Síntesis de péptidos y ciclopéptidos como posibles aplicaciones de la Biotecnología. Agentes antimaláricos, Herbicides o Inhibidores de Cianobacterias

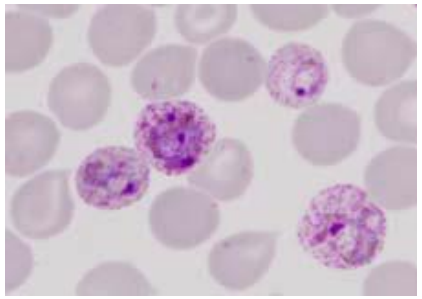
Gloria Serra, C. Fagundez, L. Posada, S. Peña, L. Scarone, D. Sellanes, C. Irabuena, H. Hojo

gserra@fq.edu.uy
Lab. de Química Farmaceútica, DQO
Facultad de Química, Universidad de la República
Uruguay

Introduction: Malaria

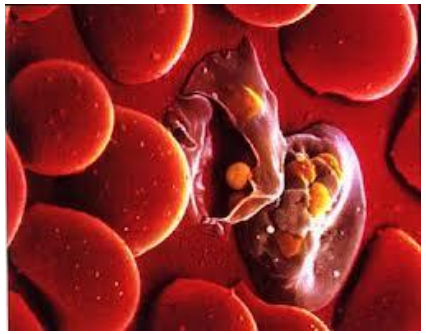
MALARIA

Plasmodium falciparum
P.vivax, P. malariae,
P. ovale, P. knowlesi



Anopheles mosquito

Fever, headache, anaemia
Liver and red blood cells infection



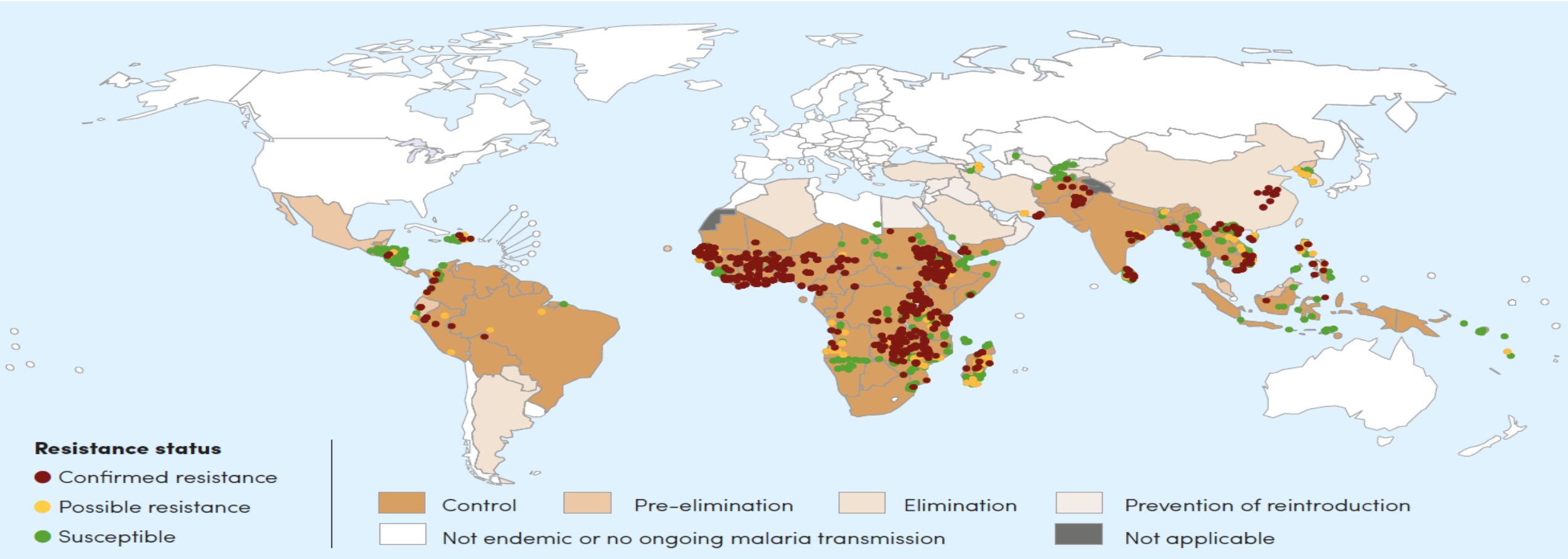
262 m cases in 2000
219 m cases in 2017
241 m cases in 2020

839, 000 deaths in 2000
558,000 deaths in 2019,
627,000 deaths in 2020
77% children under 5 years

WHO, Malaria Report **2021**

Introduction: Anopheles resistance

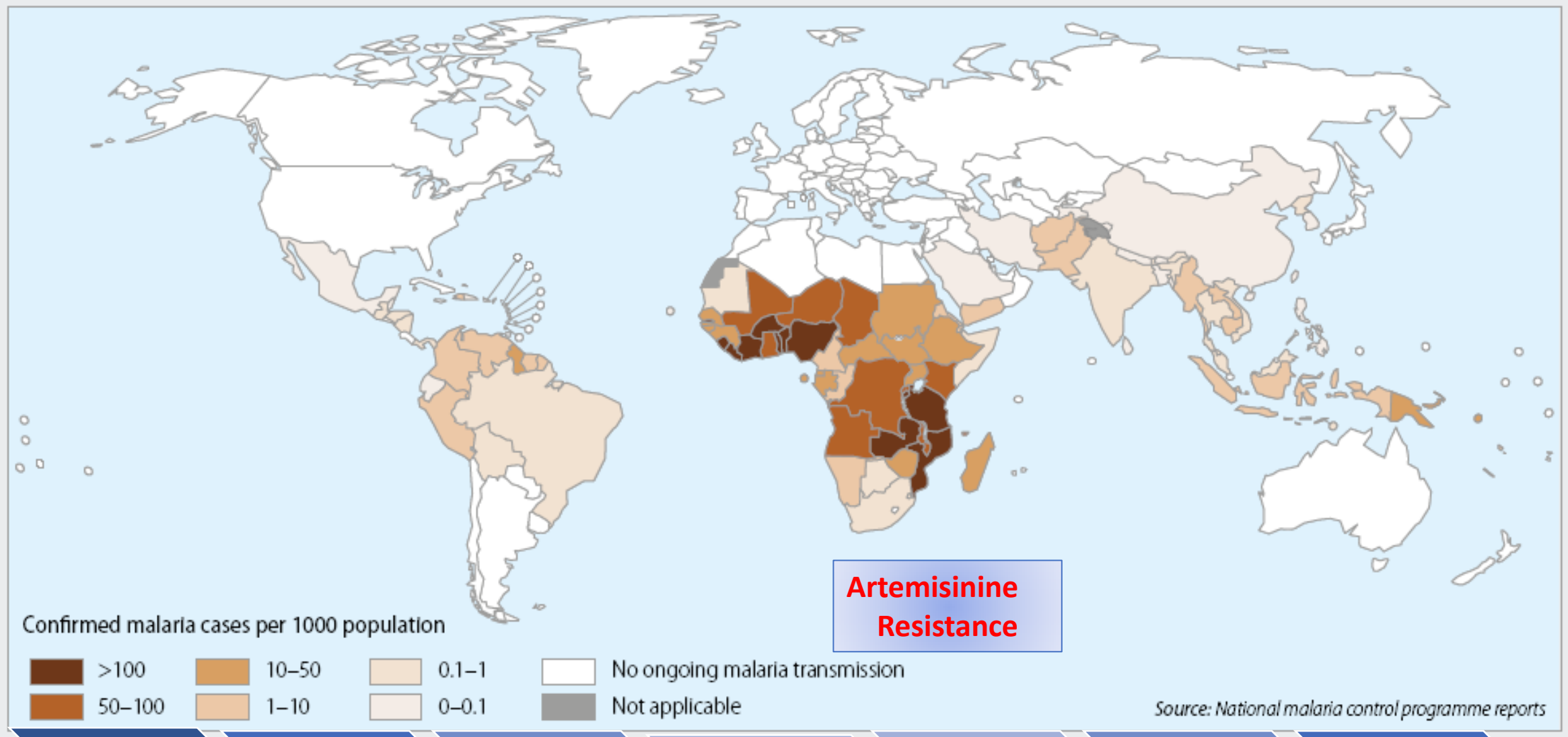
The effectiveness of insecticide-based vector control is threatened as malaria mosquitoes develop resistance to the insecticides used in **ITNs** (insecticide-treated mosquito net) and **IRS** (indoor residual spraying).



Reported pyrethroid resistance status of malaria vectors.

World Malaria Report WHO

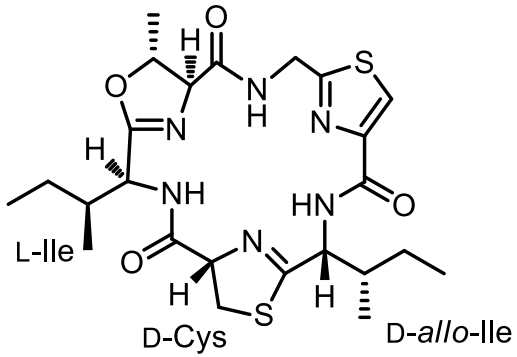
Introduction: Antimalarial Drugs



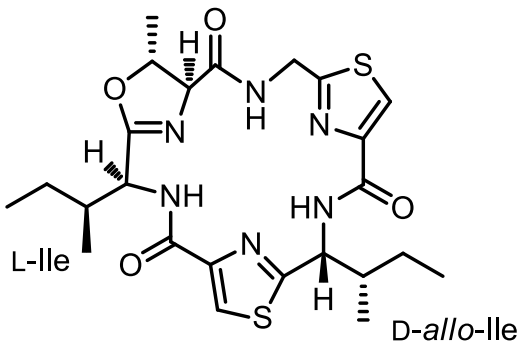
Quinine (1632) R: 1910 → Chloroquine (1945) R: 1957 → Proguanil (1948) R: 1949 → Phirymethamine (1967) R: 1967 → Mefloquine (1977) R: 1982 → Atovaquone (1996) R: 1996 → ACT (1990) R: 2009

World Malaria Report 2018, WHO

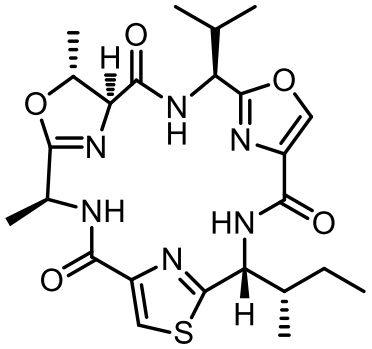
Introduction: Macrocyclic Natural Products



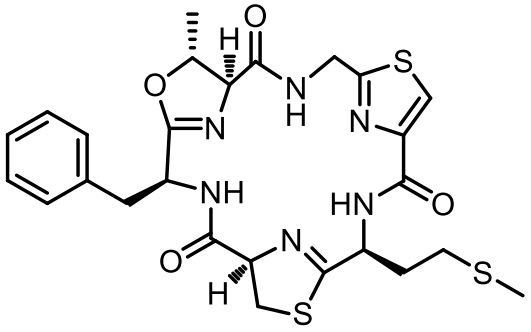
Aerucyclamide A



Aerucyclamide B



Aerucyclamide C



Aerucyclamide D

Aerucyclamides isolated from *Mycrocistis aeruginosa*

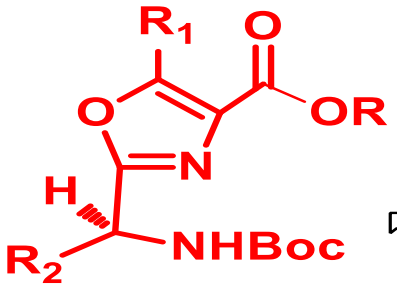
Hexacyclopeptides alternanting in hydrofobic and hydrofilic (Ser, Thr, Cys) aminoacids.

The polar AA are heterocyclized to form oxazole or thiazole rings or their reduced derivatives

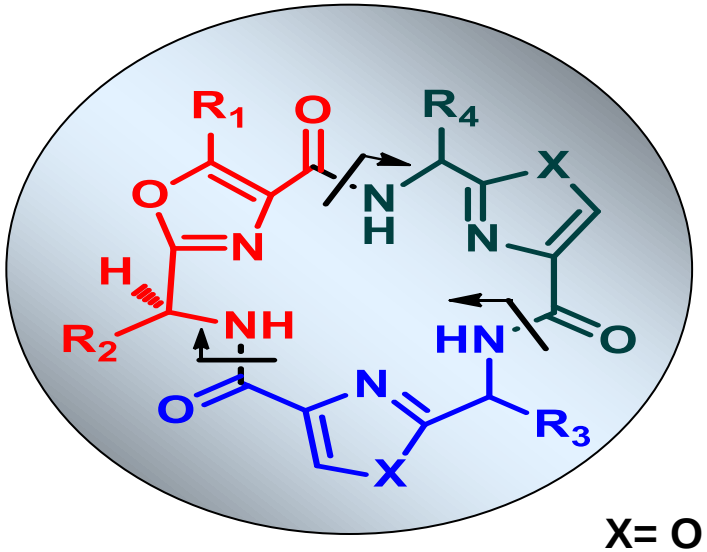
- **Aerucyclamide B: submicromolar IC_{50} value against *Plasmodium falciparum K1* (0.7 μM)**
- **Non toxic for L6 rat myoblasts**

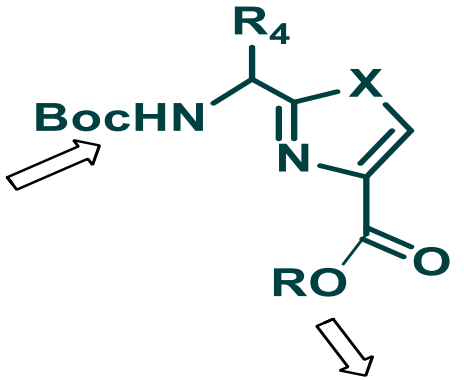
a)Portmann, C.; Blom, J. F.; Gademann, K.; Jüttner, F. *J. Nat. Prod.* **2008**, *71*, 1193. b) Portmann, C.; Blom, J. F.; Kaiser, M.; Brun, R.; Jüttner, F.; Gademann, K. *J. Nat. Prod*. **2008**, *71*, 1891

First Serie of Analogues: Retrosynthesis



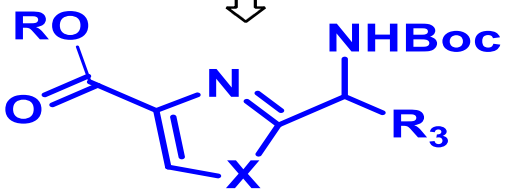
L-Ser, L-Thr, L *allo*-Thr +
L-Ile, L-Ala or L-Phe





L-Ser, Gly, L-Ile

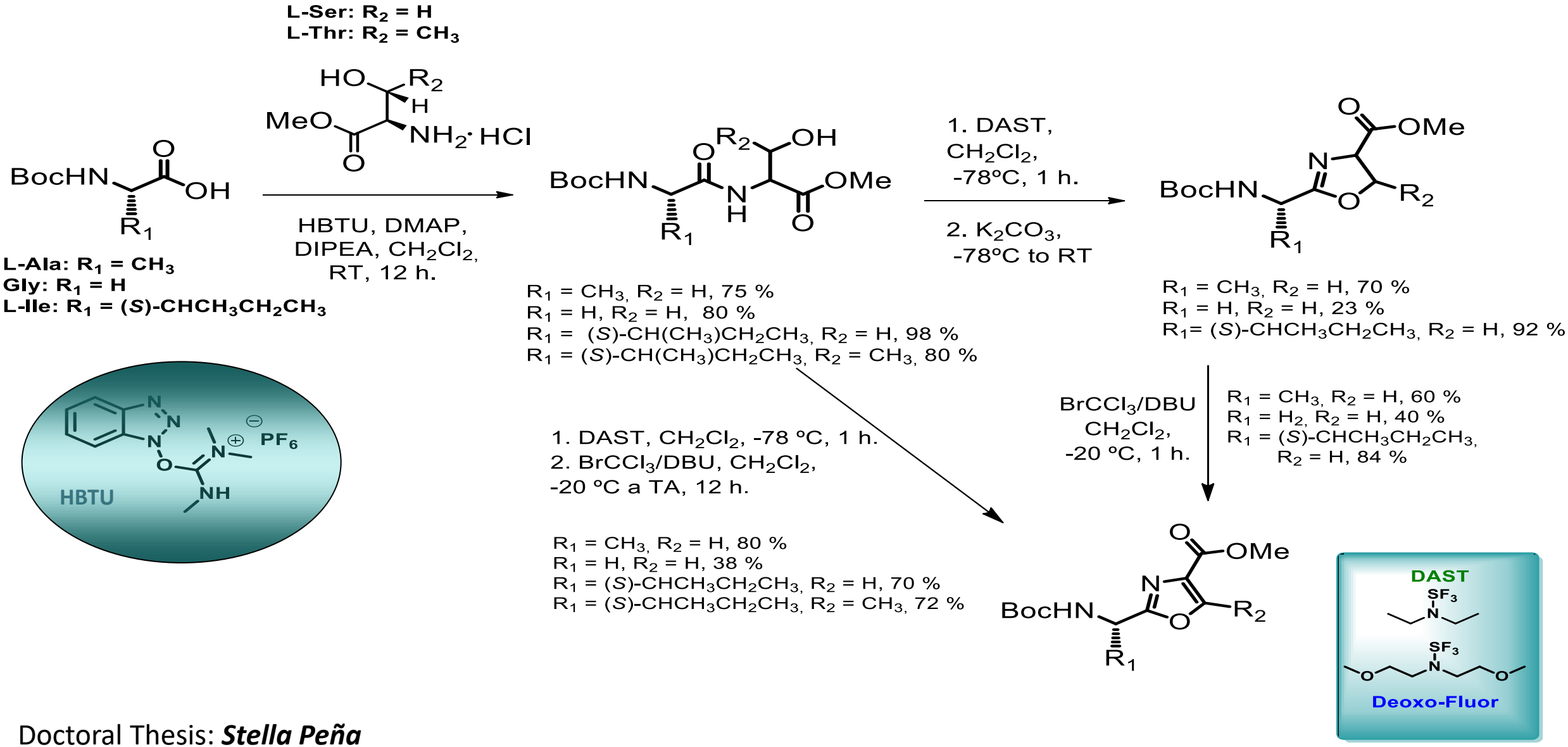
X= O or S



L-Ser+ L-Ile or D-*allo*-Ile or L-Met

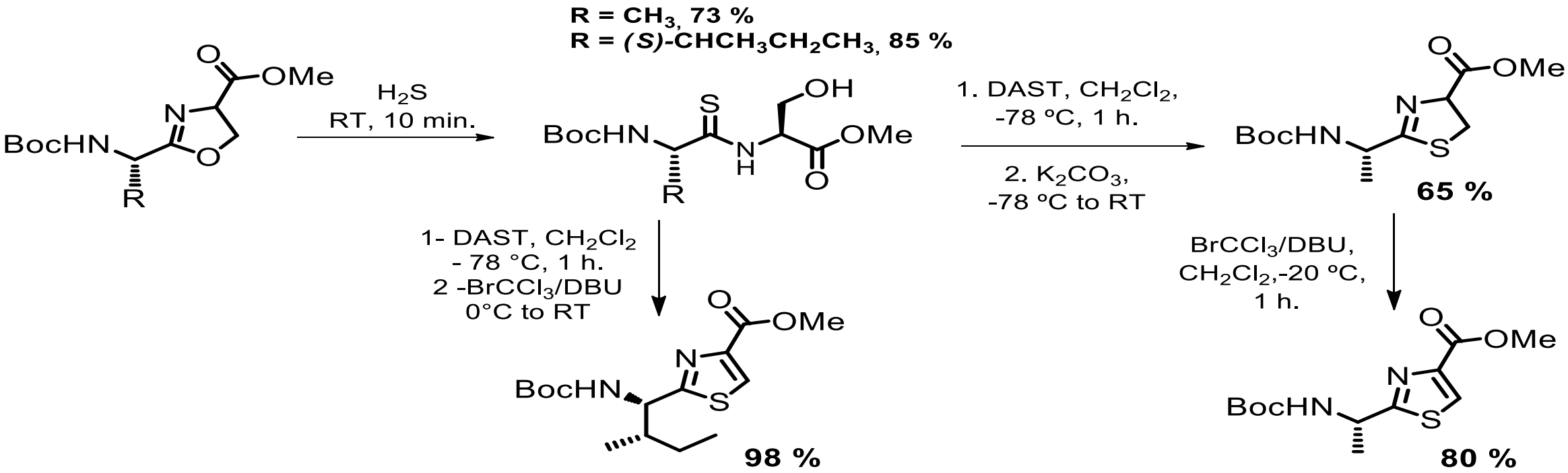
Doctoral Thesis: ***Stella Peña***

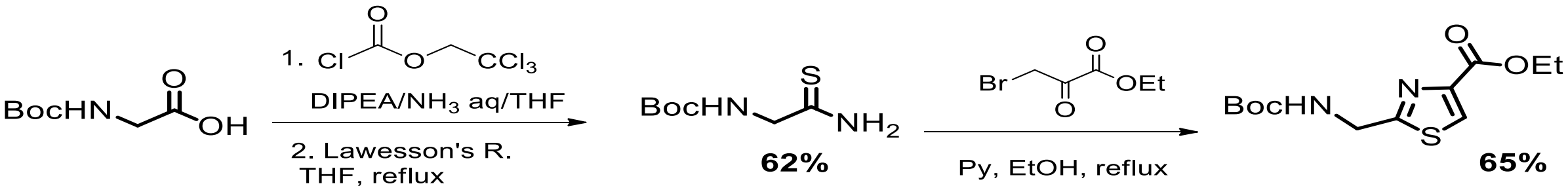
Synthesis of Building Blocks: Oxazoles



Doctoral Thesis: ***Stella Peña***

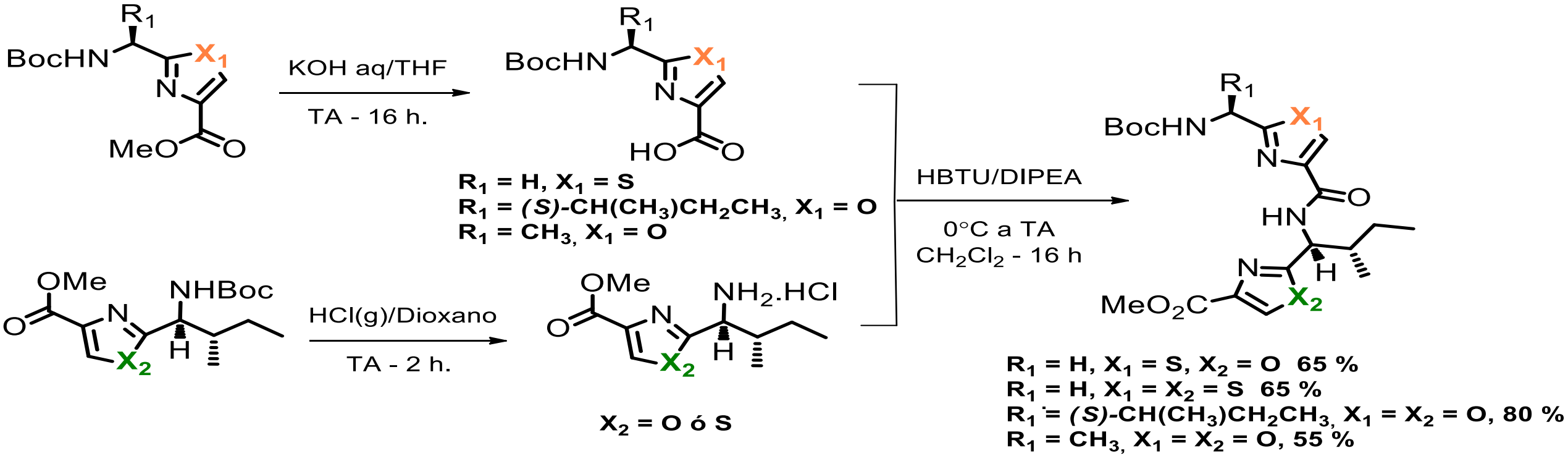
Synthesis of Building Blocks: Thiazoles





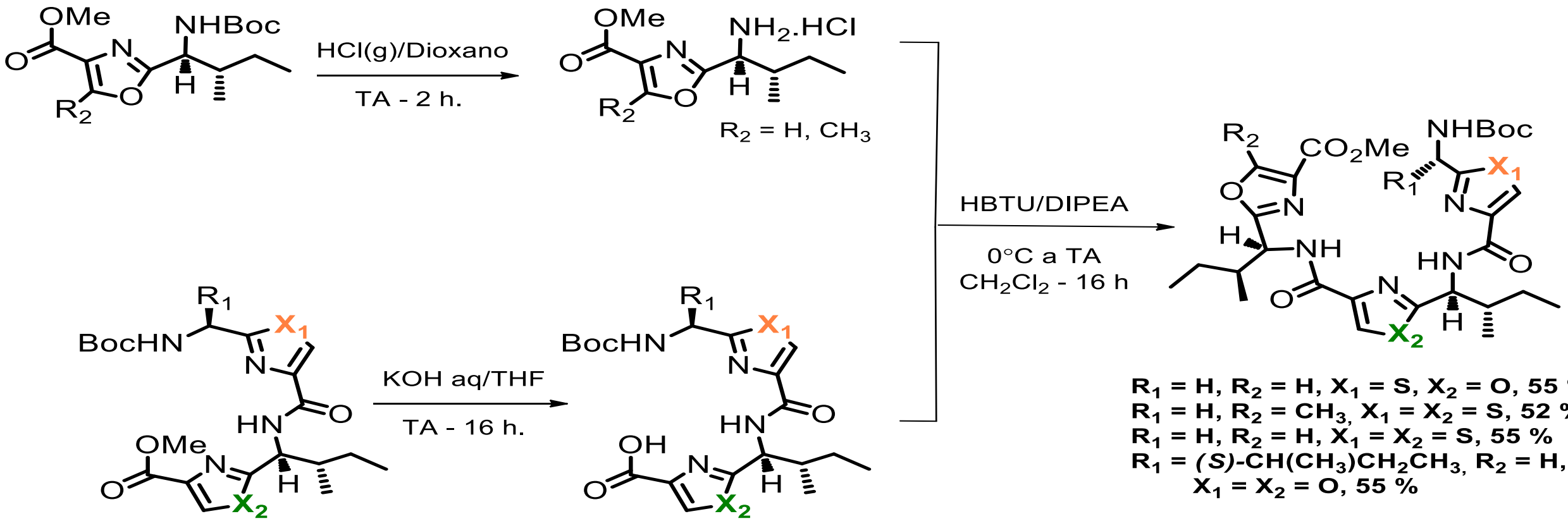
Tesis Doctoral: ***Stella Peña***

Coupling Reactions: Bis-Heterocycles



a) Peña, S.; Scarone, L.; Manta, E.; Stewart, L.; Yardley, V.; Croft, S.; Serra, G. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4994. b)Peña, S.; Scarone L.; Medeiros, A.; Manta, E.; Comini, M.; Serra, G. *Med. Chem. Comm.* **2012**, *3*, 1443.

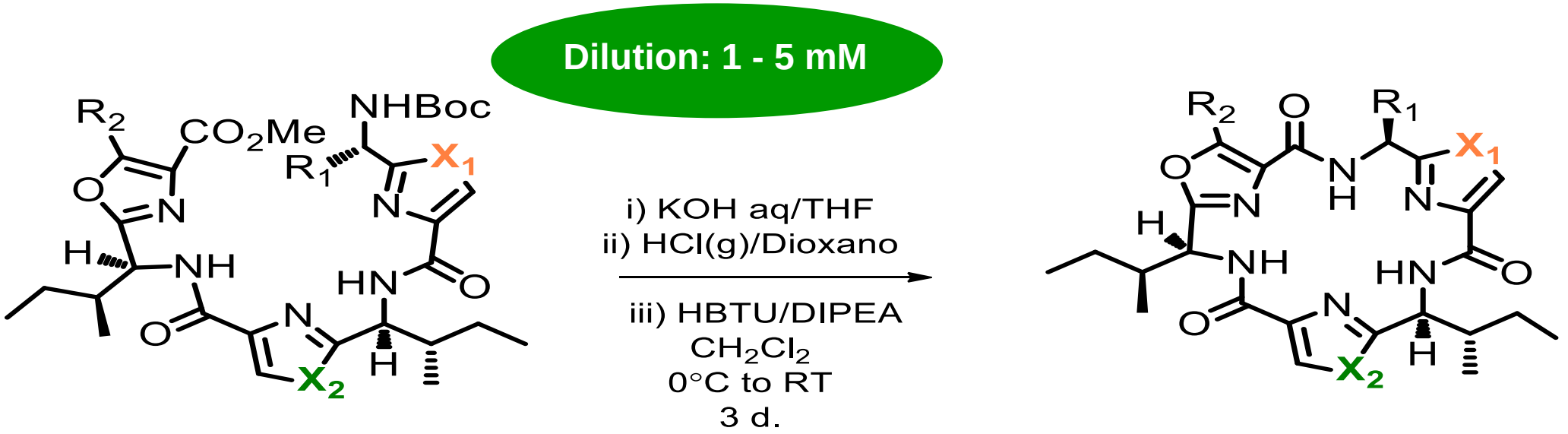
Coupling Reactions: Tris-Heterocycles



R_1 = H, R_2 = H, X_1 = S, X_2 = O, 55 %
R_1 = H, R_2 = CH_3, X_1 = X_2 = S, 52 %
R_1 = H, R_2 = H, X_1 = X_2 = S, 55 %
R_1 = *(S)*-$CH(CH_3)CH_2CH_3$, R_2 = H, X_1 = X_2 = O, 55 %

a) Peña, S.; Scarone, L.; Manta, E.; Stewart, L.; Yardley, V.; Croft, S.; Serra, G. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4994. b)Peña, S.; Scarone L.; Medeiros, A.; Manta, E.; Comini, M.; Serra, G. *Med. Chem. Comm.* **2012**, *3*, 1443.

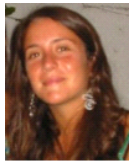
Coupling Reactions: Macrocycles



R_1 = H, R_2 = H, X_1 = S, X_2 = O, 40 %
R_1 = H, R_2 = CH_3, X_1 = X_2 = S, 60 %
R_1 = H, R_2 = H, X_1 = X_2 = S, 44 %
R_1 = *(S)*-$CH(CH_3)CH_2CH_3$, R_2 = H, X_1 =X_2 = O, 60 %

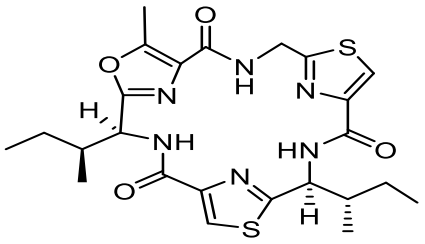
a)Peña, S.; Scarone, L.; Manta, E.; Stewart, L.; Yardley, V.; Croft, S.; Serra, G. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4994. b) Peña, S.; Scarone L.; Medeiros, A.; Manta, E.; Comini, M.; Serra, G. *Med. Chem. Comm.* **2012,** *3*, 1443.

Biological Evaluation

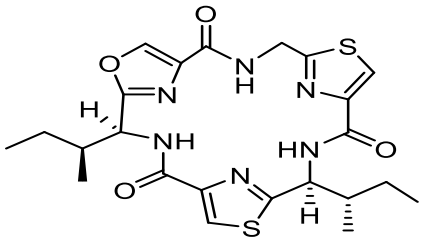


Thesis: ***Stella Peña***

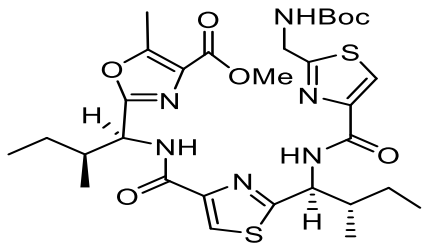
***P.f.*K1 EC_{50}= 0.18 µM**
SI > 556



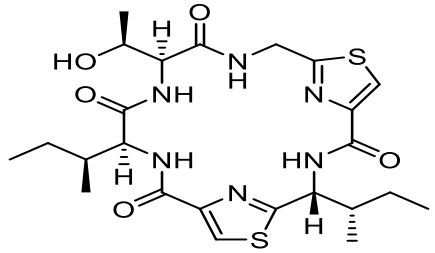
***P.f.* K1 EC_{50}= 0.62 µM**
SI = 88



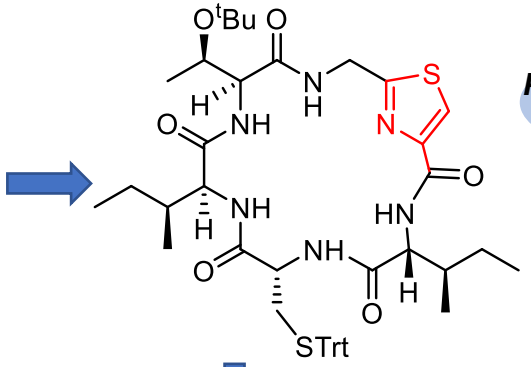
***P.f.* K1EC_{50}= 1.69 µM**



***P.f.* K1 EC_{50}= 0.18 µM**
SI > 500

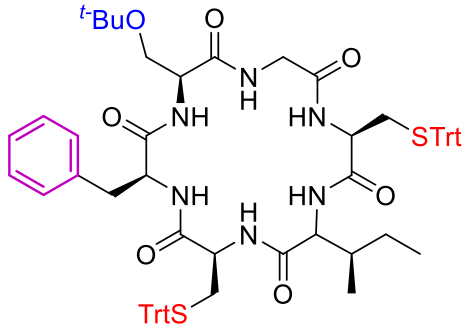


influence of the azole rings in the biological activities?

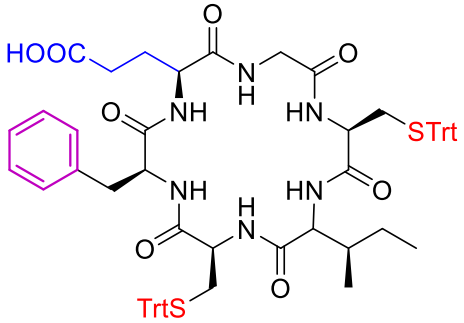


***P.f.*K1 EC_{50}= 0.19 µM**
SI > 526

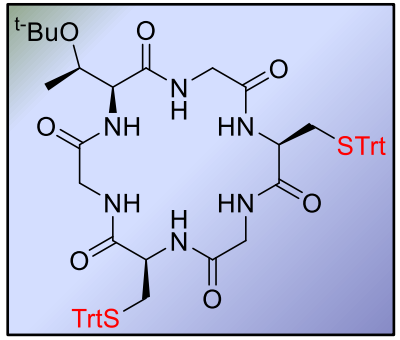
Thesis: ***Catherine Fagundez***



***P.f.* K1**
EC_{50}= 0.19 µM
SI > 1063



***P.f.*K1**
$EC_{50=}$ 0.46 µM
SI > 6200



***P.f.*K1**
$EC_{50=}$ 28 nM
SI > 8900

EC_{50} Chloroquine (*P. falciparum K1*)= 0.84 µM. EC_{50} Artemisinin (*P.falciparum K1*)= 0.03 µM

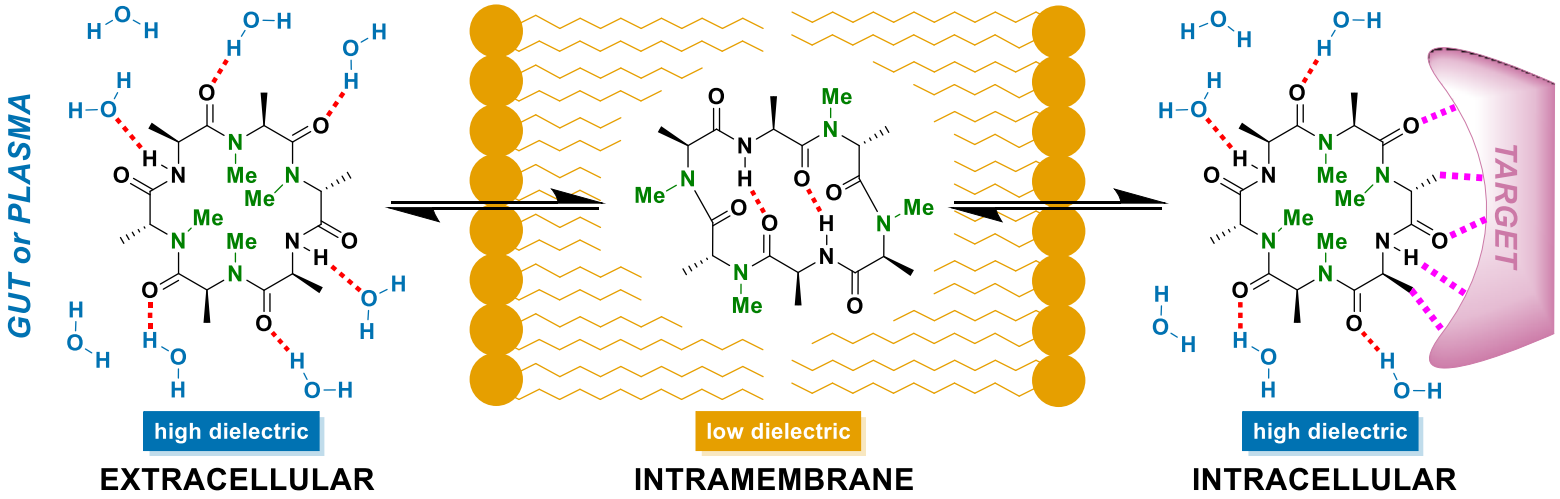
a) Peña, S.; Fagúndez, C.; Medeiros, A.; Comini, M.; Scarone, L.; Sellanes, D.; Manta, E.; Tulla-Puche, J.; Albericio, F.; Stewart, L. Yardley, V.; Serra, G *Med. Chem. Commun.* **2014**, *5,* 1309. b) Fagundez, C.; Sellanes, D.; Serra, G. *ACS Comb. Sci.* **2018**, *20*, 212.

Macrocycles: Physicochemical Barriers, Hydrocarbon Lipophilicity and Permeability

Cyclopeptides: adopt bioactive conformations
selectivity to the receptors
metabolic stability
High MW → **increased burden to maintain lipophilicity and aq. solubility**

Cyclopeptides: Molecular Chameleons

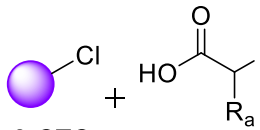
- **Simple model of passive permeation**

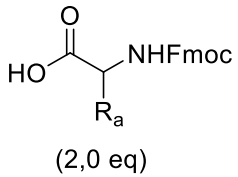


- Importance of **N-methylation** for valuable properties

Synthesis of cyclopeptides containing N-methyl amino acids to improve physicochemical properties and antimalarial activity

Synthesis: SPPS and Solution Macrocyclization

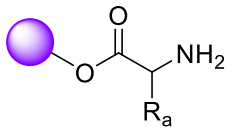




1) DIPEA (10,0 eq), DCM, 1h, T. amb.

2) MeOH, DIPEA, DCM

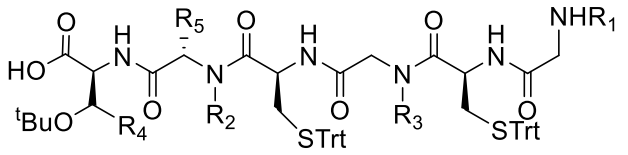
3) Piperidine 20% en DMF



1) Fmoc-AA-OH (3,0 eq); HBTU (3,0 eq)/DIPEA (6,0 eq); or HCTU (3,0 eq)/DIPEA (6,0 eq); or DIC/Cl-HOBt (3,0 eq), DMF, rt.

2) Piperidine 20%,DMF

3) TFA 1%/DCM

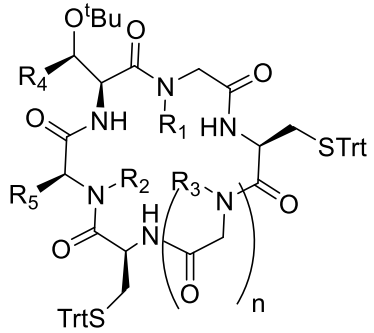


HBTU or HATU
DMAP (cat),
DIPEA ,
DCM, rt.

1-10 mM

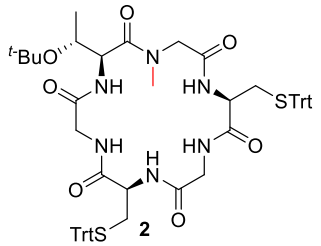
24 to 72 h

41-77% Yield

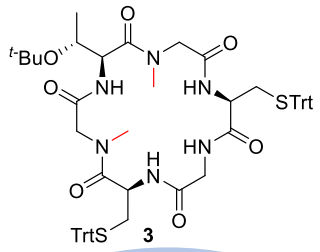


R_1, R_2, R_3, R_4: CH_3 or H

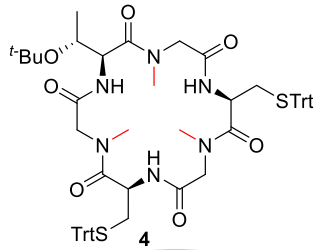
n= 0 or 1



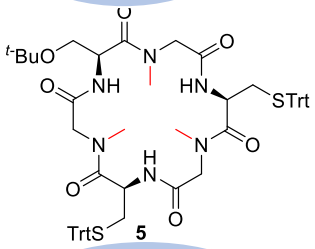
***P.f.* K1 and 3D7**
$EC_{50=}$ 8 and 3.8 nM
SI > 12000



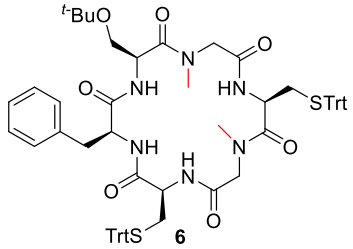
***P.f.* K1 and 3D7**
$EC_{50=}$ 0.008 and 0.25 nM
SI > 1000000



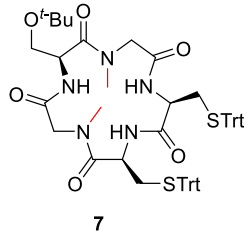
***P.f.* K1 and 3D7**
$EC_{50=}$ 0.04 and 1.0 nM
SI > 2500000



***P.f.* K1 and 3D7**
$EC_{50=}$ 0.13 and 1.4 nM
SI > 800000



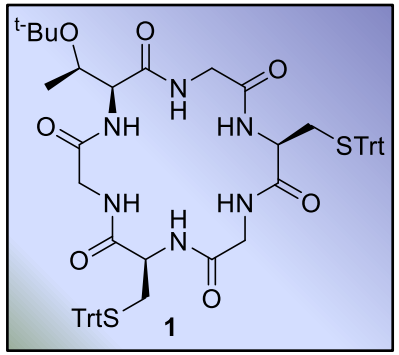
***P.f.* K1 and 3D7**
$EC_{50=}$ 9.0 and 1.8 nM
SI > 10000



***P.f.* K1**
$EC_{50=}$ 150 nM

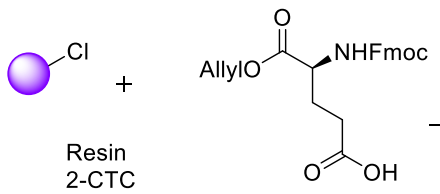
EC_{50} Artemisinin (*P.falciparum K1*)= 30 nM

***P.f.*K1**
$EC_{50=}$ 28 nM

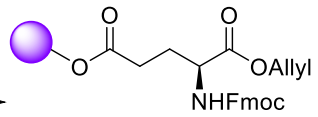


Fagundez, C.; Sellanes, D.; Peña, S.; Scarone, L.; Aguiar, A. C. C.; de Souza, J.; Guido, R. V. C.; Stewart, L.; Yardley, V.; Ottilie, S.; Winzeler, E. A.; Gamo, F-J; Sanz, L. M.; Serra, G. L. *ACS Med. Chem. Lett.* **2019**, *10*, 137.

Synthesis: SPPS and on resin macrocyclization

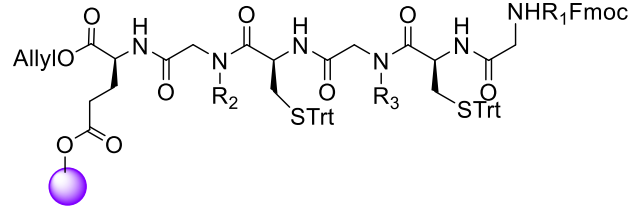


1) DIPEA, DCM
2) MeOH, DIPEA, DCM

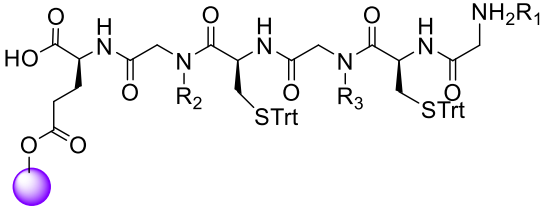


Fmoc-AA-OH;
i) HBTU /DIPEA;
ii) HCTU/DIPEA;
or iii) DIC/Cl-HOBt,
DMF,rt

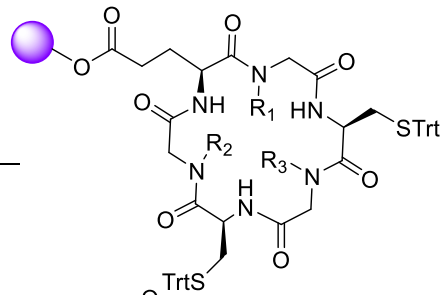
Piperidine
20%, DMF



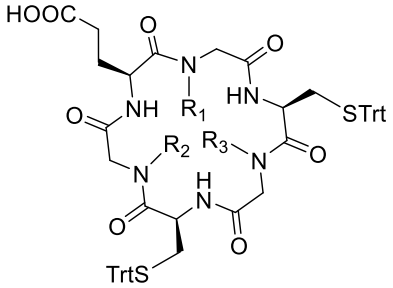
$Pd(PPh_3)_4$, Piperidine 10%,
THF, 2 h



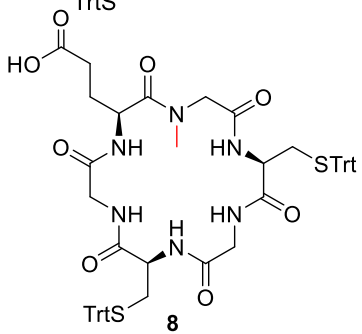
DIC/Cl-HOBt, DMAP,
DMF:DCM, rt, 18-24 h

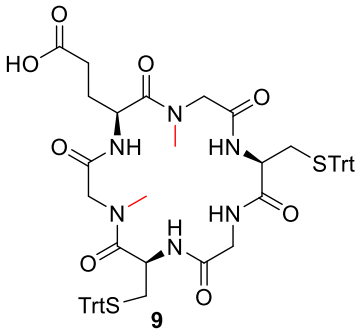


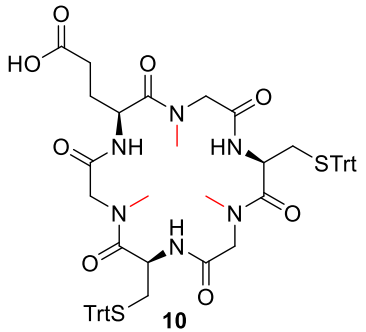
TFA 1% DCM

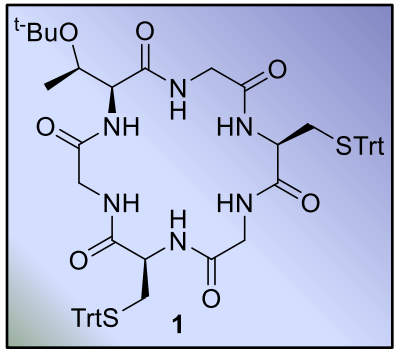


8: R_1= CH_3, R_2, R_3= H, 51%
9: R_1, R_2= CH_3, R_3= H, 54%
10: R_1, R_2, R_3= CH_3, 70%









EC_{50} Artemisinin (*P.falciparum K1*)= 30 nM

***P.f.* K1**
$EC_{50=}$ 5400 nM

***P.f.* K1**
$EC_{50=}$ 210 nM
SI >480

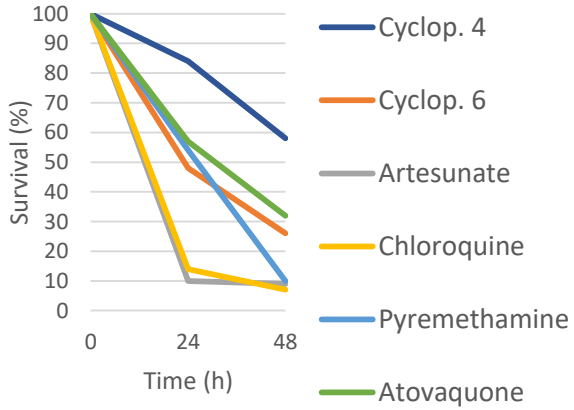
***P.f.* K1 and 3D7**
$EC_{50=}$ 0.2 and 12 nM
SI > 20000

***P.f.*K1**
$EC_{50=}$ 28 nM

Fagundez, C.; Sellanes, D.; Peña, S.; Scarone, L.; Aguiar, A. C. C.; de Souza, J.; Guido, R. V. C.; Stewart, L.; Yardley, V.; Ottilie, S.; Winzeler, E. A.; Gamo, F-J; Sanz, L. M.; Serra, G. L. *ACS Med. Chem. Lett.* **2019**, *10*, 137.

Physicochemical Properties and Biological Evaluation

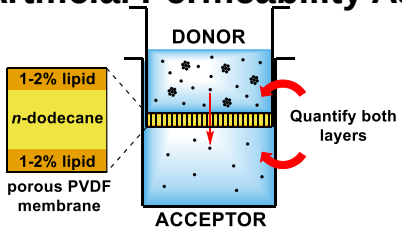
Killing rate assay

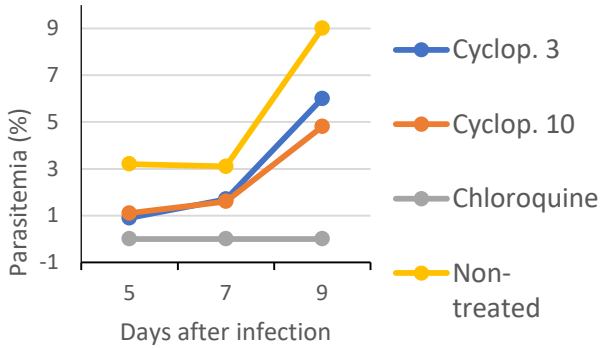


Medicines for Malaria Venture

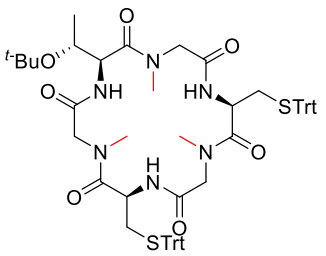
Effects over parasite viability comparable to slow agents. impair pyrimidine biosynthesis

PAMPA Artificial Permeability Assay

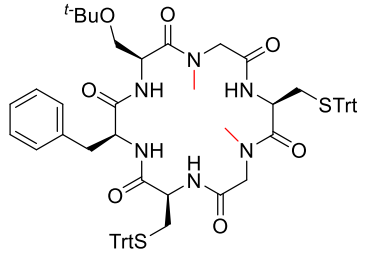




Lod D, Solubility



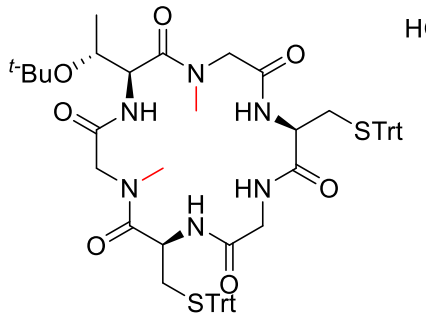
4



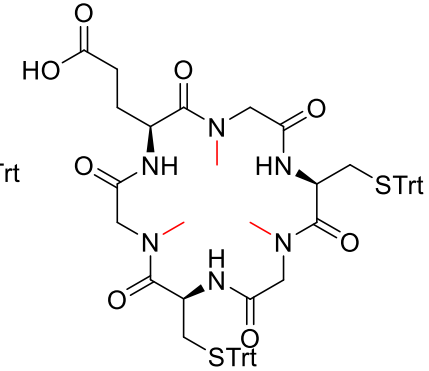
6

EC_{50} = 0.018 and 0.335 μM (**4** and **6**) in **liver stage** of the rodent *Plasmodium berghei*.

Prophylactic potential



3



10

In vivo efficacy

T_{max} (hr)	C_{max} (ng/mL)	AUC_{last} (hr*ng/mL)	AUC_{inf} (hr*ng/mL)	$T_{1/2}$ (hr)
2.00	**61.1**	314.8	390.7	**4.93**

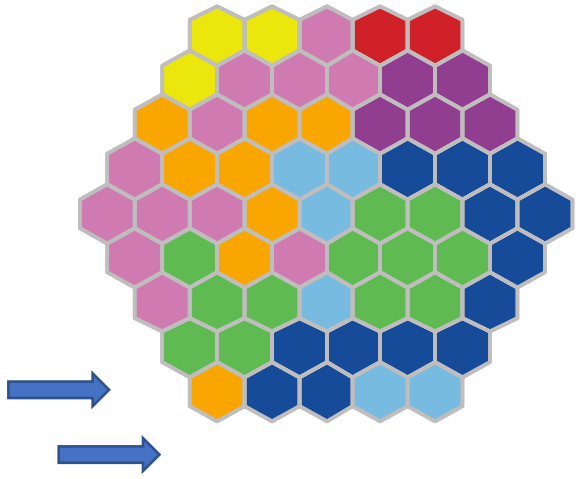
Compound **3**: Pharmacokinetic parameters oral administration (mice).

Fagundez, C.; Sellanes, D.; Peña, S.; Scarone, L.; Aguiar, A. C. C.; de Souza, J.; Guido, R. V. C.; Stewart, L.; Yardley, V.; Ottilie, S.; Winzeler, E. A.; Gamo, F-J; Sanz, L. M.; Serra, G. L. *ACS Med. Chem. Lett.* **2019**, *10*, 137.

Approach to the Mechanism of Action: Metabolomic Studies

Target Identification: *Plasmodium* parasites were incubated during 18 months with cyclopeptides 4 and 6. It was not observed any IC50 shift, therefore the parasites dit not generate resistance . The compounds were classified as **"irresistibles"**

MetaPrint Map



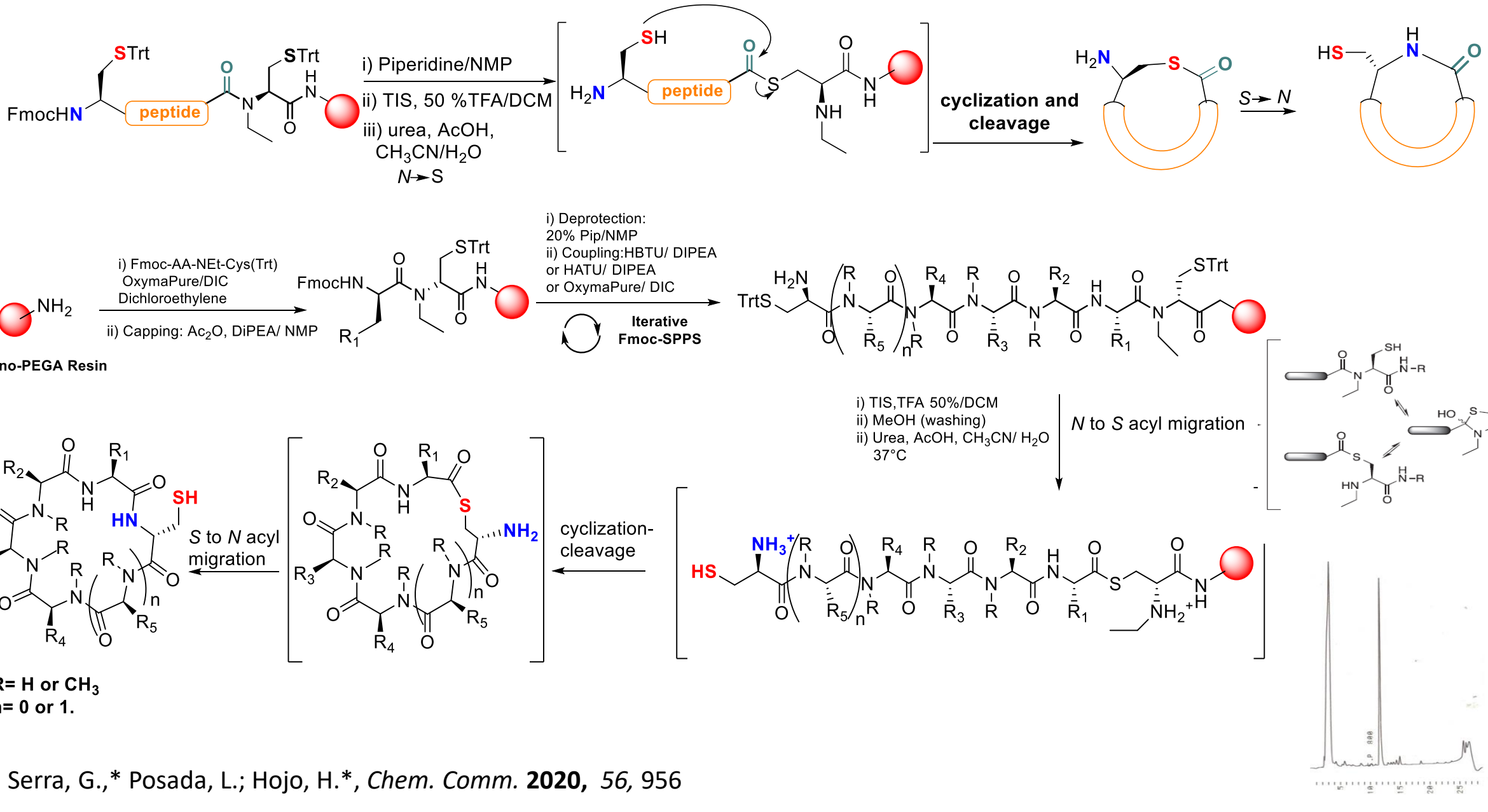
3D7 IC50's:
1: 47 nM
3: 0.25 nM
4: 1.0 nM
6: 1.8 nM

Metabolomic Studies

- **1, 4** y **6** decrease of peptides and hemoglobine catabolism
- **1, 4 and 6** decrease Pyrimidine Precursors
- **1** : decreases of nucleobases and metabolites of TCA

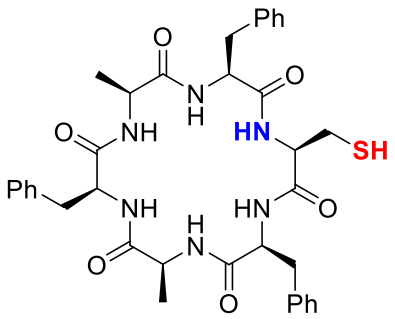
Prof Manel Llinás, PennState University

Synthesis: SPPS and on-resin macrocyclization by Native Chemical Ligation



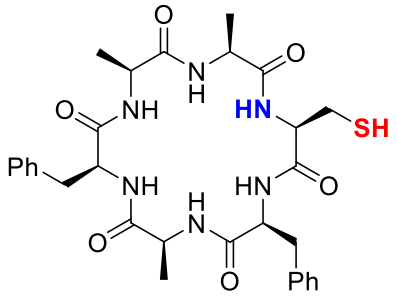
Serra, G.,* Posada, L.; Hojo, H.*, *Chem. Comm.* **2020,** *56,* 956

Synthesis: SPPS and solution macrocyclization by Native Chemical Ligation



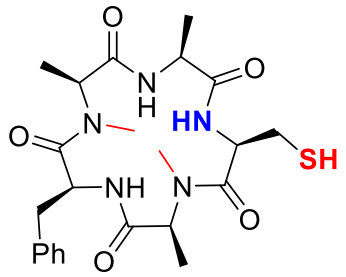
Y= 50%

P.f. 3D7
EC_{50} > 10 µM



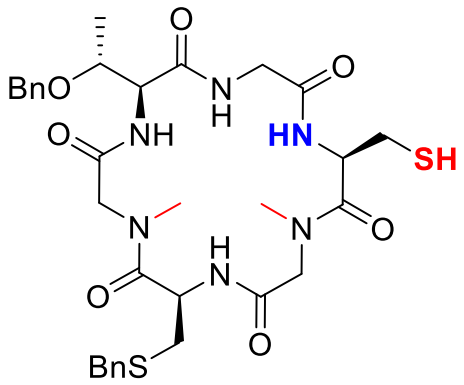
Y= 40%

P.f. 3D7
EC_{50} > 10 µM



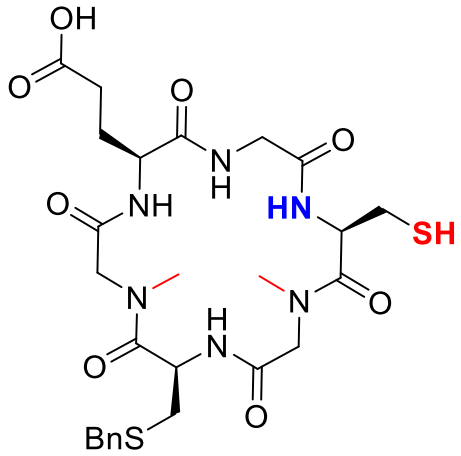
Y= 46%

P.f. 3D7
EC_{50} > 10 µM



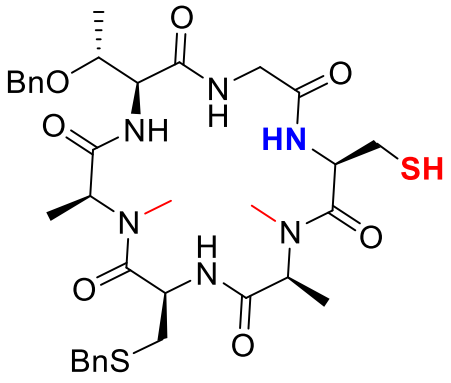
Y= 23%

P.f. 3D7
EC_{50} > 10 µM



Y= 19%

P.f. 3D7
EC_{50} > 10 µM



Y= 18%

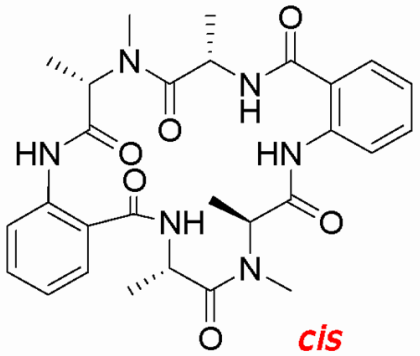
P.F 3D7
EC_{50} > 10 µM

Do not show Cytotoxicity against *P.f.* 3D7

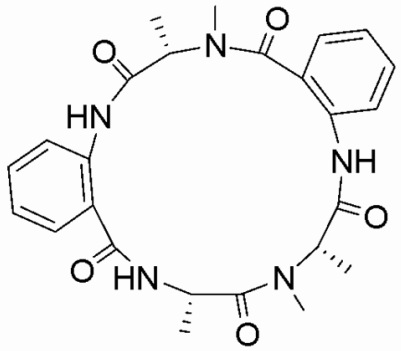
Synthesis of Versicotides A-F

2014

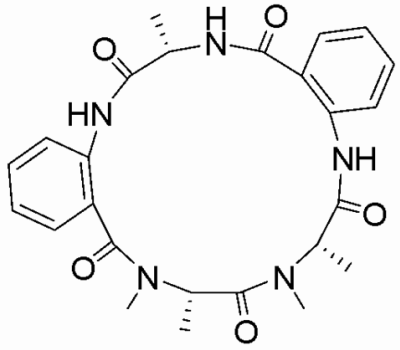
trans



Versicotide C



Versicotide A



Versicotide B

Aspergillus Versicolor *ZLN-60*

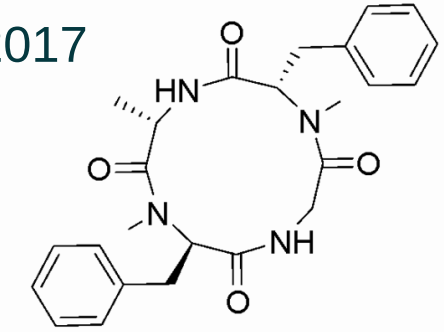
Thesis: ***Laura Posada***

Aminoácidos N-metilados: conformación *cis* o *trans*

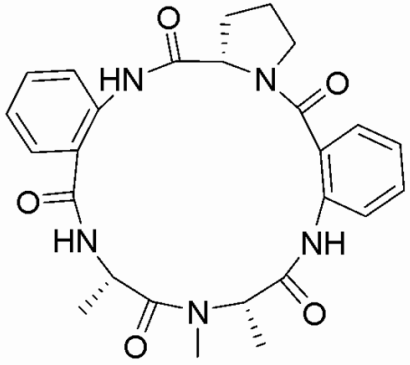
Ácido antranílico como *β* aminoácido

J. Peng, H. Gao, X. Zhang, S. Wang, C. Wu, Q. Gu, P. Guo, T. Zhu, D. Li, *J. Nat. Prod.*, **2014**, *77*, 2218.

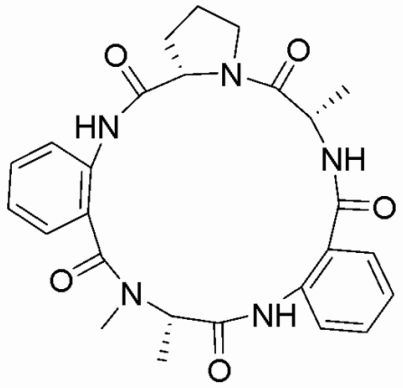
2017



Versicotide D



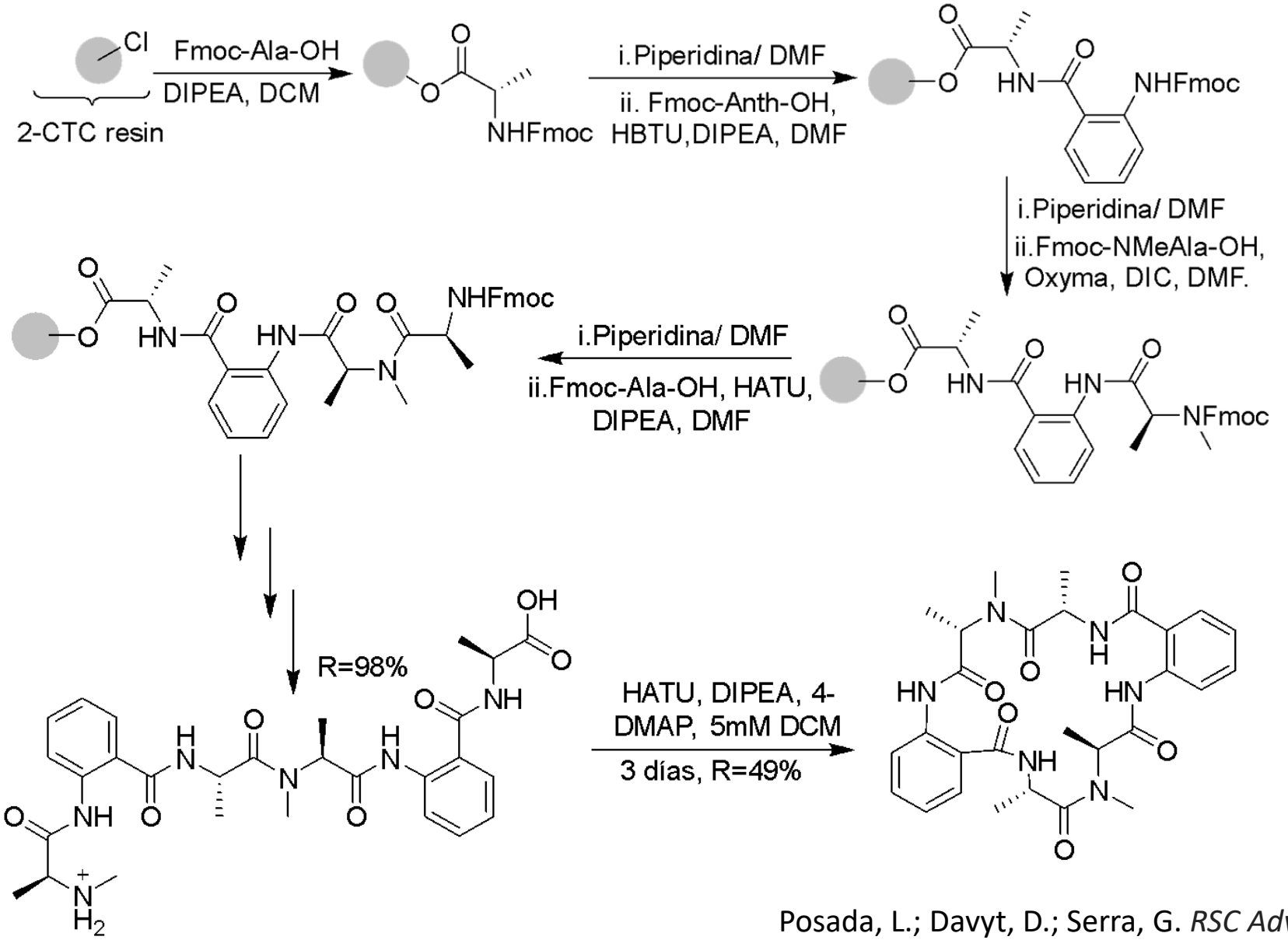
Versicotide E



Versicotide F

Chen, R.; Cheng, Z.; Huang, J.; Li, D.; Wu, C.; Guo. P.; Lin, W. ***RSC Adv.*** **2017,***7*, 49235.

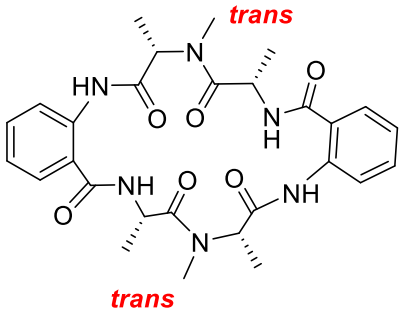
Synthesis of Versicotides C



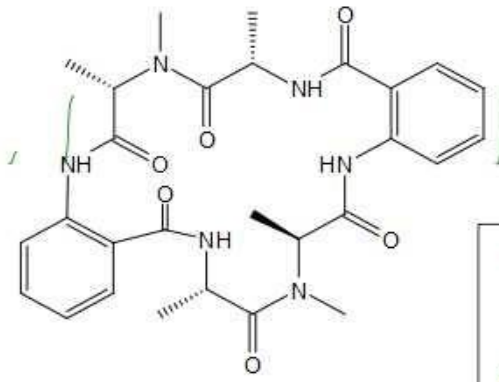
Thesis: ***Laura Posada***

Posada, L.; Davyt, D.; Serra, G. *RSC Advances*, **2020**, ***10***, 43653.

Synthesis of Versicotides C



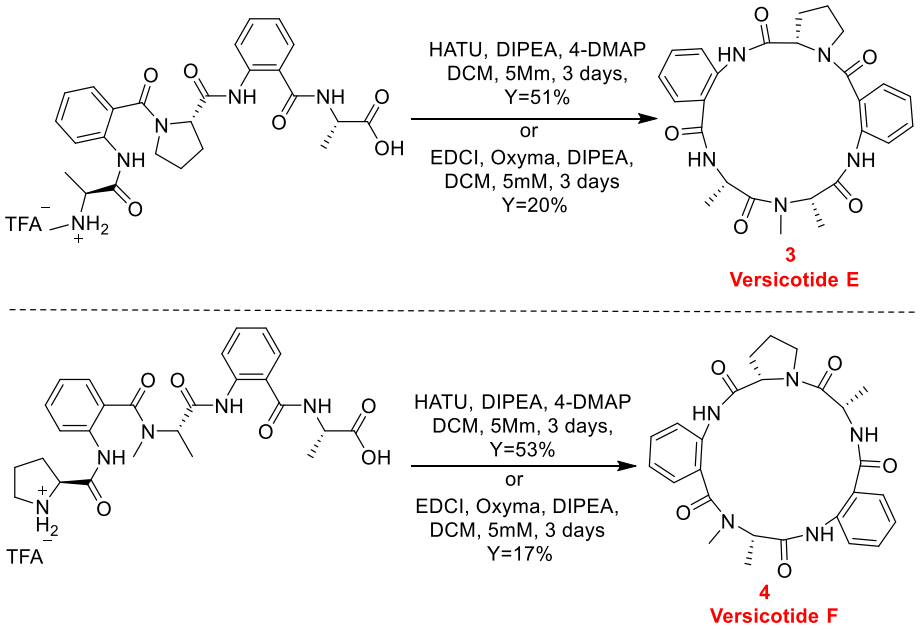
Versicotide C (**3**) main conformer in DMSO-d_6

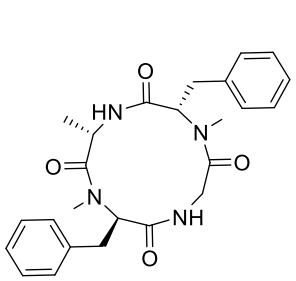


^{1}H-NMR Versicotide C

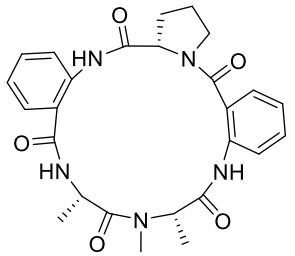
$(CD_3)_2CO$

Synthesis of Versicotides A-F

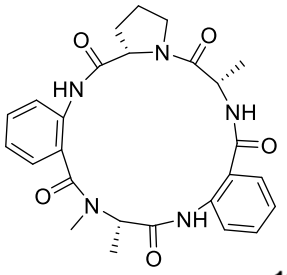




Versicotide D



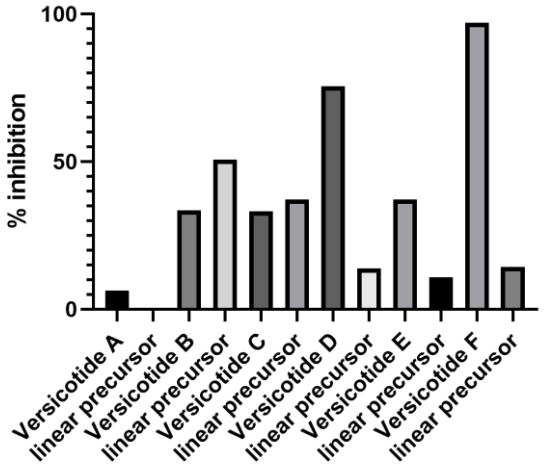
Versicotide E



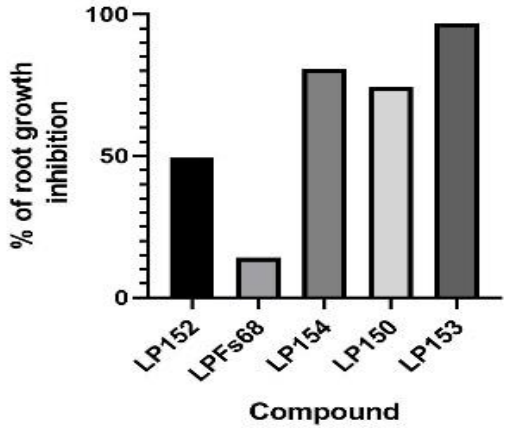
Versicotide F

***P.f.* 3D7**
$EC_{50=}$ 1.5 µM

Thesis: ***Laura Posada***



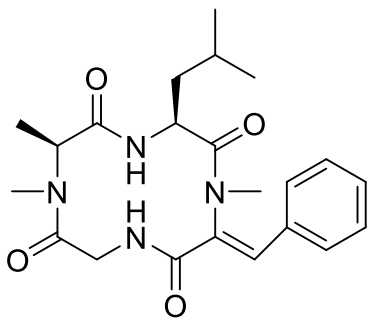
Versicotide D and F
Cianobacteria Inhibitors



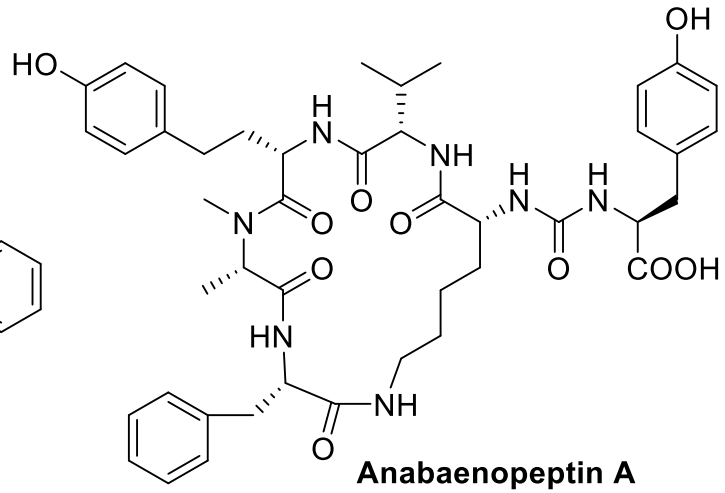
Anth compounds herbicides, insecticides

As Herbicides: Versicotides A> B> D.
Versicotides E y F (evaluation)

Synthesis od Cyclotetrapeptides and Cyclopentapeptides



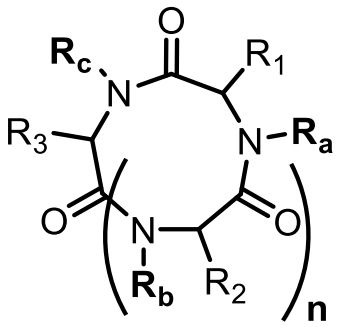
Tentoxin



Anabaenopeptin A

Camila Irabuena

Natural products with phytotoxic or cyanobactericidal activity.

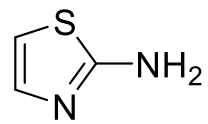


R_a, R_b,R_c: H ó CH_3

n: 2, 3 ó 4

Herbicide, Inhibitors of Cyanobacteria and Mycrocistins

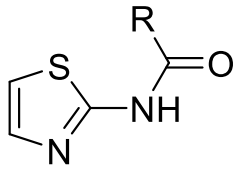
Synthesis of Nitazoxanide and Analogues



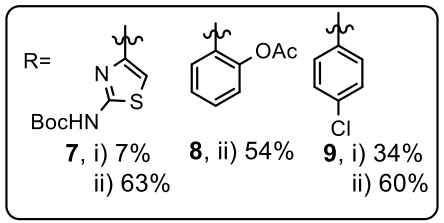
5

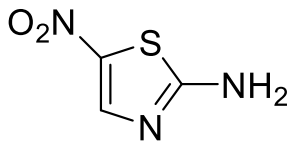
i) RCOOH, Oxyma pure, EDCI, DIPEA, DMAP, THF or

ii) RCOOH, Triphosgene, THF, 2,4,6-colidine, DIPEA, reflux



Series A

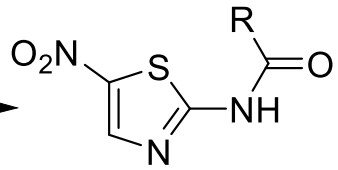




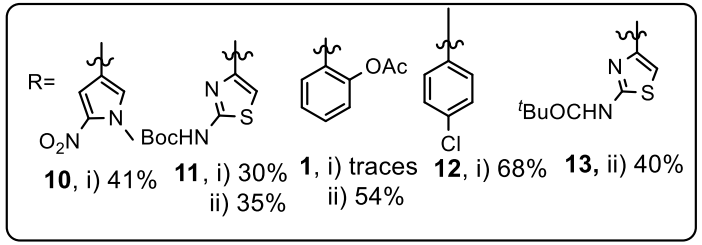
6

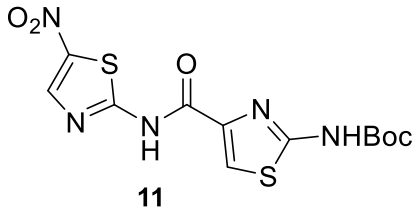
i) RCOOH, Oxyma pure, EDCI, DIPEA, DMAP, THF or

ii) RCOOH, Triphosgene, THF, 2,4,6-colidine, DIPEA, reflux



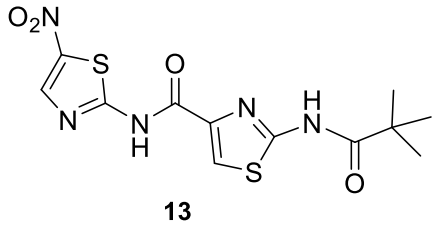
Series B





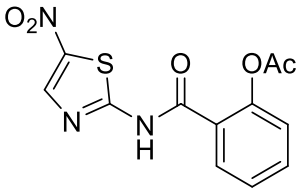
11

EC_{50} *Pf*= 5.9 μM

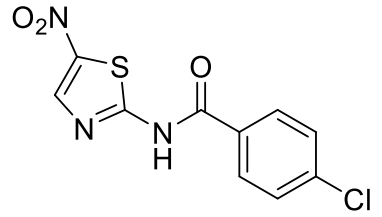


13

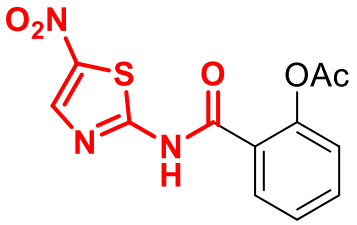
EC_{50} *Pf*= 8 μM



Nitazoxanida



Camila Irabuena



Nitazoxanida

Repurposing drug for COVID 19?

Irabuena, C.; Scarone; L.; de Souza, G. E.; Campos Aguiar, A. C; Rossi Mendes, G.; Carvalho Guido, R. V.; Serra, G. *Med. Chem. Res.* **2022.**

Conclusions

- Novel class of antimalarial cyclopeptides containing N-methyl Gly with enhanced antiplasmodial activity were obtained in good yield using SPPS and solution or on-resin cyclization.
- A novel methodology: on-resin intramolecular native chemical ligation (NCL) assisted by N-ethylcysteine using Fmoc/SPPS to obtain cyclic peptides, was described.
- Six cyclopeptides showed EC_{50} in a low **nanomolar or subnanomolar** range against *P. falciparum* K1 and 3D7. In addition, these compounds are active against the **liver stage** of the parasite showing submicromolar EC_{50}
- Cyclopeptides **3** and **10** have confirmed ***in vivo* efficacy** and **3** presents a considerable **half-life.**
- The compounds were classified as **"irresistibles"**.
- Next investigations: studies to improve PK properties.
- Exploring another concerns: **SARS-CoV2**, **herbicides**, **cyanobacteria**. The results encourage us to continue the investigations.

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Lic. Camila Irabuena

Universidad de Barcelona

Prof. Fernando Albericio

IFSC, USP, Brazil

Prof. Rafael Guido

UC, San Diego, USA

Dr. Sabine Ottilie

Prof. Elizabeth Winzeler

Diseases of the Developing World, Tres Cantos Medicines Development Campus, **Spain**

Dr. Francisco J. Gamo

Dr. Laura Sanz

Penn State University, USA

Biochemistry and Molecular Biology

Prof. Manuel Llinás.

Tarrick Qahash

LSHTM, UK

Lic. Lindsay Stewart

Dr. Vanessa Yardley

Dr. Simon Croft

IPR, U Osaka, Japan

Prof. Hironobu Hojo

Dr. Marcelo Comini

Prof. Andrea Medeiros, FMED

Dra. Estefanía Dibello, DQO, FQ

Herbicides

Prof. Juana Villalba

Ing Agr Luciana Rey

Cyanobacteria

Prof. Luis Aubriot

Sol Colombo

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Prof. Beatriz Brena

Lic. Natalia Badagian