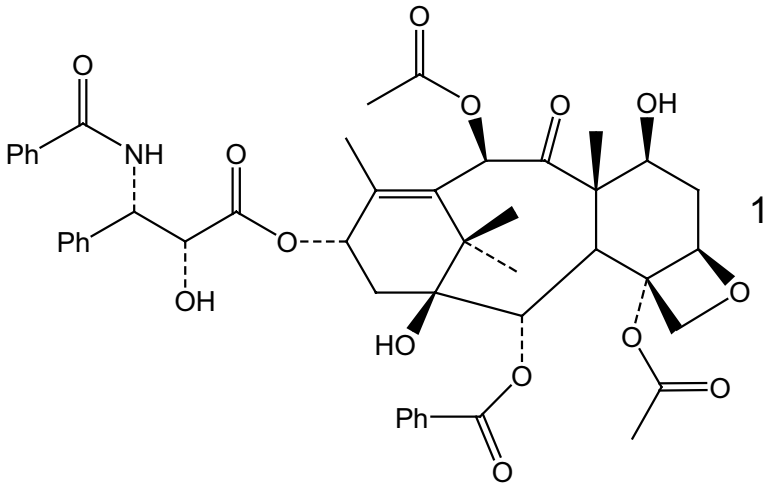
1



2

5 chiral centres

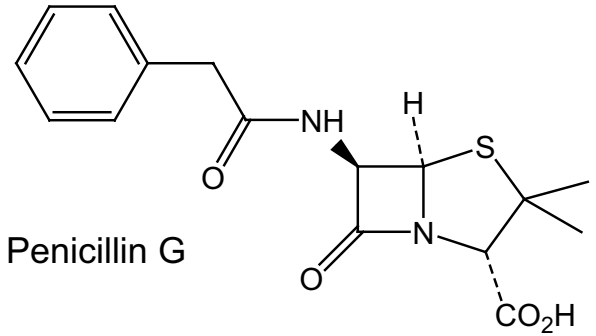
Morphine 1 and the derivative **diacetylmorphine** (heroine) **2** are vital for the control of severe pain and is isolated from the opium poppy *Papaver somniferum.*



11 chiral centres

Taxol

Taxol has been used to treat cancers such as breast and ovarian tumours for over 14 years. Taxol is a member of a family of drugs that affects microtubule formation, which is required before cell mitosis (division) can occur. In the present production process, a related compound is isolated from the leaves and needles of the European Ewe and is then chemically modified.



Penicillin G

Penicillin G is still a widely used antibiotic, despite bacterial resistance. It is manufactured by large scale fermentation of the mould, *Penicillium chrysogenum*.

All these examples have been synthesised in vitro and yet the most economic route is still to manufacture by isolation from living organisms. Obviously there is a big scope for improvement before mankind can compete with nature's synthetic processes.

Nature's high molecular weight systems

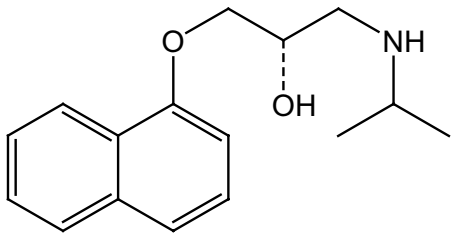
The helical structure of nature's polymeric systems (DNA/RNA) is controlled by the chirality of the sugar-phosphate backbone made up of deoxyribose or ribose.
The 3-D structure of proteins, enzymes and receptors is partly determined by the chirality of the amino-acids in the peptide chain.

Importance of asymmetric synthesis from the academic standpoint

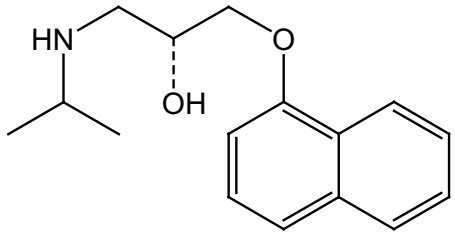
Organic chemistry is the study of carbon-based compounds, the chemistry of life. If organic chemists wish to synthesise the molecules that nature has produced then they must be able to prepare the same enantiomer as occurs naturally. Historically, the synthesis of a racemic (50:50 mixture of both enantiomers) version was accepted as a successful outcome but that is no longer the case. Synthetic chemists not only want to copy nature but to synthesise totally novel chiral structures.

Importance in Biological Systems

If the only difference between enantiomers was the direction of rotation of polarised light then asymmetric synthesis would be only of academic interest. However in living systems, chiral drug molecules interact with receptors and enzymes which are themselves chiral. The two drug receptor complexes are diastereomeric and so it is not surprising that two enantiomers can have very different effects. For example -



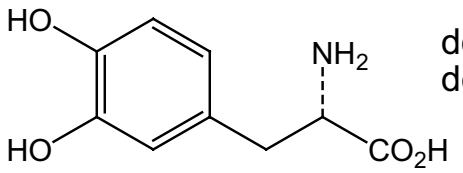
A



B

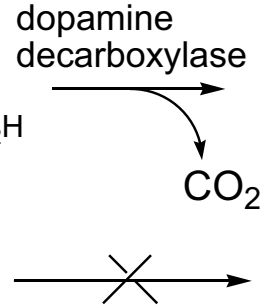
(-)-Propranolol **A** was introduced in the 1960s β-blocker used in the treatment of heart disease. The (+) enantiomer **B** is a contraceptive.

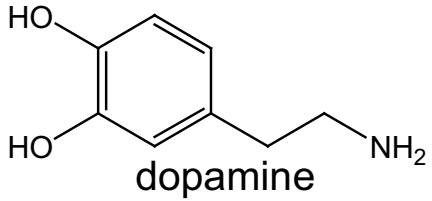
(-)-L-DOPA is used in the treatment of Parkinson's disease. The active drug is dopamine, but it cannot cross the blood brain barrier. L-DOPA can and is then decarboxylated to dopamine. (+)-D-DOPA is not decarboxylated by the enzyme and there would be a dangerous build up of the (+) form in the body.



L-DOPA

D-DOPA





dopamine

What is asymmetric synthesis? In 1971 Morrison and Mosher gave a general definition: Asymmetric synthesis is a reaction where an achiral unit is converted by a reactant into a chiral unit, such that the stereoisomeric products are formed in unequal amounts.

Optical Rotation

Enantiomers can denoted by the experimentally determined optical rotation (+) or (-) x° which is defined as:

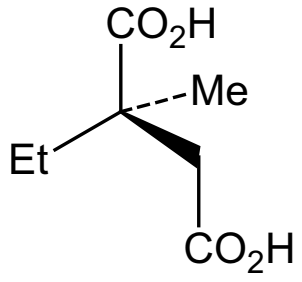
$$[\alpha]_{\lambda}^{t} = \underline{\alpha_{obs}}/l.c$$

$[\alpha]_{\lambda}^{t}$ = specific rotation, α_{obs} = observed rotation, l = cell path length (dm)

c = concentration (g/ml), t = temperature (°C), λ = wavelength of incident light (nm)

The experimental value varies considerably with temperature, concentration, and solvent and provides little reliable evidence of enantiomeric purity. Most **importantly it does not give evidence of the absolute configuration**.

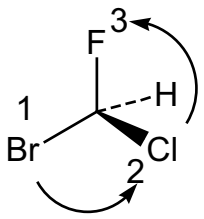
Example of specific rotation variability



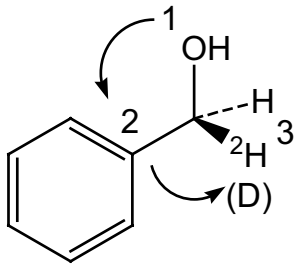
In chloroform solution:
c =0.063 g/ml $[\alpha]_D$ = 0
c >0.063 g/ml $[\alpha]_D$ = +ve
c <0.063 g/ml $[\alpha]_D$ = -ve

Stereochemistry Nomenclature

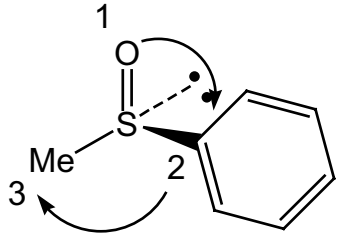
Absolute configuration of a stereogenic centre is best described using the Cahn-Ingold-Prelog rules (see previous courses for the detailed rules) used to determine (***R***) or (***S***). Some examples to help revise the rules:



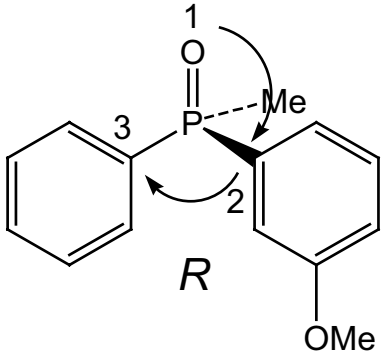
S



S



R



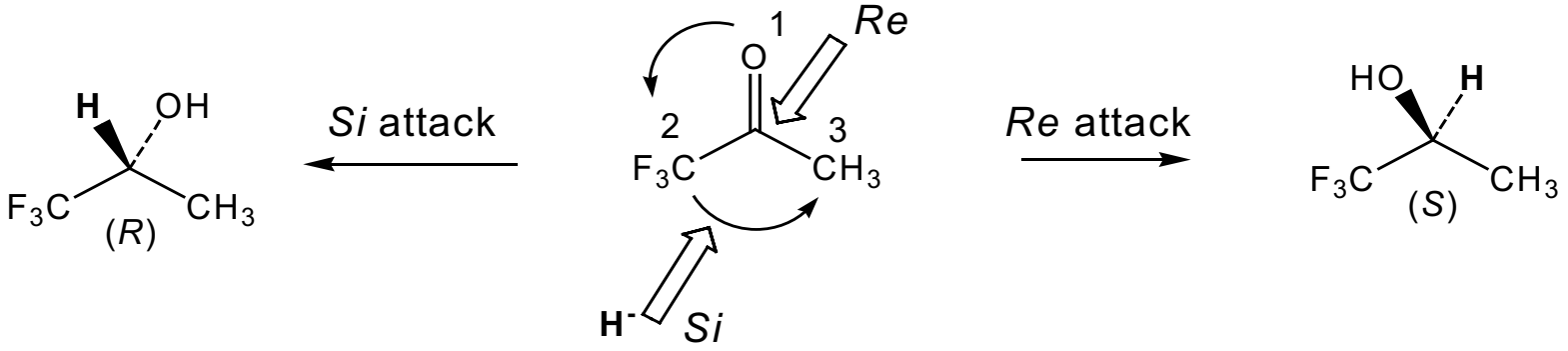
R

Topicity (face selectivity) of Enantioselective Reactions (*Re* and *Si*)

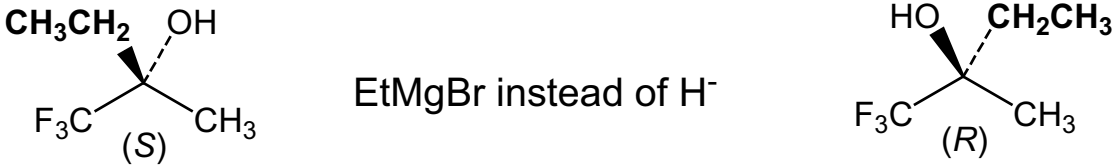
In an enantioselective reaction at a trigonal centre that generates a new asymmetric carbon, it is possible to define the face being attacked using the same Cahn-Ingold-Prelog rules. Such faces are given the term '**enantiotopic'.** Since the trigonal centre is not itself chiral, but is a potential chiral centre, the term prochiral is sometimes used instead of enantiotopic.

In this case, viewing from the side of the newly formed bond, if the groups in decreasing priority are **clockwise** it is called the ***Re* face** **anticlockwise** – ***Si* face**.

e.g hydride attack on a ketone -



Note that there is no direct connection between *Re*/*Si* and *R*/*S*. In this example, if hydride is replaced with EtMgBr, *Re* attack gives (*R*) and *Si* attack, (*S*).

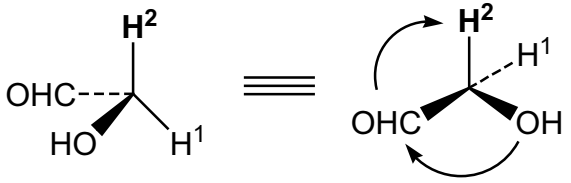


Enantiotopic atoms or groups

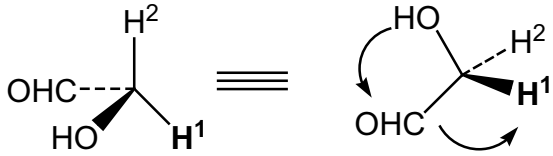
The term prochiral is also used to describe a tetrahedral group which has two enantiotopic atoms or groups i.e. C**X**$_2$WY. To differentiate the two X groups, replace one with a dummy group of higher priority.

- If dummy group gives (*S*) configuration then the atom X is pro–*S*
- If dummy group gives (*R*) configuration then the atom X is pro–*R*

This is best understood by looking at an example X = H:

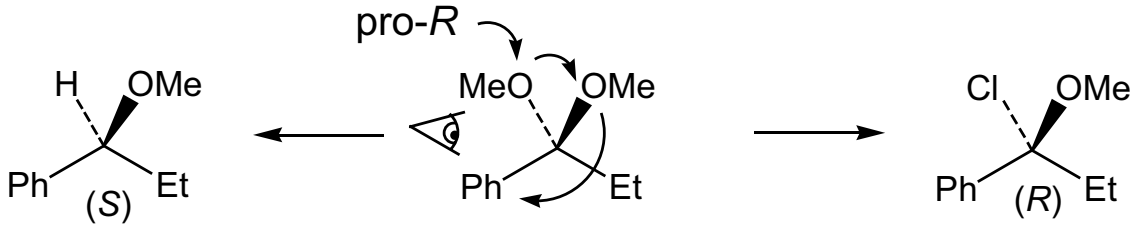


H^2 is pro-*R*



H^1 is pro-*S*

Note that there is no correlation between the designation pro-*R*, pro-*S* and the absolute stereochemistry of the product. For example – replace the pro-R methoxy in the acetal with a hydrogen gives an (*S*) isomer, replace the same methoxy with a chorine atom and the configuration is (*R*).



Determination of Enantiomeric Purity

•Measuring optical rotation is too unreliable and there is no guarantee that previously published optical rotations of the same compound will be from an enantiomerically pure source.

•The only reliable way to quantify ee is to separate the enantiomers or diasteromeric derivatives by chromatography or to distinguish them by spectroscopic means.

•Enantiomeric purity is defined by the value of the **enantiomeric excess**.

•Percentage enantiomeric excess (% ee)

$$= \frac{[R] - [S]}{[R] + [S]} \times 100 = \%\ R - \%\ S$$

Experimental techniques for separating enantiomers – In all these cases, having both enantiomers available is advisable in order to test the technique.

1. Gas chromatography using a chiral stationary phase. The enantiomers to be analysed undergo rapid and reversible diastereomeric interactions with chiral groups on the stationary phase. These **short lived diastereomeric complexes** will have **different stability** $\Rightarrow$ **separation possible**

• Compounds must be sufficiently volatile and thermally stable.

•A chiral stationary phase may only work well for certain compounds types and chiral columns are expensive.

•Quick and simple to carry out

•Measurements can be very accurate (±0.05%)

2. **Chiral HPLC – The principle is the same as for g.c.**

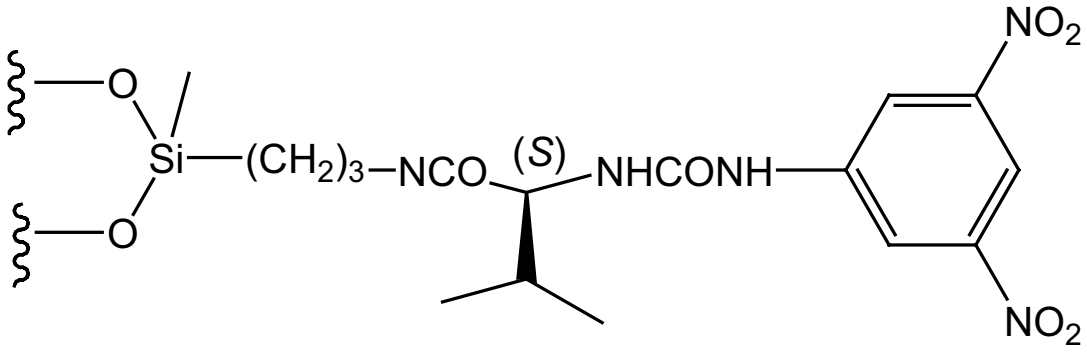
•A wider variety of compounds can be tested

•A chiral stationary phase may only work well for limited types of compounds and chiral columns are expensive, typically £1000 for a column.

•Measurements can be very accurate (±0.05%)

•Method development can be lengthy.

Example of a typical chiral stationary phase (similar to achiral reverse phase type):



Chiral group: (S)-valine linked by urea to 3,5-dinitrophenyl group

NMR Spectroscopy

Lanthanide Chiral Shift Reagents - Paramagnetic lanthanide complexes such as the chiral camphor europium complex [Eu(hfc)$_3$] bind reversibly *via* the lanthanide metal to donor sites (e.g. NH_2, OH, C=O) of the chiral molecule. This process is much faster than the NMR timescale and what is observed is an averaged downfield shift *i.e*. to higher values of δ.

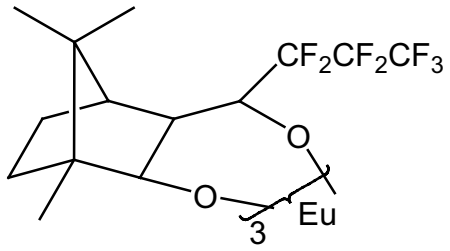
•Two enantiomers form different diastereomeric complexes and so NMR signals may be non-equivalent.

•Requires electron donor groups (lone pairs e.g. OH, NH_2, CO, COO)

•Paramagnetic complexes cause signal broadening so only add sufficient chiral shift reagent to achieve signal separation but minimum signal broadening.

•Proton NMR spectroscopy is normally used

•Simple to do but accuracy is limited to ±2%, which is the limit of NMR integration.



$Eu(hfc)_3$ + substrate ⇌ [$Eu(hfc)_3$...substrate]

Chiral Derivatising Agents (CDAs)

•Convert an enantiomeric mixture to a pair of diastereomers by attaching an enantiomerically pure (homochiral) derivative.

•Diastereomeric mixture shows a larger signal separation than for chiral shift reagents. There is no reversibility as the groups are directly bonded.

•Diastereomers can be separated on standard achiral gc and hplc columns.

•There is often a much larger peak separation on chiral gc/hplc columns.

Aspects which must be considered when using CDAs

•CDA must be enantiomerically pure.

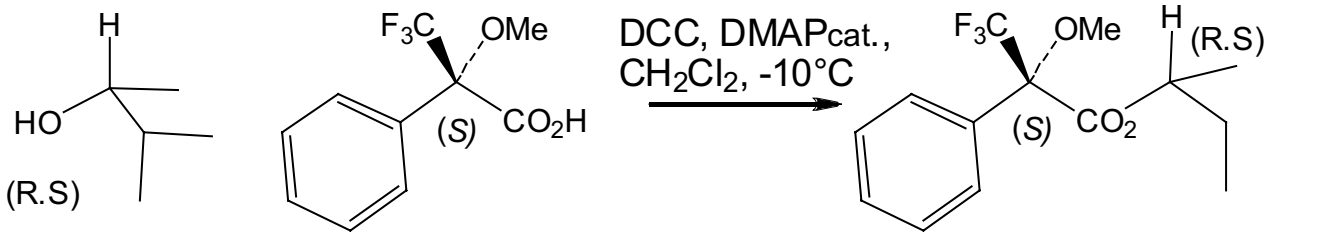
•Reaction for both enantiomers must be 100% or results will be erroneous; why care must be taken? - enantiomers can react at different rates with the CDA.

•No loss of stereochemical integrity can occur during the process, so all steps must be rigorously established.

•Limited to substrate containing: RCO_2H, RNH_2, ROH

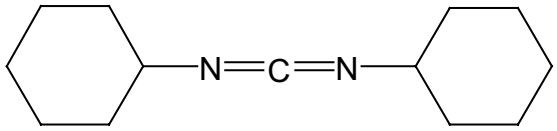
Example of a chiral derivatising agent

One of the most useful chiral derivatising agents for use with enantiomeric alcohols and amines is α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) commonly referred to as **Mosher's acid**.

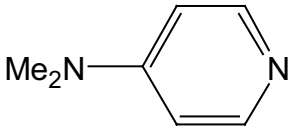


Mosher's acid in excess

a pair of diastereomeric esters



DCC - dicycohexylcarbodiimide



DMAP dimethylaminopyridine

Difference in NMR signals between diastereomers:

^{1}H NMR spectra Δδ = 0.08 (alcohol methyl signal)

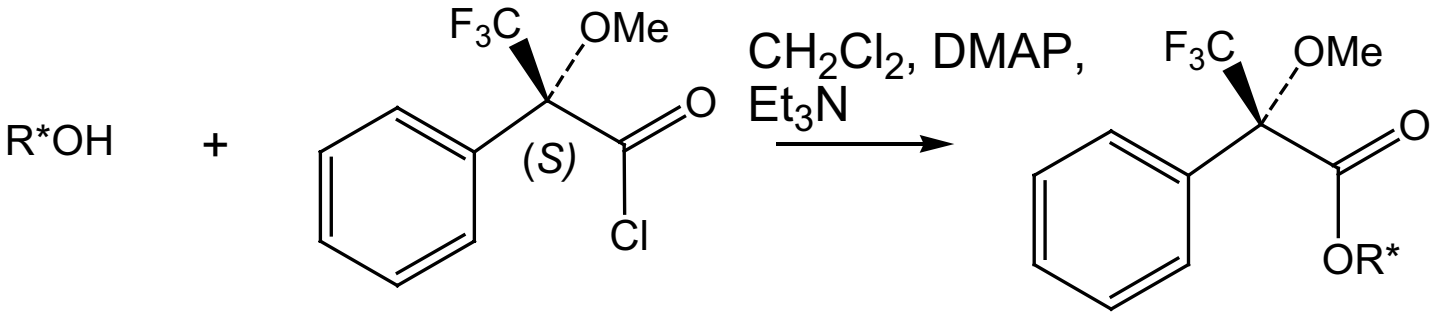
^{19}F NMR spectra Δδ = 0.17 (MTPA CF_3 signal)

- No α-hydrogen on MTPA so configurationally stable – reaction proceeds with retention of configuration.

- ^{1}H **NMR** chemical shift differences typically 0.15 ppm.
- ^{19}F **NMR** will give one signal for each enantiomer – simple to interprete.

•**Chromatography** - Diastereomers can be separated on hplc and, if they are volatile and thermally stable, on gc. Larger peak separations are normally achieved using chiral stationary phases.

In cases where the DCC/DMAP reaction is not high yielding, the more reactive acid chloride can be used.

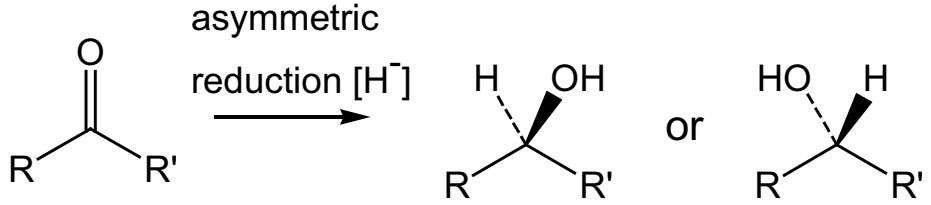


Methods of Asymmetric Synthesis

Chiral Reagents – Example – DIP chloride

Chiral reagents are consumed in the reaction; to be of practical use they must be: inexpensive, give high **ee**, high chemical yields and be convenient to use. If they fail to pass these hurdles, catalytic processes or chiral auxiliaries will always win out.

A useful reagent which has achieved widespread use in laboratory scale syntheses is **diisopinocampheylchloroborane (DIPchloride**) which is used for the **asymmetric reduction of prochiral ketones**.

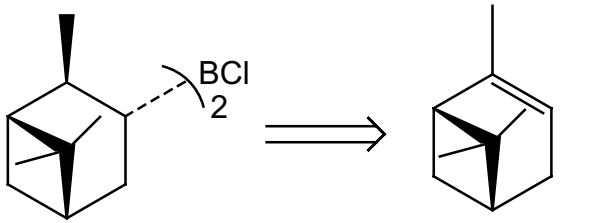


Developed by Herbert C. Brown *et al* – pioneer of organoboron chemistry - awarded the Nobel Prize in 1990 for his work in the area.

•DIP Chloride is prepared from α-pinene (cheap, produced in multi-ton quantities)

•Both enantiomers are available

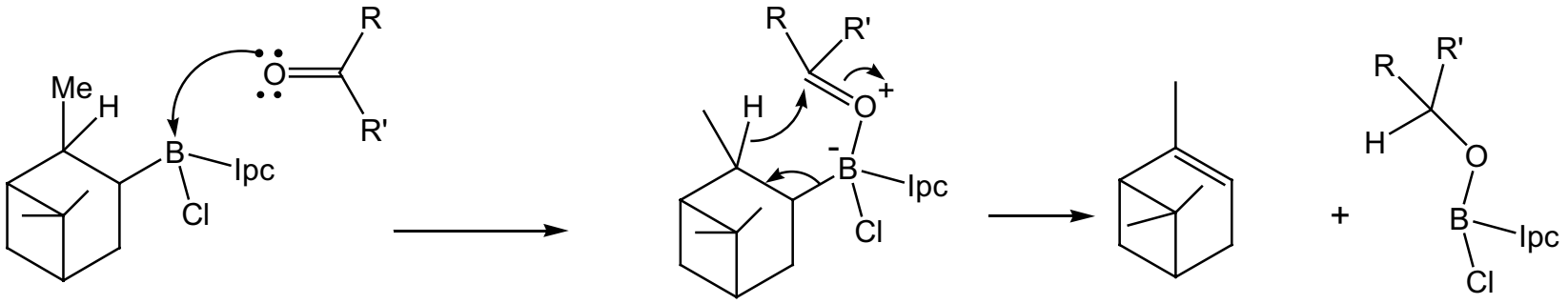
•>90% ee often achieved



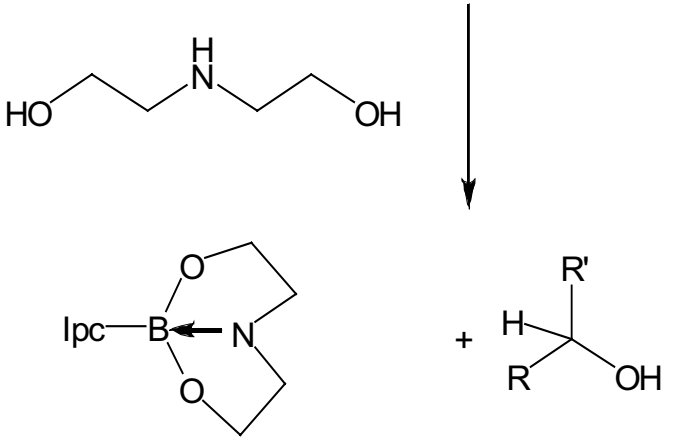
(-)-DIP-Chloride (+)-α-pinene

The reaction is carried out in diethyl ether or neat (without solvent).

The mechanism without stereochemical detail is shown:



Ipc = isopinocampheyl



crystalline
diethanolamine-boron complex
remove by filtration

Electron withdrawing chloro-substituent increases the Lewis acidity of boron.

Step 1 – carbonyl bonds to electron deficent boron (activates carbonyl to hydride attack and generation of B- activates hydride transfer

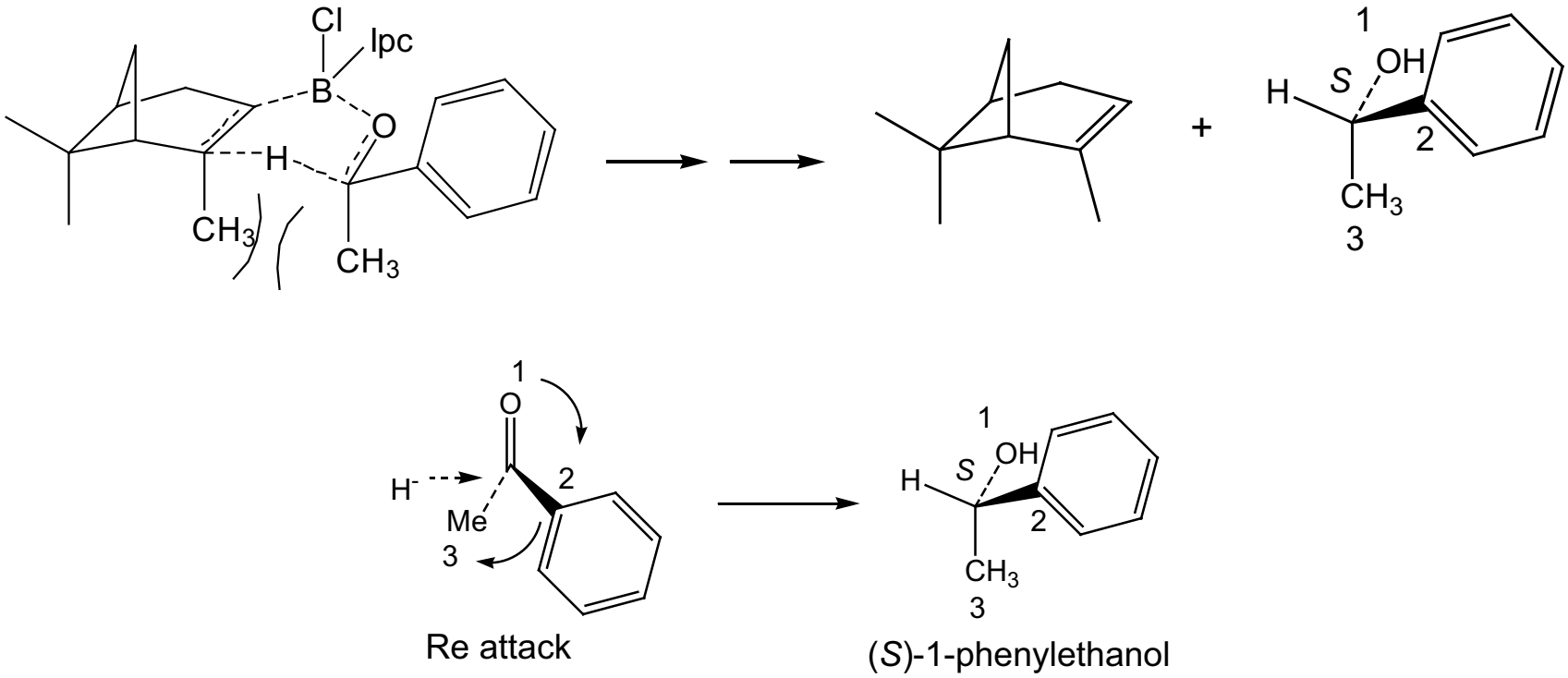
Step 2 – fast intramolecular hydride transfer with good enantioselectivity (reverse of hydroboration, see previous notes)

Step 3 – diethanolamine work-up allows easy separation of byproducts.

Enantioselectivity occurs at hydride transfer step –

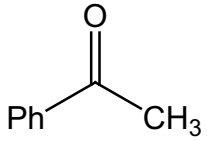
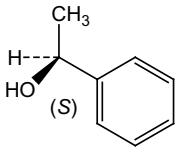
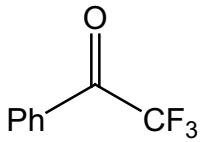
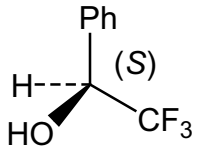
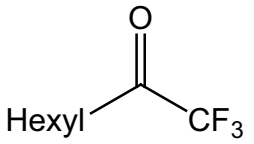
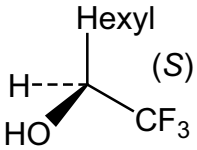
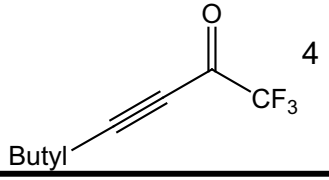
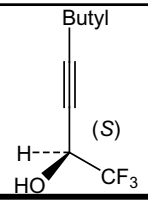
•The proposed transition state shows the ketone-borane adduct arranged such that the larger phenyl group avoids steric interaction with the pinene methyl group.

•The magnitude of ee chiefly depends on the steric differences between the groups attached to the ketone – bigger difference = high ee



Note that hydride attacks from the Re face but gives an S alcohol

Enantioselectivity data for reduction of unsymmetrical ketones with (-)-DIP Chloride

Ketone	Reaction Conditions	%ee	Product Isomer	Controlling group
 1	ether, -25°C	98		Ph
 2	ether, -25°C	90		CF_3
 3	ether, -25°C	91		CF_3
 4	ether, -25°C	>99		CF_3

Conclusions

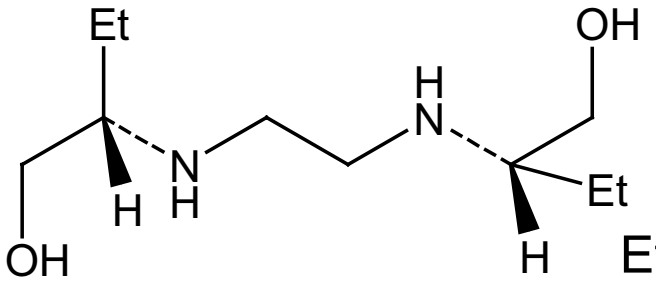
•Enantioselectivity increased by using low temperature – slower relative reaction rates for competing enantiomer

•Note that for **1** and **2** the stereochemical outcome is opposite although both are labelled *S*. This is due to the Cahn Ingold Prelog rules but the controlling groups are different.

•The steric effect of CF_3 is sometimes considered as similar to a tertiary butyl group.

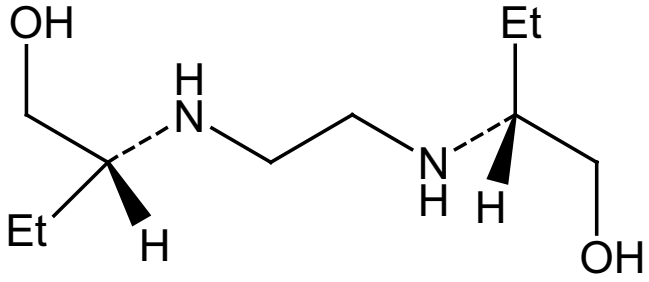
•Alkyne group is narrower than an alkyl group – greater steric difference between the trifluoromethyl and the alkyne gives higher **ee** –this provides a way to produce 2-trifluoromethylalcohols with high ee by hydrogenation of the corresponding trifluoromethylalkyne alcohols.

A Quick reminder, why asymmetric synthesis is important



Ethambutol

(*S,S*) tuberculostatic

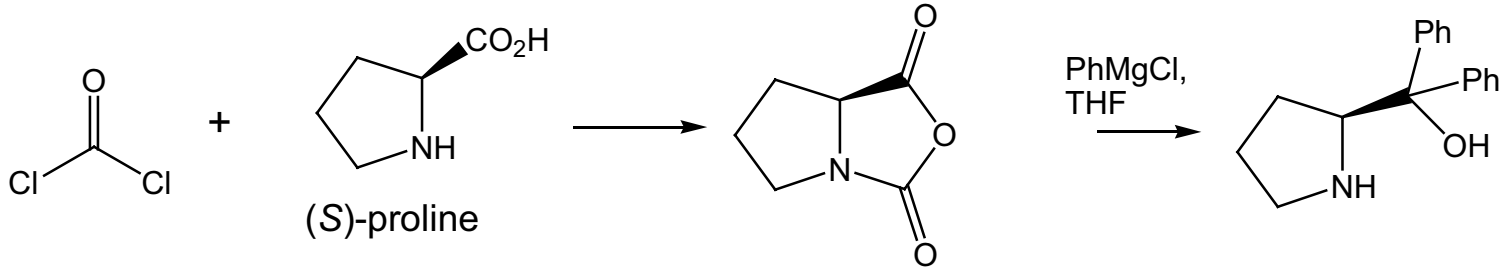


(*R,R*) causes blindness

Chiral Catalysts – Asymmetric reduction

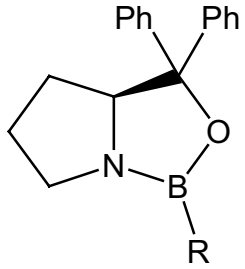
The **Corey-Itsuno oxazaborolidine catalyst** has proved a very succesful system for the catalytic asymmetric reduction of prochiral ketones - Elias J. Corey, Nobel Prize, 1990.

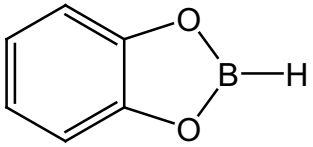
The starting material is derived from the amino-acid L-(S)-proline in two steps. Reacting the prolinol with an akylboronic acid [$RB(OH)_2$]gives the active oxazaborolidine.



- The asymmetric reduction is carried out using borane or catecholborane as the reducing agent.
- ee from 80 to 98% with 2.5 mol% of catalyst
- amenable to scale up
- catalyst can be recovered
- Catechol borane **A** (more selective) and $BH_3.SMe_2$ have also been used

oxazaborolidine

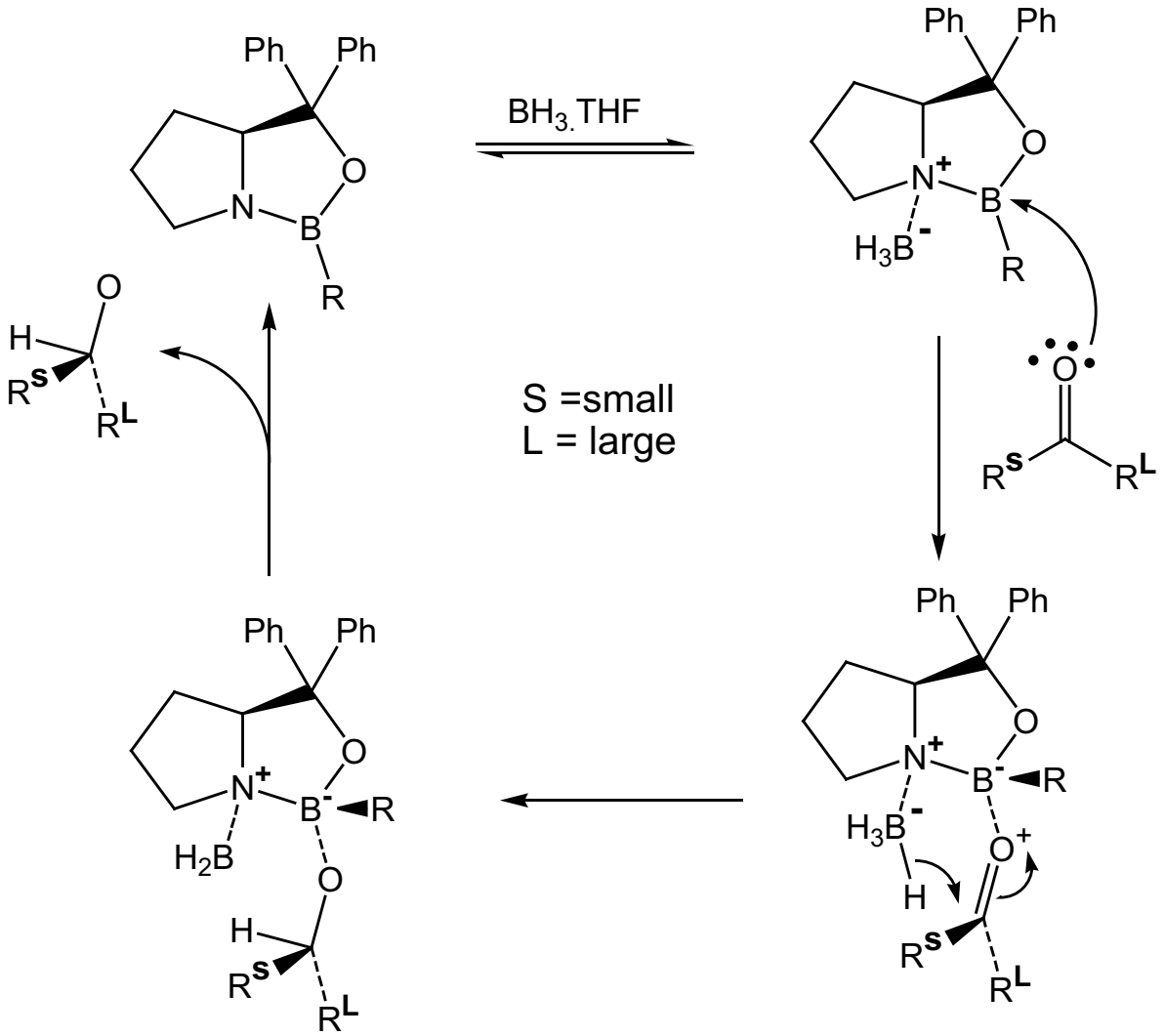


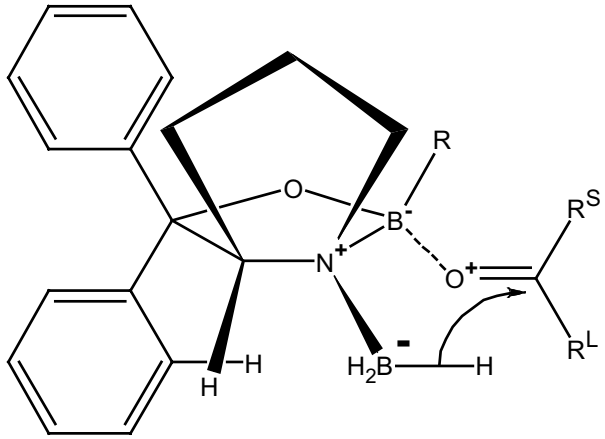


catechol borane

Mechanism for asymmetric reduction of prochiral ketones

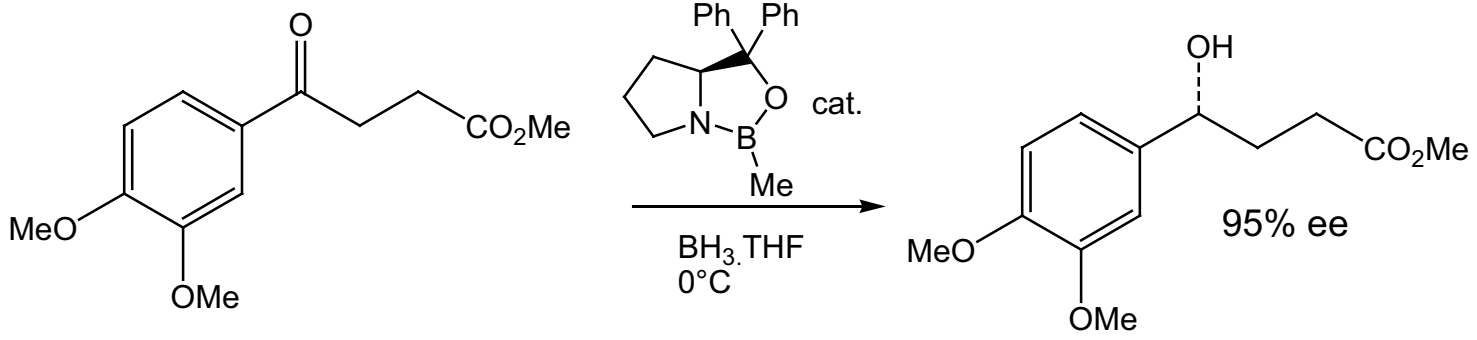
•The oxazaborolidine contains both a Lewis acid site (boron) and a Lewis base site (nitrogen lone pair). -borane bonds to to N and becomes a more reactive hydride transfer reagent -carbonyl group bonds to boron – activated to nucleophilic attack by hydride - hydride and carbonyl are positioned adjacent to each other – fast intramolecular hydride transfer is ideal for good enantio-control -large difference in size of groups attached to ketone give best enantioselectivity.





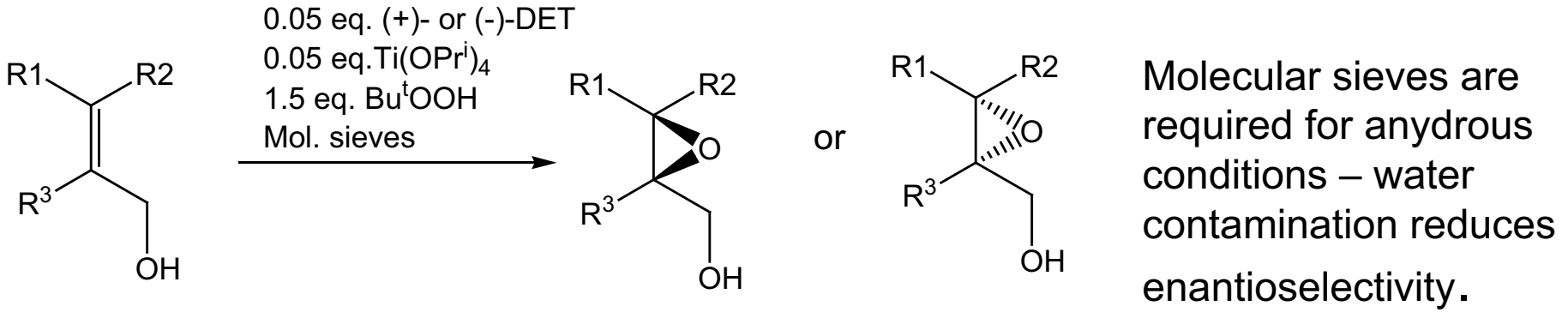
Model to explain enantioselectivity of ozazaboroldine for the asymmetric reduction of prochiral ketones. R-large lies farthest away from the other groups in an equatorial position.

Example: The following intermediate was required in a process to synthesise a platelet activating factor (PAF) inhibitor.

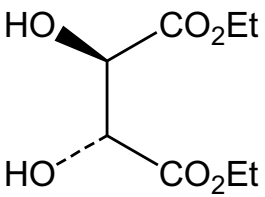


Chiral Catalysts – Epoxidation of allylic alcohols

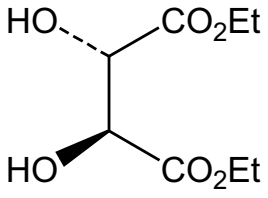
In the early 80s Barry Sharpless and coworkers developed an asymmetric epoxidation of allylic alcohols using tertiary butyl hydroperoxide as the oxidant and titanium tartrate catalysts. This method has been developed to an industrial scale – Sharpless, Nobel Prize, 2001.



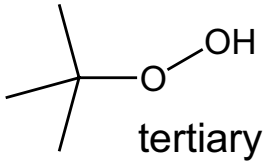
Molecular sieves are required for anydrous conditions – water contamination reduces enantioselectivity.



R,*R*-(+)-DET



S,*S*-(-)-DET



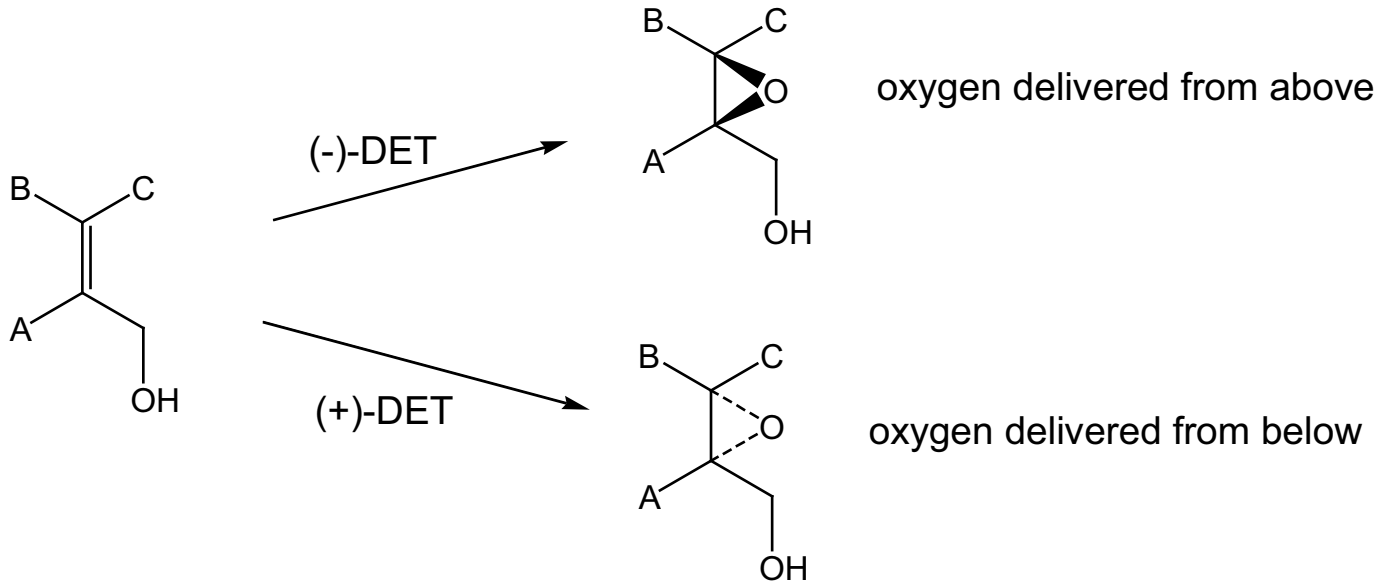
tertiary butylhydroperoxide (TBHP)

- DET = diethyltartrate from tartaric acid
- Both epoxide enantiomers can be synthesised in high enantiomeric purity.
- predictable absolute configuration
- The product, epoxy alcohols, are useful intermediates.

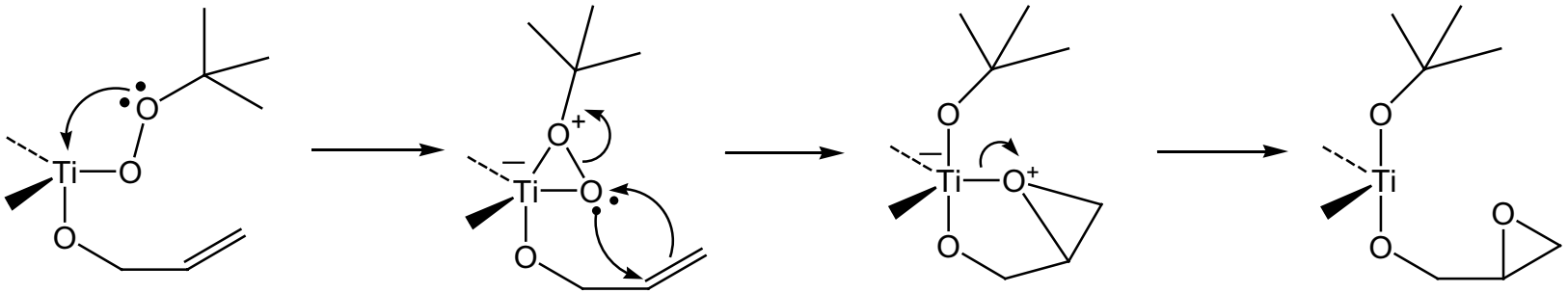
The stereochemical outcome can be predicted by drawing the allylic alcohol vertically the OH at the bottom right. In this orientation:

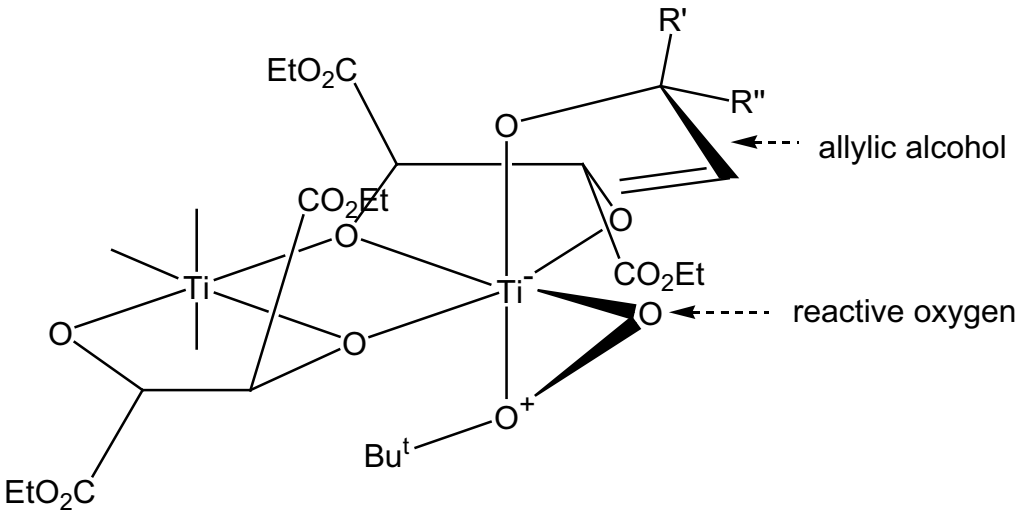
(-)-diethyltartrate (DET) delivers epoxide oxygen from above

(+)-diethyltartrate (DET) delivers epoxide oxygen from below.



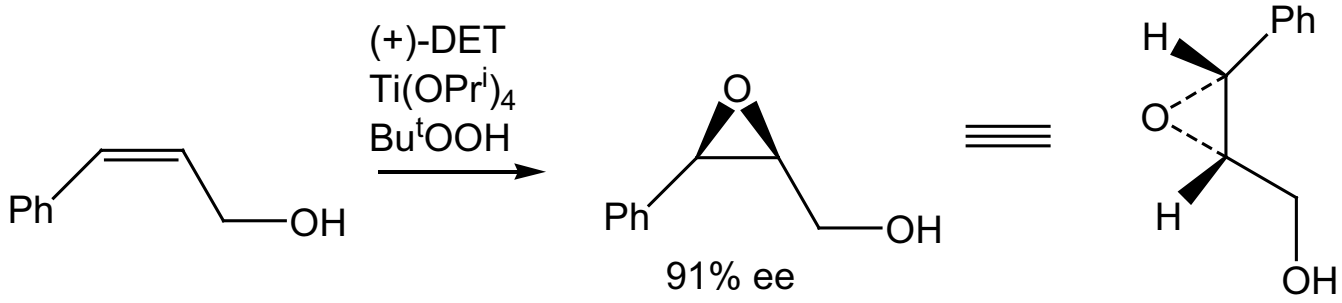
A mechanism (not including stereochemical information) shows the formation of a strained peroxonium three membered ring from the titianium tertiarybutylperoxide intermediate. This is ideally set for a rapid intramolecular epoxidation step.





A mechanism to explain the enantioselectivity is proposed to involve a di-titanium complex as shown. Mono-titanium complexes have also been proposed so this is still an area up for discussion.(**Not required to be known for examination**)

Typical example

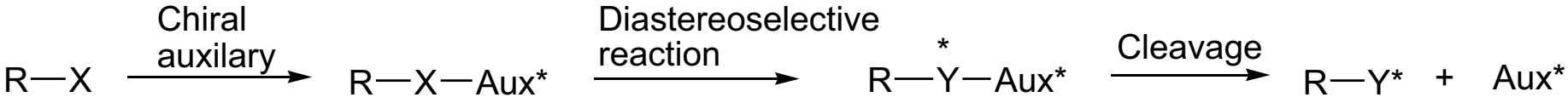


(+)-DET, attack from below

Chiral Auxiliaries

In the topics looked at so far *i.e*. chiral reagents and chiral catalysts, the enantiocontrol arises from a complex prior to the diastereoselective step. Depending on the stability of the complex there will be a reversible equilibrium to a greater or lesser extent.

A **chiral auxiliary is a homochiral group that is temporarily directly attached** to an achiral substrate (R-X) . The modified substrate undergoes a diastereoselective reaction and finally, the **chiral auxiliary is cleaved and recovered** to give a product (R-Y*) bearing a new stereogenic centre (or in some cases, several stereogenic centres).



Requirements for a chiral auxiliary

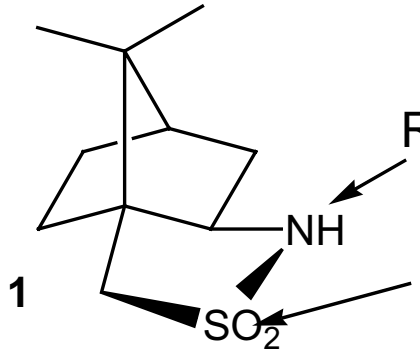
- Cleavage of R-Y*-G* must occur under mild conditions and with no racemisation.
- The auxiliary G* ideally should be low cost.
- Both enantiomers of the auxiliary should be available

Requirements for a Chiral Auxiliary continued -

•The diastereoselective step to give R-Y*-G* must proceed with very good stereocontrol and should give a crystalline intermediate. Recrystallisation of R-Y*-G* generally increases the diastereomeric purity (de) and therefore gives R-Y* with a higher ee.

Oppolzer's Camphor Sultam

Rigid tricyclic structure



Reactive group for attaching achiral substrate

Chelation site - aids stereochemical control

1

•In the late 80s, Swiss chemist Wolfgang Oppolzer developed the camphor sultam **1**, a derivative of camphor. The nitrogen is linked *via* an amide bond to the carbonyl of a substrate.

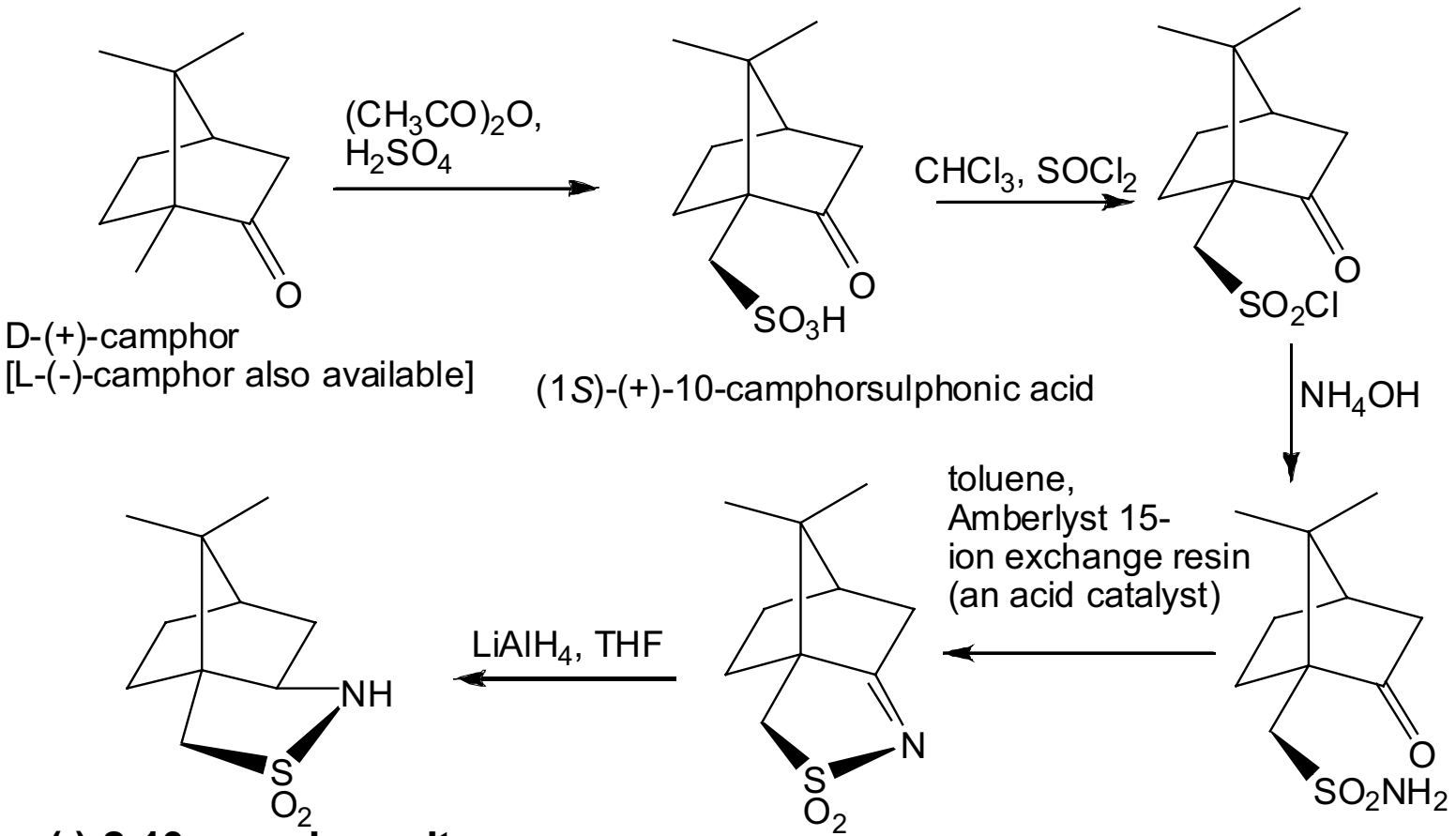
•The sultam provides a rigid tricyclic structure with bulky groups close to the site of asymmetric reaction.

•Sulphone oxygens can complex to metal ions giving further control of diasteroeselectivity.

•Nitrogen lone pair can take part in stereolectronic control in the asymmetric step.

•Several asymmetric reactions can be carried out using this system.

Synthetic Route to Oppolzer's Camphor Sultam



D-(+)-camphor
[L-(-)-camphor also available]

(1*S*)-(+)-10-camphorsulphonic acid

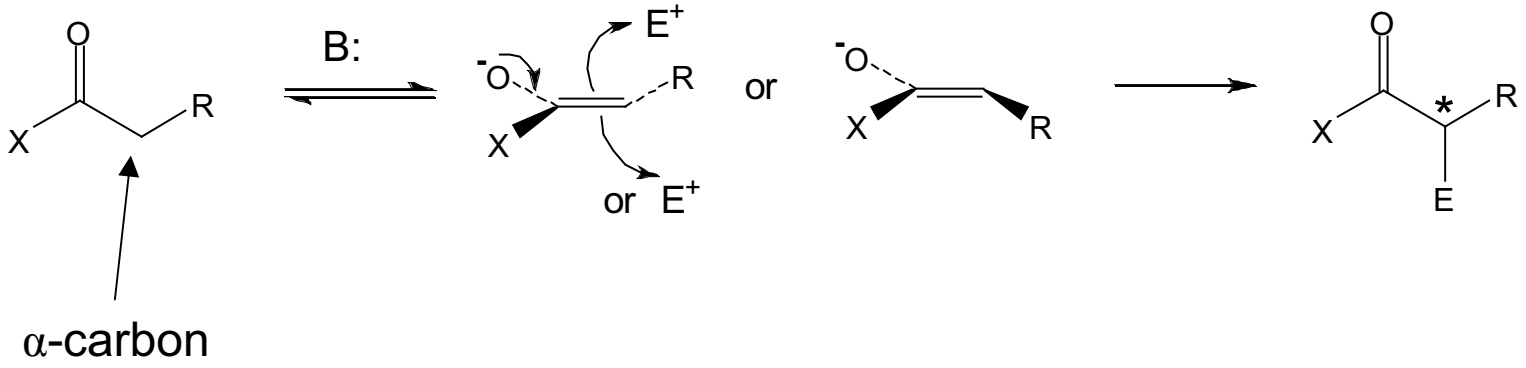
(-)-2,10-camphorsultam

Expensive to purchase the sultam but it is easily synthesised on a large scale and can be recycled with no loss of enantiomeric purity

Asymmetric syntheses using Oppolzer's camphor sultam:

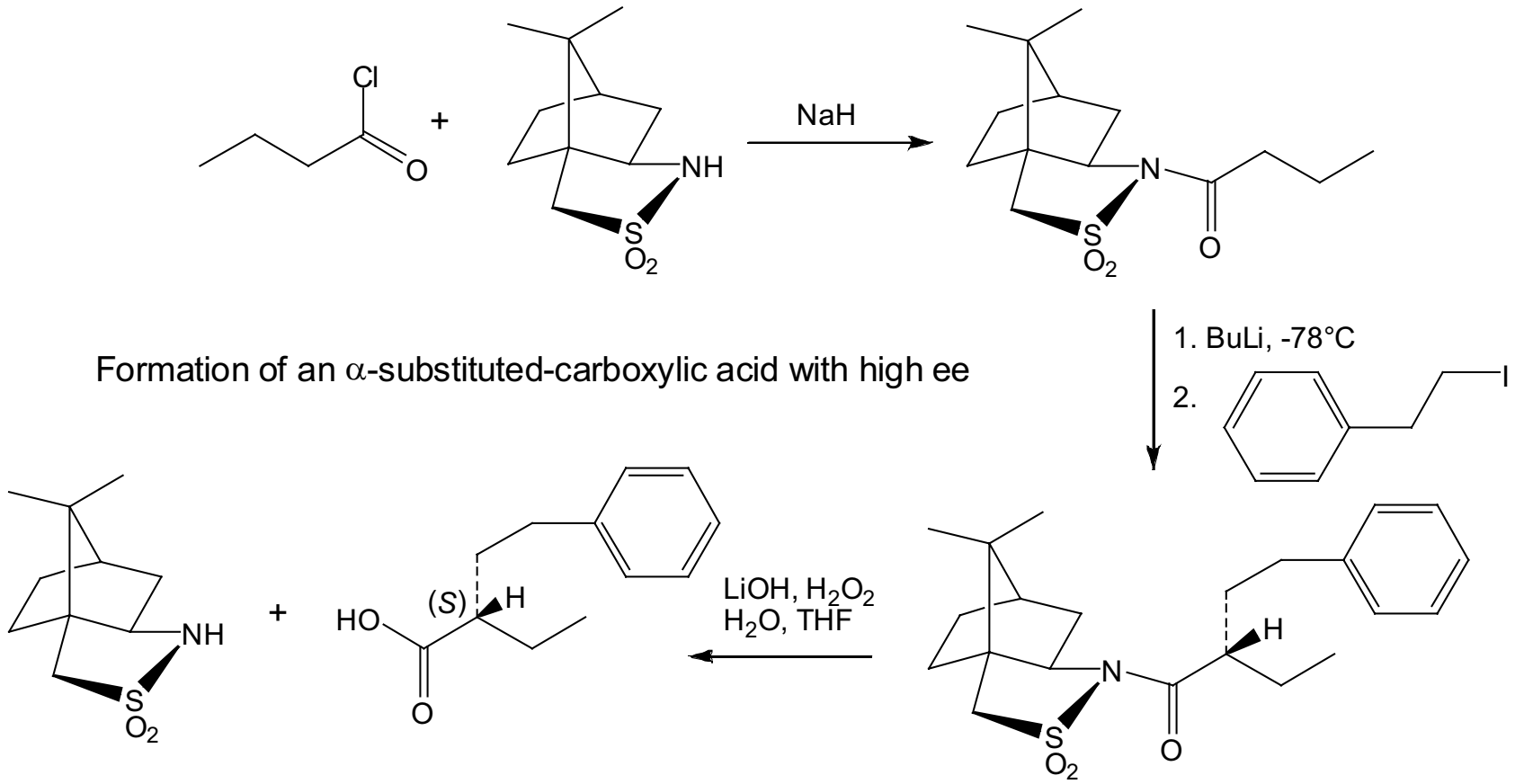
Alkylation α- to a carbonyl group

Formation of an enolate by deprotonation α- to a carbonyl group and reaction of the enolate with an electrophile is one of the most useful bond forming reactions in organic chemistry.



Enolates can have different geometries and, in addition, an electrophile can attack from or below the double bond.

This gives scope for enantioselective control of the electrophilic addition if there is a single enolate stereoisomer and it is in a chiral environment:



Formation of an α-substituted-carboxylic acid with high ee

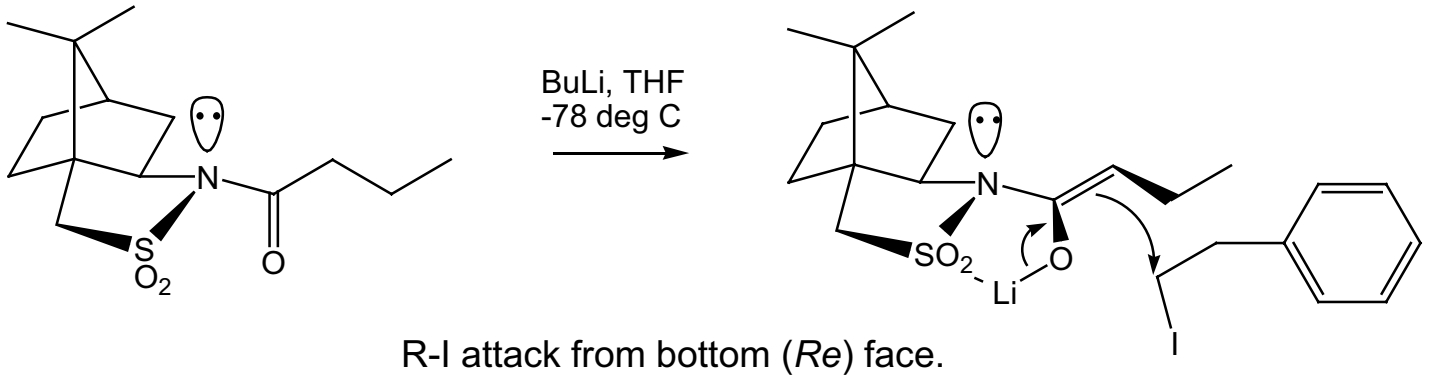
1st step: NaH deprotonates N-H prior to N-acylation

2nd step: formation of enolate using butyllithium followed by diastereoselective alkylation with an akyl halide- recrystallisation will normally increased ee

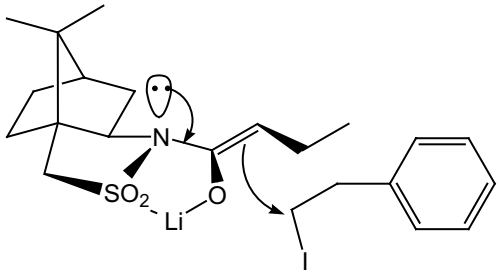
3rd step: cleavage of sultam – SO_2N-CO is easier to hydrolyse than a typical amide bond due to electron withdrawing sulphone. However, the compound is sensitive to base catalysed racemisation due to the presence of a carbonyl acidic α-proton. LiOH is more covalent than NaOH so hydroxide is less basic and more nucleophilic. Adding H_2O_2 generates HOO^- which is a much better nucleophilic than OH^- and so the cleavage can be performed at low temperature.

Rationalisation of diastereoselective enolate alkylation

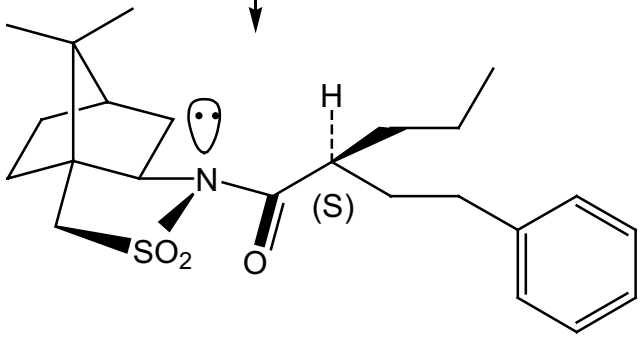
Deprotonation at low temperature gives a (*Z*)-enolate, lithium chelates to the sulphone and oxygen giving a fixed stereochemistry. Electrophilic attack comes from the bottom (Re) face. The direction of attack may be controlled by the steric hindrance of the dimethyl group or due to the nitrogen lone pair.



R-I attack from bottom (*Re*) face.

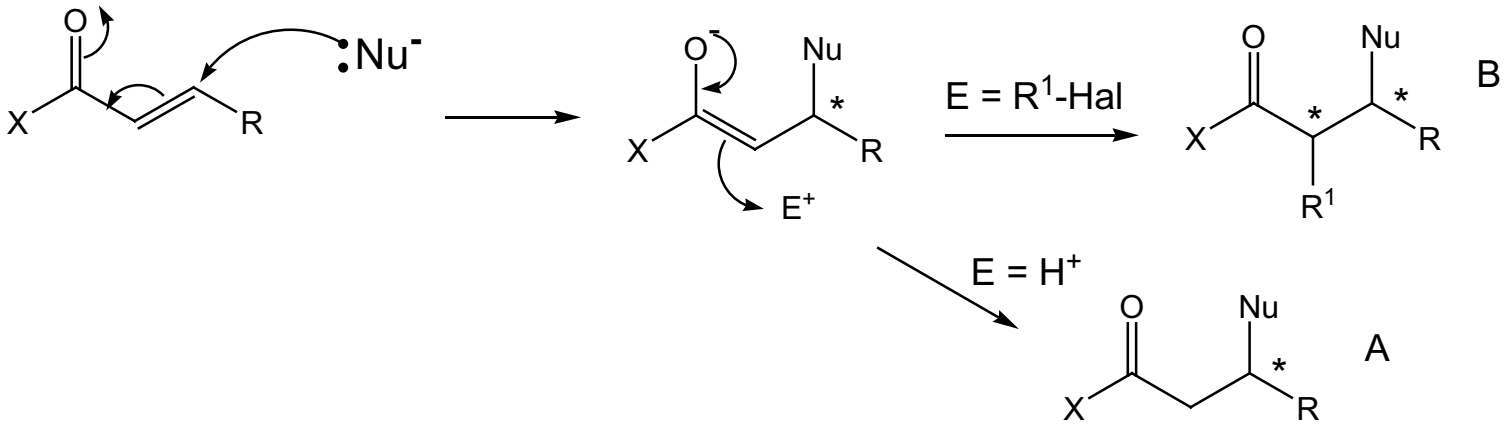


Alternative explanation of stereoelectronic control – electrophile attacks from opposite face to nitrogen lone pair



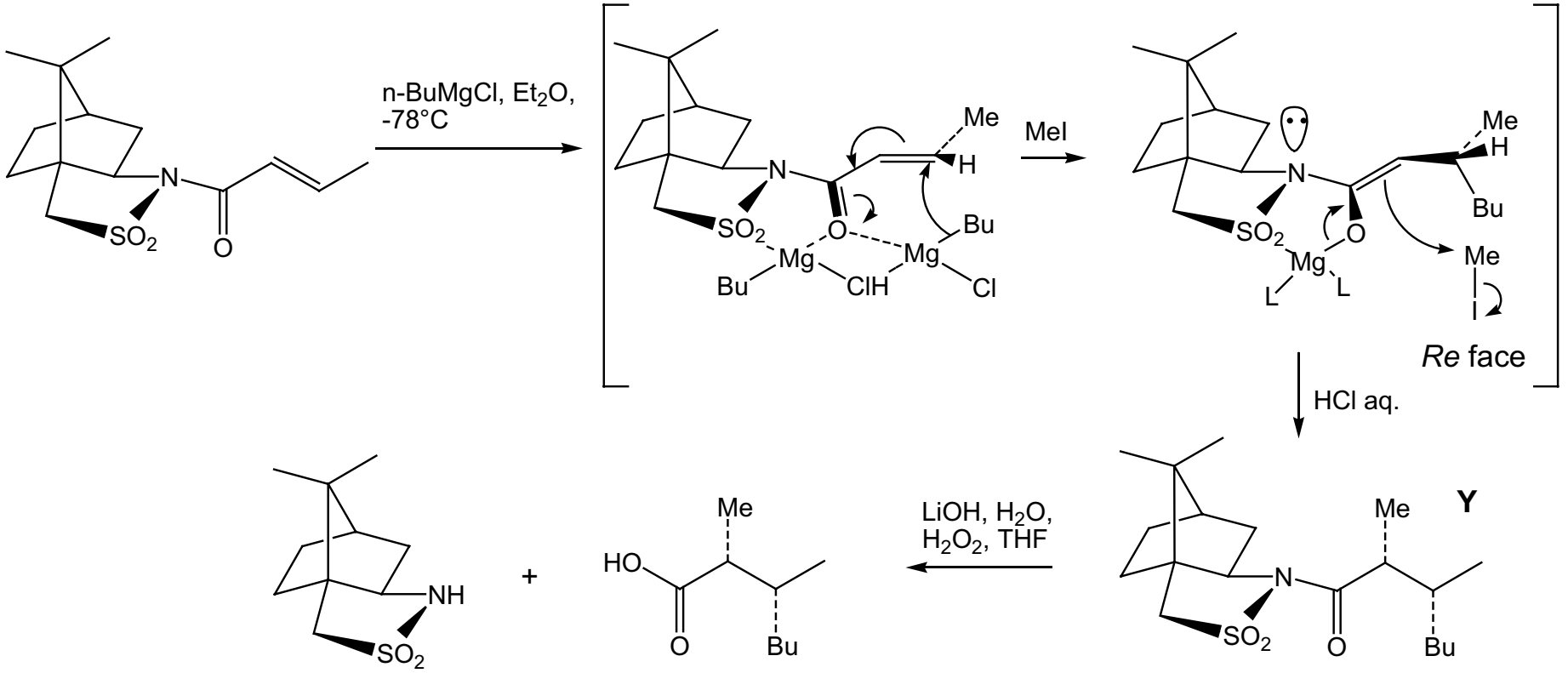
Conjugate addition to *N*-α,β-enoyl sultams

Conjugate (Michael) addition of a nucleophile to an α, β- unsaturated carbonyl group is a reaction that can be exploited using chiral auxilairies for asymmetric synthesis. Addition of the nucleophile at the β–position of the prochiral double bond can generate a new stereogenic centre. The resulting enolate may be quenched with water to give **A** or it may be trapped with an electrophile to produce a second chiral centre at the α-position i.e.**B**



The nucleophiles are normally organomagnesium e.g. Grignard or organocopper reagents. As with alkylation in the previous example, chelation to the sulphone and carbonyl oxygen plays a key role in contolling the stereochemical outcome.

Example - Grignard addition to an *N*-α,β-enoyl camphor sultam and trapping the enolate with MeI



Explanation of diastereoselectivity

•Chelation of Mg^{2+} to sulphone and carbonyl oxygen gives fixed stereochemistry.

•Intramolecular addition of n-butyl anion to lower face of (E)-double bond gives high diasteroselectivity.

•Trapping of chelated enolate at lower Re-face

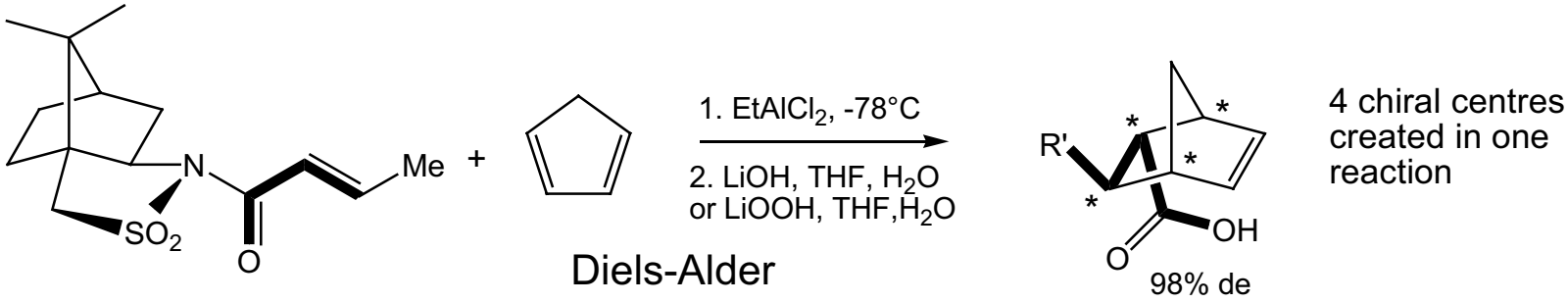
•Steric hindrance of dimethyl groups may be controlling factor in face selectivity of Me-I attack or it occurs at the opposite face to the nitrogen lone pair.

•Recrystallisation of diastereomer Y increases diastereomeric purity.

Chiral Auxiliaries - Summary

Oppolzer's camphor sultam has been exploited in many more reactions than shown in this course. Other asymmetric reactions carried out on the enoylsultam system include: Diels-Alder cycloaddition, catalytic hydrogenation (Pd/C), dihydroxylation of an alkene using osmium tetroxide, 1,3-dipolar additions. Other diastereoselective reactions of the sultam enolate include: aldol, Mannich and α-bromination.

Example

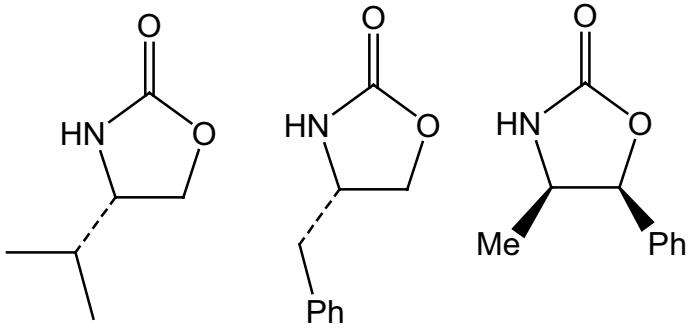


Diels-Alder

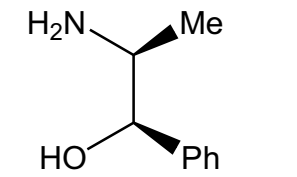
98% de

4 chiral centres created in one reaction

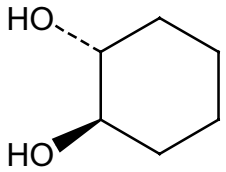
There are also many more equally noteworthy chiral auxiliaries that could have been discussed given time *e.g.*



oxazolidinones, Evans, similar applications to Oppolzer's sultam



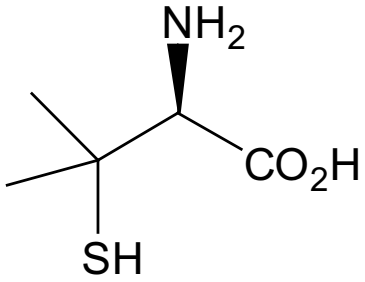
aminoalcohols
e.g. (-)-ephedrine



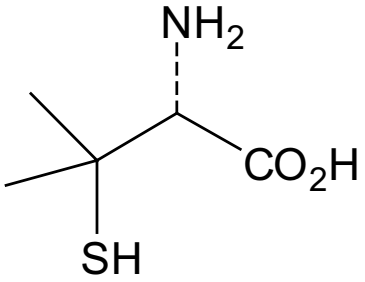
1,2-diols
with C_2 symmetry

A Quick reminder, why asymmetric synthesis is important

Penicillamine



Antidote for Pb, Au, Hg



Can cause optic atrophy - blindness

Enzymes in asymmetric synthesis

Nature's catalysts – enzymes - are proteins that have evolved, in most cases, over millions of years to carry out a particular synthetic step. **Rate increases over the uncatalysed rate of 10^{17} have been achieved by evolution**, better then any catalysts developed by human endeavour.

Pros:

•Enzymes exist to perform almost every possible reaction.
•Enzyme reactions are normally carried out in aqueous conditions – green credentials.
•However, in some cases enzyme reactions also proceed perfectly well in organic solvents.
•Enzymes are derived from poly-amino-acids so they are inherently chiral *i.e*. reactions are often highly enantioselective.

Cons:

•Only one enantiomer of an enzyme will exist.
•Enzymes sometimes limited to work with a small range of substrates.
•Modification of an enzyme to enable its use with different substrates requires gene manipulation – difficult.
•Cofactors (coenzymes) often required e.g NADPH, SAM, Coenzyme A.
•Allergic reactions sometimes occur.
•Fermenting systems – difficult to work up (emulsions) – yield by-products – cannot accept high substrate concentrations.

Classes and function of Enzymes

Enzyme major classes	**Reaction type and enzyme subclasses**
Oxidoreductases	Oxidation- reduction, transferring hydrogen *e.g* dehydrogenases (H^- transfer), oxidases (electron transfer to molecular oxygen), oxygenases (oxygen transfer from molecular oxygen) and peroxidases (electron transfer to peroxide)
Transferases	Transfer groups or atoms: amino, acetyl, phosphoryl from a donor to a suitable acceptor
Hydrolases	Hydrolytic cleavage of bonds, R-CO_2R, RCONR *e.g.* proteases, amylases, acylases, lipases and esterases
Lyases	Non-hydrolytic cleavage of small molecules from C-C, C-O, C-N by elimination to give C=C, C=O, C=N *etc, e.g.* fumarase, aspartase, decarboxylase, dehydratase, aldolase
Isomerases	Catalyse isomerisation and transfer reactions within one molecule *e.g* racemisation, epimerisation
Ligases	Catalyse the joining of two molecules *via* C-O, C-S, C-N, C-C bonds with the concomitant hydrolysis of an energy rich triphosphate (ATP)

Enzyme Cofactors and their actions

$NADP^+/NADPH + H^+$ $NAD^+/NADH + H^+$ FAD, FMN	Redox reactions and hydrogen transfer
Coenzyme A	Transfer of acyl groups
ATP	Metabolic energy, Phosphate-, pyrophosphate-transfer
Pyridoxal phosphate (PLP)	Transamination, amino acid decarboxylation
Tatrahydrofolic acid	Transfer of C1 groups
S-Adenosyl methionine, Methyl-cobalamine	Methylation

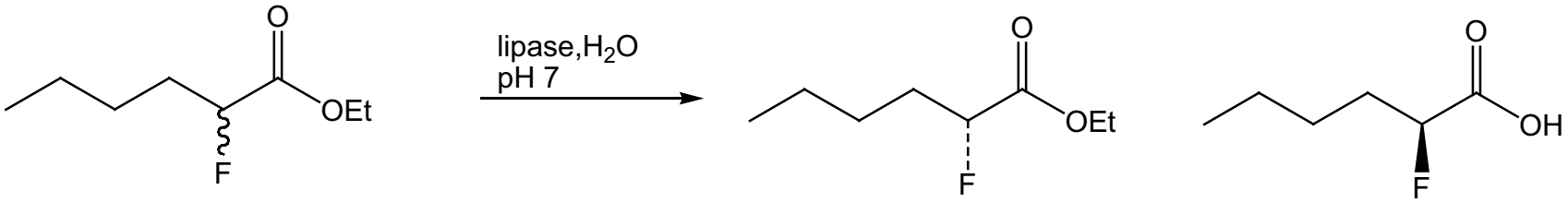
The problem of cofactors (coenzymes)

Nature uses reagents in the same way as chemists do for many reactions. If cofactors are involved, they bind to the enzyme and are intimately involved in the catalytic cycle. They are often complex molecules and as they are consumed in the reaction, there must be a recycle system

This severely limits the range of isolated enzymes that can be used as part of a simple chemical process. However recycling systems have been developed for most cofactors.

Example of a Hydrolase (Lipase PS from *Pseudomonas cepacia*, supplied by The Amano Enzyme Company)

- Lipase enzymes hydrolyse esters.
- Work on a wide range of substrates
- Often exhibit excellent steroeselectivity
- Accept nucleophiles other than H_2O (*i.e*. alcohols, amines)
- Do not require a cofactor
- Easy to use and inexpensive

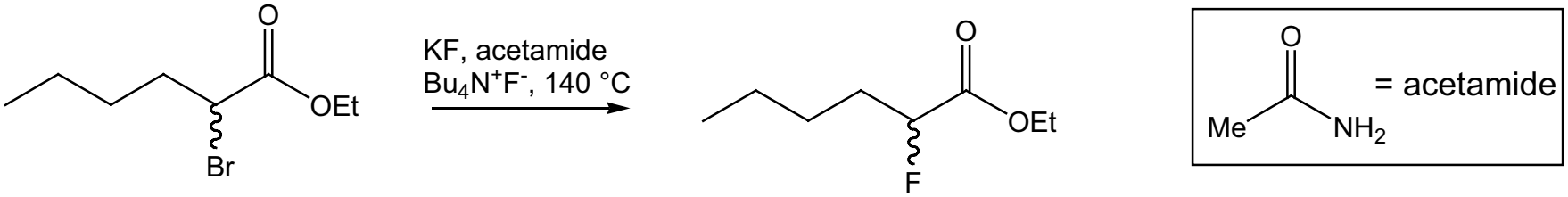


This reaction process is not strictly asymmetric synthesis but rather an enzyme catalysed kinetic resolution of a racemic ester.

- In order to achieve high **ee** the enzyme must differentiate between two groups of very similar size, **F** and **H**

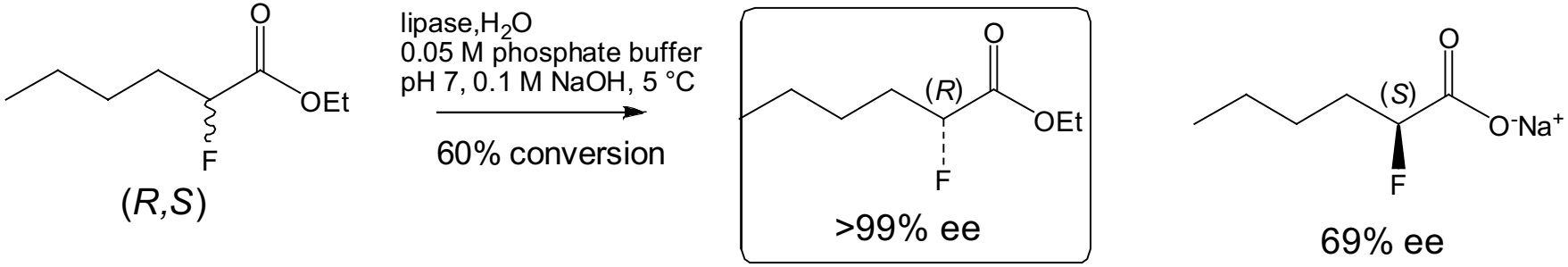
- Very unlikely that a non-enzymatic system could achieve this.

Synthesis of racemic ethyl 2-fluorohexanoate



$Bu_4N^+F^-$ is a phase transfer catalyst

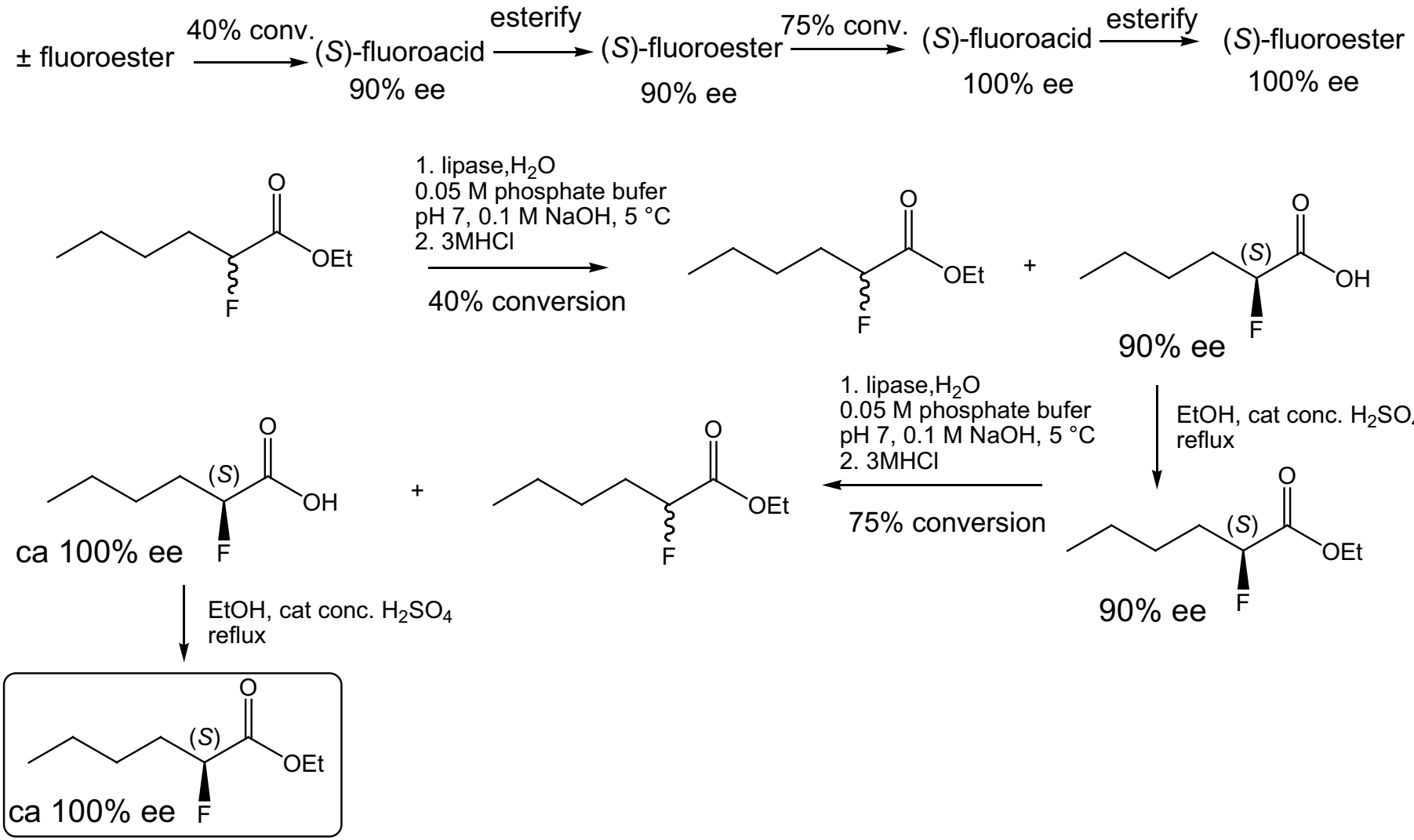
- Enzymic resolution – only requires *ca* 60 mg of enzyme for 10 g of the fluoroester.
- As the hydrolysis proceeds the formation of RCO_2H causes the pH to fall.
- 0.1 M NaOH solution is added by syringe pump (on laboratory scale) to maintain the pH at 7. This provides an accurate means of monitoring the degree of conversion.



Ethyl (*R*)-2-fluorohexanoate (99% ee) - At 60% conversion, the mixture is partitioned in an ether–water mixture, the sodium salt remains in the aqueous phase and the ethyl fluorohexanoate (99% ee) is extracted into the ether layer.

Ethyl (*S*)-2-fluorohexanoate (99% ee)

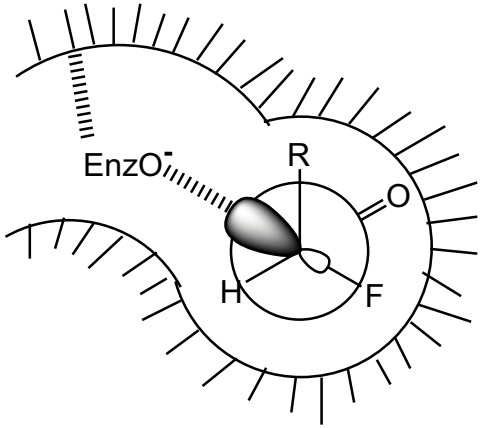
This enzyme preferentially hydrolyses the (*S*)-isomer, but selectivity is not 100%. This means that a more lengthy procedure is required to give the pure (*S*)-ethyl ester. It can be summarised as follows:

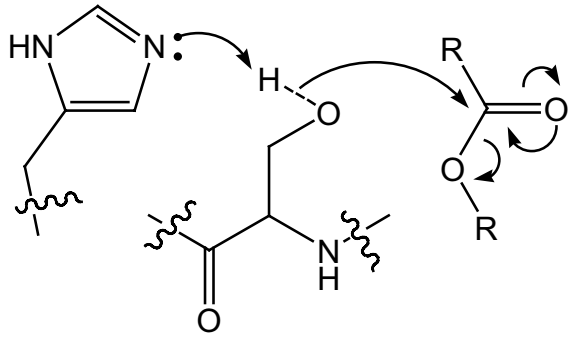


Rationale of the enantioselective hydrolysis

The similar size of H and F suggest that electronic factors must be involved, in addition to the usual steric factors, in order to give such high enantioselectivity.

During hydrolysis the ester undergoes nucleophilic attack from an alkoxide, usually from the amino acid serine. A diastereomeric complex is formed as the attack of RO^- takes place. *Ab initio* calculations show that one diasteroemeric complex is favoured by **lone pair donation of the incoming alkoxide to the σ* orbital of the C-F bond**. The result is backside attack of the alkoxide approaching antiperiplanar to the fluorine atom, before attack at the carbonyl occurs.



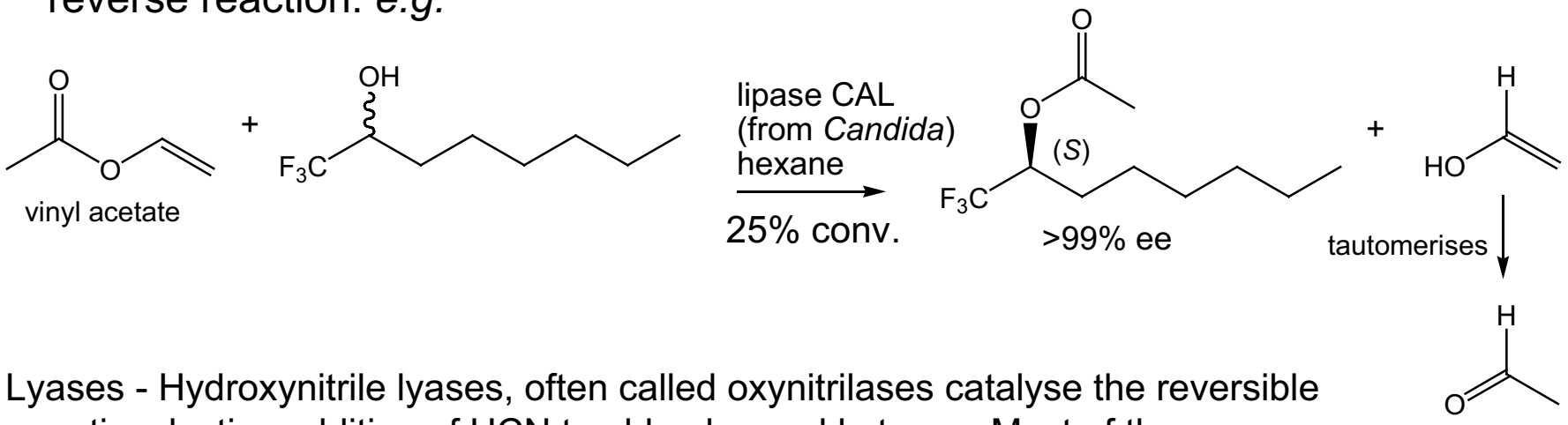


1st step of serine mediated hydrolysis

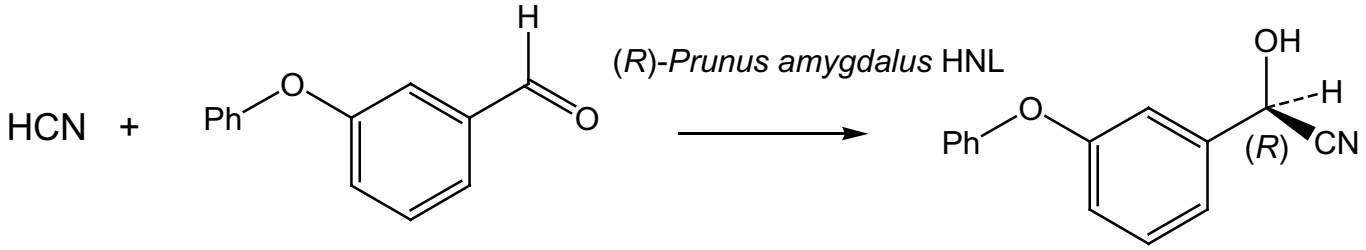
Initial histidine assisited attack of the serine OH residue on the ester carbonyl

Esterification using lipases

Lipases in organic solvents can be used to catalyse the reverse process, kinetic resolution by esterification with high enantioselectivity. Typically vinyl acetate is used as the by-product tautomerises to ethanal and prevents the reverse reaction. *e.g.*



Lyases - Hydroxynitrile lyases, often called oxynitrilases catalyse the reversible enantioselective addition of HCN to aldeydes and ketones. Most of the enzymes are obtained from plants that release HCN from cyanogenic-glycoside or -lipid. Almonds are a well known source of this enzyme. No cofactor is required

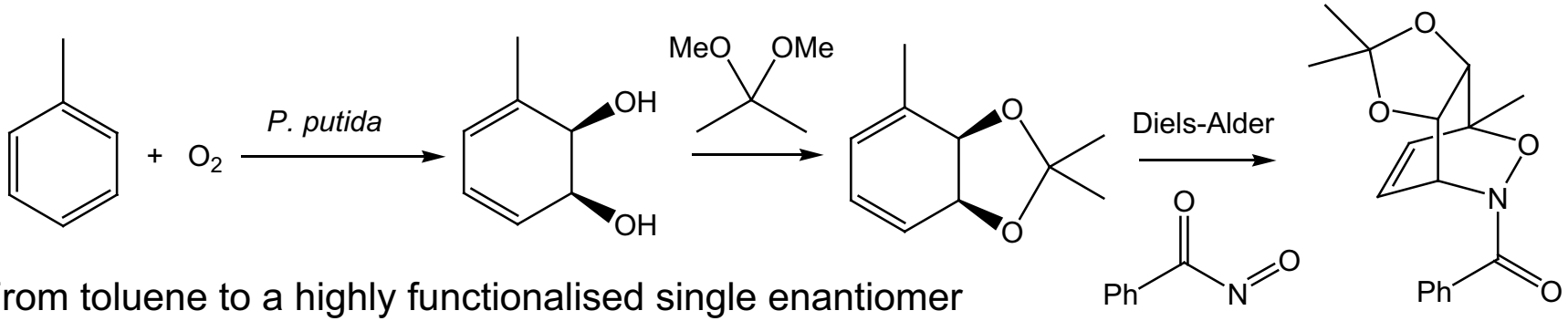


Conditions	H_2O/EtoH	yield 99%, ee 11%	Avicel is a cellulose membrane for enzyme immobilisation.
	$^{i}Pr_2O$/Avicel	**yield 99%, ee 98%**	

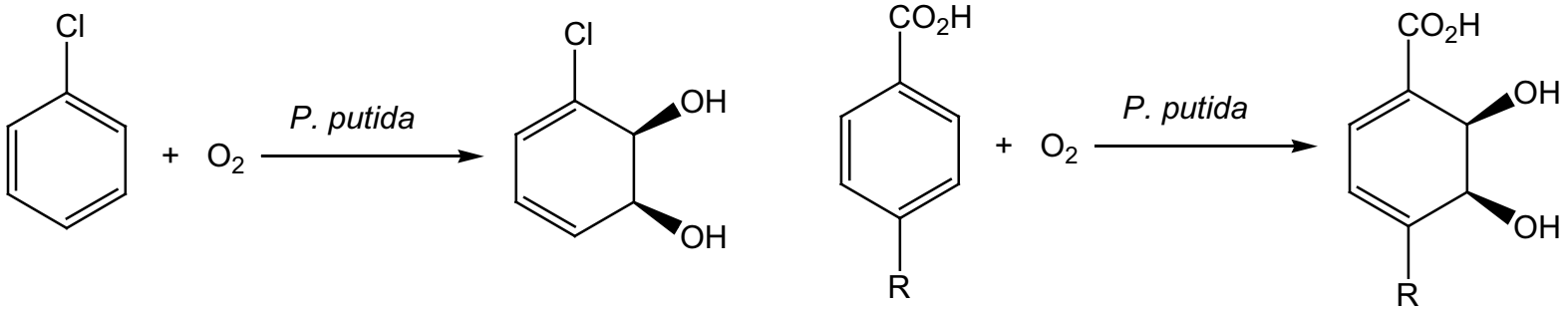
Oxoreductases

Enzymatic oxidation and reduction requires a cofactor in most cases. Nevertheless, they are still very useful and can perform reactions not readily available by conventional chemical synthesis.

For example a dioxygenase from *Pseudomonas putida* catalyses the oxidation of arenes with oxygen to *cis*-cyclohexanediols. This is an NADH/NADPH dependant system so the reactions are generally carried out with whole cells to avoid the need for added cofactors – Examples:



From toluene to a highly functionalised single enantiomer containing 4 stereogenic centres in 3 steps – other examples:



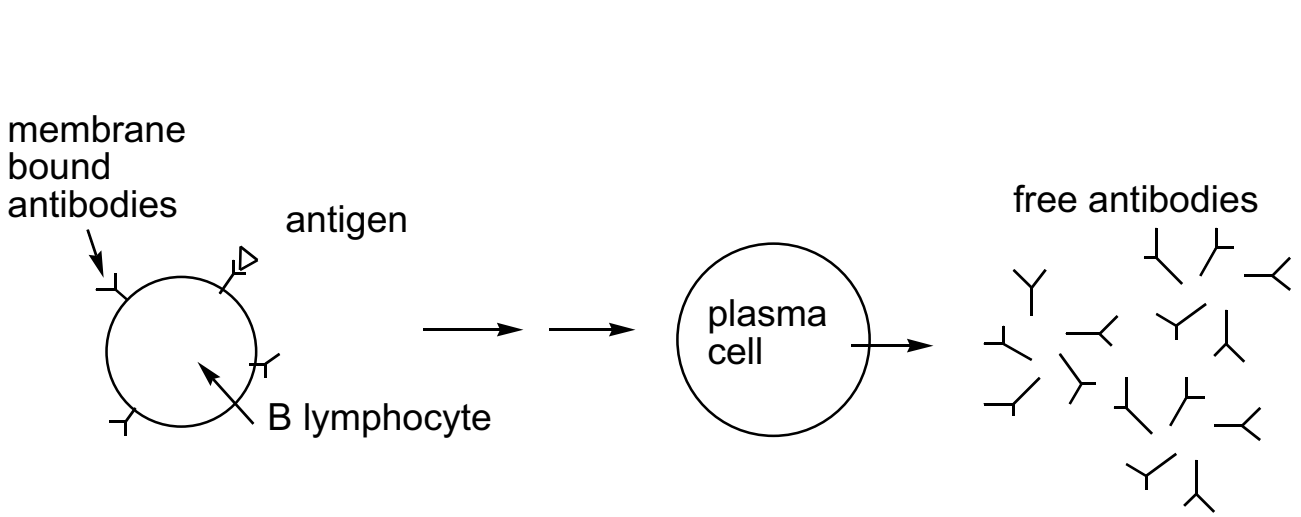
Catalytic antibodies (Abzymes)

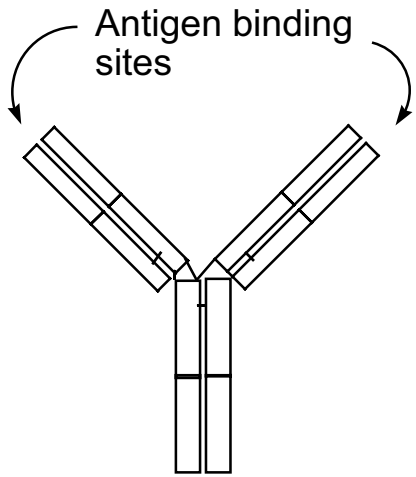
Proposal - an antibody generated to bind to a stable analogue of the transition state for the rate determining step of a chemical reaction should be a catalytic protein for that reaction.

Enzymes vs antibodies

- Enzymes evolve to bind to high energy transition states.
- Antibodies evolve to bind to molecules in their ground state.
- Enzyme evolution – millions of years
- immunological evolution of an antibody – weeks
- Antibodies can be created for both enantiomers.
- Antibodies can be created for reactions for which no known enzyme exists.

Immune system has the ability to generate more than 10^{12} unique antibodies to any antigen (foreign protein, virus, bacteria, parasite, fungi). Antibodies are glycoproteins.





Antibody protein
structure

Antibody production *in vivo*

1. Macrophage and B-lymphocytes recognise foreign antigen
2. Absorb the material into cell and cut protein into smaller peptide units - a unique system of rapid mutation called gene translocation is able to 'evolve' an antibody to any possible antigen**.** In B-lymphocytes this is catalysed by an enzyme called antibody recombinase. The mutation rate is 100,000 faster than occurs in other cells and generates membrane bound markers on the cell surface.
3. These makers bind to helper T-cells which release lymphokynes
4. Lymphokynes switch on B-lymphocytes to mature and produce antibody producing plasma cells
5. Plasma cells release large amounts of free antibodies into the serum (2000 antigen molecules per second from one cell)
6. Free antibodies-bind to antigen – quickly recognised and eliminated

How can we use immune system to generate catalysts?

•Design transition state analogue – a **hapten**

•Conjugate (link) the hapten to a larger antigen *e.g.* a protein such as BSA, bovine serum albumin or keyhole limpet hemocyanin (KLH).

•Raise antibodies

•Screen for hapten-bound antibodies

•Screen for catalytic activity

•Select monoclonal antibody and amplify production of the protein catalyst to give typically 30 mg of purified antibody.

This method required innoculation of animals. Now most of the process can be performed in vitro. This requires cloning the immunological system of a hyper-immunised mouse into a bacterio**phage** which infects *E.coli*. The result is that the enormous number of possible antibody proteins are encoded in the bacteria. In some cases the initial animal immunisation can be avoided.

A **phage** is a single strand dna virus which infects bacteria.

Concept first successfully demonstrated -1986

Examples

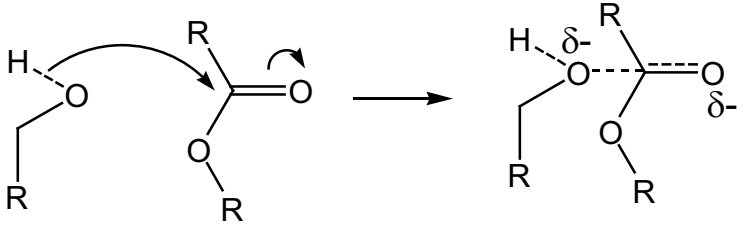
Key to success - choice of reaction and design of the hapten

Hydrolytic antibodies have achieved 10^6 x rate of uncatalysed reactions.

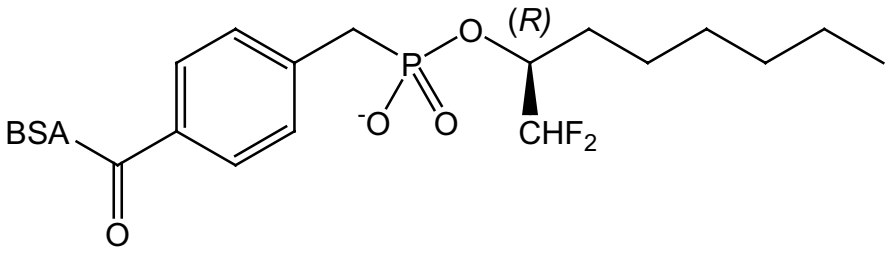
Kinetic resolution of an ester by enantioselective hydrolysis

•Hapten design – transition state has a tetrahedral shape with three oxygens, two of which have a partial negative charge.

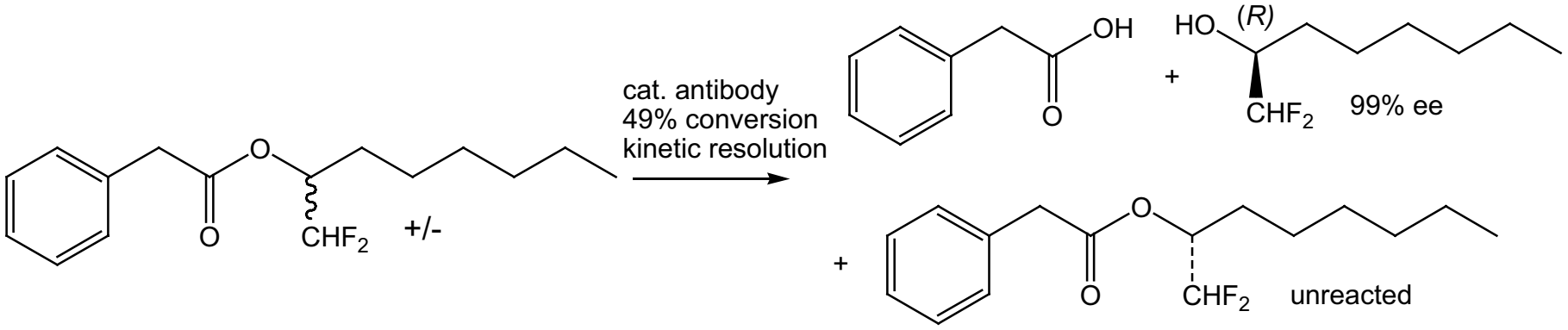
•A phosphonate ester has a similar shape, charge distribution and is stable.



RDS of ester hydrolysis



hapten – homochiral tetrahedral phosphonate ester linked to bovine serum albumin antigen

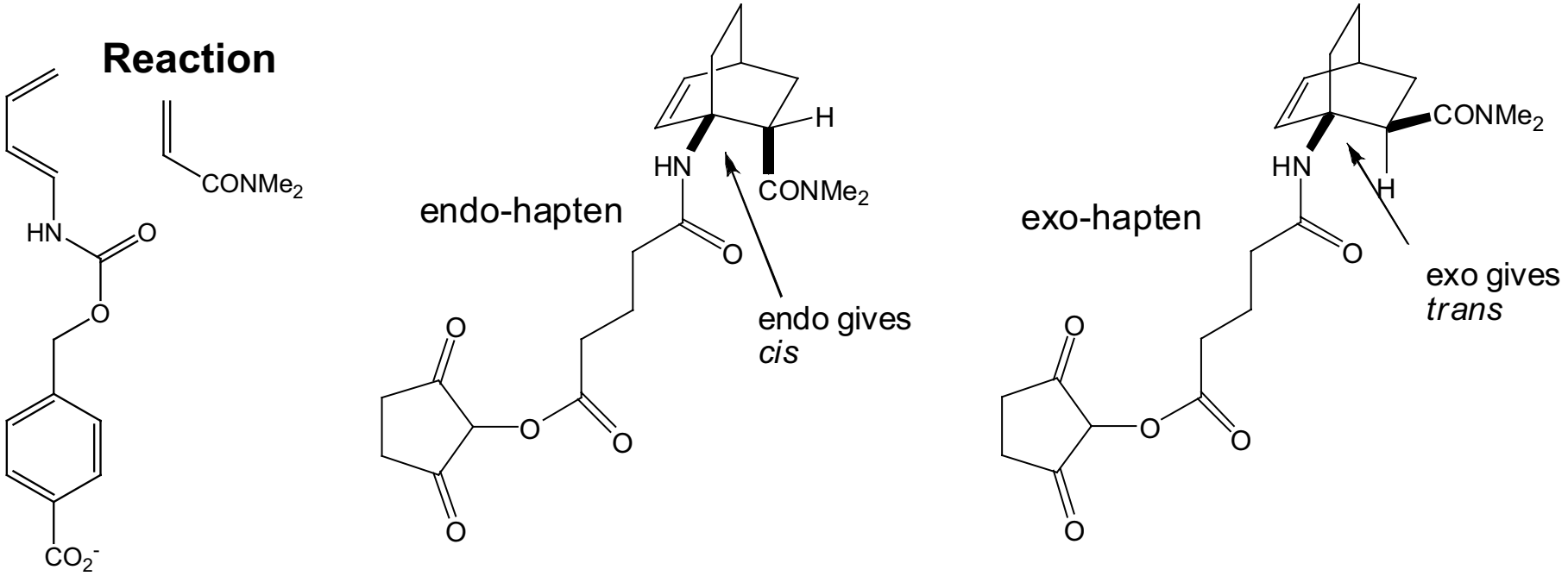


•Catalytic antibodies are not as efficient catalysts as enzymes, but are useful for reaction for which enzymes are very rare and they can be obtained for almost any substrate. **Anti-bodies for both enantiomers can be generated**.

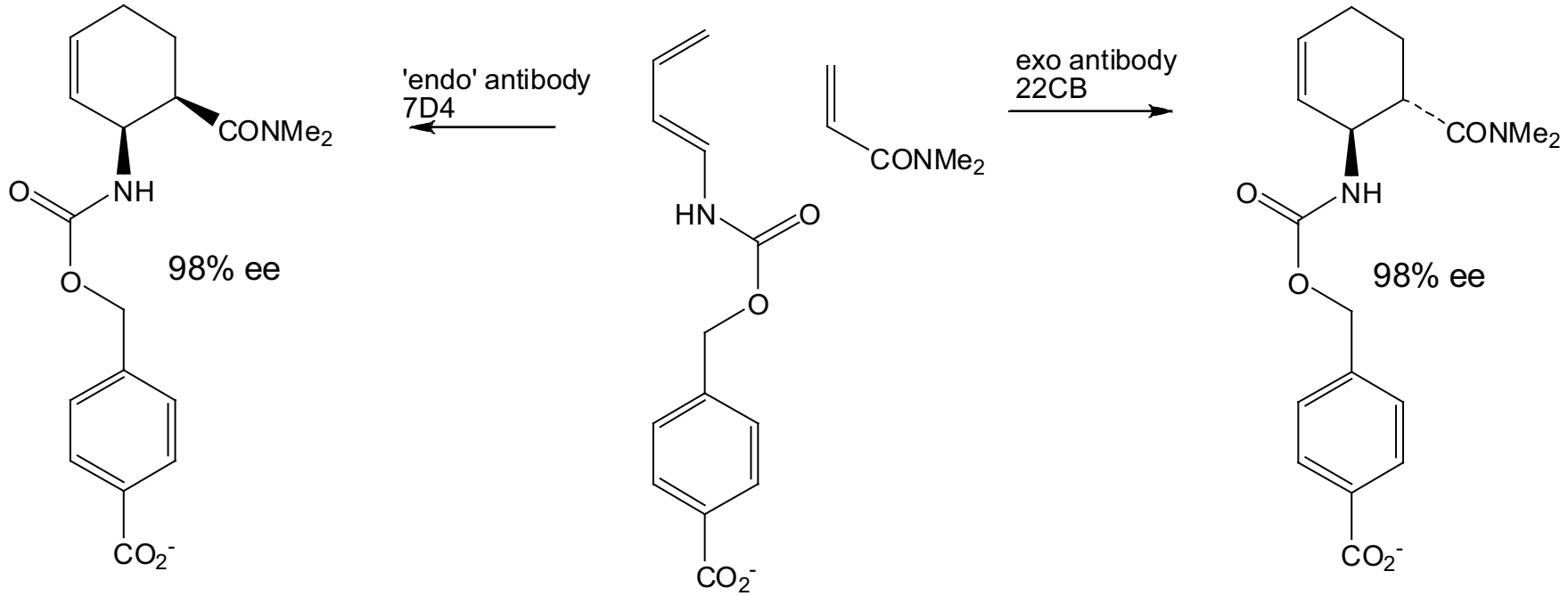
•Pericyclic reactions – almost no enzyme counterparts exist, examples have only recently been discovered.

•Example: A Diels-Alder reaction between a mono-substituted diene and a dienophile could theoretically yield 8 stereoisomers.

The aim is to control: regio- and stereo- and enantio-selectivity. Two rigid bicyclic haptens were designed, with the boat-shape of the Diels-Alder transition state. One was the **favoured endo**-transition state and the other for the **disfavoured exo**.



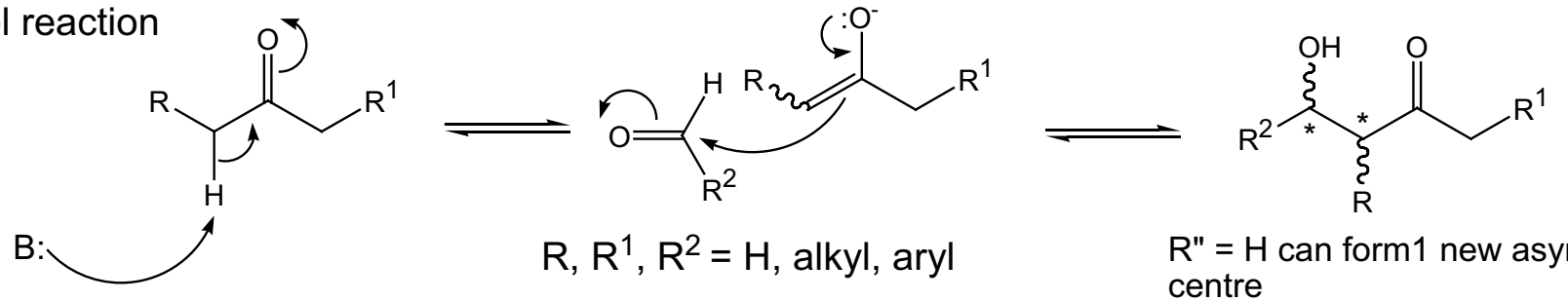
Diels Alder adducts obtained using the catalytic antibodies raised against the endo and exo haptens



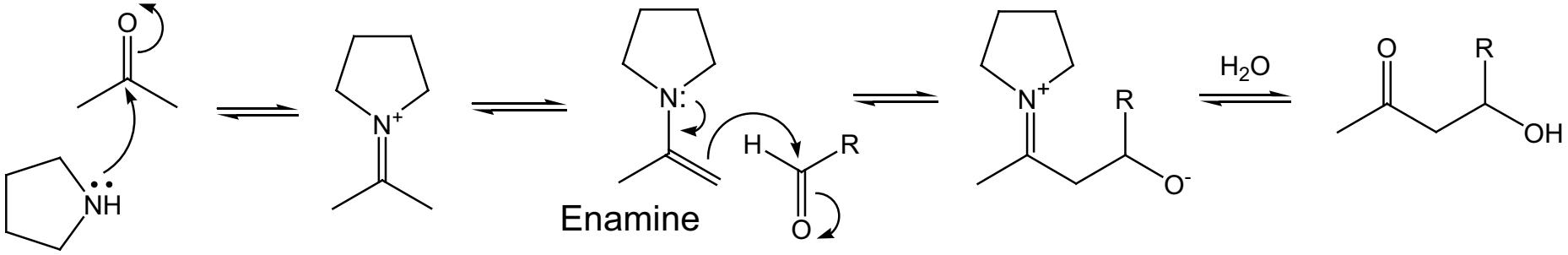
- High regioselectivity
- High stereoselectivity: relative configuration of amides, ***cis*** *(*normally favoured endo product*)* versus ***trans*** *(*normally disfavoured exo product*)*
- High enantioselectivity

Aldolase catalytic antibodies – now commercially available from Aldrich, match the activity of natural enzymes – accept a wider range of substrates.

Aldol reaction

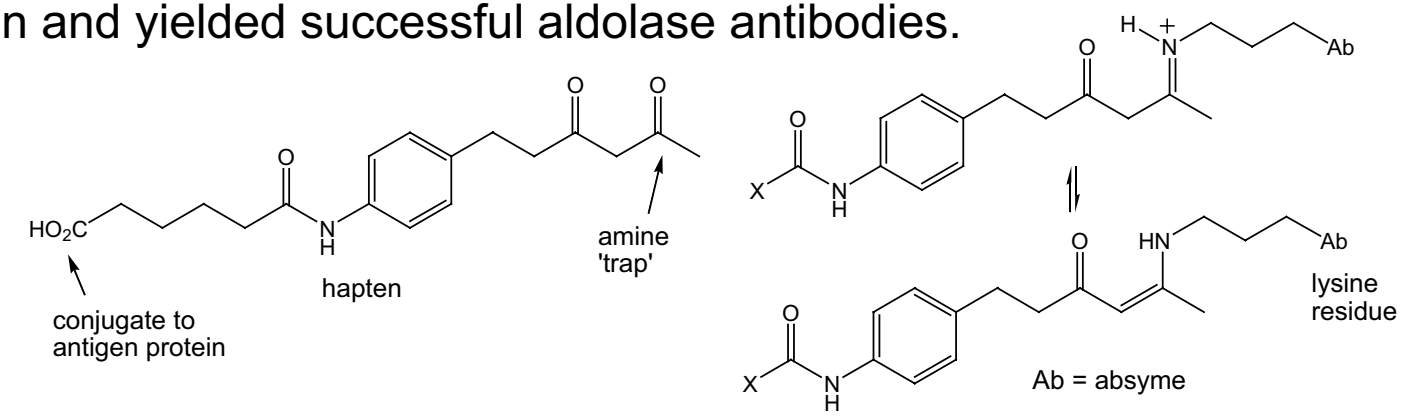


The two step aldol reaction in the lab can have problems of by-products due to cross condensation. One way round this is to prepare an enamine as a 'masked' enolate. Enzymic aldolases do the same trick. A lysine residue on the enzyme first reacts with a carbonyl and the imine residue tautomerises to an enamine. This reacts with an incoming aldehyde.

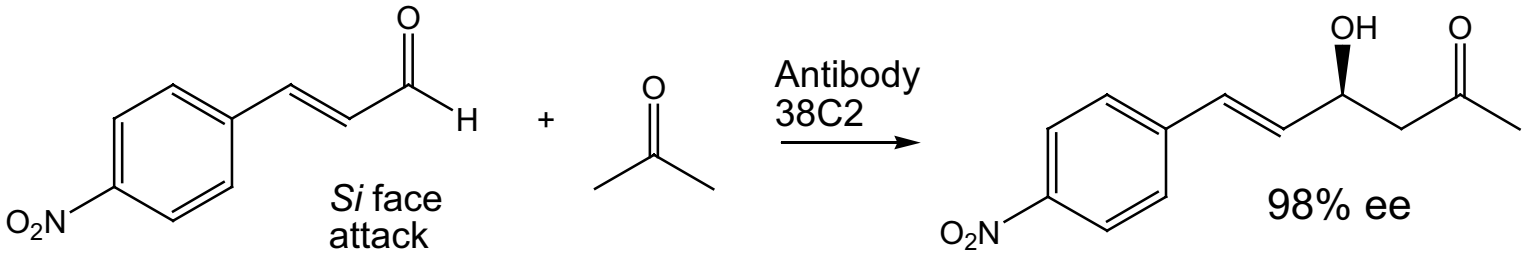


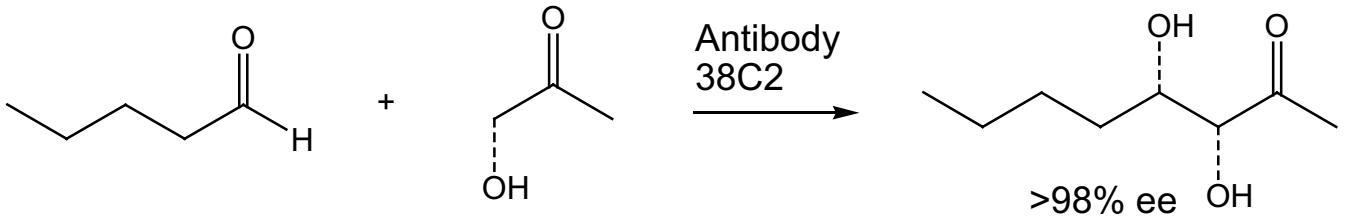
Laboratory enamine reaction

To copy nature's mechanism, '**reactive immunisation'** was used: The diketone of the hapten acts a chemical trap for lsyine residues during the antibody evolution and yielded successful aldolase antibodies.



Examples of using catalytic aldolase antibody 38C2:





Hydroxyacetone gives a regioselective reaction at the less favoured hydroxy-carbon

Separation of racemates - Resolution

- Despite the continuing advances in asymmetric synthesis techniques available, many industrial processes still employ classical resolution of a diastereomeric salt or or ‘covalent’ diastereomer.
- Important consideration that may lead to a resolution process being preferred
  - Practicality of synthesis
  - speed to market
  - economics and short effective patent life

Synthesis of enantiopure drugs at Lilly in 1993

‘Chiral pool’ or semi-synthesis	5.5 (20%)
Asymmetric synthesis	6.0 (22%)
Resolution (ionic or covalent)	15.5 (57%)
Data gathered from 40 drug candidates at phase I to phase III of development	

In any resolution the compound must have a ‘handle’ with which to form a salt or covalent link.

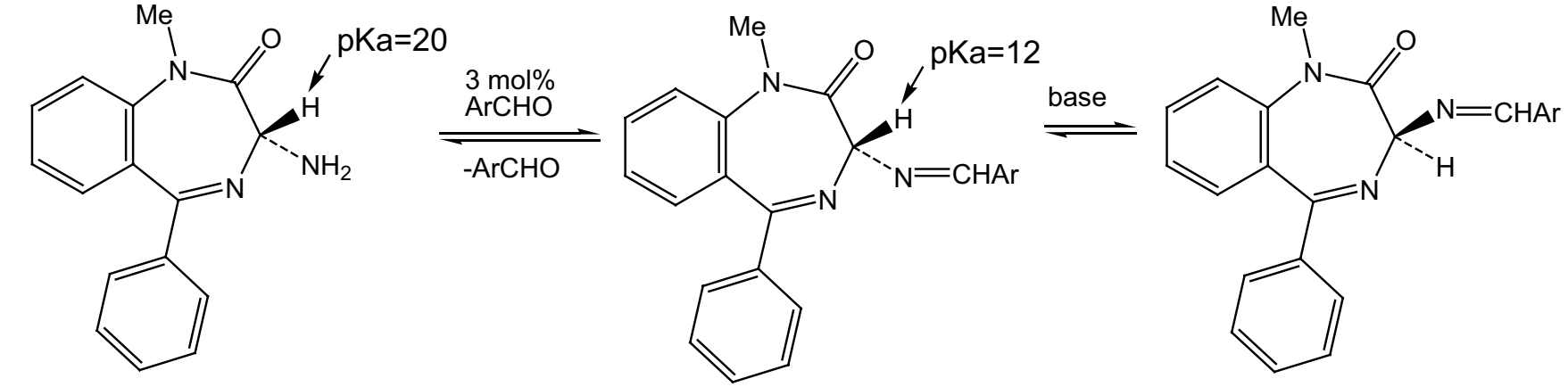
The ideal resolution system also involves an in-situ racemisation process, in which the unwanted diastereomer is soluble and the desired diasteromer crystallises out. This drives the equilibrium to give 100% of the desired diastereomer.

Merck devised an elegant in situ racemisation system:

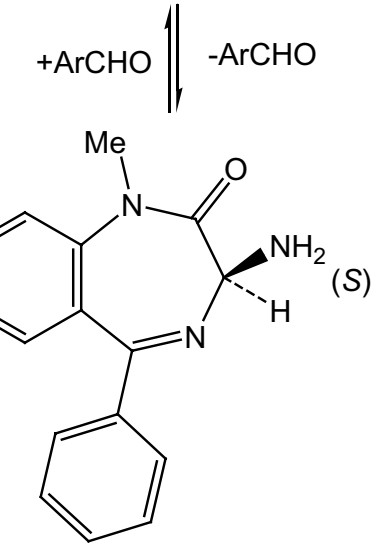
•small amount of aldehyde forms an imine, lowers pKa of acidic proton

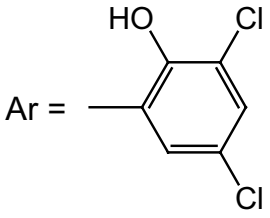
•free amine present deprotonates and thus racemises the chiral centre

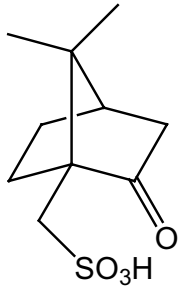
•resolving agent (+)-(*S*)-camphor-10-sulphonic acid (CSA) crystallises out the diastereomerically pure salt containing only the (*S*)-amine..



unwanted (*R*) - amine







(+) camphor-10-sulphonic acid

(+)-CSA

precipitates as (*S*)-CSA salt