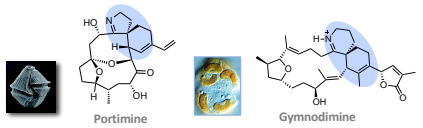
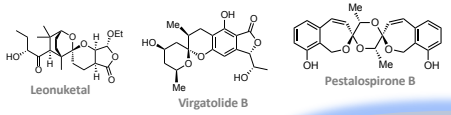
Discovery of Peptide-Based Therapeutic Agents from a New Zealand Perspective

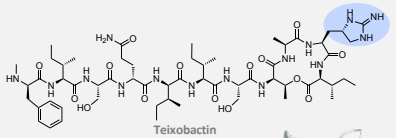
Margaret Brimble

Natural Product Synthesis



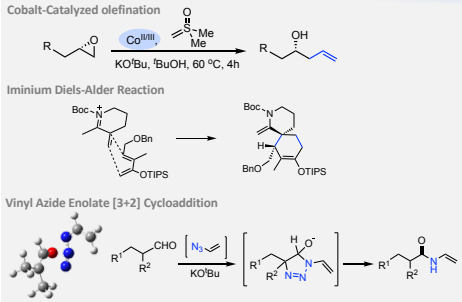


Peptide Synthesis

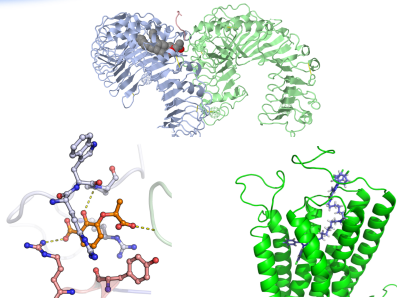


Brimble Group

New Synthetic Methods

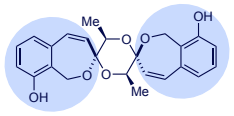


Medicinal Chemistry

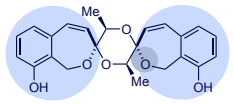


PESTALOSPIRANES

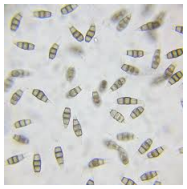
- Fungal metabolites from endophytic fungus *Pestalotiopsis virgatula*, inhabiting the plant *Terminalia chebula*
- Contain rare benzo[*c*]oxepin heterocycle
- Exhibit anti-inflammatory and analgesic activity
- Contain unprecedented 1,9,11,18-tetraoxadispiro[6.2.6.2]octadecane spiroketal
- Attractive synthetic challenge – **no total synthesis**



Pestalospirane A

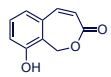


Pestalospirane B

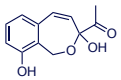


Pestalotiopsis virgatula

co-isolated with:



Xylarinol A

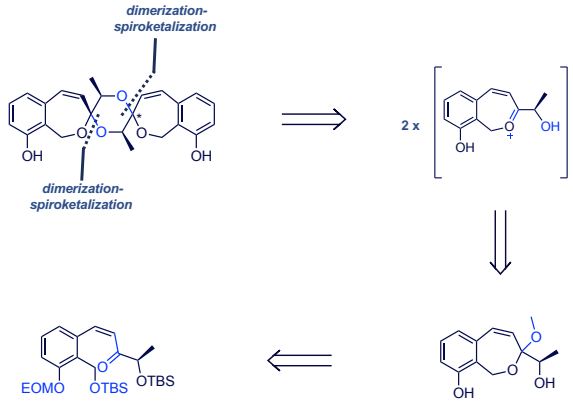


suggests related biosynthetically

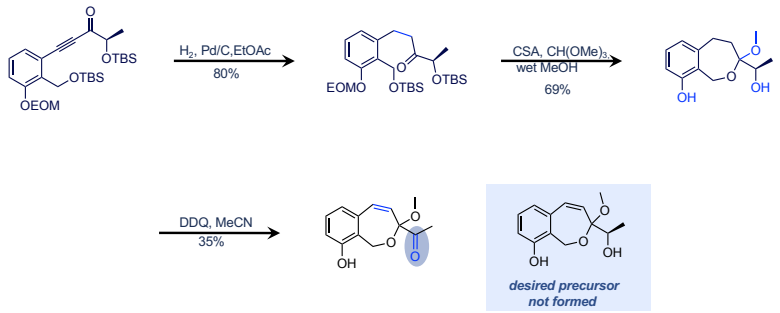
Terminalia chebula

J. R. Kesting, L. Olsen, D. Staerk, M. V. Tejesvi, K. R. Kini, H. S. Prakash, J. W. J. Jaroszewski, *J. Nat. Prod.* **2011**, *74*, 2206−2215.

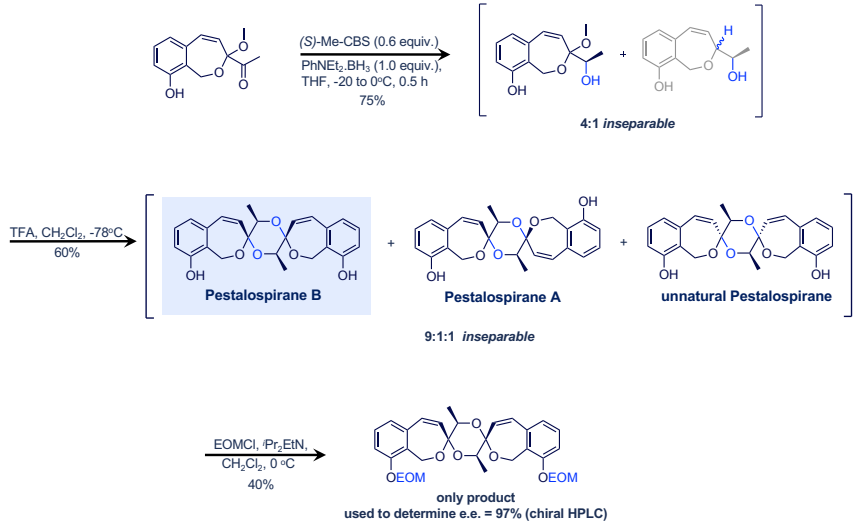
BIOINSPIRED RETROSYNTHESIS



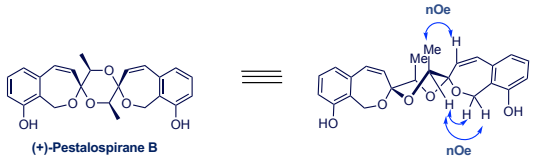
SYNTHESIS OF DIMERIZATION PRECURSOR



BIOINSPIRED TANDEM DIMERIZATION-SPIROKETALIZATION



STRUCTURE OF PESTALOSPIRANE B

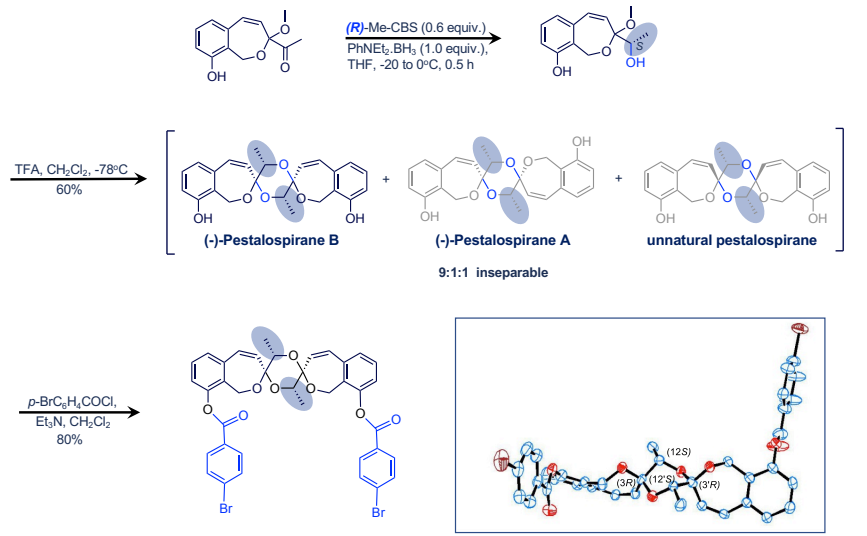


- Synthetic (+)-pestalospirane B exhibited **negative** short-wavelength Cotton effect (λ_{max} 250 nm)
- Natural product reported to exhibit **positive** Cotton effect (λ_{max} 250 nm)
- Absolute stereochemistry of natural product determined by comparing TDDFT calculations (B3LYP/TZVVP level) with ECD data
- **No optical rotation data for natural product!**

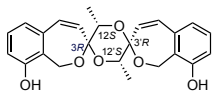
NEED TO SYNTHESIZE OPPOSITE ENANTIOMER FOR COMPARISON TO CONFIRM ABSOLUTE STEREOCHEMISTRY

S. Badrinarayanan, C.J. Squire, J. Sperry, M. A. Brimble, *Organic Letters*, **2017**, *19*, 3414-3417.

SYNTHESIS OF (-)-PESTALOSPIRANE

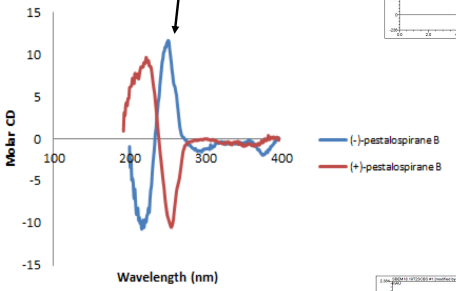


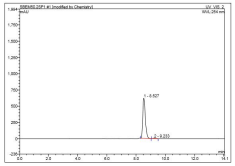
NATURAL PRODUCT IS (-)-3*R*,3'*R*,12*S*,12'*S*-PESTALOSPIRANE

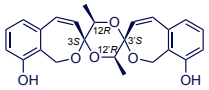


(-)-Pestalospirane B
3*R*,3'*R*,12*S*,12'*S*

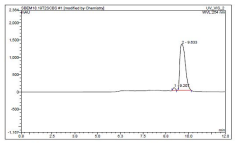
positive Cotton effect
(same as reported for natural product)





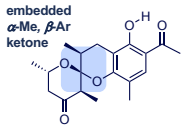


(+)-Pestalospirane B
3*S*,3'*S*,12*R*,12'*R*



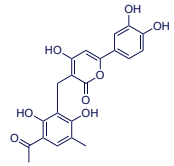
PENIPHENONE A

- (±)-Peniphenone A isolated from cultures of mangrove fungus *Penicillium dipodomycola* (strain HN4-3A), from stem of mangrove plant *Acanthus ilicifolius* in South China sea
- Isolated alongside peniphenones B/C - inhibit *Mycobacterium tuberculosis* protein tyrosine phosphate B

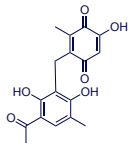


(-)-Peniphenone A

Acanthus ilicifolius



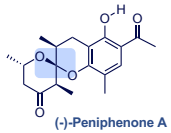
Peniphenone B
inhibits M. tuberculosis tyrosine phosphate B
(IC_{50} 0.16 μM)



Peniphenone C
inhibits M. tuberculosis tyrosine phosphate B
(IC_{50} 1.37 μM)

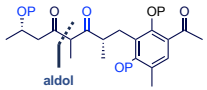
Isolation: H. Li, J. Jiang, Z. Liu, S. Lin, G. Xia, X. Xia, B. Ding, L. He, Y. Lu, Z. She, *J. Nat. Prod.* **2014**, *77*, 800-806.
Synthesis: J. T. J. Spence, J. H. George, *Org. Lett.*, **2015**, *17*, 5970-5973.

RETROSYNTHESIS

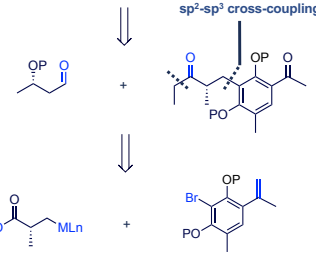


(-)-Peniphenone A

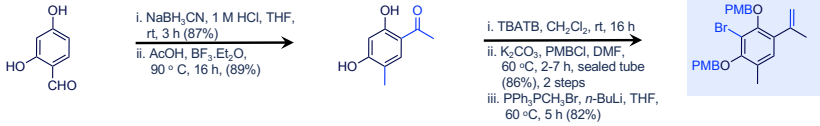
acid-catalyzed cyclization

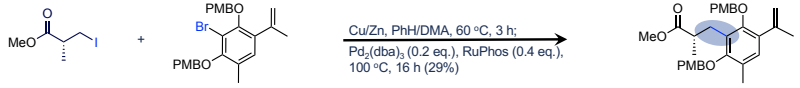


sp^2-sp^3 cross-coupling



SYNTHESIS OF α-Methyl β-Aryl MOTIF

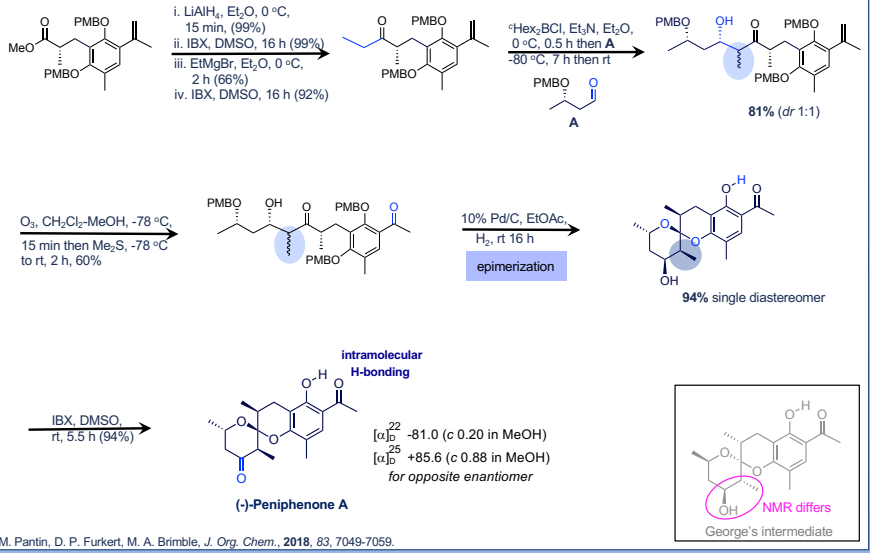




Negishi sp^2-sp^3 cross coupling
(Buchwald conditions)

J. E. Milne, S. L. Buchwald, *J. Am. Chem. Soc.* **2004**, *126*, 13028-13032.

CONVERSION TO SPIROKETAL

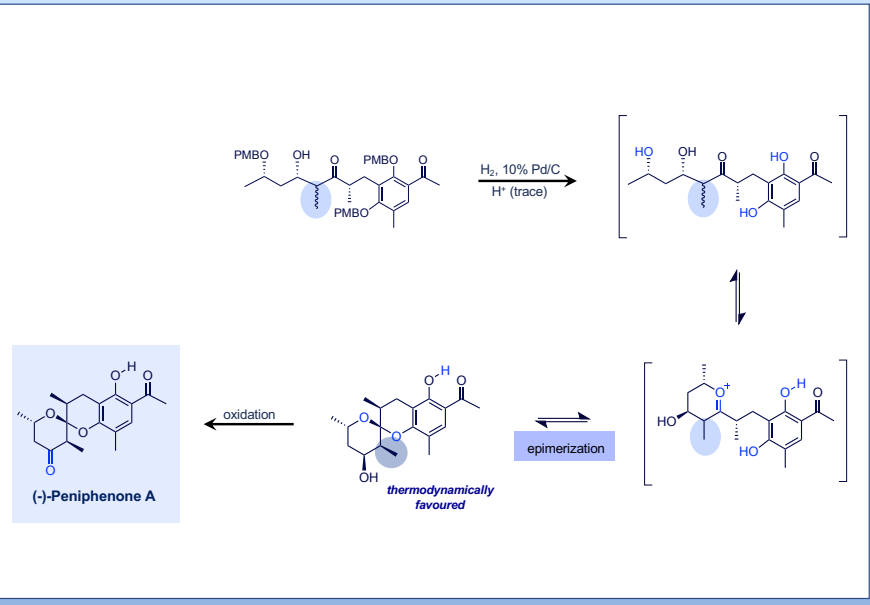


M. Pantin, D. P. Furkert, M. A. Brimble, *J. Org. Chem.*, **2018**, *83*, 7049-7059.

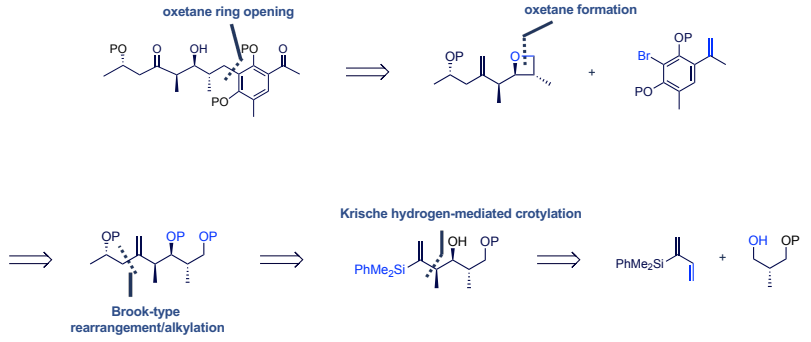
STEREOCHEMISTRY

MATHILDE PANTIN (PhD student)

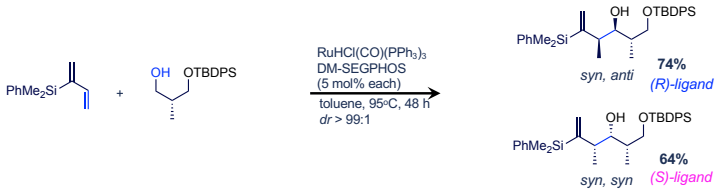
MECHANISM

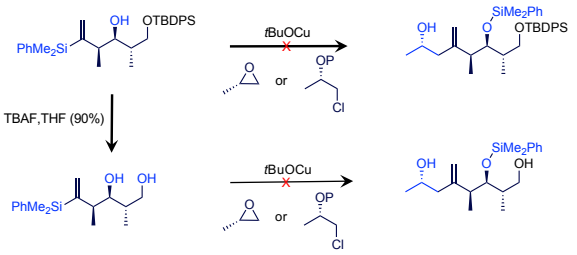


AN ALTERNATIVE APPROACH



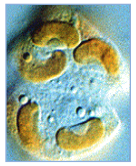
KRISCHE TYPE H-MEDIATED C-C COUPLINGS



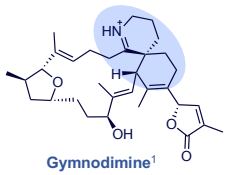


M. Panthin, D. P. Furkert, M. A. Brimble, *J. Org. Chem.*, **2018**, *83*, 7049-7059.

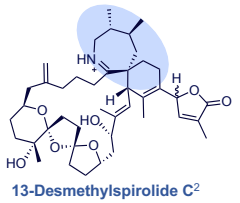
SPIROIMINE SHELLFISH TOXINS



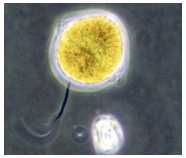
Gymnodinium mikomotai



Gymnodimine[1]

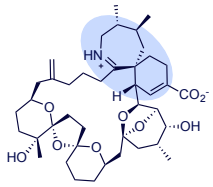


13-Desmethylspirolide C[2]

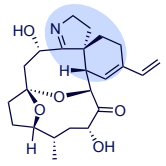


Alexandrium ostenfeldii

Pinna muricata



Pinnatoxin A[3]

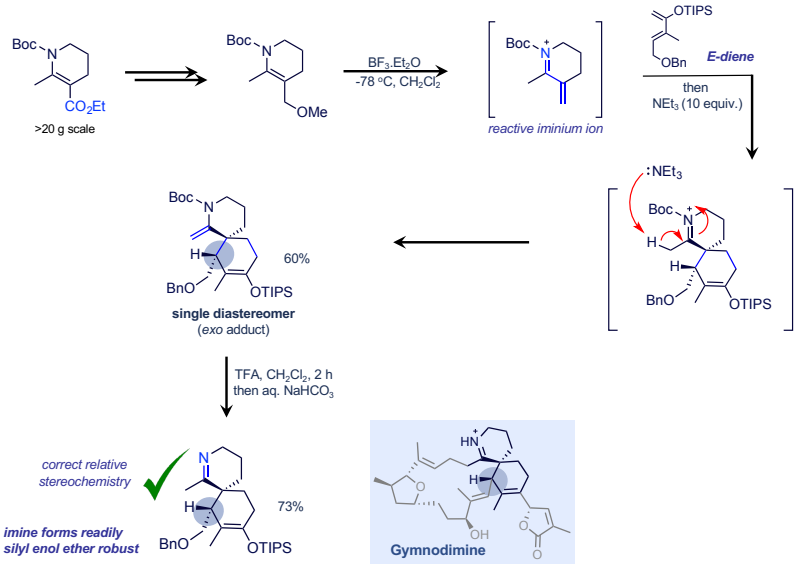


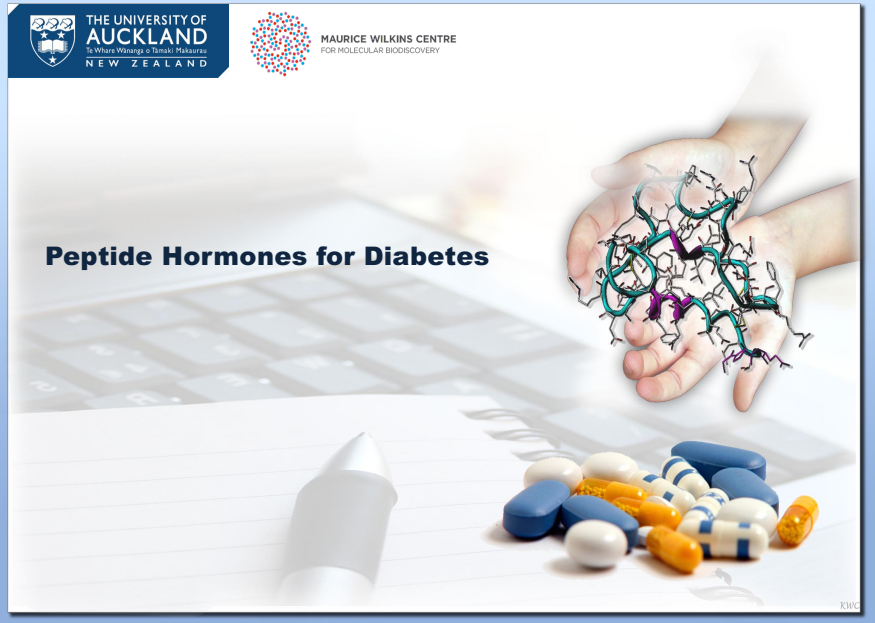
Portimine[4]

Vulcanodinium rugosum

1. T. Seki, M. Satake, L. Mackenzie, H.F. Kaspar, T. Yasumoto, *Tetrahedron Lett.*, **1995**, *36*, 7093-7096; **2.** T. Hu, I. Burton, A. Cembella, J.M. Curtis, M.A. Quilliam, J.A. Walter, J.L.C. Wright, *J. Nat. Prod.*, **2001,** *64*, 308-312; **3.** D. Uemura, T. Chuo, T. Haino, A. Nagatsu, S. Fukuzawa, S. Zheng, H. Chen, *J. Am. Chem. Soc.*, **1995**, *117*, 1155; **4.** A I. Selwood, A.L. Wilkins, R. Munday,, F. Shi, L.L. Rhodes, P.T. Holland, *Tetrahedron Lett.*, **2013**, *54*, 4705-4707.

IMINIUM DIELS-ALDER TOWARDS GYMNODIMINE



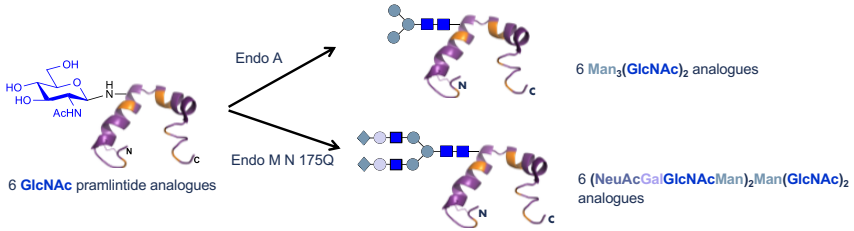


PRAMLINTIDE

- Pramlintide (Symlin®) – synthetic analogue of human amylin
- Non-aggregating, non-amyloidogenic, more soluble, equipotent to human amylin
- Used in adjunctive therapy for type 1 and type 2 diabetes
- $t_{1/2}$ 48 min; precipitates above pH 5.5; need subcutaneous injection – 3 x daily

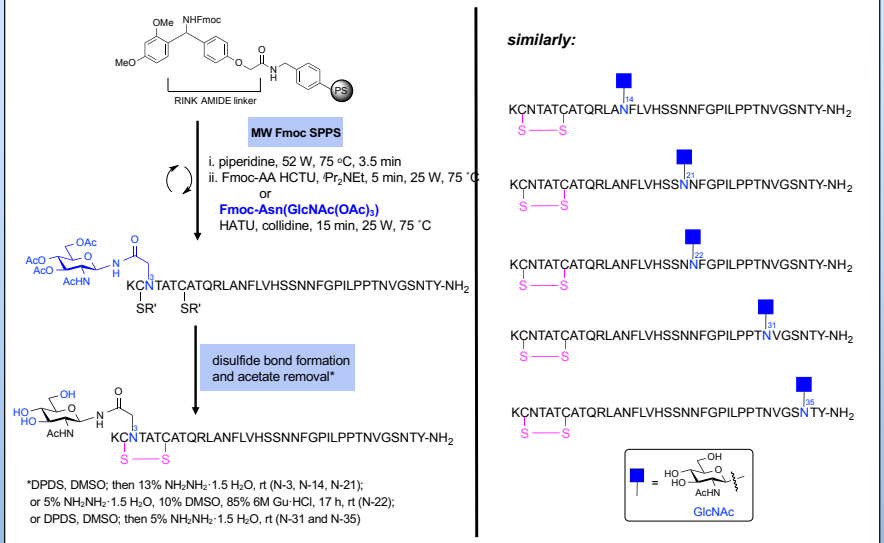
KCN-TAT-CAT-QRL-ANF-LVH-SSN-NFG-PIL-PPT-NVG-SNT-Y-NH_2

use chemoenzymatic *N*-glycosylation (N3, N14, N21, N22, N31, N35)
to improve pharmacokinetics and physiochemical properties



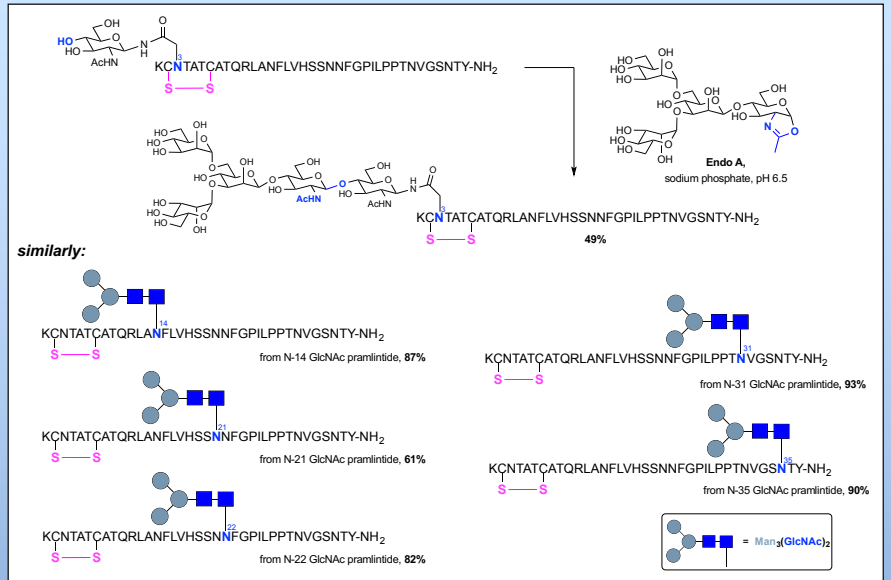
Cooper *et al.*, *Proc. Natl. Acad. Sci. U.S.A*, **1987**, *84*, 8628. Younk, *et al.*, *Exp. Opin. Pharmacother.*, **2011**, *12*, 1439.
R. Kowalczyk, M. A. Brimble, Y. Tomabechi, A. J. Fairbanks, M. Fletcher, D. L. Hay, *Org. Biomol. Chem.*, **2014**, *12*, 8142-8151.

GLYCOPRAMLINTIDES (native GlcNAc)



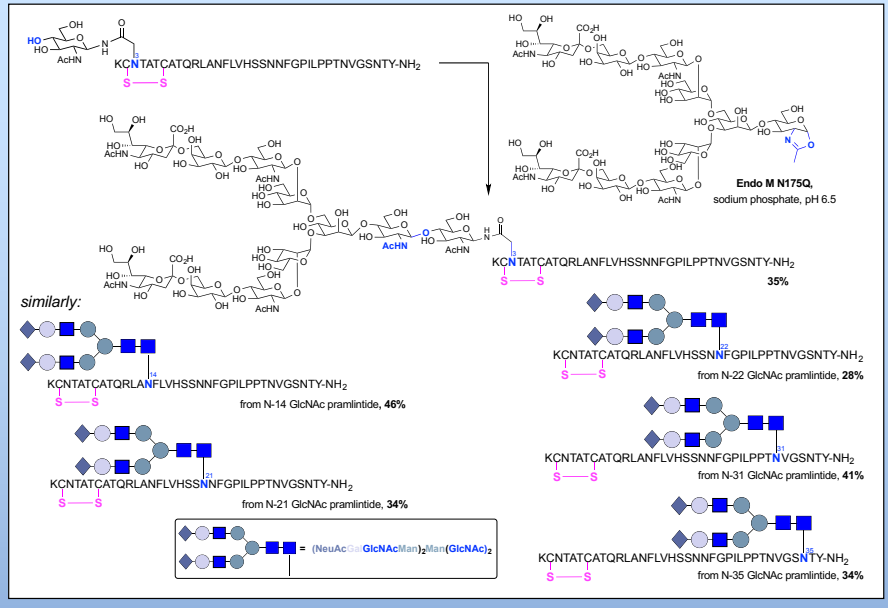
R. Kowalczyk, M. A. Brimble, Y. Tomabechi, A. J. Fairbanks, M. Fletcher, D. L. Hay, *Org. Biomol. Chem.*, **2014**, *12*, 8142-8151.

GLYCOPRAMLINTIDE PENTASACCHARIDES



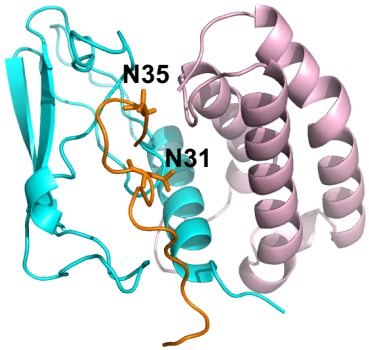
R. Kowalczyk, M. A. Brimble, Y. Tomabechi, A. J. Fairbanks, M. Fletcher, D. L. Hay, *Org. Biomol. Chem.*, **2014**, *12*, 8142-8151.

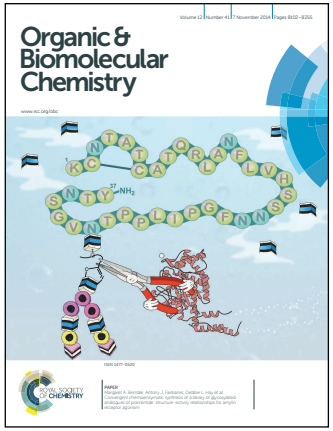
GLYCOPRAMLINTIDE UNDECASACCHARIDES



NATIVE GLYCOPRAMLINTIDES

- Agonist activity at $AMY_{1(a)}$ receptor best for **N-21, N-31 and N-35** glycopramlintides (C-term)
- **Agonist activity decreases with the size of the sugar:**
  undecasaccharide < pentasaccharide < GlcNAc
- Constructed a model consistent with these findings





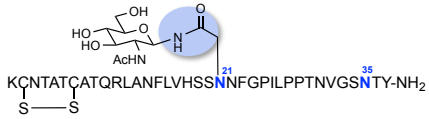
R. Kowalczyk, M. A. Brimble, Y. Tomabechi, A. J. Fairbanks, M. Fletcher, D. L. Hay, *Org. Biomol. Chem.*, **2014**, *12*, 8142-8151.

CLICK GLYCOPRAMLINTIDES

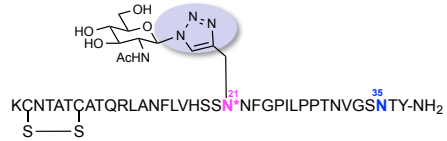
Previous study showed *N*-Glycosides at **N-21** and **N-35** most active but:

- only used single GlcNAc substitutions
- native *N*-glycosides tedious to prepare

Use click chemistry to efficiently generate glycomimetics.
Do click analogues act similarly to native N-glycosides at $AMY_{1(a)}$ receptor?

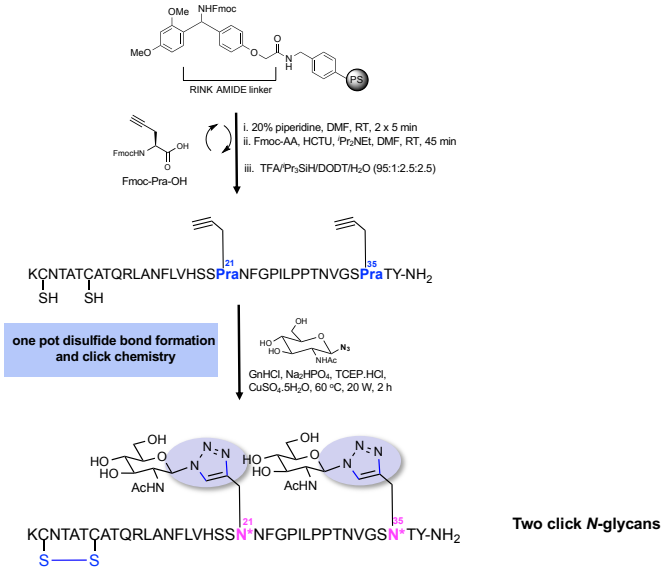


Native *N*-glycan



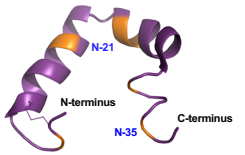
Click *N*-glycan

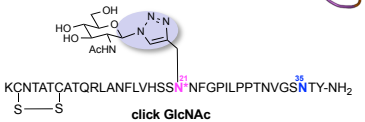
CLICK GLYCOPRAMLINTIDES - SYNTHESIS



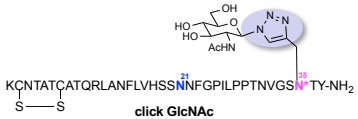
Two click *N*-glycans

NATIVE vs CLICK GLYCOPRAMLINTIDES

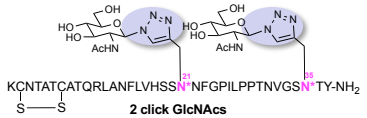




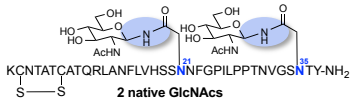
click GlcNAc



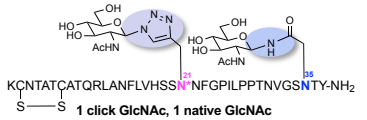
click GlcNAc



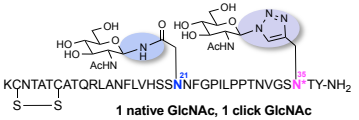
2 click GlcNAcs



2 native GlcNAcs

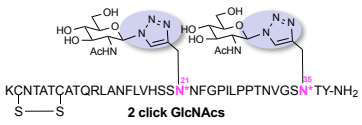


1 click GlcNAc, 1 native GlcNAc

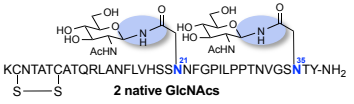


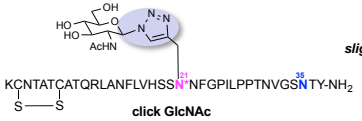
1 native GlcNAc, 1 click GlcNAc

AMYLIN RECEPTOR ACTIVITY



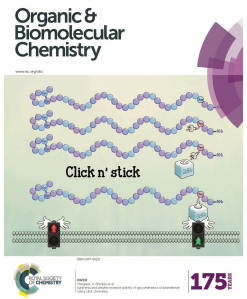
similar activity to





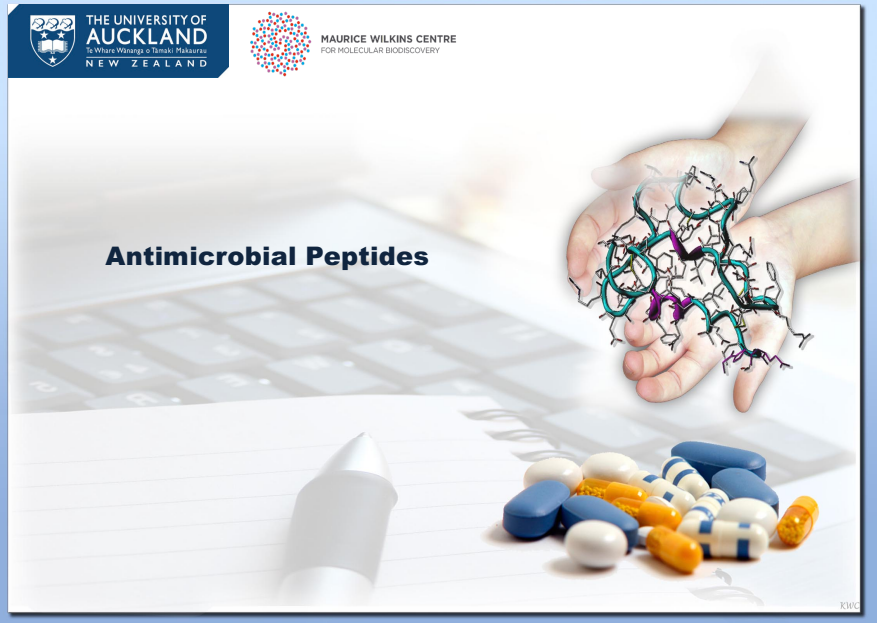
slightly more active than

AMY1$_{(a)}$ activity maintained when **2** GlcNAc moieties introduced using native GlcNAc or click GlcNAc or a combination of both



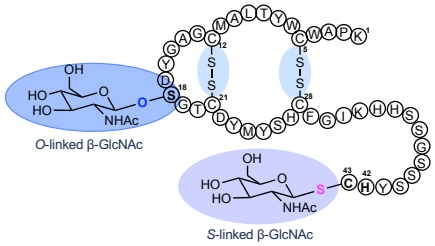
LAUREN YULE

L.R. Yule, R.L. Bower, H. Kaur, R. Kowalczyk, D.L. Hay, M.A. Brimble, *Org. Biomol. Chem.*, **2016**, *14*, 5238-5245.

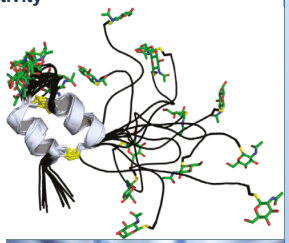


GLYCOCIN F – GLYCOSYLATED BACTERIOCIN

- Bacteriocin: ribosomally-synthesised antimicrobial peptide with narrow phylogenetic spectrum of activity
- Glycocin F – secreted by *Lactobacillus plantarum* KW30
- Rapid and sustained bacteriostasis (IC_{50} of 2 nM); **Potential probiotic**
- βGlcNAc at Ser-18, βGlcNAc at Cys-43, His-42 – **All needed for activity**



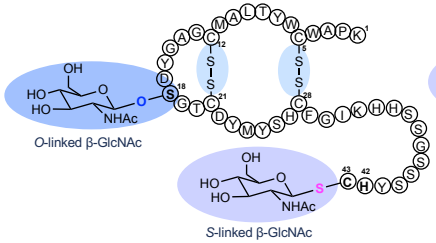
Glycocin F



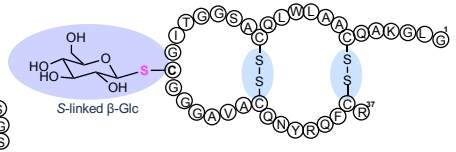
G. E. Norris *et al*, *FEBS Lett.*, **2011**, *585*, 645-650; *Biochem.*, **2011**, *50*, 2748.

S-LINKED GLYCOPEPTIDES

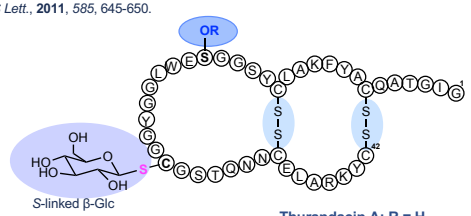
only **three** rare ***S*-linked** glycopeptides:



Glycocin F - *Lactobacillus plantarum KW30*
G. E. Norris *et al*, *FEBS Lett.*, **2011**, *585*, 645-650.



Sublancin 168 – *Bacillus subtilus 168*
H. Wang, W. A. Van der Donk,
J. Am. Chem. Soc., **2011**, *133*, 16394-16397.

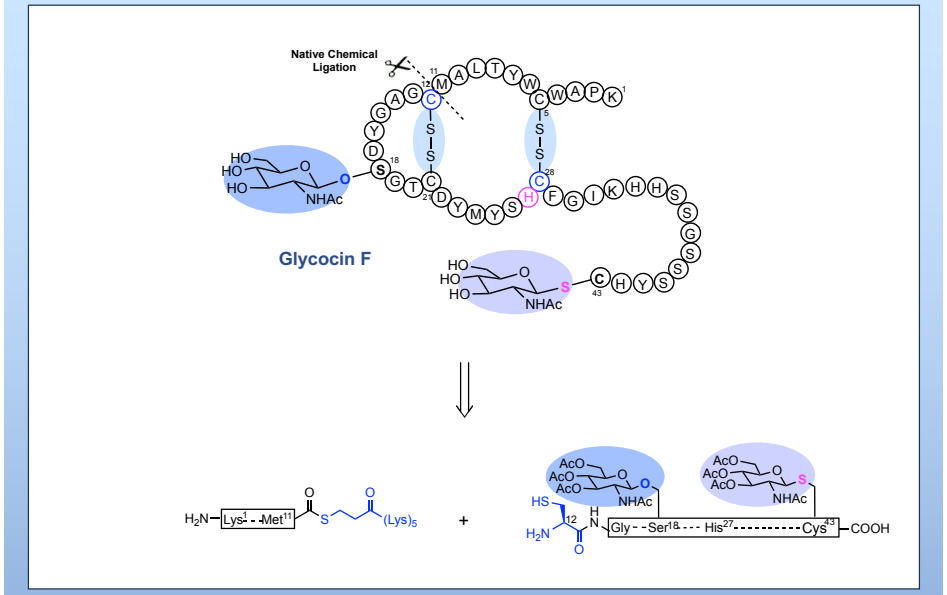


recombinant gene products from putative bacteriocin gene cluster in *Bacillus thuringiensis serovar andalousiensis BGSC 4AW1*

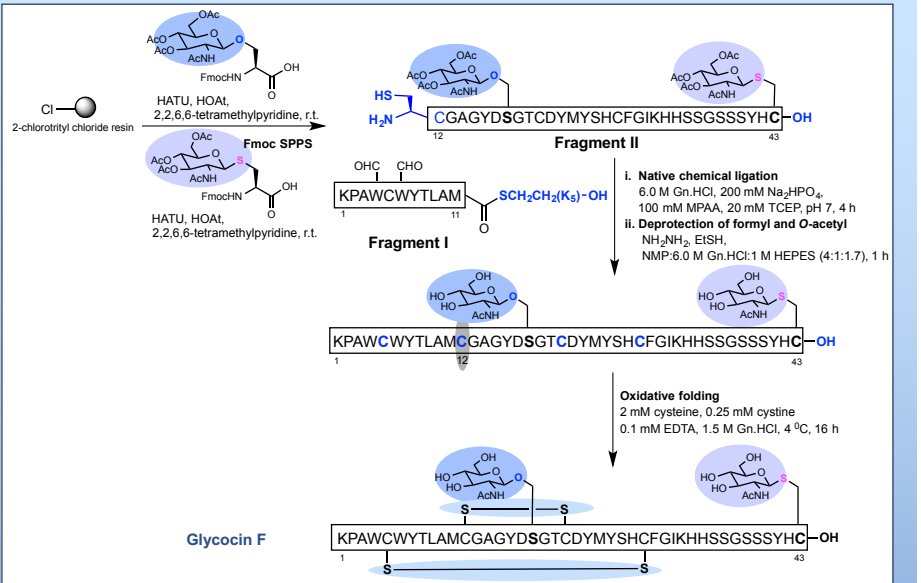
Thurandacin A: R = H
Thurandacin B: R = β-glucosyl

W. A. Van der Donk et al, *J. Am. Chem. Soc.*, **2014**, *136*, 84-87.

GLYCOCIN F – REVISED SYNTHETIC STRATEGY

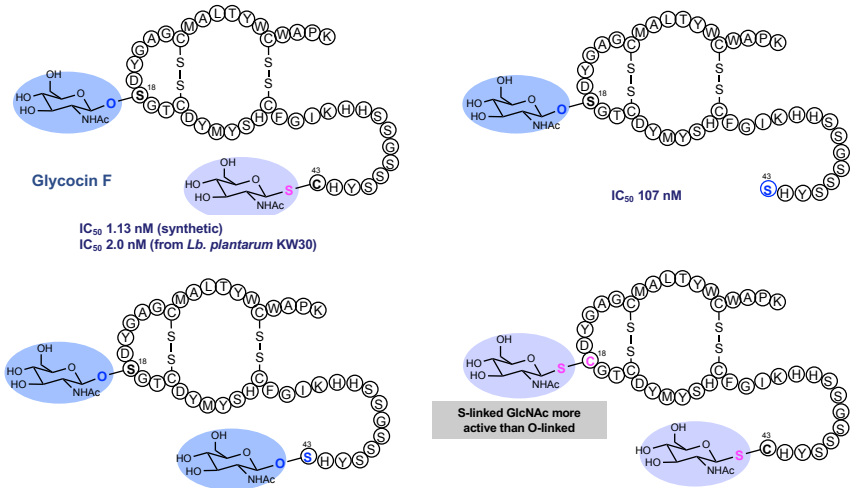


SYNTHESIS OF GLYCOCIN F



DOUBLE S-LINKED ANALOGUE

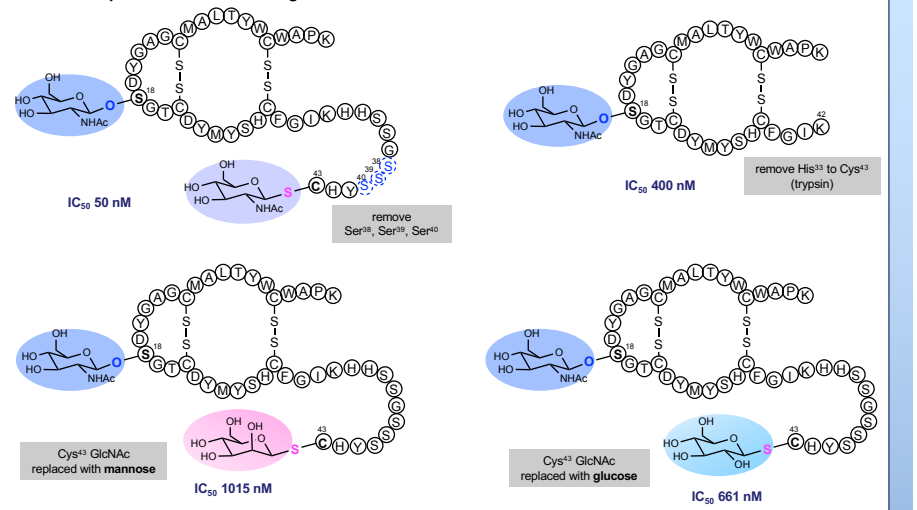
***Lactobacillus plantarum* ATCC 8014 – growth curve method**



"Total Chemical Synthesis of Glycocin F and Analogues: S-Glycosylation Confers Improved Antimicrobial Activity" Z. Amso, S. W. Bisset, S.-H. Yang, P. W. R. Harris, T. H. Wright, G. E. Norris, M. A. Brimble, *Chemical Science*, **2018**, *9*, 1686-1691.

C-TERMINAL TAIL ANALOGUES

***Lactobacillus plantarum* ATCC 8014 – growth curve method**



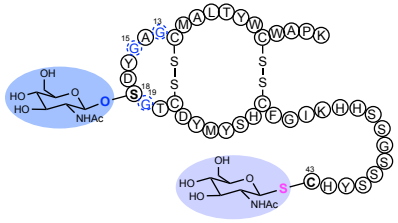
Length of C-terminal tail important for activity
Nature of C-terminal tail sugar also important for activity

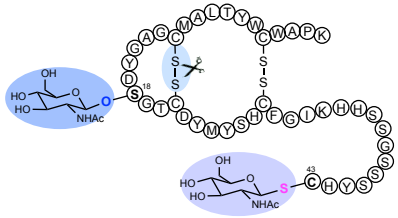
INTERHELICAL LOOP ANALOGUES

***Lactobacillus plantarum* ATCC 8014 – growth curve method**

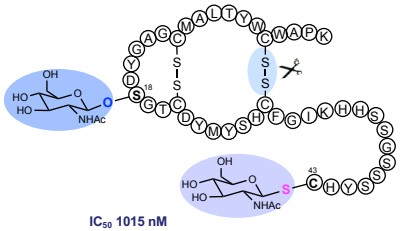
IC_{50} 57.6 nM	Gly[13] removed from loop
IC_{50} 2480 nM	Gly[13] and Gly[15] removed from loop
IC_{50} 2710 nM	Gly[13], Gly[15] and Gly[19] removed from loop

change in loop between residues 13 and 20 **reduces activity** and **decreases α-helical content** of glycocin F





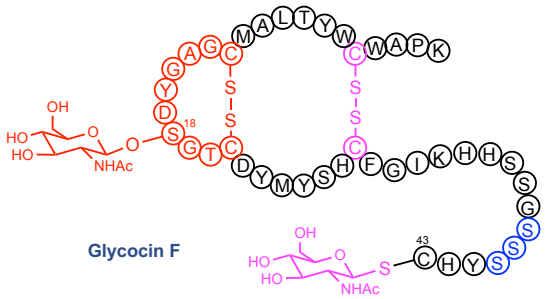
No activity



IC_{50} 1015 nM

Disulfide bonds essential for activity
– maintain stability and integrity of loop and/or hold alpha helices in specific position

GLYCOCIN F: STRUCTURE-ACTIVITY



Glycocin F

High effect on activity

Low effect on activity

SEAN BISSET
(PhD student)

ZAID AMSO
(PhD student)

GILL NORRIS
Massey University

"Using Chemical Synthesis to Probe Structure-Activity Relationships of the Glycoactive Bacteriocin Glycocin F" S. Bisset, S.-H. Yang, Z. Amso, P. Harris, M. Patchett, M. A. Brimble and G. Norris, *ACS Chemical Biology*, **2018**, *13*, 1270-1278.