Penicillin antibiotics are inactivated by beta-lactamase’s ability to perform a fast deacylation of the inhibitor-enzyme adduct.

Inhibitors of beta-lactamases:

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

\[
\begin{align*}
\text{NH}_2 & + \text{PO}_4^3- \rightarrow \text{AMP} \\
\text{H}_2\text{N} & \rightarrow \text{PO}_4^3-
\end{align*}
\]

Design of an inhibitor of alanine ligase
- inhibition is dependent on presence of ATP
- off rate \( t_{1/2} \sim 17 \text{ days} \)
- \( k_{\text{off}}^{\text{NH}} << k_{\text{off}}^{\text{PDT}} \), \( 10^8 - 10^9 \) slower off rate
- example of slow binding/slow off rate inhibitor

Enzyme performs slow transphosphorylation in presence of ATP

Better TS analog

Previously discussed antibiotic mechanisms targeting D-Ala D-Ala
- transpeptidase inhibitor
- inhibitor of alanine racemase
- inhibition 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

\[
\begin{align*}
\text{NH}_2 & \rightarrow \text{OH} \\
\text{D-Ala} & \rightarrow \text{Lactic acid}
\end{align*}
\]

Loss of H-bond and introduction of unfavorable interaction
RNA catalysts

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

Self splicing RNA in tetrahymena

General reaction:

\[
YYU_pN + G\cdot\cdot\cdotOH^3 \rightleftharpoons YYU\cdot\cdot\cdotOH^3 + G_pN
\]

(Y = pyrimidine base)

Intron splicing

DNA → RNA → Protein

pre-mRNA

| intron | exon | intron | exon | exon | exon |

Cap → mRNA → Poly-A tail

AGGGAGG

G*uaaggu-OH 3’

5’

G*UUU-OH 3’

AGGGAGG

G*uaaggu-3’

5’

AGGGAGG-UUU*G-OH3’

5’

G*uaaggu-OH 3’

5’cucucu*uaagu-3’ (ligated exons)

3’

HO-G

5’ G*UUU-AGGGAGG

O-G

5’ G*UUU-OH

AGGGAGG

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

RNA ligase-
-ACUCG-OH 3'

<table>
<thead>
<tr>
<th></th>
<th>3' HO-G</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCCCCC-OH</td>
<td>C6</td>
<td></td>
</tr>
<tr>
<td>GGGAGG-5'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ C4 + C6

(catalytic RNA unchanged)

RNA which ligates to DNA substrate-

ACUCG-OH 3'

<table>
<thead>
<tr>
<th></th>
<th>3' HO-CpG</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCCCUCC UA3UA3UA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGGAGG-5'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S

GGGAGG-5'

(catalytic RNA unchanged)

(reacts with DNA substrate)

Amplification of starting RNA with RT rxn, PCR, and in vitro transcription 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} \approx 2 \times 10^{-4} \text{ min}^{-1}

K_m \approx 7 \mu\text{M}

After selection

k_{cat} \approx 0.007 \text{ min}^{-1}

K_m \approx 2 \mu\text{M}

**Diagram:**

RNA → RT → DNA → PCR → DNA → In vitro transcription → RNA

RNA

reverse transcriptase

DNA polymerase (error prone)

T7 Promoter

T7 polymerase (amplification step)
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)

Using this strategy-
- Incubate with substrate oligo
- Wash off salt and unused substrate oligo, Elute pool from agarose column
- Pass pool through oligo affinity column
- Elute from affinity column, Reverse transcribe
- PCR amplify with selective primer
- PCR amplify with nonselective primer