1 Nucleophilic Addition Of Hard Anions To Aldehydes And Ketones

from chapter(s) _____ in the recommended text

Introduction

Focus

This chapter is about combining electrophilic carbons with nucleophiles, particularly, *C*-nucleophiles. In some cases reactions are represented as with reagents and readers must be able to recognize the nucleophilic carbon involved, but most of the examples in this section present reactions in the simplest possible way.

Reasons To Care

Synthesis of organic molecules generally involves building up carbon frameworks. Most often $C - C$ construction is achieved by adding electrophilic carbon fragments to nucleophilic ones. For instance, hypothetically pentane might be prepared via formation of the bond broken by the jagged line, hence it might be approached in the two ways represented below.

this type of arrow means "could be made from"
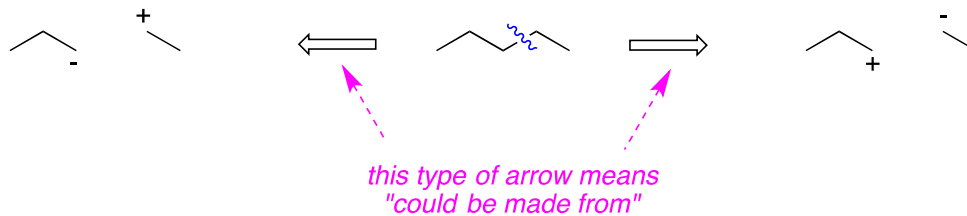
Most organic reactions are ionic processes that can be visualized in simple terms like this. When interpreting how an organic reaction works, look first for likely nucleophiles combining with electrophiles, then worry about details (*eg* byproducts, stoichiometry, and reaction conditions), just like when buying a used car it is a good idea to check the engine before the heater, radio and fuzzy dice hanging from the mirror.

Concepts

ionization • curly arrows to depict combinations of cations and anions • retrosynthesis and fundamentals of how organic molecules are formed by making $C - C$ bonds • *Re-* and *Si*-face attack

Objective

This part of the *Inquisition* encourages the people to identify and follow true path and towards overall goals, while avoiding temptations to deviate towards the superficial.

Types Of Additions To Carbonyls

*draw arrow here*

A
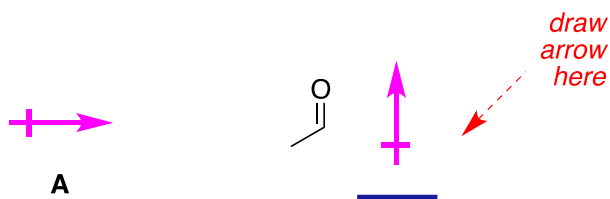
Polarity Of Carbonyls

Draw an arrow of style **A** aligned with the carbonyl group such that the crossed end is near the positive end of the dipole and the arrow-tip is near the negative end.

The carbon of a carbonyl group is the *positively* polarized.

Nucleophiles add to carbonyl *carbons* and, when they do, the electrons flow towards the most electronegative atom, which is the *oxygen*.

Reactivity Of Nucleophiles And Carbonyls At Different pH Values

Circle the best nucleophile and put a square around the best electrophile given the selection below.

CH_3^-

NH_3

O

$H-O^+$
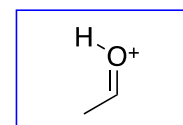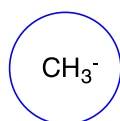
In carbonyl addition reactions, negatively charged nucleophiles tend to be *more* reactive than otherwise similar neutral ones.

Carbonyls that are protonated on the oxygen tend to be *more* reactive than ones that are not.

Anions like CH_3^- and H^- are called *hard* because the charge on these is concentrated in one place, making them highly reactive.

Protonated carbonyls *are* likely to donate H^+ to anions that are basic; therefore CO protonation *cannot* be used to enhance the reactivity of a basic anion with a carbonyl.

It is *completely wrong* to show hard, reactive basic anions adding to aldehydes and ketones under acidic conditions.

It follows that reactions of aldehydes and ketones with reactive basic anions are performed under *neutral or basic* conditions.

Additions of reactive basic anions to carbonyl compounds *cannot* be reversible if the anion involved is very stable.
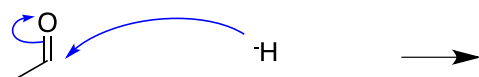
Formation Of Tetrahedral Intermediates

Draw the products of these reactions featuring attack of anions on carbonyl, showing curly arrows.
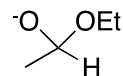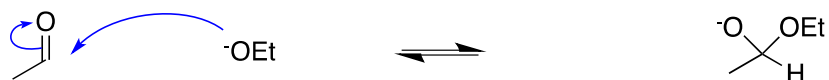
tetrahedral intermediate

tetrahedral intermediate
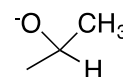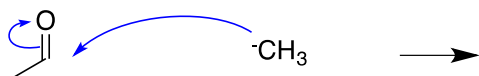
tetrahedral intermediate
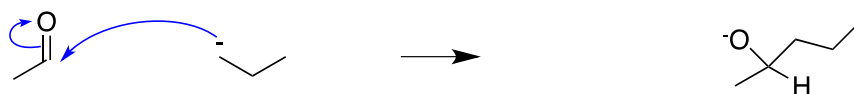
tetrahedral intermediate

Central carbons in these tetrahedral intermediates are *sp³* hybridized.

Protonation of these tetrahedral intermediates would give *alcohols*.

Addition of alkoxides to aldehydes or ketones is reversible because the reactants can surmount the energy barriers involved (this is *kinetics*), but the product and the reactants are of comparable energy (*thermodynamics*).

This process forms *one C–O* bond(s) between the nucleophile and the carbonyl carbon, and breaks *one* bond(s) of the *C=O*.

Negatively charged nucleophiles that are too stable, and do not give particularly stable products may add to aldehyde and ketone carbonyls, but the equilibrium will favor the *starting materials*.

If a nucleophile is stable but reactive it *will* be inclined to add reversibly, whereas unstable and reactive nucleophiles will tend to move "energetically downhill" to product, and stay there, *ie irreversible* addition.

Divide the following anions into relatively stable or unstable, respectively: Cl⁻, Br⁻, Me⁻, Ph⁻, CN⁻, MeO⁻, CH₂CH⁻, where methoxide is a mid point.

Cl⁻   Br⁻   CN⁻   MeO⁻   Me⁻   Ph⁻   CH₂CH⁻

relatively stable   *relatively unstable*
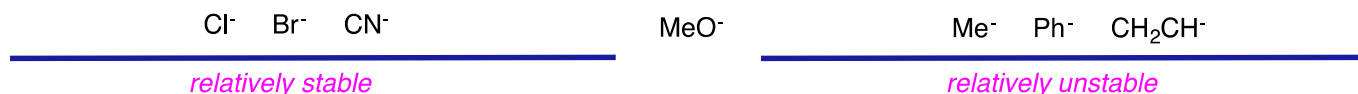
In general, anions that are weak bases tend to add *reversibly* to ketones. This chapter is mainly about nucleophiles that add irreversibly under basic conditions.

Reactions of Aldehydes And Ketones With Hydridic Reducing Agents

In each of the following reactions, assume the reagent *H⁻* is completely nucleophilic and only adds to the carbonyl. The products are formed after aqueous work-up procedures (adding water, then extraction). Show the products and indicate curly arrows to show how they are formed.

tetrahedral intermediate

product
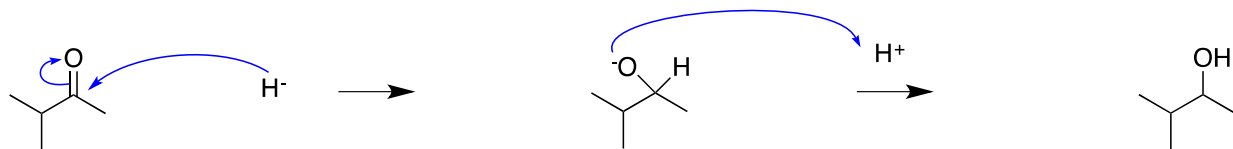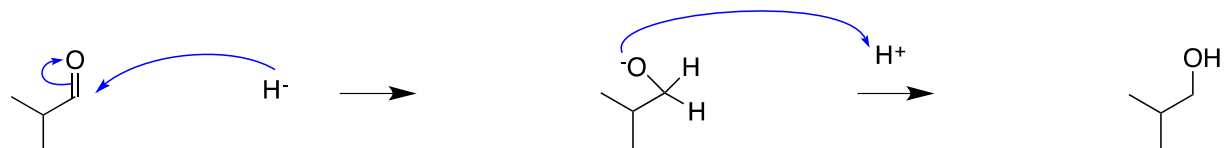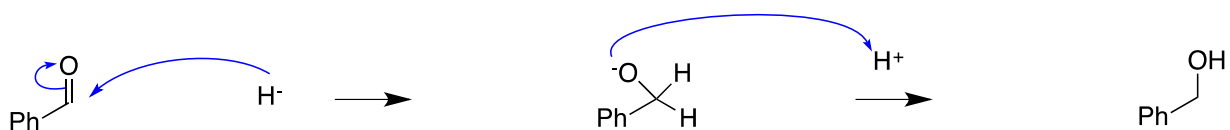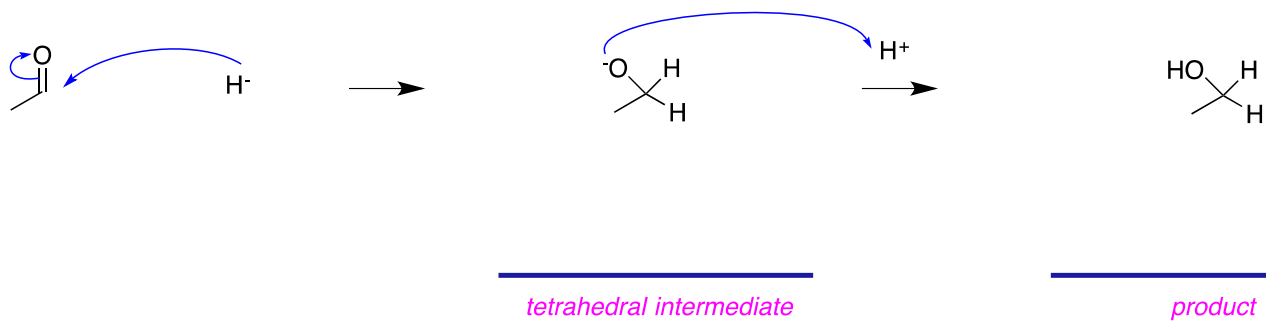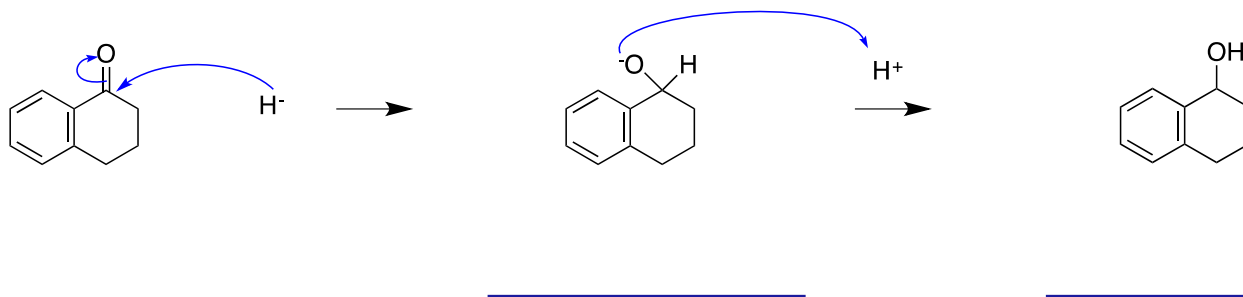
In fact, not all sources of hydride are equal. Name the following common sources of hydride. Two of them are nucleophilic, but one is *only* basic: indicate which is which.

NaBH$_4$	NaH	LiAlH$_4$
name: Sodium Borohydride	*Sodium Hydride*	*Lithium Aluminium Hydride*
nucleophilc	*basic*	*nucleophilc*

The nucleophilic reagents add hydride to the carbonyl of aldehydes and ketones to form an alkoxide that is protonated to form an alcohol on addition of acid.   The basic one *does not* do this efficiently.

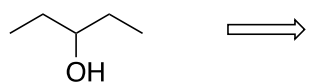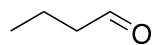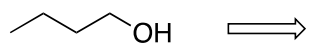Predict the products of the following reactions; an alkoxide intermediate is only shown in the first one.
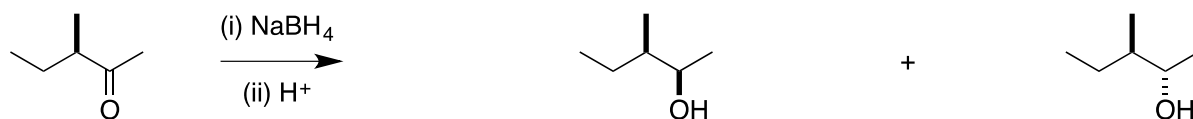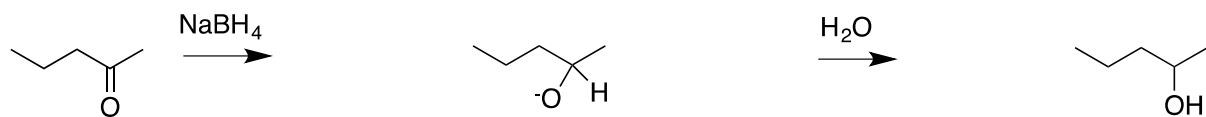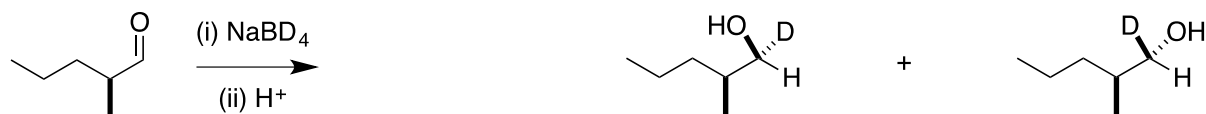
Reactions that involve addition of hydride to carbonyl compounds are *reduction* processes.
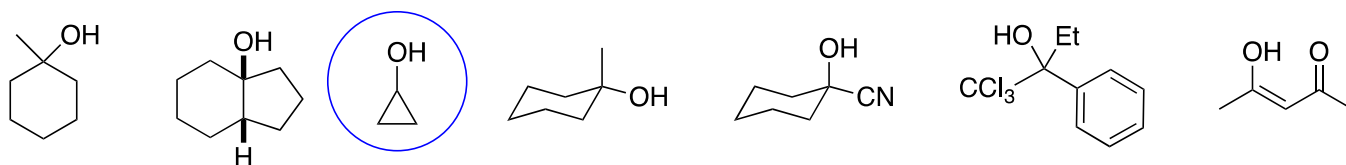
Draw aldehydes or ketones that could be used to make the following alcohols via reduction with hydrides.
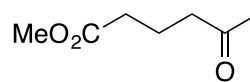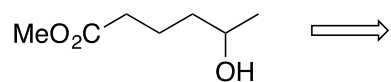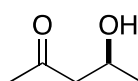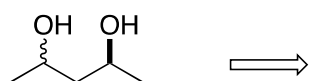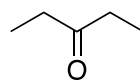
Only sodium borohydride can be used for the last reaction because:

Lithium aluminium hydride can reduce ester to alcohol, but sodium borohydride cannot.

Only one of the following alcohols can be formed by sodium borohydride reduction; circle that one.

All the following compounds except one can be formed via sodium borohydride reduction of aldehydes or ketones, circle that one.
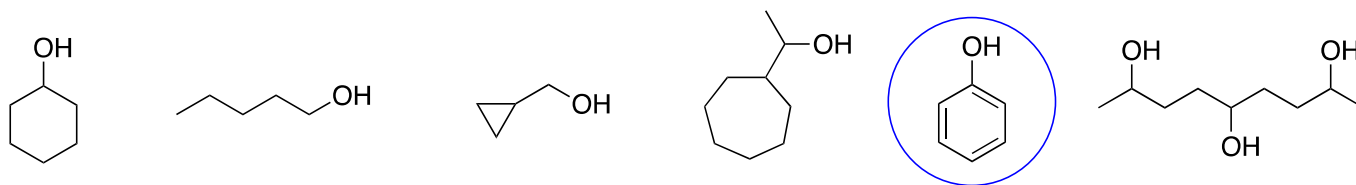
Addition Of Carbanions

Anions that would be formed in deprotonation of alkanes, alkenes, and alkynes are relatively *reactive*, hence they tend to add to carbonyls *irreversibly*.

Moreover, this tendency is reinforced since the *C–C* bond formed is *stronger* than the *C–O* π-bond that is broken.

These anions tend to react instantly with water, so they are formed and used under *anhydrous* conditions.
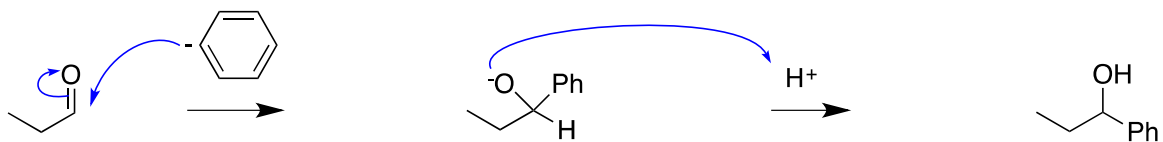
When they add to aldehydes or ketones, the product alkoxide *does not* equilibrate with starting material and remains until protons are added to the system.

Show the following mechanism with clear curly arrows.

tetrahedral intermediate
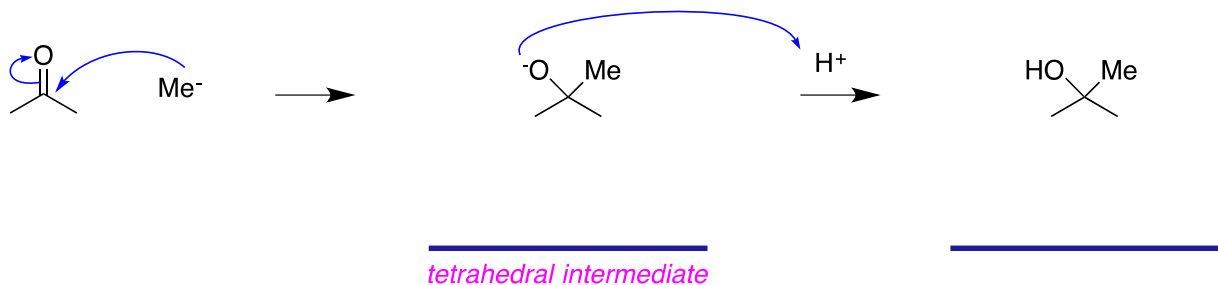
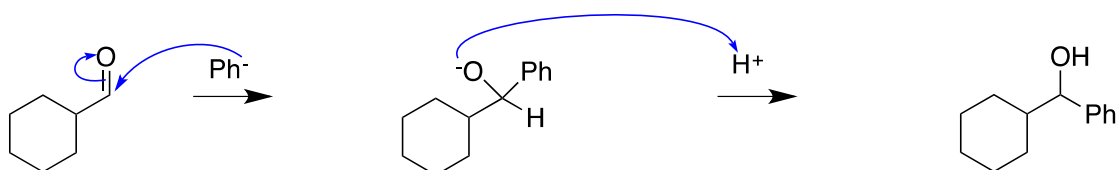Show similar mechanisms for the following reactions.
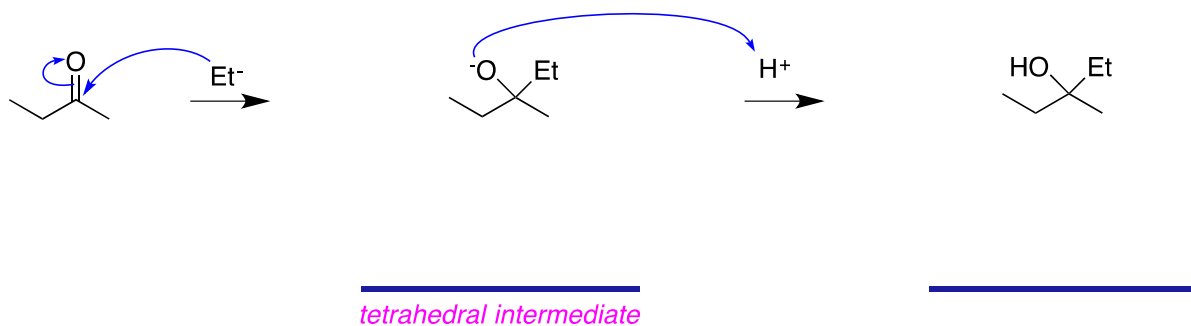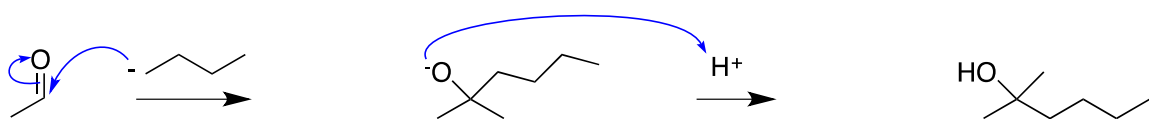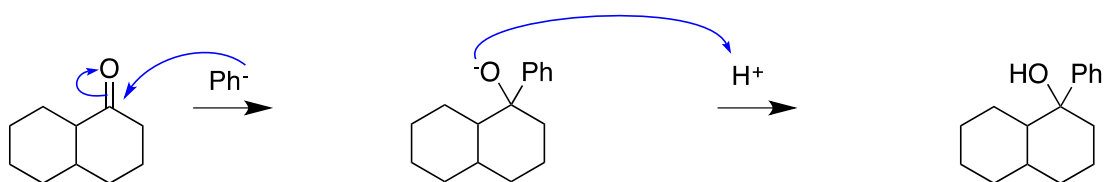
tetrahedral intermediate

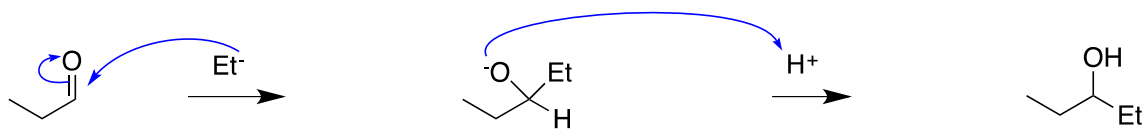*tetrahedral intermediate*

tetrahedral intermediate

tetrahedral intermediate

tetrahedral intermediate
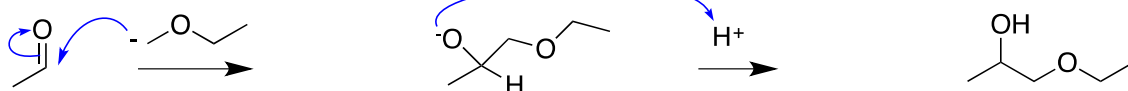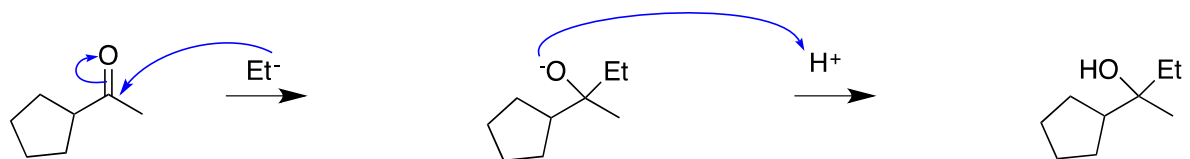
tetrahedral intermediate

tetrahedral intermediate

tetrahedral intermediate
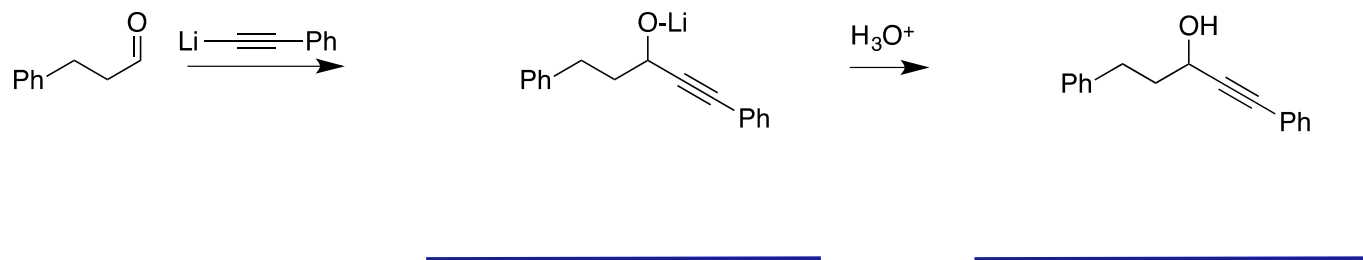
Reactions Of Carbonyl Compounds With Acetylide Anions

Circle those of the following bases that could be used to deprotonate terminal alkynes (refer to a text?)
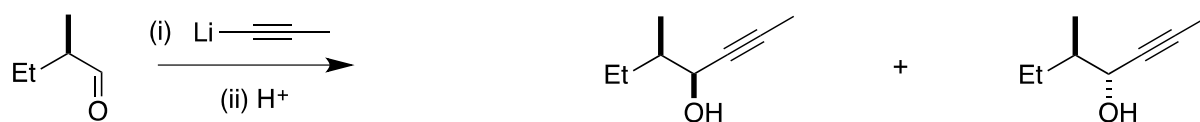
CIRCLE THE GRIGNARD TOO PLEASE

NEt$_3$ ⬭NaNH$_2$⬭   MeMgBr   HO⁻   ⬭LiN^{i}Pr$_2$⬭   ⬭LiBu⬭   Na$_2$CO$_3$

Acetylide anions add to aldehydes or ketones to form *secondary or tertiary* alcohols, respectively.

Predict the products of the following reactions.

H_2CO (i) H_2C_2, excess NaH
    ⟶
    (ii) H^+

(structure: 1,4-pentadiyn-3-ol with OH)

(structure: 2-methylbutanal, Et)   (i) Li≡≡ (lithium acetylide)
    ⟶
    (ii) H^+

(structure: Et...OH with alkyne)   +   (structure: Et...OH with alkyne)
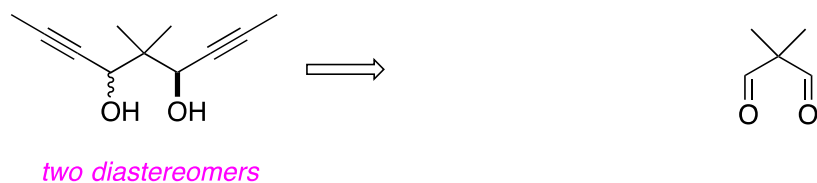
two diastereomers

Draw carbonyl compounds that could be used to make the following alcohols using acetylide anions or dianions.

(structure: 2-methyl-3-butyn-2-ol, OH)   ⟹

(structure: acetone, O)

(structure: diol with OH and HO and internal alkyne)   ⟹
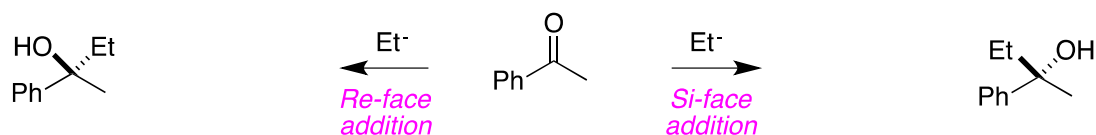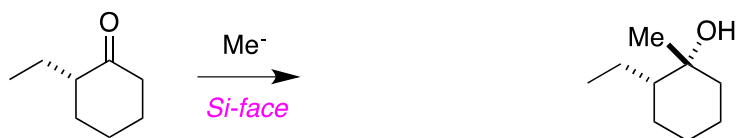
(structure: acetone, O)

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two diastereomers
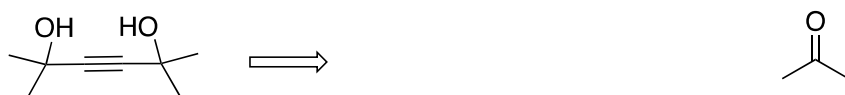
Si And *Re* Faces Revisited

Reactions on different faces of a prochiral sp^2-hybridized carbon with no existing chiral centers can give different *enantiomers*.

Approach on the face displaying a clockwise ranking of the three substituents according to the priority rules is called *Re*-face attack and addition to the other face is *Si* -face attack.

Show the products of the following reactions in which the face selectivity is somehow controlled. Recall that addition of nucleophiles to aldehydes and ketones, and on protonation can give chiral alcohols.

HO   Et
Ph

Et⁻
Re-face addition

Ph— (ketone) O

Et⁻
Si-face addition

Et   OH
Ph

_____   _____

(cyclopentanone with methyl) O

LiAlH₄
Re-face

OH
(cyclopentane with methyl, OH)

_____

(R)-Alcohols *are not* always formed from *Re*-face attack, and (S)-alcohols *are not* always formed from *Si*-face attack.

Test your reasoning by drawing 4 hypothetical examples below.
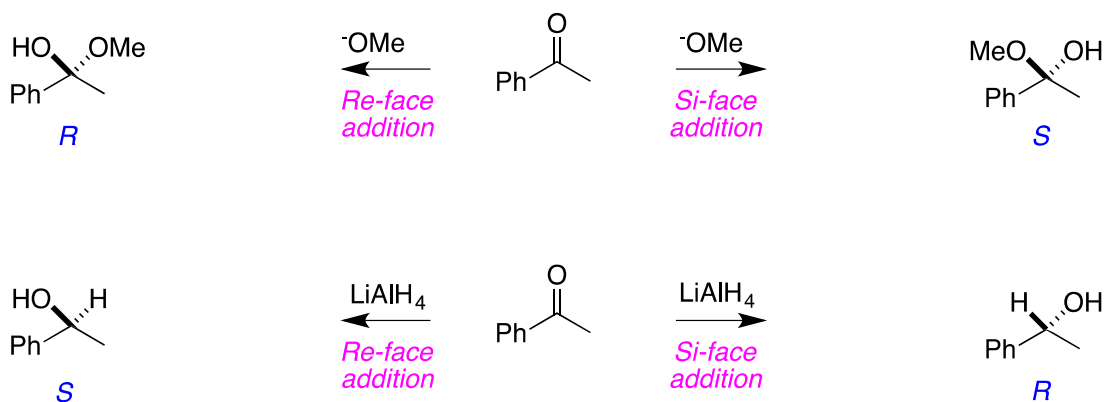
2 Addition Of Grignard Reagents To Aldehydes And Ketones

from chapter(s) _____ in the recommended text

Introduction

Focus

The last section discussed adding negatively charged agents to carbonyl compounds; this concept is more important than learning reagent names and reaction conditions. Grignard reagents, however, are special because they can be formed from a wide range of alkyl or aryl chlorides, bromides, or iodides, and because they can be used to form a huge diversity of carbon frameworks.

This section is about Grignard reagents, and ways in which they complement hydride reduction chemistry.

Reasons To Care

Grignard reagents react instantly with water or any other mildly acidic substance. Consequently, they do not exist in the body, and there is no close analog to these in biological chemistry. Grignard reagents are only directly useful in chemical laboratories and processes, but the concept of building organic molecules by matching reactive centers with opposite charges is uniformly useful, and reactions of Grignard reagents with aldehydes and ketone are a simple way to show this. These processes are also a primer for reactions of carbonyl compounds with nucleophiles under acid catalysis; those transformations *are* relevant to biological chemistry.

Concepts

curly arrows to depict reaction of carbanions with carbonyls • hydridic reduction chemistry • retrosynthesis

Objective

To understand how formation of Grignard agents converts alkyl halides (electrophiles) or aryl halides into nucleophiles for $C - C$ bond construction.
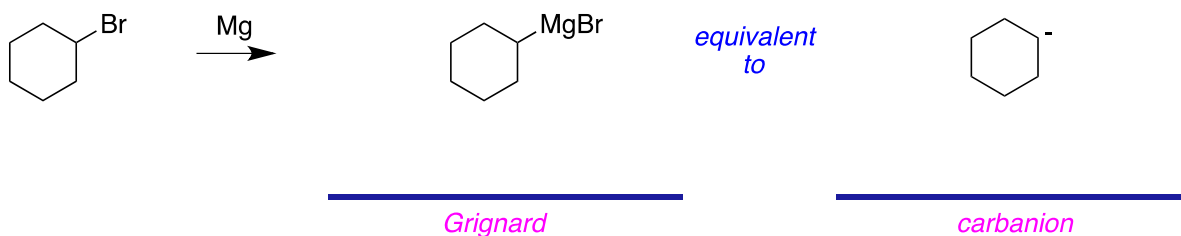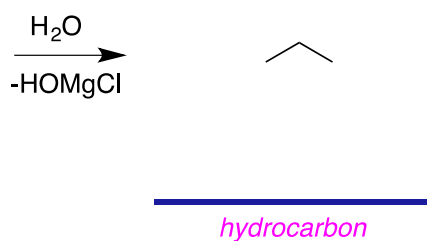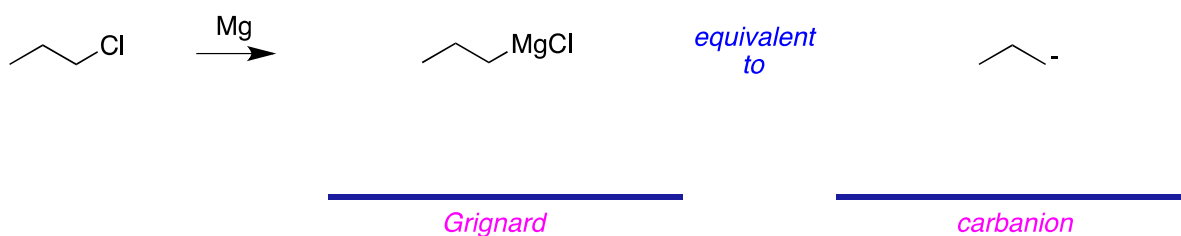
Grignard Reagents: A Type Of Carbanion Equivalents

Grignard reagents are made from alkyl halides (except fluorides) and *magnesium*; they are *strong* base carbanions.

Grignards *cannot* be formed from compounds with functional groups that would react immediately, especially acidic ones because these reagents protonate rapidly.

Grignards cannot be isolated from 1,2-dihaloalkanes because MgX$_2$ is eliminated immediately after a Grignard is formed liberating *ethyne*.
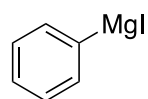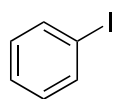
Show the products of the following reactions, and their reactions with water that illustrate how they are destroyed if not kept in a dry medium.
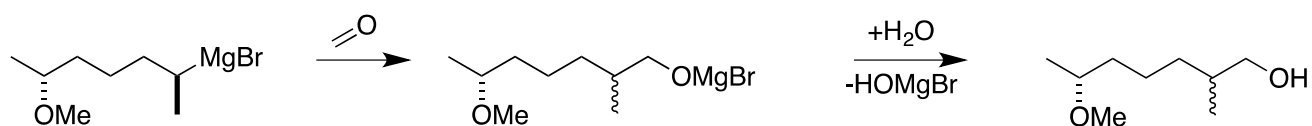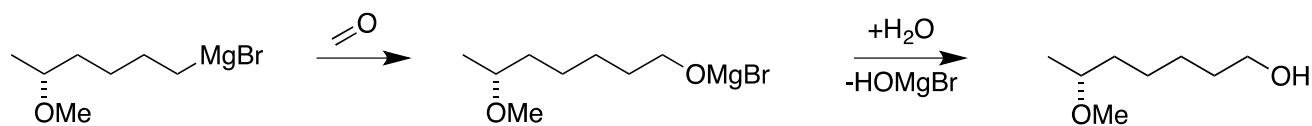
Grignard

carbanion

hydrocarbon

Grignard

carbanion

$$\xrightarrow[-\text{HOMgCl}]{\text{H}_2\text{O}}$$

hydrocarbon

$$\xrightarrow{\text{Mg}}$$

Grignard   **equivalent to**   _carbanion_

$$\xrightarrow[-\text{HOMgCl}]{\text{H}_2\text{O}}$$

hydrocarbon

$$\diagdown\diagdown\diagup CI \xrightarrow{Mg} \diagdown\diagdown\diagup MgCl \quad \textit{equivalent to} \quad \diagdown\diagdown\diagup^-$$

Grignard　　　　　　　　　　　　　　　　**carbanion**

$$\xrightarrow[\text{-HOMgCl}]{H_2O} \quad \diagdown\diagdown\diagup$$

hydrocarbon

Br   —Mg→   MgBr   *equivalent to*   ‾

_____   _____
Grignard   *carbanion*

H_2O
—————→
-HOMgCl

_____
hydrocarbon
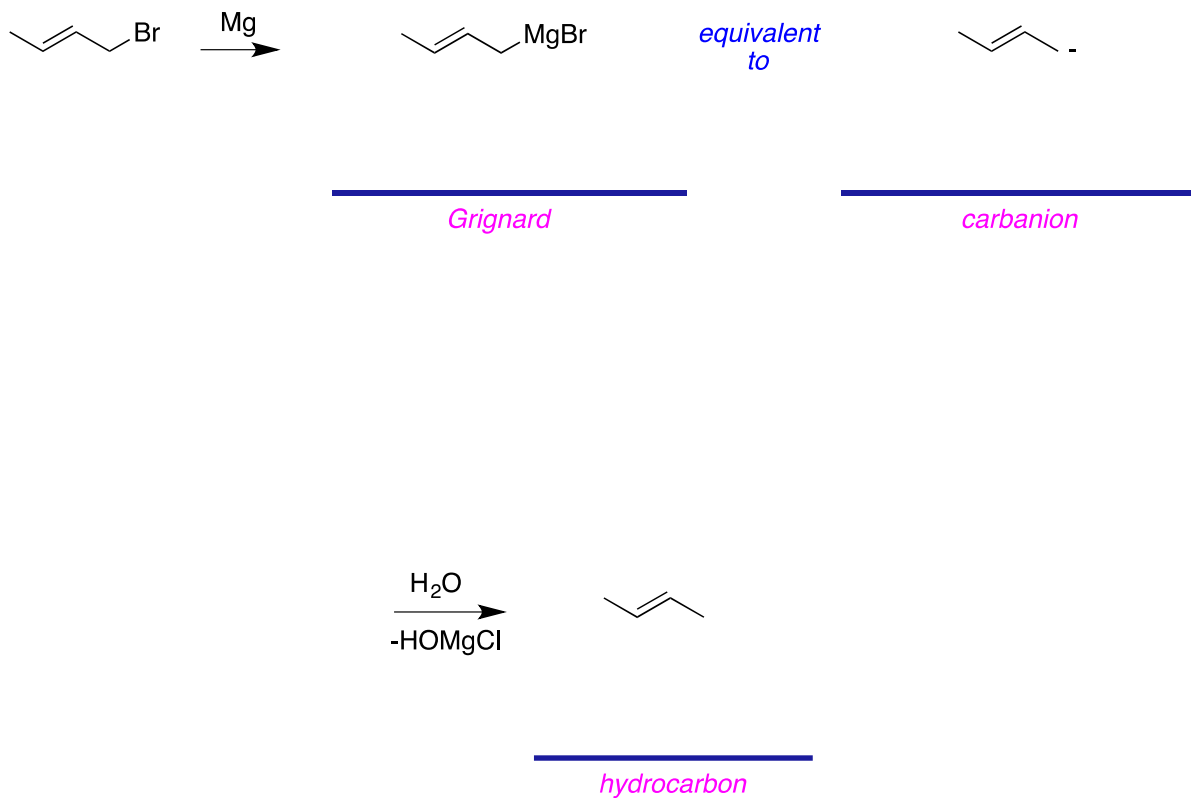
Only some of the following compounds can form Grignards; put a circle around those that *can*.

1,2-dichloroethane   (bromobenzene)   (benzyl bromide)   butane

(allyl chloride)   (2-chloropropane)   1,2-diiodopropane   2-chloroethanol

diethyl ether   fluoroethane   bromoethanal   3-iodopropanol

(chloro(methoxy)methane)   1-bromo-3-methoxy-ethanal   (bromoethane)
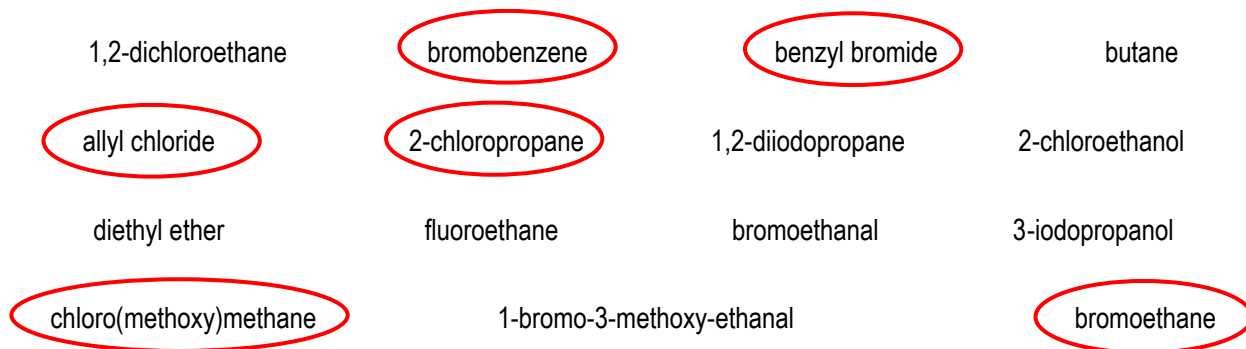
Reactions Of Methanal With Grignard Reagents

Draw formaldehyde in three different ways (try one line diagram, two formulae representations).

HCOH
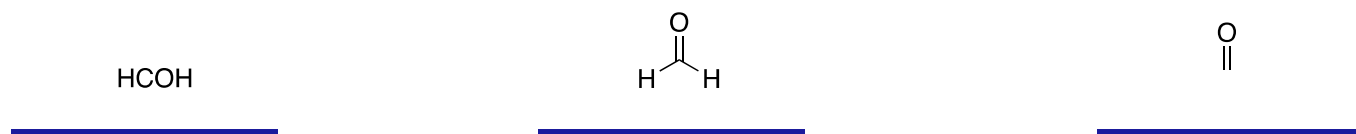
Complete the following disconnections in the same style as the first one.

Grignard
HCOH
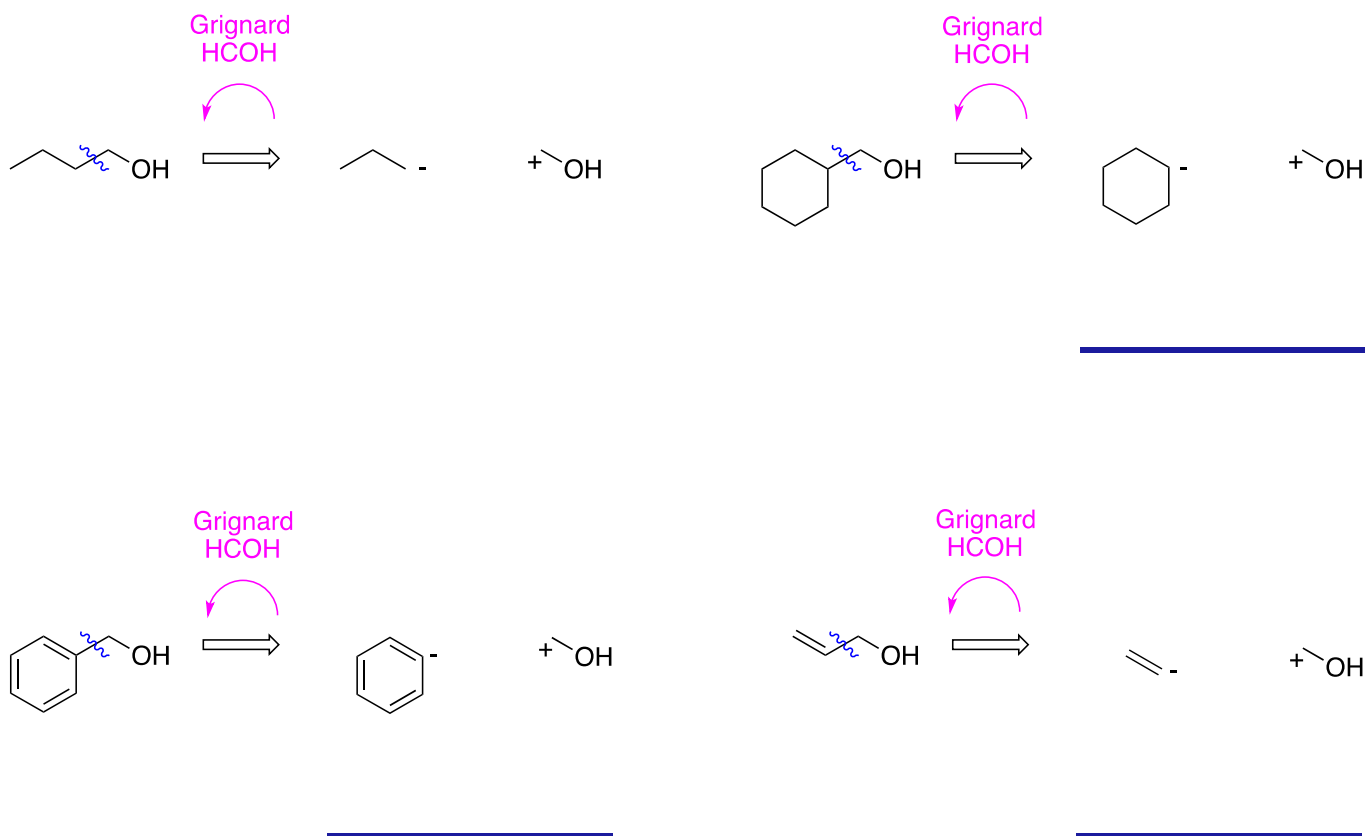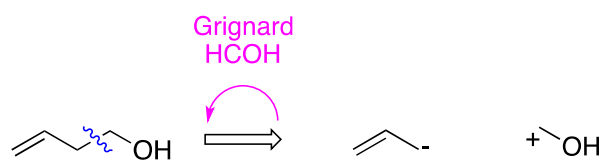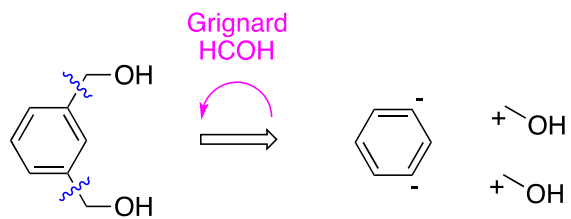
Grignard
HCOH

Draw the products of the following reaction sequence showing curly arrows to depict the electron flow.
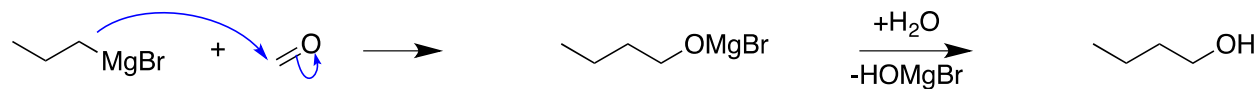
Grignard reagents with *methanal* give *primary* <u>alcohols</u> (name the functional group) on protonation.

These products have *one more* carbon than the Grignard.

Predict the products of the following reactions.

BrMg~~~~~~MgBr →(excess, =O) BrMgO~~~~~~~OMgBr →(+H₂O / -HOMgBr) HO~~~~~~~OH

_____   _____

(structure with OMe)~~~MgBr →(=O) (structure with OMe)~~~OMgBr →(+H₂O / -HOMgBr) (structure with OMe)~~~OH

_____   _____

(structure with OMe and methyl)~~~MgBr →(=O) (structure with OMe)~~~OMgBr →(+H₂O / -HOMgBr) (structure with OMe)~~~OH

_____   _____
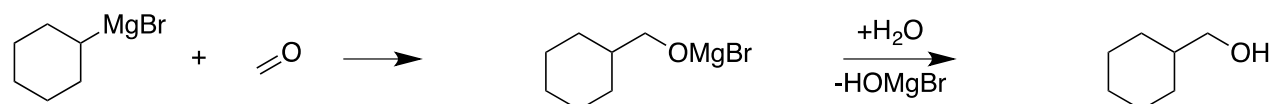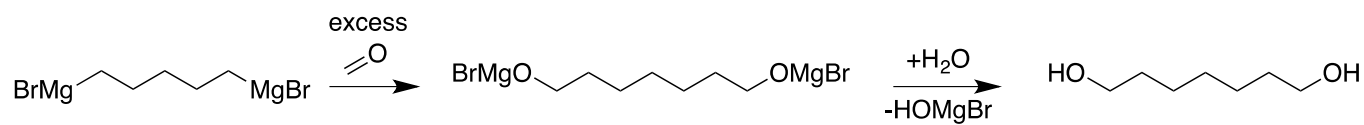
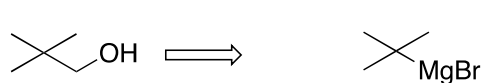Show Grignards that could be used to make the following *primary* alcohols from methanal.

$\diagup\diagup\diagup$OH $\Longrightarrow$ $\diagup\diagup\diagup$MgBr
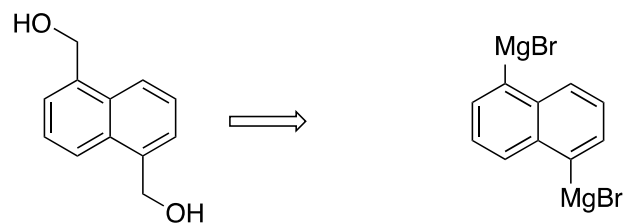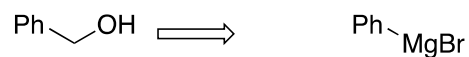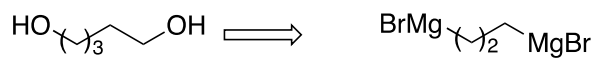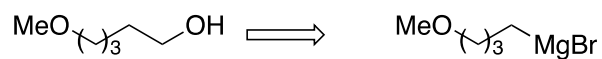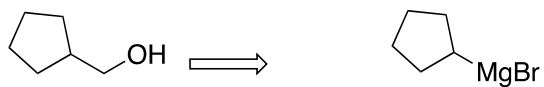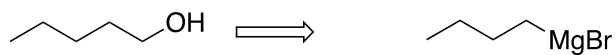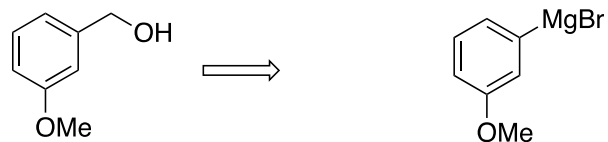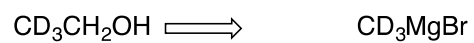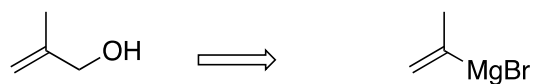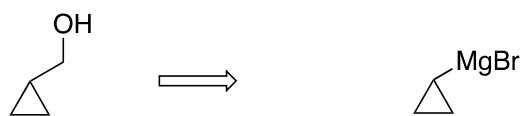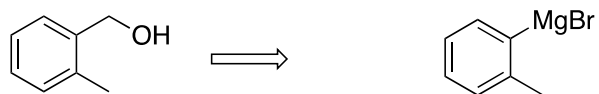
$\bigcirc$$\diagdown$OH $\Longrightarrow$ $\bigcirc$MgBr

MeO$\diagup\diagup$$_3$$\diagup$OH $\Longrightarrow$ MeO$\diagup$$_3$$\diagdown$MgBr

HO$\diagup$$_3$$\diagdown$OH $\Longrightarrow$ BrMg$\diagup$$_2$$\diagdown$MgBr

a di-Grignard

Ph$\diagdown$OH $\Longrightarrow$ Ph$\diagdown$MgBr

HO$\diagdown$ (naphthalene) $\diagup$OH $\Longrightarrow$ MgBr (naphthalene) MgBr

a di-Grignard

CD$_3$CH$_2$OH $\Longrightarrow$ CD$_3$MgBr

(benzene) OH / OMe $\Longrightarrow$ (benzene) MgBr / OMe

Grignard reagents are *more* basic than nucleophiles like methoxide, and add to carbonyl compounds *irreversibly*.

The alcohols formed from reactions of Grignards with methanal are *primary* because methanal has *two* hydrogens attached to the carbonyl group.

Reactions of Grignards with other aldehydes must give *secondary* alcohols; methanal *is* unique.
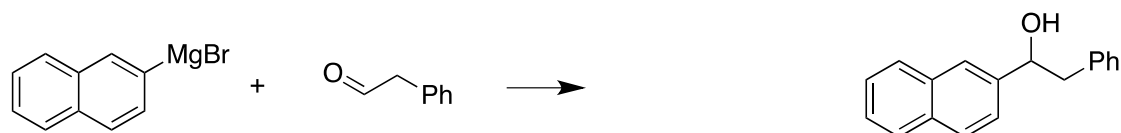
Reactions Of Other Aldehydes With Grignards
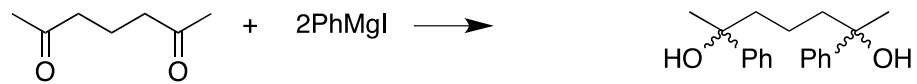
Predict the products of the following sequence showing curly arrows for the reaction flow.

MeMgBr   +   [aldehyde] ⟶ [OMgBr intermediate] $\xrightarrow[\text{-HOMgBr}]{\text{+H}_2\text{O}}$ [OH product]

_____

_____

Grignard reagents react with aldehyde *homologs* of methanal give *secondary* alcohols with *the same* carbon atoms as the combination of the Grignard and the aldehyde. Test this assertion by predicting the products of the following reactions (after protonation).

[EtMgBr]   +   [O aldehyde] ⟶ [secondary alcohol OH]

MeMgBr + EtCOH ⟶ [OH secondary alcohol]

_____

_____

excess
MeMgI

Show the Grignards and aldehydes that could be used to make the following *secondary* alcohols. If there are two ways of doing this, indicate both.

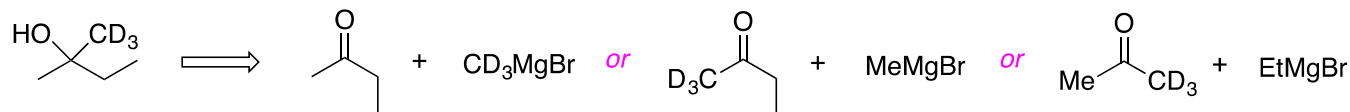Retrosynthetic analysis: 3-pentanol from ethyl MgBr + propanal

---

Retrosynthetic analysis: hexane-2,5-diol from BrMg(CH₂)₃MgBr + acetaldehyde *or* MeMgBr + pentanedial

---

Retrosynthetic analysis: 1-phenylbutanol from propyl MgBr + benzaldehyde (PhCHO) *or* butanal + PhMgBr

---

Retrosynthetic analysis: (3-methylphenyl)(cyclopentyl)methanol from 3-methylbenzaldehyde + cyclopentyl MgBr *or* (3-methylphenyl)MgBr + cyclopentanecarbaldehyde

Clue for the next problem: the Grignard must be a stable one in any synthesis proposed …..

Reactions Of Ketones With Grignards

Predict the product of the following reaction sequence.

MeMgCl + [ketone structure] → [structure with OMgCl] $\xrightarrow[\text{-HOMgCl}]{\text{+H}_2\text{O}}$ [structure with OH]

_____

_____

Grignards react with *ketones* to give *tertiary* alcohols.

The products of the reaction of a Grignard with a ketone are alcohols with *the same* carbons than the combination of the Grignard and the ketone starting materials.

Two of the alcohol product substituents *must* be the same when symmetrical ketones are used.

Predict the products of the following reactions after protonation.

[structure MgBr] + [acetone structure] → [HO structure]

_____

MeMgBr + EtCOEt → [HO structure with Et, Et]

_____

MeCOPh   +   EtMgBr $\longrightarrow$

$$\underset{Me}{\overset{HO}{\underset{Ph}{\big|}}}\!\!-\!Et$$

Indicate the components from which the following alcohols can be formed from *ketones* and Grignard reagents. If more than one way is possible, indicate all of them.

For the product above it *is* possible to draw two diastereomers of this product by varying the stereochemistry at C^5.

It *is not p*ossible to draw two diastereomers of the product below by varying the stereochemistry at C^5.

Complimentary Grignard and Hydride Reductions

Draw two ways to access these alcohols in one step (after protonation): one through hydride reduction of a ketone or aldehyde, the other through a Grignard (show reagents).

PLEASE SHOW   +NaBH4 on right

a Grignard route   *hydride route*

+ MeMgBr

or

+ MgBr

⟸   HO   ⟹

+ NaBH₄

+ MeMgBr

or

+ MgBr

⟸   OH   ⟹

+ NaBH₄

Reactions Of Carbon Dioxide With Grignards

Carbon dioxide is similar to a ketone with respect to nucleophilic reactions with Grignards. Predict the products of the following reaction sequence, and use curly arrows to show the reaction mechanism.

CO_2 adds to Grignard reagents to form the following functional group after protonation: <u>carboxylic acid</u>.

Products of addition of Grignard reagents to carbon dioxide have *one more* carbon than the Grignard.

Predict the products of the following reactions.

(i) CO_2

(ii) H_2O

COOH

(i) CO_2

(ii) H_2O

COOH

(i) CO_2

(ii) H_2O

COOH

HOOC

Identify the Grignards that can be used to make the following carboxylic acids from carbon dioxide.

Grignard reagents with the magnesium attached directly to a chiral center *almost always* react with loss of stereochemistry at that center.3 Addition Of Water And Alcohols To Aldehydes And Ketones

from chapter(s) _____ in the recommended text

Introduction

Focus

Water adds to aldehydes and ketones to form hydrates. These reactions are reversible because water can be lost easily. Similarly, *alcohols* add to ketones and aldehydes in acid-mediated reactions that can be driven to completion, and which cannot reverse unless aqueous acid is present. In the absence of water and acid, acetals and ketals, unlike hydrates, are stable because there is no proton available to reform alcohols and the parent carbonyl. This section is on formation of acetals, ketals and hydrates.

relatively unstable

hydrate

cannot reverse without acid and water

ketal

Reasons To Care

Carbohydrates form rings via formation of hemiacetals, and join to form oligomers using acetal and ketal linkages (*eg* maltose and sucrose). Understanding the mechanisms by which these are formed *chemically* facilitates appreciation of their stabilities, and how these same types of bonds might be formed in Nature.

Some natural products that are not sugars also contain acetals or ketals, *eg* spirolaxine.

acetal and ketal linkages

maltose

sucrose

spirolaxine

Concepts Involved

acids and bases • curly arrows to depict electron flow under acidic conditions • entropy

Objective

Ketones and aldehydes can be activated by protonation to add nucleophiles (*eg* water, alcohol and amines), a series of proton transfer reactions occur, then water may leave. This generic description outlines a vast number of reactions in bioorganic chemistry.

Relative Reactivities Of Aldehydes And Ketones

Hydrogen atoms are regarded as *more* electron withdrawing than alkyl groups.

Rank the reactivities of the following compounds towards nucleophiles (1 is most, 5 is least); to do this, take account of electronic *and* steric effects.

1   2   3   4   5

Addition of a nucleophile to a ketone or an aldehyde changes the carbon geometry from sp^2 planar to sp^3 tetrahedral, *ie* 120° to 109°.

When forming tetrahedral intermediates from carbonyl compounds the substituents become *closer* thus *accentuating* steric effects relative to the starting material.

Order the reactivities of the following carbonyl compounds towards nucleophiles by drawing their structures in the following diagram: benzaldehyde, diphenyl ketone, propanone, methanal, phenylethanone, 3,3-dimethylpropanal, ethanal, ethandial (glyoxal).

most reactive

least reactive

Proton Transfer Steps

Proton transfer, loss from one atom in an intermediate, followed by gain of a proton by another atom is *common* in organic mechanisms.

Illustrate the following proton transfer steps by showing curly arrows for loss of a proton from the heteroatom with the positive charge, then addition of it to the neutral one.

-H⁺

+H⁺

+H⁺

-H⁺

It is common to abbreviate loss of proton and gain or proton in one step (without curly arrows). Show this in the following examples.

HO—C(O$^+$H)(cyclohexane)—O—CH$_3$ -H$^+$, +H$^+$ ⇌   H$_2$O$^+$—C(cyclohexane)—O—CH$_3$

H$_3$N$^+$—C(OH)(CH$_3$)—CH$_2$CH$_3$ -H$^+$, +H$^+$ ⇌   H$_2$N—C(CH$_3$)(CH$_2$CH$_3$)—$^+$OH$_2$

Addition Of Water

Hydration of aldehydes and ketones is an *equilibrium* process that involves protonation of the carbonyl group, addition of water, and deprotonation.

Hydrates of methanal, and of compounds with electron withdrawing carbonyl substituents, are *strongly* favored relative to the parent carbonyls.

Draw hydration of methanal using curly arrows to show electron flow.

$+H^+$

^+OH H-OH

OH O^+H_2

protonated carbonyl

protonated hydrate

$-H^+$

OH   OH

hydrate

Draw the key intermediates for hydration of acetone using curly arrows to show electron flow.

$+H^+$

^+OH H-OH

OH O^+H_2

protonated carbonyl

protonated hydrate

-H⁺ →

OH
⟍|
/‾OH

————————————
hydrate

The equilibrium constant for hydration of acetone is 2 x 10^{-3} so when mixed with 10 equivalents of water the amount of hydrate will be: *exactly 2 %*.

On adding water, the angle between carbonyl substituents of a ketone contracts from <u>120</u>° to <u>109</u> °; consequently, formation of a hydrate is *disfavored* when the carbonyl substituents are *large*.

When aldehydes are mixed with H$_2$^{18}O, the heavy atom label *does* become incorporated into the aldehyde C=O.

Additions Of Alcohols

Draw the "hydration" acetone but using H-OMe instead of H-OH as the nucleophile.

protonated carbonyl

protonated hemiketal

The product above with one OH and one OR group attached to the carbon that was the carbonyl group, is an *hemiketal* because it is half way to the R$_2$C(OR')$_2$ form and derived from a *ketone*.

The product formed from adding one alcohol to an aldehyde is called a *hemiacetal*.

Draw a *reversible* sequence of events representing protonation on the OH group of the *hemiacetal* from hydration of *ethanal*, loss of water to give an *oxonium* intermediate, addition of another methanol nucleophile, and finally deprotonation to give an *acetal*.

protonated acetal

Acetals and ketals *do* react with excess aqueous acid to give aldehydes and ketones.

Complete the following mechanism for addition of methanol to *benzophenone* under acidic conditions to give a hemiketal by completing the structures and drawing in curly arrows.

$$
\underset{Ph}{\overset{HO}{\diagup}} \underset{Ph}{\overset{\overset{\displaystyle H}{|}}{\underset{+}{C}}} OMe
\qquad
\underset{+H^+}{\overset{-H^+}{\rightleftharpoons}}
\qquad
\underset{Ph}{\overset{HO}{\diagup}} C \underset{Ph}{\overset{OMe}{\diagup}}
$$

_____   _____

hemiketal

Draw the same process for acid catalyzed addition of methanol to butanone.

+H⁺
-H⁺

⁺OH

MeOH
-MeOH

_____

H
HO ⁺OMe

-H⁺
+H⁺

HO   OMe

hemiketal

_____   _____

Complete the following mechanism showing curly arrows.

+H⁺
-H⁺

⁺OH

MeOH
-MeOH

_____   _____

starting material?

H

HO   +OMe

$\overset{-H^+}{\underset{+H^+}{\rightleftharpoons}}$

HO   OMe

Complete the following diagram to show how *hemiacetals* and *hemiketals*, like those above, can be protonated on the hydroxyl, expel water, add another alcohol then lose a proton.

HO   O^{i}Pr   +H$^+$ H$_2$O$^+$ O^{i}Pr   -H$_2$O $^+$O^{i}Pr

Ph   Ph   ⇌   Ph   Ph   ⇌   Ph   Ph
                    -H$^+$ +H$_2$O

_____   _____
protonated on OH   *oxonium ion*

+HO^{i}Pr   H$_2$O   -H$^+$ ^{i}PrO   O^{i}Pr

⇌ ^{i}PrO $^+$O^{i}Pr   ⇌   Ph   Ph
-HO^{i}Pr   H   +H$^+$

                      Ph   Ph

                    _____
                    protonated ketal

The starting material in this sequence was formed from a ketone and *an alcohol*

Draw exactly the same sequence for the hemiacetal from ethanal and methanol.

HO   OMe   +H$^+$ H$_2$O$^+$ OMe   -H$_2$O $^+$OMe

H   ⇌   H   ⇌   H
                    -H$^+$ +H$_2$O

_____   _____   _____
                                protonated on OH   *oxonium ion*

H_2O

$\text{MeO} \quad +\text{OMe}$
over C with H below

$+\text{HOMe}$ / $-\text{HOMe}$ (equilibrium arrows)

$-H^+$ / $+H^+$ (equilibrium arrows)

$\text{MeO} \quad \text{OMe}$ with H below

_____
protonated acetal

_____
acetal

Draw exactly the same sequence for the hemiacetal from benzaldehyde and *n*-butanol (introduce the alcohol over the appropriate arrow).

$\text{HO} \quad \text{O}^n\text{Bu}$
$\text{Ph} \quad \text{H}$

$+H^+$ / $-H^+$ (equilibrium arrows)

$H_2O^+ \quad \text{O}^n\text{Bu}$
$\text{Ph} \quad \text{H}$

$-H_2O$ / $+H_2O$ (equilibrium arrows)

$+\text{O}^n\text{Bu}$
$\text{Ph} \quad \text{H}$

_____

_____
protonated on OH

_____
oxonium ion

H
$^n\text{BuO} \quad +\text{O}^n\text{Bu}$
$\text{Ph} \quad \text{H}$

$+\text{HO}^n\text{Bu}$ / $-\text{HO}^n\text{Bu}$ (equilibrium arrows)

$-H^+$ / $+H^+$ (equilibrium arrows)

$^n\text{BuO} \quad \text{O}^n\text{Bu}$
$\text{Ph} \quad \text{H}$

_____
protonated acetal

_____
acetal

Draw the same sequence for the hemiacetal from cyclopropane carboxaldehyde and ethanol, taking care to fill in the reactants around the arrows.

HO   OEt

+H⁺ / -H⁺

H₂O⁺ OEt

-H₂O / +H₂O

⁺OEt

_____

protonated on OH

oxonium ion

+HOEt / -HOEt

EtO ⁺OEt
      |
      H

-H⁺ / +H⁺

EtO   OEt

protonated acetal

acetal

Draw the same sequence for the hemiketal from 2-butanone and 1,2-ethandiol, taking care to fill in the reactants around the arrows. Draw the reagents above each arrow.

HO   O   OH

+H⁺ / -H⁺

H₂O⁺ O   OH

-H₂O / +H₂O

OH
O⁺

protonated on OH

oxonium ion

$$\rightleftharpoons$$

$$\xrightarrow[\text{-H}^+]{\text{+H}^+}$$

protonated ketal

ketal

Write the complete mechanism for formation of 2,2-dimethoxypropane from acetone and methanol.

protonated carbonyl   _protonated hemiacetal_

MeO   +OHMe   ⇌   MeO   OMe

protonated ketal   _ketal_

Write the complete mechanism for formation of a ketal from 1,2-dihydroxybenzene (show curly arrows, as usual) and cyclopentanone.

protonated carbonyl

protonated hemiketal

$\rightleftharpoons$

protonated ketal

-H$^+$

ketal

The following diagrams show one compound in a chemically related set of aldehyde or ketone, hemiacetal or hemiketal, acetal or ketal. Predict the other two compounds in the series in each case. Label each product "hemiacetal" or "ketal" as appropriate.

hemiketal

ketal

aldehyde + "diol"

acetal

ketone + "diol"　　　*hemiketal*

ketone + "diol"　　　*hemiketal*

Indicate how many alcohol and ketone *molecules* it would take to make the following acetals and ketals, and the total number of *molecules* generated (do not forget the water lost in these reactions).

a

molecules used to make 3
molecules produced 2

b

molecules used to make 2
molecules produced 2

c

molecules used to make 1
molecules produced 2

Based on entropic considerations alone, the most stable ketal to acid is *c*.

3 Addition Of Water And Alcohols To Aldehydes And Ketones

from chapter(s) _____ in the recommended text

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Some natural products that are not sugars also contain acetals or ketals, *eg* spirolaxine.

maltose   sucrose   spirolaxine

Concepts Involved

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most reactive

least reactive

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Proton transfer, loss from one atom in an intermediate, followed by gain of a proton by another atom is *common* in organic mechanisms.

Illustrate the following proton transfer steps by showing curly arrows for loss of a proton from the heteroatom with the positive charge, then addition of it to the neutral one.

It is common to abbreviate loss of proton and gain or proton in one step (without curly arrows). Show this in the following examples.

HO—O⁺(H)-cyclohexane   -H⁺, +H⁺ ⇌   H₂O⁺—O-cyclohexane

H₃N⁺—OH   -H⁺, +H⁺ →   H₂N—⁺OH₂

(Ph)(Ph)C(O⁻)(N⁺H₂)(NH₂)   -H⁺, +H⁺ ⇌   (Ph)(Ph)C(HO)(NH)(NH₂)

C(O⁻)(N⁺H₂Ph)   -H⁺, +H⁺ →   C(HO)(NHPh)

HO—O⁺(H)-cyclohexane   -H⁺, +H⁺ ⇌   H₂O⁺—O-cyclohexane

H₃N⁺—OH   -H⁺, +H⁺ ⇌   H₂N—⁺OH₂

Addition Of Water

Hydration of aldehydes and ketones is an *equilibrium* process that involves protonation of the carbonyl group, addition of water, and deprotonation.

Hydrates of methanal, and of compounds with electron withdrawing carbonyl substituents, are *strongly* favored relative to the parent carbonyls.

Draw hydration of methanal using curly arrows to show electron flow.

O
‖   +H+

protonated carbonyl

+OH
‖   H-OH

protonated hydrate

OH
O+H₂

-H+

OH
OH

hydrate

Draw the key intermediates for hydration of acetone using curly arrows to show electron flow.

O
+H+

protonated carbonyl

+OH
H-OH

protonated hydrate

OH
O+H₂

-H+
→

$\overset{OH}{\underset{OH}{\diagup}}$

_____
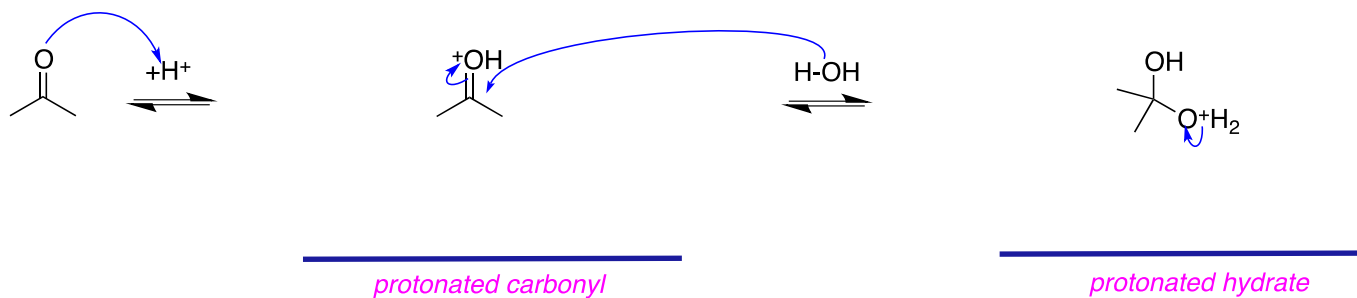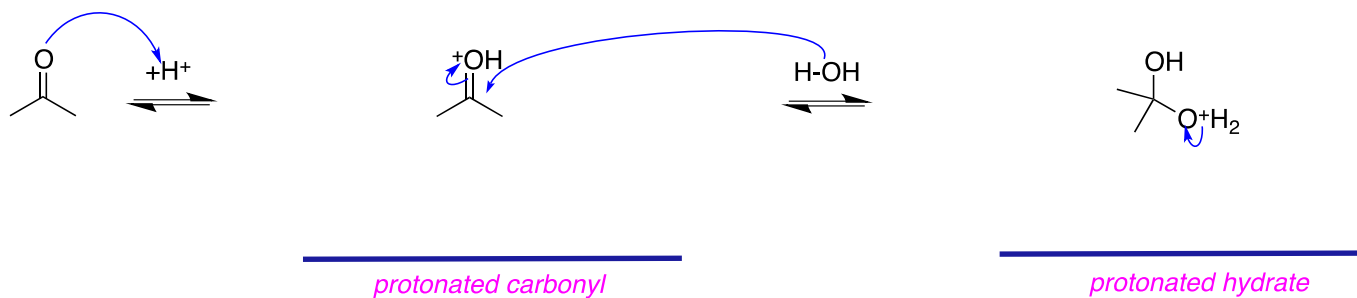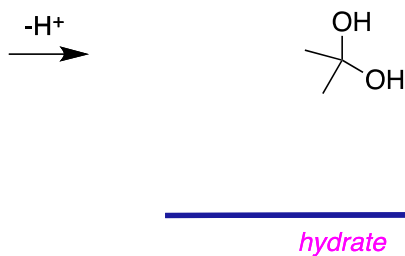
hydrate

The equilibrium constant for hydration of acetone is 2×10^{-3} so when mixed with 10 equivalents of water the amount of hydrate will be: *exactly 2 %*.

On adding water, the angle between carbonyl substituents of a ketone contracts from 120° to 109 °; consequently, formation of a hydrate is *disfavored* when the carbonyl substituents are *large*.

When aldehydes are mixed with $H_2{}^{18}O$, the heavy atom label *does* become incorporated into the aldehyde C=O.

Additions Of Alcohols

Draw the "hydration" acetone but using H-OMe instead of H-OH as the nucleophile.

protonated carbonyl   *protonated hemiketal*

The product above with one OH and one OR group attached to the carbon that was the carbonyl group, is an *hemiketal* because it is half way to the $R_2C(OR')_2$ form and derived from a *ketone*.

The product formed from adding one alcohol to an aldehyde is called a *hemiacetal.*

Draw a *reversible* sequence of events representing protonation on the OH group of the *hemiacetal* from hydration of *ethanal*, loss of water to give an *oxonium* intermediate, addition of another methanol nucleophile, and finally deprotonation to give an *acetal*.

protonated acetal

Acetals and ketals *do* react with excess aqueous acid to give aldehydes and ketones.

Complete the following mechanism for addition of methanol to *benzophenone* under acidic conditions to give a hemiketal by completing the structures and drawing in curly arrows.

hemiketal

Draw the same process for acid catalyzed addition of methanol to butanone.
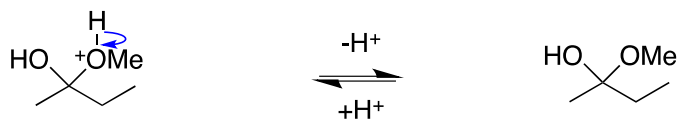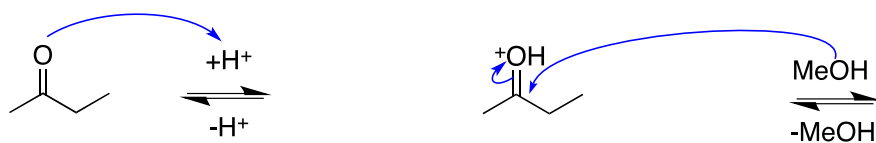
+H⁺
-H⁺

+OH
MeOH
-MeOH

_____

H
HO   ⁺OMe

-H⁺
+H⁺

HO   OMe

_____   _____

hemiketal

Complete the following mechanism showing curly arrows.

+H⁺
-H⁺

+OH
MeOH
-MeOH

_____

starting material?   _____

H
HO   ⁺OMe

-H⁺
+H⁺

HO   OMe

_____
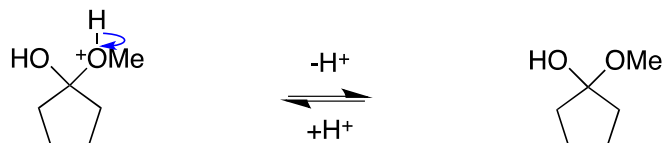
Complete the following diagram to show how *hemiacetals* and *hemiketals*, like those above, can be protonated on the hydroxyl, expel water, add another alcohol then lose a proton.
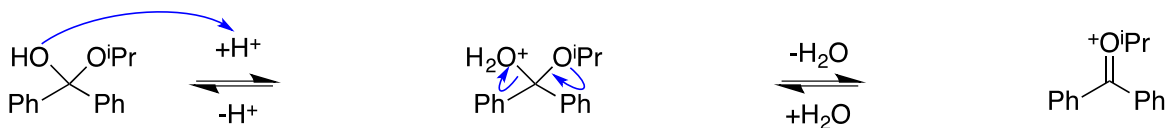
protonated on OH

oxonium ion
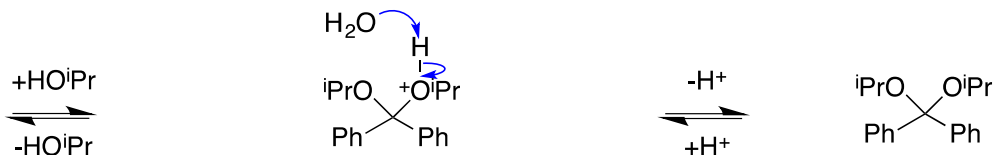
protonated ketal

The starting material in this sequence was formed from a ketone and *an alcohol*

Draw exactly the same sequence for the hemiacetal from ethanal and methanol.

protonated on OH

oxonium ion

H_2O

$MeO \quad ^+OMe$
$\quad \overset{H}{\underset{}{\bigtriangleup}}$

$\xrightarrow[-HOMe]{+HOMe}$

$MeO \quad ^+OMe$
$\quad H$

$\xrightleftharpoons[+H^+]{-H^+}$

$MeO \quad OMe$
$\quad H$

protonated acetal

acetal

Draw exactly the same sequence for the hemiacetal from benzaldehyde and *n*-butanol (introduce the alcohol over the appropriate arrow).

$HO \quad O^nBu$
$Ph \quad H$

$\xrightleftharpoons[-H^+]{+H^+}$

$H_2O^+ \quad O^nBu$
$Ph \quad H$

$\xrightleftharpoons[+H_2O]{-H_2O}$

$^+O^nBu$
$Ph \quad H$

protonated on OH

oxonium ion

$\xrightleftharpoons[-HO^nBu]{+HO^nBu}$

$^nBuO \quad ^+O^nBu$
$Ph \quad H$ (with H on top)

$\xrightleftharpoons[+H^+]{-H^+}$

$^nBuO \quad O^nBu$
$Ph \quad H$

protonated acetal

acetal

Draw the same sequence for the hemiacetal from cyclopropane carboxaldehyde and ethanol, taking care to fill in the reactants around the arrows.

protonated on OH

oxonium ion

protonated acetal

acetal

Draw the same sequence for the hemiketal from 2-butanone and 1,2-ethandiol, taking care to fill in the reactants around the arrows. Draw the reagents above each arrow.

protonated on OH

oxonium ion

$\rightleftharpoons$

+H$^+$

$\rightleftharpoons$

-H$^+$

protonated ketal

ketal

Write the complete mechanism for formation of 2,2-dimethoxypropane from acetone and methanol.

protonated carbonyl

protonated hemiacetal

protonated ketal

ketal

Write the complete mechanism for formation of a ketal from 1,2-dihydroxybenzene (show curly arrows, as usual) and cyclopentanone.

protonated carbonyl

protonated hemiketal

protonated ketal

ketal

The following diagrams show one compound in a chemically related set of aldehyde or ketone, hemiacetal or hemiketal, acetal or ketal. Predict the other two compounds in the series in each case. Label each product "hemiacetal" or "ketal" as appropriate.

hemiketal

ketal

aldehyde + "diol"

acetal

ketone + "diol"   hemiketal

ketone + "diol"   hemiketal

Indicate how many alcohol and ketone *molecules* it would take to make the following acetals and ketals, and the total number of *molecules* generated (do not forget the water lost in these reactions).

a

molecules used to make 3
molecules produced 2

b

molecules used to make 2
molecules produced 2

c

molecules used to make 1
molecules produced 2

Based on entropic considerations alone, the most stable ketal to acid is *c*.

4 Formation of Cyanohydrins, Imines, Enamines

from chapter(s) _____ in the recommended text

Introduction

Focus

Addition of hydrogen cyanide to ketones and aldehydes follows a predicable route: protonation of the carbonyl then addition of the nucleophile, cyanide. Addition of ammonia and primary amines to aldehydes and ketones

hydration of a ketone:

hydrate

no leaving group but water
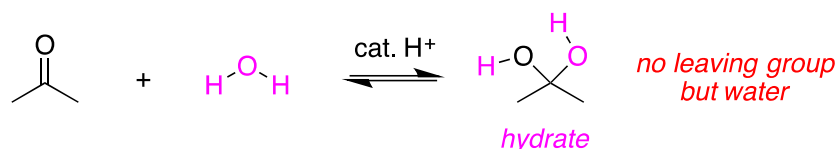
forms an intermediate very similar to hydrates, as illustrated here for methylamine. Like aldehydes and ketone hydrates, an OH group can be protonated, then water is displaced by the action of the lone pair on the other atom, now giving an imine. Reactions of secondary amines follow the same pathway, but there is no proton left on the iminium-nitrogen, so they loose another to form an enamine.

condensation with primary amine:

hydrate-like   *imine (like ketone)*
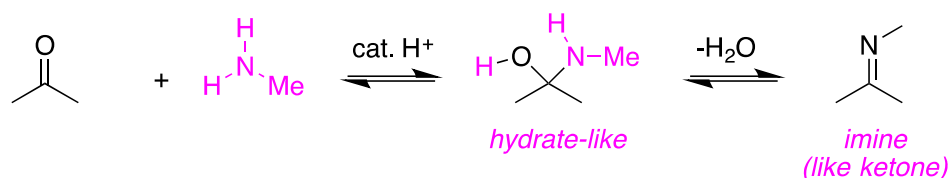
condensation with secondary amine:

hydrate-like   *iminium*   *enamine*
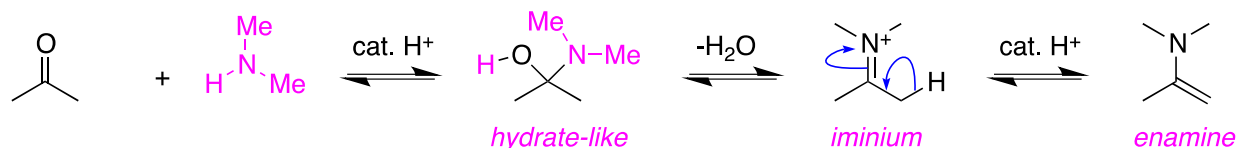
Reasons To Care

Condensations of amines with ketones are common in cell biology, *eg* in the biosynthesis of amino acids.

Concepts Involved

acids and bases • electron flow • expulsion of water in condensation reactions

Objective

Learning reaction mechanisms in isolation is quite difficult, but it is much easier when the similarities and small differences of a series of related transformations are considered. Here the objective is to relate reactions of aldehydes and ketones to mechanisms for cyanohydrin, imine, and enamine formation.

Tetrahedral Intermediates And Beyond

The four tetrahedral intermediates shown below are formed from 2-butanone and HCN, EtMgX, pyrrolidine (look up structure), or methylamine. Write the corresponding starting material below each structure.
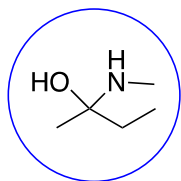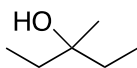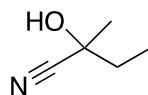
methylamine   _EtMgX_   _HCN_   _pyrrolidine_
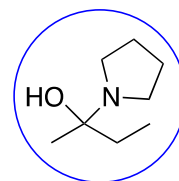
Two of these intermediates would be inclined to loose water when protonated on the oxygen _because_ a nitrogen is present to push in electrons from a lone pair. Put circles around those two intermediates.

With HCN

The pKa of HCN is 9.1; the cyanide anion is a *weak* base because it is relatively stable, so, unsurprisingly, it adds *reversibly* to aldehydes or ketones. Show the mechanism of addition of HCN to acetone illustrated with clear curly arrows.

protonated carbonyl   *cyanohydrin adduct*
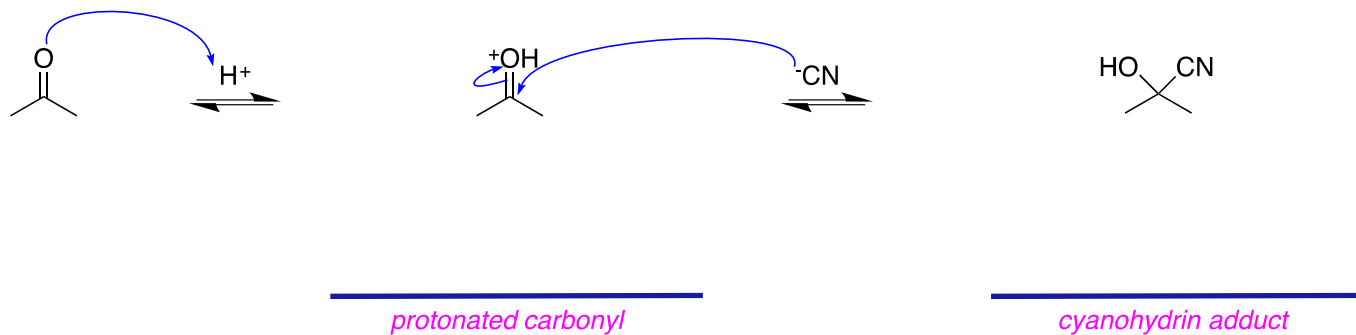
Show the same mechanism for the starting materials indicated below.
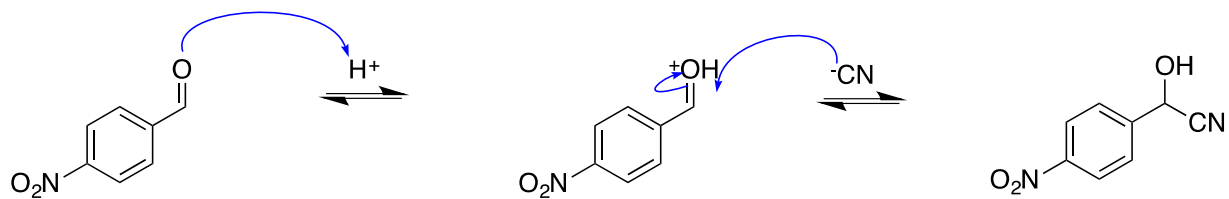
4-nitrobenzaldehyde   *protonated carbonyl*   *cyanohydrin adduct*
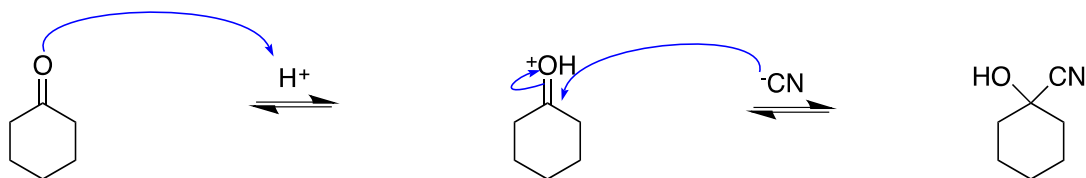
cyclohexanone
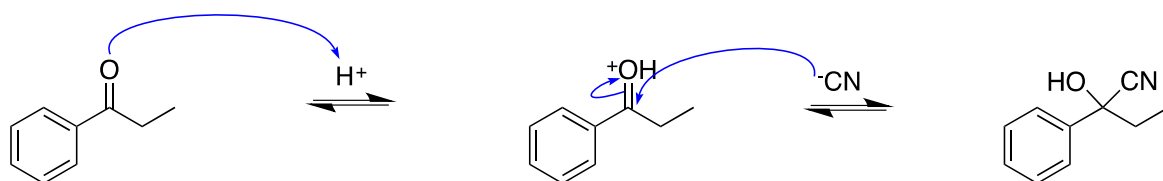
phenylethanone

Condensations Of Aldehydes Or Ketones With H$_2$N-R Or H$_2$N-X

Primary Amines Form Imines

Imines (Schiff bases) form when *primary* amines or ammonia condense (*loose* water) with aldehydes or ketones.

Show the mechanism of condensation of cyclopentanone with phenylamine.
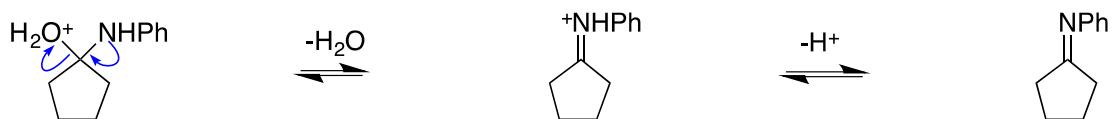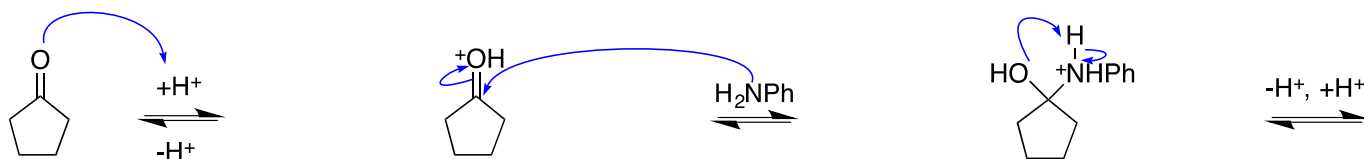
Show the same mechanism for cyclohexane carboxaldehyde.

H₂O⁺   -H₂O   ⁺NHEt   -H⁺   NEt

[reaction scheme: cyclohexyl carbinol iminium intermediates]
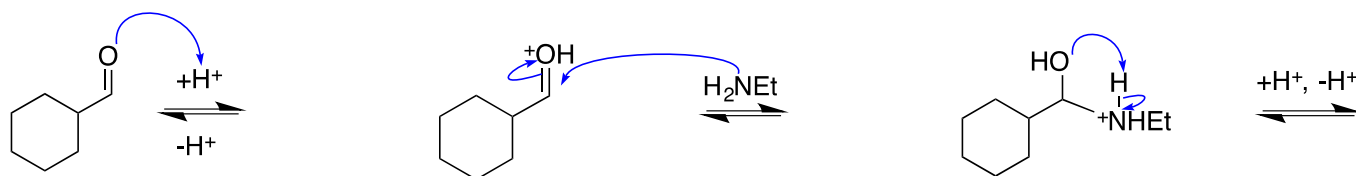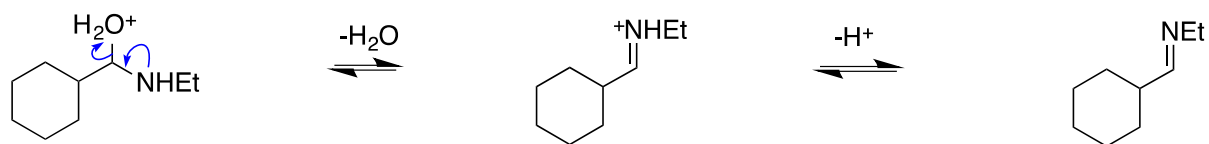
The rate of this reaction reaches a maximum at about pH 4.5 because: at this pH, the carbonyl will be protonated leading to activation of carbonyl group, facilitating the nucleophilic addition of amines. If the pH is lower than 4.5, most of the amine will be protonated making it non-nucleophilic, slowing the rate of reaction.

Draw the mechanism for reaction of benzaldehyde with phenylamine at pH 4.5 to form the corresponding imine.
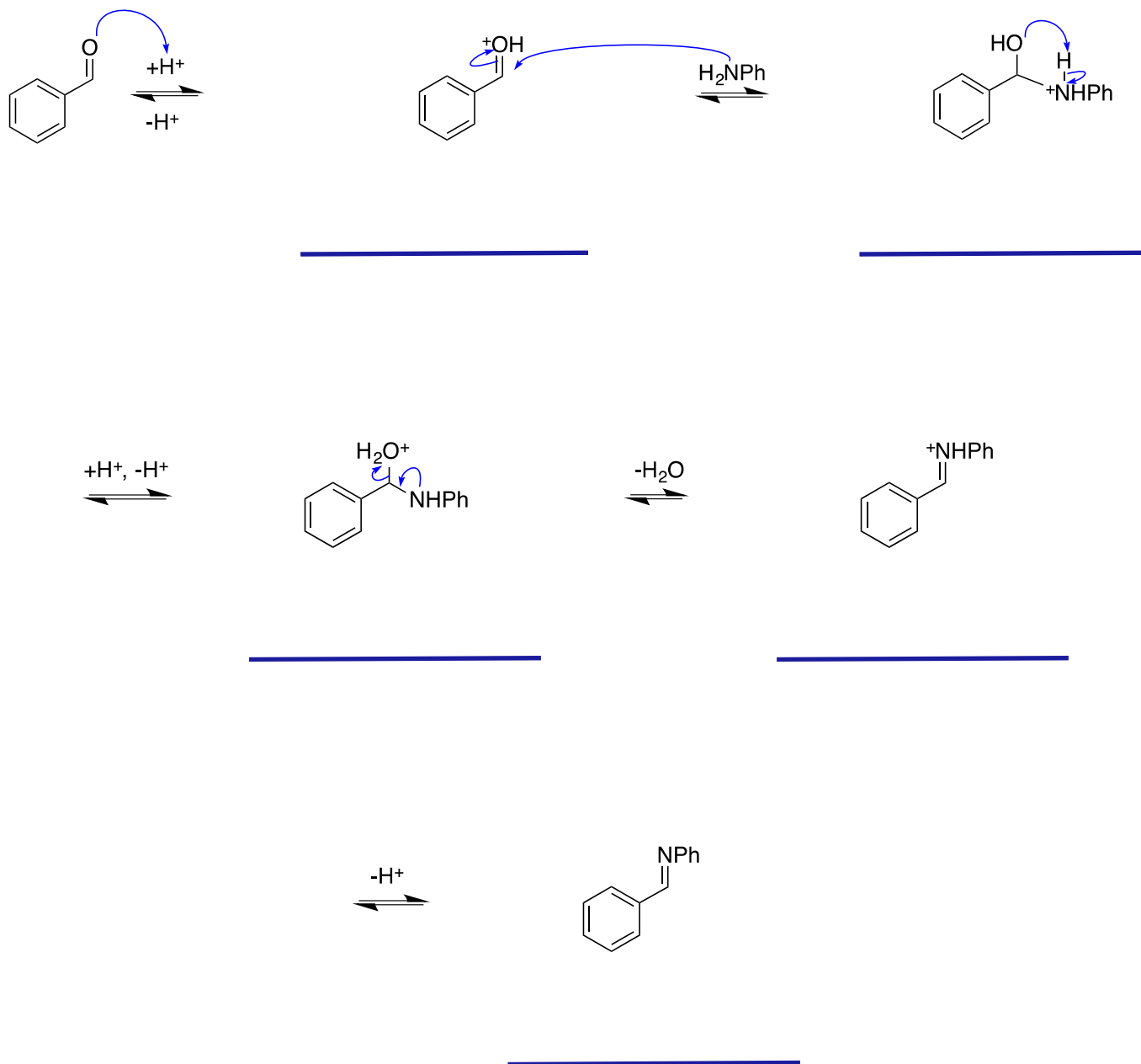
Secondary Amines Form Iminium Ions Then Enamines

Secondary amines condense with aldehydes and ketones to give *enamines* because deprotonation of the iminium intermediates must proceed through loss of protons from the *α‑carbon*.

All the steps in enamine formation are *reversible*.

Show the mechanism of enamine formation from phenylethanone and pyrrolidine.

-H+
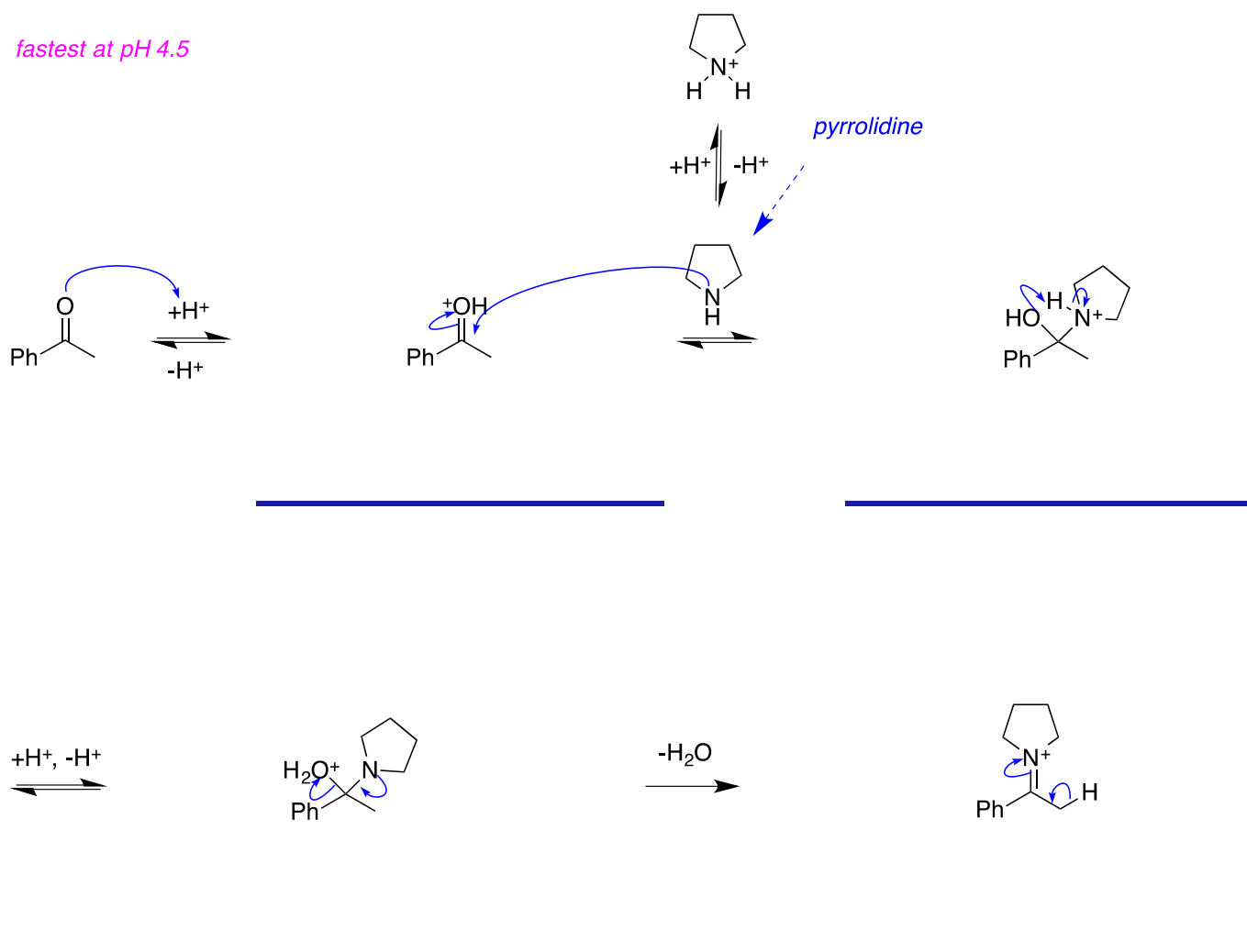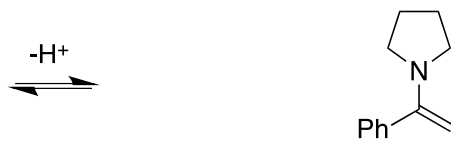
The 6-membered ring analog of pyrrolidine is called *piperidine* (check Wiki).

Show a mechanism similar to the one above but for enamine formation from cyclopentanone and piperidine.

cyclopentanone
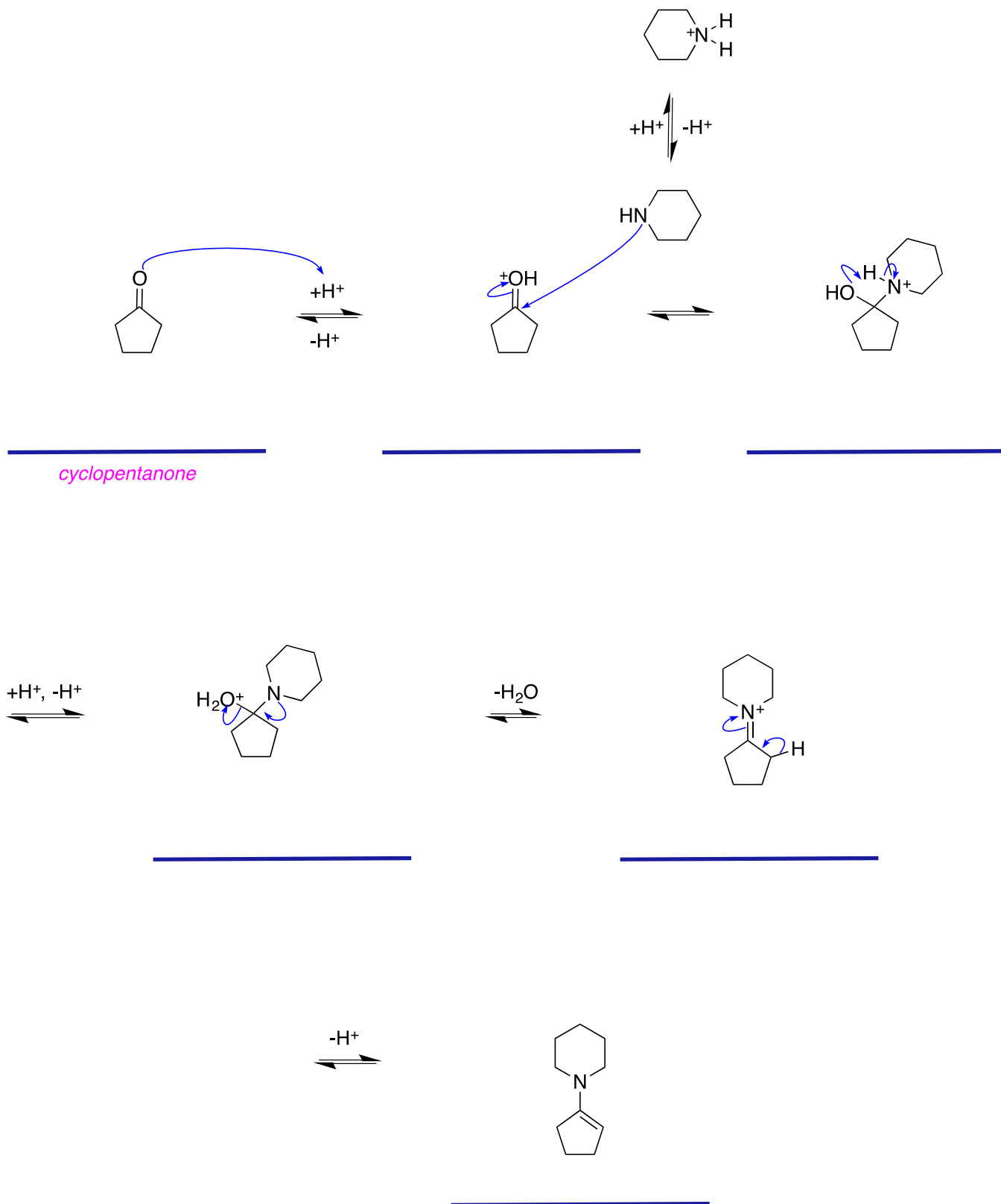
Water and acids *do* hydrolyze enamines back to the ketones and amines.

Each of the following reactions involve formation of *imines* or *enamines*; show the products.
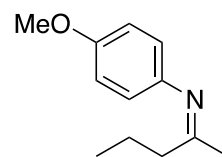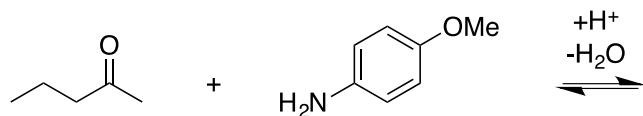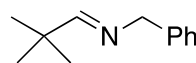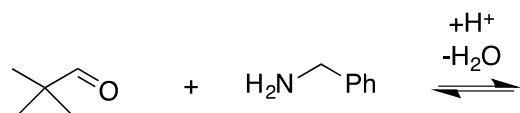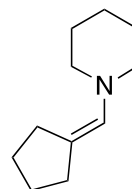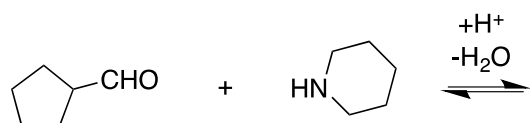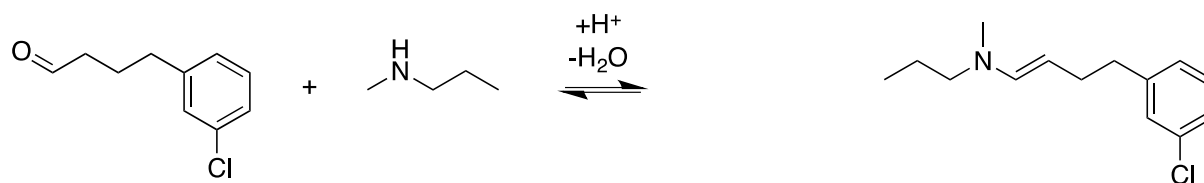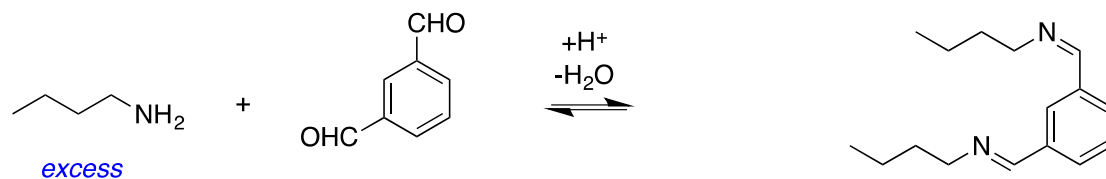
Review the mechanisms in this chapter, then state the reason why primary secondary amines react differently in condensations (to give imines and enamines, respectively) is:

Primary amines have two protons; one can be transferred to OH group then loss of water and another is removed

to neutralize iminium to form imines. On the other hand, secondary amines have only one proton involved in

dehydration step but no proton left to neutralize iminium ion. So the mechanism must involve to loosing a proton

from the α-carbon to neutralize iminium ion thus forming enamines.

Transamination

Abbreviate the following imine to R-NH₂, then draw a mechanism for addition of aspartic acid, then loss of the Lys side-chain from the enzyme (*ie* loss of N-enz).

tetrahedral intermediate

$-H^+, +H^+$

tetrahedral intermediate

$-H_3N^+enz$
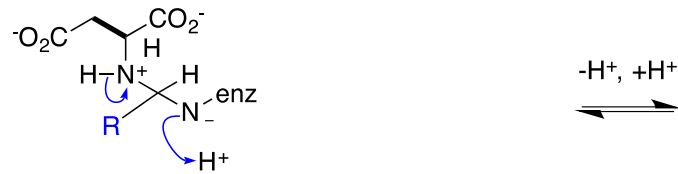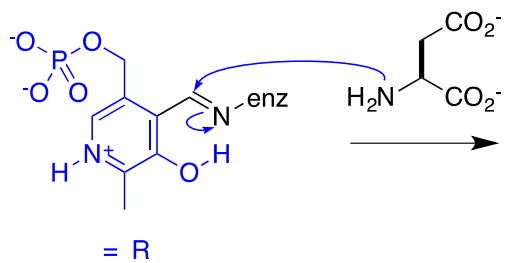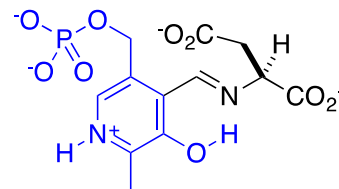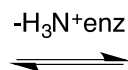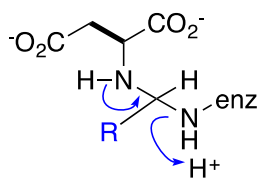
imine products

The following reaction involves a deprotonation that neutralizes the charge of the pyridinium-*N*, protonation on the benzylic carbon, then imine hydrolysis to give a product in which the amine group of Asp has been replaced by a ketone. Complete the mechanism, showing highlights of the electron flow.

pyridine intermediate

α-ketoacid
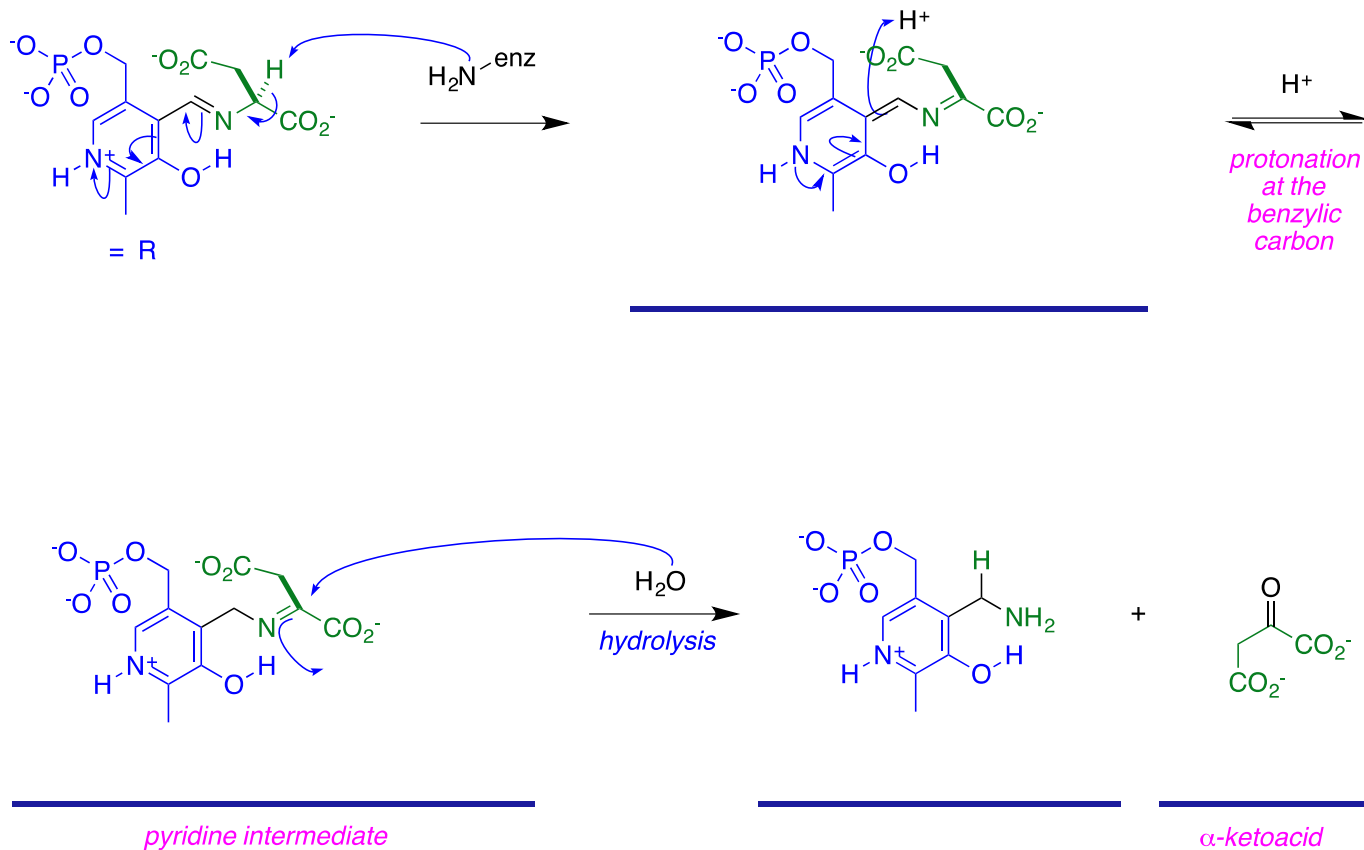
Transamination, as illustrated above, is an enzyme-mediated process for degradation of amino acids *in vivo*. Shown above is the *oxidation* of aspartic acid.

The amine formed in the process above is recycled back to the enzyme bound imine via reaction with the *amine* derived from another amino acid.

Transamination reactions *degrade one and form another.*

5 Stereochemistry Illustrated By Carbohydrates

from chapter(s) _____ in the recommended text

Introduction

Focus

Some aspects of stereochemistry were covered in the first phase of this *Inquisition*, *eg* priority rules, assigning configurations, and definitions of terms like diastereomers and *"meso*-isomers". However, these fundamentals are not as difficult to master as drawing complex chiral molecules in different ways without accidentally changing their stereochemistries. This, coincidentally, is one of the hardest aspects of carbohydrate chemistry so both together form a single focus in this chapter.

Reasons To Care

Stereochemistry in carbohydrates is important. Most carbon atoms in carbohydrates are chiral, and their configurations distinguish the different monomers (*eg* glucose, galactose, *etc*). However, the hemi-acetal (or ketal) centers in carbohydrates can sometimes open and re-form in different ways. Aldose monomers with six carbon atoms can cyclize intramolecularly to form five- or six-membered hemiacetals (furanose and hexanose forms) and other different anomeric configurations.   Acetal or ketal units link monosaccharide building blocks in oligosaccharides, and these can also have different configurations.
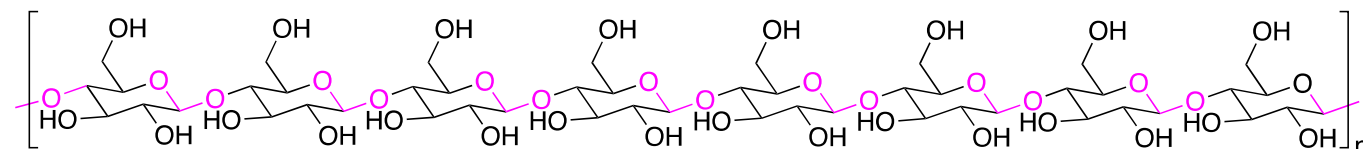
cellulose, and other oligosaccharides, are monosaccharides linked by acetal groups

Understanding carbohydrate chemistry requires the ability to manipulate complex stereochemical information.

Concepts

drawing chiral molecules accurately • Fischer projections • optical rotations • nomenclature in carbohydrate chemistry • hydrolysis of acetals and ketals • formation of hemi-ketals

Objective

This section probes and stretches minds around the torturous ways carbohydrates can equilibrate between different conformers and cyclic hemiacetals/hemiketals, then different oligomeric forms.

Assigning *R*- and *S*-Configurations

"Breakdown" representations of the following functional groups that could be used to assess their relative priorities.
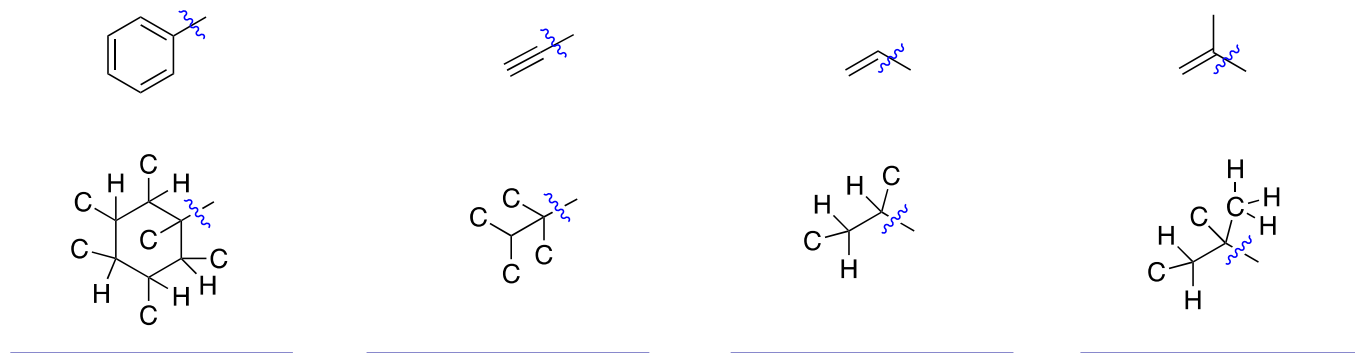
Prioritize the following fragments according to the rules for assigning configuration.

highest priority   *lowest*
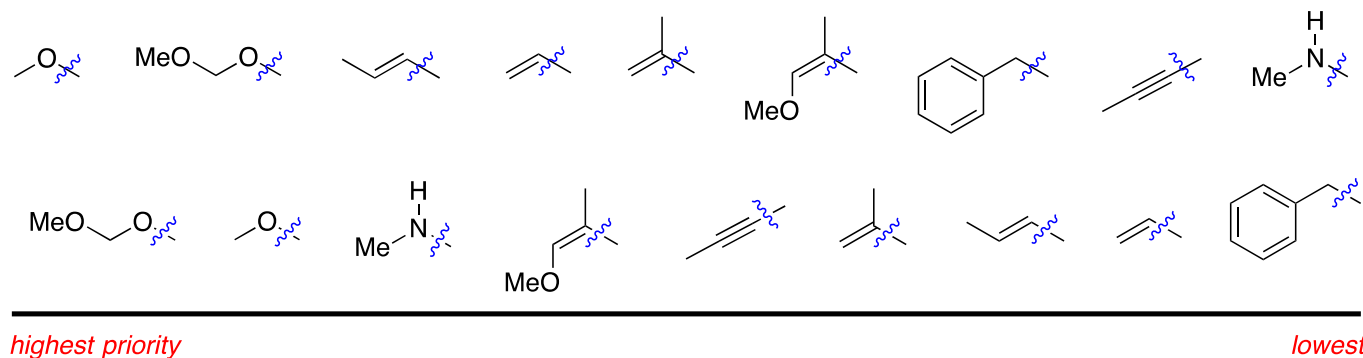
Circle which of the following compounds can be optically active
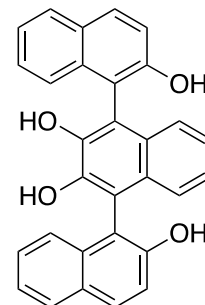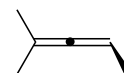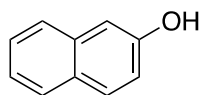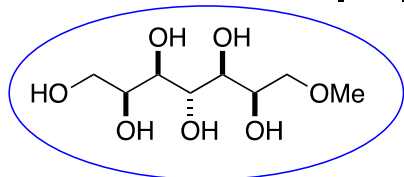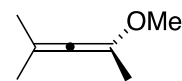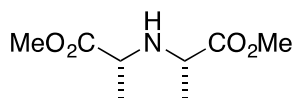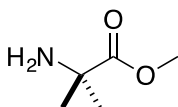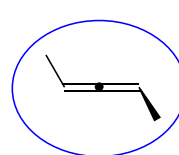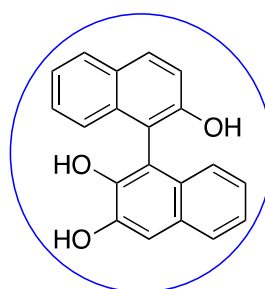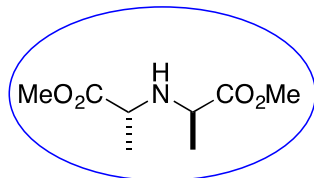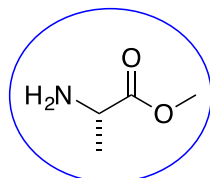
Assign the configurations of the following molecules if they are chiral. Circle any of these compounds that will not have an optical rotation, but instead use a square around those that are *meso*.

S   *R*   *R*   *R*   *S*   *R*   *S*   *S*

R / S   *R*   *R*   *R*   *S*   *R*

S   *R*   *S*   *S*   *S*

R / S   *S*   *R*   *S*   *R*   *R*   *R / S*   *S*   *S*   *S*   *R*
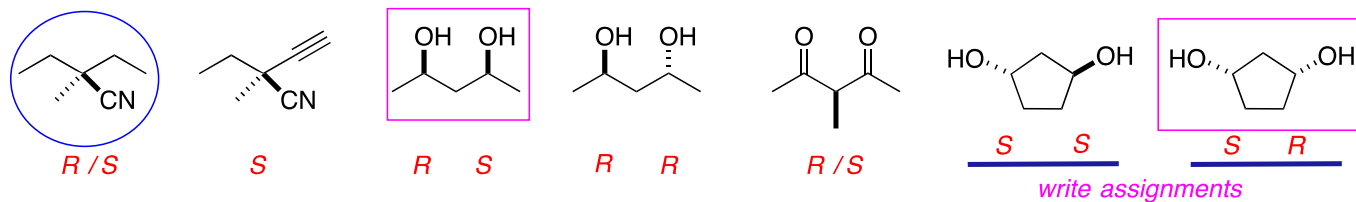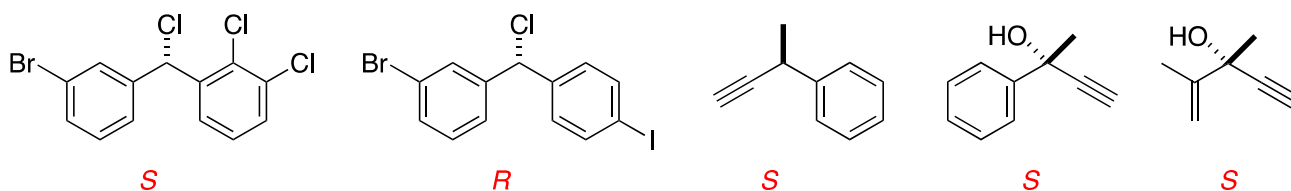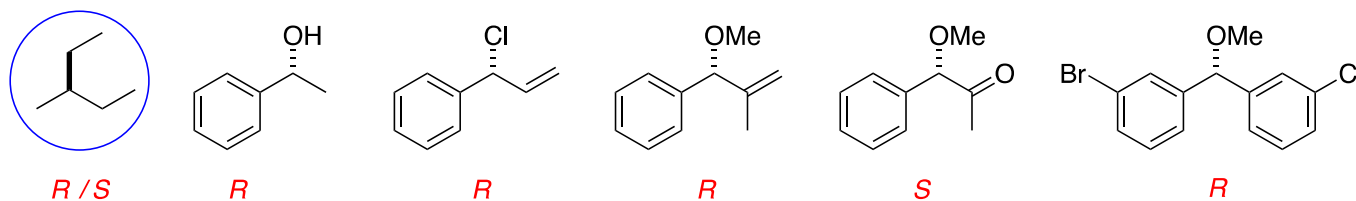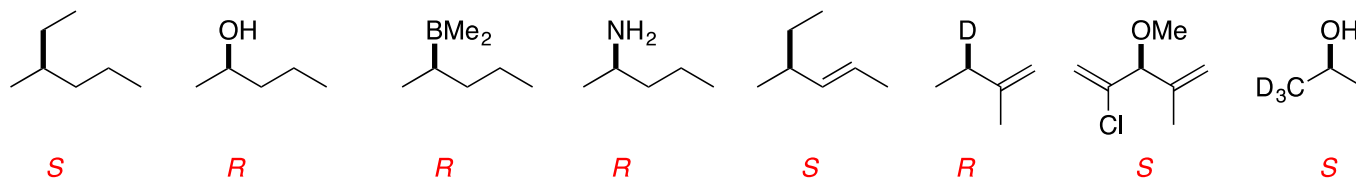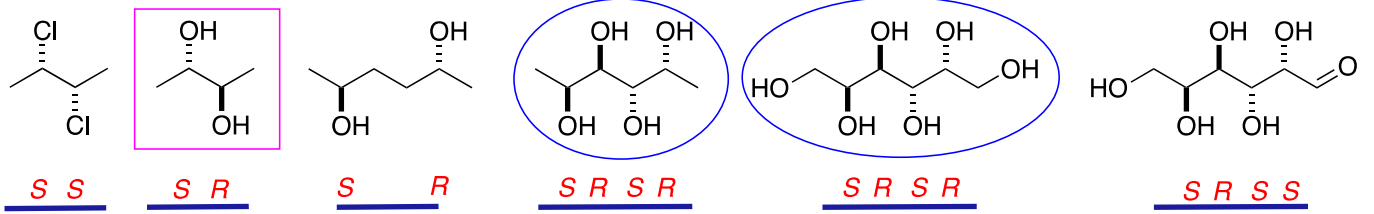
write assignments

write assignments

Stereochemical Representations Of Carbohydrates

The terms *carbohydrate, saccharide, sugar are all used to describe compounds in this series*.

Carbohydrates can be complicated structures formed from repeating units of simpler one. These units are mostly named with the suffix *"ose"* (*eg* glucose): if they contain an aldehyde they are called *aldoses* and if they contain ketones they are called *ketoses*. Classify each of these sugars as ketoses or aldoses.
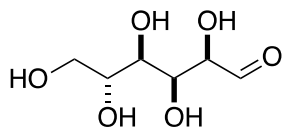
D-glucose
aldose
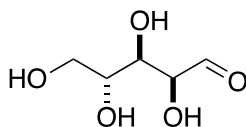
D-ribose
aldose
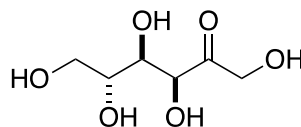
D-fructose
ketose
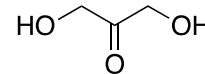
dihydroxyacetone
ketose

Stereochemistries of monomeric sugars can be represented in Fischer projections with the aldehyde at the *top* by convention.

The simplest aldose is glyceraldehyde, CHO-CH(OH)-CH$_2$OH. D-Glyceraldehyde has the OH group on the chiral center on D-right (and the less common enantiomer stammers about having it on the other side, L-, L-, L-left). Draw D-glyceraldehyde in the Fischer projection form, then as a zigzag representation.
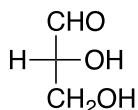
D-glyceraldehyde Fischer projection
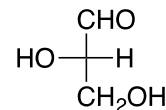
L-glyceraldehyde Fischer projection
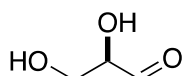
D-gylceraldehyde zig-zag
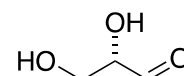
L-glyceraldehyde zig-zag

Glyceraldehyde is a *triose* because it contains *three* carbons. Monosaccharides with *five* carbons are *pentoses*, and glucose (a *six* carbon sugar) is a *hexose*.

Aldoses that share the same configuration as D-glyceraldehyde *at the end nearest the primary alcohol* are also D-. Assign these sugars as D- or L- and circle the parts that define them as such.
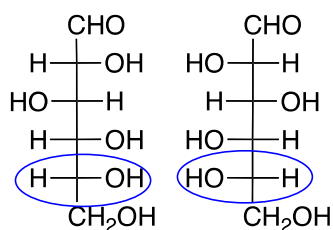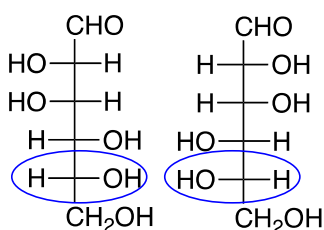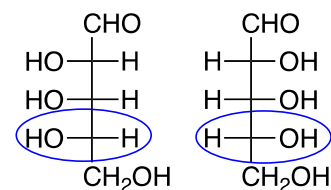
D- and L-Glucose are *enantiomers*. D-Mannose and D-glucose are *epimers*. Draw the enantiomers of the following monosaccharides by completing these Fischer projections:
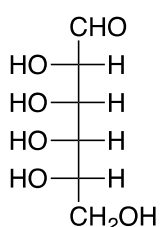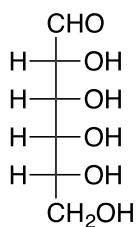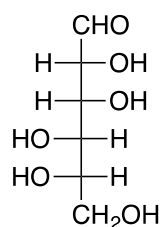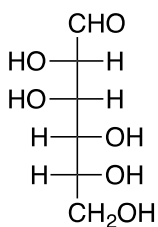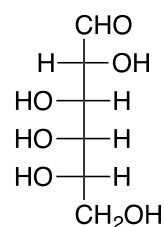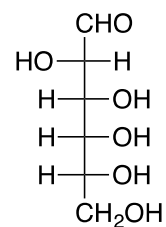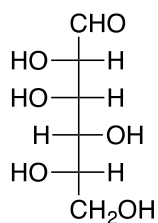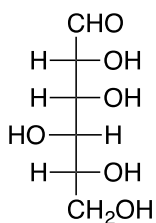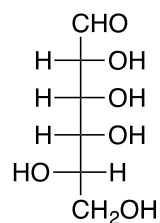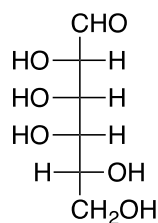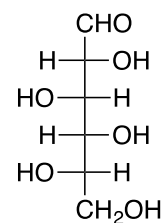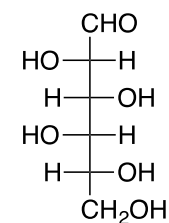
Transposing Fischer projections into zigzag structures requires careful attention to details. It helps to draw the diagrams that break down the process into steps.

Naturally occurring sugars tend to be *D-*.

Amino acids can be related to glyceraldehyde in the same way as monosaccharides. Genetically encoded amino acids are *D-*.

Complete the following zigzag representations of the Fischer projections indicated (*hard?* use models to check your answer).

CHO
HO——H
HO——H
H——OH
H——OH
CH₂OH

is equivalent to

is equivalent to
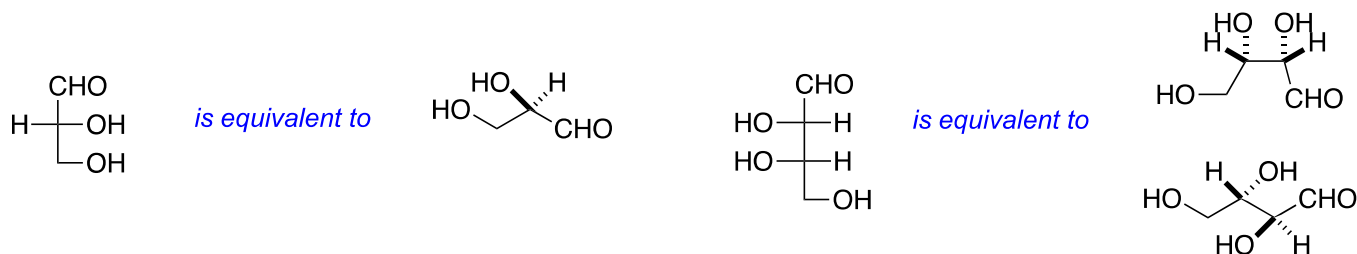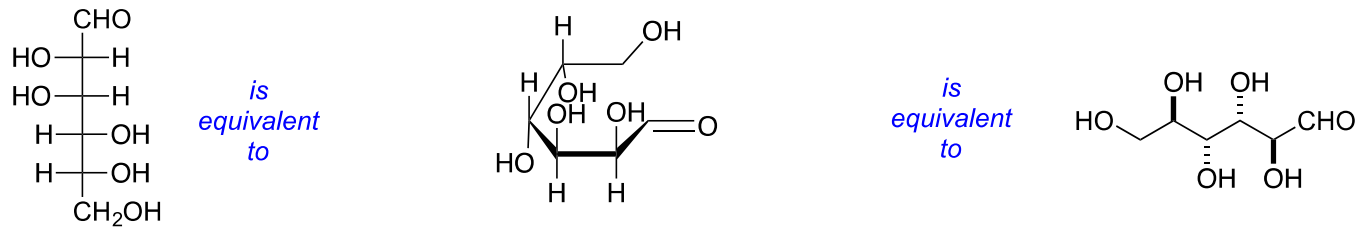
Without models it is sometimes essential to draw carbohydrates in different conformations to transpose their configurations onto other conformations. Problems like those above are much more difficult otherwise.

Carbohydrates Can Cyclize To Hemiacetals Or Hemiketals

Draw tetrahydrofuran (THF) and 4*H*-pyran (six-membered ring, C_5H_6O with saturated carbon at C^4), and tetrahydropyran.

THF　　　　　　　　*4H-pyran*　　　　　　　　*tetrahydropyran*

Much of carbohydrate chemistry relies upon being able to accurately transform a diagram of a chiral molecule into an alternative representation. This can be difficult and requires practice. Shown below are molecules drawn in an extended form, and parts of their structures re-drawn in other conformations. Complete the drawings of the molecules in the other conformations.
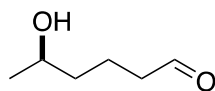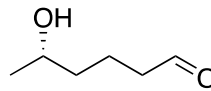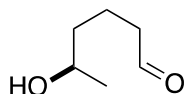
*is the
same
as*

*is the
same
as*
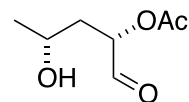
Many aldoses can cyclize into hemiacetals that resemble pyrans (*six*-membered ring) and to ones that resemble furans (*five*-membered ring). Isomerization of carbohydrates between furanose and pyranose forms proceeds via the mechanism for acid-mediated hydrolyses of hemiacetals: protonation of the endocyclic, anomeric, *-O*, ring opening, then ring closure using another hydroxyl. Draw this mechanism for the equilibrium below, showing clear curly arrows for each step.


protonated pyranose form

protonated furanose form

ring closing | *ring opening*

ring closing | *ring opening*

protonated aldehyde

protonated aldehyde redrawn poised for 5-membered ring formation
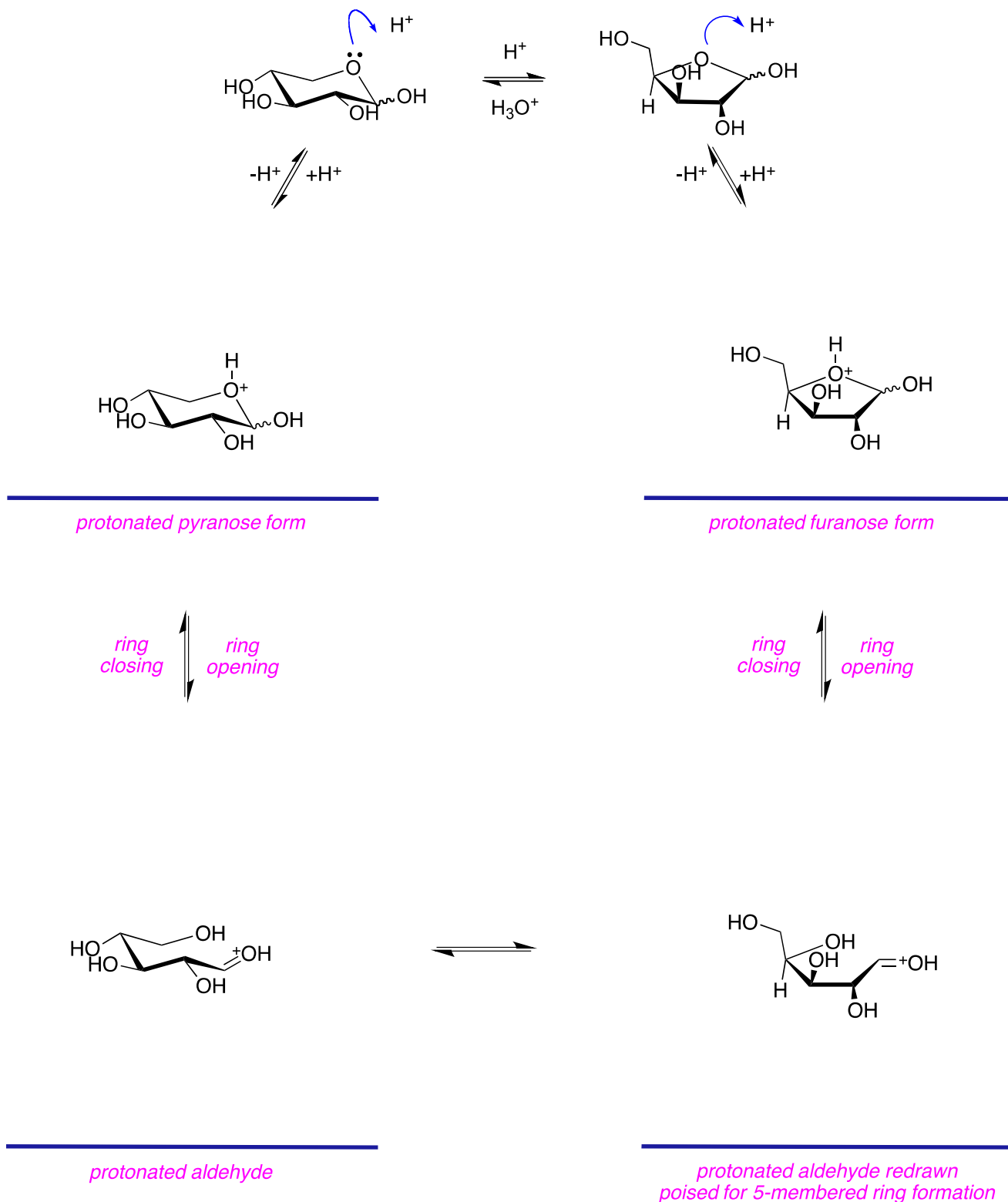
Glucose *and* ribose can exist as a mixture of furanose and pyranose forms, hence the optical rotation of this material (or these materials) is sensitive to pH and temperature.

Pyranose forms tend to exist in chair conformations, but it is best to re-draw them in artificial flat conformations to relate them to Fischer projections. Complete the following diagrams.
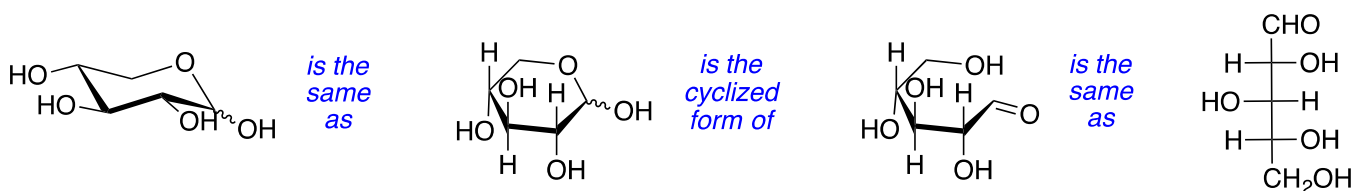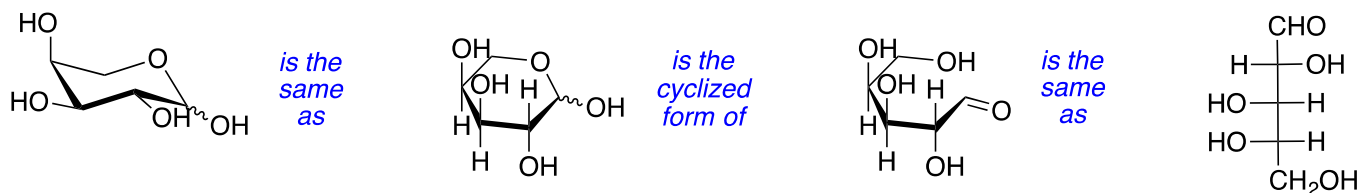
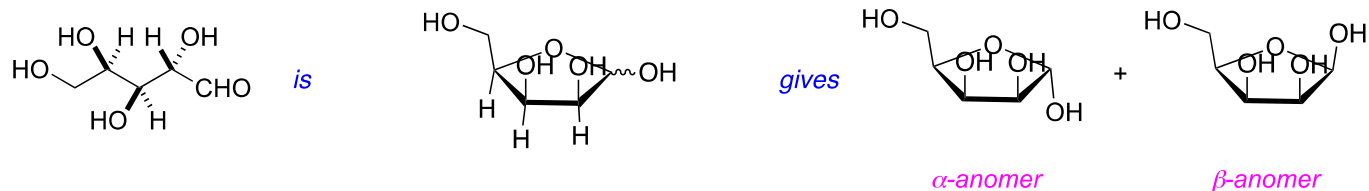Complete the following diagrams relating *pyranose* forms of *hexoses* to Fischer projections.
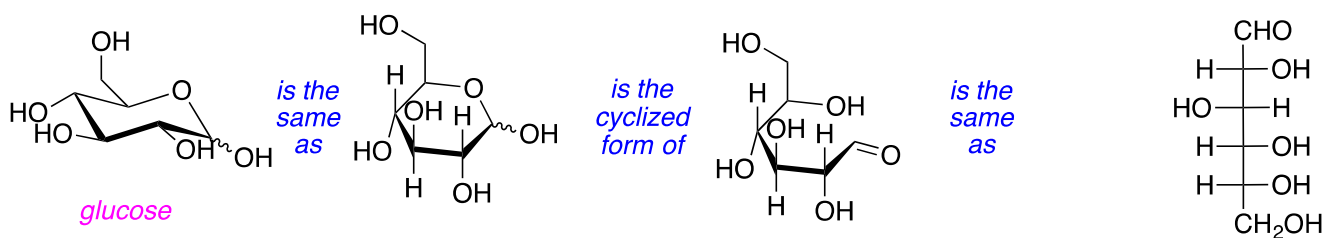
The anomer of D-glucopyranose that has all substituents in an equatorial position is **β-**.


is the same as

is the cyclized form of

is the same as
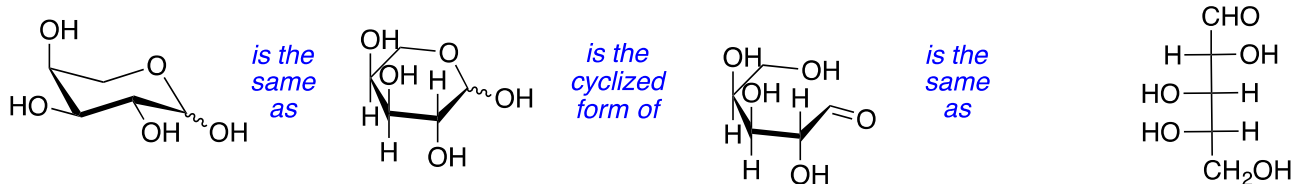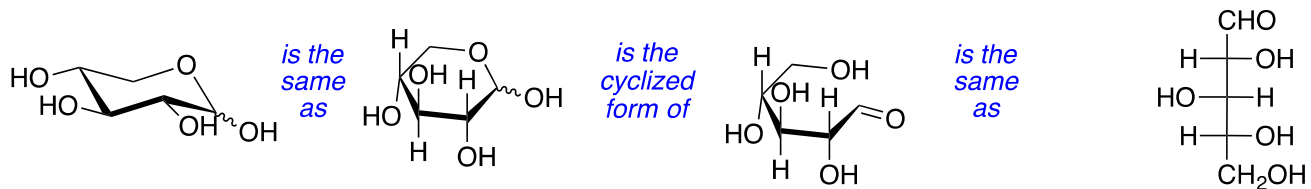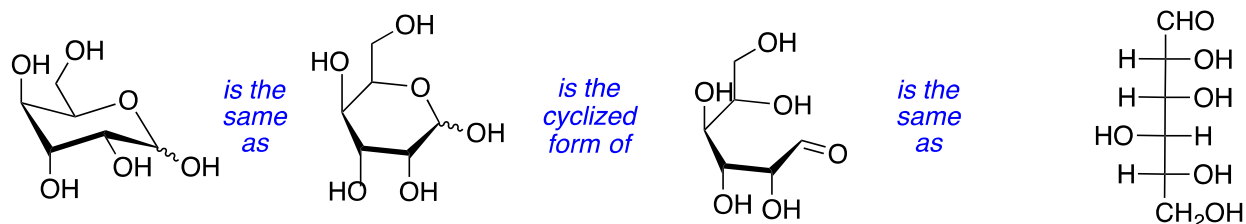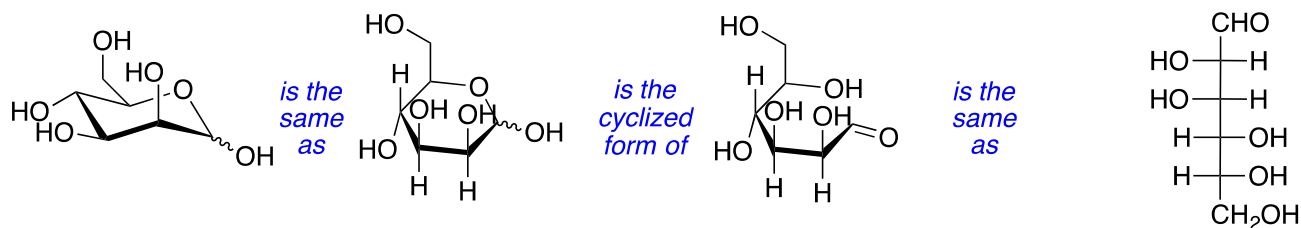
Use a similar approach to relate the following monosaccharides to their cyclic hemiketal forms. Note how α- and β-anomers are defined in the first example. By definition, the α-anomer is the one with the anomeric-*OH trans* to the -CH$_2$OH ring substituent.

is   *gives*

α-anomer   *β-anomer*

is   *gives*

α-anomer   *β-anomer*

is   *gives*

α-anomer   *β-anomer*

*is*   *gives*

α-anomer   *β-anomer*
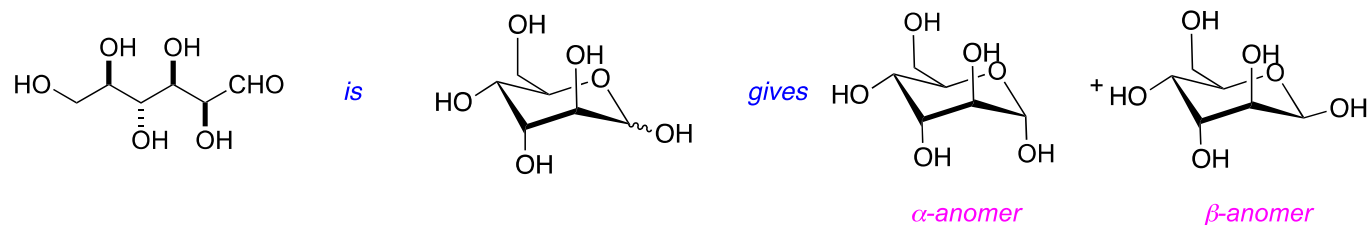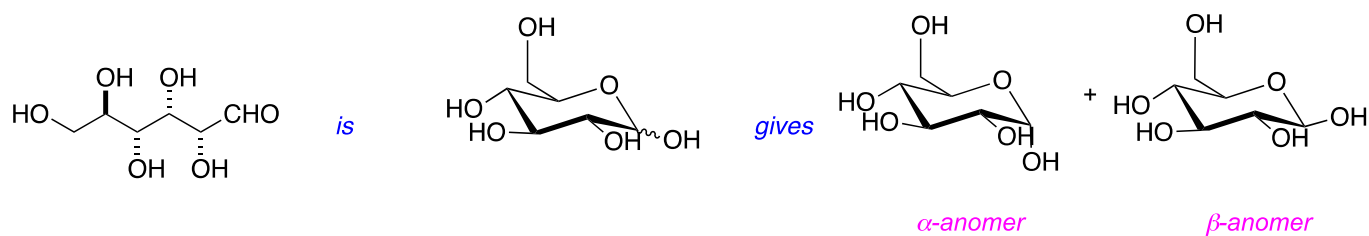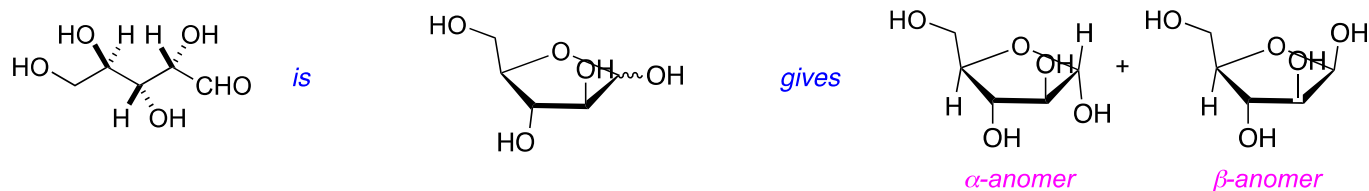
Draw pyranose forms of the following monosaccharides by completing the structures below. This question is also easy to visualize with models but hard without. The boxes are provided for drawing alternative conformations. Check answers by assigning *R*- and *S*-configurations.
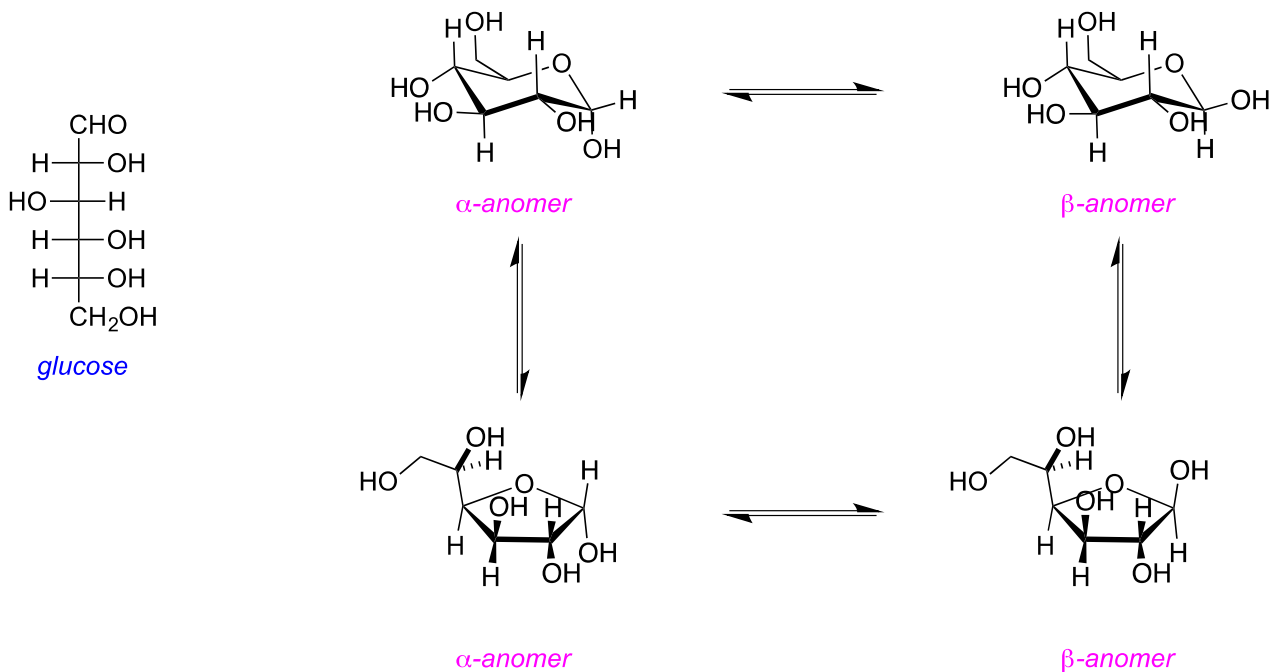

α-*anomer*   β-*anomer*

convert to the cyclized form
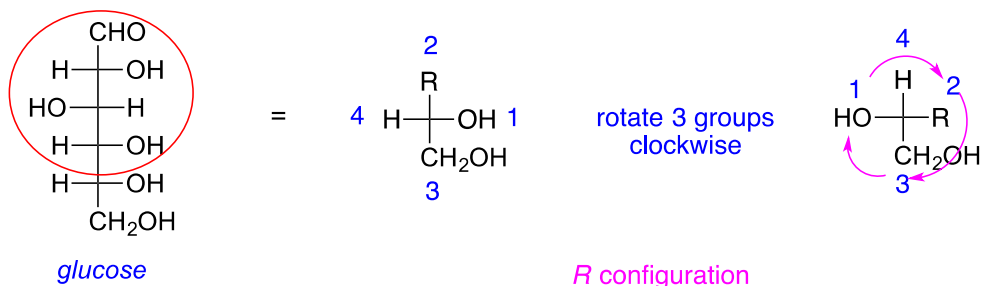

α-*anomer*   β-*anomer*
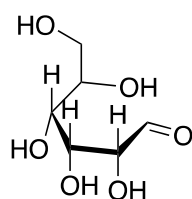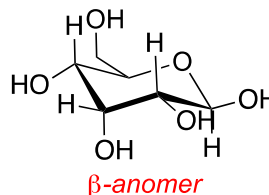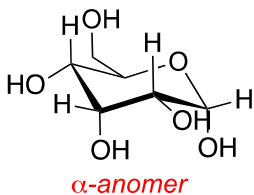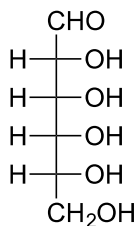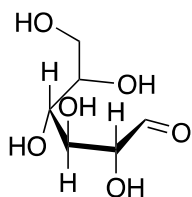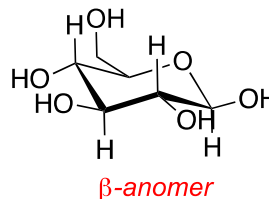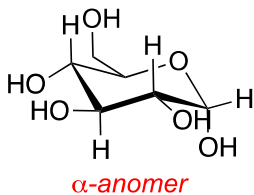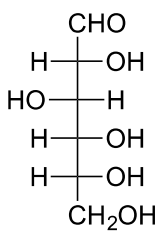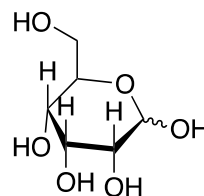
convert to the cyclized form
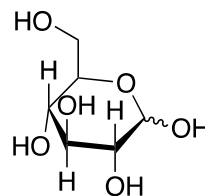
Complete the following diagram for glucose by filling in H and OH labels to indicate the correct stereochemistries.

glucose

α-*anomer*   β-*anomer*

α-*anomer*   β-*anomer*

box for reasoning, write answers above

glucose

=   4   H——OH 1
              CH₂OH
              3

2
R

R configuration

rotate 3 groups
clockwise

4
1   H   2
HO——R
     CH₂OH
        3

Homologation Of Sugars By Reaction With HCN

Monosaccharides, like other aldehydes, react with cyanide to form cyanohydrins. These cyanohydrins can be partially reduced to *imines* then hydrolyzed to form homologated *aldoses* as a mixture of epimers. Fill in the gaps in the following diagram.
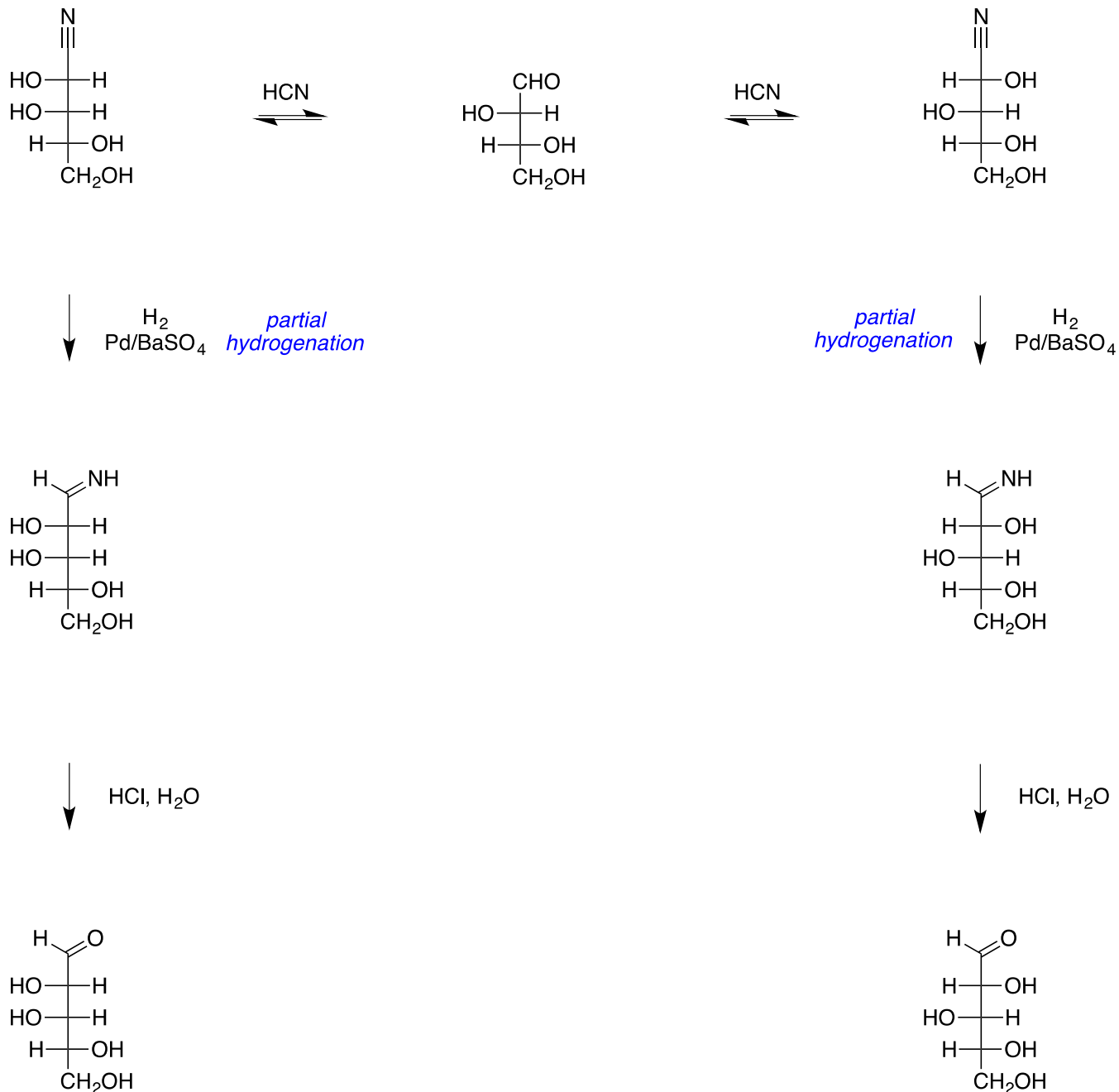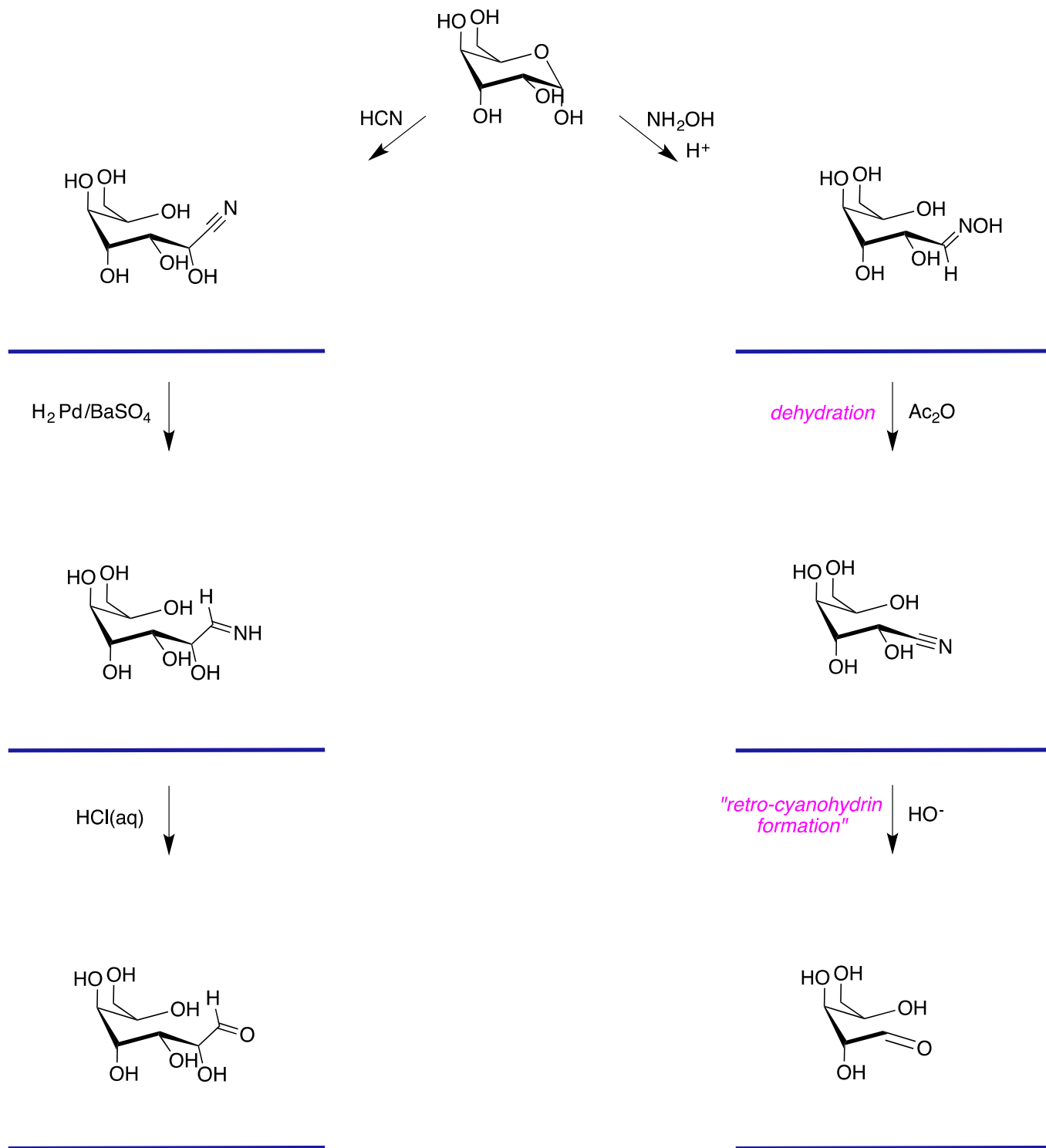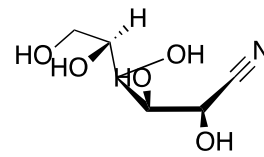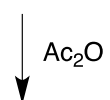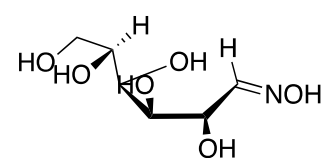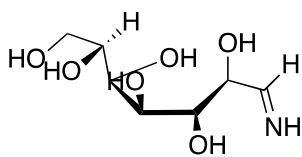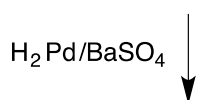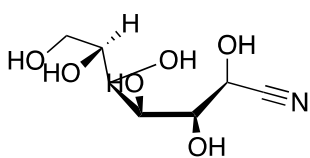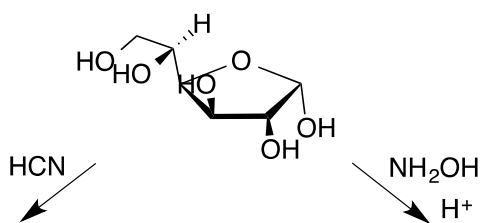

A mixture of *epimers* is produced before separation.

If the starting material was one enantiomer, then the individual, completely separated monosaccharides produced in this sequence *would not be* optically pure.
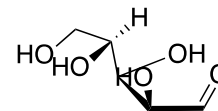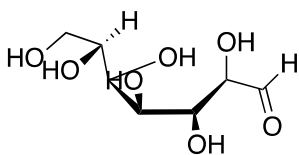
Conversion Of Aldoses To Lower Homologs

Aldehydes react with hydroxylamines to form oximes. Oximes can be dehydrated, and the cyanohydrin product can collapse back to aldehydes with one less carbon. Predict the final products of these reactions; addition of one carbon is represented on the *left* and subtraction on the *right*.
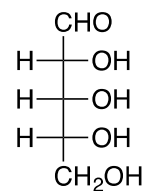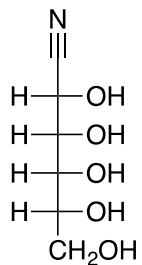
HCN

NH$_2$OH
H$^+$

H$_2$ Pd/BaSO$_4$

Ac$_2$O

HCl(aq)

HO$^-$

_____

_____

Fill in the gaps in the following sequence.

CHO
H——OH
H——OH
H——OH
H——OH
CH₂OH

→ NH₂OH →

H⎓NOH
H——OH
H——OH
H——OH
H——OH
CH₂OH

→ Ac₂O / heat →

N≡
H——OH
H——OH
H——OH
H——OH
CH₂OH

N≡
H——OH
H——OH
H——OH
H——OH
CH₂OH

⇌ HO⁻ / –HCN

CHO
H——OH
H——OH
H——OH
CH₂OH
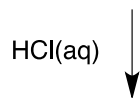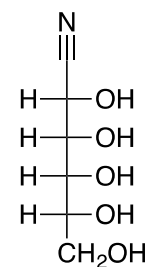
Other Reactions Of Sugars
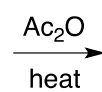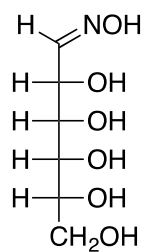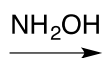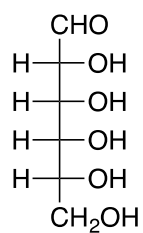
Aldoses are *oxidized* to the corresponding acids via treatment with Ag$_2$O, giving a *silver mirror*. Sugars that can precipitate silver from Ag$_2$O are called *reducing* sugars.

Like other hemiacetals, treatment with alcohols under anhydrous acid conditions will convert them to acetals. Cyclized forms are favored, and a mixture of anomers will be produced.

Draw the products of the following reactions.
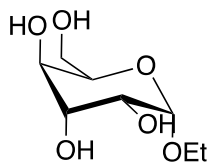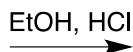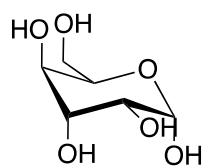
α-*anomer*

β-*anomer*
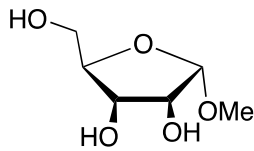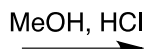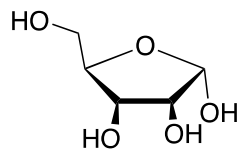
α-*anomer*
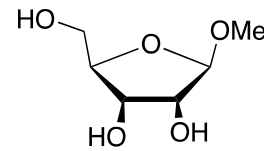
β-*anomer*

Relative Stabilities Of Anomers

Pyranosides are more stable as α-anomers because of overlap of the *axial* non-bonded lone pair on the endocyclic oxygen with the σ*-orbital of the C-O bond; this is *the anomeric effe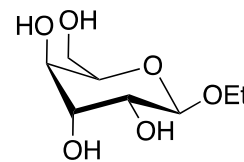ct*. Draw orbitals on 2-hydroxytetrahydropyran to represent this process, and a diagram to illustrate how they mix to give a stabilized system.
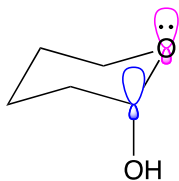
α-anomer
σ-to-σ* interactions possible
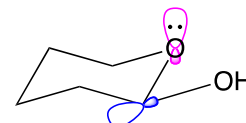
β-anomer
σ-to-σ* interactions impossible

Di- And Oligosaccharides

Connections between monosaccharides to form these polymers are made using *acetal or ketal* functional groups. These connections are then named according to the number of the carbon that uses the anomeric carbon (C^1) then a number of the carbon on which the oxygen at the second ring is attached. For example, two glucose units connected by a β-1,4-linkage is *cellobiose* but connection via an α-1,4-linkage is maltose. Draw these molecules.
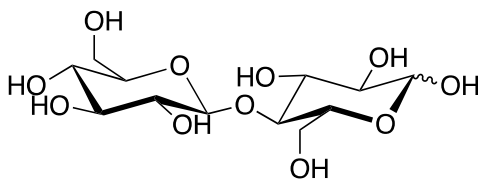
cellobiose
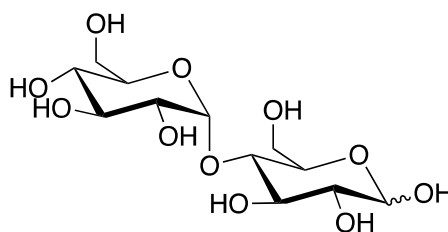
maltose

Use words to characterize the anomeric linkages in the following molecules. For sucrose call the furanose ring 1 and the pyranose ring 2, so describe the linkage as something like $\alpha 1, \alpha 2$.
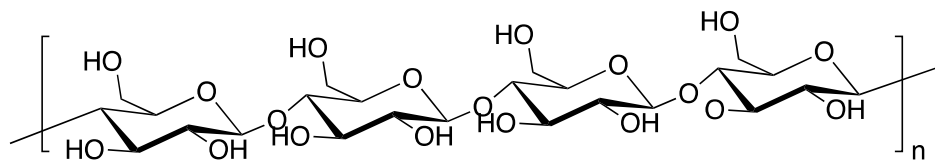
cellulose

linkages are: β-1,4
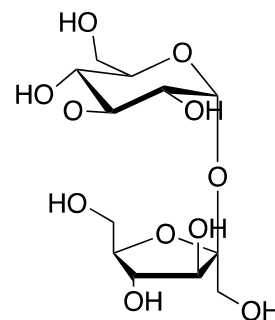
sucrose

linkage is: $\alpha 1, \beta 2$

Cellulose is a *poly*-saccharide, but sucrose is a *di*-saccharide.

Oligosaccharides can feature two anomeric linkages to one sugar (branched), sugars with amino groups (amino sugars), and sugars linked to proteins and peptides (glycoproteins and peptides).

Plants make glucose from CO_2 and water in the process called photosynthesis.

Carbohydrates In Summary

It is unproductive to learn names and stereochemistries of all the monosaccharides. However, it is valuable to know terms like *anomeric carbon*, *furanose* and *pyranose* forms, and that, in aqueous acid, α- and β-anomers can interconvert. Transposing stereochemical diagrams of carbohydrates is not trivial and takes practice. It is important to remember the most important monosaccharides:
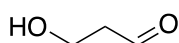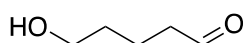
D-(+)-glyceraldehyde	*D-ribose*	*D-glucose*
D-(-)-tartaric acid	*α-D-ribofuranose*	*α-D-glucopyranose*
β-D-2-deoxyribofuranose	*β-D-ribofuranose*	*β-D-glucopyranose*
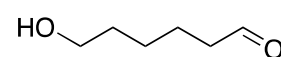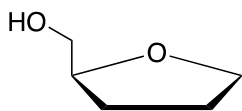
RNA is based on *β-D-ribofuranose*.

DNA is based on *β-D-2-deoxyribofuranose*

6 Heterocycles In Biological Chemistry

from chapter(s) _____ in the recommended text

Introduction

Focus

Cyclic compounds containing heteroatoms, *heterocycles*, are diverse, but some simple ring systems occur frequently in biochemistry and in biomedicinal chemistry. This section focuses on some of the most common ring systems, and on features that define their chemistry.

Reasons To Care

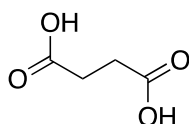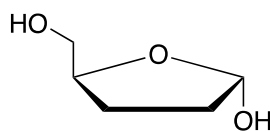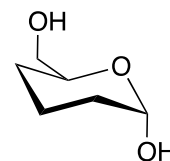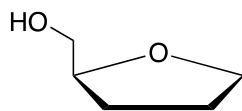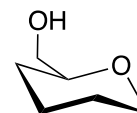Chemistry in living organisms occurs predominantly in an aqueous environment. Compounds with ring-systems containing only carbon atoms play significant roles in this chemistry, but the extent of that involvement is restricted by their poor water solubilities and limited opportunities for intermolecular interactions. Conversely, many heterocycles are basic and polar affording them greater water solubilities, especially when protonated (Figure 1). Lone pairs of electrons on the heteroatoms in heterocycles may be hydrogen-bond acceptors, or protonated to form hydrogen bond donors.

folic acid
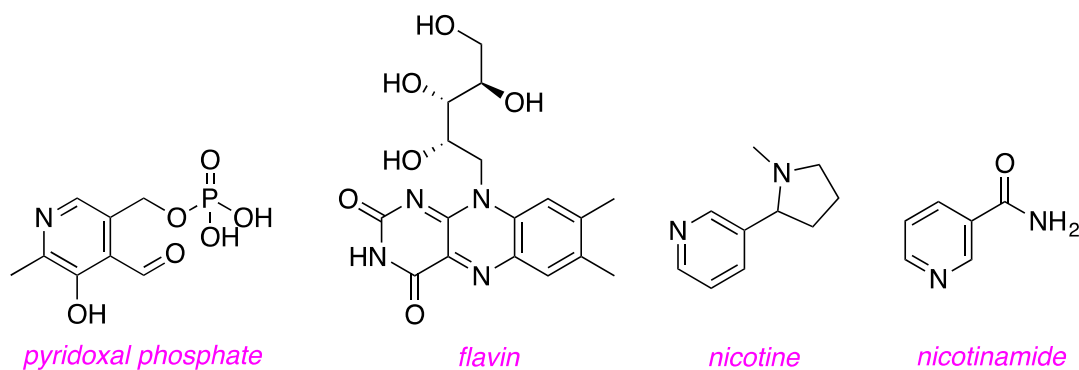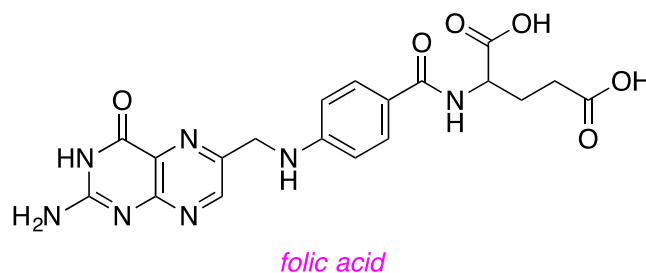
Figure 1. Some heterocycles in biochemistry.

pyridoxal phosphate   *flavin*   *nicotine*   *nicotinamide*

Concepts

aromaticity • acids and bases • electrophilic aromatic substitution

Objective

To introduce some common heterocycles, and understand how heteroatoms in a ring can influence aromaticity, basicity and reactivity.

Names

Complete the following diagram by giving whatever is missing: name or structure.

pyrrole
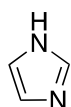
imidazole
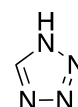
pyrrazole

tetrazole

pyridine

1,3-pyrimidine
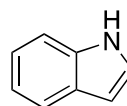
indole

thiophene
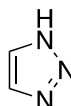
1,2,3-triazole
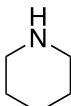
piperidine
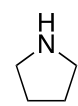
pyrrolidine

aziridine

oxirane

oxetane

furan
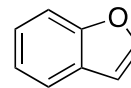
benzofuran
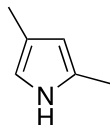
2,4-dimethylpyrrole
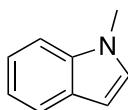
1-methylindole
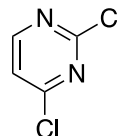
2,4-dichloropyrimidine
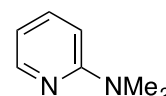
2-dimethylaminopyridine
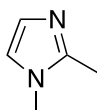
1,2-dimethylimidazole
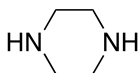
piperazine
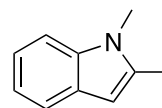
1,2-dimethylindole
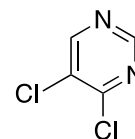
4,5-dichloropyrimidine

Name the heterocyclic core component colored in blue for each of the following pharmaceuticals.

lipitor

pyrrole

viagra

piperazine

allopurinol

pyrrazole

morphine

piperidine

imatinib
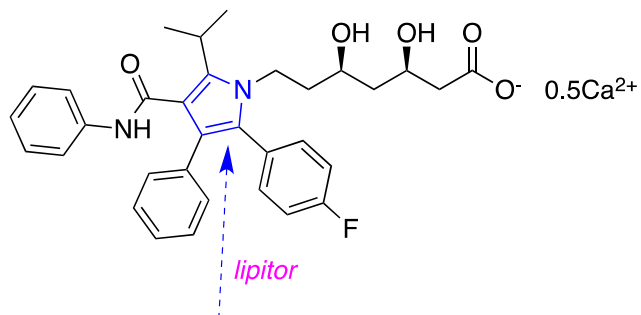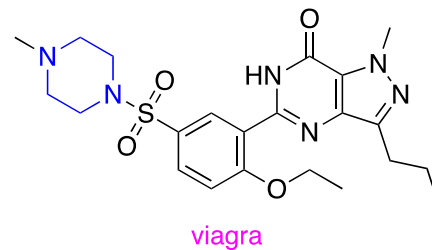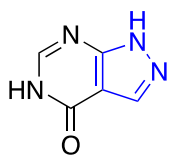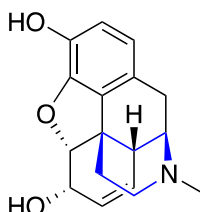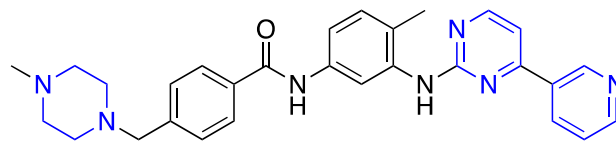
piperazine and *pyrimidine* and *pyridine*

Aromaticity And Heterocycles

Pyridines And Pyrimidines

In pyridine, the *N*-atom is *sp²* hybridized with *a lone pair* occupying one of the lobes, and there is *1* electron in the *p*-orbital that is not hybridized; this heterocycle is therefore *aromatic*.

Complete the following diagram to show the arrangement of p-orbitals, π-electrons and *N*-hybridization states in pyridine.
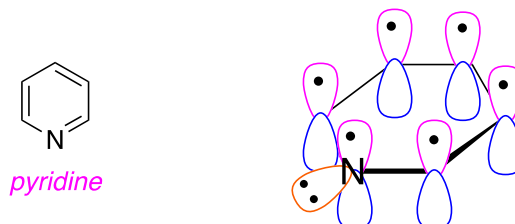
pyridine

In 1,3-pyrimidine, the *N*-atom is *sp²* hybridized with *a lone pair* occupying one of the lobes, and there is *1* electron in the *p*-orbital that is not hybridized.

1,3-Pyrimidine *aromatic*.

Complete the following diagram to show the arrangement of p-orbitals, π-electrons and *N*-hybridization states in 1,3-pyrimidine.
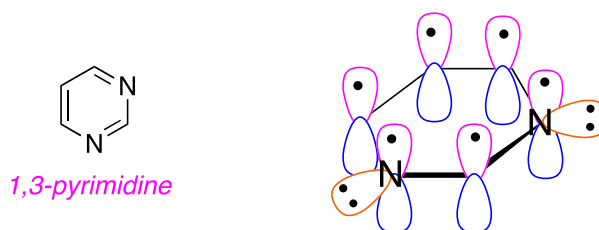
1,3-pyrimidine

In 1,4-pyrimidine, the *N*-atom is *sp²* hybridized with *a lone pair* occupying one of the lobes, and there is *1* electron in the *p*-orbital that is not hybridized.

1,4-Pyrimidine is *aromatic*.

Draw the arrangement of p-orbitals, π-electrons and *N*-hybridization states in 1,4-pyrimidine.

1,4-pyrimidine
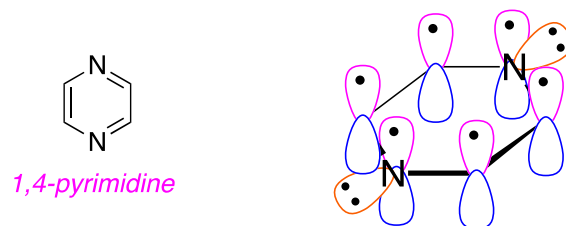
Pyrrole

In pyrrole, the *N*-atom must be *sp²* hybridized with *0 e⁻* occupying one of the lobes, and there are *2* electrons in the *p*-orbital that is not hybridized.

The pyrrole nitrogen *can* be sp³-hybridized and simultaneously give a flat conjugated aromatic system; this heterocycle is therefore *aromatic*.

Draw the arrangement of p-orbitals, π-electrons and *N*-hybridization of pyrazole.

pyrrole
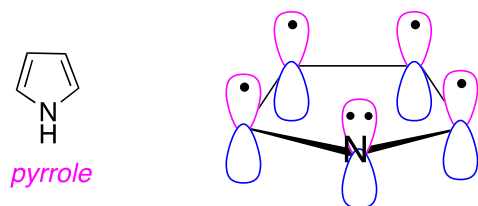
Imidazole

In imidazole there are two *different* N-atoms, which *can* interconvert via tautomerism.

The nitrogen atoms in imidazole *are* both sp^2 hybridized, and *one* contributes 2*e* to the aromatic system , consequently this heterocycle *is* basic..

Draw the arrangement of p-orbitals, π-electrons and N-hybridization of imidazole.
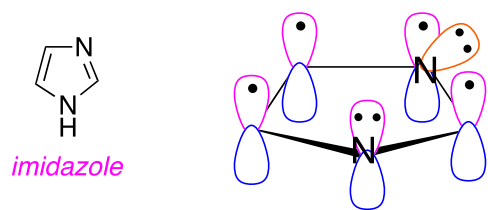
imidazole

The drive to attain aromaticity *does* influence the hybridization state and therefore the basicities of N-atoms in heterocycles like pyridine, pyrimidine, pyrrole, and imidazole.

Pyrazole

Pyrazole has two *different* N-atoms, which *cannot* interconvert via tautomerism.
Pyrazole nitrogen atoms *are* both sp^2 hybridized, and *one* contributes 2e to the aromatic system.

Pyrazole *is* easily protonated relative to pyrrole.

Draw the arrangement of p-orbitals, π-electrons and N-hybridization of pyrazole.

pyrazole
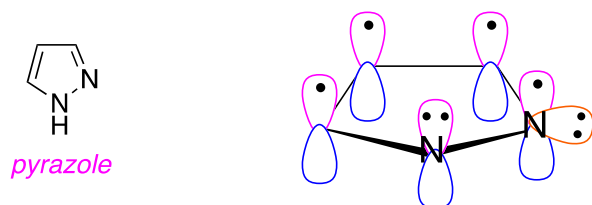
In nitrogen heterocycles like pyrrole and imidazole it is possible for a N-atom to be sp^3 hybridized. The N-atom preferentially hybridizes to the sp^2 state to gain *aromatic stabilization*.

1,3,4-Oxadiazole

In 1,3,4-oxadiazole, the N-atoms must be *sp^2* hybridized and each contributes *1 e*$^-$ to the aromatic system.

The 1,3,4-oxadiazole oxygen is *sp^2* hybridized and contributes *2 e*$^-$ to the aromatic system; this heterocycle is therefore *aromatic*.

Draw the arrangement of p-electrons and O,N-hybridization of 1,3,4-oxadiazole.

1,3,4-oxadiazole
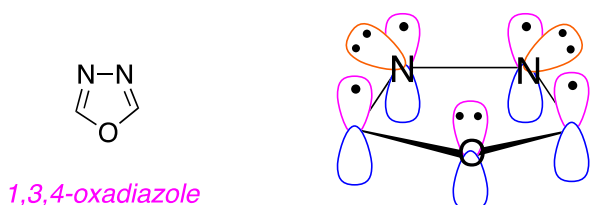
When a proton adds to a lone pair *in a sp^2-hybridized orbital* in an aromatic heterocycle it *does not* remove electrons from the 4n + 2 delocalized system.

That lone pair is therefore a relatively *good* base because the aromatic stabilization *is not* lost.

Heterocycles In Nature

For each of the heterocycles below that is aromatic, show how many πe do each of the nitrogen contribute to the aromatic system (leave blank if not aromatic).

cytosine

N^3: 0

N^2: 1

N^1: 2

thymine

N^2: 2

N^1: 2

caffeine

N^4: 2

N^3: 2

N^2: 2

N^1: 1

adenine

N^5: 0

N^4: 1

N^3: 1

N^2: 2

N^1: 1

guanine

N^5: 0

N^4: 1

N^3: 2

N^2: 1

N^1: 2
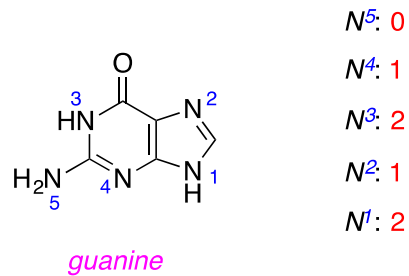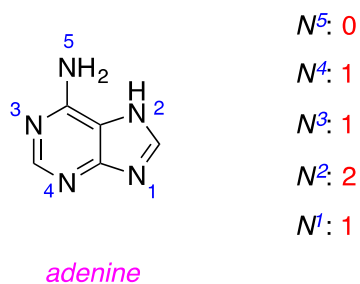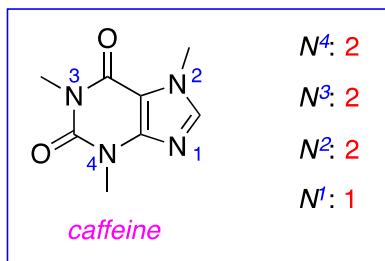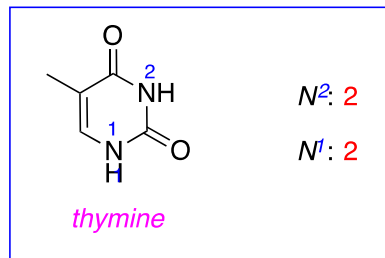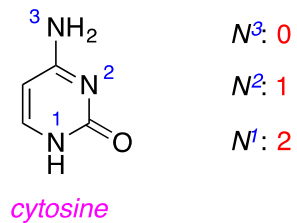
Draw a rectangle around any of these heterocycles that are *not* found in RNA.

Porphyrin ring systems involve *2* pyridine-like nitrogen atoms, *2* pyrrole-like nitrogens, and 26 π-electrons; they *are* aromatic.

In the presence of base it may be easily deprotonated *2* times to create a ligand that ideally suited to complex with transition metals in their M(2+) oxidation states.
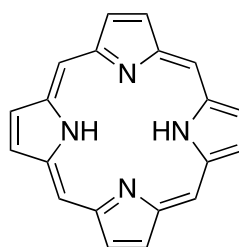
porphyrin

Drawing porphyrins takes practice! Draw the complexes of porphyrin with Fe^{2+} and with Mg^{2+} as neatly as possible, and indicate their overall charges. Porphyrin must lose two protons to form these complexes.
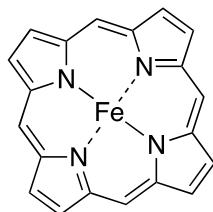
Fe²⁺ complex   overall charge 0
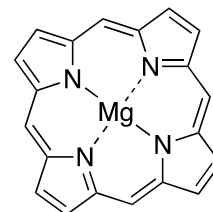
Mg²⁺ complex   overall charge 0

Iron complexes like this are found in *hemoglobin*, while the magnesium complexes feature in *chlorophyll* (circle all that apply).

Complexes like those are (circle all that apply): *strongly UV absorbing / fluorescent / capable of redox chemistry*.

Aromatic Characteristics Of Protonated Heterocycles

Indicate numbers of π-electrons and therefore the aromaticities of the following systems.
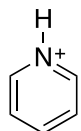
aromatic because it has
6 πe^-.
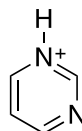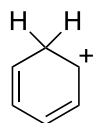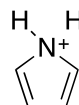
aromatic because it has
6 πe^-.

not aromatic because it has
4 πe^-.

not aromatic because it has
4 πe^-.
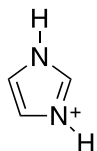
aromatic because it has
6 πe^-.
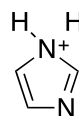
not aromatic because it has
4 πe^-.
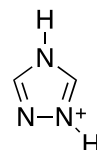
aromatic because it has
6 πe^-.
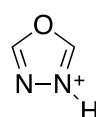
aromatic because it has
6 πe^-.
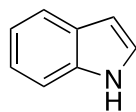
aromatic because it has
10 πe^-.
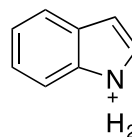
not aromatic because it has
8 πe^-.

In the last examples, the *N*-atom is, in fact, *not* where indole protonates. Can you suggest another atom to which a proton might add? C^3

Circle all the basic atoms in the following heterocycles and, to review, write their names underneath.
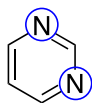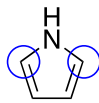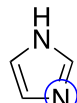
pyridine   *pyrimidine*   *pyrrole*   *imidazole*   *pyrrazole*   *oxazole*
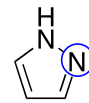
Electrophilic Attack On Pyrrole And Indole Compared

Pyrrole

The *N*-nucleophilicity of pyrrole is *low* in S_N2 reactions for the same reasons that account for the basicity of that atom.   Electrophiles tend to add to the carbon atoms of pyrrole in electrophilic aromatic substitution reactions.

Show all the resonance structures for the intermediate that would arise by adding Br^+ to pyrrole (fill in bonds and curly arrows):
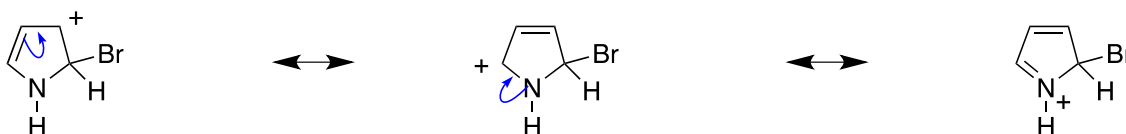
in the 2-position

complete diagrams and show arrows

in the 3 position
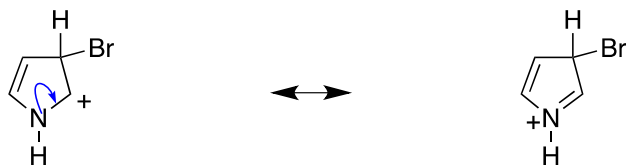
complete diagrams and show arrows

Electrophilic attack on pyrrole is favored at the *2-position* because the transition state preceding the first intermediate is more stabilized; this is a *thermodynamic* argument.

The fundamental chemical postulate that indicates transition state energies are more closely related to the entity closest to them in terms of energy is *Hammond's postulate*. This relates the kinetics of the process to the thermodynamics.

Pyrroles could undergo electrophilic aromatic substitution at either the position marked *a* or the one marked *b*. In fact, one of these is preferred because it has three resonance structures to delocalize the charge while the other has only two.

Deduce the preferred regiochemistry of electrophilic attack on pyrrole, and show the contributing resonance structures and the product.
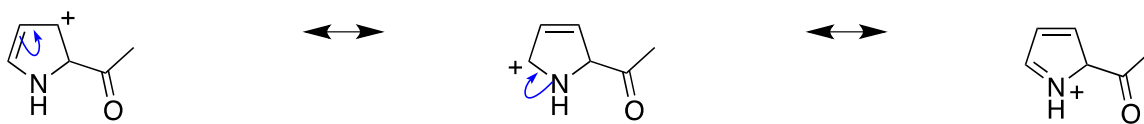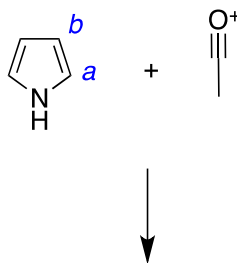
choose correct regiochemistry, show resonance structures, and electron flow that relates them using curly arrows
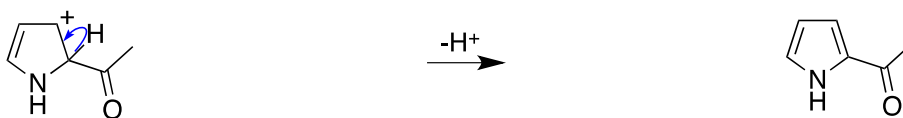
Pyrrole is *more* electron rich than benzene, hence it reacts *faster* with electrophiles.

Draw structures on the line below to rank the following aromatic compounds in order of reactivity towards electrophiles: benzene, furan, thiophene, pyrrole.
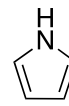
least reactive   *most reactive*

Indole

Show all the resonance structures for the intermediate that would arise by adding Br$^+$ to indole (fill in bonds and curly arrows):
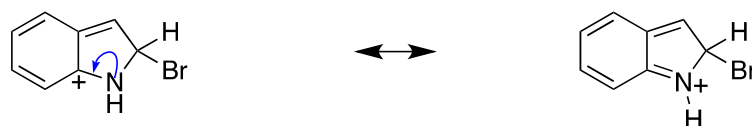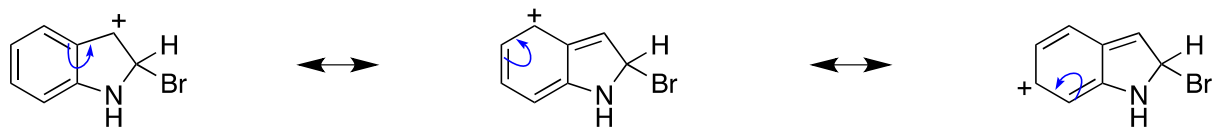
in the 2-position

donation of the N-lone pair does disrupt aromaticity of the benzene ring

in the 3 position
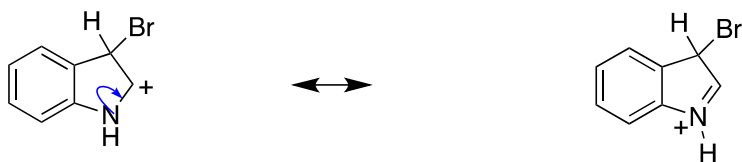
donation of the N-lone pair does disrupt aromaticity of the benzene ring

From the reasoning above, indoles preferentially add electrophiles at the *2*-position because it has four resonance structures to delocalize the charge without disrupting aromaticity of the benzene ring while substitution at the 3-position doesn't have resonance structure to delocalize the charge without disrupting aromaticity.

7 Nucleosides And Nucleotides

from chapter(s) _____ in the recommended text

Introduction

Focus

This section deals with identification of the DNA and RNA building blocks, how they base pair, and the mode of action of some nucleoside-based drugs and prodrugs.

Reasons To Care

DNA encodes the proteins, and some critical fragments of RNA, on which all forms of life are based. Pairing of the heterocyclic bases in DNA and RNA is based upon a simple set of principles. The predominant role of nucleosides and nucleotides in the body is via interactions with DNA or RNA replicated enzymes such as polymerases.

Figure 1. Interaction of Taq polymerase (red) with a modified DNA double strand (yellow).

A significant fraction of all pharmaceuticals are nucleosides, and the mode of action of these is mostly related to their interaction with DNA or RNA replicated enzymes. Some general and straightforward characteristics of nucleoside drug design are described.

Concepts

carbohydrates • heterocycles in Nature • elimination reactions • S_N1 in the context of protecting groups

Objective

This section is to describe DNA, RNA, how nucleosides may be phosphorylated, and how this impacts nucleoside drug design.

Nucleosides

Draw the purine and pyrimidine *bases* (not the carbohydrate parts) associated with deoxynucleic acid (DNA), and with RNA (ribonucleic acid).

pyrimidine
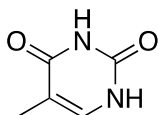
thymine
DNA
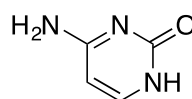
cytosine
both
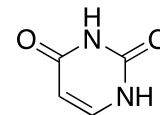
uracil
RNA
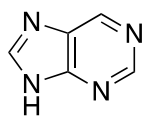
purine
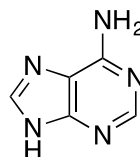
adenine
both
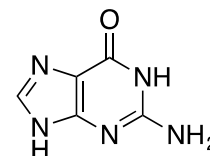
guanine
both

The sugar fragment of RNA is derived from *ribose* in a *furanose* form.

When the heteroatom at the anomeric position is *cis*-oriented to the CH$_2$OH group then that is a β-anomer.

Bases in DNA and RNA are β-anomers.

Draw the β- and α-anomers of ribofuranose, number the carbons from the anomeric position (C^1), and draw a generic structure for a *2-deoxy*ribofuranose of DNA using "B" to represent the base part, and the same for RNA.
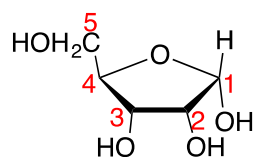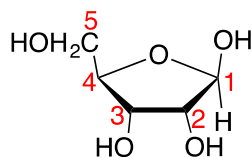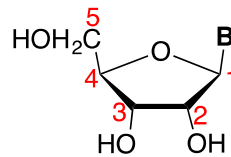
α-D-ribofuranose   *β-D-ribofuranose*   *generic RNA*   *generic DNA*
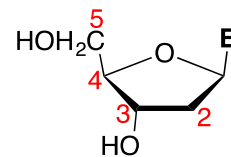
Nucleotides

Nucleoside refers to the carbohydrate and heterocyclic base component *without* a phosphate attached.

Nucleotide are nucleosides except they have the 5'-*O* or 3'-*O* incorporated into a *phosphate* esters.

Draw phospho- mono-, di- and tri-esters representing the alkyl groups attached as "R".
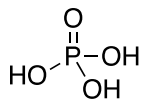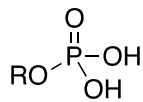
phosphoric acid   *phosphomonoester*   *phosphodiester*   *phosphotriester*
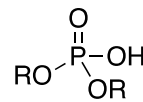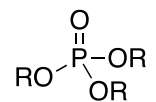
Confusingly, **A**, **T**, **U**, **C**, and **G** symbols can be used to represent just the base in nucleosides and nucleotides, or whole nucleosides or nucleotides units. Units of DNA are connected by phospho *di*-esters.

RNA bases are connected by phospho *di*-esters.

Complete the following diagram to show a DNA fragment of the sequence.

draw rest of **ATGC**
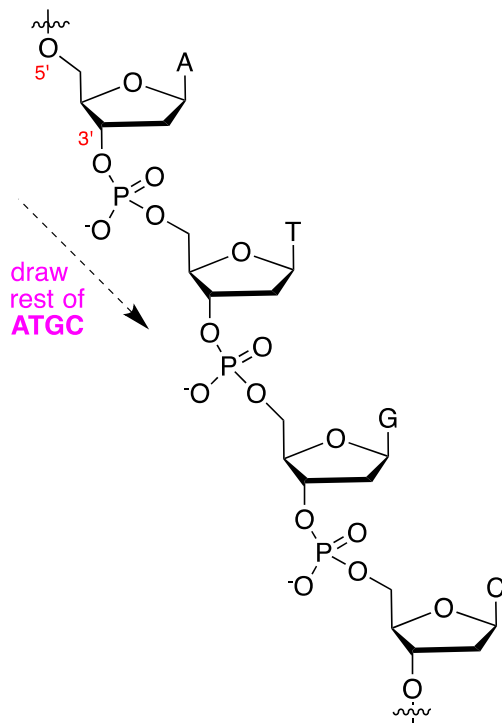
CGTA has *a different* structure to **ATGC**.

When reading DNA sequences, start at the *5'-* ends and list towards the *3'-* end.

Primes in these numbers mean they refer to *the sugar part*.

The phosphorus atoms in RNA and DNA *are not* chiral in their dibasic state.

RNA is *less* stable than DNA because it has a 2'-*OH* that can cyclize onto the phosphorus giving a 2',3'-cyclic phosphate and displacing the 5'-*O* of the next residue.

Complete the following diagram to illustrate this cyclization; push electrons from the 2-OH, and show the two products (no intermediates).
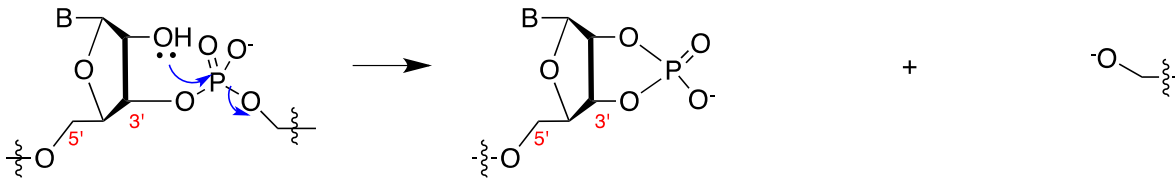
The reaction shown above cannot happen with DNA because it has no *2'-OH*.

In all organisms, excluding some viruses, genetic information is stored as deoxynucleic acid (DNA) sequences. That DNA information is *transcribed* into RNA (ribonucleic acid) when genes are switched on, then *translated* into proteins.

Complete the following (the number of *H*-bond donors and acceptors refers only to the parts highlighted).

G

H-bond donors:
2

H-bond acc's:
1

A

H-bond donors:
1

H-bond acc's:
1

C

H-bond donors:
1

H-bond acc's:
2

U

H-bond donors:
1
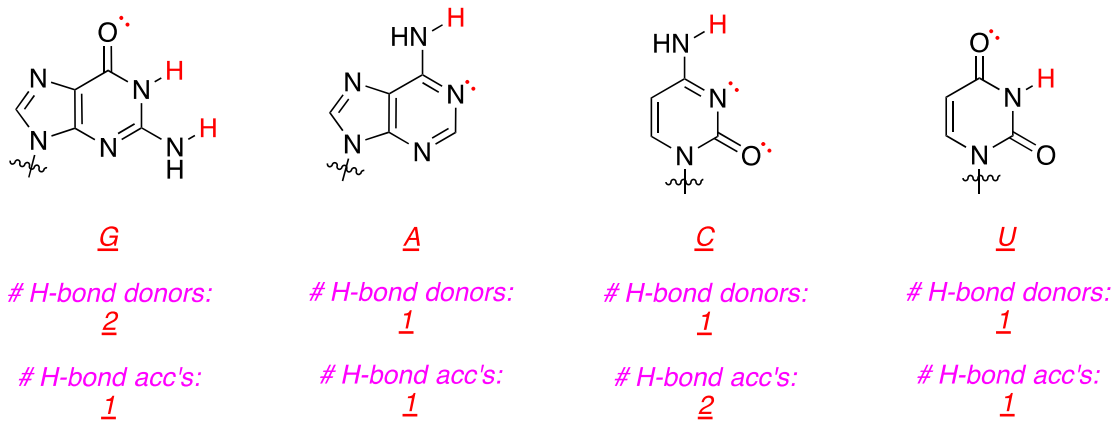
H-bond acc's:
1

refers to H-bond acceptors and donors, as indicated in structure

Due to geometric constraints in base-pairing, T and U can only use one of their H-bond acceptors. Based on the hypothesis that bases with two correctly oriented H-bond donors and one H-bond acceptor, should pair with others that have two correctly oriented H-bond acceptors and one H-bond donor:

G should *H*-bond most effectively with **C** in DNA

G should *H*-bond most effectively with **C** in RNA

A should *H*-bond most effectively with **T** in DNA

A should *H*-bond most effectively with **U** in RNA

C should *H*-bond most effectively with **G** in DNA

C should *H*-bond most effectively with **G** in RNA

T should *H*-bond most effectively with **A** in DNA

Complete the following illustration of the *H*-bonding arrangement in double stranded DNA or RNA. To do this, point the bond to the sugar residues towards the bottom right.
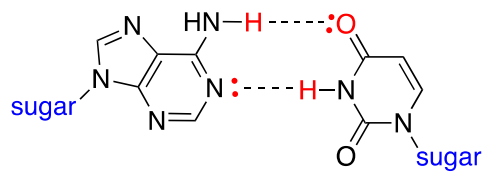
A H-bonded to U
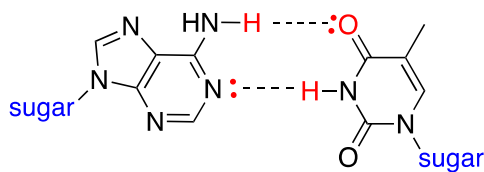
A H-bonded to T
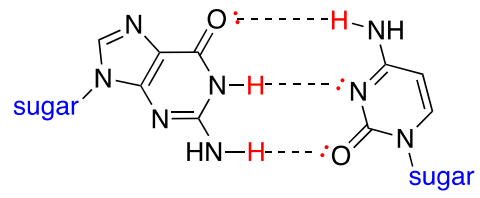
G H-bonded to *C*
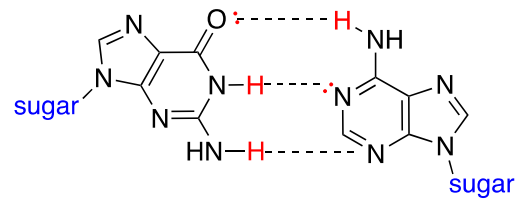
G H-bonded to *A*
does not match well

Draw the H-bonds between the DNA bases on the diagram below, and identify each base by writing A, T, G, or C beside them.
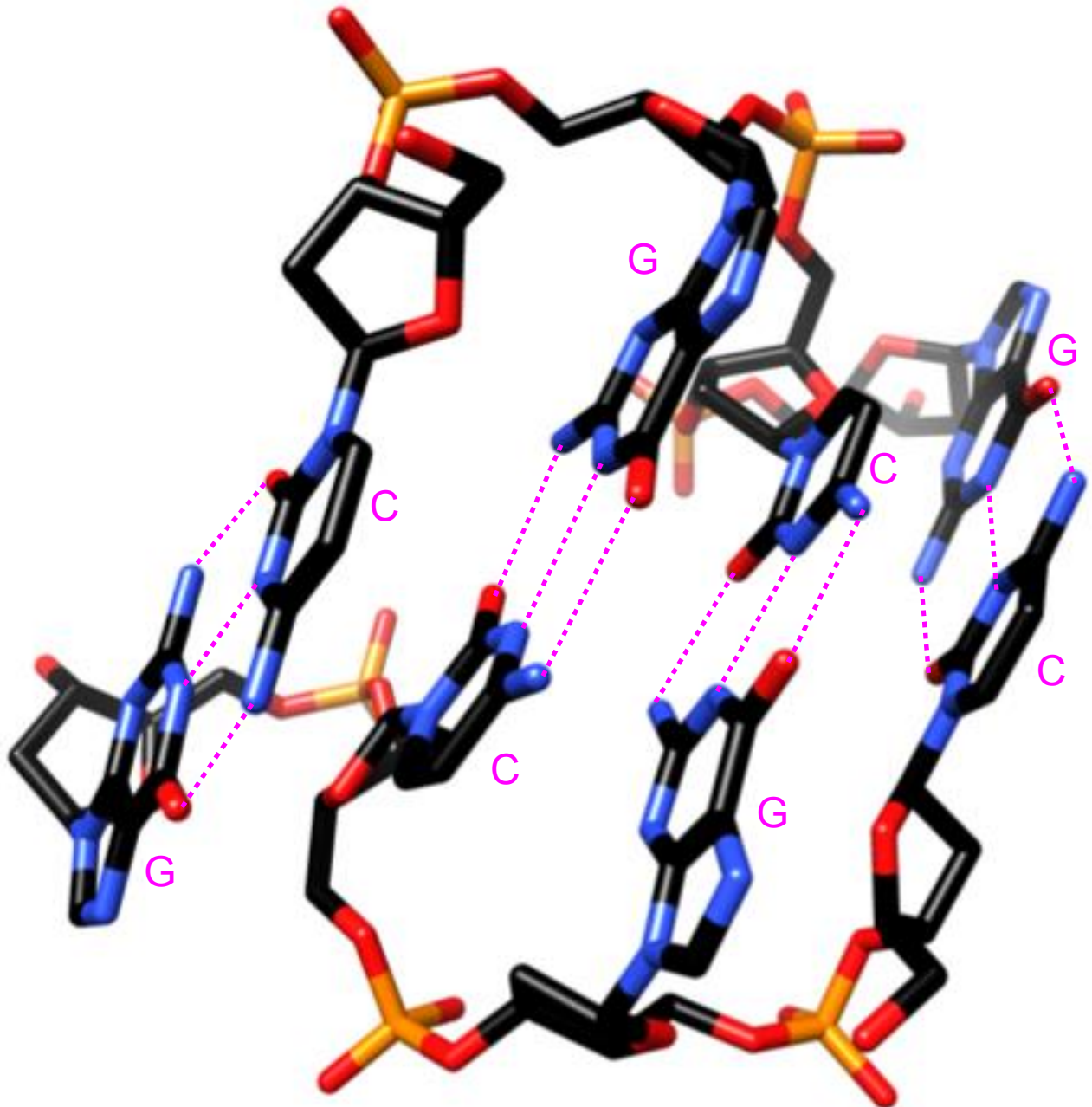
H-bonding between A and T is *weaker* than between G and C because there are *less* inter-residue H-bonds.

The diagram below is a representation of a crystal structure of a double stranded DNA. A phosphodiester linkage, various **T**, **A**, **G**, **C** nucleobases (perhaps more than once) and a ribose fragment (**R**) are highlighted in purple boxes. Label each box with one of those indications.
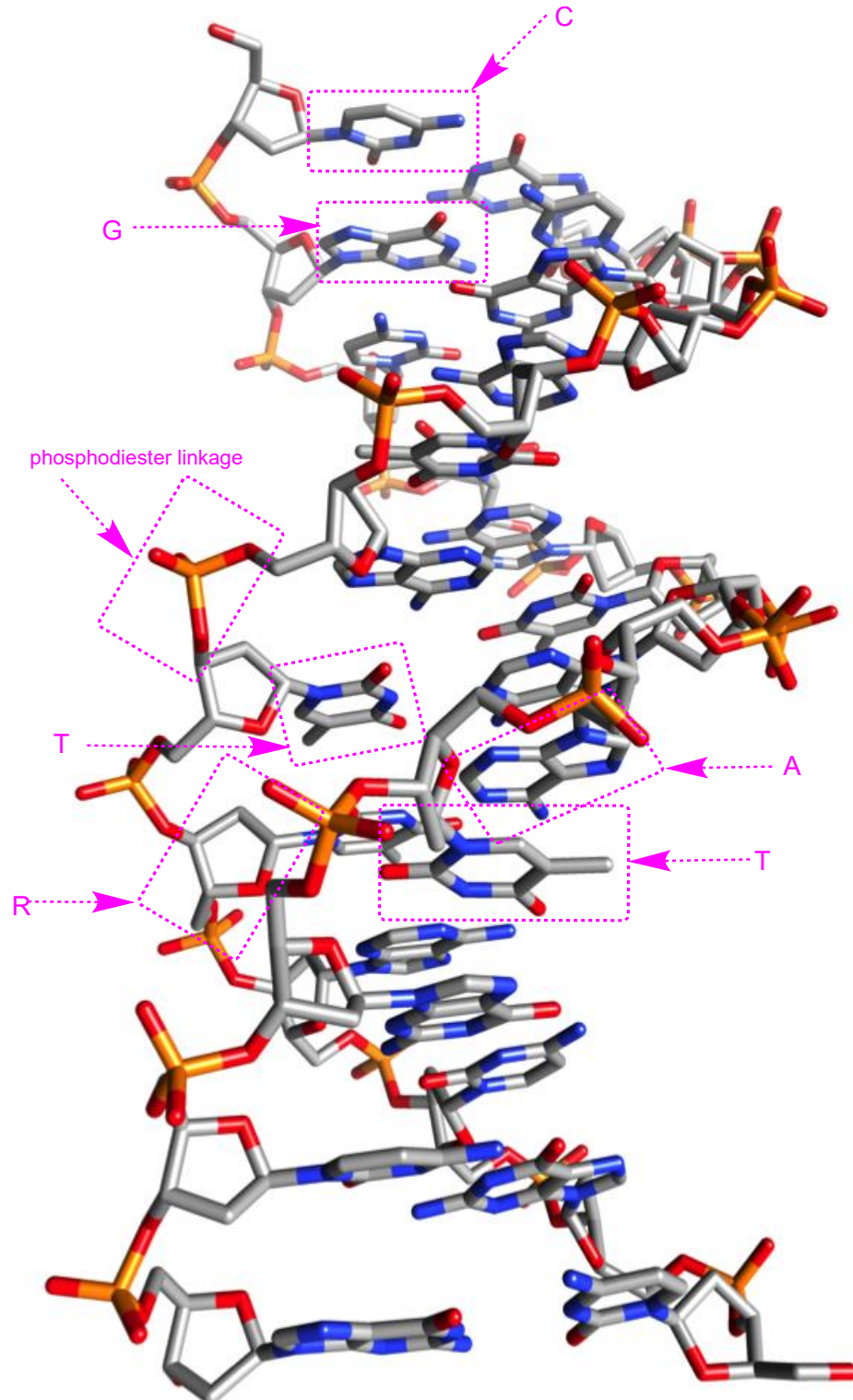
In double stranded DNA the *phosphodiesters* are on the perimeter, and the *nucleobases* form the core

Deamination in living organisms converts **C** into **U** bases.

DNA tends to exist in a *right* handed helical arrangement using the *H*-bonds outlined above.

DNA handedness is normally *the same as* helices in proteins.

RNA has *less* regularly ordered secondary structures than DNA.

Bases of *DNA* can be distinguished by the prefix "d" as in **dA**, **dT**, **dC**, and **dG**, relative to **A**, **U**, **C**, and **G** used for *RNA*.

Mono-, di-, and tri-phosphates are abbreviated to "MP", "DP", "TP" suffixes. Phosphates referred to by these abbreviations are on the 5'-*O* unless something specifically indicates otherwise. Complete the structures of the following nucleotides.
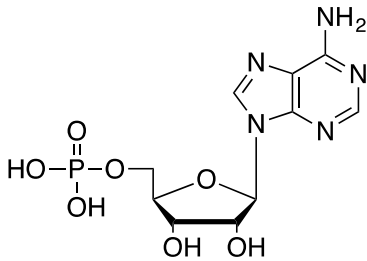
AMP
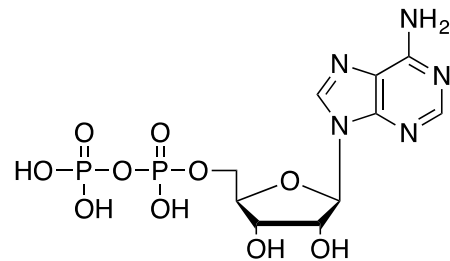
ADP
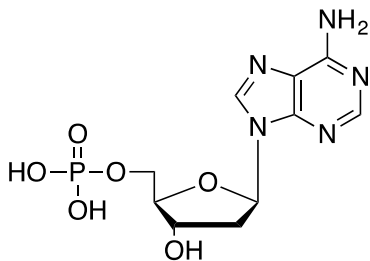
dAMP
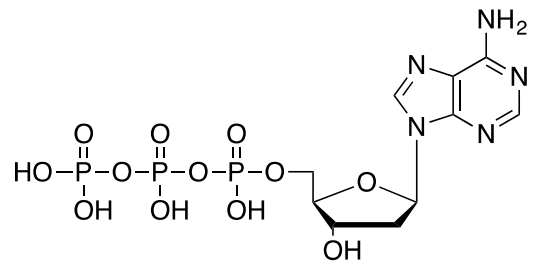
dATP
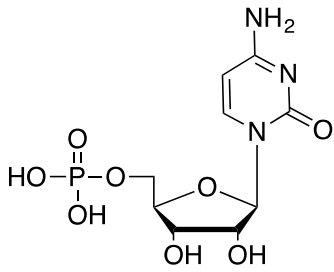
CMP
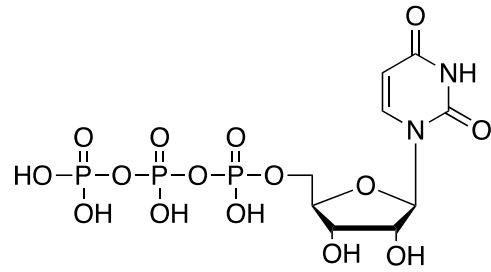
UTP
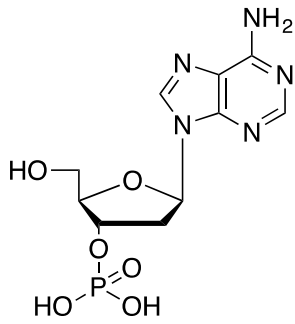
*2-deoxyadenosine
3'-monophosphate*
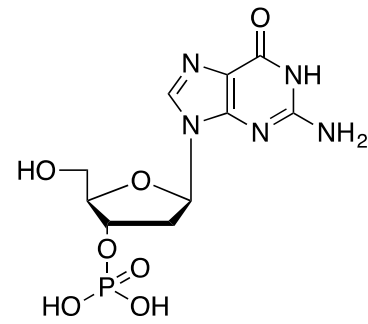
*2-deoxyguanidine
3'-monophosphate*

DNA synthesis is mediated by enzymes called *polymerases*.

These enzymes build a second *antiparallel* strand of DNA to complement the first by selecting the complementary triphosphate (dATP, dGTP, dCTP or dTTP) to pair with the next base in the sequence, and add it to the *3'-end* of the growing strand.

A *di*-phosphate is lost whenever a base is added by a polymerase.

Genes are turned on by interacting with *promoter* proteins bind to *promoting* regions of DNA sequences to initiate synthesis of a complementary strand of *messenger* RNA.

Syntheses of mRNA stops at particular three-base sequences of DNA called *stop codons*.

Exon DNA encodes for proteins, and regions that do not are called *introns*.

Non-encoding regions in the transcribed mRNA are cut out in the process of RNA *splicing*.

In the ribosome, *transfer* RNA reads three base codons on the *messenger* RNA and converts this sequence information into proteins.

Nucleoside Drugs

Viruses are particularly vulnerable to nucleoside drugs because these are somehow incorporated into replicating viral *DNA* thereby *arresting* replication in a similar way to incorporation of dsDNA in Sanger sequencing.
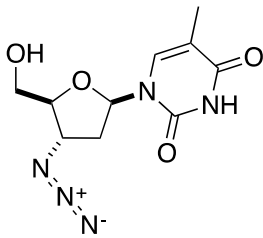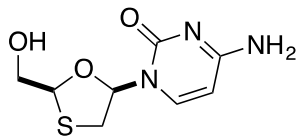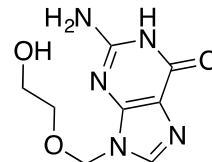
Zidovudine   *Lamivudine*   *Acyclovir*   *Penciclovir*
      treatment of HIV   *treatment of herpes*
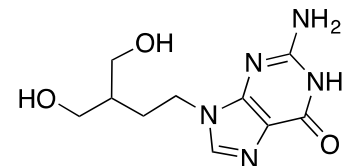
However, the substrates for DNA replicating enzymes are *nucleotide triphosphates*.

This implies the nucleosides shown above enter cells and are converted to triphosphates by *kinase* enzymes as illustrated below.
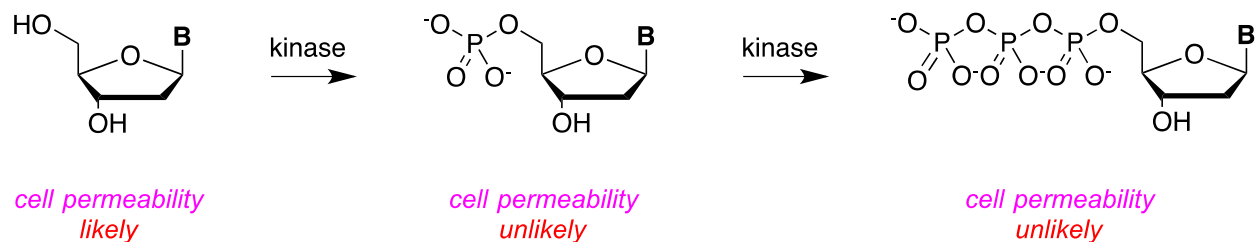
The fact that the above process does not occur for all nucleosides limits development of new anti-virals. The first step, formation of *mono*-phosphates is the difficult one in many cases.

Cell membranes are partially composed of lipids (fats) with phosphate head groups hence they tend to be impermeable to *negatively* ionized species.

Nucleotide monophosphates *do not* tend to permeate easily into cells, so these *do not* make effective anti-viral drugs.
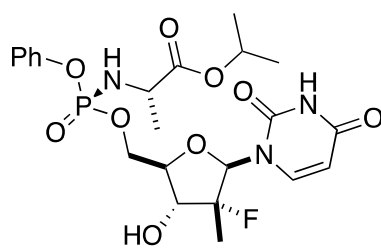
Sofosbuvir
treatment of hepatitis C

Nucleotide Prodrugs

A solution to the problem outlined above is to design nucleoside derivatives that are *neutral charged*, *cell permeable* and which form nucleoside monophosphates inside cells.

A *prodrug* is one introduced in a protected form, then converted to the active form inside the body.

One such drug is Sofosbuvir, illustrative of a class of drugs called *ProTides*.

Complete the following sequence to rationalize how drugs like Sofosbuvir are converted to their active monophosphate forms in the body.

cell permeable

esterase

-OPh
cyclization

OH$_2$

ester hydrolysis

P-amidase
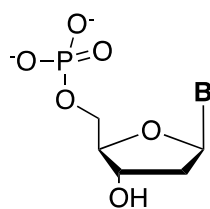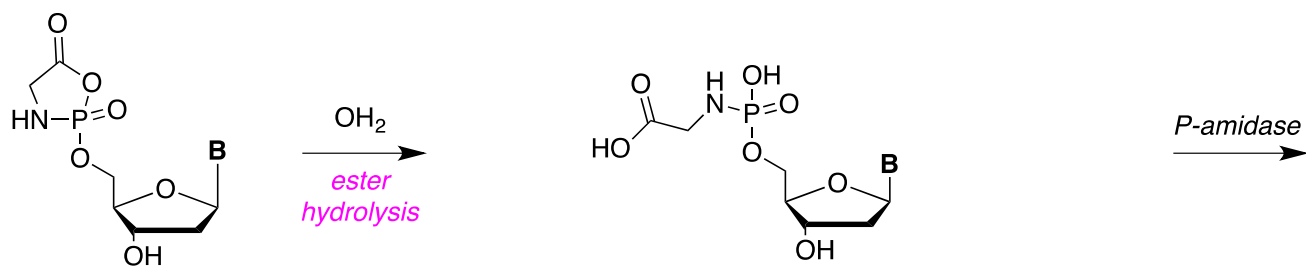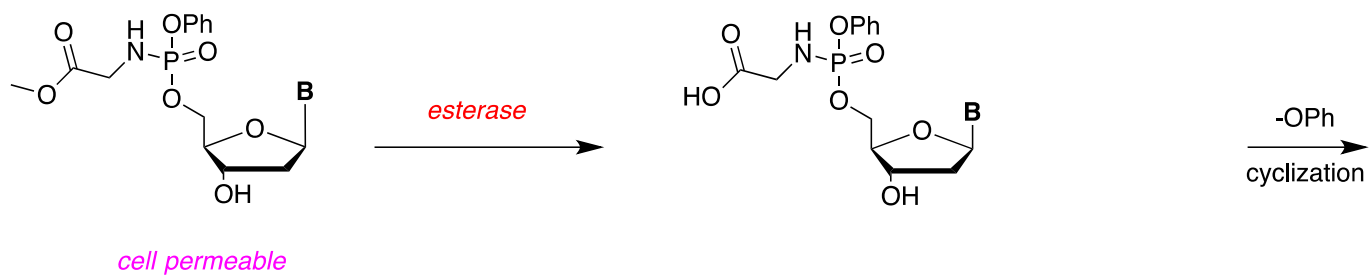
8 DNA Synthesis And Sequencing

from chapter(s) _____ in the recommended text

Introduction

Focus

This section deals with how DNA is synthesized and sequenced.

Reasons To Care

DNA encodes the proteins, and some critical fragments of RNA, on which all forms of life are based. The first step in being able to sequence DNA on a large scale was chemical syntheses of primers in polymerase-mediated reactions. This facilitated high throughput sequencing of DNA, first by methods like Sanger's, then via next generation methods like sequencing by synthesis. Now it is possible to sequence genomic DNA using methods that have become so efficient that it is feasible to correlate genetic differences in individuals with their proclivity to contract certain diseases. Now the forefront is manipulating genomic DNA using the CRISPR system featuring the endonuclease Cas9 (Figure 1). Together, these efforts have caused a paradigm shift in biomedicinal chemistry.

Figure 1. The Cas9 endonuclease complexed with single guide RNA (green) and target DNA (yellow).

Concepts

carbohydrates • heterocycles in Nature • H-bonding • elimination reactions • S_N1 in the context of protecting groups

Objective

This section is to describe DNA, RNA, and select features of DNA-synthesis, sequencing methods, and nucleotide drug design.
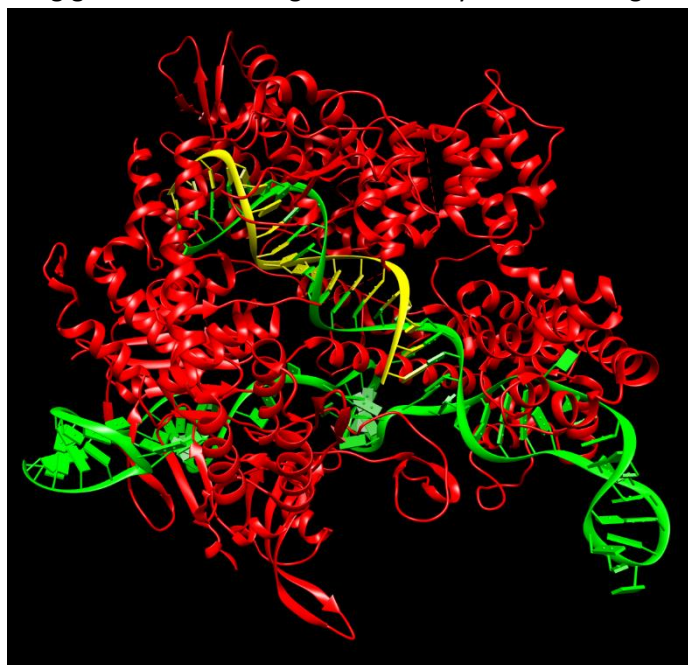
Chemical Synthesis Of DNA

DNA sequences of 100 bases, or even longer can be made so efficiently via automated syntheses that even chemists tend to buy DNA strands from commercial suppliers.

One of the most widespread methods for DNA synthesis involves protection of the 5'-O with a trityl (CPh$_3$) group and $P - O$ bonds with O-cyanoethyl.

Draw a mechanism for cleavage of a trityl ether with acid by completing the scheme below.

quenched in reaction medium
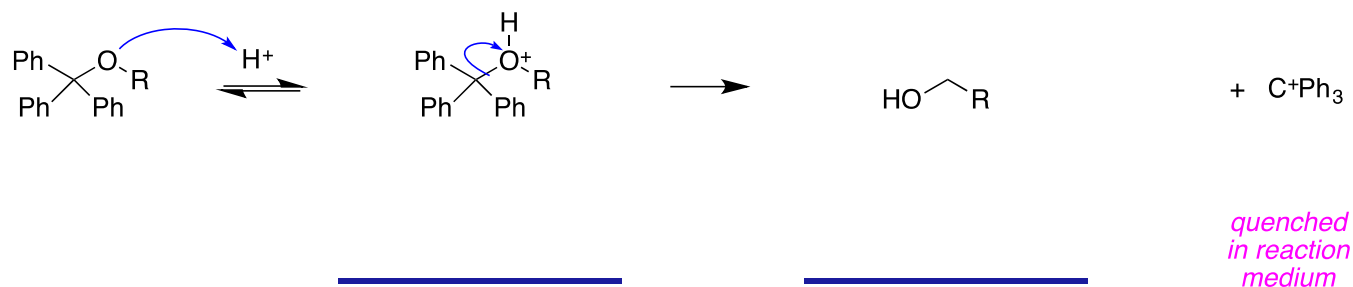
This is a S_N1 reaction, "seen through the eyes of the protecting group".

If two of the phenyl groups in the trityl were replaced with 4-methoxybenzene, (to give dimethoxytrityl, DMT) the reaction above would be *easier* because the cation formed would be *more* stabilized by resonance.

Draw a mechanism for removal of a cyanoethyl group from a phosphodiester with a base.

carbanion
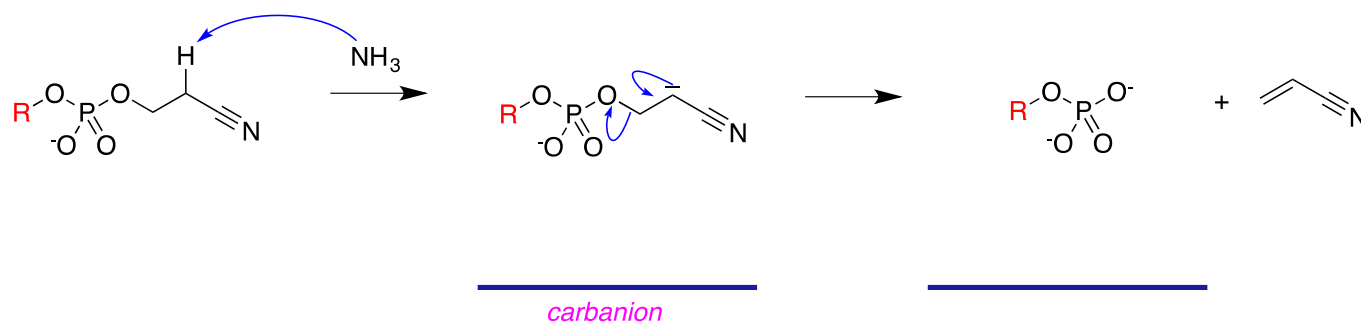
This is an *E1cb* reaction, also "seen through the eyes of the leaving group".

Circle the cyanoethyl group and put a square around the dimethoxytrityl group in the following protected nucleoside.

$$Ar = \text{(4-methoxyphenyl)} -OMe$$
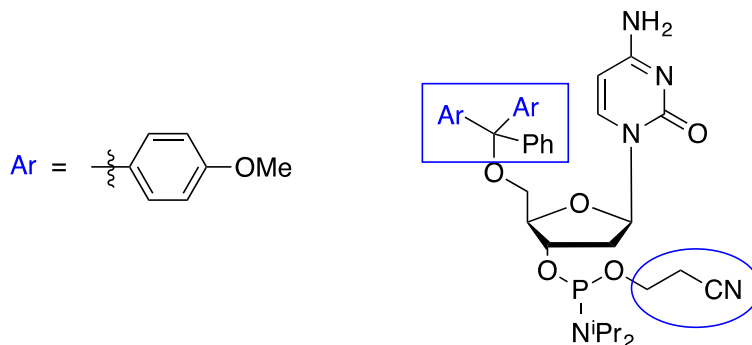
Exocyclic amine groups in the bases **G**, **A**, and **C** must be protected to withstand the conditions of DNA synthesis. Draw the following protected bases in the same style as **T** below (*ie* just show the word "sugar" and draw the base):

benzoyl
Bz

iso-butyryl
CO^{t}Bu

no protection required

*N-Bz **C***

*N-Bz **A***

*N-iso-butyryl **G***
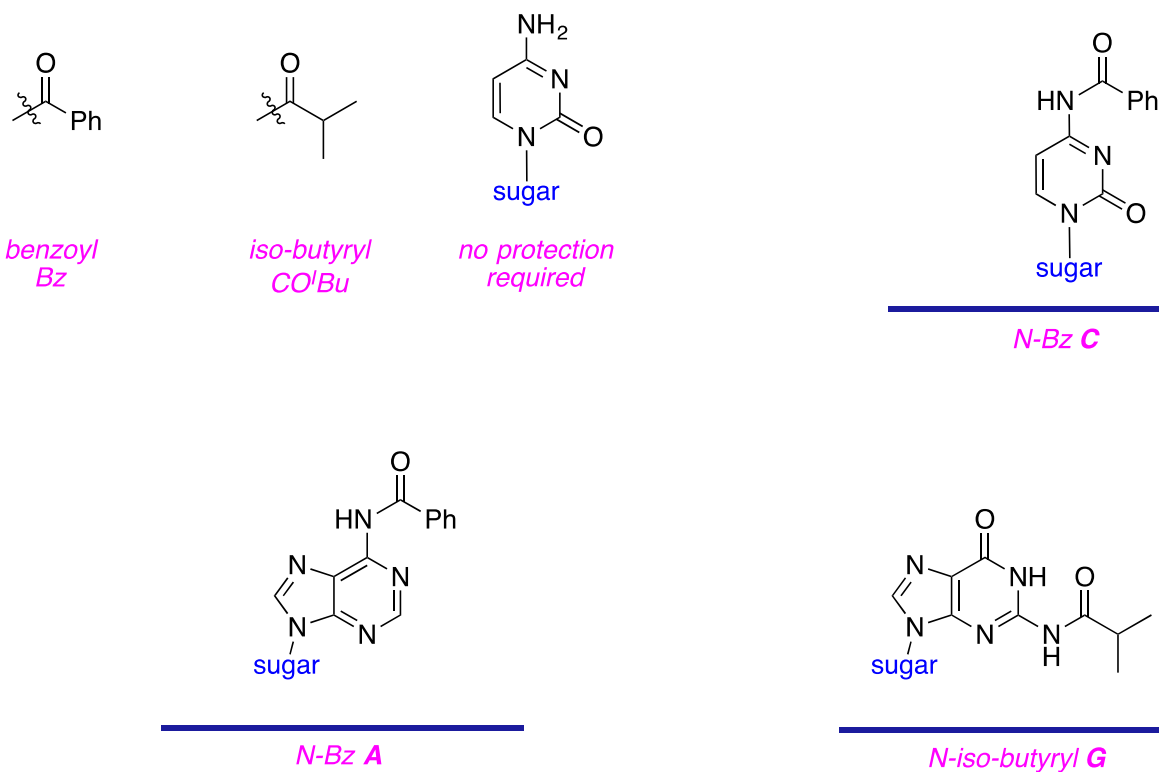
The first step in the *phosphoramidite method* is to 3'-anchor the first base to a support, typically a glass bead. Show the product of this acylation reaction, then that after removal of the 5'DMT.

X = leaving group
B' = protected nucleobase
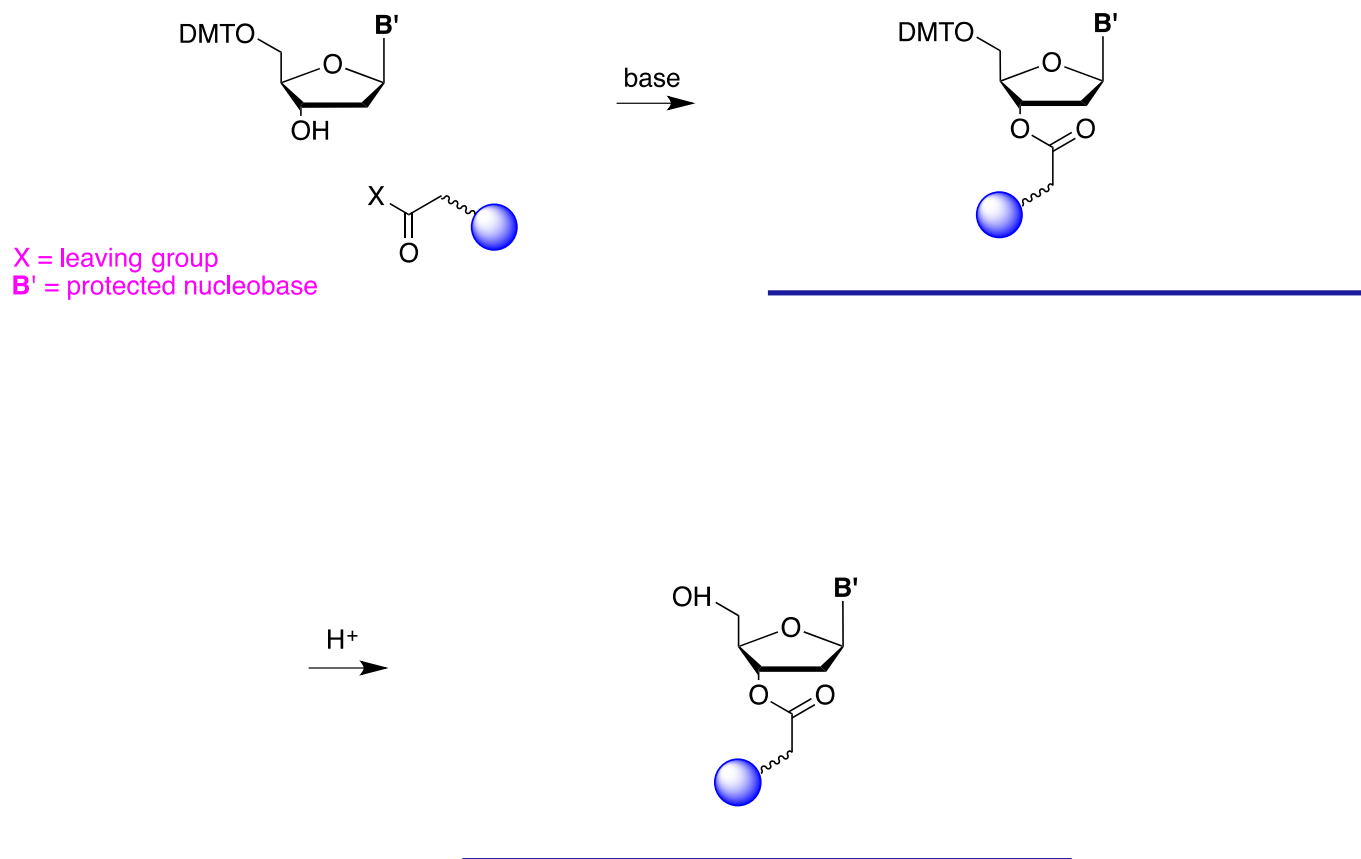
The key coupling step in the phosphoramidite method involves activation of the next phosphoramidite base to be coupled via protonation at $P - NR_2$; complete the following mechanistic outline of that process.
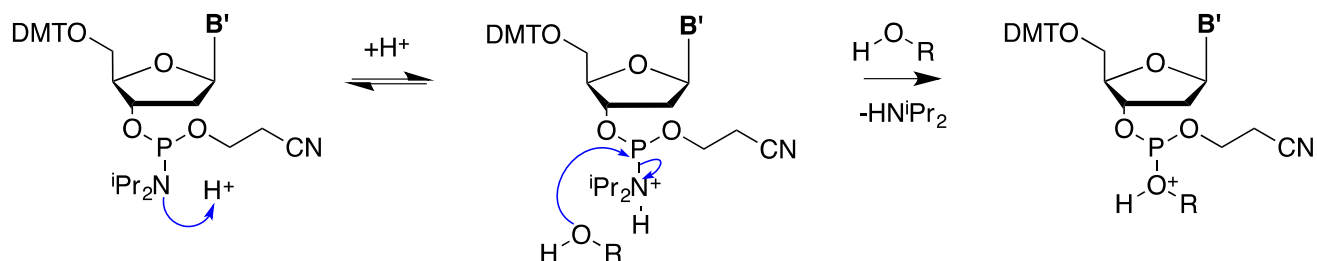
The acid used must be mild enough to promote the reaction without removing the DMT group. *Tetrazole* is a preferred acid for this step.

Coupling, as shown above, is the first step in each cycle of DNA syntheses, and the last is DMT removal. Between these comes *oxidation* of the *P*-atom in the phosphoramidite.

Complete the following structures to show that.
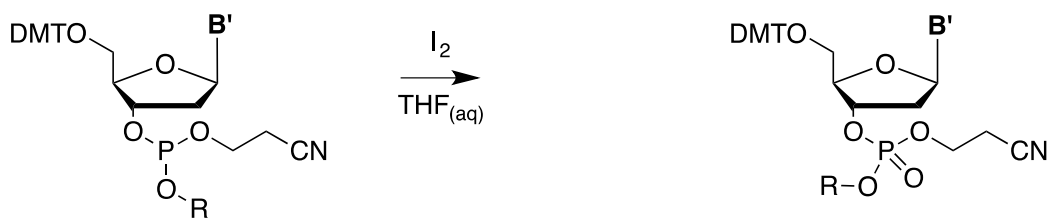
phosphotriester

The oxidation state of the starting phosphoramidite is *3*, and that of the phosphodiester product is *5*.

After repetitive couplings of phosphoramidite, oxidation, then DMT removal, DNA is cleaved from the support by treatment with ammonia; this *also removes* the *iso*-butyryl, benzoyl, and cyanomethyl protecting groups.

Complete the following diagram to show the product of this reaction (recall **B'** stands for *protected* base).
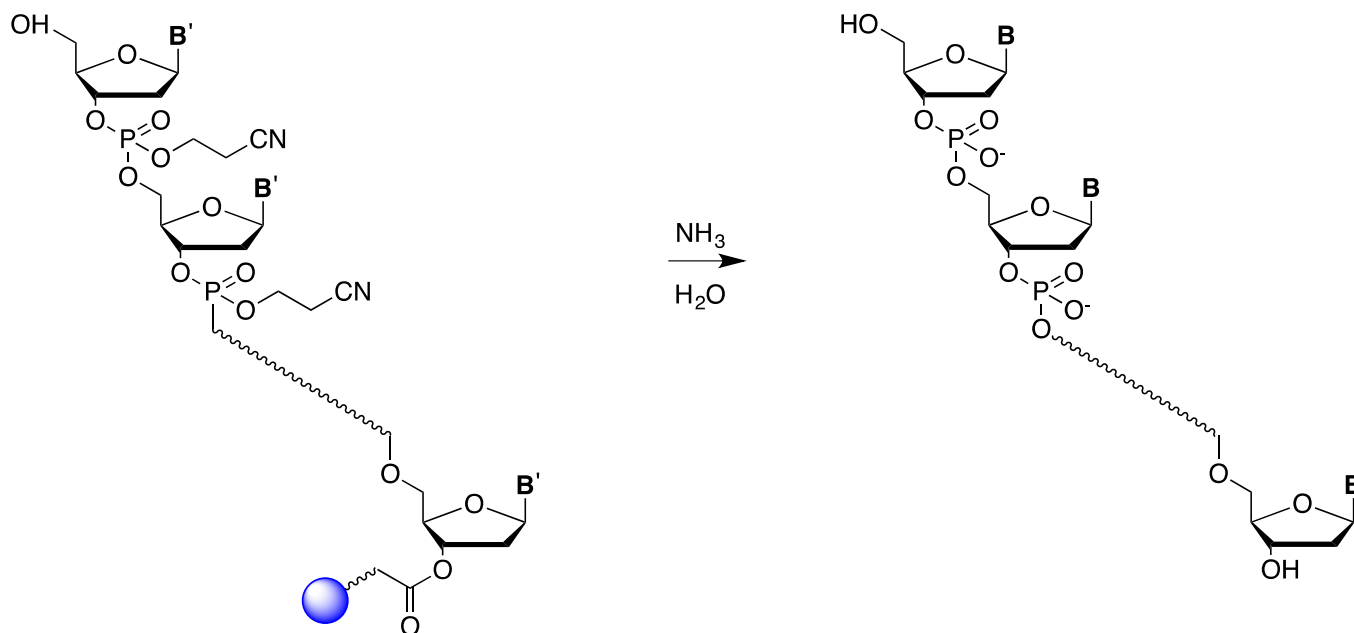
Indicate the reagents for each step in the phosphoramidite synthesis of DNA below, and show the structure of tetrazole.

show reagents throughout

n coupling oxidation deprotection cycles

tetrazole
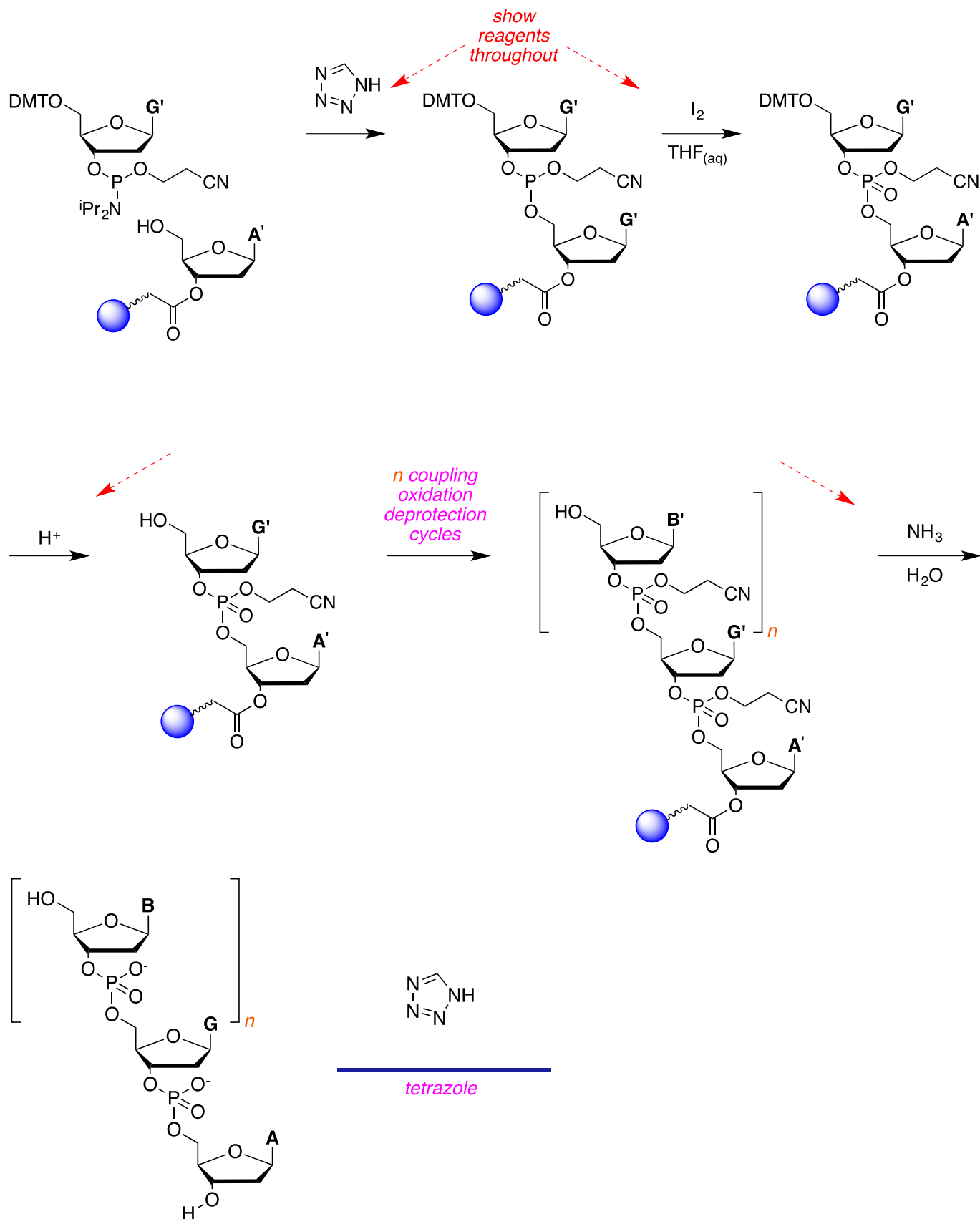
DNA Sequencing

Sanger's Method

Polymerase enzymes recognize double strand (ds) DNA in which the 5-terminus extends beyond the 3' terminus of the short strand. In this case the short strand is called a *primer* and the long strand is called the *template*.
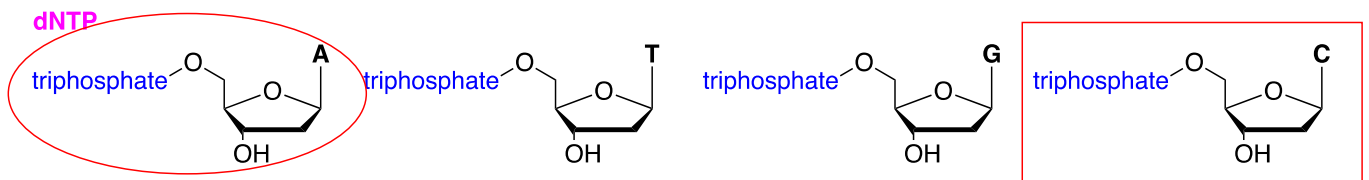
Circle the dNTP that the polymerase incorporates in the short strand opposite a **T** residue, and draw a rectangle around the one it adds when a **G** is encountered.

Imagine a polymerase that is not perfectly selective and sometimes incorporates differentially fluorescently-labeled 2',3'-didexoynucleotide triphosphates (**ddNTP**s, as shown below) *even* if the complementary dNTP is present.
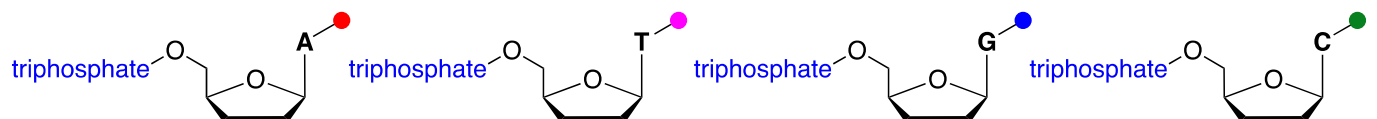
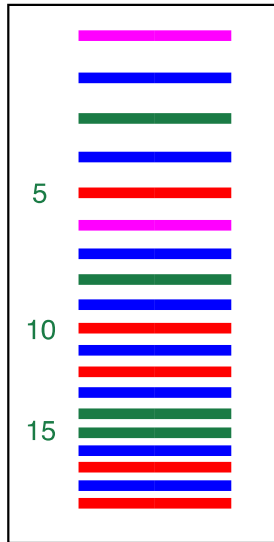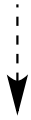If the polymerase is supplied all the **dNTP**s *and* carefully determined amounts of the labeled-**ddNTP** then it may occasionally incorporate a **ddNTP** instead of a **dNTP**, *eg* **dCTP** opposite *G* and the synthesis *cannot* progress beyond that point.

increasing
size

5
10
15

In that experiment, when the polymerase replicates the primed DNA, each terminated complementary strand produced contains *only one*.

If the terminated DNA strands are then separated according to size, *ie* to the number of nucleobases incorporated, then the order of incorporation *can* be read, and the sequence of the complement *can* be deduced.

Deduce the sequence of 19 bases incorporated as revealed by the gel to the left that separates according to size.

5'-TGCGATGCGAGAGCCGAGA-3'

Deduce the sequence of the template DNA after the primer.

5'-TCTCGGCTCTCGCATCGCA-3'

Sequencing By Synthesis

Genomic sequencing is now performed via several different *next generation* methods, of which sequencing by synthesis (SBS) is one. These methods have *higher* throughput than Sanger sequencing.

Envisage and experiment in which a polymerase, a primed DNA template (to be sequenced), and *only* the four derivatives shown below were mixed.

fluorescently labeled, blocked, dNTPs
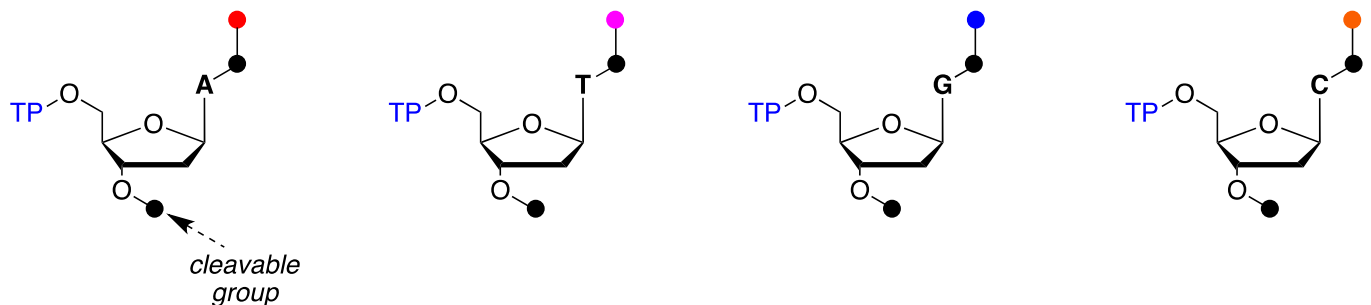
cleavable group

Further synthesis *would not* occur after addition of one base, just as in the Sanger method.

The identity of that base and its complement *could* be deduced from fluorescence.

If the cleavable group were cleaved, the fluor would be lost, and further synthesis *would* be possible because the 3'-OH was also liberated.

Incorporation, fluorescence read-out, and cleavage could be repeated until the signal disappeared into the noise for the read-out steps. If *n* bases could be sequenced via this route, and in Sanger sequencing, say, *5n* could be sequenced, then the most efficient approach would be *Sanger*.

Imagine the same very long DNA template molecules (*eg* genes), somehow anchored to a solid surface, in such a way that groups of these molecules, *or even single molecules*, had constant locations that could be remembered and uniquely addressed. These could then be treated with different primers that would anneal to *different* parts of the DNA template.

If polymerase and the four fluorescently-labeled, blocked dNTPs were added, then SBS could be performed to give *different* sequences on all the primed templates. Consequently, *y* different parts of the DNA could be sequenced at the same time.

The efficiency of Sanger sequencing 5n (see above) is greater than the efficiency of the SBS method, *ie* y x *n* if *y* is <5. However, y can be >>5 because a huge number of spatially addressable DNA templates can be supported. Consequently, SBS can be made to be much *more* efficient than Sanger sequencing.

Reduction Of Azides By Phosphines And Azidomethyl Protection

3'-Protection of the fluorescently labeled-ddNTPs described above has to be compatible with the polymerase selectivity, and it is important that dsDNA *remains annealed* when this group is removed, hence the conditions required must be *gentle*. One protection strategy is based on reduction of azides with phosphines, *ie* the *Staudinger* reaction.
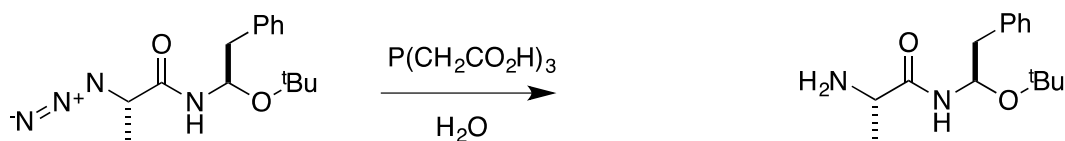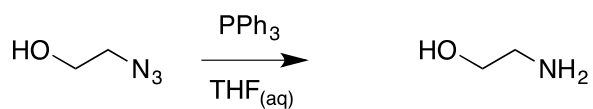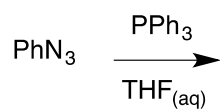
Complete the following mechanism, including curly arrows if they are not shown.
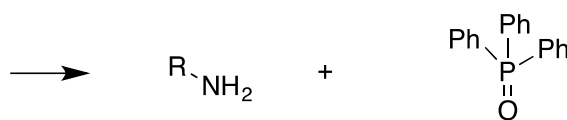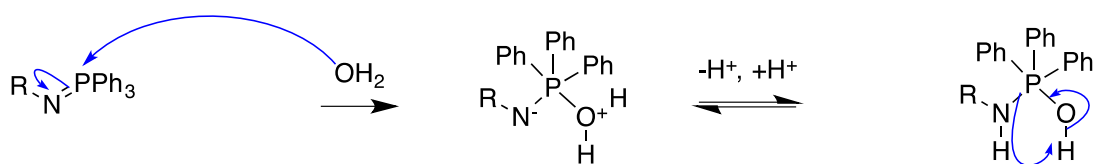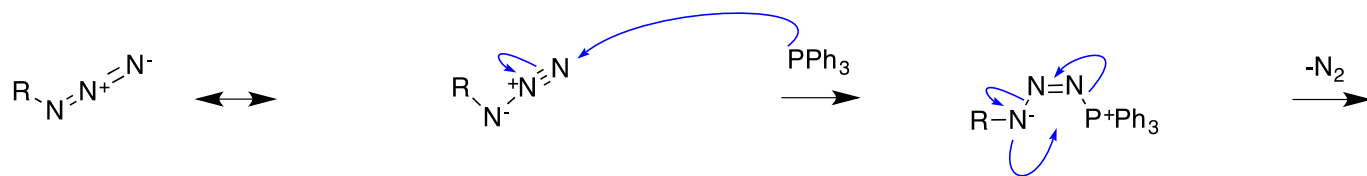
At the beginning of this reaction, in triphenyl phosphine, the *P*-atom is in the *+3*, but at the end it is *+5*, *ie* it has been *oxidized*.

It follows that the azide in this reaction has been *reduced*.

Draw the products of the following reactions.

PhN₃ $\xrightarrow[\text{THF}_{(aq)}]{\text{PPh}_3}$ PhNH₂

HO–CH₂CH₂–N₃ $\xrightarrow[\text{THF}_{(aq)}]{\text{PPh}_3}$ HO–CH₂CH₂–NH₂

$\xrightarrow[\text{H}_2\text{O}]{\text{P(CH}_2\text{CO}_2\text{H)}_3}$

Draw the intermediate in the following reaction, and superimpose curly arrows on it to express electron flow that implies the final product can form.

$\xrightarrow[\text{H}_2\text{O}]{\text{P(CH}_2\text{CO}_2\text{H)}_3}$

$\xrightarrow[\substack{-\text{H}_2\text{CO} \\ -\text{NH}_3}]{\text{H}_2\text{O}}$
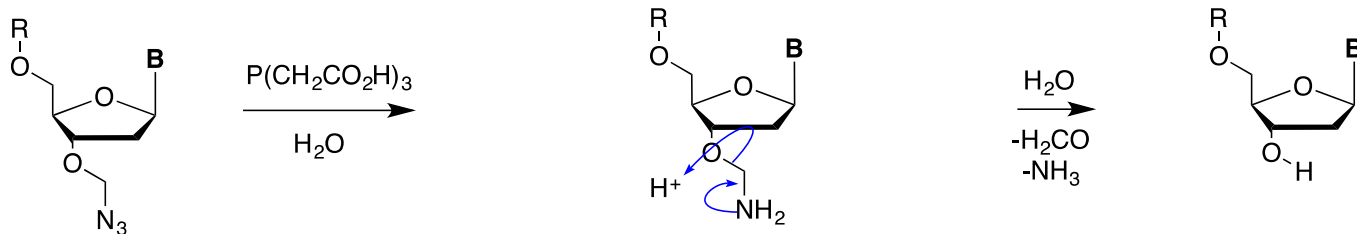
aminal

The alcohol protecting group in the last reaction, *azidomethyl,* is one frequently used in sequencing by synthesis approaches.

In that experiment, when the polymerase replicates the primed DNA, each terminated complementary strand produced contains
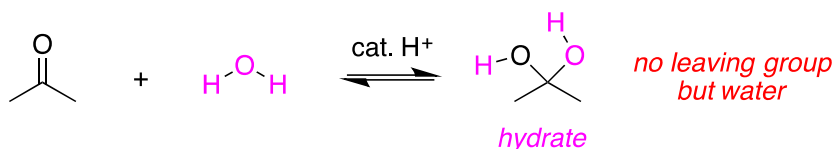
9 Ester Hydrolysis And Transesterification

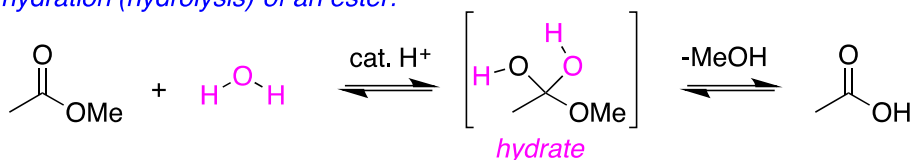from chapter(s) _____ in the recommended text

Introduction

Focus

Mechanistically, acid-mediated hydrolysis of esters is like acid mediated formation of hydrates from aldehydes and ketones, except that esters can loose alcohols, as illustrated below.

hydration of a ketone:

hydration (hydrolysis) of an ester:

This section is about ester hydrolyses, and closely related transesterification reactions.

Reasons To Care

Esterases, enzymes that catalyze ester hydrolysis, have evolved mechanisms to fine-tune substrate selectivities for key molecules in transmission of signals through the nervous system, and for digestion of lipids. Even though the details of these mechanisms are complex and intriguing, the underlying chemical process is the same.

Concepts Involved

acids and bases • curly arrows for electron flow • esterases

Objective

To show the connections between hydration of ketones and aldehydes and hydrolyses of esters.

Reactivity

Esters are *less* reactive than acid chlorides in acylation of nucleophiles.

Under neutral conditions, neutral nucleophiles, which tend to be *less* nucleophilic than similar anionic ones, *do not* tend to react with esters.

Conditions that are acidic enough to protonate the carbonyl group of esters cause it to be *more* reactive hence *more* likely to react with some neutral nucleophiles.
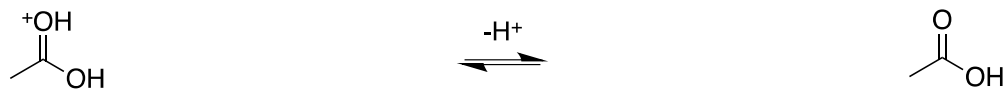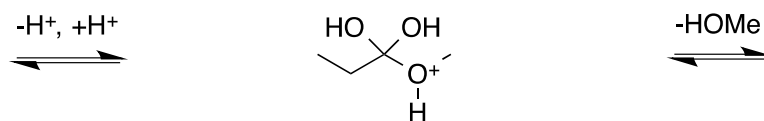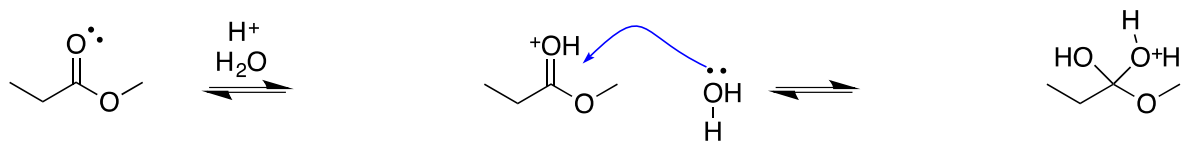
Consider whether an ester carbonyl is more or less likely than an amine to be protonated. The following step is *unlikely*.
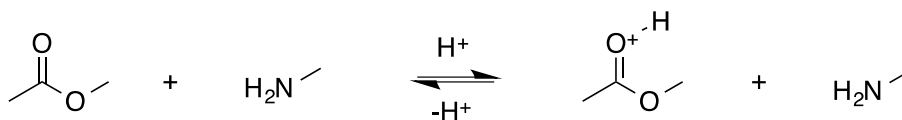
Reactions of esters with amines therefore *are not* likely to proceed under acidic conditions.

Acid-mediated Hydrolysis

Fill in the products or intermediates in the following reaction mechanism.

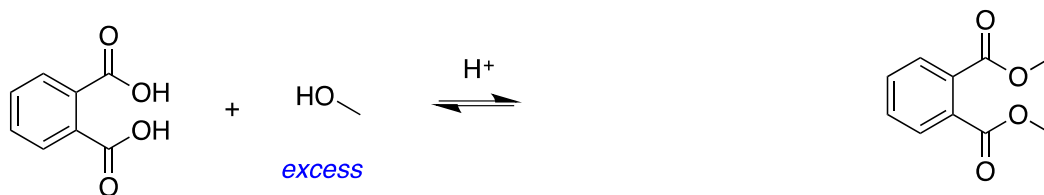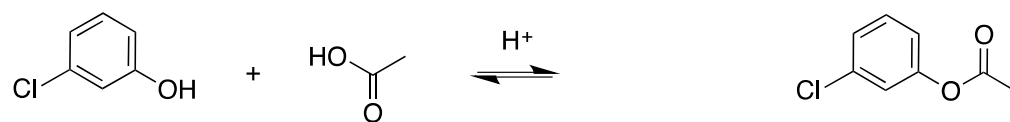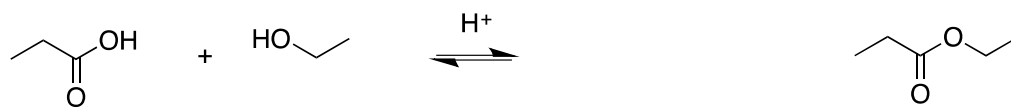Esters react with water under acidic conditions to give *carboxylic acids*; these reactions are acylation of *water*.
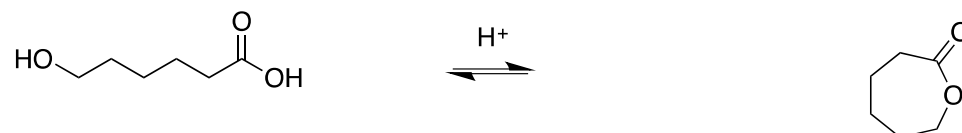
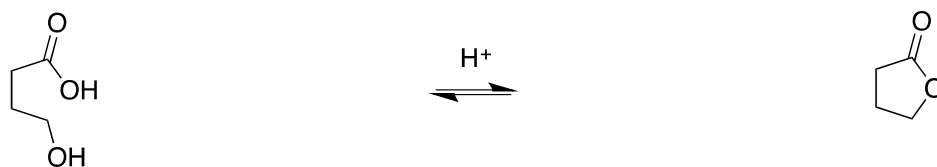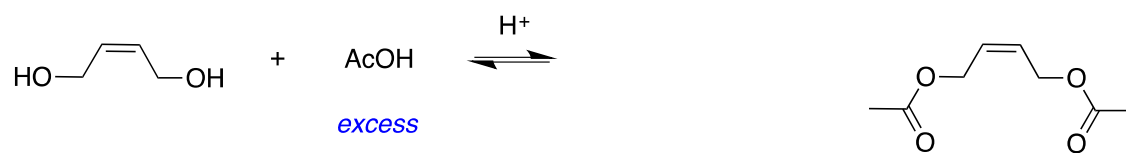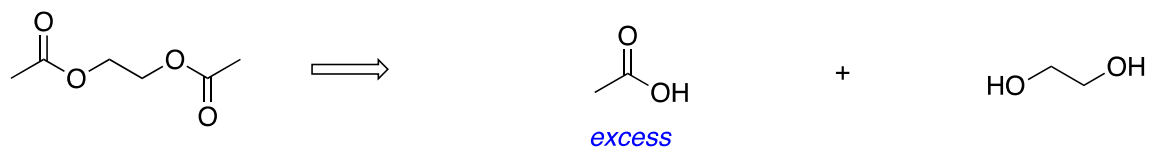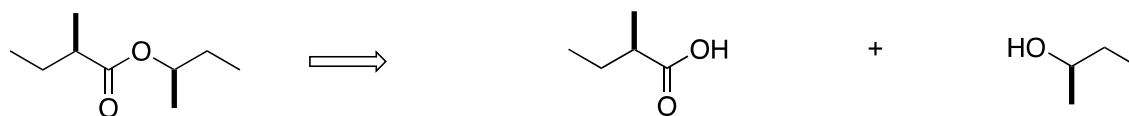Give both products of the following reactions.
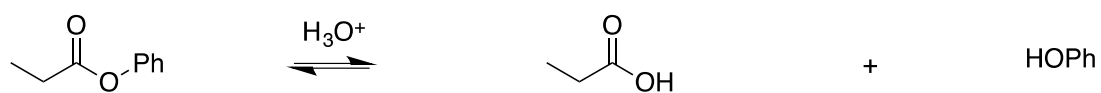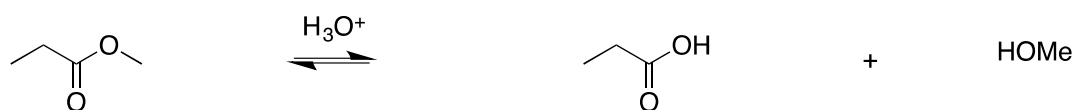
These reactions are *reversible*, so carboxylic acids can react with alcohols under acid conditions to give esters. Excess reagents are used to force the reactions in the desired direction (*eg* water or alcohol as the solvent).
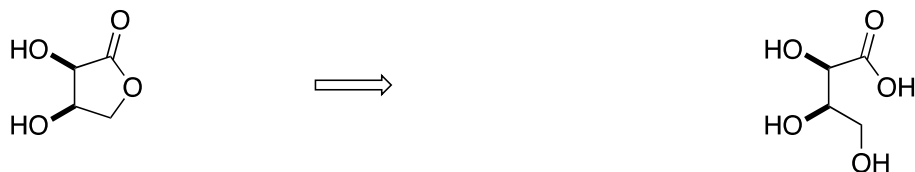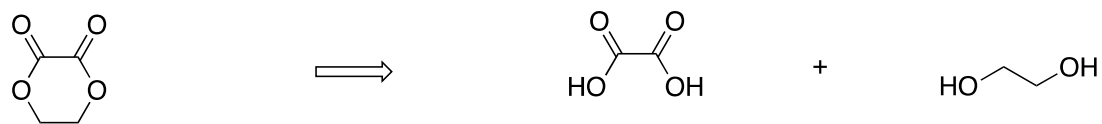
Predict the products of the following reactions when one of the starting materials is used in excess.

HO⌒⌒OH + AcOH ⇌ (H⁺) AcO⌒⌒OAc

excess

(succinic/4-hydroxybutanoic acid) ⇌ (H⁺) γ-butyrolactone

HO⌒⌒⌒COOH ⇌ (H⁺) ε-caprolactone

Show the alcohols and carboxylic acids that would be used to make the following esters.

excess

Explain why the last product is thermodynamically favored over the other possibilities: forming a five-membered ring is more favorable than three- or four-membered ring due to ring strain of sp³ carbons.

Hydrolysis Of *tert*-Butyl Esters Occurs Via A Different Mechanism

Hydrolysis of *tert*-butyl esters are special, because protonation of the carbonyl group can lead to formation of a(n) *stable* carbocation and the carboxylic acid is formed *without* attack of water on the carbonyl group.
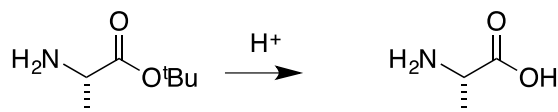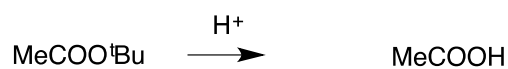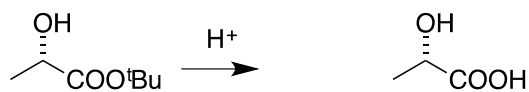
Complete the following mechanism to show this.
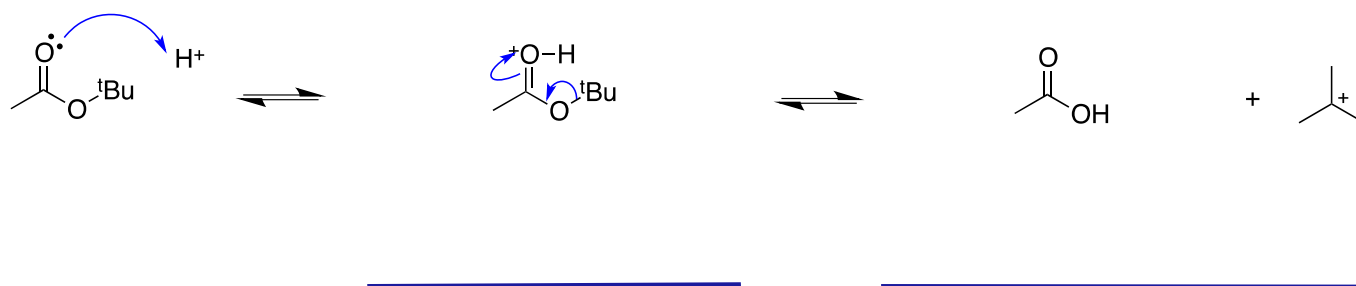
Carbocations generated in the reaction above can react in various ways; show the S*N*1 nucleophilic and E1 elimination reaction products.
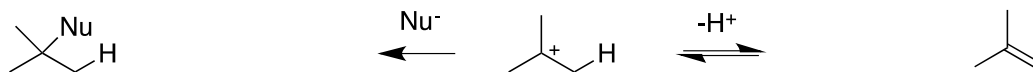
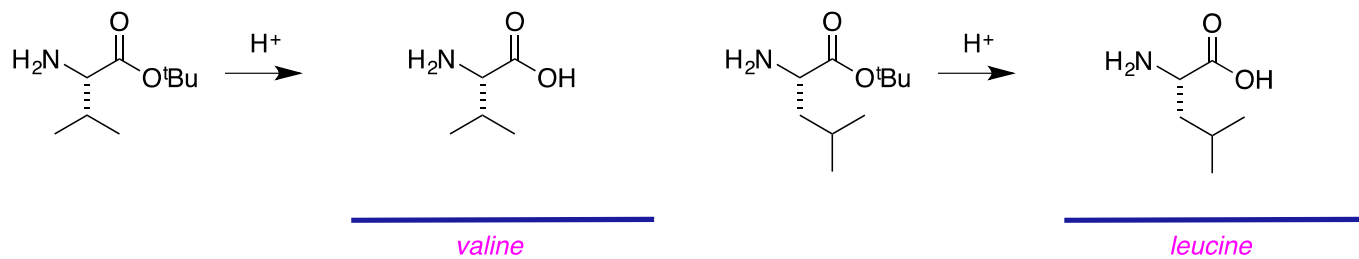Predict the acylation products of the following reactions (TFA is *trifluoroacetic acid*).

MeCOO^tBu $\xrightarrow{H^+}$ MeCOOH

alanine

lactic acid

glycine

valine

leucine
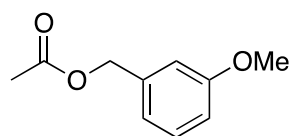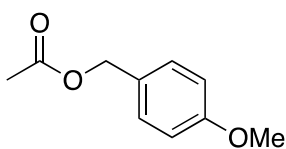
The type of mechanism illustrated above may be applicable to any ester (not exclusively ᵗBu) that can dissociate to form a stable *carbocation*.
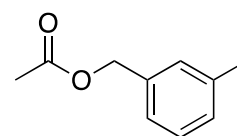
Rank the following esters in order of ease of hydrolysis to carboxylic acids under acid catalysis *via a mechanism that generates benzylic carbocations* (1 is most, 6 is least).
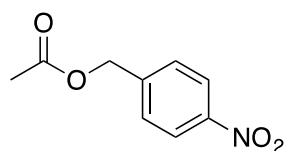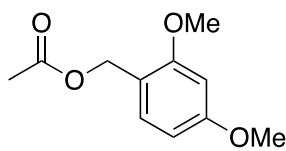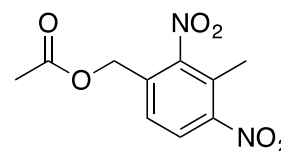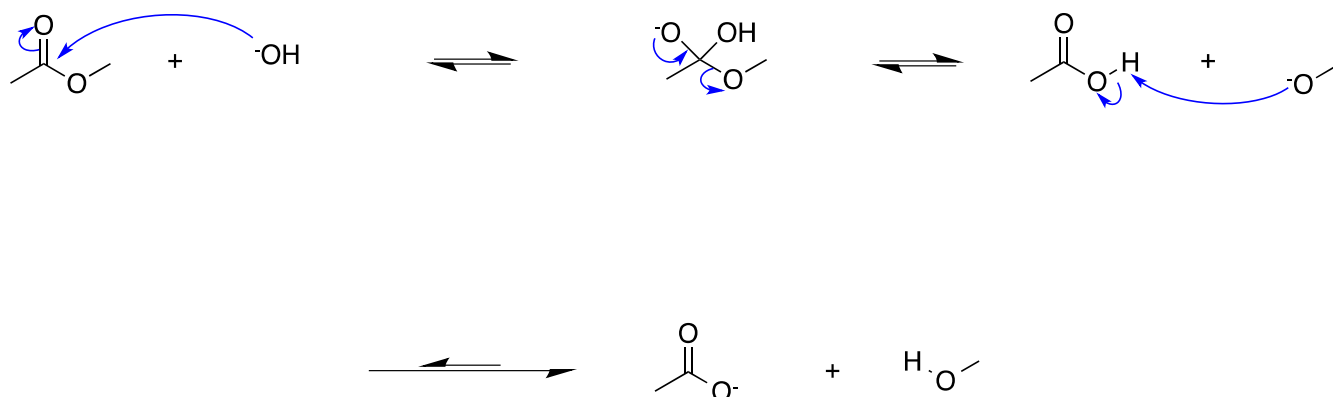

3

2

4

5


1

6

Base-mediated Hydrolysis

Mechanism

Esters hydrolyze to acids under *basic* conditions, but the mechanism is different; the carbonyl group is not protonated first, and there is no need to invoke proton transfer steps involving the nucleophile reagent. Use curly arrows in the following mechanistic pathway to show the electron flow.

Technically, the last step is reversible like all the others, but the equilibrium constant vastly favors formation of carboxylate salt and alcohol.

Base-mediated ester hydrolyses are effectively *reversible*, but *acid-mediated* ester hydrolyses are *irreversible*.
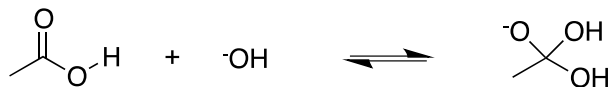
A common mistake is to show protons under basic conditions. Push arrows for the following mechanism and circle the error.
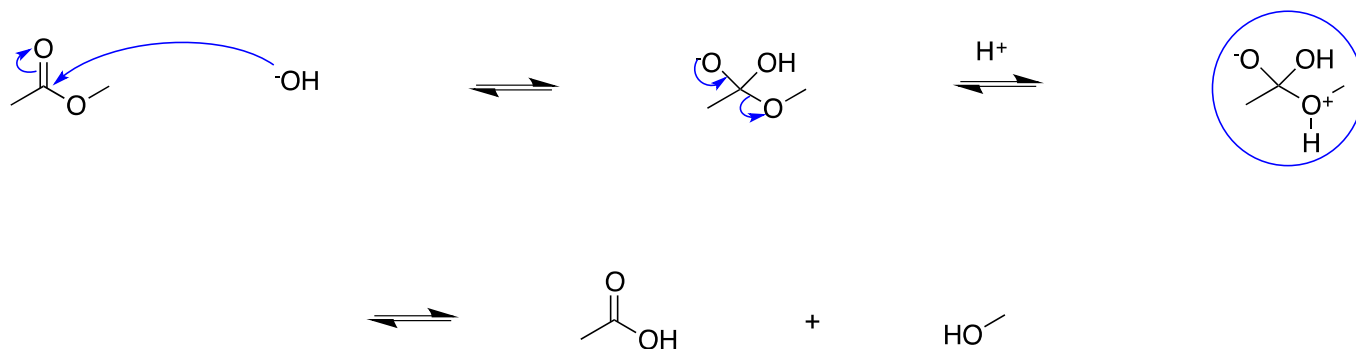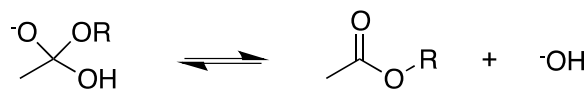
Hydroxide is a good base and a poor nucleophile so the following reaction is *likely*.
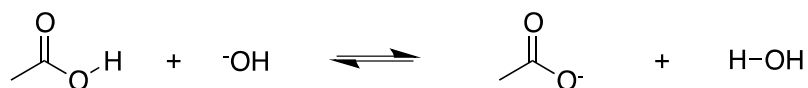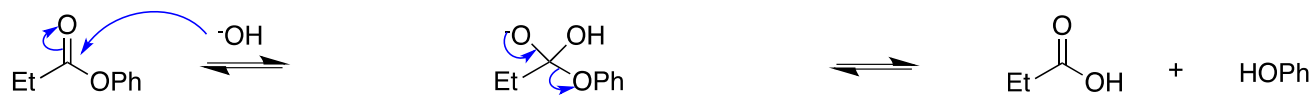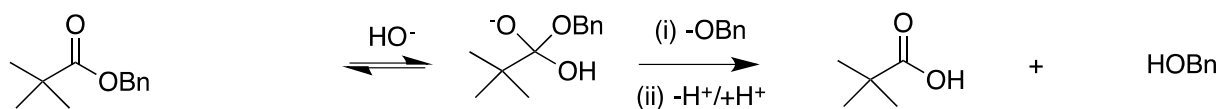
Similarly, this one is *unlikely*.
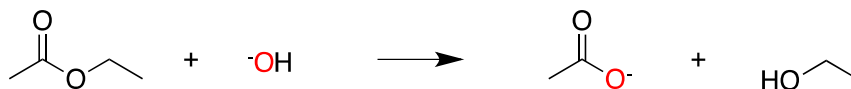
Similarly, this one is *unlikely*.

Complete the following mechanism for base-mediated ester hydrolysis by showing arrows, missing intermediates, and products.


Deduce the ester starting material and products in the following base-mediated ester hydrolysis.

Show ^{18}O in the product of the following base-mediated ester hydrolysis by shading it.

Observation of the heavy oxygen in the carboxylate *would not* be consistent with the following mechanism that features S$_N$2 displacement (shade the hydroxyl-*O* to indicate where it goes).
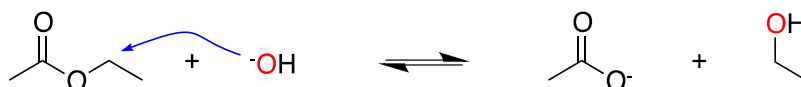
Observation of the heavy oxygen in the carboxylate *would not* be consistent with the following mechanism that features S$_N$1 dissociation (shade the hydroxyl-*O* to indicate where it goes).

Reactions involving isotopes like ^{18}O *can* be used to exclude certain mechanistic possibilities.

Hydrolysis of a glycerol triester (fats) gives a triol and three carboxylates, like this:
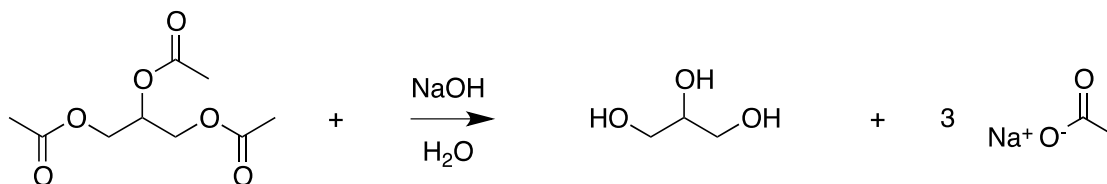
If those carboxylates have long alkyl chains they are referred to as *soaps*. Hydrolysis to give fatty acids (or their carboxylates) under basic conditions is called "saponification."

Predict the products formed from *saponification* of the following fats and oils (glycerol triesters).

Soaps in aqueous solutions form *micelles*. Describe what micelles are: Particles that aggregate in the solution with the hydrophilic "head" regions in contact with surrounding solvent and the hydrophobic single-tail regions are in the micelle center.

Draw a micelle formed from a collection of long-chain fatty acids represented like tadpoles with negatively charged heads.


cartoon of a micelle

Transesterification Reactions

Mechanism Under Acidic Conditions

Under acidic conditions, esters can undergo *transesterification* with alcohols through a process similar to acid-catalyzed hydrolysis.

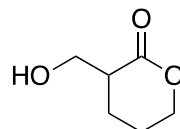Transesterification is a process wherein an ester is transformed into *another ester*.
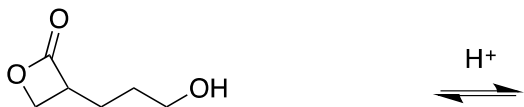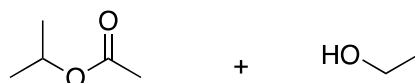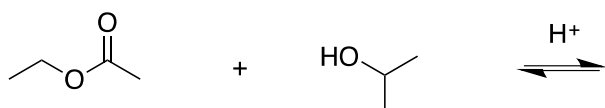
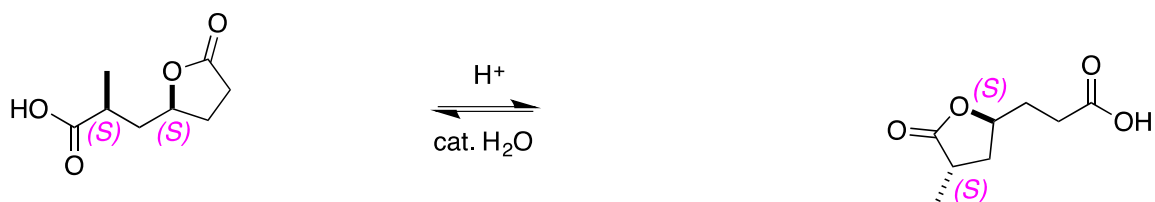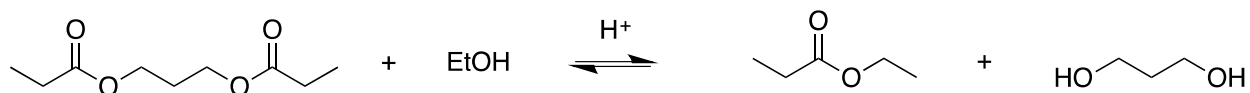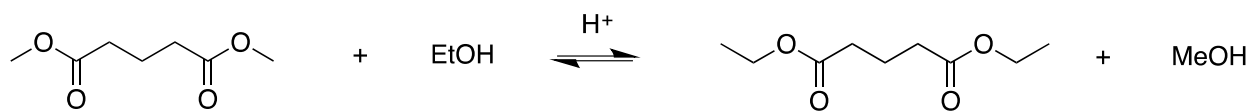Fill in the products/intermediates and arrows in the following mechanism.
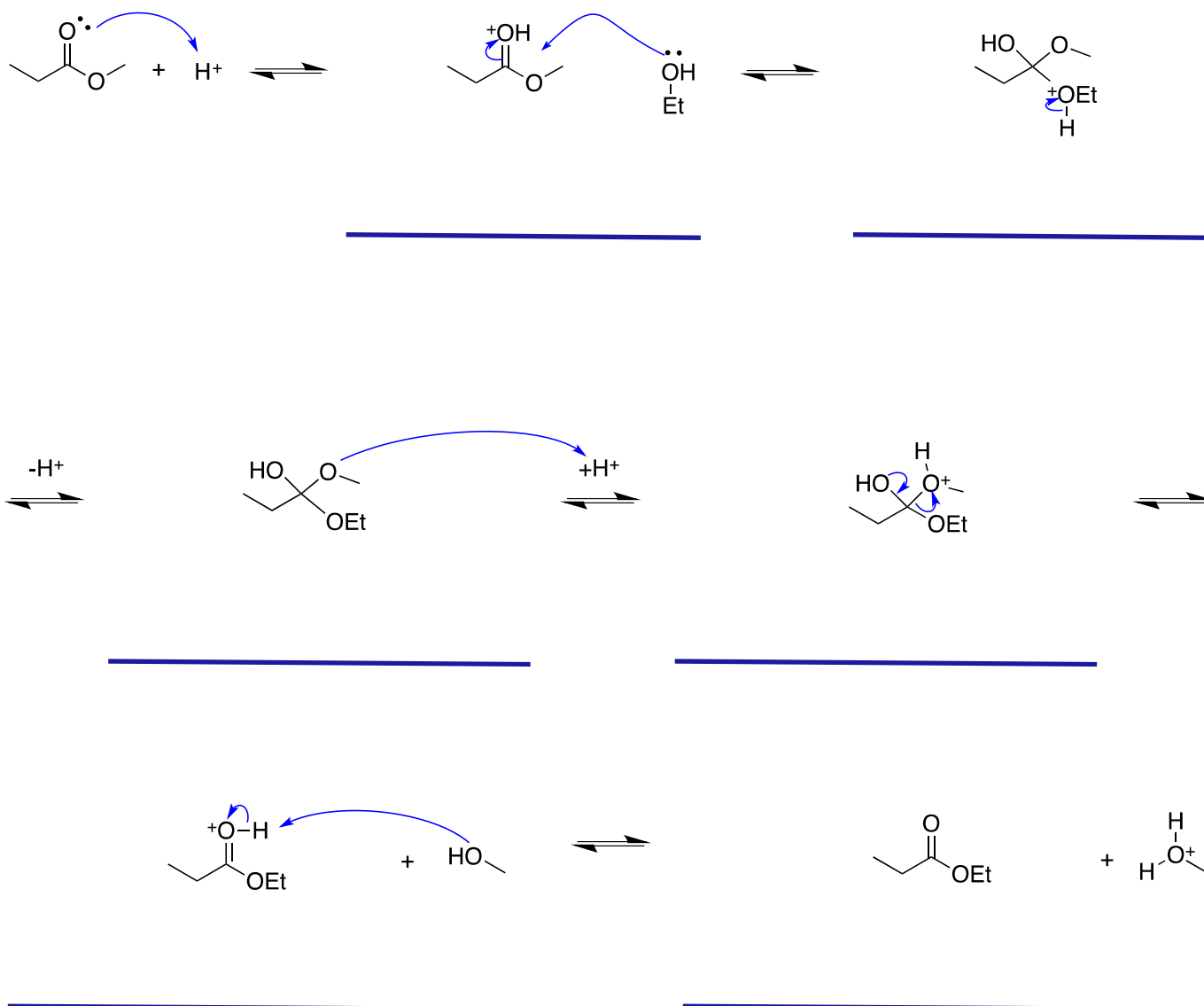
Examples Of Transesterifications

Transesterification reactions swap an alcohol for the *alkoxide* of an ester.
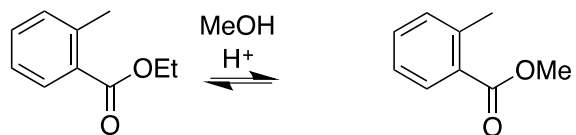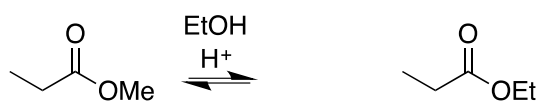
Predict the products of the following transesterification reactions where, in the intermolecular reactions, excess alcohol is used.

check by assigning (R)- or (S)-configurations

AcMe + Et¹⁷OH, H⁺ / MeOH ⇌ AcOEt (with ¹⁷O-labeled OEt)

Pent-4-en-1-ol + AcOMe, H⁺ / AcOH ⇌ pent-4-enyl acetate
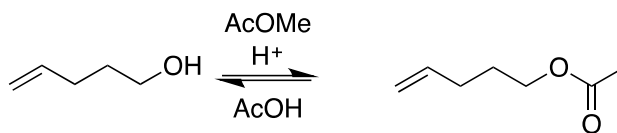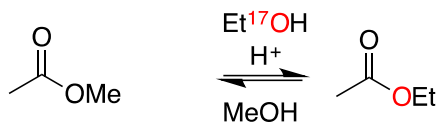
Mechanism Under Basic Conditions

Under basic conditions, transesterification occurs by reaction of the ester carbonyl with *alkoxides*.

Provide curly arrows to indicate the electron flow on the mechanism below.

Draw a similar diagram for addition of ethoxide to methyl acetate, and carefully indicate if each step is *reversible* or *irreversible*.

show arrows

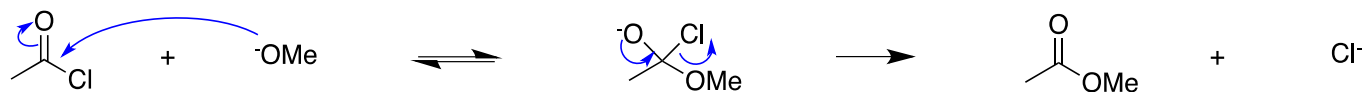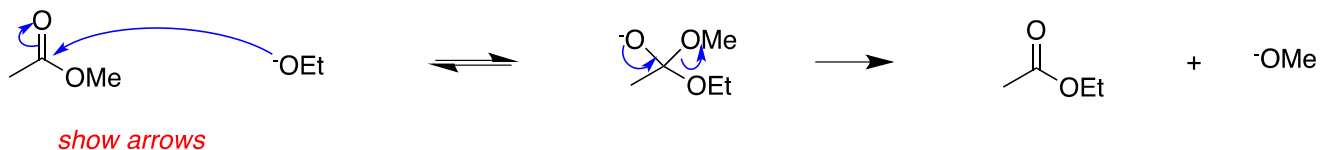# Examples Of Base-Mediated Transesterifications

Predict the products of the following reactions performed in a large excess of alcohol solvents.
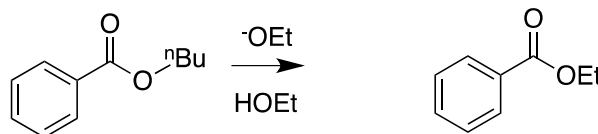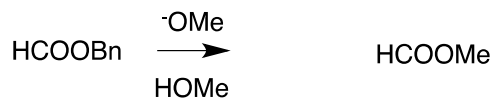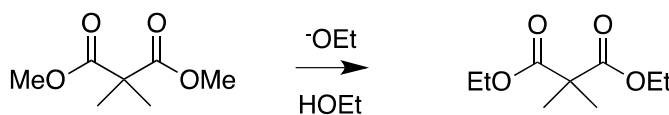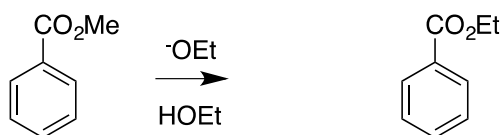
Base-mediated hydrolyses of hindered esters are slow because the reaction involves rate limiting formation of a *more* hindered *sp³*-hybridized intermediate; indicate which of the reactions above will be slow. *Acid-mediated* hydrolysis of the same ester *would not* be similarly effected.

Ester Hydrolyses In Biochemistry

In The Central Nervous System

Enzymes that hydrolyze ester bonds in biochemistry are called *esterases*.

Identify the enzyme ACHE (**a**) by considering the reaction it catalyzes, **b** (Figure 1).

ACHE stands for: acetylcholinesterase. Check your answer on the web, hence answer the following questions.

a

b

acetyl chloline
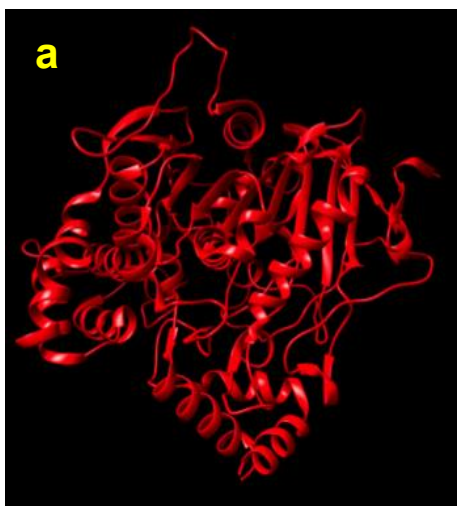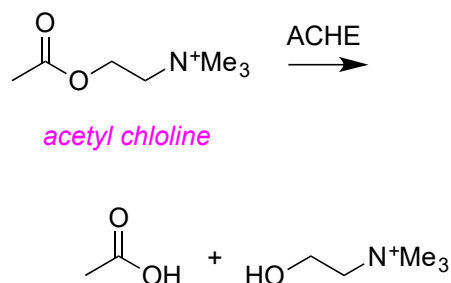
Figure 1. **A** Structure of ACHE. **b** Reaction mediated by ACHE.

In neurotransmission, presynaptic neurons liberate acetylcholine which migrates across the synaptic cleft and binds *acetylcholine receptors* on the postsynaptic neurons.

ACHE hydrolyzes bound acetylcholine giving acetic acid and *choline*.

Acetylcholine is only released into the synaptic cleft for the next neural impulse if its concentration of in the synaptic cleft is low. Consequently, if it is not cleared by *ACHE* accumulation of acetylcholine can result in *paralysis*.

Well known inhibitors of ACHE include *nerve gases*.

In The Digestive System

Search lipases on the web.

Lipids contain *long chain alkyl groups*; they feature in *triglyceride* fats.

Esterases, including lipases, are *catalysts*.

Hydrolyses and transesterification reactions featured in this section are accelerated under acidic or basic conditions. Esterases act under *neutral* conditions by placing water atoms near hydrogen-bond acceptors to make them better *nucleophiles* and carbonyls of ester substrates near hydrogen bond donors making the carbonyls better *electrophiles*.

Enzymes in general, have highly specialized active site cavities; because of this lipases, for instance, *can* hydrolyze one ester in the presence of another as shown in Figure 2b, because one ester only fits well in the active site.

Lipases play a role in *metabolism of triglycerides* from the body.
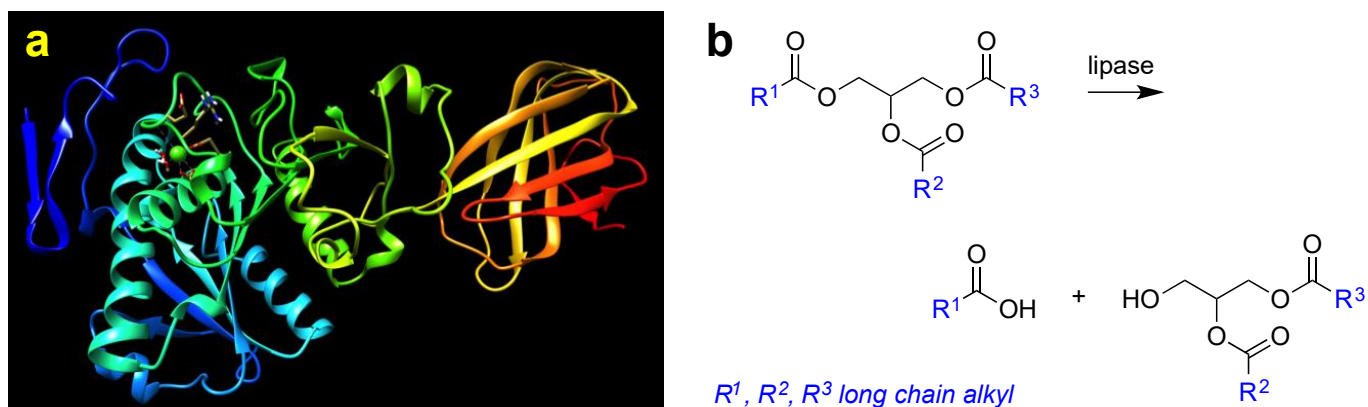
Figure 2. a Structure of a lipase. **b** Hydrolysis of triglyceride.

Some esterases retain their activities in alcohol solvents and may mediate *transesterifications*.

An example of such enzymes includes the *lipases*.

10 Hydrolysis And Dehydration Of Amides

from chapter(s) _____ in the recommended text

Introduction

Focus

This section is primarily about adding water to amide bonds to hydrolyze them into amines and acids.

Reasons To Care

Proteases cleave amide bonds in peptides and proteins. They play critical roles in many different biological pathways, and preventing their action with *protease inhibitors* is a common strategy in biomedicinal chemistry. Mechanisms by which proteases selectively catalyze cleavage of certain amide bonds are detailed and complex, but the fundamental chemical mechanism for amide hydrolysis is similar throughout.
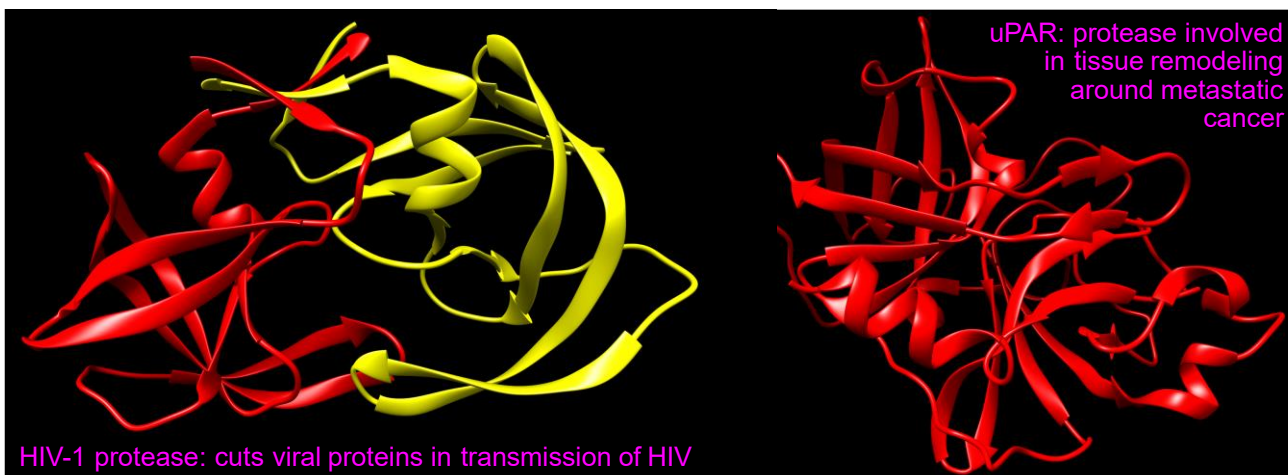
Concepts Involved

acids and bases • curly arrows to depict electron flow • protonation of amides in hydrolysis • proteases

Objective

This section covers the chemical mechanisms for hydrolysis of amides and nitriles, and how amide hydrolysis relates to proteases.

Reactivity Of Amides

Amides are *less* reactive than acid chlorides in acylation reactions because chloride is a better leaving group than NH_3 or NH_2^- (or substituted derivatives of these).

Similarly, amides are *less* reactive than esters in some acylation reactions because alkoxides are better leaving groups than amide anions, NH_2^- or NR_2^-.

Amides tend to be flat with *sp^2* hybridized nitrogen atoms, whereas the alkoxide oxygen of esters is *pyramidal* and *sp^3* hybridized; this indicates the nitrogen is *less* electron donating in an amide than the alkoxide oxygen of an ester.

For the amide and ester shown below, the energy of barrier for rotation is greater for **a** than **e**.


A ^{1}H NMR spectrum of the amide above will show *2 NCH$_3$* resonances at ambient temperature and *1* at elevated temperatures.

Under acidic conditions, however, amides are *more* easily protonated than esters, leading to activation of the carbonyl.

Amide oxygen atoms are *less* basic than the *N*-atoms because resonance stabilization is only possible when the *O*- is protonated, not the *N*-.

Complete the following diagrams, with curly arrows, to show preferred sites of protonation of esters and amides, and how the cations formed are resonance stabilized.
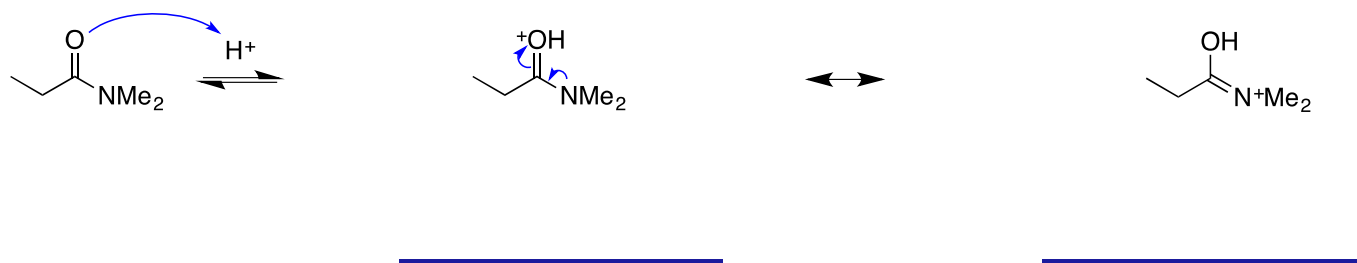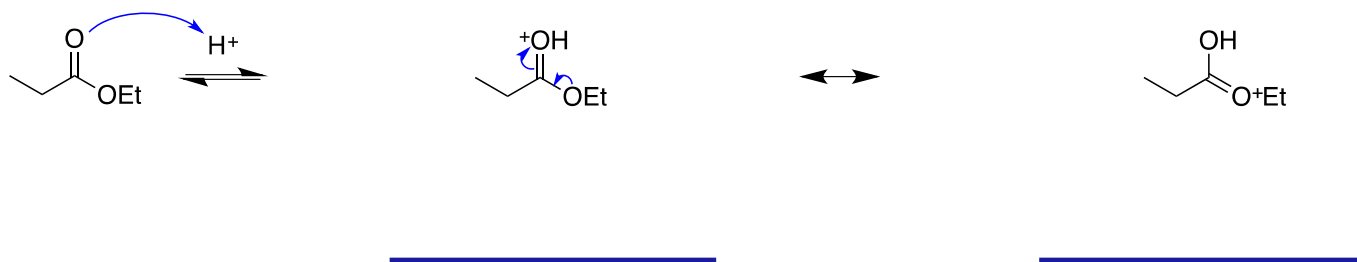
Hydrolysis Of Amides

Acid catalyzed hydrolysis of amides involves protonation of the carbonyl group, addition of water to give a *tetrahedral* intermediate, proton transfer, then loss of amine.
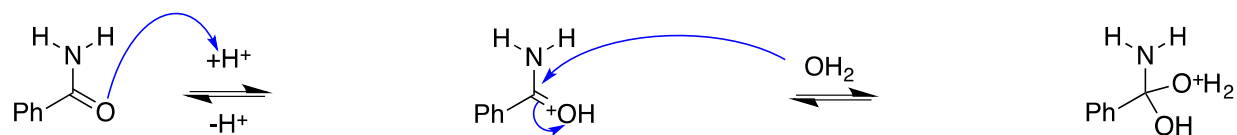
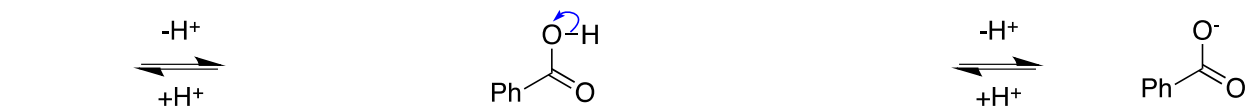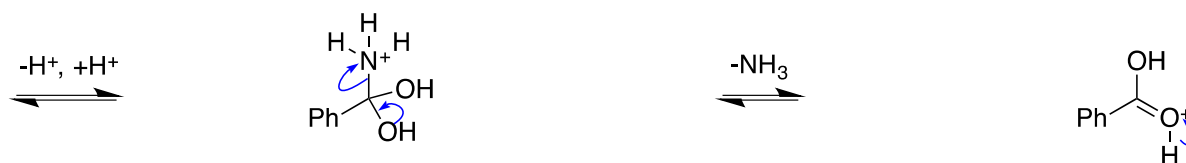Draw the mechanism for acid-mediated hydrolysis of ethanamide.
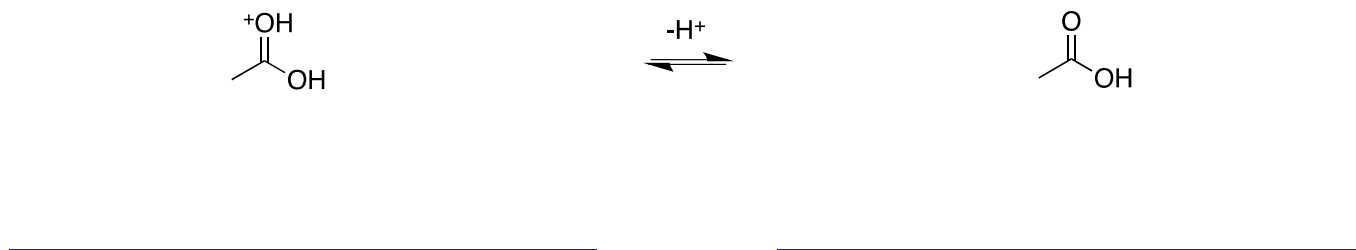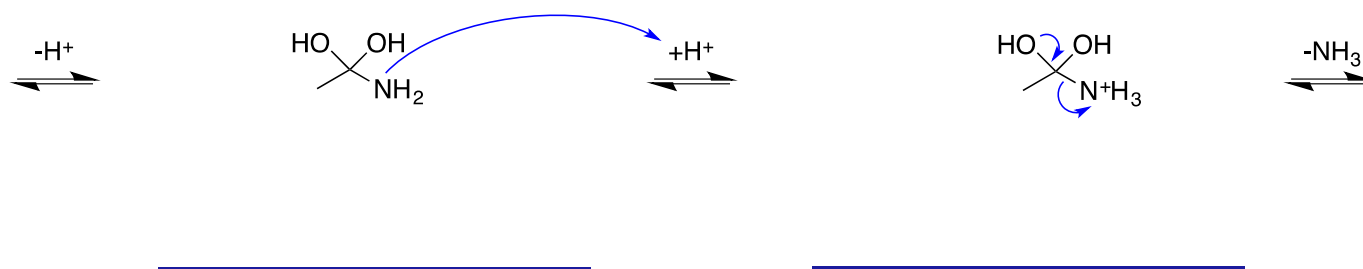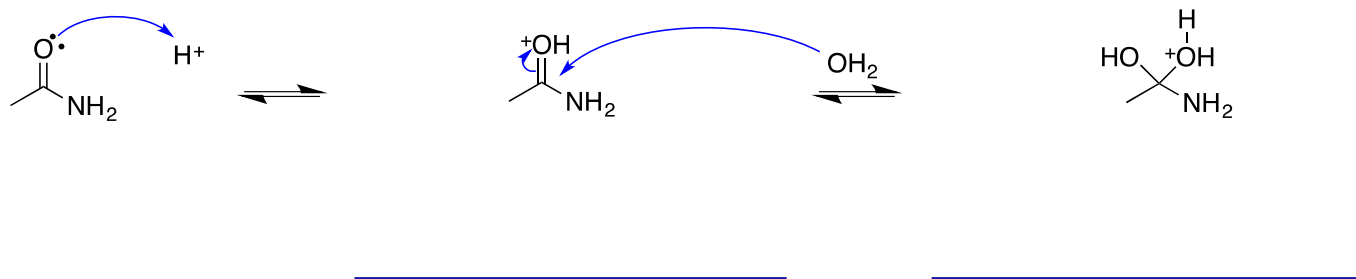
Under these acidic conditions, the by-product of the reaction above is *NH₄⁺*, and the transformation is *irreversible* because NH_4^+ is not nucleophilic and cannot attack carbonyl to form amide.

Uncatalyzed amide hydrolysis reaction cannot proceed under neutral conditions because carbonyl group on amide cannot be protonated under neutral condition, leading to inactivated carbonyl, then water cannot attach to carbonyl carbon.
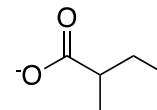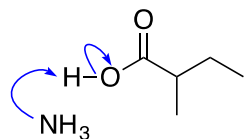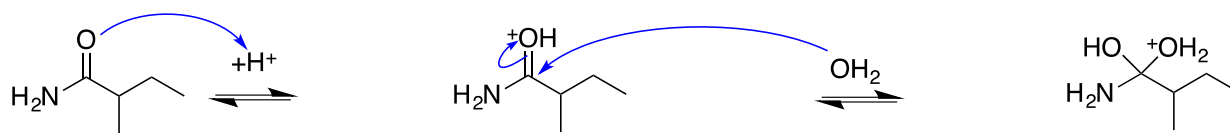
Use accurate curly arrows to show the mechanism for acid mediated hydrolysis of benzamide.

tetrahedral intermediate

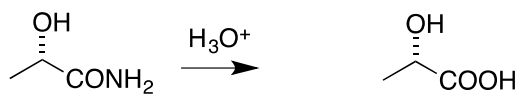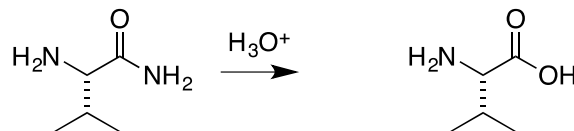Draw the mechanism for hydrolysis of 2-methylbutanamide.

-H+, +H+

-NH₃

+NH₃
-NH₄⁺

carboxylate

Show the products of the following reactions.

lactic acid

valine

Proteases

Function

Enzymes that mediate hydrolysis of esters are called *esterases* while amide hydrolysis is promoted by *proteases*.

Proteases cleave amide bonds at pH *~7* hence in living organisms they cleave peptides or proteins into fragments.

Catalytic cleavage of amide bonds by proteases in near-neutral aqueous media is possible because these *enzymes* encapsulate their substrates, placing them in environments where amide carbonyls can be activated via *hydrogen bonding* and nucleophiles can be activated by basic atoms in the cavity.

Proteases *do not* cleave every available amide bond in all proteins and peptides.

Instead, protease *active-* sites recognize shapes of the peptide or protein substrates, and the amino acid side-chain residues (like $R^1 - R^3$ in Figure 1 below) around the particular amide that is cleaved.

Figure 1a shows the structure of a protease called caspase 1. If caspase 1 selectively cleaves a peptide at the site indicated in Figure 1b, draw the hydrolysis products that would be produced.
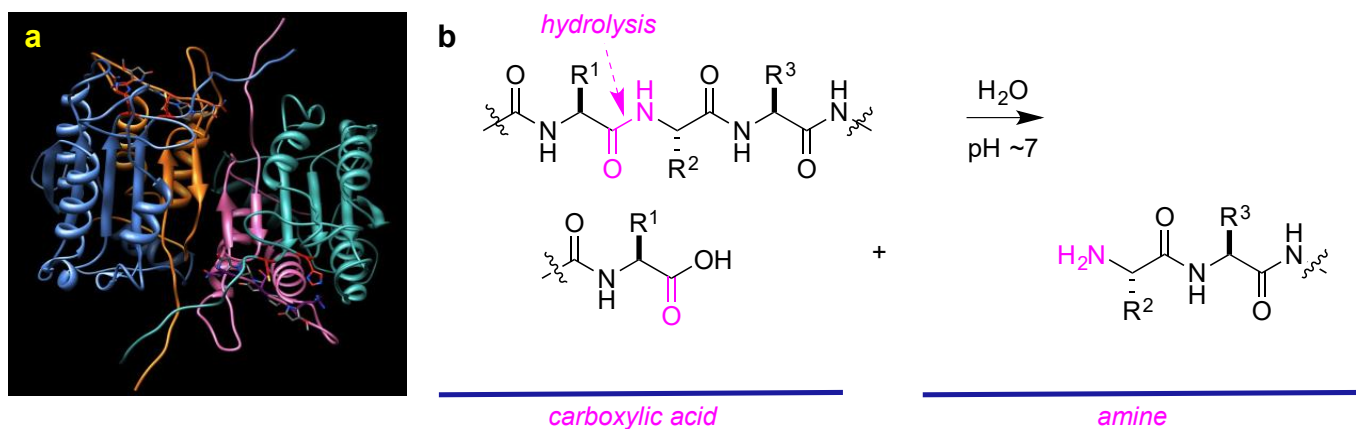
Figure 1. a Structure of caspase 1. **b** A generic substrate for proteases.

Too much caspase 1 is implicated in neuronal cell death because this enzyme *degrades* proteins that are important for cell survival.

Complete the diagram below by showing curly arrows that depict the key step in collapse of a tetrahedral intermediate in a peptide hydrolysis to amine and carboxylic acid products.

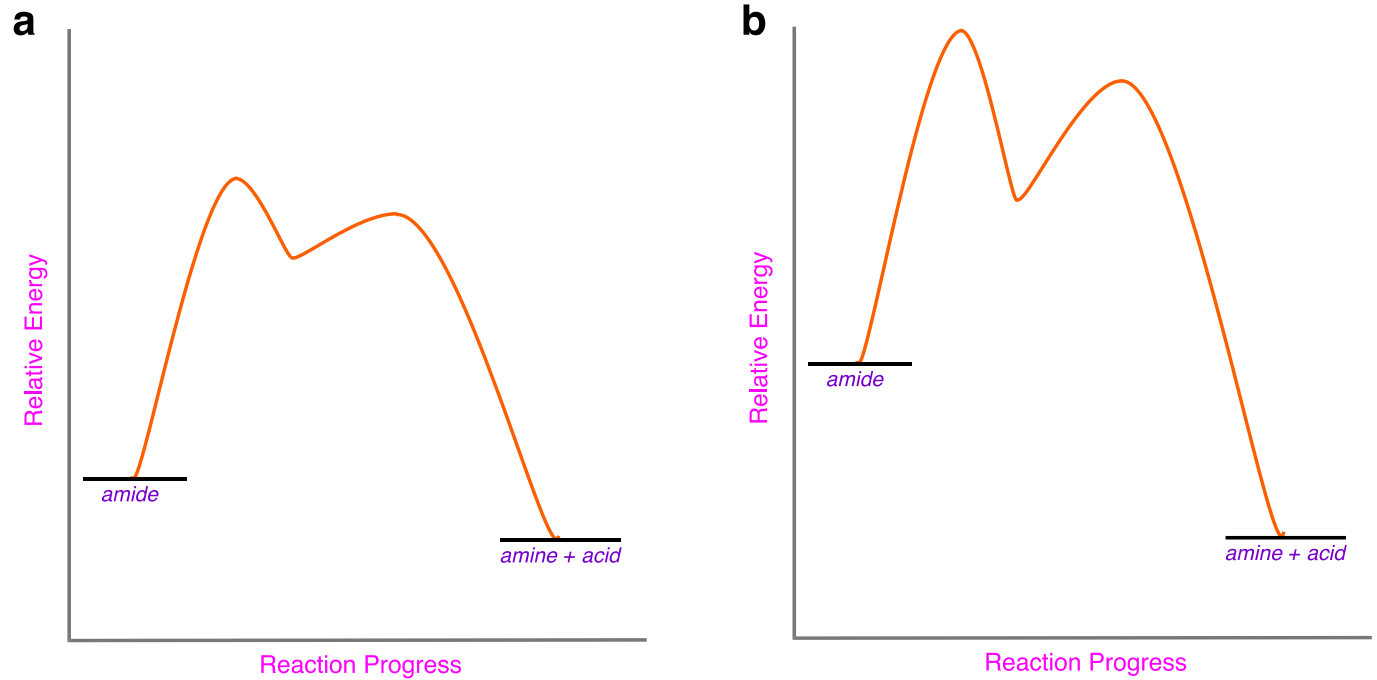carboxylic acid   amine
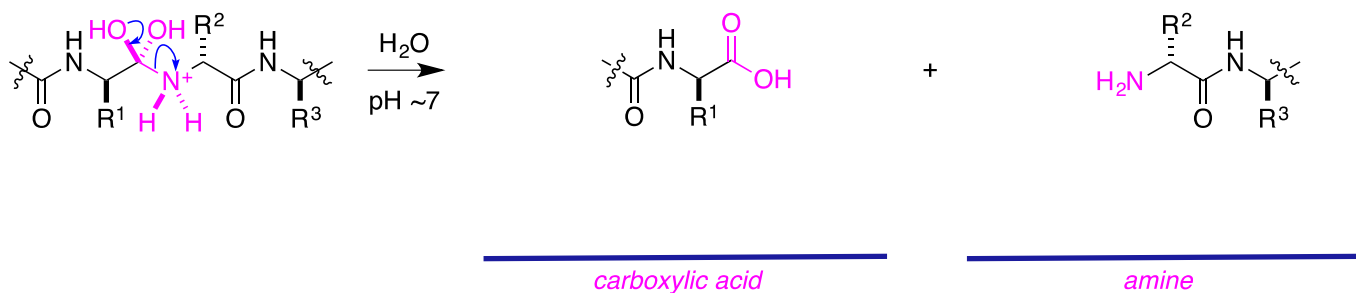
Catalysis

Catalysis is primarily a *kinetic* phenomenon.

Clearly show the *activation energy barrier*, and *intermediate* on the profile below for amide hydrolysis.
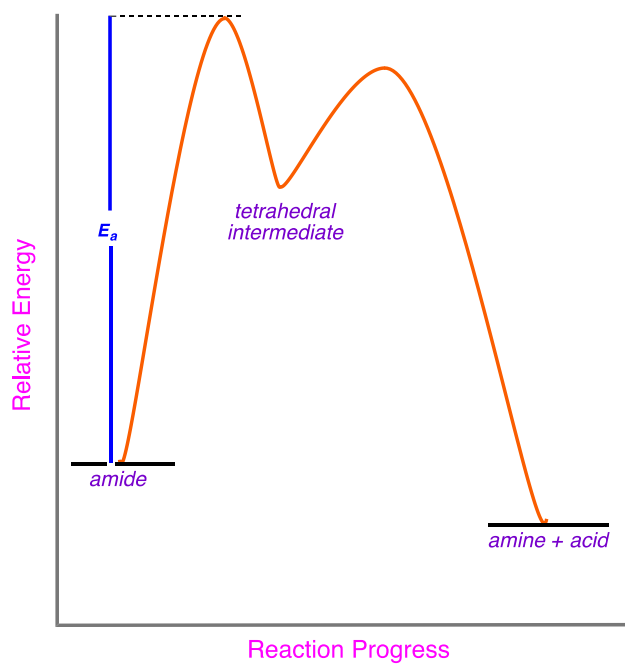
A protease could accelerate amide hydrolysis by *destabilizing the substrate* and/or *stabilizing the intermediate*.

a

b

These two ways of catalyzing amide hydrolysis are represented above. A protease that encapsulates a substrate by placing it in a cavity that stabilizes the tetrahedral intermediates in its hydrolysis is best represented by situation **b**. That *is* the way most proteases catalyze amide hydrolysis.

Protease Inhibitors

The genome of the HIV-1 virus expresses *9* proteins (search the web) and disruption of the function of any of these is likely to suppress propagation.

Nearly all *enzymes* are *proteins* but not all *proteins* are *enzymes*.

An enzyme expressed in the HIV-1 genome, HIV-1 protease, cuts other proteins into smaller, active fragments. HIV-1 protease is inhibited by the AIDS medicine crixivan.
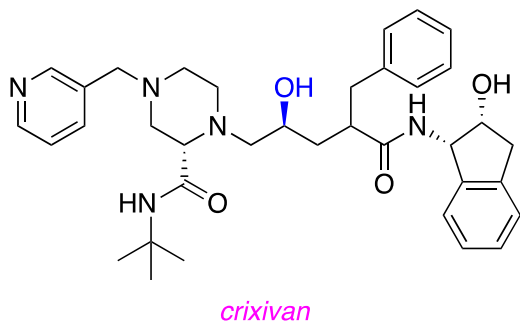
crixivan

Crixivan docks in the *active-* site of HIV-1 protease with a *high* affinity thus preventing binding of the natural protein substrates.

Crixivan can act in this way because the *alcohol* functionality shown in blue was designed to *mimic the tetrahedral intermediate in amide hydrolysis*.

Thermolysin is a protease that particular bacteria. Circle the state analog in the thermolysin

1
thermolysin inhibitor
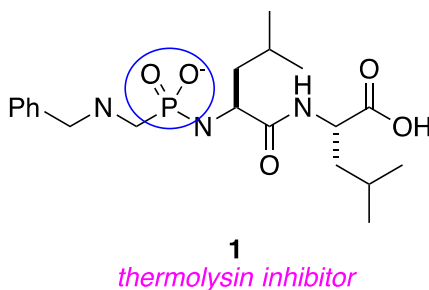
hydrolyzes certain peptides in a functionality that acts a transition-inhibitor **1**.

Detection Of Protease Substrate Selectivity

Amino coumarins dyes, like many other fluors, are fluorescent because of resonance-delocalized chromophors.

Circle the amino coumarin derivative that is most fluorescent in the following reaction.
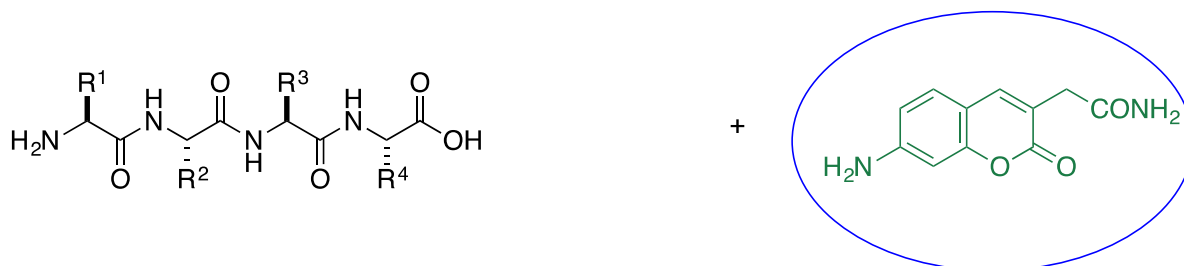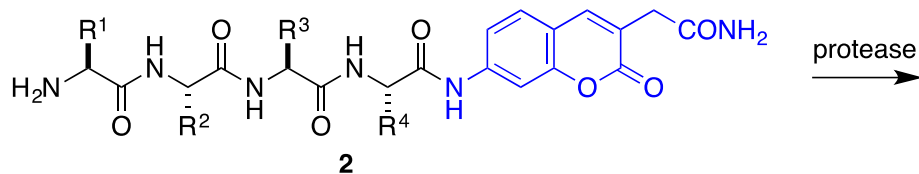

Selectivities of proteases may be determined if many substrates **2** with different amino acid side-chains $R^1 - R^4$ were exposed to the protease in reactions that could be conveniently monitored via *fluorescence*.

Hydrolysis Of Nitriles Involves Amide Intermediates

Nitriles can be hydrolyzed to amides, and amides can be hydrolyzed to *carboxylic acids*, hence hydrolysis of nitriles to amides is *partial*.
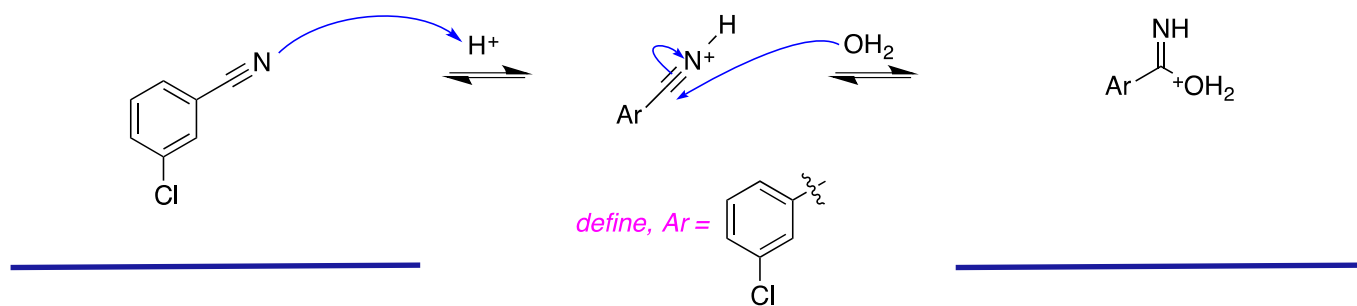
Acid-mediated hydrolysis of nitriles to amides features *N*-protonation, attack of water at the nitrile carbon, then loss of a proton. *Tautomerization* of the product leads to formation of the amide.
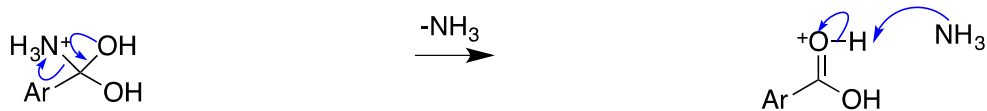
Draw these steps for acid-mediated hydrolysis of the following nitriles to *amides* (use curly arrows).

-H⁺

H₂O, HN

tautomerizarion

H₂N
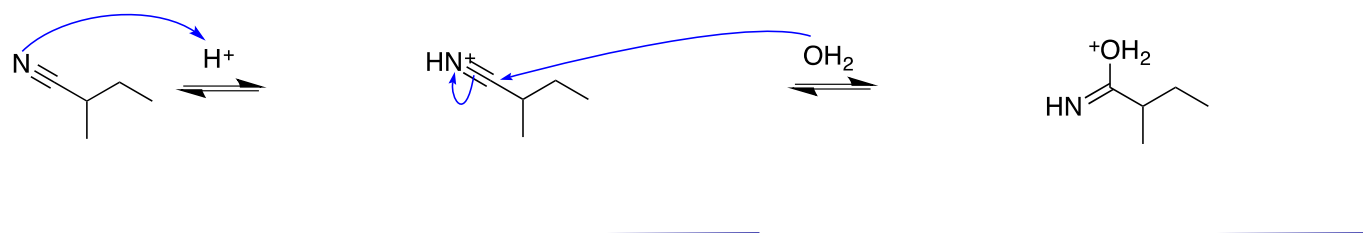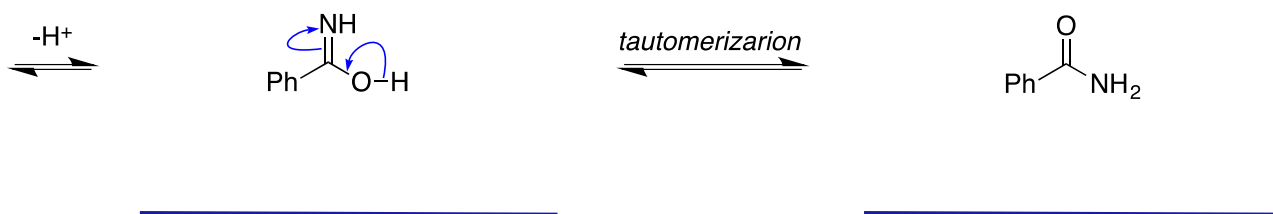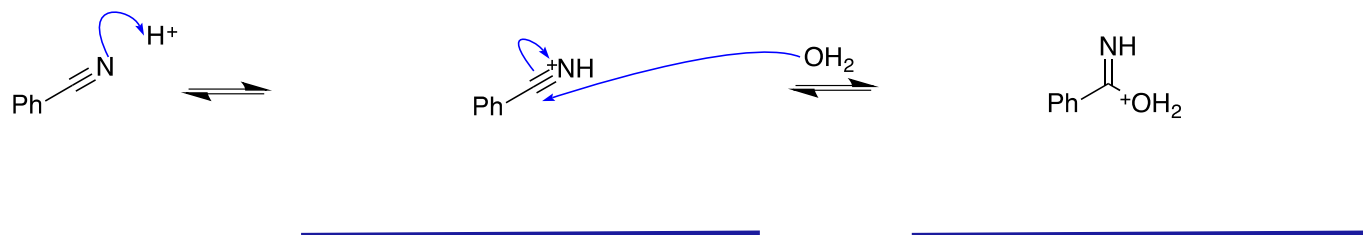
Draw the mechanism for acid-mediated hydrolysis of 3-chlorobenzonitrile *to the carboxylic acid* using clear curly arrows.

≡N

H⁺

N⁺—H, Ar, OH₂
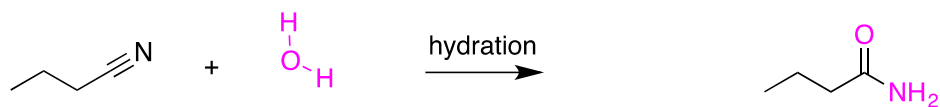
NH, Ar, ⁺OH₂

define, Ar =

Cl

-H⁺, +H⁺

⁺NH₂, Ar, OH, OH₂

H₂N, ⁺OH₂, Ar, OH

-H⁺, +H⁺

$$\xrightarrow[-NH_3]{}$$

$$\xrightarrow[-NH_4^+]{+NH_3}$$

carboxylic acid

$$\xrightarrow[-NH_4^+]{+NH_3}$$
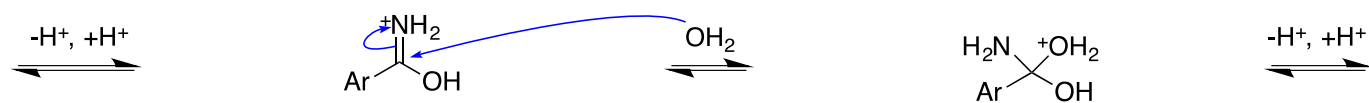
carboxylate

Complete the following cartoon:

$$\xrightarrow{hydration}$$

amide

$$+$$

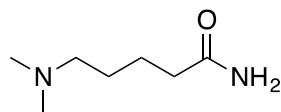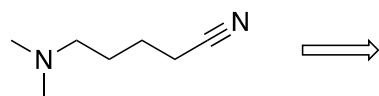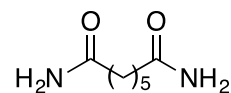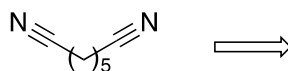$$\xrightarrow{hydration}$$

$$+ \ NH_2H$$
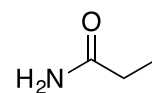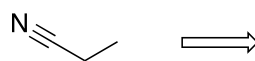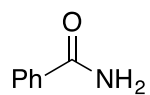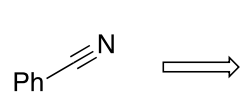
carboxylic acid

Dehydration Of Amides

Phosphorus pentoxide is a dehydrating agent because it can react with water to form phosphoric acid. Write a balanced equation for this.

$$P_2O_5 \quad + \quad 3\,H_2O \quad = \quad 2\,H_3PO_4$$

Dehydration of amides to nitriles involves loss of water to a _dehydrating reagent_. Predict the nitriles formed via the dehydration of the following amides.
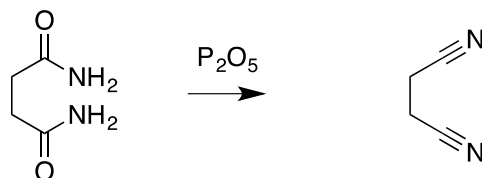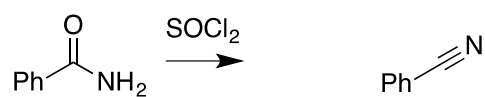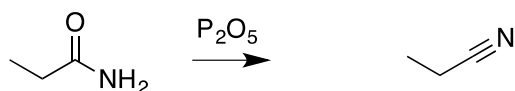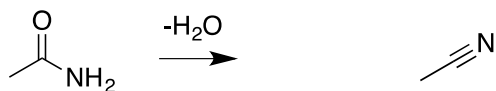
Indicate amides that could be used to make the following nitriles.

Ph—C≡N ⟹ Ph—C(=O)—NH₂

N≡C—CH₂—CH₃ ⟹ H₂N—C(=O)—CH₂—CH₃

(CH₃CH₂)₂CH—C≡N ⟹ (CH₃CH₂)₂CH—C(=O)—NH₂

N≡C—(CH₂)₅—C≡N ⟹ H₂N—C(=O)—(CH₂)₅—C(=O)—NH₂

(CH₃)(N)—(CH₂)₃—CH₂—C≡N ⟹ (CH₃)(N)—(CH₂)₃—CH₂—C(=O)—NH₂
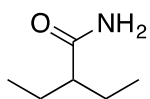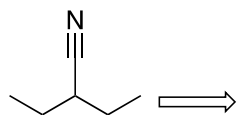
11 Reactivities Of Acylation Agents

from chapter(s) _____ in the recommended text

Introduction

Focus

This section covers concepts to rationalize why some acylation agents are more reactive than others, and the generally applicable mechanisms for acylation under basic (or neutral) and acidic conditions.

Reasons To Care

Acylation reactions are one of the most common transformations in biological chemistry (Figure 1). For instance, in protein synthesis tRNA carries activated amino acids to *acylate* growing chains of amino acids. Acetyl-CoA conveys acetyl groups to the citric acid cycle in metabolism, and to fats via fatty acid syntheses, via *acylation* reactions.
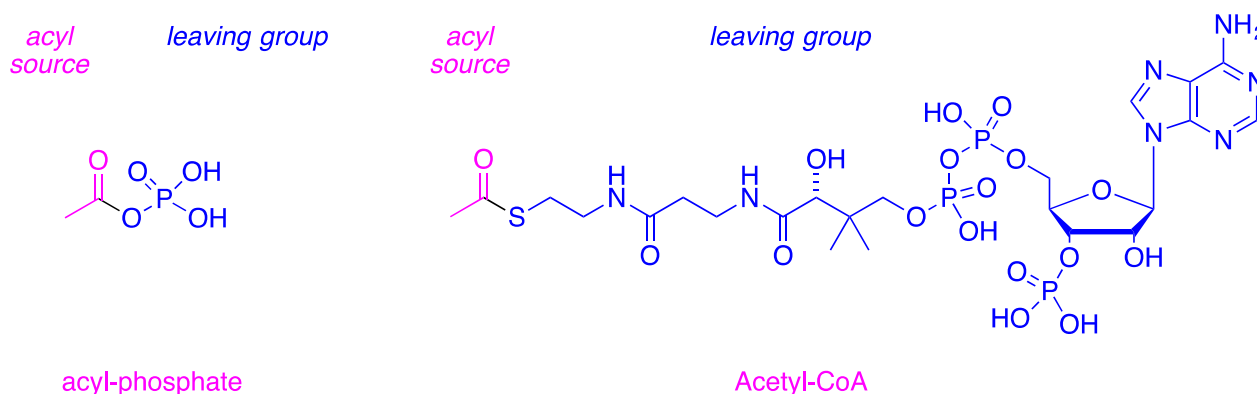
Figure 1. Two cellular acylating agents.

These "naughty secrets" make acylation reactions hard to understand:

- they can proceed under acidic, neutral, or basic conditions;

- mechanisms of acylations under acidic, and neutral or basic conditions tend to be different;

- acid halides, acid anhydrides, esters, amides, and *modified* carboxylic acids will all undergo acylation reactions, but their reactivities are wildly different;

- reactivities of nucleophiles in acylation reactions also differ; consequently,

- only some combinations of conditions, acylating agents, and nucleophiles lead to acylation products.

Concepts

electron flow • acids and bases • ester hydrolyses and transesterifications (both acylation processes)

Objective

Acylation reactions in the laboratory include syntheses of peptides and their derivatives. Biochemical acylations usually do not involve pH extremes but enzyme active sites often contain acidic and basic centers in enclosed environments. This part of the *Inquisition* outlines concepts that enable understanding reactivities in acylation reactions.

Acylation Reactions

Acylation reactions involve transfer of *RCO* groups. Only one of the following process is *not* an acylation reaction; circle that one.
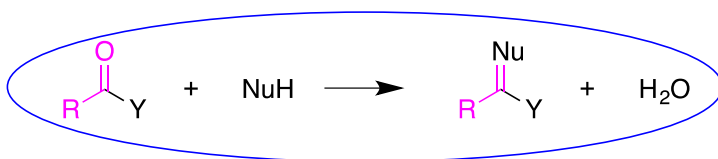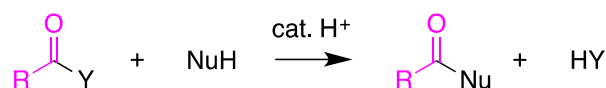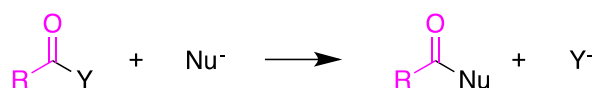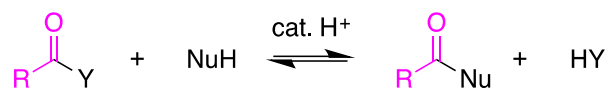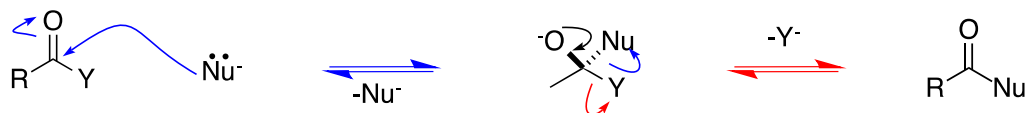

pH Dependence

Just as for reactions of aldehydes and ketones, acylation mechanisms usually depend on the acidity (pH) of the reaction mixture.

Acylations Under Basic Conditions

Good nucleophiles in acylation reactions are ones that *readily* receive acyl groups.

In the following acylation equilibrium, the leaving group Y is *displaced* by good nucleophiles Nu⁻.

Basicity of a nucleophile *does* give a rough guide to its nucleophilicity in acylation reactions.

Strong bases tend to be *good* nucleophiles (*eg OMe⁻*) whereas *weak* bases are mostly *bad* nucleophiles (*eg Cl⁻*).

examples of weak bases Y⁻:   Cl⁻, NO_3^-, HSO_4^-, H_2O, Br⁻ (add some more)

strong bases Nu⁻: Me_2N^-, HO⁻, HS⁻, ⁻OMe, CN⁻ (add some)

The group that is expelled from the pivotal *tetrahedral intermediate* (Y⁻ or Nu⁻ in the example above) tends to be the *best* able to support negative charge in the anionic form.

Nucleophilic substitution at acyl groups under *neutral* or under *basic* conditions usually involves addition of the nucleophile to a carbonyl group, then loss of a leaving group from the *tetrahedral* intermediate.

Show this mechanism in generic terms with curly arrows depicting the reaction flow for the acid chloride below.
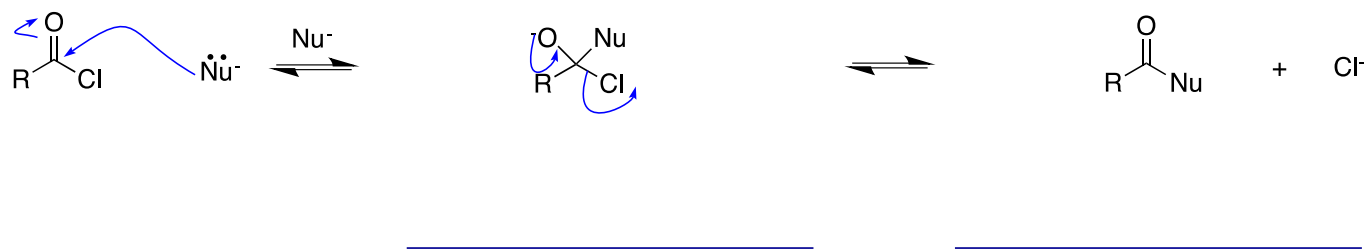

Chemistry in living organisms involves aqueous environments and complex mixtures of nucleophiles. Biological acylating agents have to be *moderately* reactive so that chemoselective reactions are possible in this environment.

In a chemistry lab, however, *more* reactive acylating agents like acid chlorides can be used.

Illustrate the pathway above for attack of OH⁻ on ethanoyl chloride (draw in arrows).

ethanoyl chloride   *tetrahedral intermediate*   *ethanoic acid*
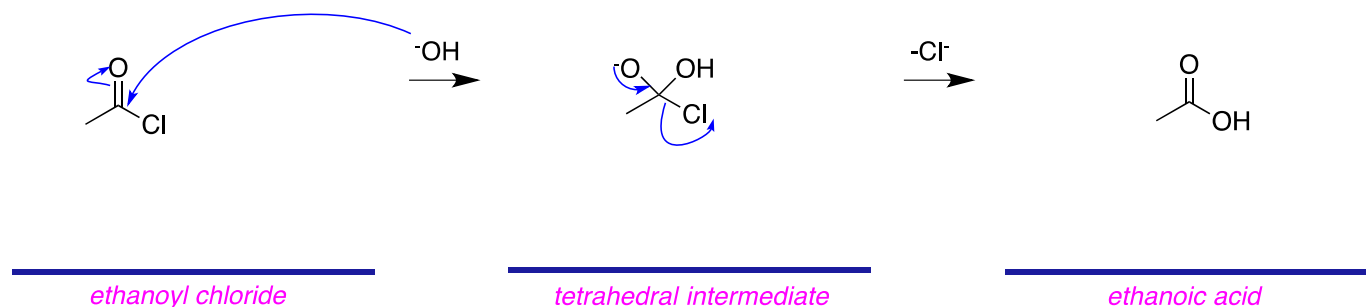
Illustrate this process for attack of methoxide on benzoyl chloride (draw in arrows).

benzoyl chloride   *tetrahedral intermediate*   *methyl benzoate*
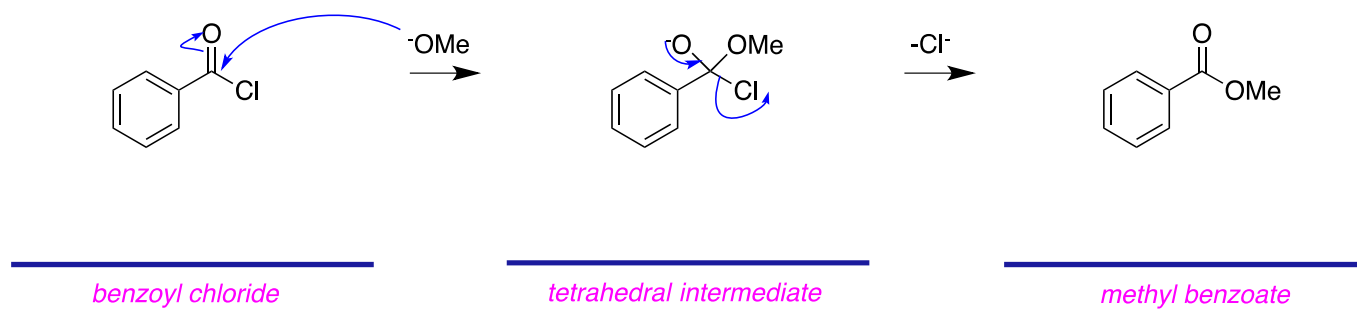
Acylations Under Acidic Conditions

Acidic conditions are used in nucleophilic acylations to increase the reactivity of the carbonyl group by protonation on the *C=O*, and/or to protonate a leaving group to enable it to depart more easily.

Protonation of the carbonyl group *increases* its reactivity towards nucleophiles. Show this for a generic, non-basic, anionic nucleophile, by completing the diagram below (show curly arrows).
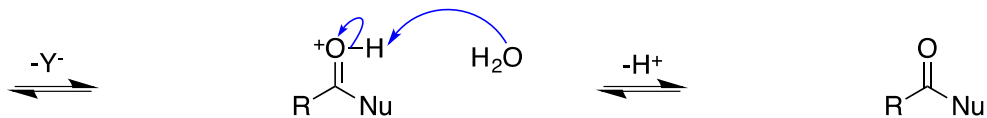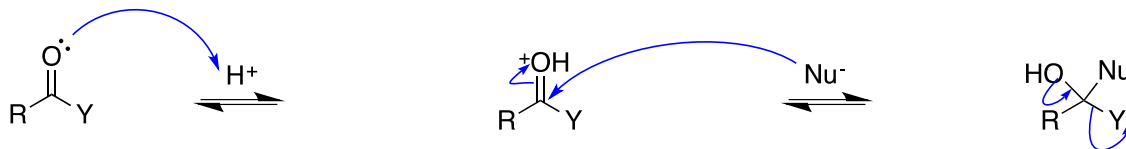
Reactivities Of Acylation Agents

Chemical Intuition

Acylating agents with relatively unstable leaving groups tend to be relatively *unreactive*.

Acyl fragments attached to good leaving groups tend to be *reactive* acylating agents.

Highly electronegative leaving groups *activate* carbonyl carbons atoms they are attached to and this tends to give *reactive* acylating agents.

Basic nucleophiles *cannot* be used under acidic conditions.

Deprotonation of an acylating agent renders it *less* reactive to nucleophiles.

Acids (protons and other Lewis acids) can complex carbonyl oxygen atoms making the associated carbonyl carbon *more* electrophilic.

Bulky leaving groups can sometimes *retard* additions of nucleophiles to acylation agents (*cf* base-mediated hydrolysis of *tert*-butyl esters is *unfavorable*).

Small nucleophiles are acylated *faster* than large ones, if all other factors are equal.

Molecular Orbital Description Of Acylation

Complete the following diagram by showing in part **a** the shapes of the carbonyl LUMO and the nucleophile HOMO, how they interact, and in **b** by filling in electrons into the bonding orbitals.

a

b

carbonyl LUMO   *transition state*   *nucleophile HOMO*
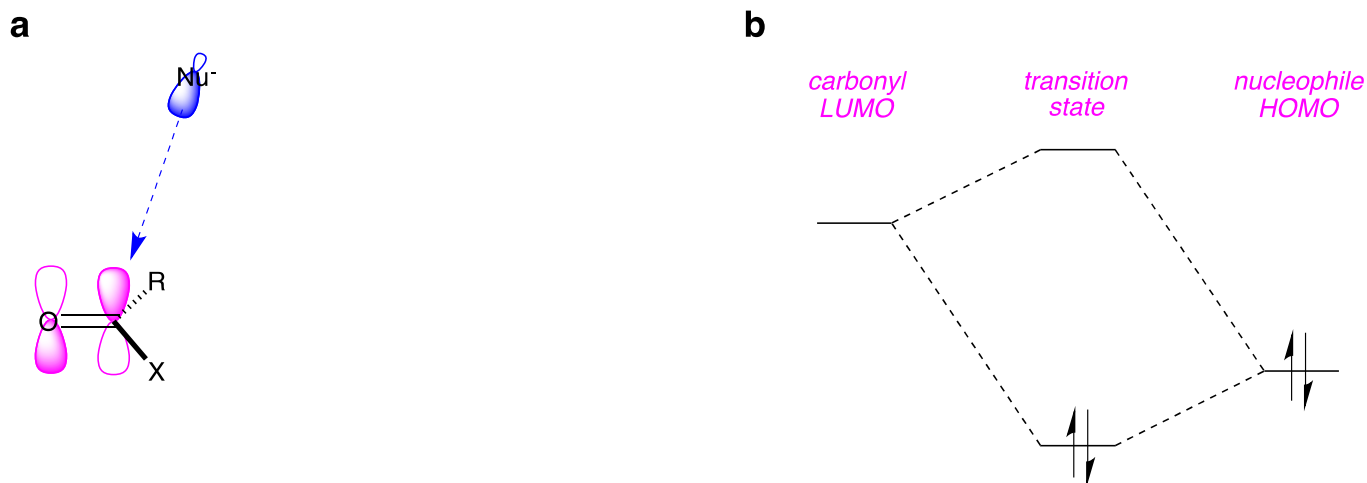
Factors that decrease the HOMO-LUMO gap *increase* the probability of combination.

Electron withdrawing groups (EWGs) attached to the carbonyl tend to *lower* its LUMO energy.

Acyl groups connected to good leaving groups are *reactive* to nucleophiles because they have relatively *low* energy LUMOs.

Good leaving groups tend to be *more* stable in the anionic form because they support negative charges well.

Delocalization of electrons into acyl substituents (as in carboxylates) *raises* the energy of the carbonyl LUMO and makes it *less* reactive to HOMOs of nucleophiles.

Reactive nucleophiles tend to have *high* energy HOMOs and this leads to *good* orbital overlap with the LUMO of the acylating agent.

Acid chlorides, bromides, and fluorides are excellent acylating agents because the halogens *lower* the LUMO of the carbonyl, and because halides are *excellent* leaving groups.

Relative Reactivities Of Functional Groups In Acylation Reactions

Carbonyl Halides (Acid Halides) Are Hot

Show the product in the following reaction.
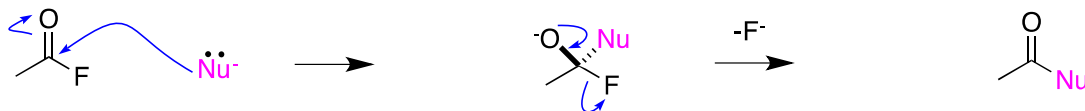
The description above shows the nucleophile is sufficiently reactive to add irreversibly under *basic* conditions.

Check you understand the parallel between reactions of aldehydes and ketones, and of acylating agents, under acidic and basic conditions.

Complete the reaction below by showing the product.
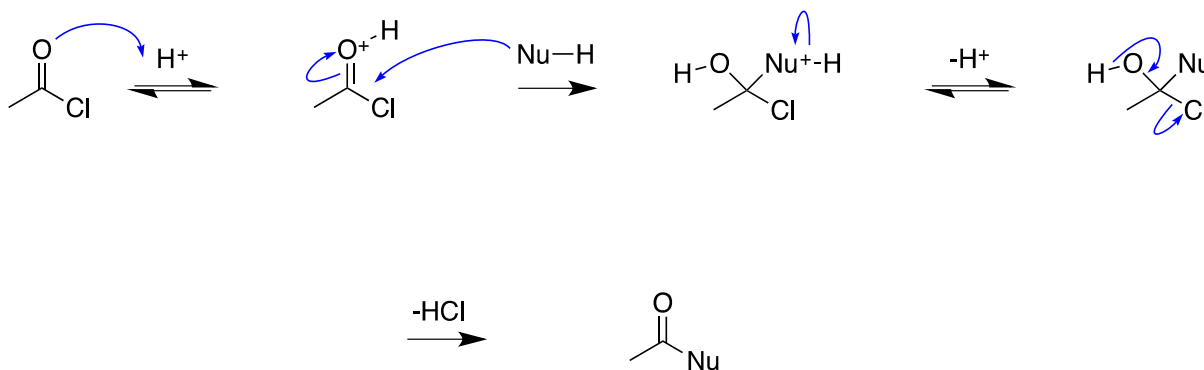
The mechanism above could only be applicable under *acid* conditions.

Carboxylic Acid Anhydrides Are Very Reactive

Carboxylic acid anhydrides are good acylating agents because carboxylates *lower* the LUMO of the carbonyl, and they are *excellent* leaving groups.

Complete the following reactions by showing the remaining curly arrows to depict electron flow, and the product.

under basic conditions …..
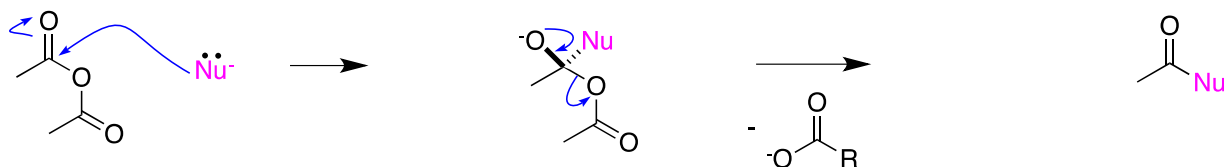
under acidic conditions …..
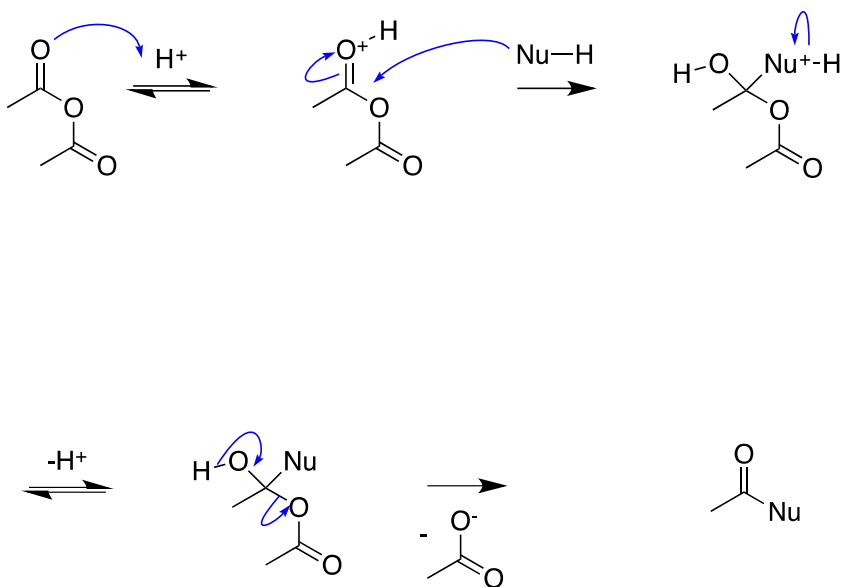
Carboxylic acid anhydrides in acylation reactions contain fragments from *2* carboxylic acid molecules.

In acylation reactions these fragments have different roles: one serves as *an electrophile*, while the other serves as a *carboxylate leaving group.*

Esters Are Not Very Reactive

Esters are relatively poor acylating agents because delocalization of electrons from alkoxide into the carbonyl *raises* the LUMO of the carbonyl; alkoxides are *inferior* leaving groups relative to halides and carboxylates.

Complete the following mechanisms showing omitted products and electron flow (throughout).

under basic conditions …..
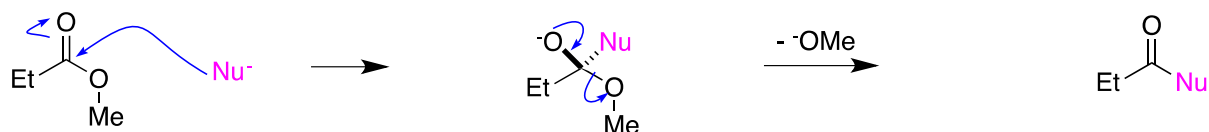
under acidic conditions …..
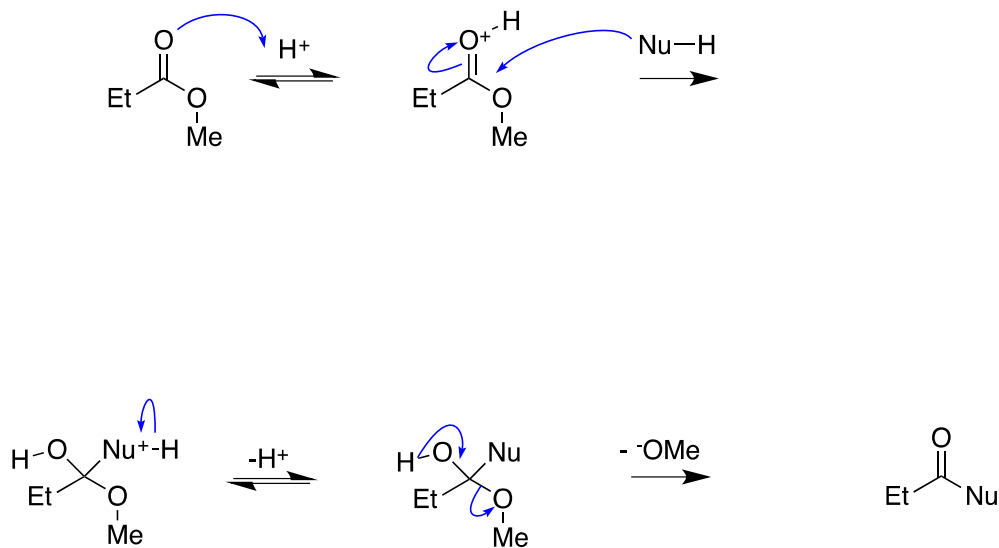
Typical nucleophiles for the reaction above are water, *ie ester hydrolysis*, and alcohols in *transesterification* reactions, but there are not so many others.

Amines *do not* tend to react with esters in this way.

Thioesters, Gentle Chemoselective Acylating Agents

Thioesters are *better* acylating agents than esters because sulfur donates *less* electrons into the carbonyl group than oxygen in these compounds.

Complete the following reactions.

under basic conditions .....

tetrahedral intermediate
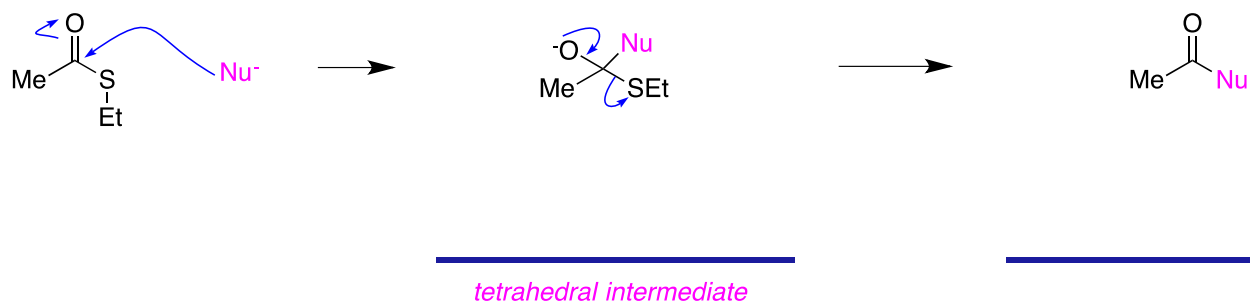
under acidic conditions .....
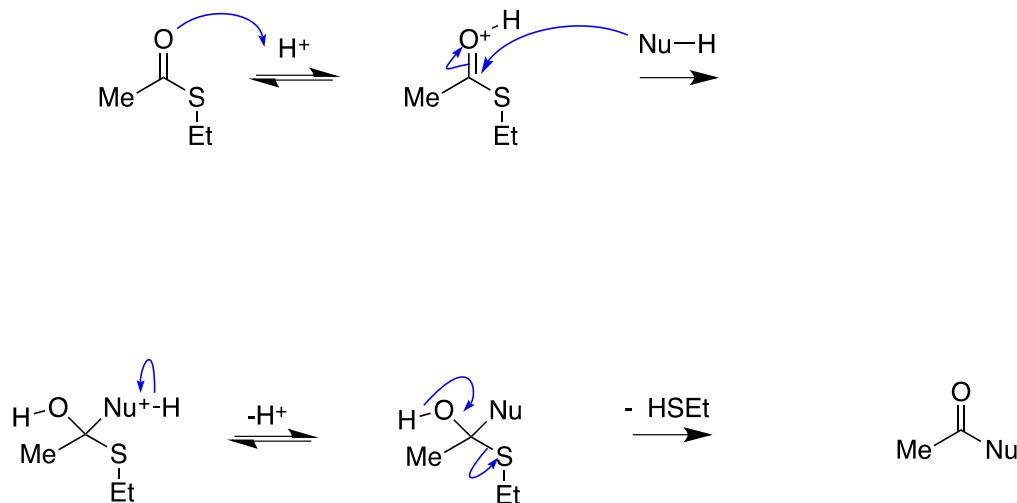
Amines *do* tend to react with thioesters via the acid catalyzed mechanism above.

Amides, Poor Acylating Agents

Amides are *worse* acylating agents than esters because amide anions (NR$_2^-$) are *poor* leaving groups.

The *N*-atom of an amide donates *more* electrons into the carbonyl group than oxygen of an ester.

Complete the following reactions.

under basic conditions …..

tetrahedral intermediate
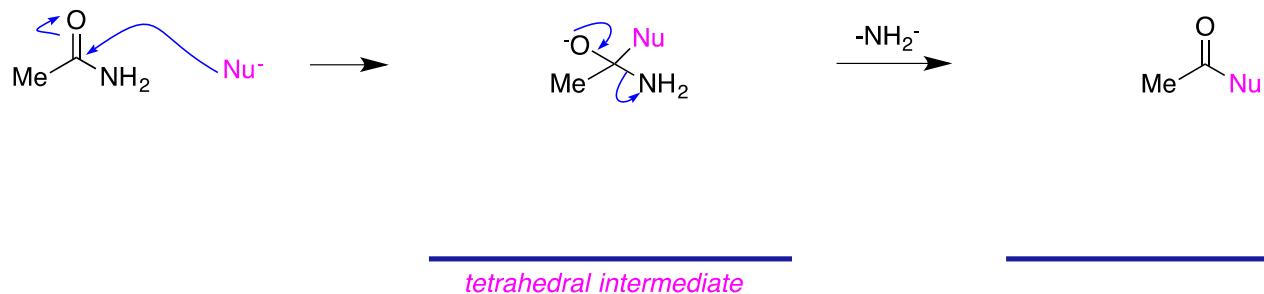
under acidic conditions …..
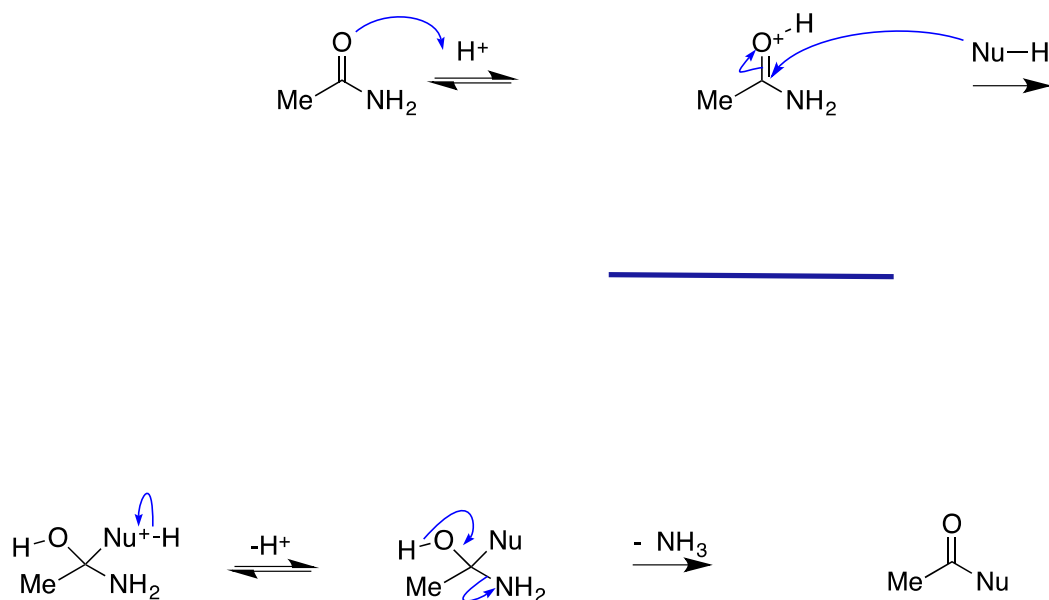
Carboxylic Acids Are Not Acylating Agents

Hydroxyl groups of carboxylic acids are poor leaving groups because:

(i)   carboxylic acids (pKa = *3 – 5*) tend to exist as carboxylate forms that repel nucleophilic attack; and,

(ii)   if a carboxylate was not deprotonated and a nucleophile did add to the carbonyl, then HO$^-$ is not a particularly good leaving group.

Completing the following graphic that summarizes the potential of unmodified carboxylic acids as acylating agents.
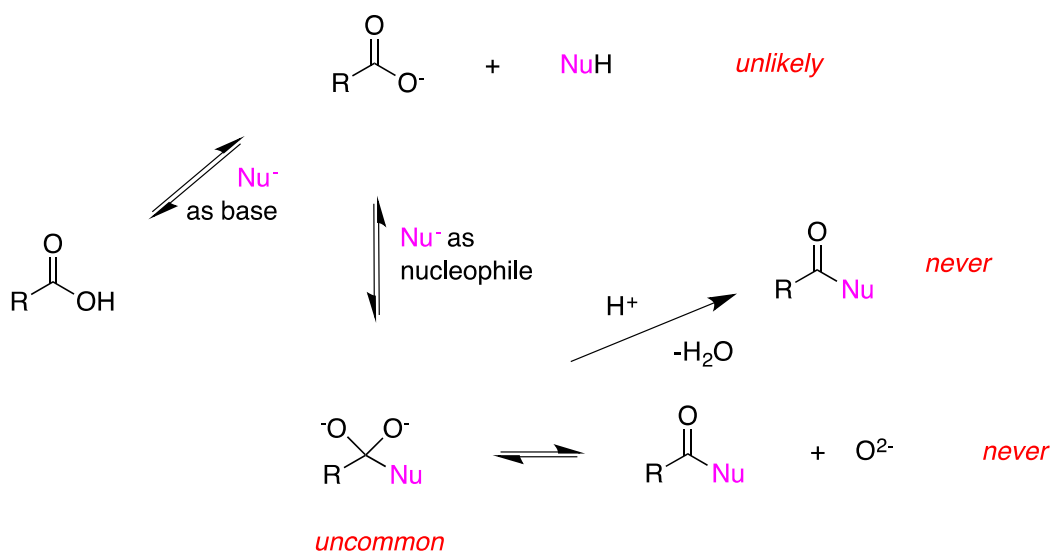
Peroxide dianion O^{2-} is *extremely* basic and a very *poor* leaving group.

Synopsis

Consider the compounds below and indicate if they are "excellent" (**E**), "sometimes suitable" (**SS**), or "inappropriate" (**I**) as acylating agents.

R—C(=O)—Br   Et—C(=O)—NH$_2$ ^{i}Pr—C(=O)—NH$_2$ (iPr)—C(=O)—Cl   (acetic anhydride)   —C(=O)—OH

E   *I*   *I*   *E*   *E*   *I*

R—C(=O)—Me ⬭   Et—C(=O)—OMe ^{i}Pr—C(=O)—H ⬭   —C(=O)—CN   —C(=O)—O—Et   —C(=O)—OPh

I   *SS*   *I*   *SS*   *SS*   *SS*
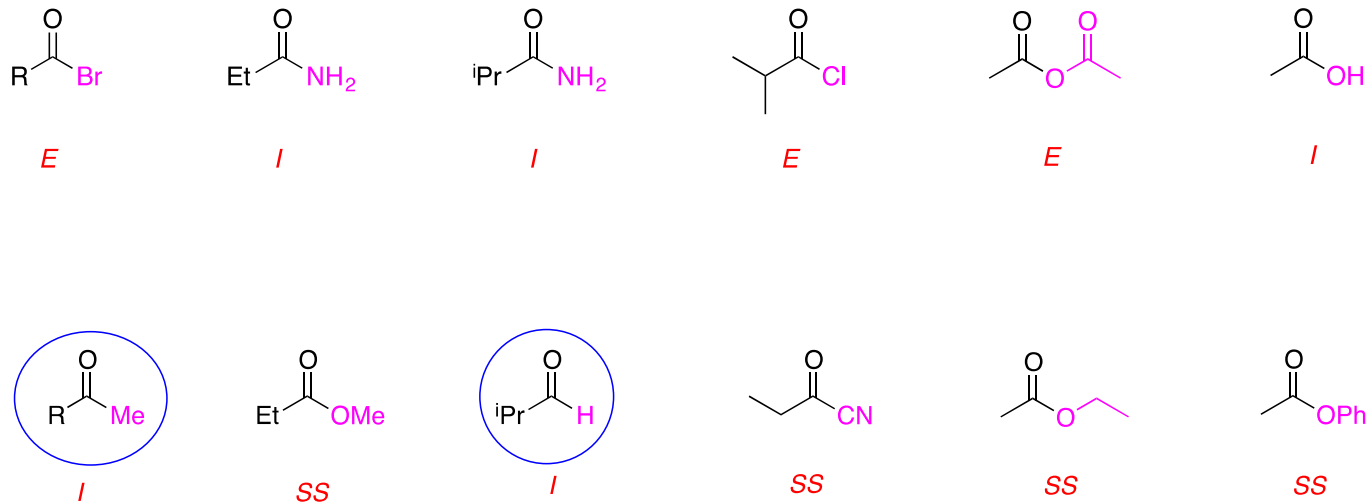
Compounds without a leaving group on the carbonyl *cannot* undergo acylation reactions. Circle two of the compounds above that fit that description.

Rank the following groups in terms of their ability to be displaced.

R—C(=O)—X   X =   OAc   OMe   O$^-$ NH$_2$ Cl   OPh
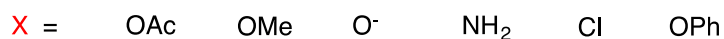
Cl	OAc	OPh	OMe	NH$_2$	O$^-$
most reactive					*least reactive*

Rank the following nucleophiles in terms of their ability to be acylated.

Nu =   (acetate anion) $^-$Me $^-$OH $^-$NH$_2$ $^-$Cl   NH$_3$ OH$_2$

| $^-$Me | $^-$NH$_2$ | $^-$OH | (acetate anion) | $^-$Cl | NH$_3$ | OH$_2$ |

most reactive   *least reactive*
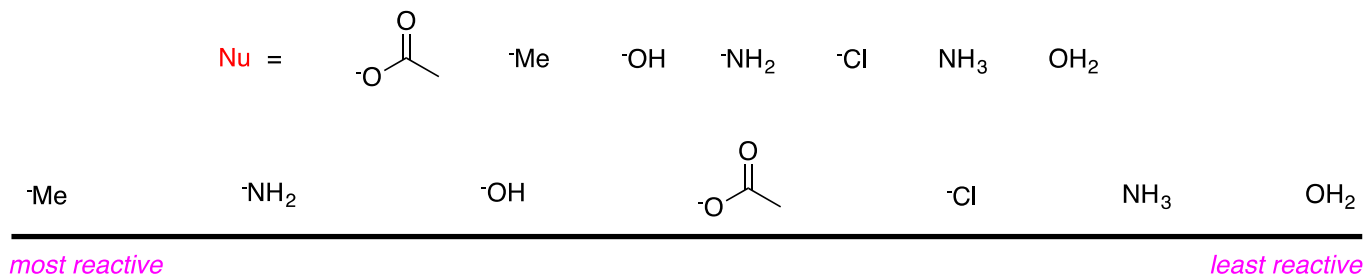
Rank the following in terms of reactivities to nucleophiles by placing a 1 under the most reactive *etc*.. If two compounds that have functional groups of the same type, use other concepts to reason which of these is the most reactive.

4

1

3

5

6

2
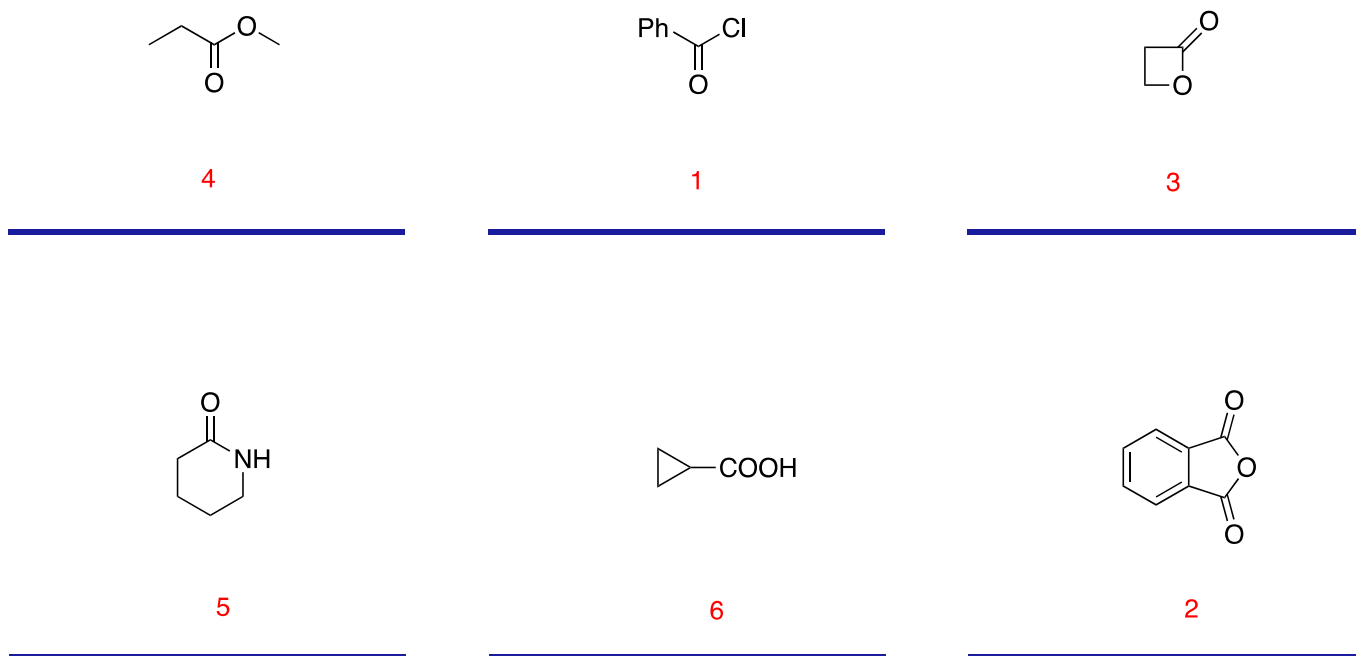
12 Acylations Via Acid Chlorides And Anhydrides

from chapter(s) _____ in the recommended text

Introduction

Reactions Of Acyl Halides

Under Basic Conditions

stable anion

many nucleophiles

essentially *irreversible*
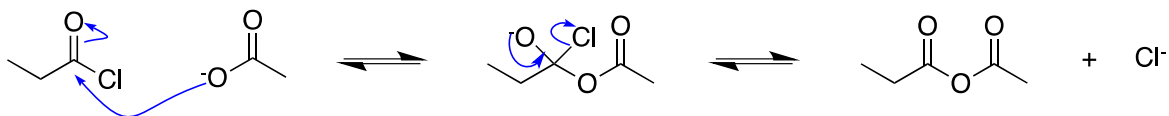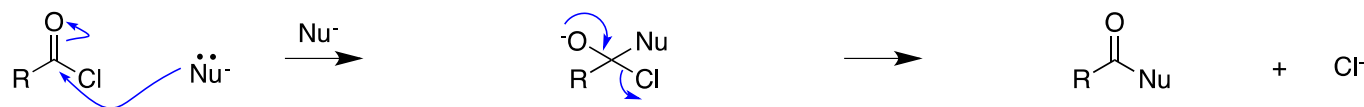
Under Acidic Conditions
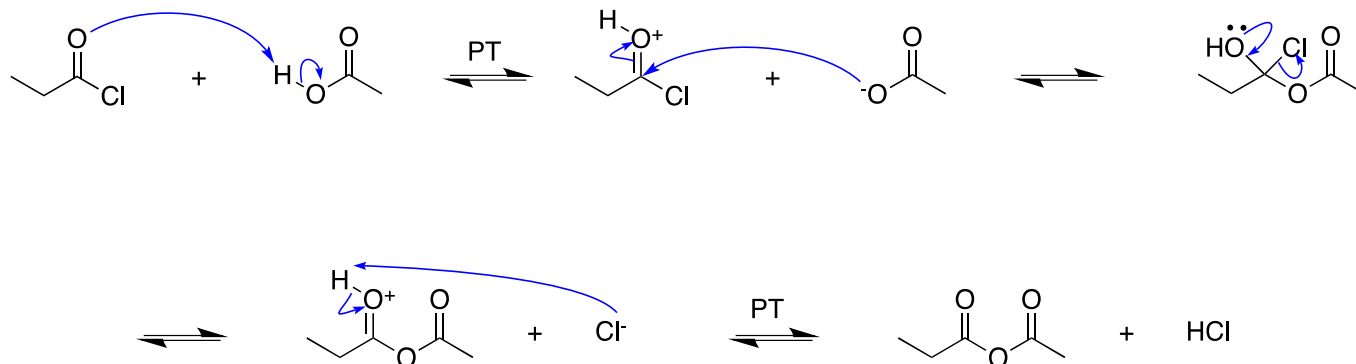
Syntheses Of Anhydrides Via Acylation Of Carboxylates
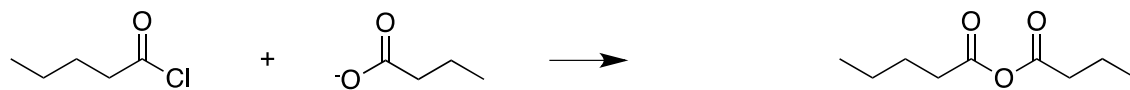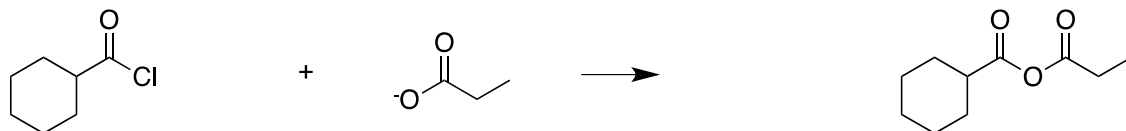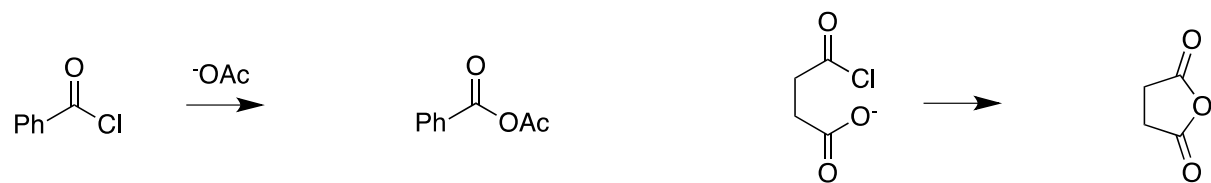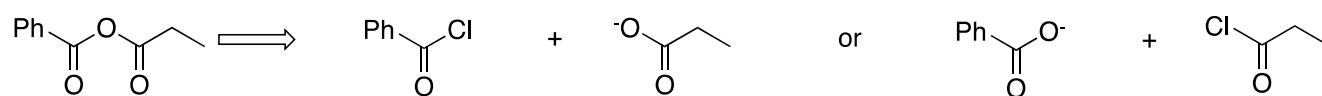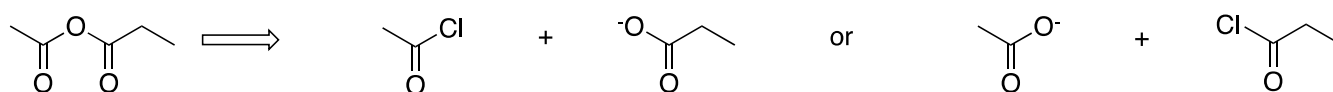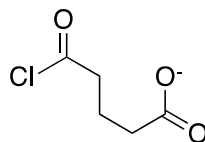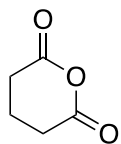
Hydrolysis Of Acid Chlorides To Form Carboxylic Acids

under basic conditions the nucleophile is *hydroxide*

under acidic conditions it is *water*

acylation of water

acidic conditions
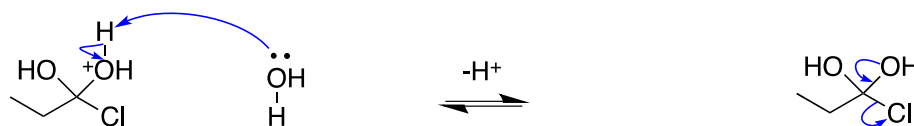
Acylation Of Alcohols To Form Esters

EtCO$_2$Bn $\Longrightarrow$ EtCOCl   +   BnOH

   $\Longrightarrow$ +
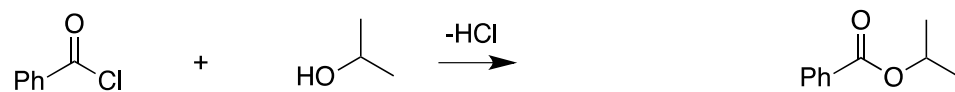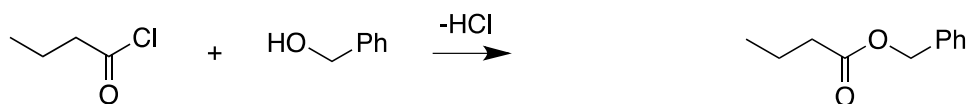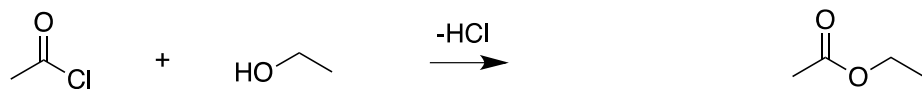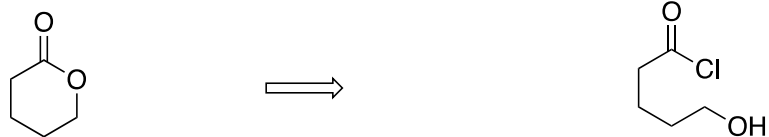
Acylation Of Amines To Give Amides
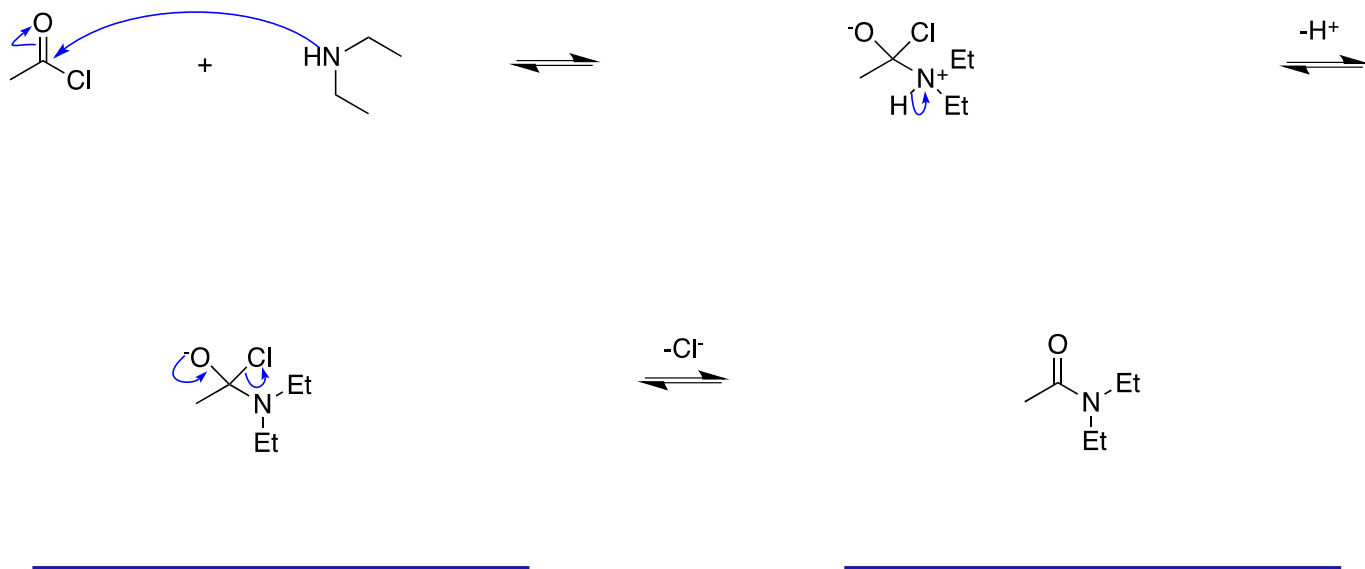
before chloride loss

shows it *after*
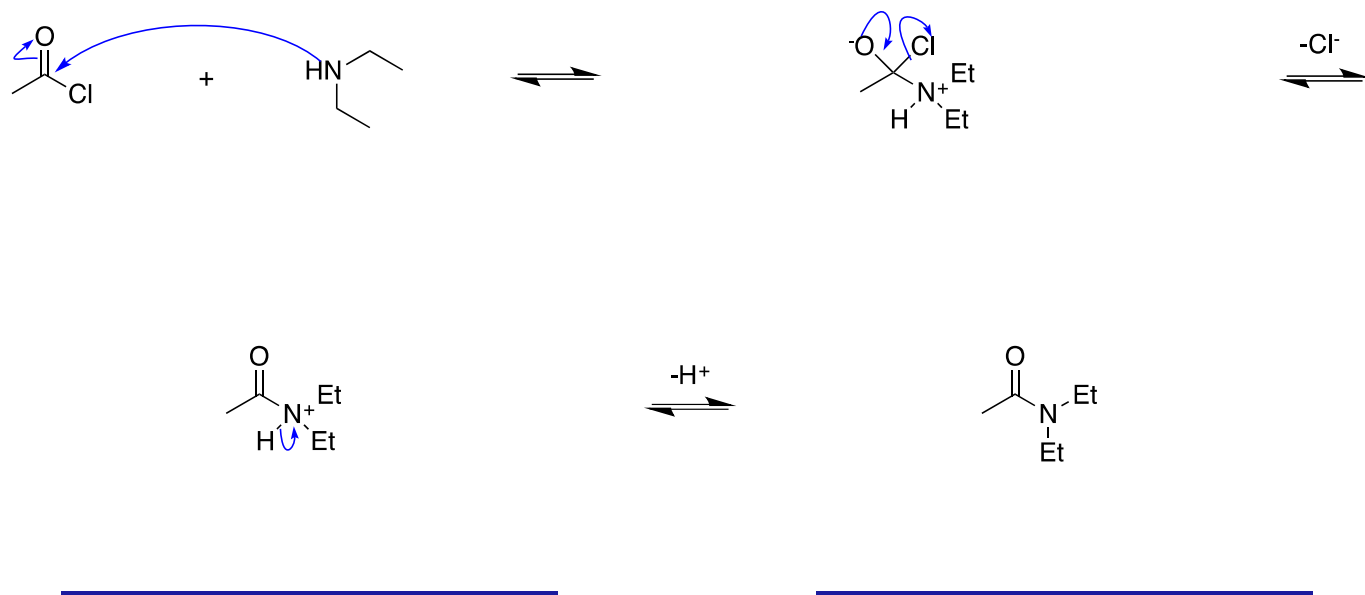
unstable products
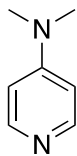
DMAP
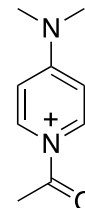
N-acetyl DMAP
good acylating agent for other nucleophiles

HNMe$_2$

NMe$_3$
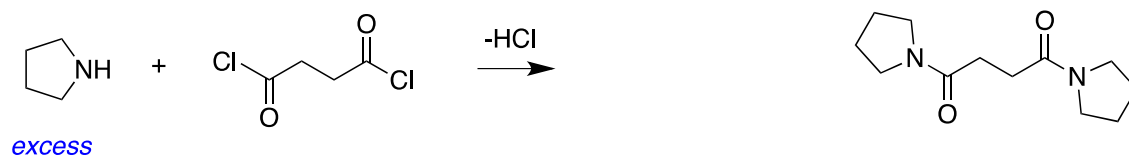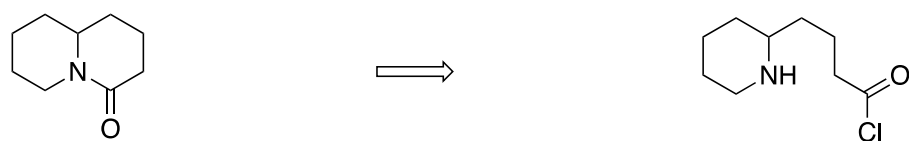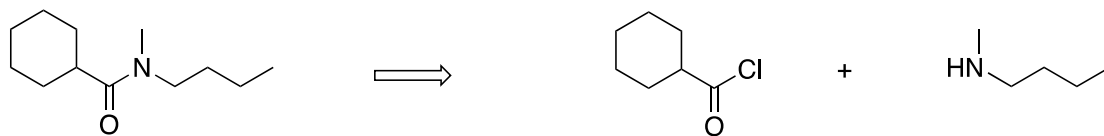
+
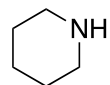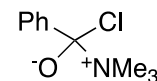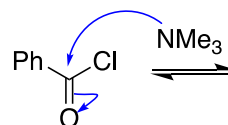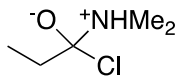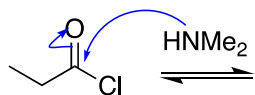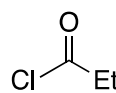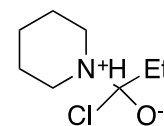
which amine?
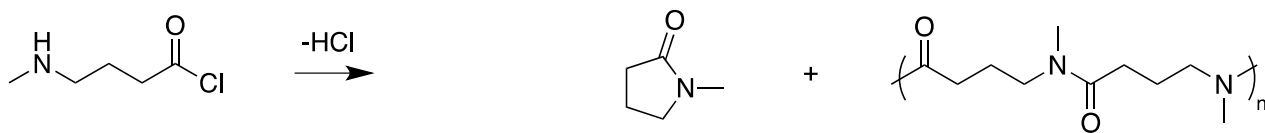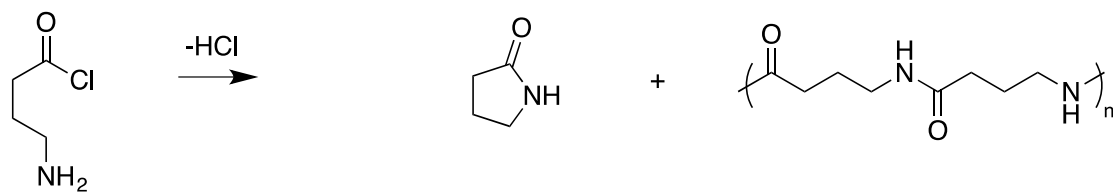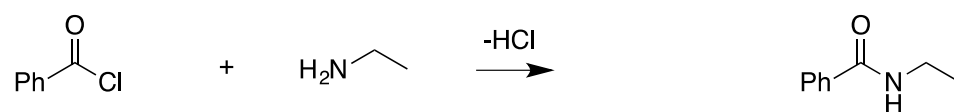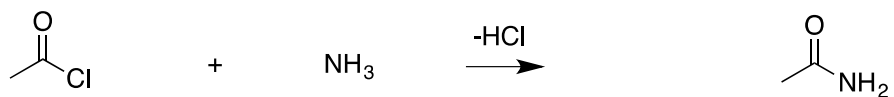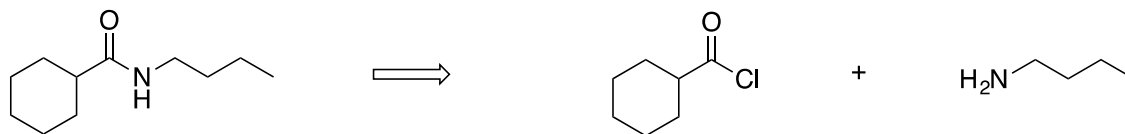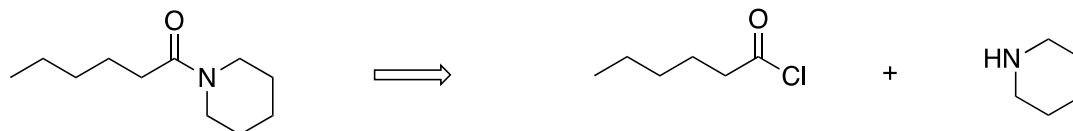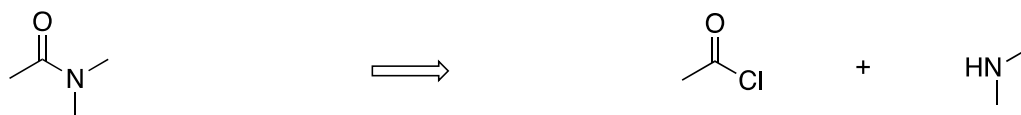
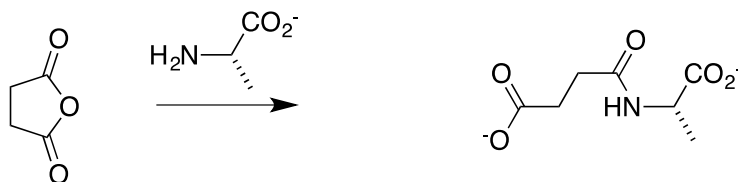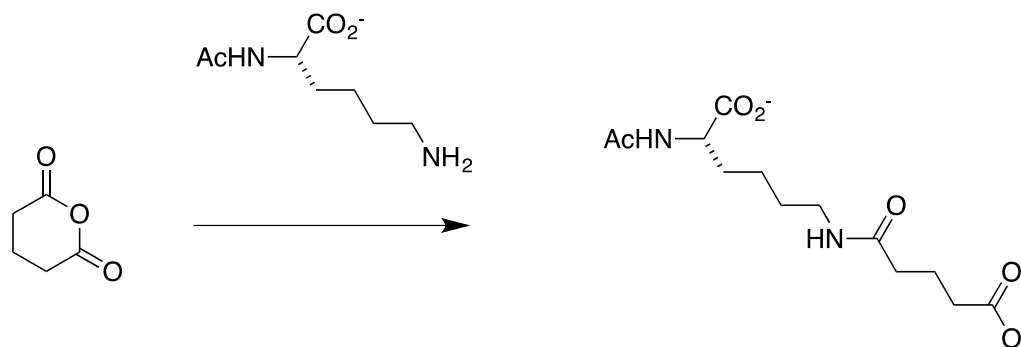alcohols to give *esters*

water to give *acids*

ammonia to give *amides*

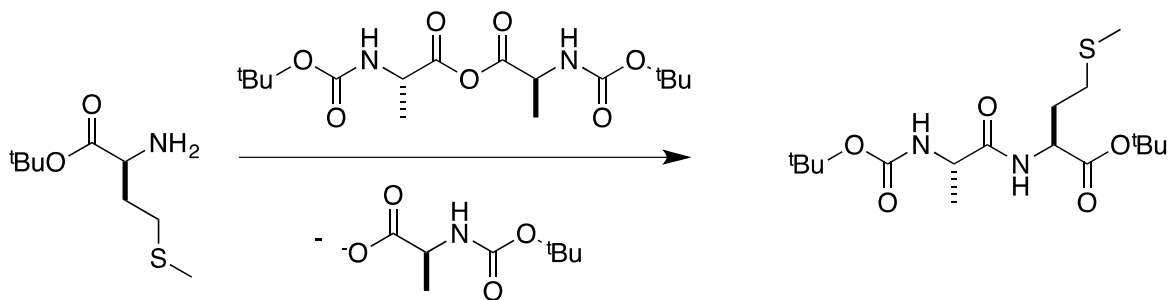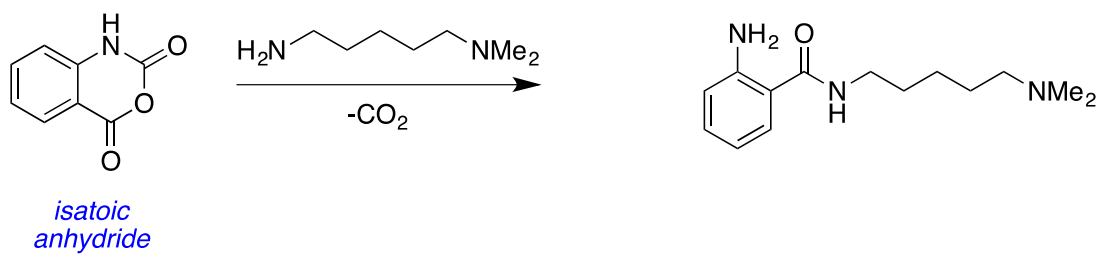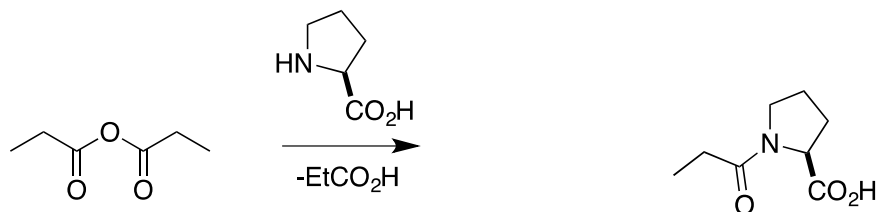## Acylation Reactions Of Carboxylic Acid Anhydrides

slightly less

be *the same*

an electrophile

would be *wasteful* because half ends up as carboxylate

isatoic anhydride

13 Activation Of Carboxylic Acids

from chapter(s) _____ in the recommended text

Introduction

Focus

Carboxylic acids do not directly react with amines and several other important nucleophiles; they often must be converted to something that is more reactive first. Chemists often will refer to this process as "activation of a carboxylic acid". Consequently, *in vivo* and in the laboratory, carboxylic acids (or functional groups that are easily converted to carboxylic acids, *eg* esters) lie dormant in a chemical environment until they are activated *when needed*.
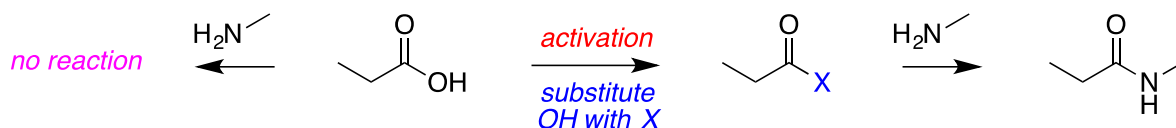
Approaches to activation of carboxylic acids are the focus of this section.

Reasons To Care

Carboxylic acids are ubiquitous in living systems and they are common starting materials for laboratory syntheses. Activation of carboxylic acids is required for many processes including ribosomal and syntheses of peptides and proteins, and construction of fatty acids and polyketide natural products *in vivo.*

Concepts Involved

substitution at acyl groups • symmetrical, unsymmetrical, and mixed anhydrides

Objective

To gain an appreciation of some of the ways by which carboxylic acids can be made into more reactive functional groups to facilitate acylation reactions.

Reactivity

Unmodified carboxylic acids are extremely *poor* acylating agents because:

(i)   hydroxide is a *mediocre* leaving group; and,

(ii) if the medium is neutral or basic then the acid exists *as a carboxylate*.

It is for these reasons that carboxylic acids are frequently converted to acid halides or acid anhydrides; both these *are* reactive to nucleophiles.
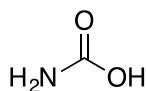
Common Carboxylic Acids Derivatives

Many of the compounds shown named below are useful for understanding this section, and all are common chemicals.
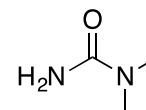
Use Wikipedia to help you draw the following carboxylic acid derivatives. Indicate if they are closely related to esters (**A**), carboxylic acids (**B**), amides (**C**), acid chlorides (**D**), or carbon dioxide (**E**).
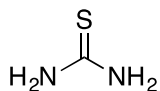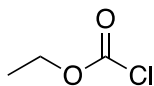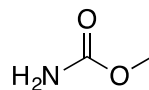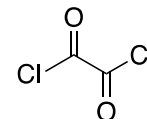
thiourea
C
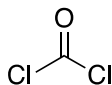
carbamic acid
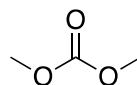**B**

N,N'-dimethylurea
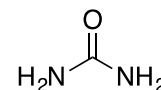**C**

ethyl chlorocarbonate
D

methyl carbamate
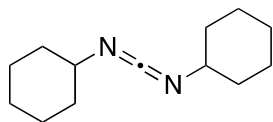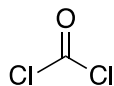**C**

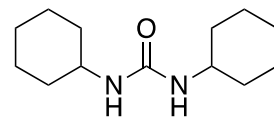*oxalyl chloride*
D

phosgene
D

dimethyl carbonate
A

urea
C

dicyclohexylcarbodiimide (DCC)
**E**

thiophosgene
C

N,N'-dicyclohexylurea
C

Activation Of Carboxylic Acids By Conversion To Acid Chlorides

Thionyl Halides: Excellent Dehydrating Agents

Thionyl chloride $SOCl_2$, and thionyl bromide (formula: $SOBr_2$), are excellent dehydrating reagents because they form only gaseous products when they react with water. Predict what those gaseous products are in the following reactions (balance the equations).

SO_2 + 2HCl

SO_2 + 2HBr
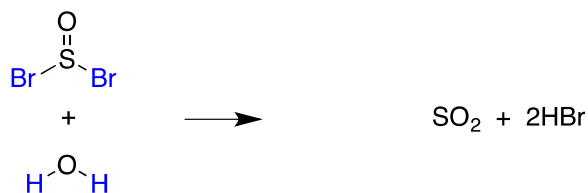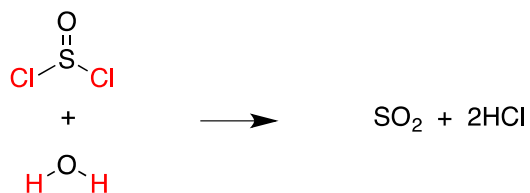
Thionyl Halide Plus Carboxylic Acid Gives Acid Halide, SO_2, And HX

Carboxylic acids *can* be reacted with thionyl chloride or -bromide giving *acid halides*.

Identify the acid halides formed from carboxylic acids and the following thionyl halides.
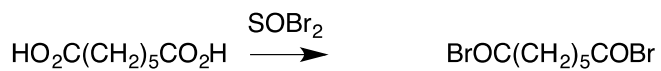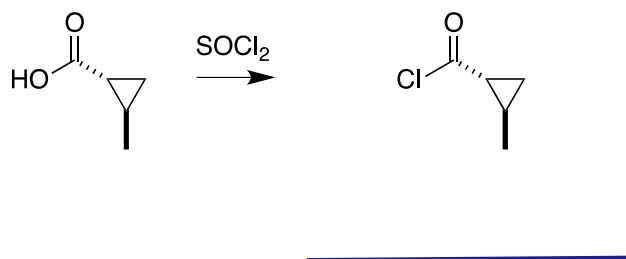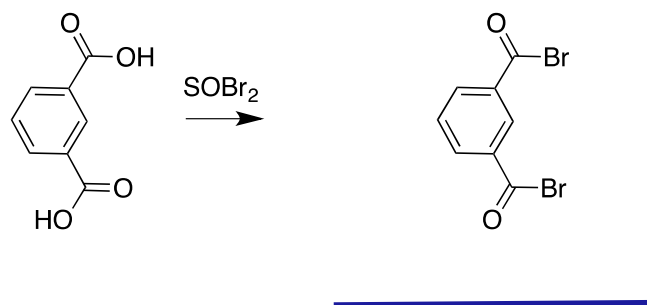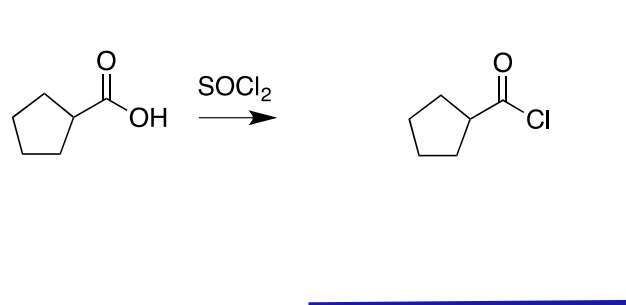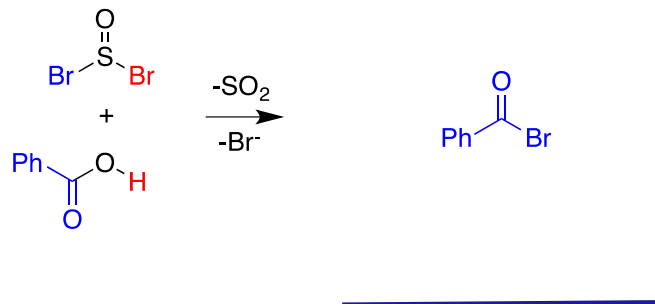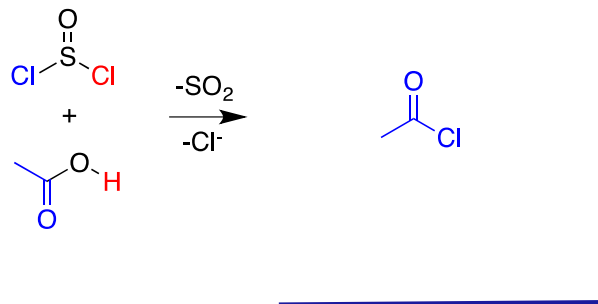
A kind of anhydride intermediate is formed in these reactions when thionyl halides react with carboxylic acids. Predict the structures of those intermediates for the following reactions.
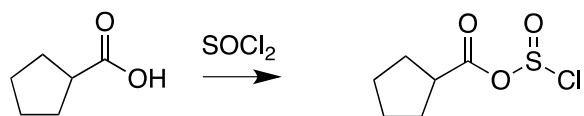
*sulfonyl anhydride
intermediate*
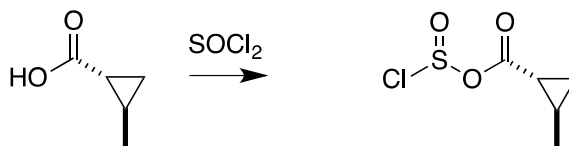
*sulfonyl anhydride
intermediate*

Intermediates in the reactions above are sulfonyl anhydrides. Draw the carboxylic acids that the following sulfonyl anhydrides can be formed from.
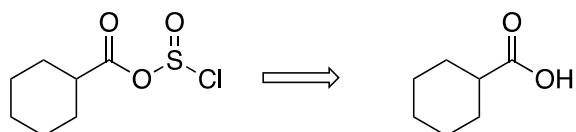
Draw the mechanism for conversion of 3-methylbenzoic acid into the corresponding benzoyl bromide with thionyl bromide.

carboxylate

*sulfonyl anhydride
intermediate*

tetrahedral intermediate

carboxylic acid bromide
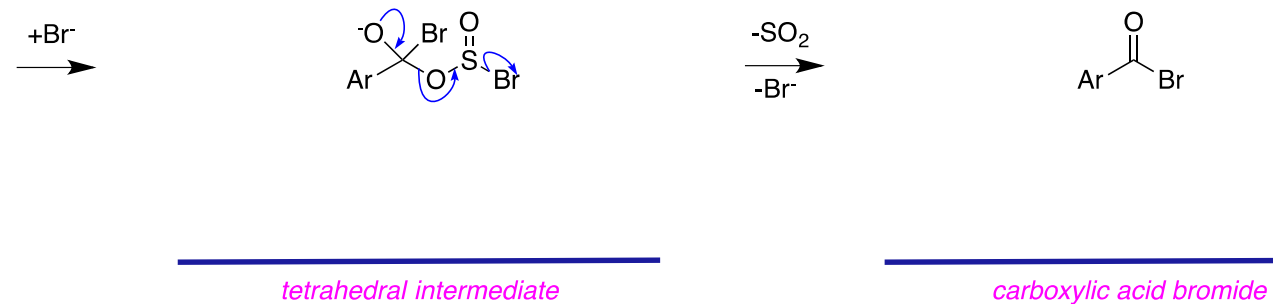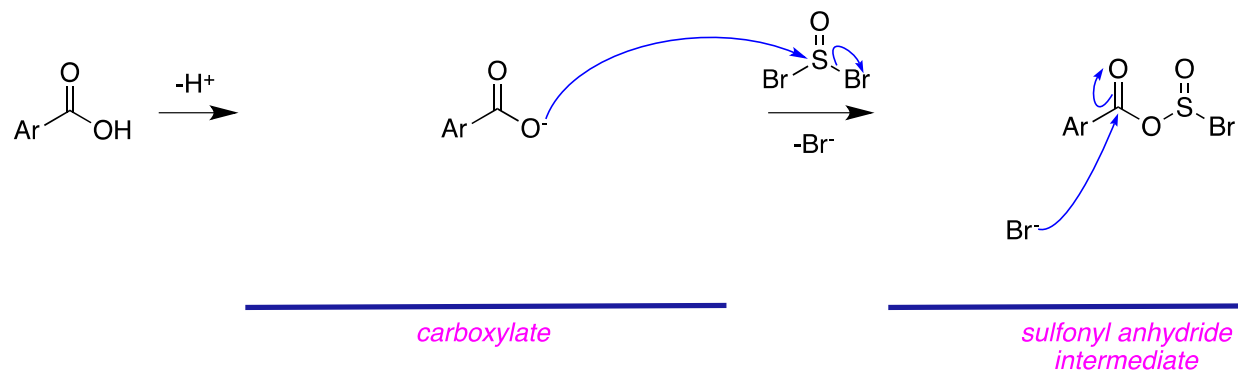
Activation By Forming Anhydrides

Symmetrical Anhydrides

Some dehydrating agents can be used to abstract one molecule of water from *2* molecule(s) of carboxylic acid to give *1* molecule(s) of a symmetrical anhydride.

Without worrying about the phosphorus-containing by-products, show the symmetrical carboxylic acid anhydrides formed in the following reactions.
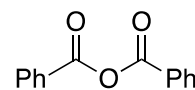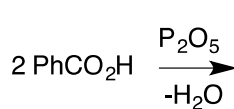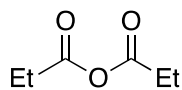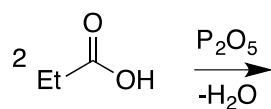
Symmetrical anhydrides can be formed by dehydrating carboxylic acids, but *unsymmetrical* ones cannot be isolated in pure form via this approach.

Show the organic products of the following reactions.
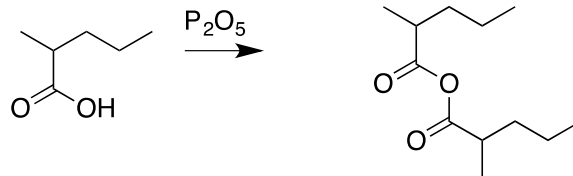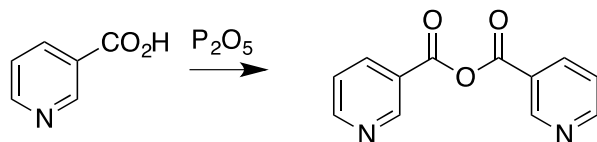
Draw acetic anhydride (which is *symmetrical*) and the *unsymmetrical* anhydride from acetic acid and propionic acid.
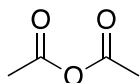
acetic anhydride
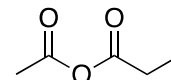
anhydride from ethanoic and propionic acids

Unsymmetrical And Mixed Anhydrides

Symmetrical anhydrides are made from *2* molecule(s) of the same carboxylic acid, but anhydrides from two different carboxylic acid molecules are called *unsymmetrical*.

Mixed anhydrides are formed from a carboxylic acid and *another type of acid.*

Unmodified carboxylic acids *are not* good acylating agents; however, they can be modified in one step, *in situ*, to give anhydride derivatives that *can* be used for effective acylation.

Draw an arrow pointing towards the most reactive carbonyl in each of the following anhydrides, circle any that are unsymmetrical, and put a box around those that are mixed.

Most unsymmetrical anhydrides could react with nucleophiles at either carbon and the outcome of those two reactions would be *different*, but reactions of mixed anhydrides tend to occur with more selectivity.
Some unsymmetrical anhydride *derivatives* enable every molecule of the starting carboxylic acid to be used as acylating agent, and the other, hopefully less interesting, side of the anhydride-like compound to be displaced.

Formation Of Unsymmetrical Anhydride Derivatives Using Carbodiimides

Even though carboxylic acids are not acylating agents, they can be changed into acylating agents simply by mixing with activating agents, of which carbodiimides are the common.

Ultimately, carbodiimides take an oxygen from the carboxylic acid to form a *urea*.

Complete the following mechanism outlines that activation of ethanoic acid with dicyclohexylcarbodiimide (DCC) then reaction with methylamine to give an amide. Complete the mechanism showing curly arrows to depict electron flow.

combination

tetrahedral intermediate
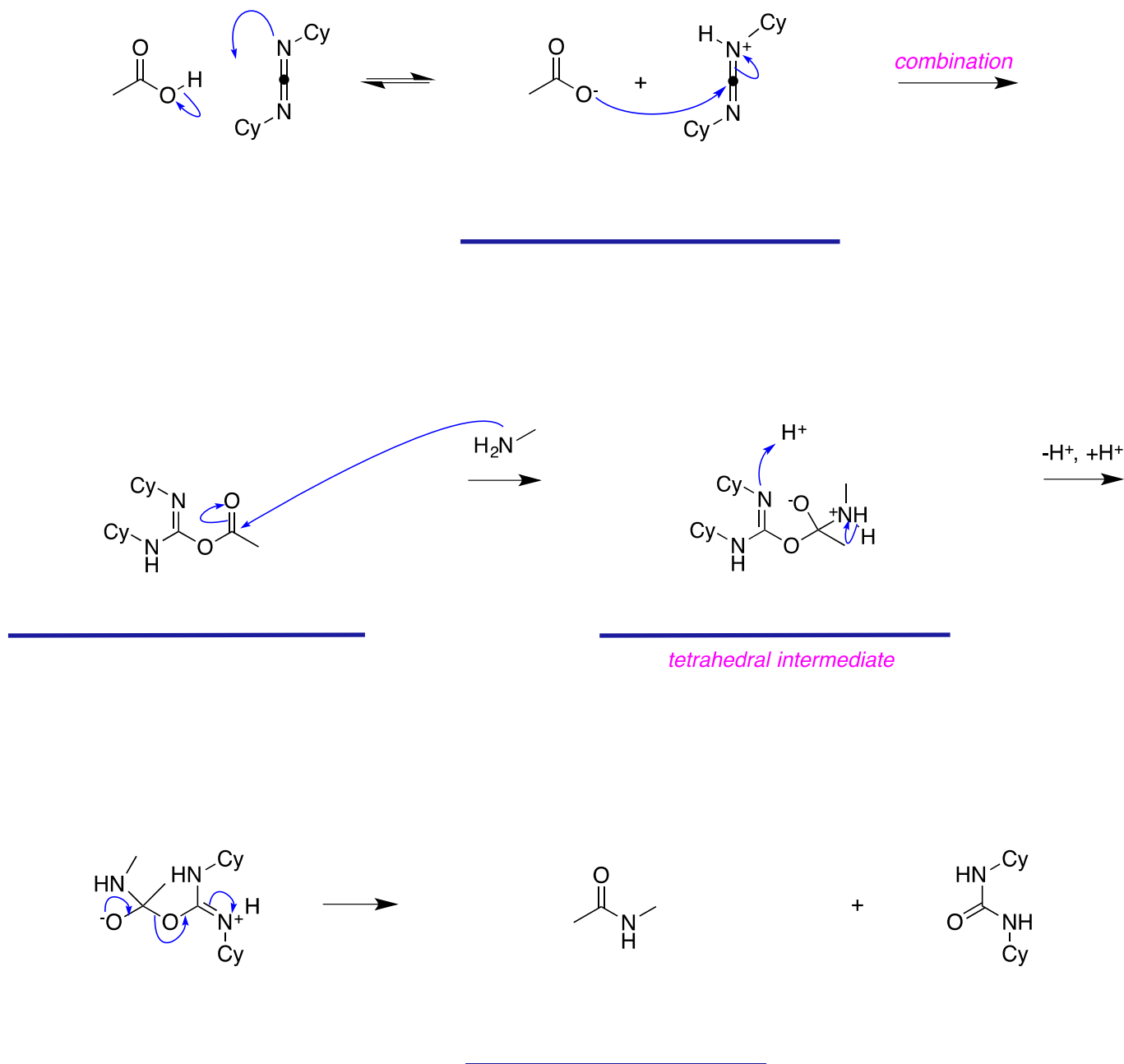
This reaction is frequently used in coupling amino acids together in syntheses of *peptides*, and many other amide syntheses.

Predict the products of the following reactions.

BOC
tert-ButylOxyCarbonyl

(i) DCC

(ii) HN

(i) DCC

(ii) H₂N—OtBu

CBZ
CarboxyBenZyl

(i) DCC

(ii) H₂NMe

(i) DCC

(ii) H₂N—OtBu
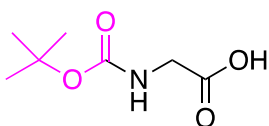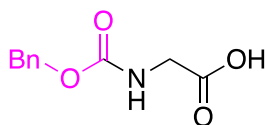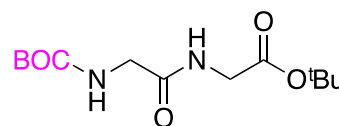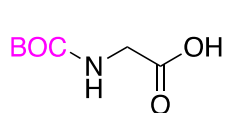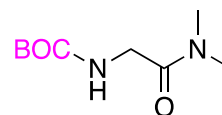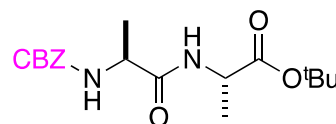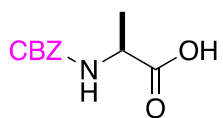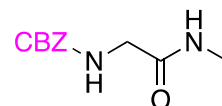
Activation Of *Phosphate* Acids In Cells Via Phosphate Anhydrides

Formation Of Mixed Anhydrides Of Phosphorus Acids

Intracellular acylation reactions can proceed via *mixed* anhydrides like acyl phosphates, derived from phosphoric acids. Complete the following diagrams.
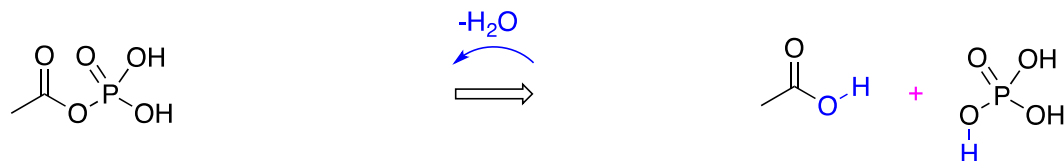
acyl phosphate

Blue coloring in the diagram above implies water attacks the *carbonyl* of the acyl phosphate; this is a reasonable hypothesis because phosphate is a *better* leaving group than acetate.

carboxylic and phosphoric acids

Hydroxyl groups in acylphosphates are acidic, so they are *protonated* in most environments.

Acyl adenylate is the mixed anhydride of acetic acid and adenosine monophosphate (AMP). Draw this compound.
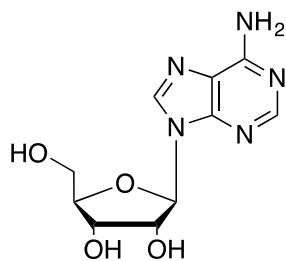
adenosine
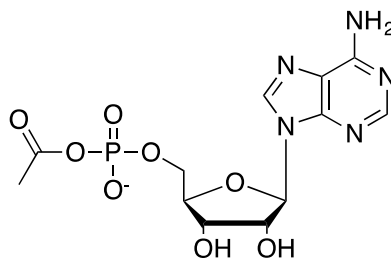
acyl adenylate

Draw adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP). Two of these, *ADP / ATP*, contain anhydrides of phosphoric acid.
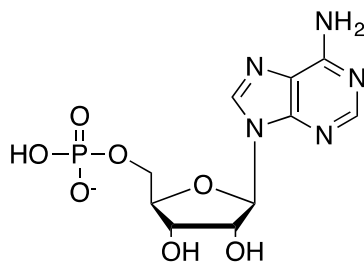
AMP
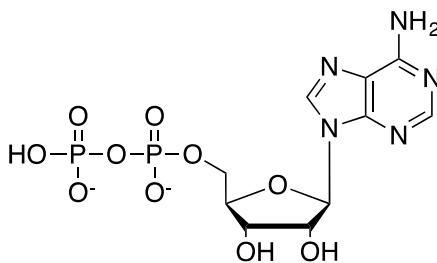
ADP
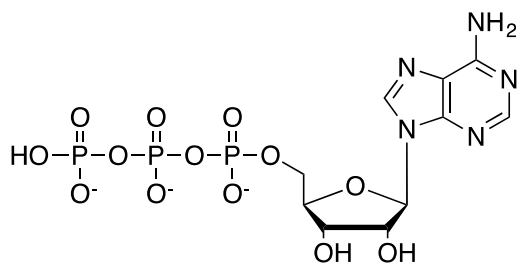
ATP

Show curly arrows to indicate the mode of attack of a carboxylate on ATP that would result in formation AMP and acyl pyrophosphate.
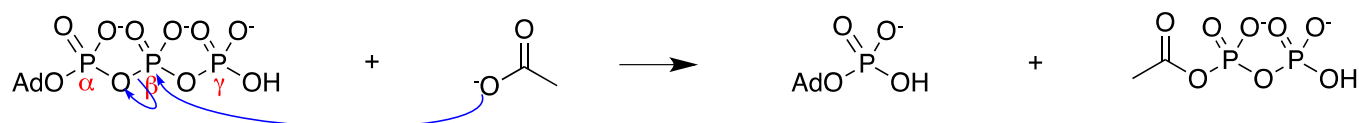
AMP and acyl pyrophosphate

The process shown above involved attack of the carboxylate on the β phosphorus. ADP and acyl {mono}phosphate would be formed from attack of the carboxylate on the γ phosphorus.

Negatively charged phosphate groups *repel* anionic nucleophiles so these reactions are *faster* if encapsulated in an enzyme active site that balances the charges by forming ion pairs.

14 Introducing, The Amino Acids!

from chapter(s) _____ in the recommended text

Introduction

Focus

Amino acids have several common features, but also each has some distinct properties because of their different side-chains.

Reasons To Care

Combinations of the 20 genetically-encoded amino acid building blocks impart diverse properties to peptides and proteins that impact some of the most interesting molecular interactions in living systems. It is necessary to know what the amino acids are, their ionization states at different pHs, a test that can be applied to detect free *N*-termini, and methods that can be used to isolate optically active samples from racemic forms.

a

b

-Arg-Gly-Asp-

H-Tyr-Gly-Gly-Phe-Met-OH
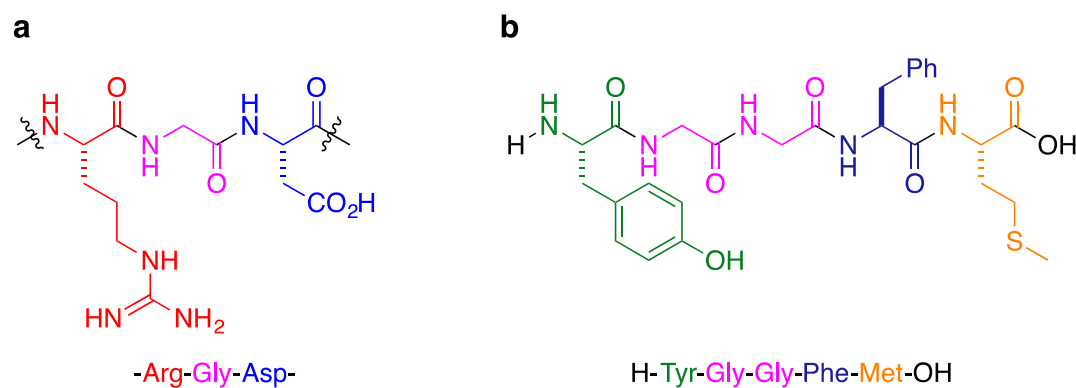
Figure 1. Even small sequences of amino acids can have important biological properties. **a** The RGD sequence involved in binding the cell surface protein family called *integrins* in cell-cell and cell-matrix recognition processes. **b** Opiate peptide *Met-enkephalin*.

Concepts Involved

acids and bases • condensation of amines with carbonyls • kinetic differentiation of enantiomers

Objective

This section introduces the amino acids as families based on similar properties of their side chains (hydrophobic, hydrophilic, acidic, basic *etc*), and lays foundations to understand syntheses and properties of peptides and proteins, as outlined in the next section.

Nomenclature And Conventions

Amino acids are best drawn with the *N*-terminus on the *left*, and the *C*-terminus on the *right*.

Gly is the simplest amino acid, $HO_2CCH_2NH_2$, because it has no side-chain.

Even though it is the convention to show amino acids and peptides in uncharged states, in reality at neutral pH they exist with a *N*-terminal *ammonium* and a *C*-terminal *carboxylate*.

Draw Gly without charges, and indicate the *N*- and *C*-termini, then show its *zwitterionic* form.
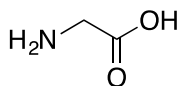
glycine, neutral form
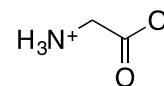
glycine, charged form

Draw *cis*- and *trans*-forms of the amide in H-Gly-NHMe and their amide bond resonance structures. Rotation about the CO-NHMe bond is *slow* compared with rotation about a RCH₂-NHMe in an amine because of *resonance*.
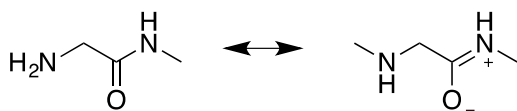
trans
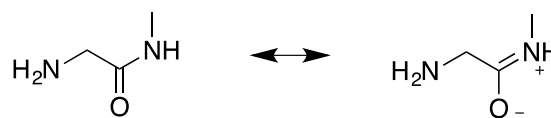
cis

The amide bond is essentially *flat* because of this resonance, and the H-Gly-NHMe has considerable *sp²* character.

In some ways, amide bonds in peptides and proteins resemble *alkenes*.

Usually, the preferred conformer of amide bonds in peptides and proteins is *trans* for steric reasons. *Cis* and *trans* nomenclature in peptides refers to the orientation of the atoms in the peptide backbone, and this *is not* the same as those set by the priority rules.

There are *20* genetically encoded amino acids in proteins.

Learning the amino acid structures requires memorization, but some concepts can help. First, draw the amino acids with their amine group on the left, begin a zigzag chain with the first bond "zagging" downwards; if this convention is followed then the configuration of the first genetically encoded amino acid in the sequence has its side chains pointing back.

Second, learn the amino acids by categories of side chains. For instance, the *four* amino acids with *aliphatic* side chains: Ala, Val (^{i}Pr side chain), Leu (^{i}PrCH$_2$), Ile {(*S*)-CHMeEt}, and Pro {which is (*S*)-pyrrolidine 2-carboxylic acid}.

The carbon between the NH and CO of an amino acid is the *Cα* and the first atom in any *side chain* attached to it is labeled *Cβ*.
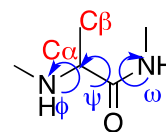
Third, note any peculiarities in each group of amino acids. In the group above, for instance, *iso*-leucine has a chiral center in the side chain, at *Cβ*.

Amino Acids With Lypophilic Side Chains

Draw the amide *cis* and *trans* forms of Me-Ala-NHMe (alanine, Ala, has a methyl side chain), label the Cα and Cβ atoms, and indicate the bond rotations that ϕ, ψ and ω describe.
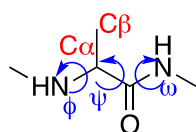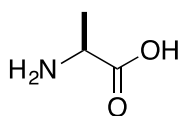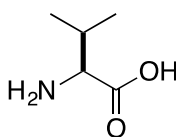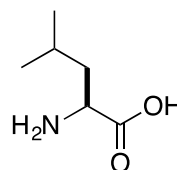

trans

cis

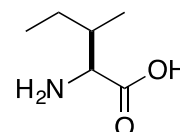Each amino acid can be represented by a three- and a one-letter code.
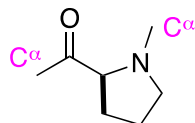

alanine, Ala, A
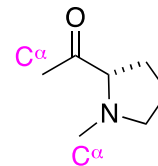
valine, Val, V

leucine, Leu, L

iso-leucine, Ile, I

All genetically encoded amino acids have ʟ-configurations; these relate to *the configuration of glyceraldehyde*.

Proline is an "odd-ball": it is the only amino acid that is a *secondary* amine.

Draw the *cis* and *trans* isomers of Me-Pro-NHMe.

<center>*trans*</center>

<center>*cis*</center>

Peptides that contain proline are *more* likely to populate conformers in solution that have *cis*-amide bonds than ones that do not have a Pro residue.

Alcohol And Thiol Amino Acids

Two amino acids contain side chains with alcohols: *Ser* (CH_2OH side chain) and *Thr* {(*R*)-CH(OH)Me}, and two contain sulfur: *Cys* (CH_2SH) and *Met* (CH_2CH_2SMe).

Draw Ser, Thr, Cys, and Met: in each case, deduce the configuration at Cα (*R* or *S*).
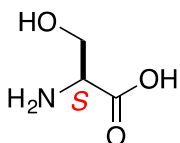
<center>*serine* *threonine* *cysteine* *methionine*</center>

One of the amino acids, *Cys*, has a different configuration than all the other genetically encoded amino acids, because sulfur atom connected to Cβ has higher priority than carbonyl group.

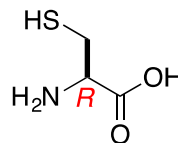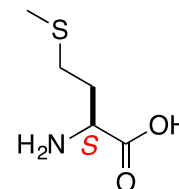## Acidic Amino Acids And Their Derivatives

Two amino acids have carboxylic acids in their side chains, Asp (CH_2CO_2H) and Glu ($CH_2CH_2CO_2H$), and *two* (Asn) and (Gln) feature the corresponding primary amides.

Draw Asp, Glu, Asn, and Gln in neutral forms (*ie* not as ammonium/carboxylates that they exist as at neutral pH), and indicate the most acidic proton in each case (*ie* side-chain or main-chain acid group?).


D   E   N   Q
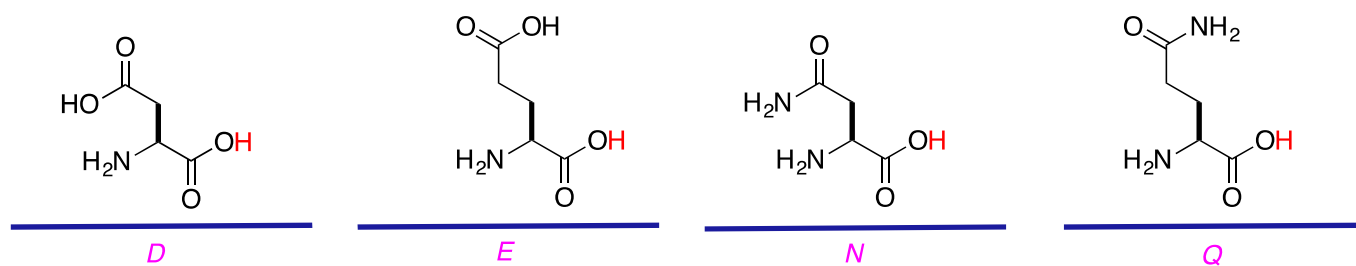
Inductive effects from the amine-*N* of Asp and Glu makes their main chain CO_2H protons *more* acidic than the carboxylic acid in the side chain.
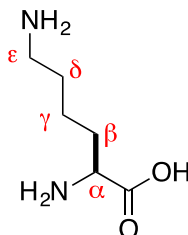
Basic Amino Acids

Three amino acids have *basic* side chains: *H* {CH_2(4-imidazoyl); pKa of conjugate acid 6.4}, *Lys* ($CH_2CH_2CH_2$ CH_2NH_2; pKa of conjugate acid 10.8), and *Arg* ($CH_2CH_2CH_2NHCNHNH_2$; pKa of the conjugate acid of the *guanidine* side chain 12.5).

Draw these amino acids in order of decreasing basicity of the side chain in the free base amino acids, and label the side chain atoms of Lys with α, β *etc*.
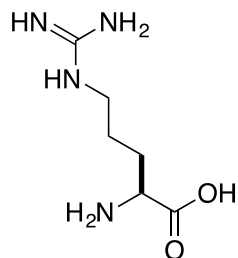
most basic   *intermediate*   *least basic*
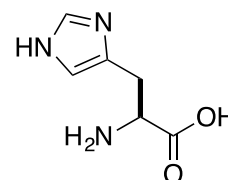
Draw diagrams describing the likely protonation states of His (pKa's 1.82, 9.17, 6.00, and 7.59) at the following pH values.
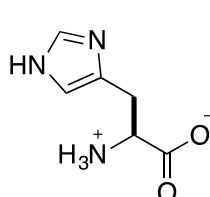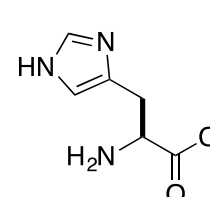
pH = 0   *4*   *8*   *12*
di-cation   *monoanion*

Aromatic Amino Acids

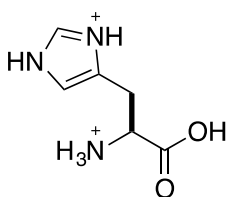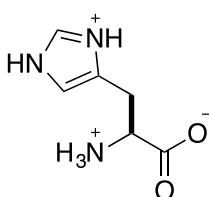Besides His, Phe (CH$_2$Ph), Tyr (CH$_2$C$_6$H$_4$OH; pKa of side chain 10.1), and Trp contain *aromatic* side chains. Draw these amino acids and indicate their one letter codes.
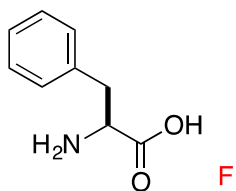
F

phenylalanine
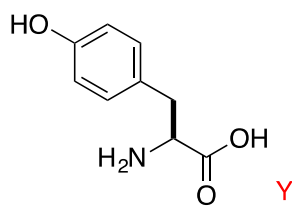
Y

tyrosine
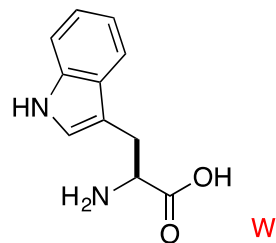
W

tryptophan

The phenolic side chain of tyrosine is a *weaker* acid than the side-chain carboxylic acids of Asp and Glu.

The *indole* *N*-atom of tryptophan *is not* significantly basic because it is involved in aromaticity.

Summary

To review, draw and name four amino acids with hydrophobic side chains, and two with acidic side chains, and two with basic side-chains. Show them in their L-configuration (the one found in genetically encoded proteins), with the amine on the left, the acid on the right, and the side-chains pointed downwards.
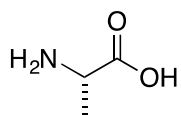
hydrophobic 1

name: alanine, Ala, A
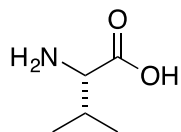
hydrophobic 2

valine, Val, V
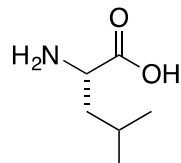
hydrophobic 3

leucine, Leu, L
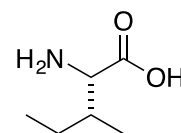
hydrophobic 4

iso-leucine, Ile, I

acidic 1

aspartic acid, Asp, D

acidic 2

glutamic acid, Glu, E

basic 1

arginine, Arg, R

basic 2

lysine, Lys, K

Isoelectric Points

The pH at which an amino acid has no net charge is called *isoelectric* point, *ie* its pI value.

Amino acids without ionizable side chains have pI values that are *midway between* the pKa values for their acid and amine groups.

Calculate the pI for alanine for which the pKa of the carboxylic acid group is 2.34, and that of the amine in its ammonium form is 9.62.
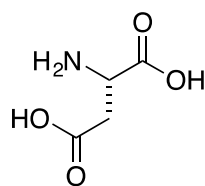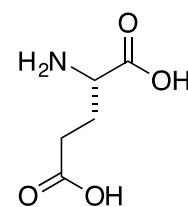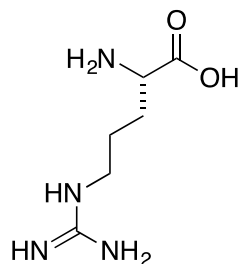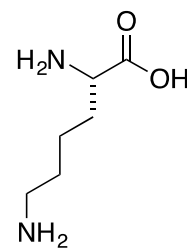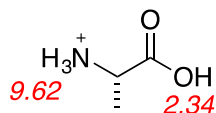
$$pI = \frac{pKa\ (\alpha\text{-COOH}) + pKa\ (\alpha\text{-NH}_3^+)}{2}$$

$$pI = (2.34 + 9.62)/2 = 5.98$$

structure of alanine indicating pKa's   *calculation*

Calculation of pI values for amino acids *with ionizable side chains* is based on the *average* pKa of the two similarly ionizing groups.

Calculate the pI of Lys. This residue has three pKa values: 2.18, 8.95, 10.79 (the side chain amine is the most basic one, show which groups these pKa levels are associated with).
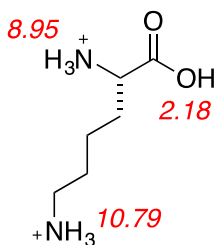
$$pI = (8.95 + 10.79)/2 = 9.87$$

structure of Lys indicating pKa's   *calculation*

Calculate the pI for glutamic acid that has pKa values of: 2.19, 4.25, 9.67 (the $C^\alpha HCO_2H$ group is the most acidic).
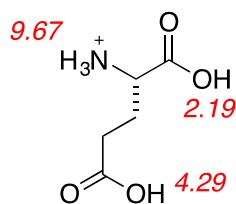
$$pI = (2.19 + 4.29)/2 = 3.24$$

structure of glutamic acid indicating pKa's   *calculation*

Classify each of the following amino acids:

Asp, *acid*   Asn, *neutral*   Arg, *basic*

Glu, *acid*   Gln, *neutral*

Ser, *neutral*   Thr, *neutral*

Select amino acids from the following sets that have:

the *highest* pI value *Arg*   the *lowest* pI value *Glu*
most *negative* charge at pH 6 *Glu*   most *positive* charge at pH 2 *Lys*

Migratory aptitude in media placed under an electrical potential tends to be proportional to *mass divided by charge*.

In electrophoresis at pH 4, *Lys* will migrate to the negative electrode faster than the others.

An amino acid pI value of 6.4 will migrate *not at all* in electrophoresis at pH 6.4.

If that same amino acid is subject to electrophoresis at pH 8 it will *migrate to the positive electrode*.

The Ninhydrin Test

Ninhydrin stains primary amines, including amino acids. Of the three carbonyls in ninhydrin the most reactive is (are) the *central* one(s).

Draw ninhydrin hydrate, and the condensation product it forms with one equivalent of a primary amine.

hydrate   *ninhydrin*   *imine*
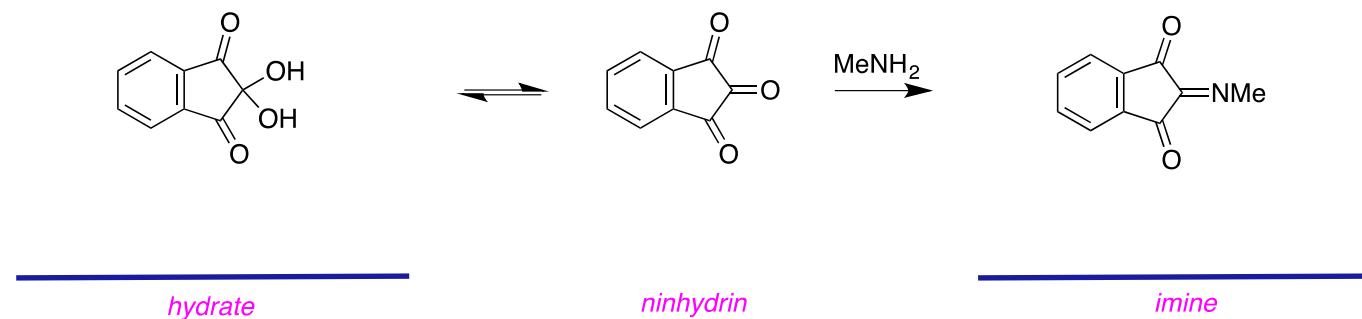
Draw the mechanism for the imine formation here:

H+

The ninhydrin test detects amino acids (*eg* after separation via electrophoresis) with primary amines (*ie* does not work well for *proline*).

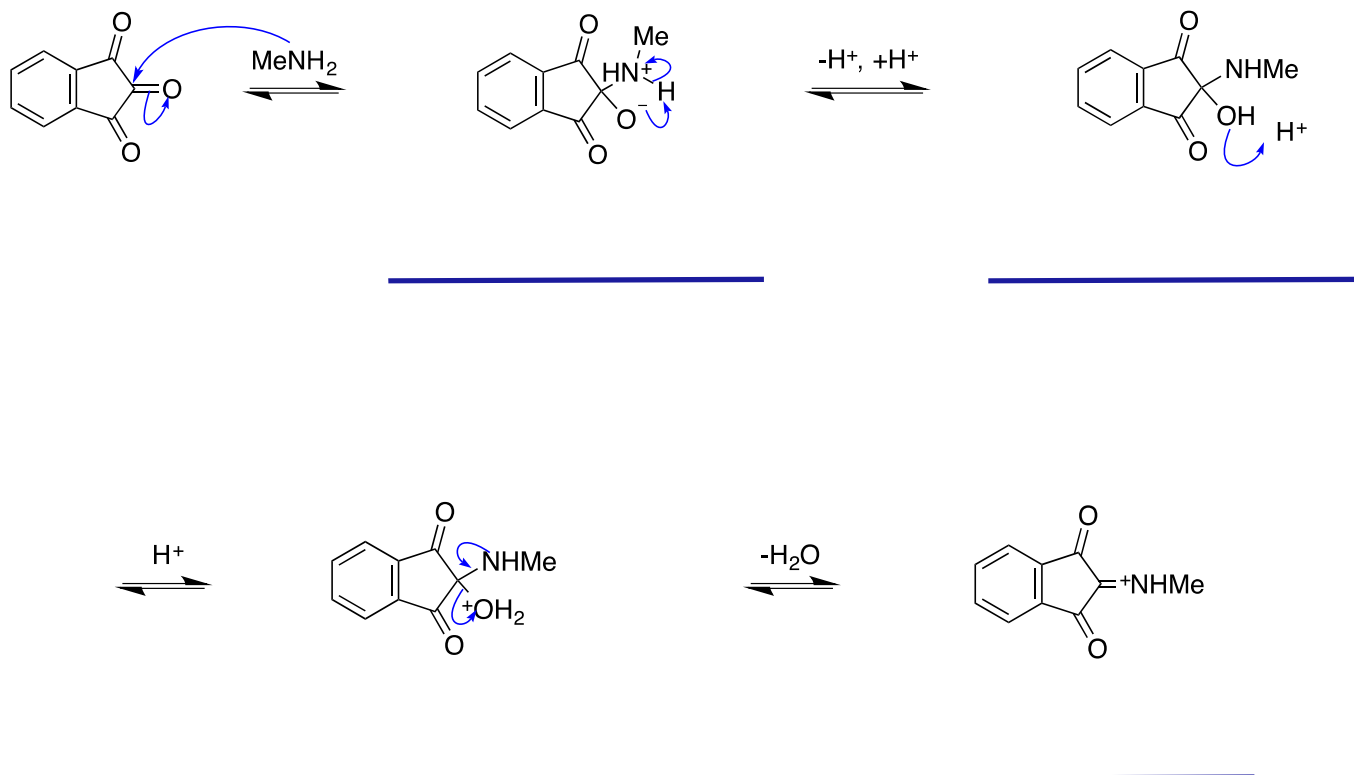In solid phase peptide synthesis, for instance, the ninhydrin test can reveal if all the *amine* groups on a supported peptide have been coupled, or if there are some unreacted ones available.

Proline *does not* have a primary amine group; it stains brown.

ninhydrin
negative
(no primary amines)

ninhydrin
positive
(primary amines)

proline
(secondary amine)
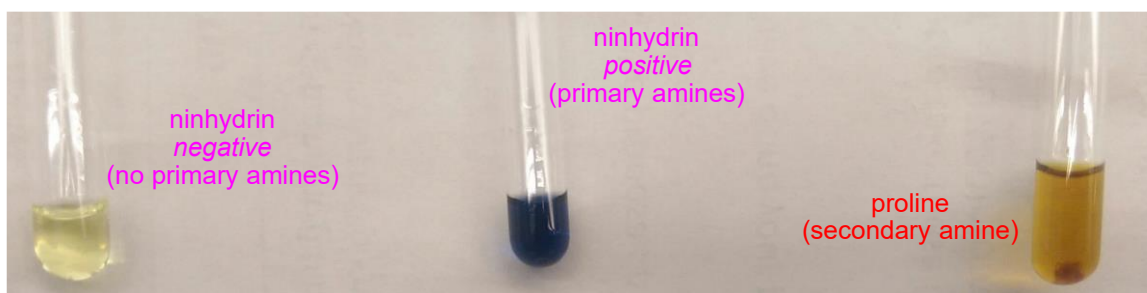
Deduce a mechanism for the formation of the purple dye illustrated below from Val (do not show the mechanism of imine formation, that is already shown in the last question).

imine

imine enolate
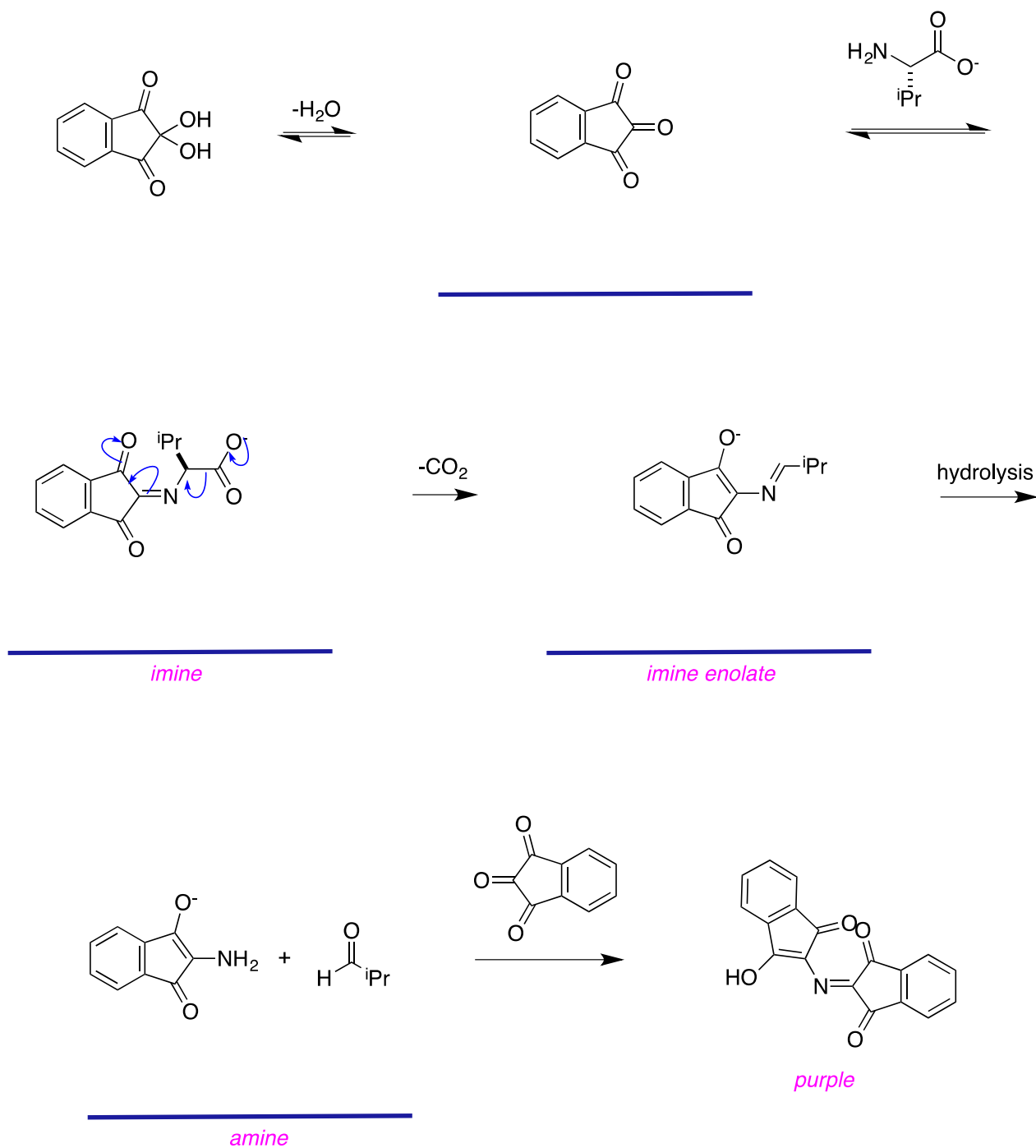
amine

purple

When a tiny amount of amino acid is reacted with ninhydrin a deep *purple* color forms immediately; that color *can be* quantified by UV.

The ninhydrin test can be used as a qualitative check from the presence of free amine at peptide *N*-termini, *and* to quantitate the amount of that amine.

Kinetic Resolutions Of Amino Acids

Kinetic resolutions involve fast reaction of one enantiomer relative to the other so that starting material enriched in one configuration may be recovered from product enriched in the other.

In a *perfect* kinetic resolution, all one antipode of the starting material would remain unreacted, while all the other would be turned into product.

Illustrate kinetic resolution of racemic *N*-acetyl Leu being preferentially hydrolyzed to the free (*S*)-amino acid by pig kidney aminoacylase.

(S)-Ac-Leu
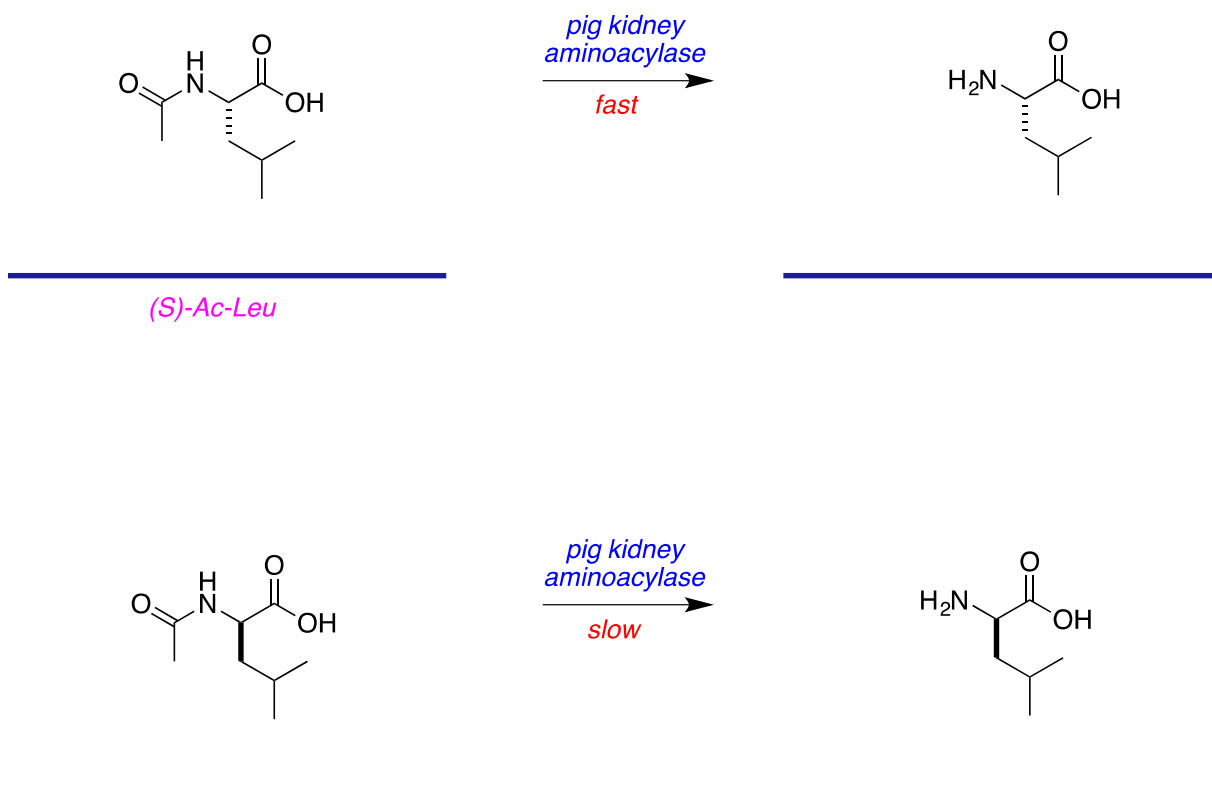
The maximum yield of almost enantiomerically pure reactant after the reaction above is *just under 50 %*.

The maximum yield of almost enantiomerically pure product in this reaction is *just under 50 %*.

The enantiomeric excess of the product in these reactions *decreases* with conversion, while that of the starting material *increases*.

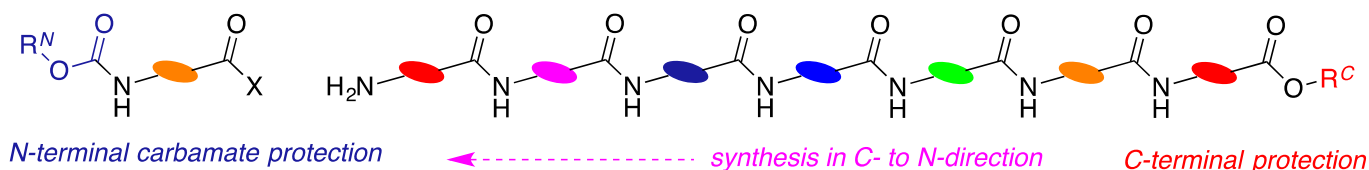# 15 Peptide Syntheses

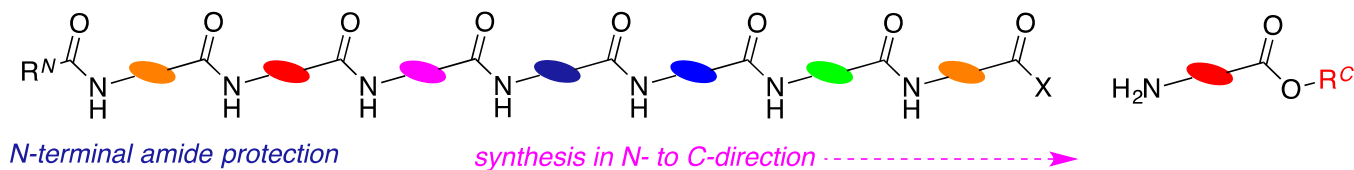from chapter(s) _____ in the recommended text

Introduction

Focus

If amino acids were *C*-activated without any *N*-protection, then they would dimerize, cyclodimerize, oligomerize, and polymerize in uncontrollable ways. Sequence-specific syntheses of peptides require coupling of appropriately *N*-protected units with *C*-protected ones.

Epimerization can be avoided by adding single amino acids to the *N*-terminus of a growing chain, but only if the protection on the amino acid is a carbamate and not an amide. Certain side-chains must be deprotected, and others must be "insulated" from reactive cations in deprotection conditions by using scavengers. Then there is the special case where the *C*-terminal protecting group on the growing chain is a polymeric support, *ie* solid phase methods. These aspects of peptide synthesis are the focus of this section.

conventional peptide synthesis can be performed like this .....

N-terminal carbamate protection   *synthesis in C- to N-direction*   *C-terminal protection*

but not like this .....

N-terminal amide protection   *synthesis in N- to C-direction*

Reasons To Care

Researchers in all branches of the biological and biomedical sciences may need peptides or modified peptides for use in their research.

Concepts Involved

activation of carboxylic acids • reactions of activated acids with amines • aromaticity

Objective

Understanding the possibilities and limitations of this aspect of organic chemistry so that it might be applied later.

Solution Phase Methods

The Need For Protection

Reactions Of Unprotected Amino Acids

Carboxylic acids *do not* combine with amines to form amides in the absence of activating agents.

If, in an attempt to make the dipeptide H-Met-Phe-OH, methionine and phenylalanine were mixed, and thionyl chloride were added this would first convert the carboxylic acids to *acid* chlorides, then four possible linear dipeptides would form; draw them using the same nomenclature (three letter codes).

H-Met-Phe-OH	H-Met-Met-OH	H-Phe-Phe-OH	H-Phe-Met-OH
dipeptide	*dipeptide*	*dipeptide*	*dipeptide*

There are also three possible cyclic byproducts, *diketopiperazines*, in the reaction above; show these:
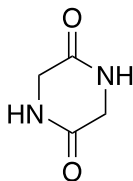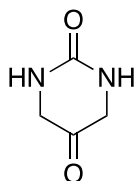
diketopiperazine   *symmetrical diketopiperazine*   *unsymmetrical diketopiperazine*
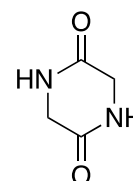
Polymeric materials *would* also be produced in this reaction.

Overall, this route would be an *impractical* synthesis of a particular linear dipeptide because of separation issues.

The problem is that the building blocks can react using the amine and/or acid functionalities. To solve this problem it is necessary to *N-* protect one of the fragments and *C-* protect the other.

Reactions Of Protected Amino Acids

Predict the products of the following reactions, where P and P' are *N*- and *C*-protecting groups.

P-Met-Cl + H-Gly-P' ⟶   P-Met-Gly-P'   P-Phe-Cl + H-Pro-P' ⟶   P-Phe-Pro-P'

_____   _____

Illustrative Protection: BOC/^{t}Bu

N-BOC Protected Amino Acids

Tert-butyloxycarbonyl, ^{t}BuOCO- or BOC-, groups are used to protect *amines* so that they no longer become nucleophilic.

BOC groups are added in much the same way as acid chlorides or acid anhydrides add to *amines*.

Draw full structures for the products of the following reactions:

_____

_____

+   H-Met-OH

H-Val-OH $\xrightarrow{(BOC)_2O}$
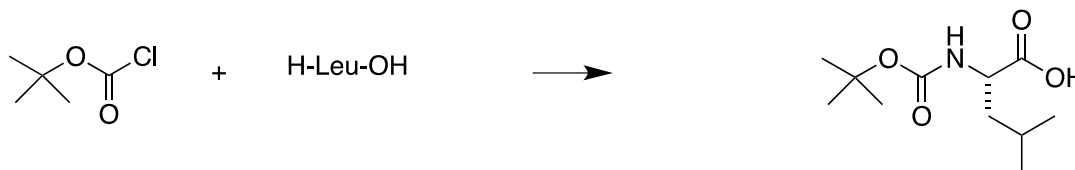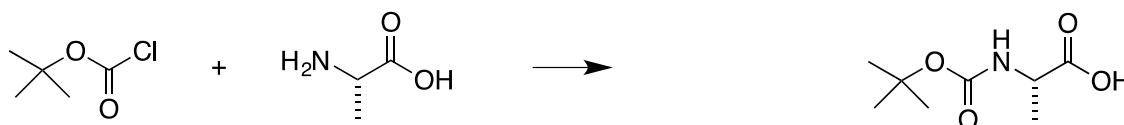
BOC groups can be removed from amines by treatment with *trifluoroacetic (TFA)* acid.

BOC removal involves protonation of the carbonyl group, then formation of a *tert*-butyl carbocation and carbon *dioxide*.

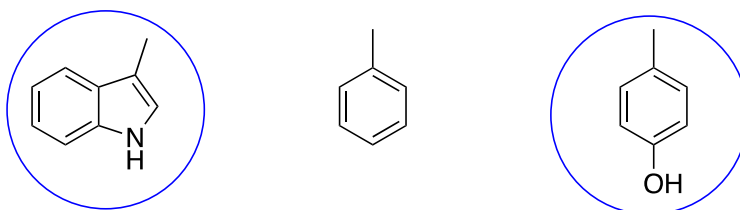Show the mechanism for BOC-deprotection of BOC-Phe-OH using TFA.

BOC-Phe-OH

unstable carbamate   amino acid   carbocation

Give the products of the following reactions

BOC-Val-Ala-OH $\xrightarrow{\text{TFA}}$ H-Val-Ala-OH
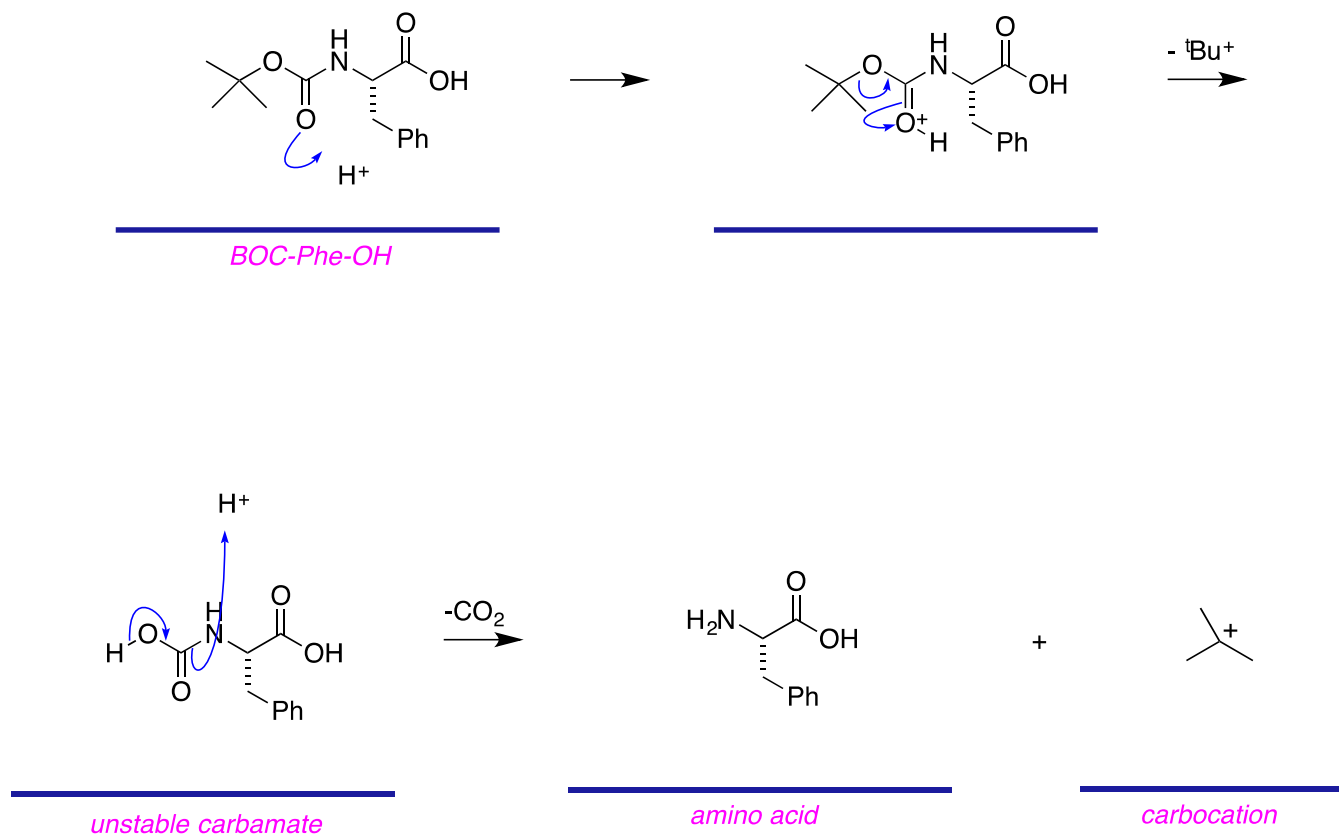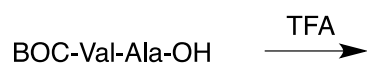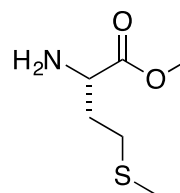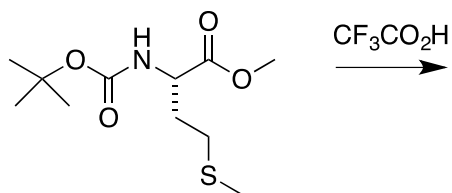
tert-Butyl cations generated in removal of BOC groups can react with amino acid starting materials or peptide products to give *undesirable* by-products.

Scavengers like *HSiEt₃* may be added in BOC removal to "mop-up" ᵗBu cations to prevent these carbocations reacting with nucleophilic groups on the peptide product.

Two of the following compounds are more reactive to electrophiles, like ᵗBu cations, in electrophilic aromatic substitution reactions than the other one; circle those two reactive aromatic systems.

Which *two* of the following amino acids have nucleophilic side chains that might most *easily* react with carbocations: *Tyr / Trp*. Circle those amino acids.

Predict the products of the following reactions (*ie* likely by-products in peptide syntheses).
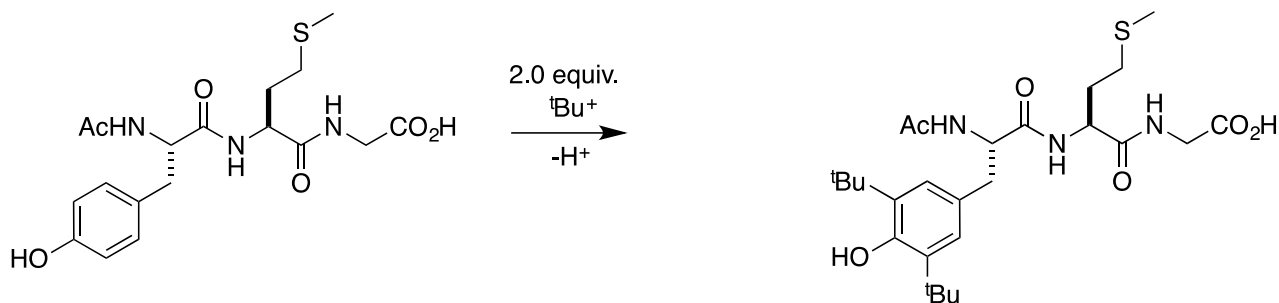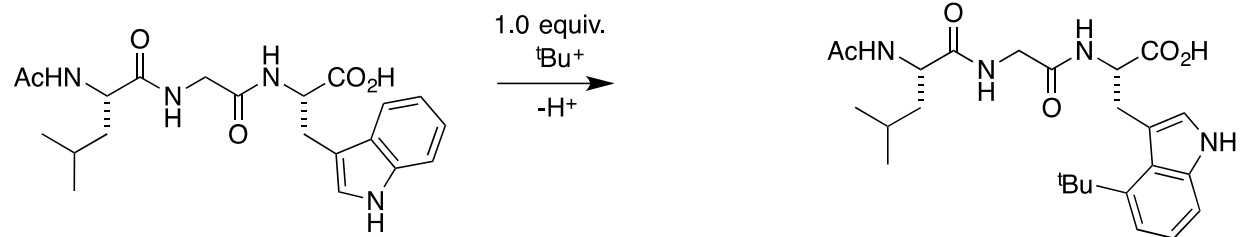
Reactions like these *usually* require a scavenger to prevent formation of byproducts.

Show the mechanism for BOC-deprotection of BOC-Try(Bn)-OH using TFA, and indicate the product formed via reaction of the ᵗbutyl cation with HSiEt₃.

BOC-Tyr(Bn)-OH

H⁺

unstable carbamate

-CO₂

amino acid   +   *carbocation*

HSiEt₃

carbocation   *by-product*
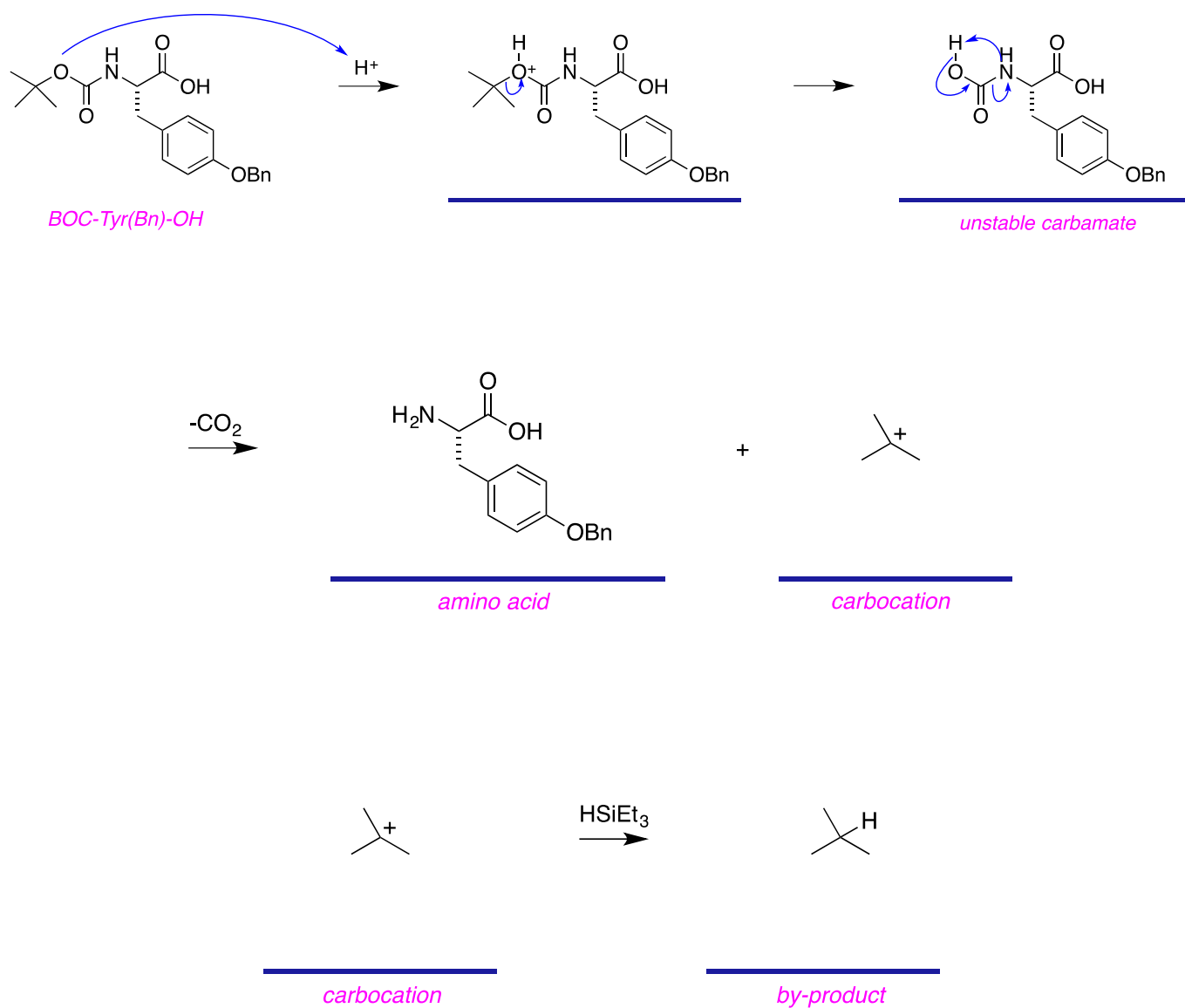
C-Protection Of Amino Acids With ^tBu-Groups

Trifluoroacetic acid also removes *tert*-butyl groups from ^tBu-esters, via protonation of the carbonyl group. Draw this mechanism for Ac-Met-O^tBu.

Ac-Met-O^tBu

by-product cation
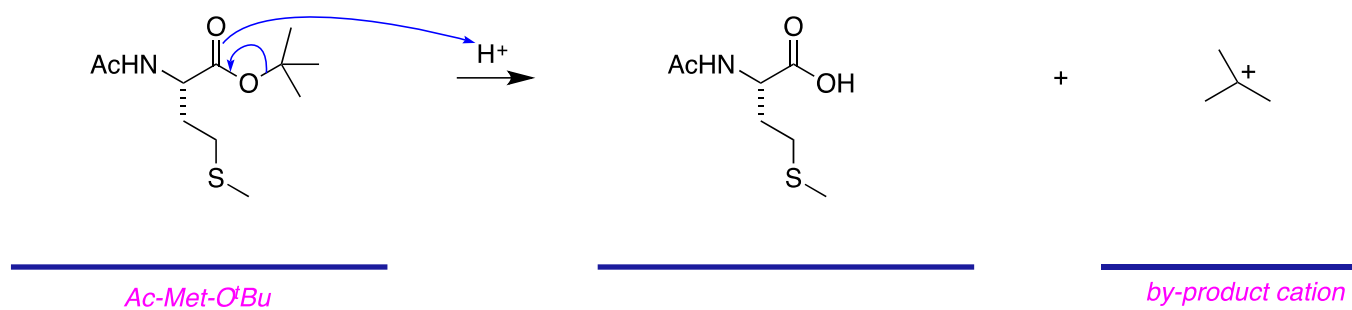
1-Adamantyl esters *cannot* be cleaved as easily under the same conditions as *tert*-butyl esters can (carbocation not as stable).

tert-Butyl carbocations are generated in these reactions, hence scavengers *are* sometimes necessary for *C*-deprotection of peptides containing nucleophilic groups.

Show the products of the following (use abbreviations and three letter codes for the amino acids).

BOC-Gly-Cl   +   H-Met-^tBu   ⟶   BOC-Gly-Met-^tBu

―――――――――

BOC-Pro-Cl   +   H-Phe-^tBu   ⟶   BOC-Pro-Phe-^tBu

―――――――――

BOC-Asp(Bn)-Cl   +   H-Thr(Bn)-^tBu   ⟶   BOC-Asp(Bn)-Thr(Bn)-^tBu

―――――――――

BOC-Asp(Bn)-Cl   +   H-His(Bn)-Thr(Bn)-^tBu   ⟶   BOC-Asp(Bn)-His(Bn)-Thr(Bn)-^tBu

―――――――――
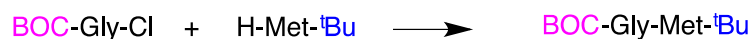
Activation Of *N*-Protected Amino Acids

In practice, amino acid chlorides are slightly too *reactive* for routine peptide syntheses.

Peptide syntheses tend to feature *in situ* activation of *N*-protected amino acids using *carbodiimide* reagents.

Unfortunately, the by-product from activating amino acids with DCC, *ie dicyclohexylurea*, is relatively insoluble in most solvents making it difficult to wash away.

Ethyl (3-dimethylaminopropyl) carbodimide, EDCI, is often used in place of DCC because *the by-products can be protonated and are water-soluble*.

Draw activation of BOC-Phe-OH (draw a structure below that shows the carboxylic acid) with EDCI, then reaction with H-Ala-O^{t}Bu, hence deduce the structure of the by-product that forms with this reagent.

Let   *be represented as*

$$\underline{\hspace{4cm}}$$

EDCI

$$\underline{\hspace{4cm}}$$

BOC-Phe-O$^-$

   +

$$\underline{\hspace{3cm}}$$ $$\underline{\hspace{3cm}}$$
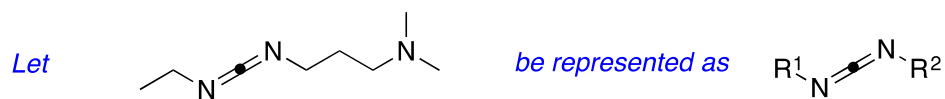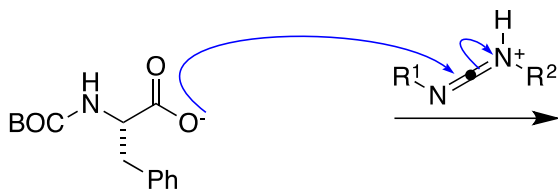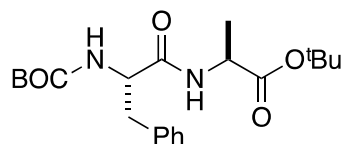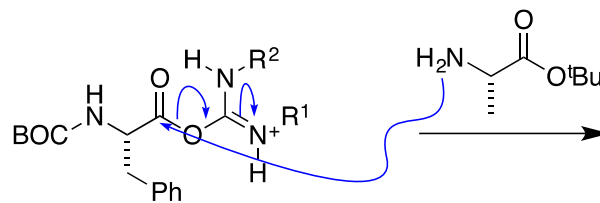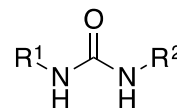
BOC-Phe-Ala-O^{t}Bu   *by-product*

The Epimerization Problem

One of the challenges in peptide synthesis is retaining the configurations of the chiral-$C\alpha$ centers. It is economically viable to begin with optically pure amino acids in peptide syntheses. However if they racemize (applies to all except Thr and Ile, which *epimerize*) in the carboxylate activation process then coupling with optically pure *C*-protected amino acids will result in the formation of *epimeric* products.

Mixtures of epimers tend to be *difficult* to separate into their isomeric components.

Racemization of activated amino acids can occur if a carbonyl group on the *N*-terminus cyclizes by attack of its *O*-atom on the activated intermediate to give an *azlactone*.

Show this cyclization for an activated form of Ac-Ala-X and for BOC-Ala-X (where X is a leaving group; draw the full structures of the amino acids), and compare the inductive effects of Me *vs* the ^{t}BuO to predict which would form the undesired cyclic intermediate faster.

azlactone
forms *rapidly*

azlactone
forms *slowly*
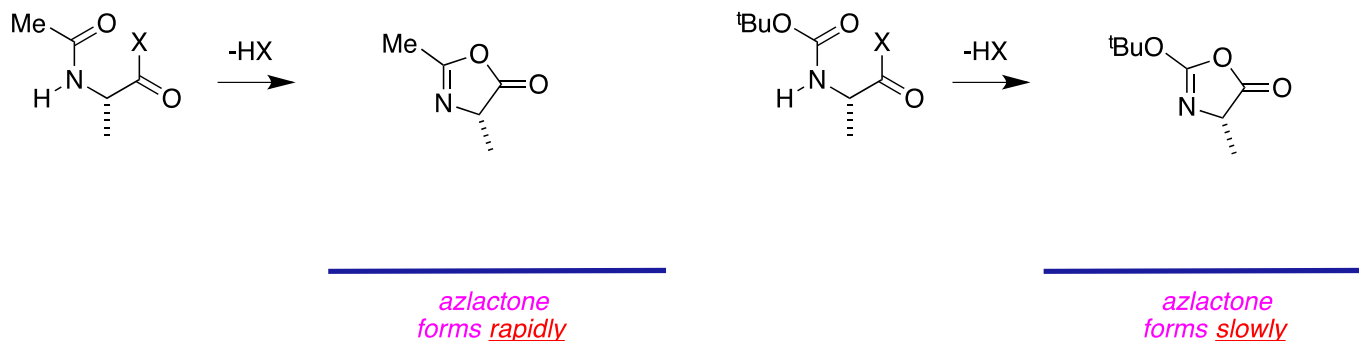
Enolization of the azlactone products *is* driven by aromatic stabilization in the product and simultaneous *loss* of amino acid stereochemistry.

The acyl group in the first example is representative of any amide bond in a peptide, but the carbonyl group of the BOC group is not an amide but a *carbamate*.

Carbonyl C=O atoms are *more* nucleophilic in amides than in carbamates.

Draw the full structures of Ac-Ala-OH and BOC-Gly-Ala-OH, and circle the amide bonds in both structures that can form azlactones.
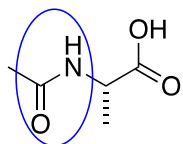
Ac-Ala-OH
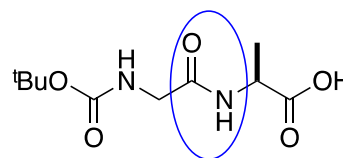
BOC-Gly-Ala-OH

Strategies In Solution Phase Syntheses That Avoid Epimerization

Boc-Gly-Ala-OH *will not* tend to epimerize when activated at the *C*-terminus.

In general, any peptide *will* tend to epimerize when activated on the *C*-terminus.

Consider the two possible solution phase syntheses of BOC-Ile-Val-Ala-O^tBu using the final couplings at the positions shown. Indicate which route is more prone to epimerization, and circle the amino acid in that structure that is most prone to epimerization.

BOC-Ile-Val-Ala-O^tBu
less prone to racemization

BOC-Ile-Val-Ala-O^tBu
more prone to racemization
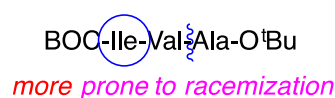
circle the one amino acid in one of these structures that is most vulnerable to epimerization

In conclusion, solution phase syntheses usually *are* best performed by adding activated *N*-carbamate-protected amino acids to a growing peptide chain.

In other words, solution-phase peptide syntheses are optimally performed in the *C- to N-* direction.

Solid Phase Peptide Syntheses

In solution phase syntheses, by-products *are* mixed with the desired product at the end of the reaction.

Purification from by-products *is* usually required at each stage of solution phase syntheses.

If one end of a peptide were attached to an insoluble polymer then this would make it *easier* to purify because the by-products could be used in excess then washed away after the reaction is complete; these are the major *advantages* of *solid phase* peptide syntheses.

Solid phase syntheses *are not* optimally performed by activating the *C*-terminus of a growing peptide chain and adding a *C*-protected amino acid ester, because of epimerization issues (see discussion of solution phase syntheses).

In solid phase peptide syntheses, it is best to attach the polymer to the *C*-terminus.

Polystyrene is often used as a support for solid phase peptide syntheses; it is modified so that some of the phenyls are *para*-substituted with chloromethyl (-CH$_2$Cl) groups. Show the structure of styrene, polystyrene, and the copolymer of styrene and (4-chloromethylphenyl)ethene.
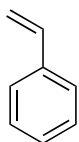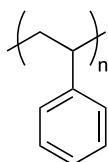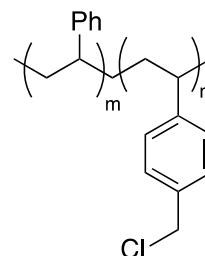
styrene   *polystyrene*   *"4-chloromethylpolystyrene"*

In typical solid phase syntheses, *N*-protected amino acids are added to "4-chloromethylpolystyrene" (Merrifield's resin) via a *S_N2* reaction to attach it to the polymer surface via a benzylic ester linkage.

Show attachment of BOC-Pro-OH to 4-chloromethylpolystyrene with curly arrows.
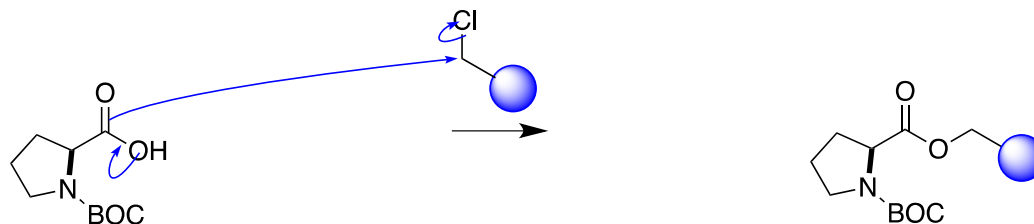
BOC-Pro-OH

The next step in a solid phase synthesis is removal of the BOC group by treatment with *TFA* often in the *presence* of a scavenger; this *does not* cleave the benzylic ester that connects the peptide to the polymer support.

Each cycle of *N*-deprotection and addition of an activated amino acid, elongates the peptide by one residue at the *N*-terminus. Show the products of the following reactions (draw unabbreviated structures).
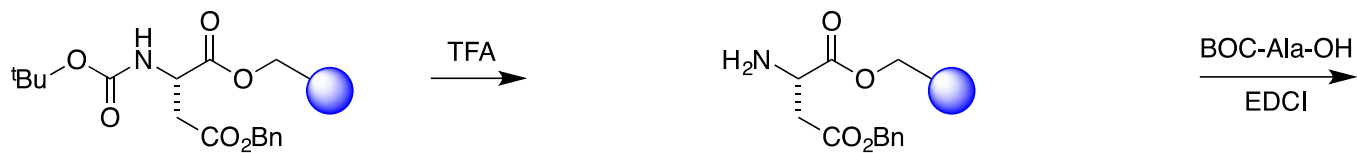
BOC-Asp(Bn)-support
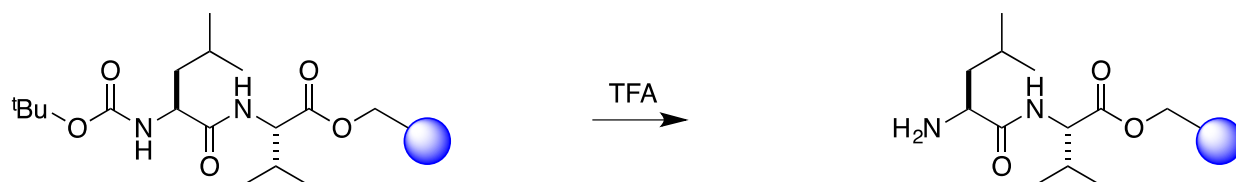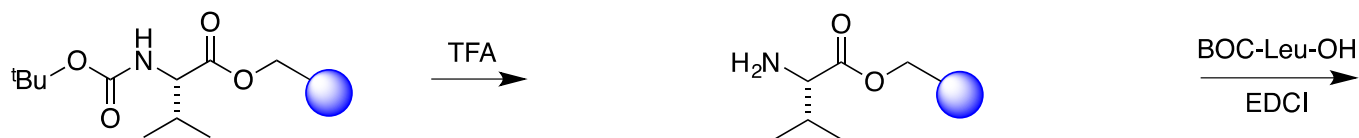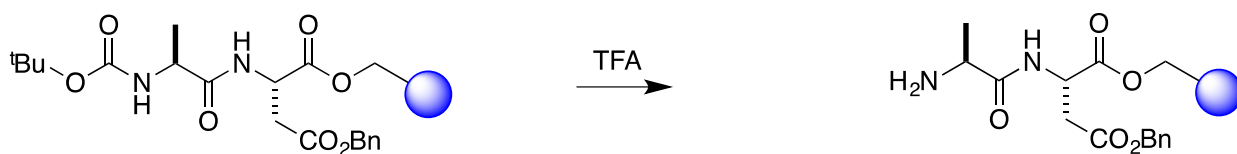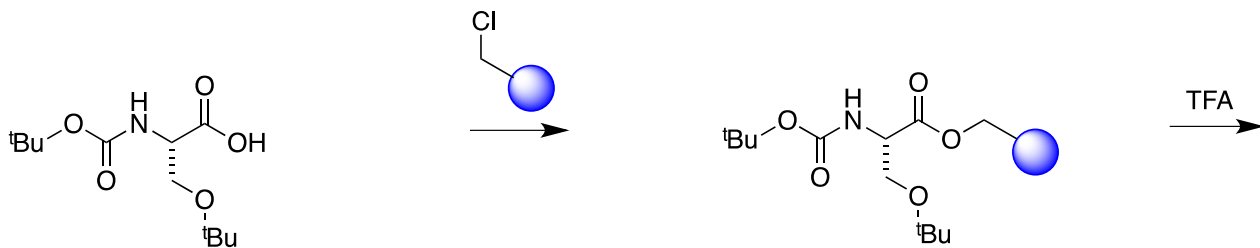
BOC-Ser(OᵗBu)-OH
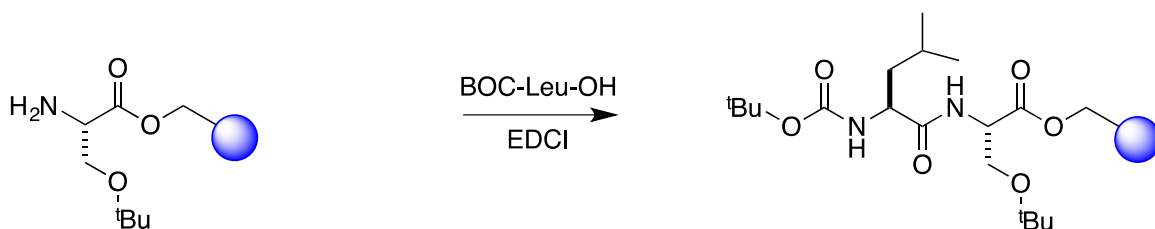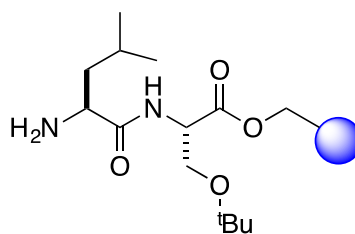
When all the amino acids in the desired sequence have been added, the last step is to cleave the desired peptide (and often the *N*-terminal BOC group). This can be achieved by treatment with a mixture containing *HF* and scavengers. Show this process for generation of the parent peptide from BOC-Gly-Ala-Met-Pro-polystyrene.

BOC-Gly-Ala-Met-Pro—O—⬤ → HF / HSiEt$_3$
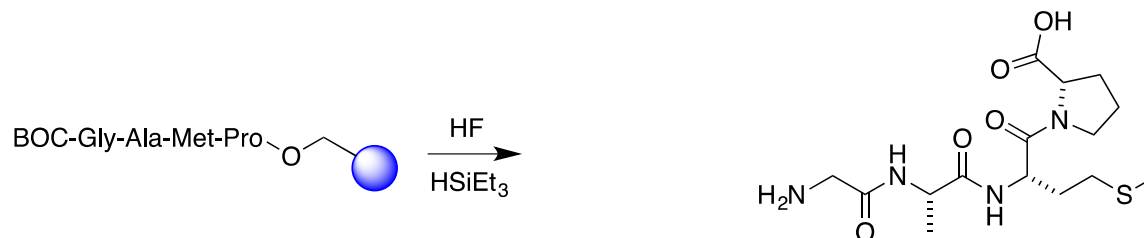
draw peptide

Illustrate how solid phase syntheses featuring poly(4-chloromethylphenylethene) could be used to make H-Leu-Gly-Gly-Phe-Met-OH (use one letter codes throughout, do not draw full structures):

BOC-M-OH   + ⬤(Cl) →   BOC-M-support   → TFA

H-M-support   → BOC-Phe-OH / EDCI →   BOC-FM-support   → TFA

$$\text{H-FM-support} \xrightarrow[\text{EDCI}]{\text{BOC-Gly-OH}} \text{BOC-GFM-support} \xrightarrow{\text{TFA}}$$

$$\text{H-GFM-support} \xrightarrow[\text{EDCI}]{\text{BOC-Gly-OH}} \text{BOC-GGFM-support} \xrightarrow{\text{TFA}}$$

$$\text{H-GGFM-support} \xrightarrow[\text{EDCI}]{\text{BOC-Leu-OH}} \text{BOC-LGGFM-support}$$

$$\xrightarrow[\text{HSiEt}_3]{\text{HF}} \text{H-LGGFM-OH}$$
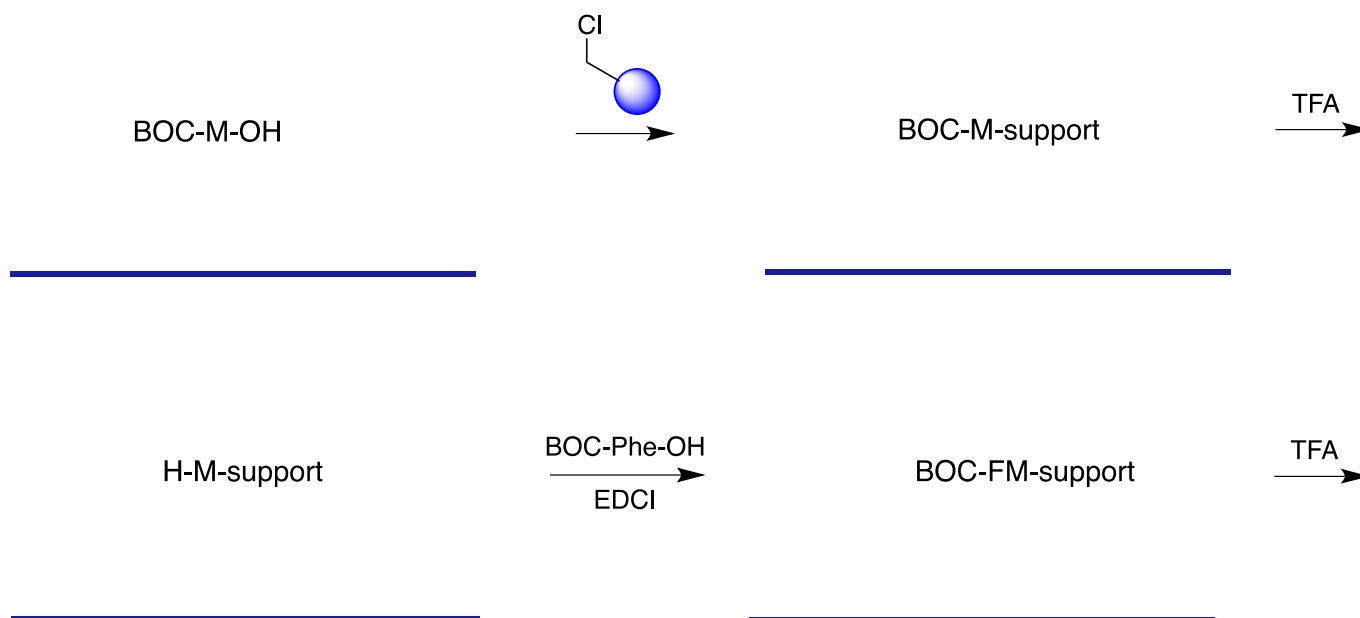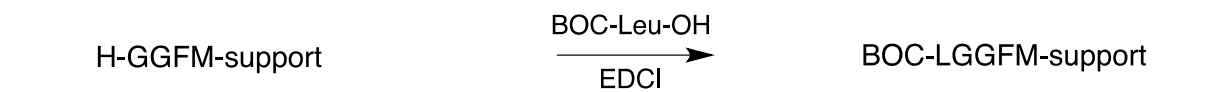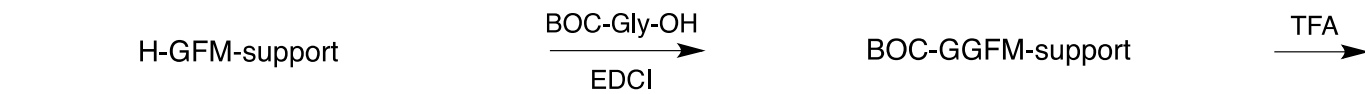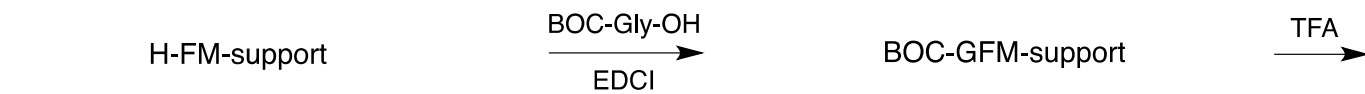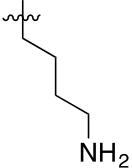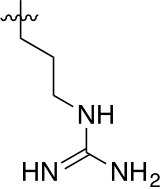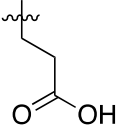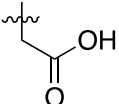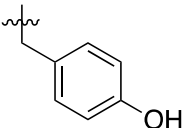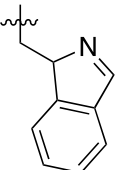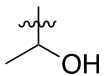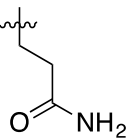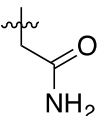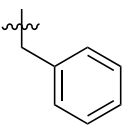
Side-chain Protection Of Amino Acids

Activated *N*-protected amino acids *may* couple to nucleophilic amino acid side chains on the growing peptide chain, hence side chain protection *is* required.

If side chain protection is used, then it is *undesirable* that those side chain protecting groups would be cleaved in removal of the main chain *N*-protecting group.

If side chain protection is used, then it is often *desirable* that those side chain protecting groups would be cleaved in removal of the peptide from the polymer support in the final purification step.

Draw the side chains for the following amino acids and indicate which ones might need to be protected during amide bond forming steps.

protection required?	*protection required?*	*protection required?*	*protection required?*
yes	*yes*	*yes*	*yes*
Lys	Arg	Glu	Asp

protection required?	*protection required?*	*protection required?*	*protection required?*
yes	*yes*	*yes*	*yes*
Tyr	Trp	Ser	Thr

protection required?	*protection required?*	*protection required?*	*protection required?*
yes	*yes*	*yes*	*no*
Cys	Gln	Asn	Phe

The FMOC Approach

The BOC-approach to peptide uses acid to remove the BOC, and *HF* to remove other protecting groups (usually benzyl-based ones) from the side-chains and *C*-termini.

FMOC *N*-protection is *base* labile, and removal of protection from side-chains and the *C*-termini is via *TFA*.

Draw the structures of these three FMOC-protected amino acids.
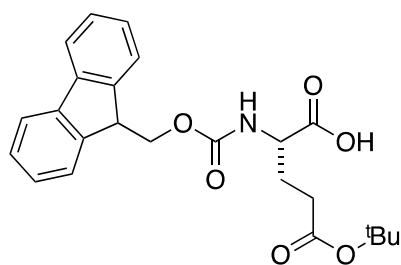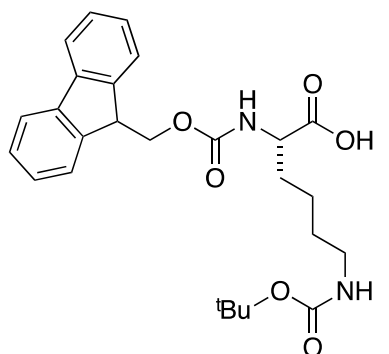
FMOC-Glu(^tBu)-OH   *FMOC-Lys(BOC)-OH*   *FMOC-Ser(^tBu)-OH*
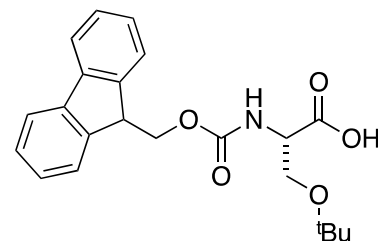
16 Peptides And Proteins

from chapter(s) _____ in the recommended text

Introduction

Focus

Short peptides do not tend to form predominant conformations in solution, but as the number of amino acids involved increases, so do the cumulative diverse inter-residue interactions. Eventually these cumulative interactions resolve into dominant conformational shapes, tertiary structures. Secondary structure motifs (*eg* helices and turns) may be recognizable within these states, and the tertiary structures may assemble into complexes having definite quaternary structures. This section is about the factors that contribute to the wonderful diversity of structures that exist for peptides and proteins.

Reasons To Care

Every year the number of protein crystal structures solved increases, and these data are having a profound impact on biology and the biomedical sciences.
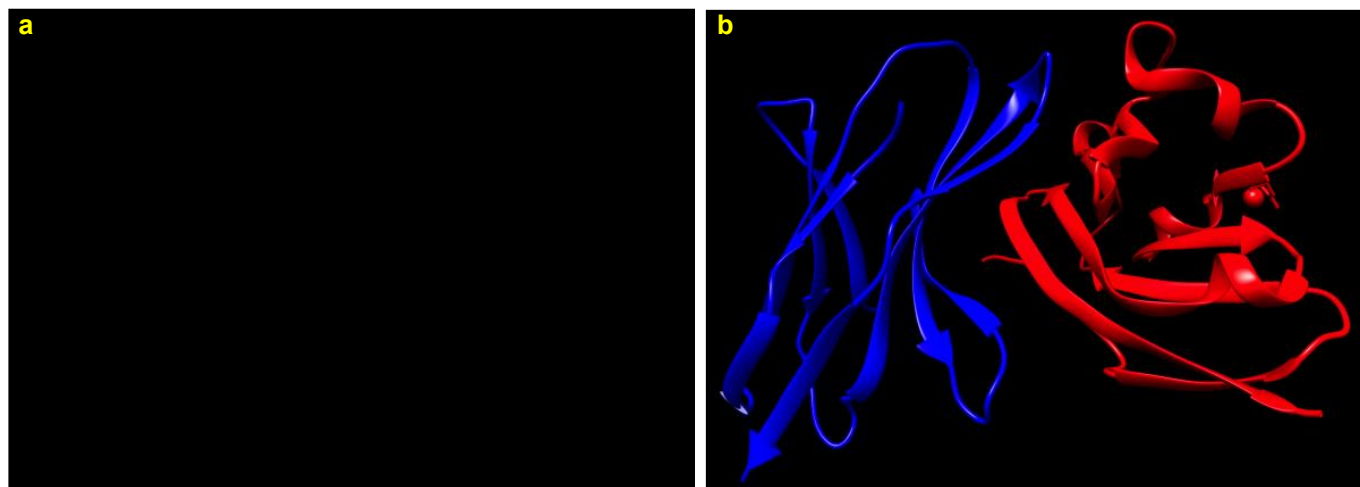
Figure 1. a Interaction between two proteins (uPAR, blue and uPA red); these proteins play key roles in cancer metastasis. uPA complexes by inserting a sheet-turn-sheet motif into a large cavity in uPAR formed by β-sheets. **b** Complex of the "checkpoint proteins" PD1 and PDL1 (blue and red) that are pivotal to some contemporary immunotherapies of melanoma and other forms of cancer.

Concepts

conformational preferences • proteases • mass spectrometry • primary, secondary, tertiary, and quaternary structures of peptides and proteins.

Objective

This section is to illustrate that the basis of protein science is chemistry.

Nomenclature And Conventions

Peptides and proteins are composed of amino acids joined by *amide* bonds.

Conventionally, peptides are drawn with *N*-termini on the *left*, and the *C*-termini on the *right*.

Draw the *tripeptide* (Gly)₃ and indicate the *N*- and *C*-termini (begin with the first bond on the left "zagging" down).

N-terminal   *C-terminal*

triglycine
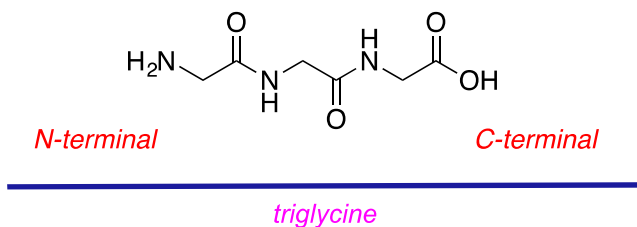
This tripeptide can be represented as H-Gly-Gly-Gly-OH where the H- indicates a primary (*ie* unsubstituted) *N*-terminal *amine*, and the -OH shows a *C*-terminal carboxylic *acid*.

Even though it is the convention to show peptide in the way indicated above, at neutral pH peptides exist with a *N*-terminal *ammonium* and a *C*-terminal *carboxylate*.

Usually, the preferred conformer of amide bonds in peptides and proteins is *trans* (based on the *peptide polyamide backbone*).

In some ways, amide bonds in peptides and proteins resemble *alkenes*.

Dihedral angles in peptides and proteins describe their *local* conformations of amino acids in those structures.

These dihedral angles are denoted by Greek symbols like ϕ (the *N-Cα* dihedral), ψ (*Cα-CO*), and ω (*CO-NH*).

Of these dihedral angles, there is *least* variation in ω because of amide resonance.

Draw the amide *cis* and *trans* forms of MeCO-Ala-NHMe (alanine, Ala, has a methyl side chain), label the Cα and Cβ atoms, and indicate the bond rotations that ϕ, ψ and ω describe.
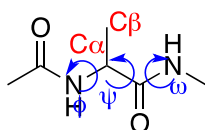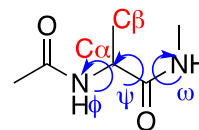
trans   *cis*

Primary Structures

Primary structures are the *sequence of amino acids* of peptides and proteins.

Primary structures of naturally occurring peptides reveal the *sequence* of DNA that encodes for their syntheses.

Homology is the word used to describe the *similarity* of protein primary sequences. Highly homologous primary sequences may indicate the proteins involved *fold into similar shapes*.

Elucidation Of Primary Peptide Structure Via The Edman Degradation

The order of amino acids in peptides and proteins is their *primary* structure.

This order can be determined via an iterative chemical procedure called *The Edman Degradation*, involving two steps. The first step is addition of the free *N*-terminus to phenyl isothiocyanate (PhNCS) to form a thiourea. Show this for H-Ala-Phe-OMe.

PhNCS   H-Ala-Phe-OMe
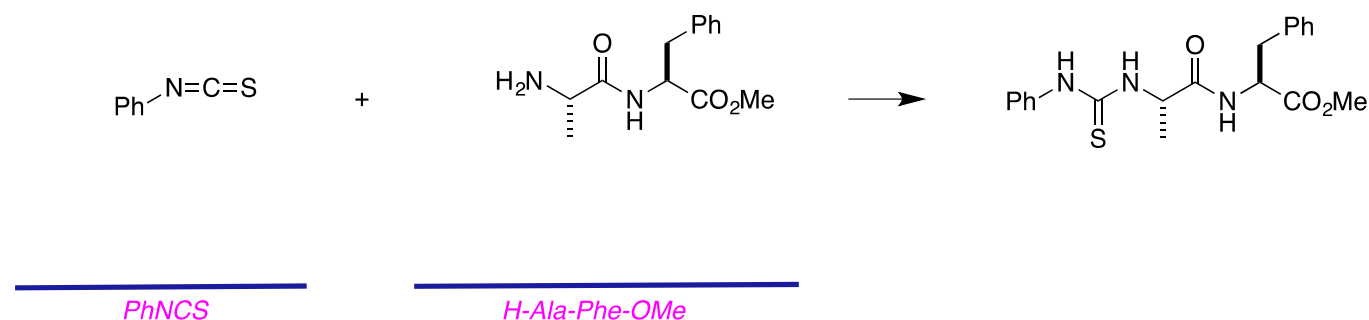
The second step in the Edman degradation involves *O*-protonation to activate the amide carbonyl causing attack of the *C=S* on the proximal amide *C=O*, formation of a tetrahedral intermediate, then collapse of this to liberate a five-membered ring heterocycle, leaving a free *N*-terminus of the peptide now containing one less amino acid. Rearrangement of the liberated heterocycle gives a thiohydantoin bearing the side-chain of the parent amino acid. Show those final products for the following:

amino acid   _H-Phe-OMe_   initial cyclization product   rearranged cyclized product
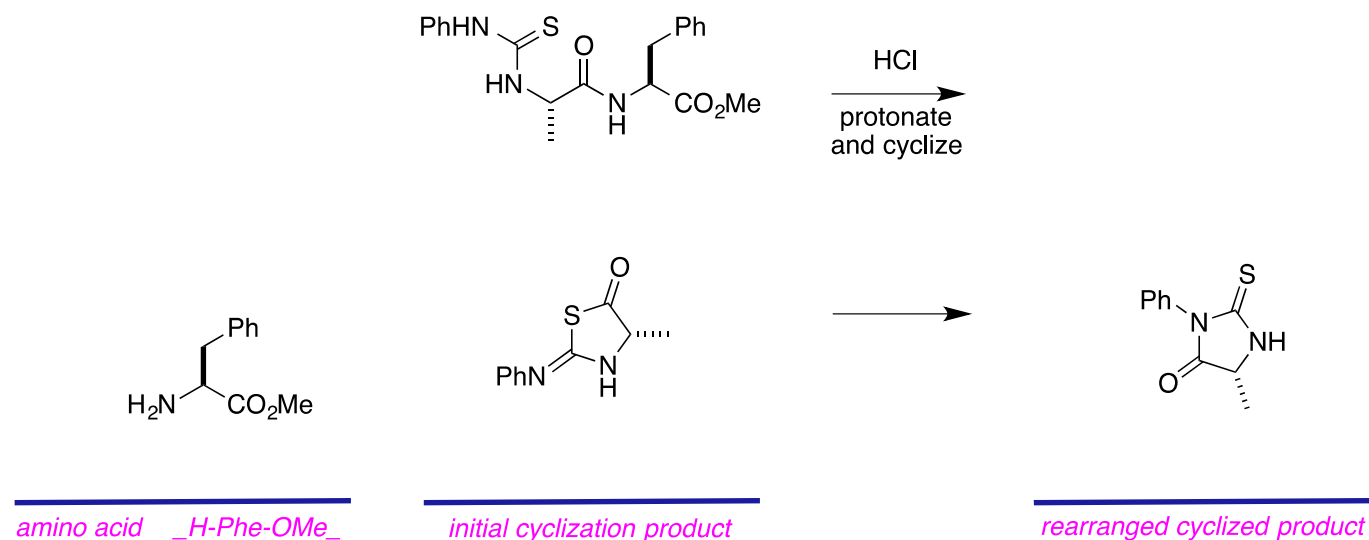
Chromatographic analysis of the liberated *thiohydantoins* is used to deduce the *N*-terminal amino acid at the start of the process (versus a set of standards with the amino acid side chains of each of the protein amino acids). Repetition of the process allows the protein to be sequenced.

Edman sequencing *does* require the *N*-terminus to be a free primary amine.

If there are disulfide linkages in the substrate, then these must first be reduced to the thio form, then reacted with an alkylating agent to allow these residues to be calibrated in the chromatographic analysis.

It *is* possible to perform an Edman degradation on *polymer supported* peptides.

It *is not* possible to perform a complete Edman degradation on *cyclic* peptides.

Draw the first three thiohydantions formed in Edman degradation of H-Met-Pro-Arg-Ala-OH.
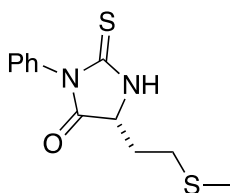
first thiohydantoin   *second thiohydantion*   *third thiohydantoin*
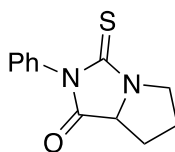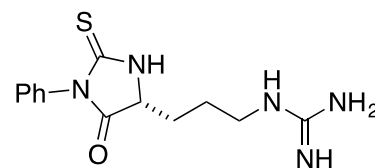
There are limits to the number of amino acids that can be sequenced in Edman degradation because small amounts of impurities accumulate increasing signal-to-noise in the HPLC analysis. Consequently, proteins are "cut" into overlapping smaller fragments, these fragments can be sequenced, and ordered by comparing regions overlap. There are two main strategies for cutting peptides: enzymatic and chemical.

Elucidation Of Primary Structure Via *Enzymatic* Cleavage And Mass Spectroscopy

Fragmentation patterns of peptides in *mass spectrometry* (particularly MS-MS) often allow their primary sequences to be elucidated.

Sequencing in this way is easier for shorter peptides than longer ones, so *proteases* are often used to cleave proteins *at predictable sites* so that the fragments can be sequenced.

Endopeptidases are enzymes that catalyze hydrolysis of peptide bonds *within* of a chain.

Positions of cleavage *vary* for different endopeptidases.

Trypsin

Trypsin tends to cleave only on the *C*-terminal side of Lys and Arg residues. Show the peptide fragments that would be formed when this enzyme is used to digest:

H-Pro-Ala-Pro-Gly-Arg-Trp-Ala-His-Gln-Met-Val-Lys-His-Lys-Pro-Trp-Pro-Ser-Tyr-Thr-Ala-OH

1

fragment 1:   H-Pro-Ala-Pro-Gly-Arg-OH

fragment 2:   H-Trp-Ala-His-Gln-Met-Val-Lys-OH

fragment 3:   H-His-Lys-OH

fragment 4:   H-Pro-Trp-Pro-Ser-Tyr-Thr-Ala-OH

Chymotrypsin

Chymotrypsin is an endopeptidase that cleaves only on the *C*-terminal side of amino acids that have side chains containing six membered rings. Predict the fragments that would be formed in exhaustive degradation of peptide **1**.

fragment 1:   H-Pro-Ala-Pro-Gly-Arg-Trp-OH

fragment 2:   H-Ala-His-Gln-Met-Val-Lys-His-Lys-Pro-Trp-OH

fragment 3:   H-Pro-Ser-Tyr-OH

fragment 4:   H-Thr-Ala-OH

Elastase

Elastase is an endopeptidase that cleaves only on the *C*-terminal side of the smallest amino acids, *ie* Gly and Ala. Predict the fragments that would be formed in elastase-mediated degradation of peptide **1**.

fragment 1:   H-Pro-Ala-OH

fragment 2:   H-Pro-Gly-OH

fragment 3:   H-Arg-Trp-Ala-OH

fragment 4:   H-His-Gln-Met-Val-Lys-His-Lys-Pro-Trp-Pro-Ser-Tyr-Thr-Ala-OH

In fact, a few of the fragments above probably would *not* be formed in appreciable amounts because those endopeptidases mentioned do not cleave amide bonds to proline. Proline is the only amino acid that contains a cyclic secondary amine, and this limits its accessibility to most proteases. Circle the fragments from the three endopeptidases above that are therefore unlikely to form.

Elucidation Of Primary Structure Via Cyanogen Bromide Cleavage And Mass Spectroscopy

Selective, *C*-terminal cleavage of *methionine* residues is effected by cyanogen bromide, Br-CN, in aqueous acid.

In this reaction, the *S*-atom of the side-chain of *methionine* adds to the nitrile and eliminates the bromide; this creates a sulfonium group (R_2S^+CN) that can be displaced (giving a sulfide) by nucleophilic attack of the Met amide *C=O* on the methionine *Cγ* atom.

Hydrolysis of the *C=N* bond of the *iminolactone* produced gives cleavage of the peptide chain, and creates a lactone on the *N*-terminal side.

Show the mechanism for cleavage of Ac-Met-Ala-NH₂ (dipeptide with *N*-terminal acyl group and *C*-terminal amide; draw out the peptide chain).

Ac-Met-Ala-NH₂

Br-CN

sulfonium salt

cyclization

iminolactone

hydrolysis

lactone

Ala-NH₂
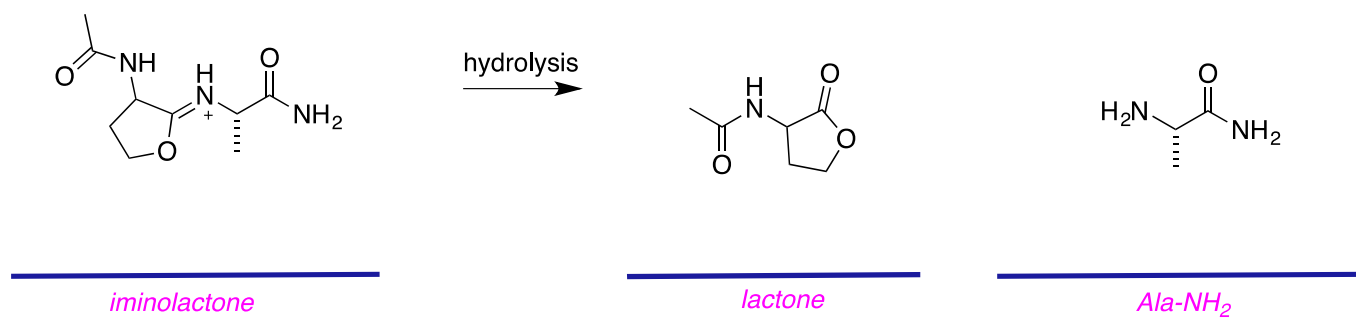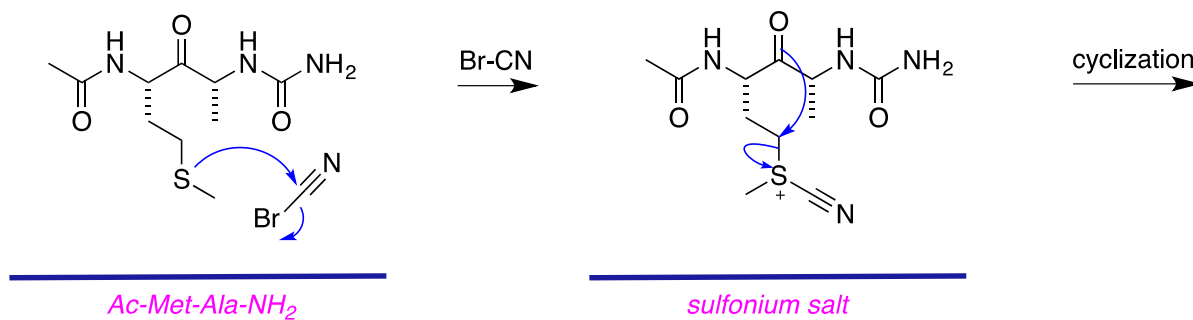
Show the products of the following reactions using three letter amino acid abbreviations, but also clearly showing the termini effected by the cyanogen bromide cleavage.

H-Pro-Ala-Met-Val-Lys-Met-Lys-NH$_2$ $\xrightarrow{\text{BrCN}}$

H-Pro-Ala–[homoserine lactone] + H-Val-Lys–[homoserine lactone] + H-Lys-NH$_2$

_____   _____   _____

H-Pro-Ala-Pro-Gly-Arg-Trp-Ala-His-Gln-Met-Val-Lys-His-Lys-Pro-Trp-Pro-Ser-Tyr-Thr-Ala-OH $\xrightarrow{\text{BrCN}}$

H-Pro-Ala-Pro-Gly-Arg-Trp-Ala-His-Gln–[homoserine lactone] + H-Val-Lys-His-Lys-Pro-Trp-Pro-Ser-Tyr-Thr-Ala-OH

_____   _____
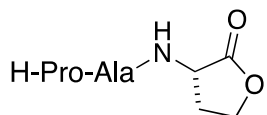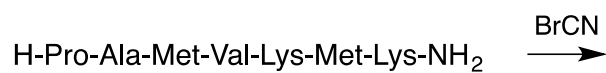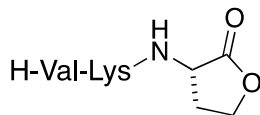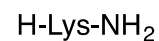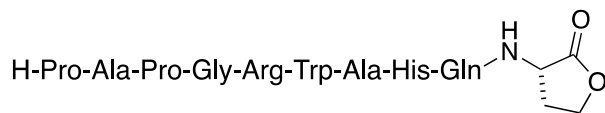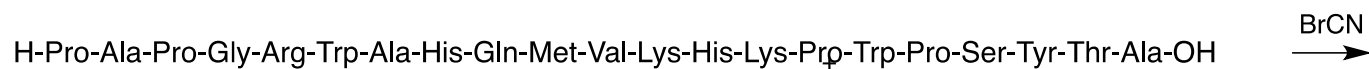
Secondary Structures

Highlight those of the following parameters that do *not* facilitate folding of peptides and proteins into strongly favored three-dimensional conformations:

hydrogen bonding between residues   shielding of hydrophobic residues from aqueous surroundings

entropy gains   placing hydrophilic residues at the core   placing hydrophilic residues at the periphery

ionic interactions between charged side-chains   stacking of aromatic rings

packing of one chain against another   overlap of orbitals containing $C\underline{O}$ lone pairs with other CO π^* orbitals

increased temperature   addition of high concentrations of guanidine hydrochloride

The folded shape of a large peptide or protein folds is called its *secondary* structure.

Within that structure there may be motifs of recognizable *primary* structures.

Types Of Helices
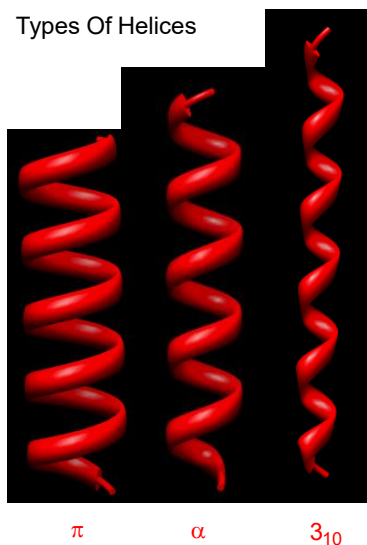
Amino acid blocks that coil into spring-like arrangements are called *helices.*

Almost invariably, these helical arrangements from L-amino acids spiral in a *right* handed corkscrew when viewed from *the N-terminus*.

Crystallographic studies reveal the "tightness" of these spring-like helical regions. Of all helical peptides, the *most* common, α-helices, have *3.6* amino acid residues per coil of the helix.

α-Helices are intermediate between π-helices that are the most compressed (least tightly wound) and 3_{10}-which are the most tightly wound. Label the three helices shown here as α, π, or 3_{10}.

Pro is rarely found in α, π, or 3_{10} helices because of the cyclic structure of this residue. However, it is formed in special polyproline helices found in *collagen*.

π α 3_{10}

Other elements of secondary structure include β-sheets. These are sandwich-like regions involving H-bonded sections of amino acids.

Parallel β-sheets have the strands running in *the same* directions.

Anti-parallel β-sheets have the strands running in *opposite* directions.

Types Of Extended Secondary Structures

Turns are secondary structure elements in which the strand loops back on itself.

If the strand forms a U-shape then they are *β-turns*, while *γ-turns* are more V-shape.

β-Turns are frequently used to connect two strands to form *antiparallel* β-sheets.
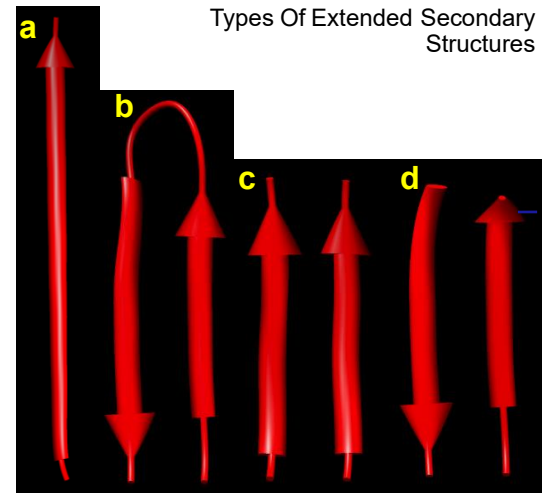
From the picture of extended secondary structures label the following from:

sheet-turn-sheet / parallel β-sheet / antiparallel β-sheet / β-strand

a __*β-strand*__   **b** ___*sheet-turn-sheet*____   **c** __*parallel β-sheet*__   **d** ___*antiparallel β-sheet*__

In the following structure, place a square around a turn region, a rectangle around a sheet region, and a circle around a helical region.
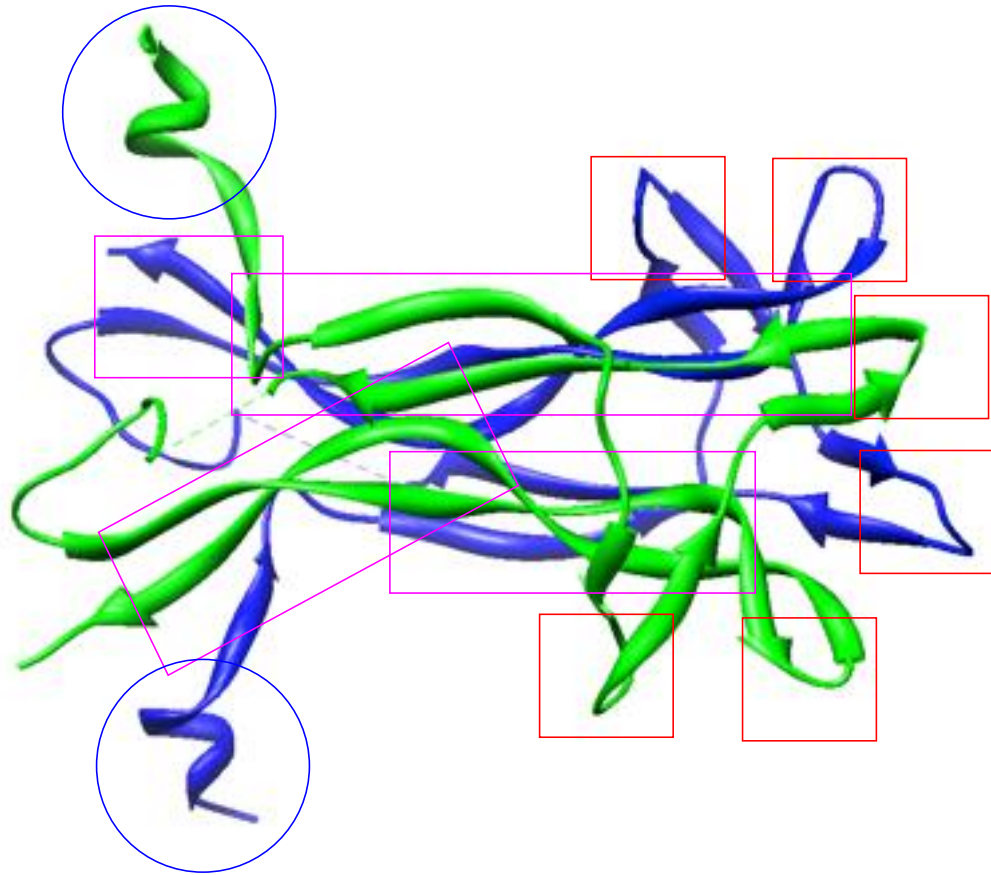
Identify the boxed structures in the following proteins as turns, helices, or sheets.

sheet	*turn*	*sheet*	*helix*
1	2	3	4

helix	*sheet*	*turn*	*helix*
5	6	7	8
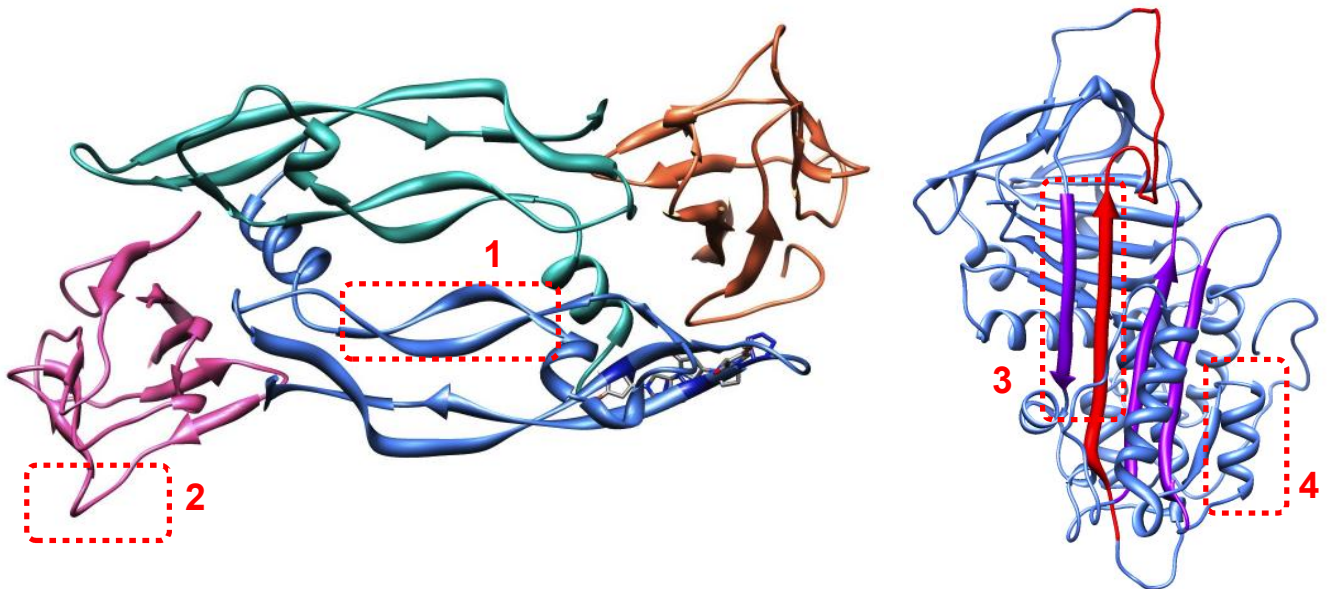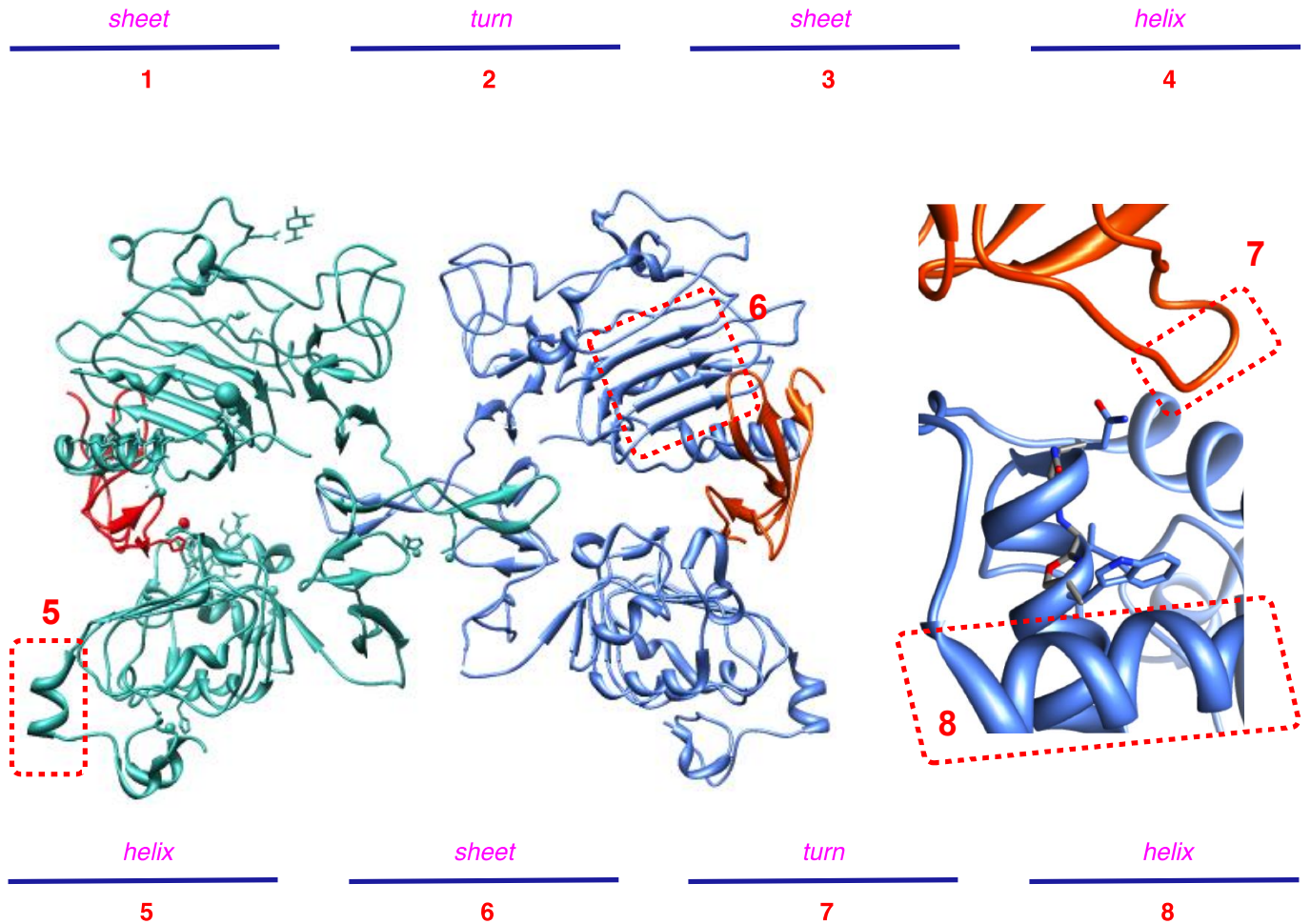
Tertiary And Quaternary Structures

Tertiary structure is the shape of a protein as a whole formed when the secondary structures assemble.
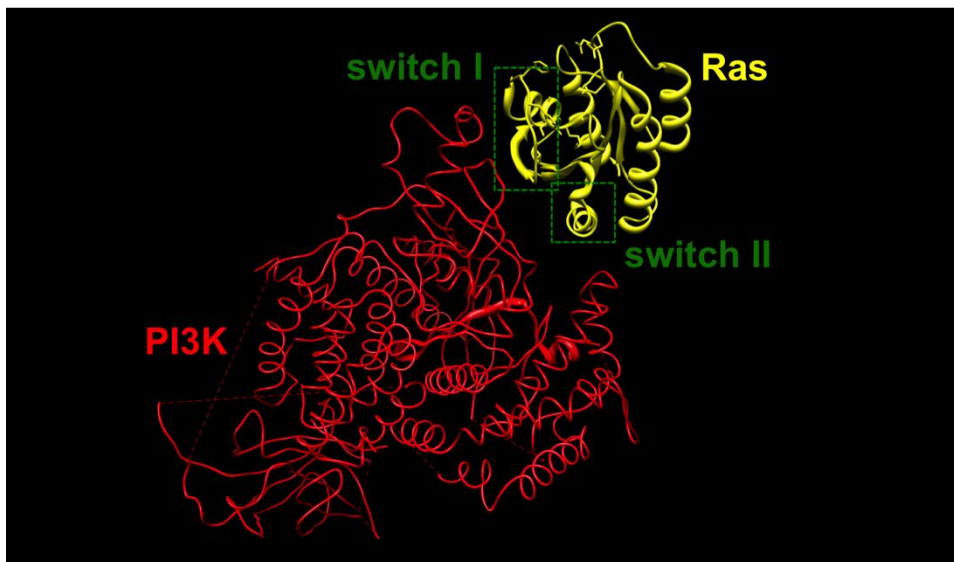
Quaternary structures form when protein units assemble into a macromolecular dimers or oligomers; these protein units usually *are not* covalently bound to each other (Figure).

Figure 1. Complex of the protein RAS with another one called PI3K. This illustrates the tertiary structures of both proteins, and the quaternary structure of the complex formed between them. RAS regions at the interface are called switch 1 and switch 2 domains. Mutations in RAS are common in cancer, and can be induced by cigarette smoke.

Constraints On Peptide And Protein Structures

Relatively unconstrained rotation about the ϕ and ψ bond angles means that most peptides of less than about 20 – 30 amino acids *do not* fold into recognizable shapes.

Some longer peptides, and many proteins can fold into different shapes. These shapes can be enforced by cyclization via an amide bond formed between the *C-* and *N*-termini. Illustrate this using amino acid abbreviations for the cyclic peptide from Orn, Leu, Phe, Pro, Val, D-Orn, Leu, Phe, Pro, Val (in that order; this is called gramicidin S. Fill in the molecular structures of L- and D-Orn as indicated.

cyclo(-Val-Orn-Leu-D-Phe-Pro-)$_2$

gramicidin S
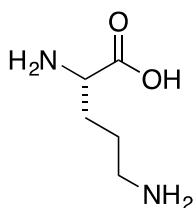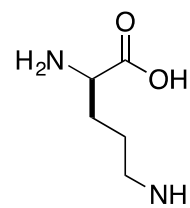
L-Orn   *D-Orn*

Another way peptides and proteins are made more rigid is via formation of *disulfide bonds* between side chains of *Cys* residues.

Chemically, disulfide bonds may be formed from thio-containing side-chains using many different mild *oxidizing* agents.

Reactions of thiols with halogens via a mechanism that involves nucleophilic attack of thiolates on the halogen, then nucleophilic attack on the halo-sulfur compound formed are easy to envisage. Draw the product of BOC-Cys-Cys-O^{t}Bu oxidation (use the BOC and ^{t}Bu abbreviations, but do not abbreviate the amino acids).

I$_2$
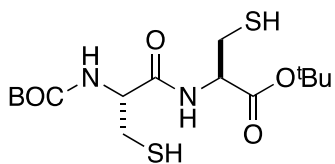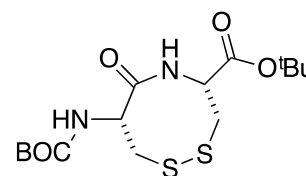
BOC-Cys-Cys-O^{t}Bu   *BOC-Cys-Cys-O^{t}Bu*

Use nomenclature like in the last question (*ie* using three letter codes) to denote the structure of H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH$_2$ after disulfide bond formation.

```
          S————————————S
          |            |
H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH₂
```
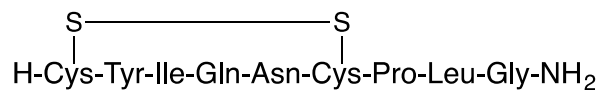
Indicate all the intramolecular disulfide linked peptides that might arise from oxidizing Ac-Cys-Ala-Cys-Ala-Cys-Ala-Cys-OH (use one letter codes; we count four including two diastereomers where the same Cys residues are linked).

```
    S————S   S————S
    |    |   |    |
Ac-Cys-Ala-Cys-Ala-Cys-Ala-Cys-OH
```

```
Ac-Cys-Ala-Cys-Ala-Cys-Ala-Cys-OH
    S———————|————S       |
            S————————————S
```

```
    S————————————————S
    |                |
Ac-Cys-Ala-Cys-Ala-Cys-Ala-Cys-OH
        |        |
        S————————S
```

```
Ac-Cys-Ala-Cys-Ala-Cys-Ala-Cys-OH
    S————————|————S       |
             S————————————S
```
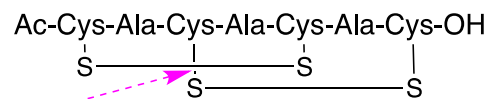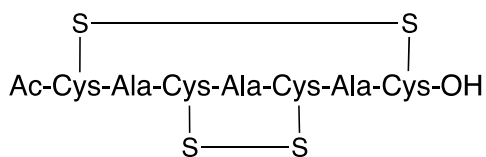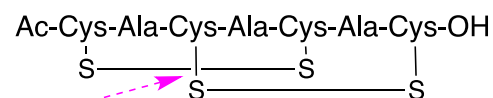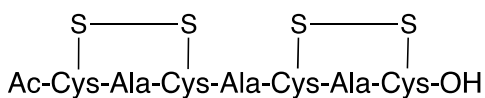
It *is* necessary to control disulfide bond formation when forming compounds like this.

Control of disulfide bond formation *could* be done by using different Cys *S*-protecting groups, chemoselective deprotection, and separate oxidation steps.

17 Hydridic Reductions

from chapter(s) _____ in the recommended text

Introduction

Focus

In the presence of the right catalysts, hydrogen tends to add across non-polarized double bonds (*eg* alkenes and alkynes) easily. However, if the bond is polarized then stepwise addition of hydride then proton is usually the favored reduction strategy. This section focuses on reductions of polarized functional groups via hydridic mechanisms.
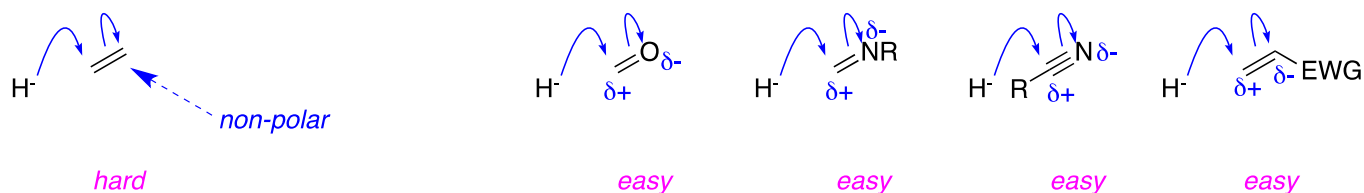
all give net addition of H_2 after protonation

(EWG = Electron Withdrawing Group)

Reasons To Care

Many organic reactions involve oxidation state changes and net addition of hydrogen is one of the most common reduction pathways. Sometimes in the lab, and almost invariably *in vivo*, it is not practical to achieve reduction using hydrogen gas. Organic chemists therefore achieve many reduction reactions by addition of hydridic agents then acidic work-up, while NADH and similar "gentle" reducing agents are used in Nature.

Concepts Involved

additions of nucleophiles to aldehydes and ketones • acylation reactions • oxidation and reduction processes

Objective

Hydridic reactions can be made to appear quite complex if we try to draw arrows to explain how a reagent "lets go" of a hydride and what becomes of the reagent residues. However, these issues are unimportant relative to the basic idea: addition of hydride placing the negative charge on an electronegative atom, then protonation. This section is designed to convey that simple concept

Mechanism

Stepwise reduction of unsaturated bonds can occur via addition of hydride, then a proton. Draw this generic process for the following substrates using clear curly arrows.
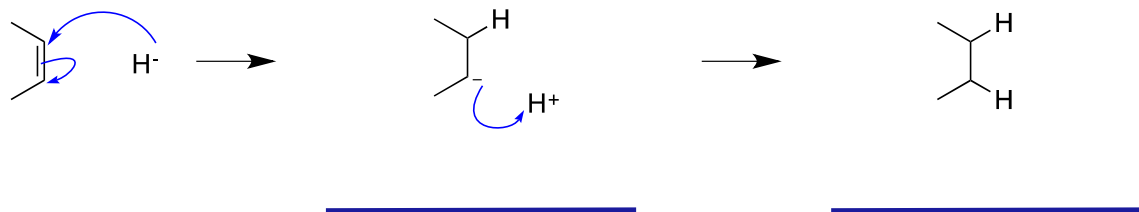
hard
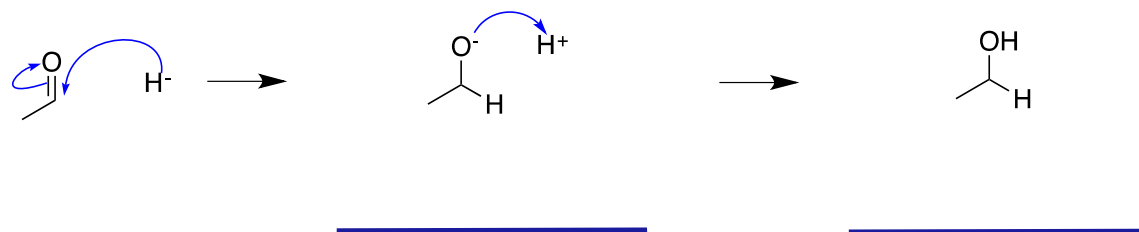
easy
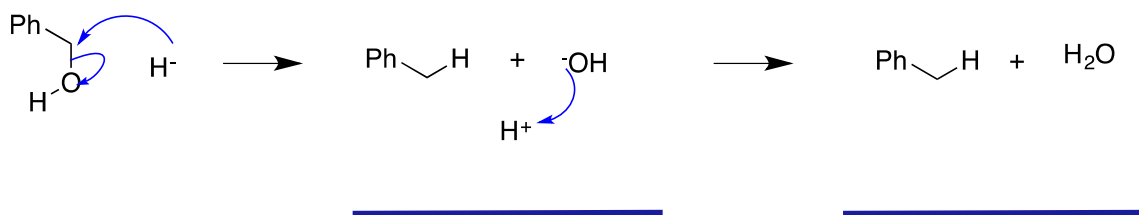
hard
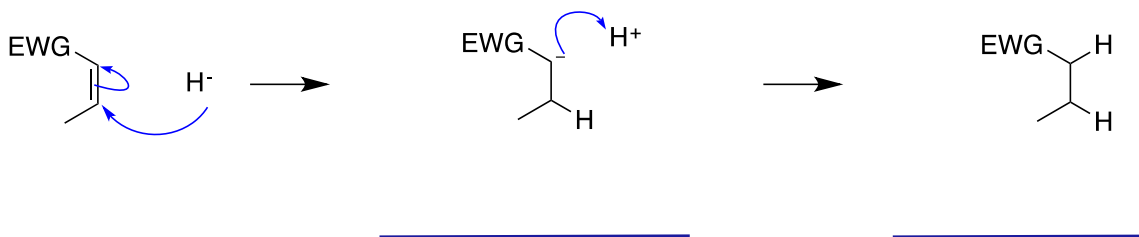
easy
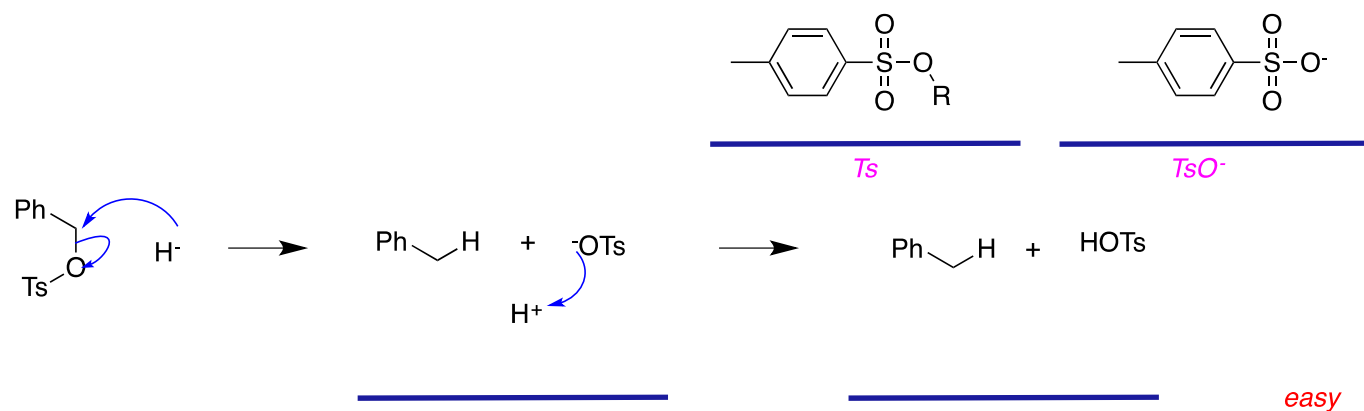
Three of the processes shown above are easy relative to the other two, indicate which ones. It follows that it is *easy* to reduce aldehydes and ketones to alcohols, but *hard* to reduce them to alkanes using hydridic agents.

Write the structures of a tosyl group (Ts) and a tosylate (TsO⁻).

tosylates are relatively good leaving groups in nucleophilic substitutions but *tosyl groups* are not.

Hydrogenations and hydrogenolysis reactions are basically *ionic* processes favoring different types of substrates, so it is usually possible to use these and hydridic agents to selectively reduce functional groups that tend to favor homolytic or heterolytic additions, respectively.

This is the basis of many *chemoselective* reductions in organic chemistry.

Just as there are many heterogeneous catalysts for adding hydrogen, there are many homogeneous reagents for adding hydride and they can have widely different properties.

Some simple metal hydrides, LiH, NaH and KH *do not* tend to be suitable for hydridic reductions.

For this section, assume an appropriate hydridic agent is used to deliver the number of hydrides indicated.

Substrate Scope

One Reduction

Fill in the gaps for the following mechanisms, and write clear curly arrows to describe the reaction flow.

alkoxide

alkoxide
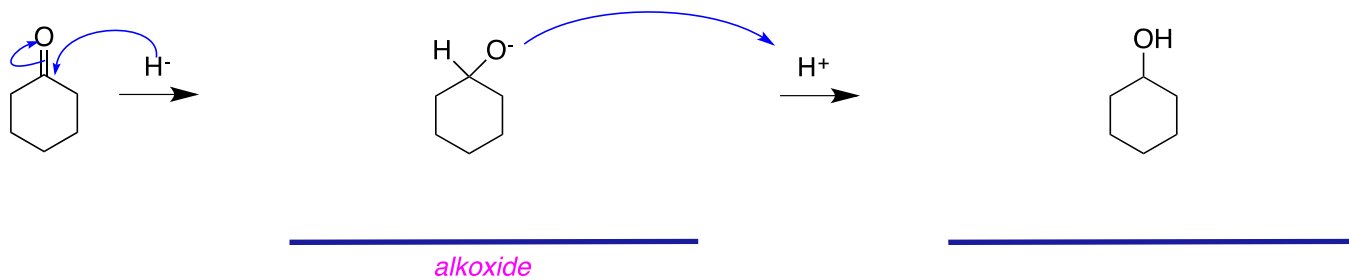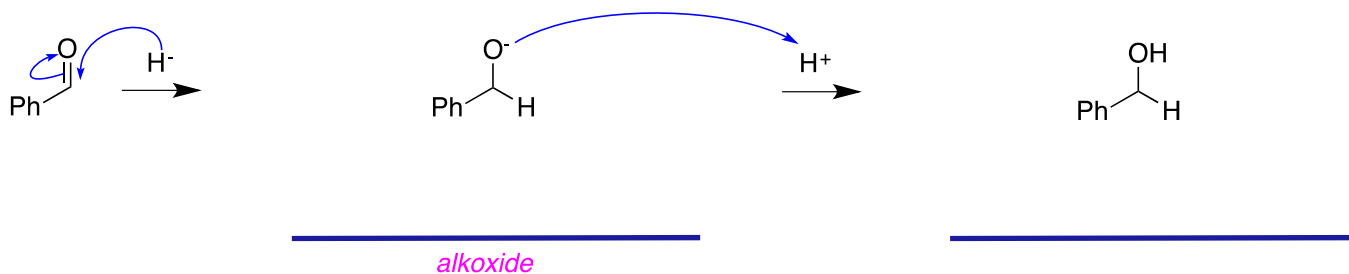
The preferred reagent for hydridic reduction of aldehydes and ketones is, in fact, *NaBH₄* because it is sufficiently reactive but not so much that it interacts violently with water.

amide anion

alkoxide
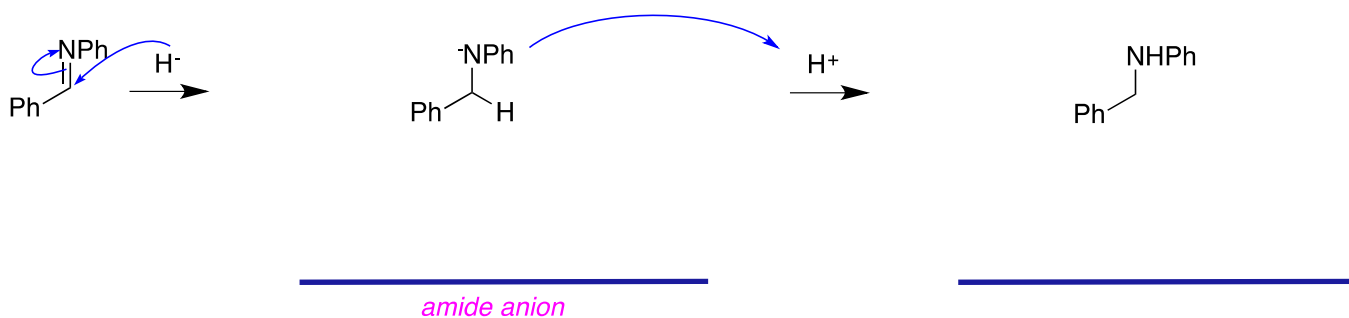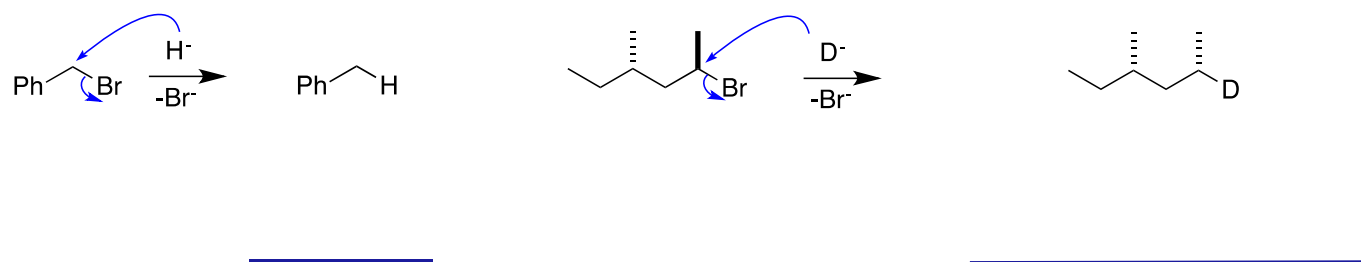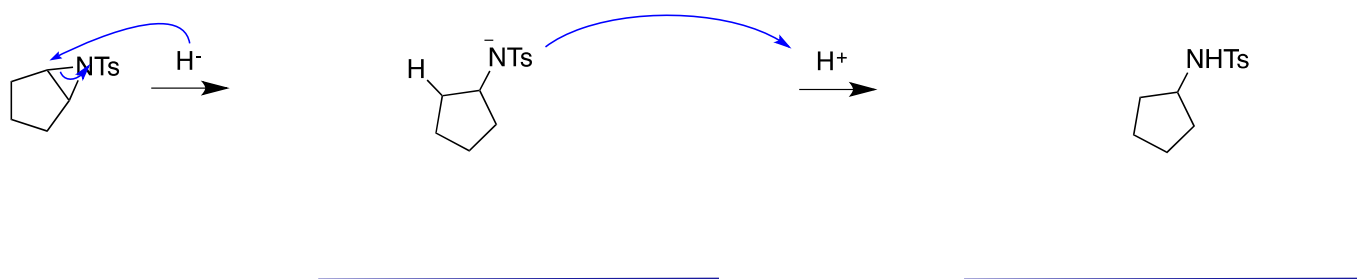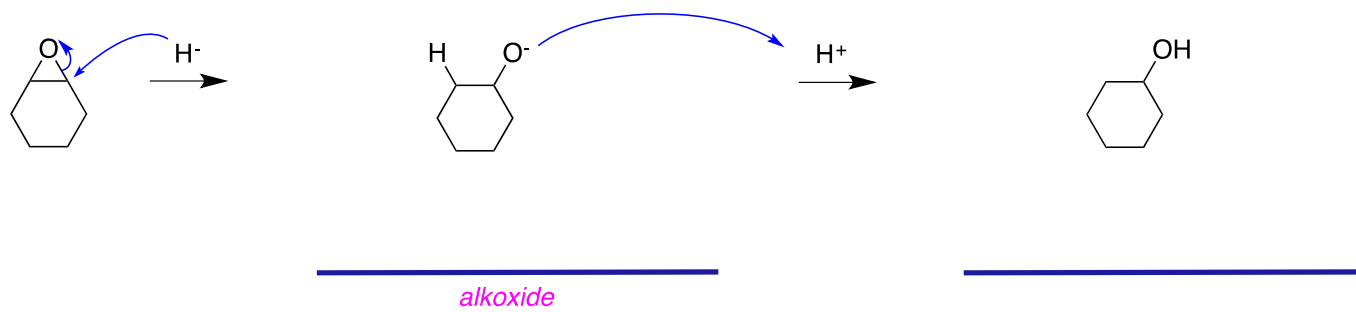
It takes *2* reduction steps to bring functional groups at the acid oxidation level down to the alcohol one.

Two Step Reductions

Show the mechanisms of the following reactions, using clear curly arrows. The true leaving group in many of these reactions will not be a free hydroxide, alkoxide, *etc.*, but the mechanisms are simplified to convey the concept of hydridic reduction.
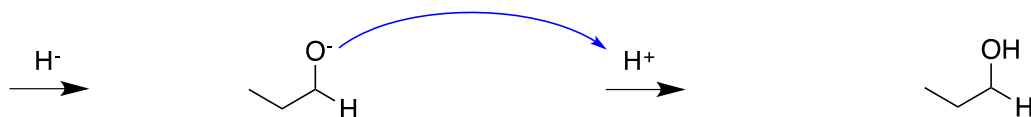
alkoxide
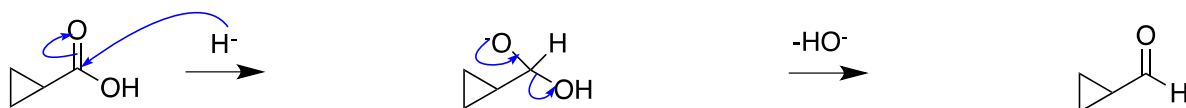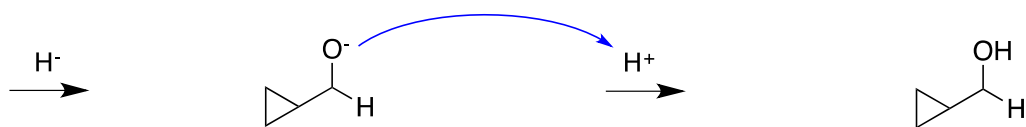
H⁻ →   O⁻   → H⁺ →   OH

_____   _____
          alkoxide

H⁻ →   ⟋N → propyl-N⁻ → H⁺ →   ⟋NH

_____   _____

H⁻ →   ⟋NH⁻ → H⁺ →   ⟋NH₂

_____   _____

Predict the products of the following reactions.

(i) H⁻ → (ii) H⁺

(i) 2H⁻ → (ii) 2H⁺

sulfone

(i) H⁻ → (ii) H⁺

imine

(i) H⁻ → (ii) H⁺

(i) H⁻ → (ii) H⁺

(i) 2H⁻ → (ii) 2H⁺

(i) 2H⁻ → (ii) 2H⁺

(i) 2H⁻
(ii) 2H⁺ →
-MeOH

(i) 2H⁻ reaction — γ-butyrolactone → HO—CH₂CH₂CH₂CH₂—OH

(i) H⁻ → HO—CH₂CH₂CH₂CH₂—CHO

(i) 2H⁻
(ii) 2H⁺
-OH₂   butyric acid → butanol

(i) 2H⁻
(ii) 2H⁺
-MeOH   Ph-CO-OMe → Ph-CH₂-OH

(i) 2H⁻
(ii) 2H⁺
-HCl   butyryl chloride → butanol

(i) 2H⁻
(ii) 2H⁺
-HCl   Ph-CO-Cl → Ph-CH₂-OH

(i) 4H⁻
(ii) 4H⁺

→   ∕∕OH

(i) 2H⁻
(ii) 2H⁺
-OH₂

Ph—NH₂

Ph⌒NH₂

(i) 4H⁻
(ii) 4H⁺
-2H₂O

⌒⌒OH   +   HO⌒

(i) 2H⁻
(ii) 2H⁺
-OH₂

(i) 4H⁻
(ii) 4H⁺
-2H₂O

HO⌒⌒⌒OH

(i) 2H⁻
(ii) 2H⁺
-H₂O

⌒⌒NH₂
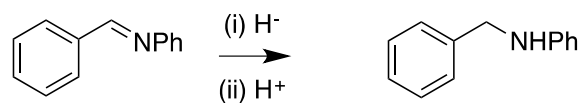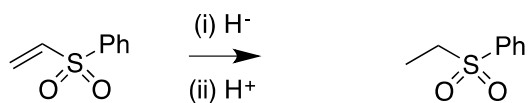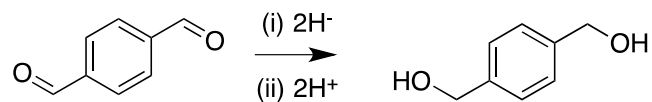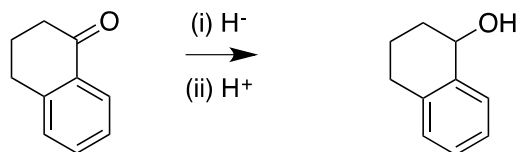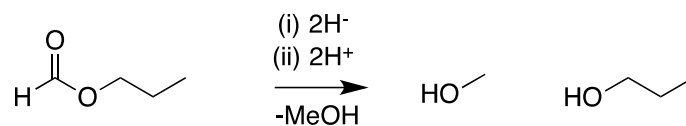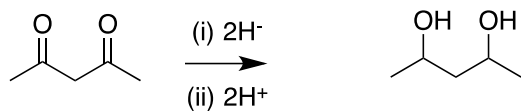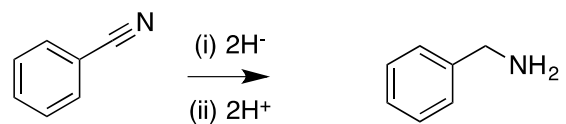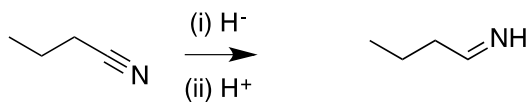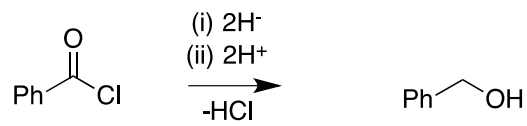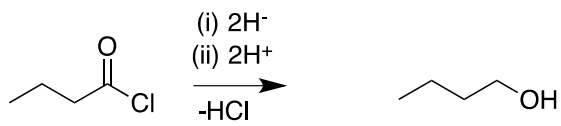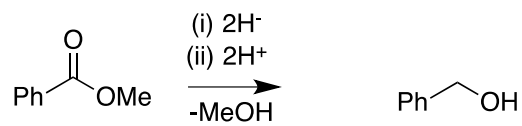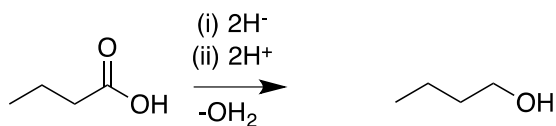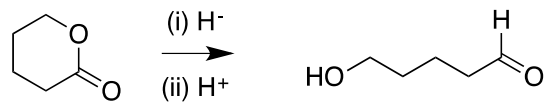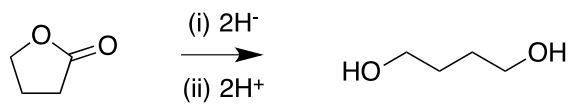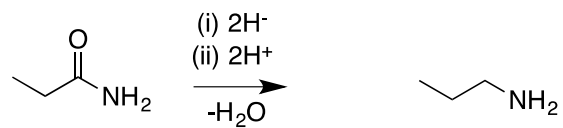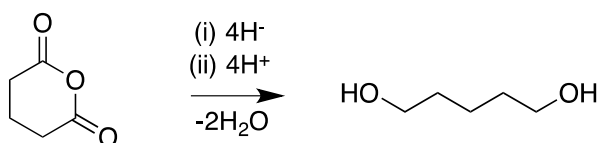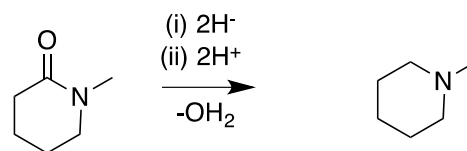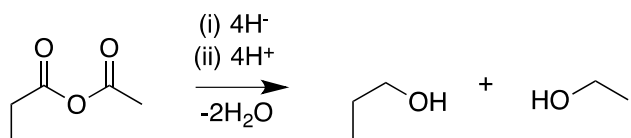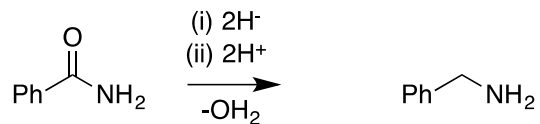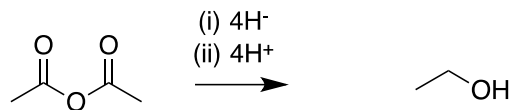
Difference Between Hydridic Reductions Of Amides And Esters

Outline mechanisms for the following hydridic reductions using clear curly arrows to depict electron flow.

a

tetrahedral intermediate   *aldehyde*

alkoxide

b

tetrahedral intermediate   *imine*

H⁻ →   [structure with H, N⁻]   H⁺ →   [amine structure with N, H]

amine

c

[structure: propanoyl amide with O, N, H] H⁻ → ([tetrahedral intermediate: O⁻, H, N, H] -MeHN⁻ → [aldehyde structure O, H with H⁻])

tetrahedral intermediate

H⁻ → [structure: O⁻, H] H⁺ → [structure: OH, H]

alcohol
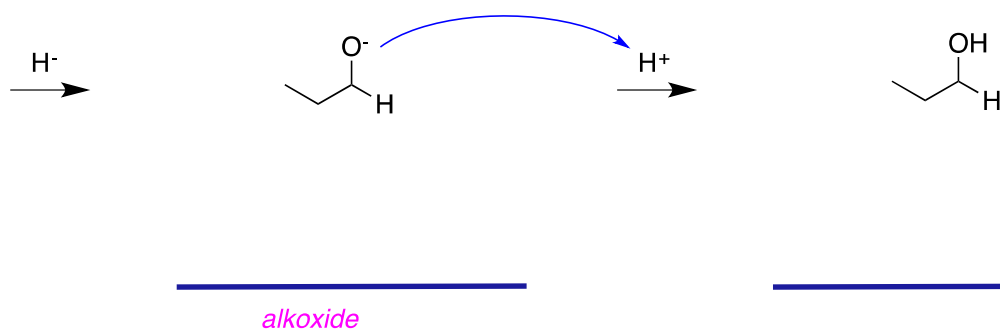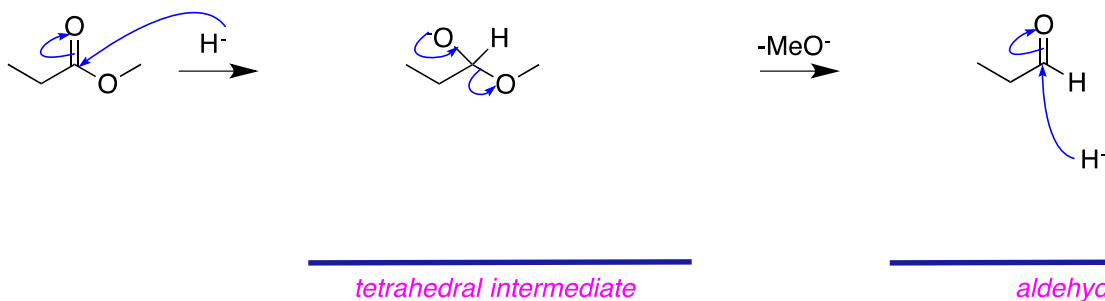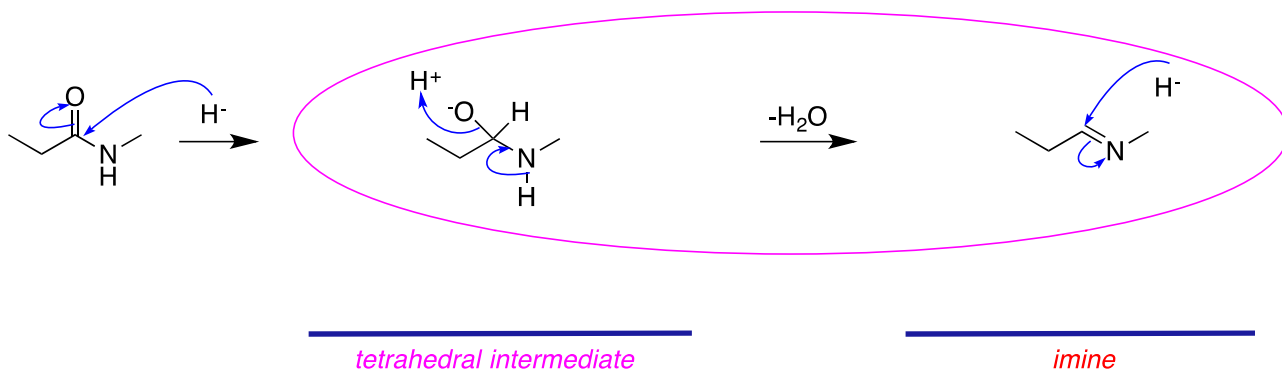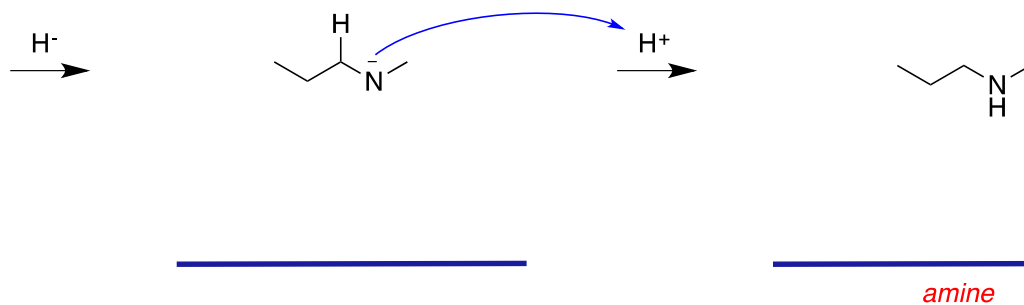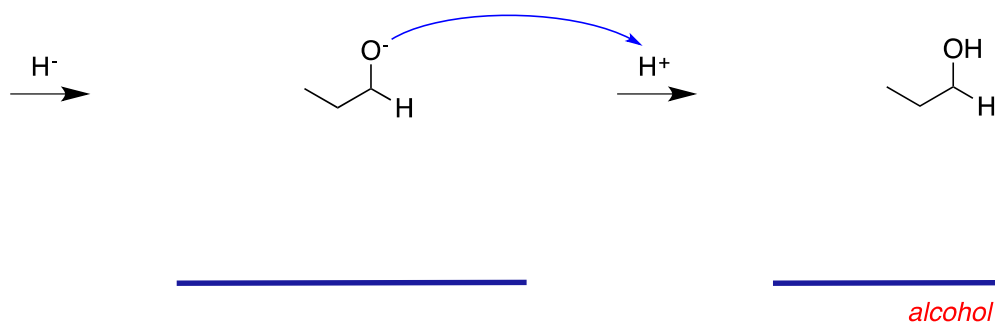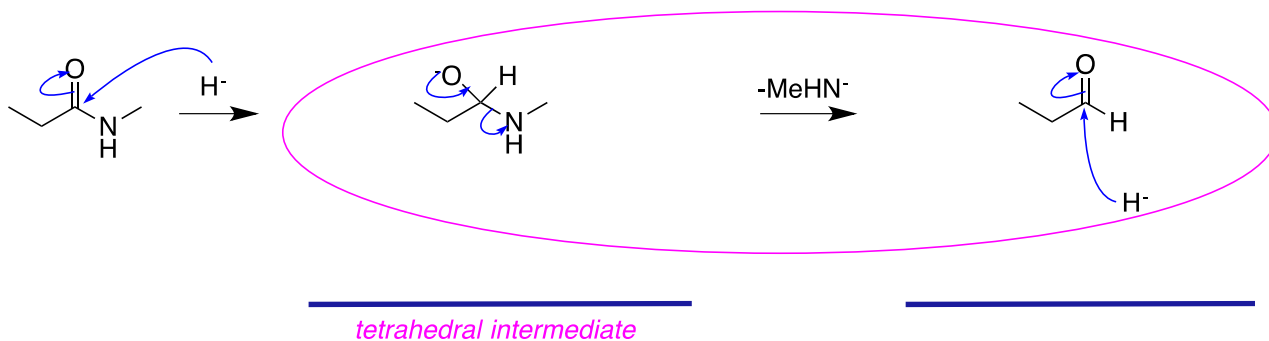
Pathway **a** is the one followed in reductions of esters. If reductions of amides followed the same route then they would proceed via pathway **c**; however, mechanism **b** is an alternative that differs from that for reduction of esters.

Highlight the first point of divergence in the mechanism above by circling that step.

In fact, hydridic reduction of amides tends to proceed via mechanism **_b_**. Suggest reasons:

Because the amine anion is not a good leaving group.

NADH: A Hydride Source *In Vivo*

Research the full name and structure of NADH, hence complete the following diagram.
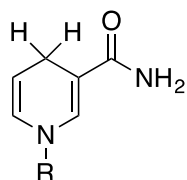
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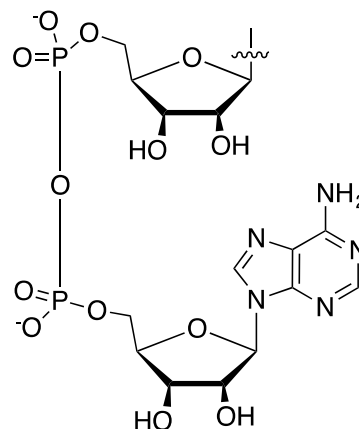
NADH full name: Reduced nicotinamide adenine dinucleotide

Draw arrows to express how NADH might donate a hydride to a protonated ketone.

+

by-product full name:
Nicotinamide adenine
dinucleotide (NAD)

The by-product in this reaction is *NAD*.

Draw the product of this ketone reduction.

pro-S
addition

+H+
-NAD
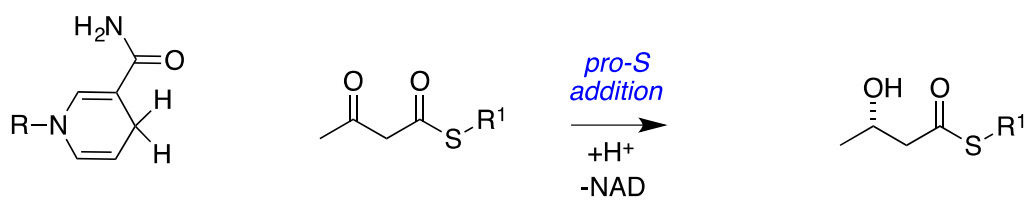
18 Reductions Via Electrons And Radicals

from chapter(s) _____ in the recommended text

Introduction

Focus

The first workbook in this series, and in this book up until here, has dealt almost exclusively with mechanisms that involve flow of two electrons. This section focuses on reactions involving movements of single unpaired electrons, *ie* radicals. The simplest radical is an electron, generated in, for instance, solutions of alkali metals in ammonia. Neutral radicals in organic chemistry may be involved in many processes characterized by a series of initiation, propagation, and (non-productive) chain termination steps. The focus of this section is on illustrative types of both processes.

electron + neutral gives radical anion   *radical + neutral gives radical*   *radical anion + neutral gives radical anion*
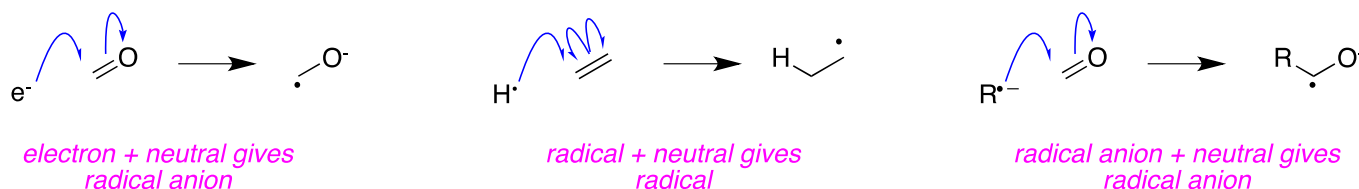
Reasons To Care

Most organic reactions in biological systems involve flow of electrons in two electron pairs, but some do not. For instance, formation of "reactive oxygen species" in and around cells leads to multiple complex reactions that can be detrimental to cell survival when the cell is stressed (*eg* during inflammation). In drug metabolism, the cytochrome p450 enzymes tend to use a battery of radical reactions to remove alien organic molecules from the system.

Concepts

fish-hook curly arrows • reductions and oxidations • chain reactions

Objective

This section uses some synthetic radical reactions that are well controlled and give predominant products to illustrate how.

Reductions Via Free Electrons

Addition Of One Electrons

An electron, e^-, has nothing to pair with, so in isolation it is an unpaired electron. Radicals are entities with an unpaired electron, and an isolated electron is a radical. However, it might more accurately be called a *radical anion* since it is not neutral.

Isolated electrons are different to hydrogen radicals that have nuclei consisting of a *proton* orbited by a single unpaired electron.

If an electron adds to a carbonyl group in a neutral molecule, then the product will be a *radical anion*. Show this process carefully using fishhook arrows.

e^-

e^-
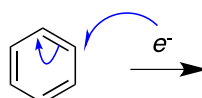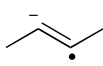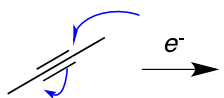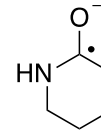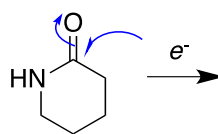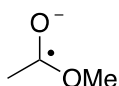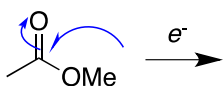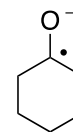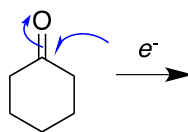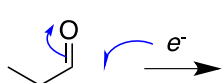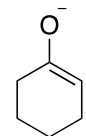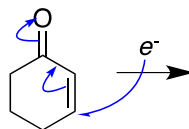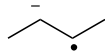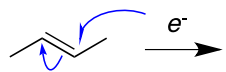
Addition Of One Electron Then Protonation

If a proton adds to a radical anion then the product is *a radical*. Show this for the following steps.
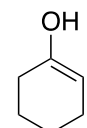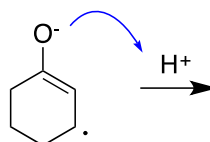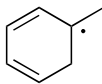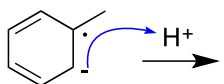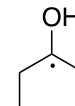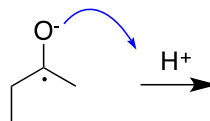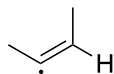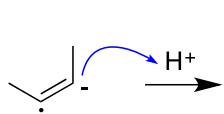
If an electron were to add to a radical anion then the product would be a *dianion*, but this addition is unlikely because of the electrostatic repulsion between the electron and the radical anion. This mechanism is shown in some books, but it is probably incorrect.

When a free electron adds to a radical the product is *an anion*. Show this for the following, showing clear fishhook arrows to depict the electron flow.
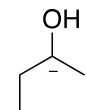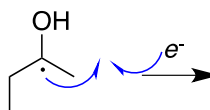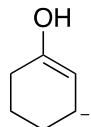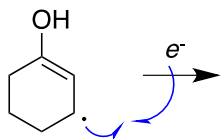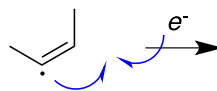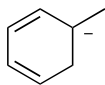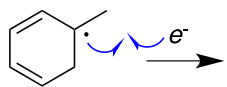
Overall, if double bonds undergo addition of an electron, a proton, an electron, then a proton again, then the net effect is *addition of two hydrogens*.

Show this mechanism for the following reductions.

e⁻ →

O

O⁻
•

H⁺ →

OH
•

e⁻

_____
radical anion

_____
radical

e⁻ →

OH
⁻

H⁺ →

OH

_____
anion

_____
product
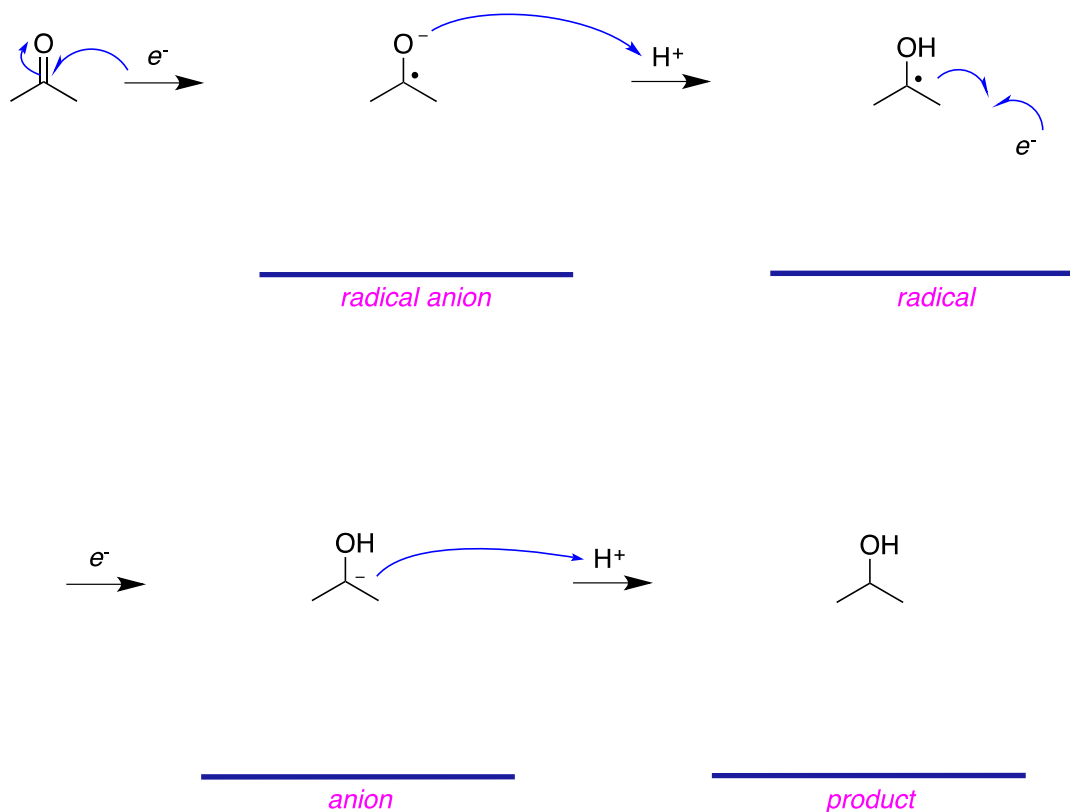
In fact, the reaction may take a different course if, for instance, the radicals self-couple before an electron adds, but these outcomes are dependent on the source of the electrons, and the conditions.

A way to generate single electrons is to use salts like samarium diiodide, SmI_2, that readily *oxidize* to Sm(3+) salts in this particular case.

Another way to generate single electrons is to dissolve sodium in mercury to give *sodium amalgam* where electrons liberated from the sodium are dispersed in the metallic solvent. This form of sodium can be used in the presence of protic solvents.

Amines and ammonia are not ideal media for substrates that may react with them (*eg* aldehydes), but for others it is ideal.

Sodium dissolves in liquid ammonia giving an intense *inky-blue* solution.

Ammonia is protic, so it is conceivable that electrons in ammonia would be protonated by the solvent; draw fishhook arrows to show this.

$$\text{Na} \xrightarrow[-\text{Na}^+]{\text{NH}_3} e^- \xrightarrow{\text{H}-\ddot{\text{N}}\text{H}_2} \text{H}^\bullet \quad + \quad \text{}^{\bullet\bullet}\text{NH}_2$$

hydride radical *radical anion*
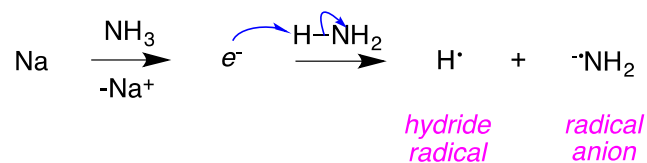
This process is *not* the dominant one; presumably formation of hydrogen radicals and *N*-centered radical anions is kinetically or thermodynamically unfavorable. However, when an electron adds to a double bond, then the product is a radical *anion*, and this *can* be protonated by ammonia.

Cycles Of Electron Addition Then Protonation

Reduction of internal alkynes in sodium/ammonia is interesting because the intermediate radical anion adopts a *trans*-geometry, and this is maintained in the product of the reaction.

Reduction of alkynes via stepwise addition of electrons is *equivalent* to hydrogenation with respect to double bond geometry of the alkene product.

Show mechanisms for the following reductions.

radical anion

radical

anion

product

$\xrightarrow{e^-}$

$\xrightarrow{\text{H-NH}_2}$

_____   _____
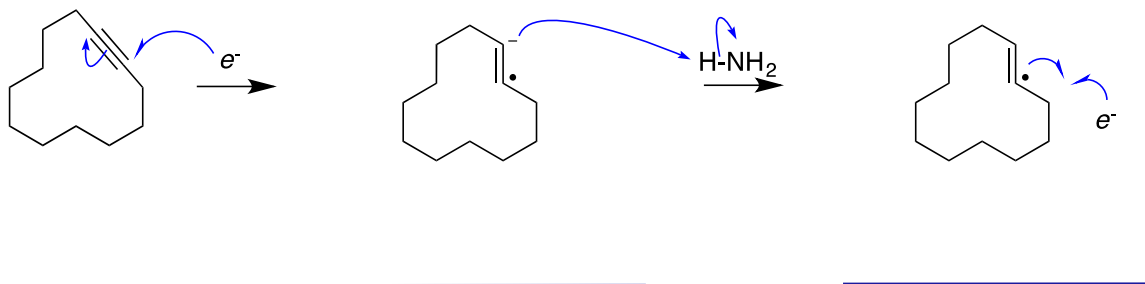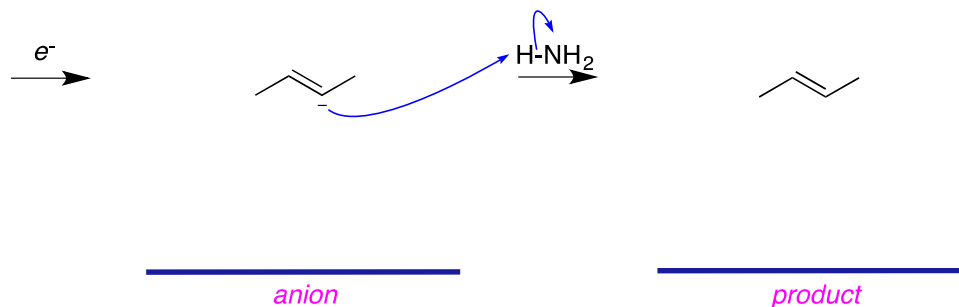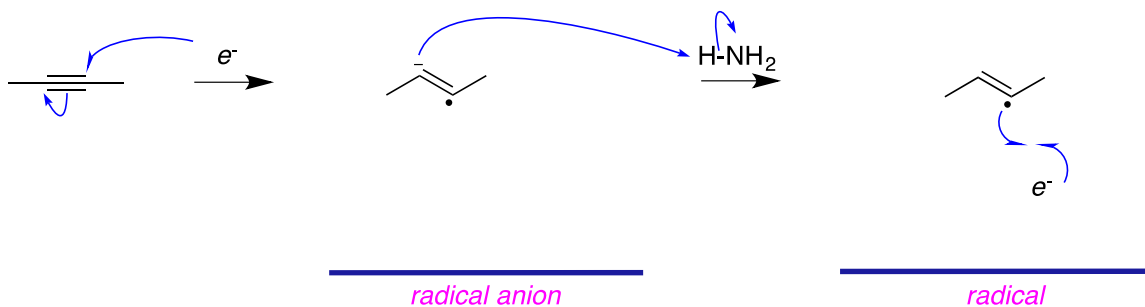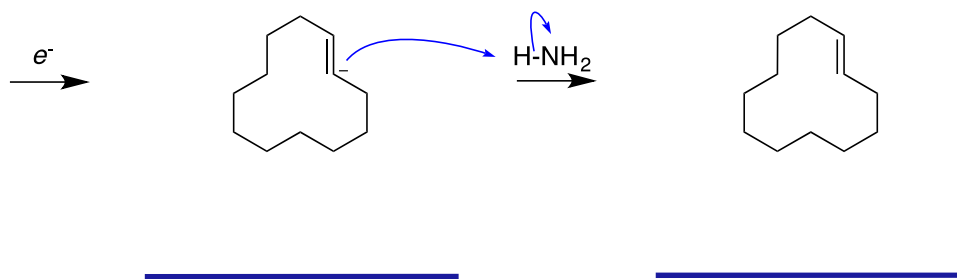
One electron reduction of aromatic compounds in liquid ammonia gives cyclohexadienyl radical anions.

Subsequently, they can add a proton to form *a radical*, then another electron to form *an anion* then finally a proton to achieve overall *2* electron reduction.

Show this process by carefully adding arrows to the following.

radical anion

radical   *anion*
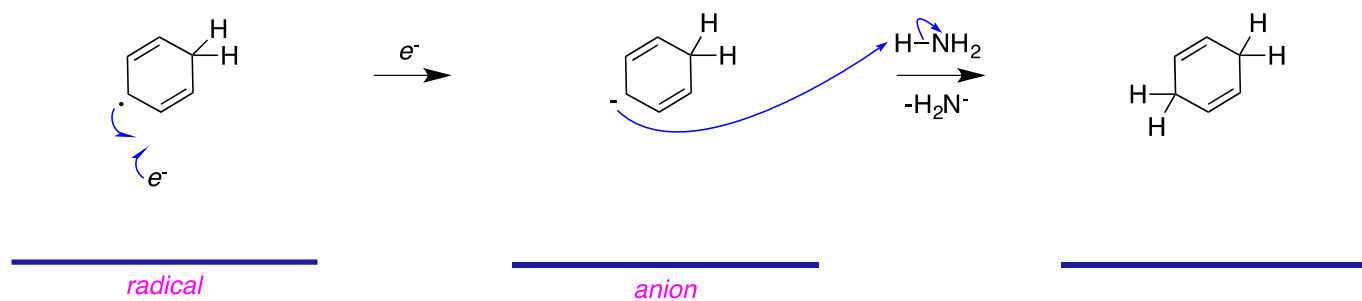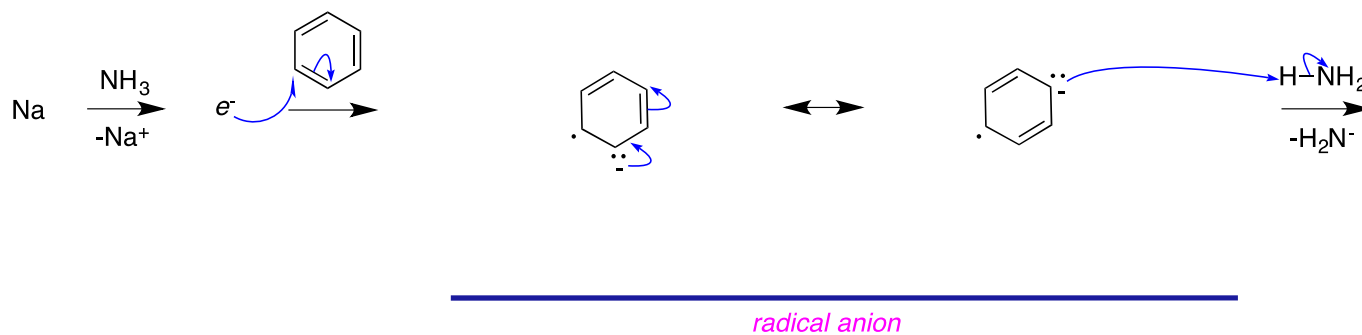
Protonation on the central carbon of the pentadienyl anion fragment is presumably favorable because that is where the *HOMO* has the greatest orbital size.

This process, the Birch reduction, produces *non-conjugated* dienes from aromatic compounds.

Complete the following, for dissolving metal reduction of 1,4-dimethylbenzene, consider the stability of the two possible radical anion and anion intermediates, hence predict the regioselectivity of the reduction.

radical anion

radical

anion
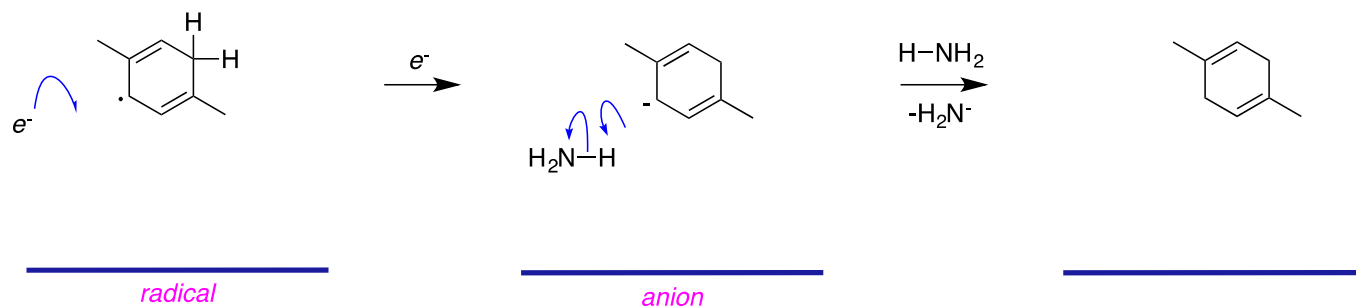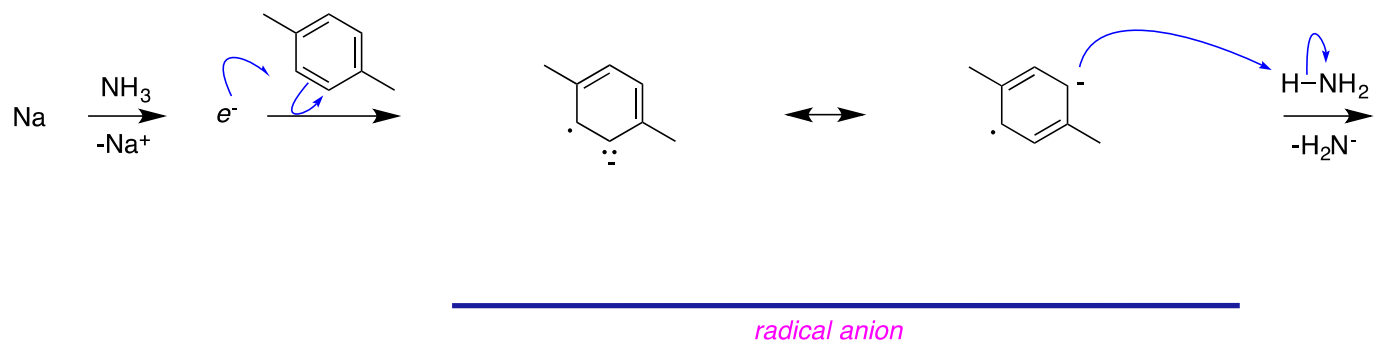
Reductions Via Radicals

Reductions can also be achieved using radical reactions. Imagine a hypothetical process wherein a hydrogen radical attacks iodoethane *on the iodine* to give a *C*-based radical. If that radical then combined with another hydride the overall effect would be net *reduction* of the substrate.

Show this by placing clear fishhook arrows on the following diagram.
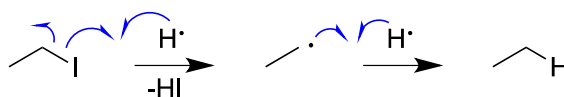
This reaction does *not* work efficiently. Outcomes of radical reactions are dominated by relative reaction rates, *ie kinetic* effects.

Catalytic amounts of hydrogen radical are required for the reaction above, and at such concentrations they would irreversibly recombine to hydrogen.

Reagents that can dissociate to form hydrogen radicals are used to overcome the problem outlined above. In the case of trialkyltin hydrides, the $R_3Sn\bullet$ that is formed can remove the iodide.

Complete the following mechanism showing clear curly arrows; label each step from the choices indicated.

$$H-SnR_3 \xrightleftharpoons{\text{homolytic fission}} H^\bullet \quad + \quad {}^\bullet SnR_3$$

chain initiation

_____

chain propagation

_____   _____
radical   *products*

Consumption of one trialkyltin radical by reaction with the iodide leads to generation of *another tin radical*.

Every SnR₃• that enters the process via the first transformation can be *regenerated many times*.

The overall reaction is temporarily slowed when hydrogen atoms collide with SnR₃•, or when two SnR₃• radicals combine; it is permanently retarded when hydrogen radicals combine to give hydrogen, but this will happen rarely because their concentration is *low*. Complete the following.

$$H^\bullet \quad {}^\bullet SnR_3 \xrightleftharpoons{\text{homolytic fusion}} H-SnR_3$$

$$R_3Sn^\bullet \quad {}^\bullet SnR_3 \xrightleftharpoons{\text{homolytic fusion}} R_3Sn-SnR_3$$

_____   _____

Show the mechanism for the following reaction.

chain initiation

H−SnR$_3$ ⇌ *homolytic fission* → H• + •SnR$_3$

_____

chain propagation

_____   _____
radical   *products*

chain termination

H• + •H ⇌ *homolytic fusion* → H−H

_____

$$H^{\bullet} \quad {}^{\bullet}SnR_3 \xrightarrow{\text{ homolytic }}_{\text{ fusion }} H-SnR_3$$

$$R_3Sn^{\bullet} \quad {}^{\bullet}SnR_3 \xrightarrow{\text{ homolytic }}_{\text{ fusion }} R_3Sn-SnR_3$$

The chain initiation and termination steps *would not* change as the substrate is varied.

Biosynthesis Of Prostaglandin H$_2$ (PGH$_2$)

Complete the following mechanism showing clear curly arrows throughout.

arachidonic acid

prostaglandin H$_2$ (PGH$_2$)
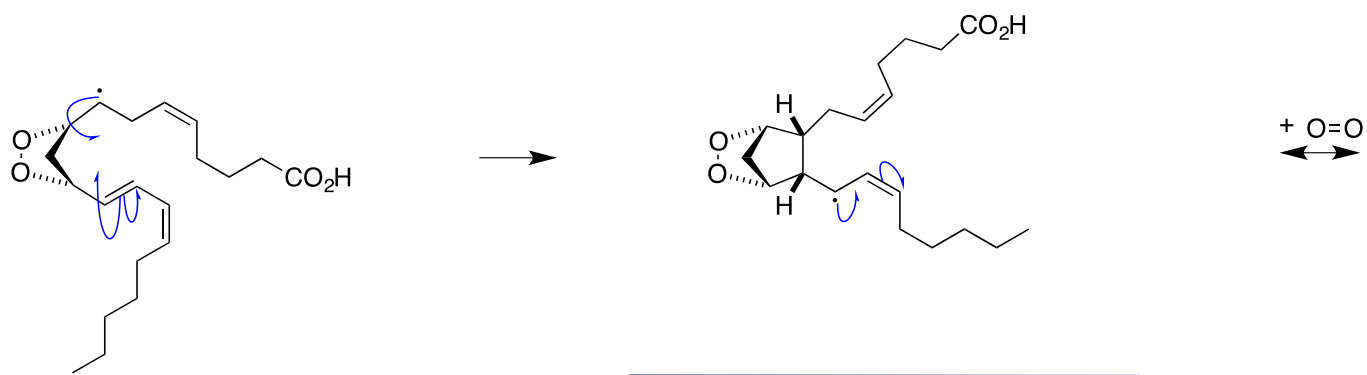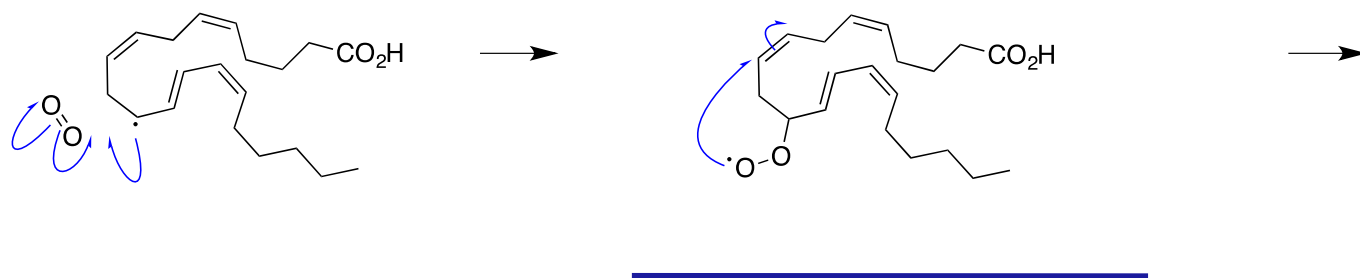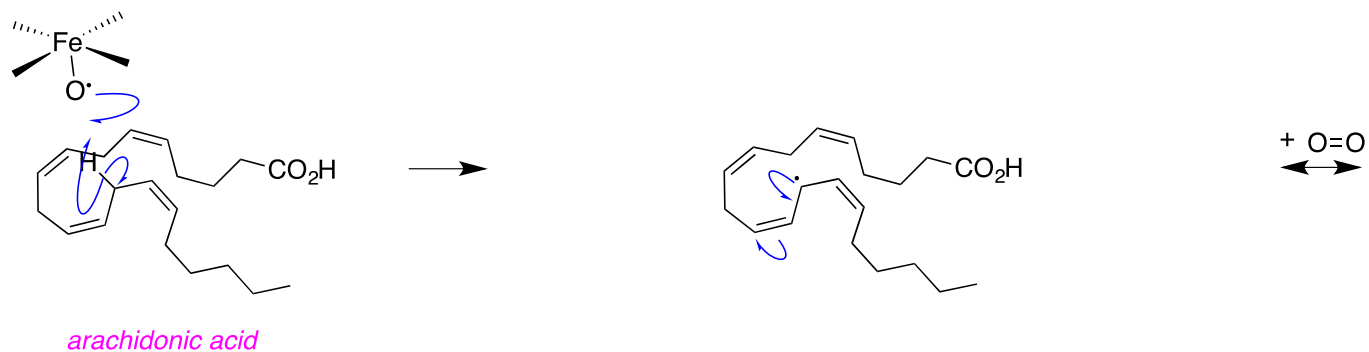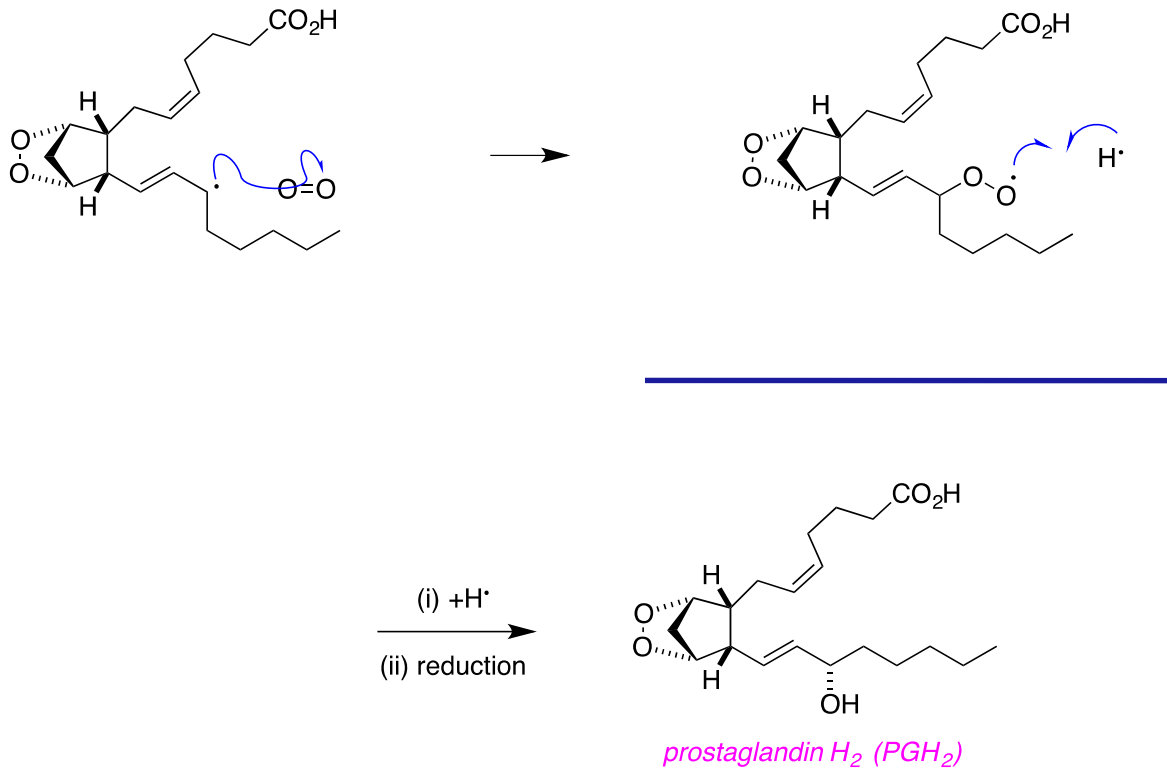
Prostaglandins *dilate blood vessels and are secreted in seminal fluid from the prostate gland* (circle all that is true).

Reactive Oxygen Species

Write mechanisms for the following reactions using clear curly arrows.

O=O $\xrightarrow{e^-}$ ·O–O⁻
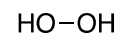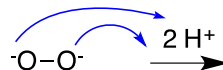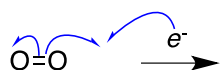
superoxide, radical anion

⁻O–O⁻ $\xrightarrow{2\ H^+}$ HO–OH

·O–O⁻ $\xrightarrow{e^-}$ ⁻O–O⁻

peroxide anion

·O–O⁻ $\xrightarrow{H^+}$ ·O–OH
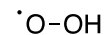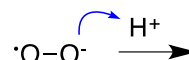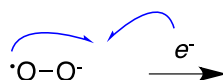
perhydroxyl radical

Reactive oxygen species are produced in the _endoplasmic reticulum and mitochondria and peroxisomes_ of cells (circle all that apply).

19 Oxidations

from chapter(s) _____ in the recommended text

Introduction

Oxidation reactions are diverse, but share some characteristics. Many involve addition of oxygen atoms; successive additions of oxygen atoms to amines reveal there is a logical correlation between oxidation and stepwise addition of [O], but it is somewhat obscured by loss of water in only some steps. All oxidations involve reduction of something else (usually a reagent, or sometimes an electrochemical cathode). Alcohol oxidations often involve formation of an "alkoxide" with a metal or some non-metallic elements in high oxidation states; deprotonation and elimination of the entity attached creates a *C=O* bond. Mechanisms can be proposed for these reactions that resemble *trans*-antiperiplanar E2 processes, or intramolecular synperiplanar eliminations. Aldehyde hydrates can be oxidized in much the same way as alcohols (formation of "alkoxides" then elimination), but this pathway is not open to ketones because they do not have hydrogen atoms attached to the hydrate carbon. In general, oxidations of ketones are harder because they involve cleavage of *C-C* bonds.

some oxidations have steps that resemble E2 eliminations

Reasons To Care

Nearly all the reactions discussed in this section are laboratory synthetic methods, but they are a primer for being able to recognize oxidations in all branches of the biological sciences.
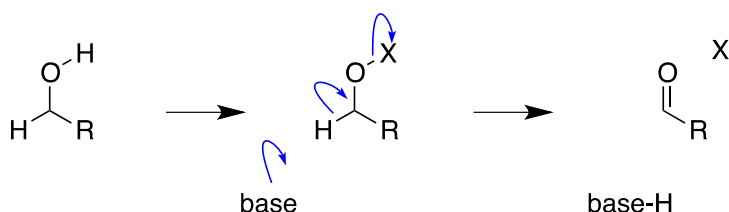
others involve migration reactions with a similar electron flow
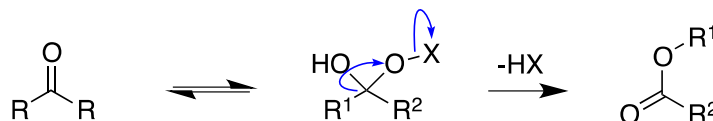
Concepts Involved

oxidation and reduction • E2 • *anti*- and *syn*-periplanar • epoxidation • migration reactions

Objective

This section is to highlight common mechanistic features of oxidation reactions, particularly oxidations of alcohols, aldehydes, and ketones.

Amine Oxidations

Whereas reductions in organic chemistry usually feature adding hydrogens, oxidations involve replacing hydrogen with a heteroatom. There is considerable scope for the types and connectivities of heteroatoms added in oxidation reactions, so there are a lot of different kinds.

Many oxidation reactions involve *increasing* the number of oxygen atoms.

Ammonia

Complete the following reaction sequences for oxidation of ammonia to nitric acid showing the intermediates. It involves inserting an oxygen atom between *N - H* bonds. Note that if there are two hydroxyls joined to nitrogen, the compound sometimes *dehydrates* to a more stable form, *ie HO-N-OH* often extrudes *water*.

Draw out each of the following products in three dimensions.

NH_3 $\xrightarrow{[O]}$ $HO-NH_2$ $\xrightarrow{[O]}$ $HO-\overset{H}{N}-OH$

_____   _____
hydroxylamine   *dihydroxylamine or azinic acid*

$HO-\overset{OH}{\underset{OH}{N}}$ $\xrightarrow{-H_2O}$ $O\!\!\nwarrow^{N}\!\!\searrow OH$ $\xrightarrow{[O]}$ $O\!\!\nwarrow\overset{OH}{\underset{}{N^+}}\!\!\searrow O^-$
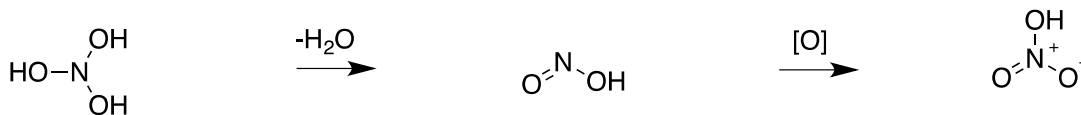
_____   _____   _____
trihydroxyamine   *nitrous acid*   *nitric acid*

Dihydroxylamine and azinic acid are *tautomers*.

A similar pathway is conceivable for *C-N* bonds with elimination of alcohols; in fact, amine *C-N do* tend to be oxidized in the same way as *H-N*, as shown in the following section.

Organic Amines

Deduce the consequences of oxidation of ethylamine in a series of steps in which oxygen atoms are inserted into one or more *N-H* bonds or simply added to *N*-atoms.
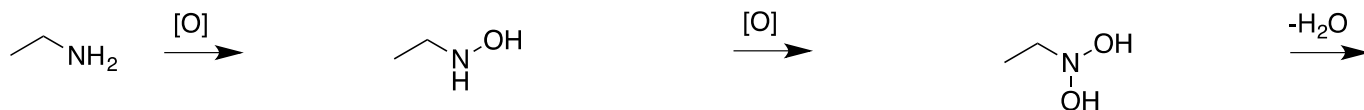
hydroxylamine   *intermediate*
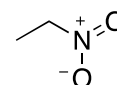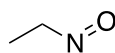
nitroso compound   *nitro compound*

Show the consequences of similar transformations for the secondary amine, piperidine, and the tertiary amine *N*-methylpiperidine, by completing the diagrams below.
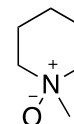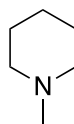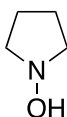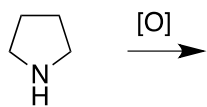
hydroxylamine   *N-oxide*

Overall, it is *easier* to oxidize bonds to hydrogen than to carbon.

Oxidations Of Alkenes And Alkynes Via Additions Of Heteroatoms

Modification of $C - C$ unsaturation by forming more than one $C -$ heteroatom bonds, or forming $C - C$ multiple bonds, is *oxidation*.

For the following additions, show the increase or decrease in oxidation levels at one of the carbons involved in the change (*eg* oxidation of an alcohol to a ketone would be +1, but reducing it to an alkane would be -1).
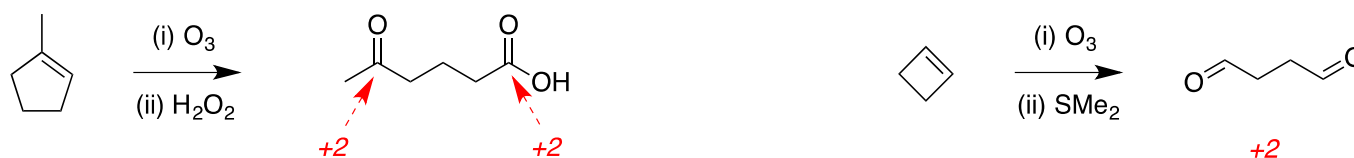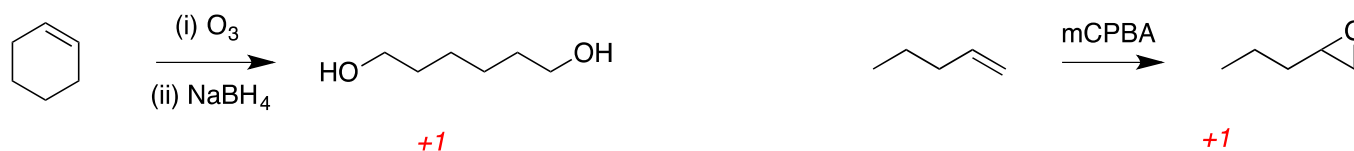
Oxidation States Of Common Oxidants

Write the oxidation state of the red atoms in the reagents below.

+5   +4   +6   +6   +6   +7   +7   +1
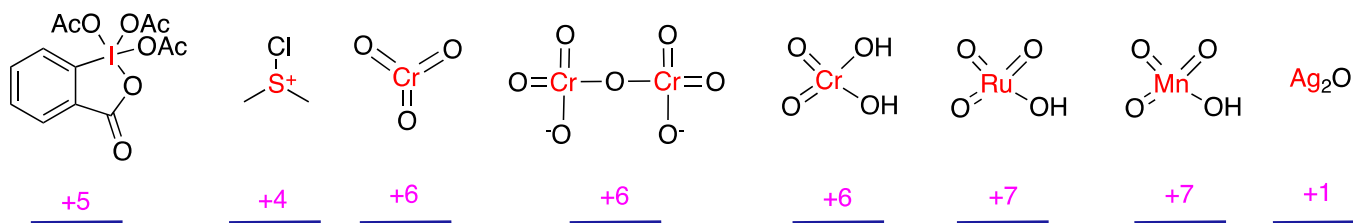
These are relatively *high* oxidation states for these elements.

Dehydrogenation Reactions

Dehydrogenation of alkanes is an *oxidation* reaction.

Catalysts lower the activation barrier for reactions, and *do not* influence the thermodynamic states of the reactants and starting materials.

If a hydrogenation catalyst lowers the activation energy barrier for addition of hydrogen to an alkene, it *does* lower it for the reverse reaction.

This is called *The Principle of Microscopic Reversibility*.

In practice, catalysts that mediate hydrogenation of alkenes can also promote dehydrogenation of the alkane products, but only if the *kinetic* barrier for the reverse reaction can be surmounted under those conditions.

If the kinetic barrier can be surmounted, dehydrogenation products will only be formed in a ratio that reflects the energy difference of the products (including hydrogen) and the starting materials. Dehydrogenation reactions become easier if hydrogen is *enclosed* from the system.

It is *easier* to find practical catalysts for hydrogenation reactions than dehydrogenation reactions because the energy barrier to the process involving loss of hydrogen is *greater*.

Heat Of Hydrogenation

Energy is *liberated* when hydrogen is added across a *C=C* bond, because alkenes are *less* stable than the parent hydrocarbons.

The amount of energy liberated on hydrogenation of different compounds to give *the same* product *can* be used to gauge the relative stabilities of the starting materials; energies involved in such processes are called heats of *hydrogenation*.

Complete the following diagram to show the relative energies of cyclohexane, cyclohexene, and 1,3-cyclohexadiene, given that their heats of hydrogenation are 0, -118, and -230 kJ•mol^{-1}, respectively

energy
kJ•mol^{-1}

200

-230 kJ/mol

-118 kJ/mol

100

0 kJ/mol

0
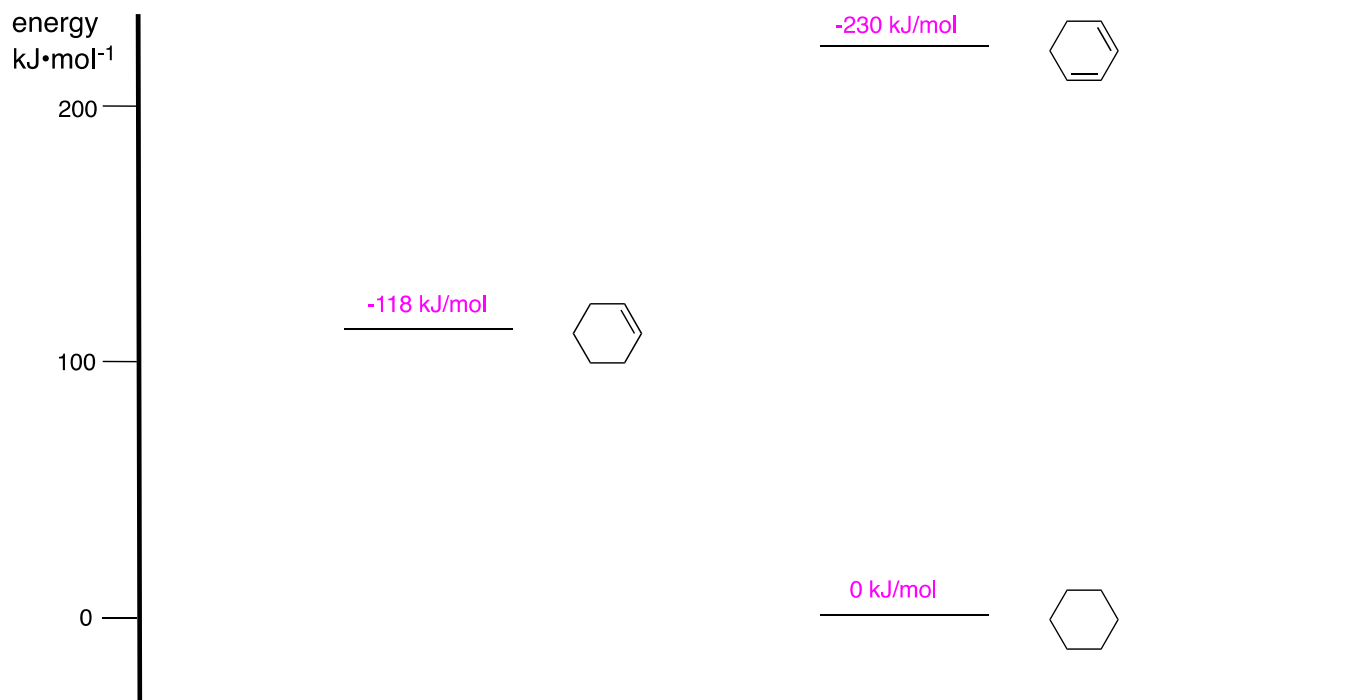
Based on heat of hydrogenation data, it is thermodynamically *downhill* to dehydrogenate an alkane to an alkene.

Dehydrogenation reactions can be achieved by simultaneously reacting the hydrogen formed with something else in the same reaction mixture (*eg* another alkene).

Dehydrogenation reactions are *oxidation* reactions; they are less common in laboratory scale organic chemistry than reductions.

Oxidation Of Alcohols

Catalytic Dehydrogenation

There are heterogeneous catalysts that will dehydrogenate, and therefore *oxidize* alcohols to the corresponding aldehydes or ketones.

Complete the following diagrams.
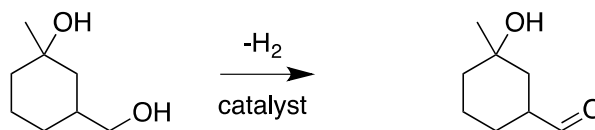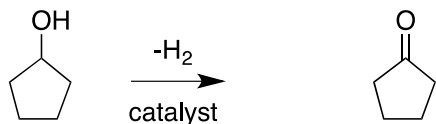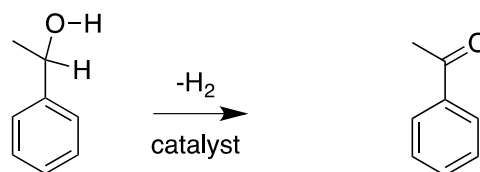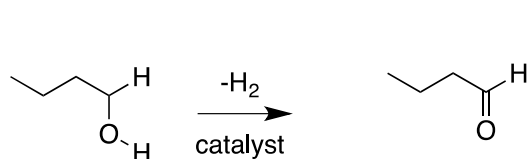
Alcohols have to be *primary / secondary* and not *tertiary* for this reaction to be possible (circle all that apply).

In general, and not just for catalytic dehydrogenation reactions, it is much harder to oxidize *tertiary* alcohols than *primary / secondary* ones.

In laboratory scale chemistry, catalytic dehydrogenations of alcohols are rarely used because there are numerous convenient alternatives that involve elimination from alkoxides.

Elimination From Alkoxides: A Mechanistic Commonality Between Many Alcohol Oxidations

Imagine a process in which an alcohol interacts with a metal salt (dicationic, for example) to form an alkoxide. Elimination then occurs via loss of a *CHOH* proton to form a carbonyl as follows.
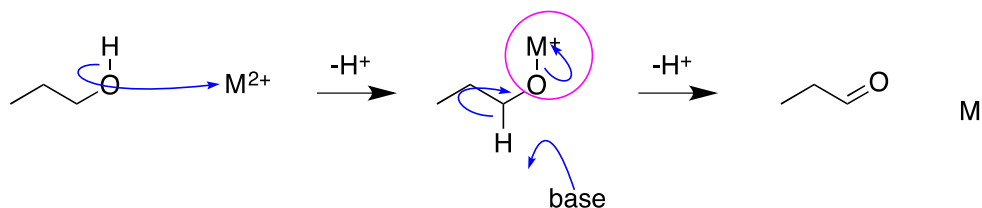
The process shown above is similar to the *E2* mechanism.

In this process, the metal is *reduced*; circle the key arrow that is responsible for that.

Based on that arrow, good oxidants in this process probably involve metals in *high* oxidation states, so that it is most easily *reduced*.

It is *harder* to change Cr(2+) into Cr(0), that it is to convert Cr(6+) into Cr(4+), for example.

In practice, the "metal" can be a transition metal like manganese, chromium, or ruthenium, but it can also be a non-metallic element in a *high* oxidation state.

For the following hypothetical reactions, show clear curly arrows, intermediate and the products, including the heteroatom used to make the alkoxide; it is important to show its oxidation state at the end of the reaction (*eg* what becomes of the S^{2+} in the first reaction).
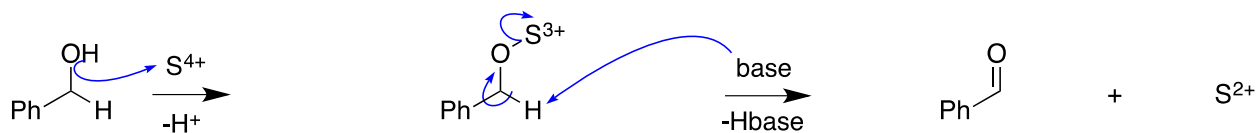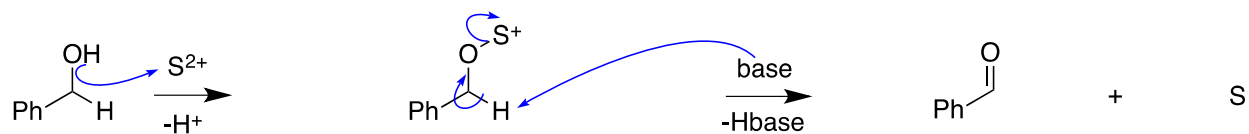
alkoxide
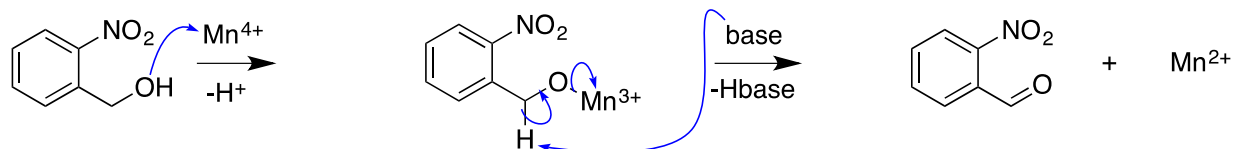
alkoxide
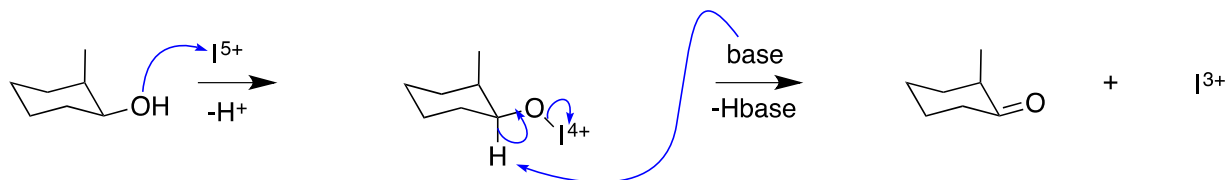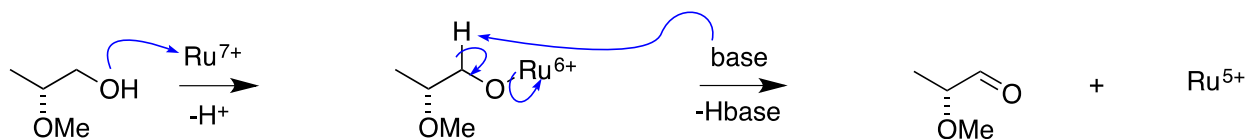
alkoxide

alkoxide

Draw curly arrows and predict products from oxidation reactions that involve the following key steps; identify for each if the step that removes the proton is intramolecular or intermolecular. In many cases it is uncertain if the reaction is intra- or inter-molecular, so both are shown here.
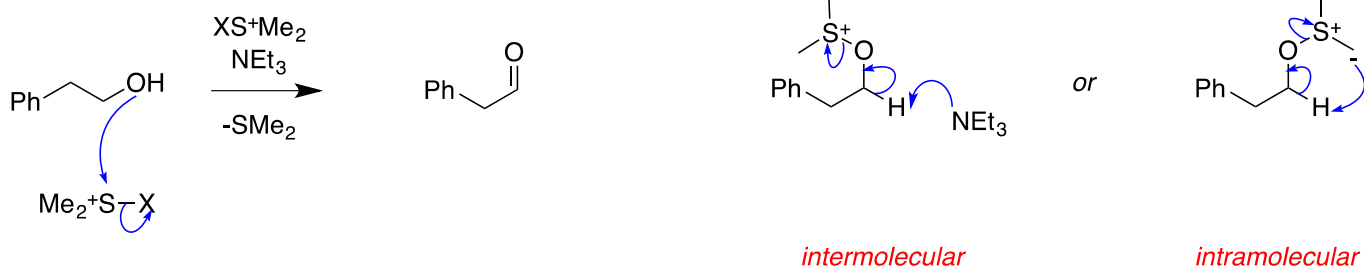
intermolecular   *intramolecular*
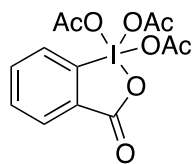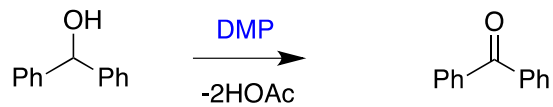
DMP
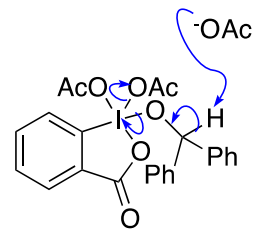
intermolecular
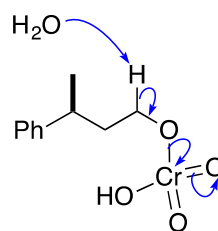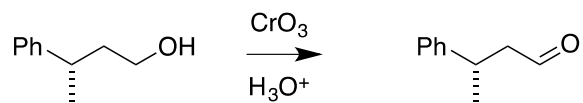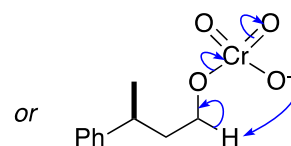
intermolecular   *intramolecular*

The last example, below, is special because the metal is not reduced, but a ketone ligand is. The initial product is an alkoxide of the product alcohol

Al(O^{i}Pr)$_3$
MeCOMe
-HO^{i}Pr
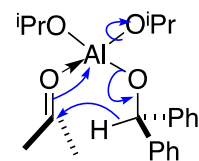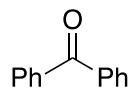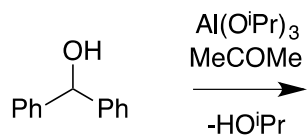
intramolecular

Oxidation Of Aldehydes

Aldehydes and ketones often are oxidized under conditions that favor an equilibrium amount of hydrate formation; alkoxides formed from these hydrates may eliminate just as in the previous section, except that the product automatically forms in the acid oxidation state.

Show this process, with clear curly arrows, for the following reactions.

hydrate

alkoxide

indicate what happens to the metal here

hydrate

alkoxide

$$\xrightarrow[\text{-Hbase}]{\text{base}}$$

Ph–CH$_2$CH$_2$–C(=O)OH   +   Mn^{5+}

_____
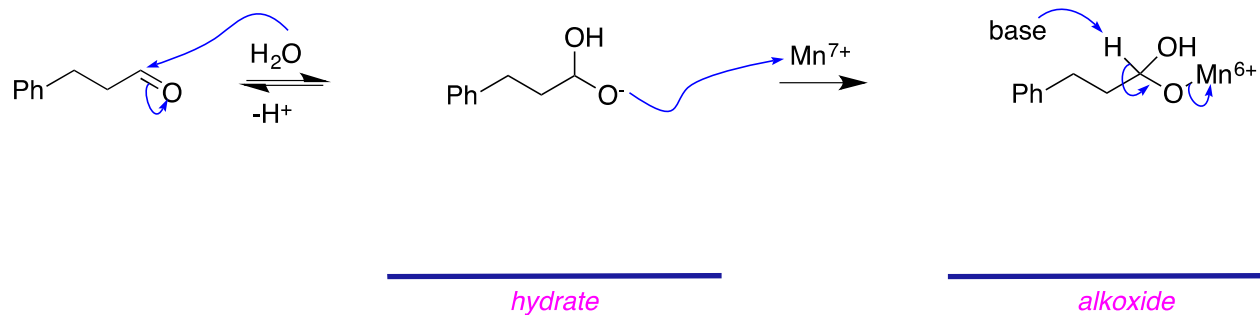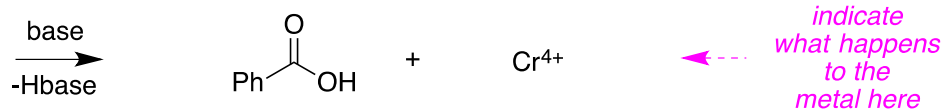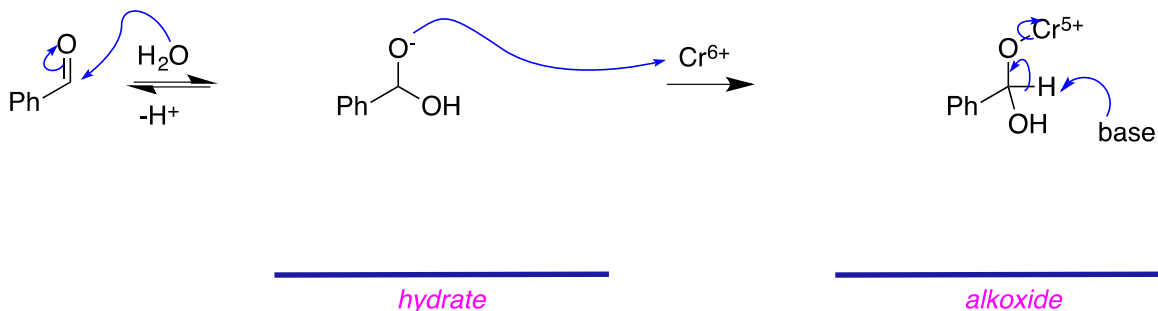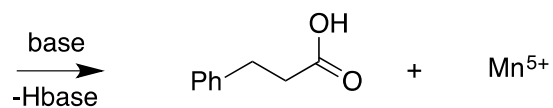
Ketones *cannot* be oxidized in a similar way because they *do not have* α-C*H* atoms.

The following reaction does *not* involve hydration, but it has a similar feature. Show the mechanism with clear curly arrows.

$$\underset{\text{HCN}}{\rightleftarrows}$$

$$\xrightarrow[\text{-Cr}^{4+}]{\text{Cr}^{6+}}$$

alkoxide

$$\xrightarrow[\text{-Hbase}]{\text{base}}$$

$$\xrightarrow[\text{-CN}]{\text{H}_2\text{O}}$$
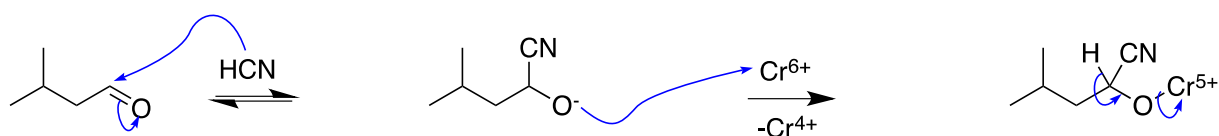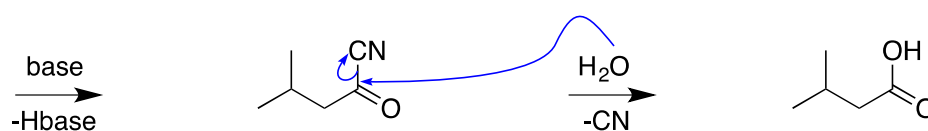
acyl nitrile

Write the structures of chlorous acid HOClO, and hypochlorous acid, HOCl.

$$O=\overset{Cl}{\underset{O}{}}{\overset{}{O}}-H \qquad\qquad H-\overset{O}{\underset{Cl}{}}$$
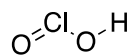
<u>*chlorous acid*</u>
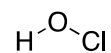
<u>*hypochlorous acid*</u>

Deduce the mechanism of oxidation of the following aldehyde by chlorous acid that involves intramolecular abstraction of an α–C<u>H</u> by an oxygen attached to a chlorine.
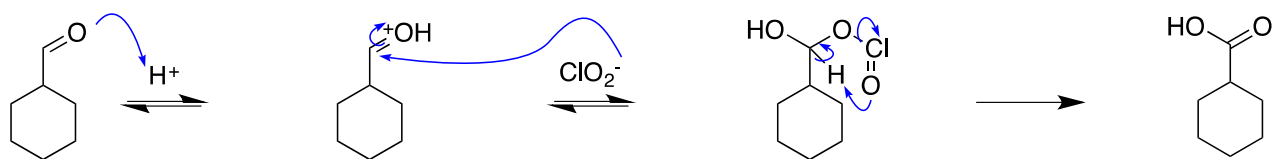
<u>*"alkoxide"*</u>

Oxidation Of Ketones

Ketones can be oxidized via attack of peroxide then rearrangement via alkyl migration. This reaction is similar to oxidation of boranes with hydrogen peroxide. Draw curly arrows on the following reactions to illustrate the electron flow.
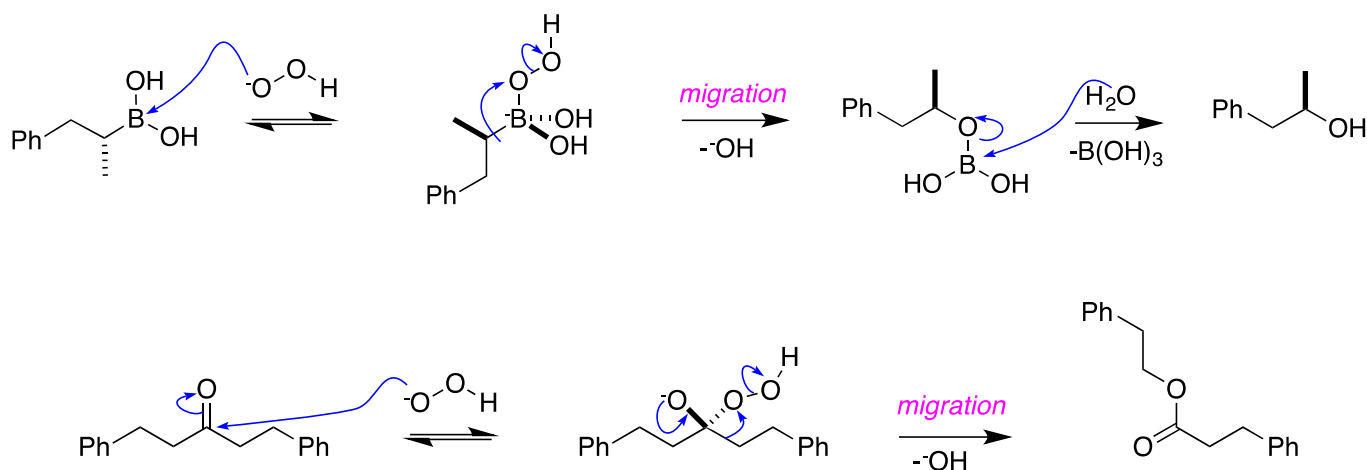
The alignment of the migrating group and the bond cleaved is *anti*-periplanar in these migrations.

Draw mechanism and predict products for the following reactions where the group best able to support positive charge migrates, and with retention of stereochemistry.
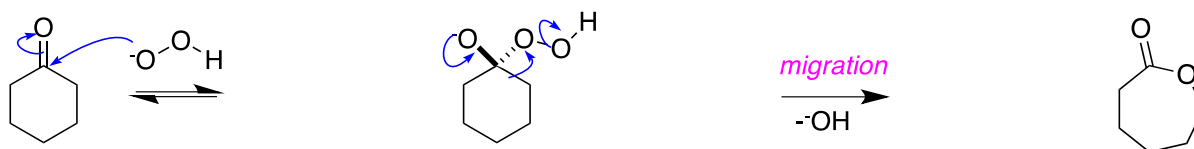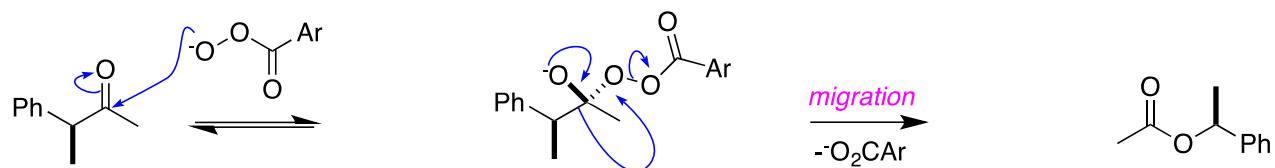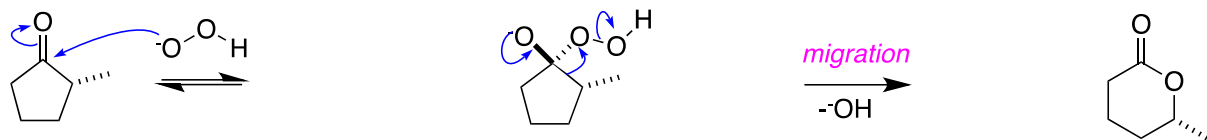
20 Characteristics Of Enols and Enolates

from chapter(s) _____ in the recommended text

Introduction

Focus

Enols and enolates may form under acidic and basic conditions from many compounds that have a *CH* bond adjacent an electron-withdrawing group. Those electron-withdrawing groups can be selected from numerous different functionalities. Enolization can impact chemical reactivity via deuterium exchange, racemization or epimerization, and isomerism.
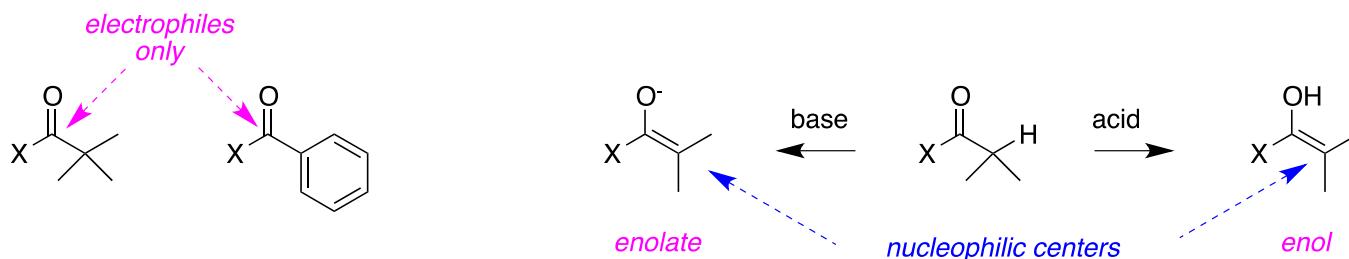
Reasons To Care

Nature and laboratory syntheses use enolates milder alternatives for constructing *C-C* bond frameworks than more reactive hard-anion reagents like Grignards. Enols and enolates are a gateway to electrophilic attack on those α-*C* atoms. Reactions of enolates (dealt with after this section) are key to *in vivo* construction of huge numbers of natural products.

Concepts

Grignard reagents • acids and bases • resonance • tautomerism

Objective

This section introduces structures of enols and enolates, and the mechanisms by which they might form.

Enols Form Under Acidic Conditions

Mechanism Of Formation

Reactions of aldehydes and ketones under acidic conditions tend to involve *protonation* of the carbonyl oxygen as the first step.

Protonation of the carbonyl facilitates removal of an α-C*H* because that enables the electrons to flow directly into the carbonyl oxygen. Consequently, the base used can be as weak as *O*-lone pairs of water, hence this proton loss can occur under *acidic* conditions, resulting in formation of an *enol*.

Draw the structures in the enolization of the simple aldehydes and ketones below.

protonated carbonyl   *enol form*

protonated carbonyl   *enol form*

enol

d

H$^+$

$^+$OH   H

OH$_2$
-H$_3$O$^+$

OH
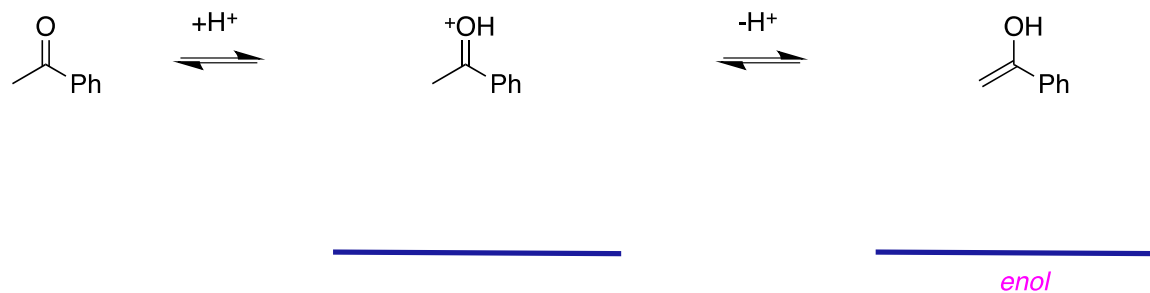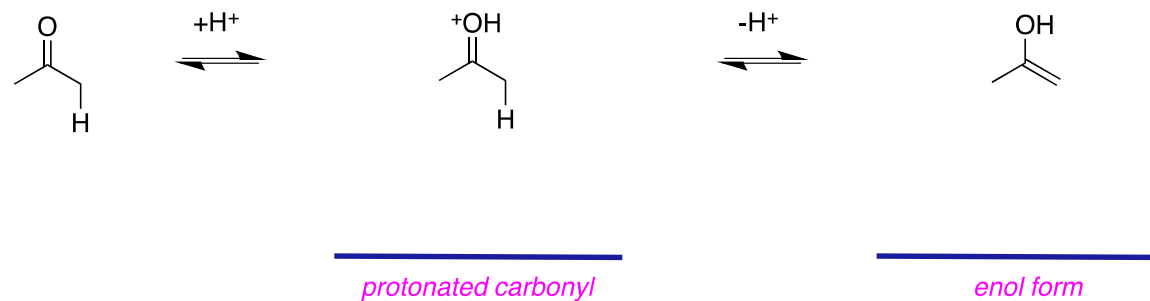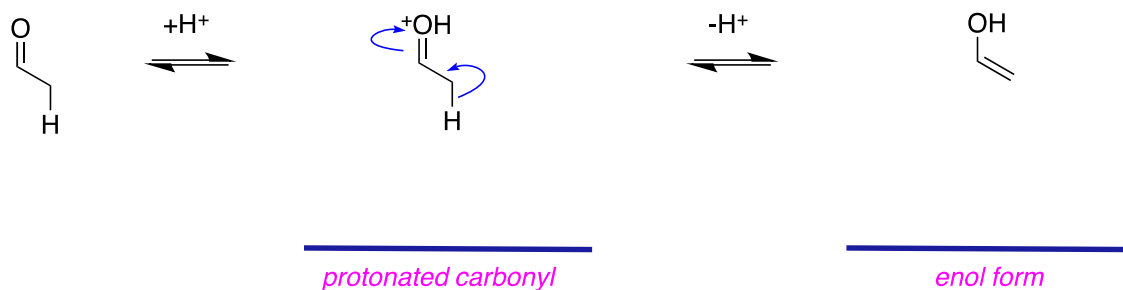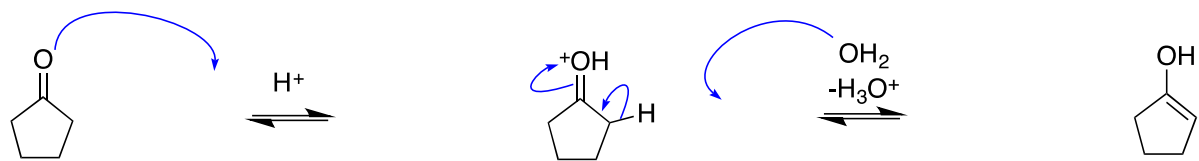
Deuterium Exchange

Ketones and aldehydes with enolizable hydrogens exchange these for deuterium when exposed to *deuterons*.

Illustrate how deuterium is incorporated onto *C*-atoms into the following compounds when they are exposed to D⁺.
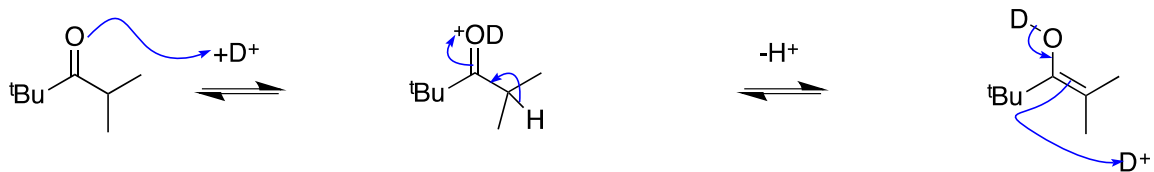
deuterated carbonyl   *enol form*

deuterated product

$+D^+, -D^+$
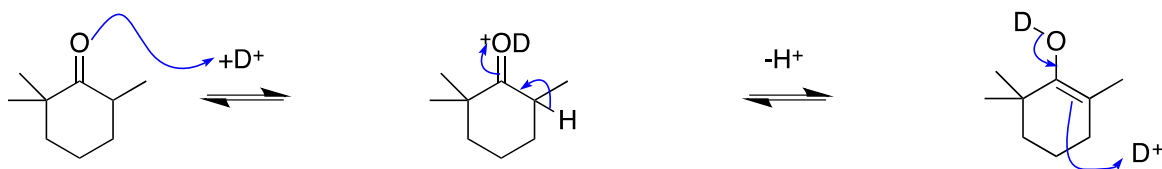
Some enols and enolates can have *E*- and *Z*-isomers. Often mixtures are formed and this can be represented using a wavy line bond; do this in the following example.

$+D^+$

$-H^+$
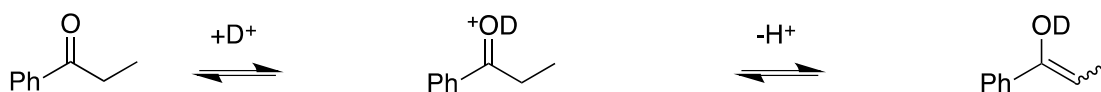
O-deuterated carbonyl

O-deuterated enol

$+D^+, -D^+$

$+D^+$

$-H^+$
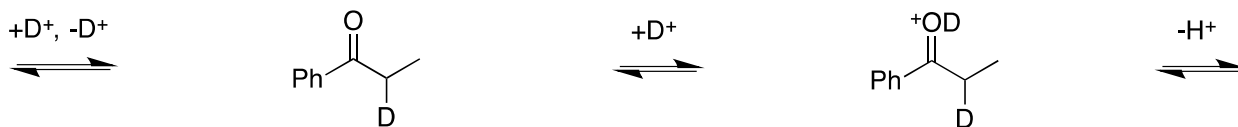
C-deuterated ketone

O-deuterated carbonyl

$+D^+, -D^+$
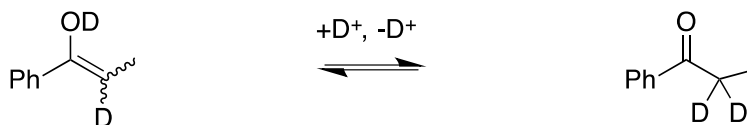
enol form

dideuterated ketone

Circle carbons in the following molecules that bear H that may exchange with excess D⁺.
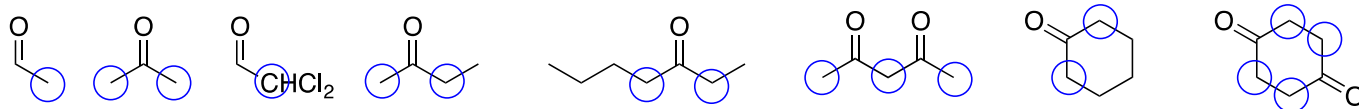
In general, when isomers interconvert by changing the position of a hydrogen atom and nothing else, such as going from carbonyl to enol form, this is called *tautomerism*; it can be achieved via acid or base catalysis.

Enols Of 1,2- And 1,3-Dicarbonyl Compounds

For simple aldehydes and ketones the *enol* form is more abundant in solution.

For acetone the keto : enol ratio is about $10^6 : 1$.

The *enol* form is much more prevalent for some other types of carbonyl compounds. Fill in the structures below and suggest reasons why their enols are more favored than that of acetone.

protonated carbonyl   *enol form*
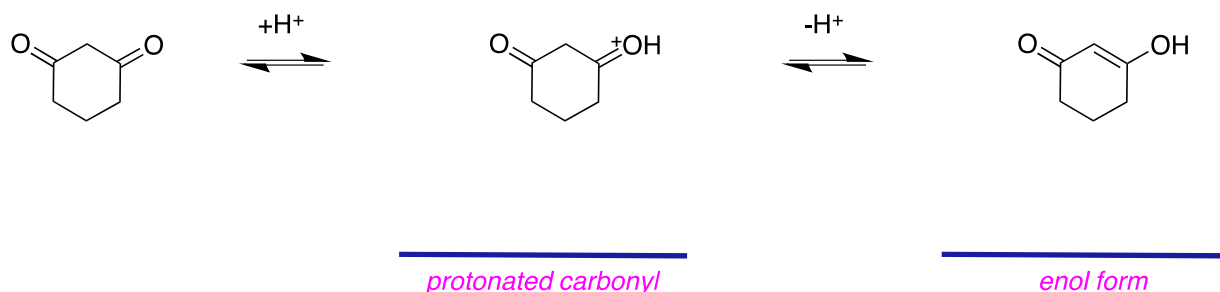
reason: Enolization of 1,3-cyclohexanedione forms conjugation between carbonyl and C=C which is stabilized by

resonance, while the acetone does not have resonance effect.
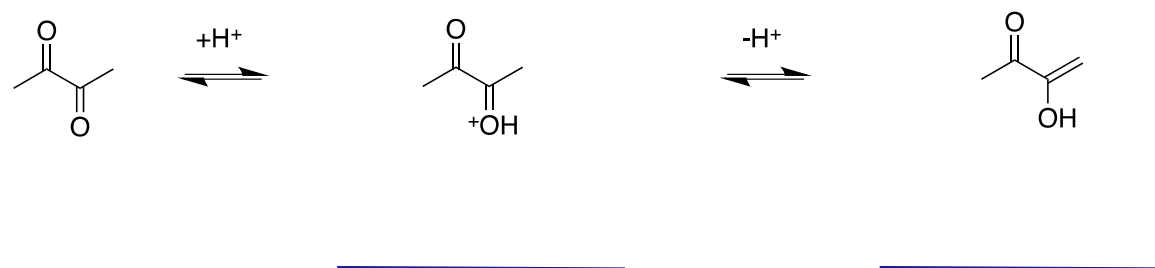
reason: Compared to acetone, one carbonyl group in the 2,3-butanedione acts as electron withdrawing group that

enhances acidity of α-H.

Enolization of 1,3-dicarbonyl *conjugates* one carbonyl with a double *C=C* bond and sometimes enables *H*-bond stabilization.

Draw enol forms of the following compounds having one or both those attributes.
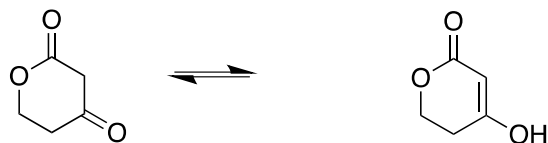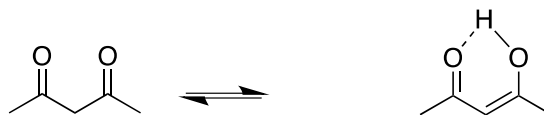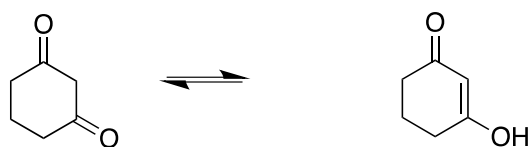
Show the 1,3-dicarbonyl compounds associated with the following.
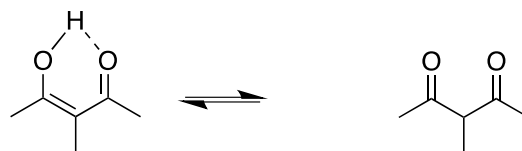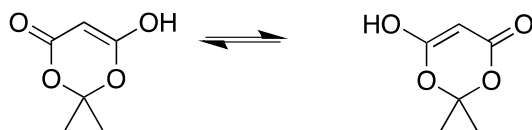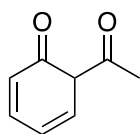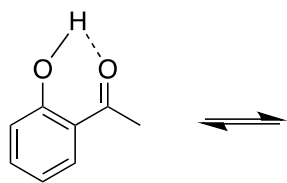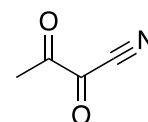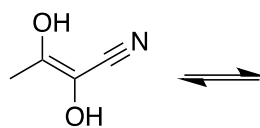
Enols Of Other Carbonyl Compounds

Draw the formation of the following enols with clear arrows. Use a wavy line bond to indicate mixtures of *E*- and *Z*-isomers, or show the configuration if only one is formed.

HO—C(=O)—CH₂CH₂CH₃   H⁺   ⇌   HO—C(⁺OH)—CH(H)—CH₂CH₃   OH₂ / -H₃O⁺   ⇌   HO—C(OH)=CH—CH₂CH₃
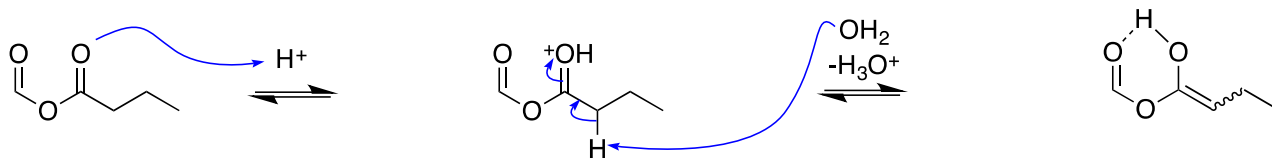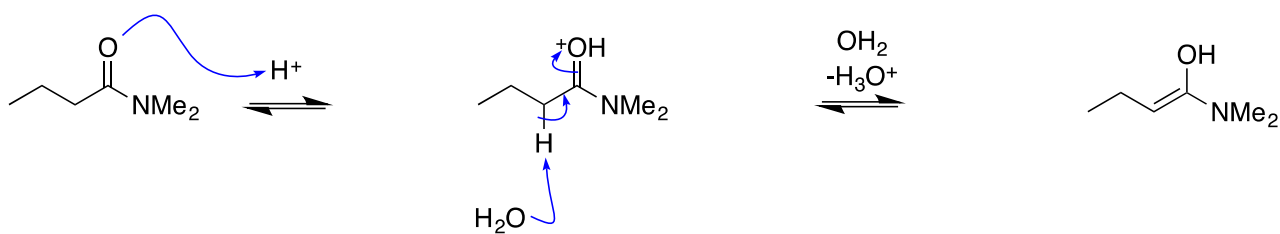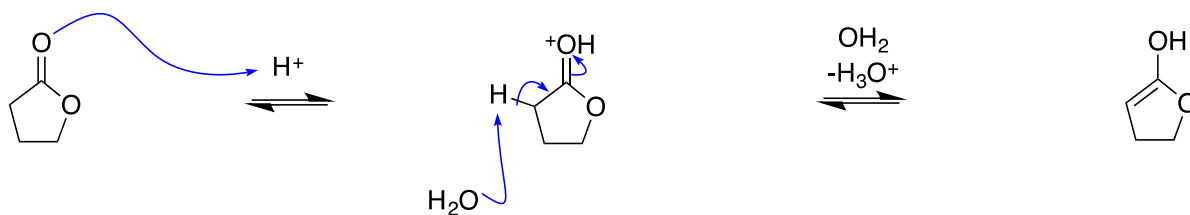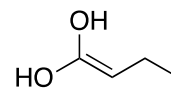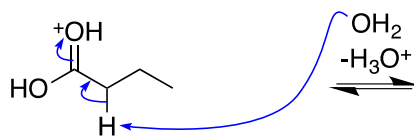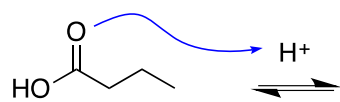
Keto-Enol Tautomers Of Other Compound Types

Draw tautomers of the following compounds, and indicate if they are *keto* or *enol* forms.
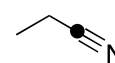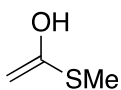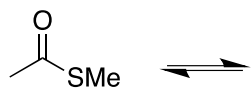
enol   _keto_
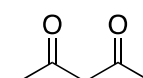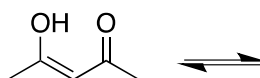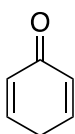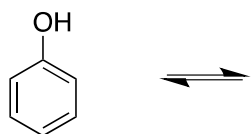
keto   _keto_
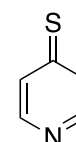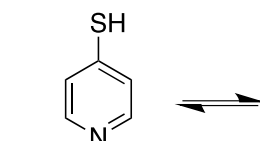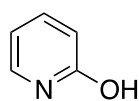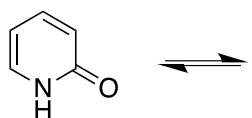
enol   _keto_
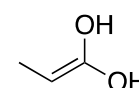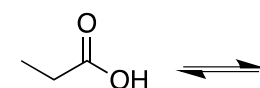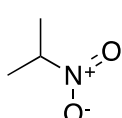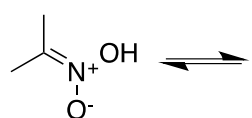
keto   _enol_

It would be *incorrect* to relate the keto and enol forms above with a double-headed arrow

(⟷) because they are not resonance structures.

Enolates Form Under Basic Conditions

Mechanism Of Formation

Base-mediated formation of enolates involves *C*-deprotonation then *O*-protonation. Draw this mechanism for formation of the enol form of ethyl acetate.
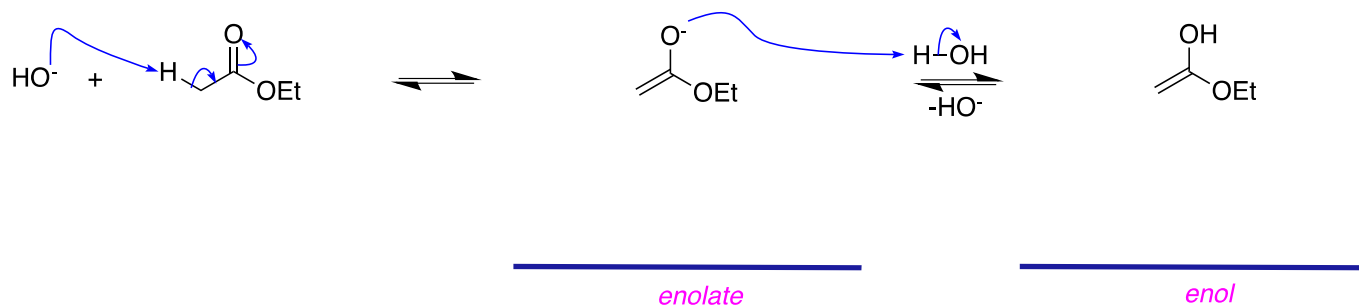
enolate   *enol*

Draw similar processes for the following transformations using wavy lines to indicate mixtures of *E*- and *Z*-isomers, or show the configuration if only one is formed.

Na⁺ H⁻ + H—⎯CO₂Me → ⎯⎯O⁻⎯OMe   H—OH ⇌ -HO⁻   OH⎯OMe

Na⁺ NH₂⁻ + H—CN, Ph → Ph—•—N⁻   H—OH ⇌ -HO⁻   Ph—•—NH

Na⁺ NH₂⁻ + H—C(=O)NMe₂, Ph → Ph—C(O⁻)=NMe₂   H—OH ⇌ -HO⁻   Ph—C(OH)=NMe₂
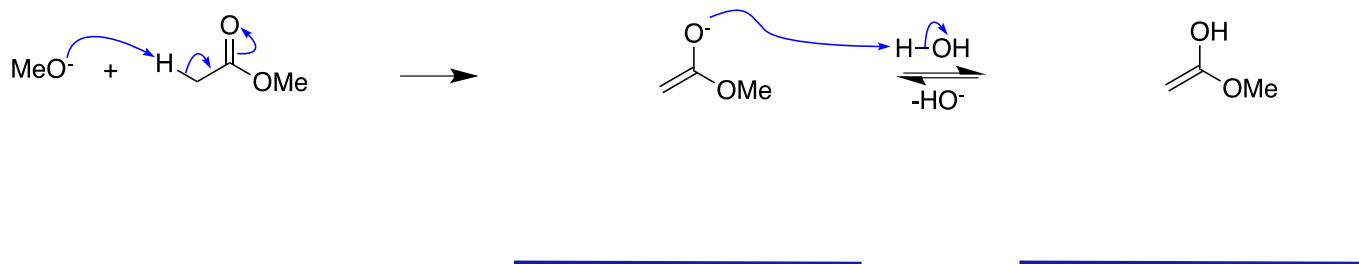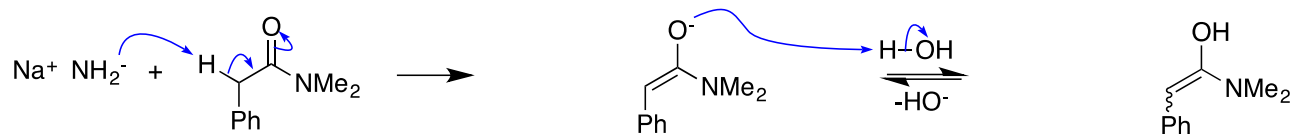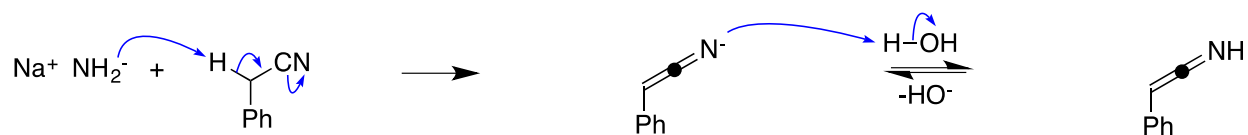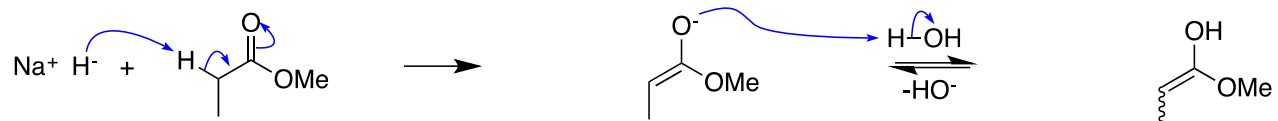
Resonance Structures Of Enolates

Draw resonance structures of the following anions.
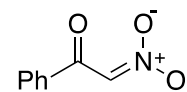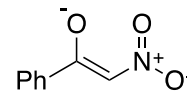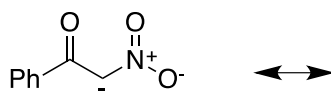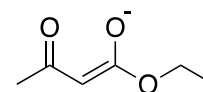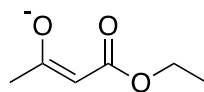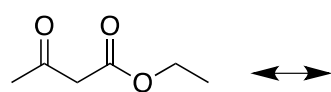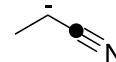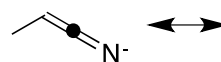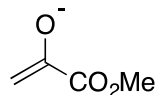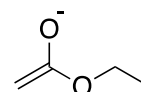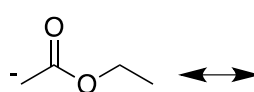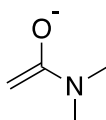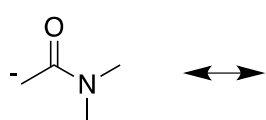
Nitrogen is *more* electronegative than carbon so *more* able to support a negative charge.

Circle the most acidic hydrogen(s) in the following structures.

It *is not* easy to form *C*-enolates from compounds like the last three from the right shown here because they form *N*-anions.
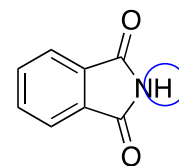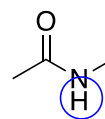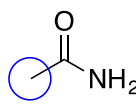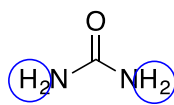
Effects Of Enolization

Racemization

Draw enol forms of the following optically active molecules, and indicate if these are also optically active.
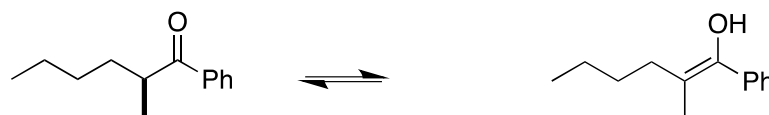
optically active

achiral
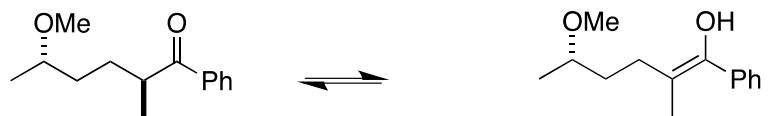
optically active
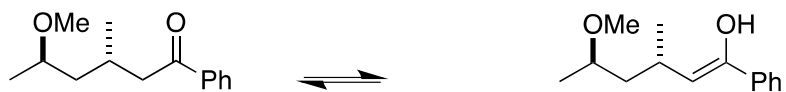
optically active

In general, α-chiral ketones and aldehydes *can* racemize or epimerize in the presence of acid or base.

Complete the following to determine if other carbonyl compounds can undergo the same type of process.

ibuprofen
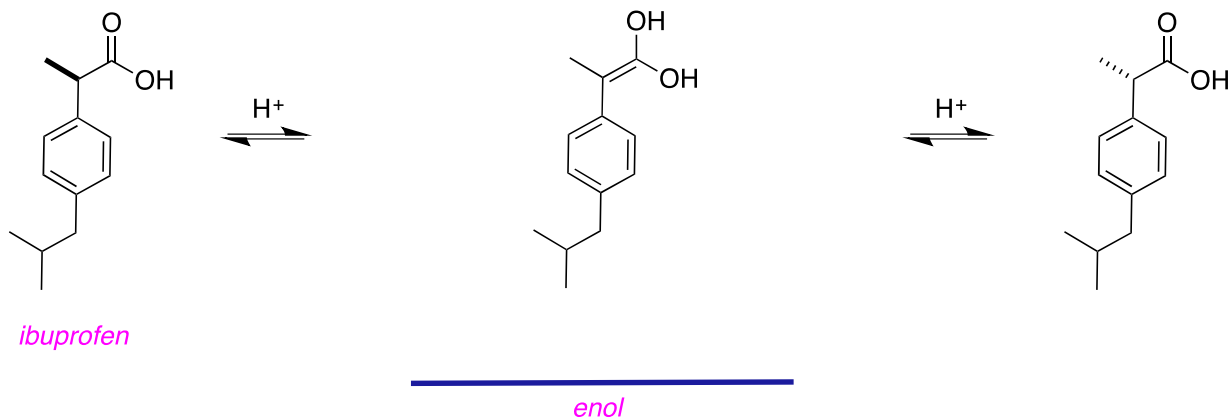
enol

Double Bond Migration

The following compounds can form enolates via deprotonation at the $C\alpha$ then form keto forms by adding protons back to $C\gamma$; the consequences of this is *migration*. Complete the following diagrams:

enol   *keto*

enol

keto
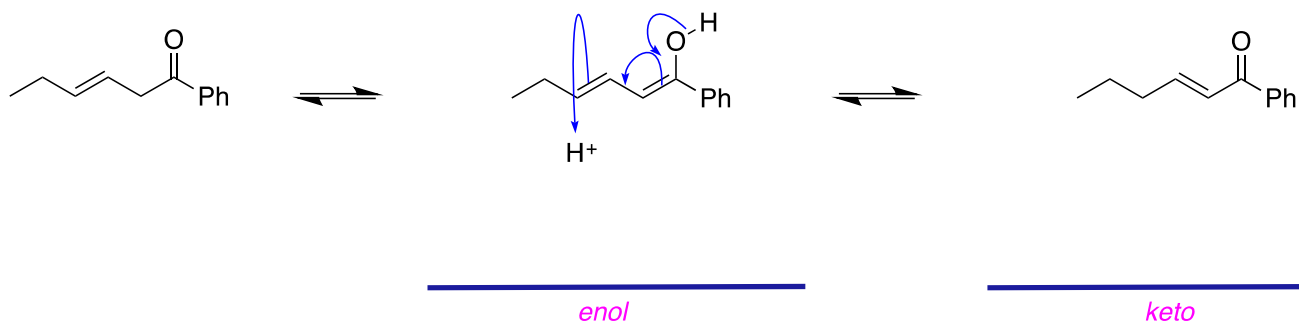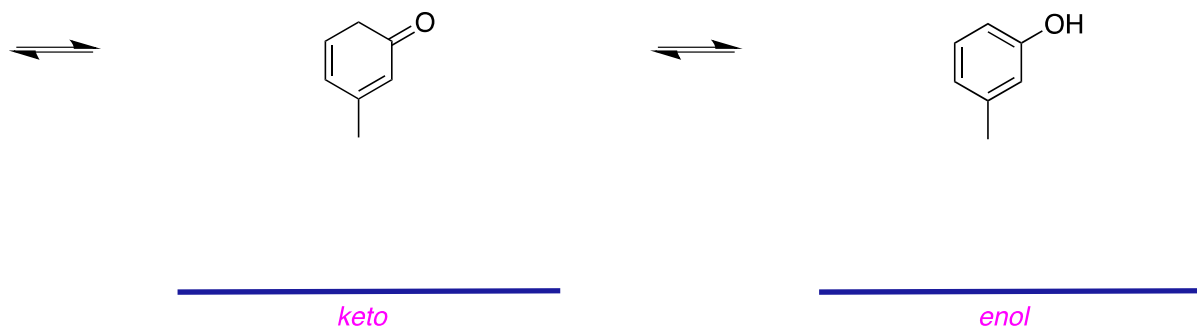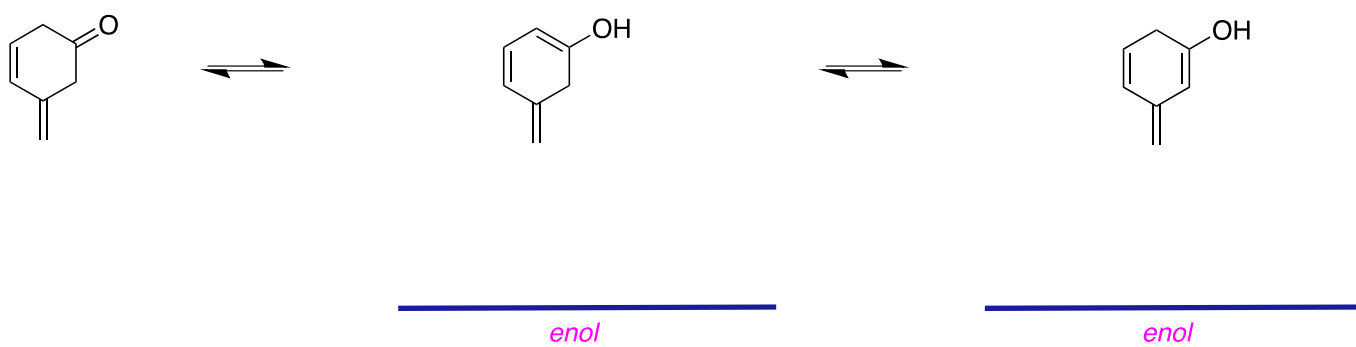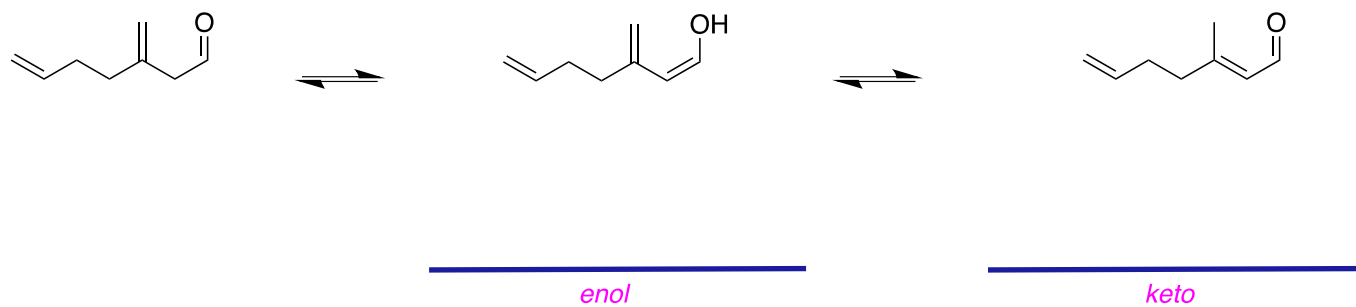
enol

enol

keto

enol

Draw a keto form of ascorbic acid (vitamin C) to indicate why *C*β may have some carbonyl character.

keto
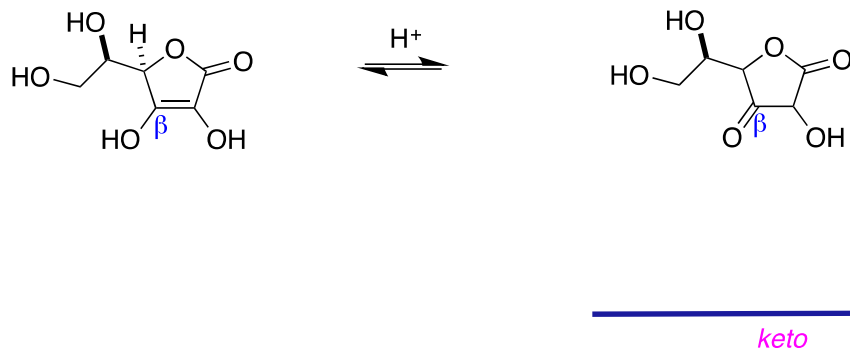
Draw the enol form that would enable glucose to be isomerized into fructose.

glucose

enol

fructose
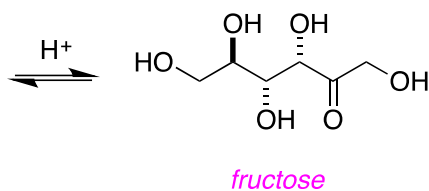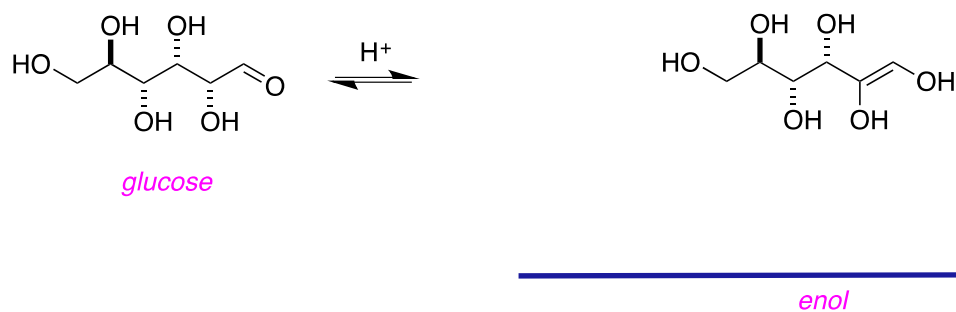
21 Halogenation Of Enolizable Carbonyl Compounds

from chapter(s) _____ in the recommended text

Introduction

Focus

Just as halogenation of alkenes is one of the simplest electrophilic addition mechanisms, halogenation of enols and enolates is also straightforward. However, unlike the corresponding alkene reactions, this is a substitution, not an addition, and the mechanism, and outcomes, are different under acidic and basic conditions.
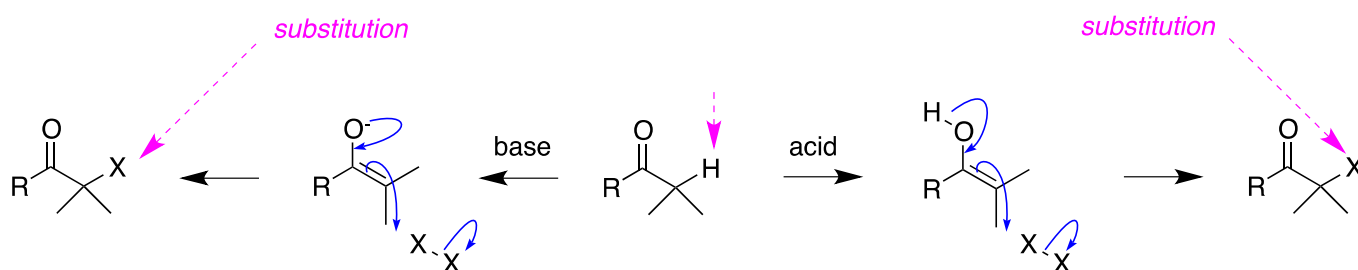
This section explains why monohalogenation predominates under acidic conditions, but polyhalogenation is possible under basic conditions. Methyl ketones, in fact, can react further losing trihalomethyl anions, CX_3^-, generating carboxylic acids, *ie* "the haloform reaction".   The halogenation processes featured here illustrate how simple electrophiles add to enols and to enolates, and why the outcome is not always the same.

Reasons To Care

Halogenation of enolates is a process that does occur in some living systems, and these transformations are widely used in laboratory syntheses.

Concepts

halogenation of alkenes • resonance • tautomerism • enols and enolates

Objective

Rationalization of halogenation of enols and enolates in terms of the reactivities of the initial product formed, and a gentle introduction into the wider set of reactions of enols and enolates with electrophiles.

Halogenation Via Enols (Acidic Conditions)

Acidic Conditions Give Monohalogenation

Enols tend to form under neutral or *acidic* conditions, whereas enolates form under *basic* conditions.

Enols are *less* reactive than *enolates* towards electrophiles because they do *not* have a negative charge.

Show the reaction of phenylethanone with bromine via dissociation of a proton from the enol-*OH* while bromine simultaneously adds to the α-carbon.

enol   *bromoketone*
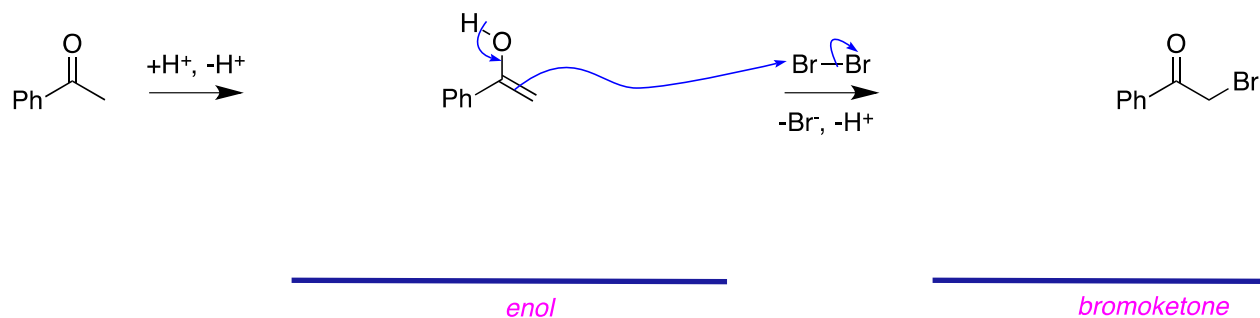
Another way to represent this reaction is: *O*-deprotonation (rather than just dissociation) with the strongest base available (the solution is neutral or acidic, so this will not be a strong base) and simultaneous reaction with the electrophile.

Draw the same reaction shown above but with bromide deprotonating the enol-*OH* at the same time the carbon of the enol attacks the bromine.
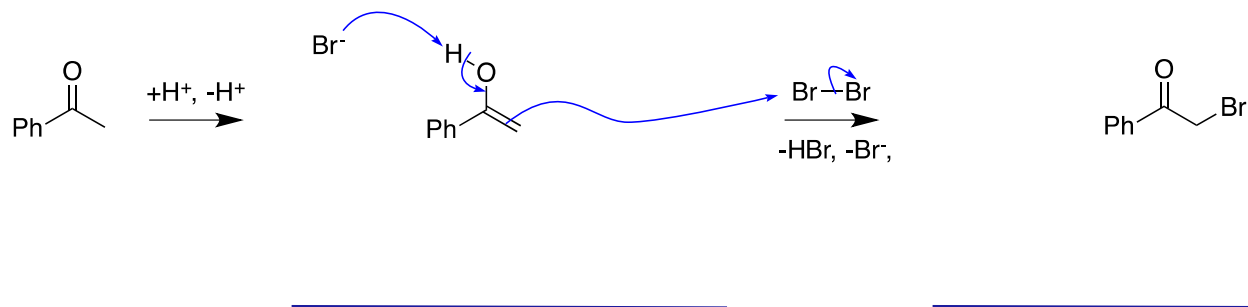
Consider how likely it is for three friends walking from three different directions to accidentally bump into each other at *exactly* the same time. By analogy, the second representation is *less* likely because the process would involve three molecules coming together simultaneously, which is *less* statistically favorable.

Complete the following diagram by showing the products; the nitrosylation reaction adds NO⁺ as an electrophile. NOCl is a pseudo- *halide*.

I_2 / H^+

Cl_2 / H^+

NOCl

monosubstitution

monosubstitution

nitroso form

oxime tautomer
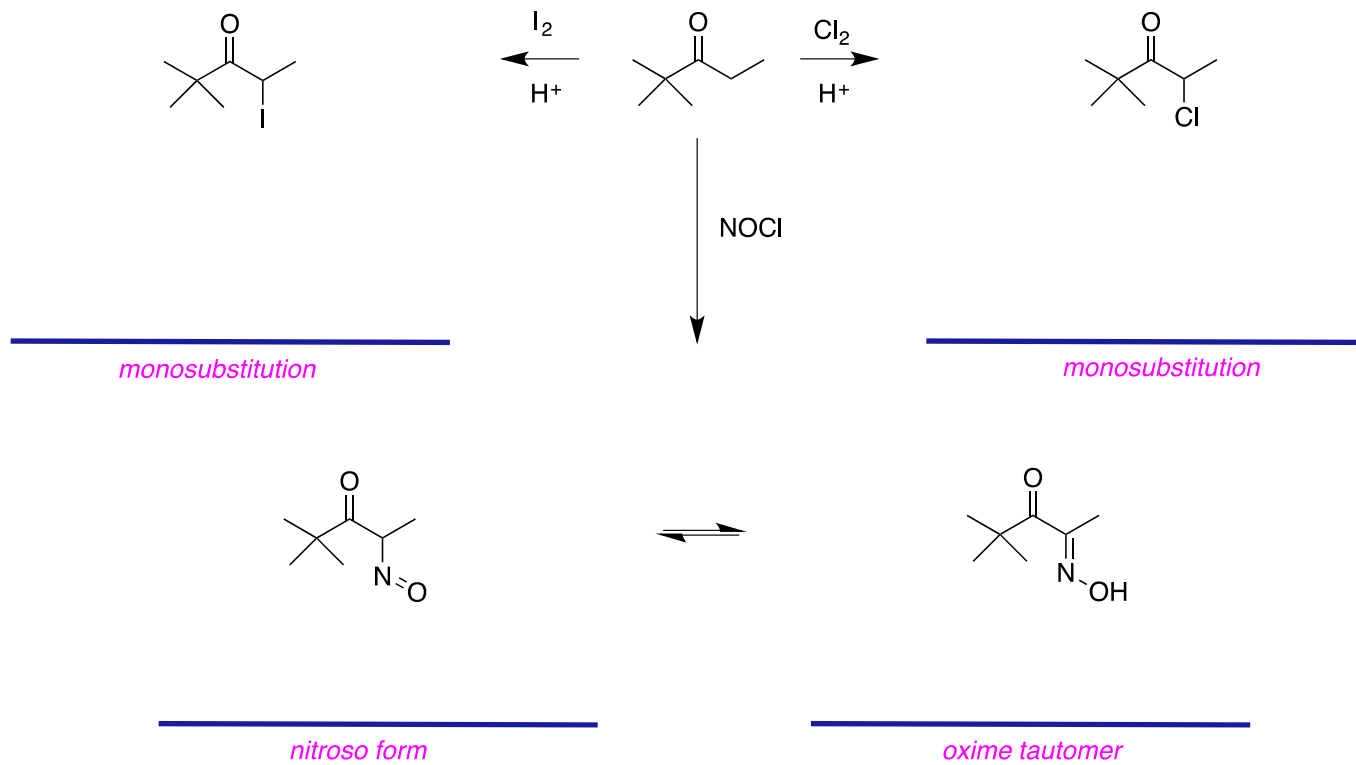
Halogenation Via Enolates (Basic Conditions)

Basic Conditions Facilitate Substitution Of More Than One Halogen

Acid- and base-promoted halogenations of carbonyl compounds are mechanistically *different*.

Rank the following enolates in order of decreasing stabilities by showing their resonance forms and circling **1** for the most stable, **2** next, *etc*.

3

1

2

4
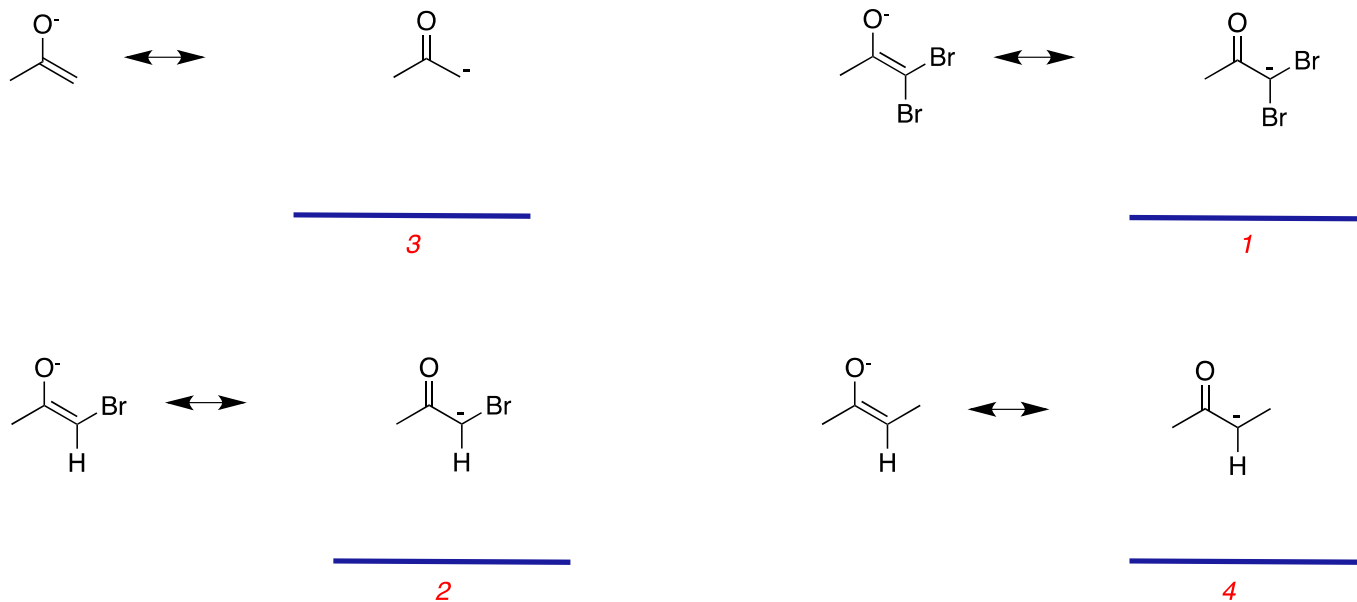
Under *basic* conditions, haloketones are *more* acidic than the starting materials due to inductive stabilization of the enolate.

Monohaloketone products under basic conditions are *more* likely to enolize and react with more halogen electrophile than ones formed under acidic conditions.

Show the mechanism of base-mediated bromination of 2,2-dimethylpentan-3-one to the corresponding dibromoketone.
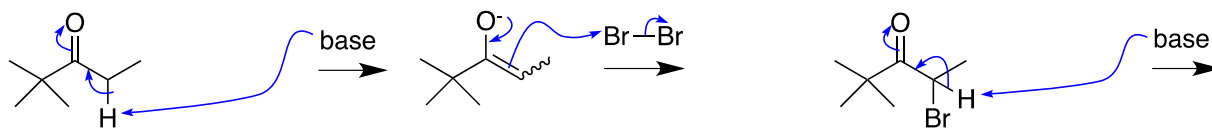
2,2-dimethylpentan-3-one

more likely to form enolate than starting ketone
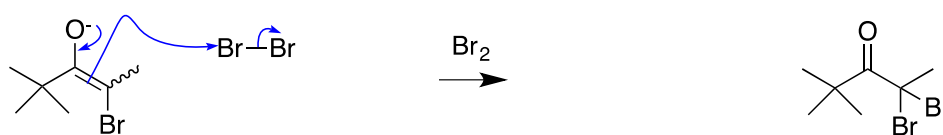
enolate

2,2-dibromo-4,4-dimethylpentan-3-one

Based on the above, methyl ketones under basic conditions *would* react with *three* equivalents of base and halogen to form 1,1,1-trihalomethylketones.

In fact, the final product of the reaction above is *not*, in fact, 1,1,1-trihalomethylketones. This is because after each substitution the carbonyl group becomes *more* electrophilic.

After three substitutions the base can add to the carbonyl and the bulky, inductively stabilized trihalomethyl anion is expelled; this is the *haloform* reaction.

Outline the mechanism for reaction of 1-phenylethanone with excess bromine and base, by completing the following diagram.

enolate

monobromination

enolate

dibromination

enolate

tribromination

tetrahedral intermediate
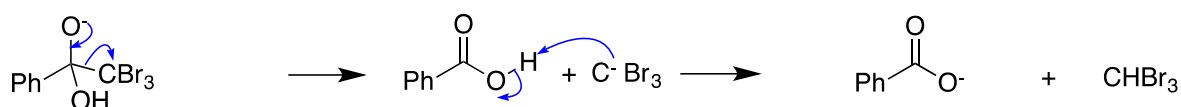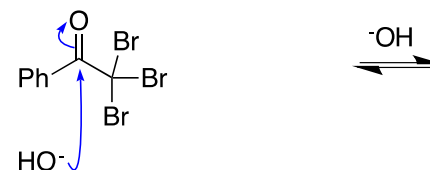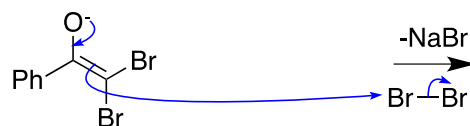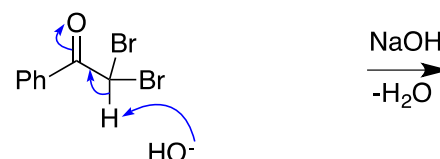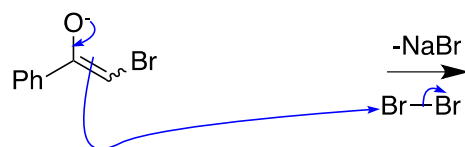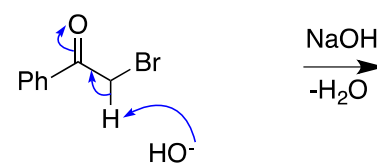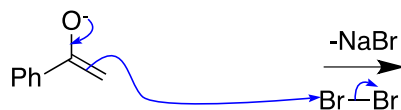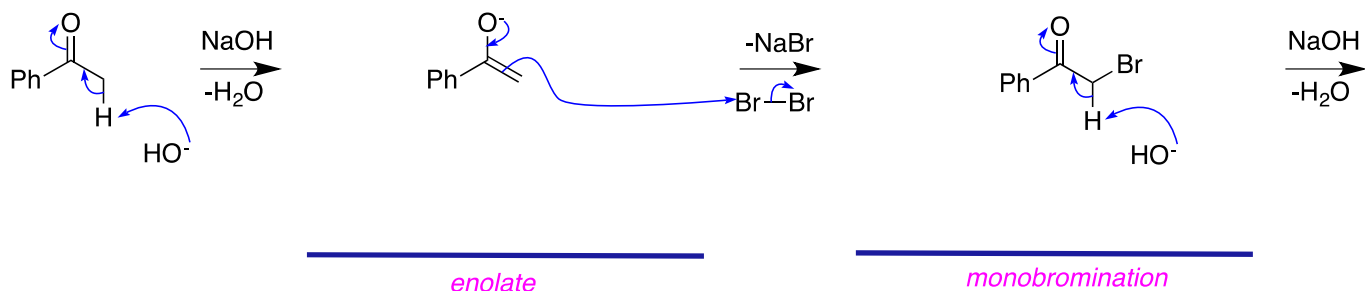
Iodoform, I₃CH, is a crystalline solid that precipitates from many organic solvents so treatment with hydroxide and iodine *could* be used as a visual test for methyl ketones.

Outline the mechanism of the following iodoform-generating reaction.
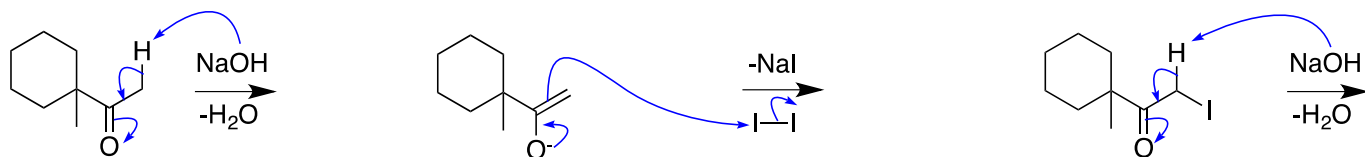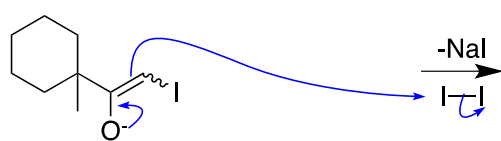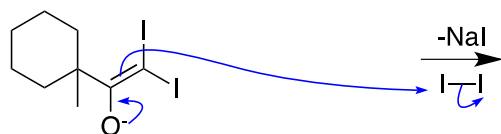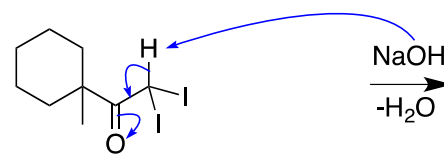
enolate

triiodination
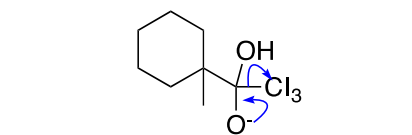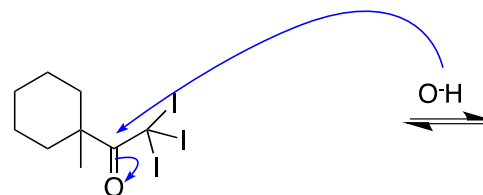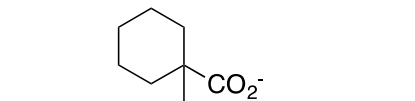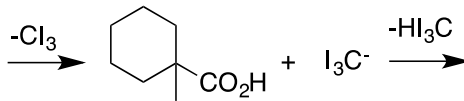
tetrahedral intermediate

Under _acidic_ conditions enolization of the monohaloketone product is _slower_ than the ketone starting material because the carbonyl oxygen is less basic than in the starting material.

Which of the following ketones could give iodoform when treated with iodine and hydroxide?

yes / no   yes / no   yes / no   yes / no   yes / no   yes / no   yes / no
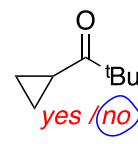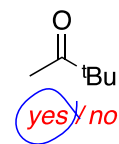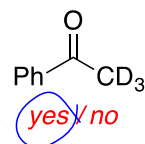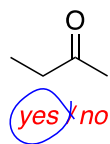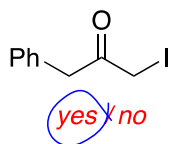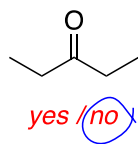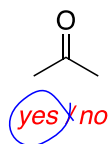
Choosing Acidic Or Basic Conditions For Halogenations

Predict the products of the following halogenation reactions.

Br_2, H^+

Br_2, HO^-

Br_2, H^+

Br_2, HO^-
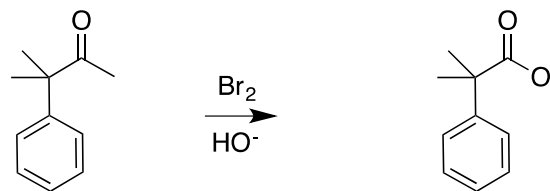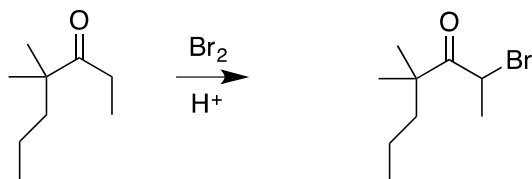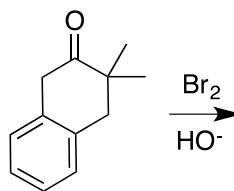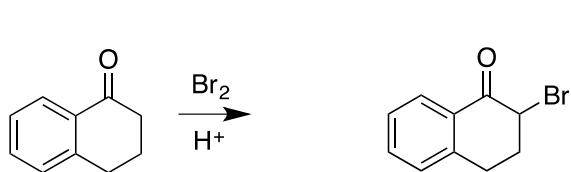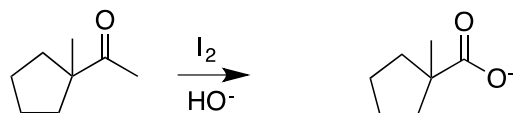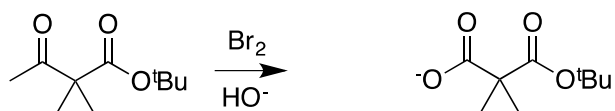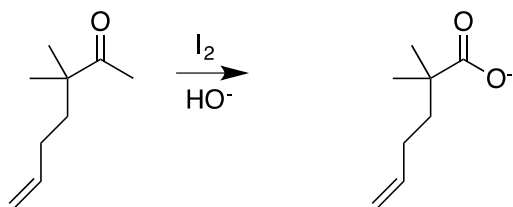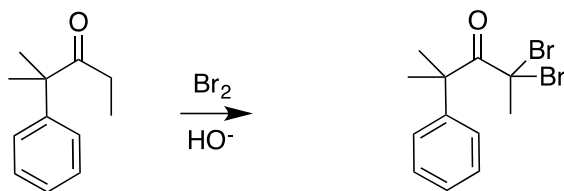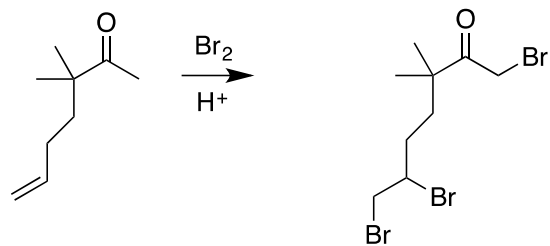
Identify the conditions and starting materials for preparation of the following products; all these reactions require halogenation.

Br$_2$
H$^+$
indicate conditions

Br$_2$
HO$^-$

Br$_2$

Br$_2$

Br$_2$
CH$_2$Cl$_2$

Br$_2$
FeBr$_3$
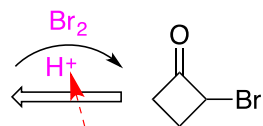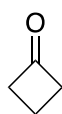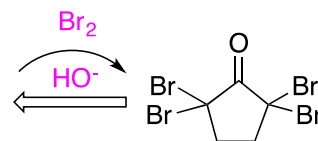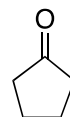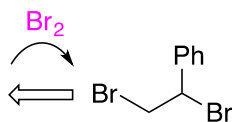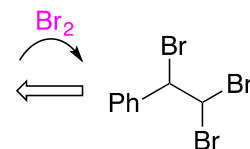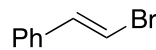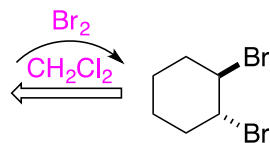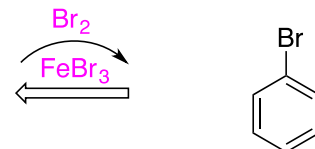
Alkene bromination followed by *E1cb* eliminations are operative in the following reactions (1 equiv of bromine is used). Show starting materials and indicate conditions.

i) Br$_2$

ii) base

i) Br$_2$

ii) base

i) Br$_2$

ii) base

(i) Br$_2$

(ii) base
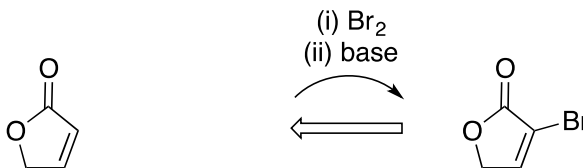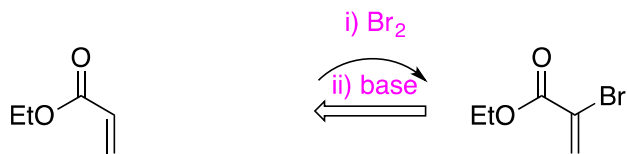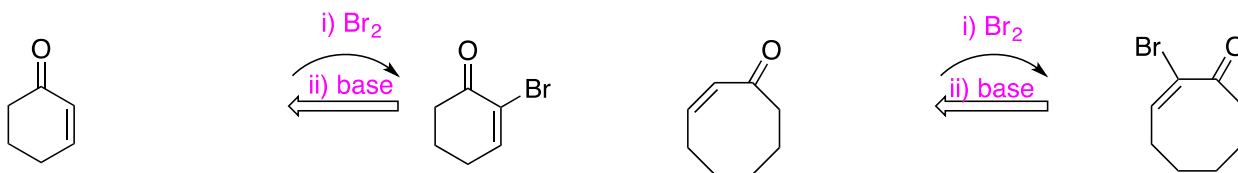
α-Halogenation Of Carboxylic Acids

Mechanism

Carboxylic acids react with PBr₃ via: (i) displacement of bromide from the PBr₃; (ii) formation of an acyl bromide; and, (iii) reaction of this acyl bromide with bromine formed *in situ* from phosphorus tribromide.

Show the mechanism of bromination of phenylacetic acid with PBr₃.

activated carbonyl   *acyl bromide*

protonated carbonyl   *enol*   *acyl bromide*
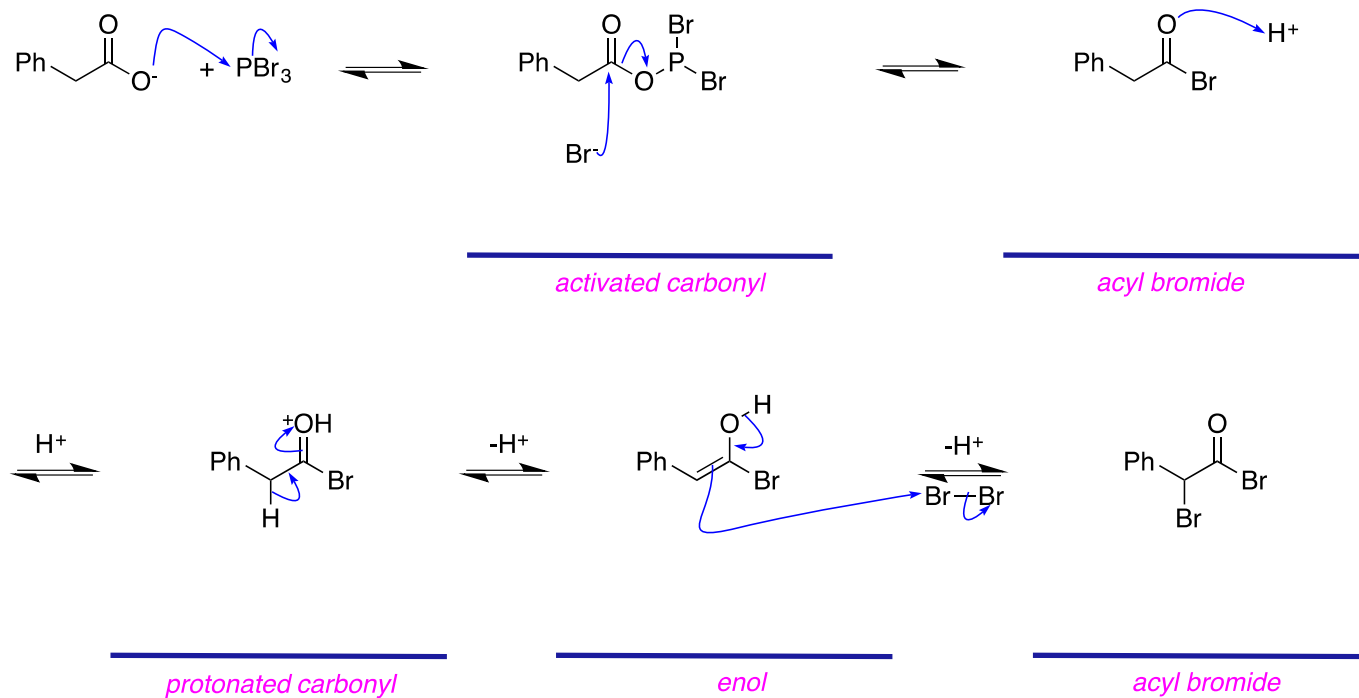
Show the products of bromination of the following acids under the conditions described above.
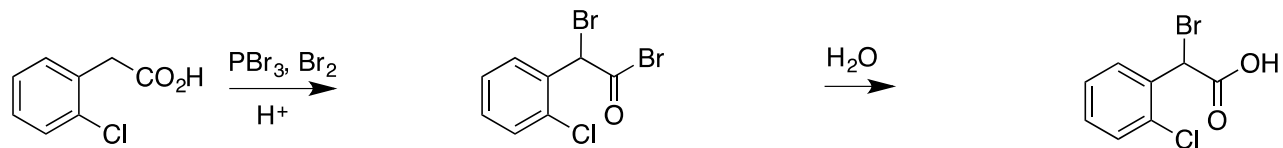
bromoacid bromide
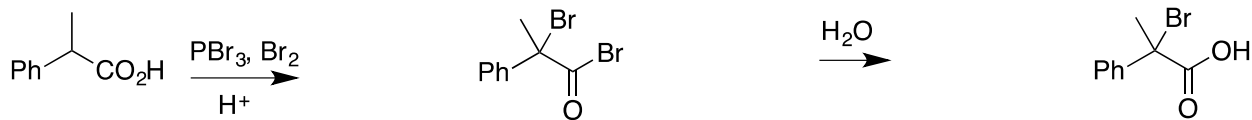
bromoacid bromide

Syntheses Featuring α-Bromo Acids

α-Bromination of ketones facilitates S_N2 addition of nucleophiles to the α-C carbon.

Indicate how each of the following compounds could be made via nucleophilic attack on bromocarbonyl compounds.

indicate nucleophile
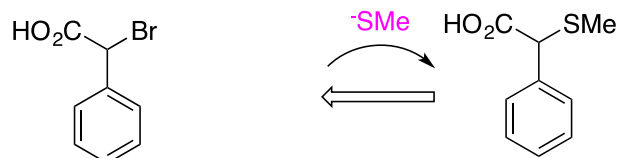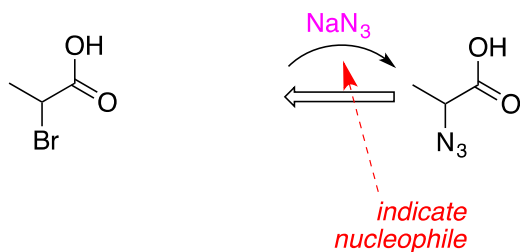
Show halogenated ketones, aldehydes, esters or amides and the nucleophiles that could be used to make these products. It is *not* necessary to show reaction conditions.

indicate nucleophile
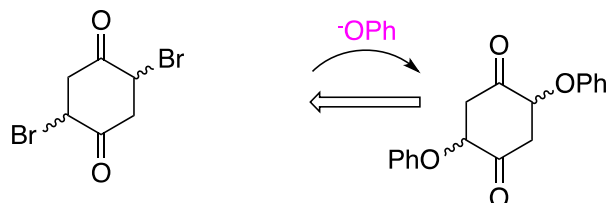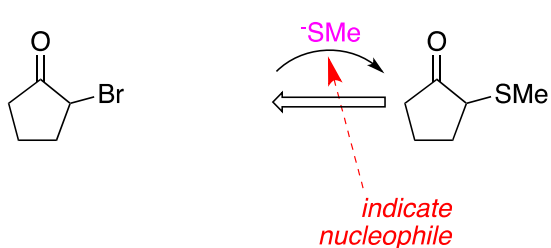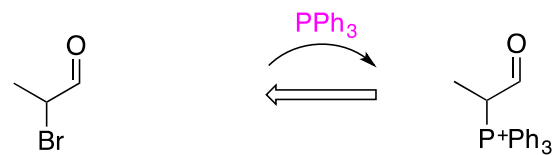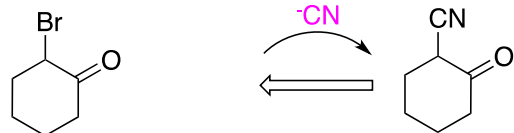
22 Reactions Of Enolizable Compounds With *C*-Electrophiles

from chapter(s) _____ in the recommended text

Introduction

Focus

Grignard reagents can be used to build-up carbon frameworks in organic molecules by adding nucleophiles to carbonyl compounds (and some other electrophiles). In addition to this, enolizable carbonyl compounds can be converted to enols, enolates, or some equivalent of these then *C*-alkylated. The fact that enolizable carbonyl compounds can react as electrophiles (at the carbonyl) or nucleophiles (on the α-*C* carbon) makes them extremely versatile intermediates in synthetic pathways.
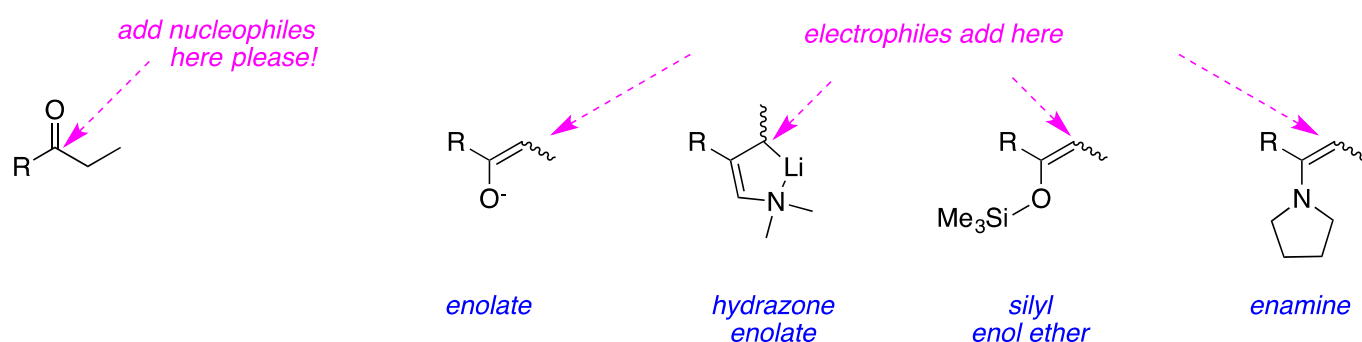
Reasons To Care

Alkylation of enolates are used in laboratory syntheses and biosynthesis, but the other strategies are mostly for synthesis laboratories. All these methods, however, illustrate ways in which enolate reactivities may be tuned to give α-*C* alkylation, as an alternative to nucleophilic additions to carbonyl groups.

Concepts

enolates • enamines • electrophilic attack • construction of the carbon framework of organic molecules

Objective

Practitioners should understand the concept of enolate alkylation, some of the limitations of that approach, and strategies that have been adopted to overcome some of those restrictions.

α-Alkylation Of Carbonyl Compounds Under Strongly Basic Conditions

C-Alkylation Of Ketones

Stronger bases than HO⁻ drive most ketones to exist predominantly as enolates, but may also be *nucleophilic* enough to react at the carbon of carbonyl groups.

Deprotonation of simple carbonyl compounds in preference to addition to the carbonyl group of these compounds, requires a base that is *strong* and *non-nucleophilic*.

One strategy to favor deprotonation of carbonyl compounds is to use *hindered* alkali metal amides.

Draw products of the following reactions.

mostly

tertiary alcohol
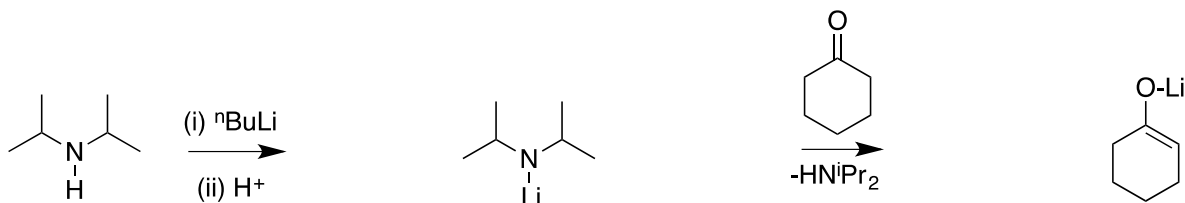
lithium diisopropylamide LDA
pKa 35

enolate

Z-enolate

draw E-enolate
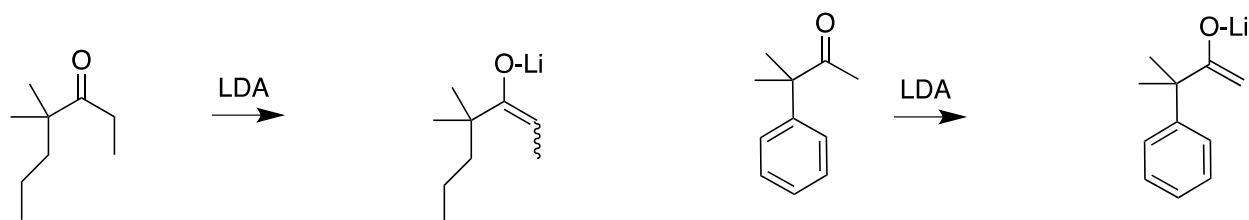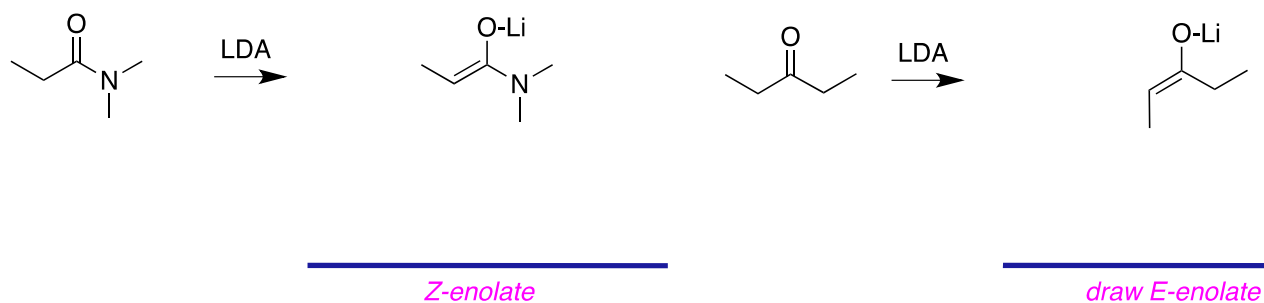
Draw the two possible products of monoalkylation of 2-methylcyclopentanone with ethyl bromide.

2,5-dialkyl product

2,2-dialkyl product
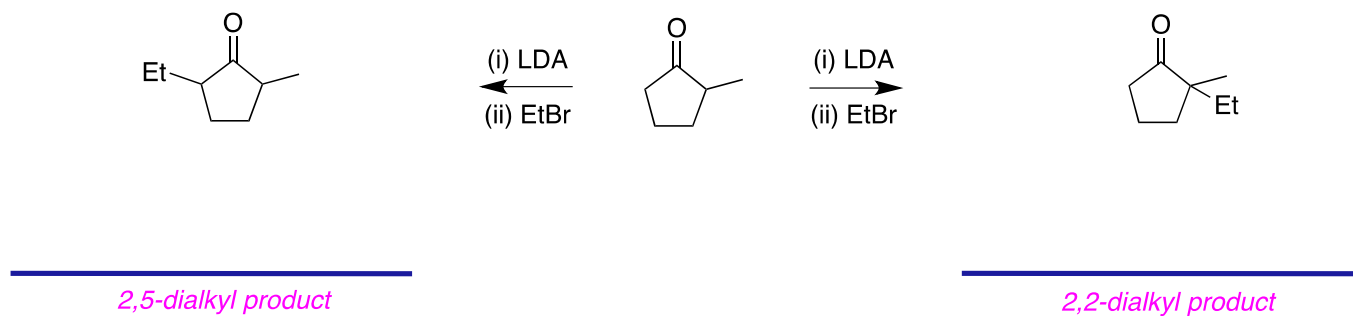
It is *difficult* to design conditions so that one of these products is significantly favored over the other.

Ways to overcome this problem include alkylation of anions from hydrazones, from silyl enolates, or to use an enamine and alkylate that under neutral conditions.

Alkylation Of Hydrazones

Hydrazones can have *syn* and *anti*-forms. Under thermodynamic conditions, the most stable (usually the least hindered) isomer is formed.

Show the isomeric hydrazones from 2-methylcyclopentanone.

	+	
syn less stable		*anti more stable*
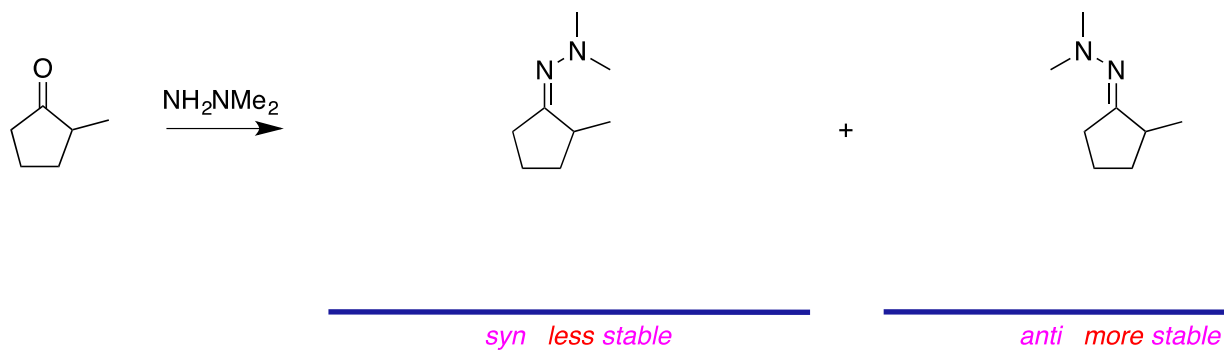
Alkylation of the following *N,N*-dimethylhydrazone-derived anion gives almost exclusively 2,5-dialkyl products, because the hydrazone is mostly *anti* and the lithium cation becomes coordinated at the least hindered site. This is an example of *thermodynamic* control.

Illustrate this by completing the diagram below.
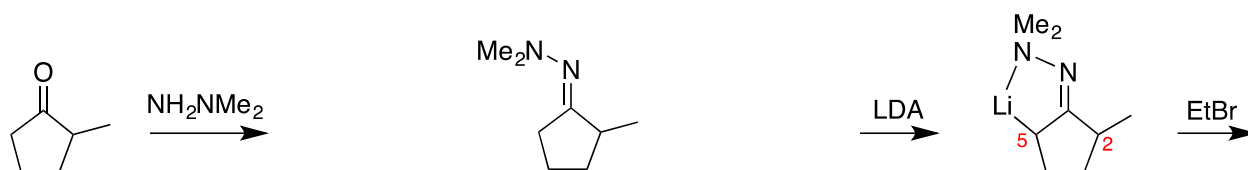
anti hydrazone
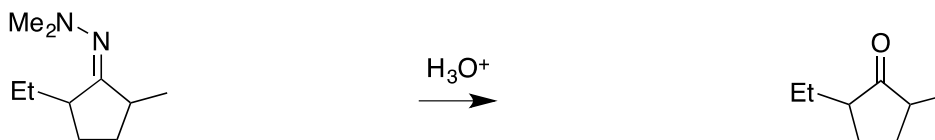
alkylation at least hindered carbon

2,5-dialkylation product

Silylation and α-Of Aldehydes And Ketones

Silicon – oxygen bonds are relatively *strong* so *Si*-based electrophiles tend to silylate on *O*-atoms of enolizable carbonyl compounds.

Silylation of ketones and aldehydes tends to be so facile that only a weak base is required (*eg* a tertiary amine), but stronger bases can be used.

Draw the products of the following reactions.
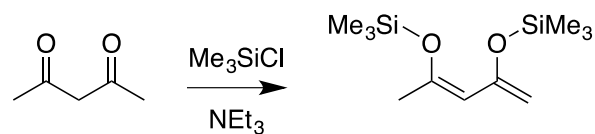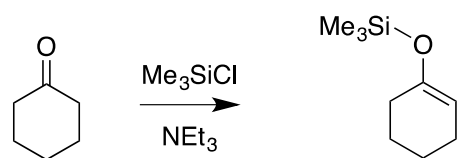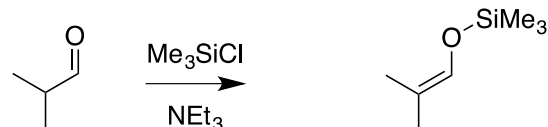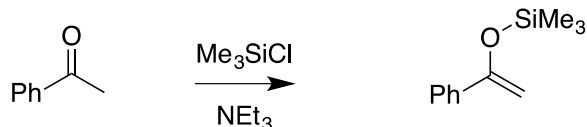
disilyl enol ether
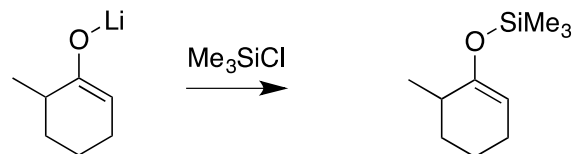
C-based electrophiles preferentially add to enolates at *carbon*.

However, in *polar aprotic* solvents that do not solvate oxygen anions well then it is possible to favor *O*-alkylation using *hard* electrophiles (hard electrophiles have *concentrated* positive charges).

Draw the *O*-alkylation products of the following reactions.

(i) KOᵗBu
DMSO
──────────→
(ii) Me₃O⁺ BF₄⁻

(i) KOᵗBu
DMSO
──────────→
(ii) Me₃O⁺ BF₄⁻

(i) KOᵗBu
DMSO
──────────→
(ii) Me₃O⁺ BF₄⁻

Ph

(i) KOᵗBu
DMSO
──────────→
(ii) Me₃O⁺ BF₄⁻

Ph

(i) KOᵗBu
DMSO
──────────→
(ii) Me₃O⁺ BF₄⁻
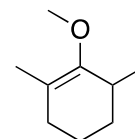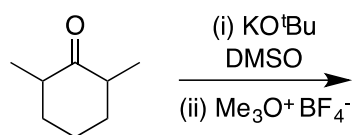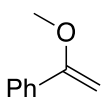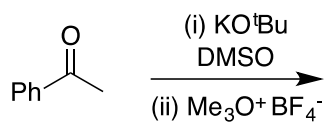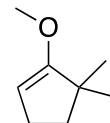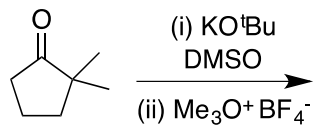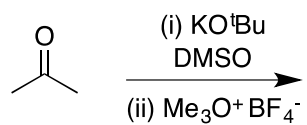
Simple electrophiles tend to add to the *C*-atoms of enol ethers, then the best nucleophile available will remove the silyl group.

Draw mechanisms for the following reactions.
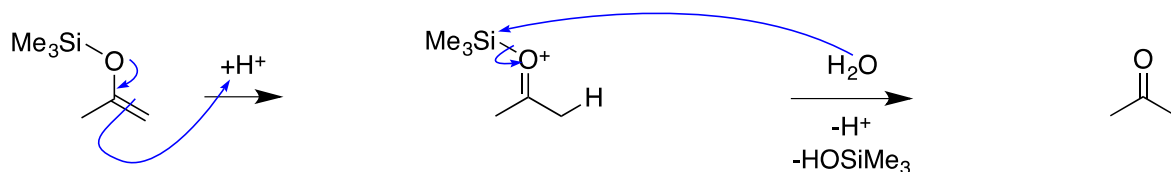
C-protonated form
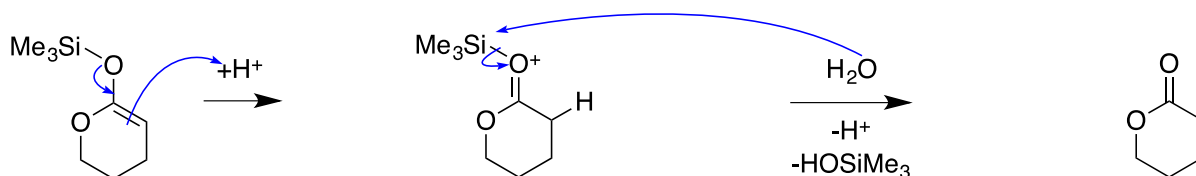
C-protonated form

addition of a PhS⁺ electrophile
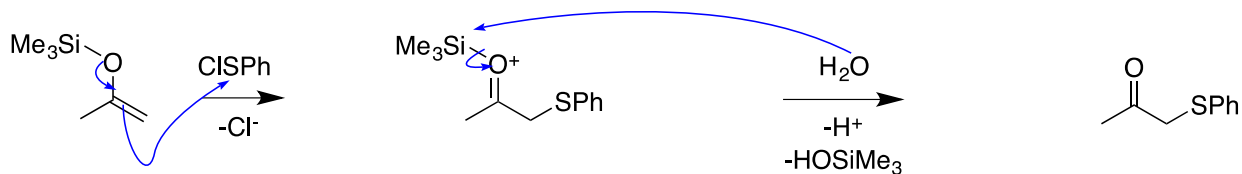
thioether silyloxonium ion   *thioether*

Reactions of dihydropyran with alcohols in the presence of anhydrous acid gives THP-protected (THP tetrahydropyran) alcohols.

The functional group in THP ethers is a(n) *acetal*; it *is* chiral leading to diastereomeric products if the R group is chiral.

protonation then quench of the oxonium ion with an alcohol
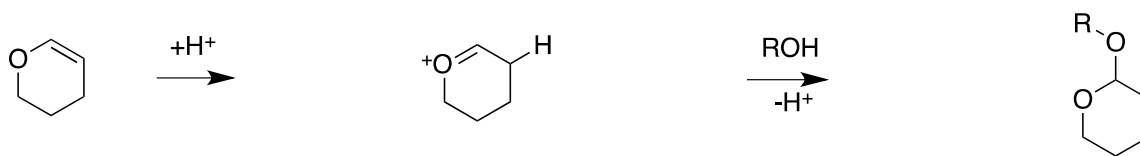
dihydropyran   *C-protonated form*   *THP-protected alcohol*

This process can be reversed by treating the product with *aqueous* acid.
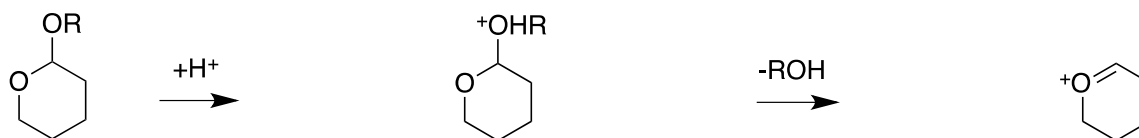
THP ether

α-Alkylation Of Carbonyl Compounds Under Near-neutral Conditions

Enamines From Ketones and Aldehydes

α-Alkylation can be achieved via formation of enamines, then reaction of these with electrophiles.

Donation of *N*-electron density in enamines through the alkene and through the α-carbon to electrophiles results in *iminium ions*, which are easily hydrolyzed on workup.

 Show the mechanism of the following enamine-mediated alkylation.

least hindered enamine

iminium

tetrahedral intermediate

ammonium intermediate
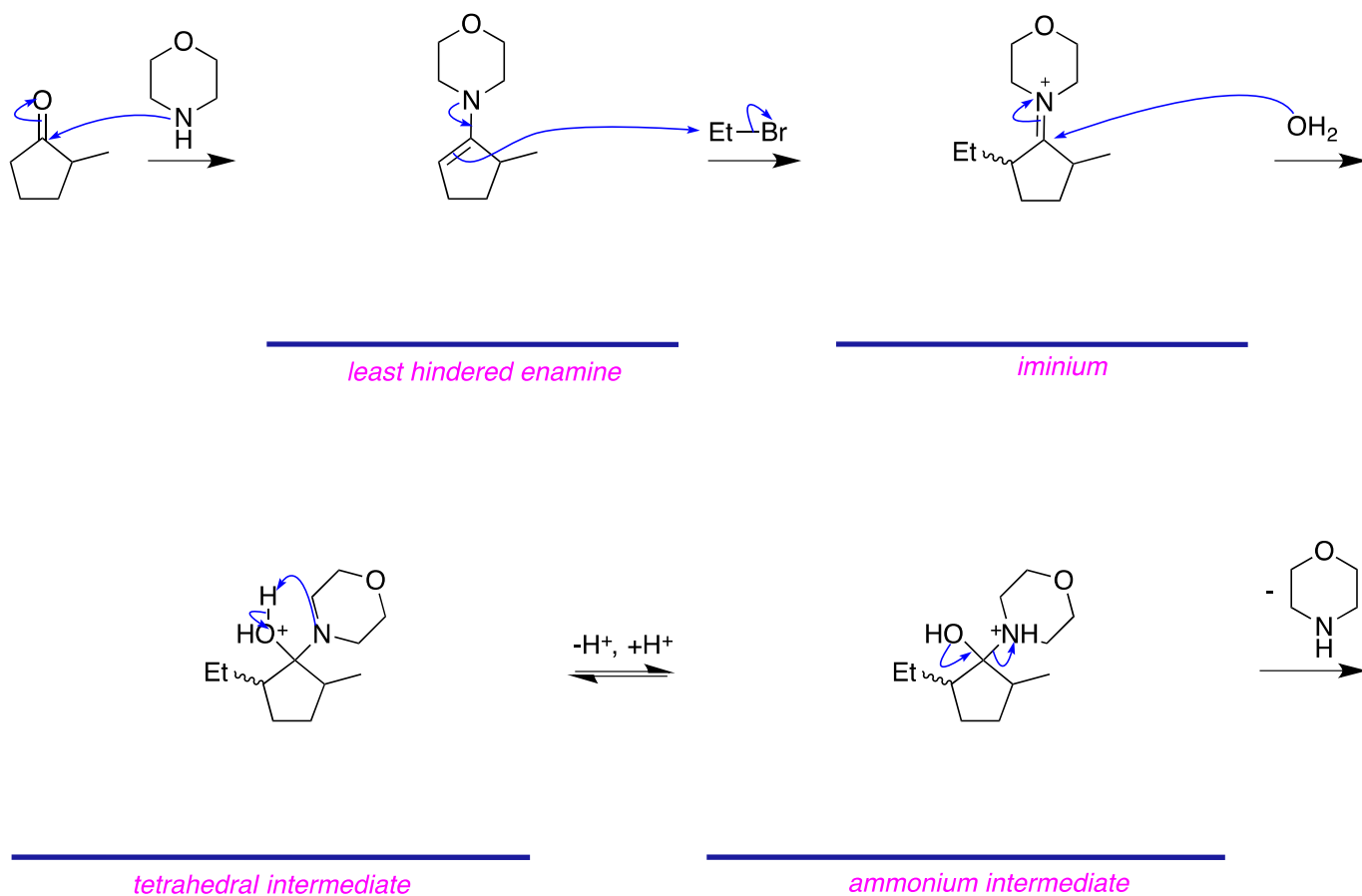
protonated carbonyl

Synthesis of enamines from unsymmetrical ketones could give *2* possible product(s); the enamine with the least hindered alkene is preferred, so enamine chemistry to preferentially form the corresponding alkylated products at the *least* substituted α-carbon is convenient.

Formation of enamines with four groups on the alkene part is disfavored because steric clashes occur if one of the *N*-substituents is co-planar with any alkene substituent.

Show the products of the following reactions.

_____
enamine

_____
4-methyl-3-hexanone

_____
enamine

_____

_____

_____
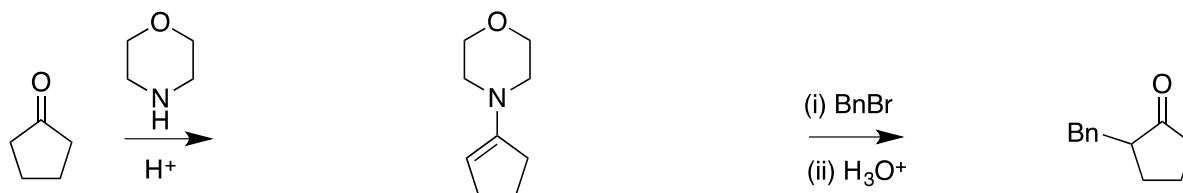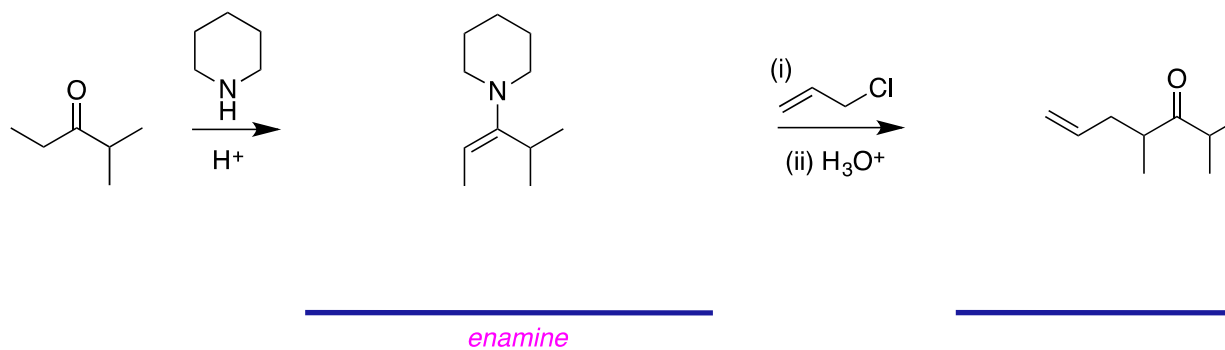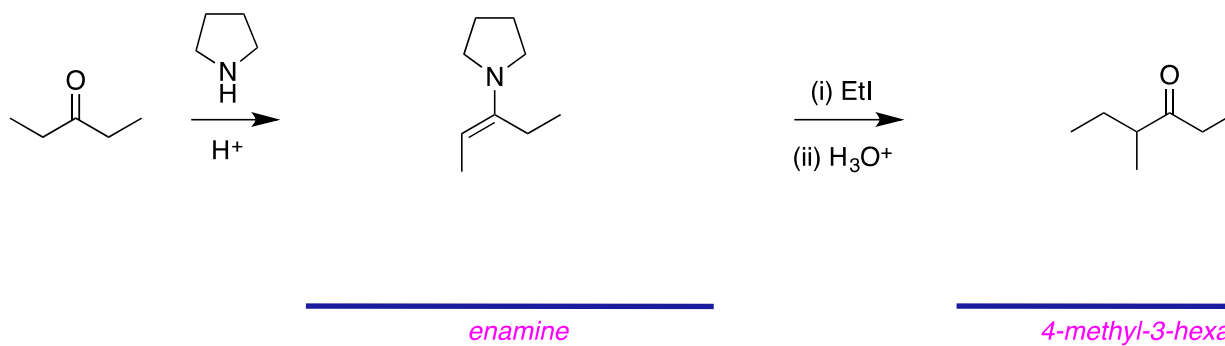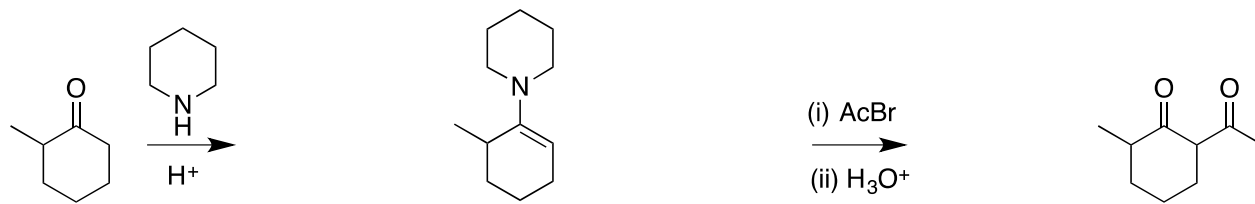
23 Reactions Of Ester Enolates With Carbonyl Compounds

from chapter(s) _____ in the recommended text

Introduction

Focus

Halogens and alkylating agents are not the only electrophiles that can add to enolates. Enolates can be *acylated* and, even though esters are not particularly good acylating agents, they *do* tend to react with enolates. Thus an ester enolate can react with the ester it was formed from, and a mixture of two esters may cross-react under basic conditions, though a complicated mixture of products is formed if *both* esters are enolizable.

Reasons To Care
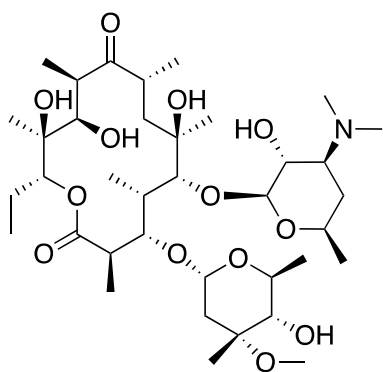
Besides carbohydrates, nucleic acids, and proteins, living organisms produce a huge number of diverse organic molecules, "natural products", some of which have medicinal properties and a subset of these can be used as pharmaceuticals. Like carbohydrates, nucleic acids, and proteins, these natural products are assembled *in vivo* from naturally occurring building blocks. However, the building blocks used to obtain these compounds are diverse, and their assembly is augmented by a series of reactions to modify them. For instance, a common pathway that Nature follows to make natural products involves combinations of the following steps: acylation of enolates (particularly, thioenolates with thioester electrophiles), carbonyl reductions, dehydration, and addition of hydrogen across alkenes. Those steps may be performed iteratively, where some are not always used in each cycle, to build materials that display functional groups in irregular three-dimensional shapes, often evolved for specialized functions *in vivo.*
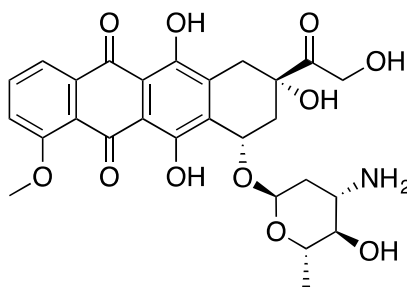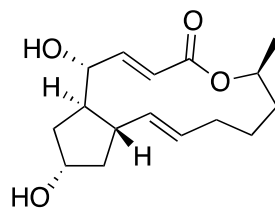
erythromycin   *doxorubicin*   *brefeldin A*

Concepts Involved

enolates • electrophilic attack • condensation reactions • thioesters

Objective

Sophomore organic chemistry, or any other course, cannot present a simple rationale for how all natural products are formed, because there is not one. However, the objective of this part of the course is to cover some of the most important reactions that are used in syntheses and biosyntheses of natural products

Enolates With Ester Electrophiles (Claisen Condensations)

Homocoupling Of Esters

Each of the following structures has *one or two* predominantly nucleophilic or electrophilic *C*-atom(s); circle it and indicate if it is a nucleophile or an electrophile.
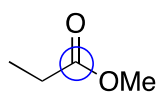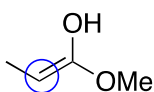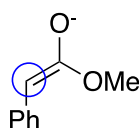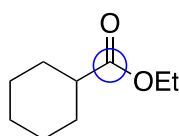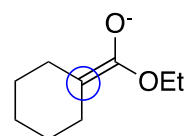
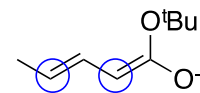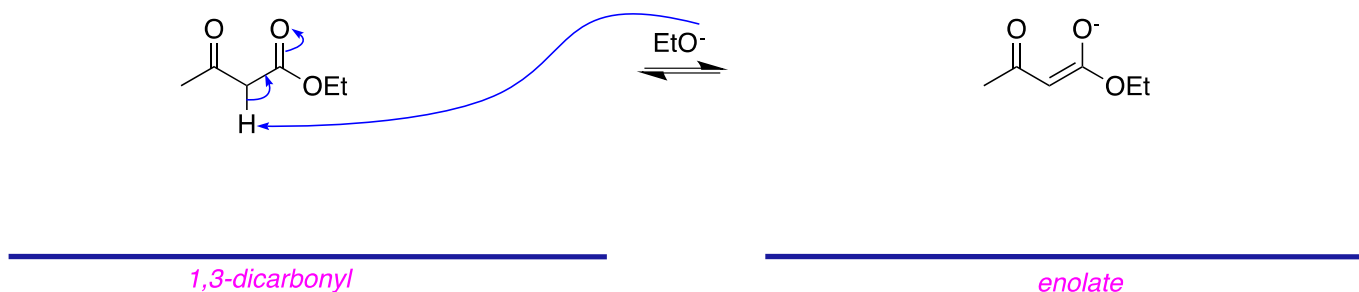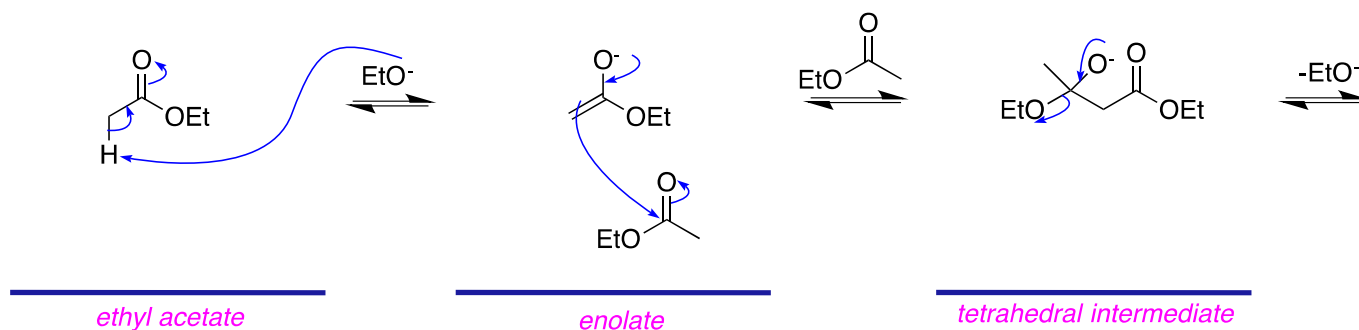*electrophile*   *nucleophile*   *nucleophile*   *electrophile*   *nucleophile*   *nucleophile*

Claisen condensations involve reaction of *enolates* with esters.

Show the electron flow for this process for self-condensation of ethyl acetate.

ethyl acetate   *enolate*   *tetrahedral intermediate*

1,3-dicarbonyl   *enolate*

In the reaction sequence above, the 1,3-dicarbonyl product is *more* acidic than the starting material.

The amount of base needed in these reactions is *catalytic*.

Alkoxide bases are frequently used in these processes. It is desirable to use the alkoxide that corresponds to the alkoxy group in the ester to avoid *transesterification*.

Give the products of the following homocoupling reactions of esters.

Ph⟶OMe (with C=O)   (i) MeO⁻ → (ii) H⁺   Ph⟶(C=O)⟶CH(Ph)⟶(C=O)OMe

1,3-dicarbonyl

(allyl)CH₂CH₂(C=O)OEt   (i) EtO⁻ → (ii) H⁺   product: ester with OEt, adjacent C=O and allyl groups

(propanoyl allyl ester)   (i) ⁻OCH₂CHCH₂ → (ii) H⁺   product: 1,3-dicarbonyl allyl ester with methyl

How could Claisen condensations be used to make these products?
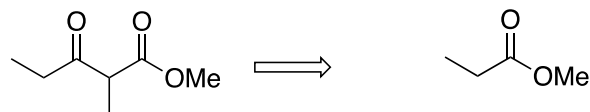
Uncontrolled Cross-Claisen Condensations

The following cross coupling reaction *cannot* give predominantly one product; if only one material was desired then separation might be difficult because two of the products are constitutional isomers that probably have similar polarities and boiling points. Show all possible products of the following reaction.
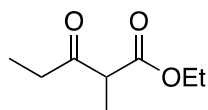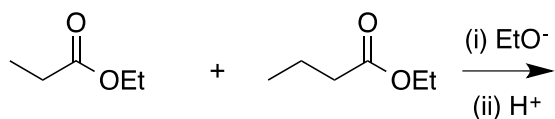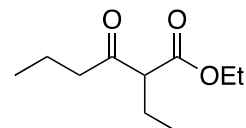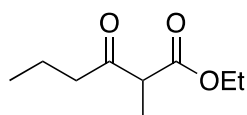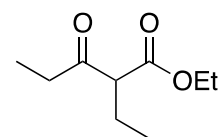
constitutional isomers

Controlled Cross-Claisen Condensations

Only three of the eight carbonyl compounds shown below can form enolates. Draw the enolates of those three.
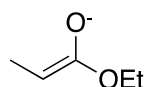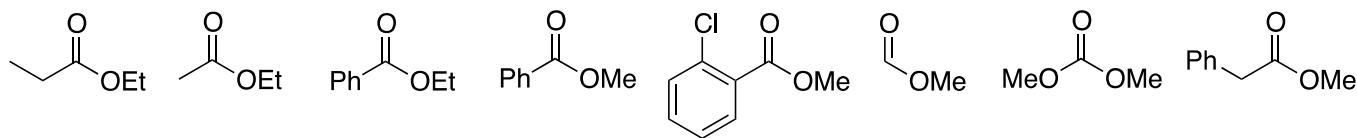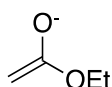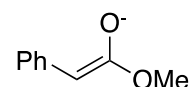
enolate 1   *enolate 2*   *enolate 3*

Cross coupling reactions between esters can give predominantly one product if *only one* of the components has/have enolizable carbons. *Only* three of the six reactions below can give exactly *one* cross coupling product; identify those three, and draw the products.
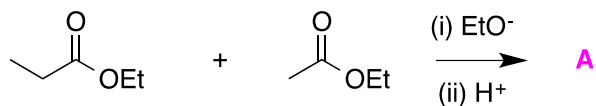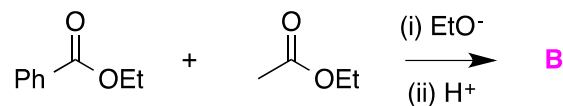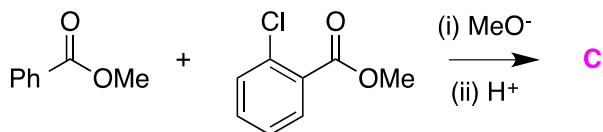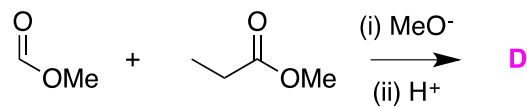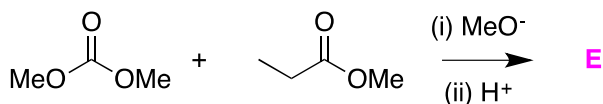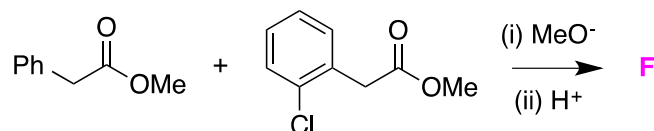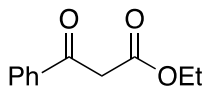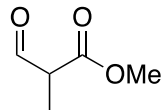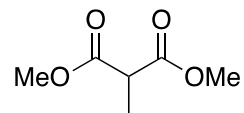
letter __**B**___   letter __**D**___   letter __**E**___

Identify starting materials from which the following cross Claisen products could be made.
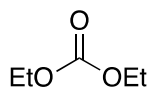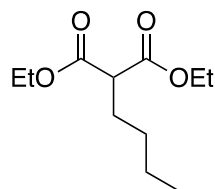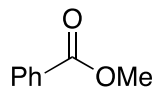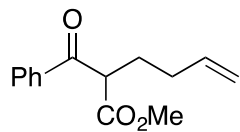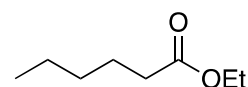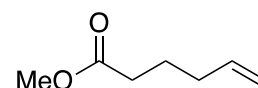
Intramolecular Reactions Of Ester Enolates With Esters (Dieckmann Reactions)

Intramolecular condensations of a diester where the ester groups are identical can give predominantly one product.

Show how this 1,6-diester can undergo intramolecular cyclization to form a substituted cyclopentanone.

enolate

enolate in conformation for cyclization

-MeO⁻

a cyclopentanone
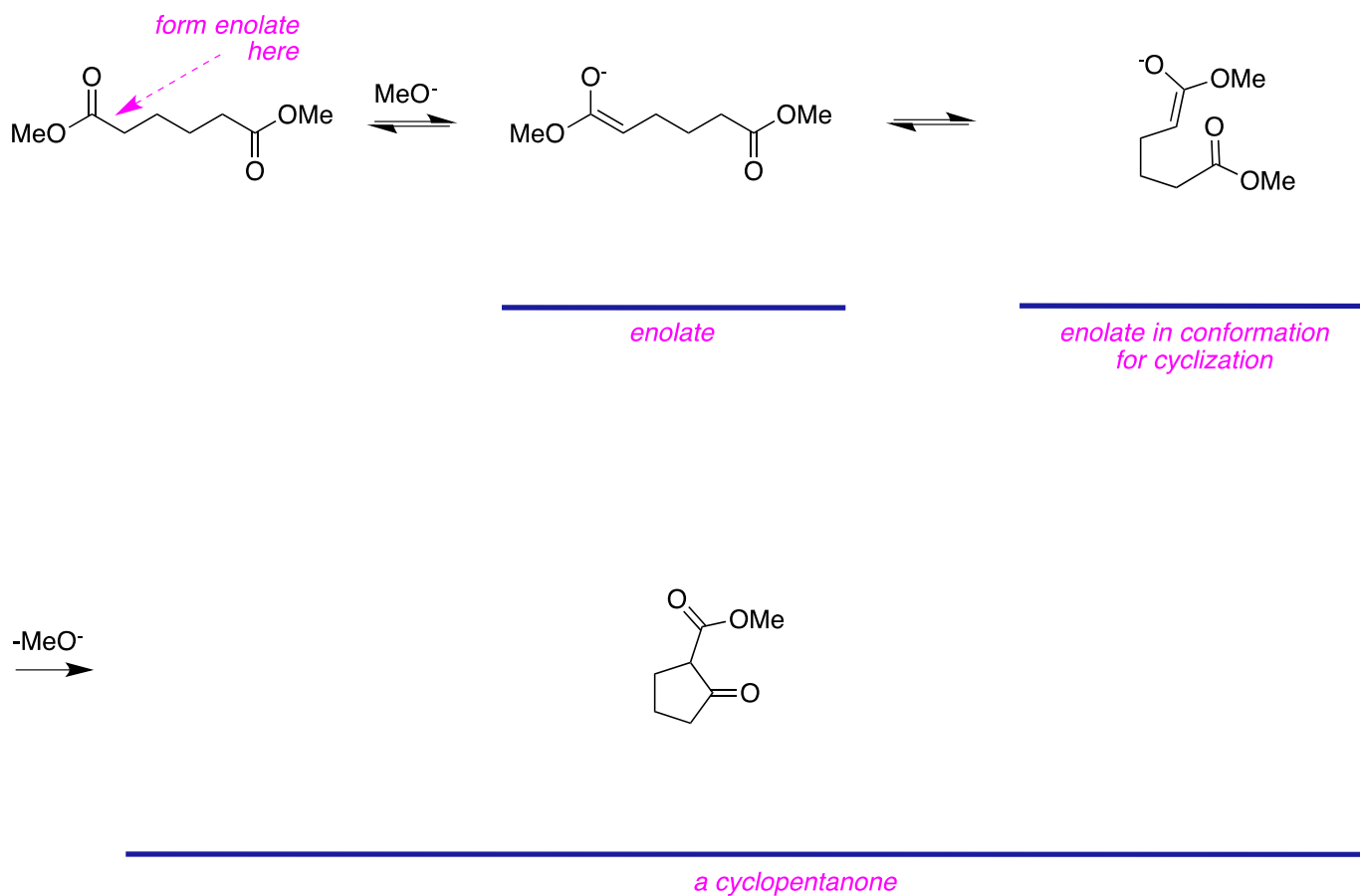
Predict the products of the following reactions (the left hand one goes through a ketone enolate).
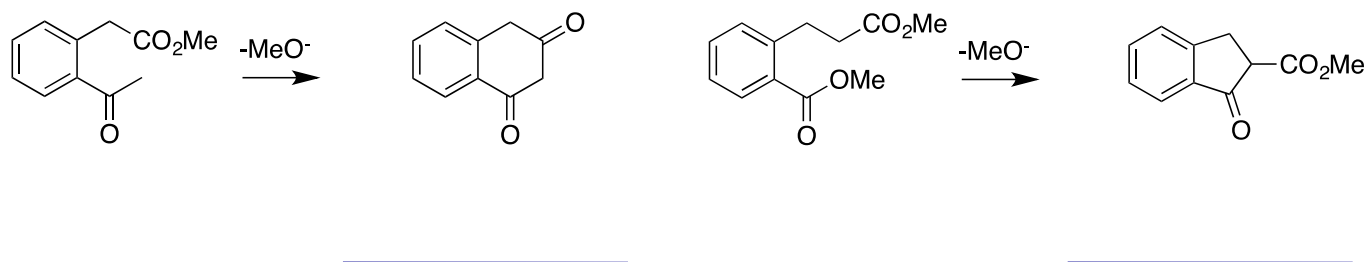
Spirocyclic atoms are ones that *form a single point of contact between two rings*.

Draw the product of the following reaction and encircle the spirocyclic atom.
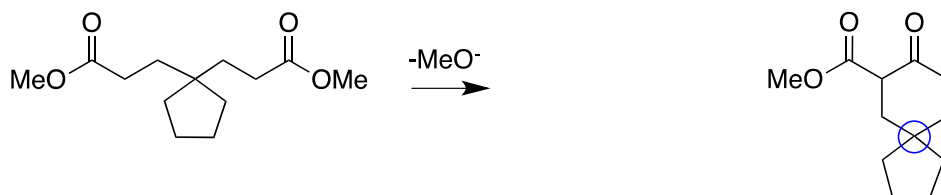
Similar cyclizations that involve two inequivalent, enolizable esters can give *more than one* cross-coupling product.
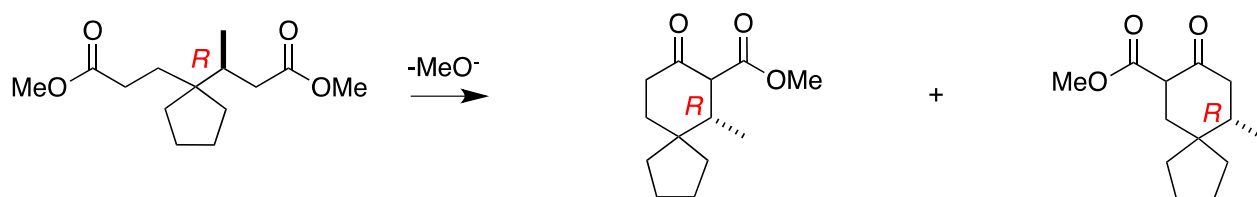
These are _less_ practical strategies to obtain cyclic products than the ones shown above because the two products would have to be separated and the yield of the desired one would be _diminished_ in the isolation.

Draw all the possible products of the following reactions.
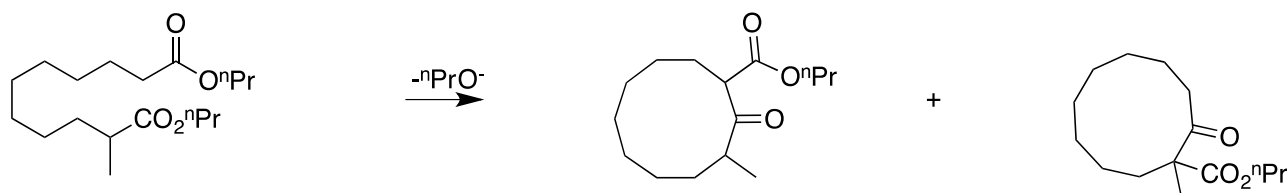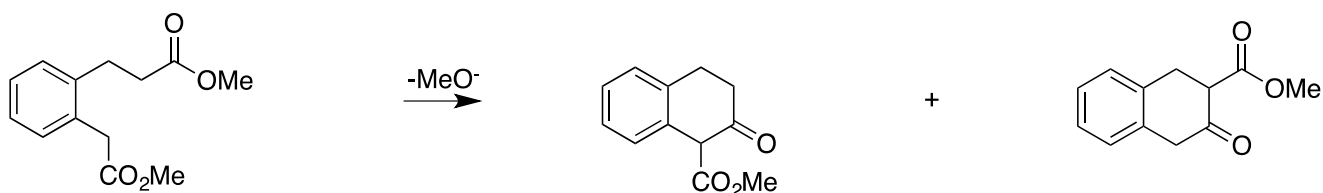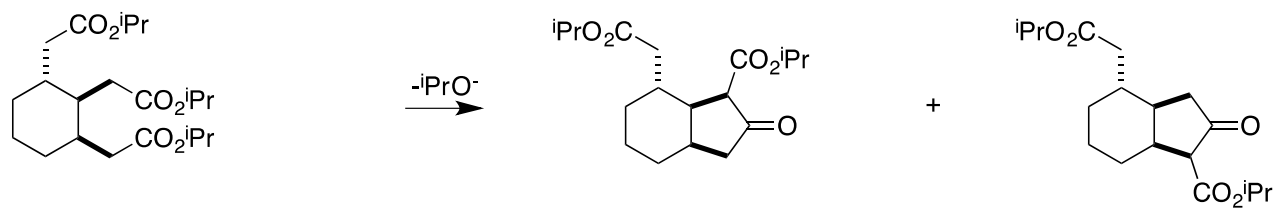
assume trans-fused 6,5-rings are not formed since they are less stable

Check your answer to the last question has the correct stereochemistry by assigning *(R)*- or *(S)*-configuration to the starting material and to the product; the chiral center should have _the same_ configurations.

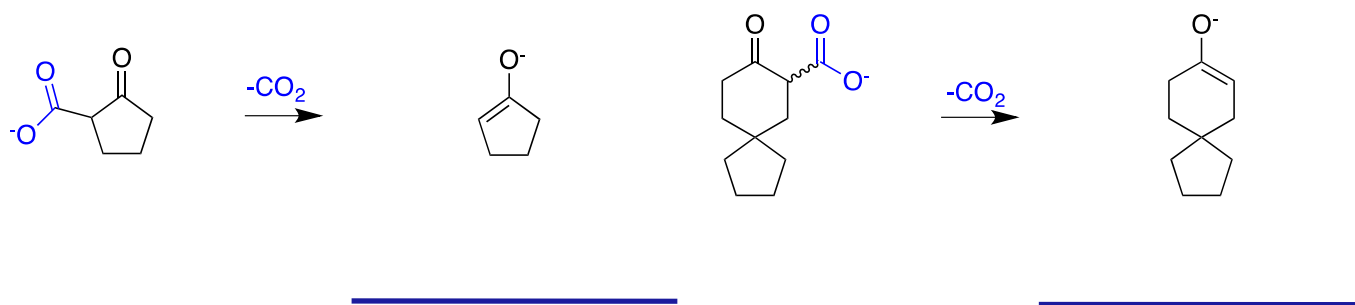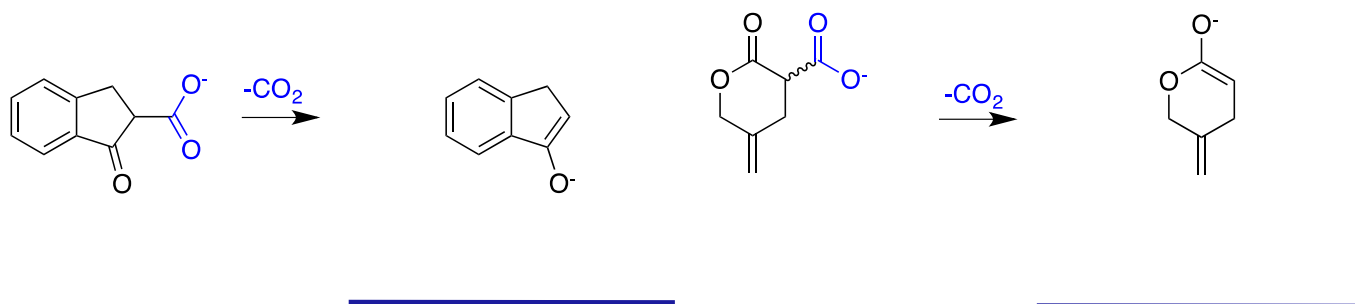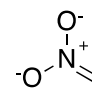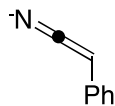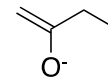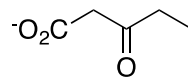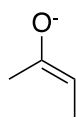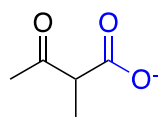# Decarboxylation Of 3-Oxocarboxylic Acids

From Carboxylates

β-Keto carboxylates (*eg* from Claisen reactions) tend to decompose quickly to form an enolate, with release of CO_2.

Draw arrows to show loss of carbon dioxide from 3-oxobutanoate *anion* to give the enolate of acetone.
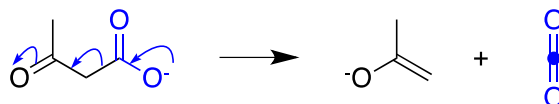
Use the concept shown above to predict the enolates formed from loss of CO_2 in the following reactions.

-CO$_2$

-CO$_2$

-CO$_2$

-CO$_2$
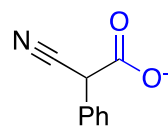
From Carboxylic Acids

Enolates and enols are similar to *carboxylates* and *carboxylic acids*, respectively.
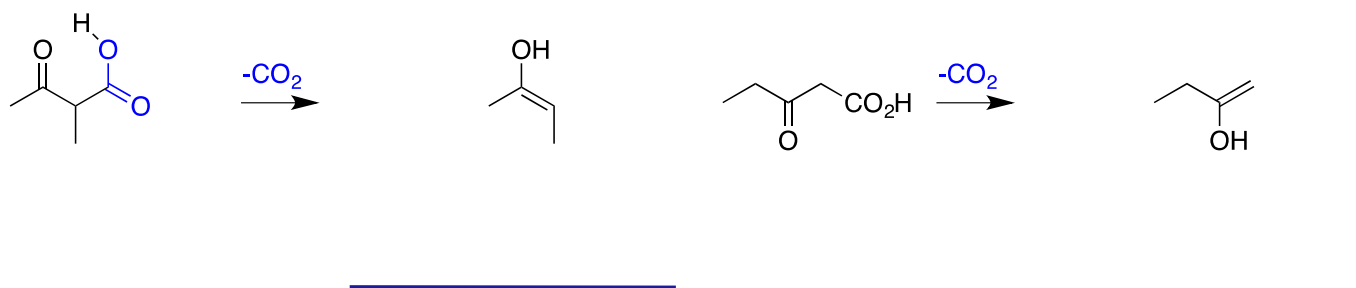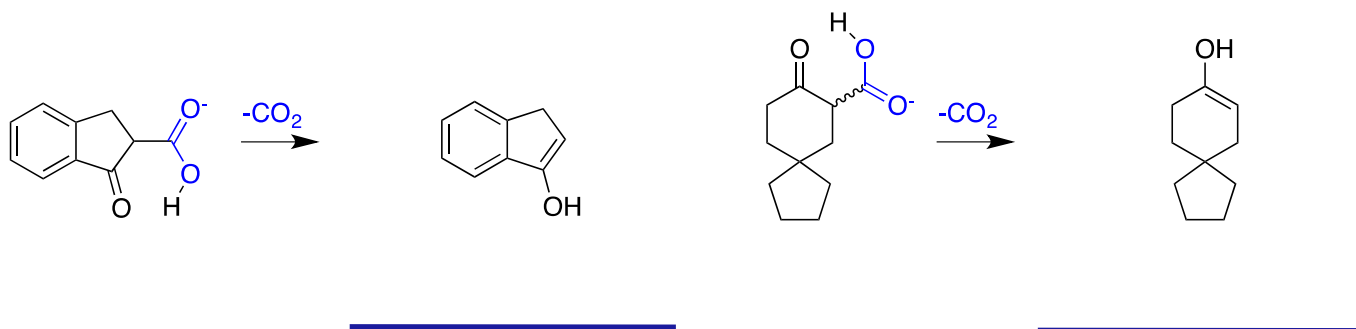
Like β-keto carboxylates, β-keto *acids* can lose CO_2 but they directly generate *enols* (whereas *carboxylates* produced *enolates*).
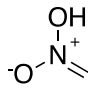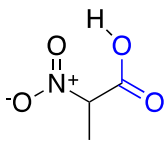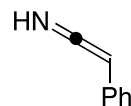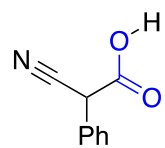
These processes are facilitated by intramolecular hydrogen bonding in the starting material that makes them possible with minimal movement of atoms.

Complete the following drawing of an intramolecularly *H*-bonded conformer of 3-oxobutanoic acid by adding arrows to illustrate loss of CO_2 with concurrent formation of the *enol* of acetone.
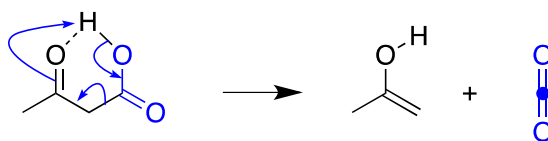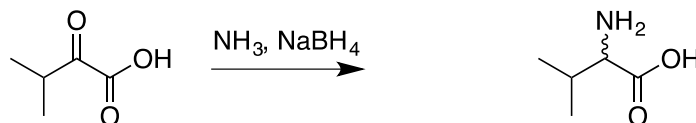

Use this concept to predict the products of the following reactions.

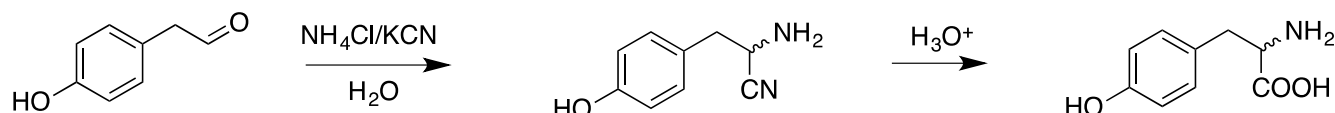$$N\equiv C-\underset{\underset{Ph}{|}}{CH}-CO_2H \quad \xrightarrow{-CO_2} \quad HN=C=CH-Ph$$

$$O_2N-\underset{\underset{CH_3}{|}}{CH}-CO_2H \quad \xrightarrow{-CO_2} \quad \underset{O^-}{\overset{OH}{\underset{|}{N^+}}}=CH-CH_3$$
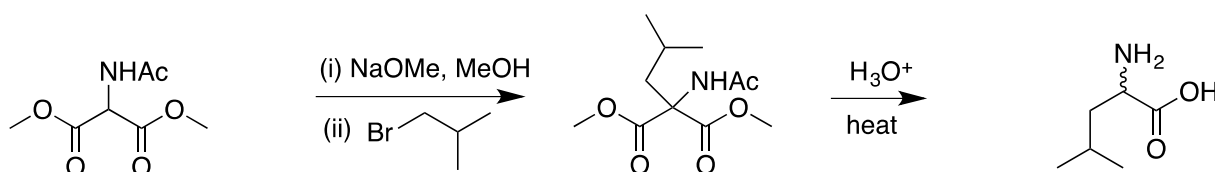
Classical Syntheses Of Amino Acids

Draw and name the amino acid formed in each of the following syntheses.
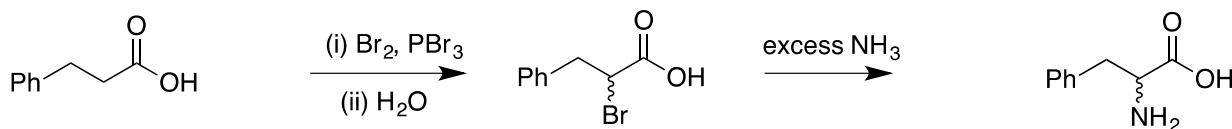

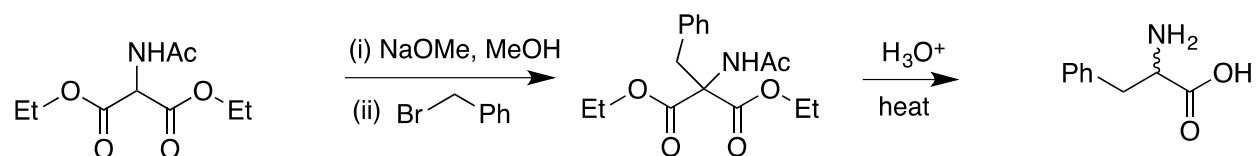*amino acid* ____valine_____

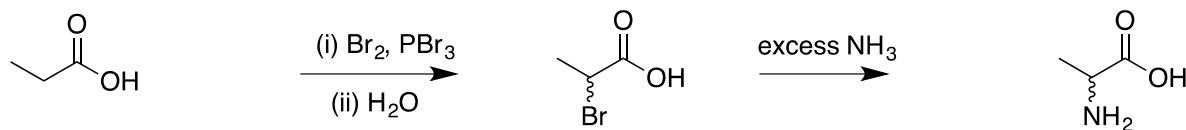*amino acid* tyrosine

amino acid leucine

amino acid phenylalanine

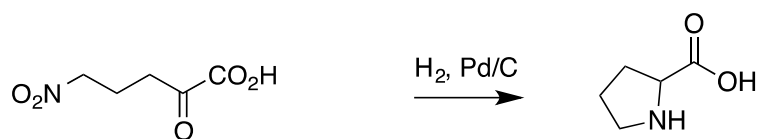Amino acids formed in these syntheses will be *racemates*.

Suggest a synthesis of (±)-Phe from diethyl acetamidomalonate and benzyl bromide.

Suggest a synthesis of (±)-Ala from propionic acid.

Suggest a synthesis of (±)-Pro from the following nitro compound.

Thioesters Are More Reactive Than Esters
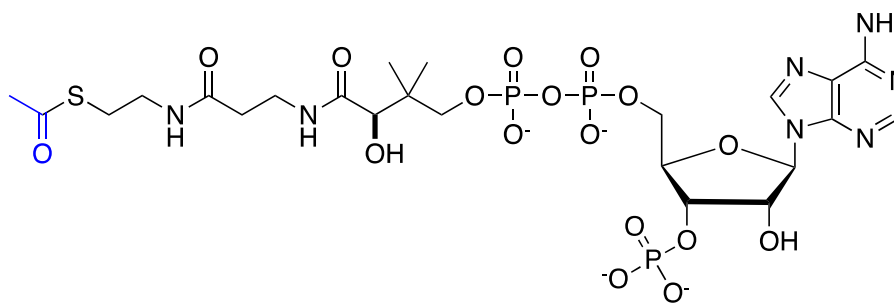
Nature uses *thio*esters for intracellular acylation.

The pKa of thioethanol is 10.5 whereas that of ethanol is 15.9. EtSH is therefore a *stronger* acid than ethanol, EtS⁻ is a *better* leaving group than EtO⁻, and thioesters will undergo reactions in cells under *milder* conditions than esters would.

Look up the structures of coenzyme A and acyl-CoA and write them below.
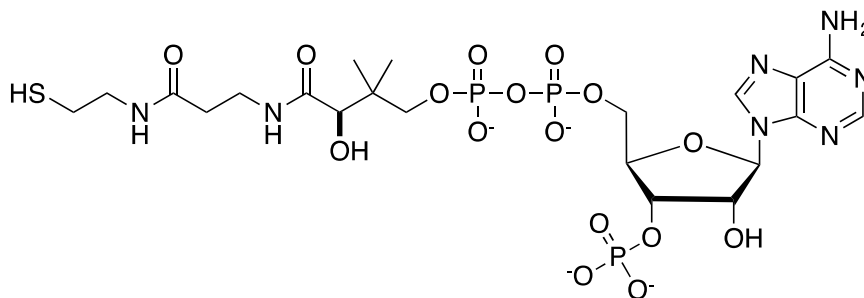

coenzyme A or CoASH

acyl coenzyme A or acyl-CoA

The leaving group in these molecules is *huge* compared to acyl.

Thioesters are *better* electrophiles than esters.

Nature's Equivalent To Claisen Condensations

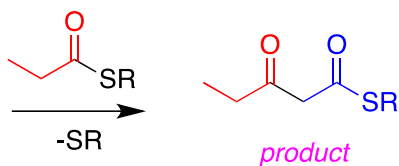Malonyl-CoA is the intracellular equivalent of malonic esters in the lab. This transesterifies with endogenous thiols and the product reacts with thioesters, then reacts with thioesters in a reaction driven by loss of CO_2 from the malonate derivative. Outline this process in general terms.
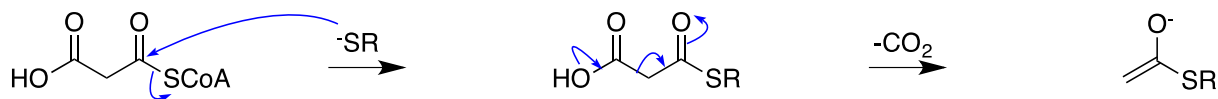
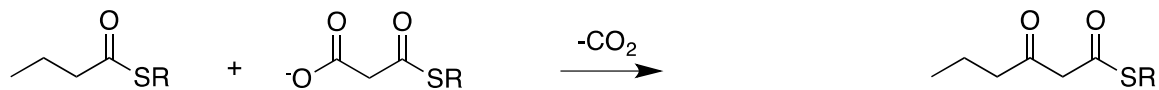*malonyl-CoA*   *transthioesterification product*   *enolate of thioester*
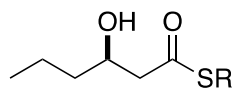

product

Fatty acids are constructed by hydridic reduction of the carbonyl in the products, followed by combinations of dehydration and hydrogenation reactions.
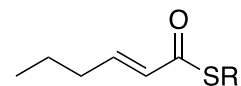
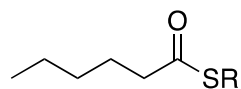Draw an outline of this process in general terms in the space below.

24 Aldol and Aldol Condensation Reactions

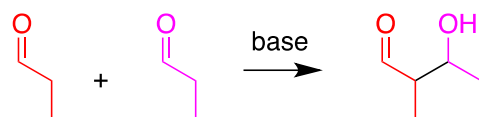from chapter(s) _____ in the recommended text

Introduction

Focus

The last section was about reactions of ester enolates with esters that give 1,3-dicarbonyl compounds. This one is primarily about analogous reactions of aldehyde or ketone enolates with aldehydes or ketones, and it mirrors transformations of esters, but one oxidation state below them.
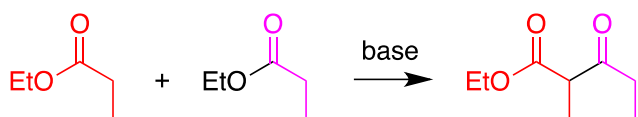
esters condensing with esters: Claisen   *aldehydes condensing with aldehyde: aldol*

Aldol reactions are inherently more complicated than condensations between esters for several reasons. Firstly, mixtures of diastereomers can be formed (*syn* and *anti*) because there are two chiral centers in most aldol products, not one as in most ester/ester condensations. Secondly, aldol product may dehydrate to α,β-unsaturated carbonyl compounds.

Reasons To Care

Both aldol and ester condensations are useful in construction of larger carbon skeletons in natural products.

Concepts Involved

enols and enolates • ester-ester condensations • stereochemistry • alkene hydration reactions (reversed)

Objective

To illustrate that aldol reactions are not hard to learn for students who already understand ester condensations. Aldol reactions have some different characteristics, but these are logical, and not as important as appreciating the parallel shown in the diagram above.
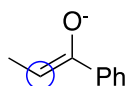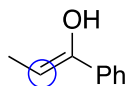
Acidities Of Carbonyl Compounds

Aldehydes and ketones (and other carbonyl compounds) may act as electrophiles, and, if they have acidic α-*CH* atoms, as nucleophiles too. Each of the following structures has at least one carbon that is nucleophilic or electrophilic; circle that center(s) in each case and indicate if it is a nucleophile or an electrophile.
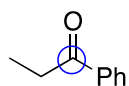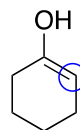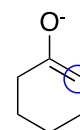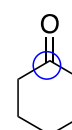
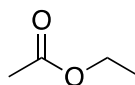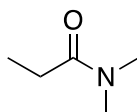*electrophile*   *nucleophile*   *nucleophile*   *nucleophile*   *nucleophile*   *electrophile*

Draw the following compounds in the spaces provided, in order of increasing acidity of their α-*CH*s based on the pKa values indicated: *N,N*-dimethylpropionamide (~30), ethyl acetate (24.5), ethanal (17.0), acetone (19.2), ethanonitrile (25).

increasingly acidic α*CH*

These compounds are *less* acidic than water (pKa *ca* 16).

Electron withdrawing groups (EWGs) increase the acidity of α-*CH*s in a compound by stabilizing its deprotonated form. Two EWGs can provide more stabilization than only one EWG. Arrange the following doubly activated compounds in order of increasing acidities of the α-*CH*s.
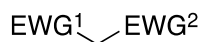
EWG^1 EWG^2 where EWG^1 = EWG^2 = CO$_2$Et; COMe; NO$_2$
and where EWG^1, EWG^2 = MeCO, CHO; MeCO, CO$_2$Et

EtO$_2$C CO$_2$Et   MeOC CO$_2$Et   MeOC COMe   MeOC CHO   O$_2$N NO$_2$
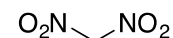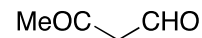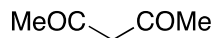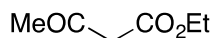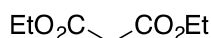
increasingly acidic α*CH*

Some of these compounds are *more* acidic than water. In the presence of one equivalent of hydroxide they exist mainly in their *deprotonated* forms.

Aldol Reactions

An *aldol reaction* occurs when a carbonyl compound (aldehyde or ketone) in the enol or enolate form, *ie* acting as a(n) *nucleophile* combines with a second carbonyl compound acting as a(n) *electrophile*.

Show the aldol products of the following reactions (without dehydration).

Enolates are produced from carbonyl compounds under basic conditions. Consequently, aldol reactions such as the ones above may occur simply by adding base to the carbonyl; some of the carbonyl is converted to the enolate form that may add to molecules that have not yet enolized. Thus these types of reactions may be expressed in the following way (show products).
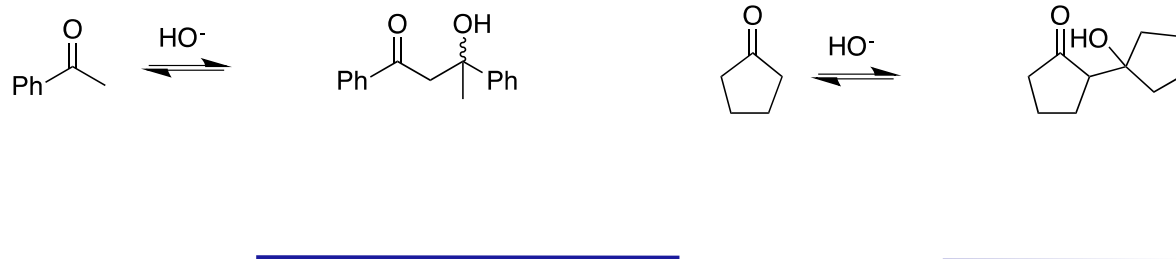
These are *homo*-coupling reactions because they involve combinations of the same units.

Substituted enolates can react with their parent carbonyl compounds to give two diastereomers: *syn* and *anti* (and their en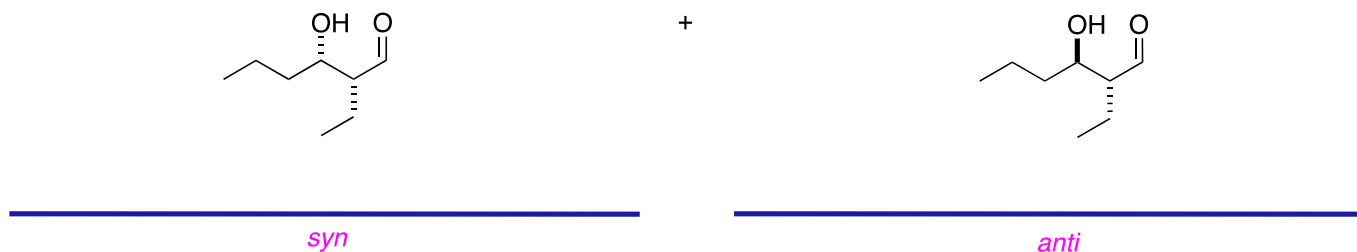antiomers). Draw the *syn* and *anti* isomers of the following aldol reactions. These diastereomers will be formed in *equal* amounts because the products, and the transition states that precede product formation, have the *same* free energies.
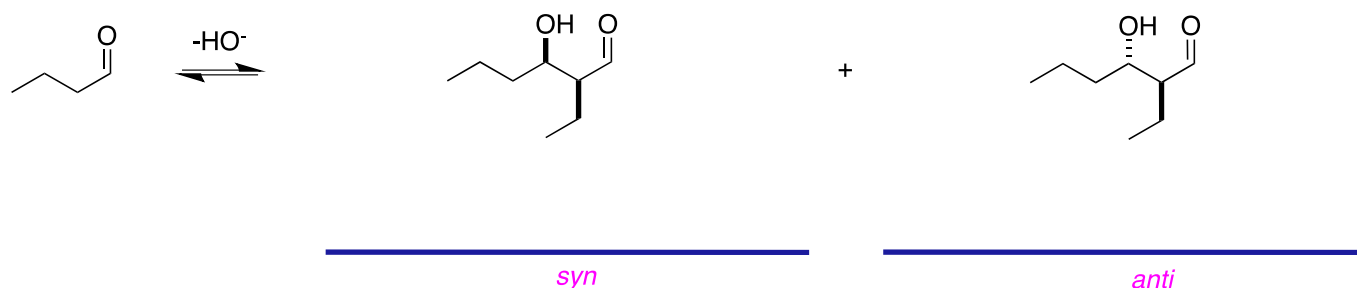
syn   +   *anti*

Draw enantiomers of the products above. The ratio of the enantiomeric *syn* products in this reaction will be *exactly* 1:1. The ratio of the *syn* and *anti* forms of the two products above will be *the same* as to the enantiomers drawn below.

*syn*   *anti*

In reactions such as these, it is common to draw just one set of *syn* and *anti* diastereomers, and ignore their enantiomers. Using this approach, draw the aldol products of the following reaction.

*syn*   *anti*
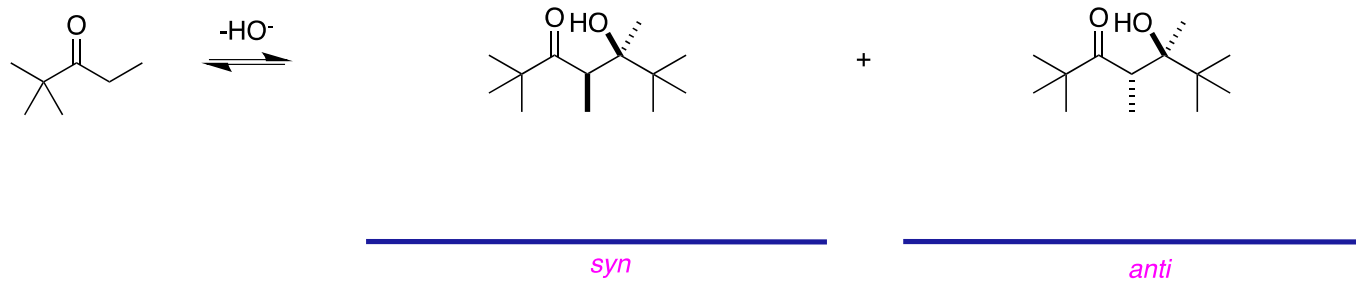
Intramolecular Aldol Reactions

Intramolecular aldol reactions can occur between two ketone or aldehyde groups in *the same molecule*. Draw the products of the following intramolecular reactions.
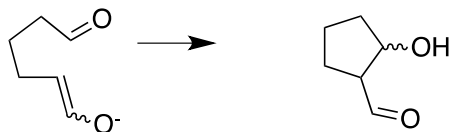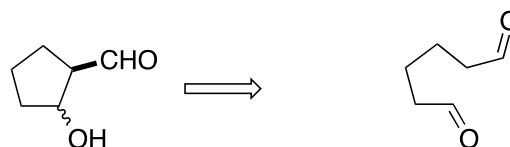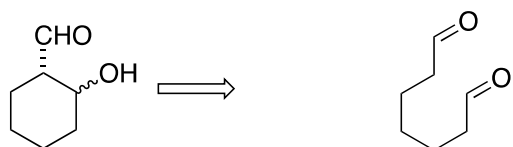

In organic chemistry it is often helpful to draw starting materials in conformations that are predisposed to giving products. Try this for the following cyclization reaction.
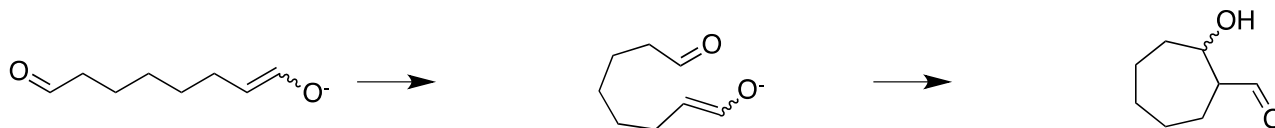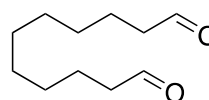

starting material re-drawn   *product*

Predict the dialdehyde starting materials that were used to form these cyclic products via aldol reactions.
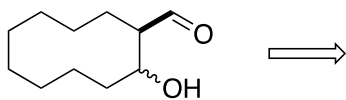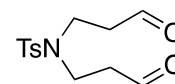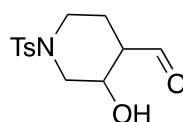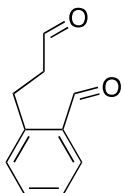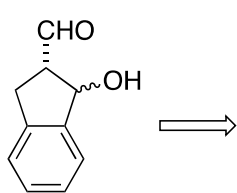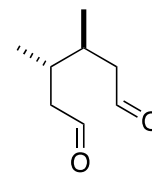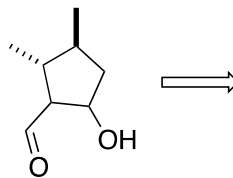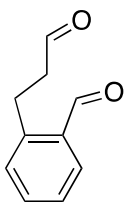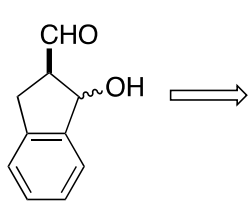
Ten-membered rings may be drawn by sketching decalin in a 2D representation, then removing the edge-shared C – C bond. Similarly, an easy way to draw 14-membered rings is removing the two shared C – C bonds from the structure of three linearly fused cyclohexanes.

Draw the following cyclic molecules in two dimensions.

trans-decalin
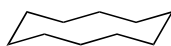
cyclo-$C_{10}H_{20}$
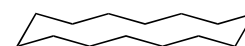
cyclo-$C_{14}H_{28}$
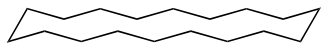
cyclo-C$_{18}$H$_{36}$
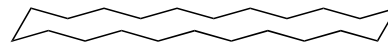
cyclo-C$_{22}$H$_{44}$

Under reversible conditions, a cyclization process at equilibrium favors the thermodynamic product. Illustrate why base treatment of 2,5-cyclohexadione leads to 3-hydroxyl-3-methylcyclopentanone in preference to (2-hydroxy-2-methylcyclopropyl)ethanone.

internal enolate
(extended conformation)

terminal enolate
(extended conformation)

internal enolate
(conformation to give cyclopropane)

terminal enolate
(conformation to give cyclopentane)

kinetic alkoxide

thermodynamic alkoxide
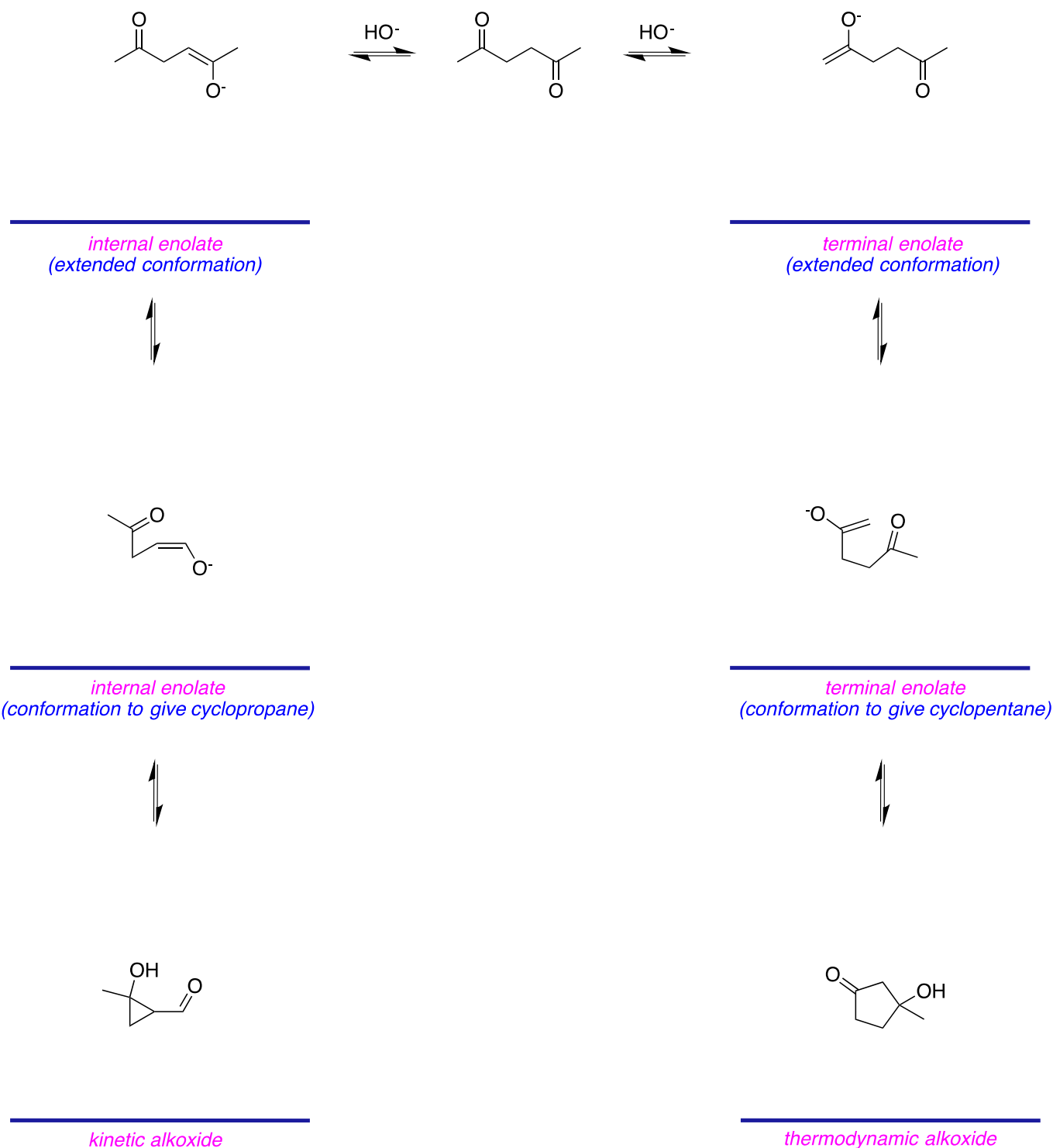
By analogy with the questions on the previous page, explain why 3-hydroxyl-3-methylcycloheptanone is *not* the predominant product from base mediated cyclization of 2,7-octanedione.

*internal enolate
(extended conformation)*

2,7-octanedione

*terminal enolate
(extended conformation)*

*internal enolate
(conformation to give
5-membered ring)*

*terminal enolate
(conformation to give
7-membered ring)*

alkoxide from a cyclopentanol

alkoxide from a cycloheptanol
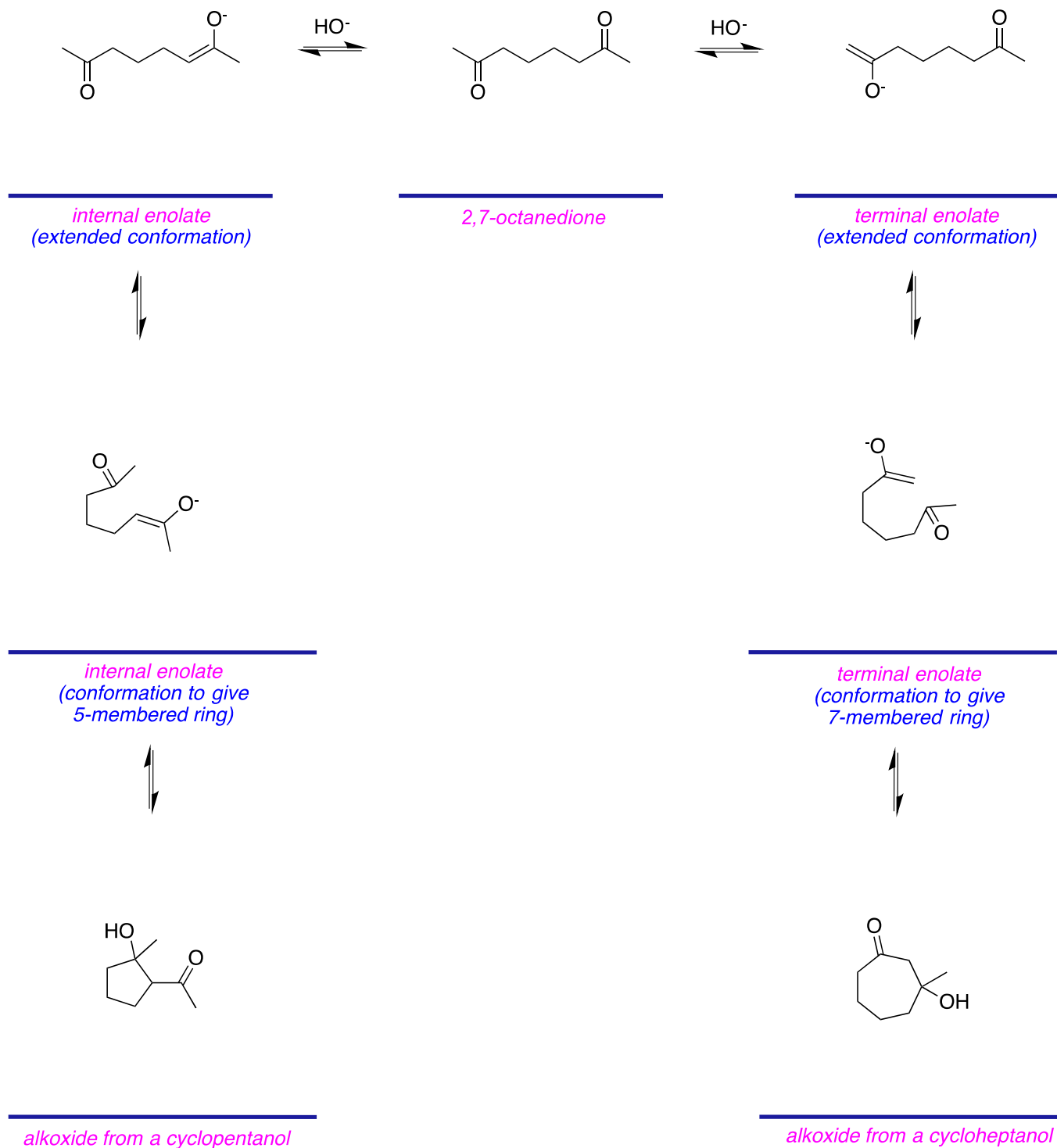
3-Hydroxyl-3-methylcycloheptanone is *not* the predominant product from base mediated cyclization of 2,7-octanedione because it is the *neither the thermodynamic or the kinetic product*.

Predict the alcohol products (after aqueous work-up) of the following *intramolecular* aldol reactions. It may help to first: (i) count the carbon atoms, (ii) deduce which two become connected to give favorable ring sizes; and, (iii) re-draw the starting material with those atoms close to each other.

starting material re-drawn

*product
(edge shared
7 and 5-membered rings)*

starting material re-drawn

product

starting material re-drawn
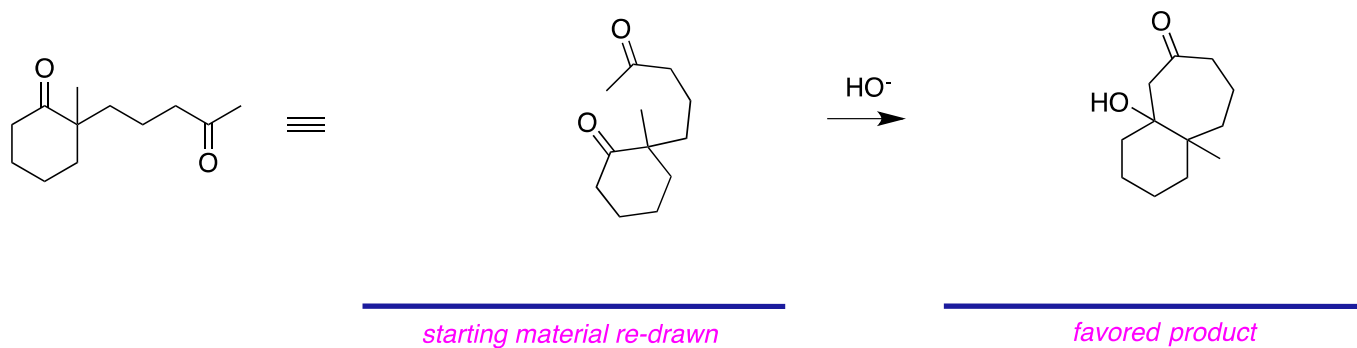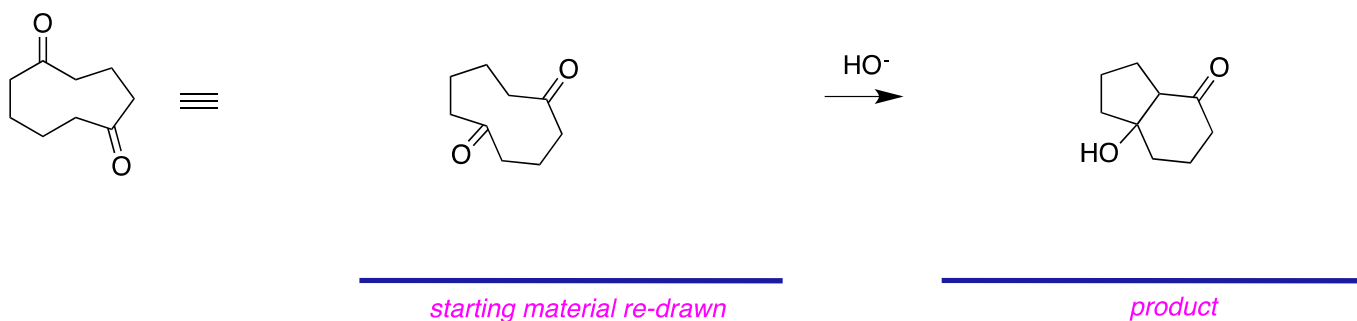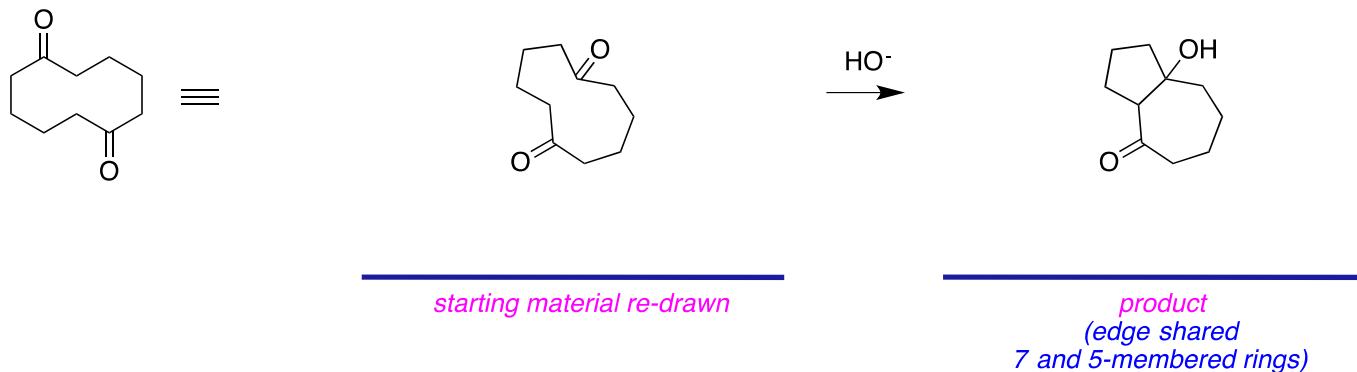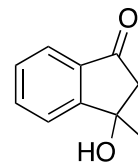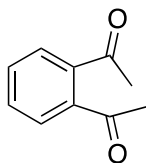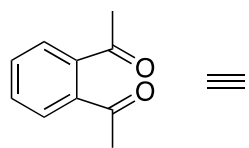
favored product

starting material re-drawn

favored product

Dehydration Of Aldol Products: Aldol Condensations

Homocouplings

Draw the aldol product that would form in the following reaction, then show a mechanism to yield the dehydration product.

*aldol product after
protonation with water*   *oxonium*   *enone*
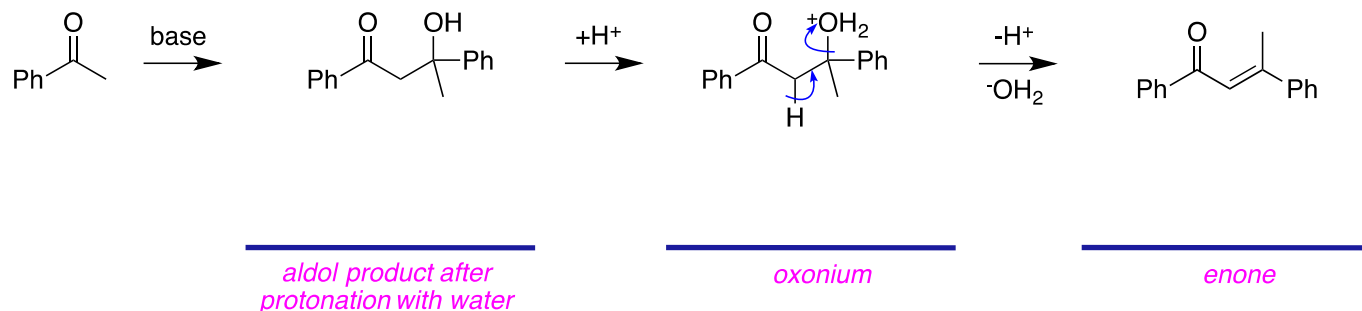
Similarly, draw the product from base-mediated self-condensation of one molecule of acetone with one molecule of itself, then acid-mediated dehydration to give an enone.

*aldol product after
protonation with water*   *oxonium*   *enone*
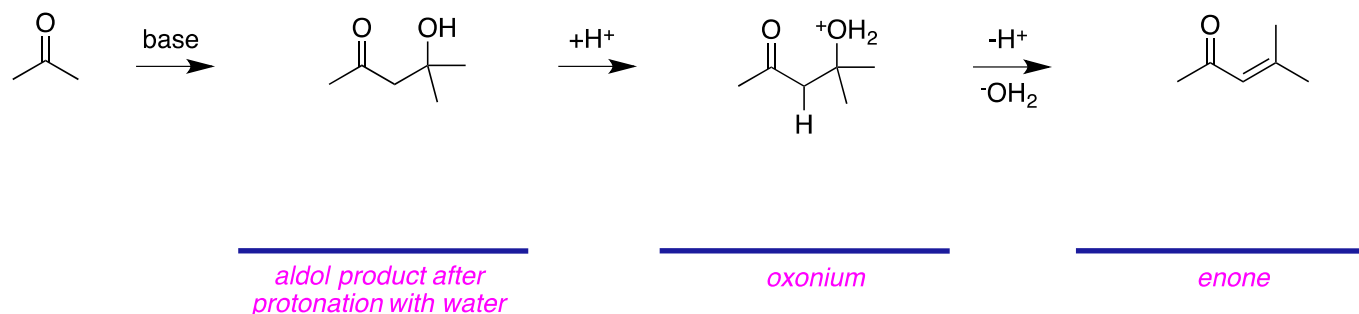
Cross Condensations

Featuring One Enolizable Component

Some *intermolecular* reactions between two different carbonyl compounds can give only one aldol condensation product and only one possible dehydration product. Show the product that may arise via coupling of the following carbonyl compounds.

intermolecular cross aldol   *enone*
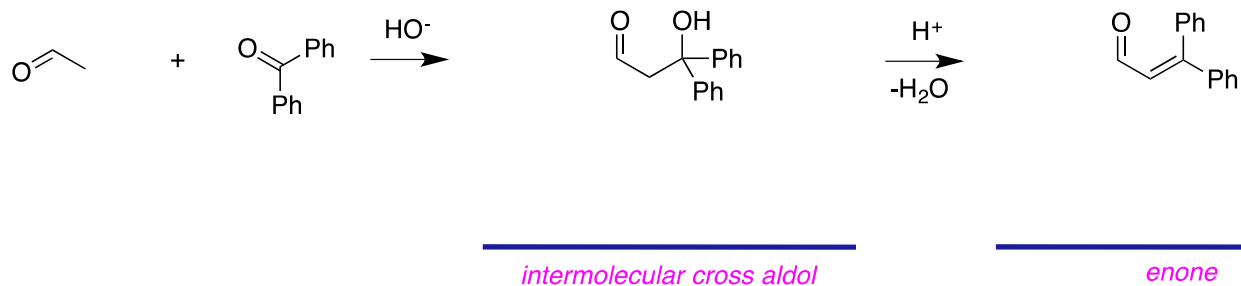
Ethanal is a *more* reactive electrophile than benzophenone, so the reaction above will tend to lead to self-condensation of the aldehyde, but there are no such ambiguities for the following reactions; show the products.

intermolecular aldol   *dehydration to enone*

intermolecular aldol   *dehydration to enone*
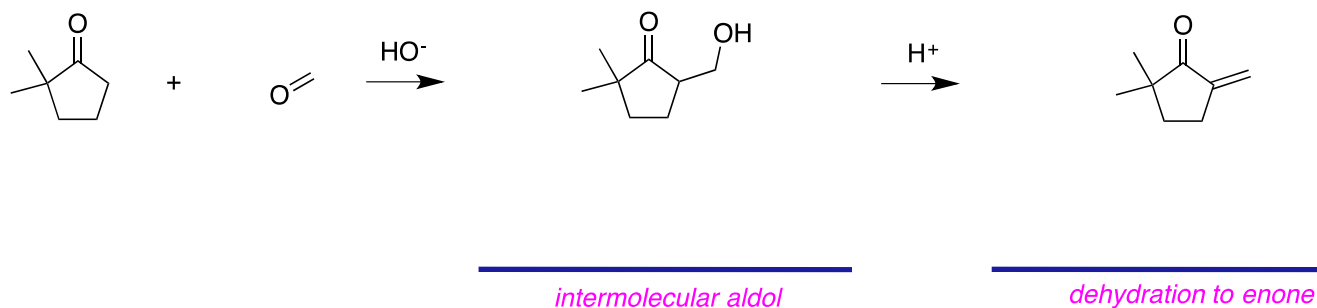
One different enolates can be formed from the starting materials in these transformations. In general, *highly selective* cross-coupling reactions occur when *one* of the components is enolizable.

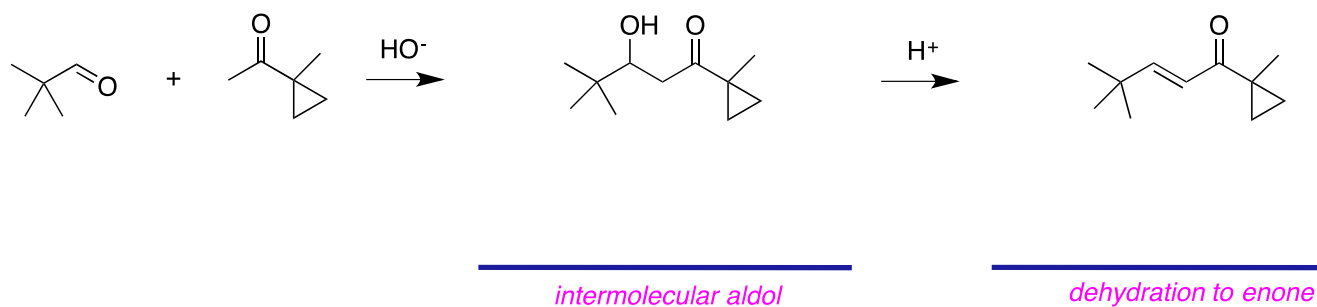If only one of the components can be enolized then only *two* products are possible: one from self-combination and the other from *cross coupling*. Show this for the reaction of ethanal with benzaldehyde.

Ph—CHO   +   CH₃CHO   —base→   homocoupling product   +   heterocoupling product

benzaldehyde   +   ethanal   →   homocoupling product   +   heterocoupling product (ie cross coupling)
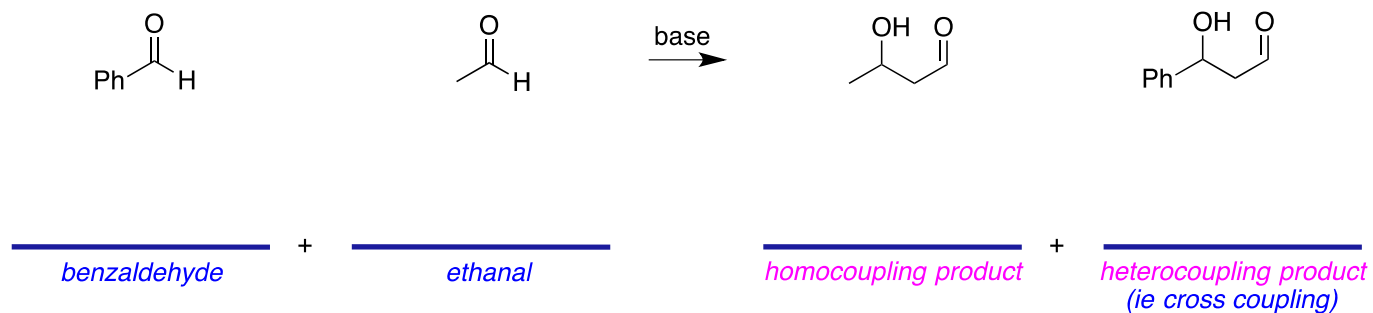
It is possible to suppress *homocoupling* and favor formation of the cross-coupling product by using one carbonyl component in excess, and adding the other one to it slowly. To achieve this the component used in excess would be *benzaldehyde* and the one added slowly to this would be *ethanal*.

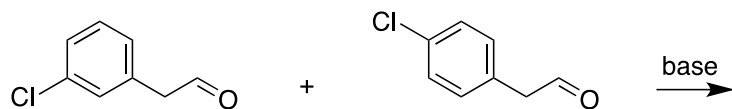Aldol cross-coupling reactions of aldehydes are extremely difficult to control if the aldehydes are similarly enolizable and reactive. Only in some of the cases below can reaction conditions be designed to predominantly afford the cross-coupling product. Draw the corresponding product *for only those cases.*
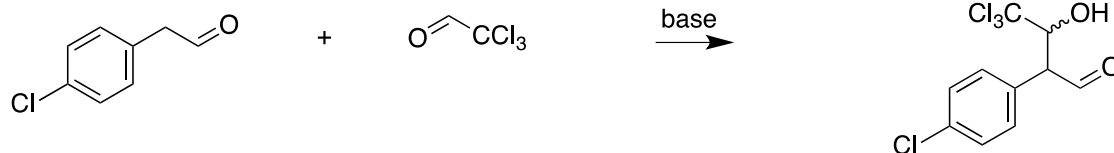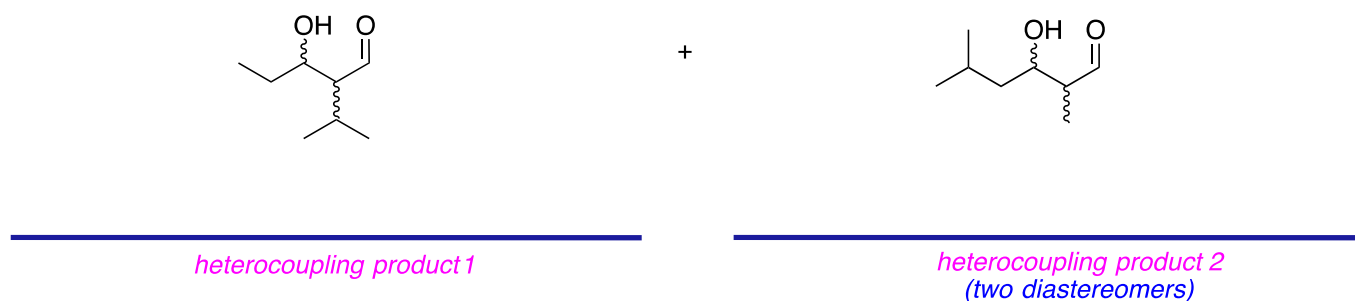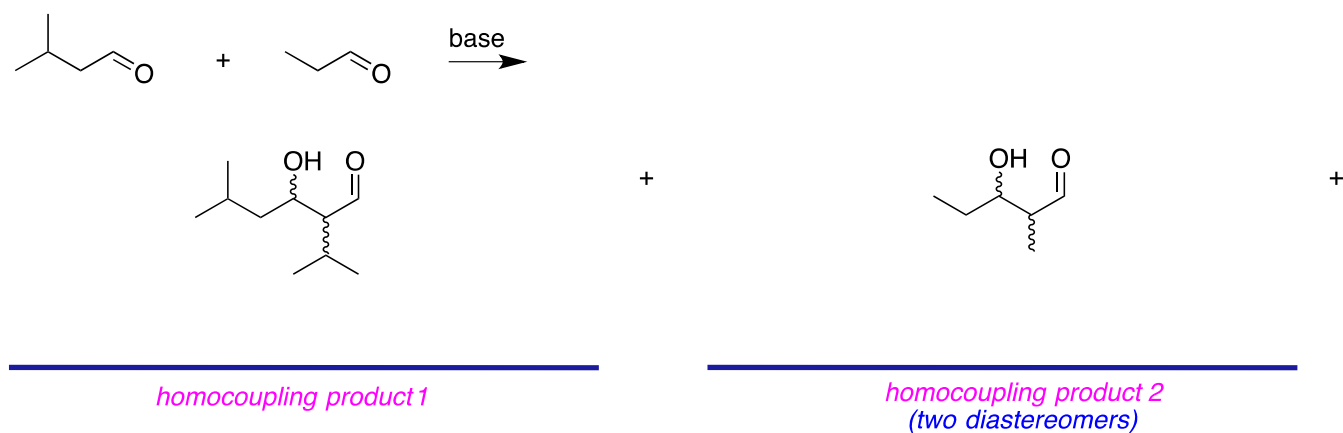

base

heterocoupling product
(ie cross coupling)
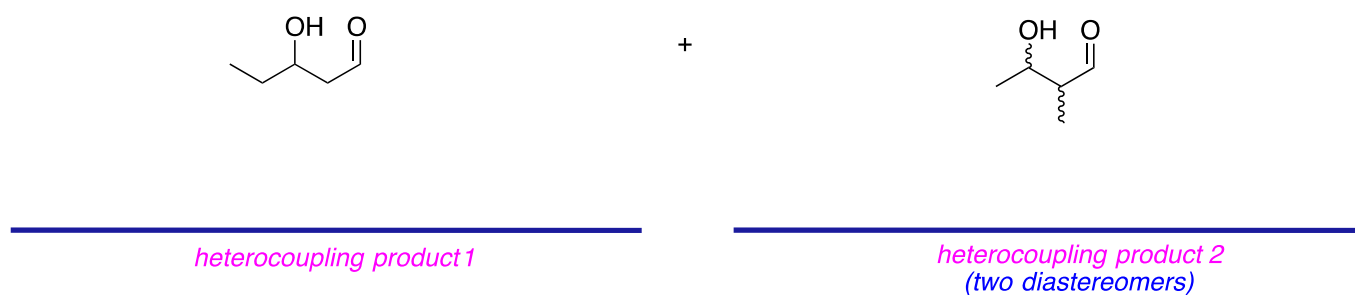

base
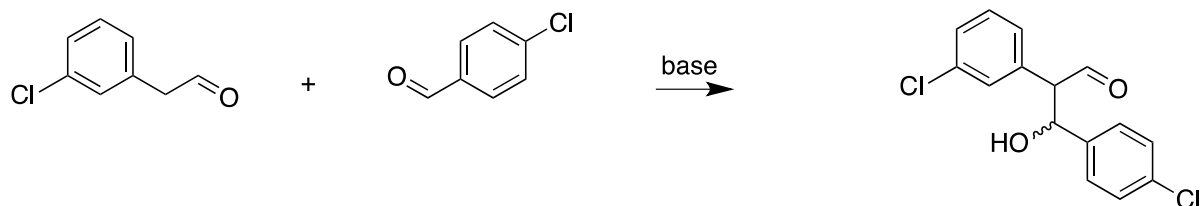
mixture

heterocoupling product
(ie cross coupling)

base

heterocoupling product
(ie cross coupling)

Aldol Condensations Are Hard To Control When Two Enolizable Fragments Are Used

When two different enolizable aldehydes are mixed in the presence of base then either could be both the nucleophile and the electrophile resulting in two self- and two cross-aldol reactions. Show the products (without indicating relative stereochemistry, or show their enantiomers, *ie* use squiggly lines).

homocoupling product 1

homocoupling product 2
(two diastereomers)

heterocoupling product 1

heterocoupling product 2
(two diastereomers)

homocoupling product 1

homocoupling product 2
(two diastereomers)

heterocoupling product 1

heterocoupling product 2
(two diastereomers)
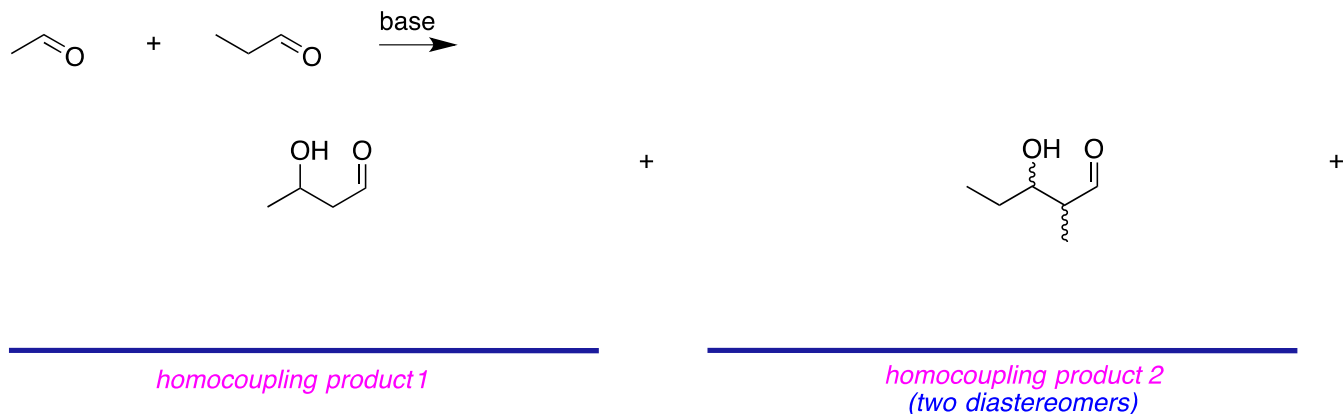
Intramolecular Condensations

The predominant products formed in these types of reactions result from formation of unstrained rings, usually 5- or 6-membered. Draw the predominant product you would expect from each of the following intramolecular aldol-dehydration reactions.


(i) HO⁻

(ii) H_3O^+

(i) HO⁻

(ii) H_3O^+

(i) HO⁻

(ii) H_3O^+
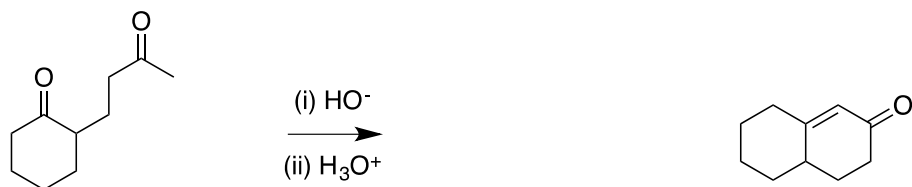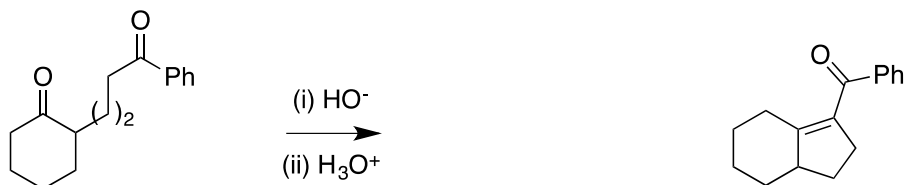
25 Conjugate Additions

from chapter(s) _____ in the recommended text
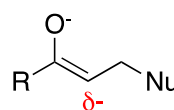
Introduction

Focus

Esters, aldehydes, and ketones can act as electrophiles at their carbonyl atoms, and, if they have α-*CH* atoms, as enolate nucleophiles. α,β-Unsaturated carbonyl compounds can act as electrophiles at the carbonyl group *and* at the β-carbon. Moreover, when α,β-unsaturated carbonyl compounds react with nucleophiles at the β-carbon this produces an enolate that may then combine with another electrophile.
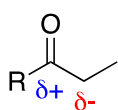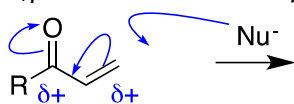
saturated carbonyls   *unsaturated carbonyls in conjugate additions*

Thiols, amines, alkoxides, and even peroxides readily undergo conjugate addition reactions. In cases where the nucleophile is a delocalized enolate, the enolate that is produced may combine with other electrophiles in both inter and intramolecular reactions.

Electron deficient aromatic systems can also accommodate nucleophiles in conjugate addition reactions. The rate determining step in these processes is disruption of the aromaticity. Conjugate additions of this kind are given a special name: nucleophilic aromatic substitutions, S$_N$Ar reactions.

Reasons To Care

α,β-Unsaturated carbonyl compounds are formed after ester-ester couplings, reduction and dehydration, or after aldol reactions followed by dehydration. Their ability to undergo conjugate additions reactions adds a useful facet to their reactivity profiles.

Concepts Involved

ester-ester condensations • aldol reactions and aldol condensations • epoxidation

Objective

This section illustrates how α,β-unsaturated carbonyl compounds have two electrophilic centers, and a masked enolate that is exposed when a conjugate addition occurs.

Polarization Of α,β-Unsaturated Carbonyl Compounds

Draw resonance structures of the following alkenes that put a negative charge on the most electronegative atom of the electron-withdrawing group (EWG).
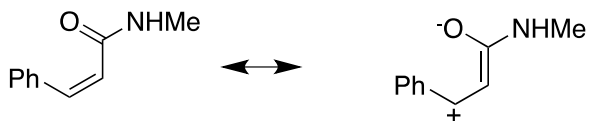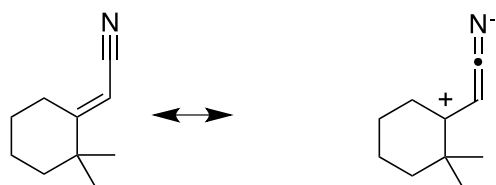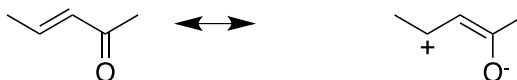
The *positive* charge of these resonance structures *is* always on the β-carbon; the charge simply indicates how the polarity of the substrate kinetically drives nucleophiles to add at this position.

In molecular orbital theory, the *LUMO* at that β-carbon would have a large coefficient (*ie* a big orbital).

Enolates are formed by adding nucleophiles to the β-carbon hence the products are *more* stable than ones which could conceivably be formed in similar reactions of non-activated alkenes.

Mechanisms Of Conjugated Addition

Anionic nucleophiles add to α,β-unsaturated carbonyl compounds to give enolates directly, and these are then protonated (usually when water is added to quench the reaction) to give the products.

Draw curly arrows and the missing structures to complete this mechanism.
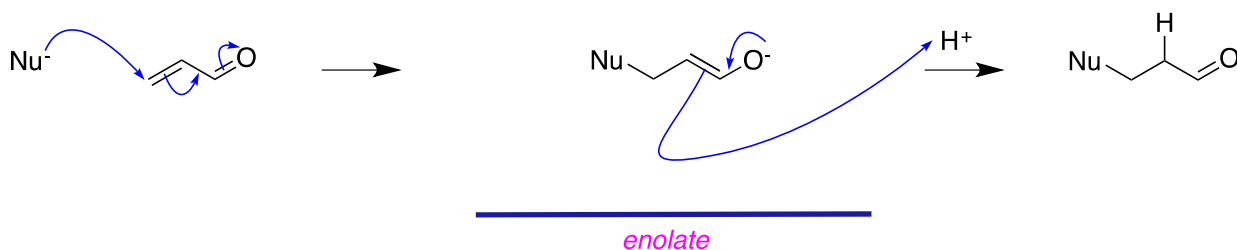
enolate

If the nucleophile is neutral, then the mechanism must involve loss of a proton from the nucleophilic site after the addition, and gain of a proton to protonate the enolate.

Show the following mechanism.

enolate
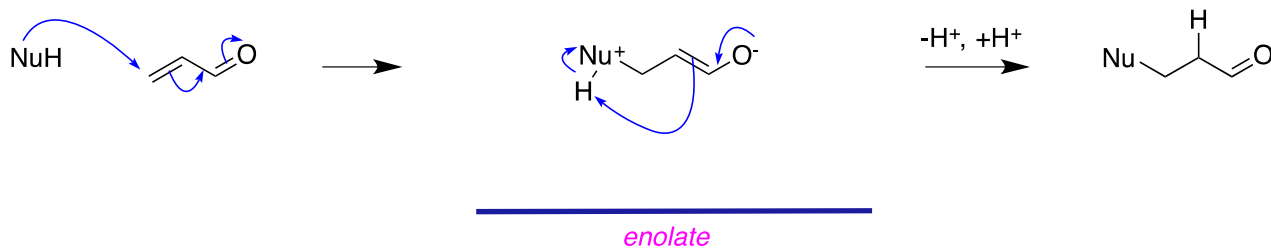
Examples Of Conjugate Additions

Amines And Thiols

Other conjugate additions typically involve thiolates and amines. The products can be referred to as 1,4-addition products because the *nucleophile* adds to the 4-position, and the *proton* adds to the oxygen giving an enolate.

Draw mechanisms for the following conjugate additions.

enolate intermediate

1,4-addition product

enolate intermediate

1,4-addition product
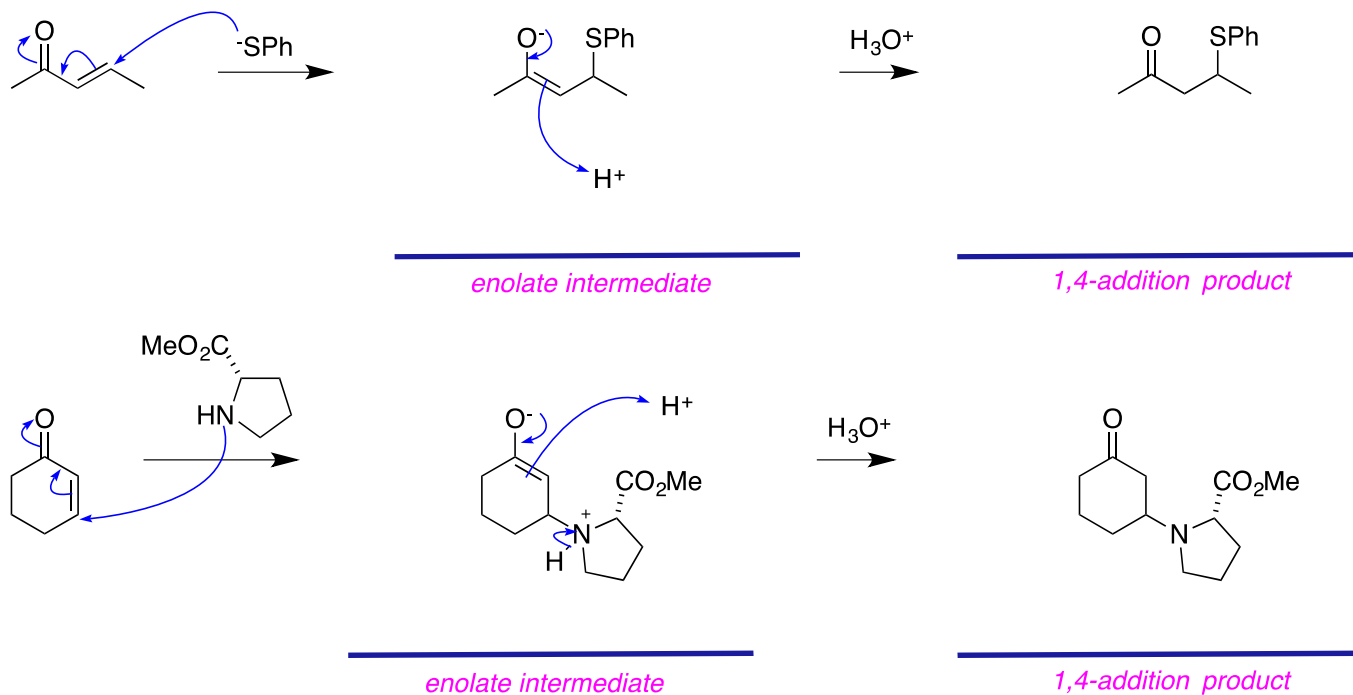
Predict the products of the following *conjugate* addition reactions to alkenes substituted with EWGs that are *not* aldehydes or ketones.
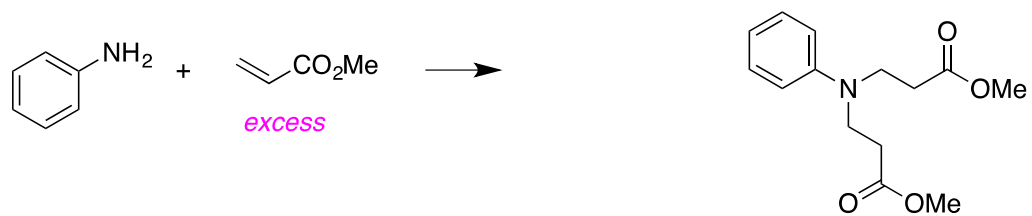
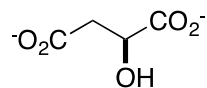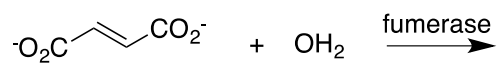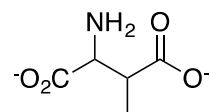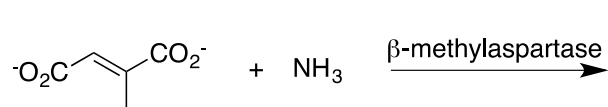## Enzyme-mediated Conjugated Additions

Predict the products of the following enzyme-mediated conjugate addition reactions.

^-O_2C ⟍⟍ CO_2^-　+　NH_3　$\xrightarrow{\beta\text{-methylaspartase}}$

^-O_2C ⟍⟍ CO_2^-　+　OH_2　$\xrightarrow{\text{fumerase}}$

S-product

Thiol nucleophiles can undergo conjugate addition reactions of cysteine-containing peptides and proteins. Give a mechanistic explanation below to show how Cys-nucleophiles could mediate *cis-trans* isomerization of the substrate shown via a reversible conjugate addition.

adduct

rotamer of initial adduct

retro-1,4-addition

isomerized product
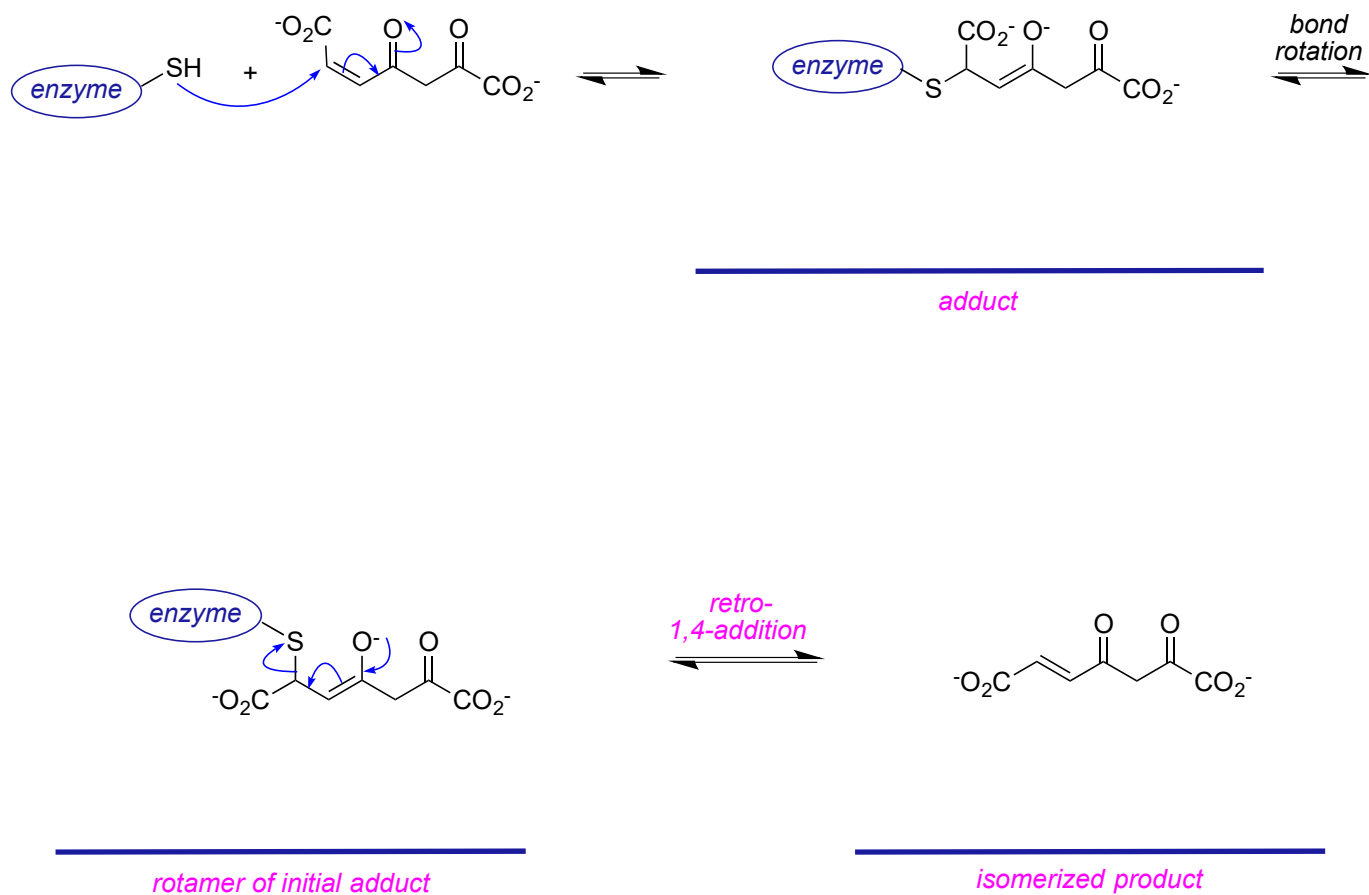
Stabilized *C*-Anion Nucleophiles

Stabilized enolates undergo conjugated addition reactions with electron deficient alkenes. For each of these reactions, the base is required in *stoichiometric* quantities.
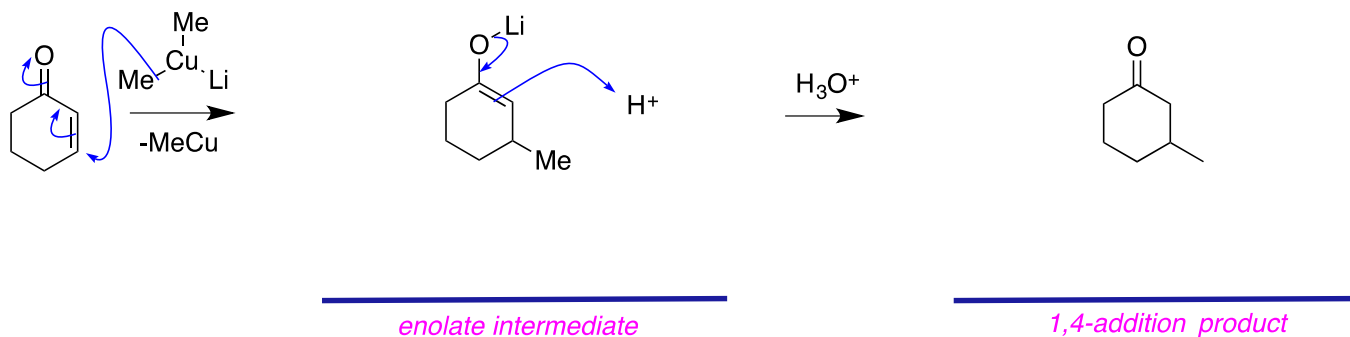
Draw the products of the following reactions.
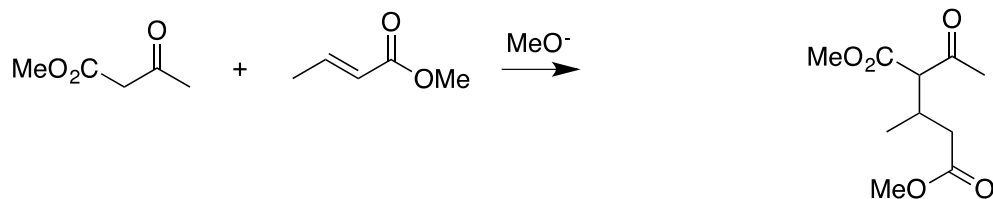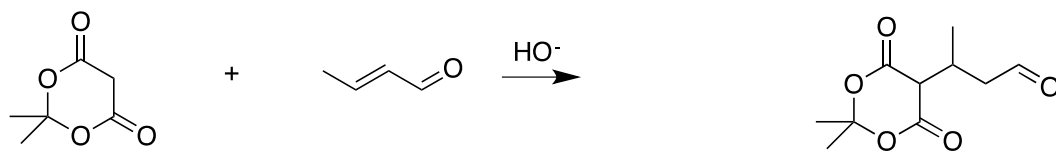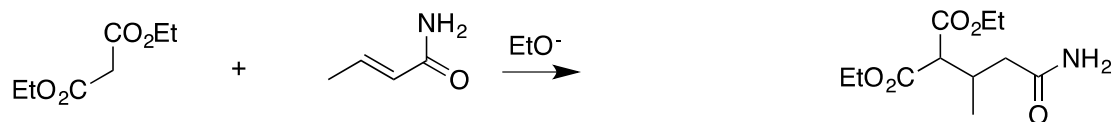
Organometallic Agents In Laboratory Chemistry

Cuprates (R$_2$CuLi) are typical nucleophiles for conjugate additions in synthetic chemistry; they are sources of "soft" R$^-$ groups that do not add to aldehydes or ketones.

Complete the following diagrams showing illustrative curly arrows.
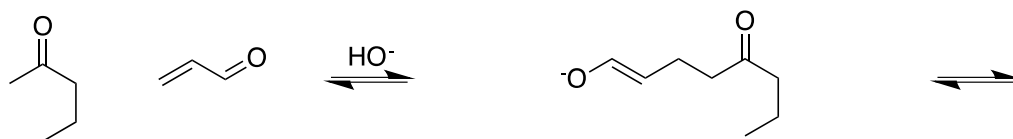

enolate intermediate   _1,4-addition product_

Show the product of the following reaction.

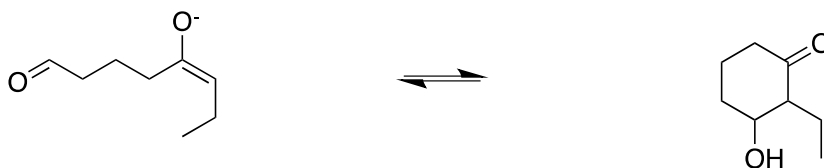Ph$_2$CuLi   +   [alkene]≡N   ⟶   Ph—[chain]≡N

Conjugate Addition Then Aldol Condensation

In the following example the conjugate addition product cyclizes via an intramolecular aldol reaction. *Note* under appropriate basic conditions all the possible enolates are in equilibrium.
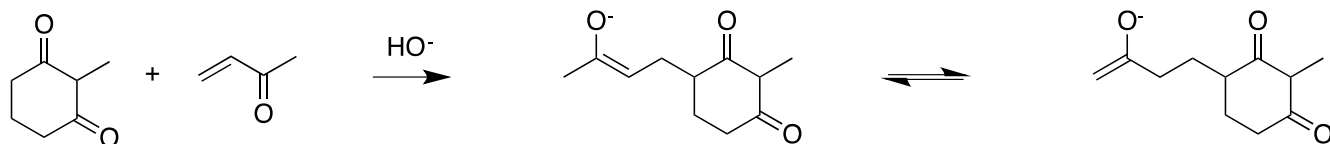

conjugate addition product

an enolate that can cyclize easily

cyclization product

cyclic aldol/dehydration product

Predict the products of the following reactions.
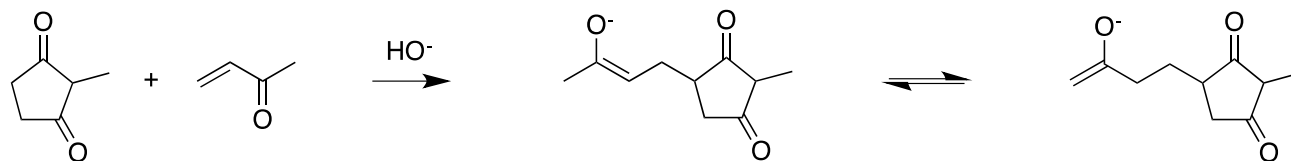

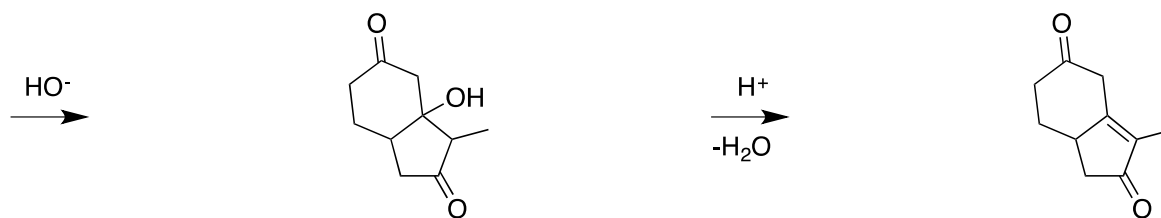*enolate from conjugate addition*   *terminal enolate*

intramolecular cyclization product   *enone*
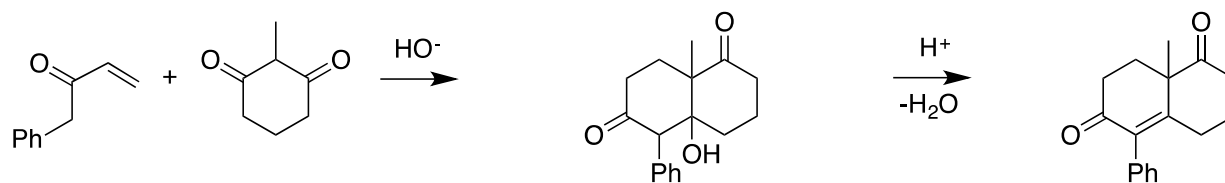

enolate from conjugate addition   *terminal enolate*
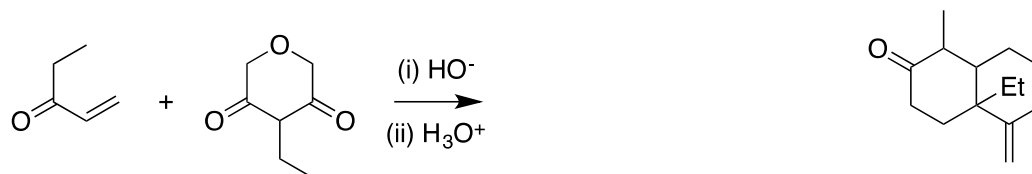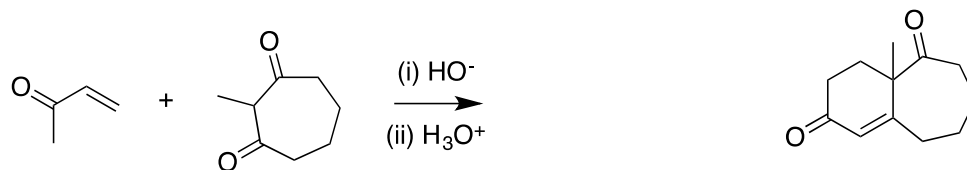
intramolecular cyclization product

enone

The whole process outlined above can be performed in one pot (the _Robinson Annulation_). Show the products of the following reactions.


intramolecular cyclization product

enone

Nucleophilic Epoxidation

Just as hydrazine is more nucleophilic than ammonia, the hydroperoxide anion is more nucleophilic than hydroxide; this is called the *α-effect*.

Hydrogen peroxide is also *more* acidic than water (pK$_a$ 11.6 and 15.4, respectively) so the hydroperoxide anion forms in aqueous solutions of hydrogen peroxide and base.

Activated alkenes in this medium may add hydroperoxide to give an enolate; the enolate then acts as a *C*-nucleophile to displace hydroxide. Complete the following mechanisms showing clear curly arrows.
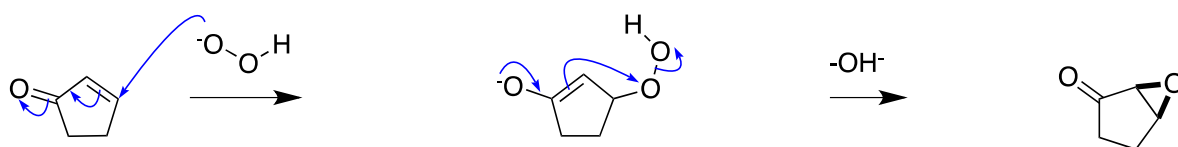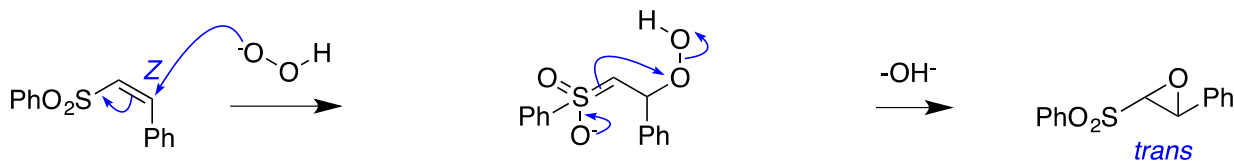
enolate intermediate   *epoxide*
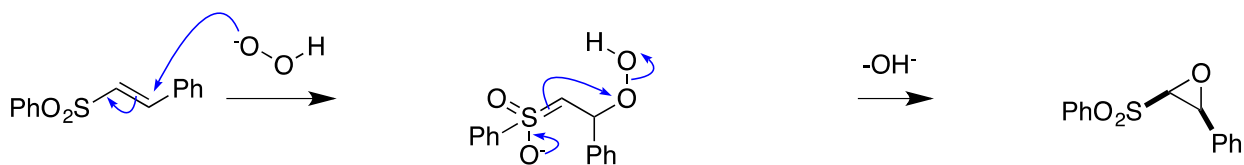
enolate   *epoxide*

enolate intermediate

It *is not* possible to form the *cis*-epoxide of the product above via this route.

Addition Elimination Reactions

Addition-elimination reactions of activated alkenes are mechanistically similar to those outlined above, except the enolate displaces a β-leaving group. Complete the mechanisms of the following reactions using clear curly arrows to depict the electron flow.

enolate

product

enolate

product

enolate

product

enolate

intermediate

base

enolate

product
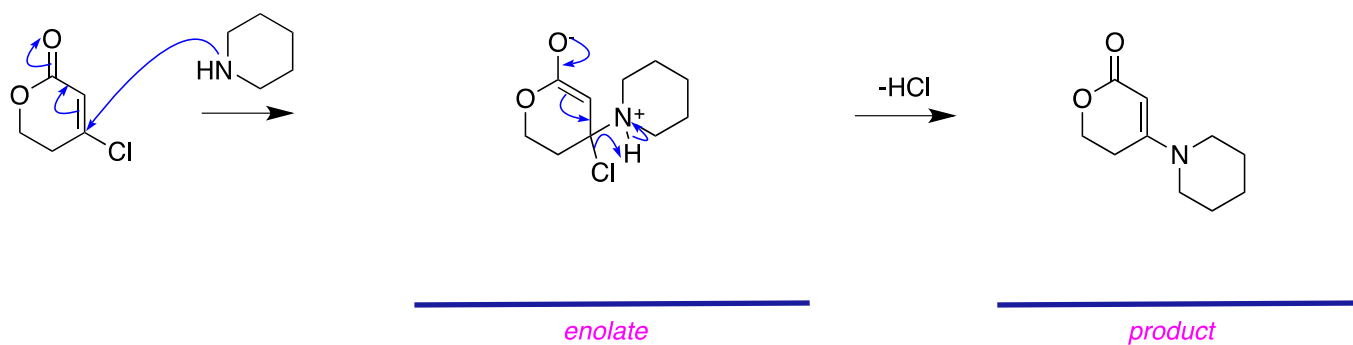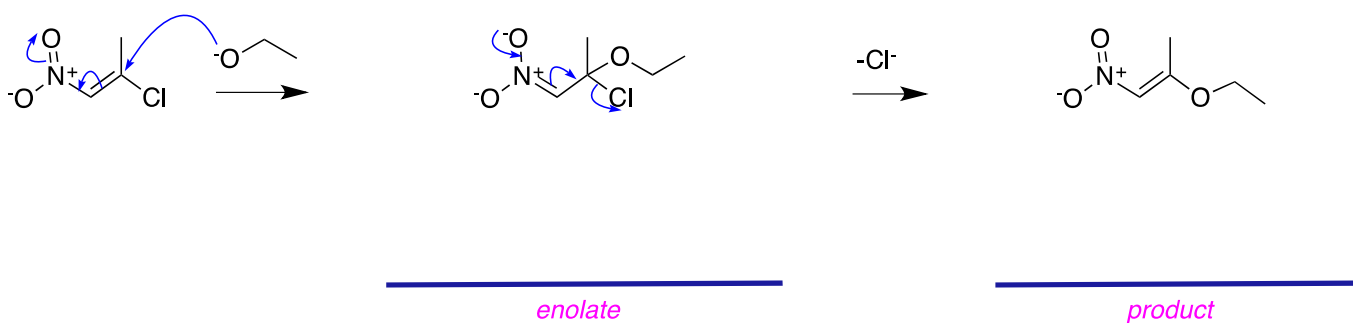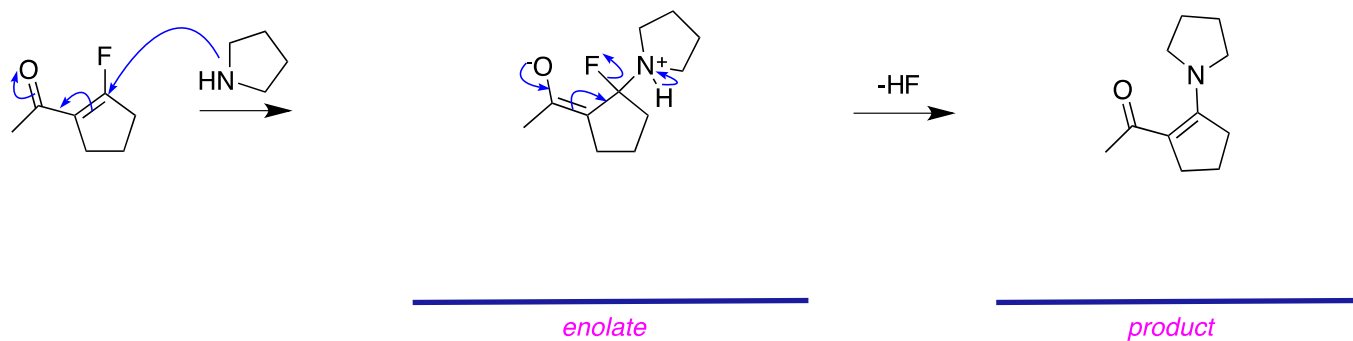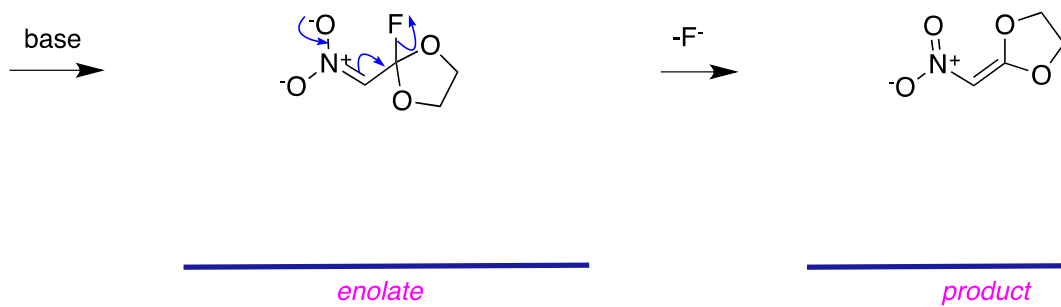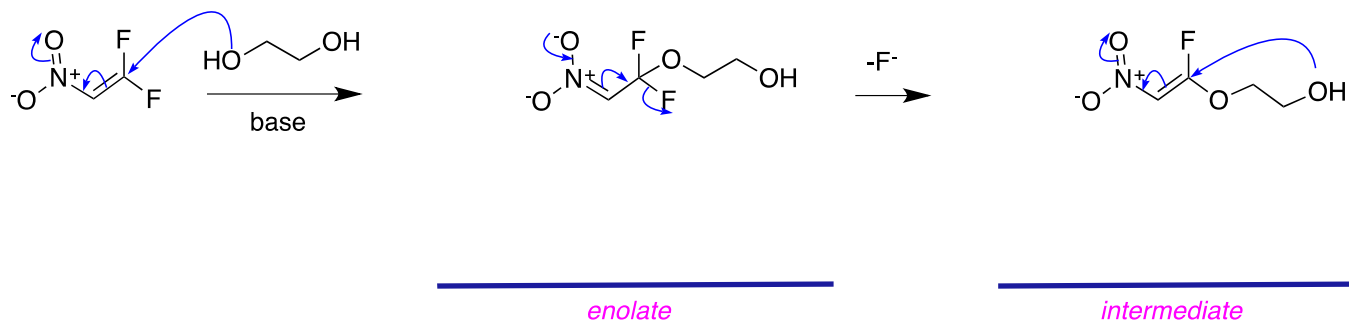
Formation Of αBromo Enones

The following reaction is not quite the same, but is mechanistically related. Fill in the gaps.

Br₂, base →

dibromide intermediate

→

enolate

-HBr →

monobrominated product
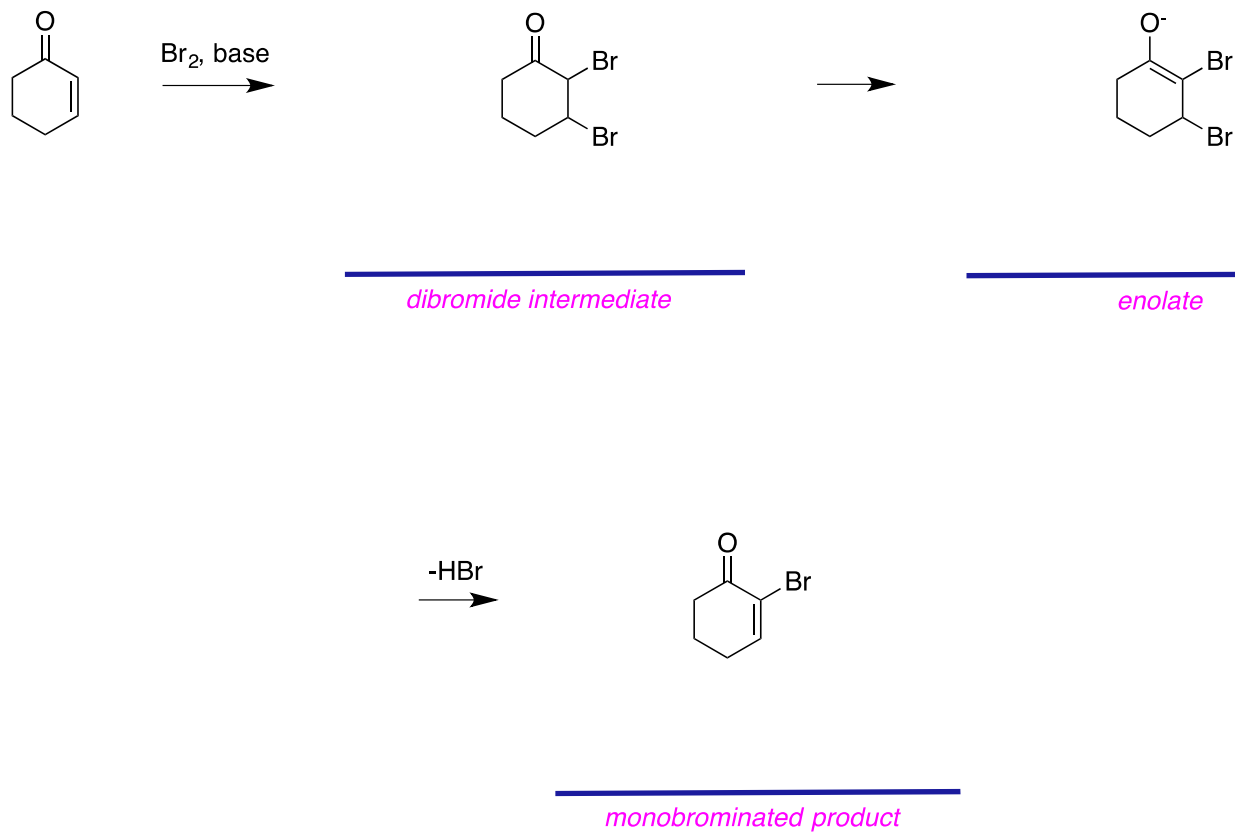
Nucleophilic Aromatic Substitution

Nucleophilic aromatic substitution reactions are called *S$_N$Ar* processes.

They involve *rate limiting* addition of nucleophiles giving *anionic* intermediates with one *sp^3* hybridized *C*-atom.

Draw this addition process for attack of cyanide on 2-fluoropyridine, and arrows to indicate how 2-cyanopyridine can be produced via expulsion of fluoride from the intermediate.
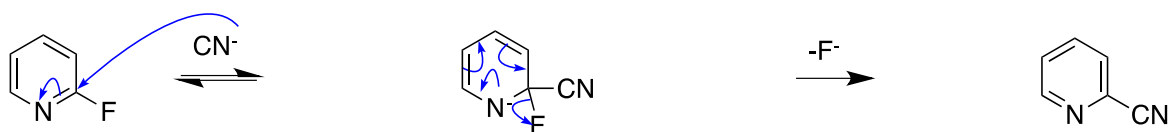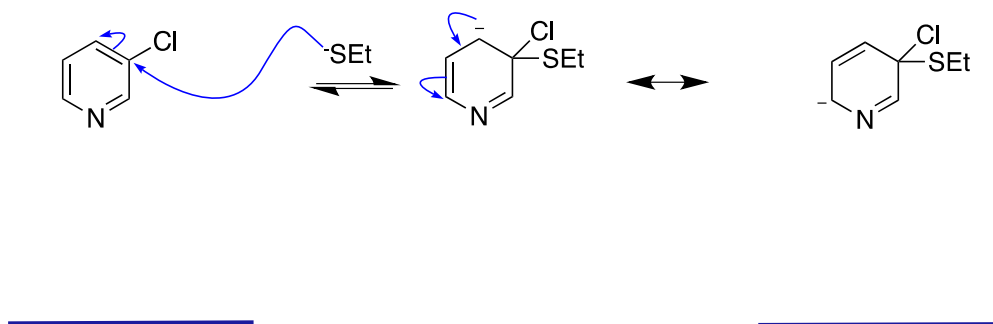

Draw all the resonance structures for the intermediate formed by addition of thioethoxide to:

2-chloropyridine

3-chloropyridine

[Reaction scheme: 3-chloropyridine with Cl reacting with ⁻SEt to form intermediate resonance structures]

_____   _____
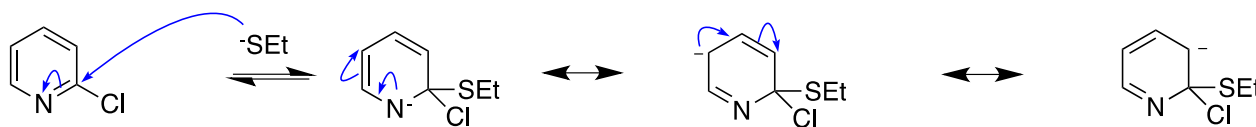
4-Chloropyridine in these types of reactions will behave similar to the *2-* isomer.

Addition occurs fastest for the *2- and 4*-isomers because the intermediates have negative charges on the *N*-atoms.

Draw the intermediates (as most stable resonance form) and products for the following S$_N$Ar reactions. One *does not proceed* under convenient conditions, and the other two may be ranked as *slow*, and *fast*. Show that ranking in each case.
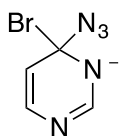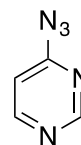
2-chloro-1,3-pyrimidine reacted with cyanide

[Structure: intermediate with N, CN, Cl groups]
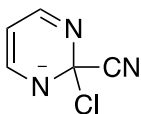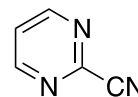
_____   _____
intermediate   *product*   *fast*

4-bromo-1,3-pyrimidine reacted with azide

[Structure: intermediate with Br, N₃ groups]

_____   _____
intermediate   *product*   *slow*
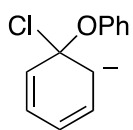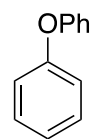
chlorobenzene reacted with phenoxide

Cl   OPh

_

intermediate

OPh

product

slow