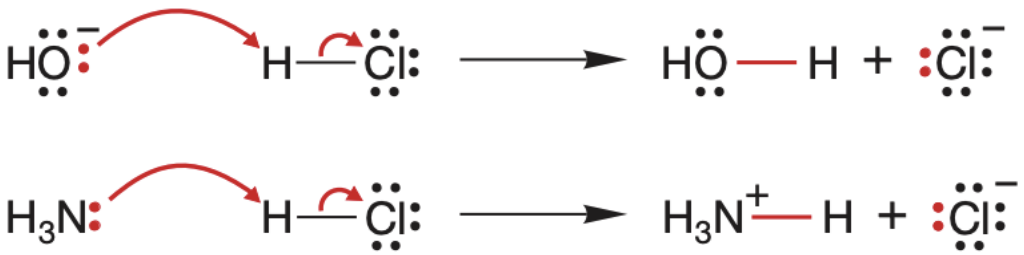
Bioorganic Chemistry

Lecture 6

Reaction mechanisms – Make & Break

Curved Arrow Formalism vs. Reaction Mechanism: The curved arrow formalism helps track electron flow in bond-making and bond-breaking but does not fully describe the reaction mechanism, which includes intermediates, transition states, and molecular interactions.

Electron Flow in Bond Formation and Breaking: Chemical bonds are depicted as forming from electron pairs moving from a donor to an acceptor, sometimes displacing other electrons.



Examples of Electron Flow: Hydroxide ion acts as a Brønsted base to break the H-Cl bond, and ammonia donates electrons to react with hydrogen chloride, illustrating how electron movement drives reactions.

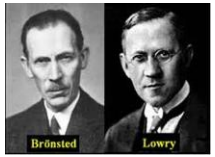
Lewis Acids and Bases

- Book p. 337
- Clayden p. 180 ff.

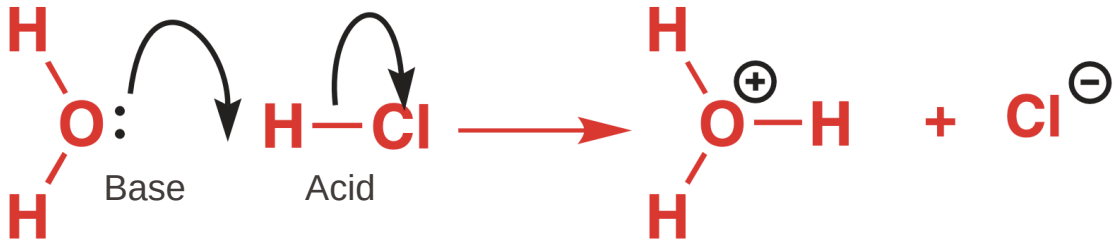
Resonance Stabilization

Lewis (1924)
Acid = Electron-pair Acceptor
Base = Electron-pair Donor

So far



Brønsted Lowry (1923)
Acid = Proton Donor
Base = Proton Acceptor

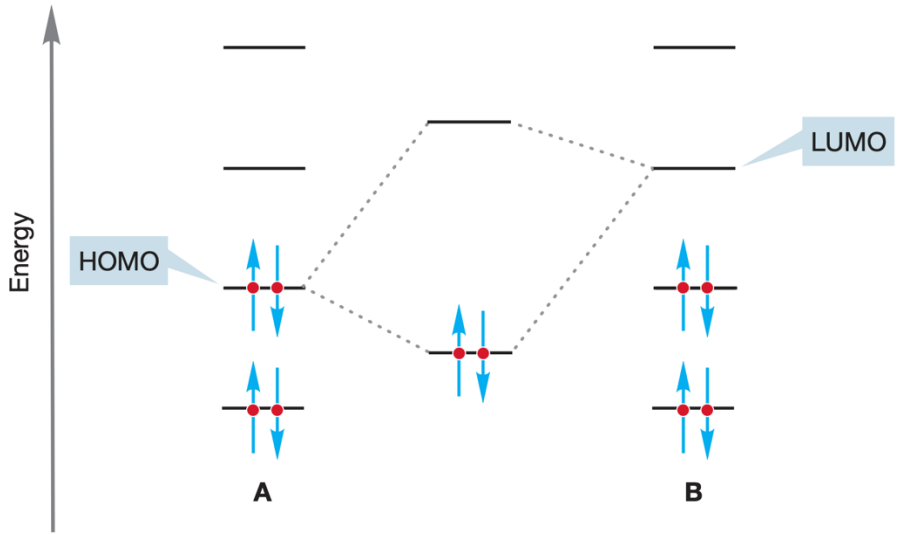


→ All Brønsted acids are also Lewis acids, but not all Lewis acids (only protic ones) are also Bronsted acids

Lewis Acids and Bases

Orbital Overlap Explanation: Lewis base electrons (from a filled orbital) overlap with an empty orbital on a Lewis acid, stabilizing the interaction

Reversibility of Reactions: All reactions can theoretically proceed in both directions, with thermodynamics determining the favored direction rather than an inherent "forward" or "backward" preference.

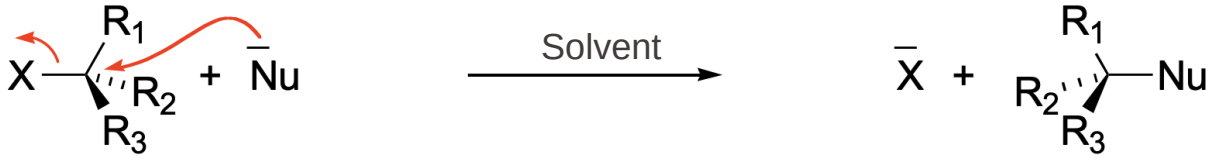


HOMO–LUMO Interactions: The strongest stabilizing interactions occur between the highest occupied molecular orbital (HOMO) of a nucleophile and the lowest unoccupied molecular orbital (LUMO) of an electrophile.

Nucleophile substitutions

A **nucleophile** is an electron pair donor, meaning it has a free electron pair. It can be **anionic (negatively charged)** or **neutral**.

In a nucleophilic substitution reaction, a nucleophile (**Nu^- or Nu**) attacks a molecule (**$R_3C–X$**) and replaces a functional group (**X**), which is expelled as **X^- or X**.

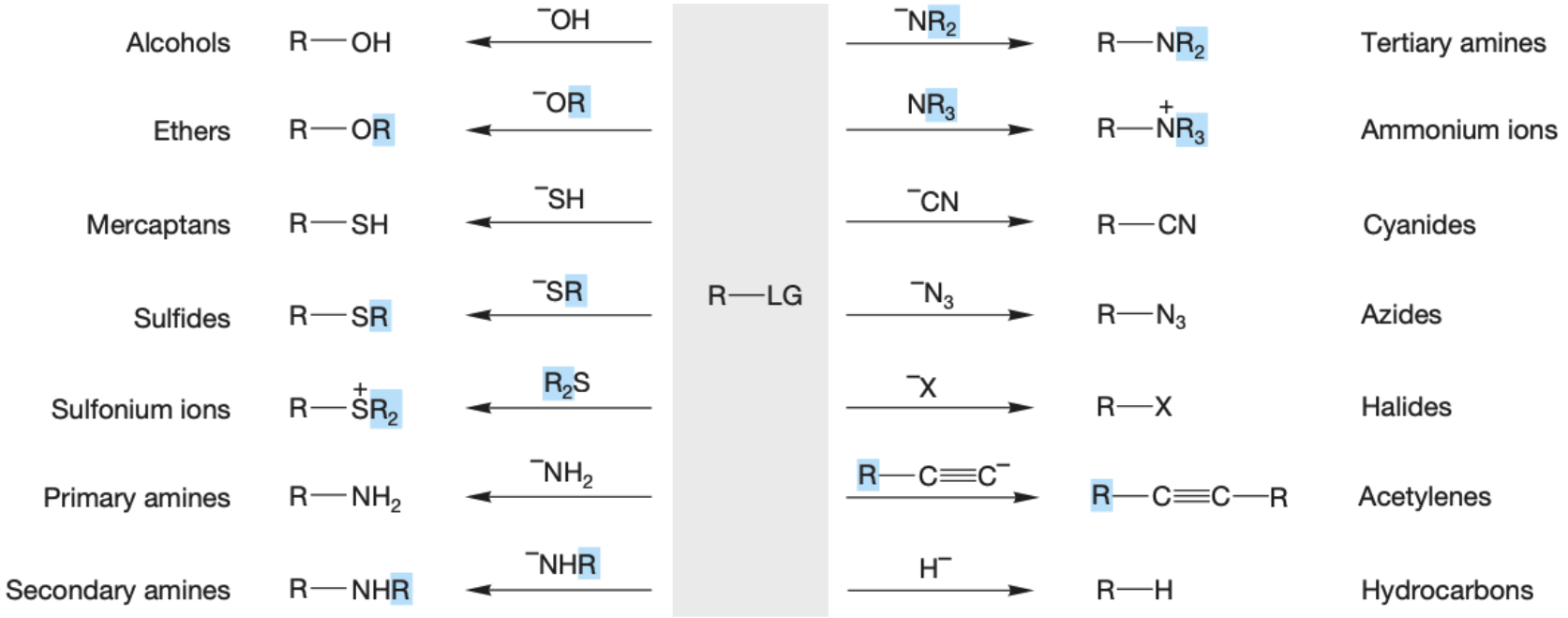


In a nucleophilic substitution reaction, a nucleophile (**Nu^- or Nu**) attacks a molecule (**$R_3C–X$**) and replaces a functional group (**X**), which is expelled as **X^- or X**.

Formulate with Lewis Acid-Base:

All substitution reactions involve competition between two Lewis bases for a Lewis acid. A substitution reaction consists of a **nucleophile (Nu, the incoming Lewis base)** and a **leaving group (LG/X, the departing Lewis base)**. All substitution reactions involve competition between two Lewis bases for a Lewis acid.

Substitutions as a great toolbox for chemists



Let's look at some reactions

Williamson Ether Synthesis (1852)

Finkelstein Reaction (1910)

Solvolysis

Let's look at some reactions

Williamson Ether Synthesis (1852)

Finkelstein Reaction (1910)

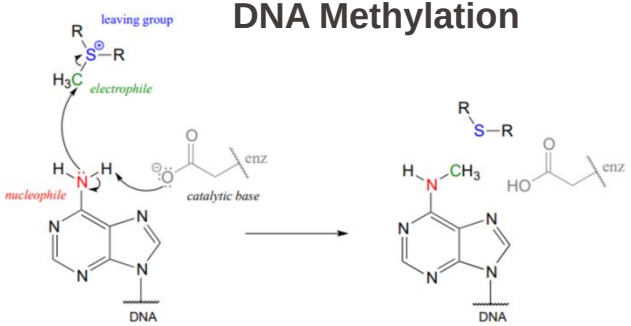
Solvolysis

Remember:
All substitution reactions involve competition between two Lewis bases for a Lewis acid.

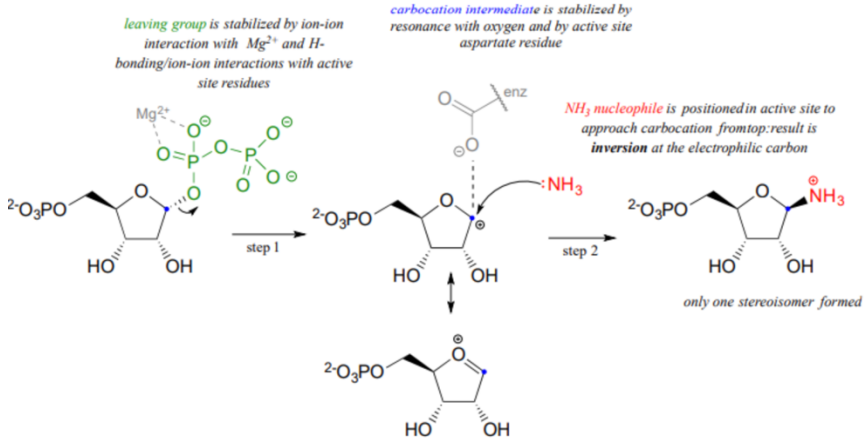
-> Acetone is used as the solvent to precipitate NaCl, while NaI is soluble. We therefore drive the reaction forward. Can be used to detect alkyl chlorides and bromides

Nucleophile Substitutions in biology

DNA Methylation



Nucleotide Biosynthesis



Allow the interconversion of functional groups!

S-Adenosylmethionine

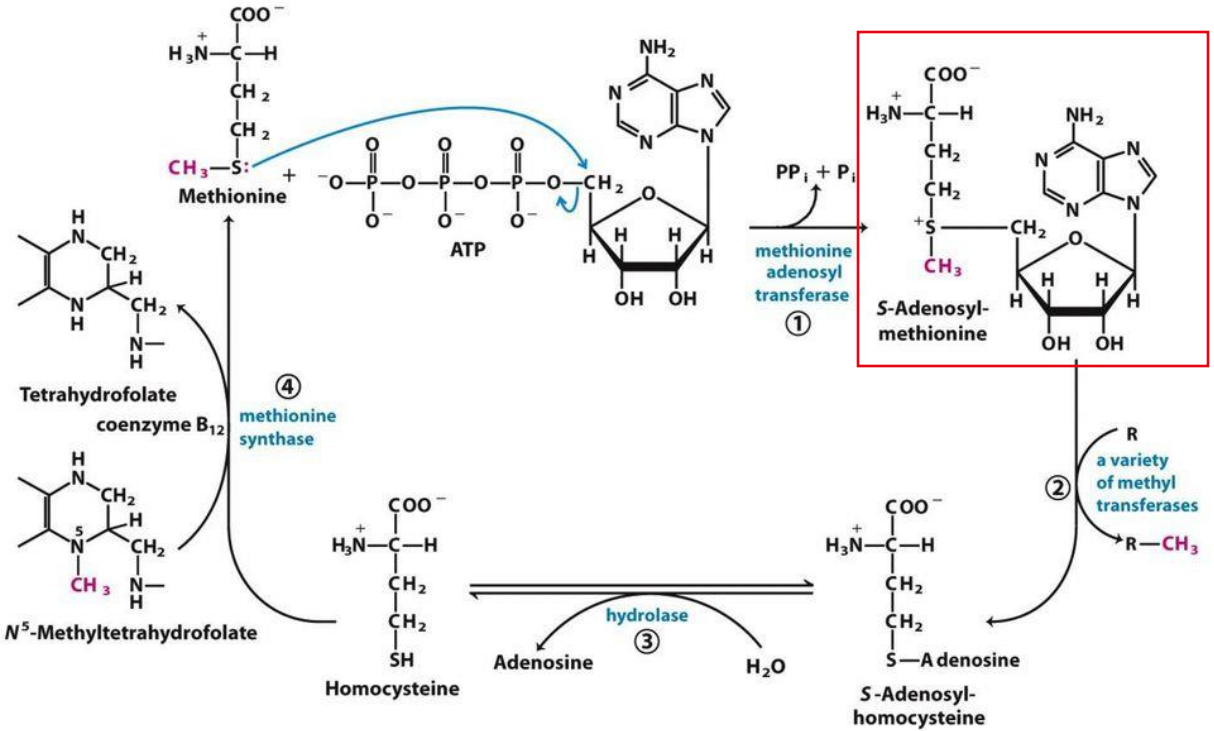


Figure 18-18
Lehninger Principles of Biochemistry, Fifth Edition

S-Adenosylmethionine as a methyl-donor

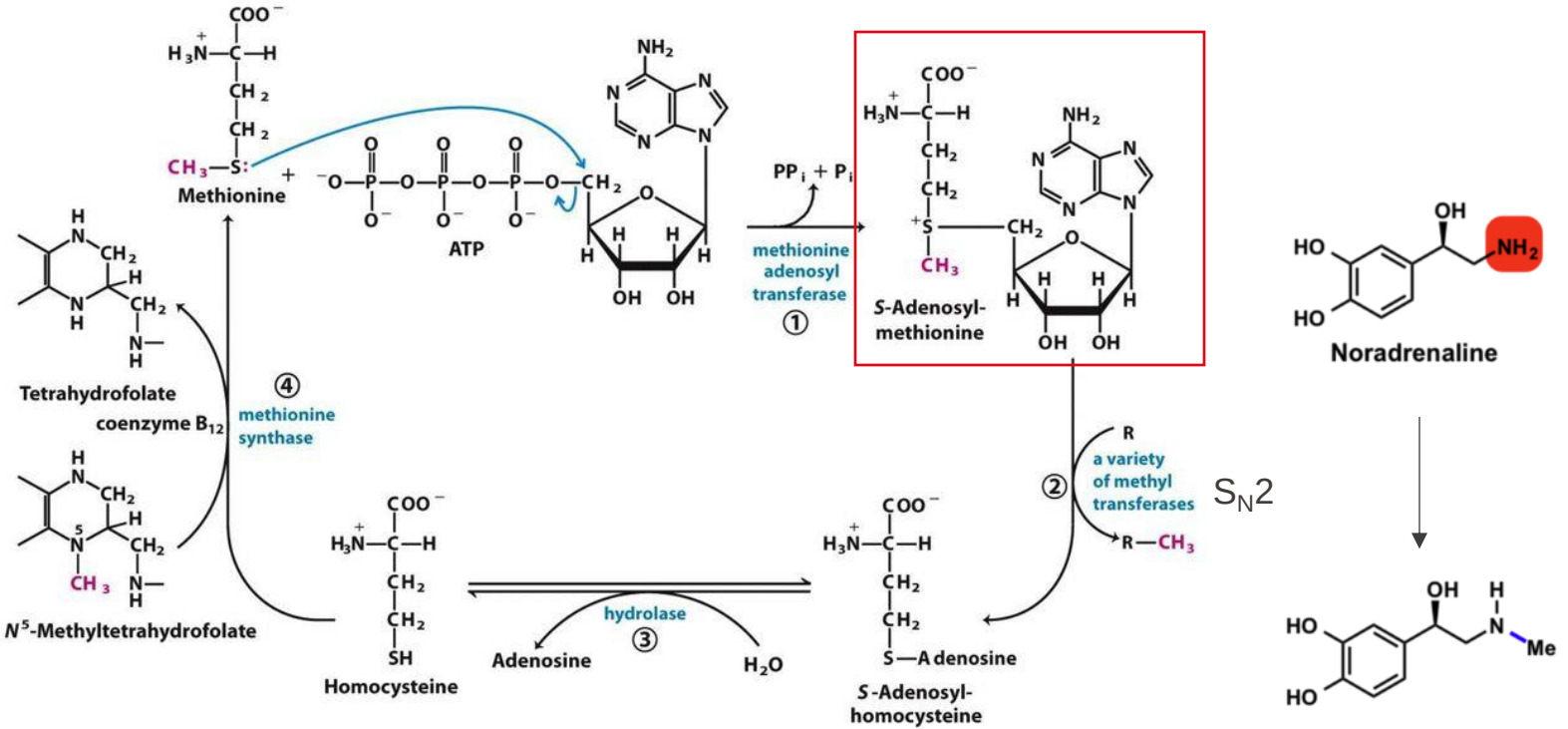


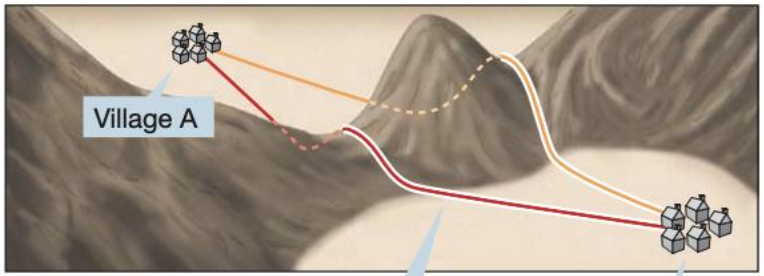
Figure 18-18
Lehninger Principles of Biochemistry, Fifth Edition

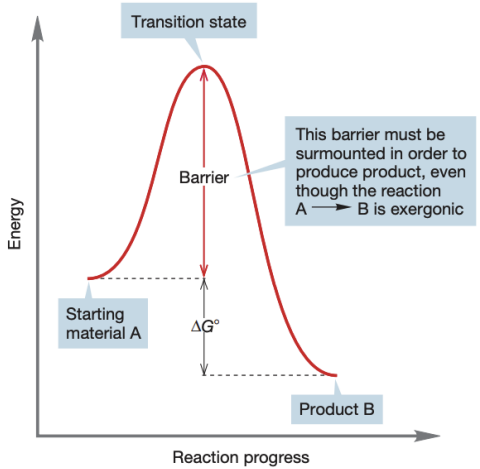
How to prove a mechanism in Chemistry

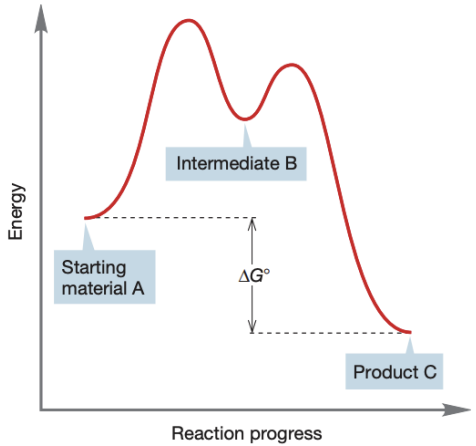
-> Proving with Experiment and theory: **Mostly** by disproving the alternatives
"…when you have excluded the impossible, whatever remains, however improbable, must be the truth" (Sherlock Holmes)

Problem: You need to catch them **all**

First, lets look at the energy path of the reaction







Speaker

Let's begin from scratch

Break Bond (**D**issocitation)

Make Bond (**A**ssociation)

Combinations:

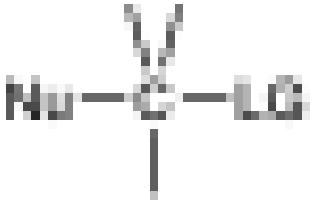
D then **A**

S_N1

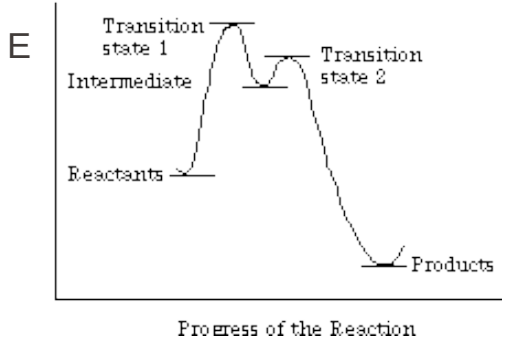
Trivalent *Intermediate*

A then **D**

Happens, but not with carbon

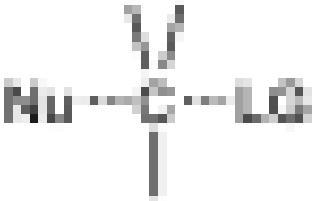


Pentavalent *Intermediate*

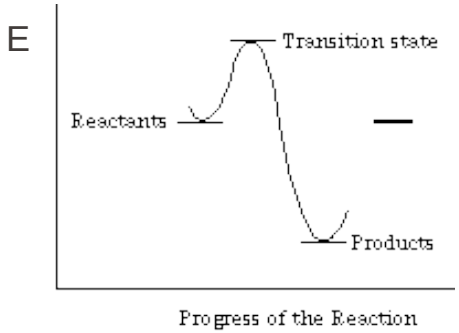


Simultaneous "**Concerted**" (make as you break)

S_N2



Transition State



Let's begin from scratch

Break Bond (**D**issocitation)

Make Bond (**A**ssociation)

Combinations:

D then **A**

S_N1

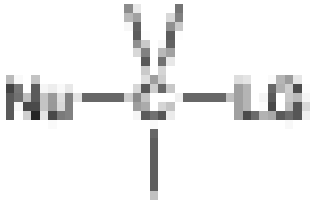
achiral

Trivalent *Intermediate*

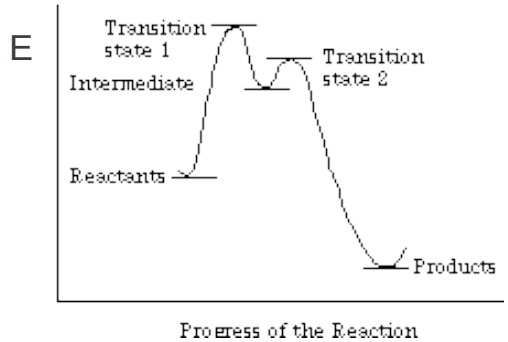
A then **D**

Happens, but not with carbon

chiral



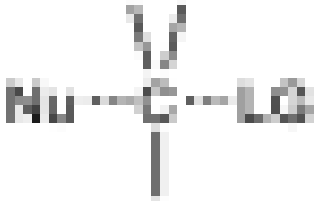
Pentavalent *Intermediate*



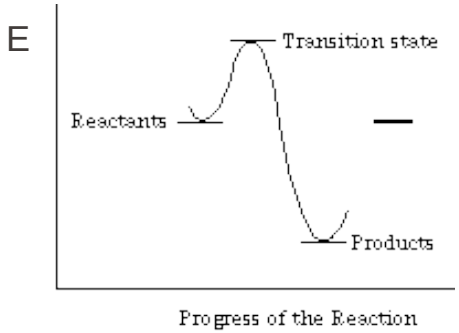
Simultaneous "**Concerted**" (make as you break)

S_N2

chiral

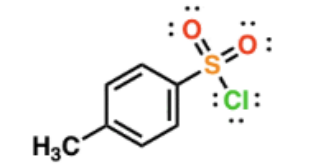


Transition State



We can use stereochemistry

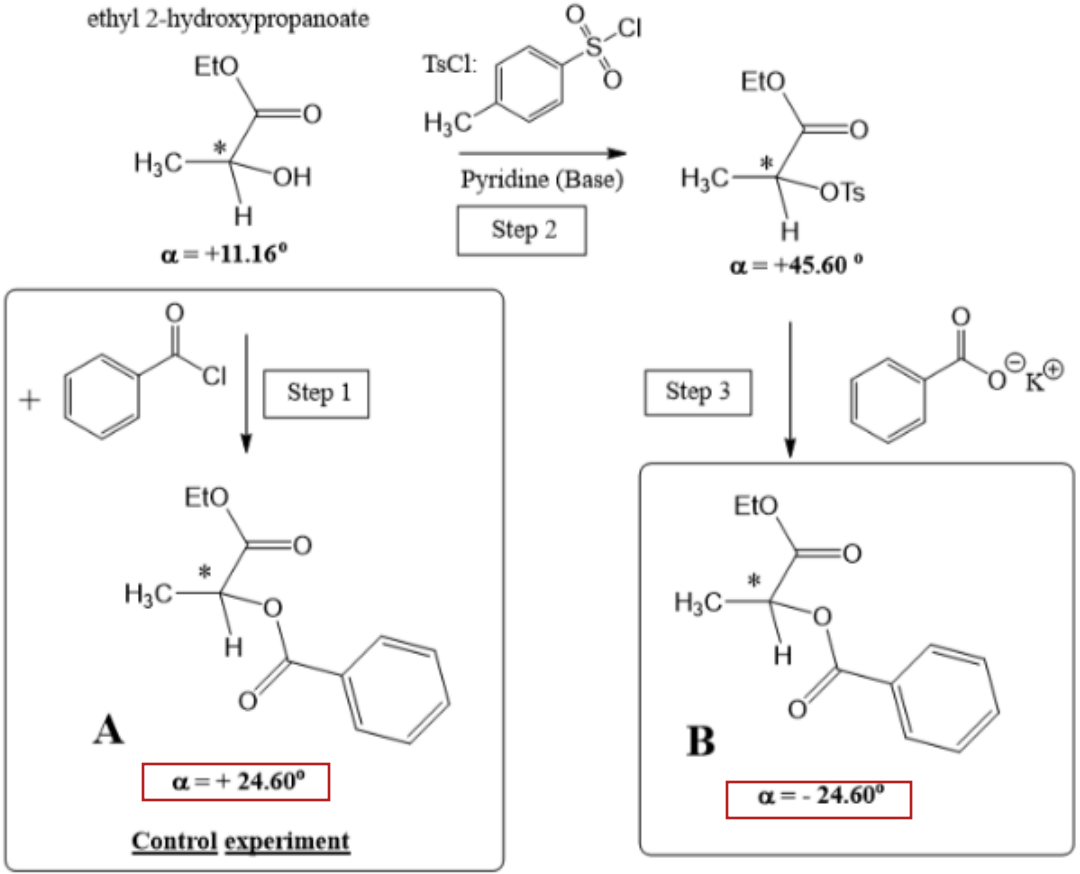
In the 1920s and 1930s Kenyon and Philips carried out experiment to find out how this inversion works



p-toluenesulfonyl chloride
("tosyl chloride")

TsCl: Turns OH groups into great leaving groups

Compounds that rotate the plane of polarized light are termed **optically active**. Each enantiomer of a stereoisomeric pair is optically active and has an equal but opposite-in-sign specific rotation. A 50:50 mixture (**racemate**) of enantiomers has no observable optical activity



We could use this to disprove a mechanism

Break Bond (**D**issocitation) | Make Bond (**A**ssociation)

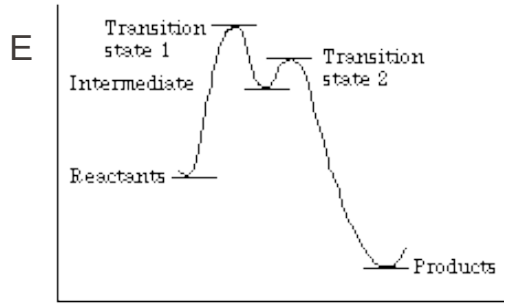
Combinations:

D then **A**

S_N1

achiral

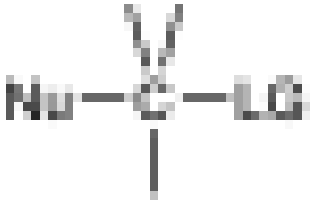
Trivalent *Intermediate*



A then **D**

Happens, but not with carbon

chiral

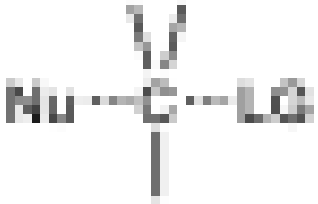


Pentavalent *Intermediate*

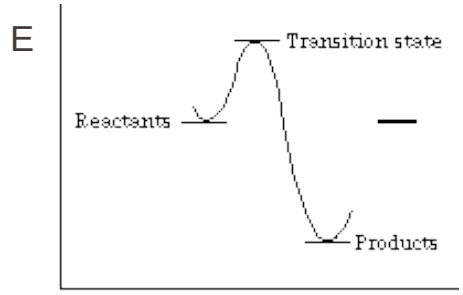
Simultaneous "**Concerted**" (make as you break)

S_N2

chiral



Transition State



We could use this to disprove a mechanism

Break Bond (**D**issocitation) | Make Bond (**A**ssociation)

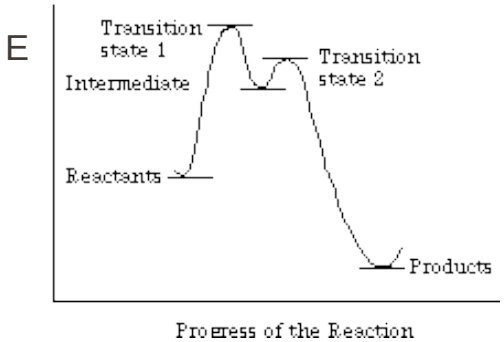
Combinations:

D then **A**

S_N1

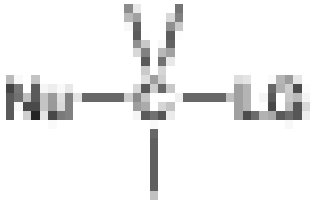
unimolecular

Trivalent *Intermediate*



A then **D**

Happens, but not with carbon

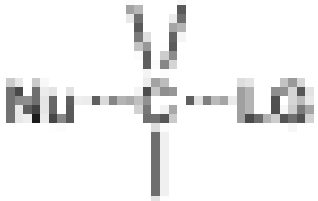


Pentavalent *Intermediate*

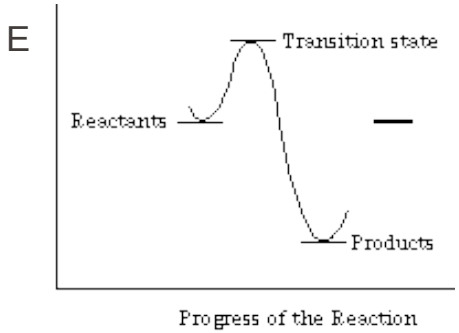
Simultaneous "**Concerted**" (make as you break)

S_N2

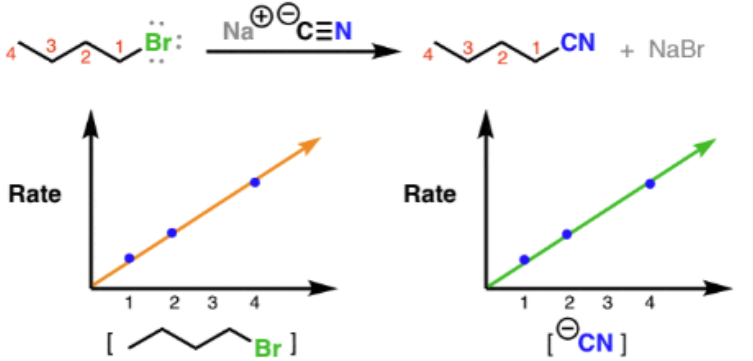
bimolecular



Transition State



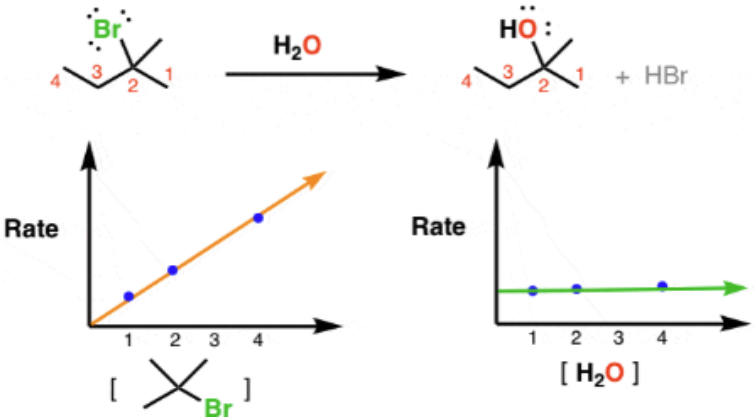
We can use the rate law



Follows **second-order kinetics**, meaning the rate depends on the concentration of both the **substrate** and **nucleophile**. This is characteristic of an **S_N2 reaction** (bimolecular nucleophilic substitution), where both species participate in the rate-determining step.

$$\text{Rate} = k[A][B]$$

$$\text{in units, } \underbrace{\left(\frac{\text{mol}}{\text{L}}\right) \times (\text{s})^{-1}}_{\text{Rate} = \text{concentration/time}} = \underbrace{k}_{\text{(rate constant)}} \times \underbrace{\left(\frac{\text{mol}}{\text{L}}\right)}_{\text{(concentration)}} \times \underbrace{\left(\frac{\text{mol}}{\text{L}}\right)}_{\text{(concentration)}}$$



Follows **first-order kinetics**, meaning the rate depends only on the concentration of the **substrate**. This is typical of an **S_N1 reaction** (unimolecular nucleophilic substitution), where the rate-determining step is the **formation of a carbocation intermediate**, independent of the nucleophile concentration.

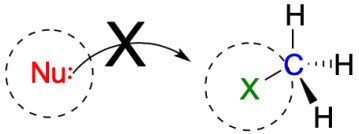
$$\text{Rate} = k[A]$$

$$\text{in units, } \underbrace{\left(\frac{\text{mol}}{\text{L}}\right) \times (\text{s})^{-1}}_{\text{Rate} = \text{concentration/time}} = \underbrace{k}_{\text{(rate constant)}} \times \underbrace{\left(\frac{\text{mol}}{\text{L}}\right)}_{\text{(concentration)}}$$

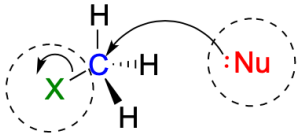
Bimolecular nucleophilic substitution (S_N2)

Mechanism and stereochemical outcome

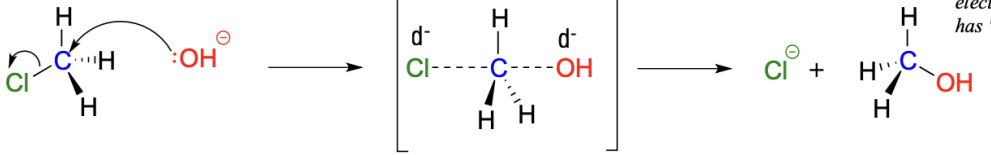
-Backside attack – S_N2 reaction can only occur when the nucleophile collides with the electrophilic carbon from the opposite side relative to the leaving group



nucleophilic attack is blocked from the front side . . .

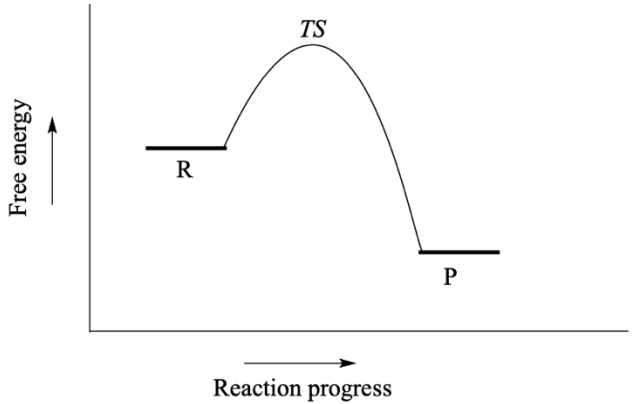


. . .so attack occurs from the back side



transition state

electrophilic carbon has 'inverted'

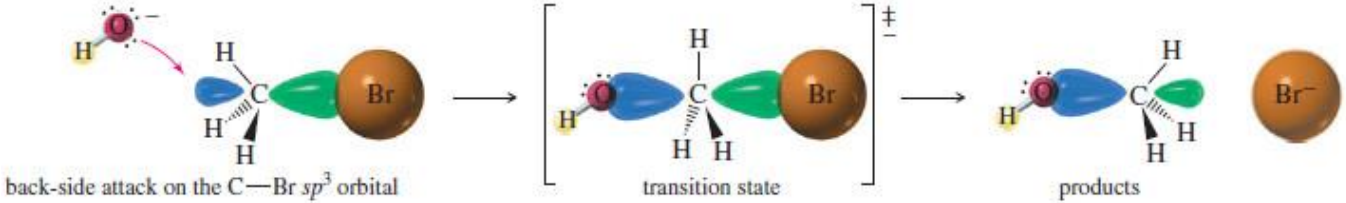


Walden - Inversion

There is a strong preference for inversion in the SN2 reaction. Indeed, there is no authenticated example of retention of configuration in this process, despite a great deal of searching by some very clever people

Paul Walden

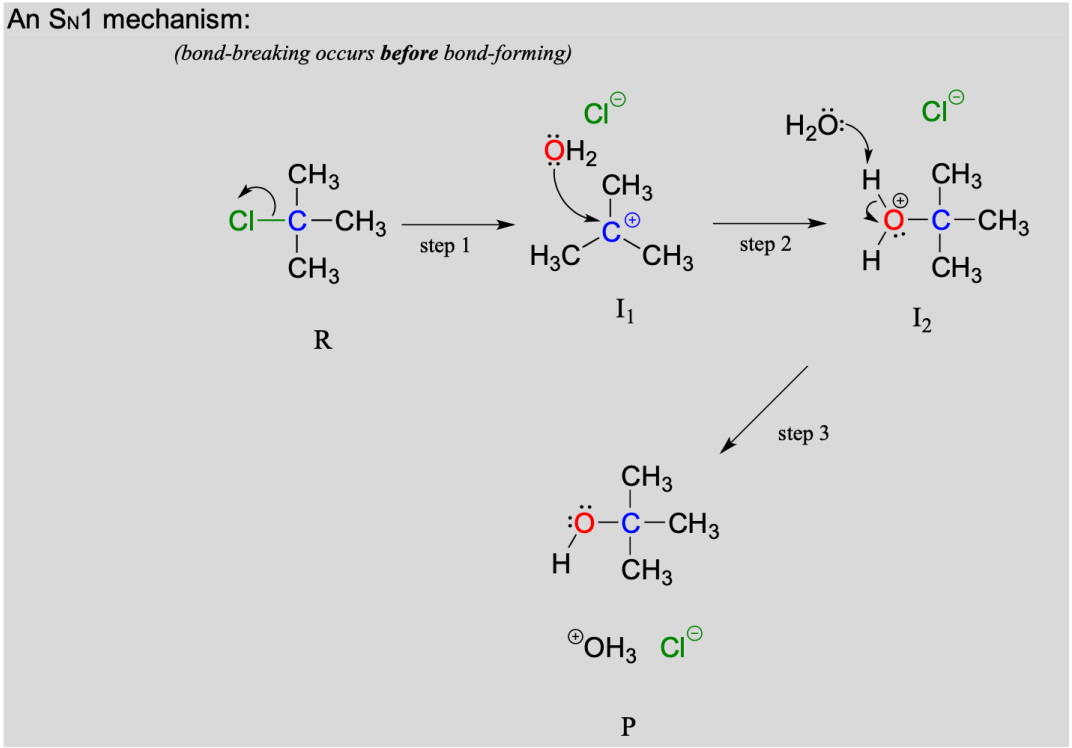
In 1897, Paul Walden demonstrated this effect by converting bromosuccinic acid into both L-malic acid and D-malic acid. He observed that the introduced substituent takes a different position at the chiral carbon than the leaving group, resulting in an inversion of the molecule's stereochemistry rather than forming a racemic mixture.



Unimolecular nucleophilic substitution (S_N1)

Overview

S_N1 – 1 indicates that the rate determining step of the reaction is unimolecular



-carbon leaving group breaks first before the nucleophile approaches resulting in formation of a **carbocation intermediate**

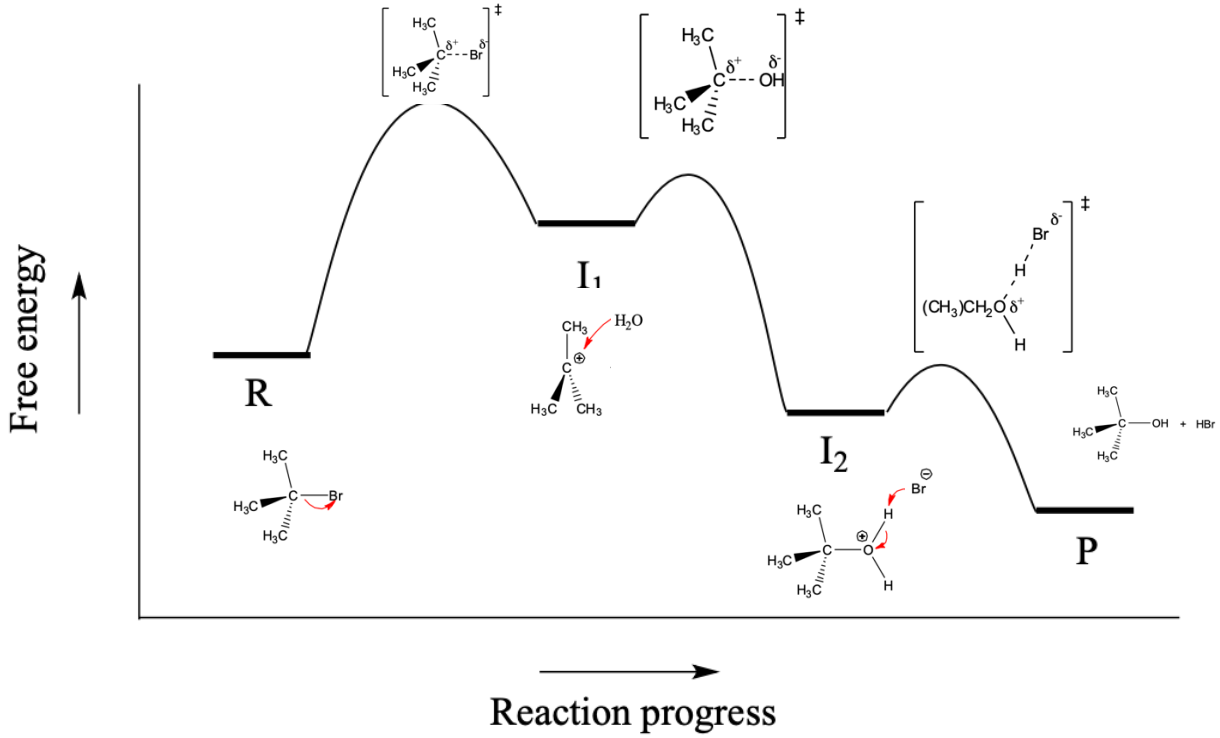
-carbocation is a powerful electrophile which is “electron-hungry”

-step 2 a lone pair of electrons on the water nucleophile fills in the empty orbital of the carbocation to form new bond

-acid base step leading to the alcohol product

Unimolecular nucleophilic substitution (S_N1)

Mechanism



-bond-breaking step is the slowest
Becoming rate-determining step

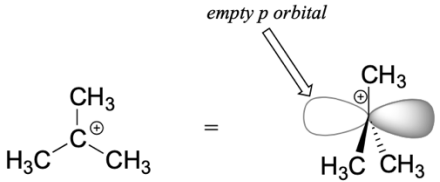
-highest activation energy and
highest-energy species

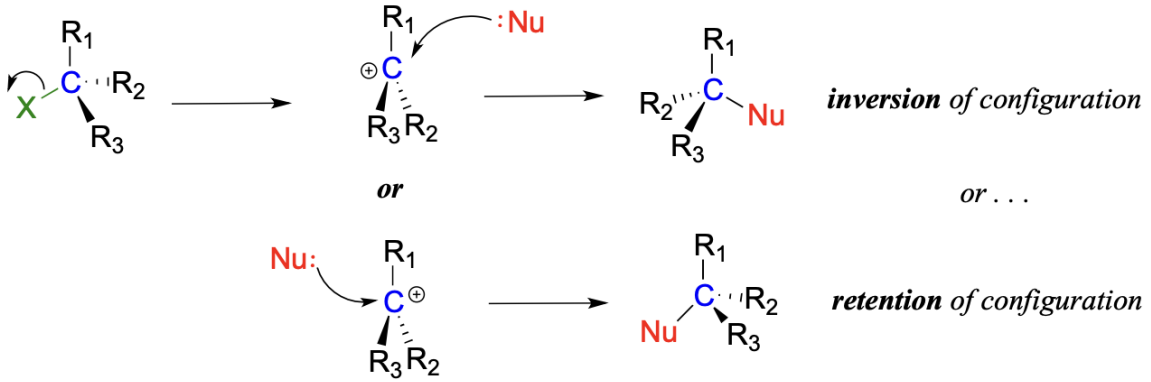
Unimolecular nucleophilic substitution (S_N1)

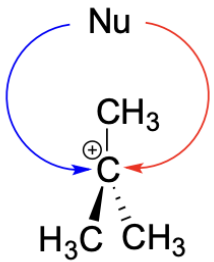
Stereochemical Outcome

-Remember that S_N2 reactions result in inversion of stereochemical configuration at the carbon center

-Carbocation is sp2–hybridized leaving different attack possibilities for the nucleophile



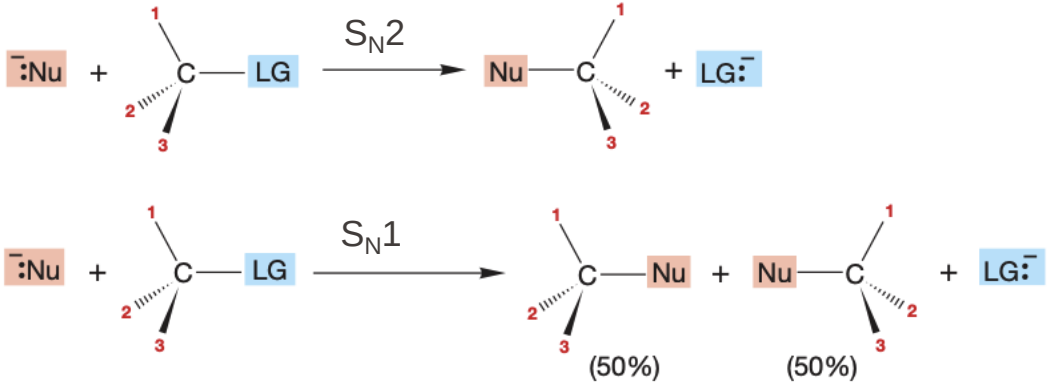




S_N1 reactions can occur with either retention or inversion of the configuration at the electrophilic carbon, leading to racemization if the carbon is chiral

Predicting what happens

Often times, we want to look at the educts and products and predict what will happen.
But there are many factors influencing the mechanism and rate of a reaction.

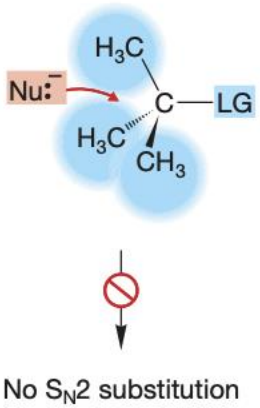


Effects of Substrate Structure: The R Group (1,2,3)
Effect of the Nucleophile (Size and Nucleophilicity)
Effect of the Leaving Group
Effect of Solvent

Effects of Substrate Structure: The R Group (S_N2)

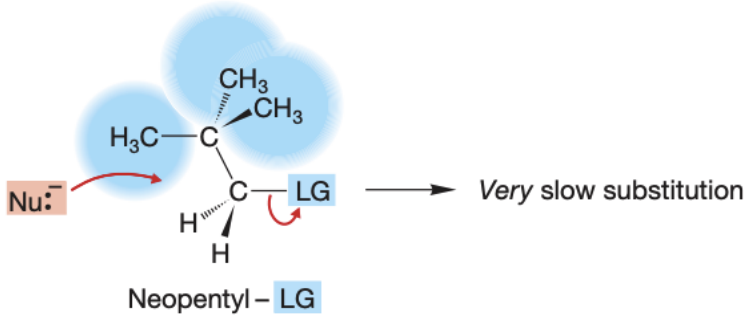
- **Primary and secondary substrates** undergo SN2 reactions effectively, while **tertiary substrates do not**.
- The rate of SN2 reactions with **tertiary substrates is negligible** due to **steric hindrance**. The **small size of hydrogen** allows space for **backside attack** by the nucleophile
- In tertiary substrates, **three alkyl groups** block the **backside attack**, preventing nucleophilic approach.

Reaction rate trend:
Methyl > Primary > Secondary > Tertiary (negligible rate)



No S_N2 substitution

R	Average Relative Rate
$CH_2{=}CHCH_2$	1.3
CH_3	1
CH_3CH_2	0.033
$CH_3CH_2CH_2$	0.013
$(CH_3)_2CH$	8.3×10^{-4}
$(CH_3)_3CCH_2$	2×10^{-7}
$(CH_3)_3C$	~0



Neopentyl – LG

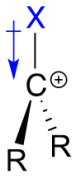
Effects of Substrate Structure: The R Group (S_N1)

- The **rate of SN1 reactions** is highest for **tertiary substrates**, lower for **secondary substrates**, and **negligible for primary and methyl halides**.
- **Carbocation formation** is the key step in SN1 reactions, so **only substrates that form stable carbocations** react via this mechanism.
- **Carbocations are generally unstable**, as **carbon does not easily accommodate a positive charge**.
- **More alkyl groups = more stability**, due to **inductive effects and hyperconjugation**.

Carbocation stability trend:
Tertiary (most stable) > Secondary > Primary > Methyl (least stable).

Entry	Cation	Substitution	ΔH_f°, kcal/mol (kJ/mol)	
1	$^+CH_3$	Methyl	261.3 (1094)	Least stable
2	$^+CH_2CH_3$	Primary	215.6 (902.7)	
3	$^+CH_2CH_2CH_3$	Primary	211 (833)	
4	$^+CH_2CH_2CH_2CH_3$	Primary	203 (850)	
5	$(CH_3)_2\overset{+}{C}H$	Secondary	190.9 (799.3)	
6	$H_3C\overset{+}{C}HCH_2CH_3$	Secondary	183 (766)	
7	$(CH_3)_3C^+$	Tertiary	165.8 (694.2)	Most stable

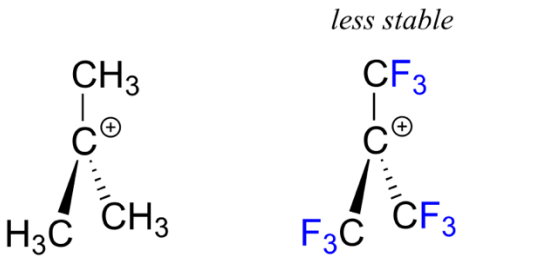
electron donating group: stabilizes a carbocation

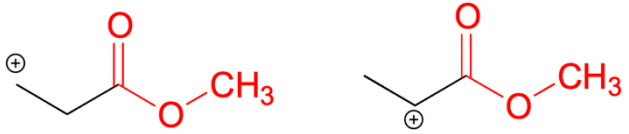


*electron withdrawing group: **de**stabilizes a carbocation*

Effects of Substrate Structure: The R Group (S_N1)

Destabilization of carbocation through inductive effect

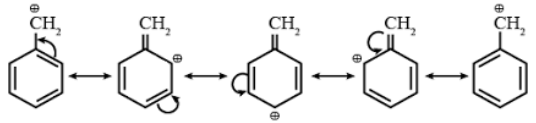




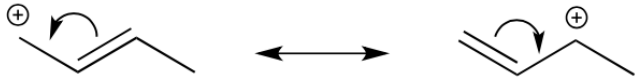
A: more stable

B: less stable

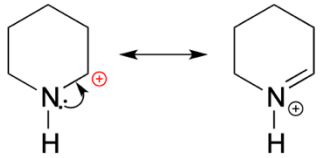
Stabilization of carbocation through resonance effects



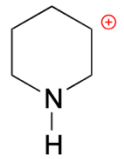
Allylic carbocation



Heteroatoms



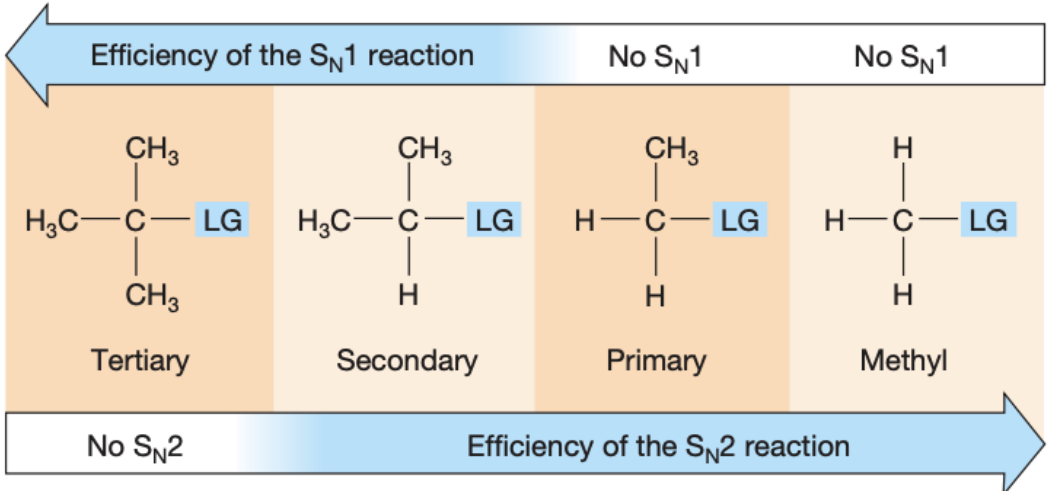
more stable



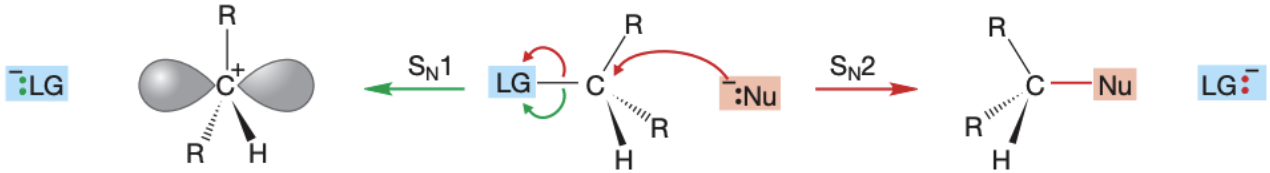
less stable
(no resonance delocalization)

- Be aware: No Substitution occurs at an sp2 hybridized Carbon!

Effects of Substrate Structure: The R Group



Secondary

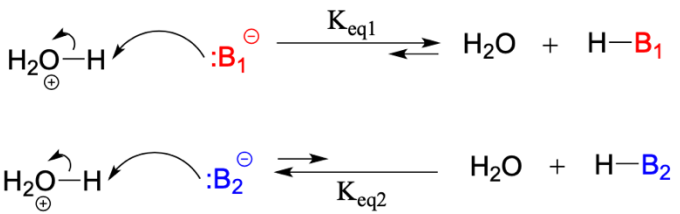


Be aware: These are idealized considerations. Real chemistry is often more ”grey”

Effect of the Nucleophile (Size and Nucleophilicity)

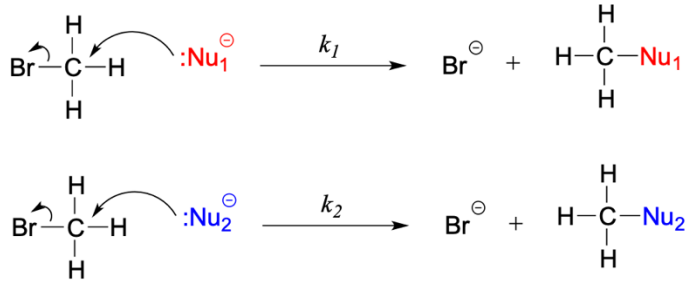
Nucleophile is an atom or a functional group with a pair of electrons that can be shared

This is similar with the Bronsted-Lowry bases we saw last lecture



In acid-base reactions “we think” thermodynamics

$Base_1$ stronger than $Base_2$ – equilibrium lies further to the right in reaction with a common acid $K_{eq1} > K_{eq2}$



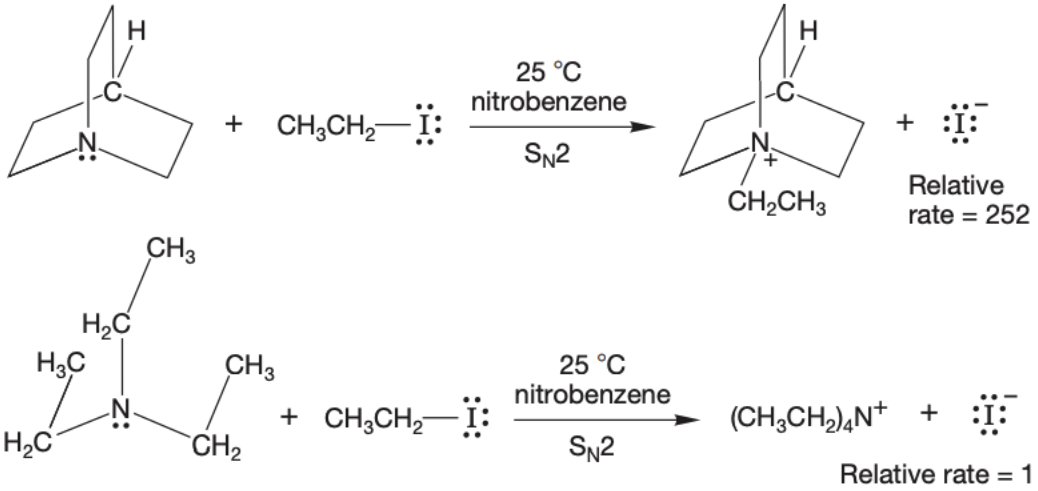
In nucleophilic reactions “we think” kinetics

$Nucleophile_1$ more powerful than $Nucleophile_2$ – reacts faster

$k_1 > k_2$

Effect of the Nucleophile - Size (S_N2)

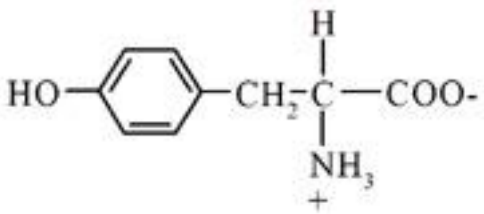
- **Nucleophiles vary in effectiveness** at displacing a leaving group in SN2 reactions.
- A **good nucleophile** competes effectively for the **carbon 2p orbital (Lewis acid)**.
- **Size matters**: Large R groups slow or prevent SN2 reactions due to **steric hindrance**.
- Similarly, **large nucleophiles** may struggle to **overlap effectively** with the substrate.



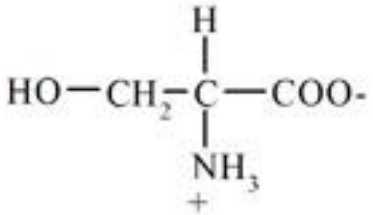
Triethylamine is effectively larger (freely rotating) than the cage compound in which the alkyl groups are tied back.

Let's Discuss

Which amino acid has the more nucleophilic side chain – serine or tyrosine ?



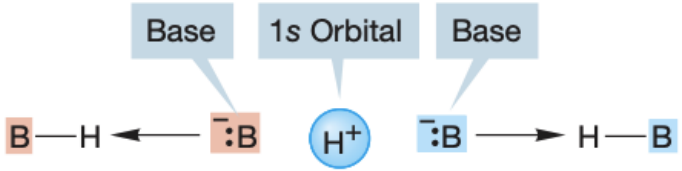
Tyrosine
(Tyr)

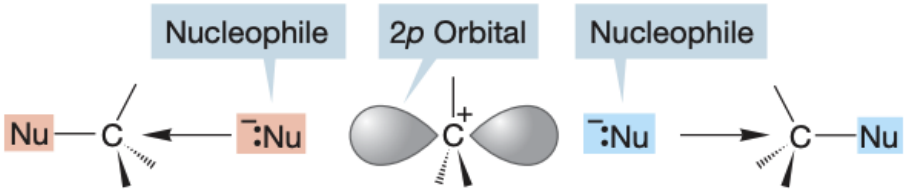


Serine
(Ser)

Effect of the Nucleophile - Nucleophilicity (S_N2)

- **Nucleophilicity (Lewis basicity)** measures how well a nucleophile competes for an **empty carbon 2p orbital** while **Brønsted basicity** measures how well a base competes for an **empty hydrogen 1s orbital**.
- **Nucleophilicity and basicity are related** but not identical, as **1s and 2p orbitals differ in energy and shape**.
- **Stronger bases often make stronger nucleophiles**, but exceptions exist due to **solvent effects, steric hindrance, and resonance stabilization**.





Based on charge

$^-NH_2 > NH_3$

$^-SH > SH_2$

$^-OH > OH_2$

Based on electronegativity

$^-NH_2 > ^-OR > ^-OH$

$H_2Se > H_2S > H_2O$

$R_3P > R_3N$

$I^- > Br^- > Cl^- > F^-$

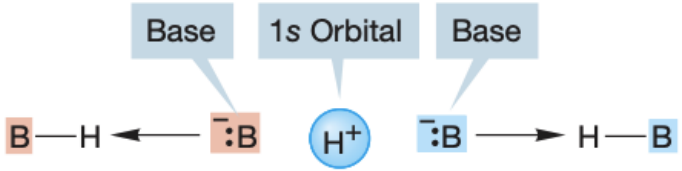
Species	Name	Relative Nucleophilicity
	Excellent Nucleophiles	
NC^-	Cyanide	126,000
HS^-	Mercaptide	126,000
I^-	Iodide	80,000
	Good Nucleophiles	
HO^-	Hydroxide	16,000
Br^-	Bromide	10,000
N_3^-	Azide	8,000
NH_3	Ammonia	8,000
NO_2^-	Nitrite	5,000
	Fair Nucleophiles	
Cl^-	Chloride	1,000
CH_3COO^-	Acetate	630
F^-	Fluoride	80
CH_3OH	Methyl alcohol	1
H_2O	Water	1

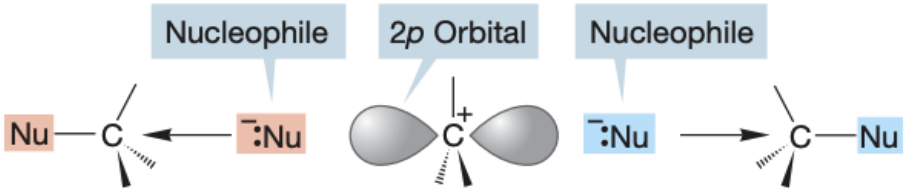
Be careful!

Relative nucleophilicity depends, for example, on the identity of the reaction partner, which is always an electrophile (Lewis acid), and on the nature of the solvent

Effect of the Nucleophile - Nucleophilicity (S_N2)

- **Nucleophilicity (Lewis basicity)** measures how well a nucleophile competes for an **empty carbon 2p orbital** while **Brønsted basicity** measures how well a base competes for an **empty hydrogen 1s orbital**.
- **Nucleophilicity and basicity are related** but not identical, as **1s and 2p orbitals differ in energy and shape**.
- **Stronger bases often make stronger nucleophiles**, but exceptions exist due to **solvent effects, steric hindrance, and resonance stabilization**.





Based on charge

$^-NH_2 > NH_3$

$^-SH > SH_2$

$^-OH > OH_2$

Based on electronegativity

$^-NH_2 > ^-OR > ^-OH$

$H_2Se > H_2S > H_2O$

$R_3P > R_3N$

$I^- > Br^- > Cl^- > F^-$

- But wait…. This doesn't make sense?

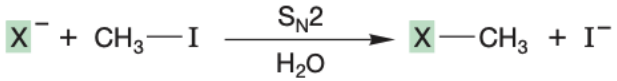
Species	Name	Relative Nucleophilicity
	Excellent Nucleophiles	
NC^-	Cyanide	126,000
HS^-	Mercaptide	126,000
I^-	Iodide	80,000
	Good Nucleophiles	
HO^-	Hydroxide	16,000
Br^-	Bromide	10,000
N_3^-	Azide	8,000
NH_3	Ammonia	8,000
NO_2^-	Nitrite	5,000
	Fair Nucleophiles	
Cl^-	Chloride	1,000
CH_3COO^-	Acetate	630
F^-	Fluoride	80
CH_3OH	Methyl alcohol	1
H_2O	Water	1

Be careful!

Relative nucleophilicity depends, for example, on the identity of the reaction partner, which is always an electrophile (Lewis acid), and on the nature of the solvent

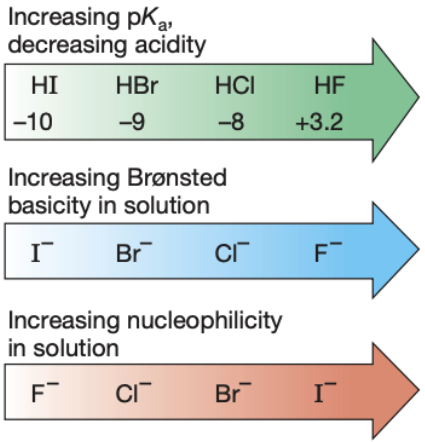
Effect of the Nucleophile - Nucleophilicity (S_N2)

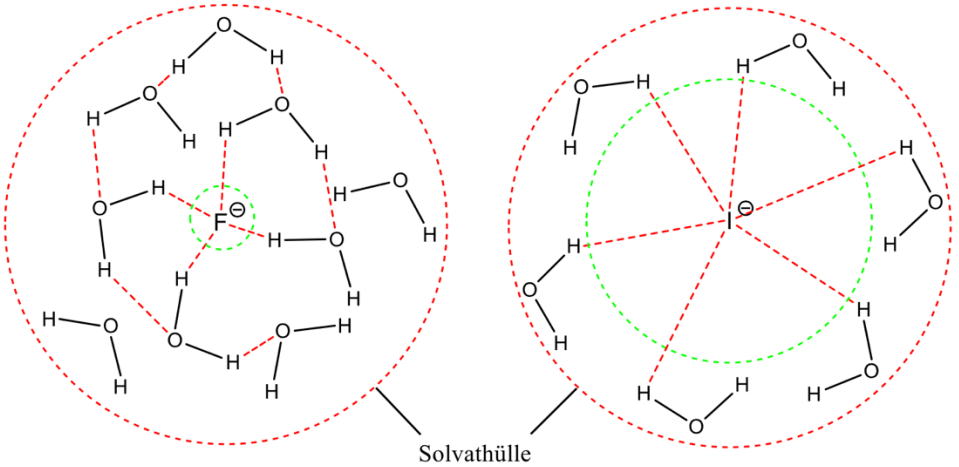
Based on charge	Based on electronegativity
$^-NH_2 > NH_3$	$^-NH_2 > ^-OR > ^-OH$
$^-SH > SH_2$	$H_2Se > H_2S > H_2O$
$^-OH > OH_2$	$R_3P > R_3N$
	$I^- > Br^- > Cl^- > F^-$



Relative Rates			
I	Br	Cl	F
160	14	1	–

But wait…. This doesn't make sense according to "a good Brønsted base is a good nucleophile



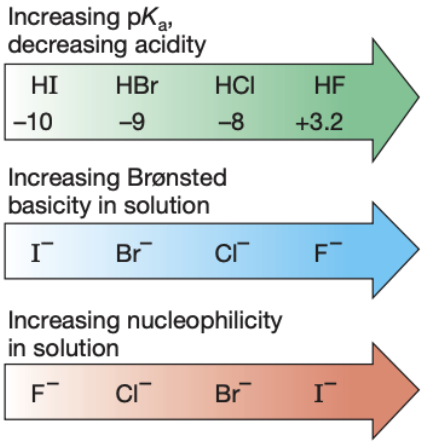


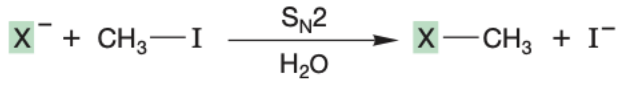
Halides can act as Brønsted bases by interacting with **protic solvents**.
Cl^- and Fluoride F^- are **stronger bases** than I^-.
Stronger base = stronger hydrogen bonding to **protic solvents**.

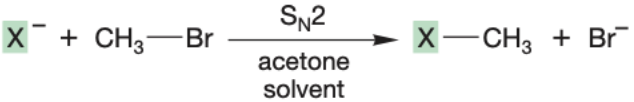
Effect of the Nucleophile - Nucleophilicity (S_N2)

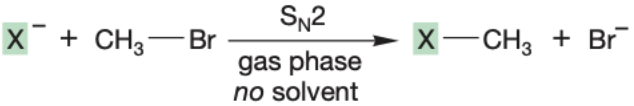
Based on charge	Based on electronegativity
$^-NH_2 > NH_3$	$^-NH_2 > ^-OR > ^-OH$
$^-SH > SH_2$	$H_2Se > H_2S > H_2O$
$^-OH > OH_2$	$R_3P > R_3N$
	$I^- > Br^- > Cl^- > F^-$

But wait…. This doesn't make sense according to "a good Brønsted base is a good nucleophile"







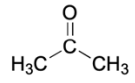


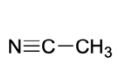
Relative Rates			
I	Br	Cl	F
160	14	1	–
1	5	11	–
–	<0.015	0.02	1

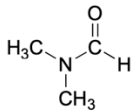
X = halogens

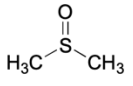
As the polarity of the solvent decreases, fluoride becomes more competitive with iodide -> In gas phase we see the expected trend

- Polar aprotic solvents

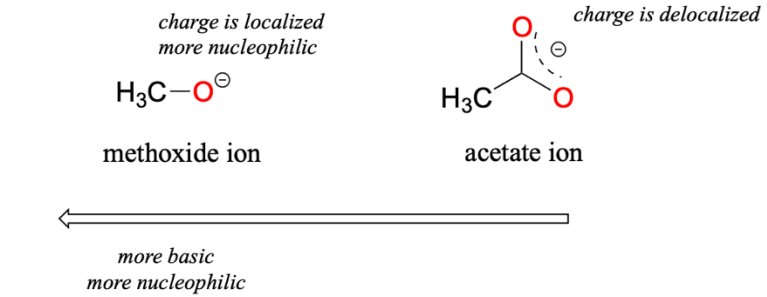

acetone

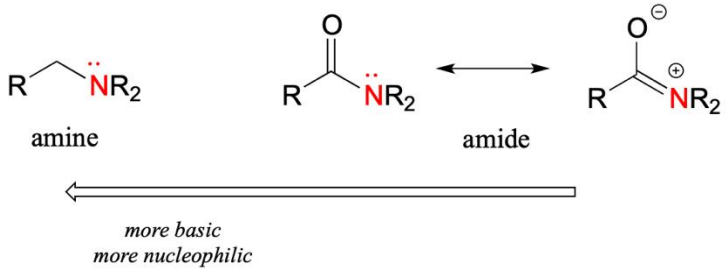

acetonitrile


dimethylformamide (DMF)


dimethylsulfoxide (DMSO)

Effect of the Nucleophile - Nucleophilicity (S_N2)





Same reasoning as for basicity

If the lone pair is delocalized by resonance it is inherently less reactive

Less nucleophilic

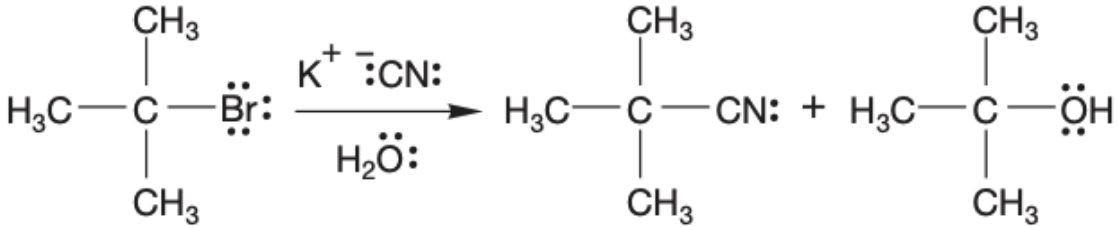
Effect of the Nucleophile - Nucleophilicity (S_N1)

SN1 reactions are first-order, meaning the **nucleophile does not affect the reaction rate**.
However, the **nucleophile determines the product structure** by reacting with the carbocation intermediate.

Rate-determining step ≠ product-determining step:

- **Slow step**: Ionization of the substrate to form a carbocation.
- **Fast step**: Capture of the carbocation by the nucleophile.

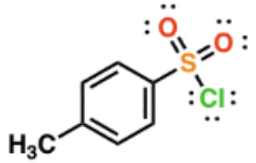
Example: In the reaction of **tert-butyl bromide in water with cyanide**, cyanide (CN^-) competes with water despite its lower concentration because it is a **much stronger nucleophile**.



For S_N1, the Nucleophile is not as important

Effect of the Leaving Group (S_N2)

- **Most leaving groups (LG^-) depart as anions** during substitution reactions.
- **Better leaving groups = more stable anions (LG^-)** after departure.
- Leaving group stability is **linked** to the **pKa of its conjugate acid (H–LG)**:
  Low pKa (strong acid) → **Good leaving group** (weak base).
  High pKa (weak acid) → **Poor leaving group** (strong base).

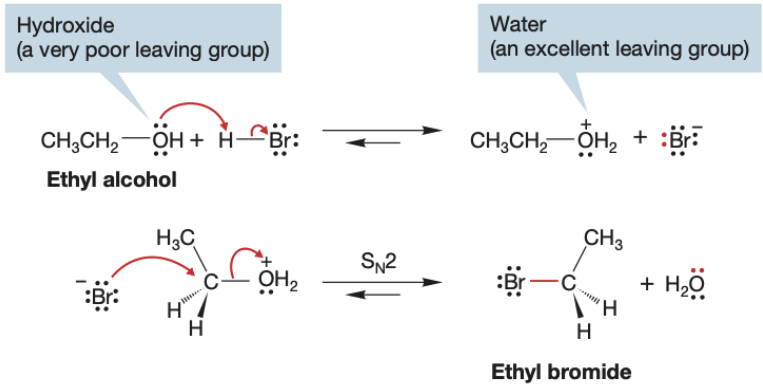


p-toluenesulfonyl chloride
("tosyl chloride")

$$Nu:^- + R{-}LG \rightarrow Nu{-}R + LG:^-$$

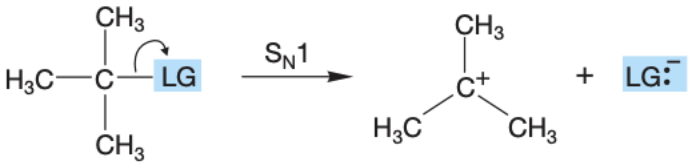
Acid	pK_a	Leaving Group	Name
		Good Leaving Groups	
HI	−10	^-I	Iodide
HBr	−9	^-Br	Bromide
HCl	−8	^-Cl	Chloride
$HOSO_2R$	−3	$^-OSO_2R$	Sulfonate
H_3O^+	−1.7	OH_2	Water
		Poor Leaving Groups	
HF	+3.2	^-F	Fluoride
H_2S	+7.0	^-SH	Thiolate
HCN	+9.4	^-CN	Cyanide
H_2O	+15.7	^-OH	Hydroxide
$HOCH_2CH_3$	+15.9	$^-OCH_2CH_3$	Ethoxide
HOR	+16 to 18	^-OR	Alkoxide

Remember Kenyon and Philips
-> We can make LGs better



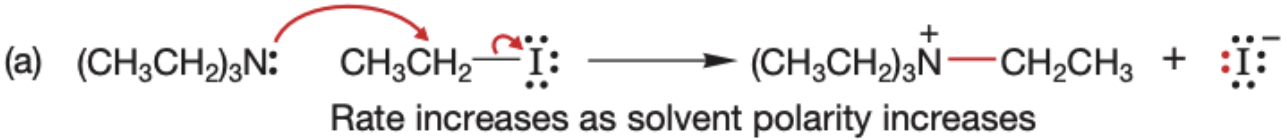
Effect of the Leaving Group (S_N1)

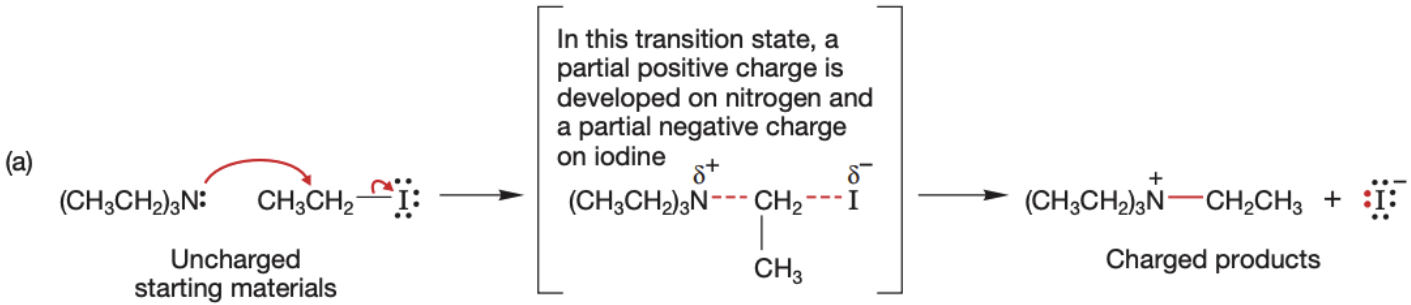
- **Leaving Group Influence on SN1 Reactions**: The rate of an SN1 reaction is significantly affected by the stability of the leaving group, as it impacts the ease of ionization.
- **Carbocation vs. Leaving Group Stability**: While carbocation stability is crucial, the stability of the departing anion (LG^-) also plays a key role—more stable anions are lost more easily.
- **Trends in Leaving Group Quality**: Good leaving groups stabilize the negative charge well, whereas poor leaving groups make ionization more difficult



Effect of the Solvent (S_N2)

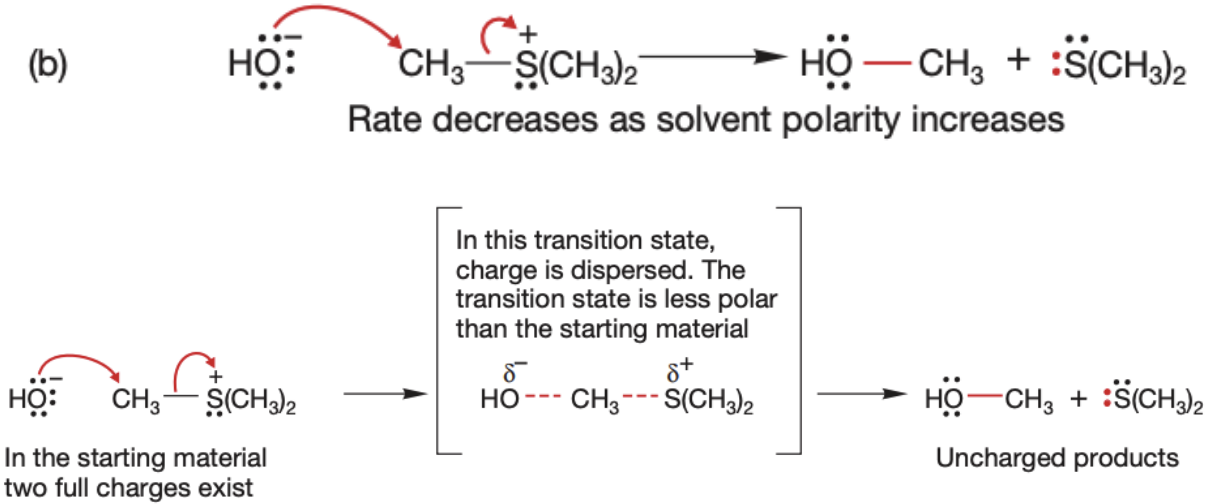
At first, the behaviour of the SN2 reaction as the solvent polarity is changed is perplexing. As the solvent polarity is increased, some SN2 reactions go faster, some slower





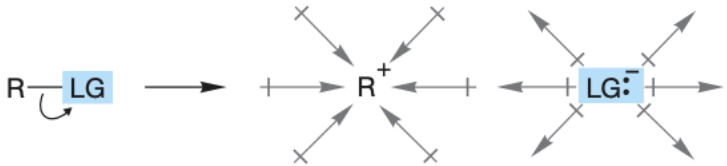
Effect of the Solvent (S_N2)

At first, the behaviour of the SN2 reaction as the solvent polarity is changed is perplexing. As the solvent polarity is increased, some SN2 reactions go faster, some slower



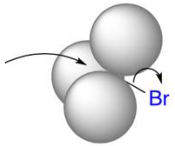
Effect of the Solvent (S_N1)

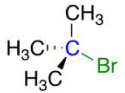
Polar vs. Nonpolar Solvents: Polar solvents enhance SN1 reaction rates by stabilizing intermediates, whereas nonpolar solvents provide little stabilization.



S_N1 vs S_N2
Predicting the mechanism

tertiary alkyl halide





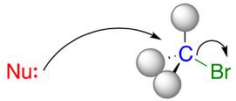
Factors favoring the S_N1 pathway:

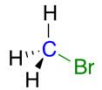
hindered electrophile
potential for a tertiary, secondary, or resonance-stabilized carbocation intermediate
uncharged nucleophile
protic solvent such as water

Factors favoring the S_N2 pathway:

Unhindered (methyl or primary) electrophile
powerful, anionic nucleophile
polar aprotic solvent

methyl halide

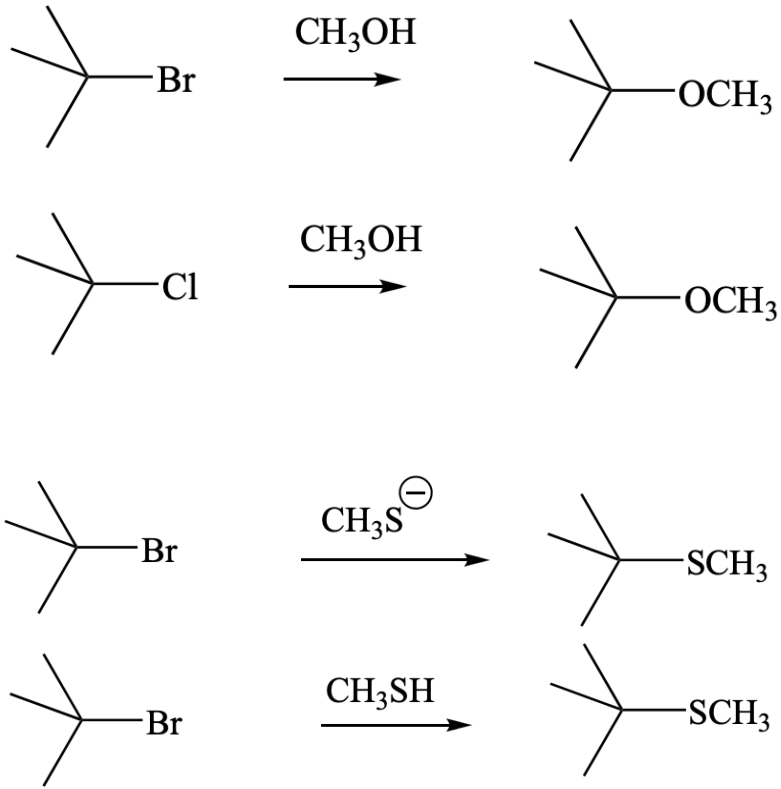




It is important to remember that many of these reaction pathways are just conceptual models and many reactions will fall somewhere in between.

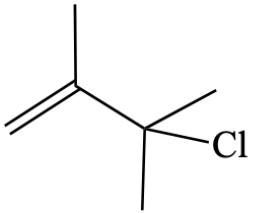
Let's do some together

For each of the following pairs, which reaction, if either, will be faster?



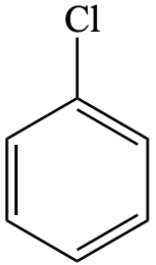
Let's do some together

For each of the following reactions, give the structure of the product and indicate whether the mechanism is likely to be SN1, SN2, both or neither.



CH_3CH_2OH

3˚Carbon
Allyl carbocation
Steric Hindrance

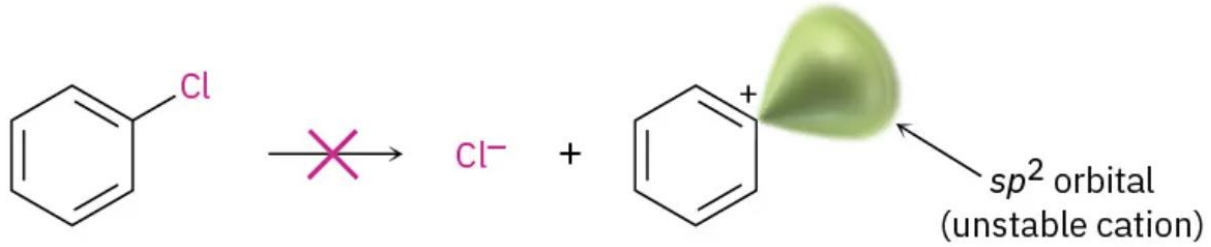


NaOH

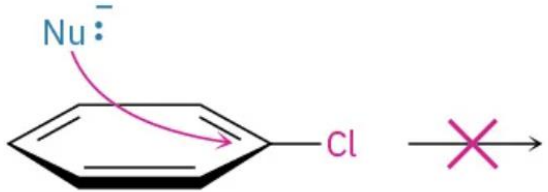
Sp2 hybridized carbon
-> no substitution possible

Let's do some together

For each of the following reactions, give the structure of the product and indicate whether the mechanism is likely to be SN1, SN2, both or neither.



Dissociation reaction does not occur because the aryl cation is unstable; therefore, no S_N1 reaction.



Backside displacement is sterically blocked; therefore, no S_N2 reaction.

What to know

- Draw the mechanisms for nucleophilic substitution reactions
- Understand considerations about reaction mechanisms
- Understand stereochemistry
- Understand kinetics
- Discuss effects of different Substituents
- Discuss different Nucleophiles and their properties
- Discuss solvent effects
- Discuss how different leaving groups influence the substitution
- Make considerations if SN1 or SN2 is preferred and what products are formed

Questions?

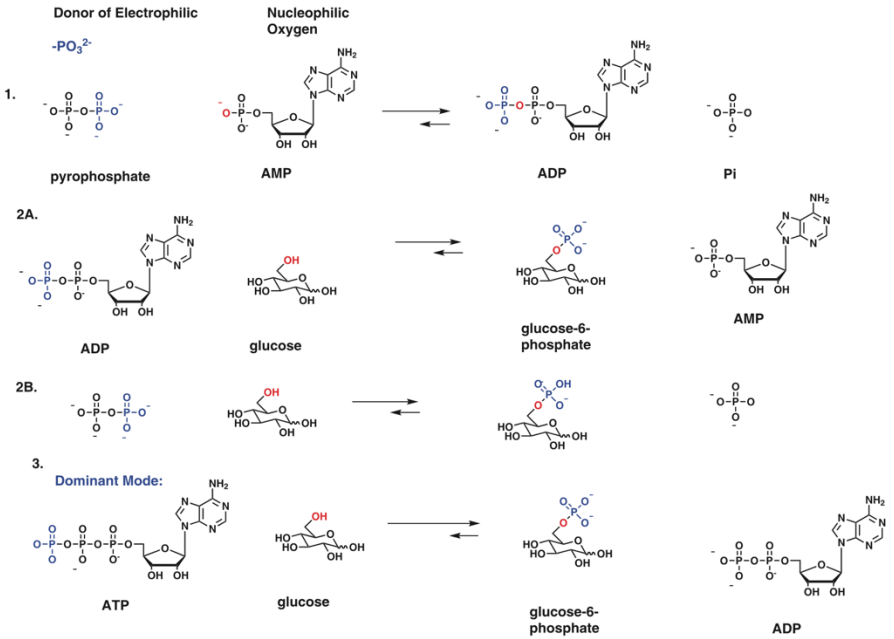
Phosphates in biology

Table 1. Examples of phosphates in biochemistry.

Phosphate	Acid derivative
DNA	Diester of phosphoric acid
RNA	Diester of phosphoric acid
ATP	Anhydride of phosphoric acid
Creatine phosphate	Amide of phosphoric acid
Phosphoenolpyruvate	Enol ester of phosphoric acid
Pyridoxal phosphate	Phenol ester of phosphoric acid
Nicotine adenine dinucleotide	Ester and anhydride of phosphoric acid
Fructose 1,6-diphosphate	Ester of phosphoric acid
Glucose-6-phosphate	Ester of phosphoric acid
Isopentenyl pyrophosphate	Ester of pyrophosphoric acid
Ribose-6-phosphate-1-pyrophosphate	Ester of phosphoric and pyrophosphoric acids

Why Nature Chose Phosphates

F. H. WESTHEIMER



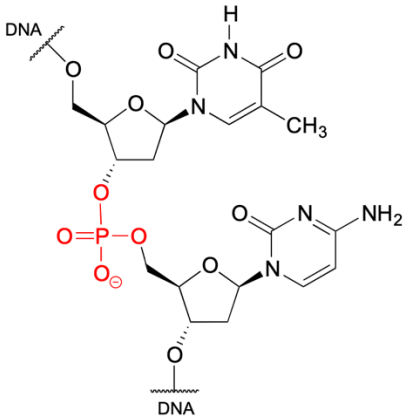
Why Phosphorylation?

1. Electrostatics: two negative charges
2. Specificity: >3 highly directional hydrogen bonds
3. Thermodynamic stability: -12 kcal/mol
4. Signal can be amplified
5. Adjustable kinetics (seconds to hours)
6. Availability: high intracellular ATP concentration

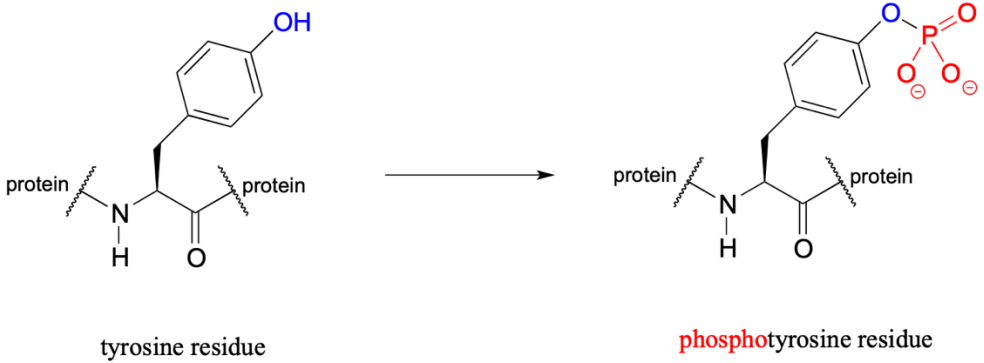
Great historic paper:
Westheimer: Why Nature Chose Phosphates (1987) Science, 235 (4793),1173-8

Phosphates in biology

Base-pair linkage



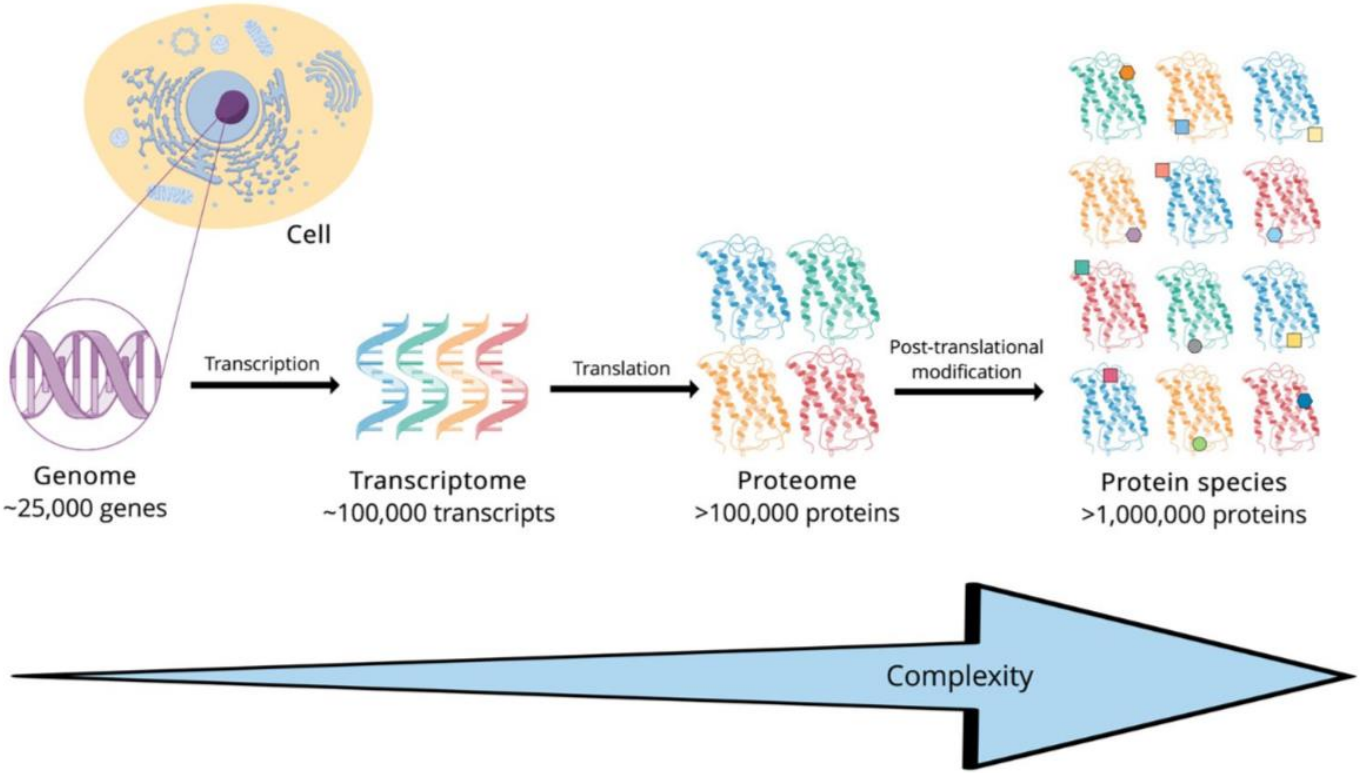
Controlling protein activity



tyrosine residue

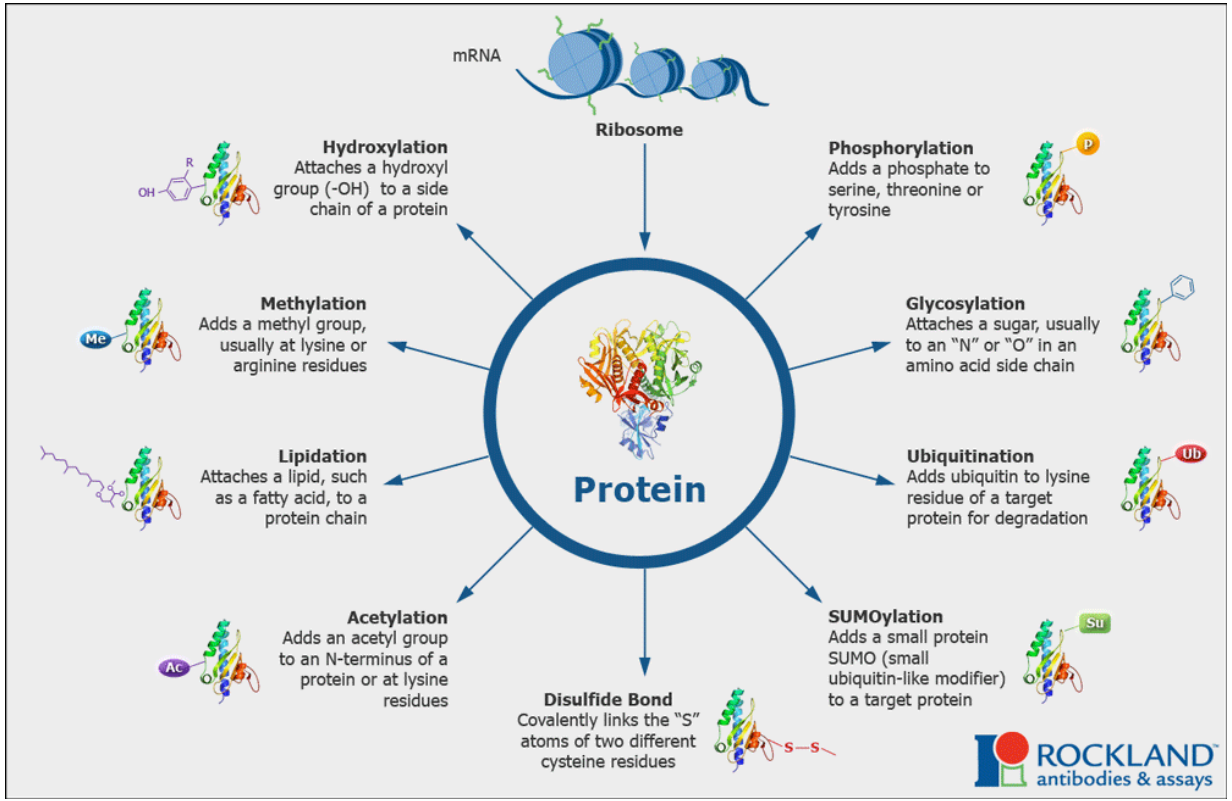
phosphotyrosine residue

What are post-translational modifications

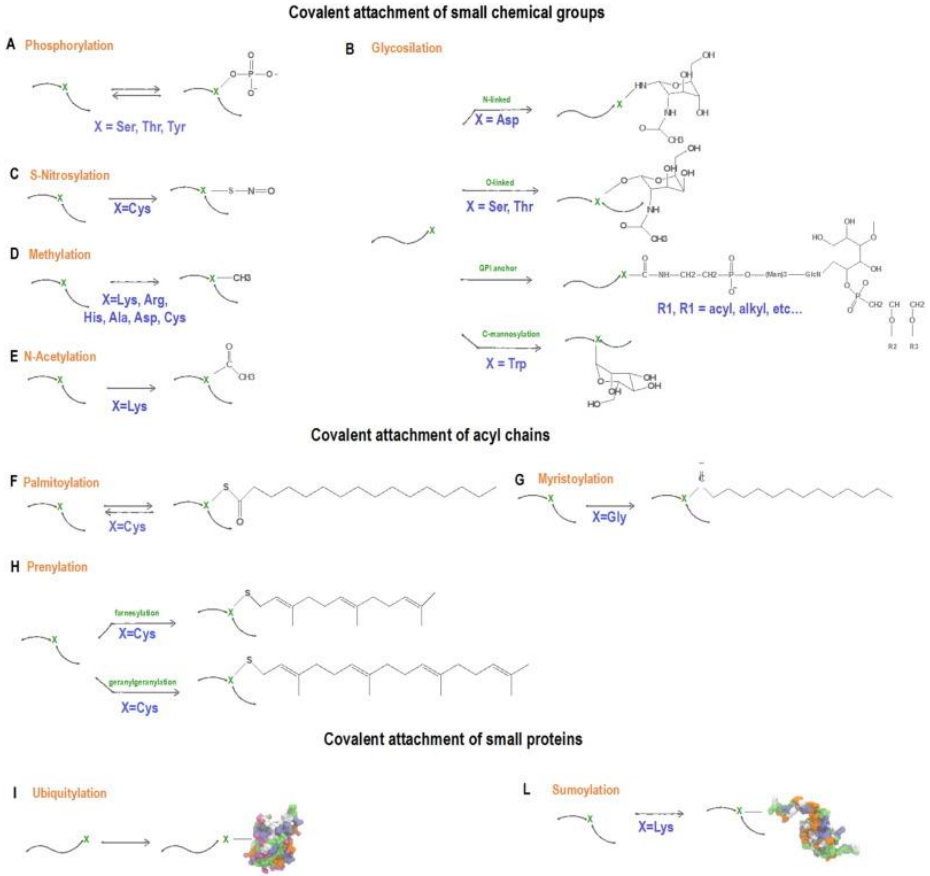


The sidechains of certain amino acids can be modified

Most Common Post-translational Modifications	
Reversible	**Irreversible**
disulfide bridge	cofactor binding
cofactor binding	proteolysis
glycosylation	ubiquitination
phosphorylation	peptide tagging
acylation	lysine hydroxylation
ADP-ribosylation	methylation
carbamylation	
N-acetylation	



Chemically defined modifications are performed to the aminoacids



PERSPECTIVE
PUBLISHED ONLINE: 14 FEBRUARY 2018 | DOI: 10.1038/NCHEMBIO.2576
nature chemical biology

How many human proteoforms are there?

Ruedi Aebersold[1], Jeffrey N Agar[2], I Jonathan Amster[3], Mark S Baker[4], Carolyn R Bertozzi[5], Emily S Boja[6], Catherine E Costello[7], Benjamin F Cravatt[8], Catherine Fenselau[9], Benjamin A Garcia[10],

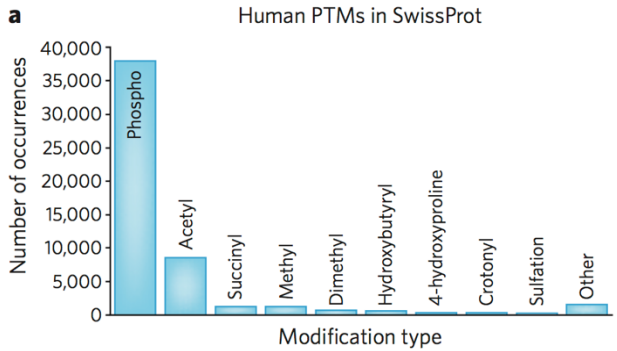
Prof. Dr. Ruedi Aebersold

Contact
ETH Zurich
Inst. f. Molekulare Systembiologie

Prof. Dr. Rudolf Aebersold
HPT E 78
Auguste-Piccard-Hof 1
8093 Zürich
Switzerland

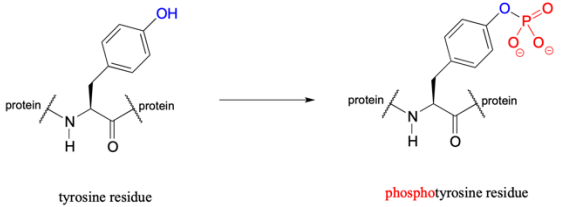
+41 44 633 31 70
E-mail
V-Card (vcf, 1kb)

- 19,587–20,245 genes in the human genome
- ~70,000 transcript variants (due to alternative splicing)
- >100 different PTMs: many hundreds of thousands of protein variants



Protein Phosphorylation: General

- Most common post-translational modification
- Occur on hydroxyl (OH-) groups in Ser, Thr or Tyr (in eukaryotes)
- Also on His (but much less well studied)
- At least 30% of all human proteins are known to be phosphorylated at at least one residue



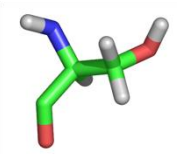
tyrosine residue

phosphotyrosine residue

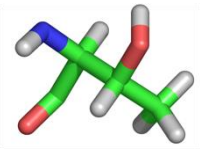
Theoretical considerations on phosphorylation:

- average length of protein is ~400 amino acids
- ~10.000 different proteins in a cell
- ~15% are Ser (6.6%), Thr (5.3%) or Tyr (2.9%) residues (see BCI)
- then there are ~600,000 potential phosphorylation sites!

Serine

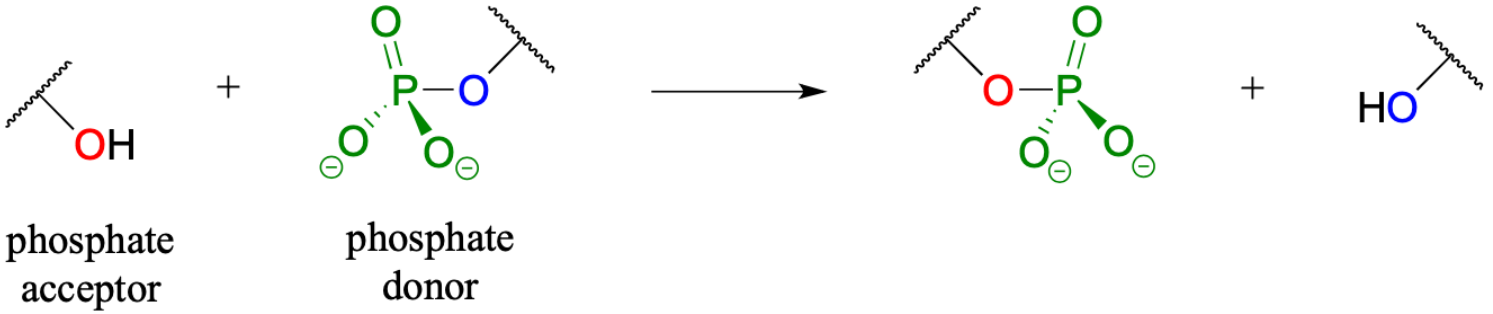


Threonine

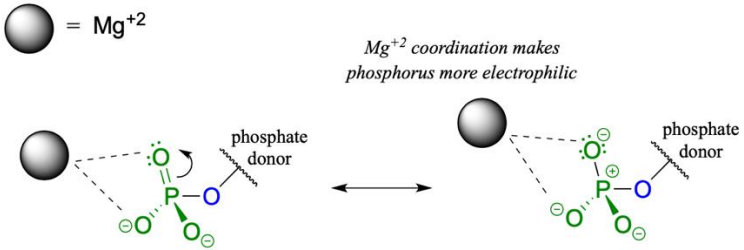


Tyrosine

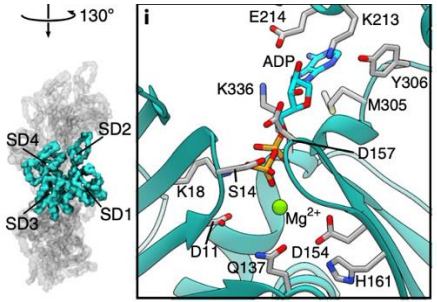
Phosphate transfer reactions – overview



-Electrophilicity of the phosphorus atom is enhanced by magnesium ions



-Magnesium ion pulls electron density away from the phosphorus making it more electrophilic



Remember

Break Bond (**D**issocitation)

Make Bond (**A**ssociation)

Combinations:

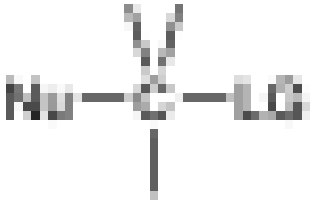
D then **A**

S_N1

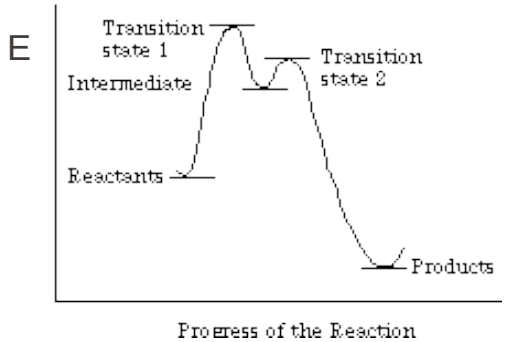
Trivalent *Intermediate*

A then **D**

Happens, but not with carbon

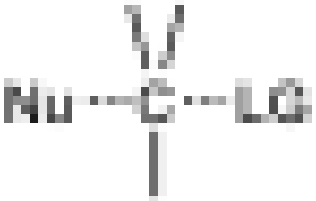


Pentavalent *Intermediate*

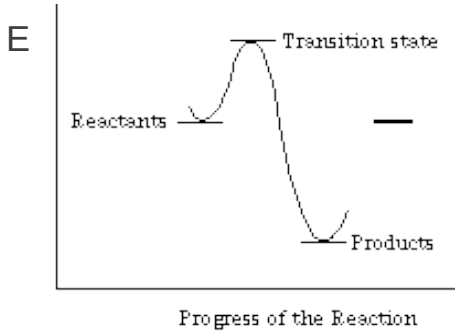


Simultaneous "**Concerted**" (make as you break)

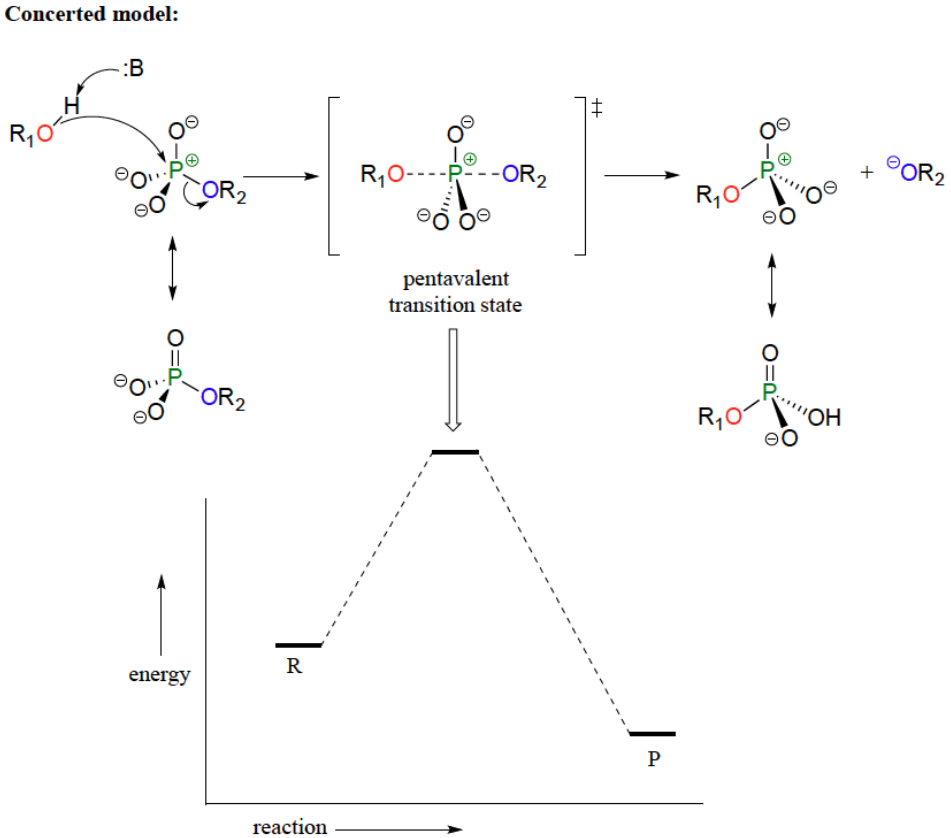
S_N2



Transition State



Mechanism of Phosphotransfer



-Phosphate transfer reaction can be thought of much like an SN2 reaction

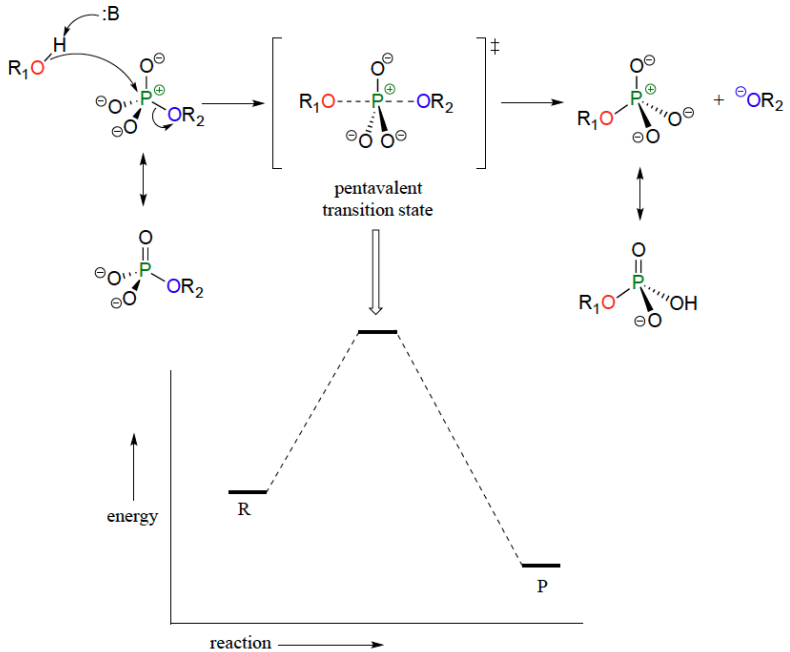
-Nucleophile attacks the phosphorus atom from the opposite side

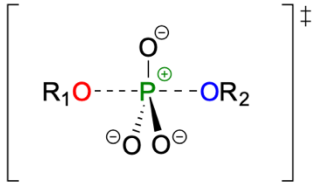
- **geometry** around the phosphorus atom shifts from **tetrahedral to trigonal bipyramidal** at the transition state with five bonds.

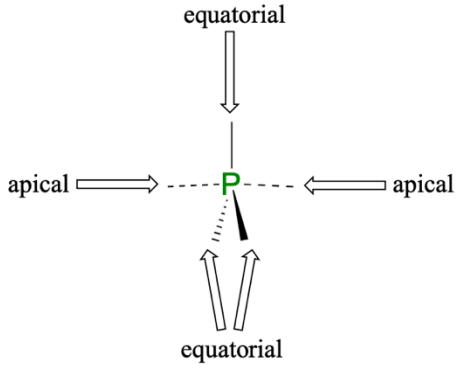
-Phosphorus undergoes a temporary change in bonding, shifting back to its initial tetrahedral state after the nucleophile and leaving group alteration.

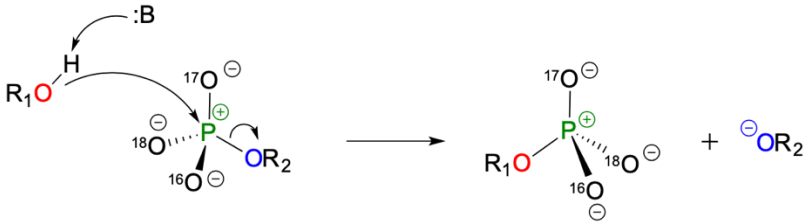
Mechanism of Phosphotransfer

Concerted model:







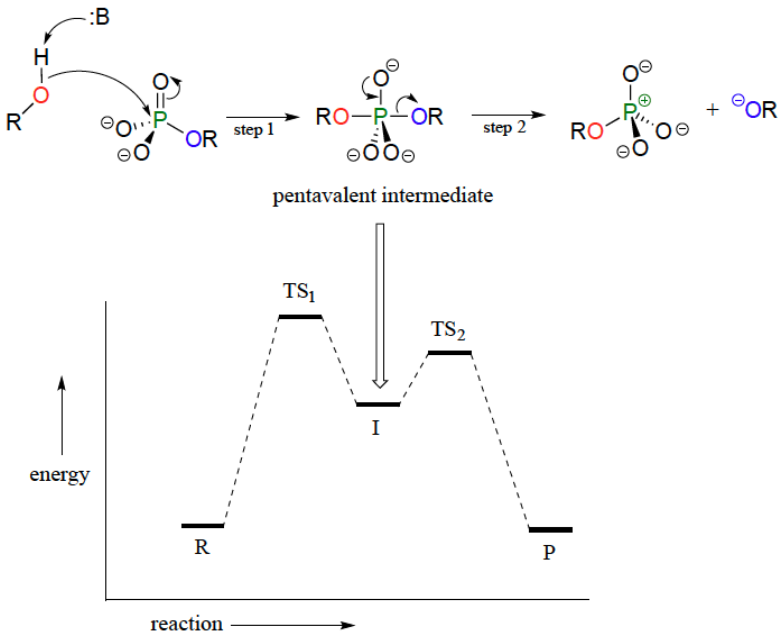


phosphate has *S* configuration

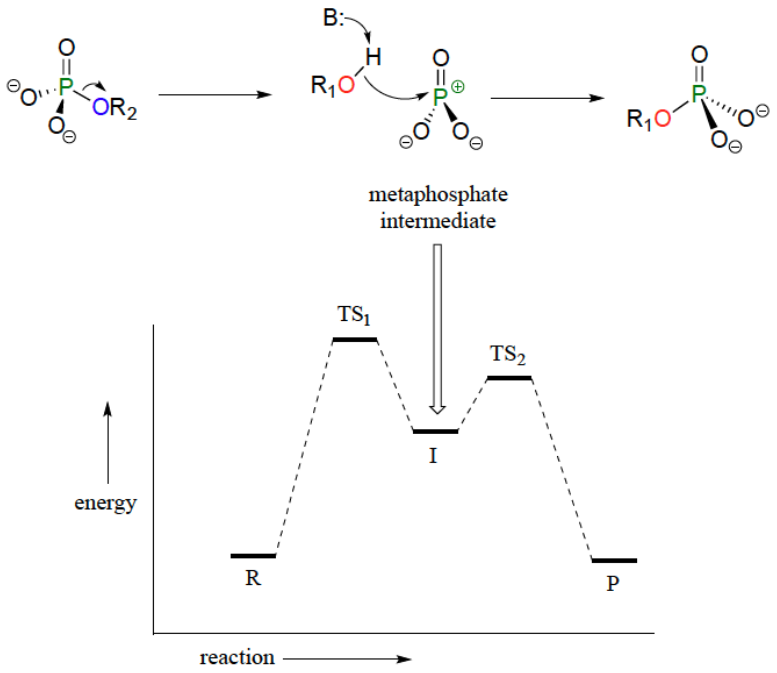
phosphate has *R* configuration

Mechanism of Phosphotransfer

Addition-elimination model:



Elimination-addition model:



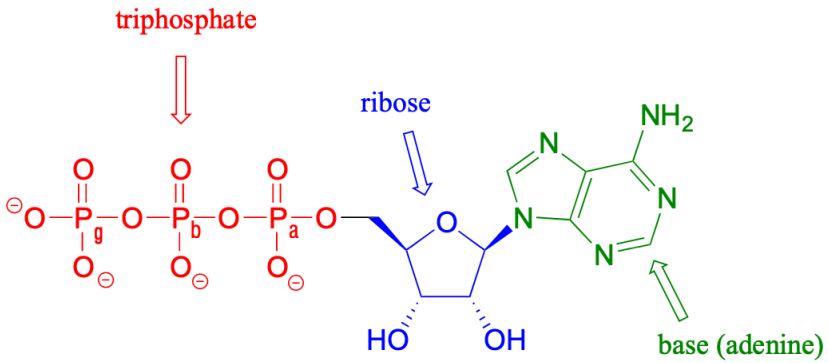
- There is still debate to which real mechanism is happening in the phosphor transfer reaction
- For now we ill accept the concerted model as the one that is occurring

The reaction of life: ATP as a biological Phosphate Donor

-The most important phosphate donor is a adenosine triphosphate

-ATP is used as the energetic currency in biological processes

-ATP hydrolysis is one of the most important reactions in biology



One can represent these molecules in different ways