

Fighting the Resistance:

Controlling Antibacterial Activity with Visible Light

Lluita contra les resistències: control de l'activitat antibacteriana amb llum visible

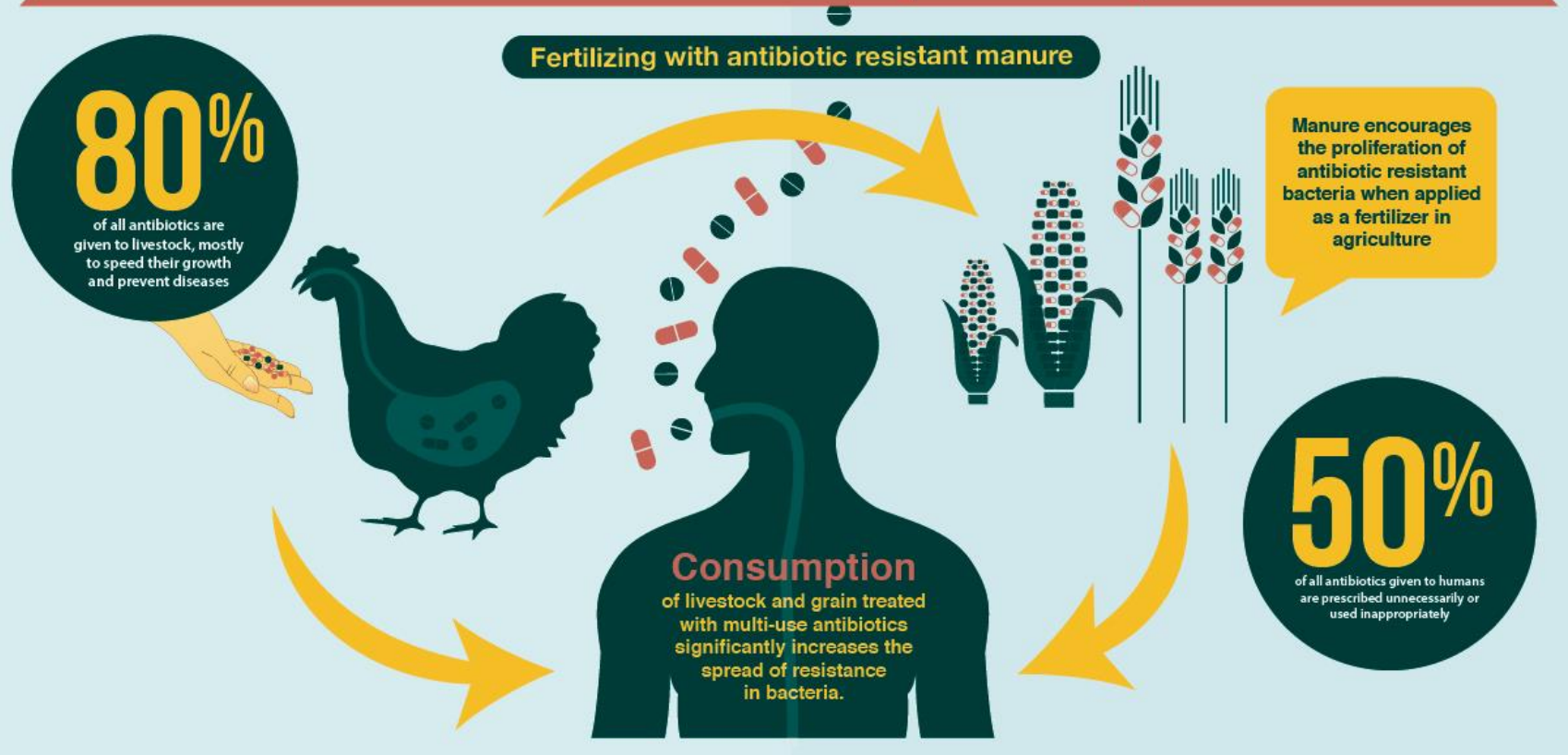


Dr. Xavier Just Baringo
28 d'abril de 2022

ANTIBIOTIC RESISTANCE

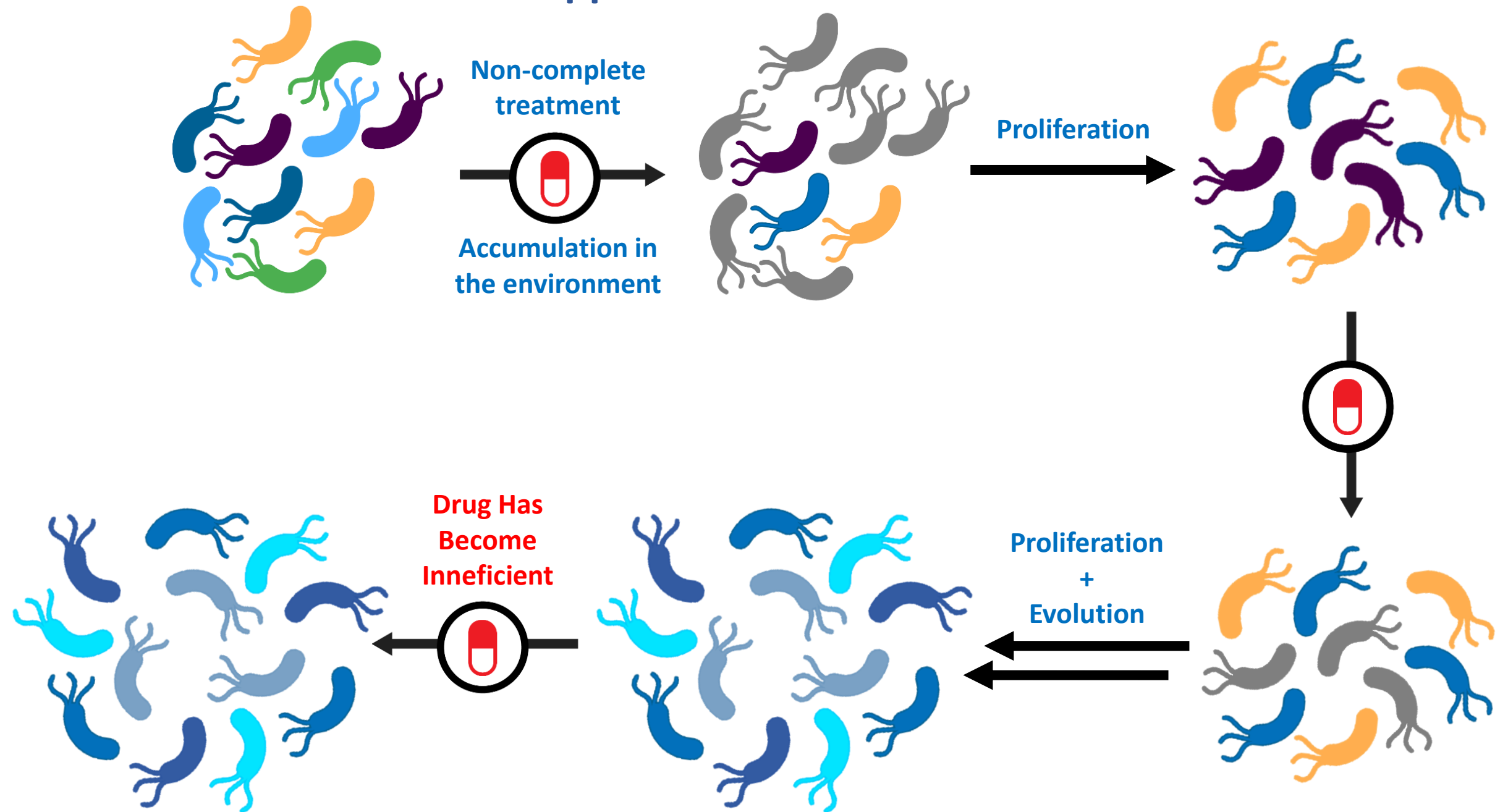
Will Kill More People Than Cancer and Diabetes Combined By 2050

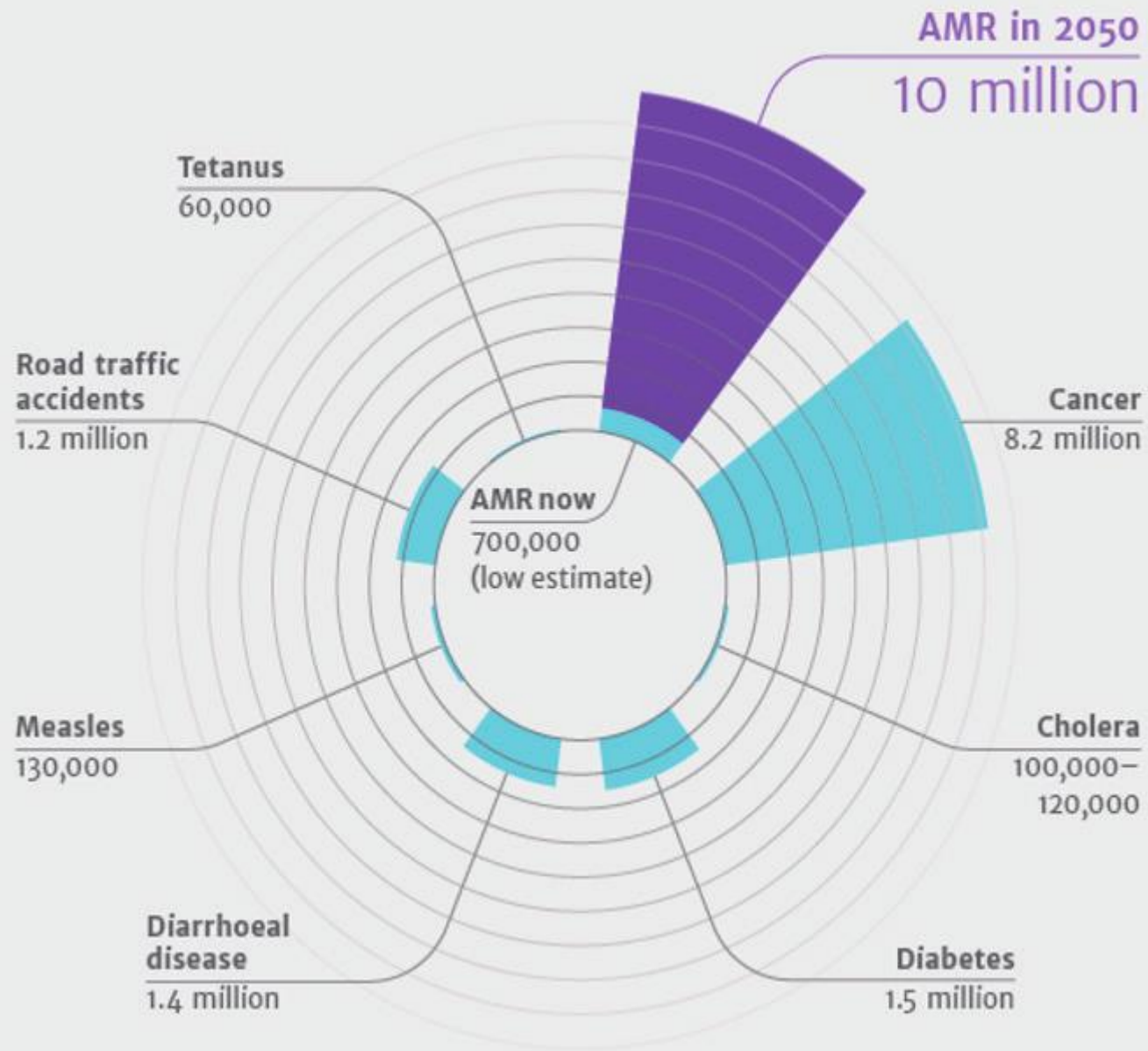
How Resistance Develops and Spreads



**More than 750,000 deaths a year worldwide caused by resistant bacteria.
Estimated to raise to 10,000,000 deaths by 2050.**

Appearance of Resistances

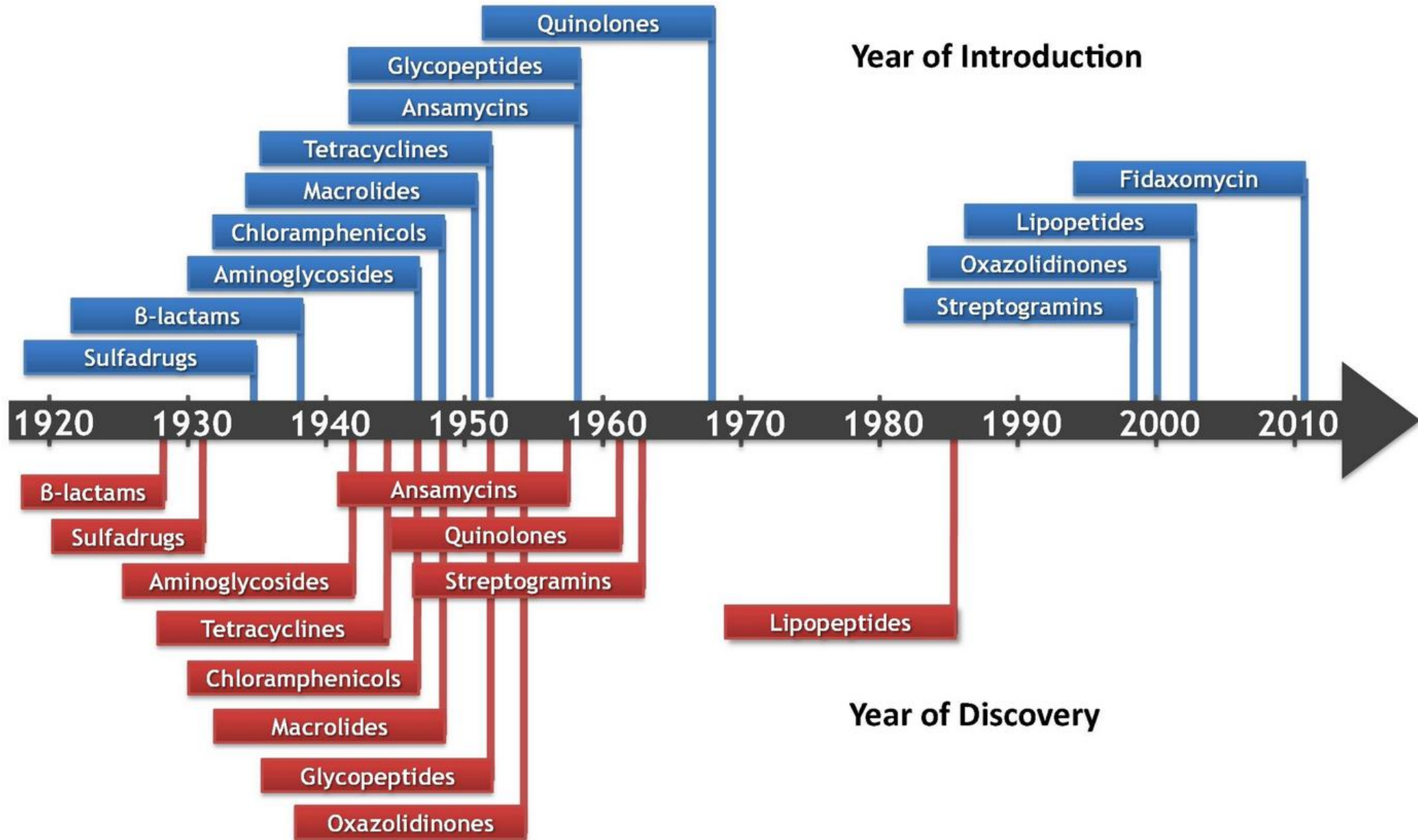




Estimated annual deaths by AMR in 2050
VS.
Other major causes of death.

Review on Antimicrobial Resistance. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations. London, UK: Wellcome Trust; **2014**. p. 20.

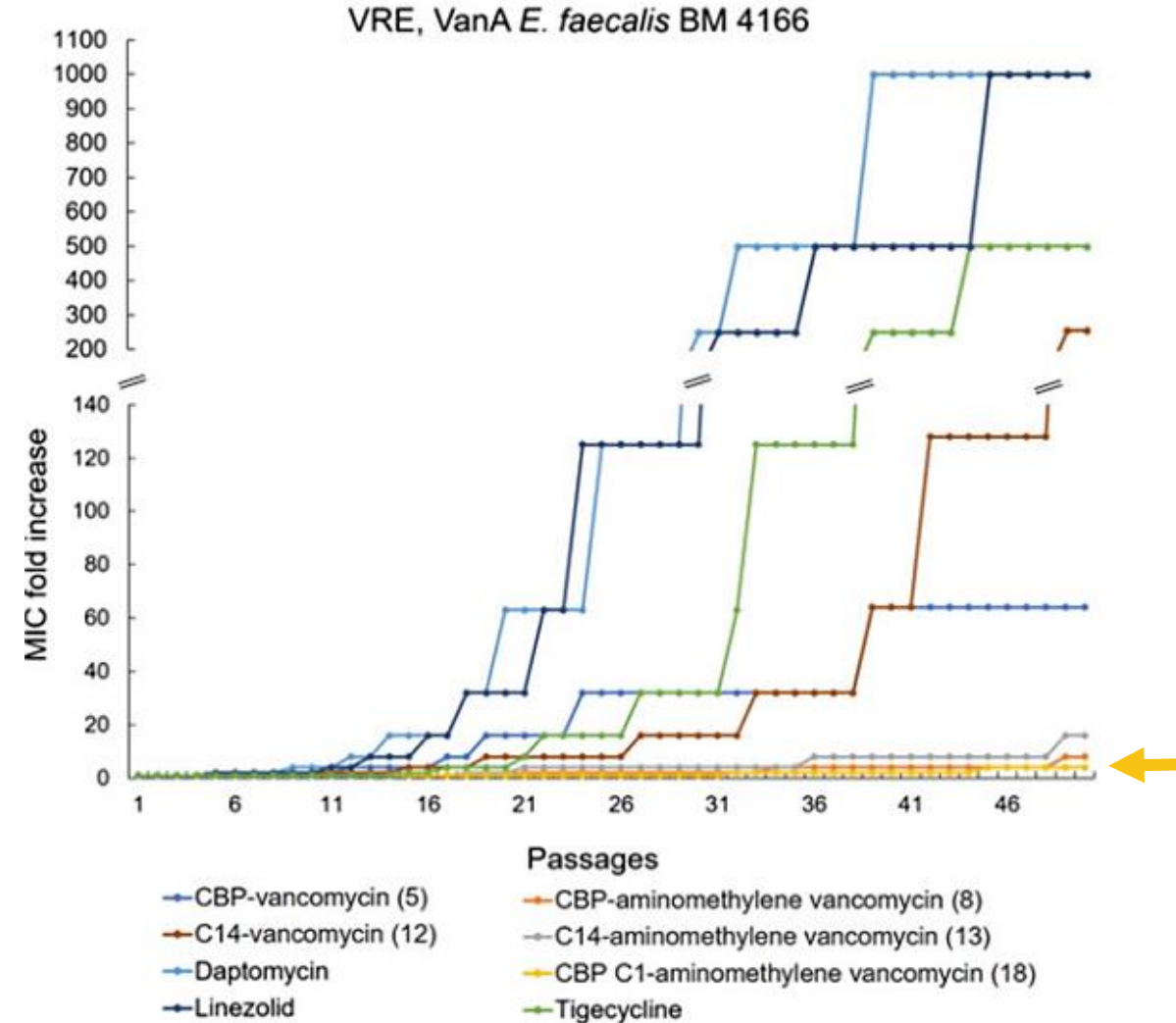
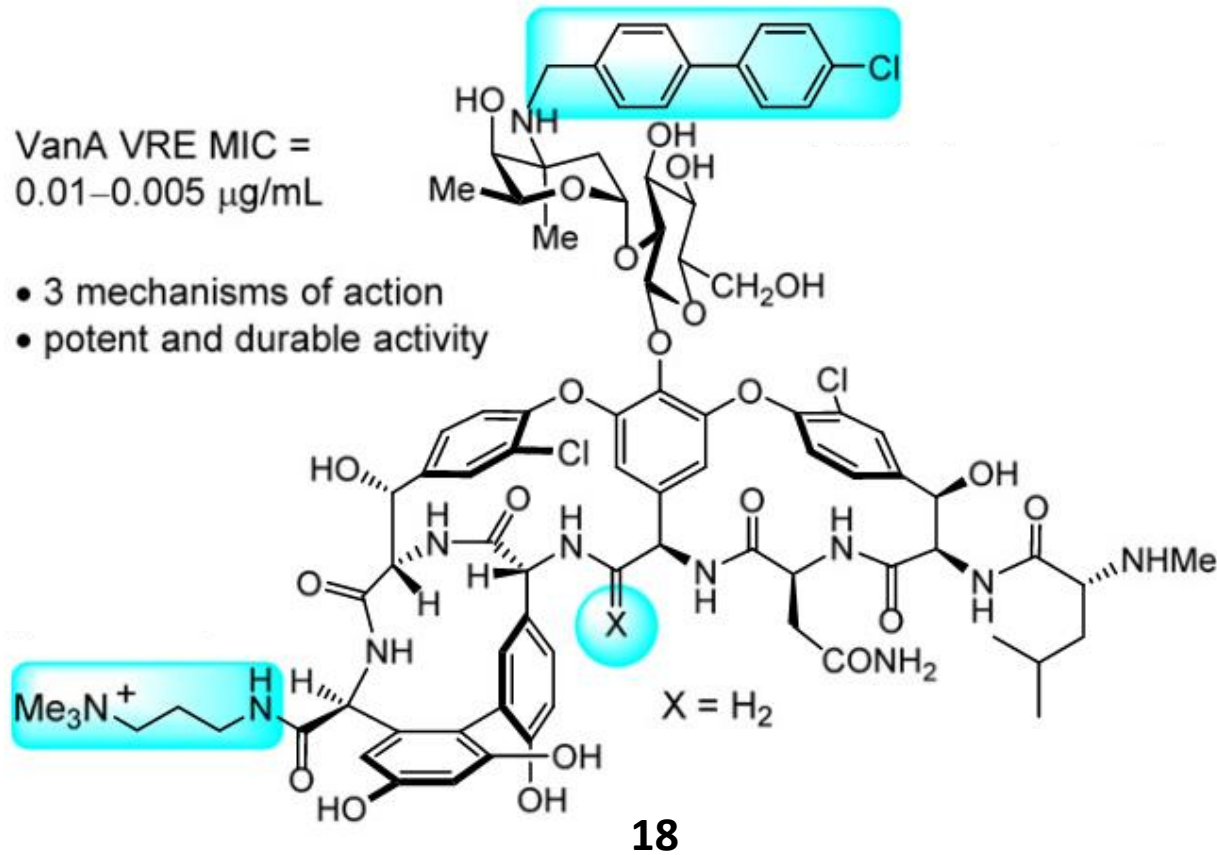
On the Hunt for New Antibiotics



Engineered Antibiotics!

VanA VRE MIC =
0.01–0.005 $\mu\text{g}/\text{mL}$

- 3 mechanisms of action
- potent and durable activity



“Peripheral Modifications with Added Synergistic Mechanisms of Action Provide Durable and Potent Antibiotics”

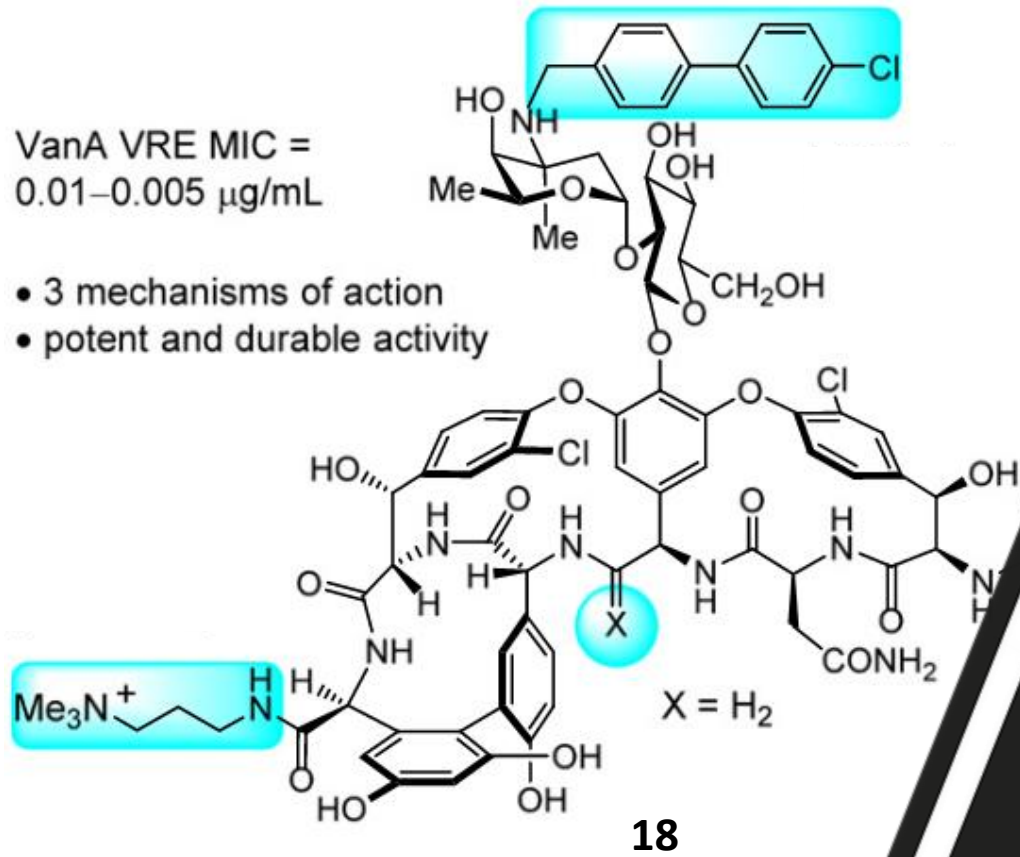
Boger and co-workers *Proc. Natl. Acad. Sci. U.S.A.* **2017**, E5052.

Engineered An

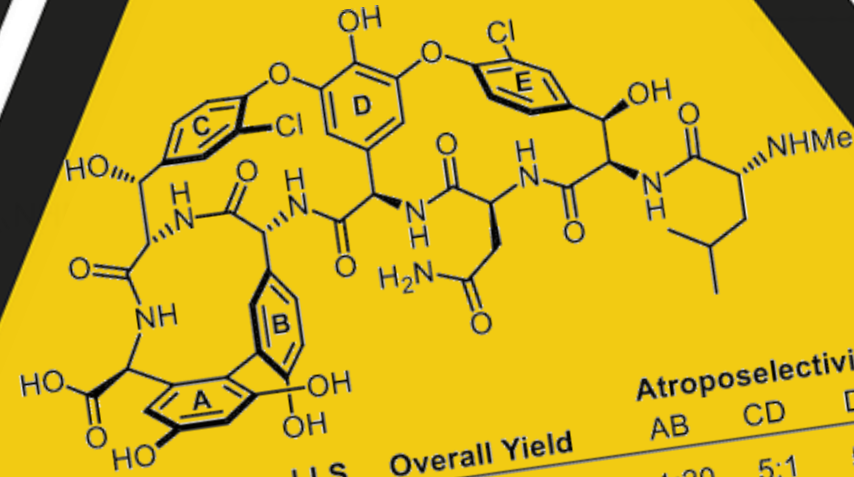
VanA *E. faecalis* BM 4166

VanA VRE MIC =
0.01–0.005 µg/mL

- 3 mechanisms of action
- potent and durable activity



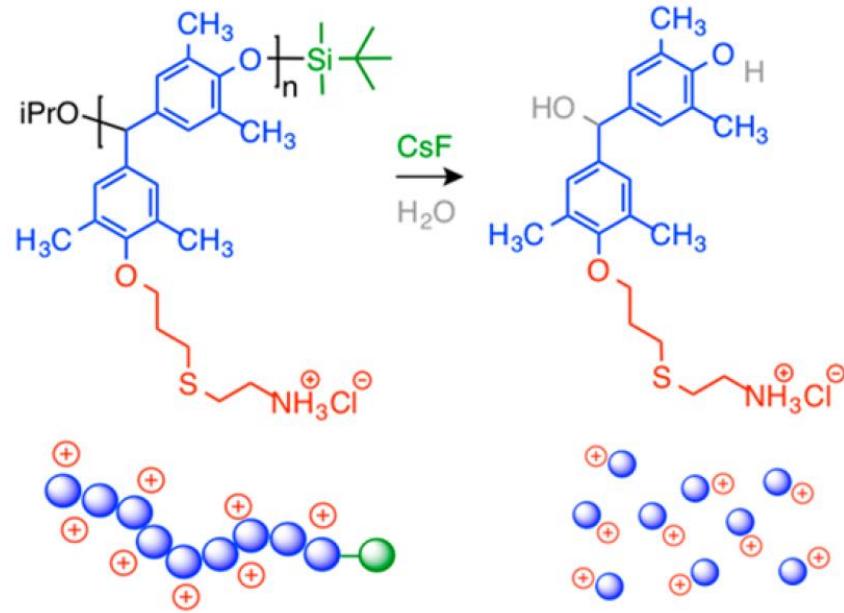
Total synthesis
of the aglycon
(efficient access
to engineered
antibiotics?)



Total Syntheses	LLS	Overall Yield	Atroposelectivity		
			AB	CD	DE
Evans (1998)	32	0.4%	<1:20	5:1	5:1
Nicolaou (1998)	24	0.05%	2:1	1:1	1:3
Boger (1999)	25	0.2%	1:1	1:1	8:1
Boger (2020)	17	5%	>20:1	8:1	14:1

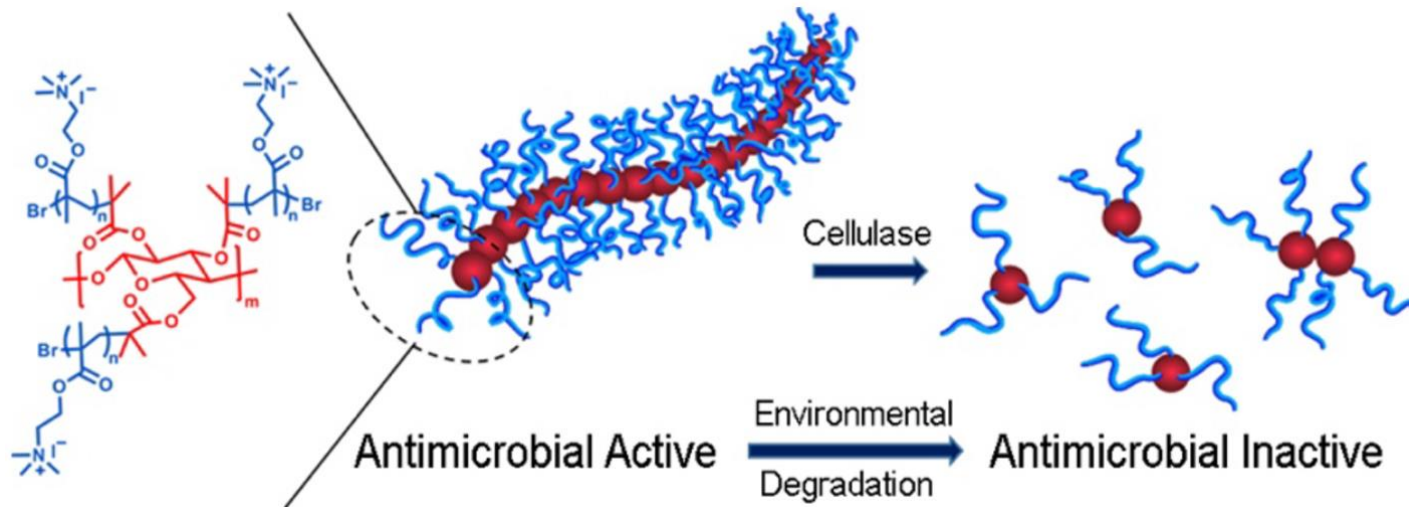


Paradigm Change: Self-Immolative Antimicrobials



“Cationic Polybenzyl Ethers as Self-Immolative Antimicrobial Polymers”

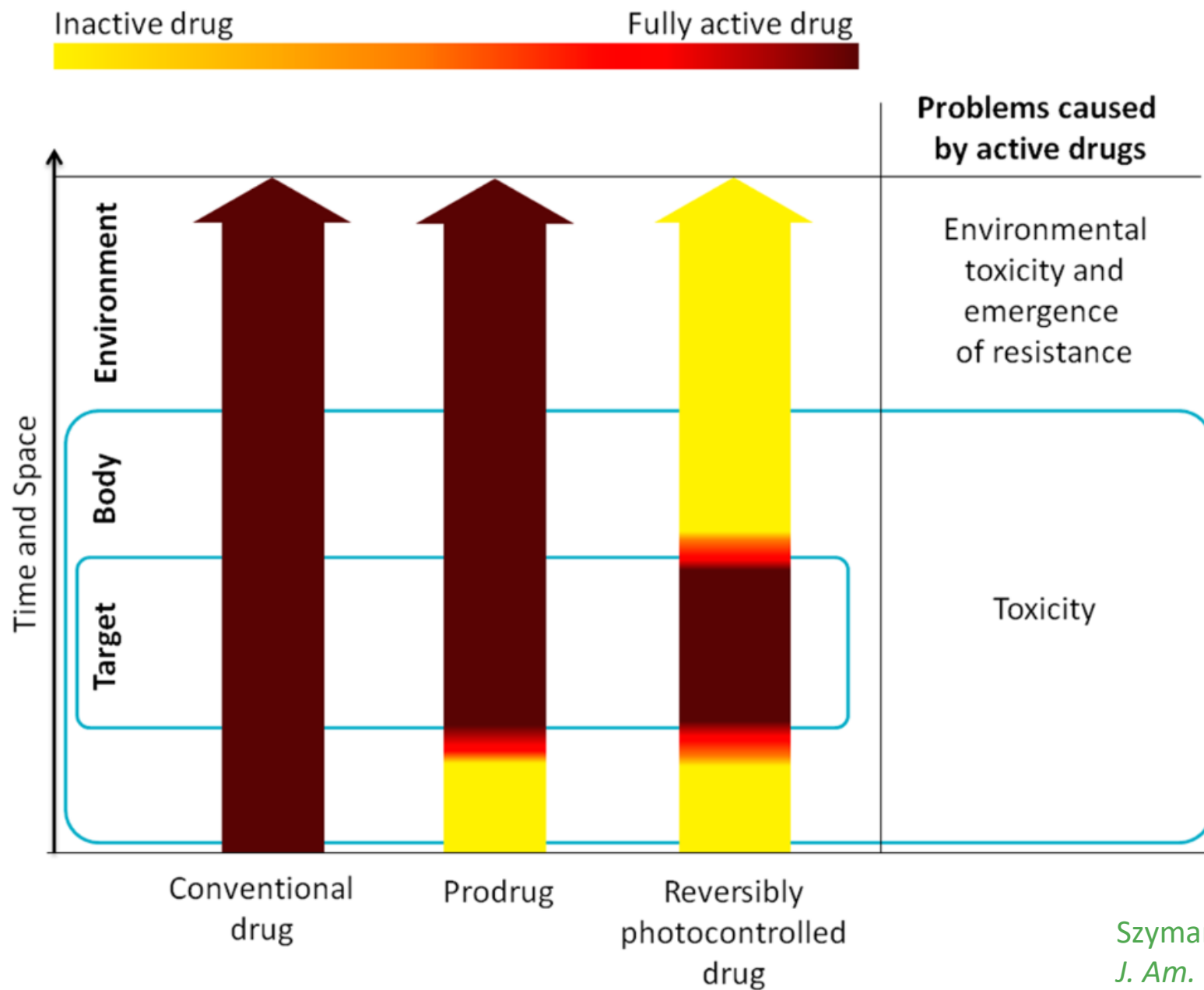
Palermo and co-workers
Biomacromolecules **2017**, *18*, 3400.



“Built in Deactivation Switch”

Liang and co-workers
Biomacromolecules **2020**, *21*, 2187.

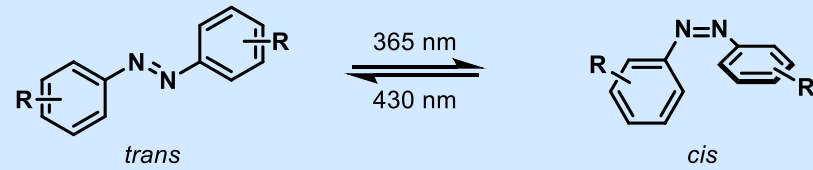
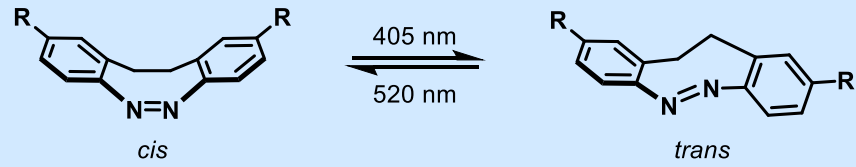
Photopharmacology



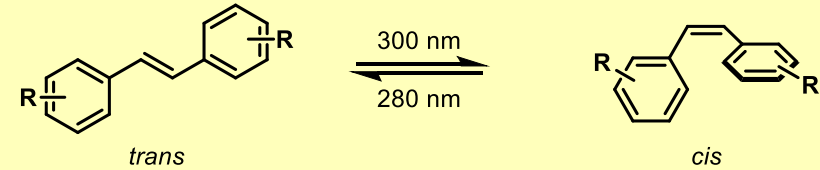
Azobenzenes and Other Photoswitches

cis/trans isomerisations

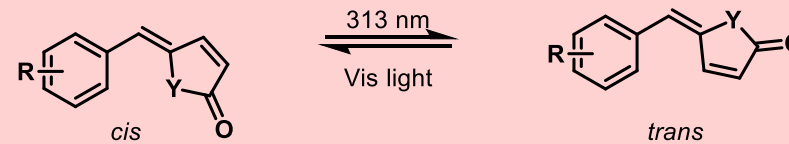
Azobenzenes



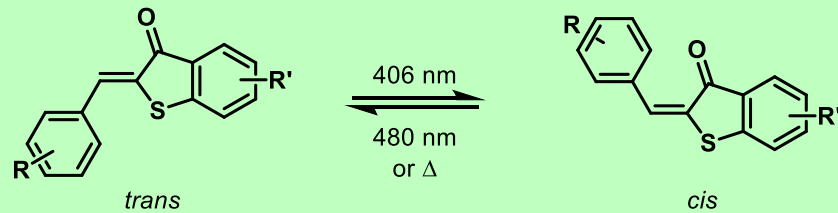
Stylbenes



Other

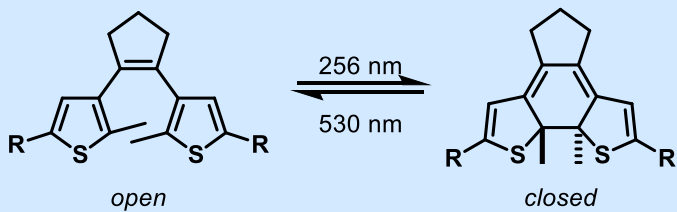


Hemithioindigo

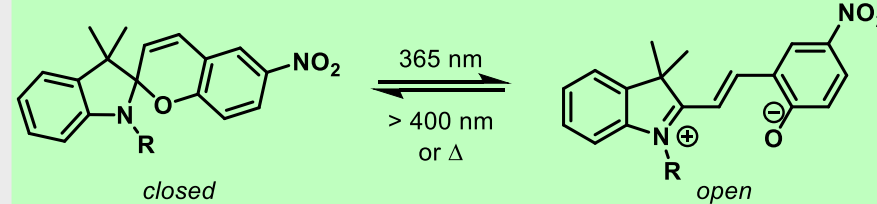


open/closed isomerisations

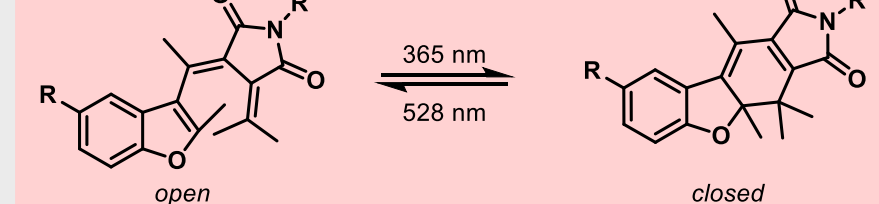
Diarylethanes



Spiroyrans

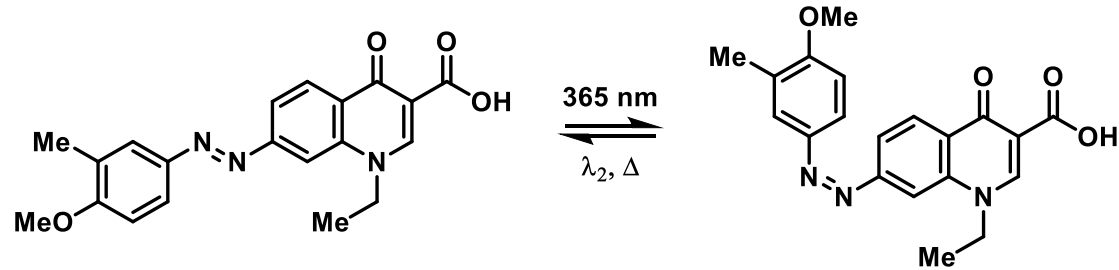


Fulgimide



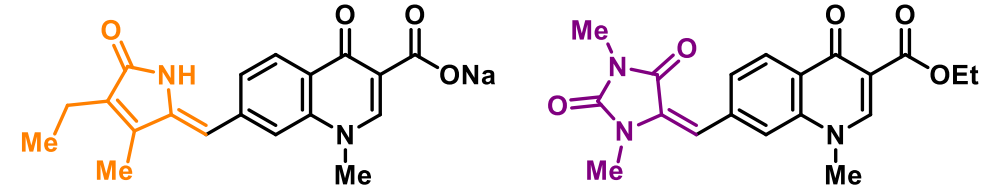
Photoswitchable Antibiotics

Photoswitchable Quinolones: the First Light-Regulated Antibiotics:



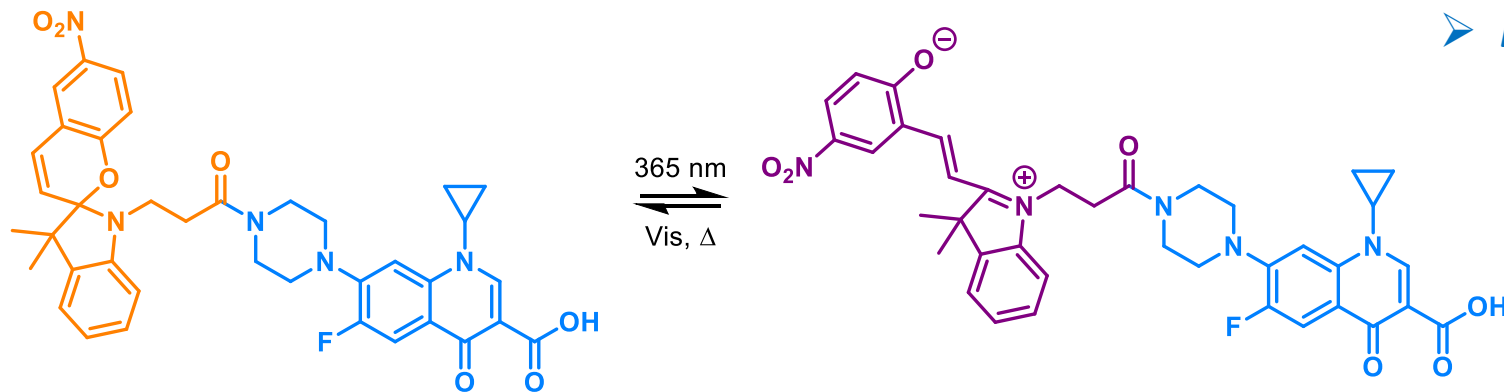
Feringa and co-workers
Nat. Chem. **2013**, 5,
924.

- Up to 3-fold increase in potency upon irradiation.



Sampedro and co-workers:
Eur. J. Org. Chem. **2017**, 4719.

Engineered Antibiotic:



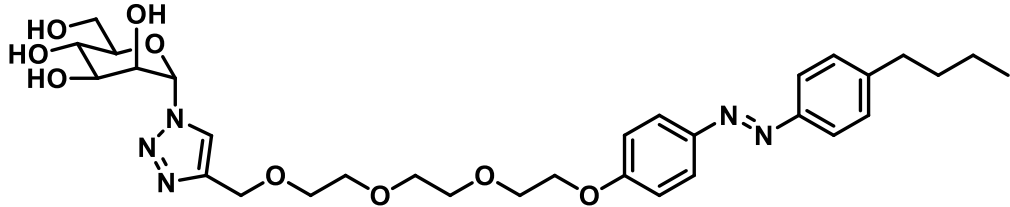
- Low isomerisation ratios.

Feringa and co-workers
Bioconjugate Chem. **2015**, 26, 2592.

- Only up to 1-fold change in potency between irradiated and not-irradiated compound.
- An azobenzene photoswitch did not allow control of antibacterial activity in this case.

Photoswitchable Antibiotics

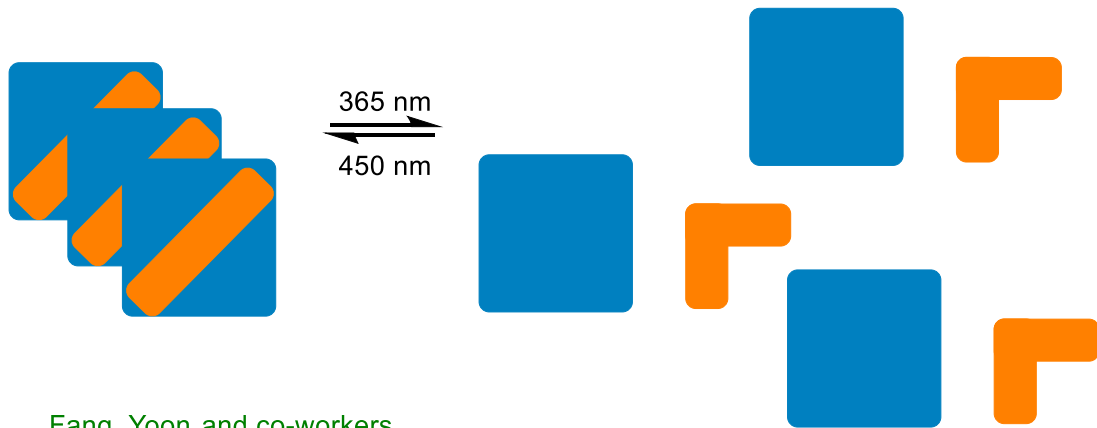
Photoswitchable Carbohydrate-Based Surfactants:



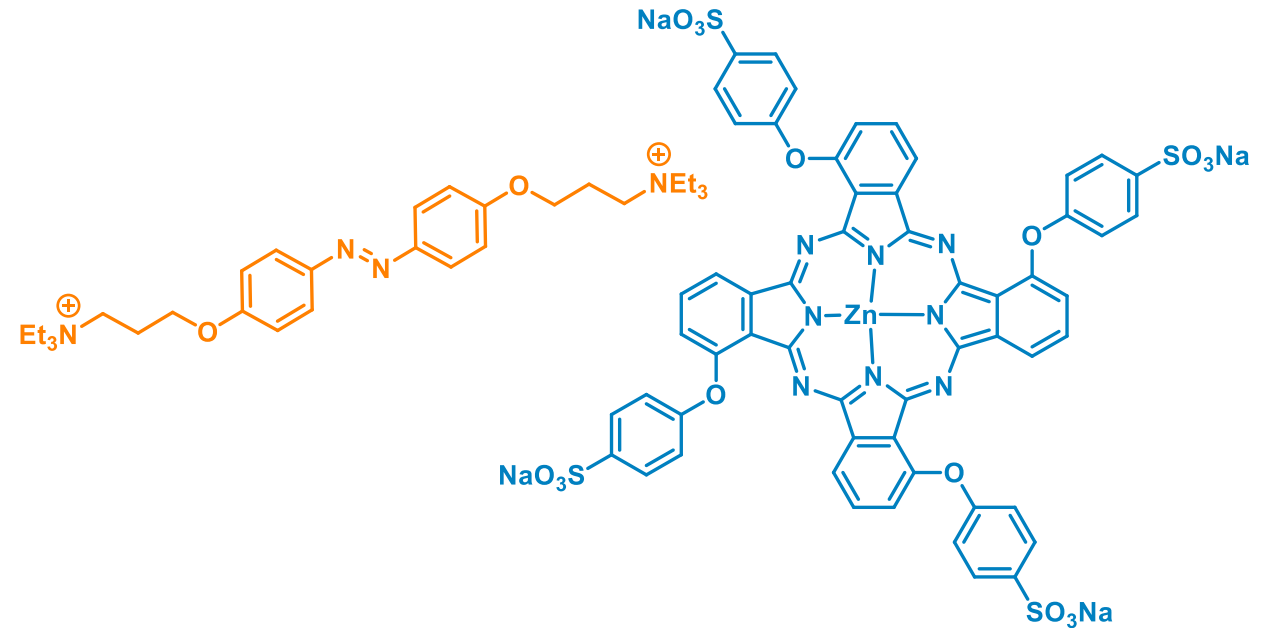
Bansal, Wilkinson and co-workers
Aust. J. Chem. **2015**, 68, 1880.
Chem. Sci. **2016**, 7, 6628.

- **Biofilm growth modulation (strain dependent).**
- **Limited impact of isomerisation.**

Photoswitchable assembly of nanoparticles:



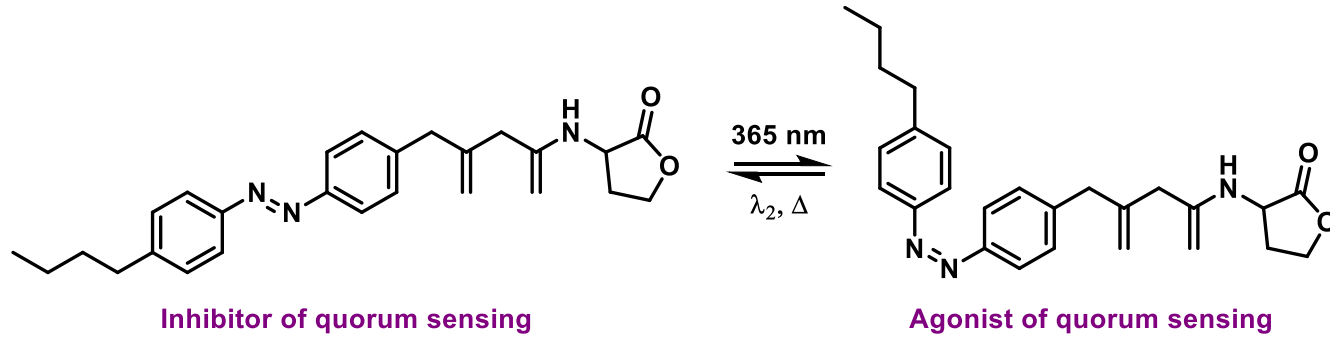
Fang, Yoon and co-workers
Chem. Commun. **2019**, 55, 12316.



- **Molecules stack when azobenzene is in trans configuration.**
- **Light-mediated disassembly triggers generation of ROS (reactive oxygen species, such as $^1\text{O}_2$).**

Photoswitchable Antibiotics

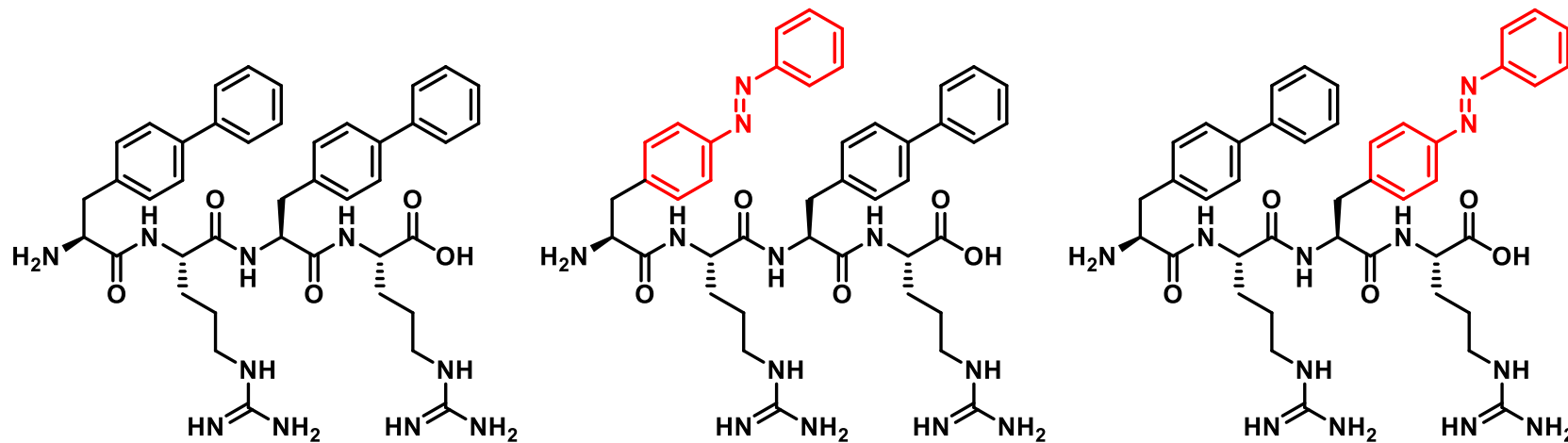
Quorum sensing regulation:



Feringa and co-workers
Chem. **2019**, 5, 1293.

- Light-induced configurational change swaps between inhibitory or agonist activity.
- 700-fold change in activity.

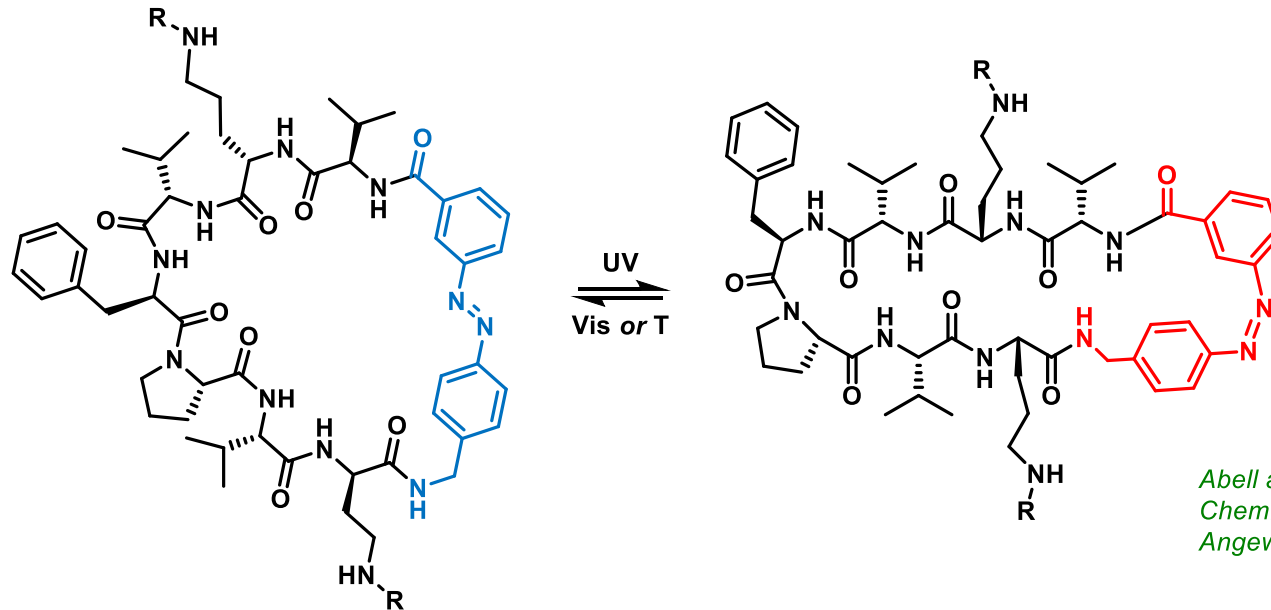
Photoswitchable short peptide antibiotics:



Abell and co-workers:
ChemMedChem **2020**, 15, 1505.

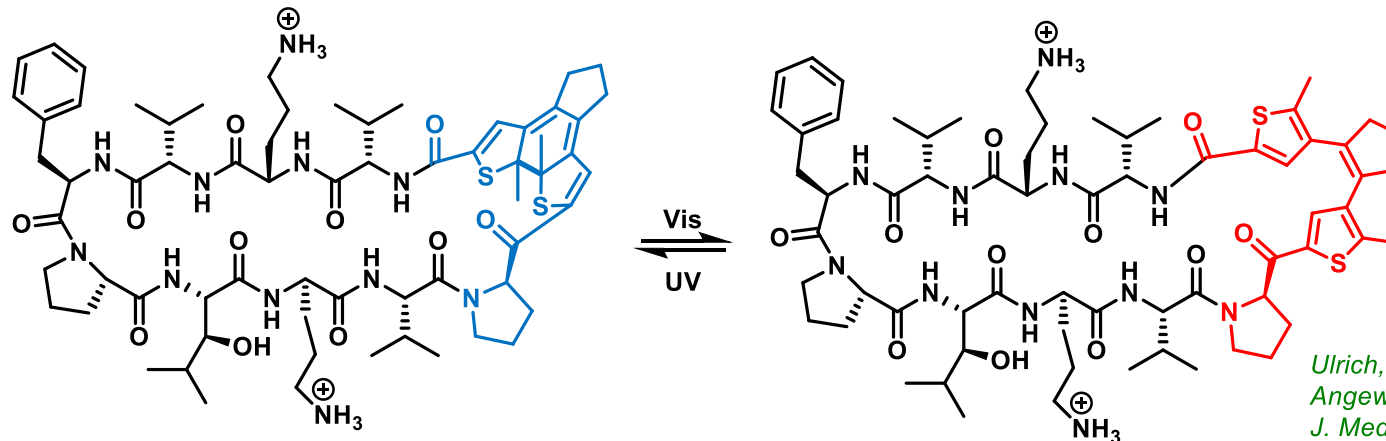
- 3-fold potency increase upon activation.

Photoswitchable Gramicidins



Abell and co-workers:
ChemBioChem **2018**, 19, 2591.
Angew. Chem. Int. Ed. **2020**, 59, 22554.

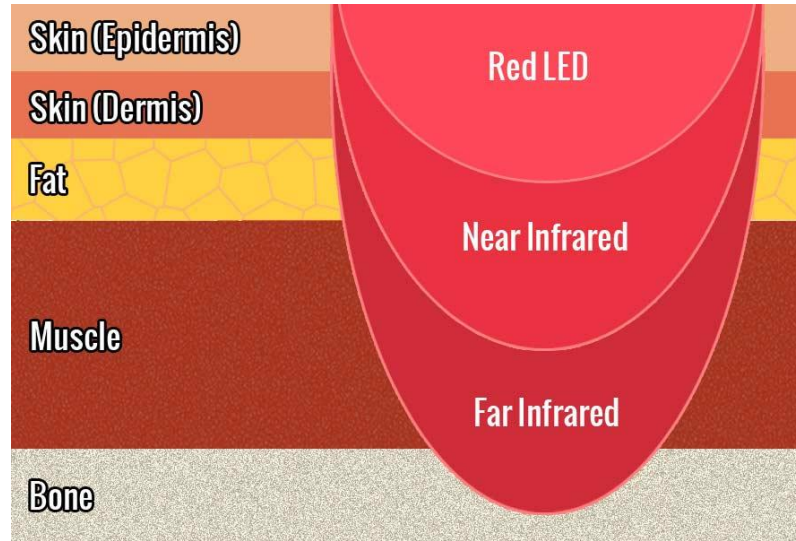
- **UV light needed for activation.**
- **Great loss of activity and low trans/cis discrimination.**
- **Deactivates within minutes on its own.**



Ulrich, Komarov et al.
Angew Chem. Int. Ed. **2014**, 53, 3392.
J. Med. Chem. **2018**, 61, 10793.

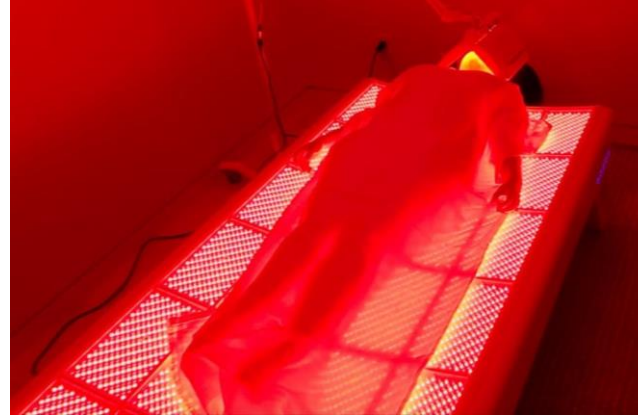
- **Non-Reversible Isomerisation under Thermal Conditions**
- **Requires UV irradiation for deactivation.**

Advantages of Visible Light Photoswitchable Drugs

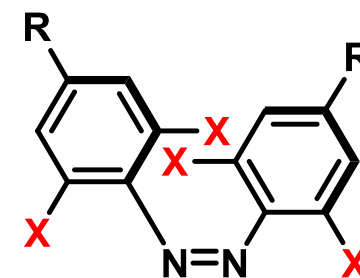
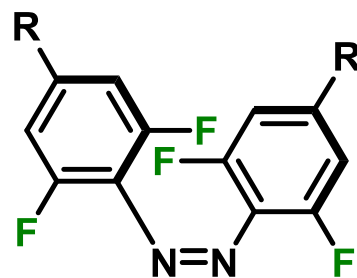
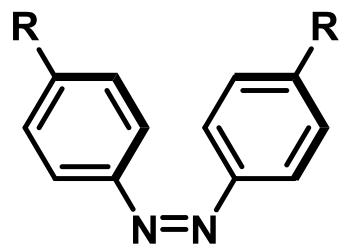
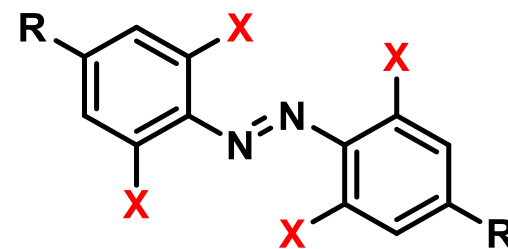
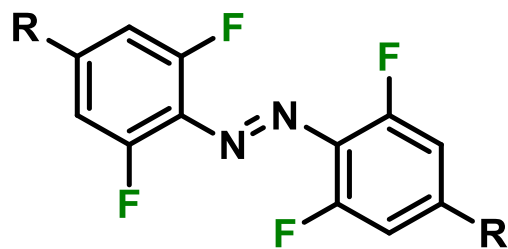
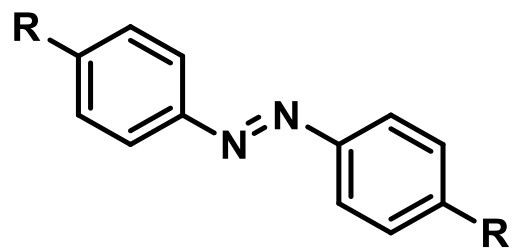


- ***Non-Harmful (as opposite to UV).***
- ***Red Light and IR penetrate tissue without harming it.***
- ***Sunlight is thoroughly available (can we use it?).***

Using Red Light



Use of red light is thoroughly used in cosmetic and therapeutic devices.



X = Cl, OMe



INACTIVE DRUG



ON



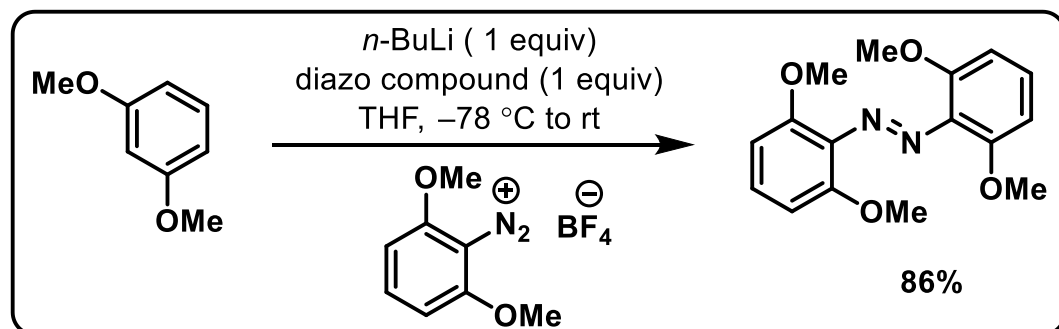
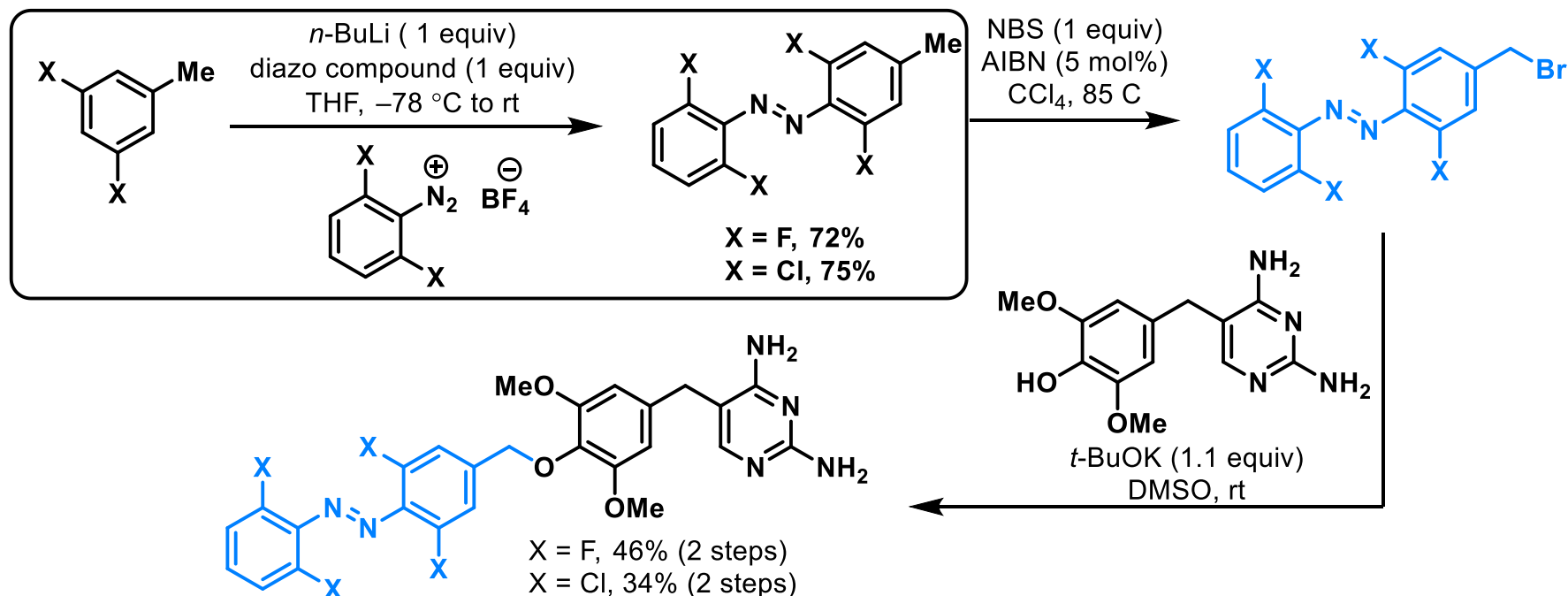
ACTIVE DRUG



OFF



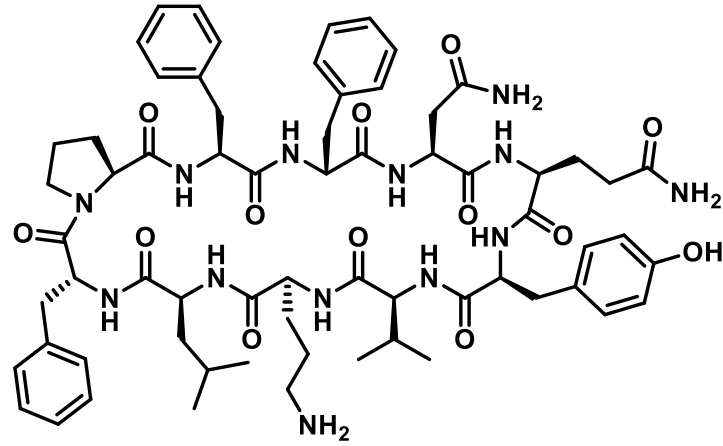
Visible Light Photoswitchable Antibiotics



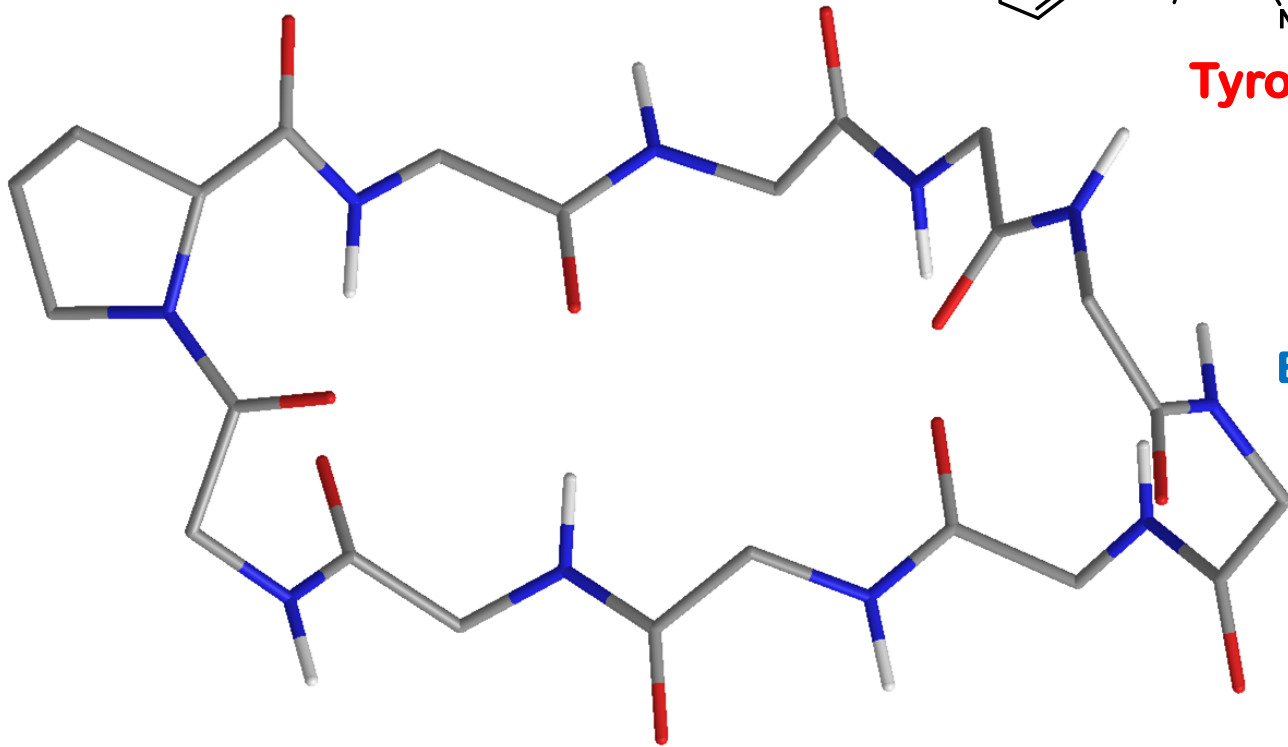
- **Breakthrough in tetra-ortho-substituted azobenzenes.**
- **Highly limited functional group tolerance.**

Feringa and co-workers:
J. Am. Chem. Soc. **2017**, 139, 17979.
Angew. Chem. Int. Ed. **2016**, 55, 13514.

Engineered Antibiotic



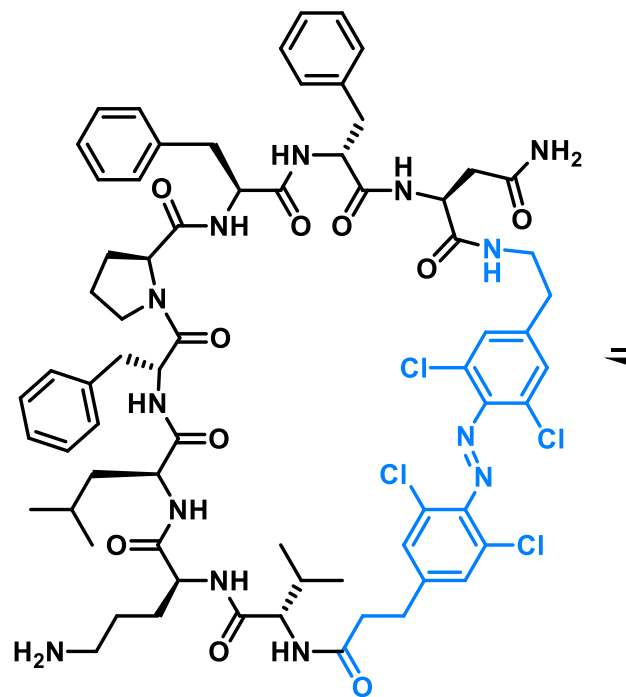
Tyrocidine A



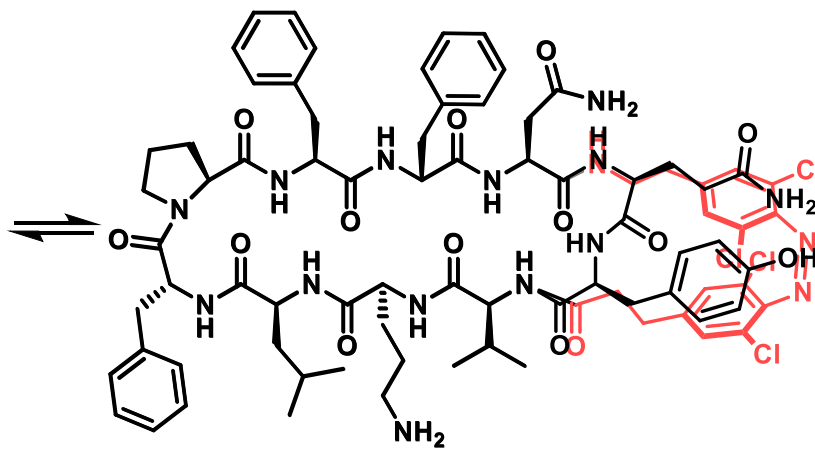
Backbone of Tyrocidine A from crystal structure

Biochim. Biophys. Acta 2014, 1838, 1199.

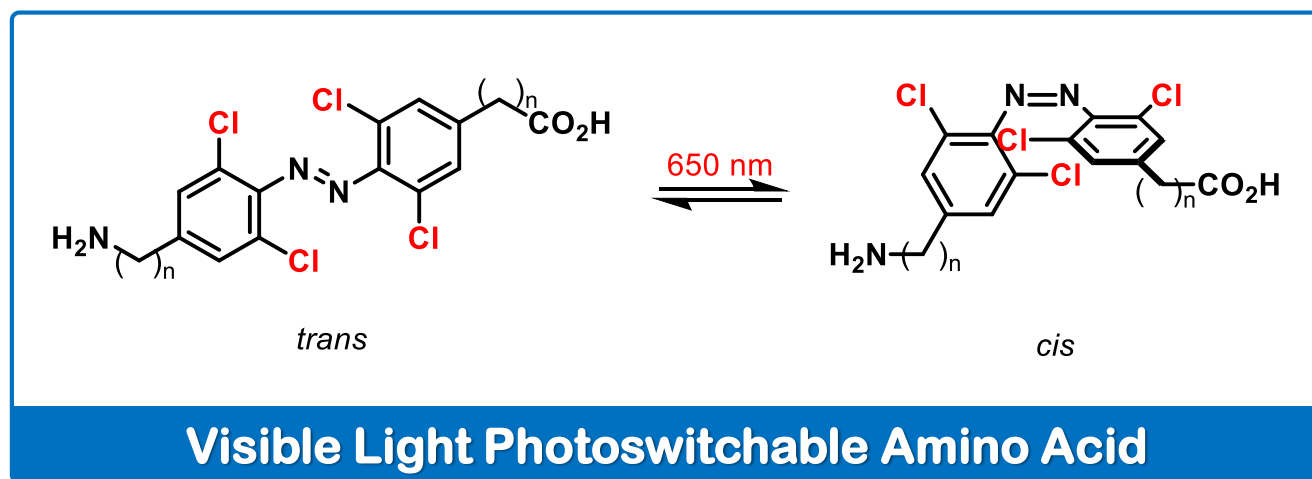
Engineered Antibiotic: Hypothesis



trans-Tyrocidine PS



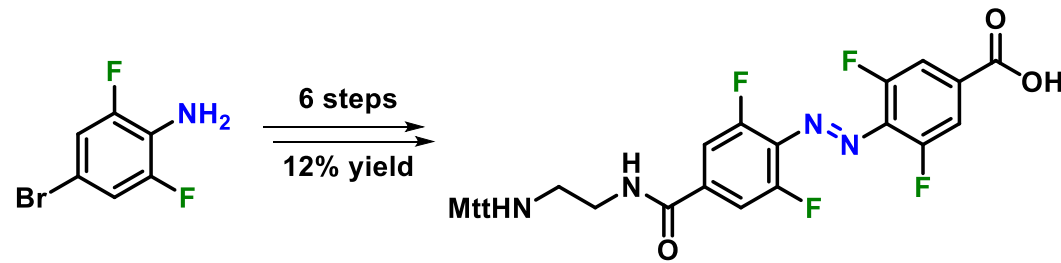
cis-Tyrocidine PS



Visible Light Photoswitchable Amino Acid

Visible Light-Operated Azobenzene Amino Acid(s)

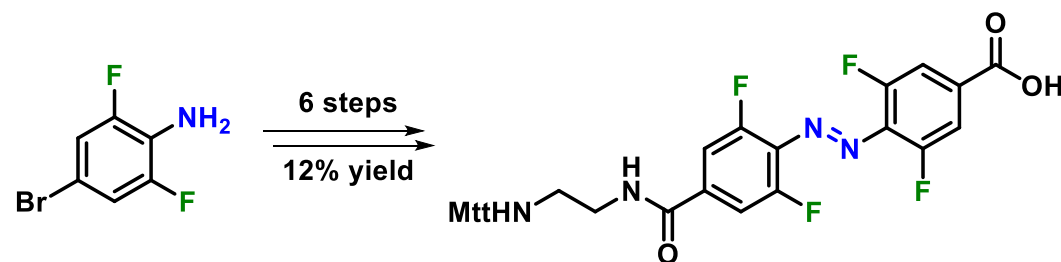
Only previous Visible Light-Operated Azobenzene Amino Acid:



Vázquez, O. and co-workers *ChemBioChem* **2019**, *20*, 1417.

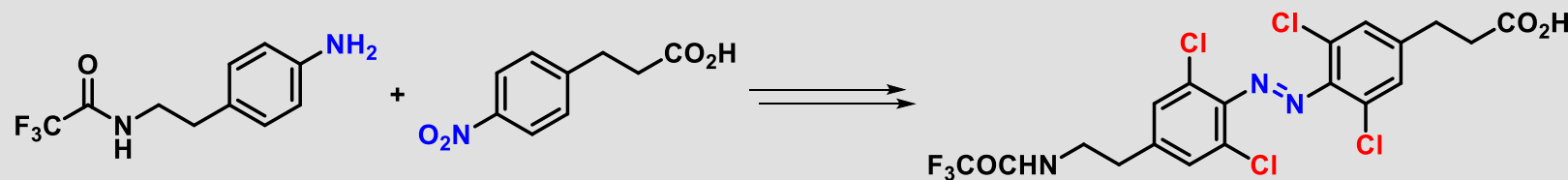
Visible Light-Operated Azobenzene Amino Acid(s)

Only previous Visible Light-Operated Azobenzene Amino Acid:



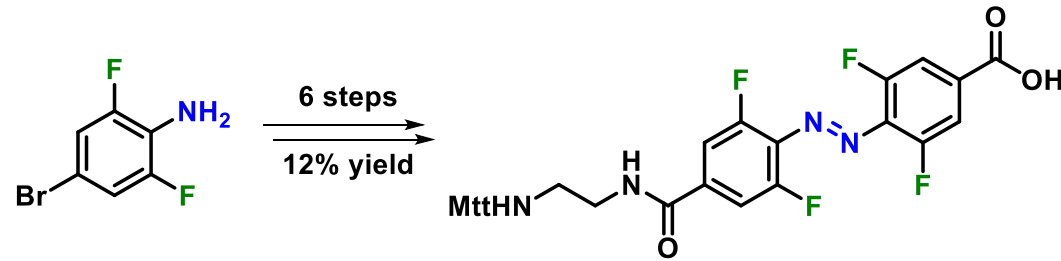
Vázquez, O. and co-workers *ChemBioChem* **2019**, *20*, 1417.

Our new Photoswitch:



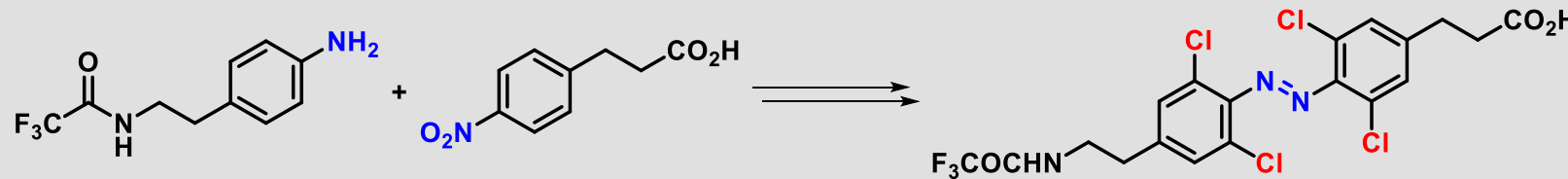
Visible Light-Operated Azobenzene Amino Acid(s)

Only previous Visible Light-Operated Azobenzene Amino Acid:

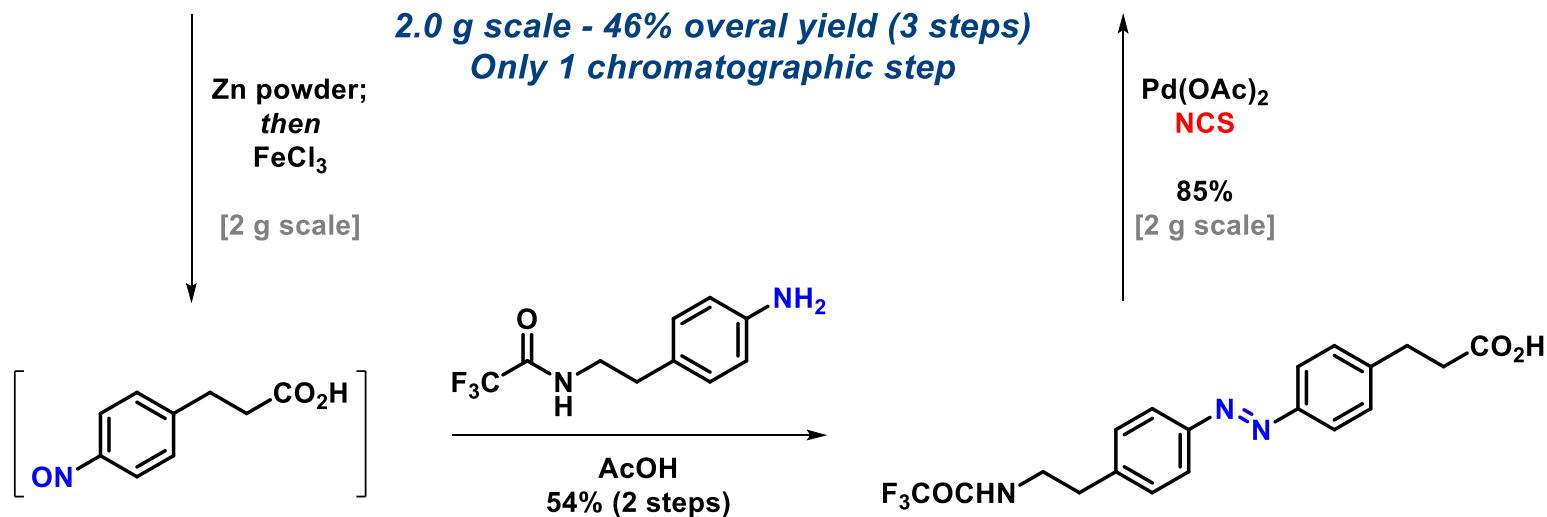


Vázquez, O. and co-workers *ChemBioChem* **2019**, *20*, 1417.

Our new Photoswitch:

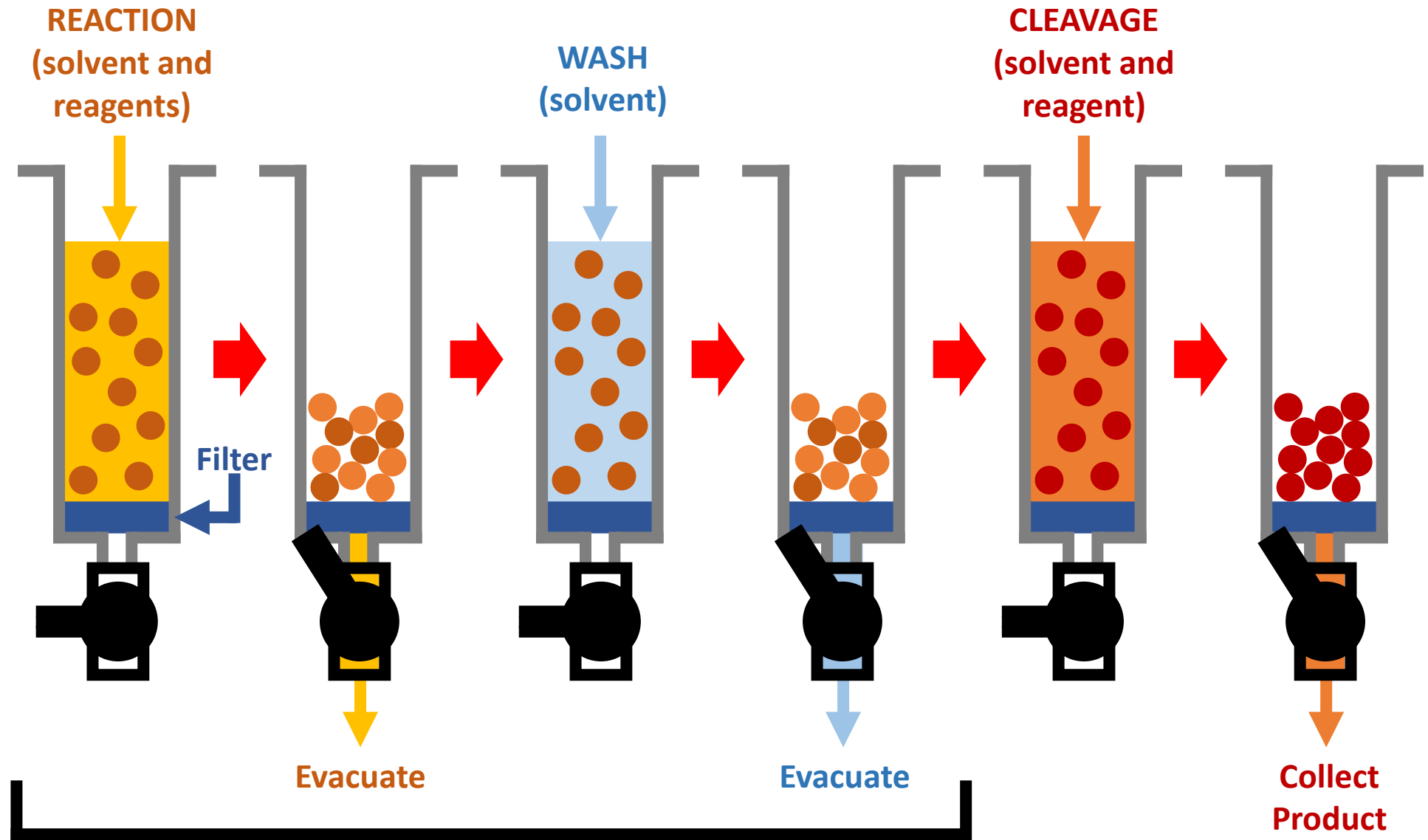


✓ Ready for SPPS.



Solid Phase Peptide Synthesis

● = Functionalised resin bead



ITERATIVE SYNTHESIS
(repeat for each reaction)

Automated Solid Phase Peptide Synthesis



vs.



vs.



Manual Synthesis
~2 h per amino acid
(coupling + deprotection)

10-Aa peptide in ~3 days

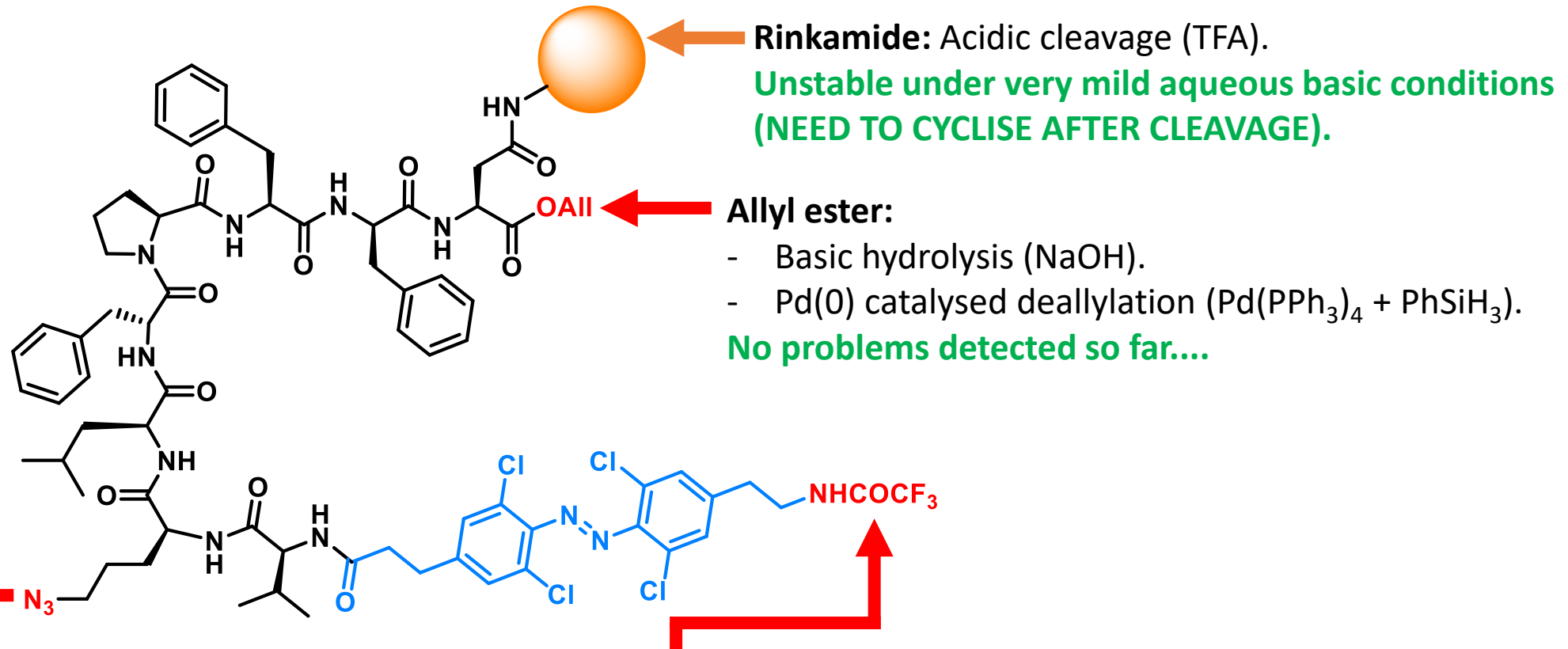
Automated Synthesis
~2 h per amino acid
(coupling + deprotection)

10-Aa peptide in ~1 day

Microwave-Assisted Synthesis
~20 min per amino acid
(coupling + deprotection)

10-Aa peptide in ~3 hours

Custom Made Strategy (AKA Wisdom through Challenge)



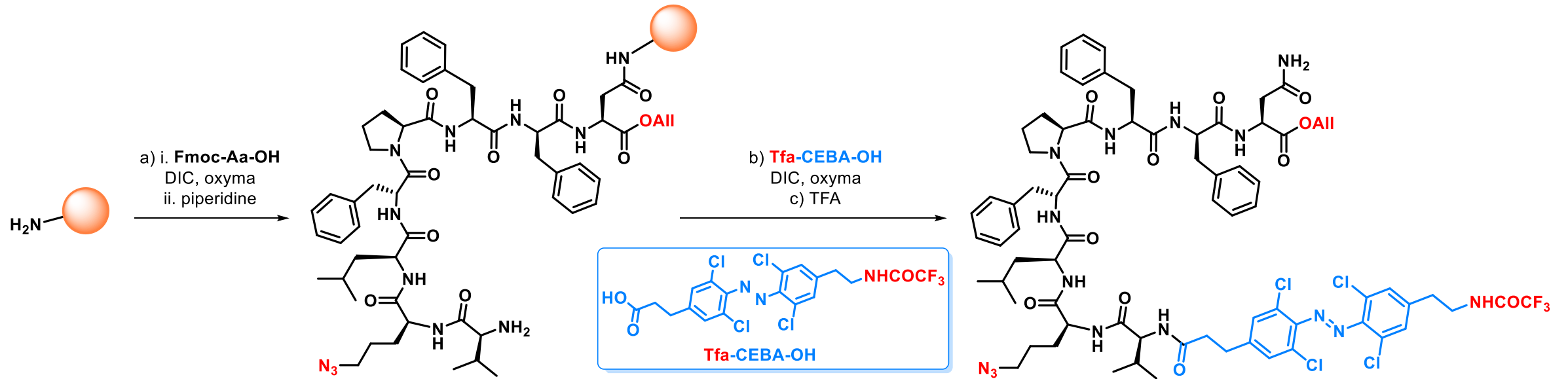
Azide: Mild Staudinger reduction (H₂O and PMe₃).

- Cannot use most common protecting group (Boc) if cyclisation on resin is not possible.
- No precedents of base-free Staudinger reduction on resin (and it didn't work!).
- Need to deprotect after cleavage and cyclisation.

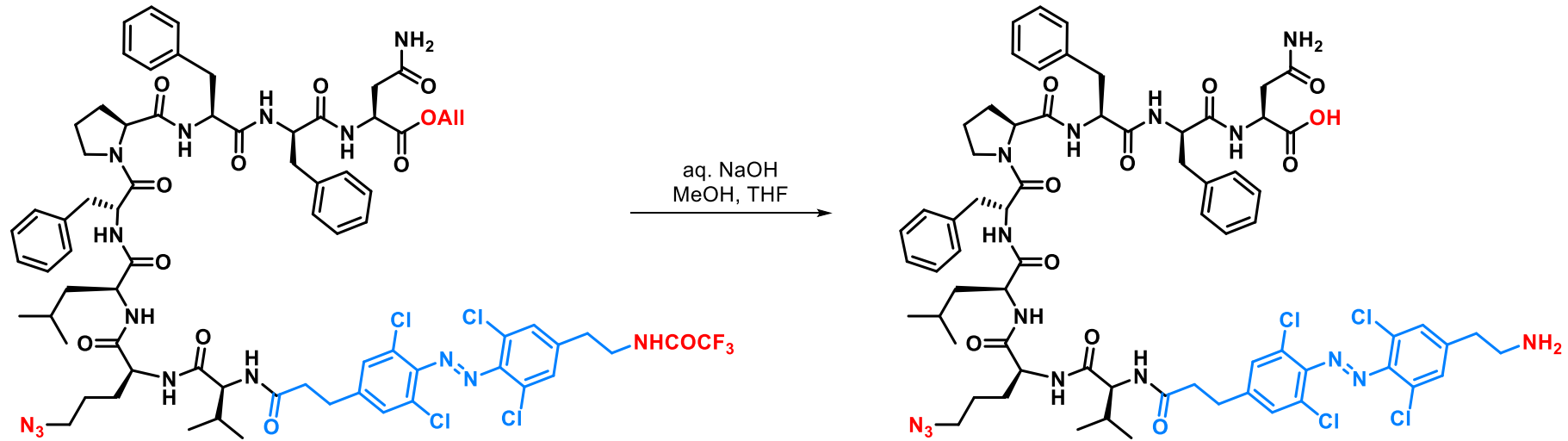
Trifluoroacetamide: Basic hydrolysis (NaOH).

- Cannot use Fmoc group (PIPERIDINE ATTACKS PHOTOSWITCH).
- Deprotection conditions incompatible with Rinkamide resin
(NEED TO CYCLISE AFTER CLEAVAGE).

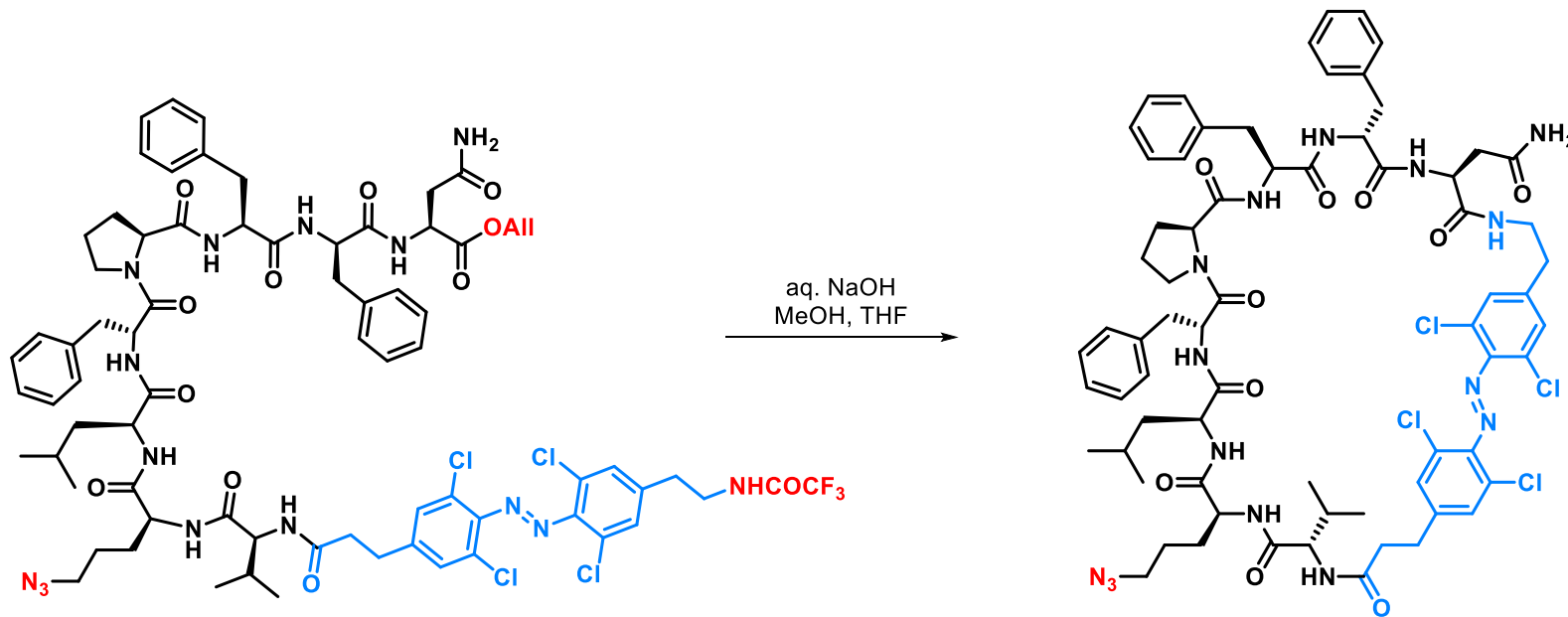
Synthesis of Photoswitchable Tyrocidine A



By-Product Formed During Basic Hydrolysis

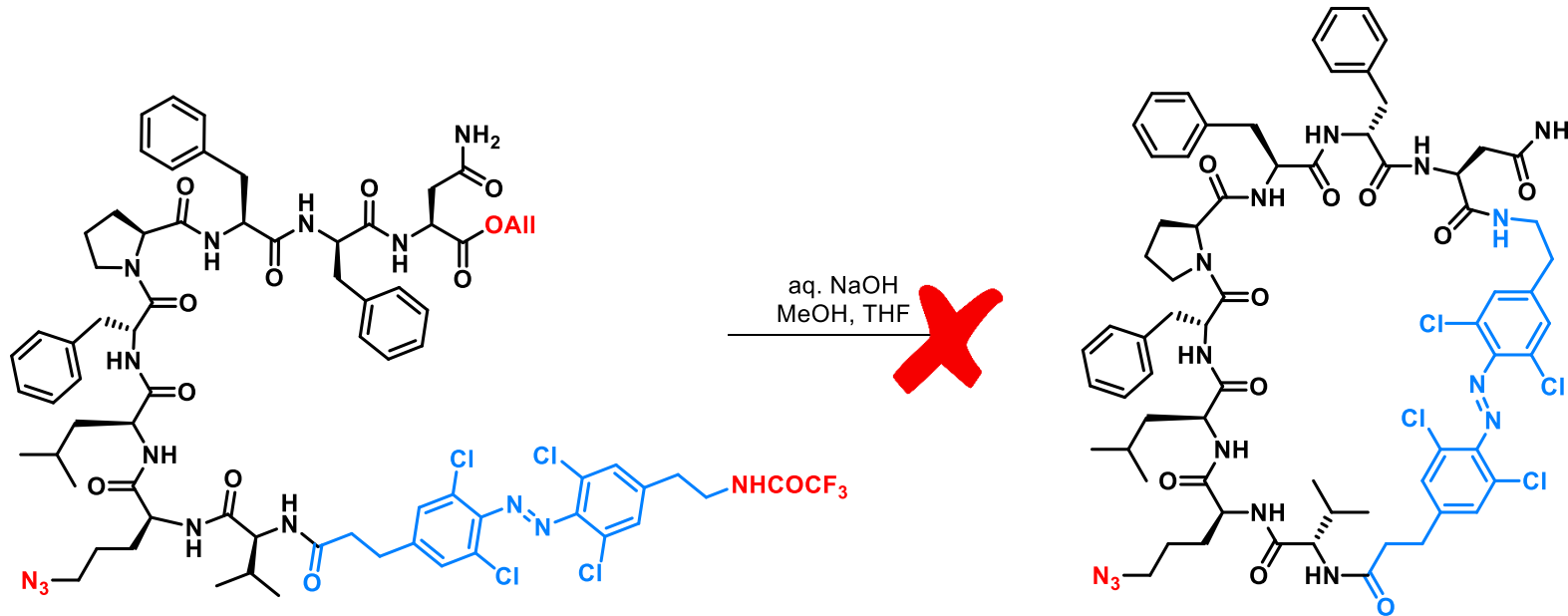


By-Product Formed During Basic Hydrolysis



➤ *MS of cyclic product detected instead of the expected hydrolysis product.*

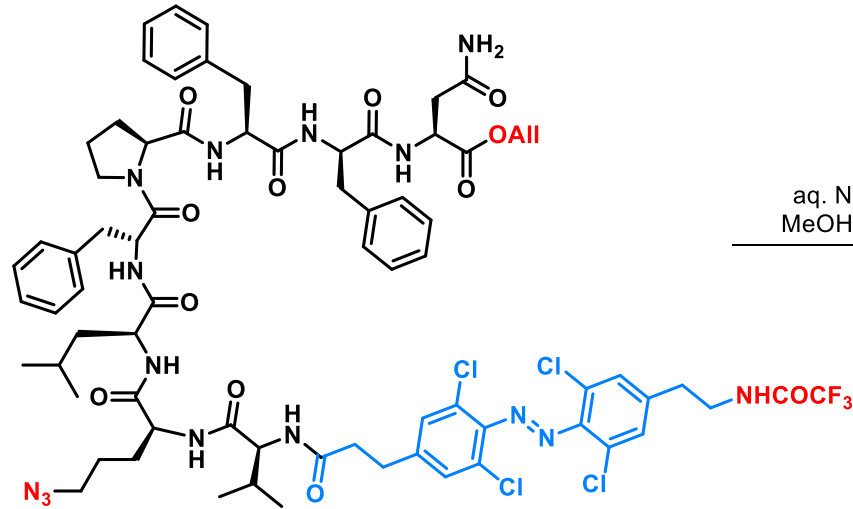
By-Product Formed During Basic Hydrolysis



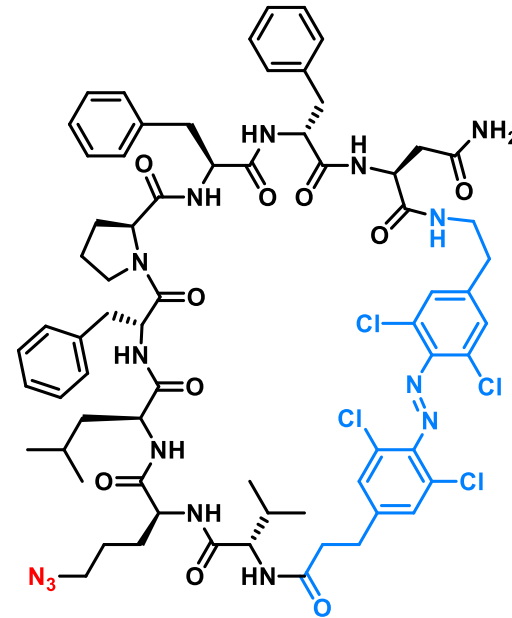
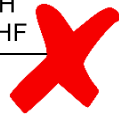
➤ *MS of cyclic product detected instead of the expected hydrolysis product.*

➤ *Characterisation discarded the cyclic product.*

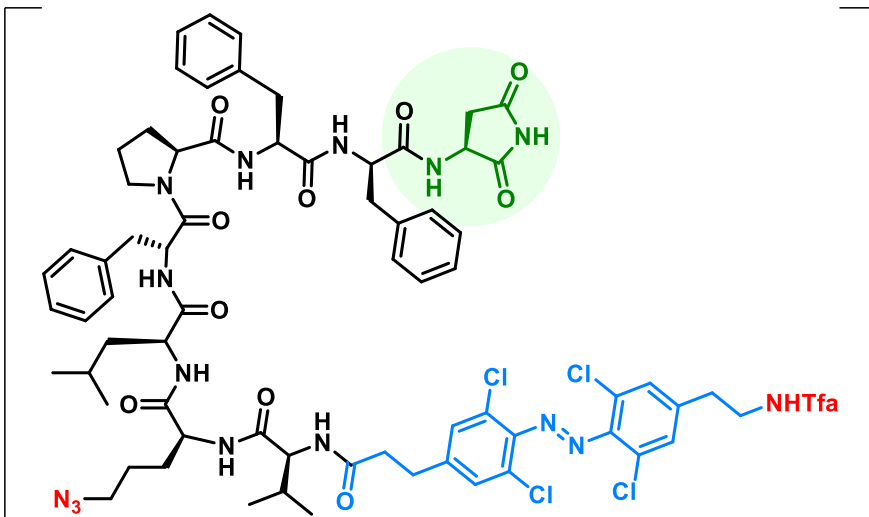
By-Product Formed During Basic Hydrolysis



aq. NaOH
MeOH, THF



succinimide formation
—FAST—

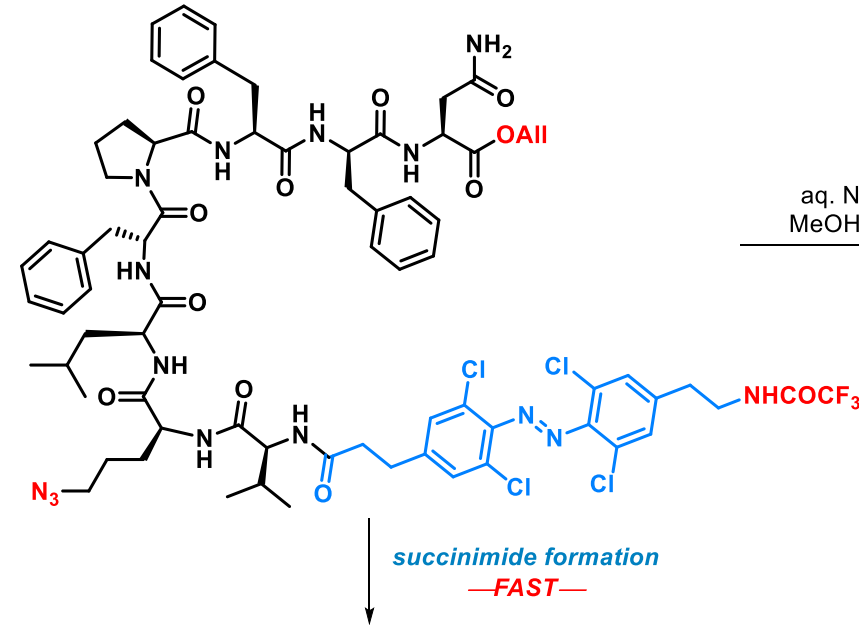


m/z of intermediate detected by UPLC-MS

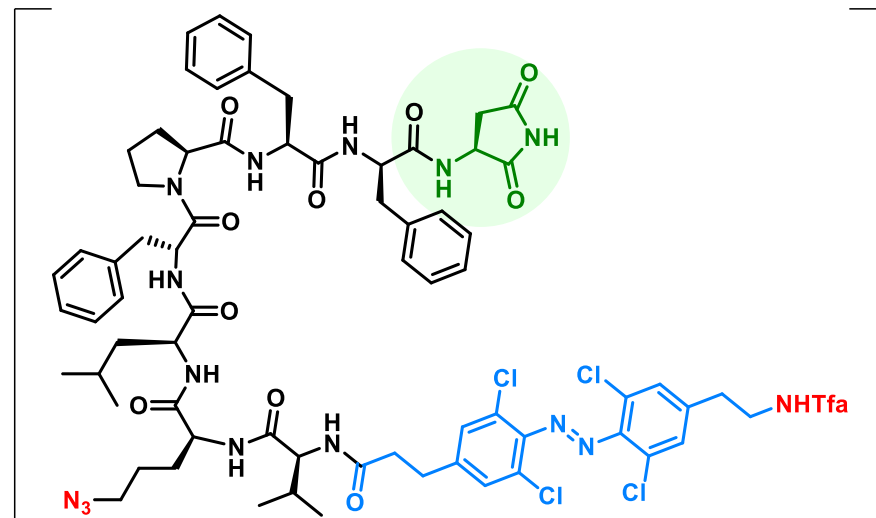
➤ *MS of cyclic product detected instead of the expected hydrolysis product.*

➤ *Characterisation discarded the cyclic product.*

By-Product Formed During Basic Hydrolysis

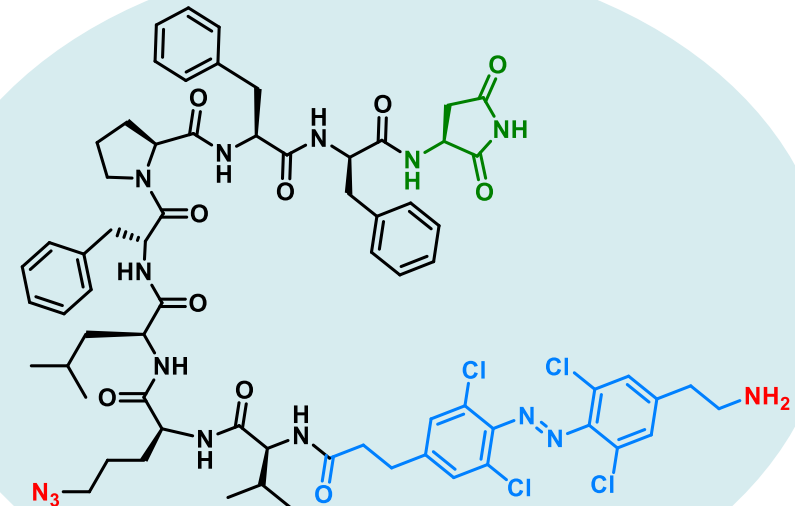


- Same MW as cyclic product.
- **Ninhydrin test positive.**
- Mechanistic studies confirm reaction of Asp side chain.

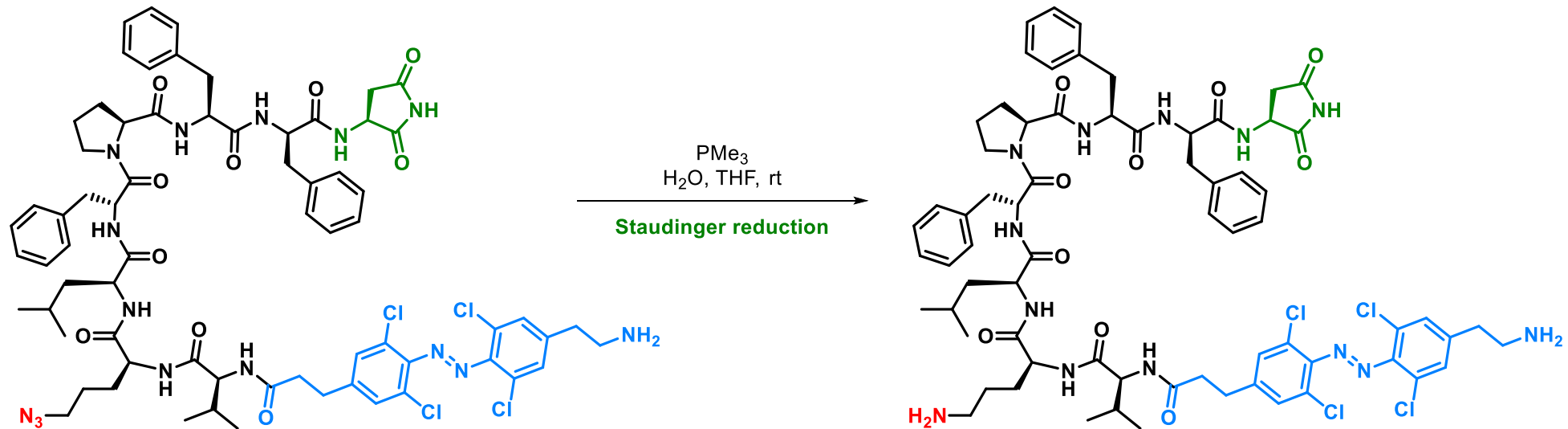


m/z of intermediate detected by UPLC-MS

trifluoroacetamide hydrolysis
—slow—
Rate Limiting Step

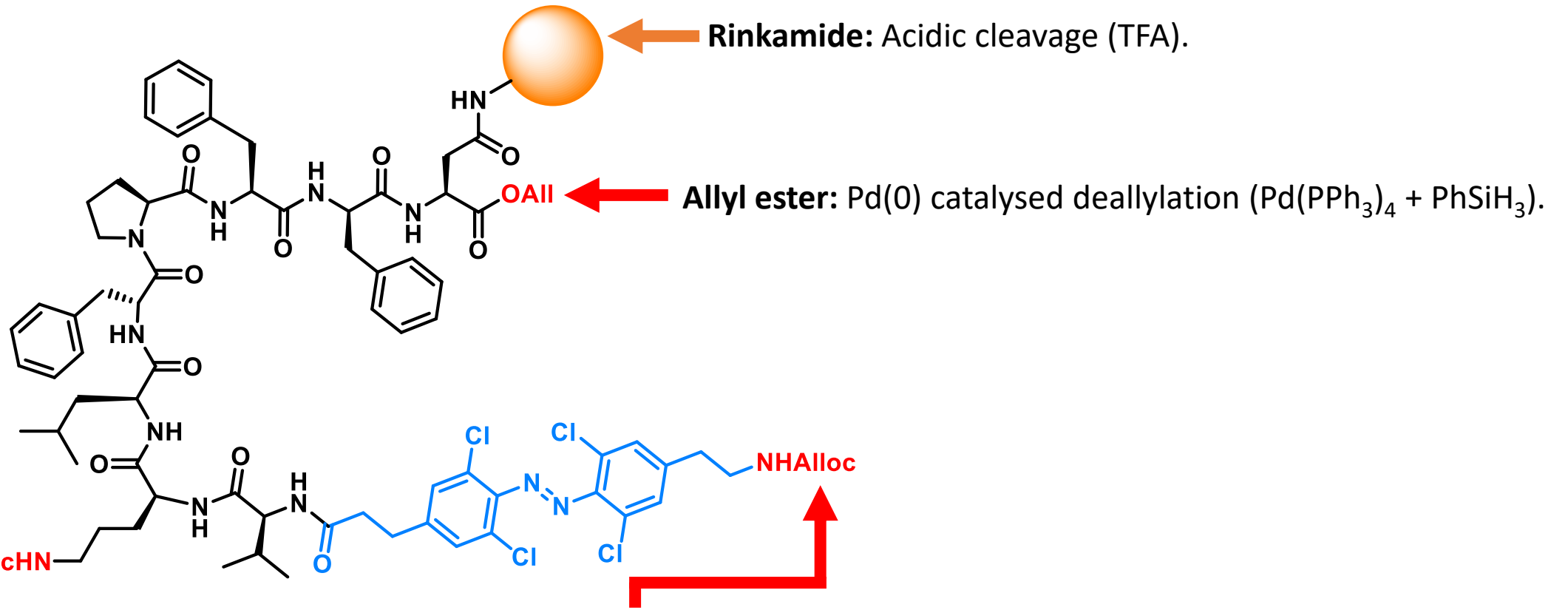


Synthesis of Photoswitchable Tyrocidine A



- Use of PMe_3 allows mild conditions for the Staudinger reaction (RT and no NaOH).
- ~20% isolated yield after the whole synthetic route.

Custom Made Strategy (AKA Wisdom through Challenge)



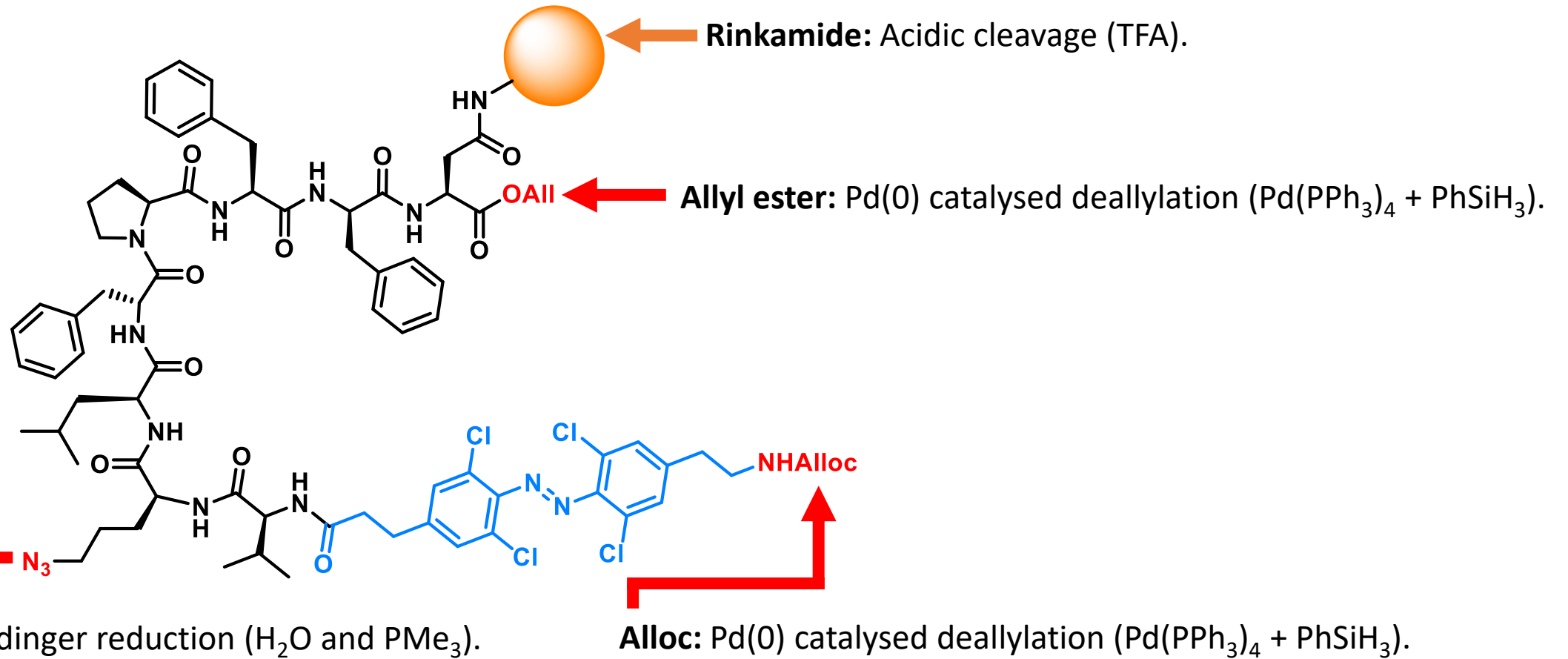
Boc: Acidic deprotection (TFA).

Cyclisation on resin doesn't work! Need to change for a group that avoids Pd(0), acids, amine nucleophiles or aqueous base.

Alloc: Pd(0) catalysed deallylation ($\text{Pd}(\text{PPh}_3)_4 + \text{PhSiH}_3$).

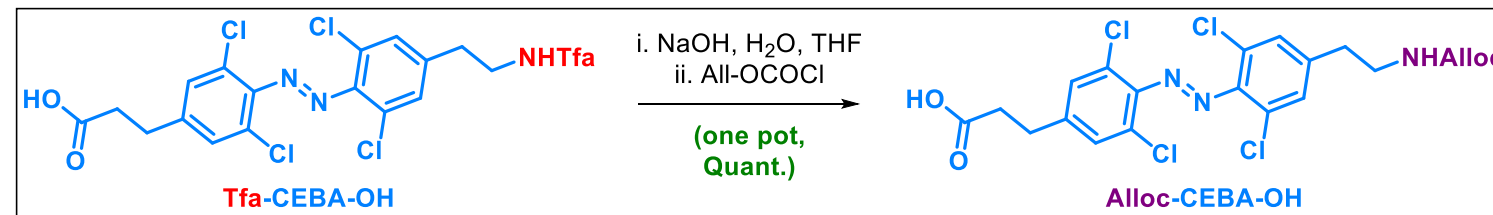
Need to change PG on photoswitch.

Custom Made Strategy (AKA Wisdom through Challenge)

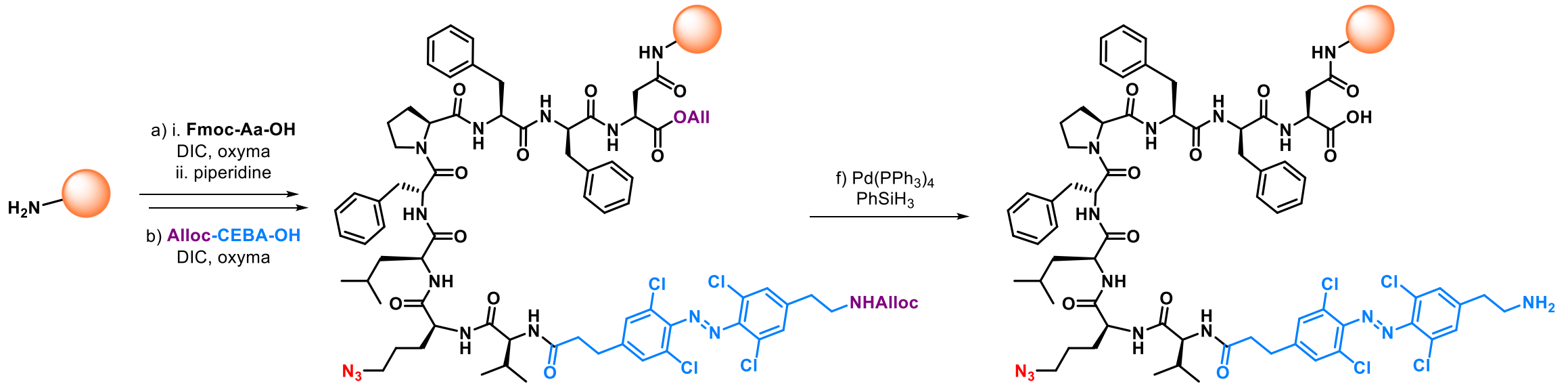


Alternative Sequence Towards Cyclic Photoswitchable Tyrocidine A

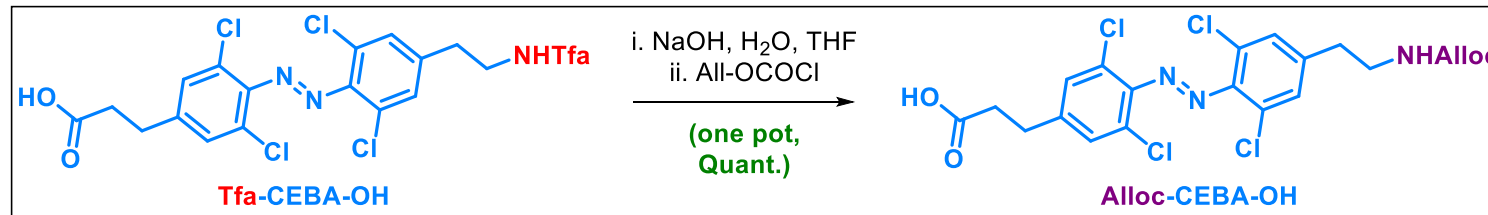
One-pot and Quantitative Protecting Group Exchange:



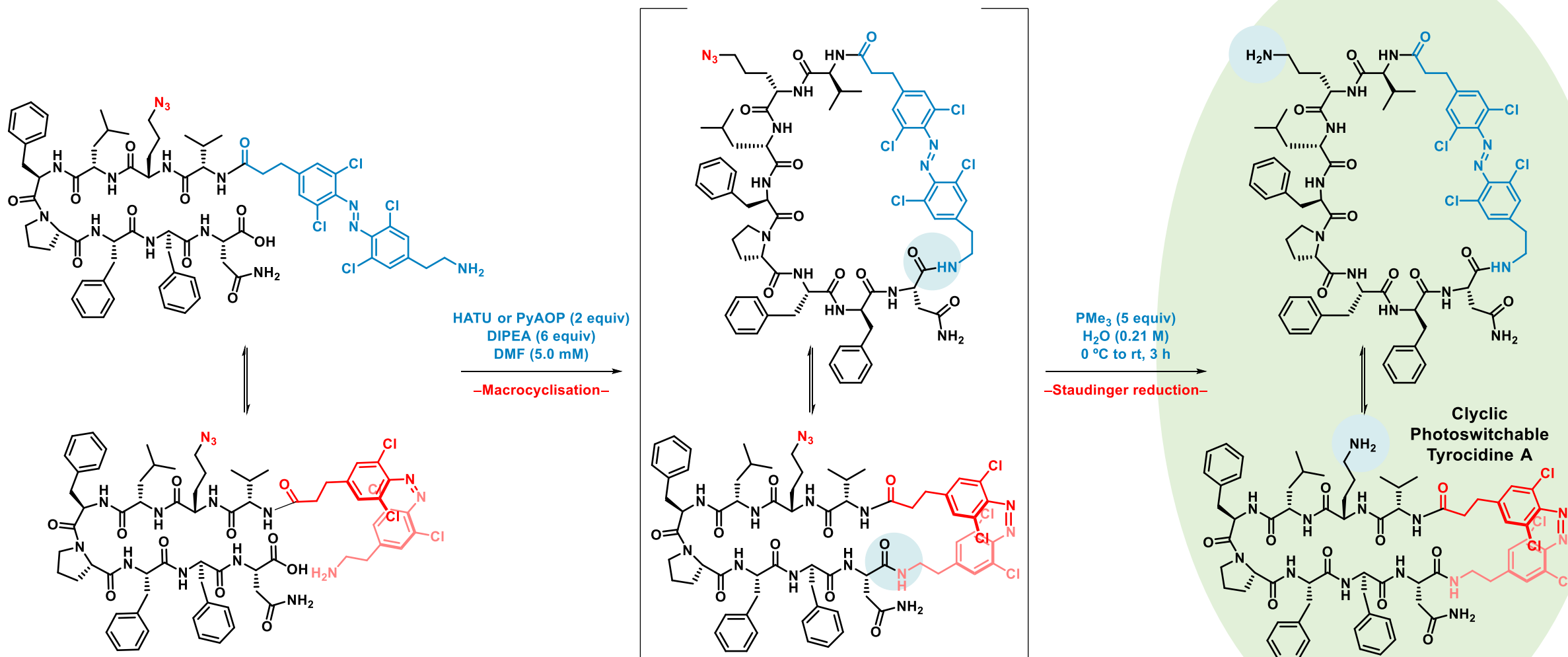
Alternative Sequence Towards Cyclic Photoswitchable Tyrocidine A



One-pot and Quantitative Protecting Group Exchange:



Synthesis of Cyclic Photoswitchable Tyrocidine A

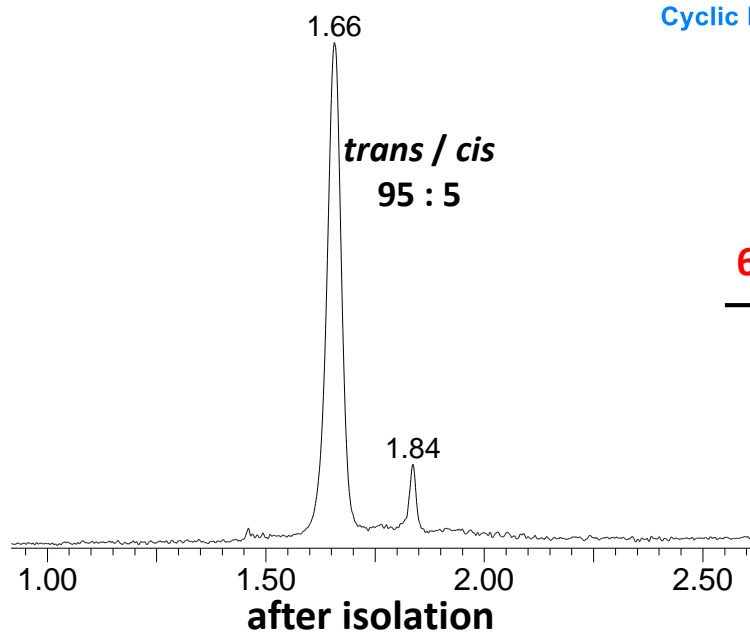
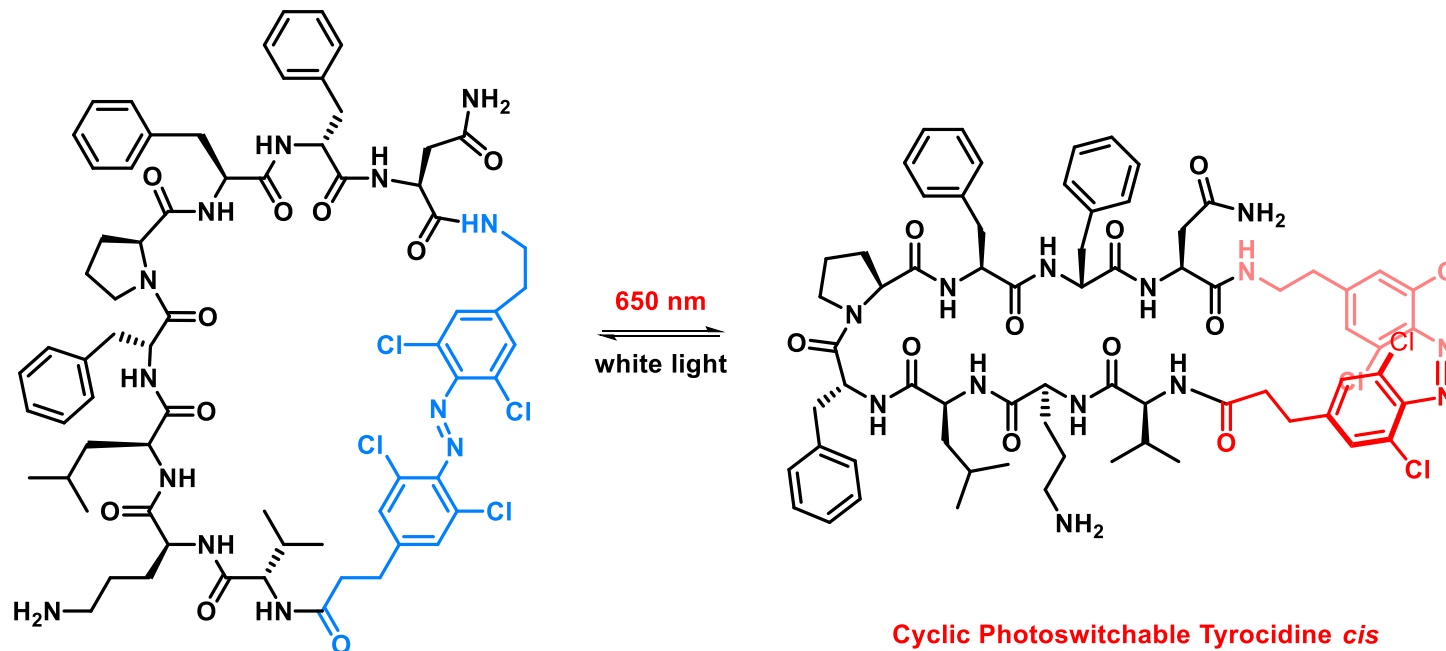


- Reactions have been optimised to be compatible with a one-pot procedure.
- Irradiation of the cyclisation reaction mixture did not affect the outcome.

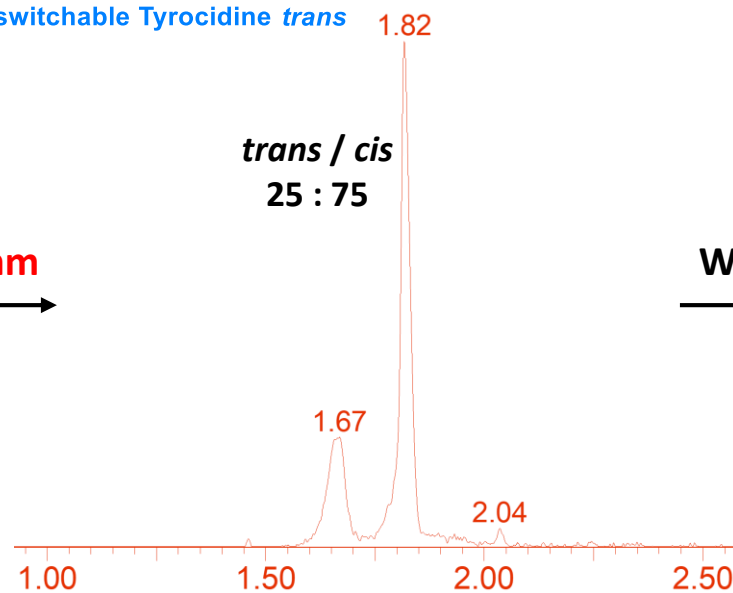
Visible Light Isomerisation – Cyclic Compound



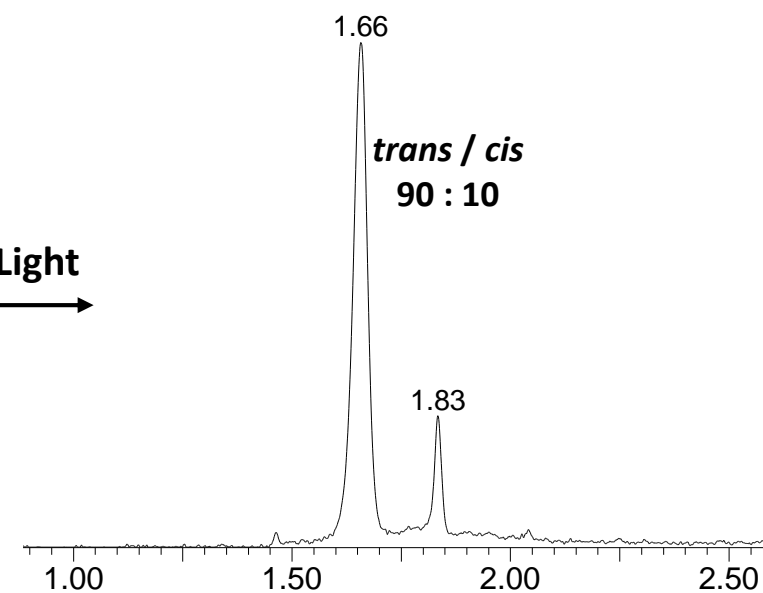
650 nm, 369 W·m⁻²



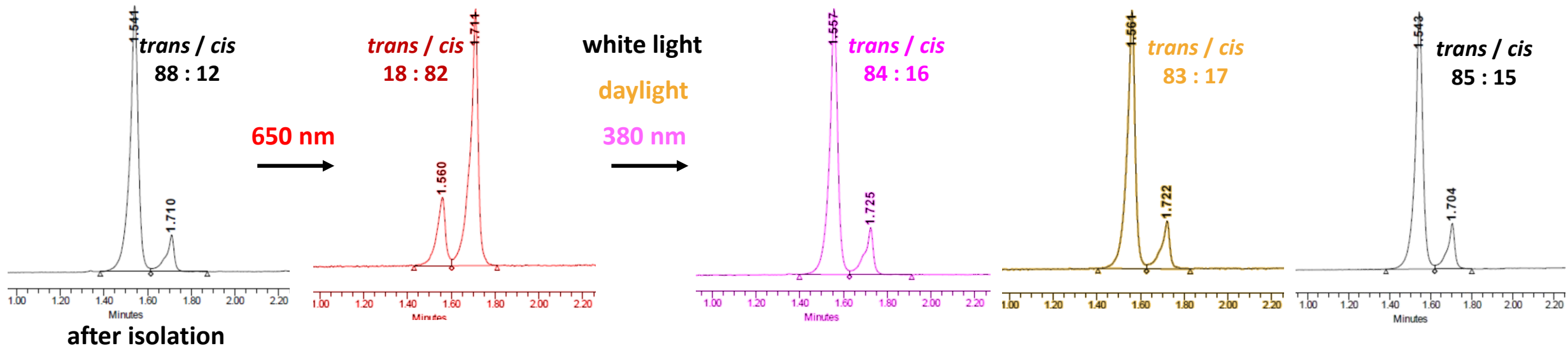
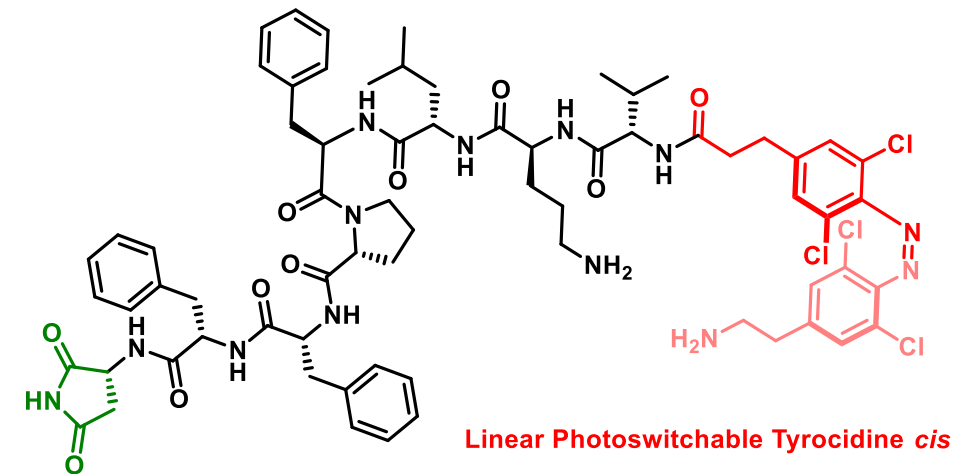
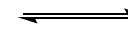
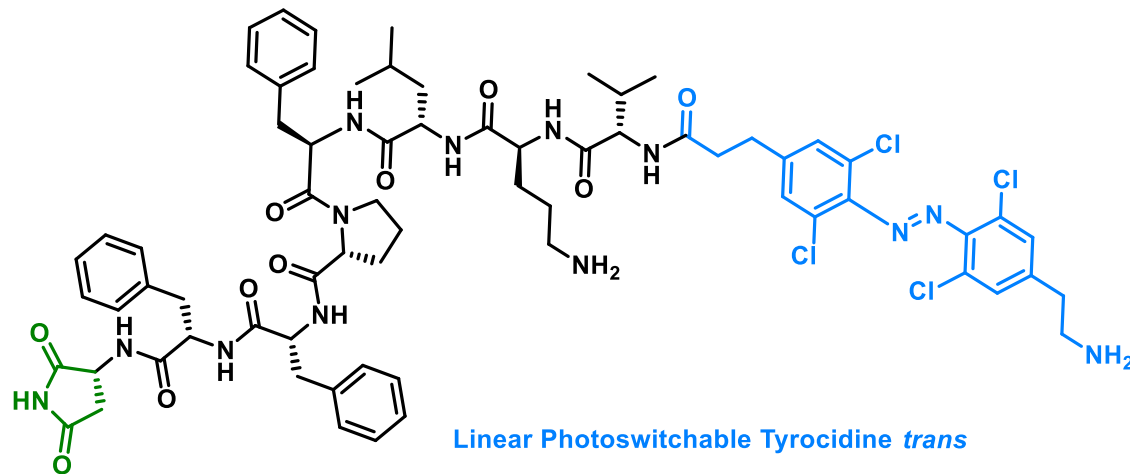
650 nm



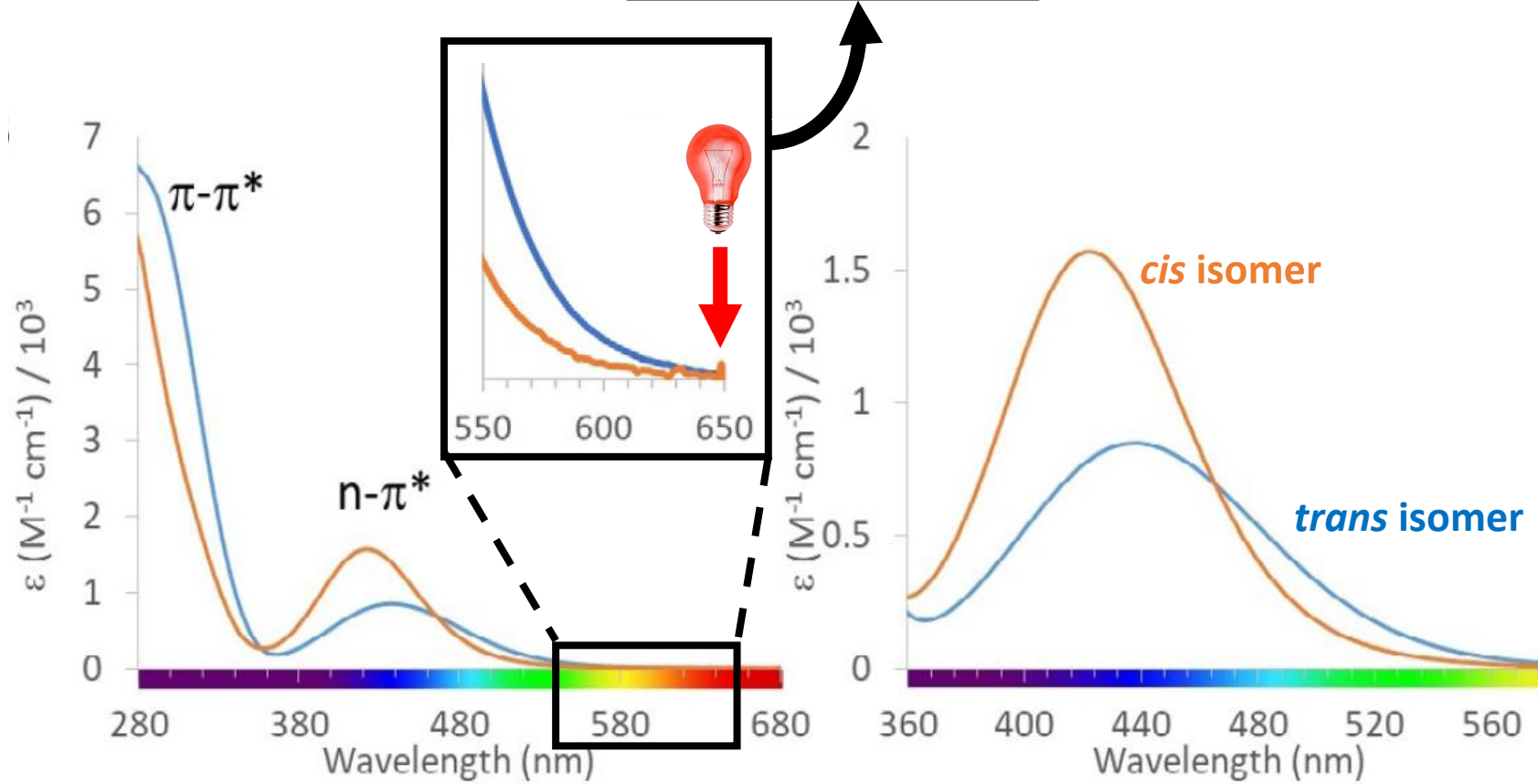
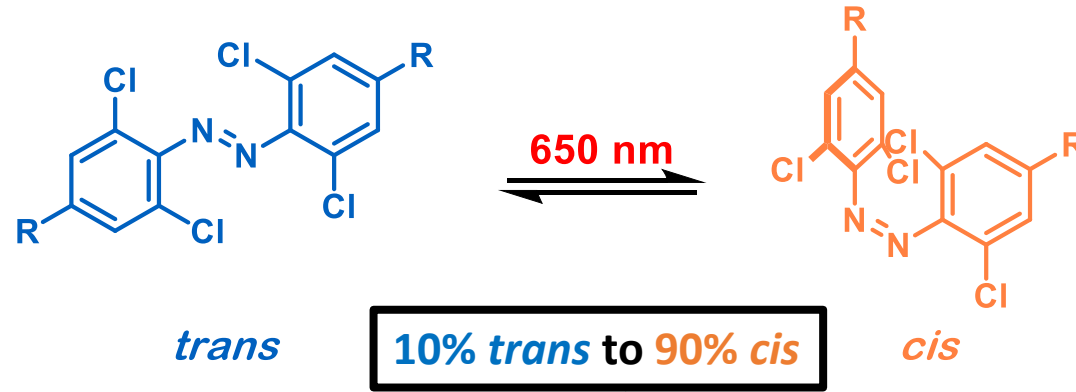
White Light



Visible Light Isomerisation – Linear Compounds



Azobenzenes



10% *trans* to 90% *cis*

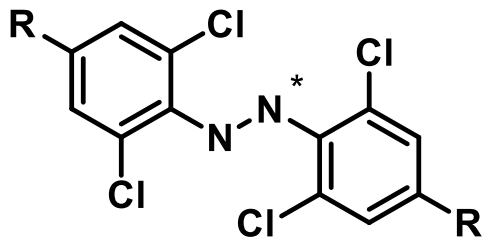


Photo-excited *trans* isomer
(most stable)

Photo-isomerisation (FAST)

Excitation breaks double bond.

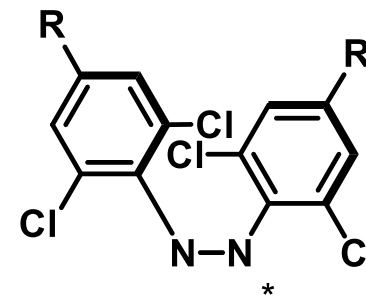
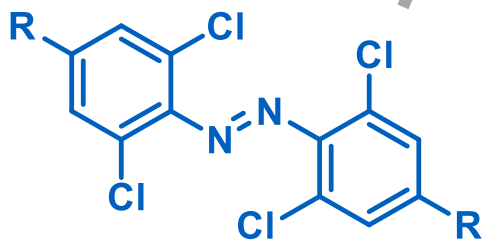


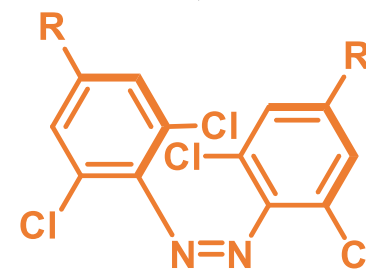
Photo-excited *cis* isomer
(least stable)



Thermal *trans* isomer
(most stable)

Thermal isomerisation (slow)

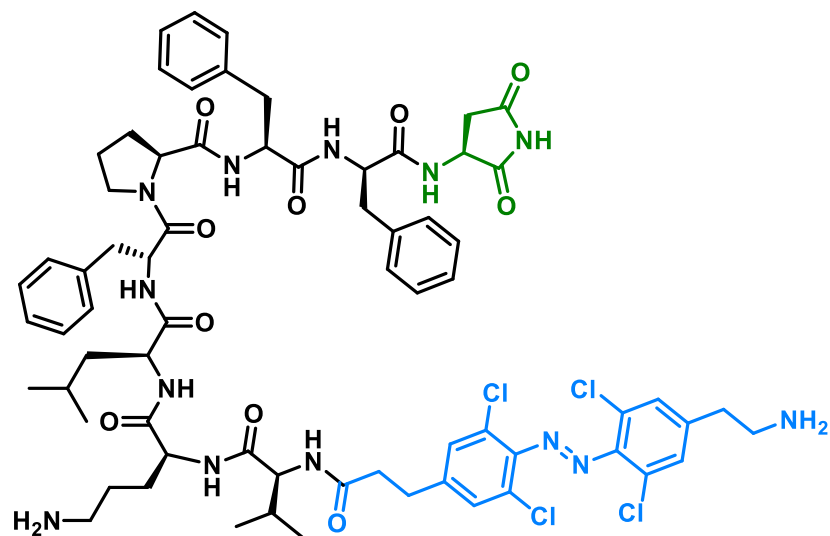
Double bond cannot rotate.



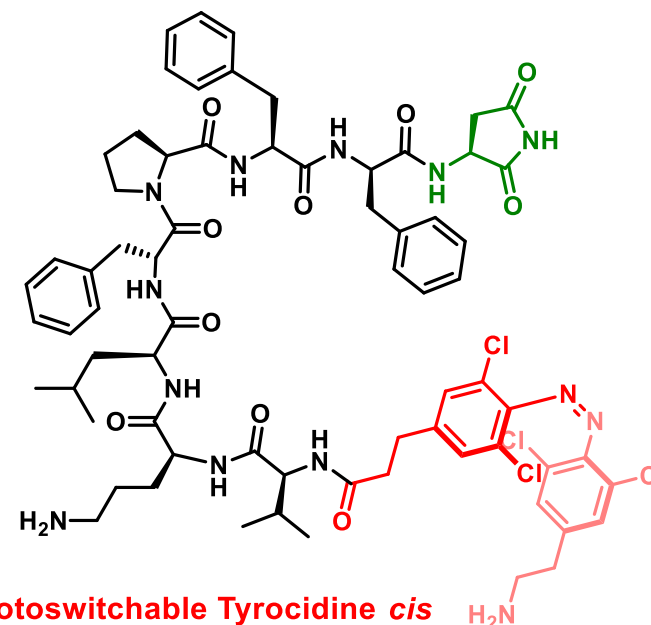
Thermal *cis* isomer
(least stable)



Isomerisation Kinetics



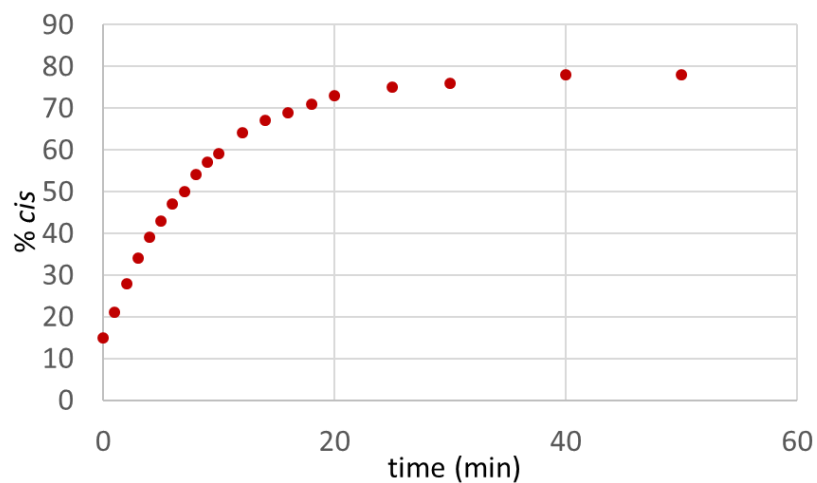
Linear Photoswitchable Tyrocidine *trans*



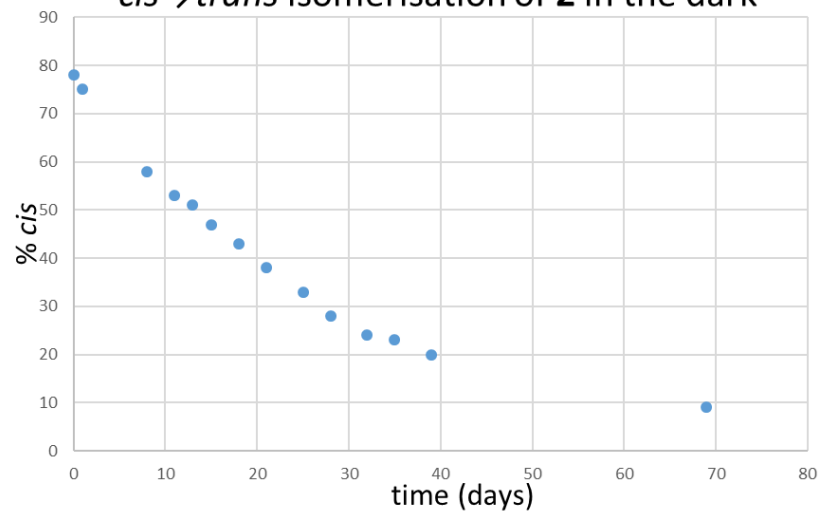
Linear Photoswitchable Tyrocidine *cis*

➤ **Excellent response for photoactivation and deactivation and slow isomerisation in the dark!**

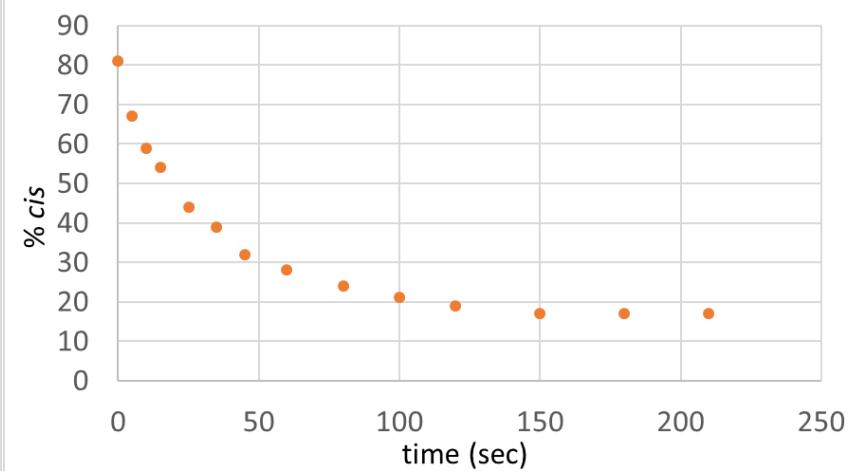
trans→*cis* isomerisation of **2** at 650 nm



cis→*trans* isomerisation of **2** in the dark



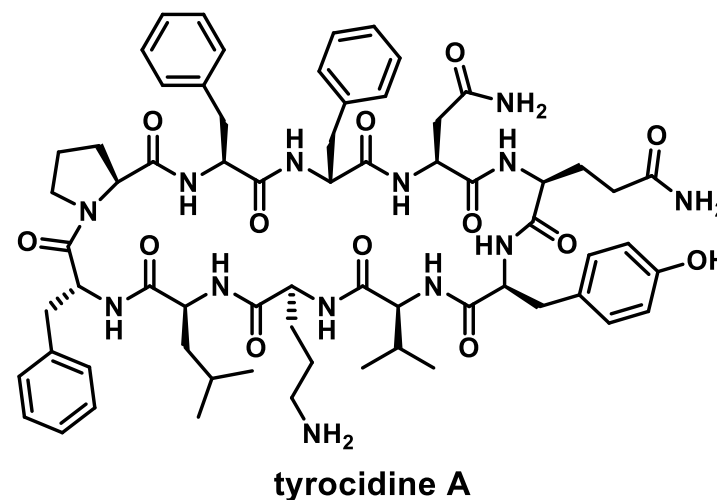
cis→*trans* isomerisation of **2** under daylight



in vitro Results – Antibacterial Activity

		TA	
		NoIrr	Irr ^[b]
Gram-positive	<i>Staphylococcus aureus</i> ATCC 25923 ^[c]	8	8
	<i>Bacillus subtilis</i> ^[c]	4	4
	<i>Staphylococcus epidermidis</i> F652012 ^[c]	8	8
	<i>Streptococcus pyogenes</i> 016 ^[c]	4	4
	<i>Enterococcus faecium</i> VancoR ^[c]	4	4
Gram-negative	<i>Pseudomonas aeruginosa</i> ATCC 27853 ^[d]	>64	>64
	<i>Escherichia coli</i> ATCC 25922 ^[d]	>64	>64
	<i>Acinetobacter baumannii</i> ATCC 19606 ^[d]	16	16
	<i>Acinetobacter baumannii</i> CR17 ^[d]	32	16
	<i>Acinetobacter nosocomialis</i> 256 ^[d]	4	4

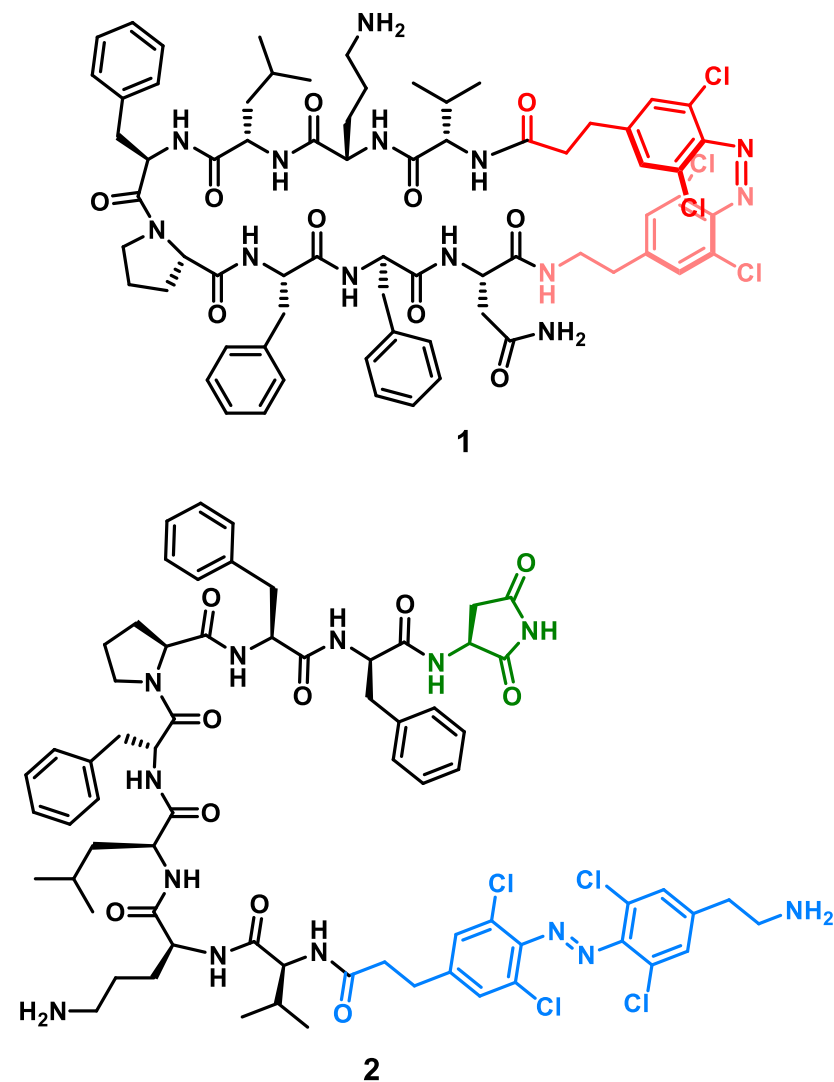
Minimum Inhibitory Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)



in vitro Results – Antibacterial Activity

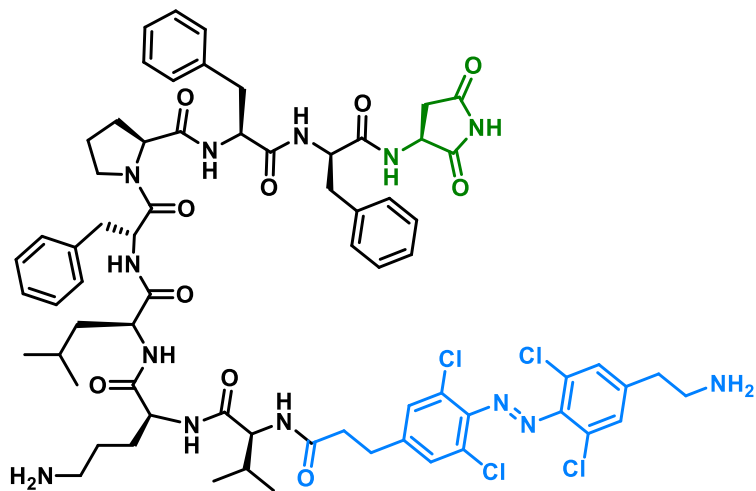
	TA		cyclic		linear	
			1		2	
	NoIrr	Irr ^[b]	NoIrr	Irr ^[b]	NoIrr	Irr ^[b]
Gram-positive	<i>Staphylococcus aureus</i> ATCC 25923 ^[c]	8	8	8	8	8
	<i>Bacillus subtilis</i> ^[c]	4	4	4	8	2
	<i>Staphylococcus epidermidis</i> F652012 ^[c]	8	8	8	8	4
	<i>Streptococcus pyogenes</i> 016 ^[c]	4	4	4	4	4
	<i>Enterococcus faecium</i> VancoR ^[c]	4	4	8	8	4
Gram-negative	<i>Pseudomonas aeruginosa</i> ATCC 27853 ^[d]	>64	>64	>64	>64	>64
	<i>Escherichia coli</i> ATCC 25922 ^[d]	>64	>64	>64	>64	64
	<i>Acinetobacter baumannii</i> ATCC 19606 ^[d]	16	16	>64	>64	64
	<i>Acinetobacter baumannii</i> CR17 ^[d]	32	16	>64	>64	>64
	<i>Acinetobacter nosocomialis</i> 256 ^[d]	4	4	2	4	4

Minimum Inhibitory Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)

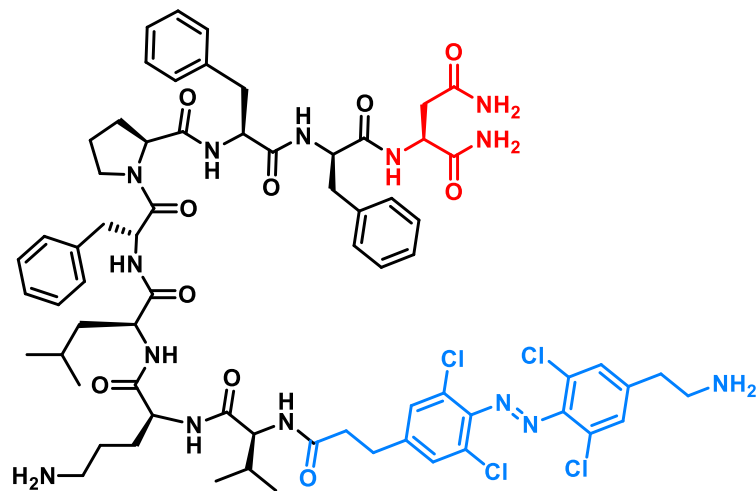


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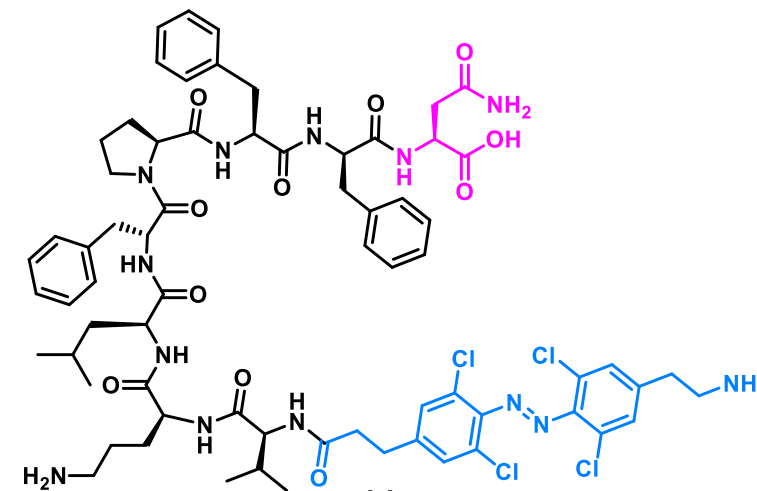
Analogues to Interrogate *N*- and *C*-Termini of Linear Analogues



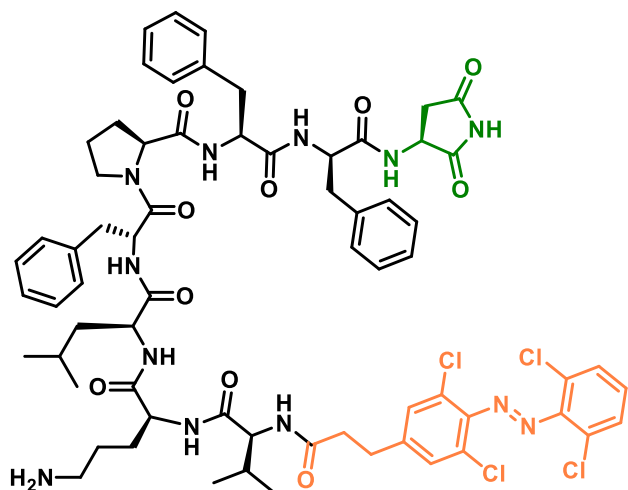
C-terminus: succinimide
N-terminus: PS-(CH₂)₂NH₂



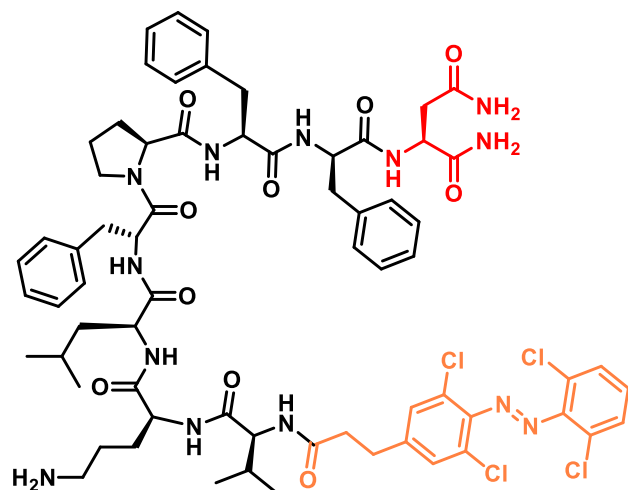
C-terminus: Asn-NH₂
N-terminus: PS-(CH₂)₂NH₂



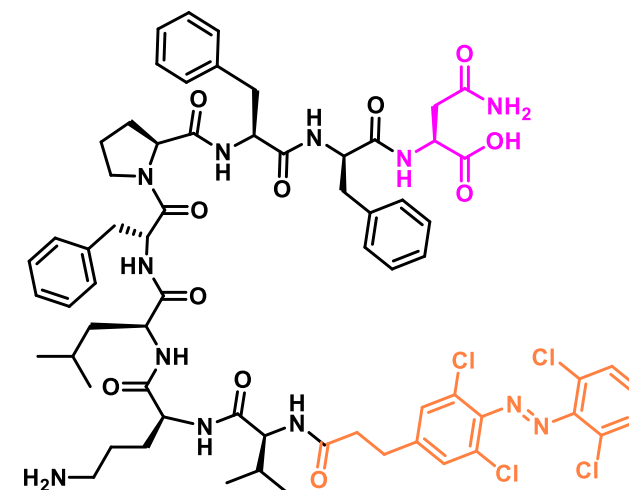
C-terminus: Asn-OH
N-terminus: PS-(CH₂)₂NH₂



C-terminus: succinimide
N-terminus: PS-H

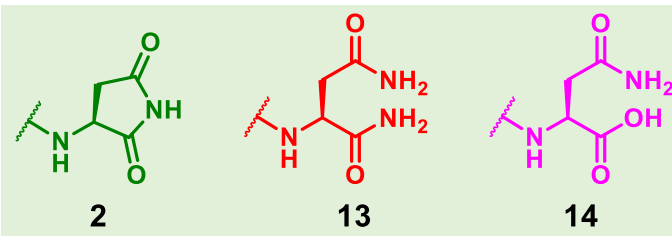


C-terminus: Asn-NH₂
N-terminus: PS-H



C-terminus: Asn-OH
N-terminus: PS-H

in vitro Results – Antibacterial Activity

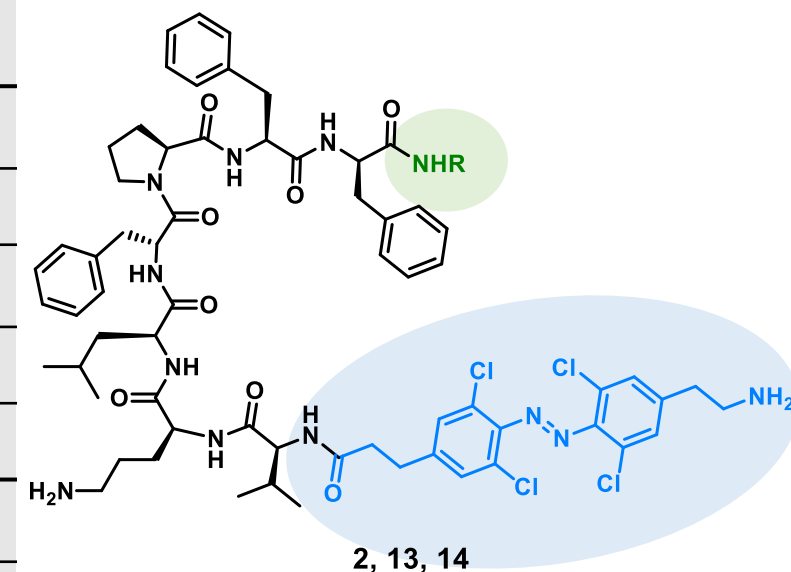


cyclic

linear - amine in PS

TA		1		2		13		14	
Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]

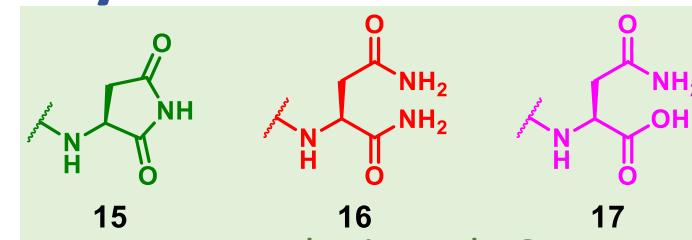
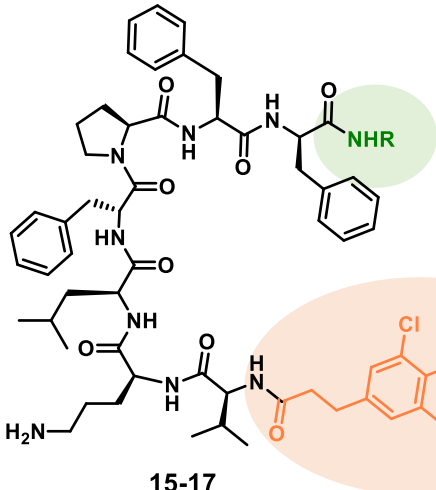
Gram-positive	Strain	TA		1		2		13		14	
		Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]
	<i>Staphylococcus aureus</i> ATCC 25923 ^[c]	8	8	8	8	8	8	4	4	8	8
	<i>Bacillus subtilis</i> ^[c]	4	4	4	8	2	2	1	2	4	16
	<i>Staphylococcus epidermidis</i> F652012 ^[c]	8	8	8	8	4	4	4	4	8	8
	<i>Streptococcus pyogenes</i> 016 ^[c]	4	4	4	4	4	4	4	4	8	4
	<i>Enterococcus faecium</i> VancoR ^[c]	4	4	8	8	4	4	4	4	8	8
Gram-negative	<i>Pseudomonas aeruginosa</i> ATCC 27853 ^[d]	>64	>64	>64	>64	>64	>64	>64	64	>64	>64
	<i>Escherichia coli</i> ATCC 25922 ^[d]	>64	>64	>64	>64	>64	64	>64	32	>64	>64
	<i>Acinetobacter baumannii</i> ATCC 19606 ^[d]	16	16	>64	>64	64	8	8	8	>64	>64
	<i>Acinetobacter baumannii</i> CR17 ^[d]	32	16	>64	>64	>64	32	16	8	>64	>64
	<i>Acinetobacter nosocomialis</i> 256 ^[d]	4	4	2	4	4	8	2	4	4	4



Minimum Inhibitory Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)

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in vitro Results – Antibacterial Activity



TA	<u>cyclic</u>		<u>linear - amine in PS</u>								<u>unsubstituted PS</u>						
	1		2		13		14		15		16		17				
Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]	Nolrr	Irr ^[b]		
Gram-positive	<i>Staphylococcus aureus</i> ATCC 25923 ^[c]	8	8	8	8	8	8	4	4	8	8	>64	64	64	32	>64	>64
	<i>Bacillus subtilis</i> ^[c]	4	4	4	8	2	2	1	2	4	16	>64	>64	64	64	>64	>64
	<i>Staphylococcus epidermidis</i> F652012 ^[c]	8	8	8	8	4	4	4	4	8	8	>64	32	64	64	64	64
	<i>Streptococcus pyogenes</i> 016 ^[c]	4	4	4	4	4	4	4	4	8	4	>64	64	>64	16	>64	16
	<i>Enterococcus faecium</i> VancoR ^[c]	4	4	8	8	4	4	4	4	8	8	64	64	32	32	64	64
Gram-negative	<i>Pseudomonas aeruginosa</i> ATCC 27853 ^[d]	>64	>64	>64	>64	>64	>64	>64	64	>64	>64	>64	>64	>64	>64	>64	>64
	<i>Escherichia coli</i> ATCC 25922 ^[d]	>64	>64	>64	>64	>64	64	>64	32	>64	>64	>64	>64	>64	>64	>64	>64
	<i>Acinetobacter baumannii</i> ATCC 19606 ^[d]	16	16	>64	>64	64	8	8	8	>64	>64	>64	>64	>64	>64	>64	>64
	<i>Acinetobacter baumannii</i> CR17 ^[d]	32	16	>64	>64	>64	32	16	8	>64	>64	>64	>64	>64	>64	>64	>64
	<i>Acinetobacter nosocomialis</i> 256 ^[d]	4	4	2	4	4	8	2	4	4	4	32	32	16	16	64	64

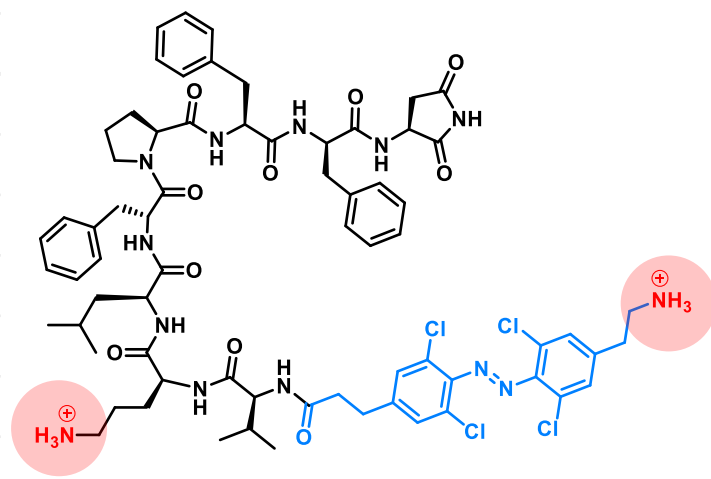
Minimum Inhibitory Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)

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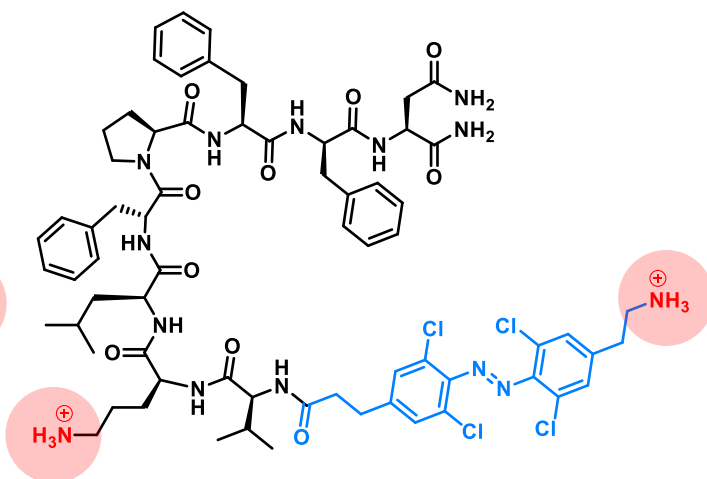
in vitro Results - Toxicity

Haemolysis (IC50)

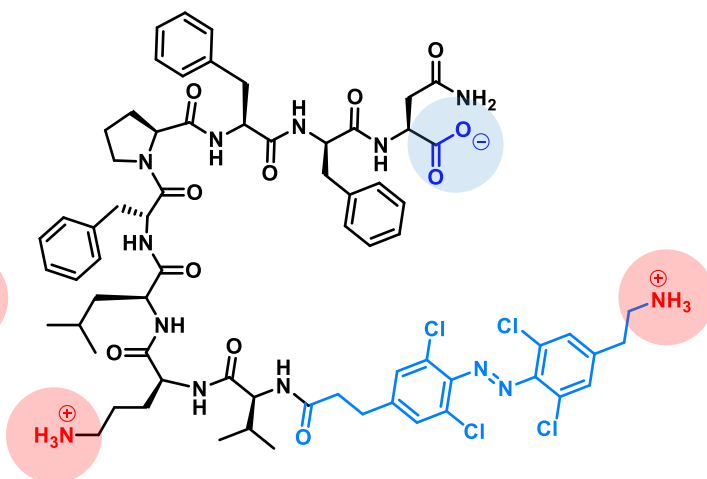
	IC50 ($\mu\text{g}\cdot\text{mL}^{-1}$)	
	Nolrr	Irr ^[b]
TA	25	24
1	21	26
2	32	15
13	36	13
14	25	33
15	39	33
16	23	25
17	133	173



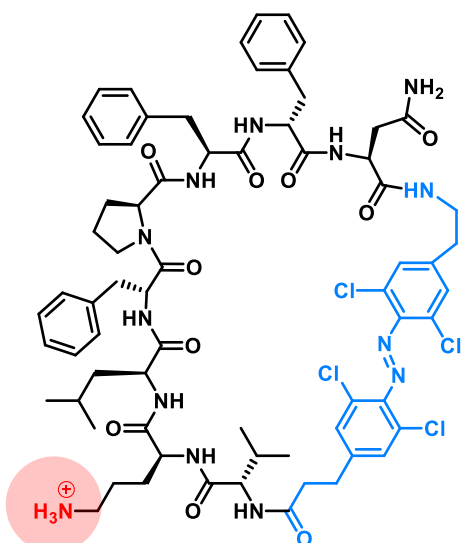
2, net charge: +2



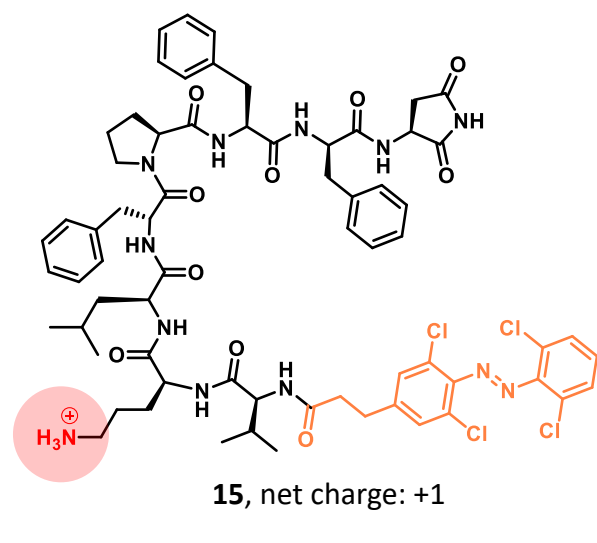
13, net charge: +2



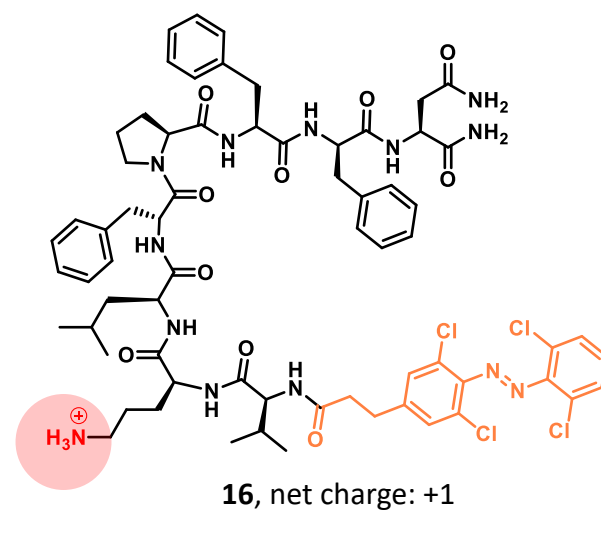
14, net charge: +1



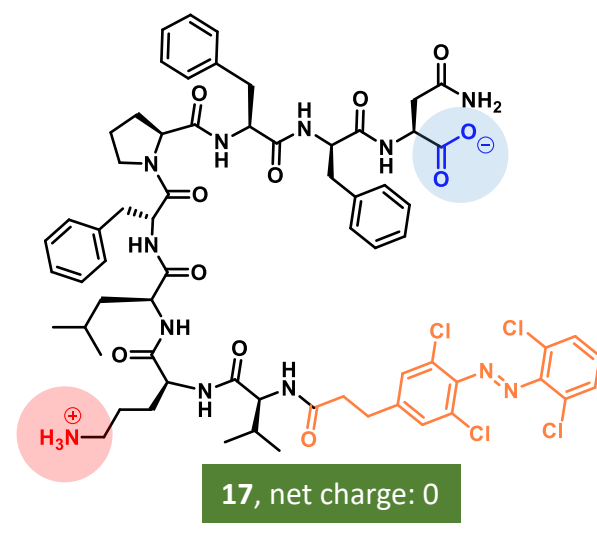
1, net charge: +1



15, net charge: +1

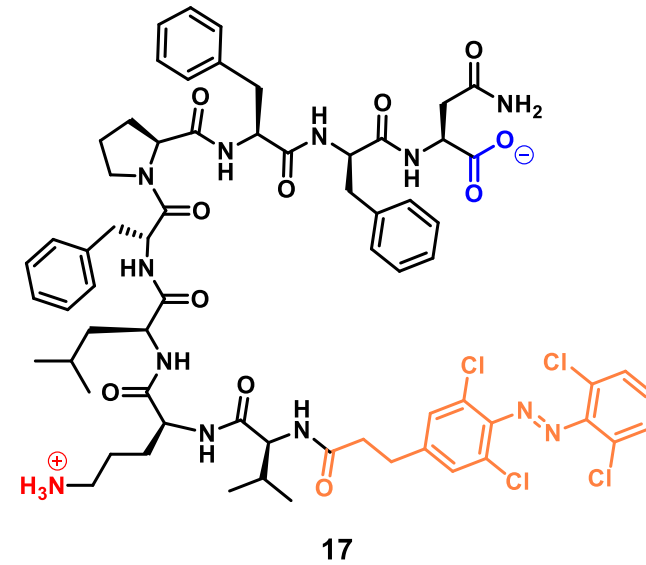
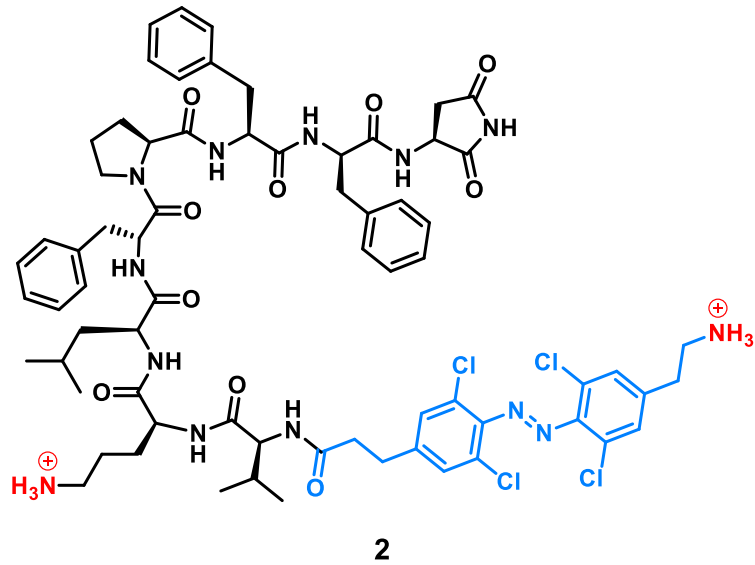


16, net charge: +1



17, net charge: 0

Photoswitchable Antimicrobials: Hits



	2		17	
	NoIrr	Irr	NoIrr	Irr
<i>S. Pyogenes</i> 016	4	4	>64	16
<i>A.baumannii</i> ATCC 19606	64	8	>64	>64
IC50 ($\mu\text{g}\cdot\text{mL}^{-1}$)	32	15	133	173

Acknowledgements

Current Team



Prof. Mercedes Amat and all members of Sintefarma

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Alejandro Yeste



Hospital Clínic de Barcelona / ISGlobal



Javier Moreno



Prof. Jordi Vila



Dr. Clara Ballesté



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