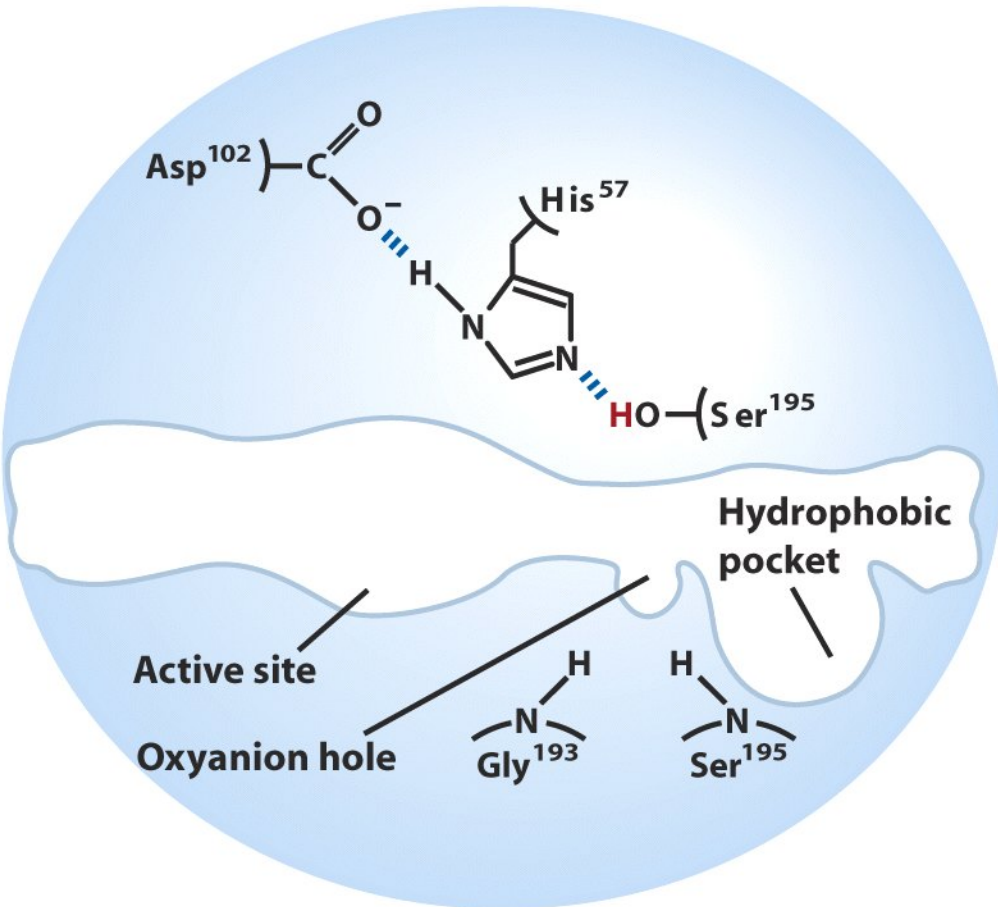
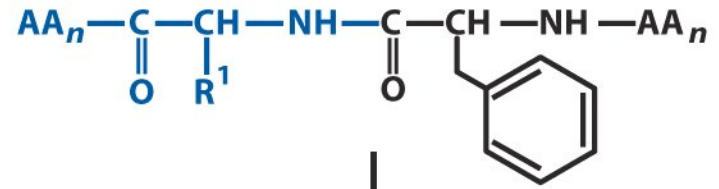


# Chymotrypsin (free enzyme)



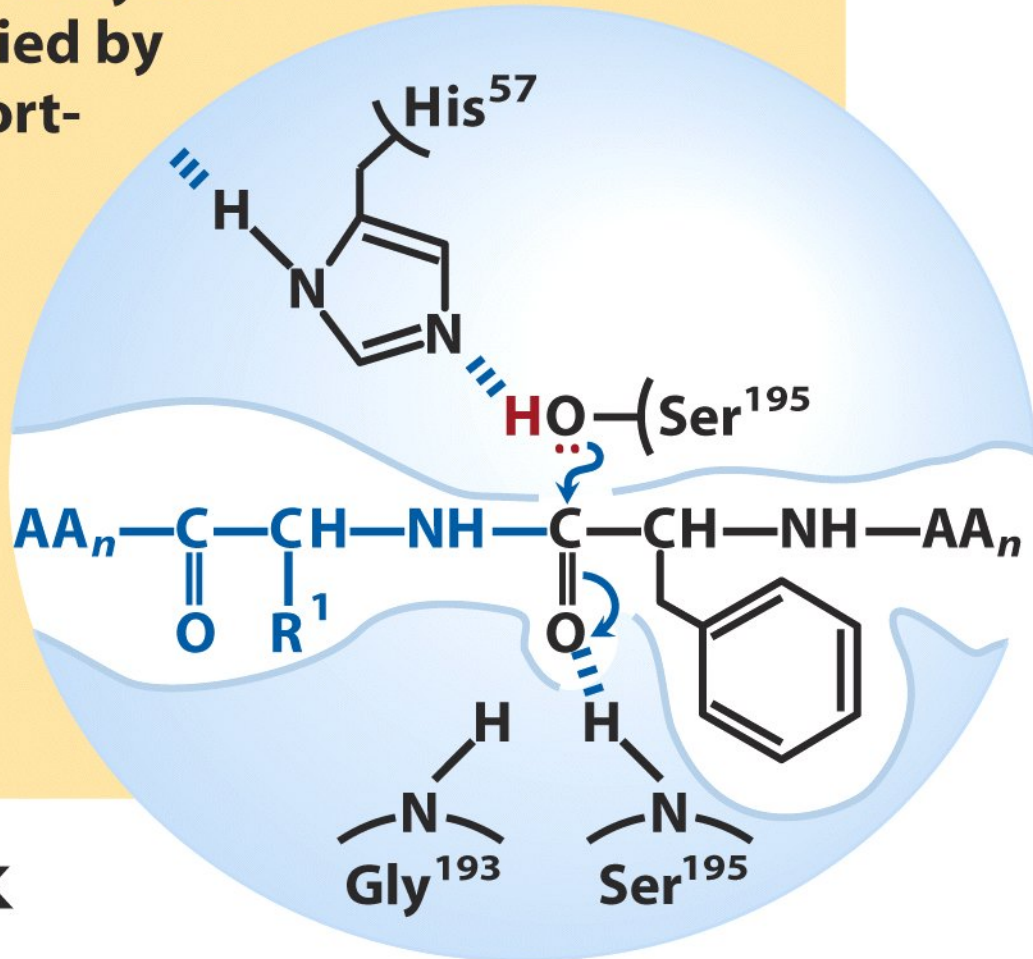
## Substrate (a polypeptide)



When substrate binds, the side chain of the residue adjacent to the peptide bond to be cleaved nestles in a hydrophobic pocket on the enzyme, positioning the peptide bond for attack.

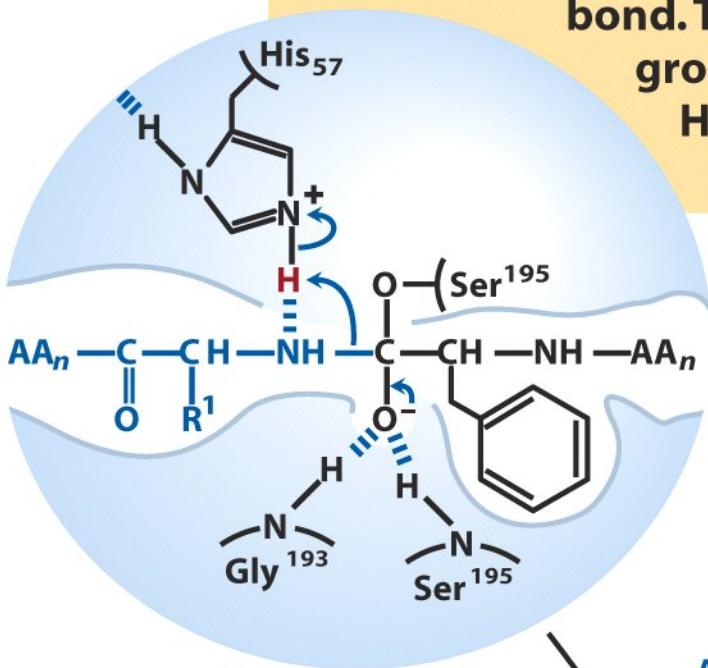
Interaction of Ser<sup>195</sup> and His<sup>57</sup> generates a strongly nucleophilic alkoxide ion on Ser<sup>195</sup>; the ion attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme.

This is accompanied by formation of a short-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bonding in the oxyanion hole.



**ES complex**

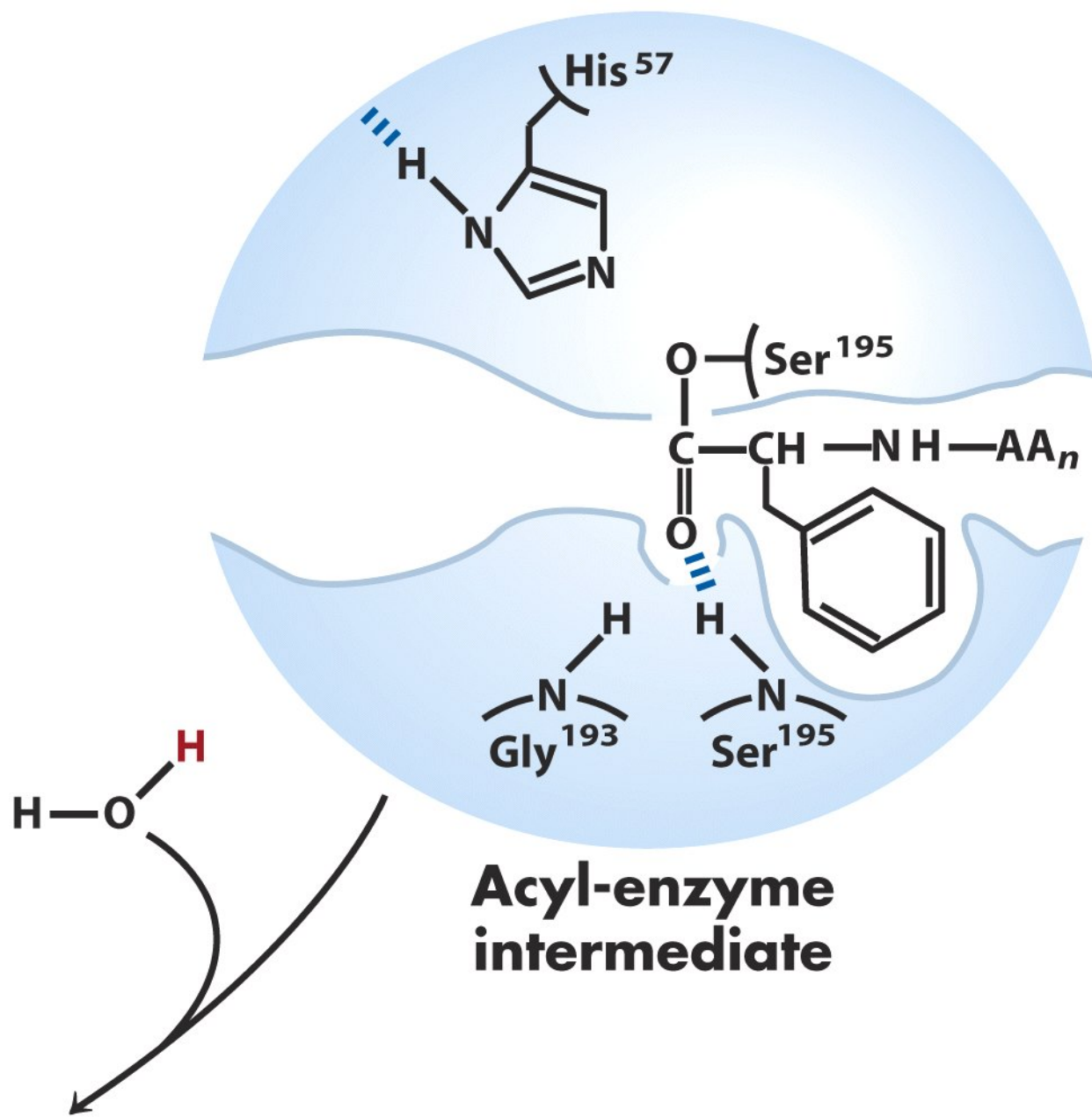
Instability of the negative charge on the substrate carbonyl oxygen leads to collapse of the tetrahedral intermediate; re-formation of a double bond with carbon displaces the bond between carbon and the amino group of the peptide linkage, breaking the peptide bond. The amino leaving group is protonated by His<sup>57</sup>, facilitating its displacement.



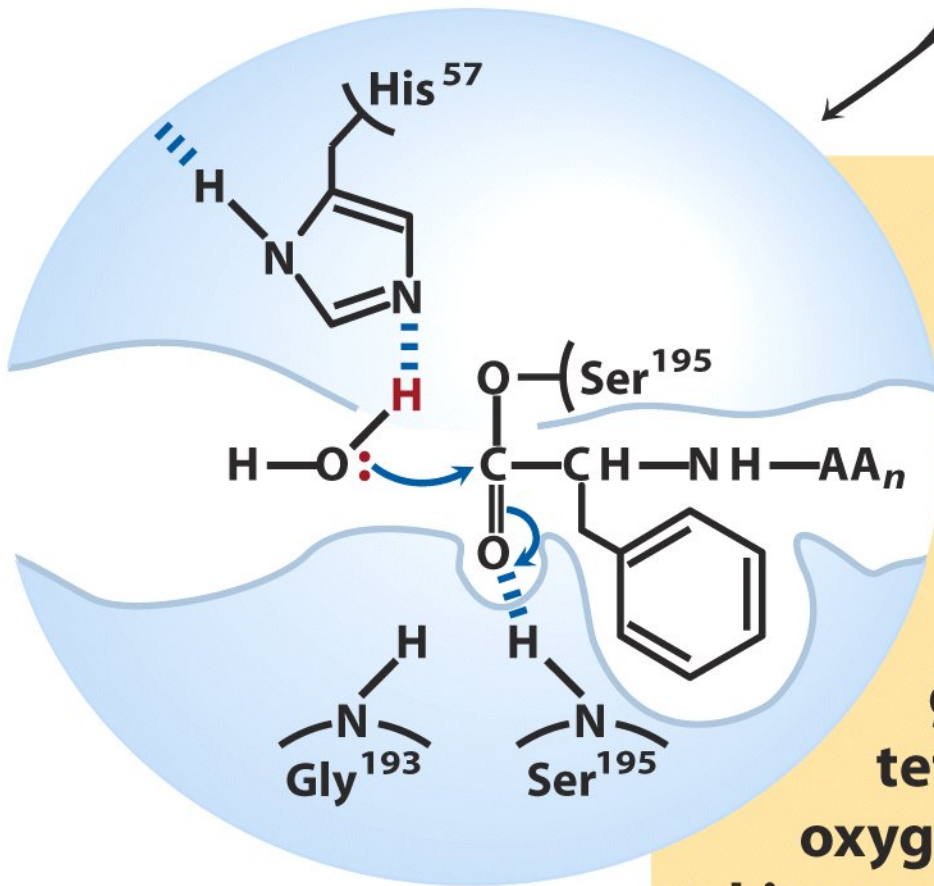
**Short-lived intermediate (acylation)**

**Product 1**



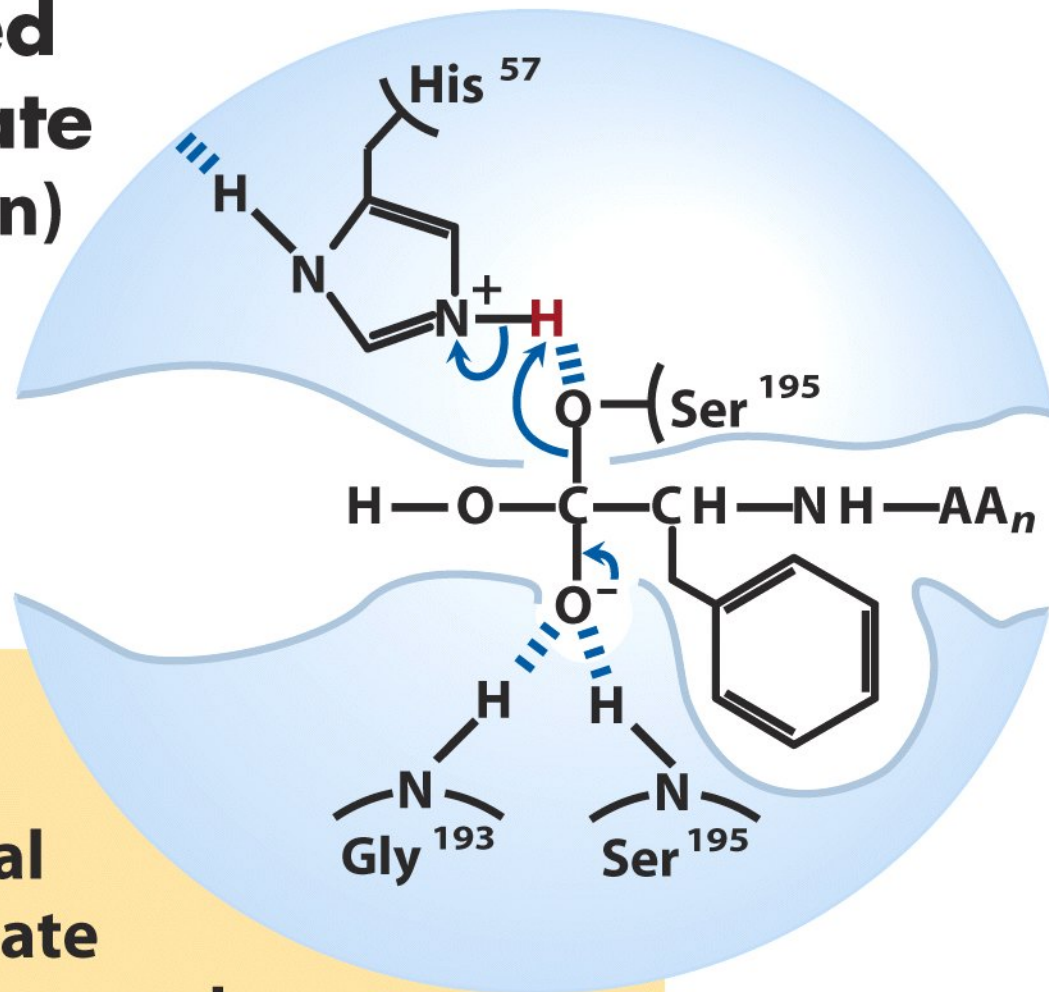


# Acyl-enzyme intermediate



An incoming water molecule is deprotonated by general base catalysis, generating a strongly nucleophilic hydroxide ion. Attack of hydroxide on the ester linkage of the acyl-enzyme generates a second tetrahedral intermediate, with oxygen in the oxyanion hole again taking on a negative charge.

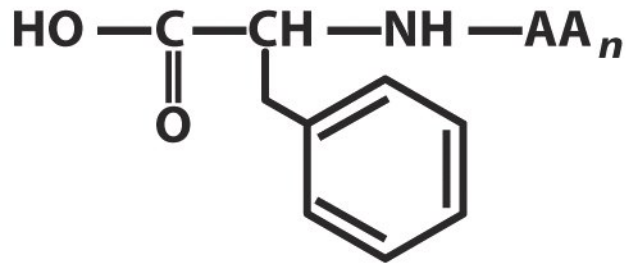
# Short-lived intermediate (deacylation)



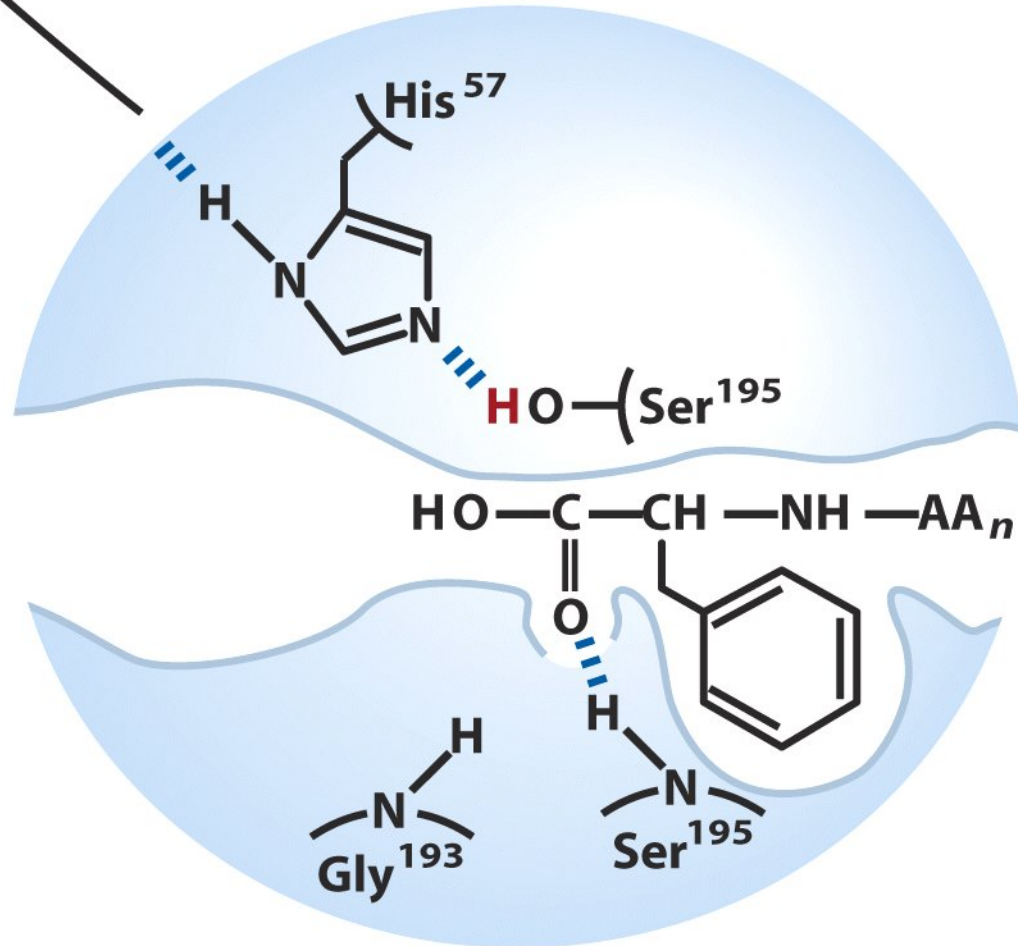
**Collapse of the tetrahedral intermediate forms the second product, a carbohydrate anion, and displaces Ser<sup>195</sup>.**

## Enzyme-product 2 complex

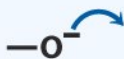
Product 2



Diffusion of the second product from the active site regenerates free enzyme.



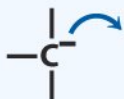
# Nucleophiles



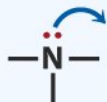
Negatively charged oxygen (as in an unprotonated hydroxyl group or an ionized carboxylic acid)



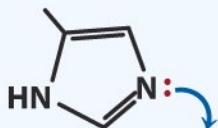
Negatively charged sulfhydryl



Carbanion



Uncharged amine group

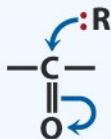


Imidazole

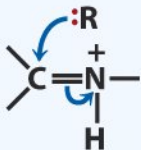


Hydroxide ion

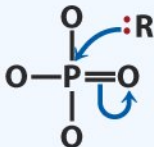
# Electrophiles



Carbon atom of a carbonyl group (the more electronegative oxygen of the carbonyl group pulls electrons away from the carbon)



Protonated imine group (activated for nucleophilic attack at the carbon by protonation of the imine)



Phosphorus of a phosphate group



Proton