The Guanidino Lipoglycopeptides: Semisynthetic glycopeptides with best-in-class potential

VHIG congres 2021
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prof. Nathaniel Martin
Biological Chemistry
Leiden University

The History of Antibiotic Discovery

1908
Salvarsan
(arsenical)

1932
Prontosil
(sulfonamide)

1940-1950
- Gramicidin (peptide)
- Penicillin (ß-lactam)
- Neomycin (aminoglycoside)
- Streptomycin (aminoglycoside)
- Cephalosporin (ß-lactam)

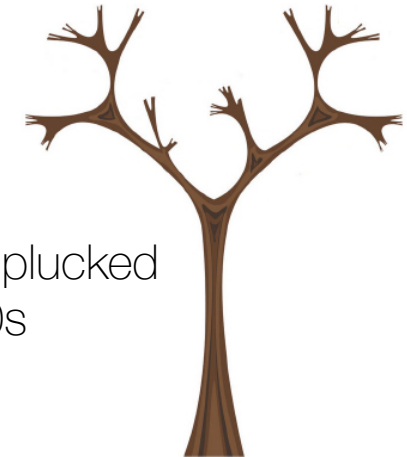
1950-1960
- Chloramphenicol (phenylpropanoid)
- Chlortetracycline (tetracycline)
- Polymyxin (lipopeptide)
- Erythromycin (macrolide)
- Vancomycin (glycopeptide)
- Virginiamycin (streptogramin)
- Rifamycin (ansamycin)

1962
Nalidixic acid
(quinolone)

2000
Linezolid
(oxazolidinone)

2003
Daptomycin
(lipopeptide)

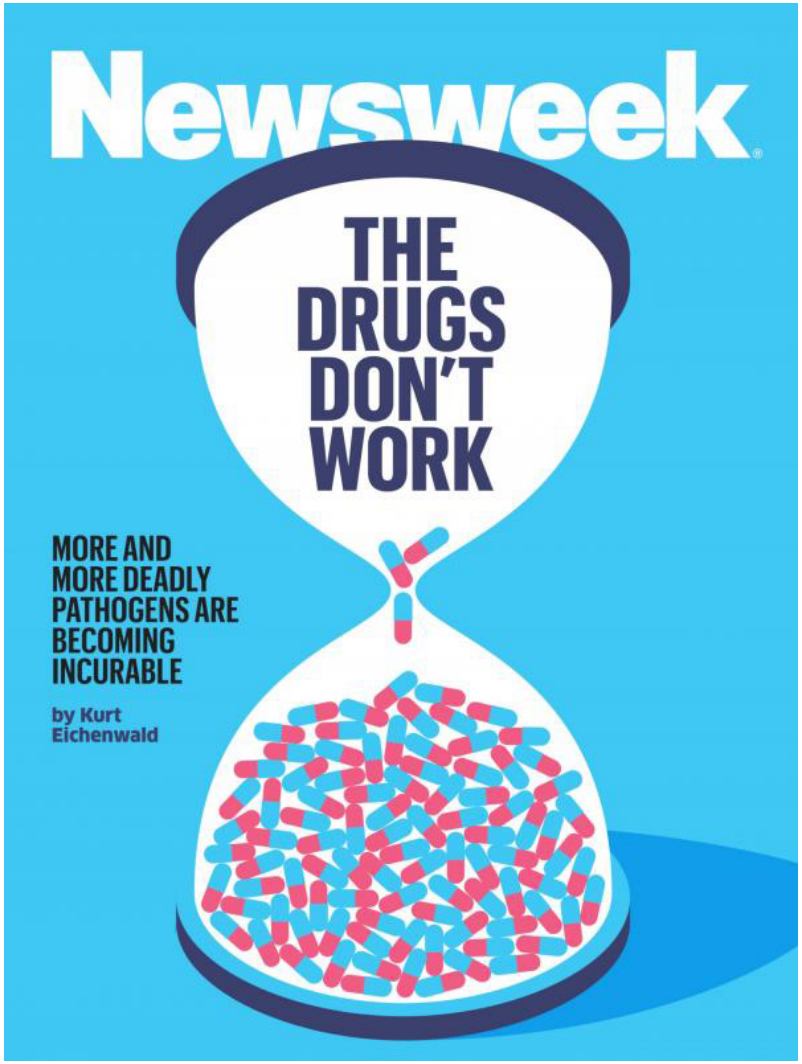
1920 1930 1940 1950 1960 1970 1980 1990 2000 2010



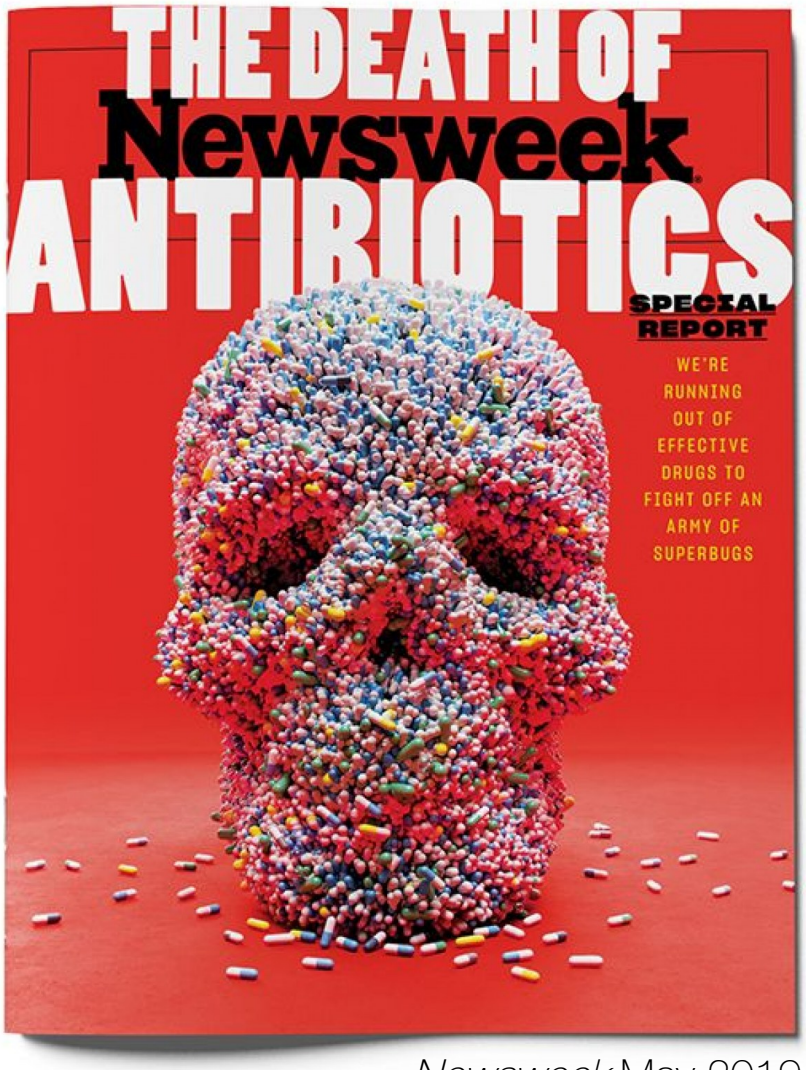
"Low hanging fruit" plucked by end of the 1960s

A problem emerges…

Antibiotic Resistance



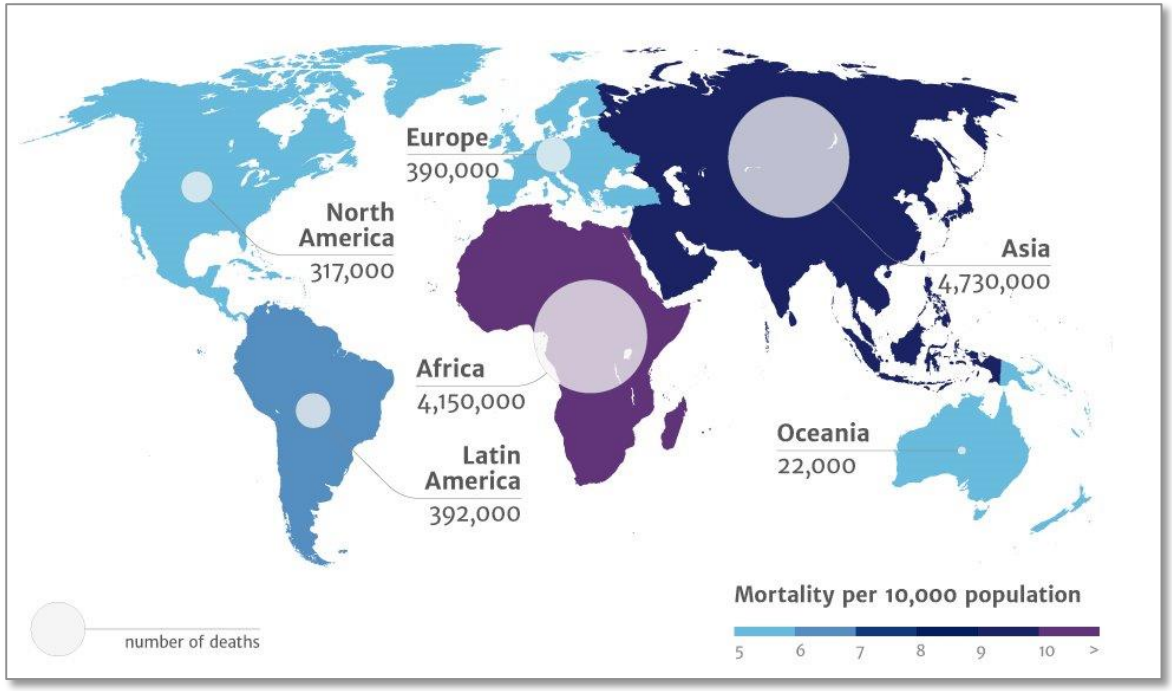
Newsweek October 2013



Newsweek May 2019

Antibiotic Resistance

- Drug resistant bacteria cause 2M infections in the US each year
- Globally 700K annual deaths attributed to resistant bacteria (estimated to reach 10M by 2050 if unchecked)



The "ESKAPE" pathogens pose a major threat:

- *E. faecium*
- *S. aureus*
- *K. pneumoniae*
- *A. baumanii*
- *P. aeruginosa,*
- *Enterobacter* species

Gram-positive pathogens remain a serious threat

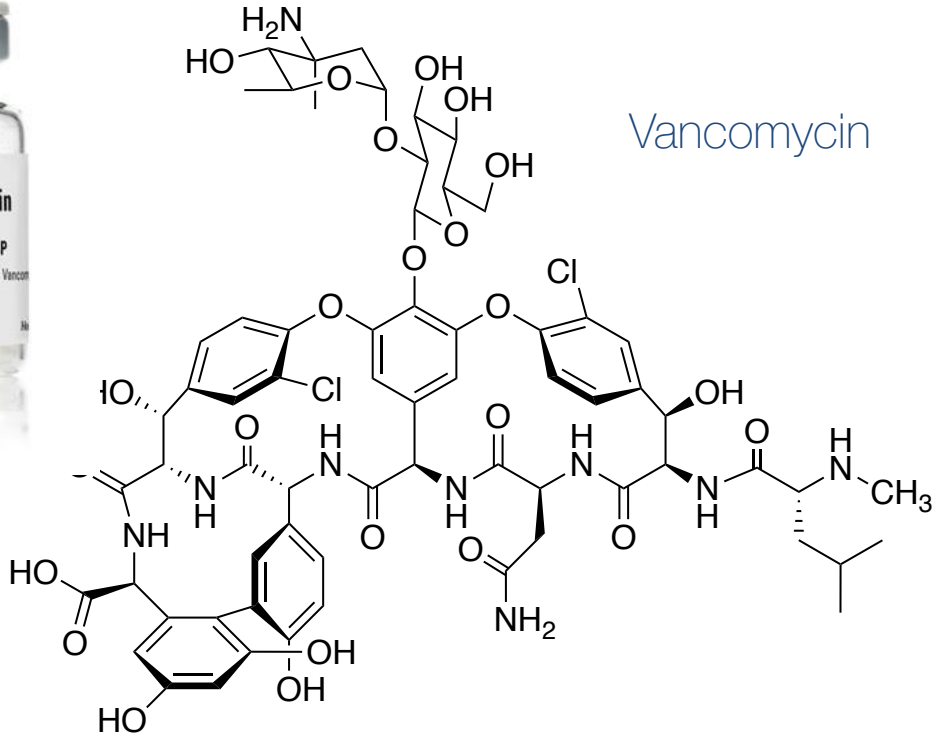
- MRSA and VRE account for half of all AMR-associated deaths in USA and Europe

- 2018 global MRSA market worth US$ 922.2M (by 2026 estimated to grow to US$ 1,3B)
- Global VRE market estimated between US$ 280 and 440M

CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.

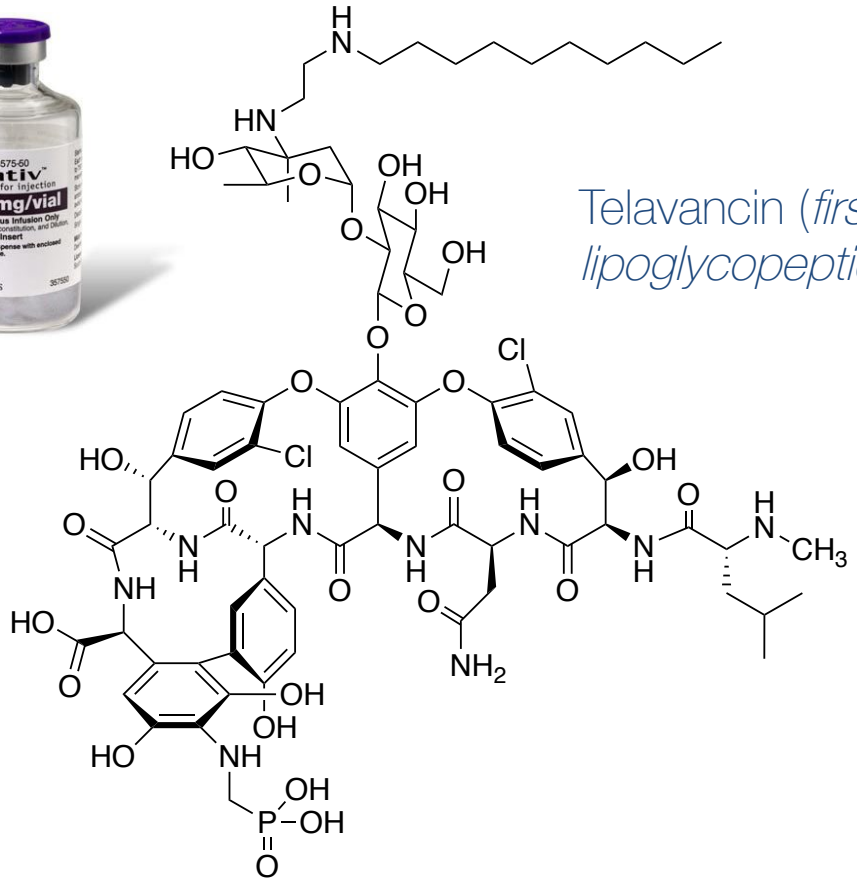
Glycopeptide antibiotics

- Since the 1970's vancomycin used in treatment of serious infections, including acute bacterial skin and skin structure infections (ABSSSI), due to staphylococci and enterococci
- Resistance now a serious problem



Lipoglycopeptide antibiotics

- Vancomycin resistance led to development of the lipoglycopeptides (e.g. teicoplanin, telavancin, dalbavancin, and oritavancin)
- Increased activity but also renal toxicity and unusual PK ($t_{1/2}$ up to 300hr)



Telavancin (*first semisynthetic lipoglycopeptide brought to market*)

FDA BLACK BOX WARNING

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There is a growing unmet need for novel antibiotic compounds with activity against resistant pathogens and improved safety profiles.

Introducing the guanidino lipoglycopeptides

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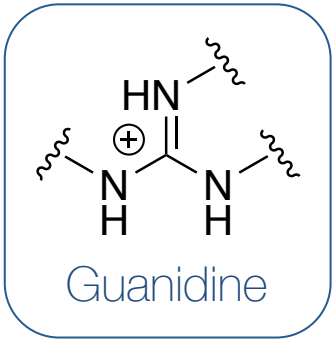
- The guanidine group carries a strong positive charge
- In 2019 the Martin group (Leiden University) discovered the guanidino lipoglycopeptides:



Guanidine

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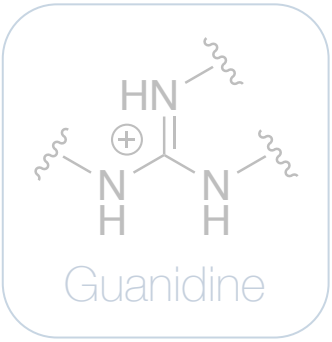
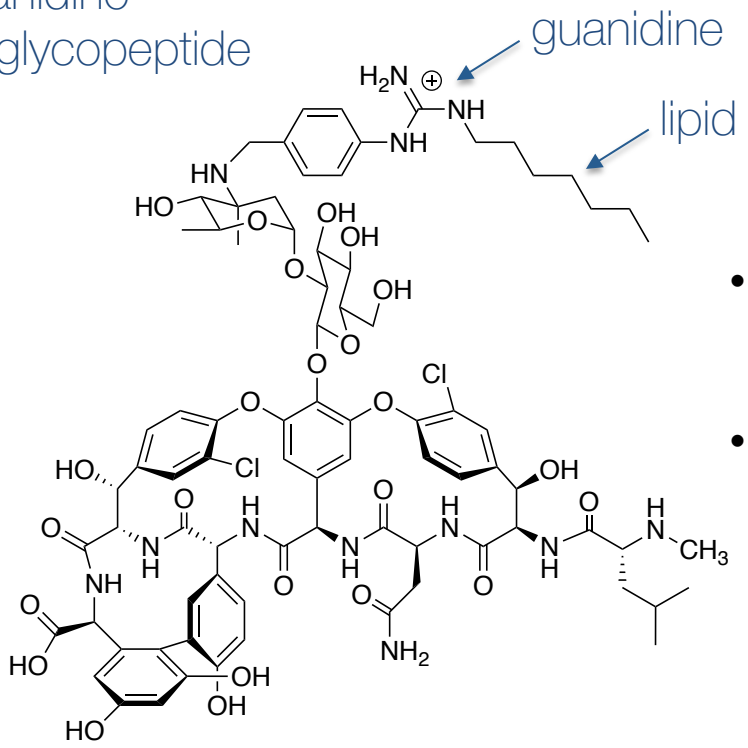


- Recently the Martin group (Leiden University) discovered the guanidino lipoglycopeptides:

Guanidino lipoglycopeptide

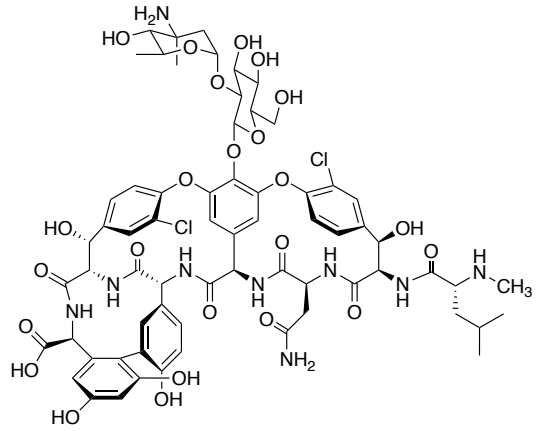


- Readily synthesized from vancomycin (scalable and cost effective)
- Extremely potent antibiotics

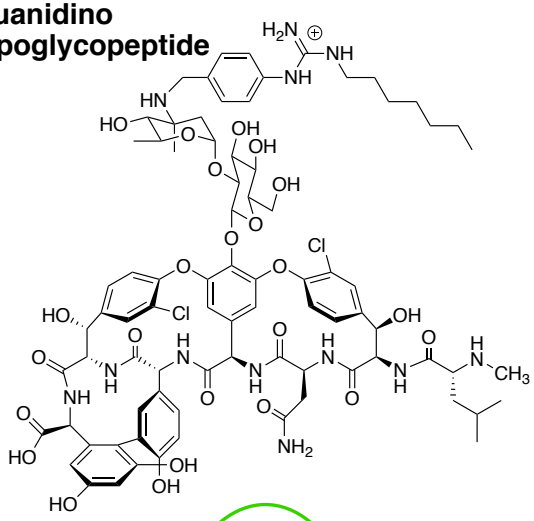
In vitro activity

- The guanidino lipoglycopeptides exhibit potent antibacterial activity (very low MIC values) superior to vancomycin:

Vancomycin



Guanidino Lipoglycopeptide



Strain	Vancomycin	Guanidino Lipoglycopeptide
MSSA	1	≤0.0078
MRSA	1	0.0156
VISA	8	0.0313
VRSA	>128	1
VSE	0.5	≤0.0078
VRE (vanB)	128	≤0.0078
VRE (vanA)	>128	2
SP	0.5	≤0.0078

Potent antibacterial effect

MSSA ATCC29213; MRSA USA300; VISA LIM-2, NR-45881; VRSA HIP13419, NR-46413; VSE 980; VRE 7314 (vanB); VRE 155 (vanA); *S. pneumoniae* 153

Cell-based assays

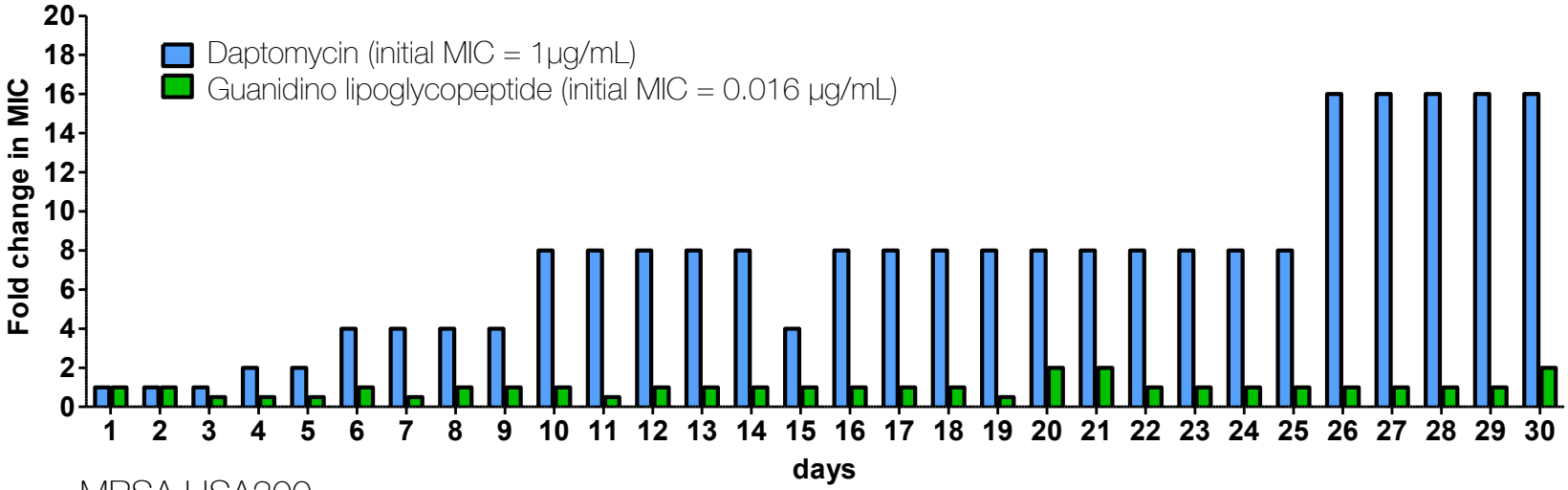
- Guanidino lipoglycopeptide found to be non-haemolytic up to 1000x MIC while clinically-used oritavancin exhibits significant hemolysis (>40% at 1000x MIC)
- Guanidino lipoglycopeptide not toxic to kidney cells at 100 μM while clinically-used oritavancin and telavancin both demonstrate toxicity:

Compound	**Cytotoxicity CC_{50} (μM)**
Vancomcyin	>100
Guanidino lipoglycopeptide	>100 ✓
Telavancin	24
Oritavancin	3.5

MTT assay with HEK293 cells, 24-hour incubation.

Resistance studies

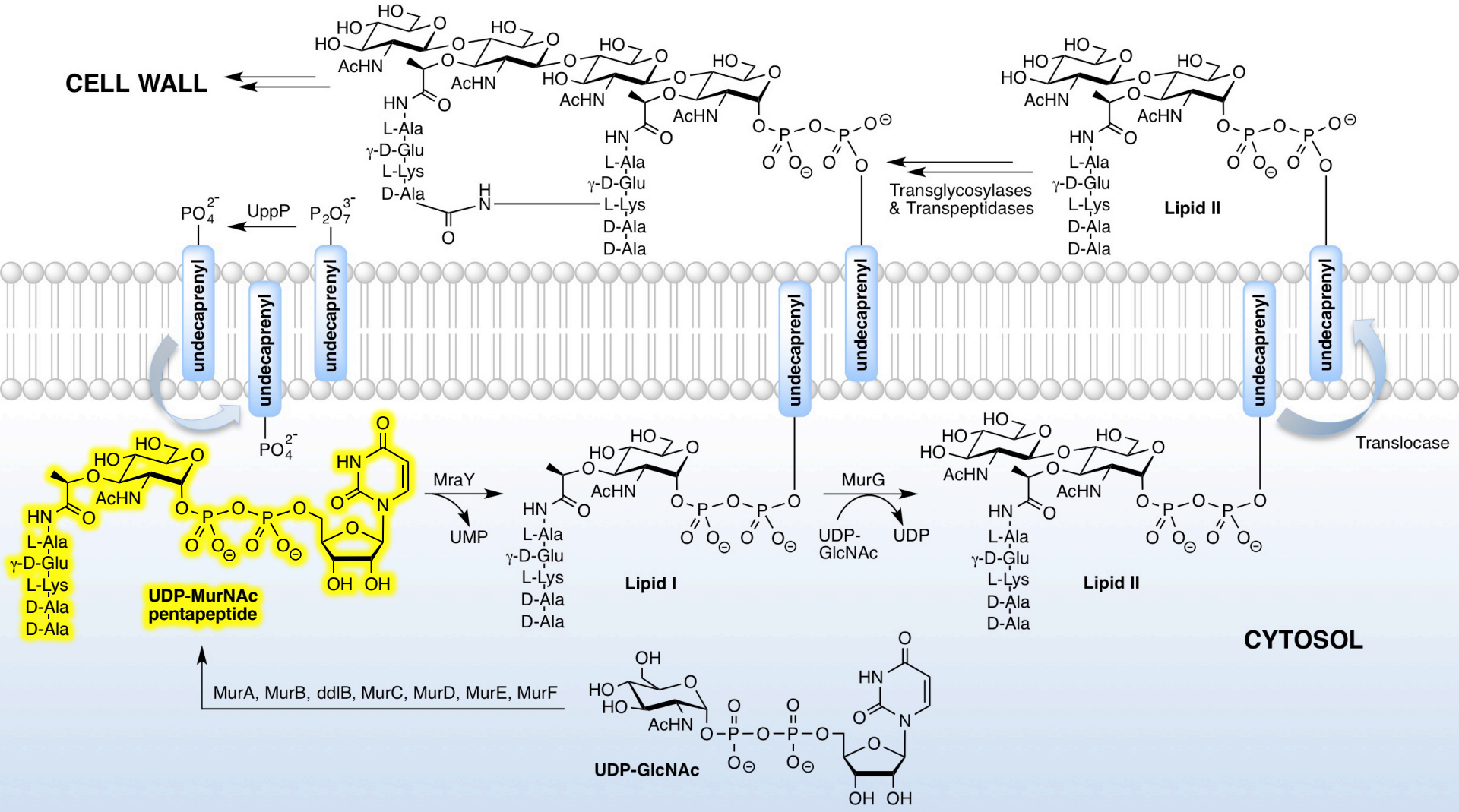
- Serial passage assays indicate guanidino lipoglycopeptides have low propensity to induce resistance compared with the clinically-used daptomycin:



MRSA USA300

Mechanistic studies: Inhibiting cell wall biosynthesis

- UDP-MurNAc pentapeptide accumulation assay:



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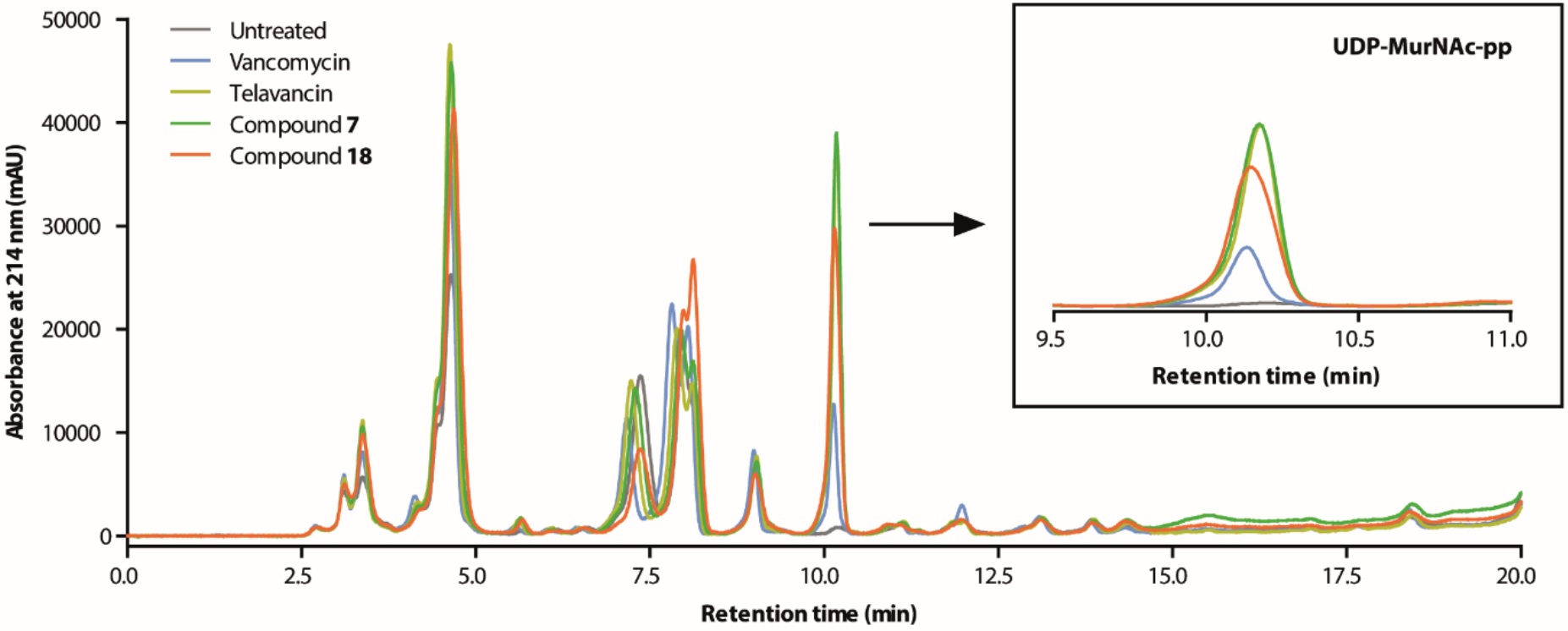
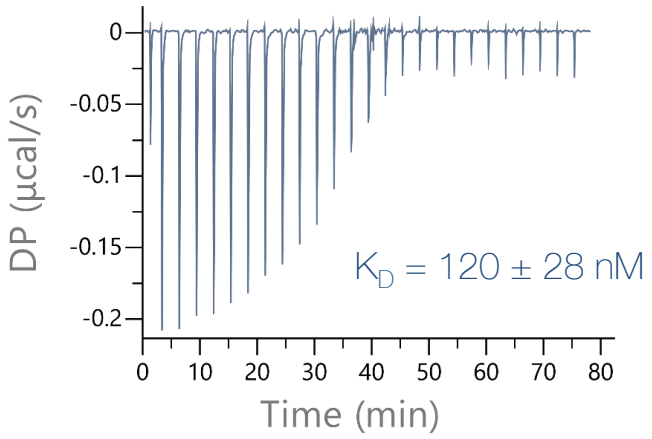


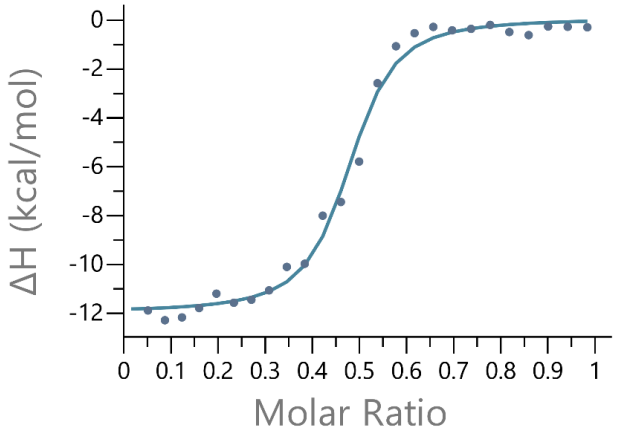
Treatment of S. aureus 29213 with glycopeptide antibiotics results in accumulation of UDP-MurNAc-pentapeptide.

Mechanistic studies: Lipid II binding

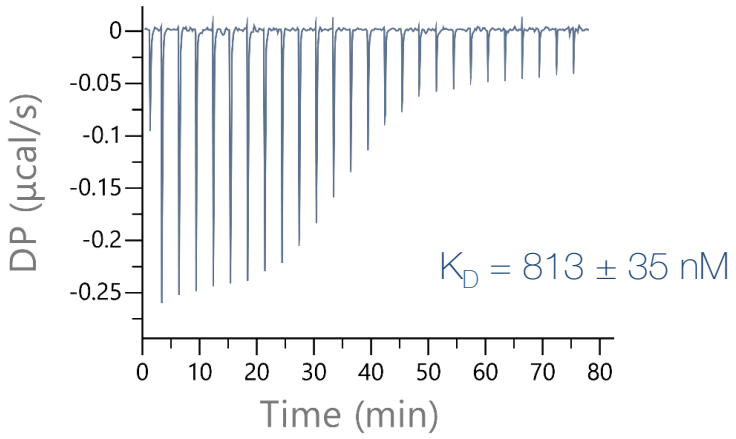
- The guanidino lipoglycopeptides bind both wild-type Lipid II as well as the D-Ala-D-Lac variant found in vancomycin resistant strains:

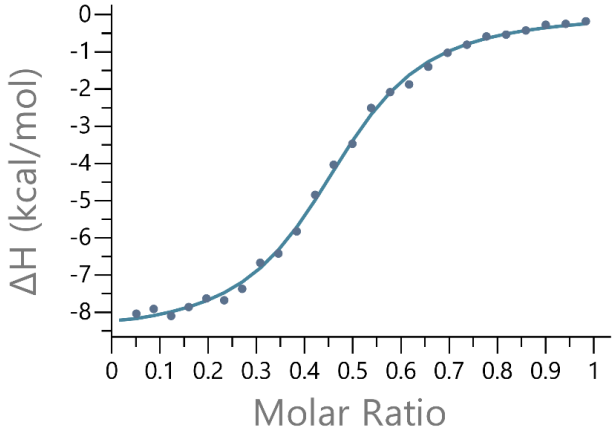
Guanidino analogue + WT Lipid II





Guanidino analogue + D-Ala-D-Lac Lipid II





In vivo studies: Tolerability, PK & efficacy

- Guanidino lipoglycopeptide well tolerated in mice as high as 100 mg/kg
- Favorable pharmacokinetics ($t_{1/2}$ = 1.22 hours) with blood concentrations maintained above MIC >8 hours when dosed at 3 mg/kg

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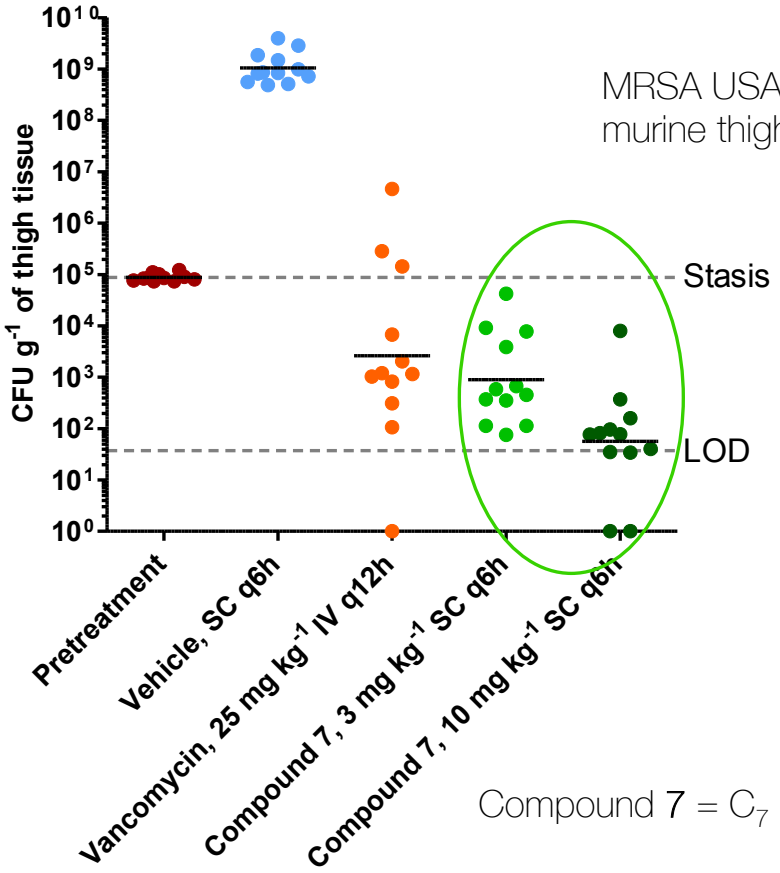
- Favorable pharmacokinetics ($t_{1/2}$ = 1.22 hours) with blood concentrations maintained above MIC >8 hours when dosed at 3 mg/kg
- *In vivo* efficacy mirrors *in vitro* data:



MRSA USA300 neutropenic murine thigh infection model

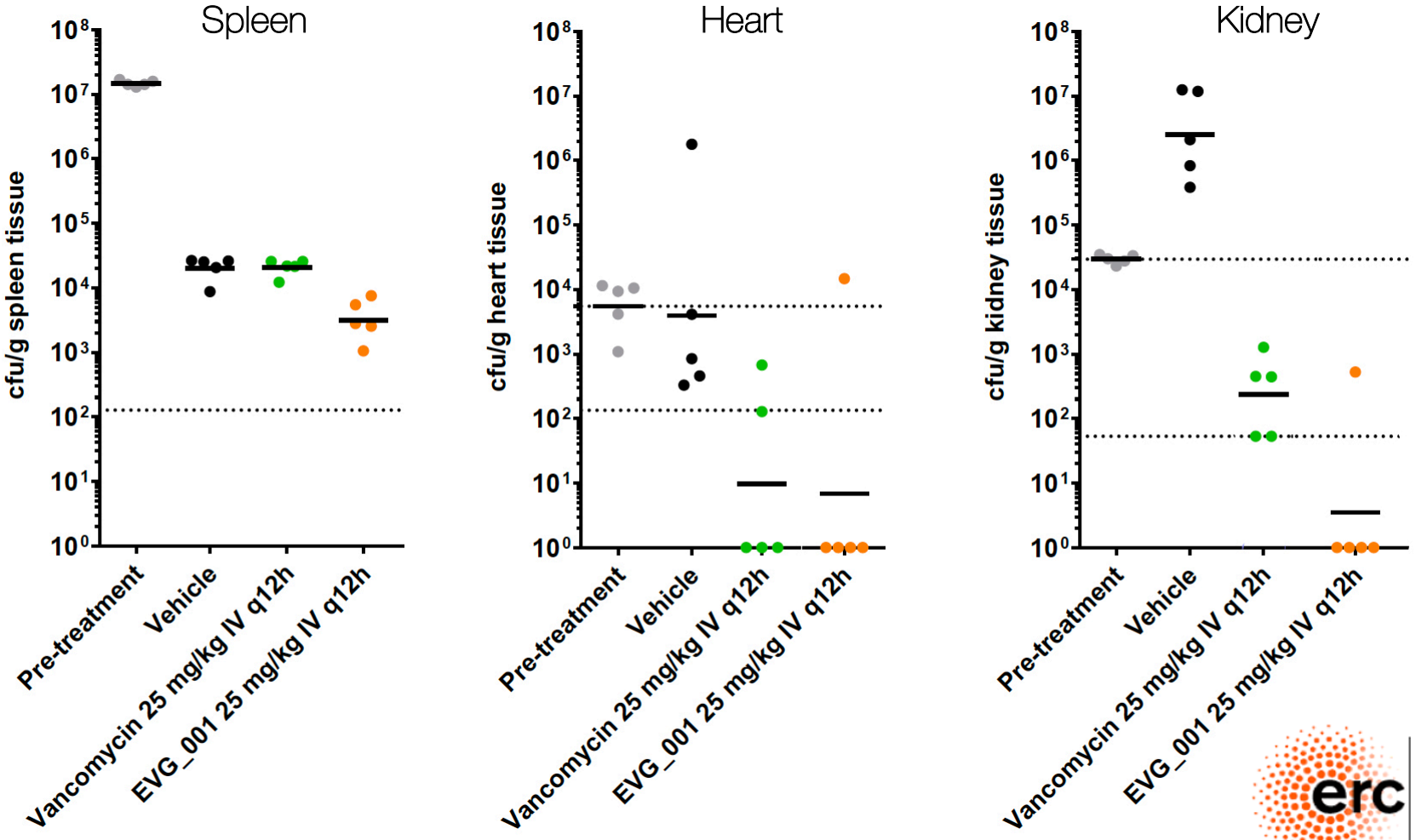
Guanidino lipoglycopeptides significantly more effective than vancomycin in vivo

Compound 7 = C_7 guanidino lipoglycopeptide

nadp netherlands antibiotic development platform

In vivo studies: Organ specificity

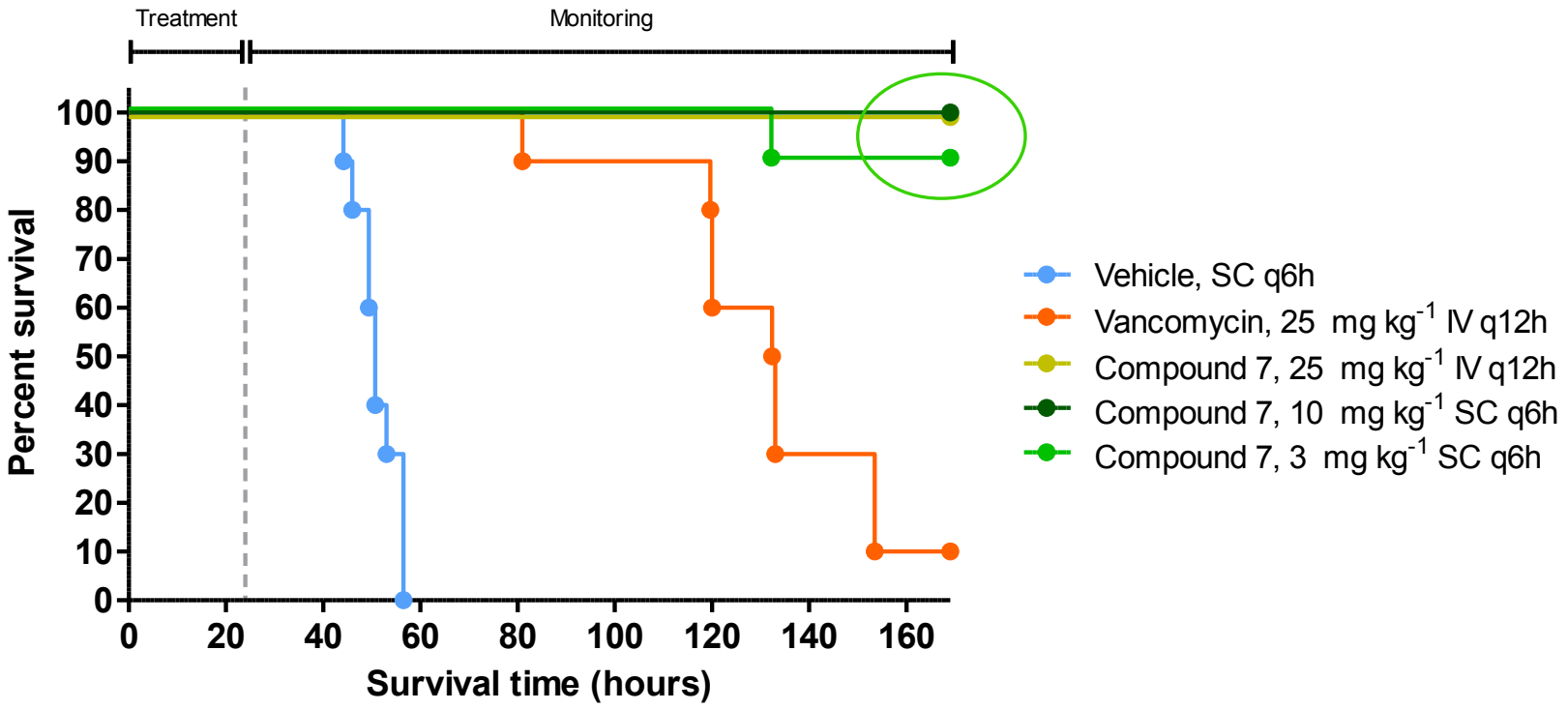
- Organ specific infection burden reduced in mice treated with guanidino lipoglycopeptide vs vancomycin in *S. aureus* sepsis study



European Research Council

In vivo studies: Sepsis survival study

- 7-day survival study with immunocompetent mice infected with *S. aureus* demonstrates superiority of Martin group's guanidino lipoglycopeptide vs vancomycin



Compound 7 = C_7 guanidino lipoglycopeptide

European Research Council

Next steps

- Guanidino lipoglycopeptides outperform vancomycin and the other clinically used glycopeptides = *best in class potential*

Enhanced Activity + Lower Toxicity = Greater Therapeutic Window

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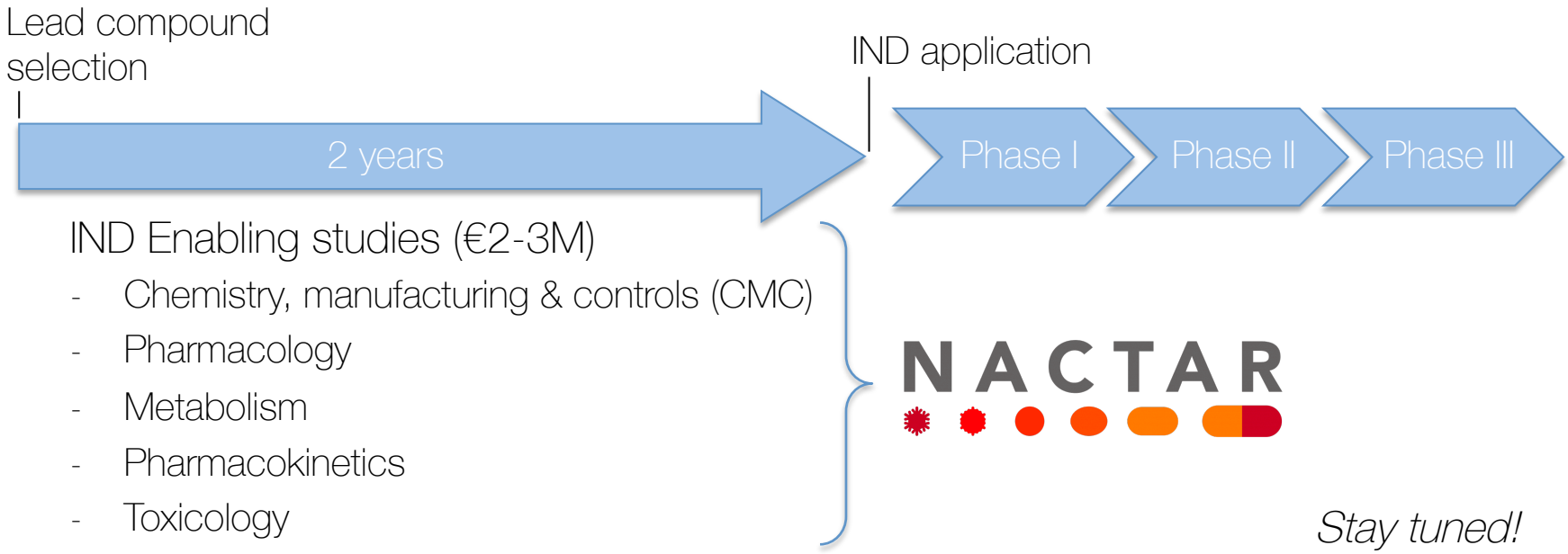
Enhanced Activity + Lower Toxicity = Greater Therapeutic Window

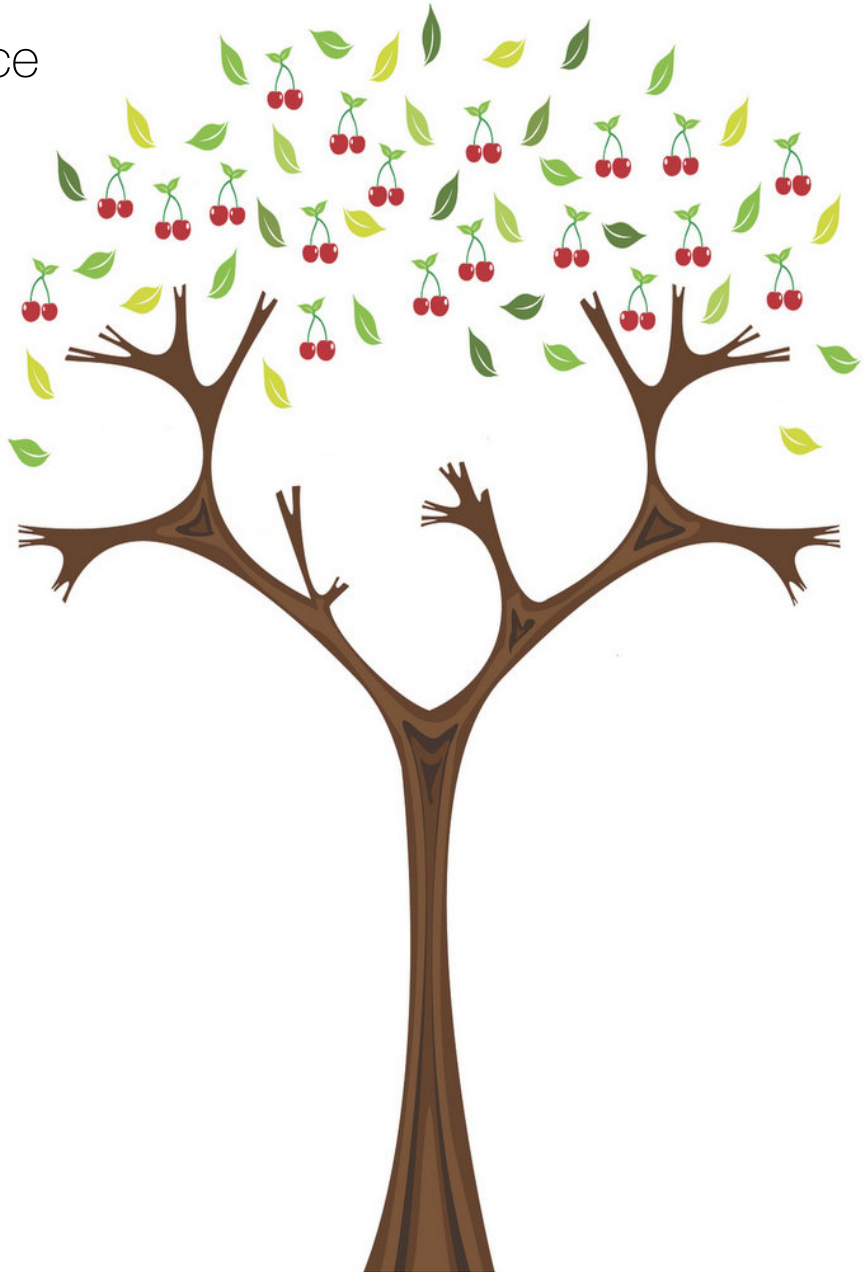
- Preclinical investigations required prior to IND application:



Stay tuned!

Conclusion

- Natural products remain valuable resource in developing new antibiotics
- (Semi)synthesis to enhance potential or provide access
- Low hanging fruit may be gone but plenty available a little higher up



Acknowledgements

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- Stefania De Benedetti (Bonn)
- Melina Arts (Bonn)
- Stephen Cochrane (Belfast)
- Ross Ballantine (Belfast)

E-mail: n.i.martin@biology.leidenuniv.nl
Twitter: @natmart_chembio

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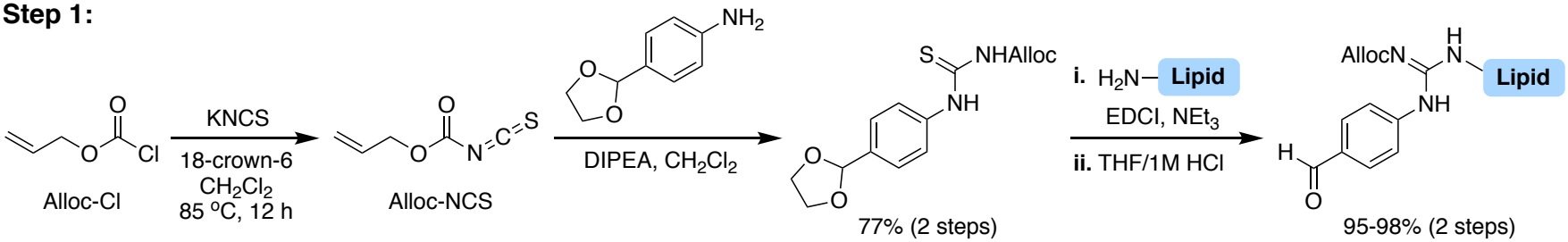
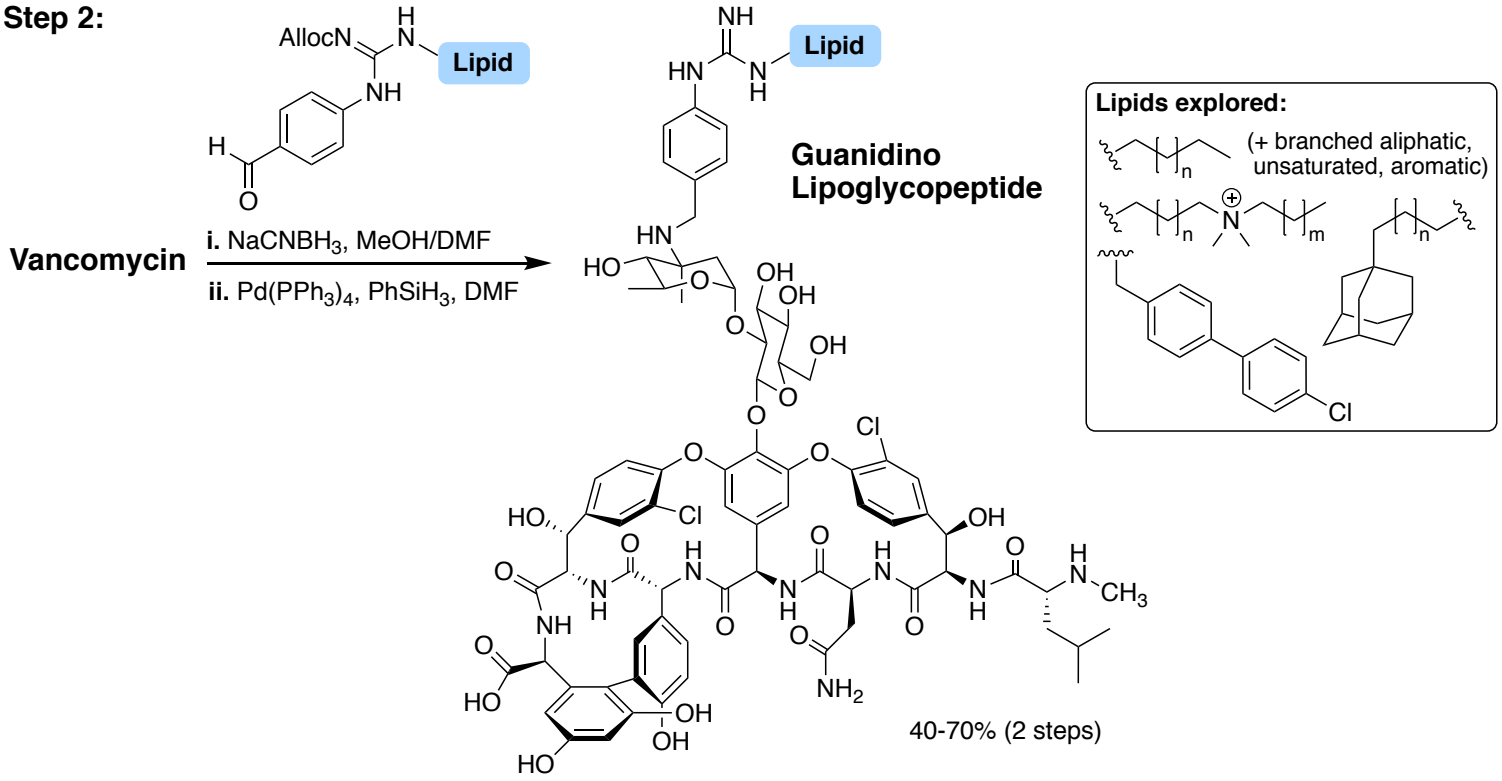
Questions?

Supplemental: Chemistry

Step 1:



Step 2: