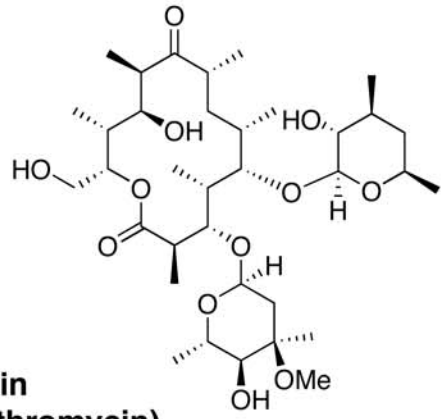
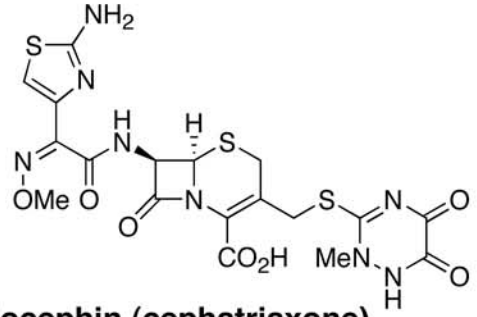
5.451 F2005
Natural Product Biosynthesis

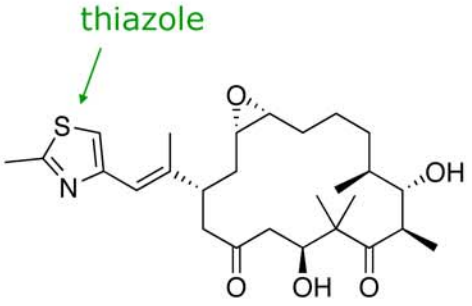
Examples of pharmacologically important natural products



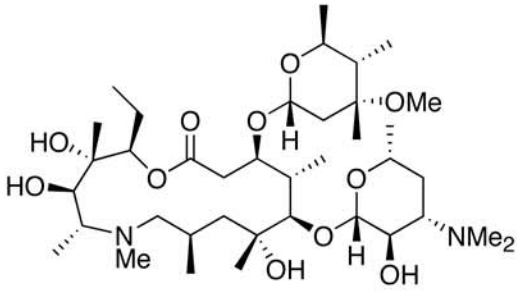
Biaxin
(Erythromycin)
Polyketide (acetate)



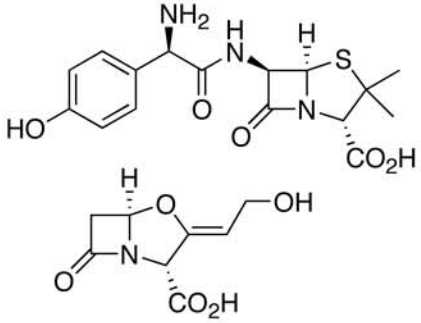
Rocephin (cephatriaxone)
peptide (amino acids)



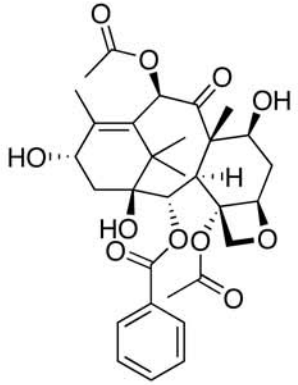
Epothilone
polyketide/peptide



Zithromax (Azomycin)
polyketide

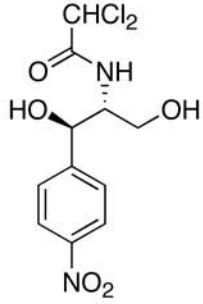


Augmentin
peptide



10-deacetylbaccatin III
terpene (isoprene)

taxol



Chloramphenicol
peptide

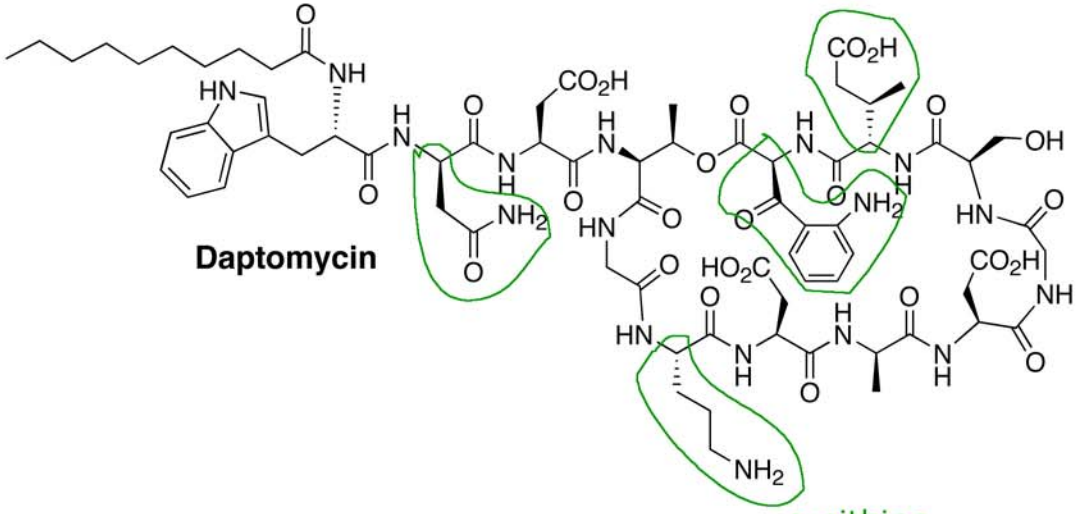
Relevance of natural products in the pharmaceutical industry (see Table 2 in Butler J. Nat. Prod. 2004, 67, 2141-2153.))

Table removed due to copyright reasons.

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides

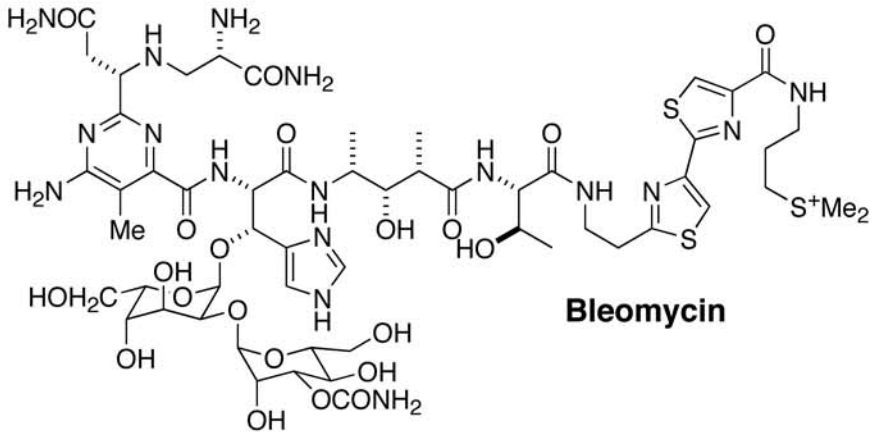
Dewick: pp. 421-454
Chem. Rev. 2005, 105, 715-738.

- **small (10-20 residues)** (ribosomal much longer)
- **unusual amino acids**
- **stereochemistry D (R) and L (S)**
- **cyclic**

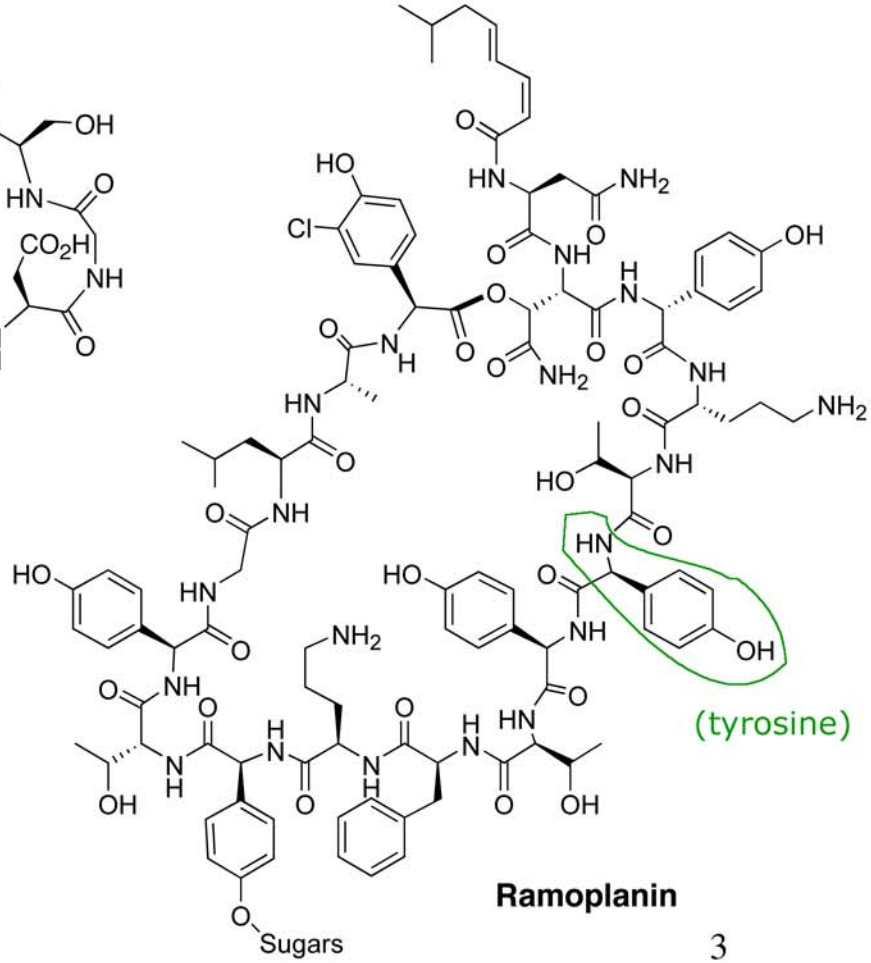


Daptomycin

ornithine



Bleomycin



Ramoplanin

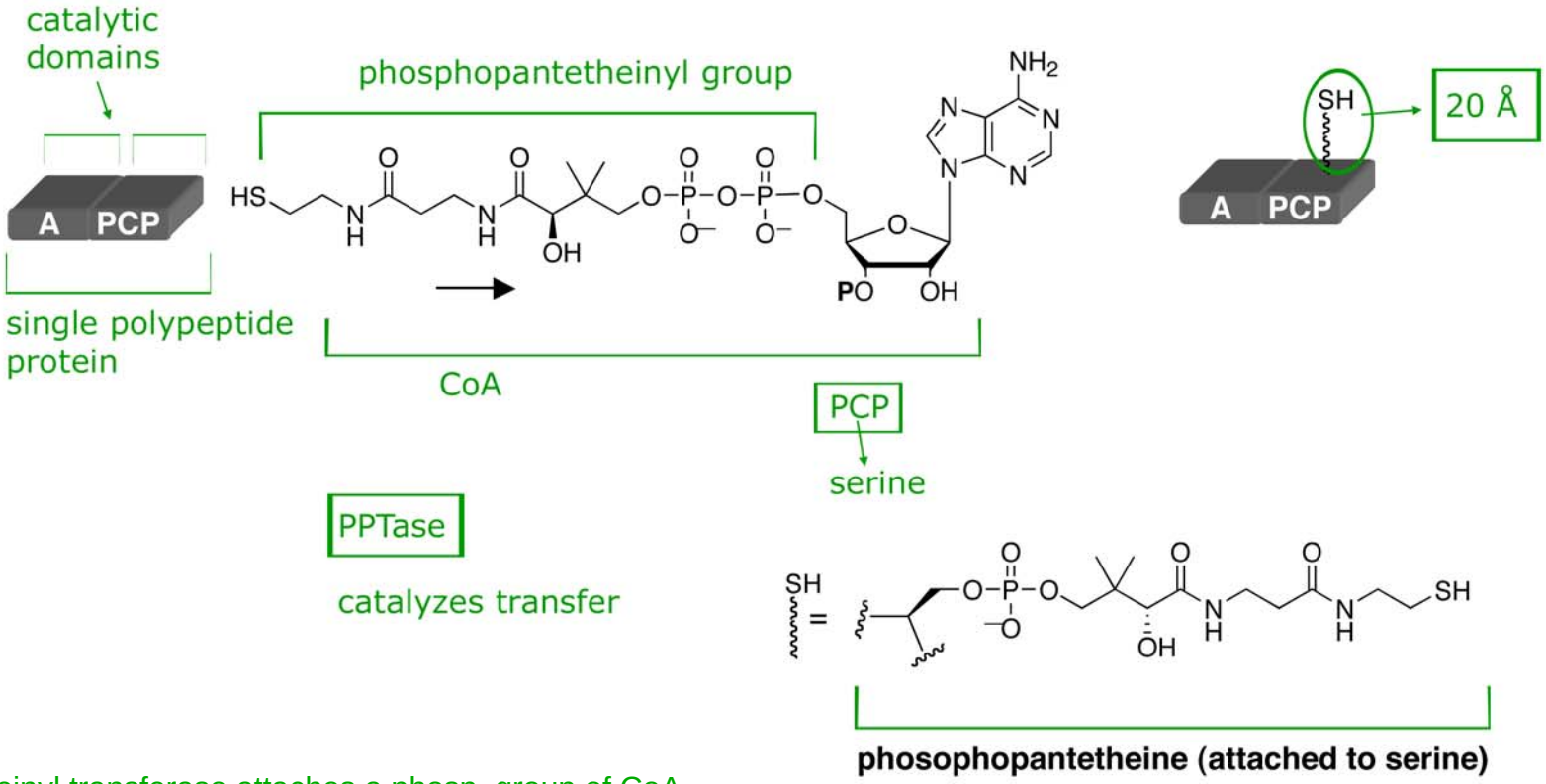
(tyrosine)

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides

PCP --> Peptidyl Carreir Protein
T --> thiolation domain

Catalyzed by Non-Ribosomal Synthetases
4 Major Steps:
Priming, Initiation, Elongation, Termination

Priming



* a phosphopantetheinyl transferase attaches a phosp. group of CoA to serine in PCP (or T)

* provides a long (20 Å) thiolate handle

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides

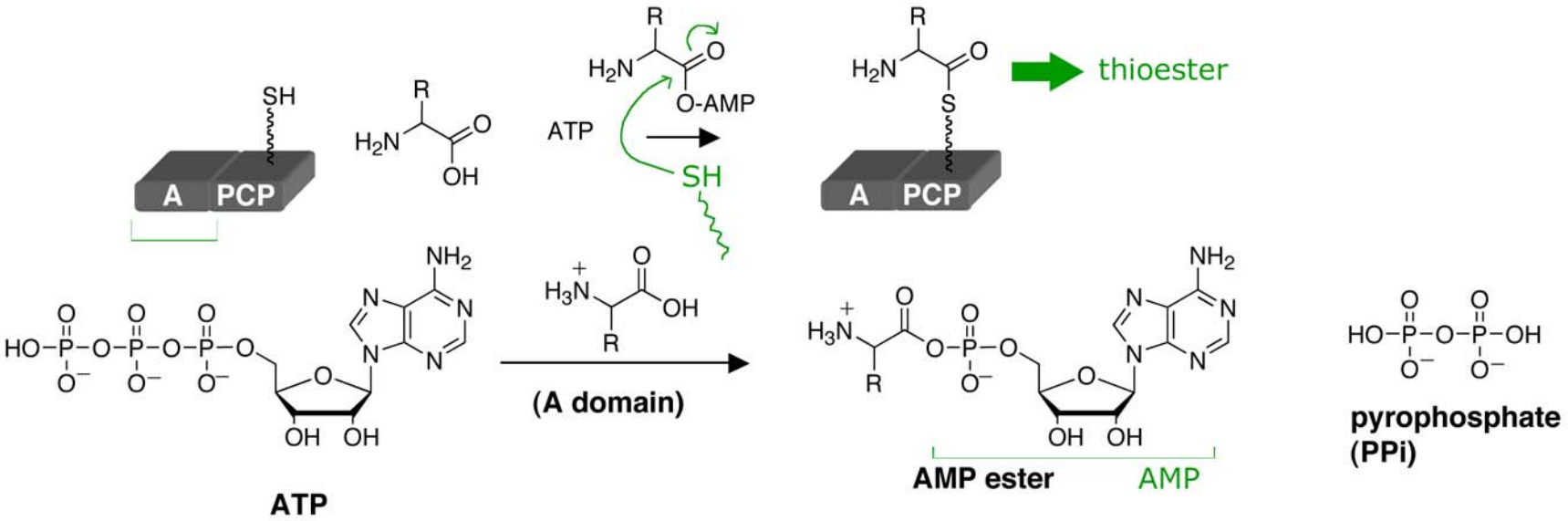
The specificity of the amino acid is encoded into the A domain protein sequence
Chem. Biol. (1999) 6, 493-505.
Chem. Biol. (2000) 7, 211-224.

Initiation - Installation of first amino acid.

An A (Adenylation) domain will activate the amino acid as the AMP ester (activating group).

Sequester it --> thiol arm can attach

Initiation



Free energy of hydrolysis (ΔG) of ATP to AMP + PPi = -32 kJ/mol

Nature uses AMP as activating group for amide bond formation

The A domain needs to pick out the AA in great specificity.

Adenylation domain is responsible for substrate specificity.

A domain kind of like a codon in Ribosomal system

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides

The specificity of the amino acid is encoded into the A domain protein sequence
Table 1 in Chem. Biol. (1999) 6, 493-505.
Chem. Biol. (2000) 7, 211-224.

Redesign efforts:
J. Comp. Biol.
2005, 12, 740-761.

1) "swap" A domains from another pathway

--> interferes w/ protein iinteractions found in remainder of pathway

2) point mutations. (SDM).

Phe --> Leu
Asp --> Asn
Glu --> Gln

*Phe A domain crystallized --> contacts w/substrate noted

10 protein aa's that made contect

*can predict which aa activated by substrate

Table removed due to copyright reasons.

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides

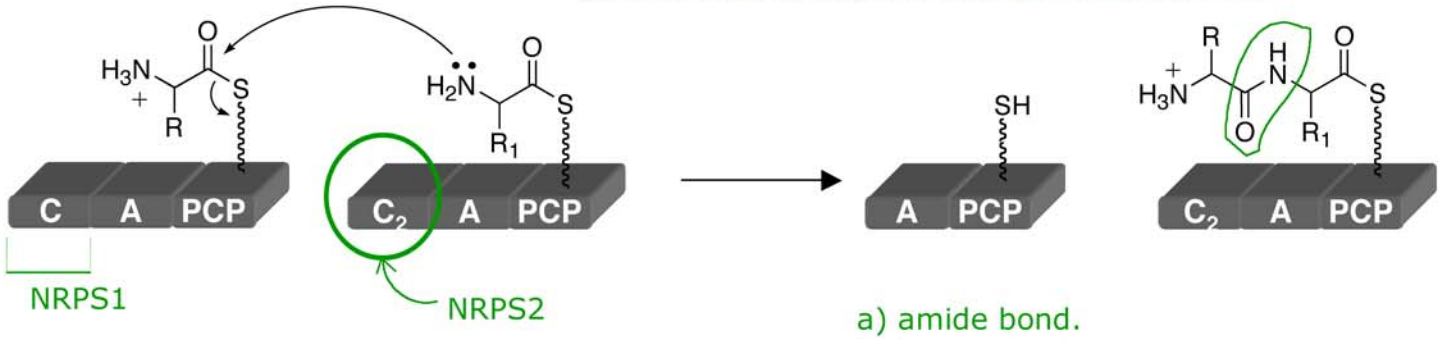
C = Condensation Domain. --> Amide Bond Formation.

Protein Sequence: His (His) Xxx Xxx Xxx Asp Gly

general base to deprotonate amino acid amine

3) **Elongation**

Amide Bond Formation



a) amide bond.

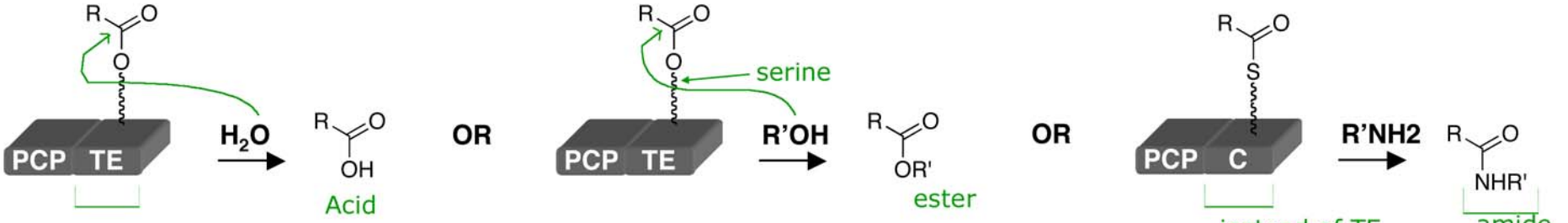
b) sequentially transfering peptide down chain of NRPS

Amide bond formation catalyzed by C (condensation domain)

4) **Termination**

TE = thioesterase.

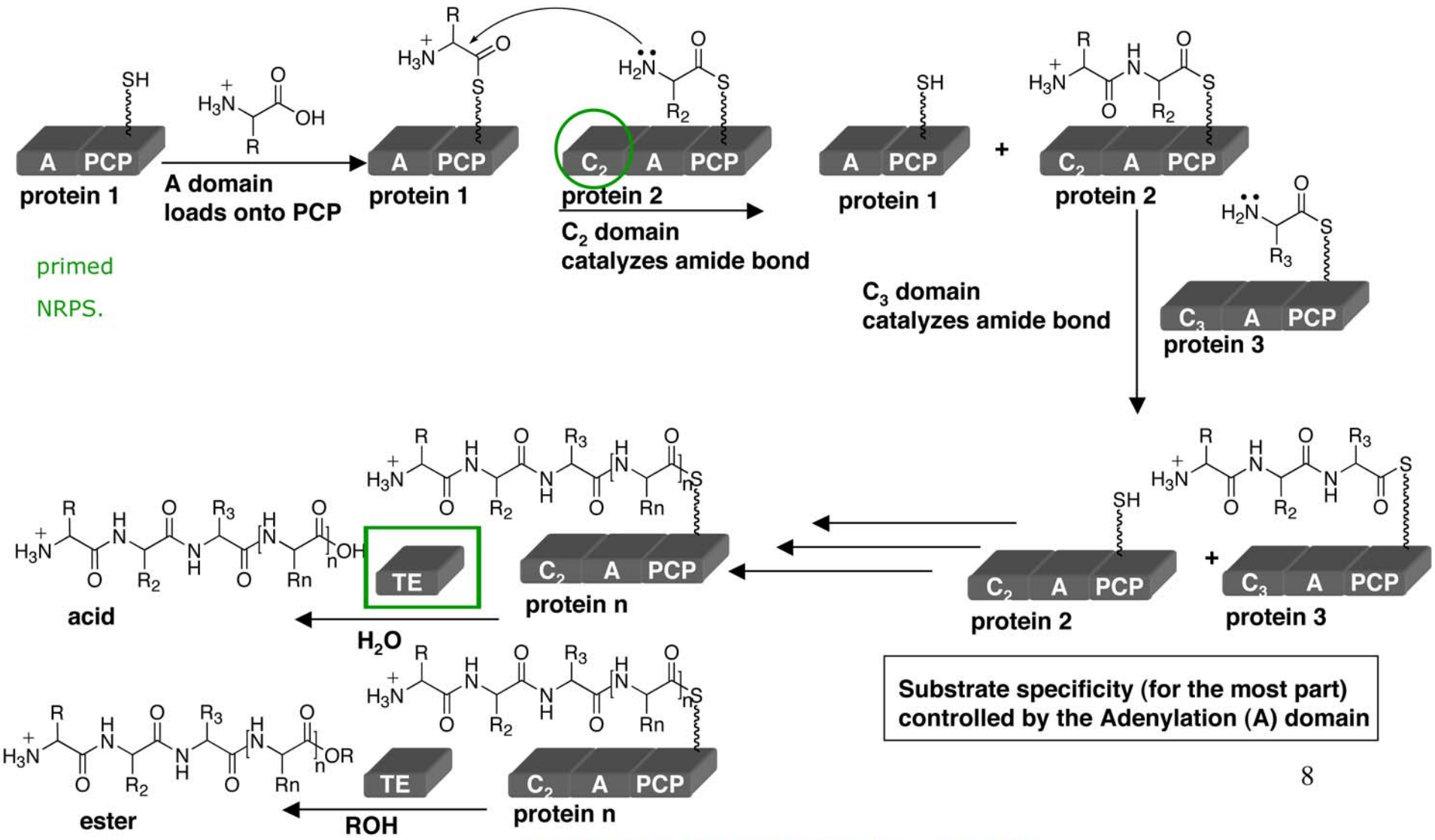
thioester linkage --> ester attached to a serine on the TE



Transfer to active site serine of thioesterase (TE) domain (thioester to ester chemistry)
Hydrolysis to form acid; or
Cyclization with hydroxyl to form cyclic ester (lactone); or
Termination can also be mediated by last C domain and amine to make amide (or lactam)

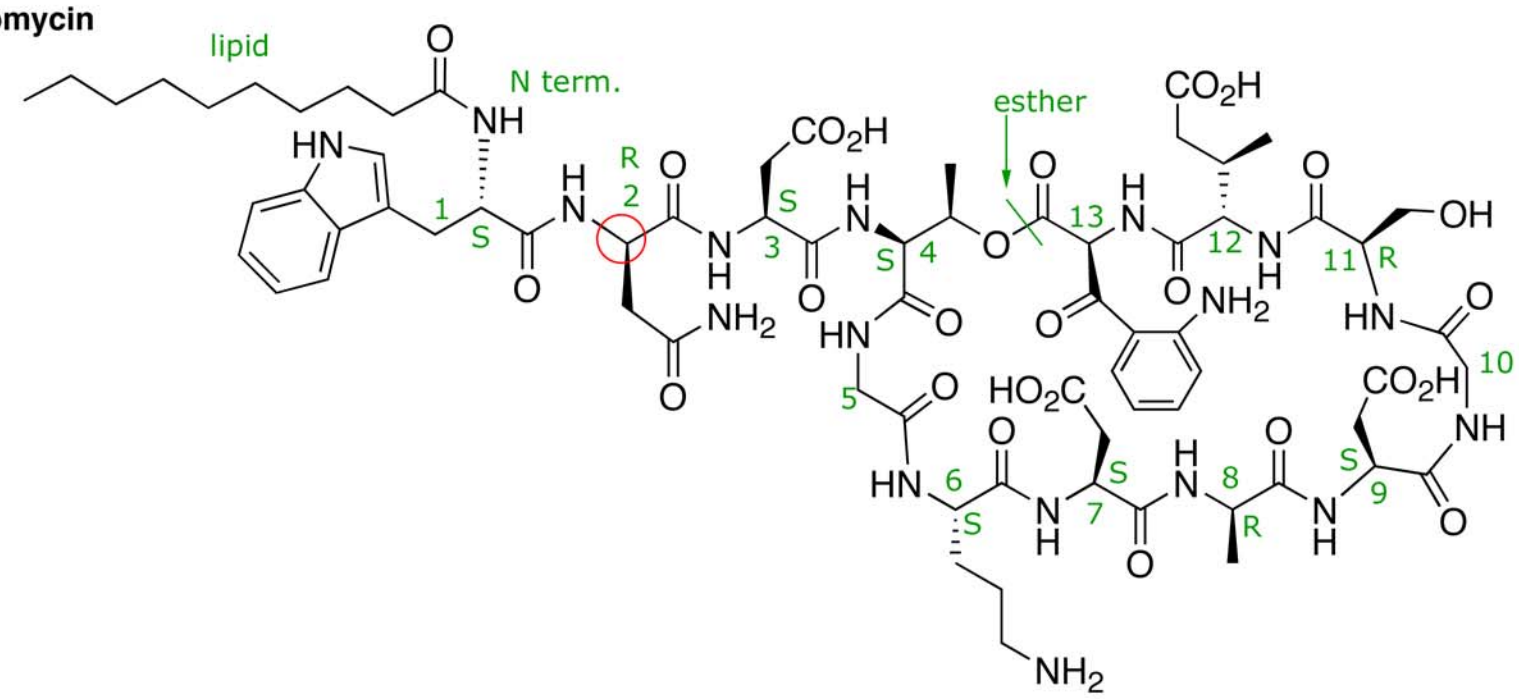
5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides

An "assembly line" that is synthesized by large enzymes with various catalytic domains (represented by a box)
Recent review: Science (2004) Vol 303, 1805-1810



5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptide Biosynthesis: Can a biosynthetic pathway be predicted from the structure???

Daptomycin



W	N	D	T	G	K	D	A	D
AT	CATE	CAT	CAT	CAT	CAT	CAT	CATE	CAT
1	2	3	4	5	6	7	8	9

G	S	"U"	"U"	
CAT	CATE	CAT	CAT	TE
10	11	12	13	

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptide Biosynthesis: Can a biosynthetic pathway be predicted from the structure???

1. Recognize the amino acid building blocks and trace out the amide-bond backbone chain.
2. The peptide natural product is most likely made by the nonribosomal peptide synthetases if:
• Its cyclic. Look for the point of cyclization-
may be an ester. may be an amide bond formed with an amine side chain (I.e. Lys)
• Its a small peptide (less than 20 residues)
• It has unusual amino acids (A domains can incorporate unusual amino acids) or D amino acids

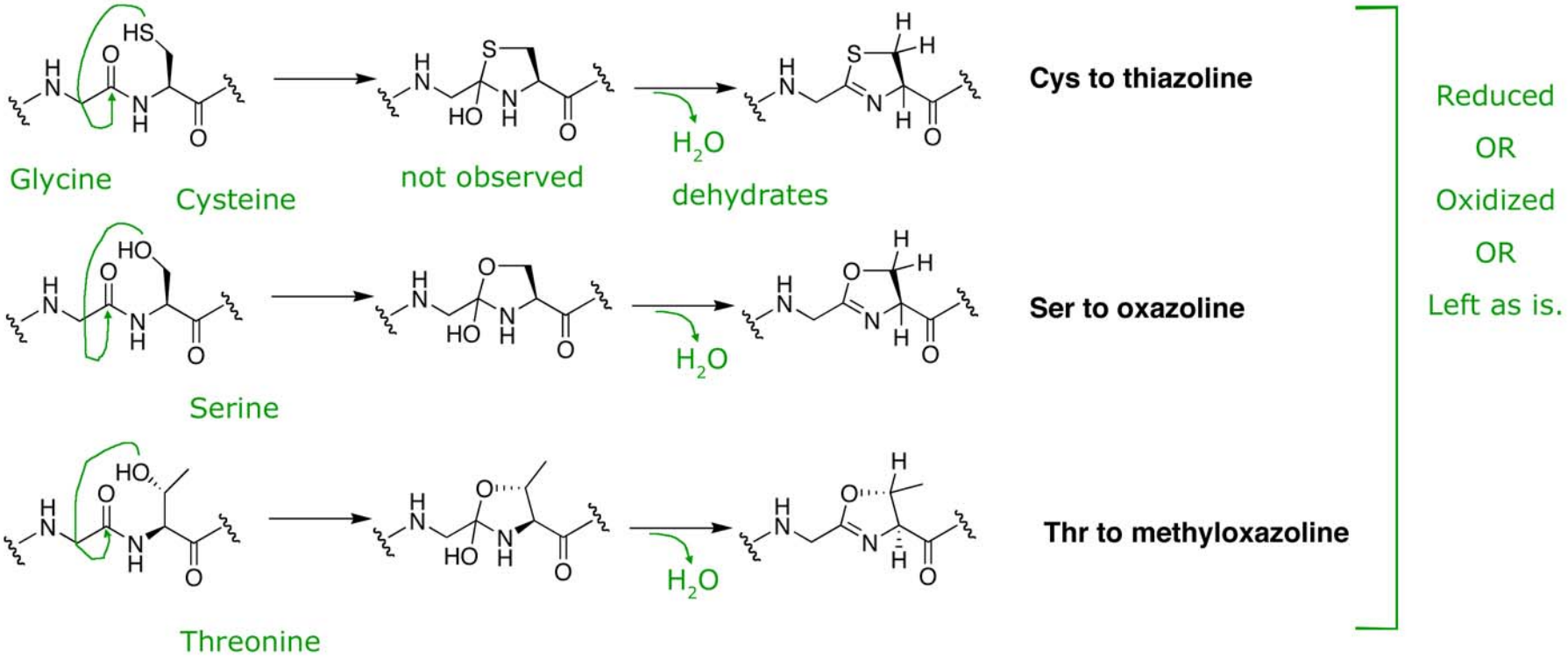
3. Identify the modifications to the basic peptide structure.
The most common modifications are:
Cyclization of cysteine, serine, threonine to thiazoline, oxazoline (Cy domain that replaces C domain)
Oxidation of thiazoline/oxazoline to thiazole/oxazole (Ox domain)
Reduction of thiazoline/oxazoline to thiazolidone/oxazolidone (Red domain)
N-methylation of amide amine(MT domain)
Epimerization of L amino acid to D amino acid(E domain)
Hydroxylation at the beta carbon position of the amino acid side chain (Fe containing Ox domain)

The protein domains that catalyze these reactions are typically adjacent to the C-A-PCP core domains that make the amide bond

Addition of lipids, fatty acids, sugars happen after the core of the peptide has been formed, and the peptide has been released from the peptide synthetase by the thioesterase (TE) domain.
We will discuss glycosylation later

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases
Catalytic Domains added into the primary amino acid sequence

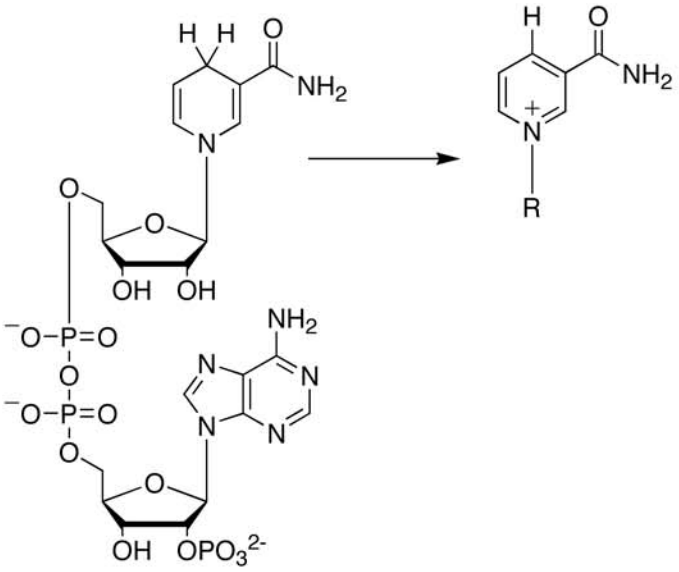
Cyclization (Cy) domain replace C domain (His-> Asp mutation) (no extra domain.)



mechanistic difference between C and Cy domain???

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases
Catalytic Domains added into the primary amino acid sequence

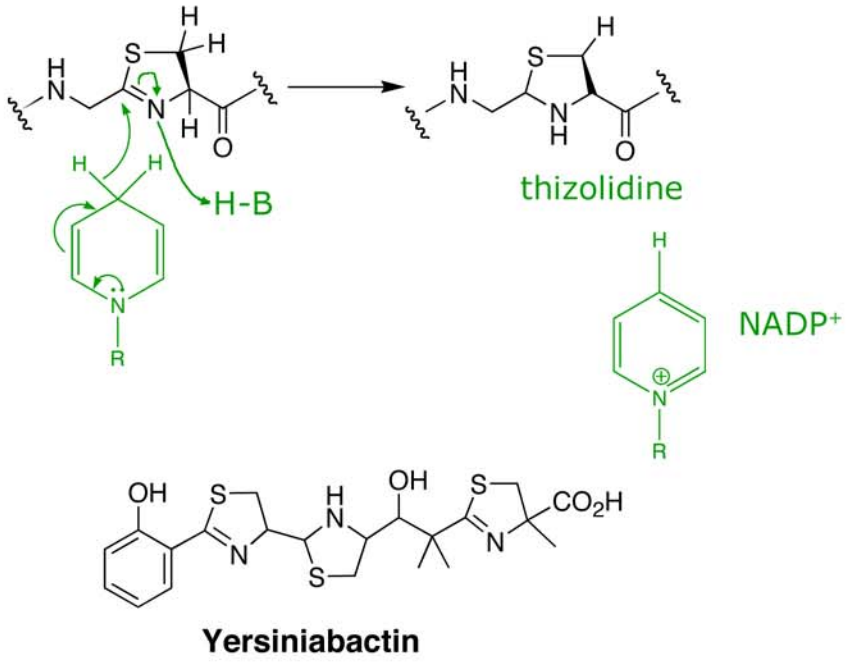
Reductase (Red) domain
utilizes NADPH cofactor to reduce
(J. Bact. (2001) *183*, 813.)



NADPH **NADP+**

hydride

($NaBH_4$, $NaCNBH_4$)



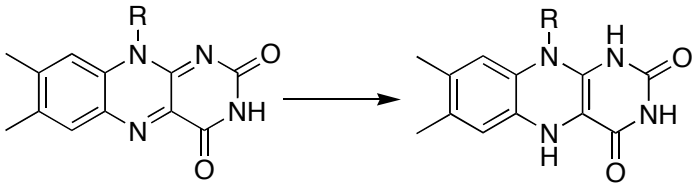
Yersiniabactin

thiazoline to thiazolidine (oxazoline to oxazolidine)
note: uses NADPH as reducing agent

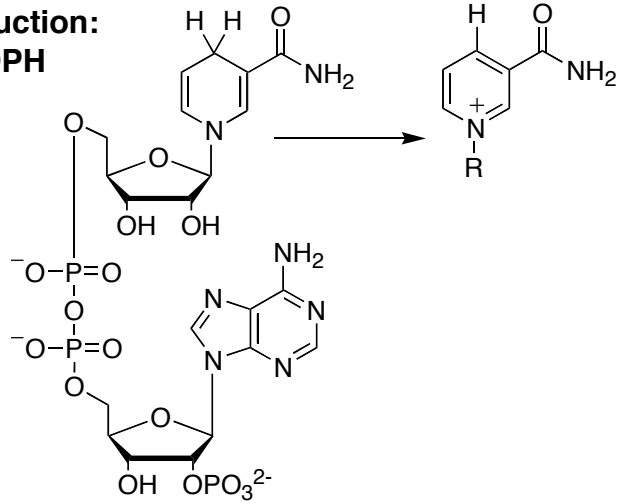
5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides

Small molecules that are attached to proteins that facilitate particularly difficult chemistry
Cofactors to recognize and the reactions they catalyze:

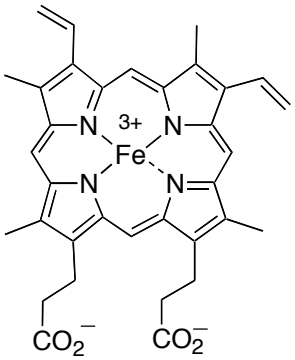
Oxidation:
flavin



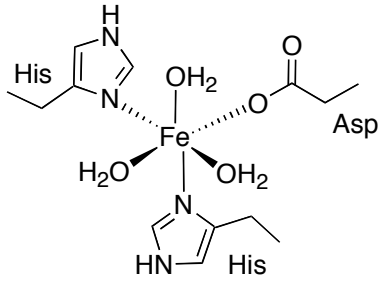
Reduction:
NADPH



Oxidation:
Iron, with the appropriate ligands

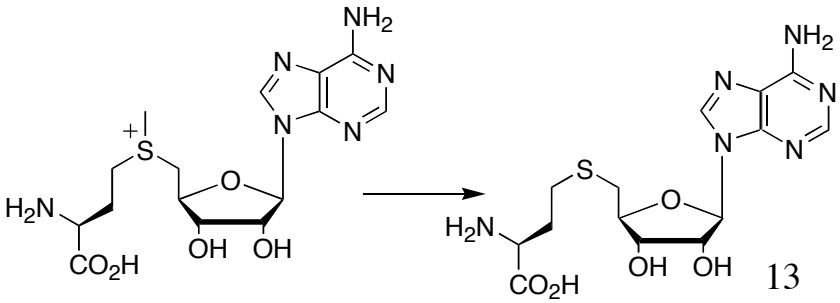


Heme



Non-Heme

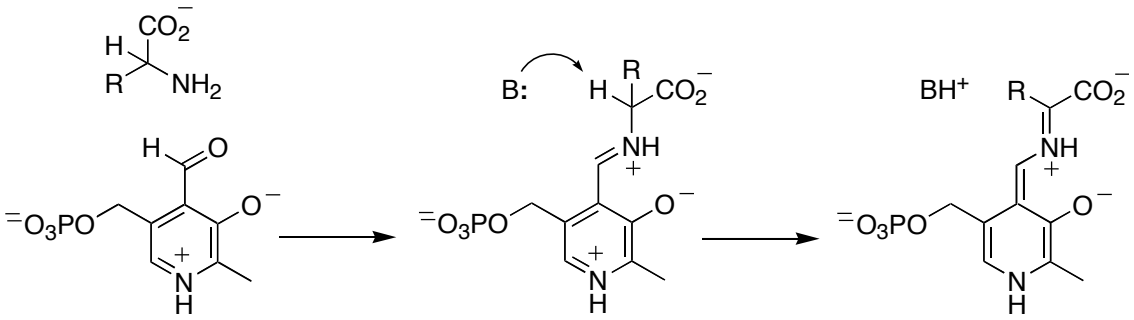
Methylation:
SAM



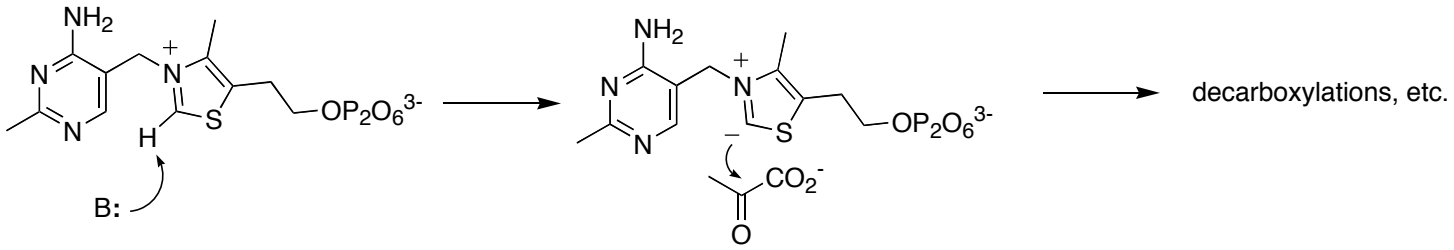
5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides

Small molecules that are attached to proteins that facilitate particularly difficult chemistry
Cofactors to recognize and the reactions they catalyze:
(not used in NRPS but see in other 2° metabolic pathways- will encounter later)

Transamination:
pyridoxal phosphate

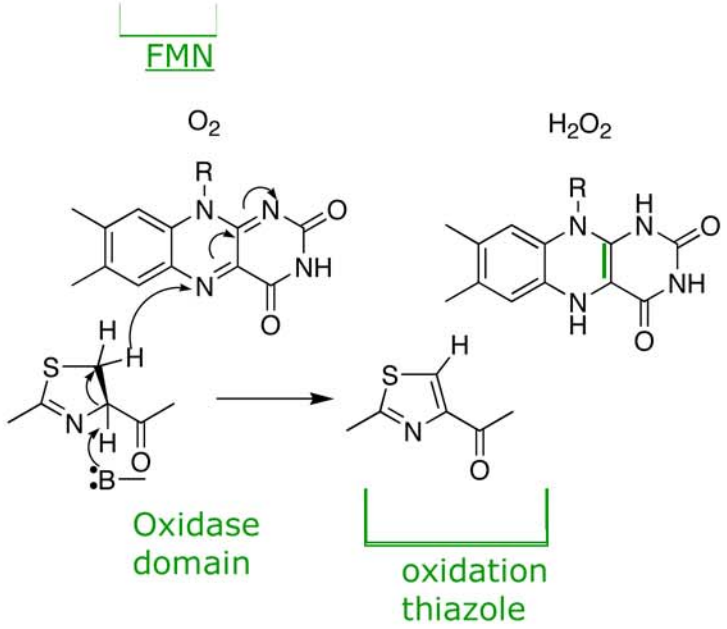


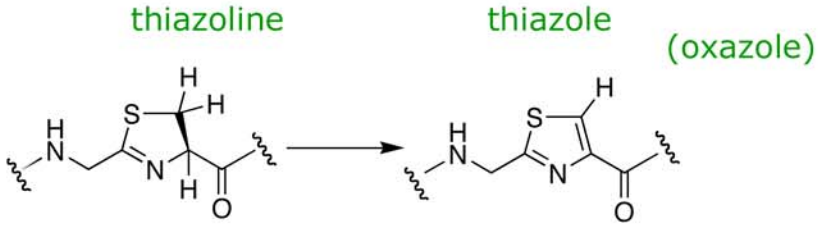
Carbanion for decarboxylations:
thiamine-PP (TPP)



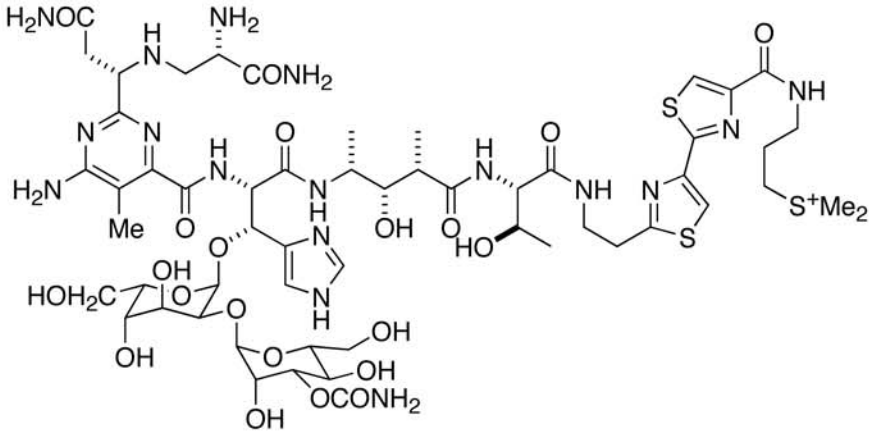
5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases
Catalytic Domains added into the primary amino acid sequence

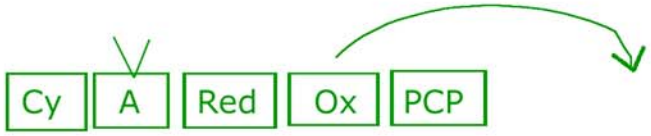
Oxidase (Ox)) domain
utilizes flavin cofactor to reduce





examples: bleomycin, epothilone

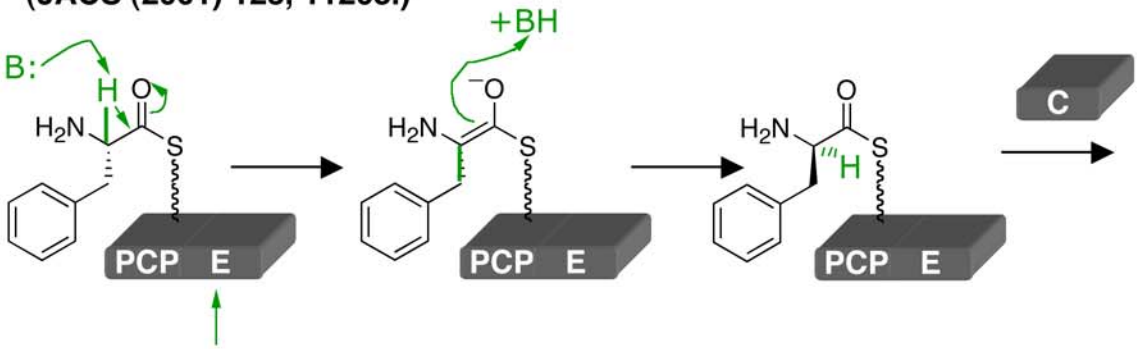


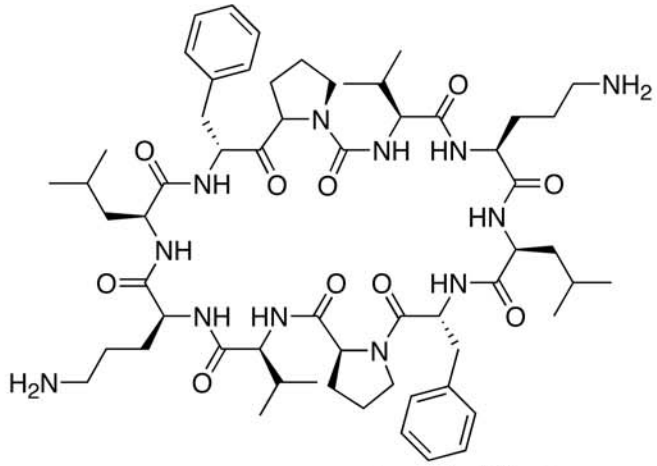


thiazoline to thiazole (oxazoline to oxazole)
note: uses FMN as oxidizing agent

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases
Catalytic Domains added into the primary amino acid sequence

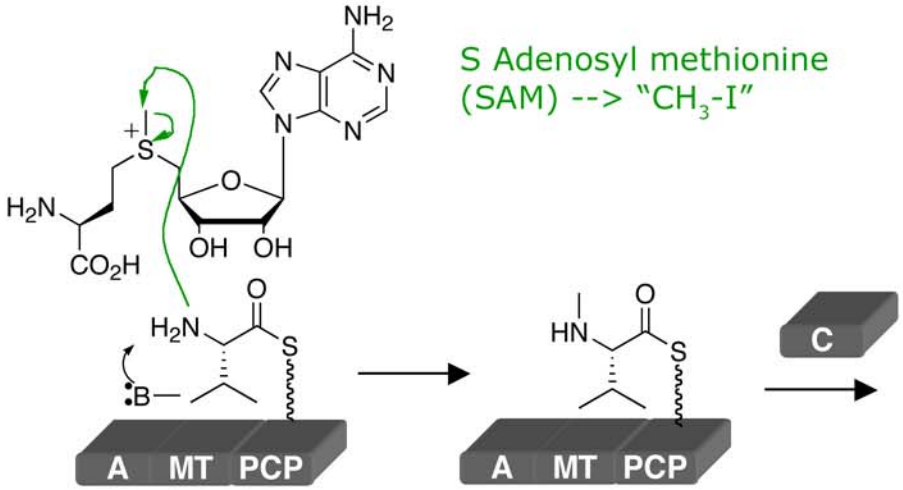
Epimerase (E) domain
(JACS (2001) 123, 11208.)

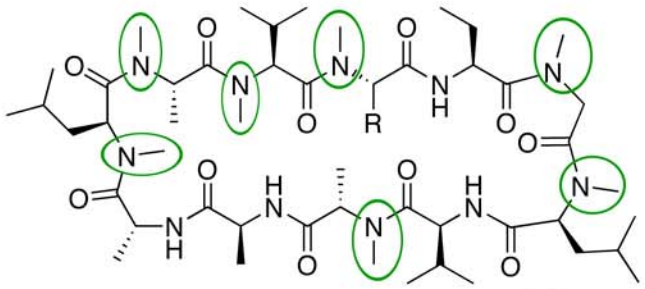




Gramicidin S

N Methyl transferase (MT) domain



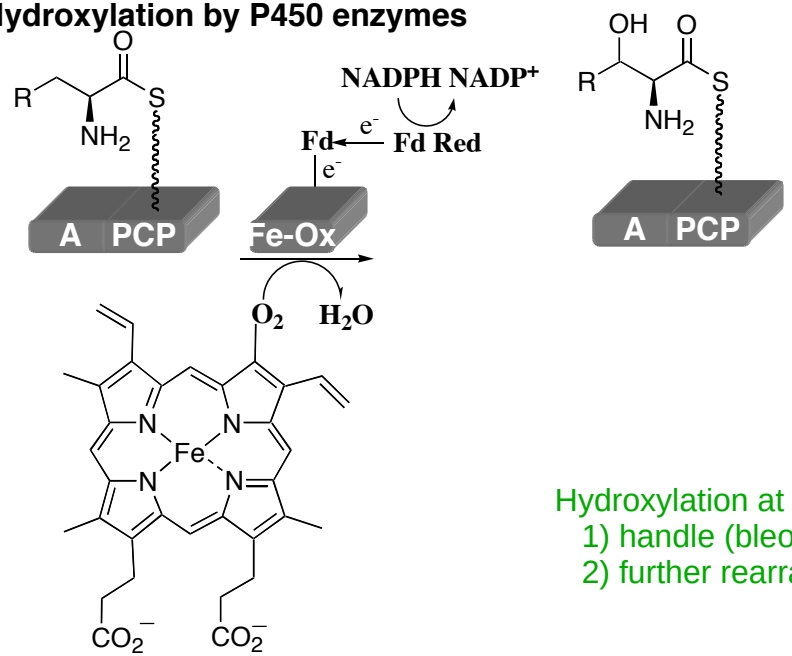


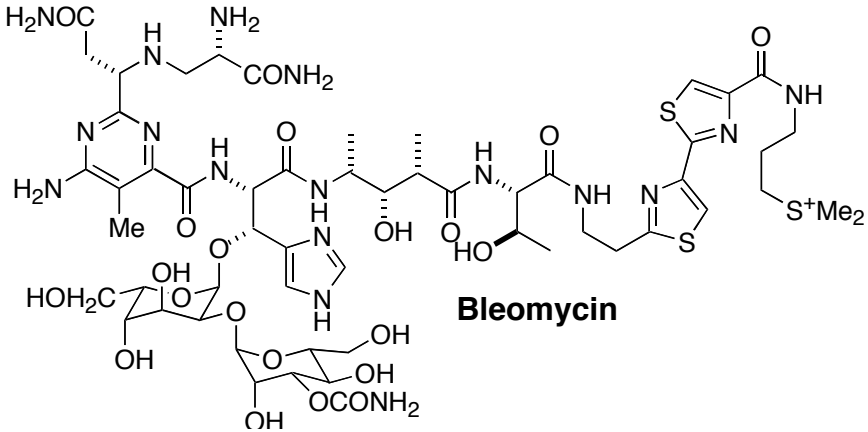
Cyclosporin A

mid 1950s isolated enzyme from human lines --> added CO, UV absorbance shifted to 450 --> hence P450

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases
Catalytic Domains added into the primary amino acid sequence

Hydroxylation by P450 enzymes

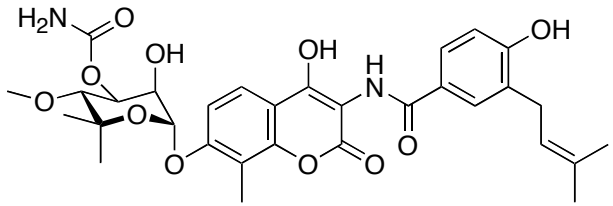




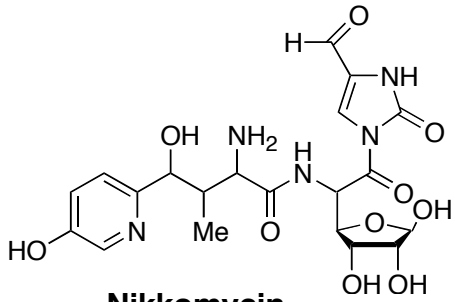
Bleomycin

Hydroxylation at beta carbon
1) handle (bleo)
2) further rearrangement --> novobiocin

Hijacking of the NRPS/hydroxylation machinery to make non-peptide structures
Using a thioester tethered system to make a dedicated pool of non-proteogenic amino acids



Novobiocin



Nikkomycin

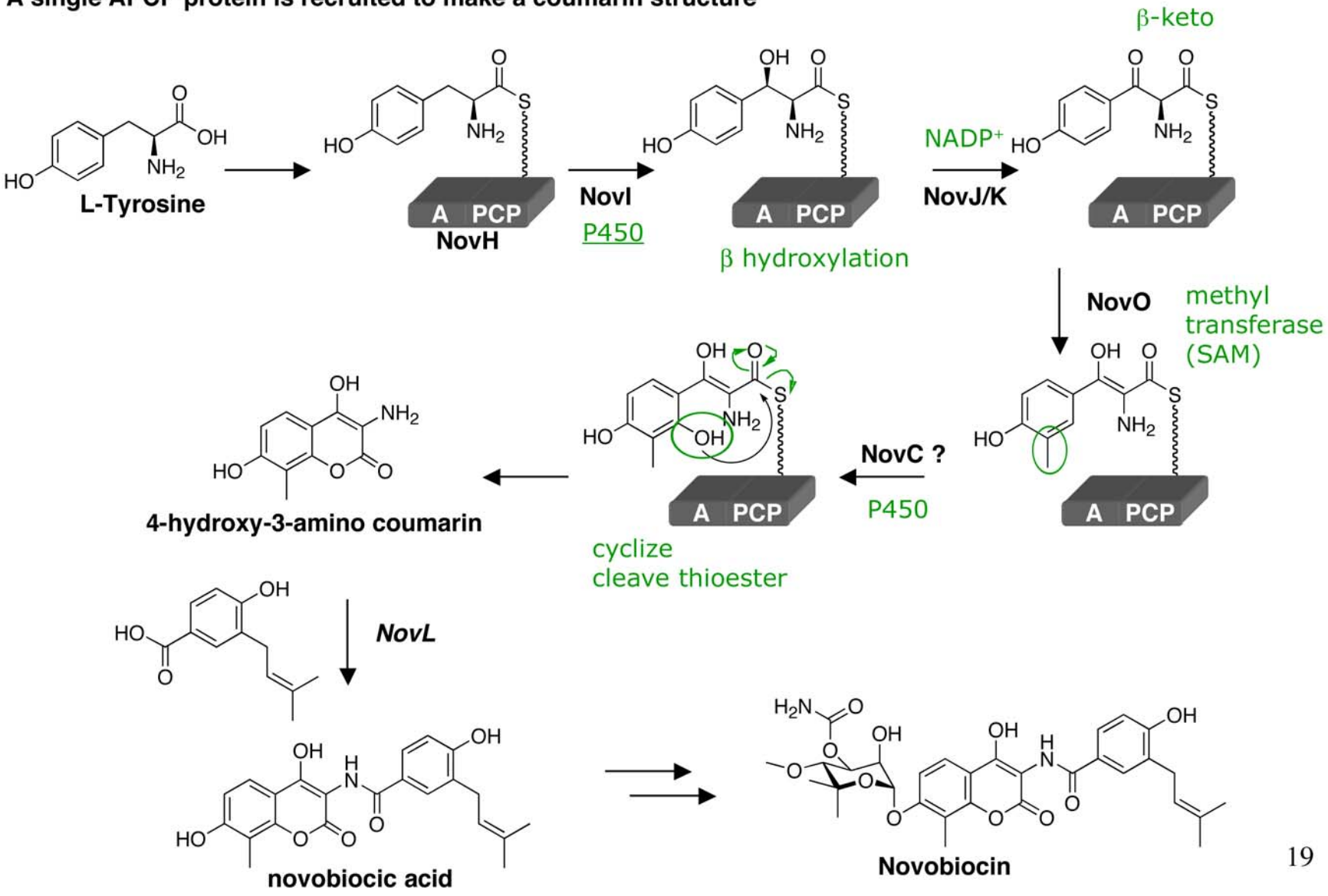
5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases
Catalytic Domains added into the primary amino acid sequence

Hydroxylation by P450 enzymes: Basic idea of how heme works

Figure removed due to copyright reasons.

Scheme 4.27. Silverman, The Organic Chemistry of Enzyme Catalyzed Reactions.

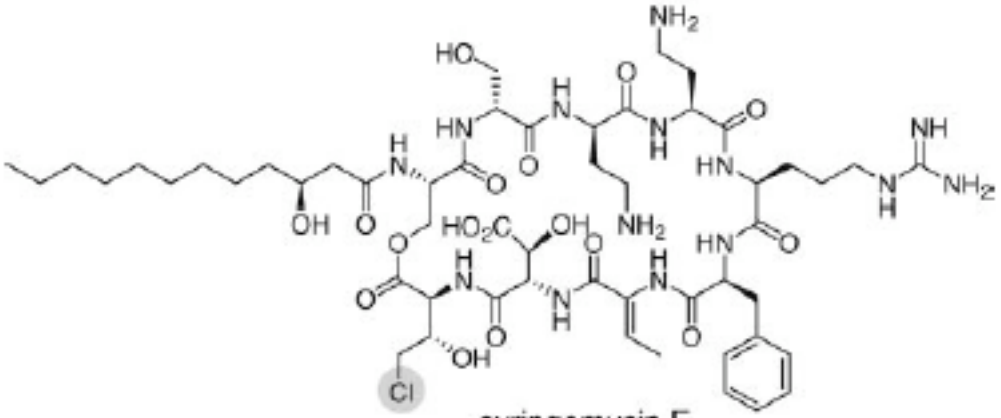
5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases
A single APCP protein is recruited to make a coumarin structure



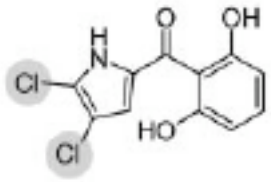
Chlorination most common
Bromination in marine based Nat. Products
Limited fluorinases

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides
Catalytic Domains added into the primary amino acid sequence

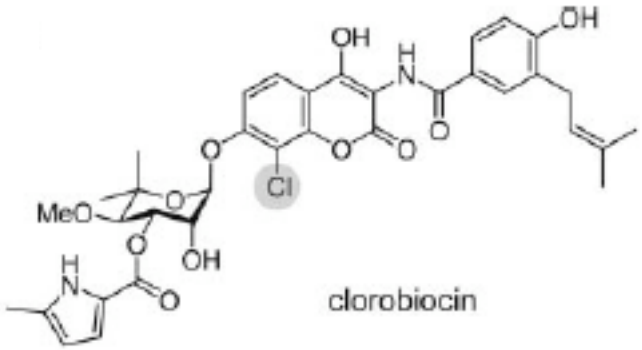
Halogenation (Chlorination)
PNAS, 2005, 102, 10111-10116



syringomycin E



pyoluteorin

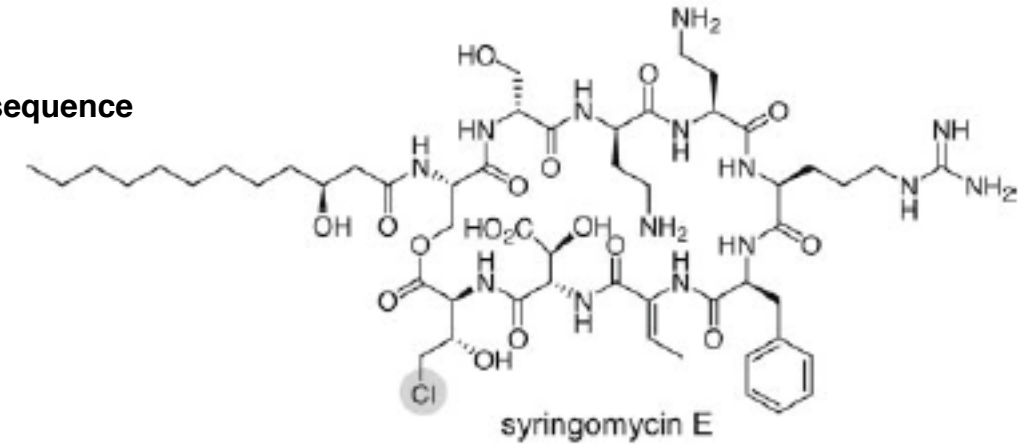


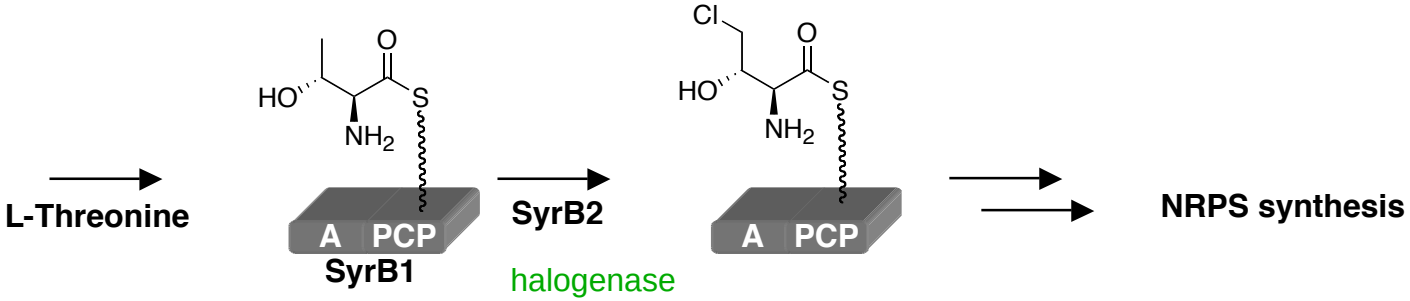
clorobiocin

Fluorinase
Nature (2002) 416, 279

5.451 F2005
Peptide Biosynthesis
Non-Ribosomal Peptides
Catalytic Domains added into the primary amino acid sequence

Halogenation (Chlorination)
PNAS, 2005, 102, 10111-10116





halogenase
iron containing protein
non heme iron

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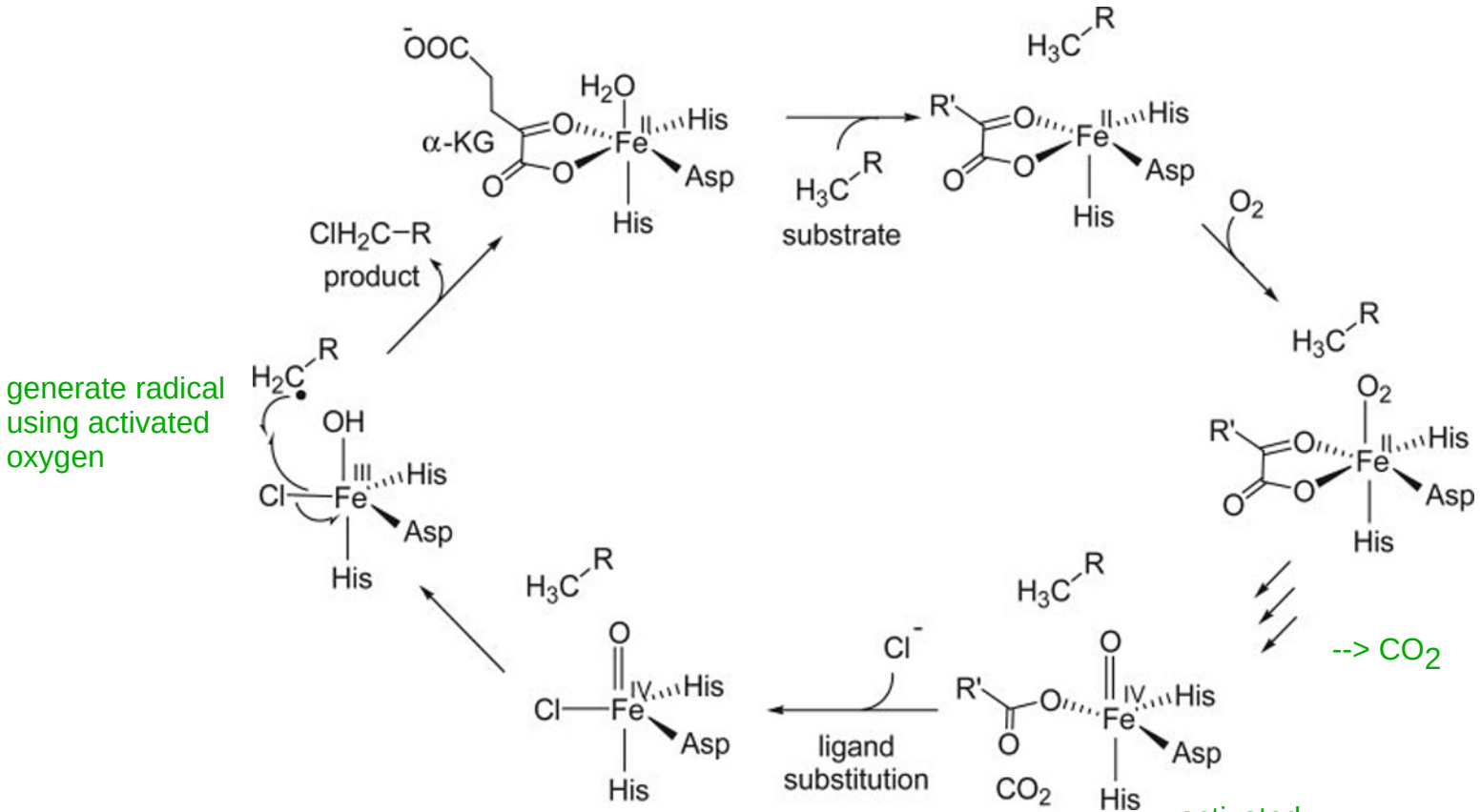
Peptide Biosynthesis
Non-Ribosomal Peptides
Catalytic Domains added into the primary amino acid sequence

Halogenation (Chlorination)
PNAS, 2005, 102, 10111-10116

cofactor
alpha keto glutarate



oxygenase --> hydroylates
oxidadase --> oxidation (not necessarily hydroxylation)

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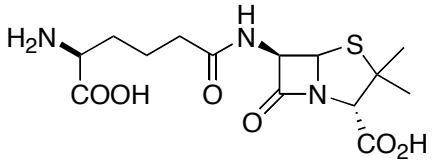
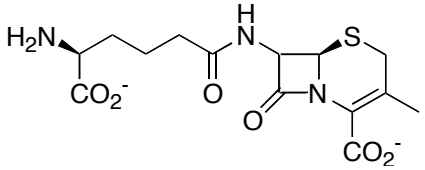
Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases

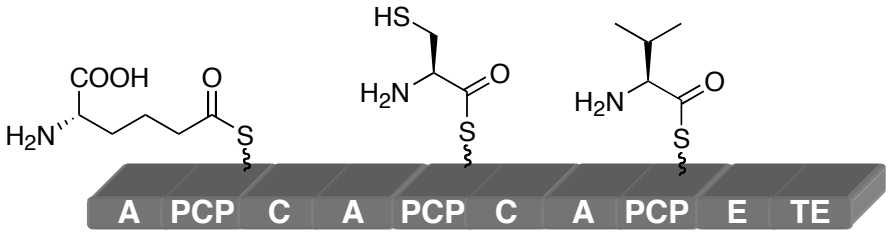
β-lactam

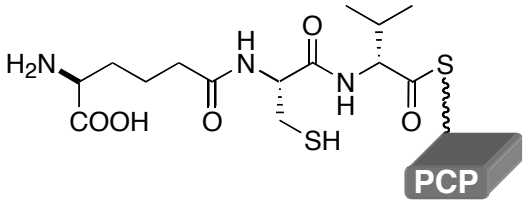


Isopenicillin N

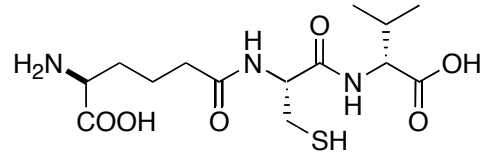


Deacetoxycephalosporin C

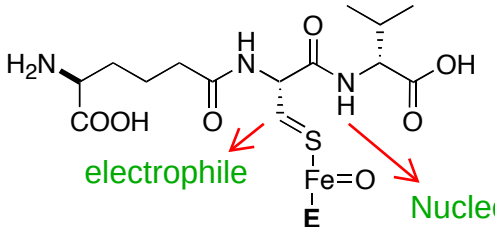


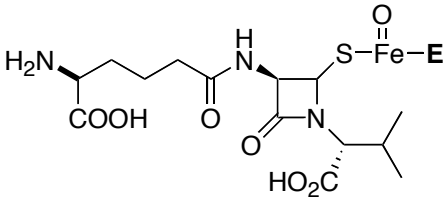


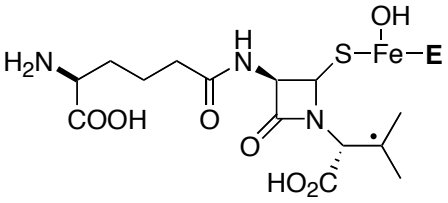
TE

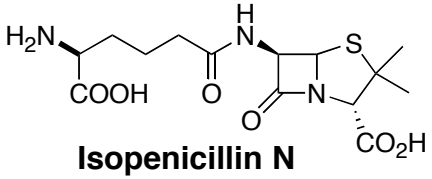


INS









Isopenicillin N

Nature (1998) *394*, 805.
Nature (1999) *401*, 721.

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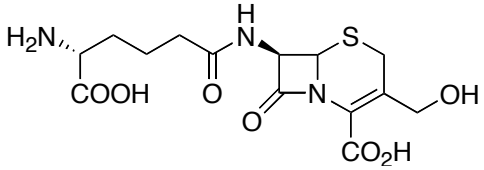
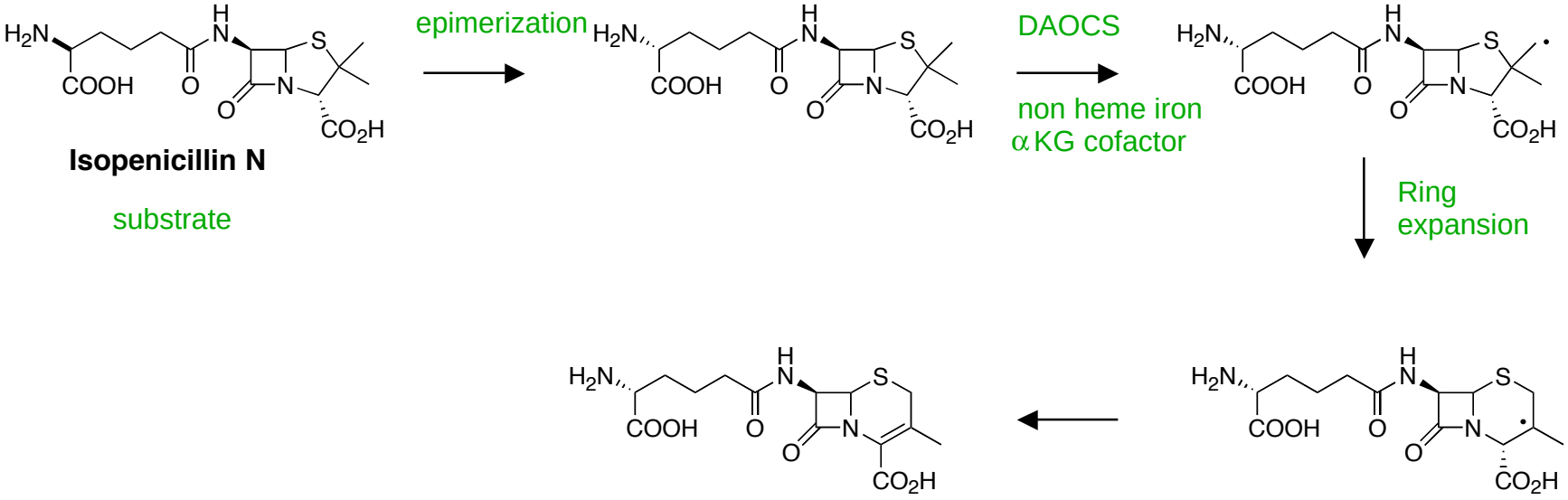
Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases



Deacetoxycephalosporin C



Isopenicillin N

substrate

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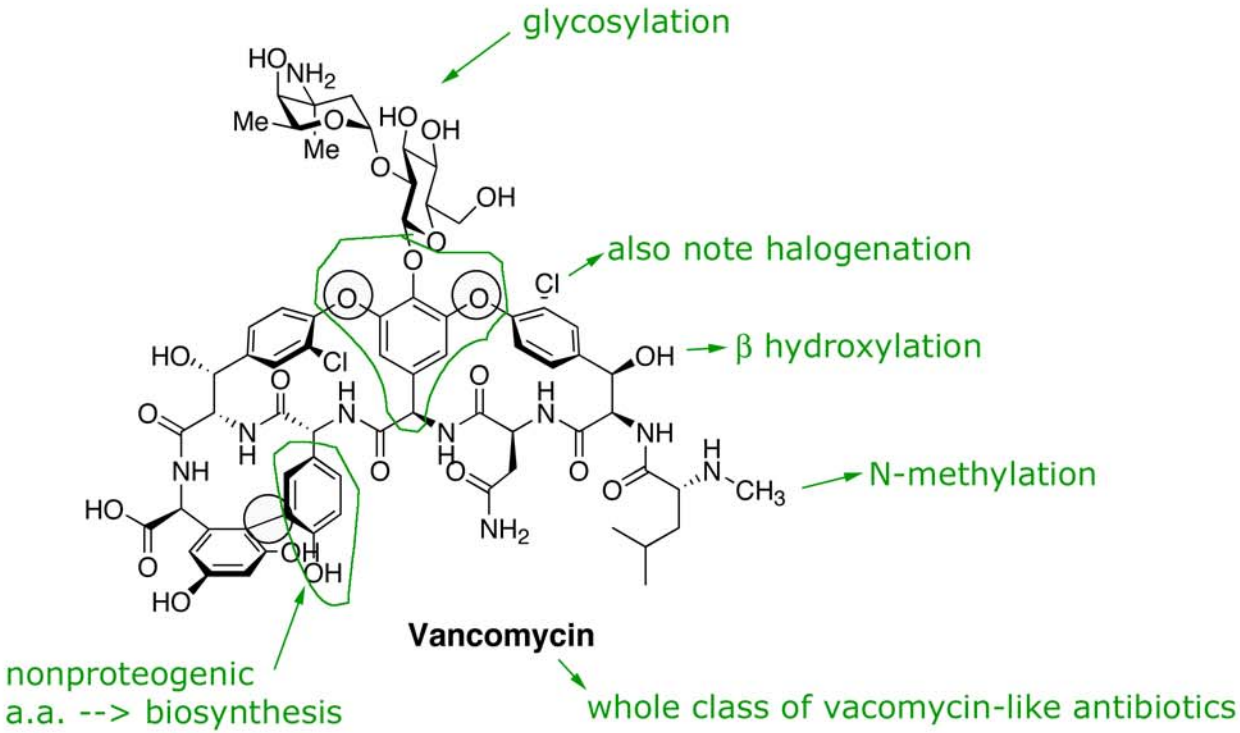
Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases



Vancomycin

P450 enzymes also catalyze C-O and C-C bond formation:
Oxidative Crosslinking

Angew. Chem. Intnl. Ed. (2003) *42*, 730.

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Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases

P450 enzymes also catalyze C-O and C-C bond formation:
Oxidative Crosslinking

Figure removed due to copyright reasons.

See Scheme 24 in Angew. Chem. Intnl. Ed. (2003) *42*, 730.

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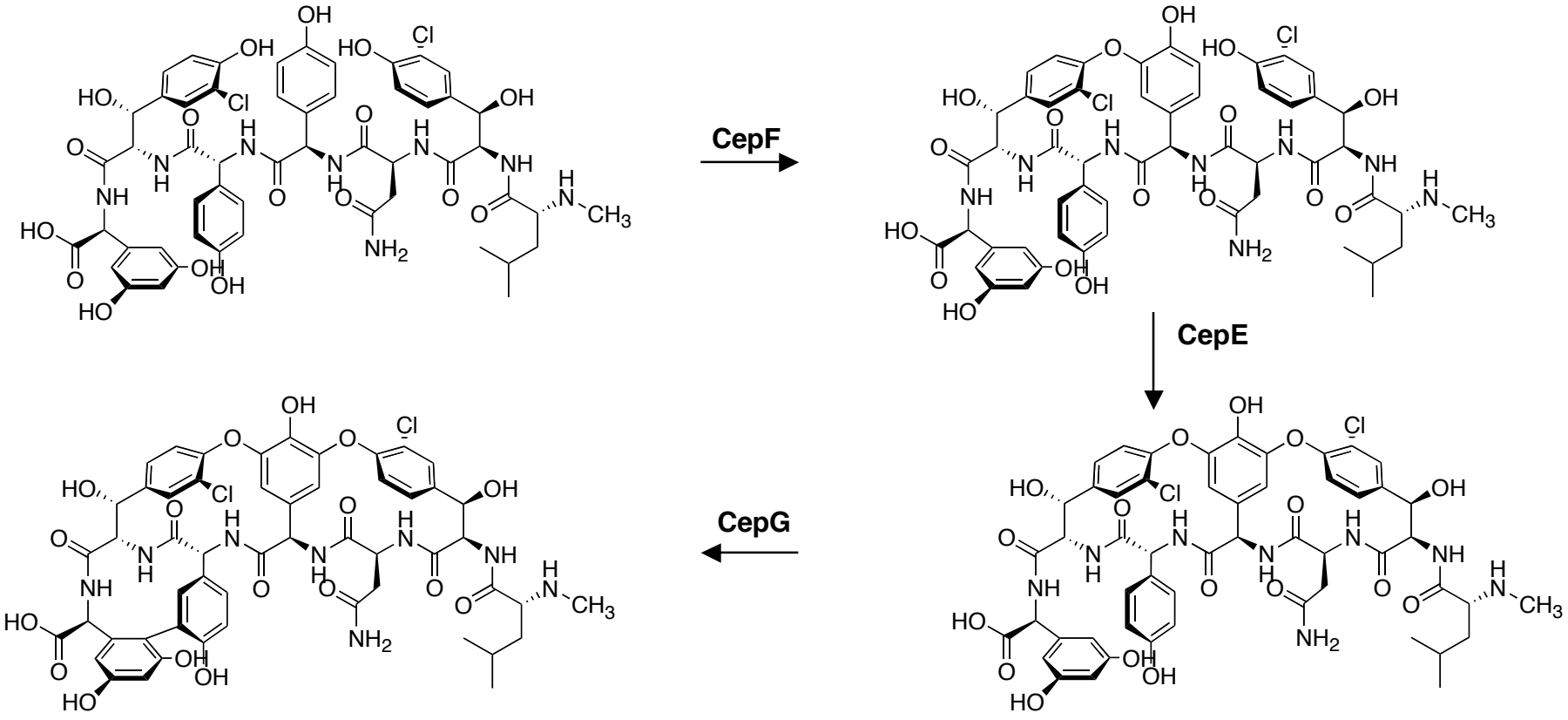
Peptide Biosynthesis
Non-Ribosomal Peptides
Elaborating the structures produced by the peptide synthetases



Never reconstituted in vitro
Timing unclear --> scale up
revealed lots of truncated peptide products

Genetic knockouts --> analyze culture broth for products

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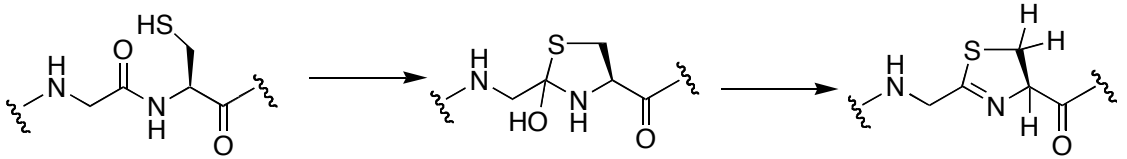
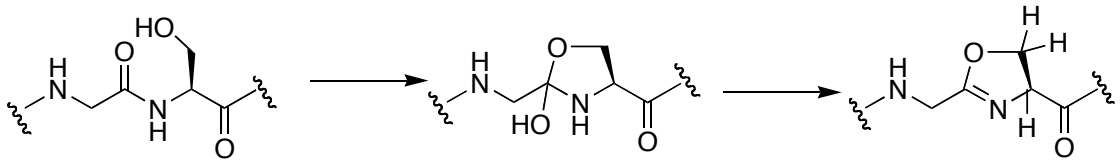
Peptide Biosynthesis
Non-Ribosomal Peptides
Review of Peptide Modifications

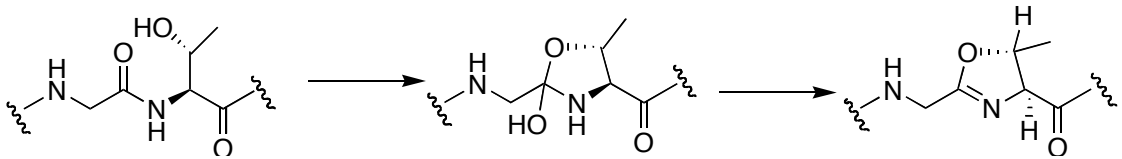
1. Cyclization (replaces C domain): amide bond formation (not shown) and then cyclization/dehydration



Cys to thiazoline **example: yersiniabactin**

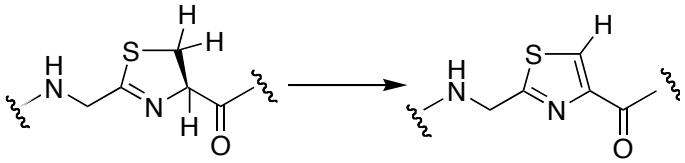


Ser to oxazoline



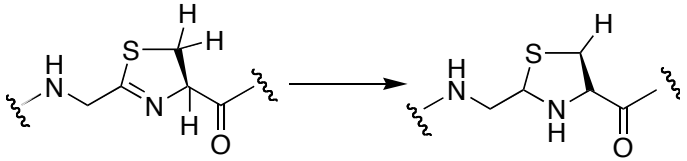
Thr to methyloxazoline

2. Oxidation



thiazoline to thiazole (oxazoline to oxazole)
note: uses flavin as oxidizing agent
examples: bleomycin, epothilone

3. Reduction



thiazoline to thiazolidine (oxazoline to oxazolidine)
note: uses NADPH as reducing agent
example: yersiniabactin

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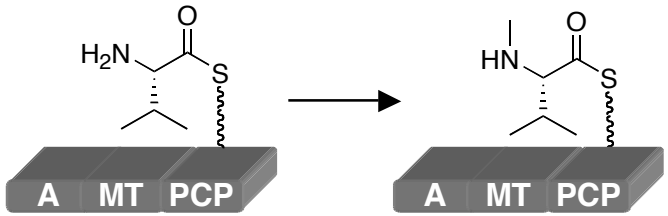
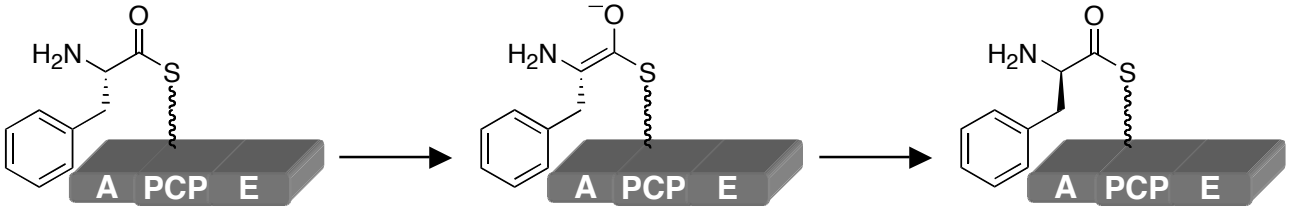
Peptide Biosynthesis
Non-Ribosomal Peptides
Review of Peptide Modifications

4. N-Methylation (MT)



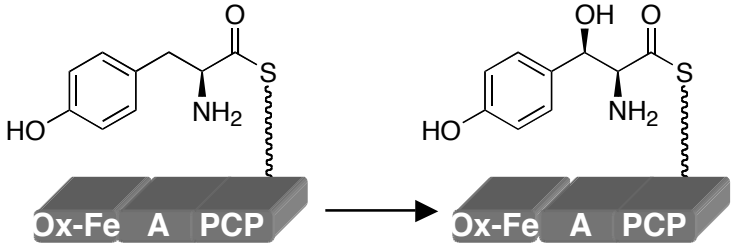
note: uses SAM as methyl source
example: cyclosporin

5. Epimerization



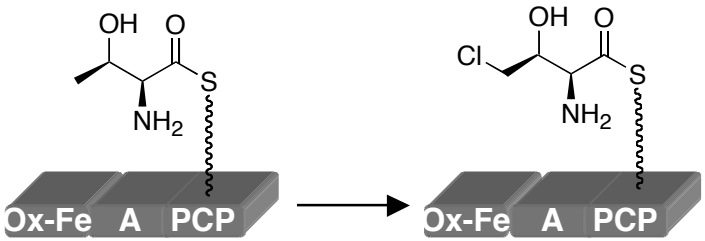
example: gramicidin

6. Hydroxylation



note: uses heme-chelated iron as oxidant
example:bleomycin, ramoplanin, novobiocin

7. Halogenation (chlorination most common)



note: non-heme-chelated iron as oxidant
example:syringomycin

Major changes to the peptide backbone structure are typically carried out after peptide biosynthesis
Most important examples:

Oxidative crosslinking in vancomycin biosynthesis
β-lactam formation in penicillin/cephlasporin biosynthesis

Also occuring after peptide biosynthesis:
Glycosylation of peptide (will be discussed in detail later)
Derivatization with lipids

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Peptide Biosynthesis
Ribosomal Peptides

• standard ribsosome-based protein synthesis of standard linear peptide
• proteolysis and post-translational modification occurs after protein is translated
• examples are microcin and lantibiotics
(most peptide based natural products are synthesized "non-ribosomally")

Lantibiotics
Chem. Rev. 2005, 105, 633-683.

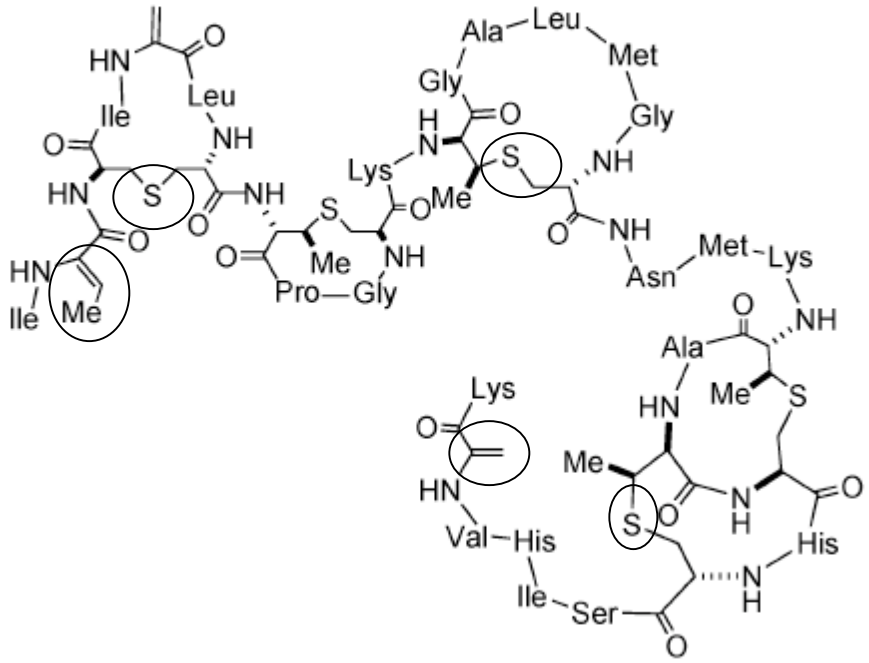

Figure 2. The structure of nisin A.

standard peptide biosynthesis on ribosome

Figure removed due to copyright reasons.
Please see Figure 4 in *Chem Rev* 105 *(*2005): 633-683.

Figures removed due to copyright reasons.
Please see Figures 22 and 23 in *Chem Rev* 105 *(*2005): 633-683.

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Peptide Biosynthesis
Non-Ribosomal Peptides

What is a gene cluster?

• The genes that encode the biosynthetic enzymes next to each other on a chromosome

• Gene = open reading frame (ORF) = ATG XXX XXX XXX … … TAA
start/stop codon "in frame" (multiple of 3)

prokaryotes (i.e. bacteria)
many yeast/fungi (simple eukaryotes)

Figure removed due to copyright reasons.

Figure 3 in Microbiology, 2005, 151, 1507-1523.
Daphomycin Biosynthesis