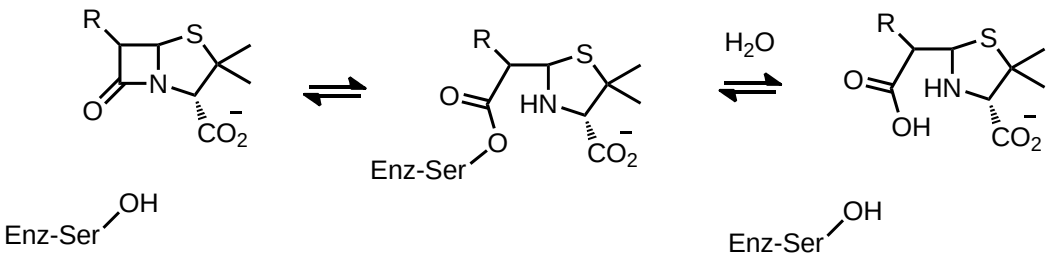
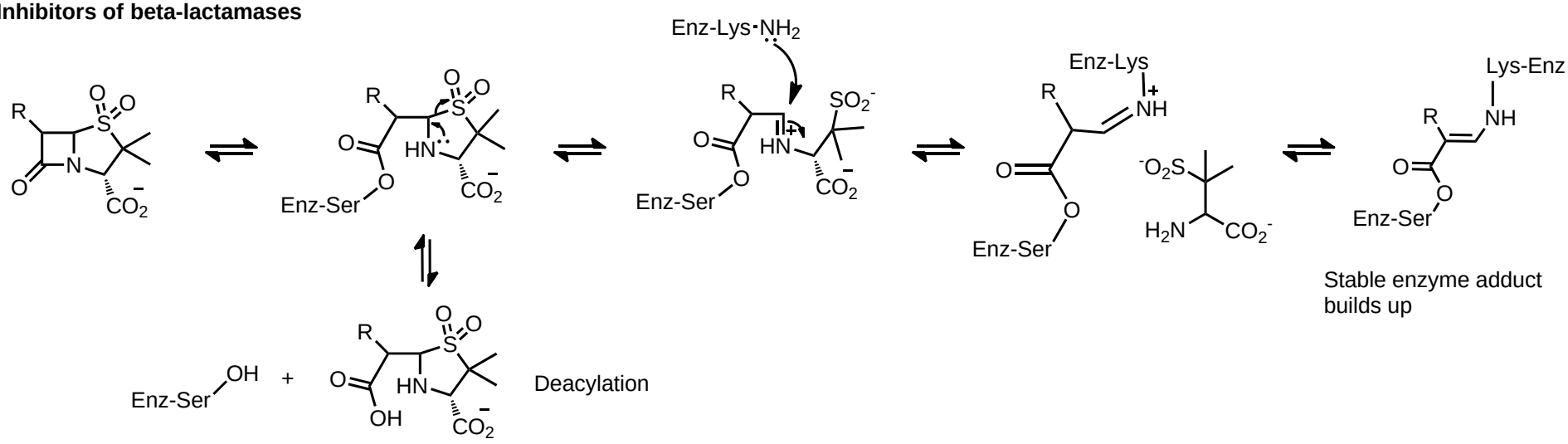
11.10.15 - Lecture 15- Antibiotics and RNA catalysts

Penicillin antibiotics-

-inactivated by beta-lactamase's ability to perform a fast deacylation of the inhibitor-enzyme adduct

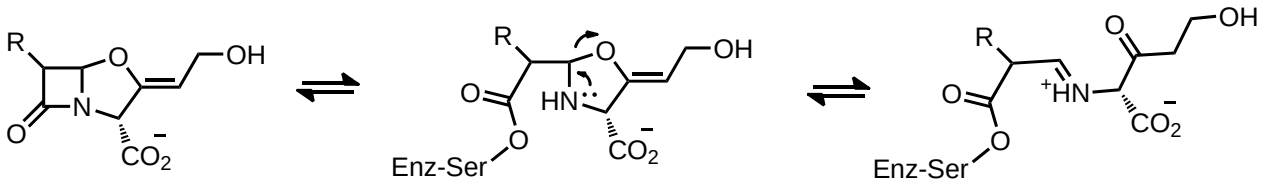


Inhibitors of beta-lactamases



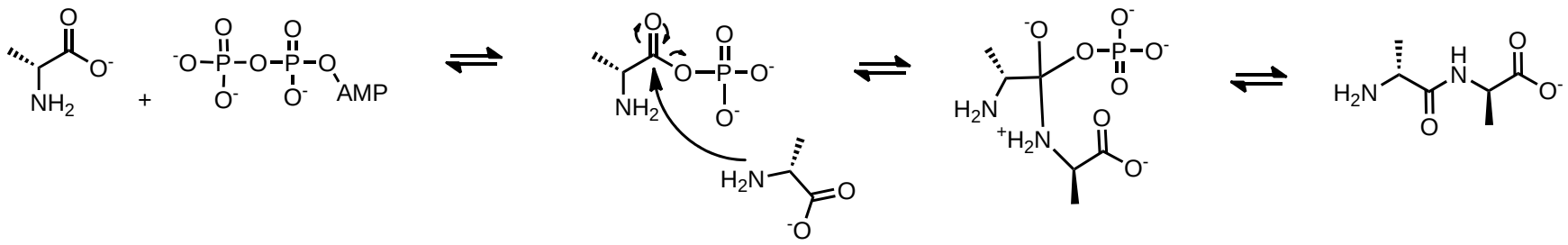
Stable enzyme adduct builds up

Using an alternative leaving group-



In general,beta-lactam antibiotics are not affected by drug pumps as their target resides in the periplasmic space

Targeting D-Ala D-Ala ligase



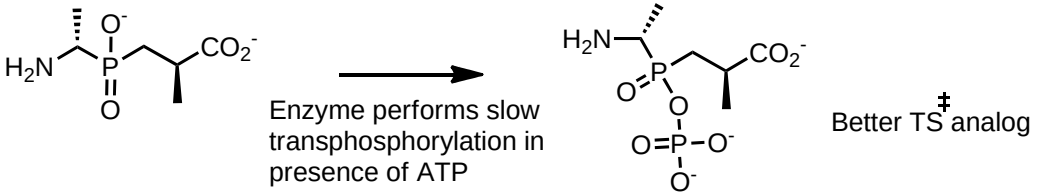
Design of an inhibitor of alanine ligase

-inhibition is dependent on presence of ATP

- off rate $t_{1/2}$ ~17 days

- $k_{off}^{INH} << k_{off}^{PDT}$, 10^8-10^9 slower off rate

-example of slow binding/slow off rate inhibitor



Previously discussed antibiotic mechanisms targeting D-Ala D-Ala

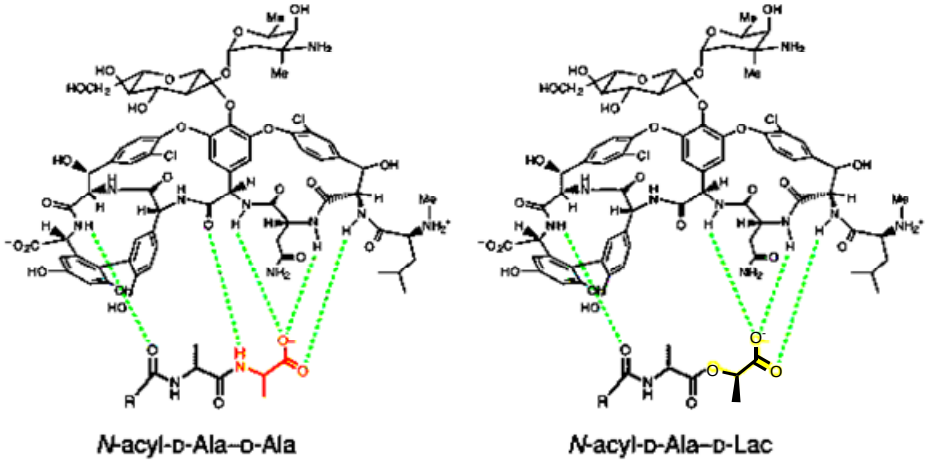
-transpeptidase inhibitor

-inhibitor of alanine racemase

-inhition of D-Ala ligase

Vancomycin-

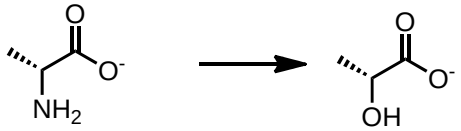
-a natural product 'receptor' which specifically binds to D-Ala-D-Ala via a network of H-bonding and blocks transpeptidase



Vancomycin Resistance

-bacteria use lactic acid instead of D-Ala

-ester linkage is much less favorable, repulsive interactions between oxygen lone pairs, loss of H-bond

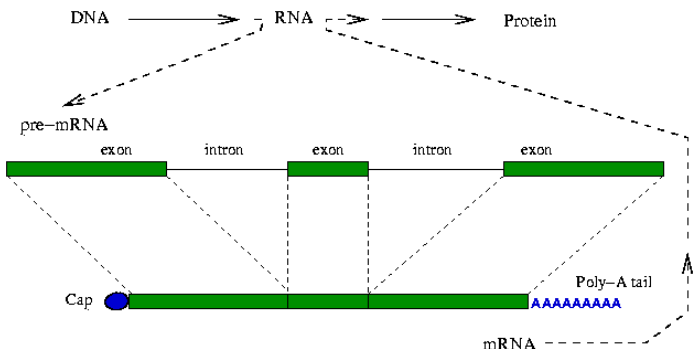


Loss of H-bond and introduction of unfavorable interaction

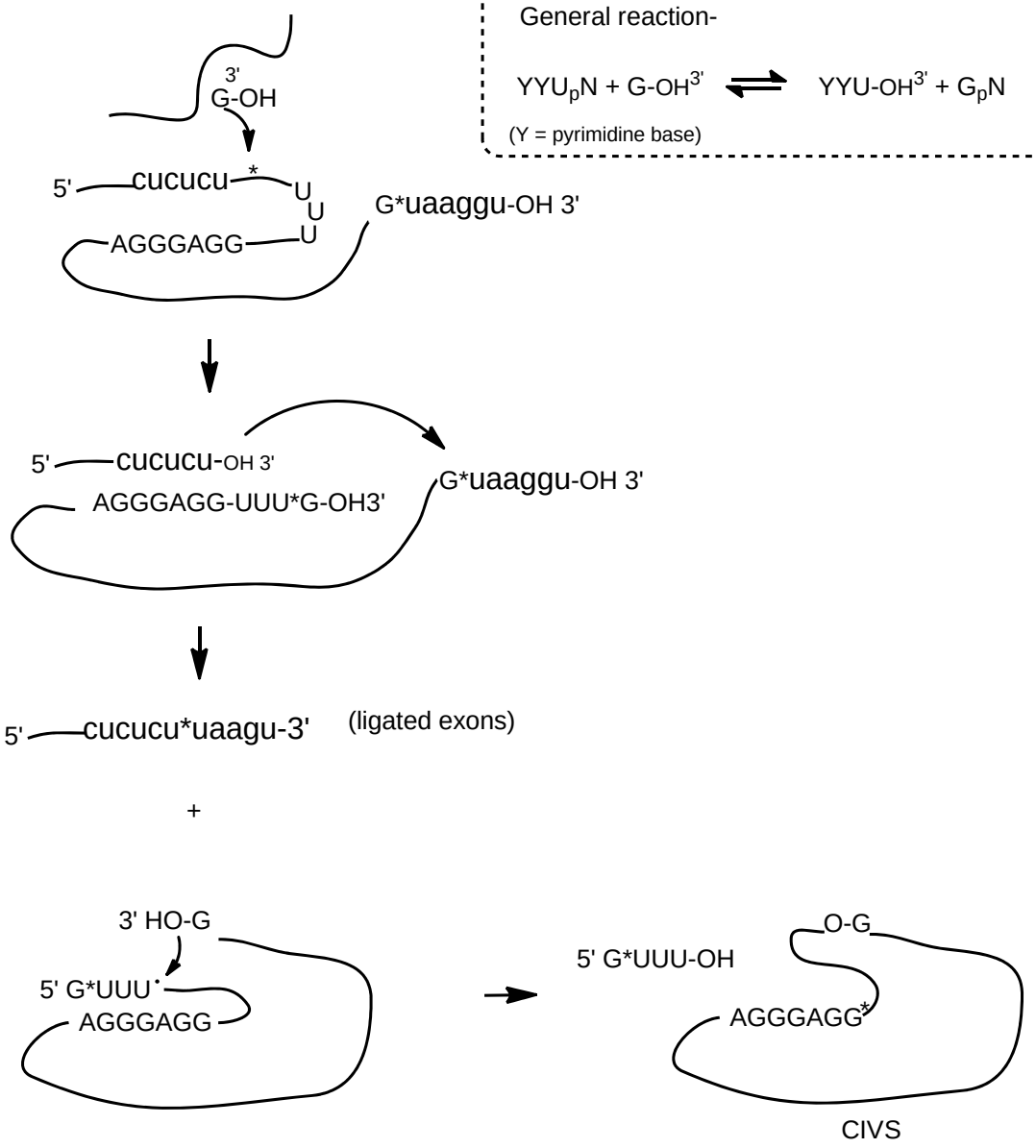
RNA catalysts

-RNA can carry out catalysis
-most often dependent on presence of Magnesium

Intron splicing



Self splicing RNA in tetrahymena

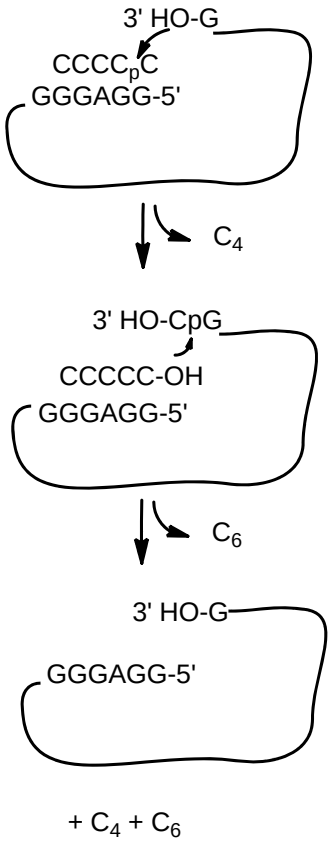


General reaction-

$YYU_pN + G\text{-}OH^{3'} \rightleftharpoons YYU\text{-}OH^{3'} + G_pN$

(Y = pyrimidine base)

Catalytic RNA
-in order to be catalytic, the catalyst molecule must remain unchanged at the end of the reaction

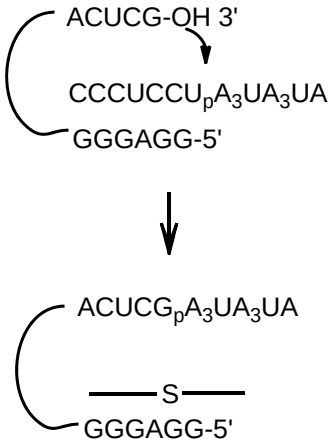


+ C_4 + C_6

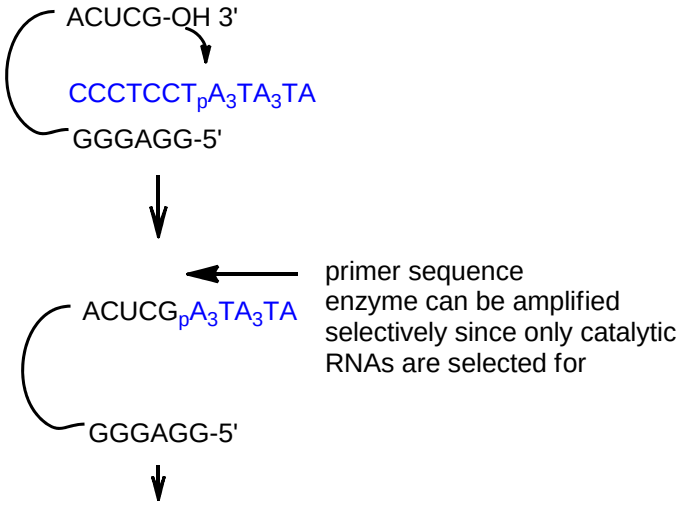
(catalytic RNA unchanged)

(catalyzes disproportionation reaction)

RNA ligase-



RNA which ligates to DNA substrate-

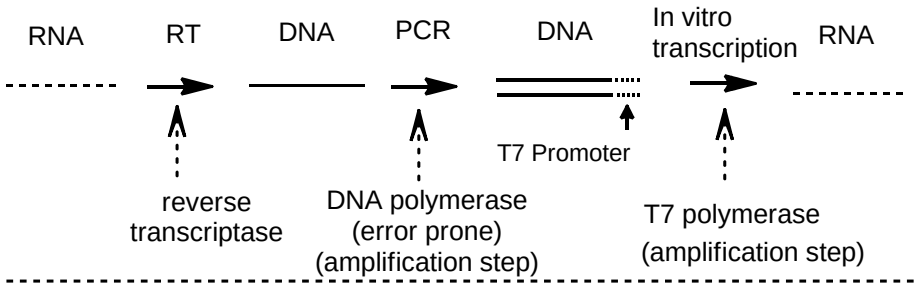


primer sequence enzyme can be amplified selectively since only catalytic RNAs are selected for

Amplification of starting RNA with RT rxn, PCR, and in vitro transcption with T7 polymerase. Sequence diversity generated by error prone PCR conditions or using an error prone DNA polymerase

RNAs with increased catalytic activities

Starting RNA	k_{cat} - $2x10^{-4}$ min^{-1}	Km ~7μM
After selection	k_{cat} - 0.007 min^{-1}	Km ~2μM



An RNA ligase selected from randomized sequence

-randomize 220 bases
-library will be incomplete (4220 potential members possible); must assume many potential solutions

Using this strategy-
an RNA enzyme was discovered with a $t_{1/2}$ of 5 minutes vs. 33 years compared to the uncatalyzed reaction (10^7 rate acceleration)