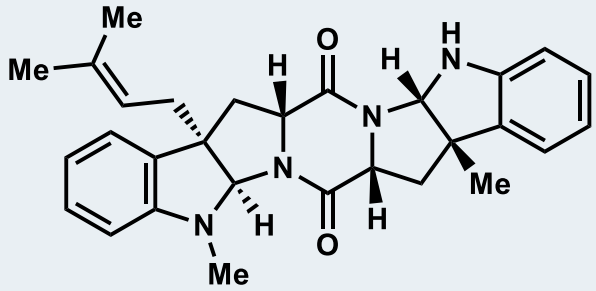

Describing the Biosynthesis of Nocardioazine B

Presentation by Jaren Lobb

Nocardioazine B Biosynthesis

nature communications

Article

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Unveiling an indole alkaloid diketopiperazine biosynthetic pathway that features a unique stereoisomerase and multifunctional methyltransferase

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Check for updates

Garrett Deletti[1,6], Sajan D. Green[1,6], Caleb Weber[1], Kristen N. Patterson[1,5], Swapnil S. Joshi[2], Tushar M. Khopade[2], Mathew Coban[3], James Veek-Wilson[1], Thomas R. Caulfield[3,4], Rajesh Viswanathan[1,2] ✉ & Amy L. Lane[1] ✉

The 2,5-diketopiperazines are a prominent class of bioactive molecules. The nocardioazines are actinomycete natural products that feature a pyrro-

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- *B.S., Chemistry, Indiana state University*
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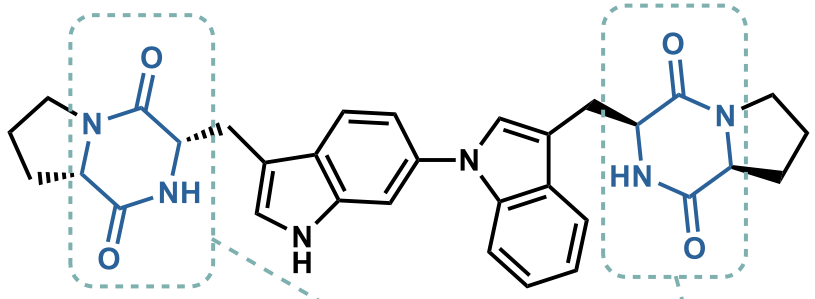
- *Current Whereabouts Unknown*

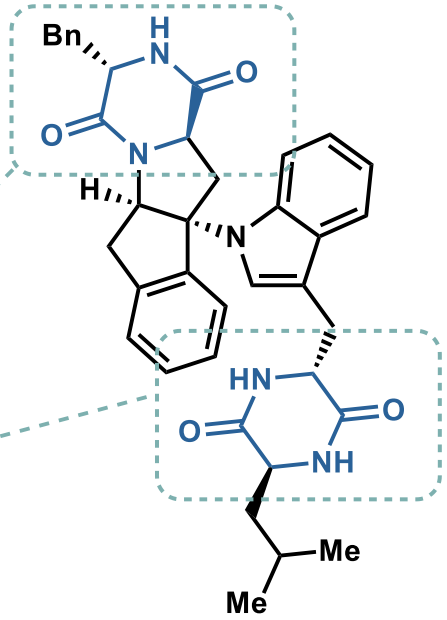
Sajan Green

- *ACS Organic Chemistry Awardee 2022*
- *BS., Chemistry, University of North Florida*
- *3rd Year PhD Candidate, University of Florida, Loesgen Lab*

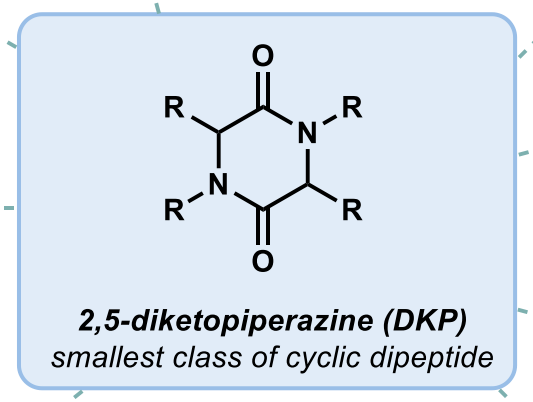
Overview of DKPs in Natural Products



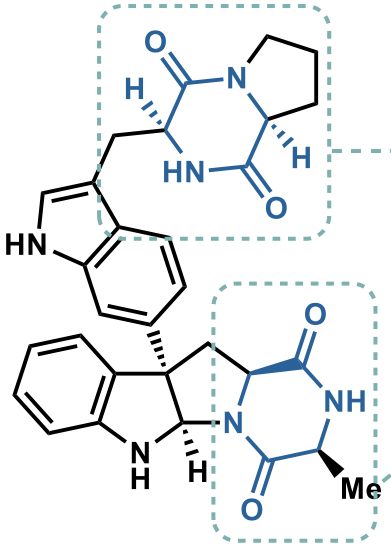
(+)-pestalazine B
Pestalotiopsis theae



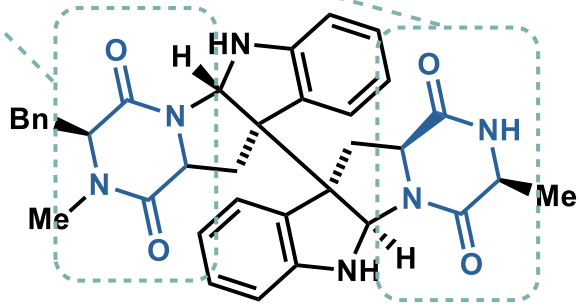
(+)-aspergilazine A
Apergillus taichungensis



2,5-diketopiperazine (DKP)
smallest class of cyclic dipeptide

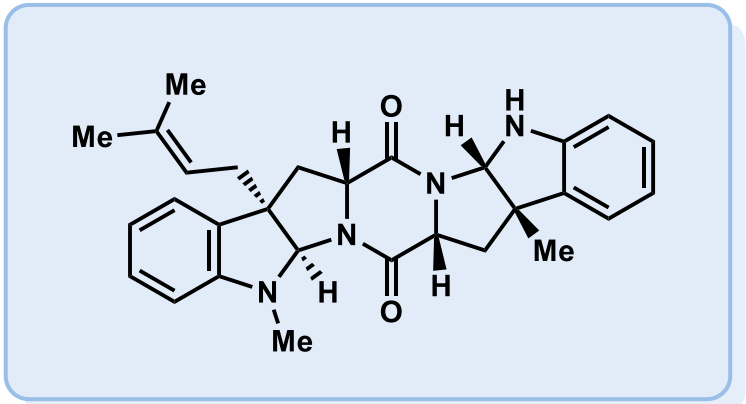


(+)-naseseazine A
Streptomyces



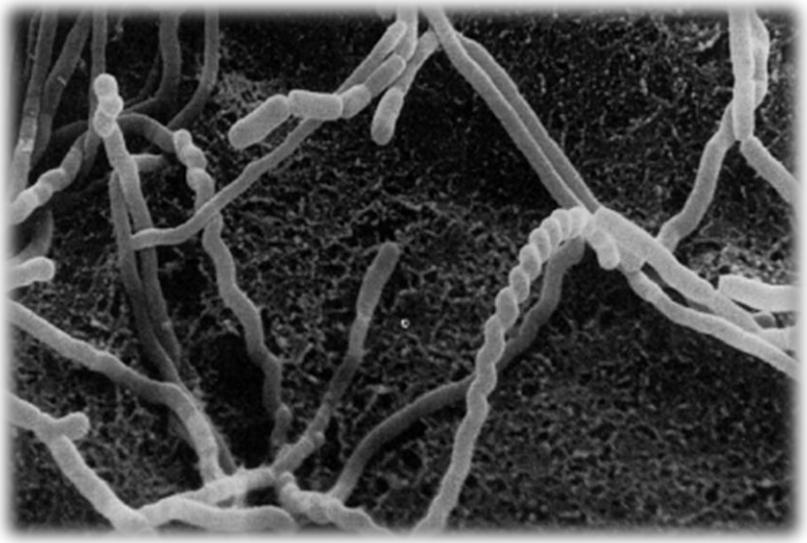
(-)-ditryptophenaline
Aspergillus flavus

Overview of Nocardioazine B



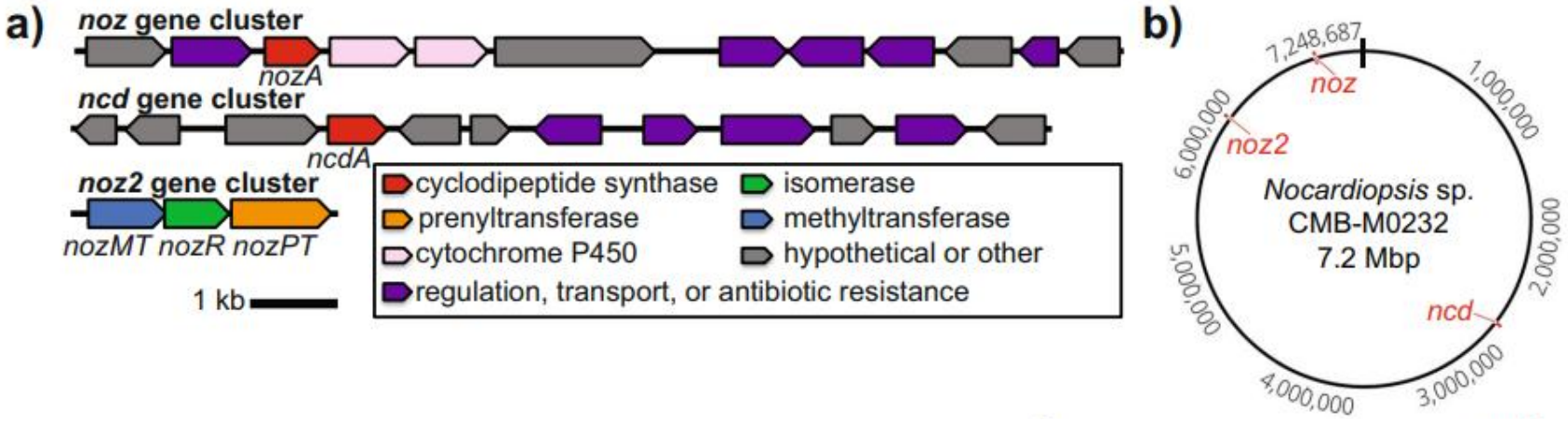
- ***2,5-diketopiperazine natural product isolated from Nocardiopsis* sp. *CMB-M0232***
  - *5 chiral centers, 2 quartenary*
  - *C3 methyl and C3' prenyl groups establish asymmetric structure of this di-tryptophan DKP*

- ***Demonstrated strong anti-cancer activity***
  - *Non-cytotoxic P-glycoprotein inhibitor*
  - *Reverses drug resistance in cancer cells*

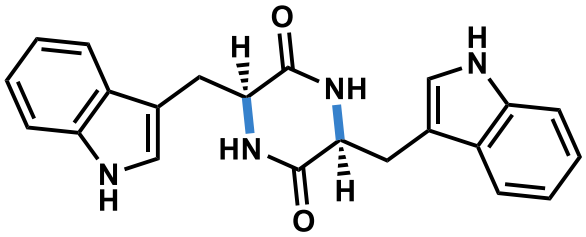


Nature Communications | **(2023)** 14:2558

Unusual Gene Cluster Distribution

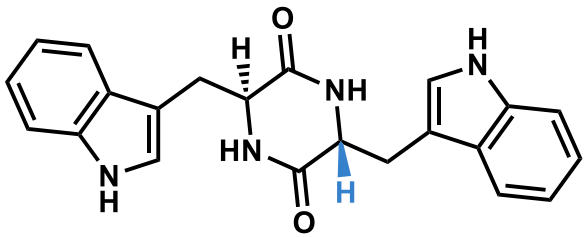


Biosynthesis Overview



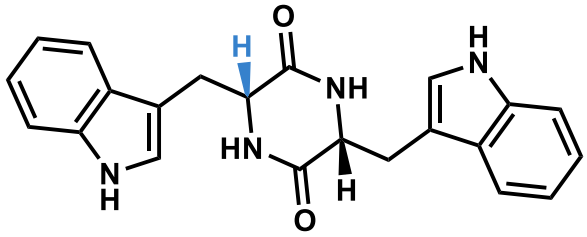
(1) *cyclo-L-Trp-Trp*
from tryptophanyl-tRNA^{Trp} via ***NozA or NcdA CDPS***

NozR



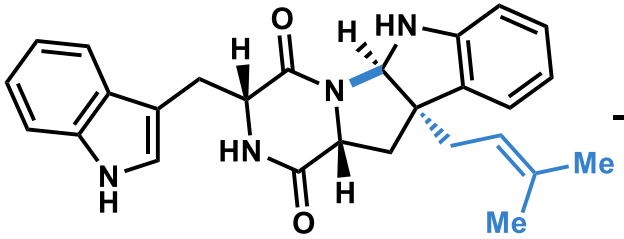
(2) *cyclo-D-L-Trp-Trp*
isomerase product I

NozR



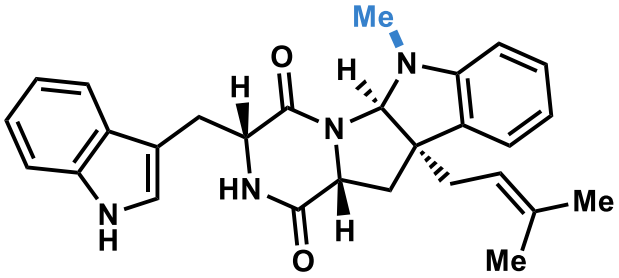
(3) *cyclo-D-D-Trp-Trp*
isomerase product II

NozPT
DMAPP



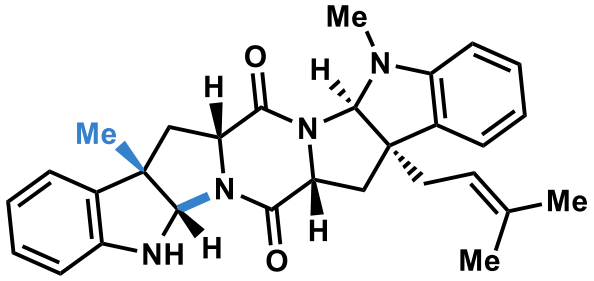
(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*
prenyltransferase product

NozMT
SAM



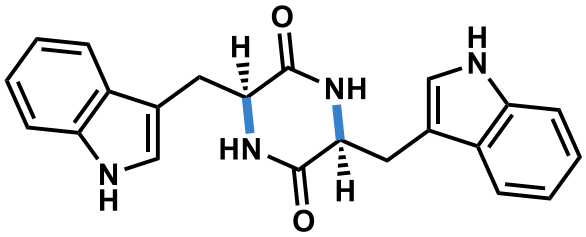
(5) *cyclo-D-Trp-N1'-methyl-C3'-prenyl-D-Trp*
methyltransferase product I

NozMT
SAM



(6) *Nocardioazine B*
methyltransferase product II

Biosynthesis Overview



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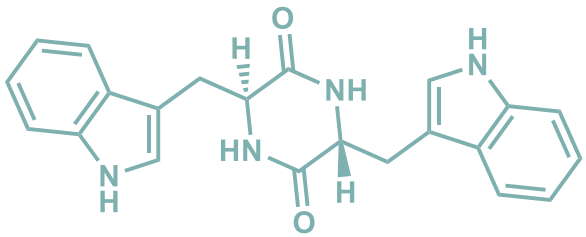
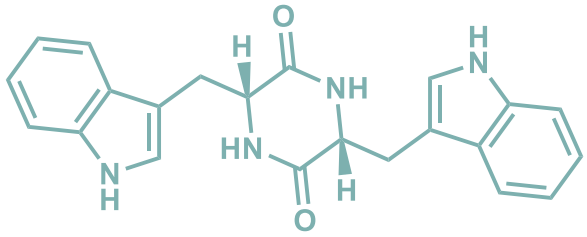
NozR



(2) *cyclo-D-L-Trp-Trp*

isomerase product I

NozR

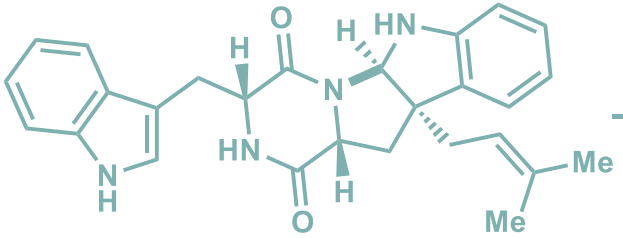


(3) *cyclo-D-D-Trp-Trp*

isomerase product II

NozPT

DMAPP

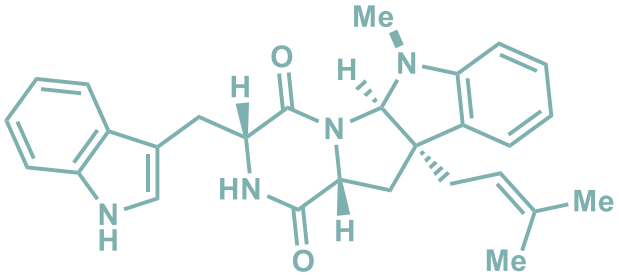


(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*

prenyltransferase product

NozMT

SAM

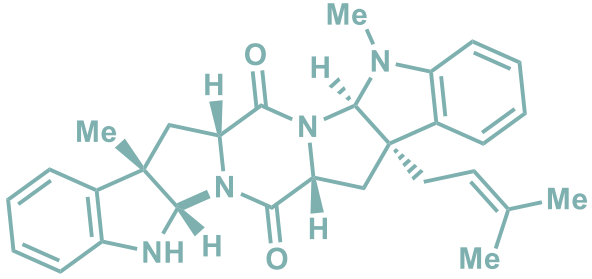


(5) *cyclo-D-Trp-N1'-methyl-C3'-prenyl-D-Trp*

methyltransferase product I

NozMT

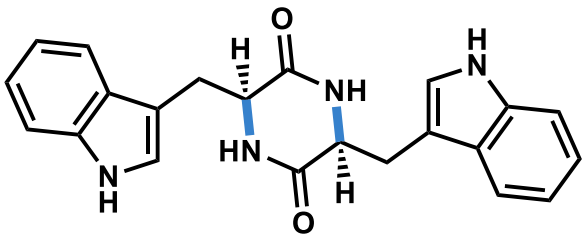
SAM



(6) *Nocardioazine B*

methyltransferase product II

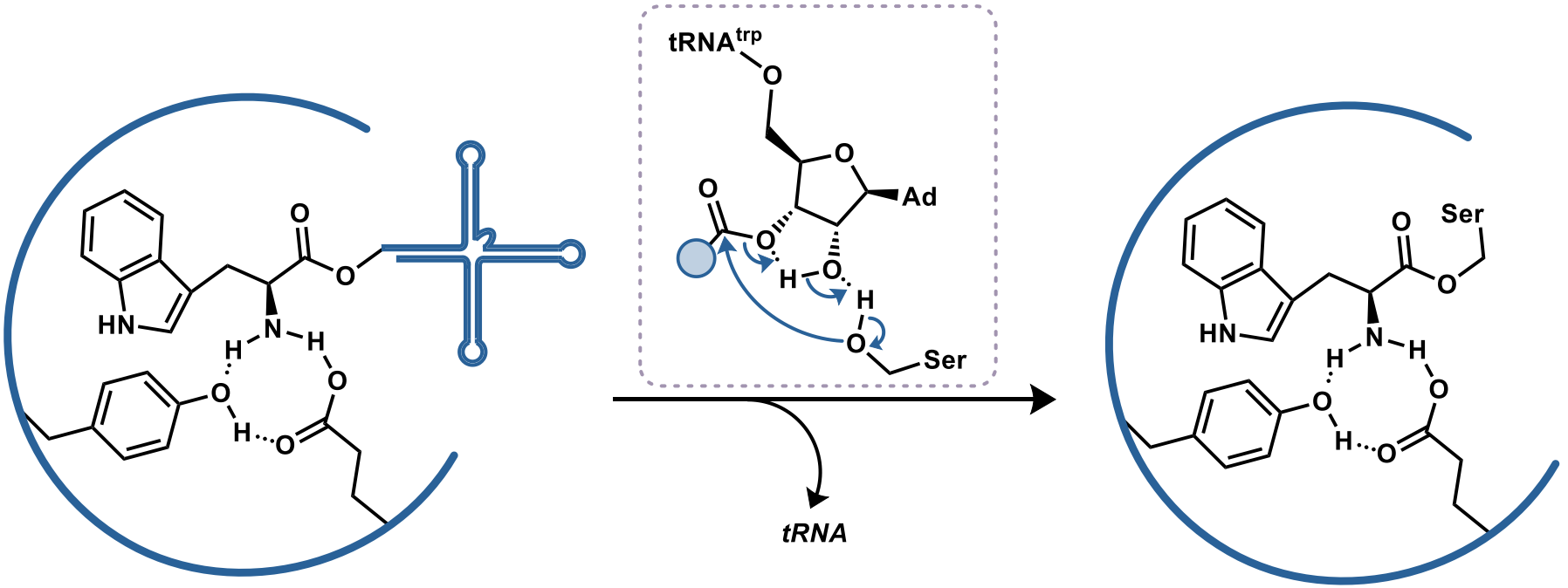
NozA/NcdA Cyclodipeptide Synthases



(1) *cyclo-L-Trp-Trp*

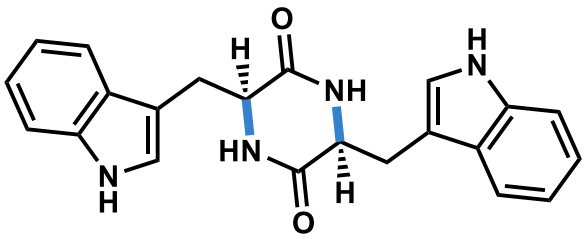
from tryptophanyl-tRNA^{Trp} via ***NozA or NcdA CDPS***

Cyclodipeptide Synthases are an example of non-ribosomal peptide synthetases (NRPSs)



ACS Synth. Biol. 5, 547–553 **(2016).**

NozA/NcdA Cyclodipeptide Synthases



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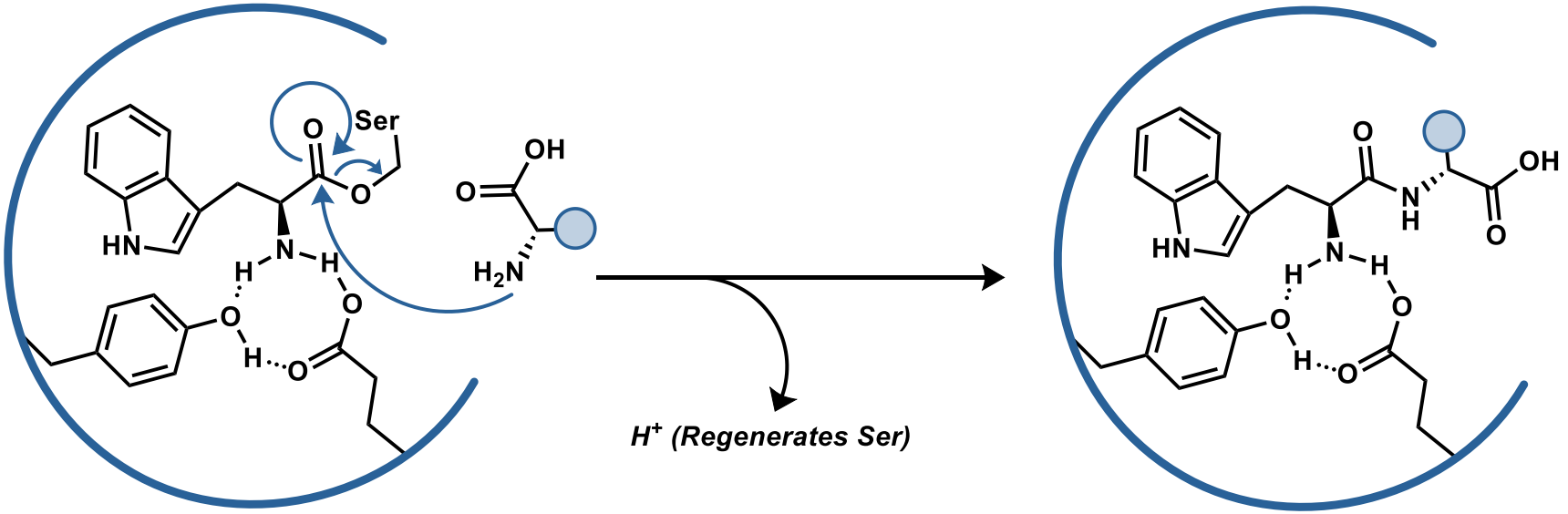

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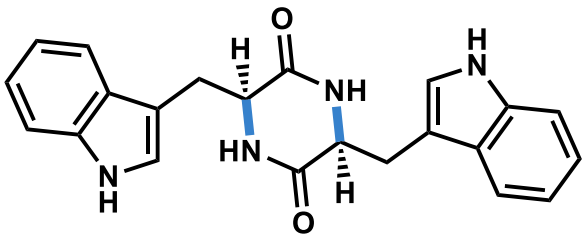
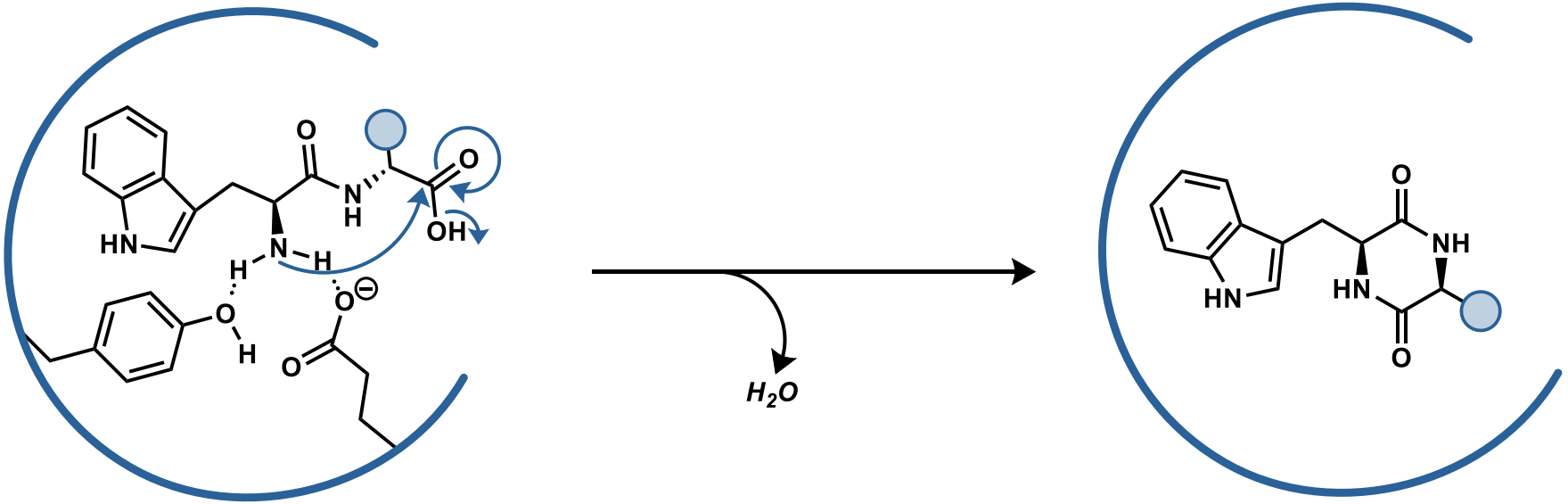
NozA/NcdA Cyclodipeptide Synthases



(1) *cyclo-L-Trp-Trp*

from tryptophanyl-tRNA^{Trp} via ***NozA or NcdA CDPS***

Cyclodipeptide Synthases generate DKPs from amino acyl tRNA through serine mediated hydrolysis and amide formation



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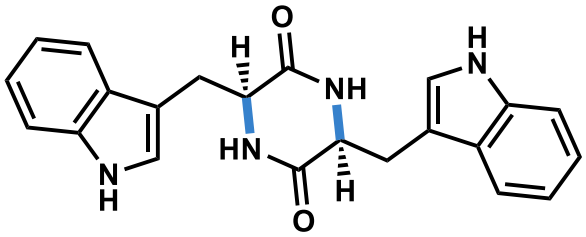
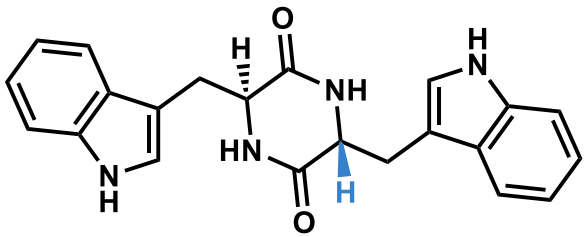
Biosynthesis Overview



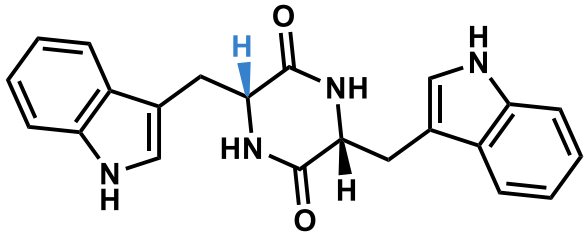
(1) *cyclo-L-Trp-Trp*
from tryptophanyl-tRNA^{Trp} via ***NozA or NcdA CDPS***

NozR



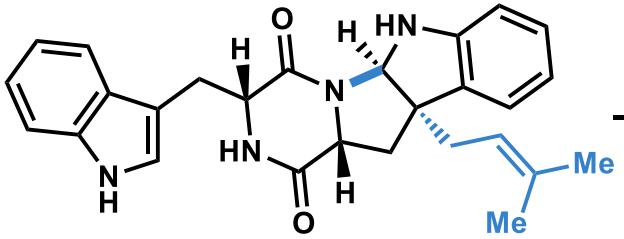
(2) *cyclo-D-L-Trp-Trp*
isomerase product I

NozR



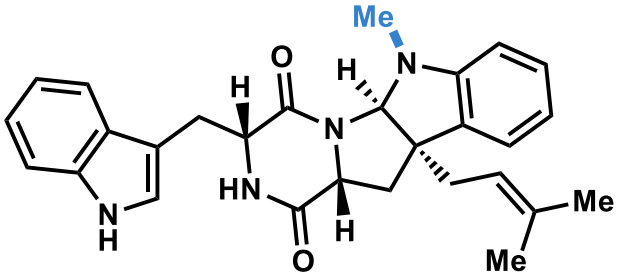
(3) *cyclo-D-D-Trp-Trp*
isomerase product II

NozPT
DMAPP



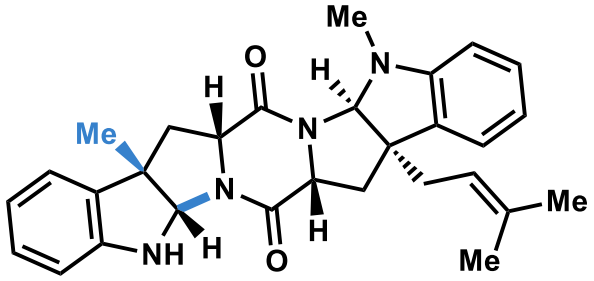
(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*
prenyltransferase product

NozMT
SAM



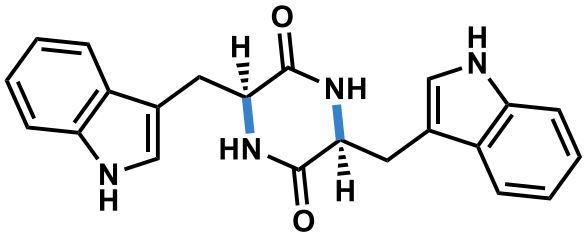
(5) *cyclo-D-Trp-N1'-methyl-C3'-prenyl-D-Trp*
methyltransferase product I

NozMT
SAM



(6) *Nocardioazine B*
methyltransferase product II

Biosynthesis Overview



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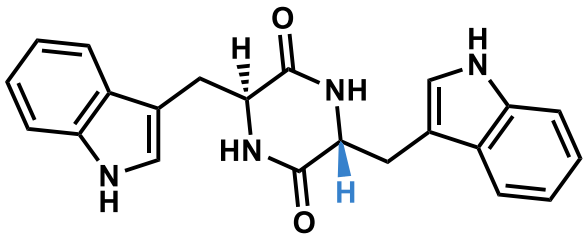
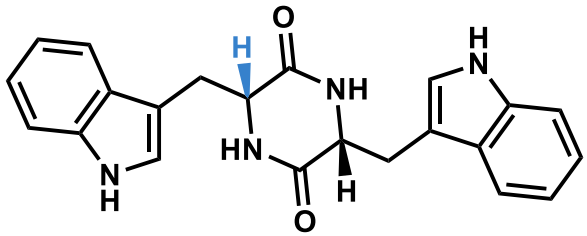
NozR



(2) *cyclo-D-L-Trp-Trp*
isomerase product I

NozR



(3) *cyclo-D-D-Trp-Trp*
isomerase product II

NozPT
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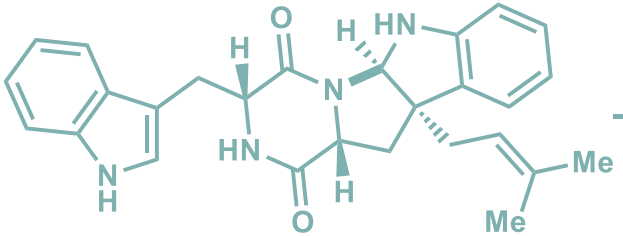
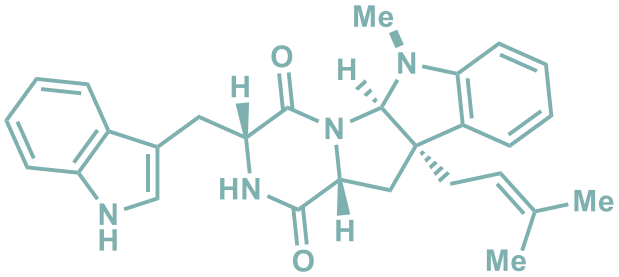


(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*
prenyltransferase product

NozMT
SAM



(5) *cyclo-D-Trp-N1'-methyl-C3'-prenyl-D-Trp*
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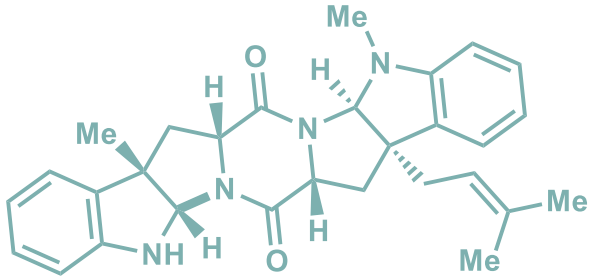
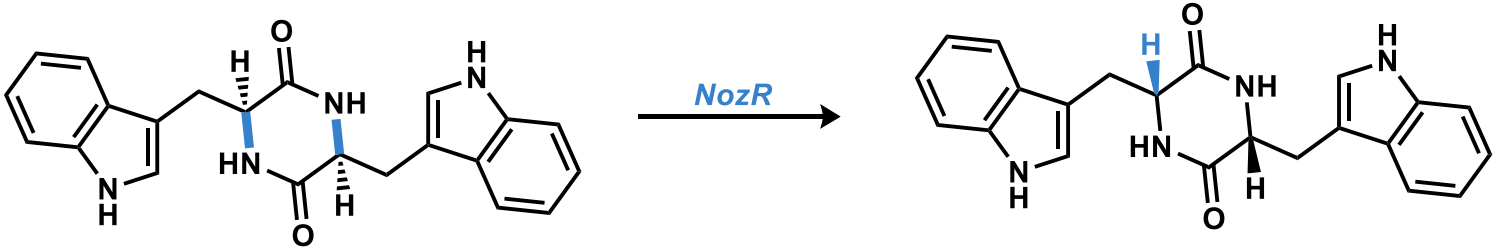
NozMT
SAM



(6) *Nocardioazine B*
methyltransferase product II

NozR DKP Isomerase



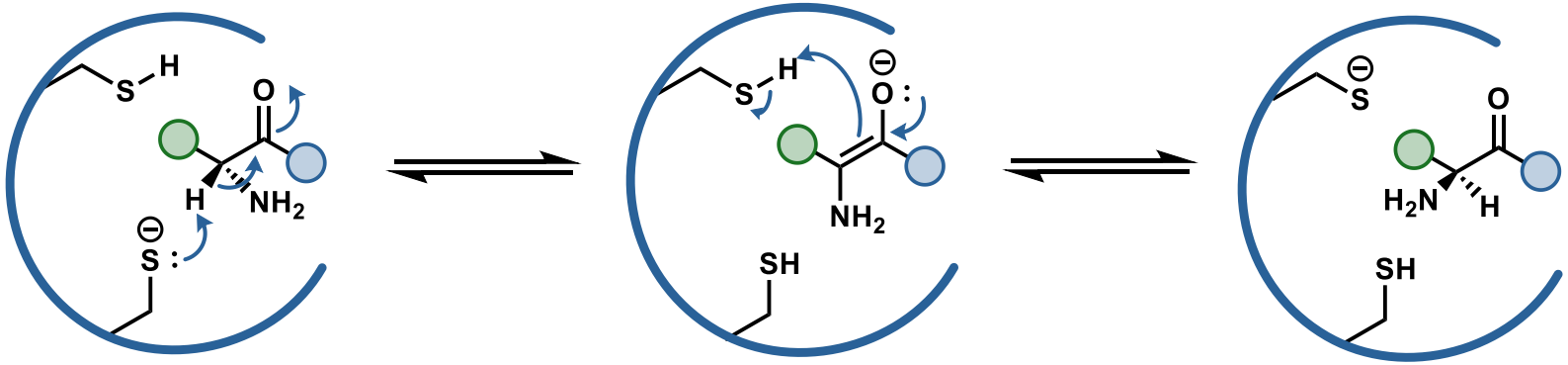
(1) *cyclo-L-Trp-Trp*

from tryptophanyl-tRNA^{Trp} via ***NozA or NcdA CDPS***

(3) *cyclo-D-D-Trp-Trp*

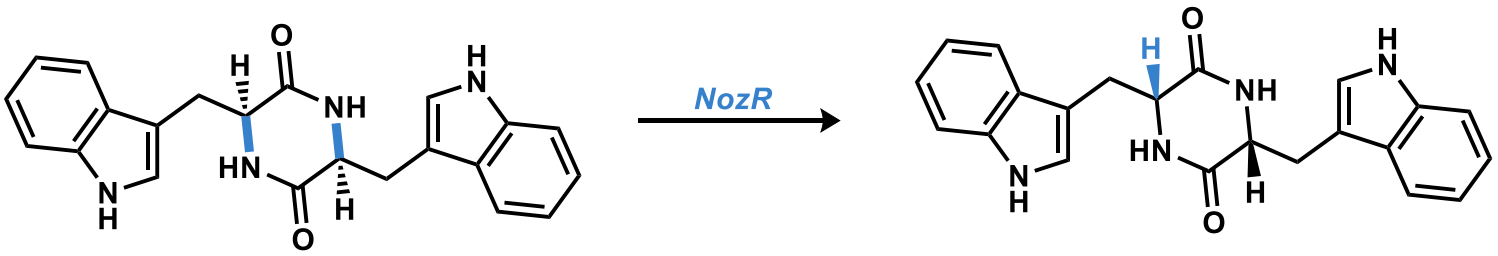
isomerase product II

NozR is a member of the Asp/Glu Racemase family which catalyze the general isomerization shown below:



Isomerization between enantiomers occurs readily through this cysteine-cysteine catalytic dyad

NozR DKP Isomerase



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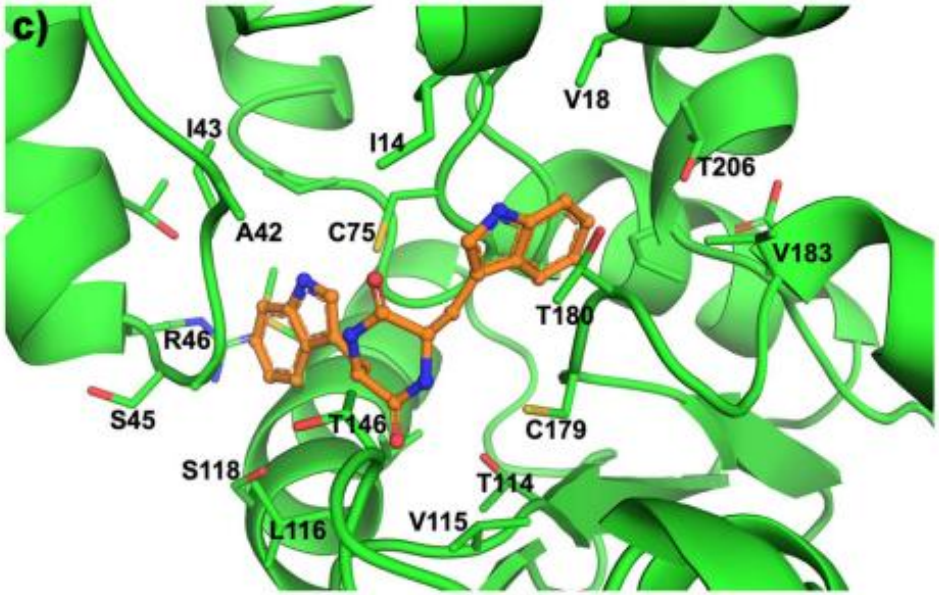
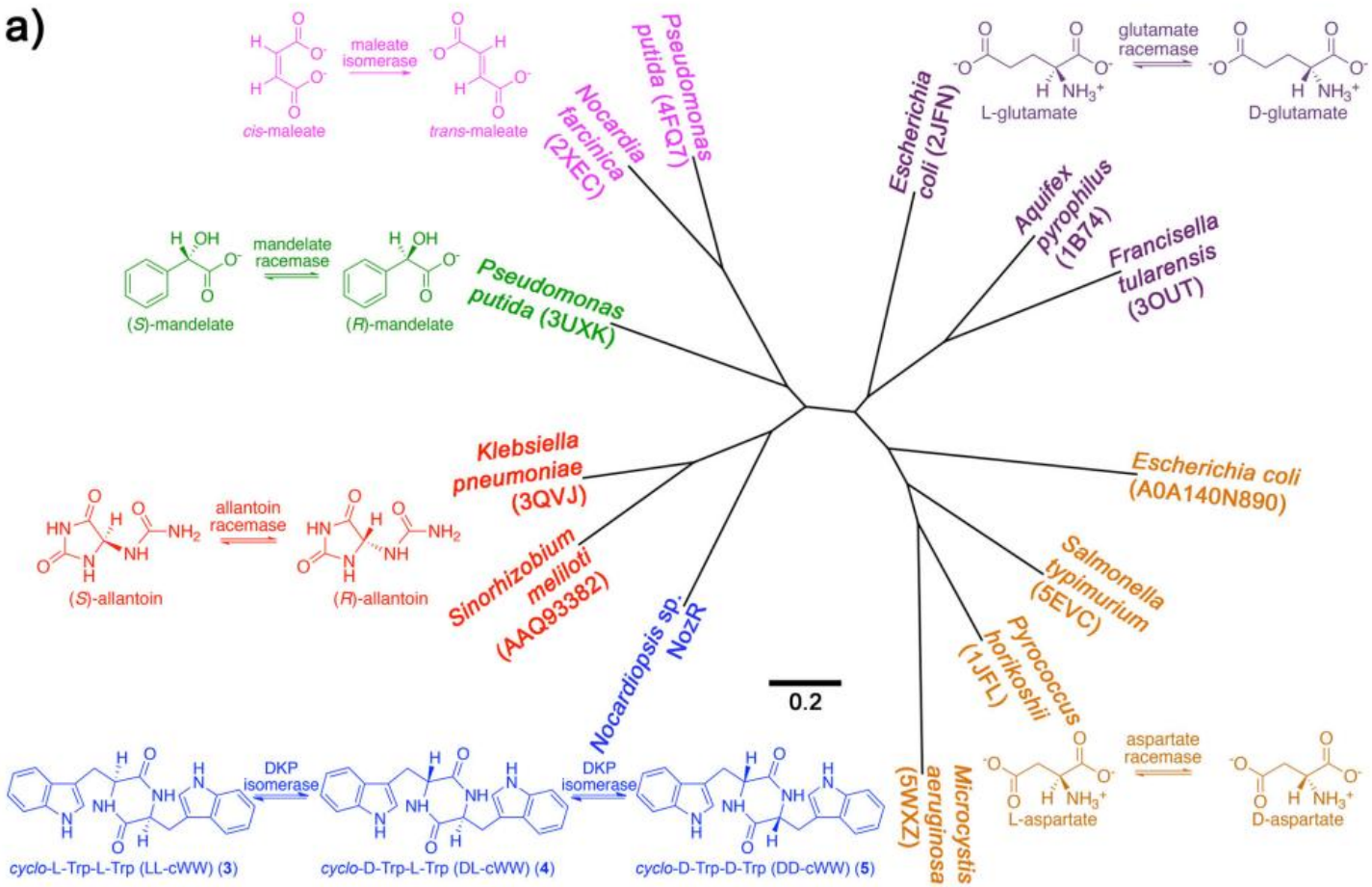
isomerase product II

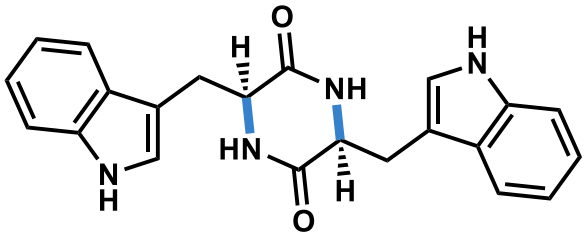


NozR active site in complex with substrate ***1***

Asp/Glu Racemase Phylogeny



Biosynthesis Overview



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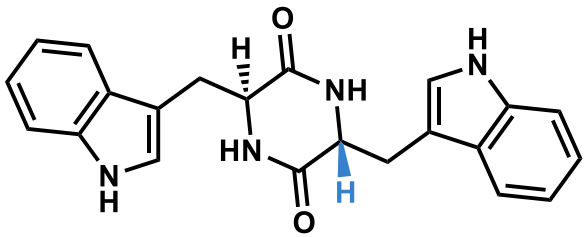
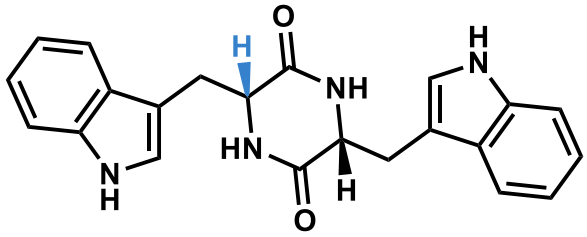
NozR



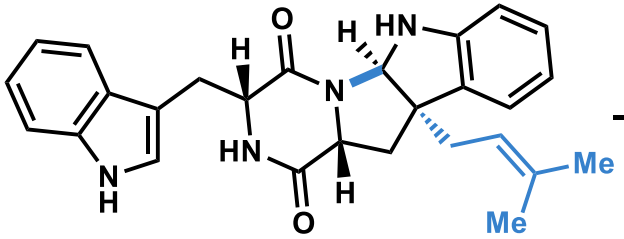
(2) *cyclo-D-L-Trp-Trp*
isomerase product I

NozR



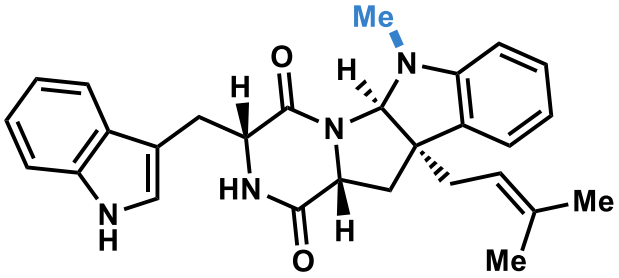
(3) *cyclo-D-D-Trp-Trp*
isomerase product II

NozPT
DMAPP



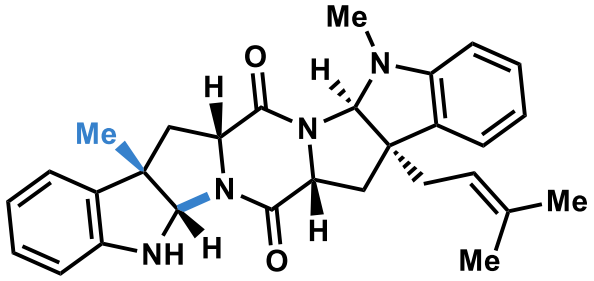
(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*
prenyltransferase product

NozMT
SAM



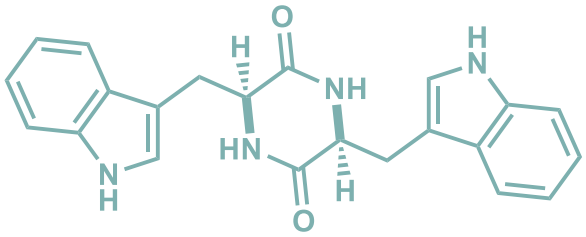
(5) *cyclo-D-Trp-N1'-methyl-C3'-prenyl-D-Trp*
methyltransferase product I

NozMT
SAM



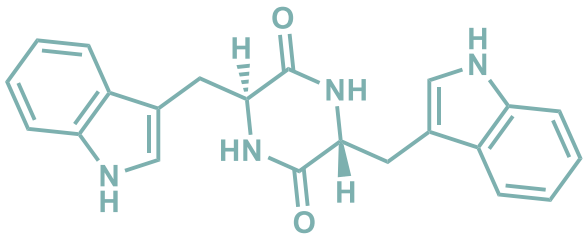
(6) *Nocardioazine B*
methyltransferase product II

Biosynthesis Overview



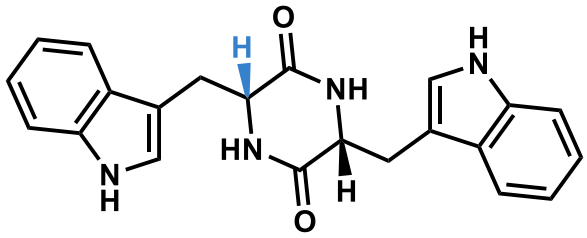
(1) ***cyclo-L-Trp-Trp***
from tryptophanyl-tRNA^{Trp} via ***NozA or NcdA CDPS***

NozR



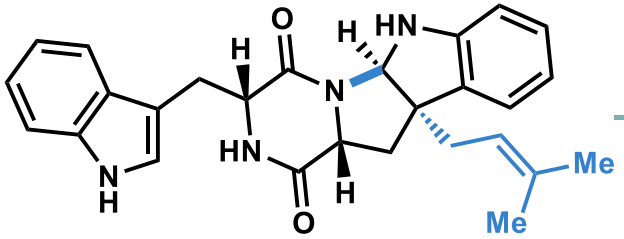
(2) ***cyclo-D-L-Trp-Trp***
isomerase product I

NozR



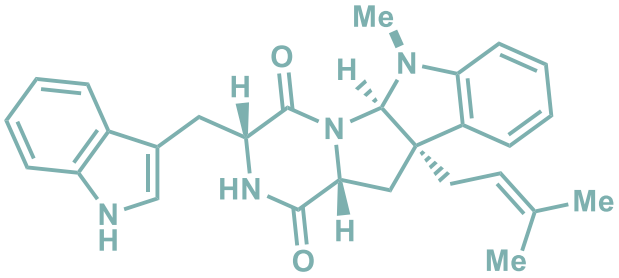
(3) *cyclo-D-D-Trp-Trp*
isomerase product II

NozPT
DMAPP



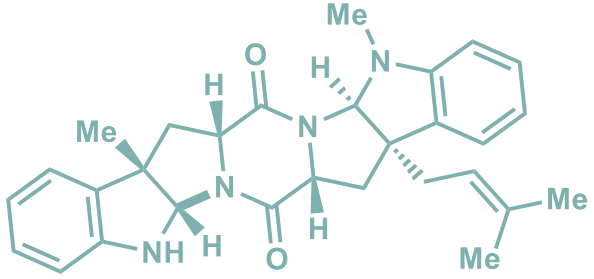
(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*
prenyltransferase product

NozMT
SAM



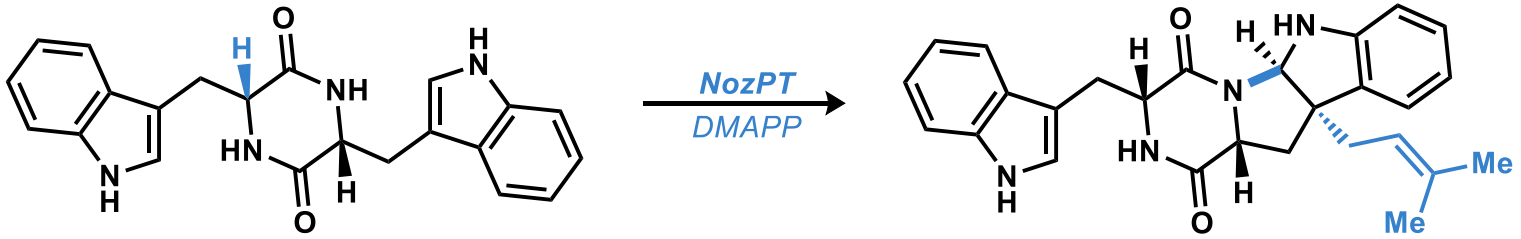
(5) ***cyclo-D-Trp-N1'-methyl-C3'-prenyl-D-Trp***
methyltransferase product I

NozMT
SAM



(6) ***Nocardioazine B***
methyltransferase product II

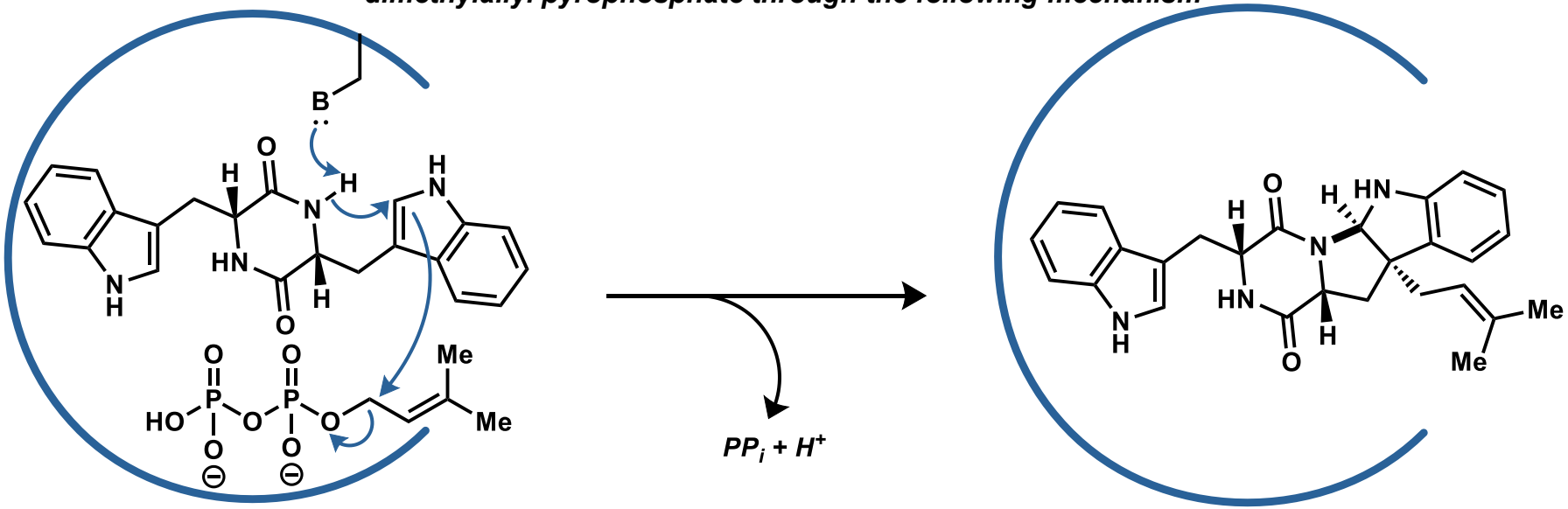
NozPT Prenyltransferase



(3) *cyclo-D-D-Trp-Trp*
isomerase product II

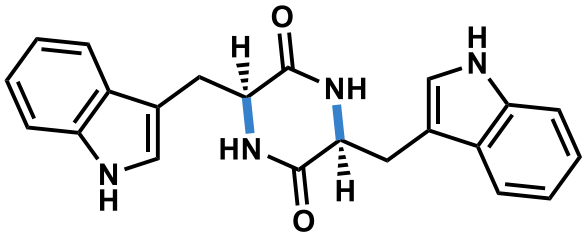
(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*
prenyltransferase product

NozPT Prenyltransferase facilitates the interaction between cyclo-D-D-Trp-Trp and dimethylallyl pyrophosphate through the following mechanism



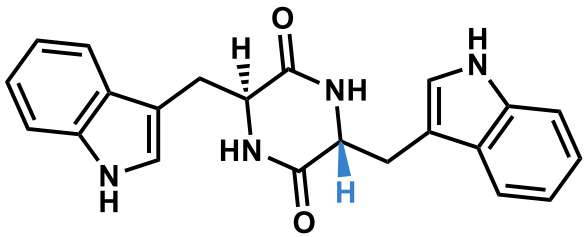
Note that the base residue involvement in this transformation is not elucidated in this work, but is included for mechanistic completeness

Biosynthesis Overview



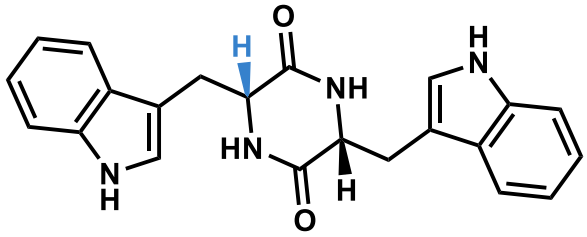
(1) *cyclo-L-Trp-Trp*
from tryptophanyl-tRNA^{Trp} via ***NozA or NcdA CDPS***

NozR



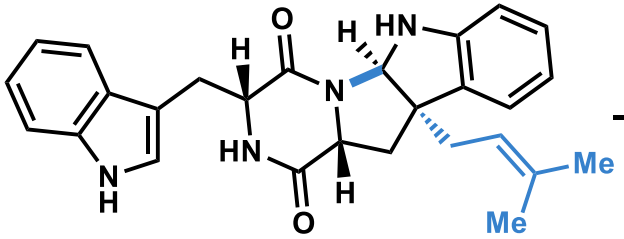
(2) *cyclo-D-L-Trp-Trp*
isomerase product I

NozR



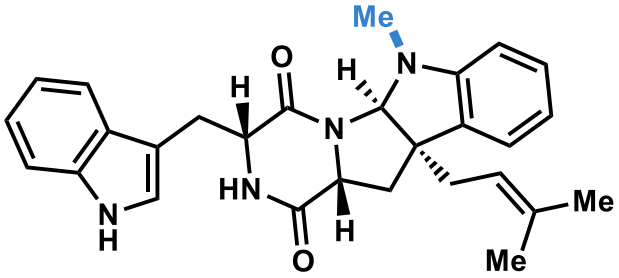
(3) *cyclo-D-D-Trp-Trp*
isomerase product II

NozPT
DMAPP



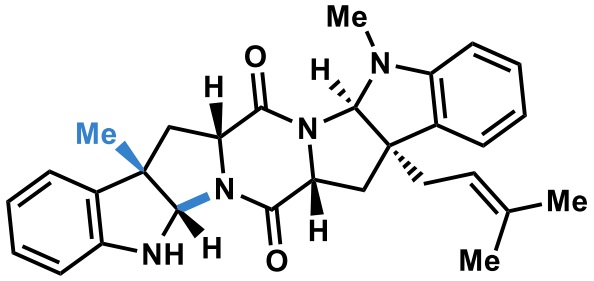
(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*
prenyltransferase product

NozMT
SAM



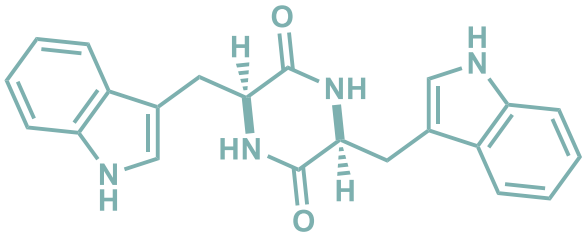
(5) *cyclo-D-Trp-N1'-methyl-C3'-prenyl-D-Trp*
methyltransferase product I

NozMT
SAM



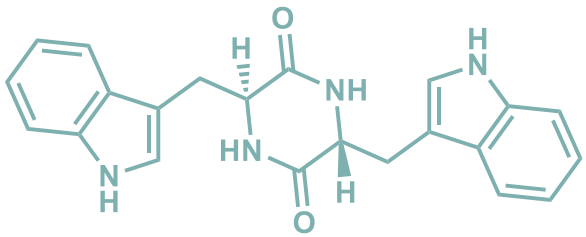
(6) *Nocardioazine B*
methyltransferase product II

Biosynthesis Overview



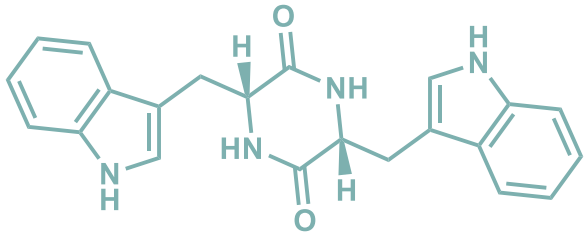
(1) ***cyclo-L-Trp-Trp***
from tryptophanyl-tRNA^{Trp} via ***NozA or NcdA CDPS***

NozR



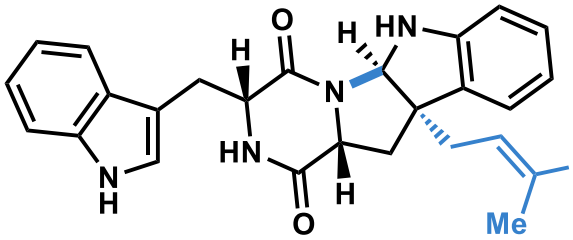
(2) ***cyclo-D-L-Trp-Trp***
isomerase product I

NozR



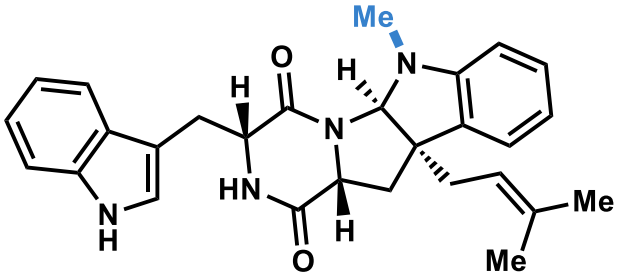
(3) ***cyclo-D-D-Trp-Trp***
isomerase product II

NozPT
DMAPP

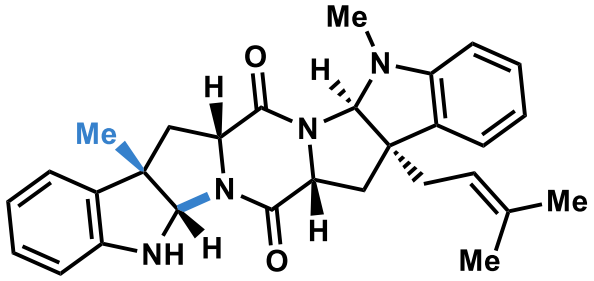


NozMT
SAM

(4) ***cyclo-D-Trp-C3'-prenyl-D-Trp***
prenyltransferase product



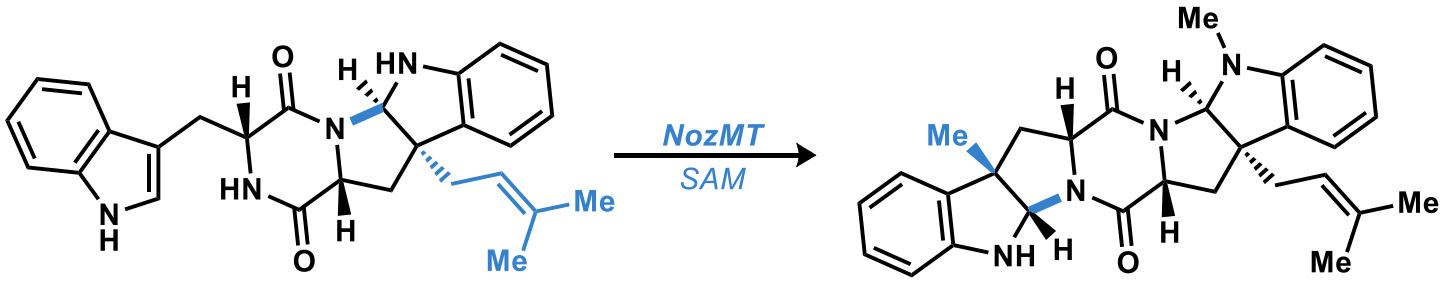
NozMT
SAM



(5) ***cyclo-D-Trp-N1'-methyl-C3'-prenyl-D-Trp***
methyltransferase product I

(6) ***Nocardioazine B***
methyltransferase product II

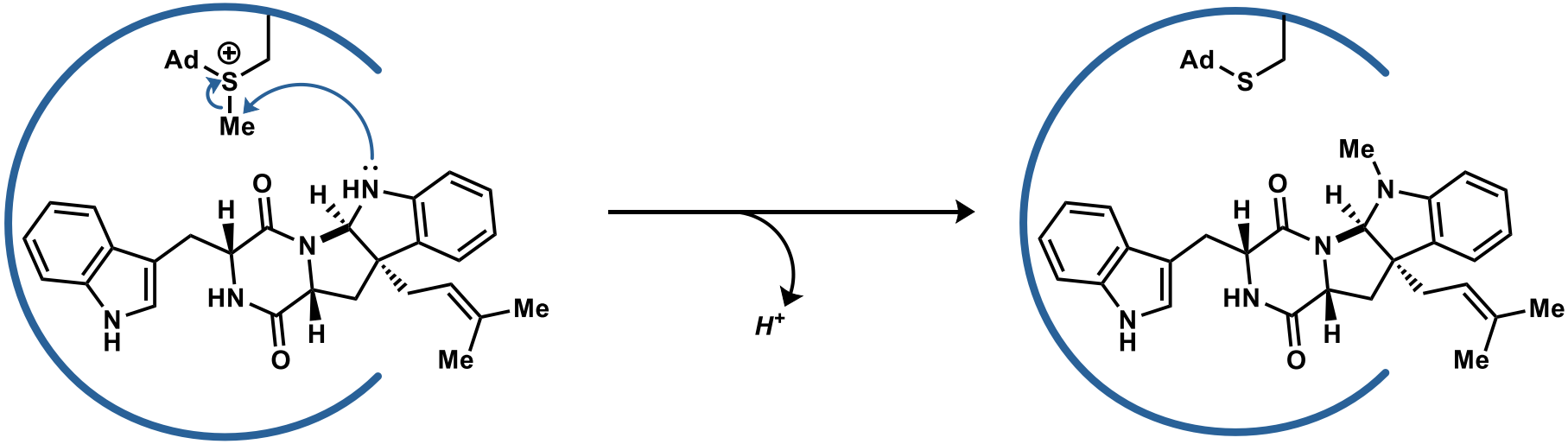
NozMT SAM-Dependent Methyltransferase



(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*
prenyltransferase product

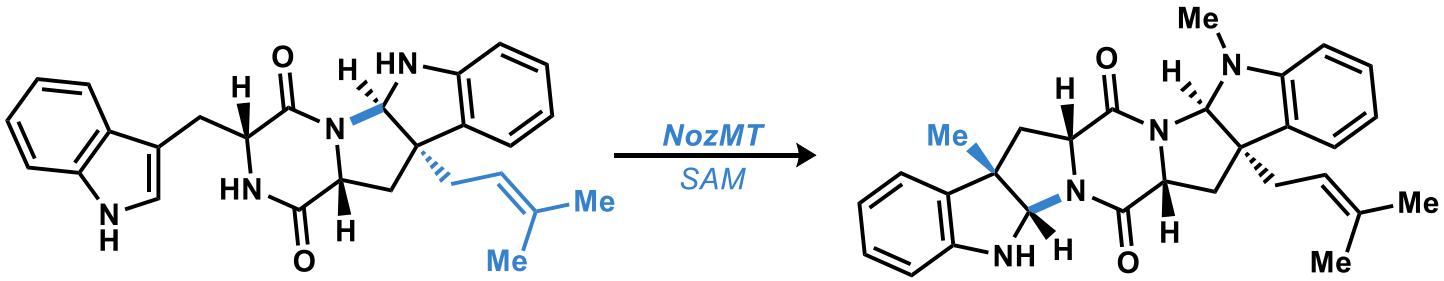
(6) *Nocardioazine B*
methyltransferase product II

S-Adenosyl Methionine is responsible for an electrophilic methyl transfer by the following mechanism



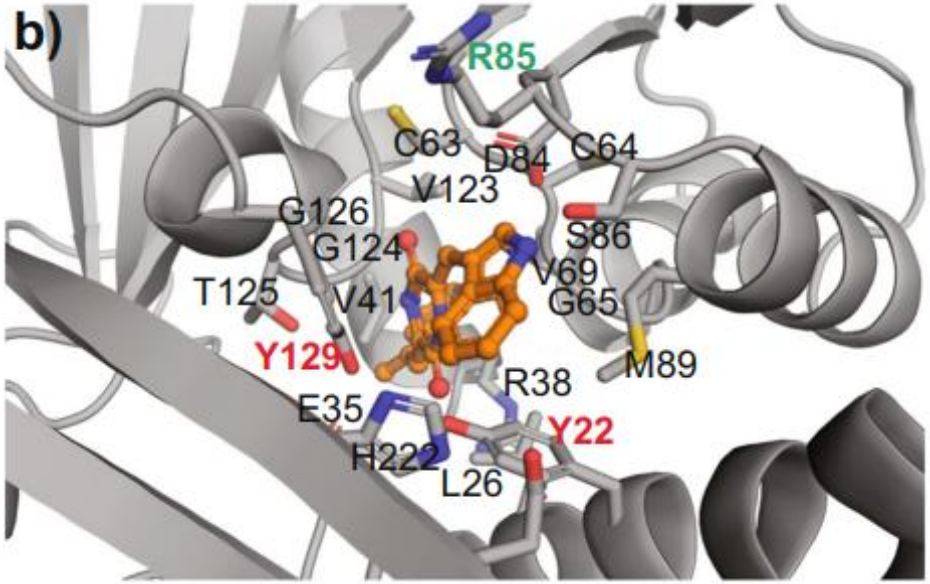
Cofactor regeneration is described in a later slide

NozMT SAM-Dependent Methyltransferase



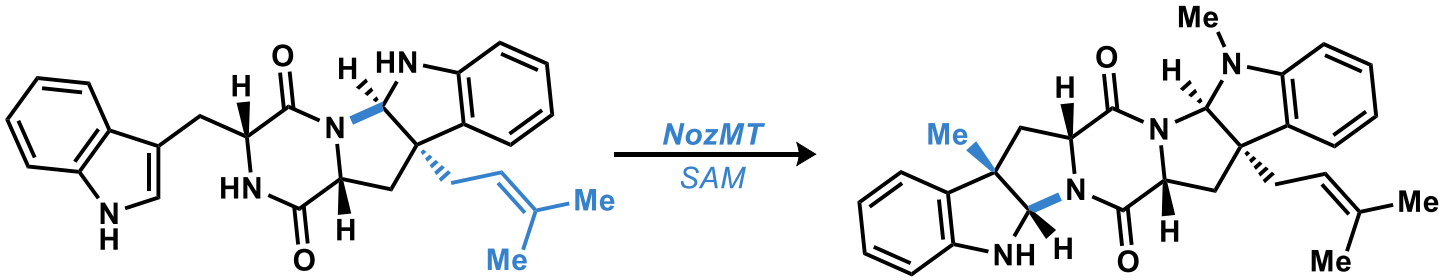
(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*
prenyltransferase product

(6) *Nocardioazine B*
methyltransferase product II



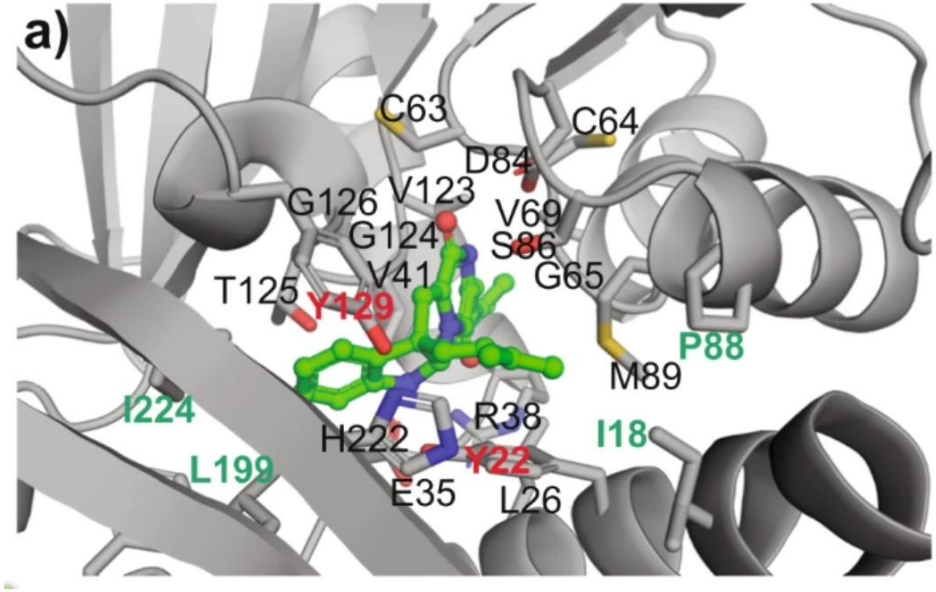
*NozMT active site in complex with substrate **3** (rejected)*

NozMT SAM-Dependent Methyltransferase



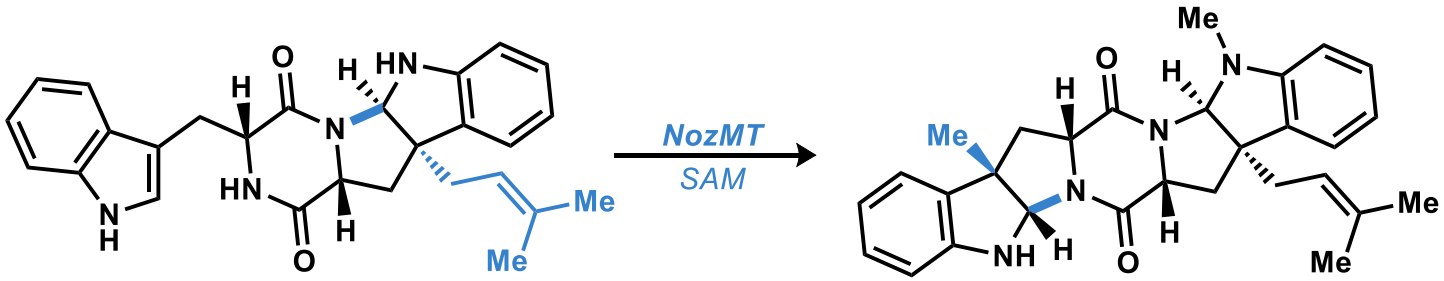
(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*
prenyltransferase product

(6) *Nocardioazine B*
methyltransferase product II



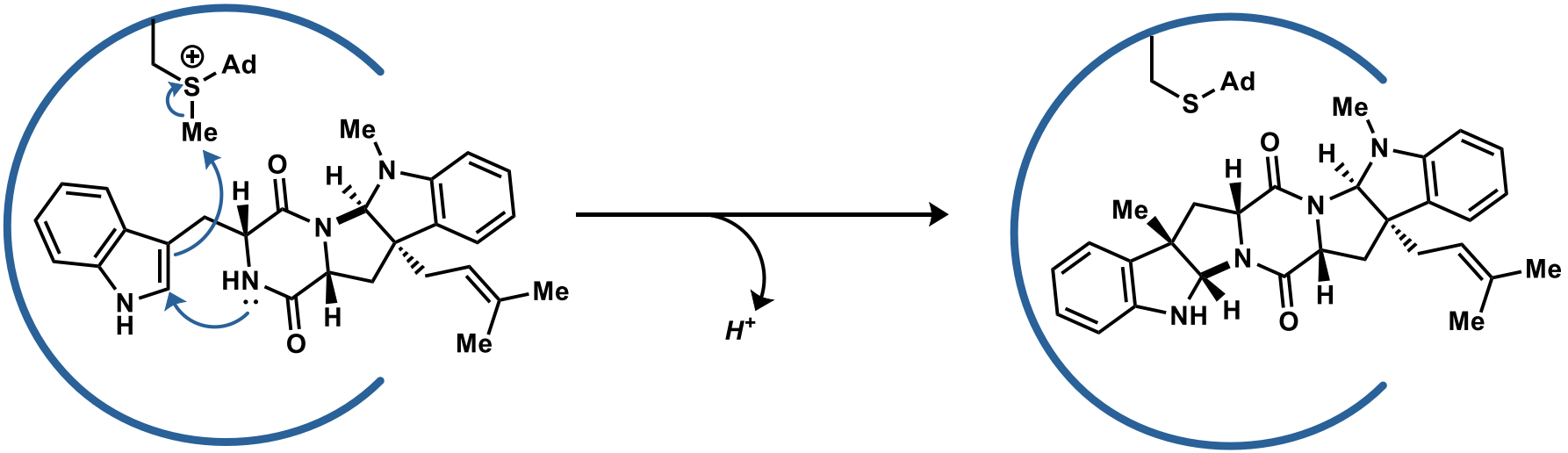
*NozMT active site in complex with substrate **4***

NozMT SAM-Dependent Methyltransferase

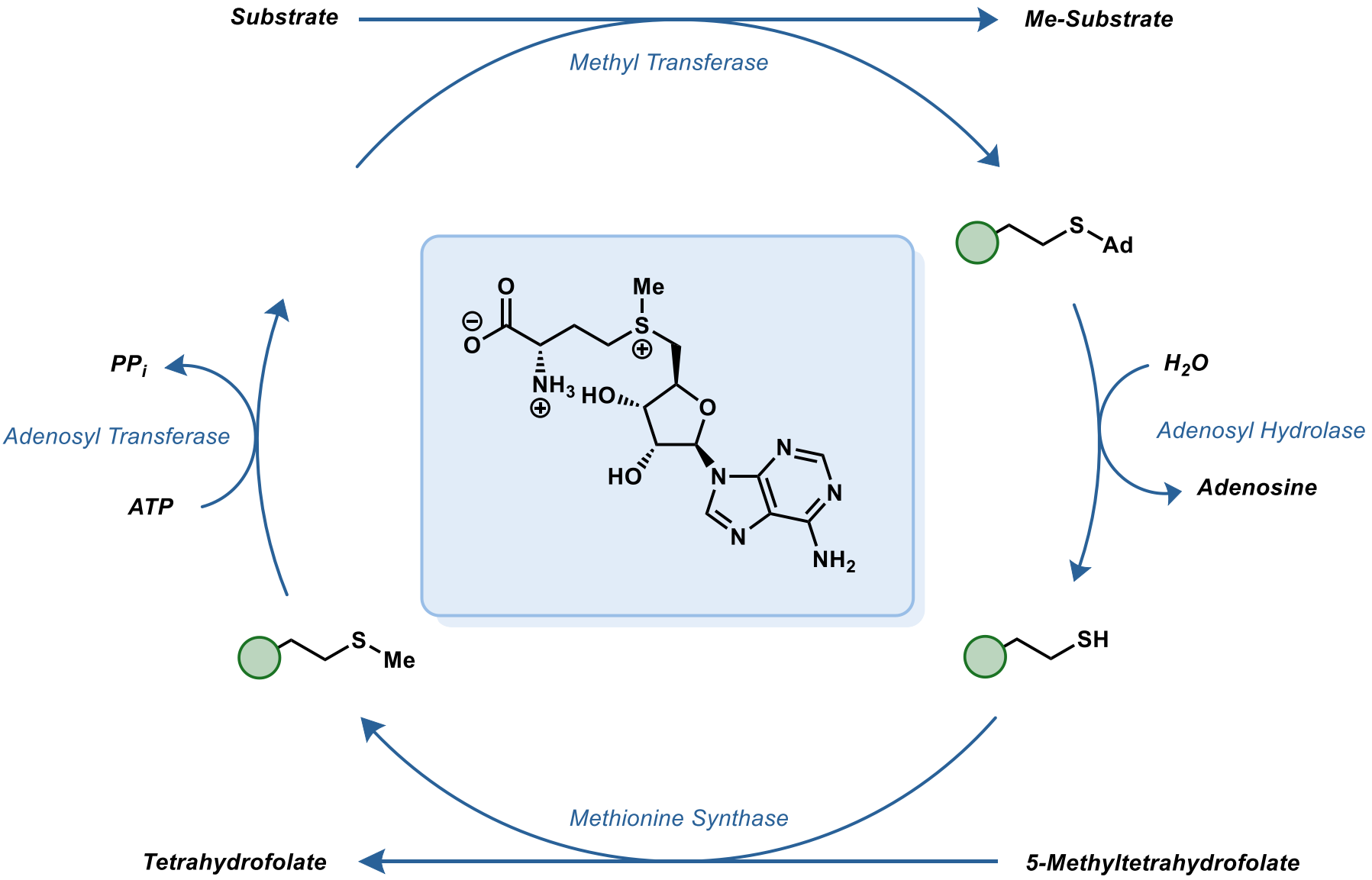


(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*
prenyltransferase product

(6) *Nocardioazine B*
methyltransferase product II



S-Adenosyl Methionine (SAM) Cofactor Regeneration



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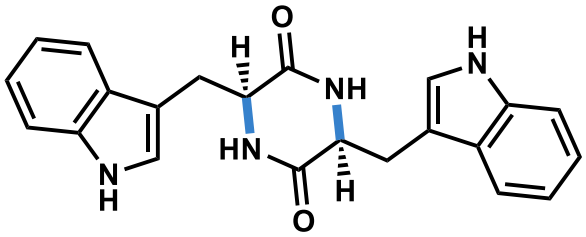
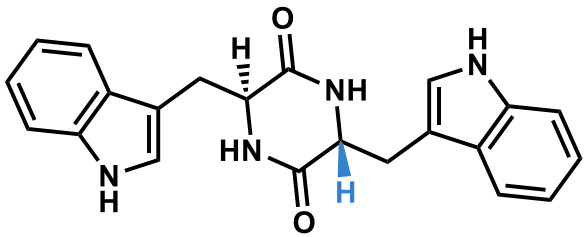
Conclusions



(1) *cyclo-L-Trp-Trp*

from tryptophanyl-tRNA^{Trp} via ***NozA or NcdA CDPS***

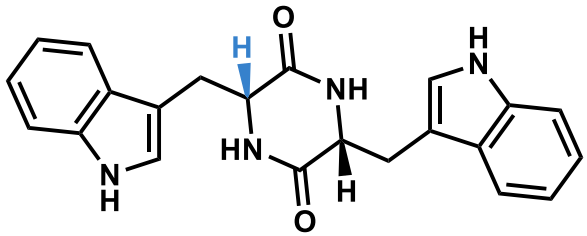
NozR



(2) *cyclo-D-L-Trp-Trp*

isomerase product I

NozR

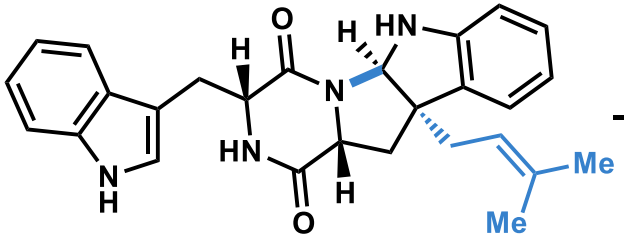


(3) *cyclo-D-D-Trp-Trp*

isomerase product II

NozPT

DMAPP

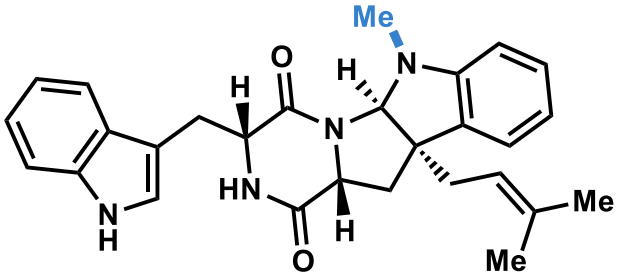


(4) *cyclo-D-Trp-C3'-prenyl-D-Trp*

prenyltransferase product

NozMT

SAM

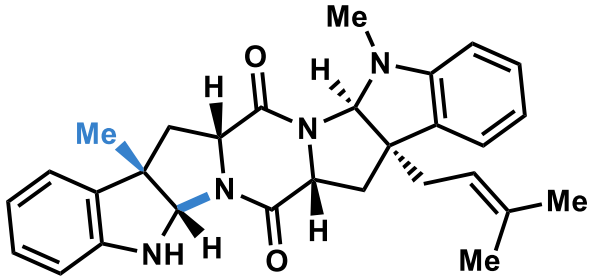


(5) *cyclo-D-Trp-N1'-methyl-C3'-prenyl-D-Trp*

methyltransferase product I

NozMT

SAM



(6) *Nocardioazine B*

methyltransferase product II