Search for New Antibacterials Using Diversity-Oriented Synthesis

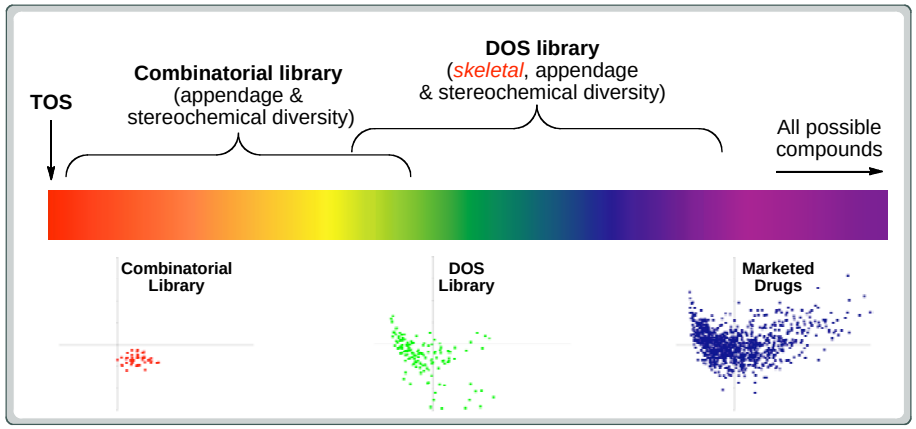
David R Spring
spring@ch.cam.ac.uk

Department of Chemistry

UNIVERSITY OF CAMBRIDGE

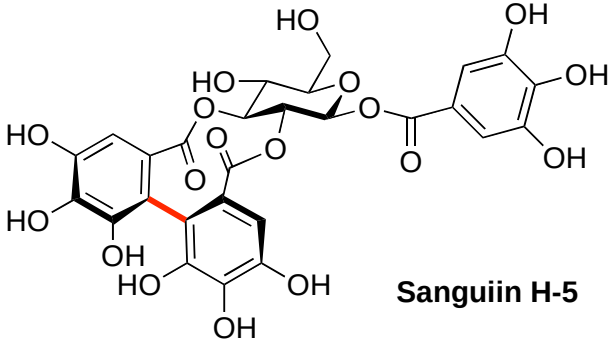
Spring Group Research

Diversity-Oriented Synthesis



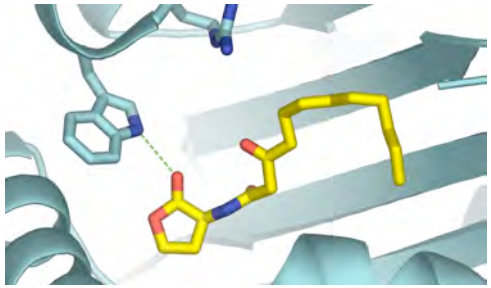
High Throughput Synthesis & Screening

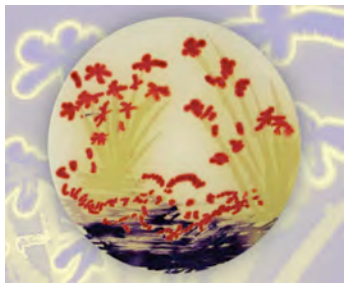
New Methodology, Target-Oriented Synthesis



Sanguiin H-5

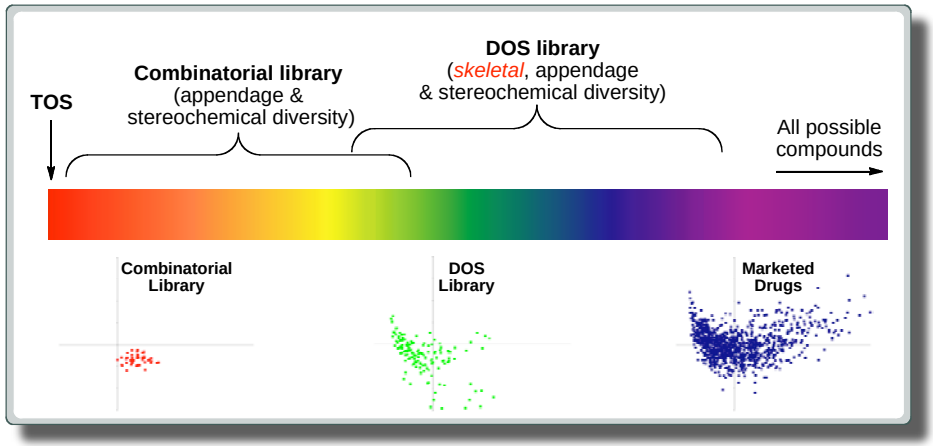
Chemical Biology, Antibacterial Discovery





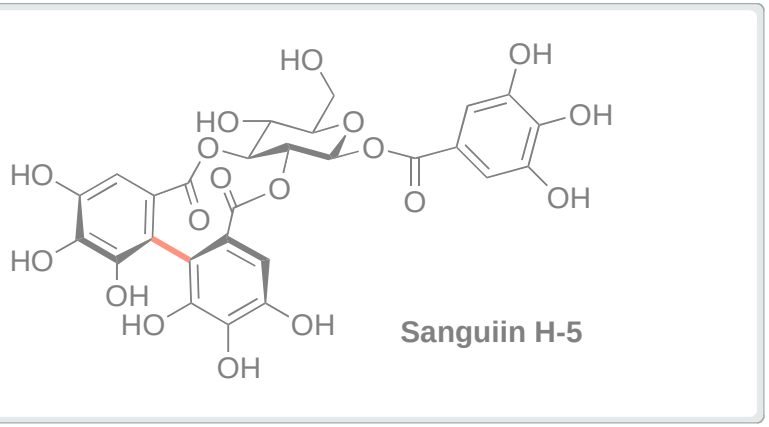
Spring Group Research

Diversity-Oriented Synthesis

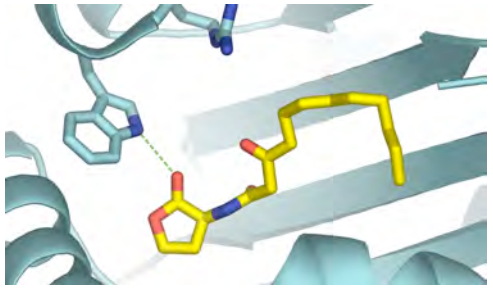


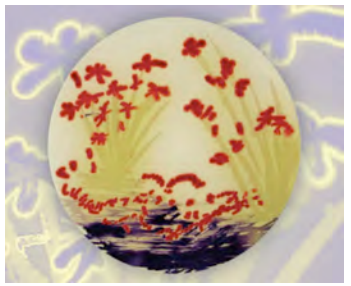
High Throughput Synthesis & Screening

New Methodology, Target-Oriented Synthesis



Chemical Biology, Antibacterial Discovery





HOSPITALS FEAR OVER SUPERBUG EPIDEMIC

JACKO HAS SUPERBUG

'MRSA' infection spreads all over his skin

SUPERBUGS KILL 20,000 A YEAR

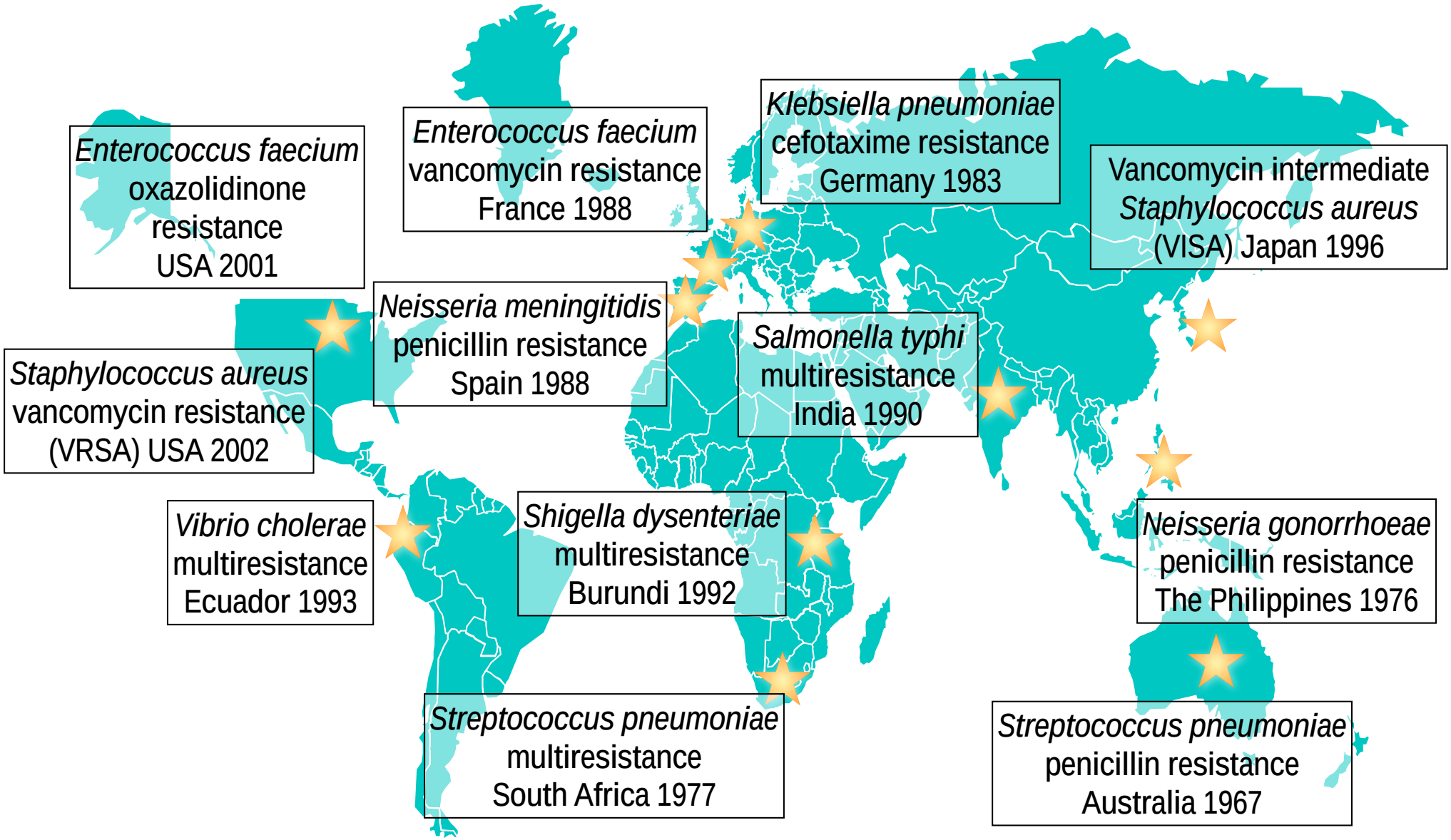
Hospital halts transplants after killer virus strikes

BY JOHN VINCENT

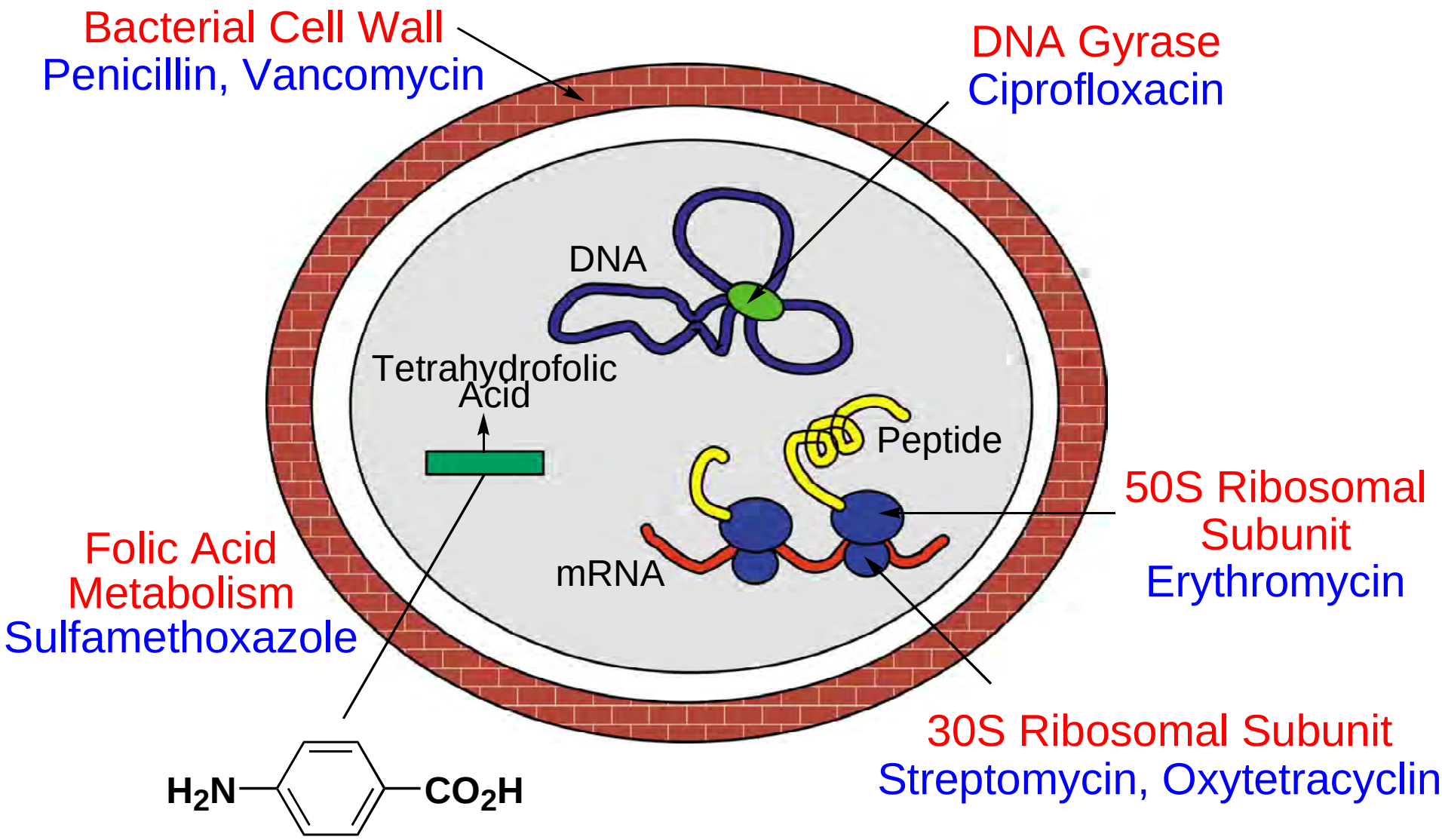
THE death of a patient after the outbreak of a highly resistant wound infection has forced cancellation of the adult liver transplant programme at Addenbrooke's Hospital in Cambridge.

The MRSA virus, linked with dozens of hospital deaths vulnerable after surgery. The MRSA virus contributed directly to the death but, according to doctors, a second patient's death could not be attributed directly to the virus. Keith Day, director of administration, said: "We would like to assure people

Global Antibiotic Resistance



Current Antibacterials



Current Antibacterials

Due to multidrug-resistant bacterial pathogens infectious disease mortality in the developed world is **increasing**!

Some pharmaceutical companies are not investing in research into *new* antibacterial agents.

Current Antibacterials

How to tackle multidrug-resistant bacterial pathogens?

1. Target resistance mechanisms

2. Search for new antibacterial agents with novel modes of action

Current Antibacterials

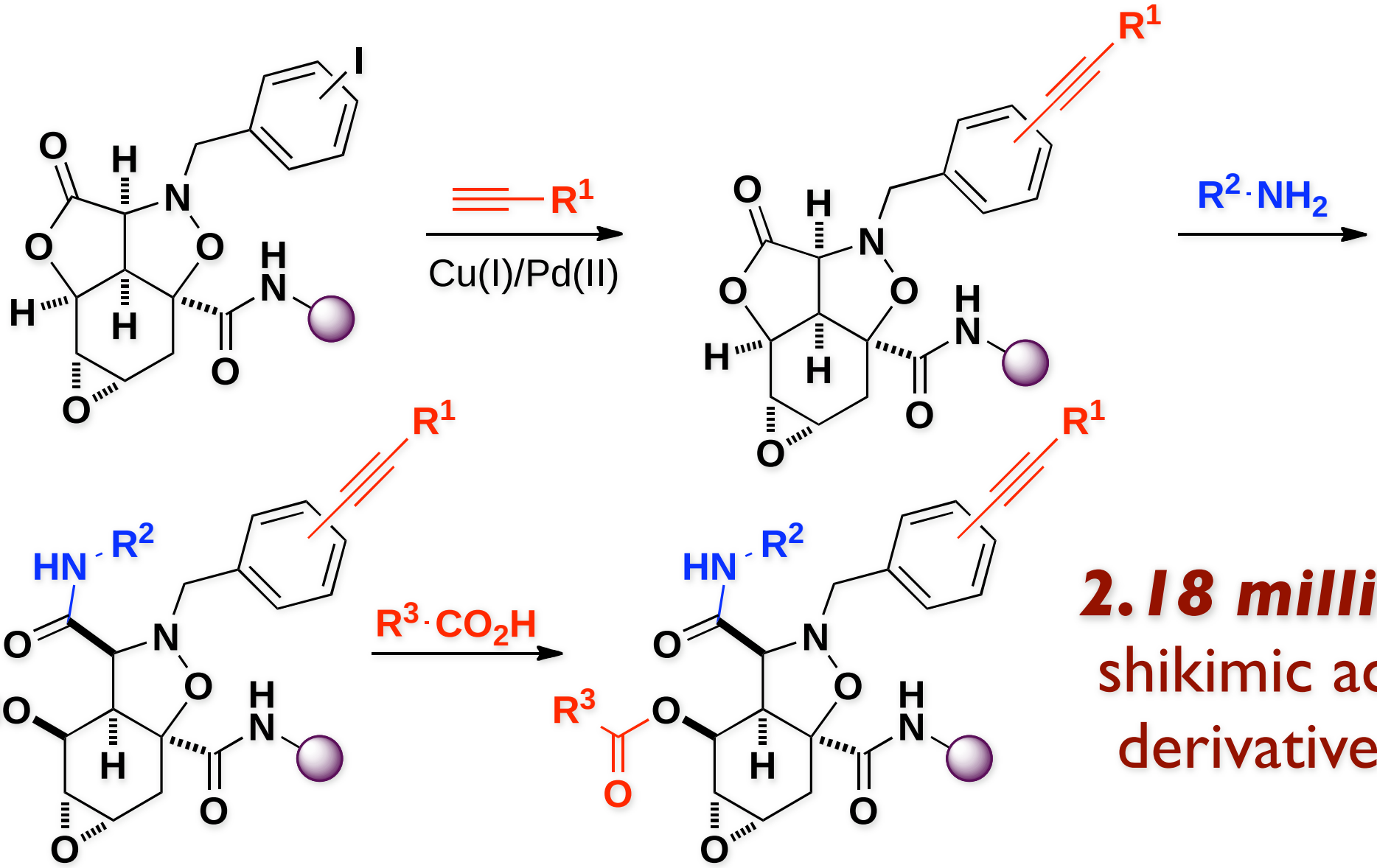
How to tackle multidrug-resistant bacterial pathogens?

1. Target resistance mechanisms

2. Search for new antibacterial agents with novel modes of action

We need a source of small molecules

Split-Pool Combinatorial Synthesis



2.18 million shikimic acid derivatives.

S. L. Schreiber *et al. J. Am. Chem. Soc.* **1999**, *121*, 9072.

Small Molecule Challenge

The number of possible "drug-like" molecules has been calculated to be

RS Bohacek, *et al. Med. Res. Rev.* **1996**, *16*, 3; MJ Owen *Biotech Advantage* **2002**, 6.

Small Molecule Challenge

The number of possible "drug-like" molecules has been calculated to be

astronomic!

(10^{62} to 10^{200})

RS Bohacek, *et al. Med. Res. Rev.* **1996**, *16*, 3; MJ Owen *Biotech Advantage* **2002**, 6.

Small Molecule Challenge

- ***Quality*** *and, but not just, quantity counts.*
- *Display functional groups in* ***three dimensions****.*
- *There are* ***many answers*** *to every (biological) problem.*

Structurally-Diverse
Small Molecule Collections

Structurally Diverse Small Molecule Collections

- Natural Products
- Commercial Collections
- Diversity-Oriented Synthesis

Small Molecule Collections

Natural Products

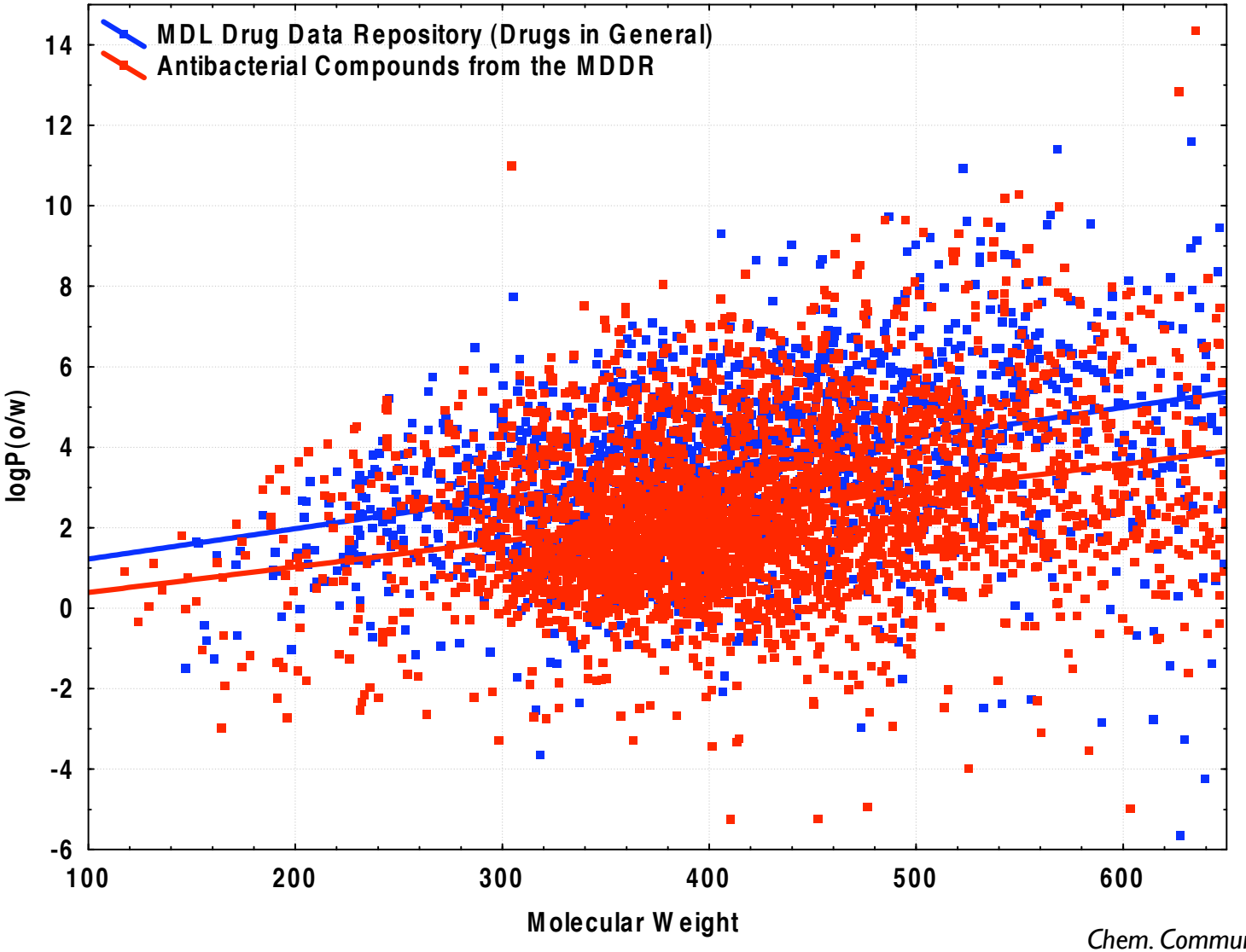
- Often mixtures, supply problem
- Identify active component
- Chemical derivatization difficult

Commercial Collections

- e.g. ChemBridge & ChemDiv
- Low molecular weight (*ca.* 350 Da)
- Few stereocentres

Small Molecule Collections

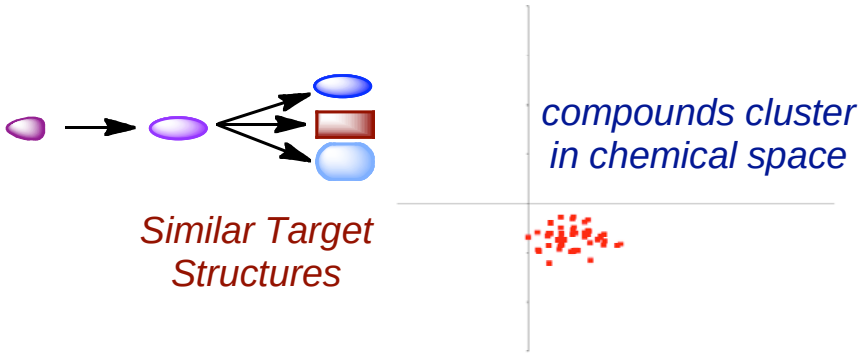
Antibacterials are different



Chem. Commun. **2006**, 2446-2462

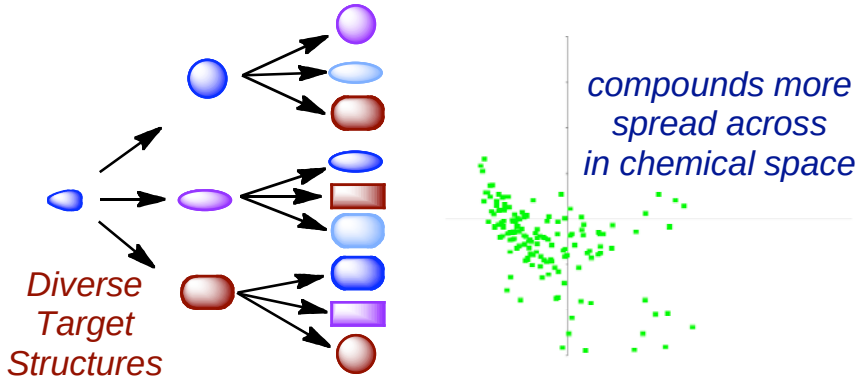
Diversity-Oriented Synthesis

Combinatorial Library Synthesis



Simple ← **Retrosynthetic Analysis** → Complex & Similar

Diversity-Oriented Synthesis

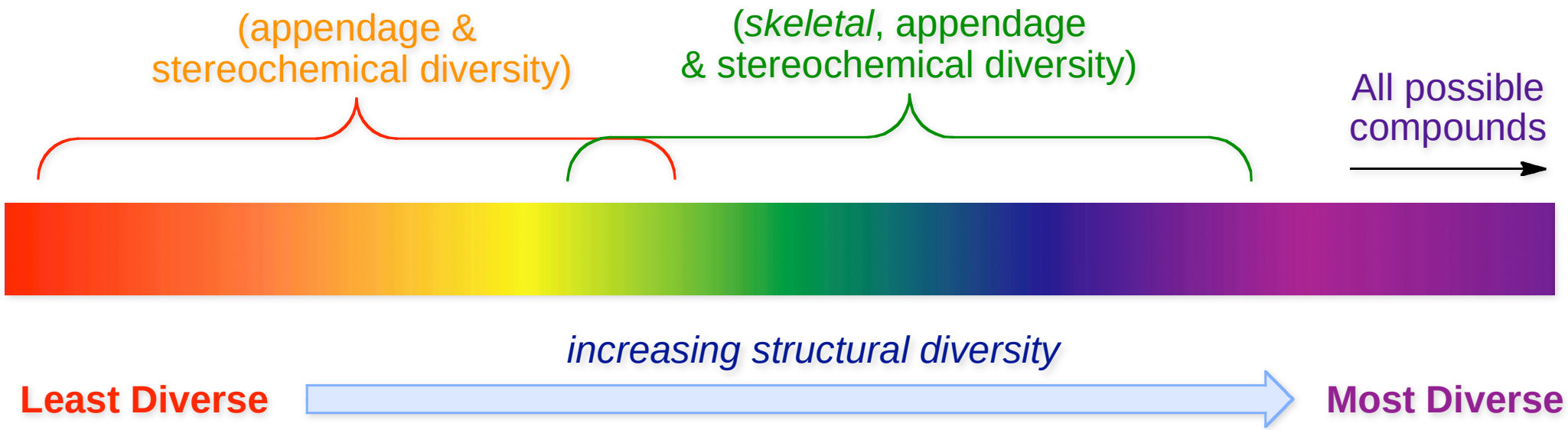


Simple & Similar → **Forward Synthetic Analysis** → Complex & Diverse

Nature Commun. **2010**, *1*, 80; *Org. Biomol. Chem.* **2008**, *6*, 1149-1158; *Science* **2000**, *287*, 1964

Diversity-Oriented Synthesis

Combinatorial Library Synthesis ***Diversity-Oriented Synthesis***



Nature Commun. **2010**, *1*, 80; *Org. Biomol. Chem.* **2008**, *6*, 1149-1158; *Science* **2000**, *287*, 1964

Diversity-Oriented Synthesis

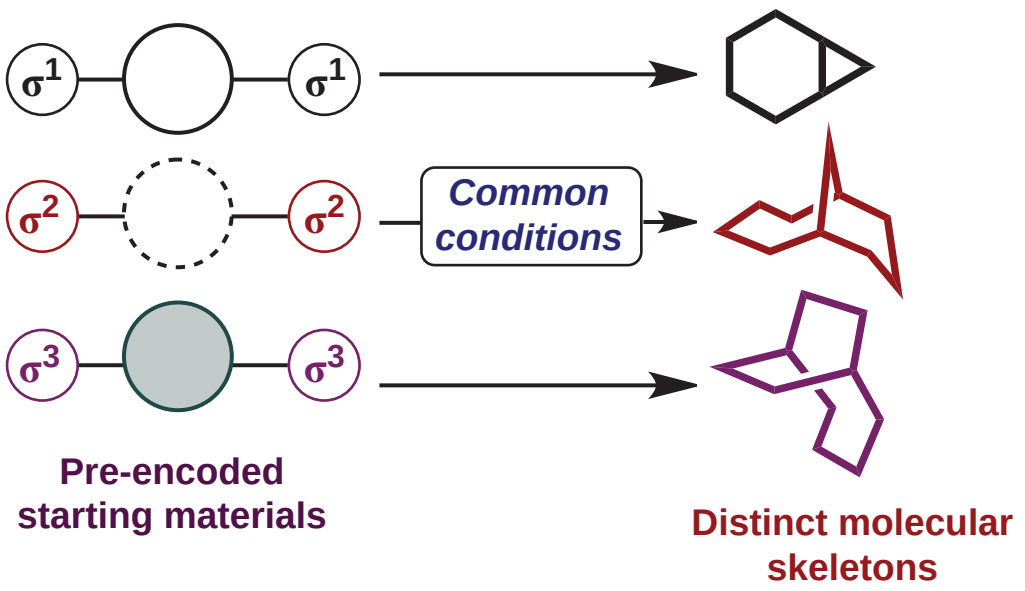
Generating Scaffold Diversity

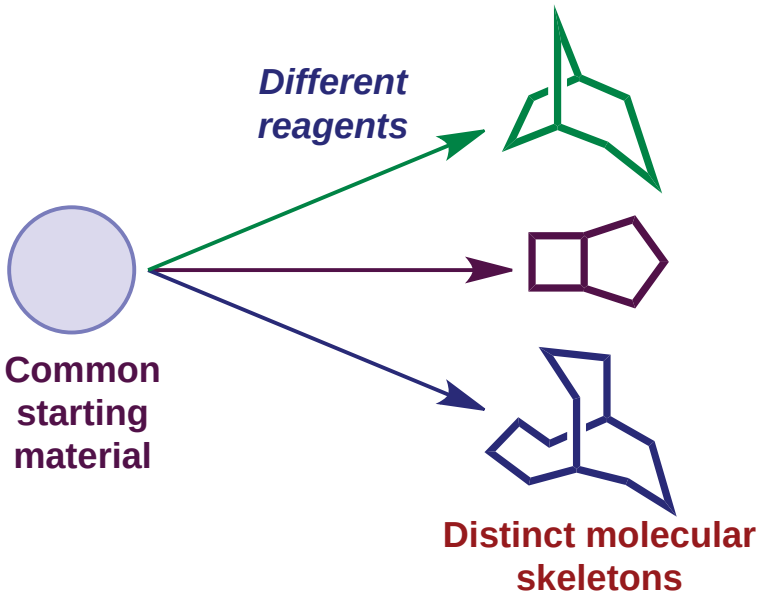
- Substrate-Based Approach
- Reagent-Based Approach

Nature Commun. **2010**, *1*, 80; *Org. Biomol. Chem.* **2008**, *6*, 1149-1158

Diversity-Oriented Synthesis

Generating Scaffold Diversity





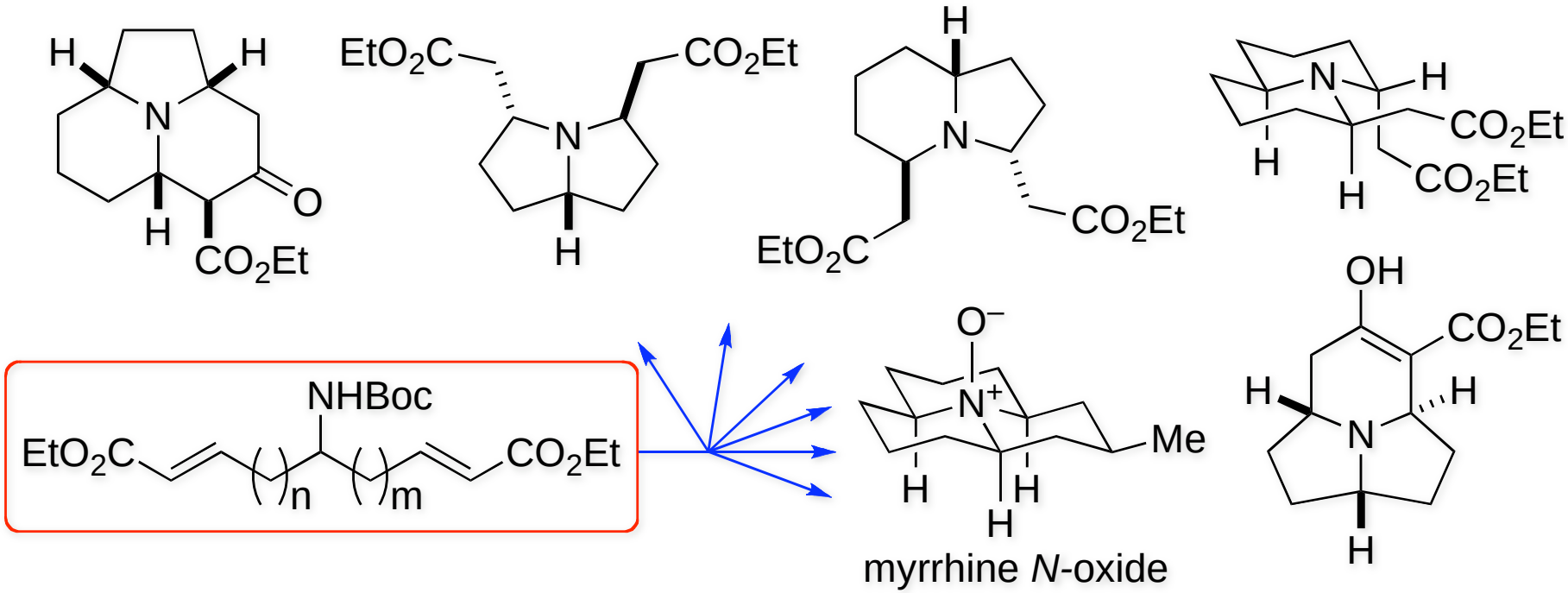
Substrate-Based Approach

Reagent-Based Approach

Nature Commun. **2010**, *1*, 80; *Org. Biomol. Chem.* **2008**, *6*, 1149-1158

Diversity-Oriented Synthesis

- Substrate-Based Approach

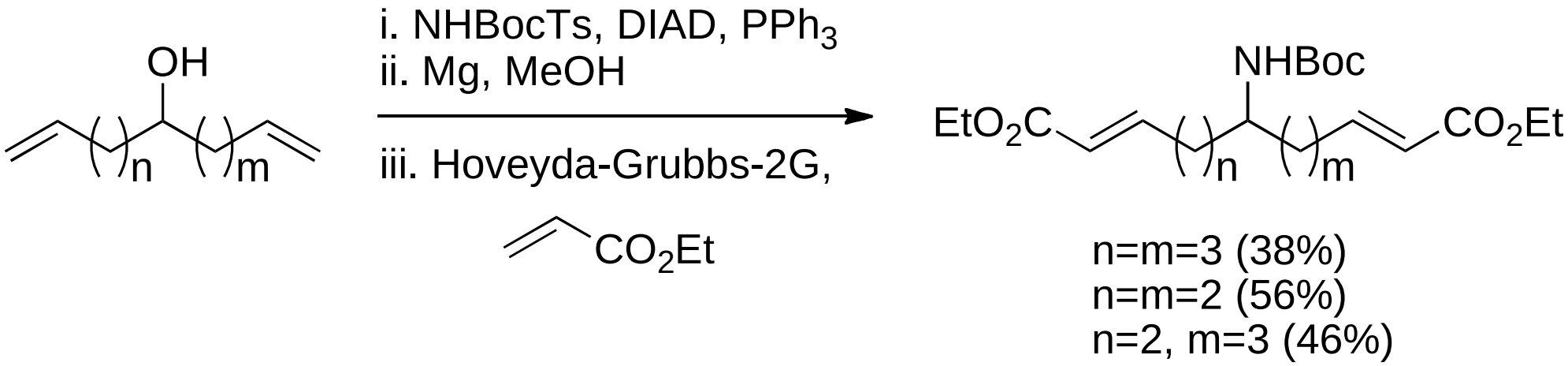


Monica Diaz Gavilan, Warren Galloway, Kieron O'Connell, James Hodgkinson

Chem. Commun. **2008**, 4962-4964

Diversity-Oriented Synthesis

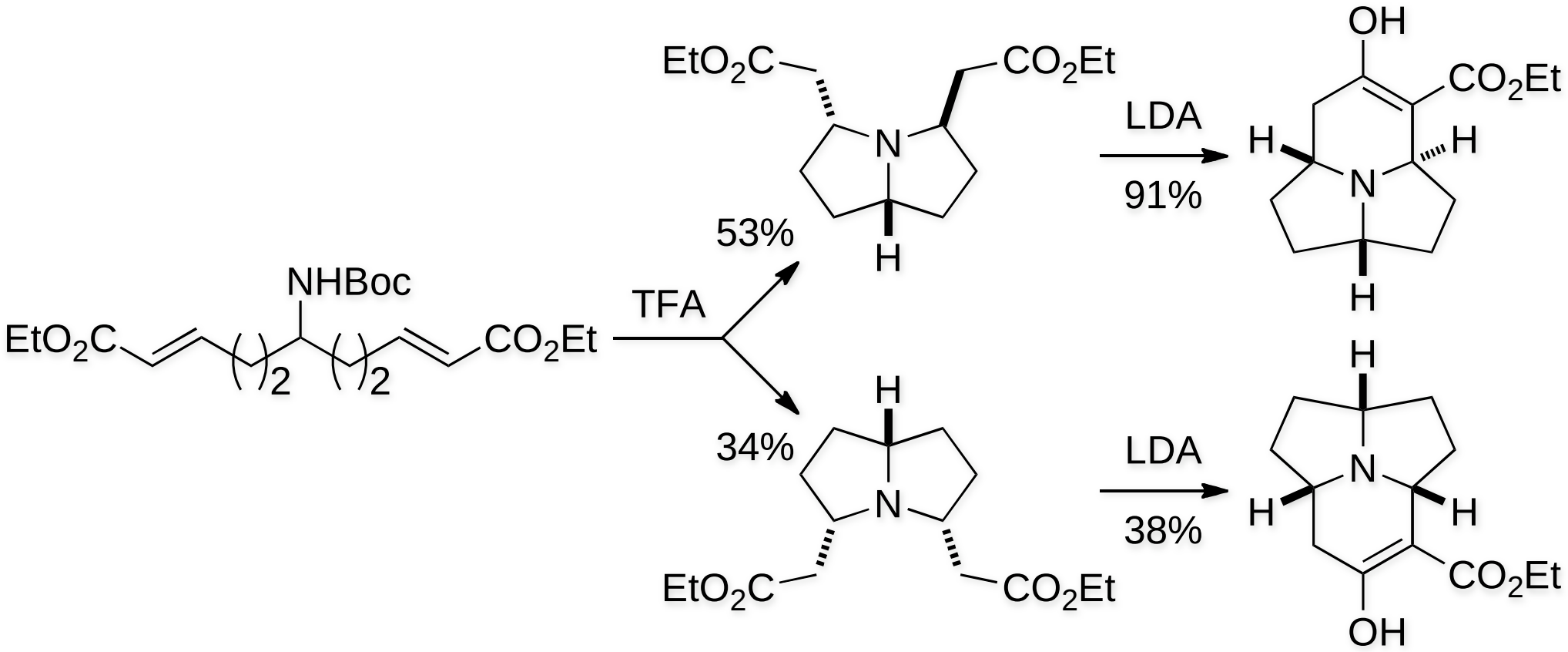
- Substrate-Based Approach



Monica Diaz Gavilan, Warren Galloway, Kieron O'Connell, James Hodgkinson *Chem. Commun.* **2008**, 4962-4964

Diversity-Oriented Synthesis

- Substrate-Based Approach

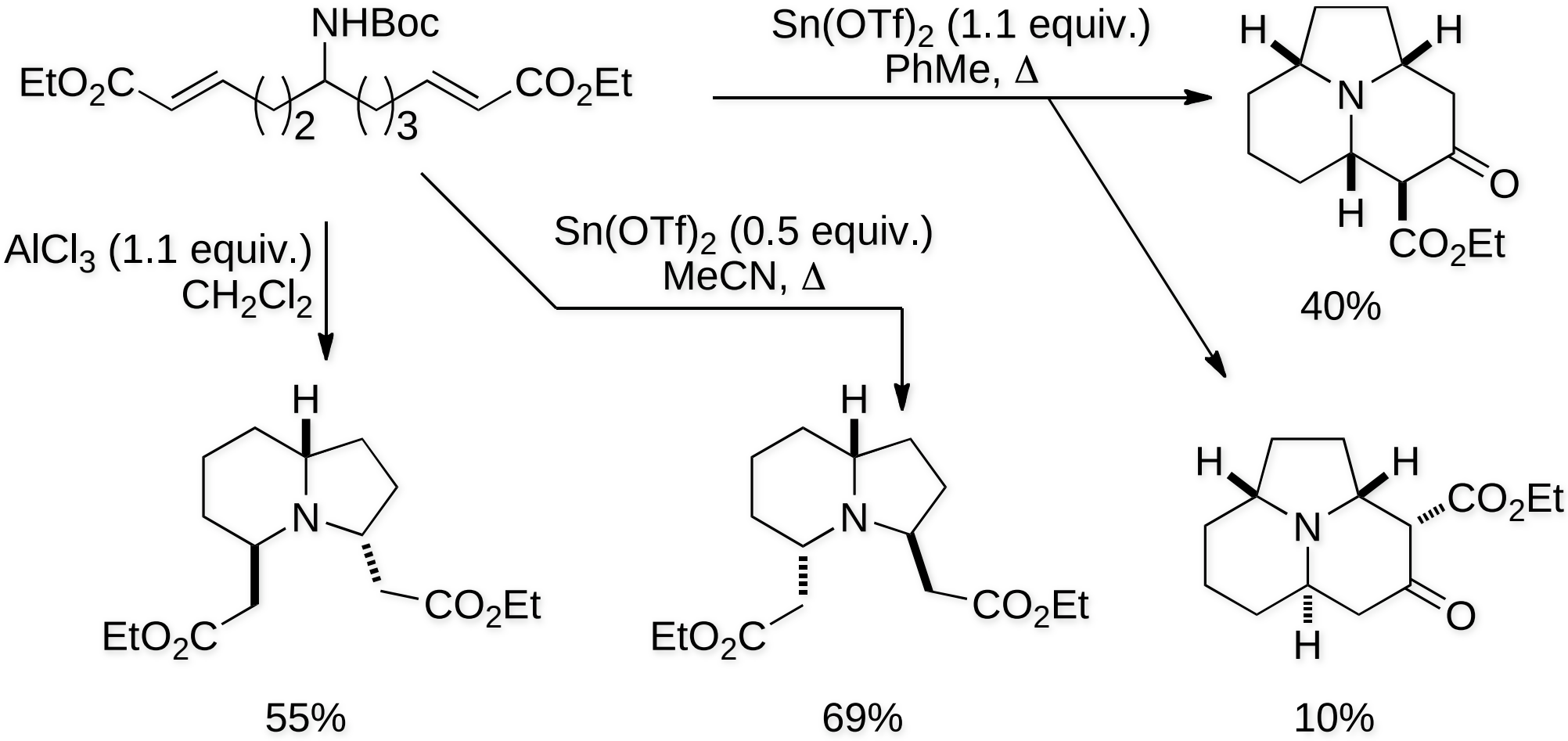


Monica Diaz Gavilan, Warren Galloway, Kieron O'Connell, James Hodgkinson

Chem. Commun. **2008**, 4962-4964

Diversity-Oriented Synthesis

- Substrate-Based Approach

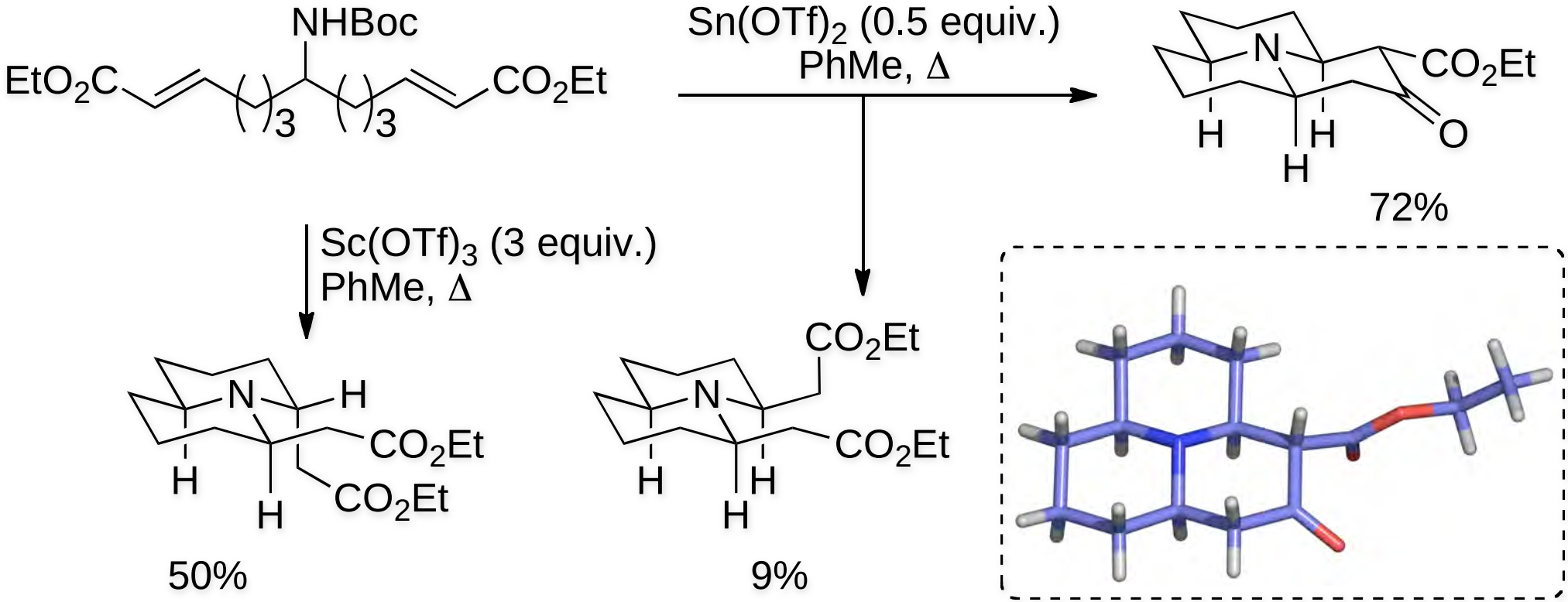


Monica Diaz Gavilan, Warren Galloway, Kieron O'Connell, James Hodgkinson

Chem. Commun. **2008**, 4962-4964

Diversity-Oriented Synthesis

- Substrate-Based Approach

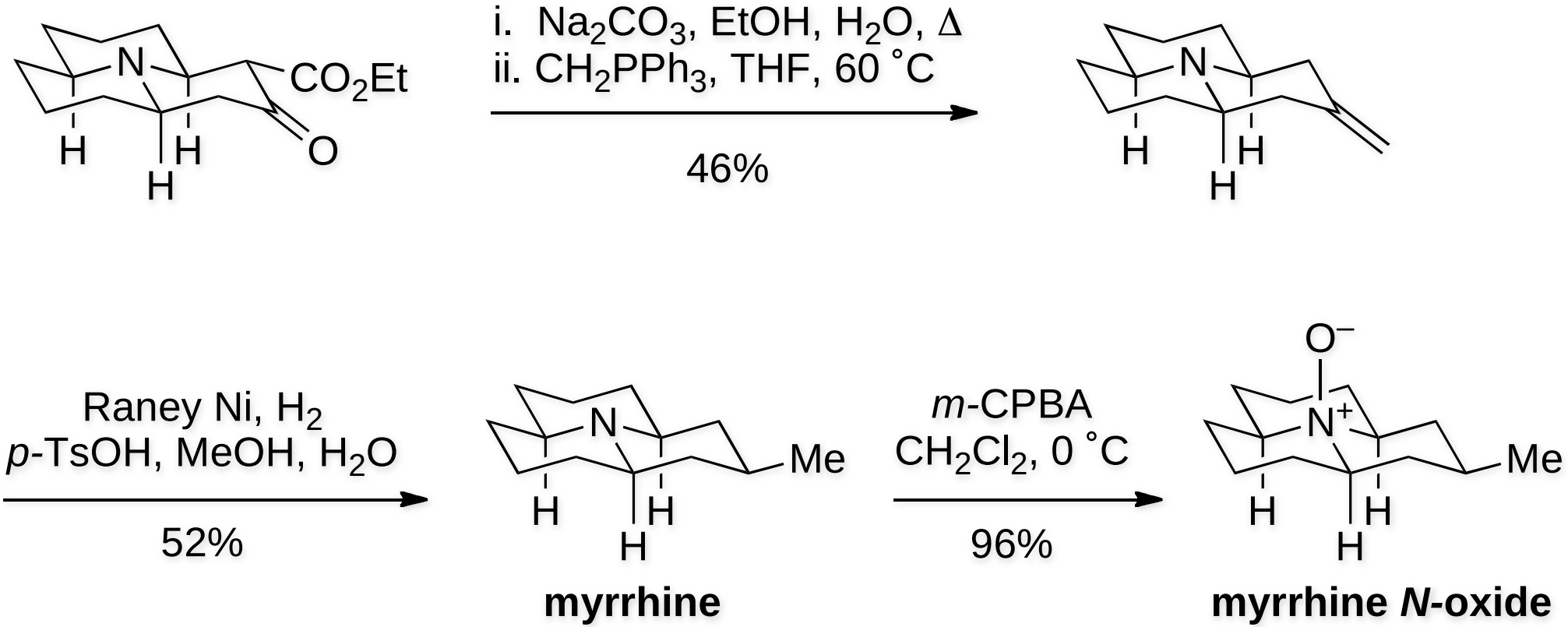


Monica Diaz Gavilan, Warren Galloway, Kieron O'Connell, James Hodgkinson

Chem. Commun. **2008**, 4962-4964

Diversity-Oriented Synthesis

- Substrate-Based Approach



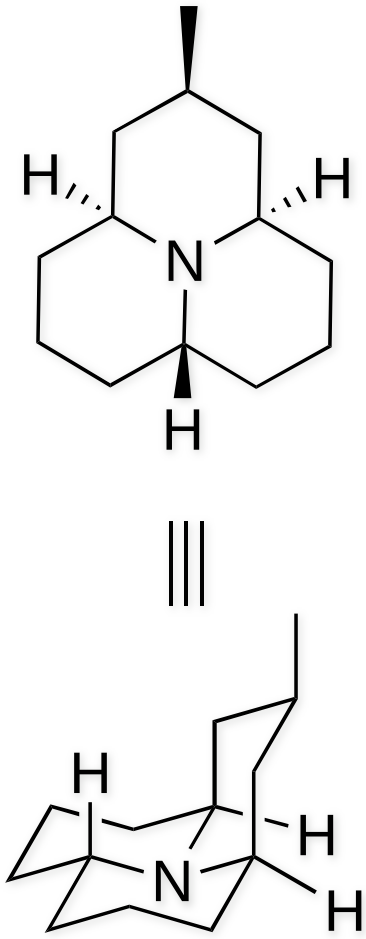
myrrhine

myrrhine *N*-oxide

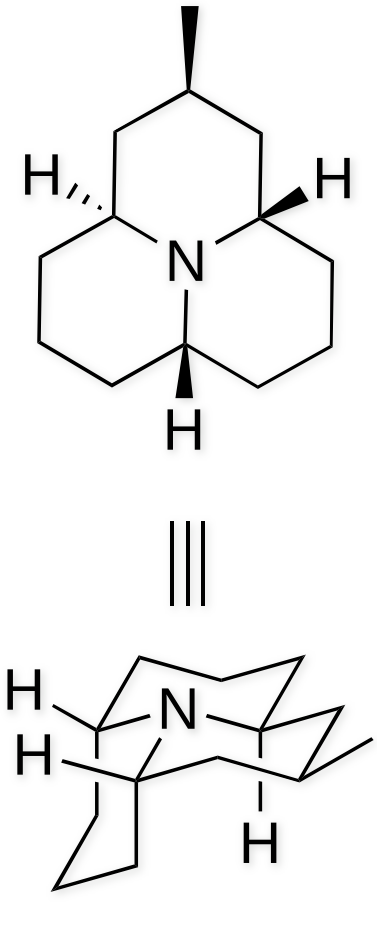
Monica Diaz Gavilan, Warren Galloway, Kieron O'Connell, James Hodgkinson

Chem. Commun. **2008**, 4962-4964

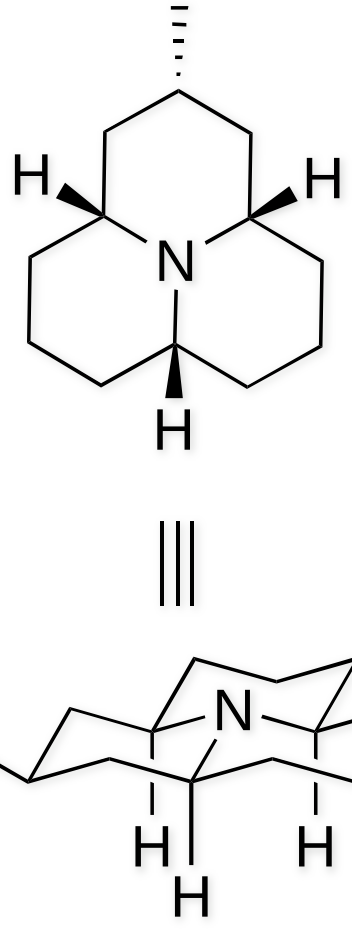
Diversity-Oriented Synthesis



Precoccinelline
N-Oxide: Coccinelline



Hippodamine
N-Oxide: Convergine



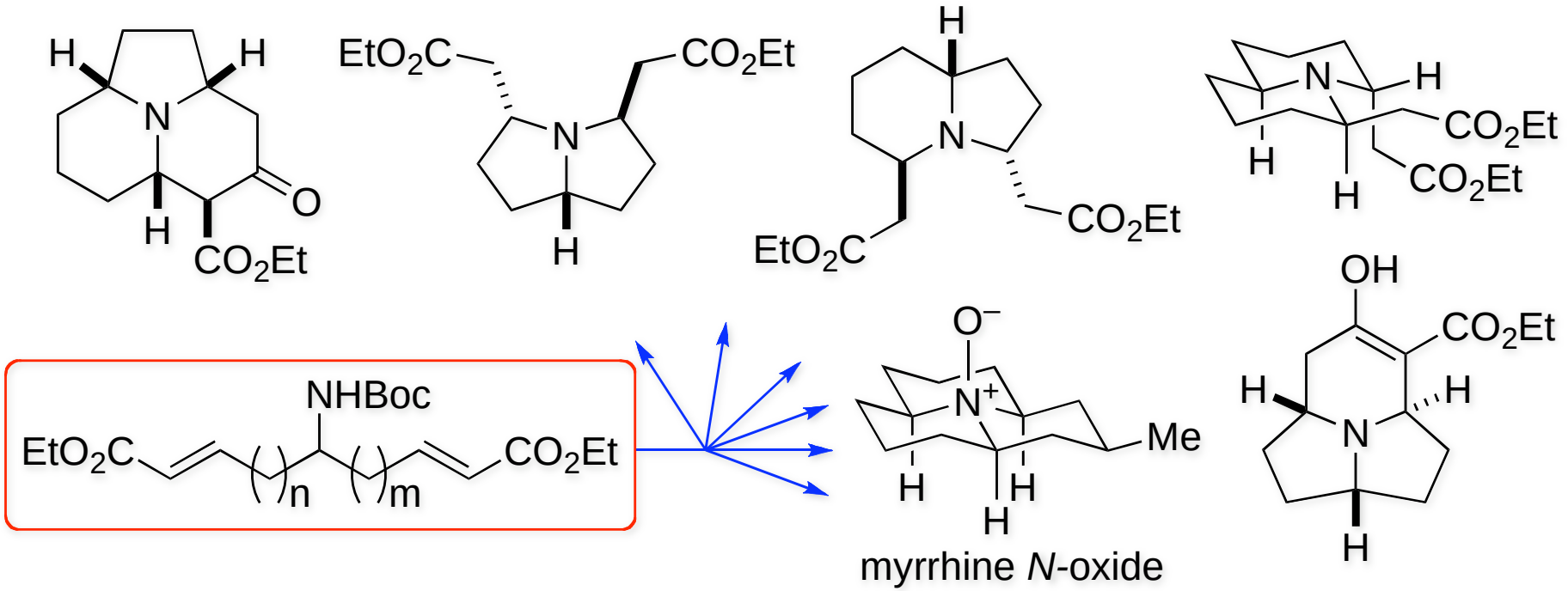
Myrrhine
N-Oxide: Unknown

Monica Diaz Gavilan, Warren Galloway, Kieron O'Connell, James Hodgkinson

Chem. Commun. **2008**, 4962-4964

Diversity-Oriented Synthesis

- Substrate-Based Approach



14 compounds, 7 molecular scaffolds, synthesized in 23 steps

'Steps per scaffold efficiency'= 3.3 steps per scaffold

Monica Diaz Gavilan, Warren Galloway, Kieron O'Connell, James Hodgkinson *Chem. Commun.* **2008**, 4962-4964

Diversity-Oriented Synthesis

Generating Scaffold Diversity

- Substrate-Based Approach
- Reagent-Based Approach

Nature Commun. **2010**, *1*, 80; *Org. Biomol. Chem.* **2008**, *6*, 1149-1158

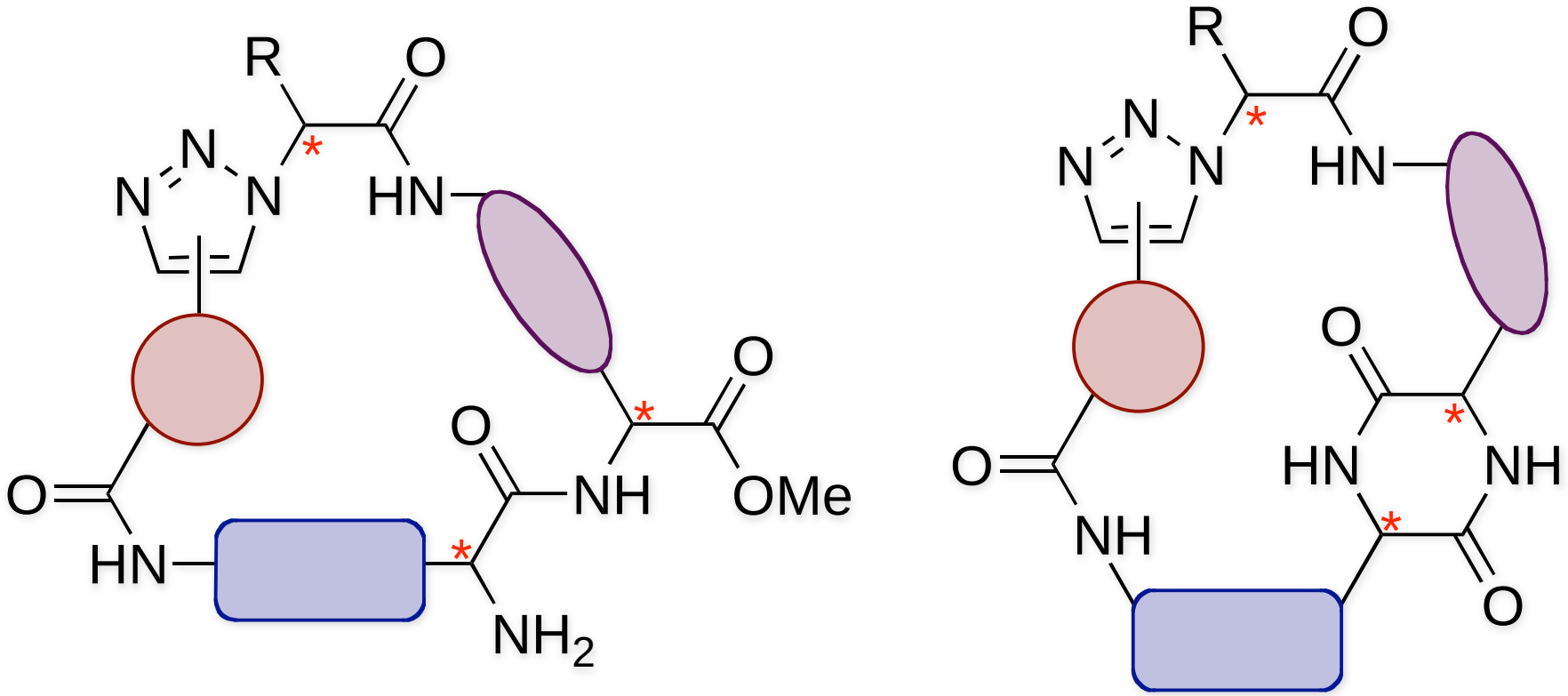
Diversity-Oriented Synthesis

- Reagent-Based Approach
  - Densely Functionalized Molecule
  - Pluripotent Functional Group

Nature Commun. **2010**, *1*, 80; *Org. Biomol. Chem.* **2008**, *6*, 1149-1158

Diversity-Oriented Synthesis

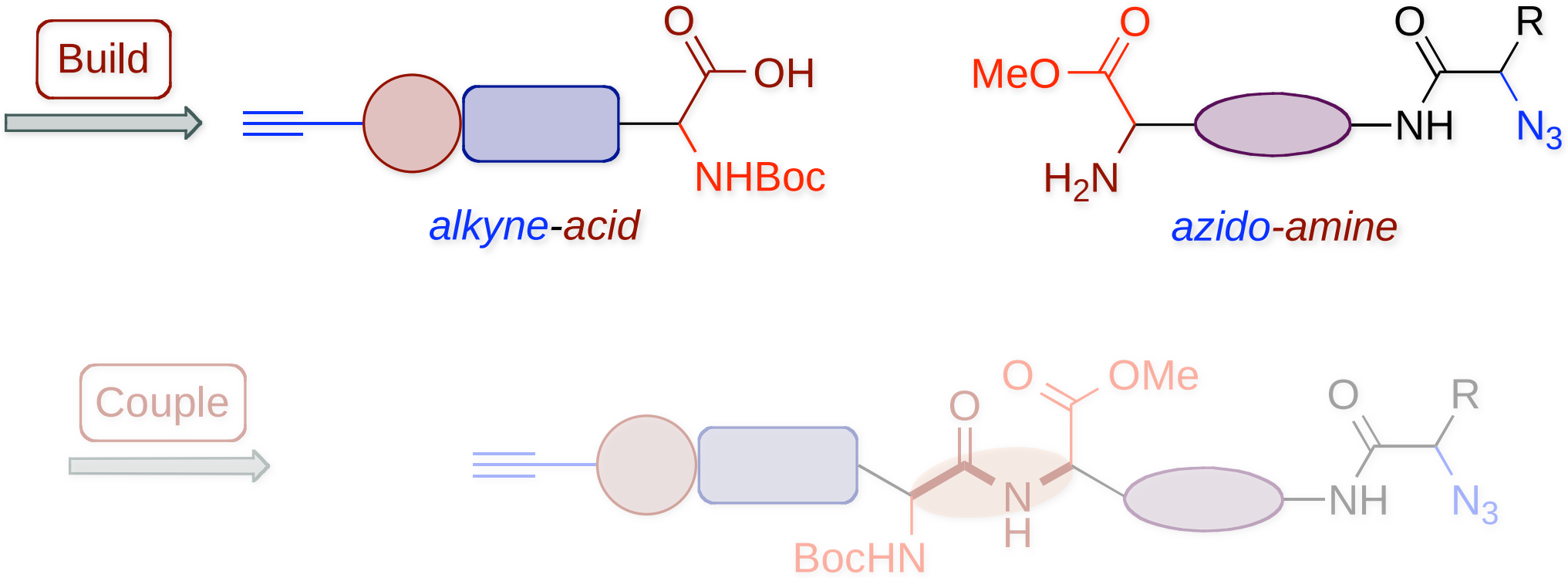
- Densely Functionalized Molecule Strategy



Albert Isidro Llobet, Tiffanie Murillo, Paula Bello, Agostino Cilibrizzi, Warren Galloway, James Hodgkinson *Proc. Natl. Acad. Sci. USA* **2011**, *108*, ASAP

Diversity-Oriented Synthesis

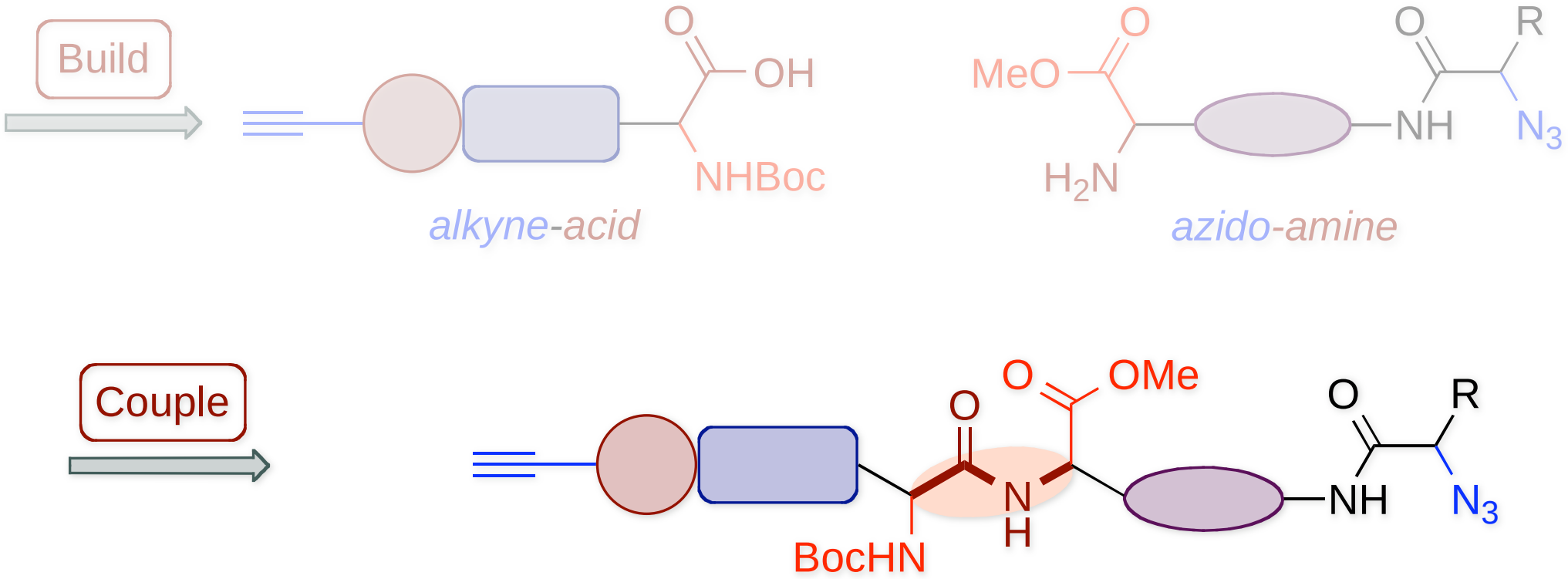
- Densely Functionalized Molecule Strategy



Albert Isidro Llobet, Tiffanie Murillo, Paula Bello, Agostino Cilibrizzi, Warren Galloway, James Hodgkinson *Proc. Natl. Acad. Sci. USA* **2011**, *108*, ASAP

Diversity-Oriented Synthesis

- Densely Functionalized Molecule Strategy



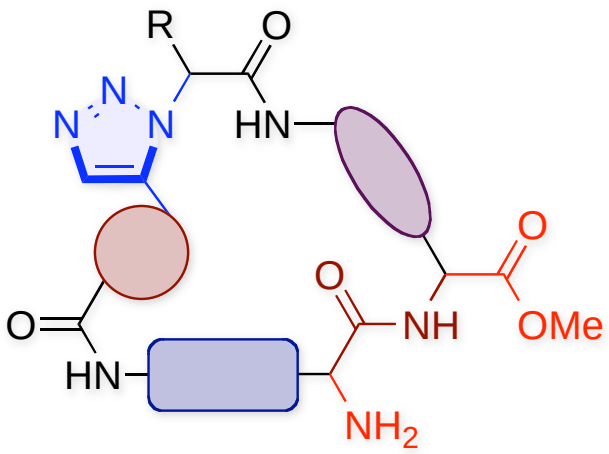
Albert Isidro Llobet, Tiffanie Murillo, Paula Bello, Agostino Cilibrizzi, Warren Galloway, James Hodgkinson *Proc. Natl. Acad. Sci. USA* **2011**, *108*, ASAP

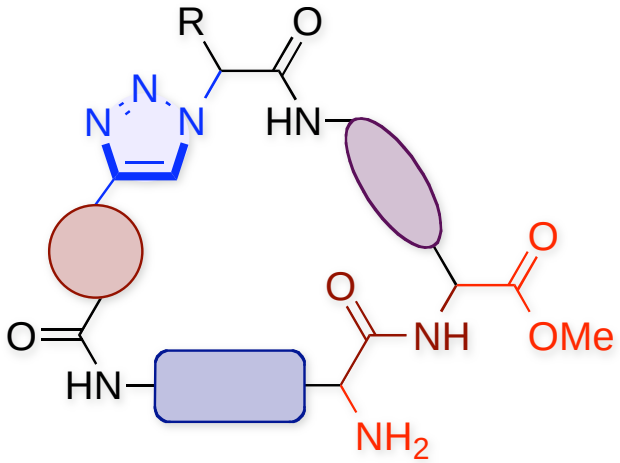
Diversity-Oriented Synthesis

- Densely Functionalized Molecule Strategy

Pair

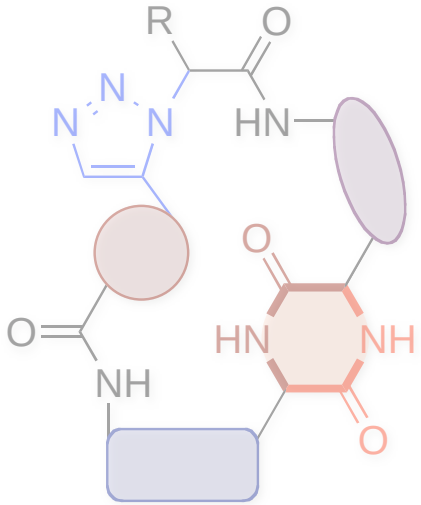
1,3-dipolar cycloaddition

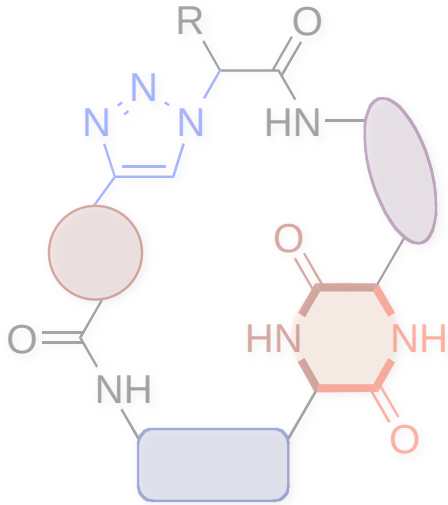




Pair

DKP formation



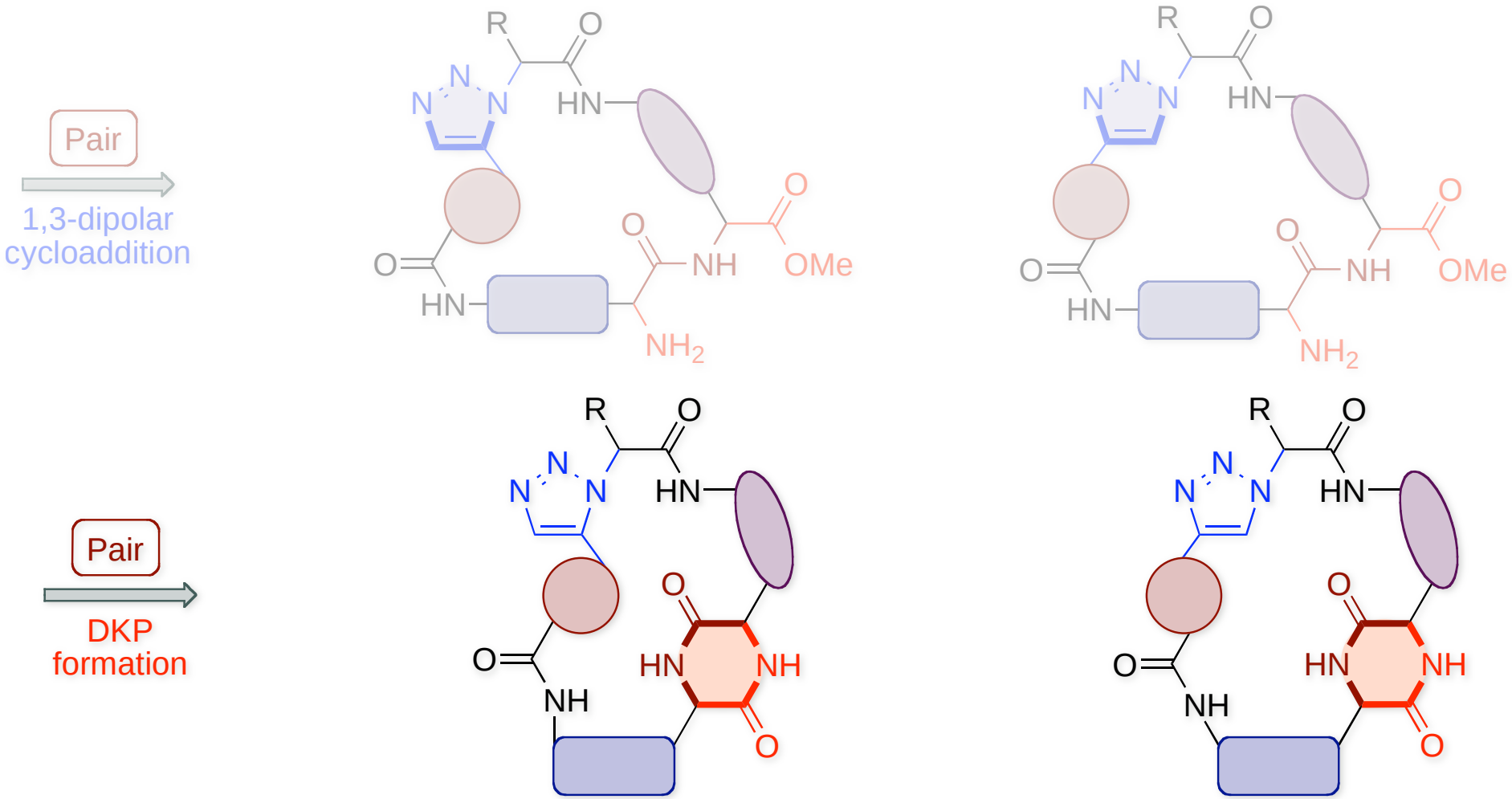


Albert Isidro Llobet, Tiffanie Murillo, Paula Bello, Agostino Cilibrizzi, Warren Galloway, James Hodgkinson

Proc. Natl. Acad. Sci. USA **2011**, *108*, ASAP

Diversity-Oriented Synthesis

- Densely Functionalized Molecule Strategy



Albert Isidro Llobet, Tiffanie Murillo, Paula Bello, Agostino Cilibrizzi, Warren Galloway, James Hodgkinson

Proc. Natl. Acad. Sci. USA **2011**, *108*, ASAP

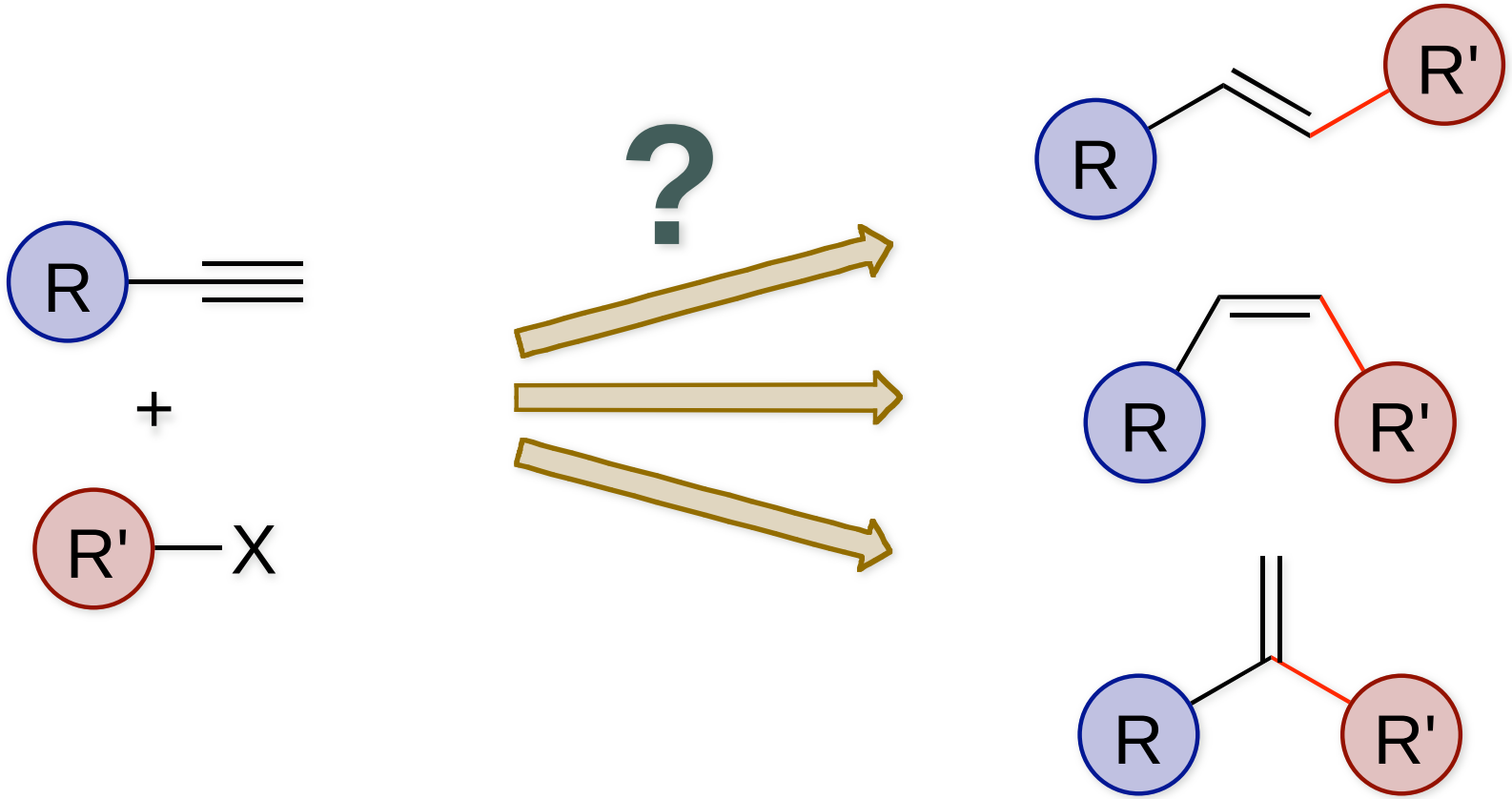
Diversity-Oriented Synthesis

- Reagent-Based Approach
  - Densely Functionalized Molecule
  - Pluripotent Functional Group

Nature Commun. **2010**, *1*, 80; *Org. Biomol. Chem.* **2008**, *6*, 1149-1158

Diversity-Oriented Synthesis

- Pluripotent Functional Group Strategy

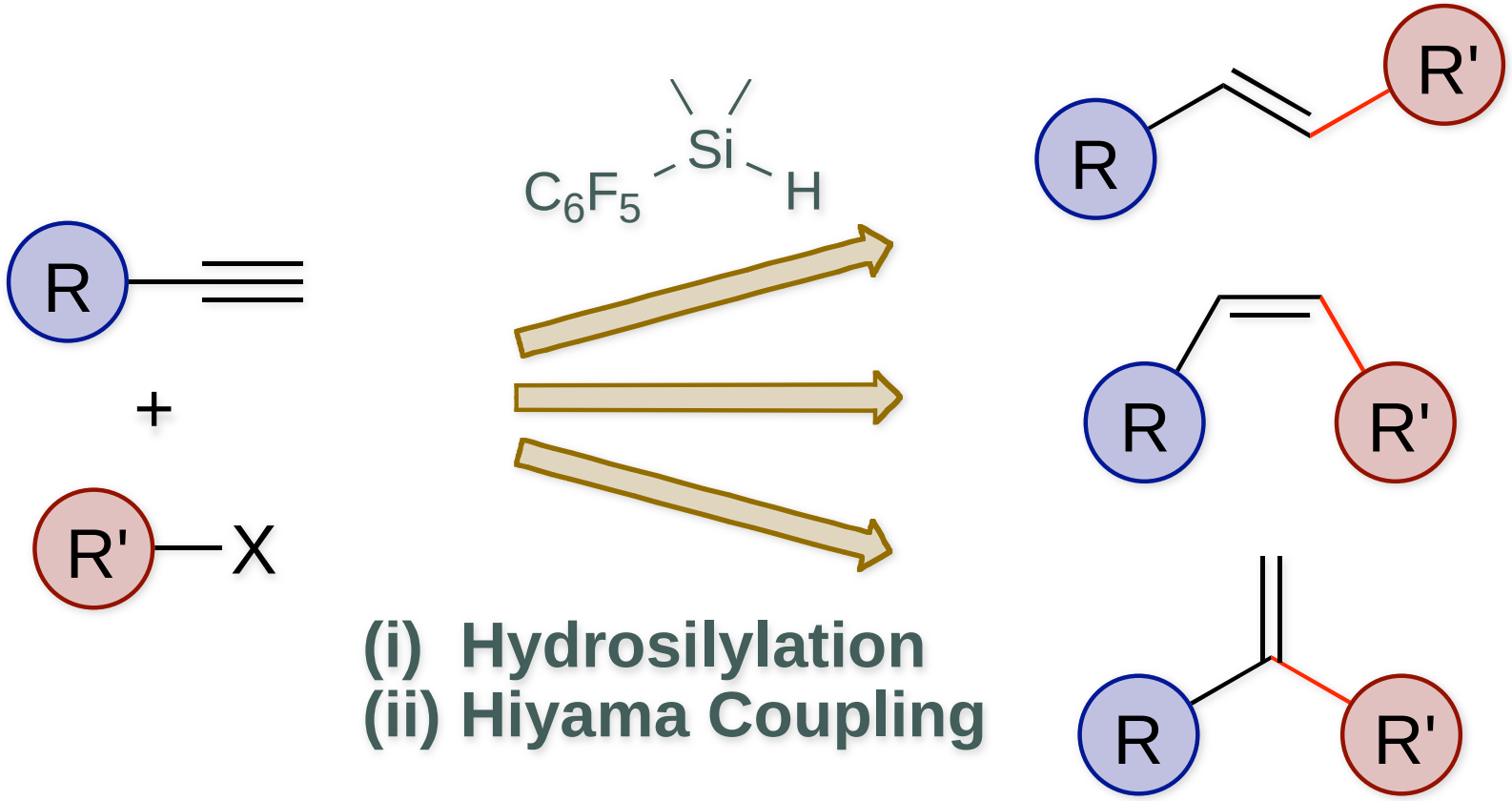


Hannah Sore, Christine Böhner, David Blackwell, Luca Laraia, Matthew Scott, Patrizia Logoteta, Cora Prestinari, Katherine Williams, Warren Galloway

Org. Lett. **2010**, *12*, 2806-2809; *Org. Biomol. Chem.* **2011**, *9*, 504-515.

Diversity-Oriented Synthesis

- Pluripotent Functional Group Strategy

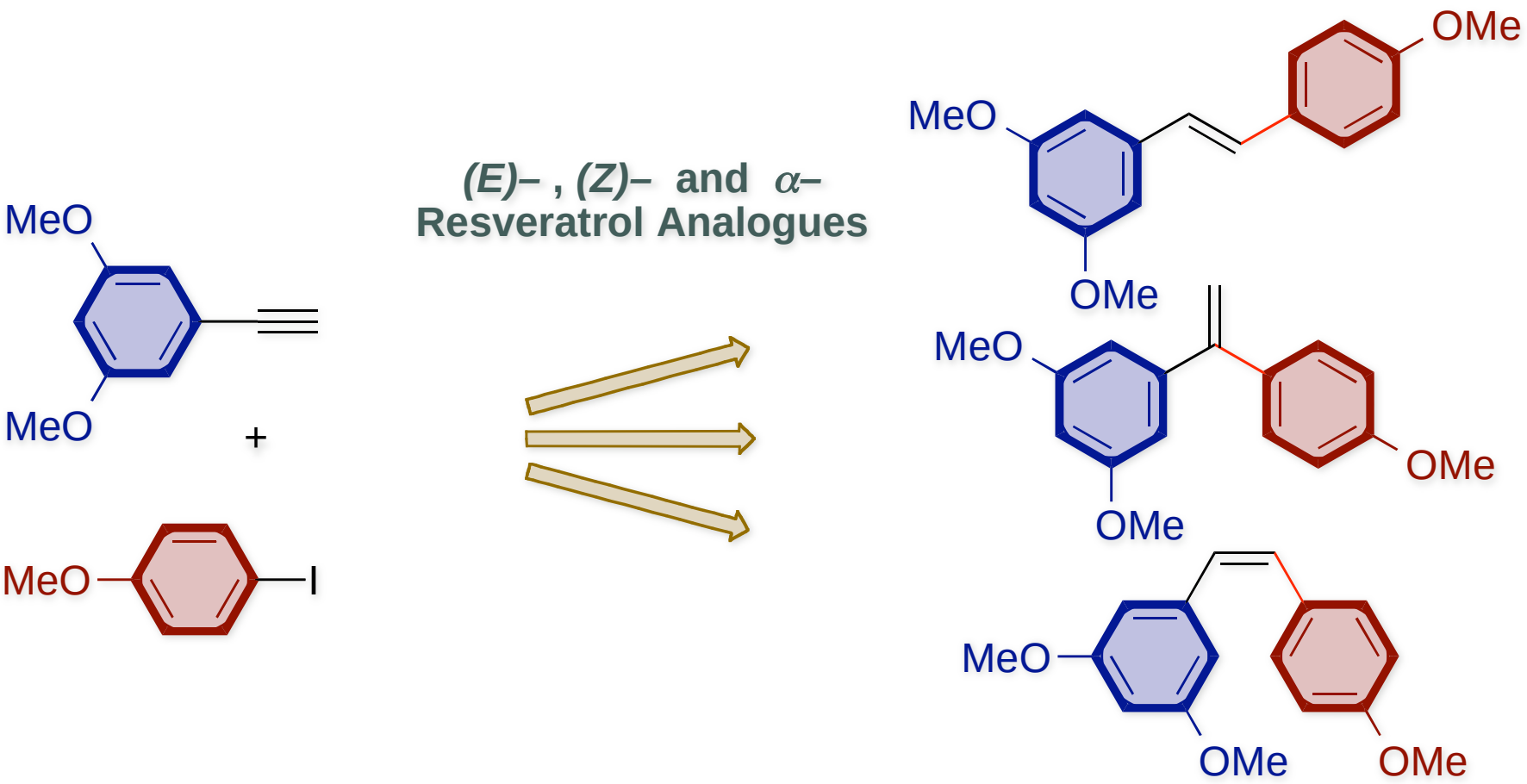


Hannah Sore, Christine Böhner, David Blackwell, Luca Laraia, Matthew Scott, Patrizia Logoteta, Cora Prestinari, Katherine Williams, Warren Galloway

Org. Lett. **2010**, *12*, 2806-2809; *Org. Biomol. Chem.* **2011**, *9*, 504-515.

Diversity-Oriented Synthesis

- Pluripotent Functional Group Strategy

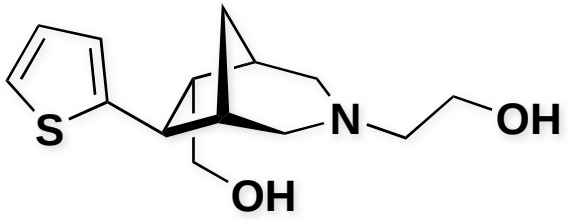


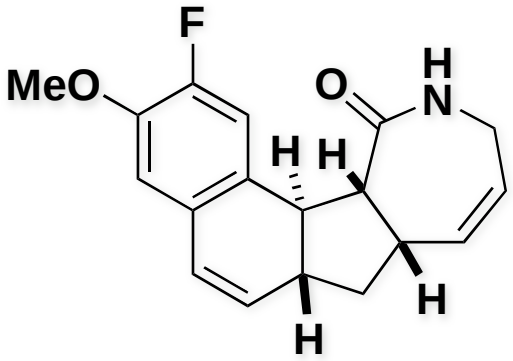
Hannah Sore, Christine Böhner, David Blackwell, Luca Laraia, Matthew Scott, Patrizia Logoteta, Cora Prestinari, Katherine Williams, Warren Galloway

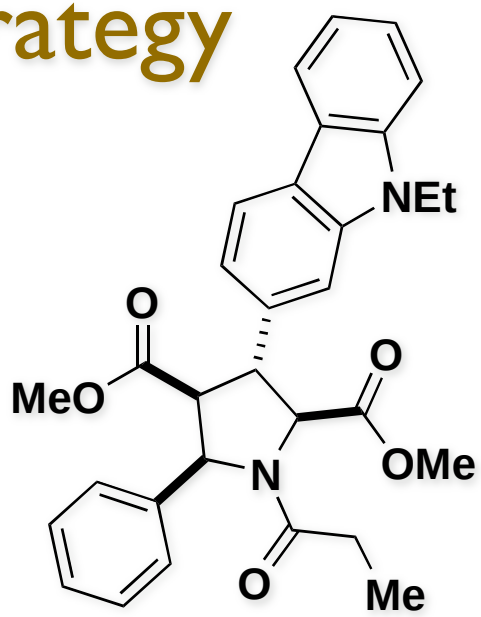
Org. Lett. **2010**, *12*, 2806-2809; *Org. Biomol. Chem.* **2011**, *9*, 504-515.

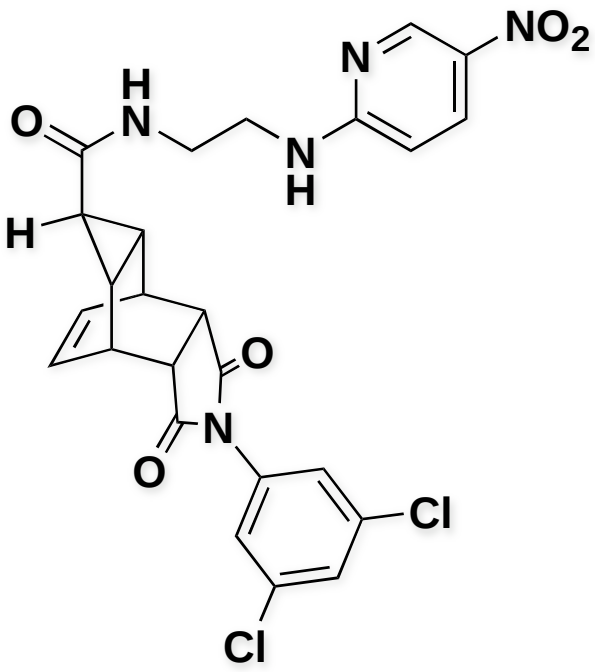
Diversity-Oriented Synthesis

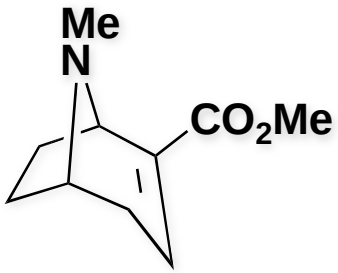
- Pluripotent Functional Group Strategy

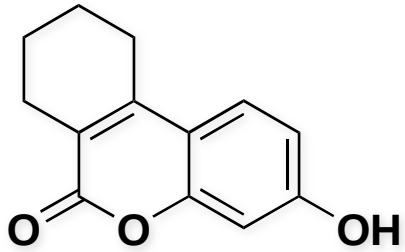


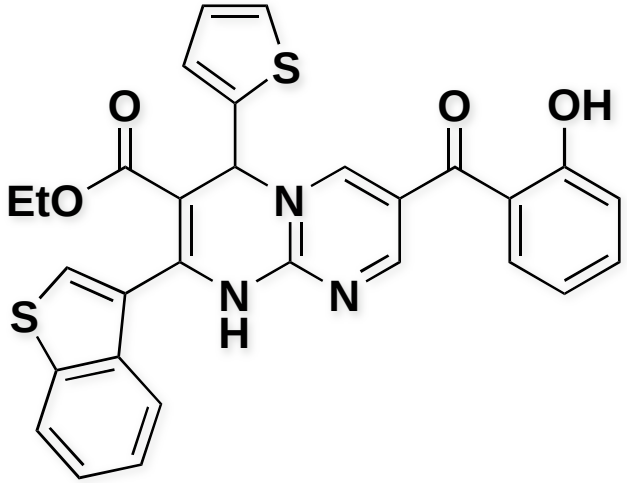




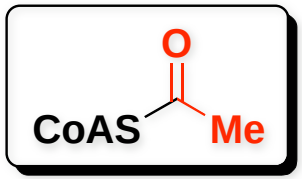








Diversity-Oriented Synthesis



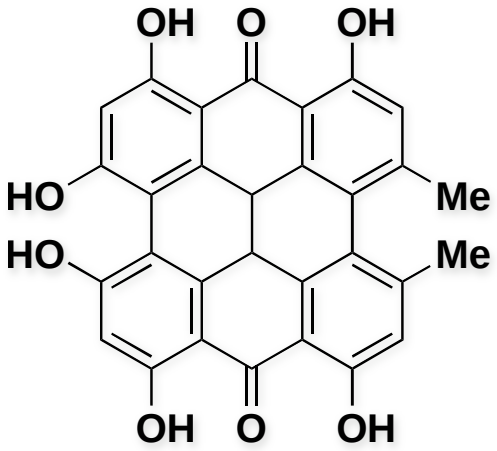
Acetyl coenzyme A
biosynthetic
'two-carbon' starting unit

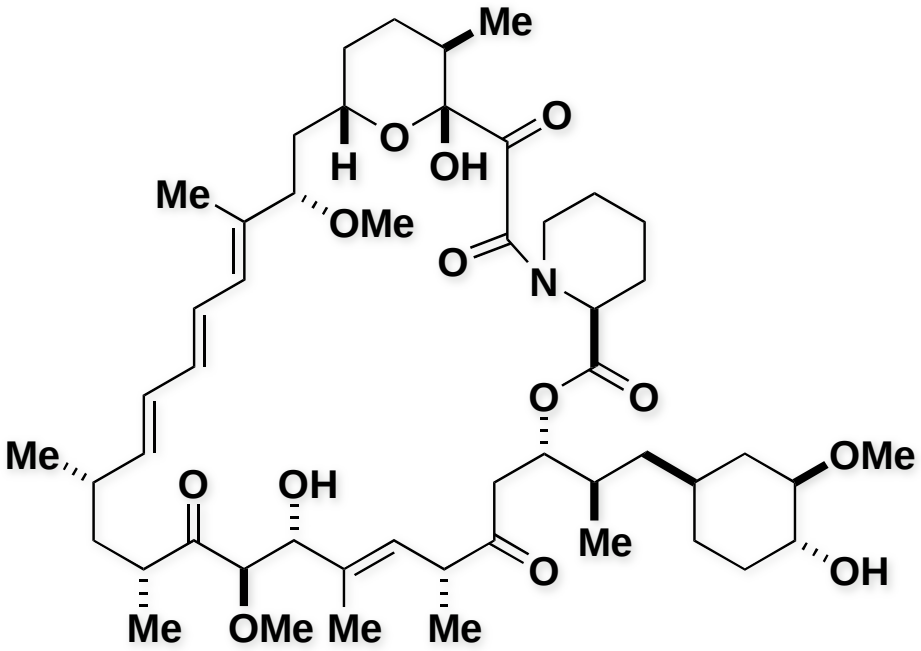
>20 steps

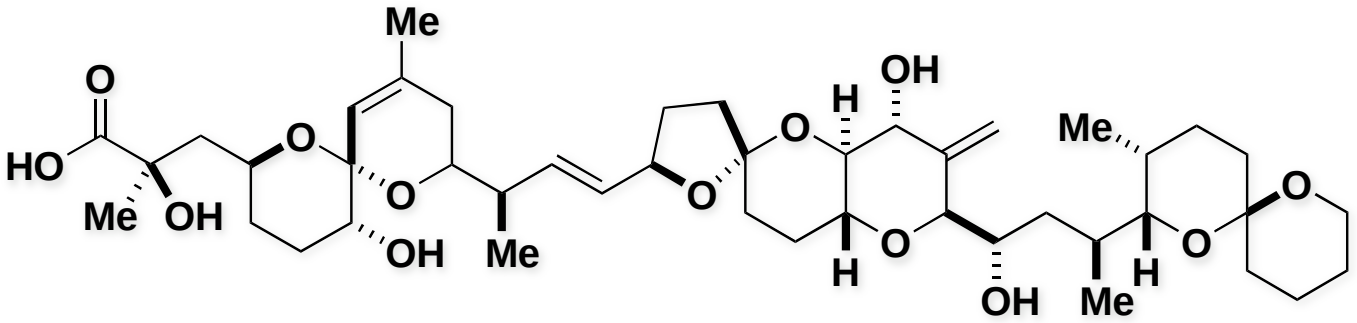
fatty acids
polyketides
steroids
terpenes

e.g.

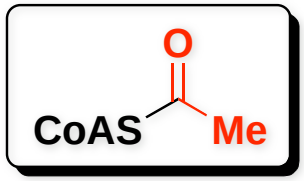
palmitic acid
rapamycin
cholesterol
camphor







Diversity-Oriented Synthesis



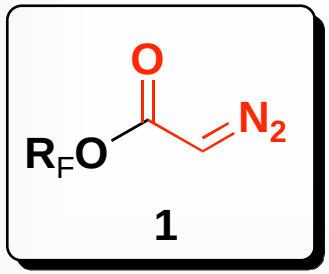
Acetyl coenzyme A
biosynthetic
'two-carbon' starting unit

>20 steps

fatty acids
polyketides
steroids
terpenes

e.g.

palmitic acid
rapamycin
cholesterol
camphor



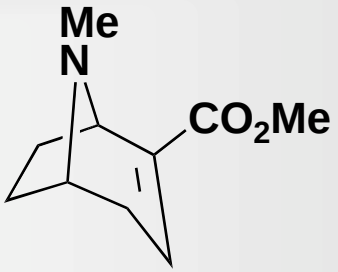
Fluorous–tagged diazoacetate
diversity–oriented synthetic
'two-carbon' starting unit

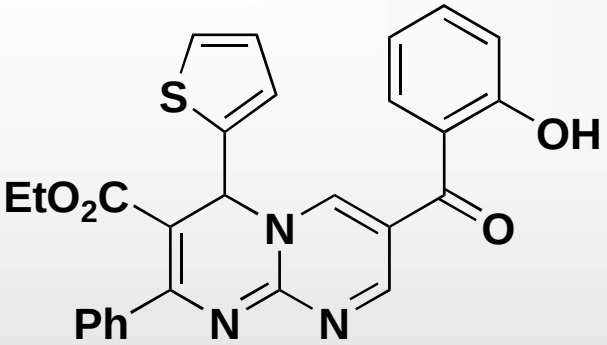
$\mathbf{R_F}$ = Fluorous tag

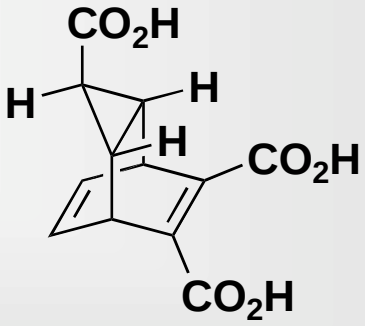
2–4 steps

alkaloid scaffolds
polycarbocyclic scaffolds
heterocycles
chiral centres

e.g.

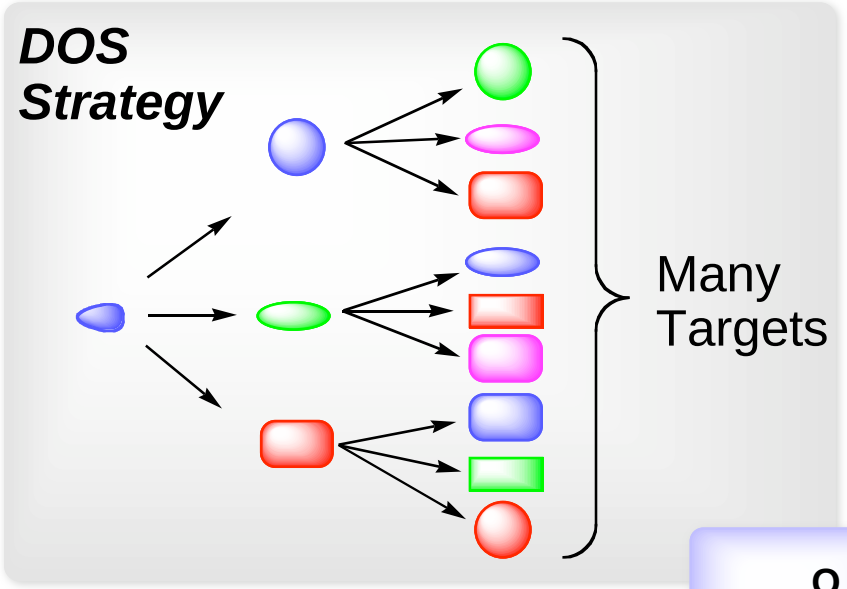


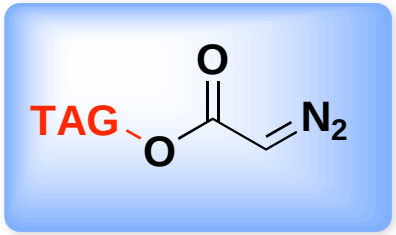


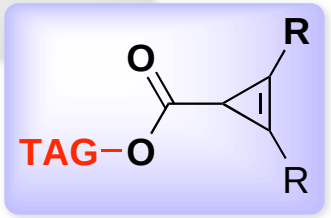


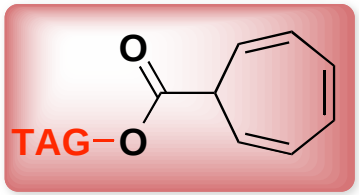
Emma Wyatt, Warren Galloway & Suzanne Fergus

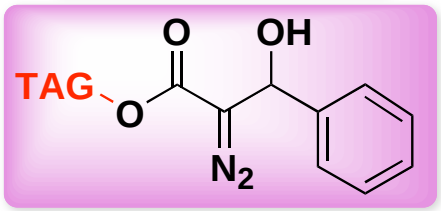
Diversity-Oriented Synthesis



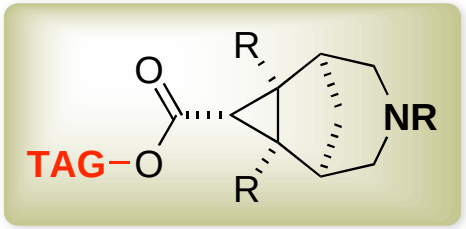


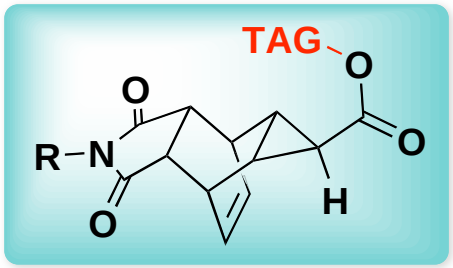


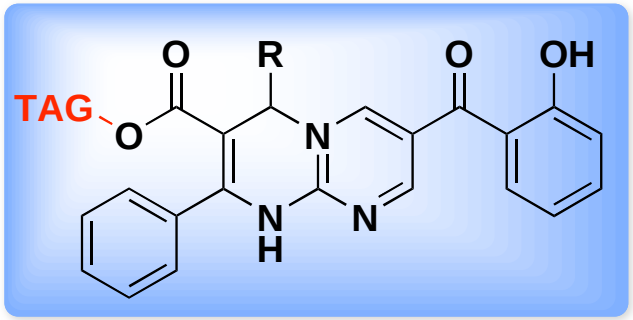




TAG– = $C_6F_{13}CH_2CH_2$–

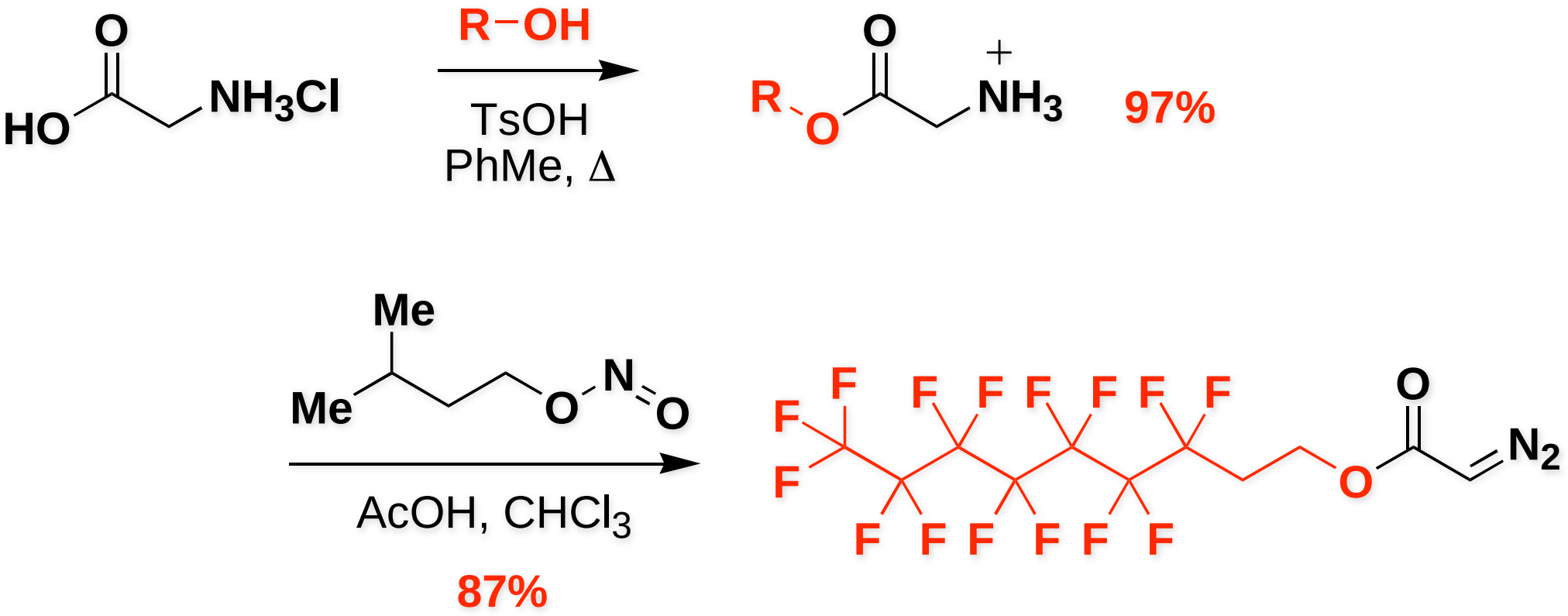






Emma Wyatt, Warren Galloway & Suzanne Fergus

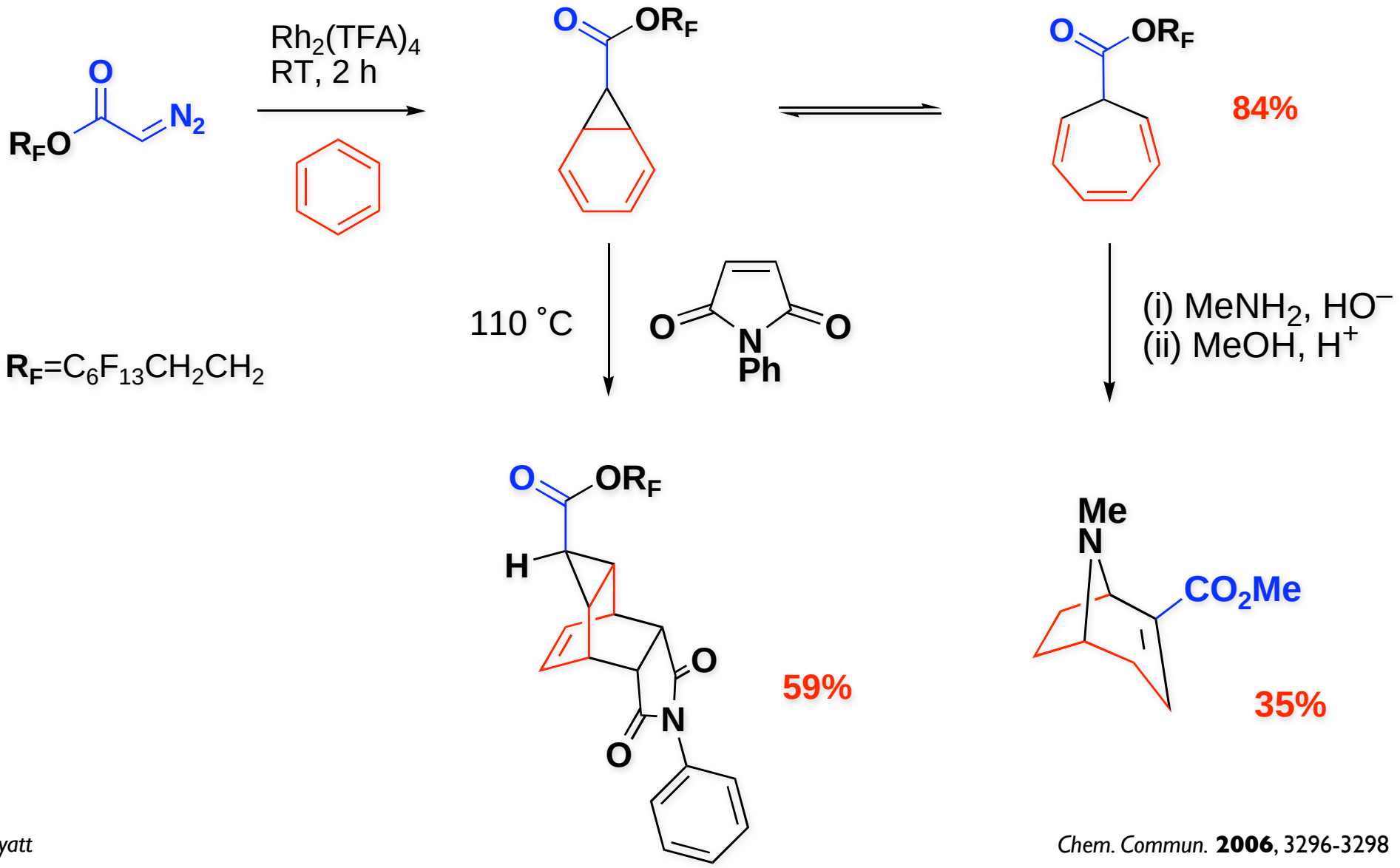
Diversity-Oriented Synthesis



Suzanne Fergus

Chem. Commun. **2006**, 3296-3298

Diversity-Oriented Synthesis

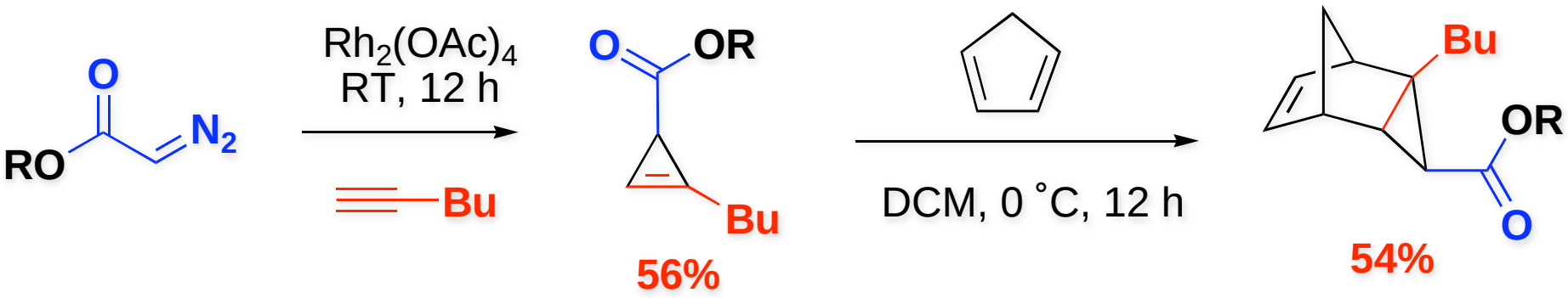


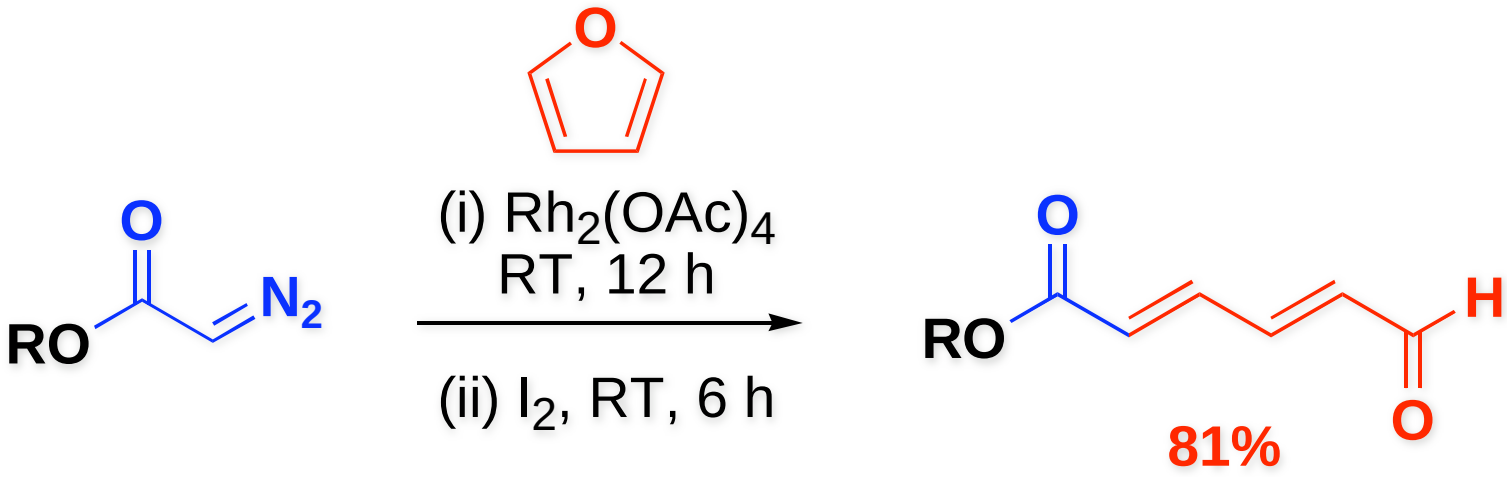
$R_F = C_6F_{13}CH_2CH_2$

Emma Wyatt

Chem. Commun. **2006**, 3296-3298

Diversity-Oriented Synthesis

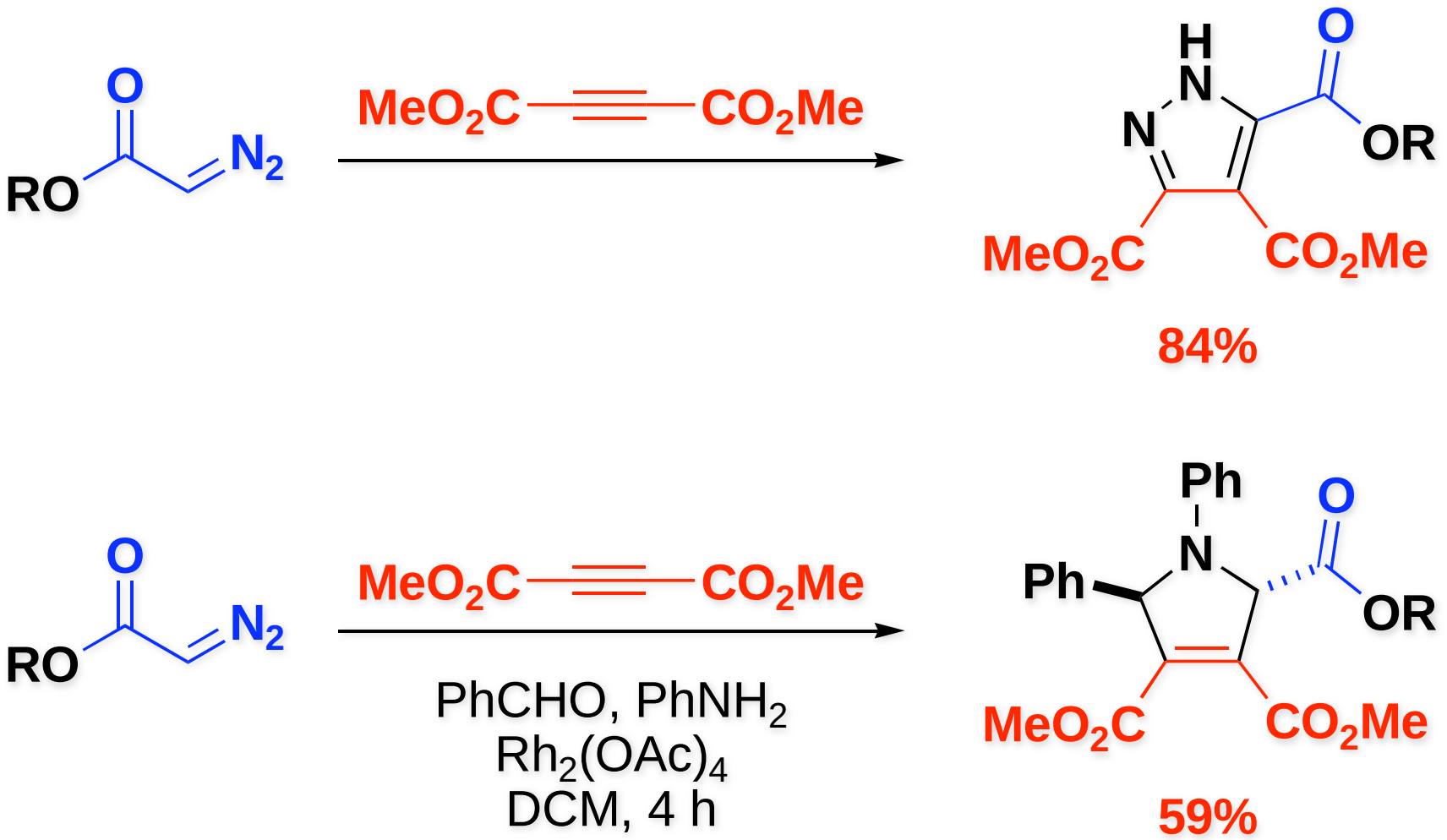




Emma Wyatt, Warren Galloway

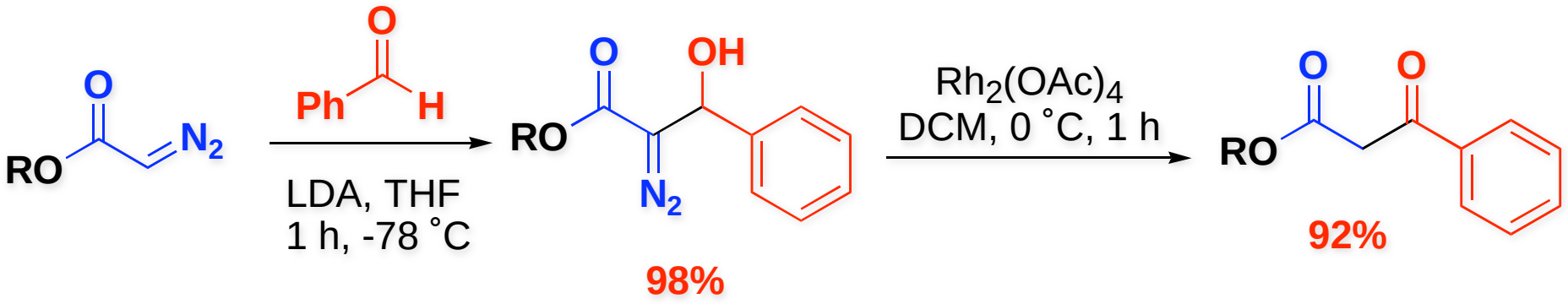
Chem. Commun. **2006**, 3296-3298

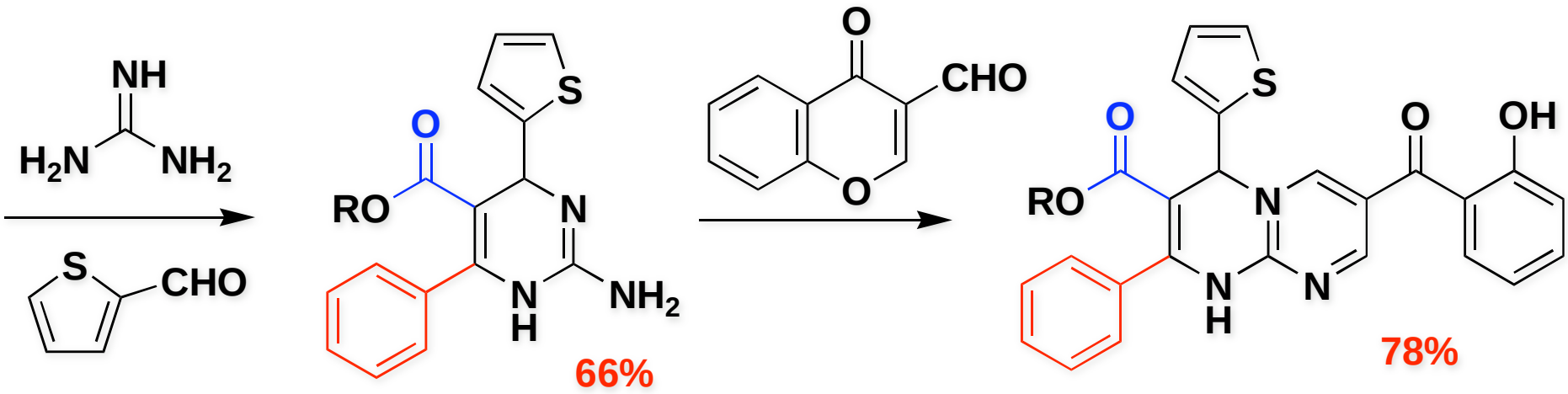
Diversity-Oriented Synthesis



Chem. Commun. **2006**, 3296-3298

Diversity-Oriented Synthesis

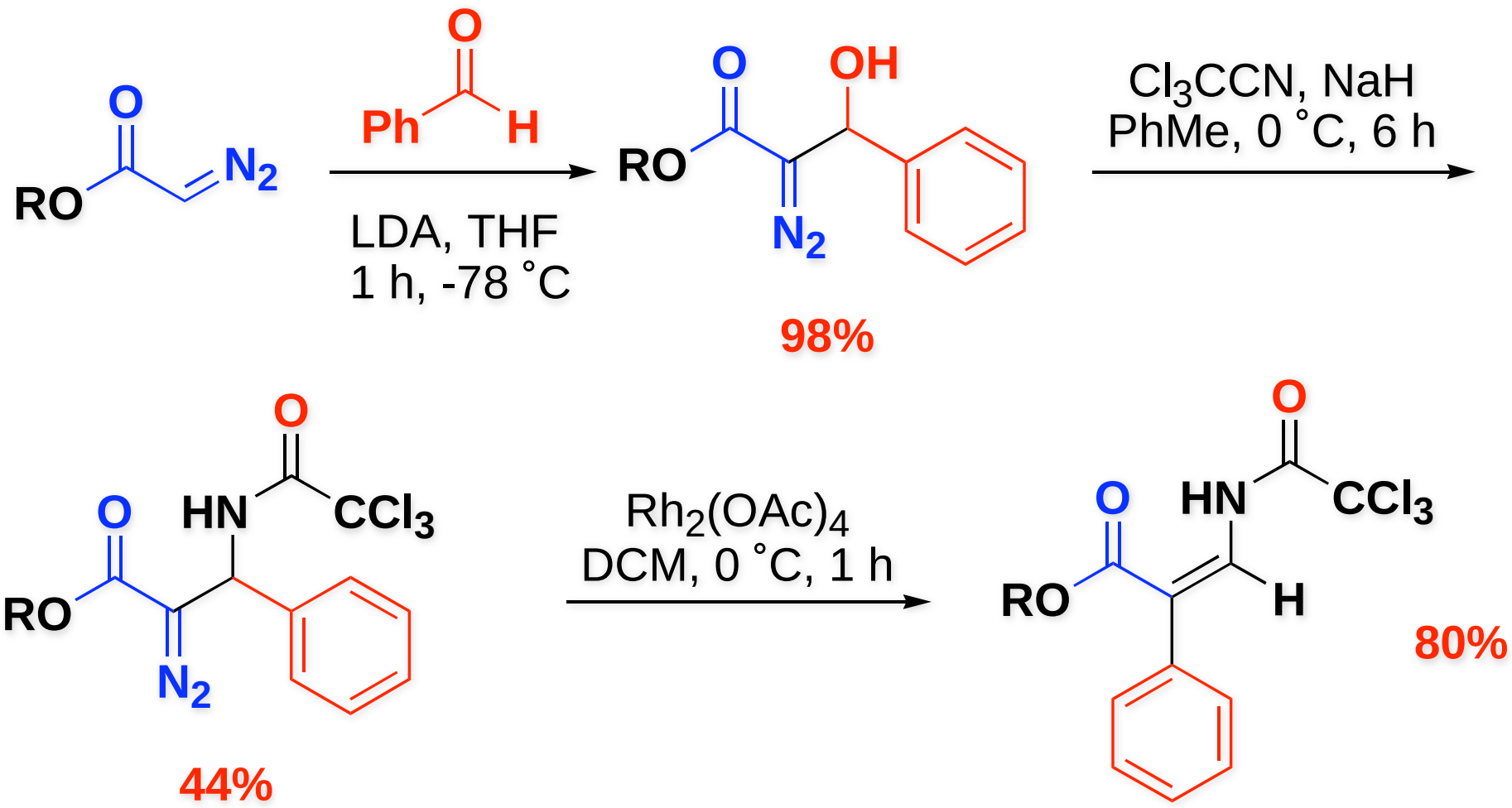




Emma Wyatt

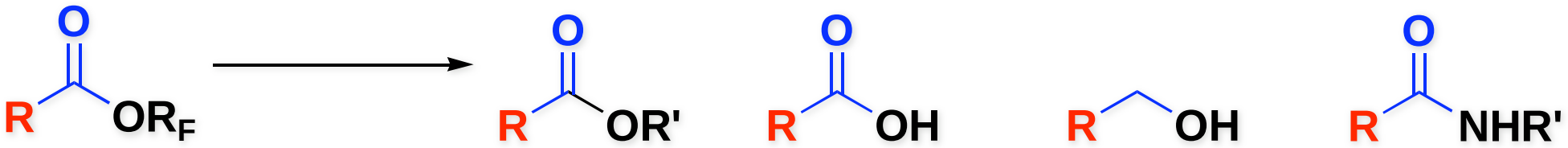
Chem. Commun. **2006**, 3296-3298

Diversity-Oriented Synthesis



Chem. Commun. **2006**, 3296-3298

Diversity-Oriented Synthesis



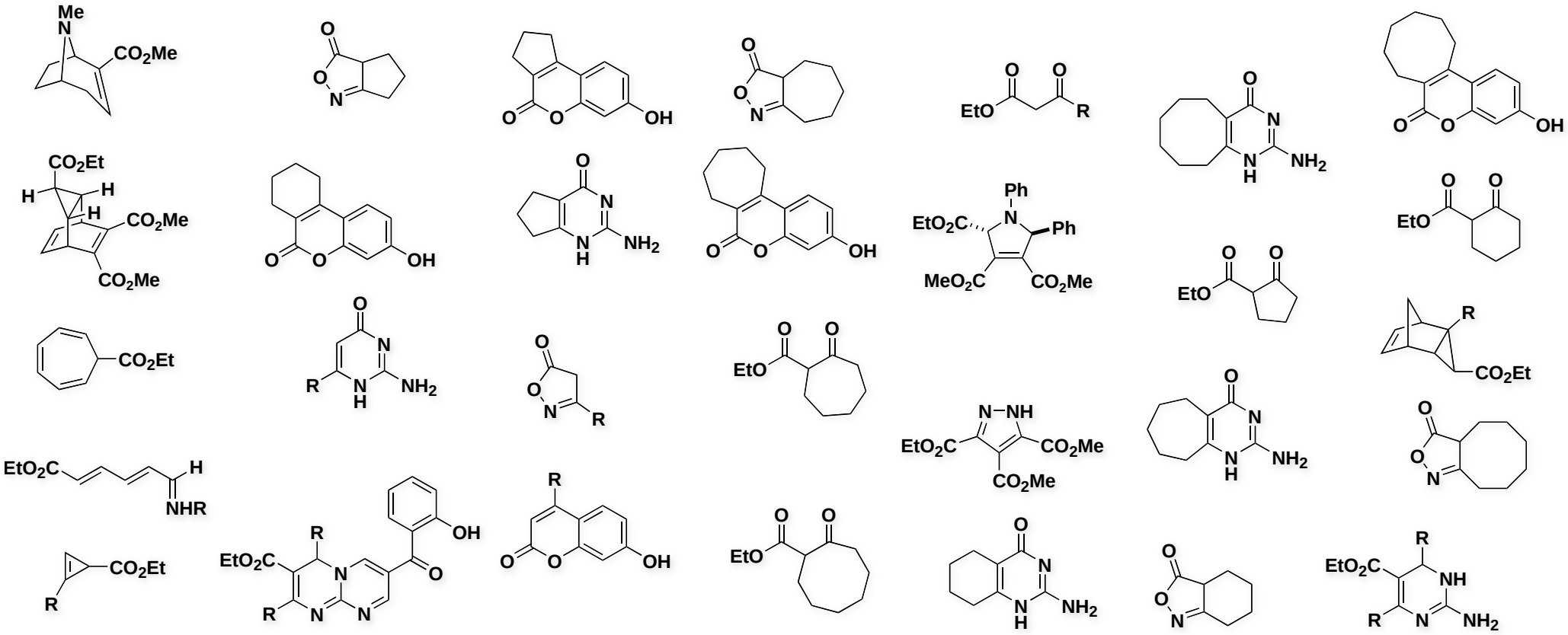
Parallel library synthesis of 223 compounds

quantity range 2-15 mg
MW range 140-614
purity > 90%

Emma Wyatt, Warren Galloway & Suzanne Fergus

Diversity-Oriented Synthesis

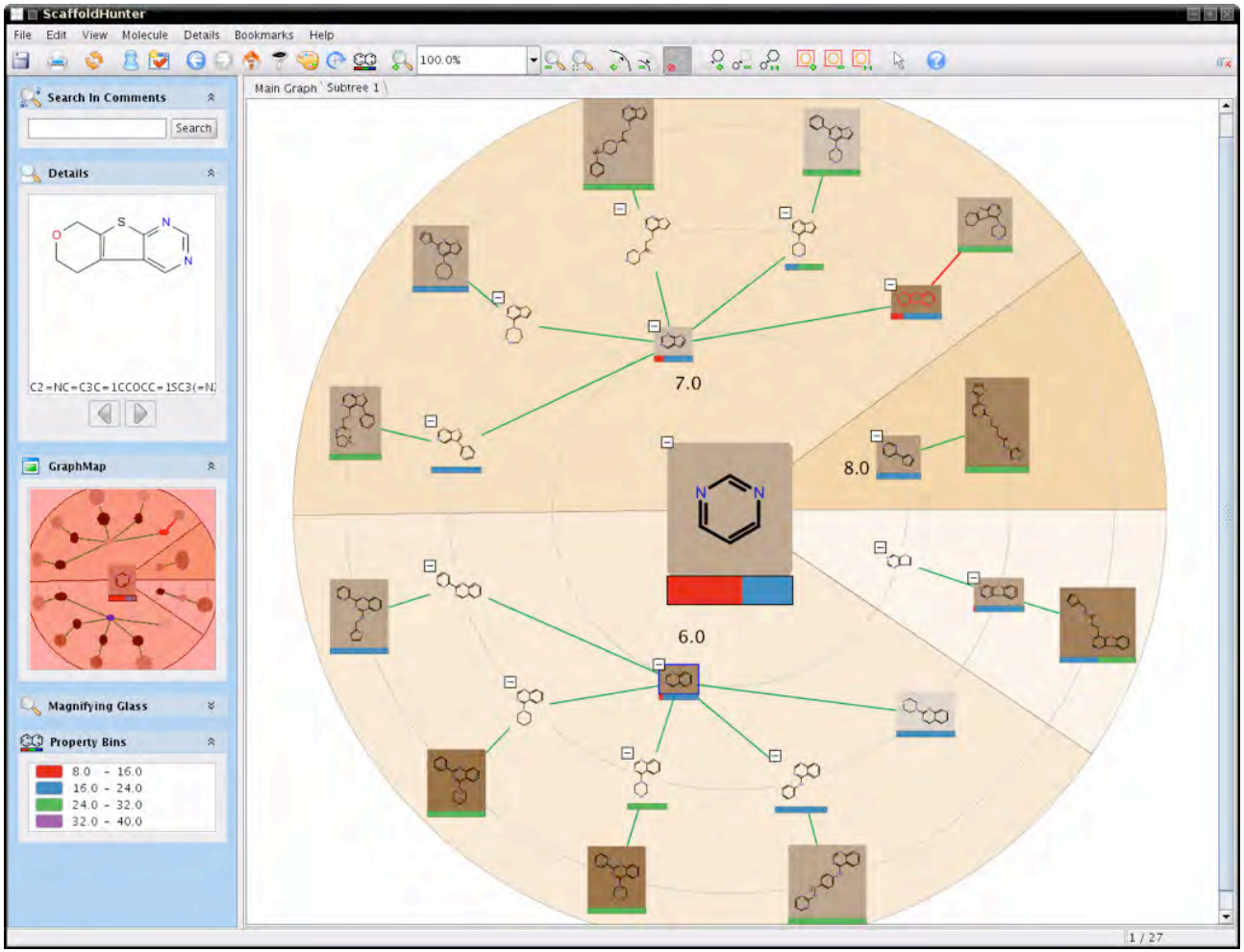
Library of 223 compounds includes
30 discrete molecular frameworks.



Emma Wyatt, Warren Galloway & Suzanne Fergus

Chem. Commun. **2006**, 3296-3298

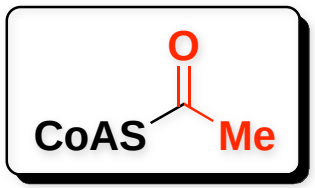
Scaffold Hunter



JAVA-based software tool for the analysis of structure-related biochemical data.
Available as open source software distributed free of charge under the GNU General Public License.
http://scaffoldhunter.sourceforge.net/index.html

Stefan Wetzel, Karsten Klein, Steffen Renner, Daniel Rauh, Tudor I Oprea, Petra Mutzel, Herbert Waldmann *Nature Chem. Biol.* **2009**, *5*, 581-583

Diversity-Oriented Synthesis



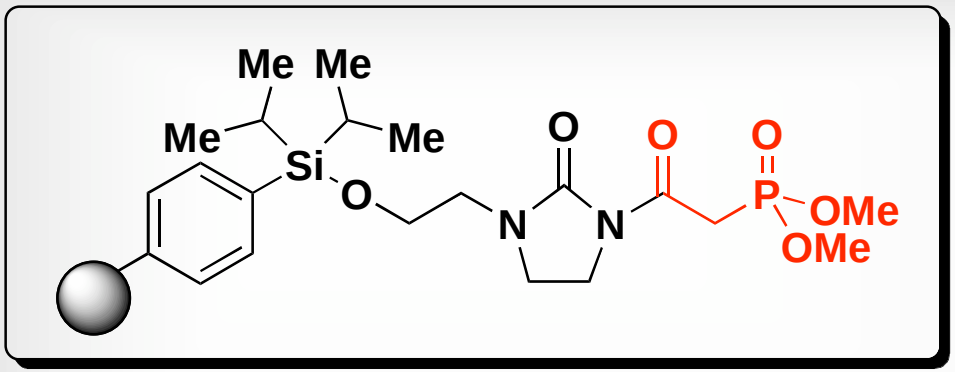
Acetyl coenzyme A
biosynthetic
'two-carbon' starting unit

>20 steps

fatty acids
polyketides
steroids
terpenes

e.g.

palmitic acid
rapamycin
cholesterol
camphor



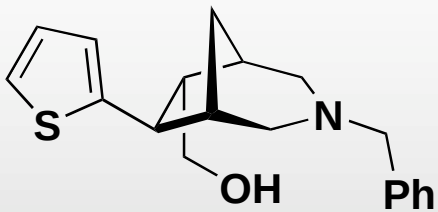
Solid phase phosphonate
diversity–oriented synthetic
'two-carbon' starting unit

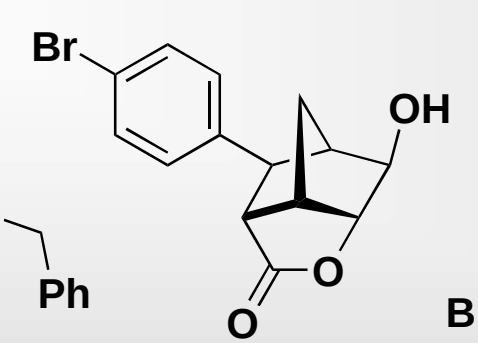
= Crosslinked polystyrene

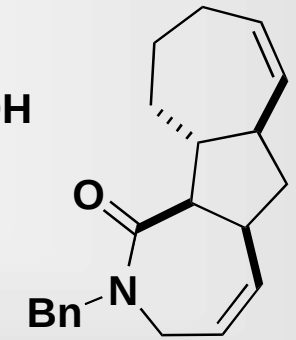
2–5 steps

alkaloid scaffolds
polycarbocyclic scaffolds
terpene scaffolds
chiral centres

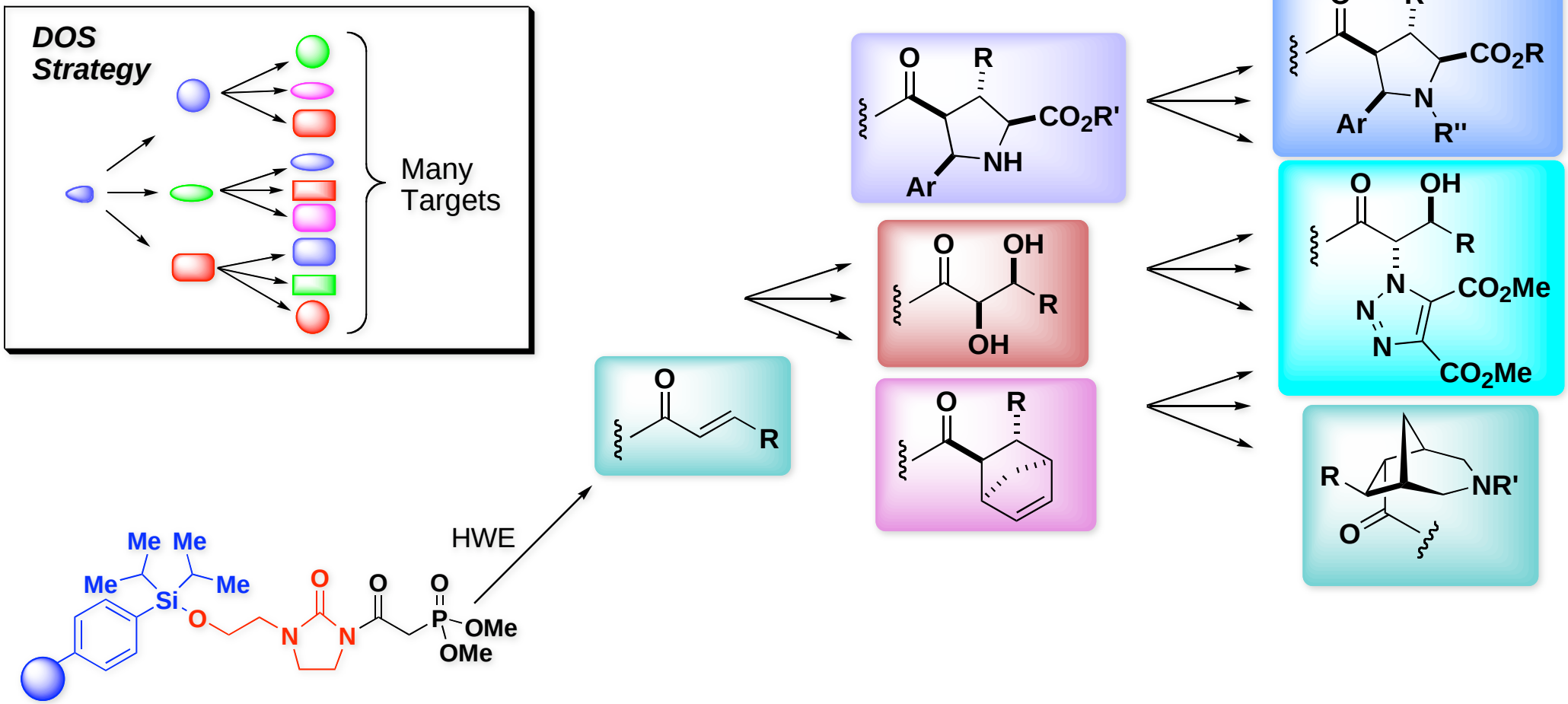
e.g.





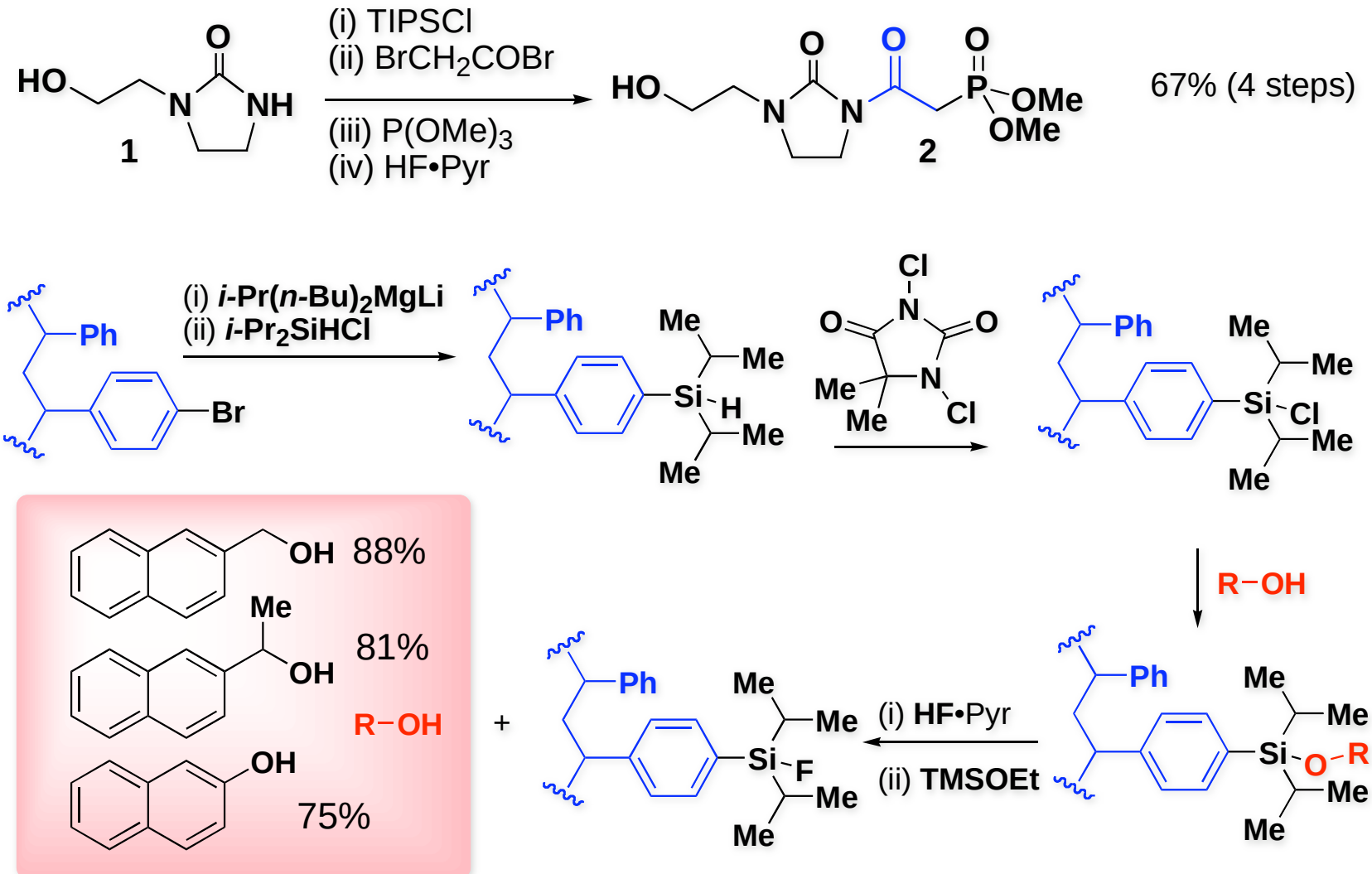


Diversity-Oriented Synthesis



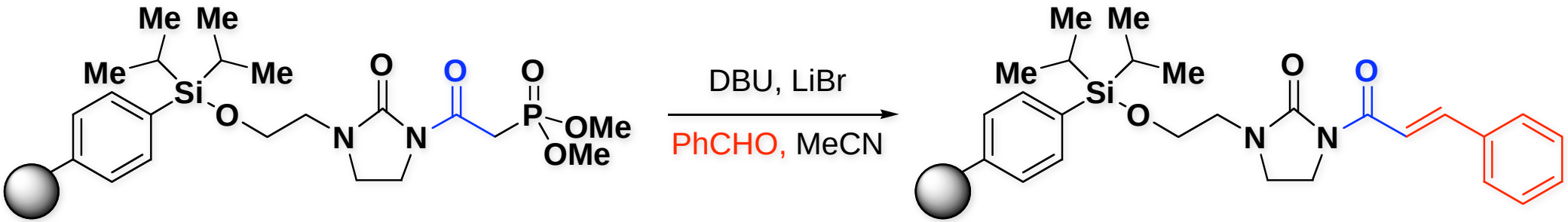
Gemma Thomas, Freija Glansdorp, Richard Spandl

Diversity-Oriented Synthesis

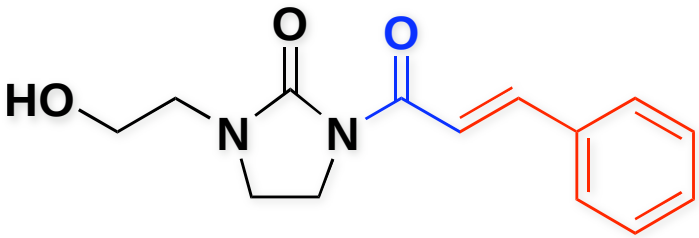


Org. Biomol. Chem. **2004**, *2*, 1679-1681

Diversity-Oriented Synthesis



(i) HF•Pyr, THF
(ii) TMSOEt

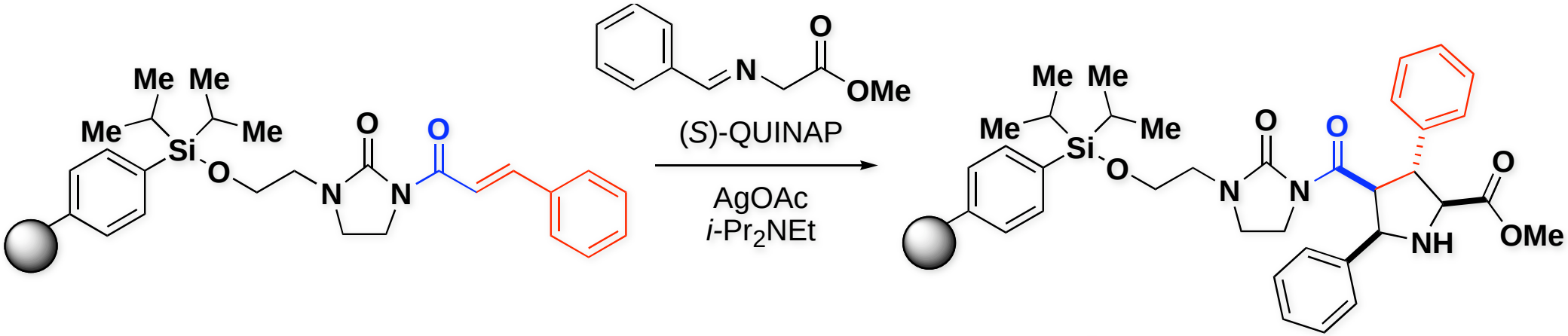


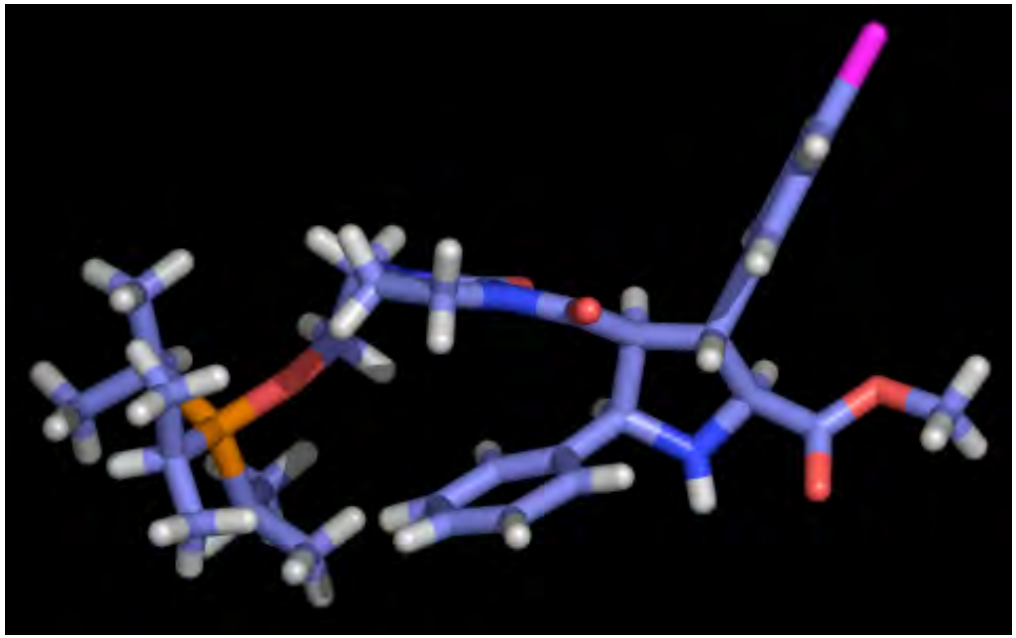
77%
E:Z **>50:1**

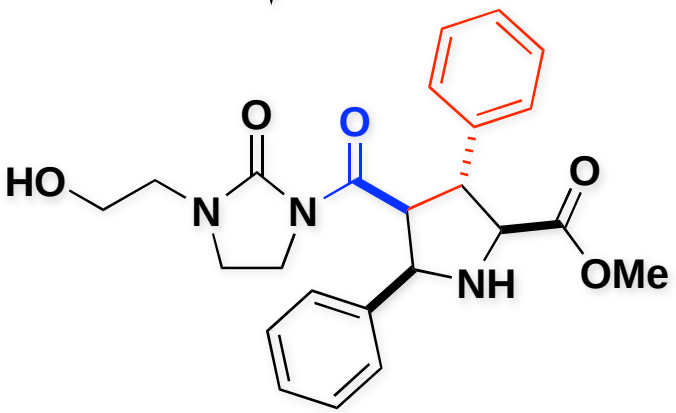
Gemma Thomas

Angew. Chem. Int. Ed. **2008**, 47, 2808-2812

Diversity-Oriented Synthesis





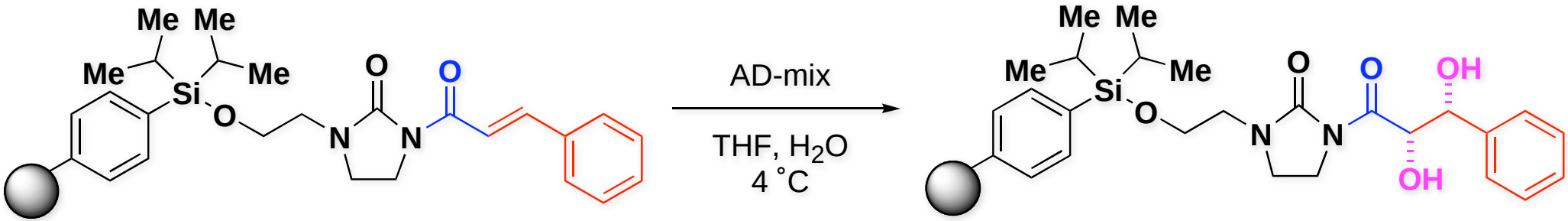


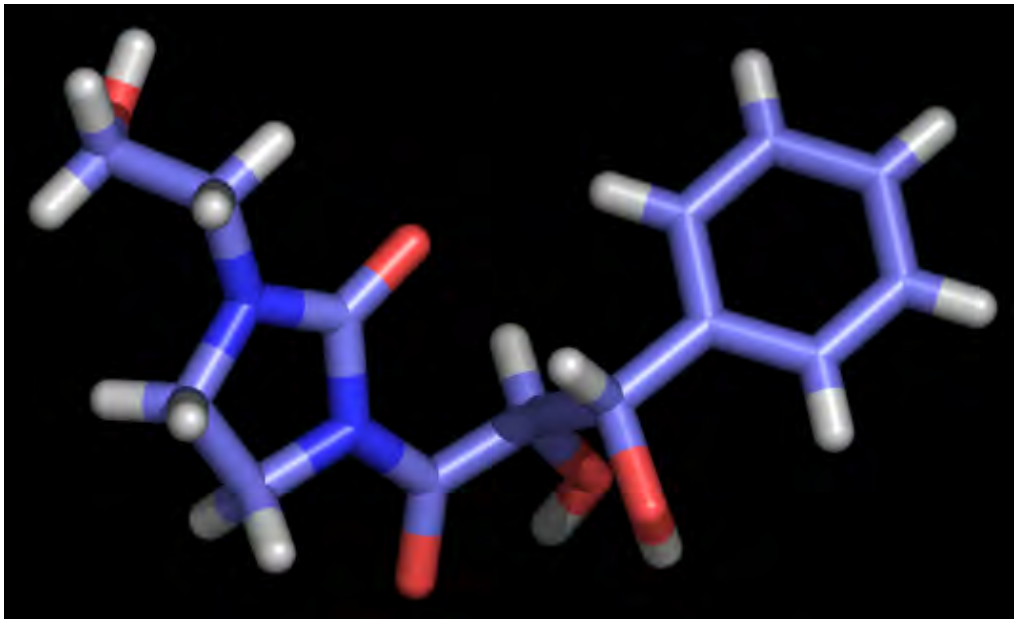
91%
endo:exo = 8:1
endo ee: 70%

Freija Glansdorp

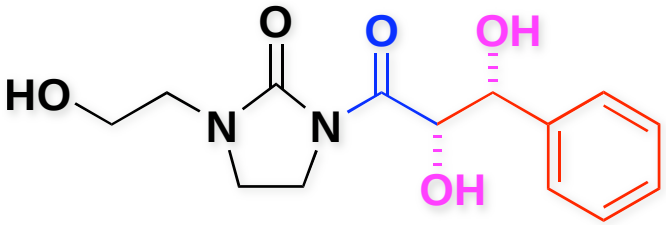
Angew. Chem. Int. Ed. **2008**, 47, 2808-2812

Diversity-Oriented Synthesis





(i) HF•Pyr, THF
(ii) TMSOEt

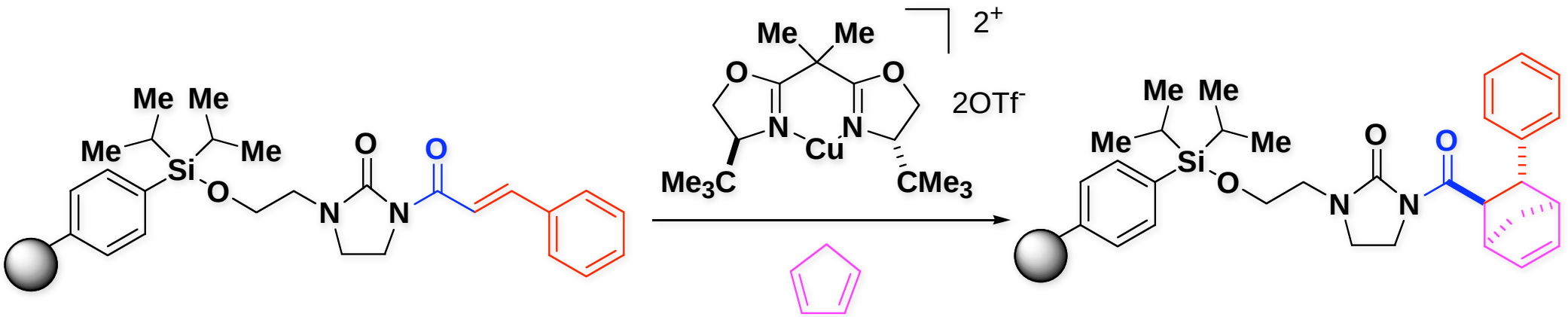


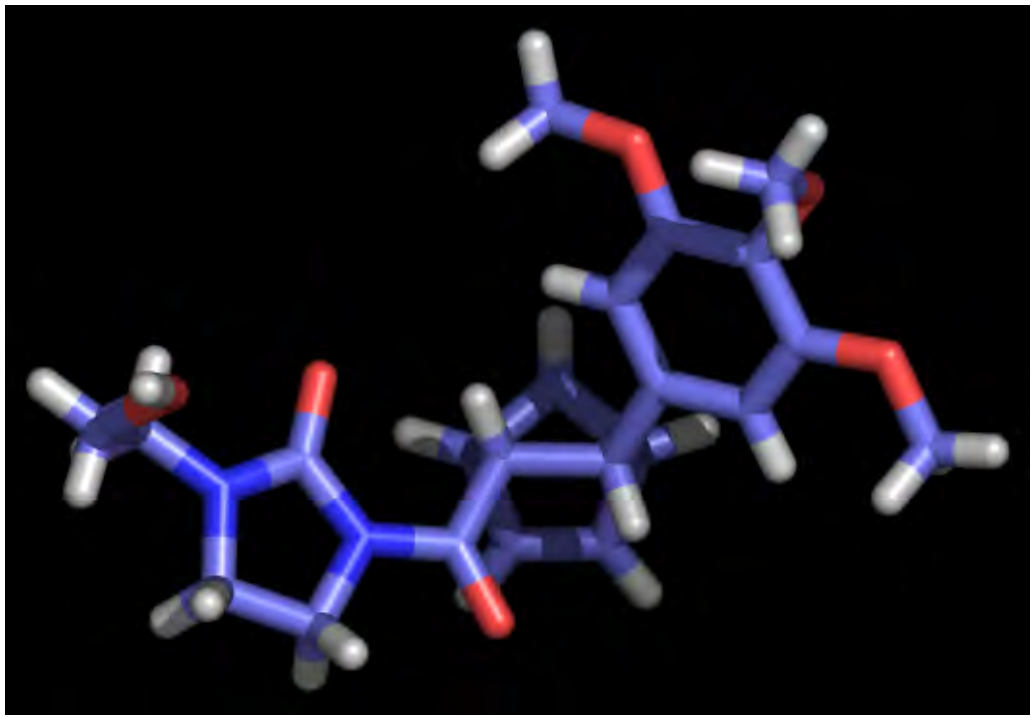
> 75%
ee α = 90%
ee β = 91%

Richard Spandl

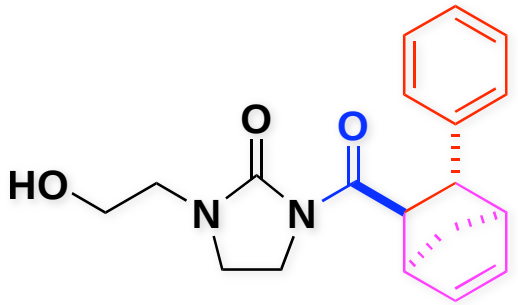
Angew. Chem. Int. Ed. **2008**, 47, 2808-2812

Diversity-Oriented Synthesis





(i) HF•Pyr, THF
(ii) TMSOEt

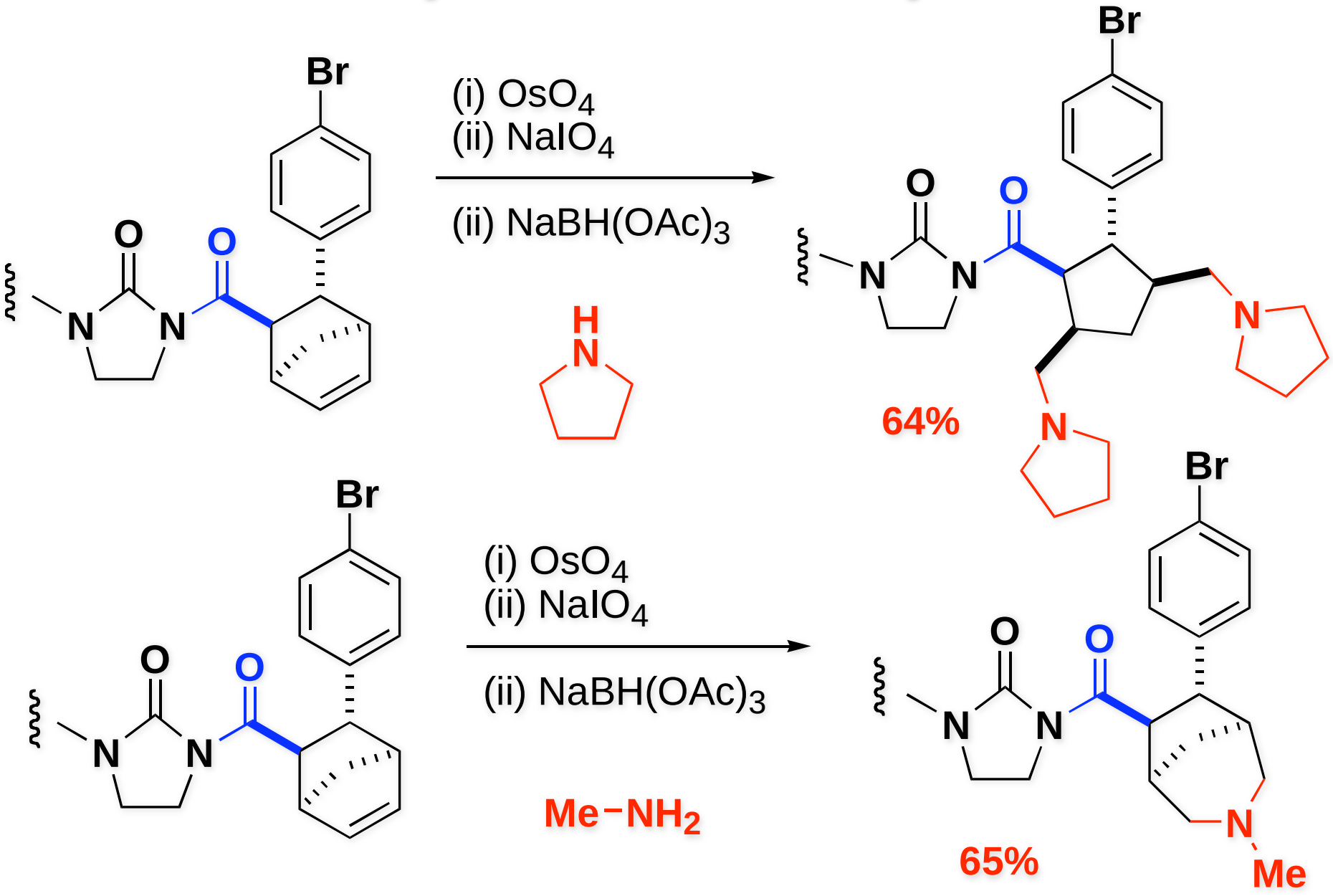


77%
endo:exo = 87:13
endo ee: 96%
exo ee: 90%

Gemma Thomas

Angew. Chem. Int. Ed. **2008**, 47, 2808-2812

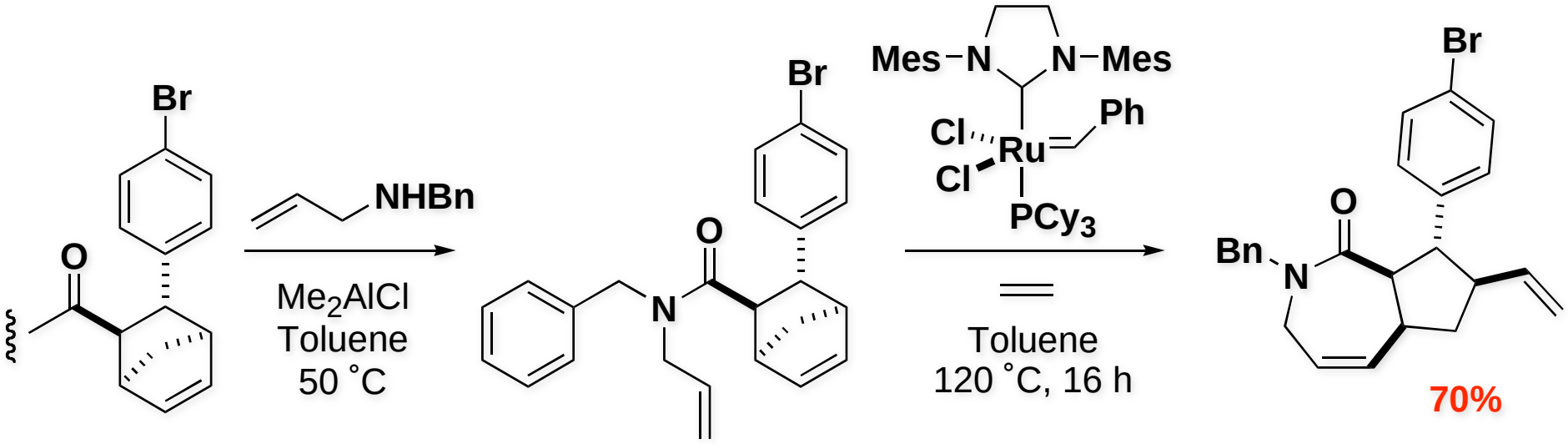
Diversity-Oriented Synthesis

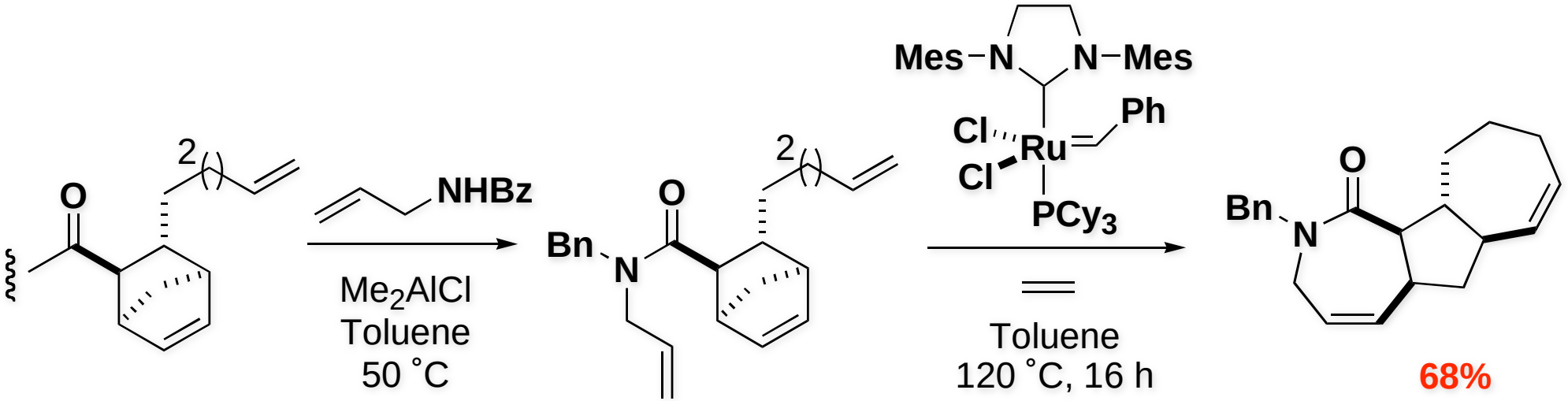


Gemma Thomas

Angew. Chem. Int. Ed. **2008**, 47, 2808-2812

Diversity-Oriented Synthesis



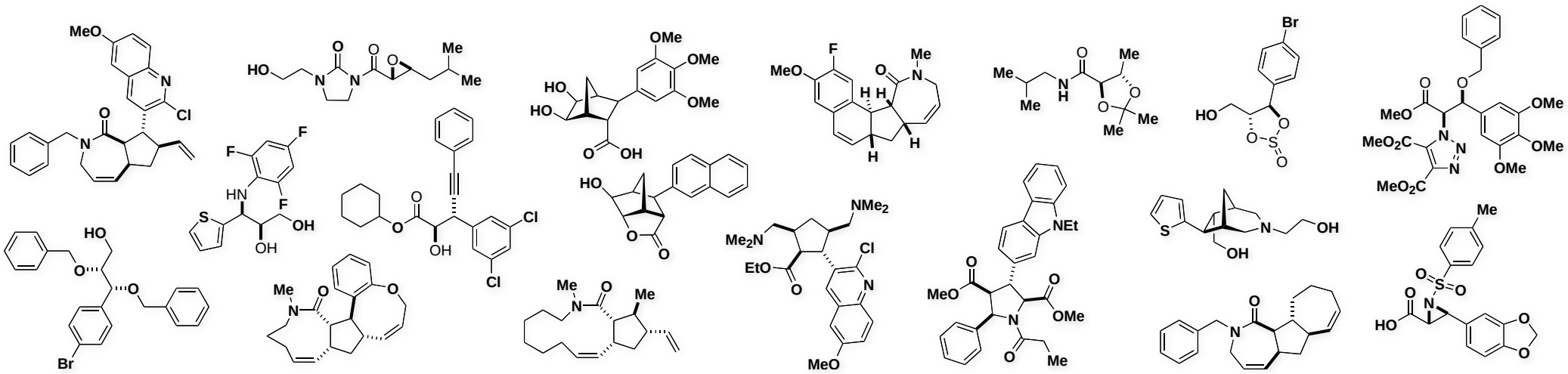


Angew. Chem. Int. Ed. **2008**, 47, 2808-2812

Diversity-Oriented Synthesis

Parallel library synthesis of 261 compounds

quantity range 1-20 mg
MW range 152-730
purity > 80%
molecular frameworks **25**



Angew. Chem. Int. Ed. **2008**, 47, 2808-2812

Diversity-Oriented Synthesis

Assess physicochemical and topological diversity.

Calculate 184 **molecular descriptors**, e.g.

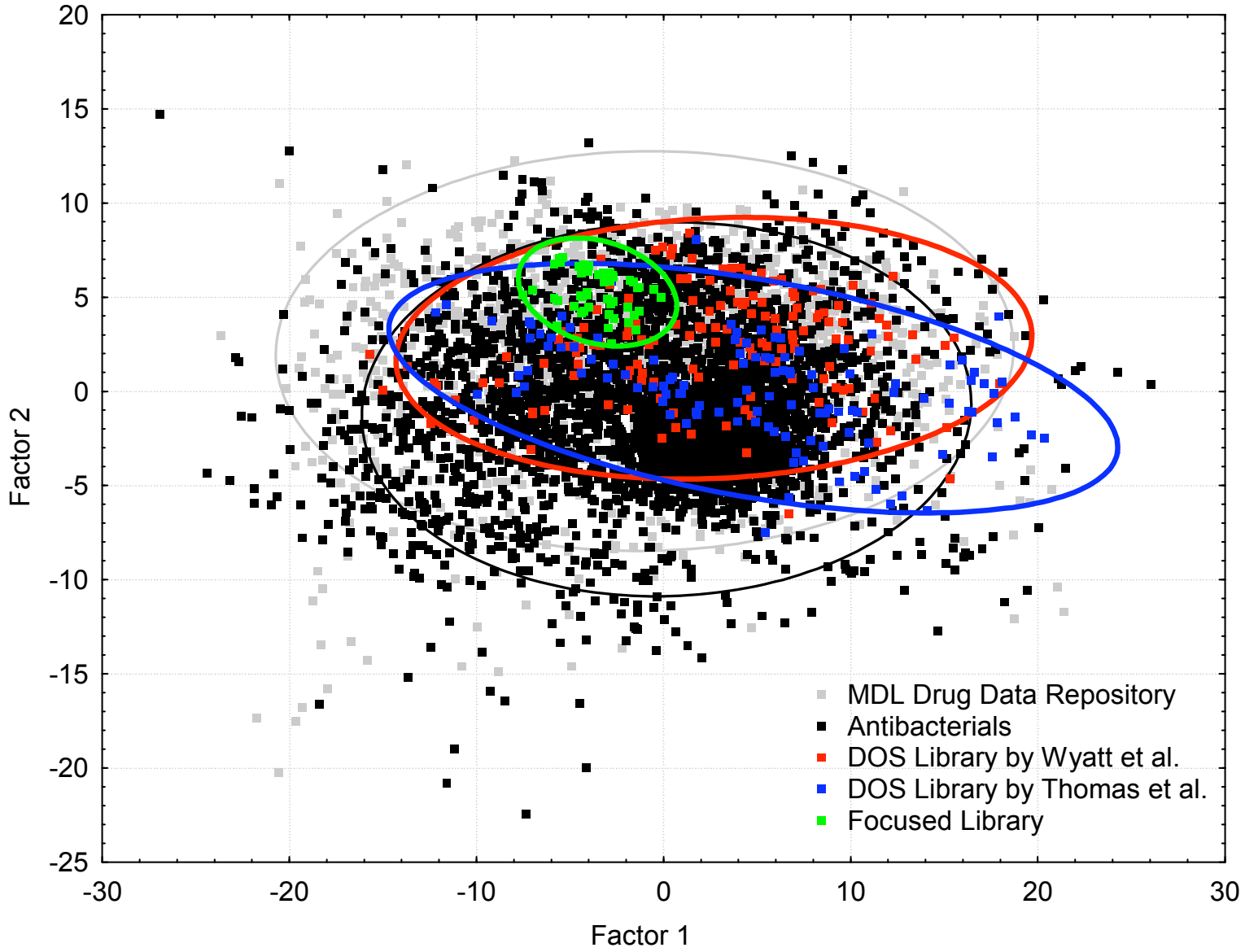
Size
Polarity
Charges
Degree of Branching…

Use **principal component analysis** to visualize.

Andreas Bender

Angew. Chem. Int. Ed. **2008**, 47, 2808-2812

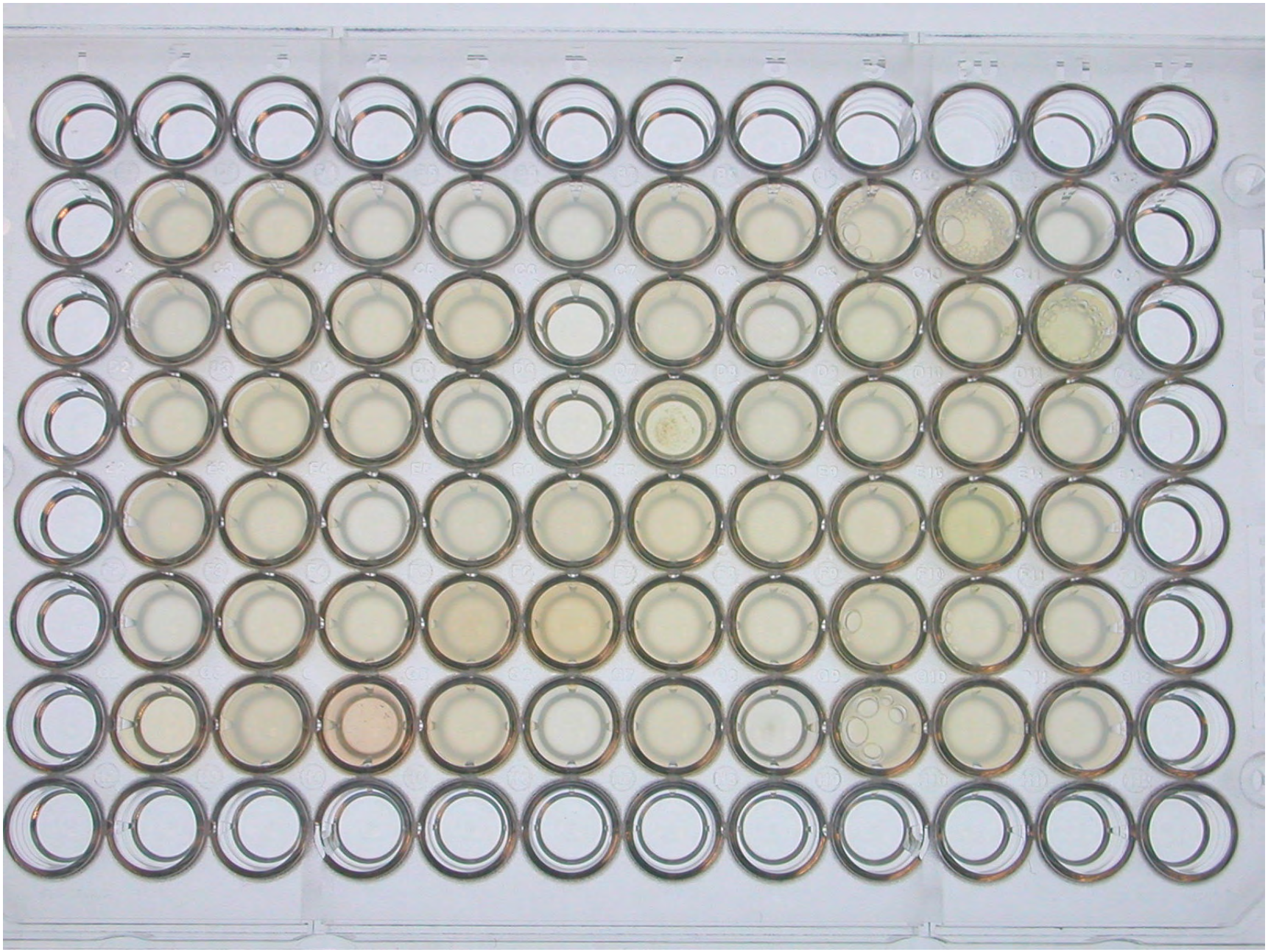
Diversity-Oriented Synthesis



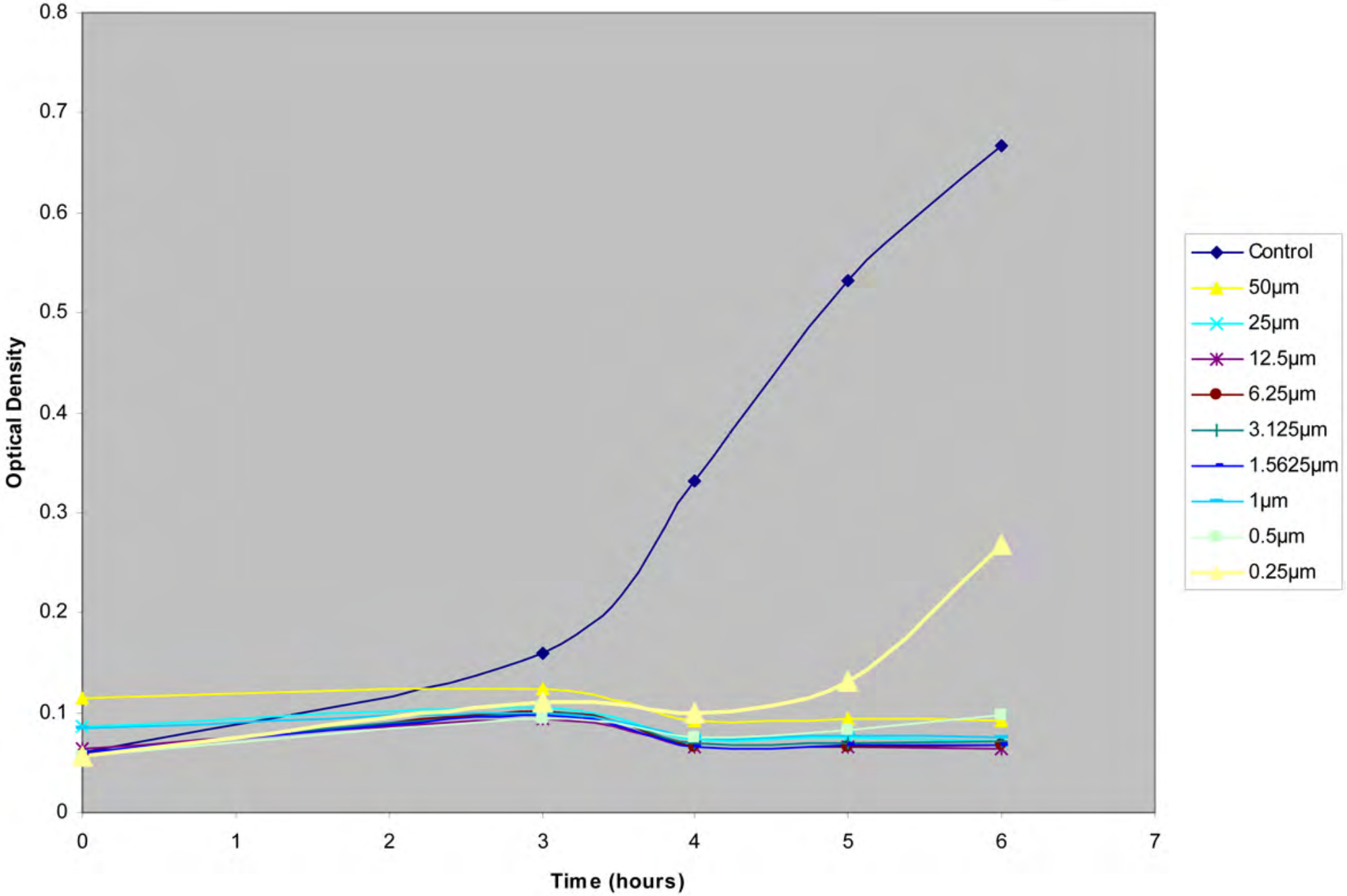
Andreas Bender

Angew. Chem. Int. Ed. **2008**, 47, 2808-2812

Antibacterial Screening



Antibacterial Screening

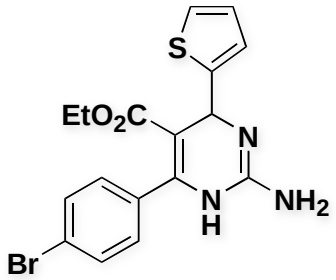


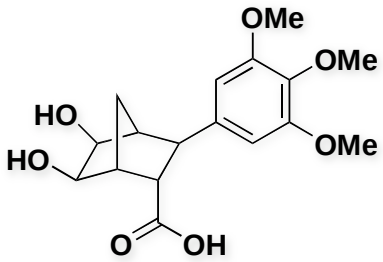
Antibacterial Screening

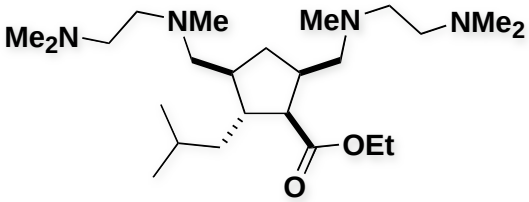
Gemma Thomas, Emma Wyatt, Suzanne Fergus

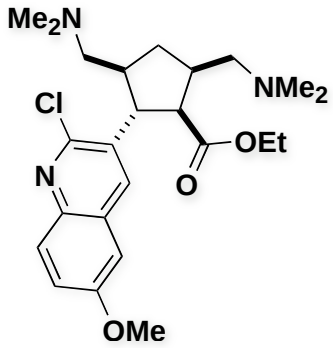
Antibacterial Screening

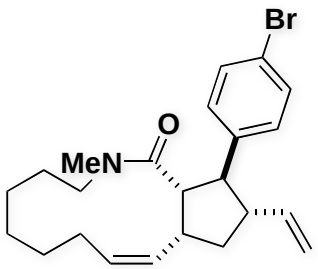
20 hits at 100 μM concentration, e.g.

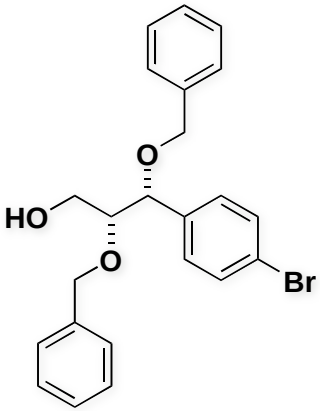


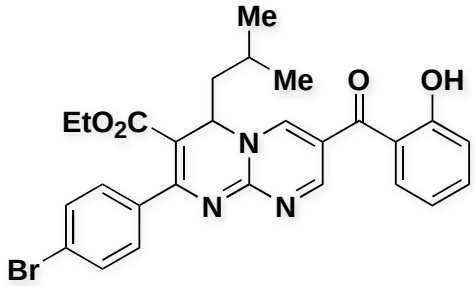










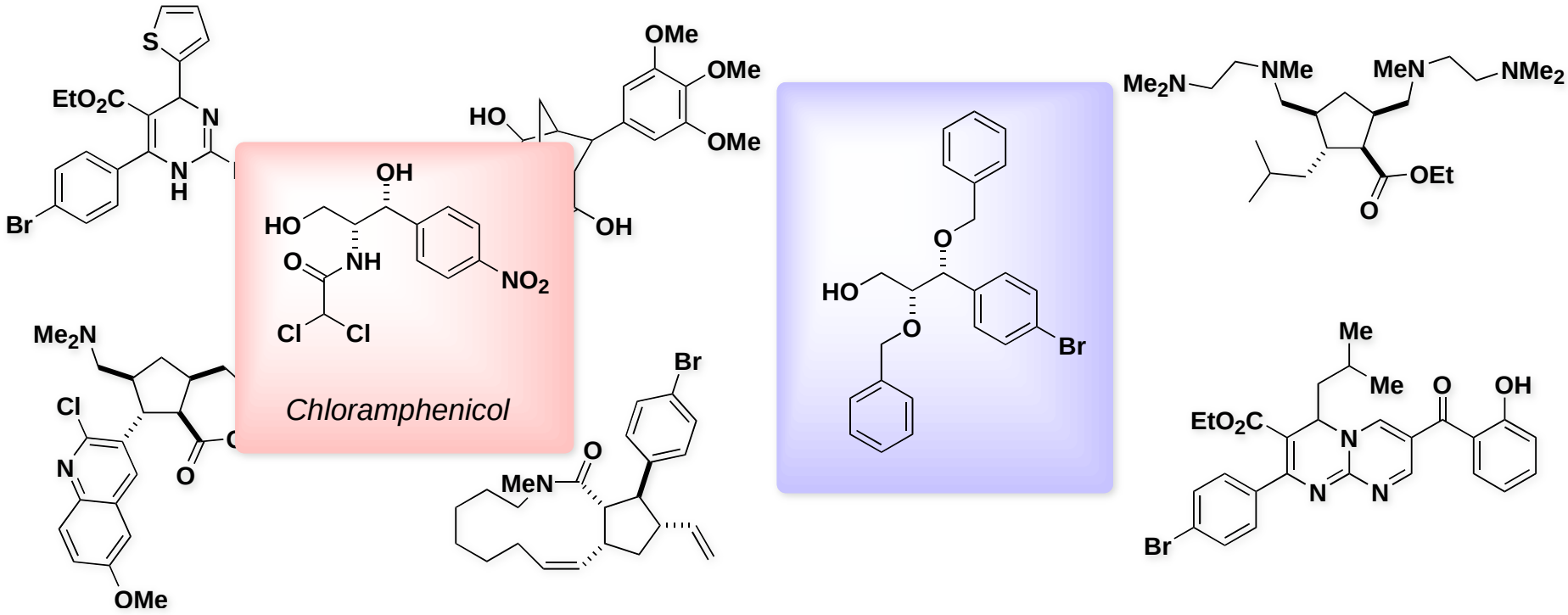


Gemma Thomas, Emma Wyatt, Suzanne Fergus

Unpublished Results

Antibacterial Screening

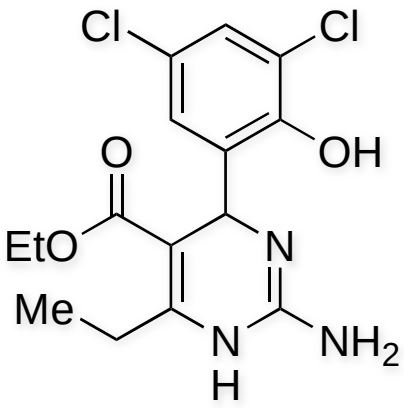
20 hits at 100 μM concentration, e.g.



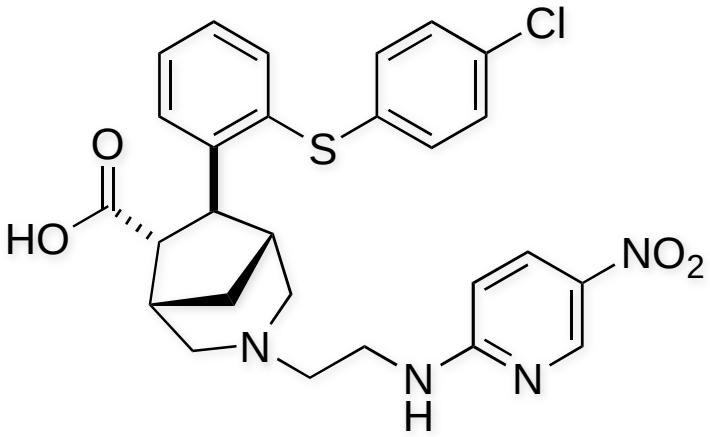
Gemma Thomas, Emma Wyatt, Suzanne Fergus

Unpublished Results

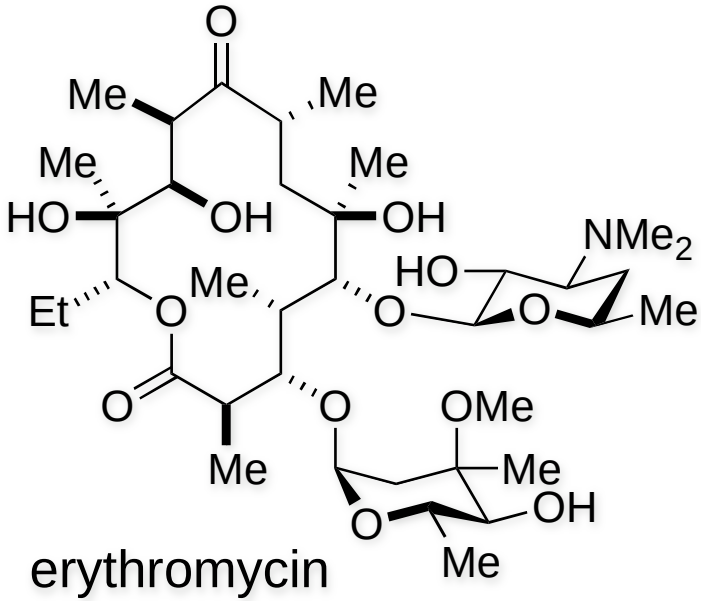
Antibacterial Screening



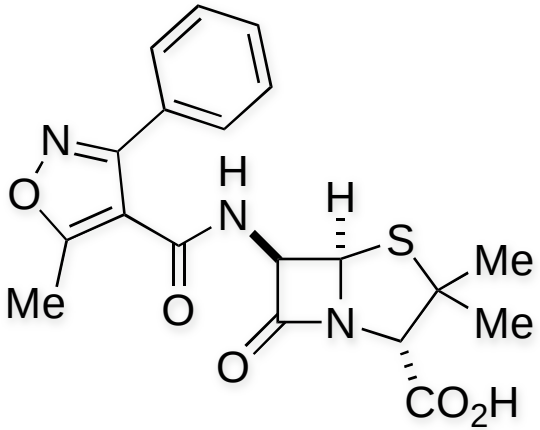
emmacin



(–)-gemmacin



erythromycin



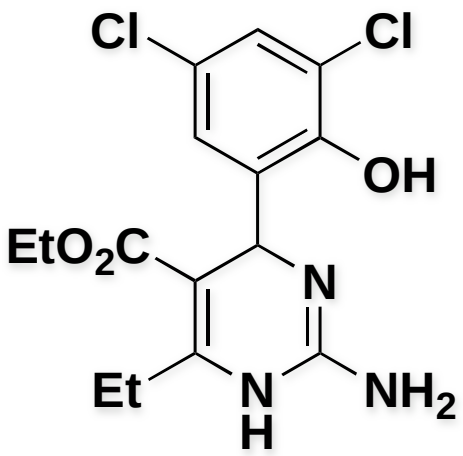
oxacillin

	MIC_{50} (μg ml^{-1})		
	MSSA	EMRSA 15	EMRSA 16
emmacin	2	9	9
(–)-gemmacin	2	8	16
erythromycin	0.5	>64	>64
oxacillin	0.5	>32	>32

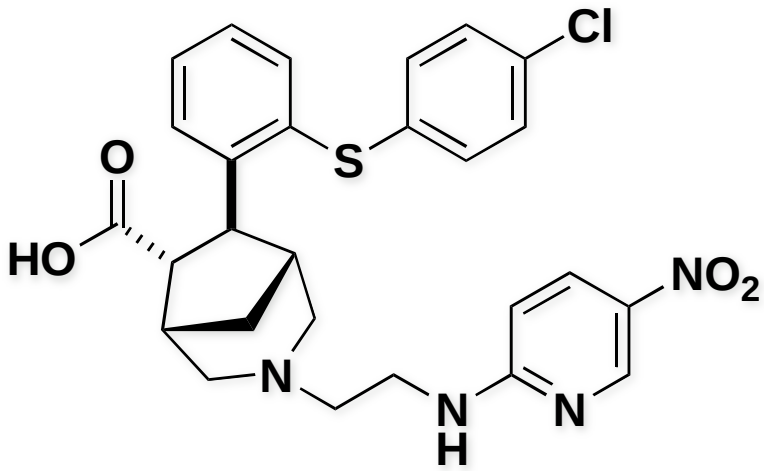
Chem. Commun. **2008**, 4962-4964; *Angew. Chem. Int. Ed.* **2008**, 47, 2808-2812

Antibacterial Screening

*Emmacin & gemmacin only affect cell growth of **human** epitheleal cells at concentrations above 250 μM.*



emmacin

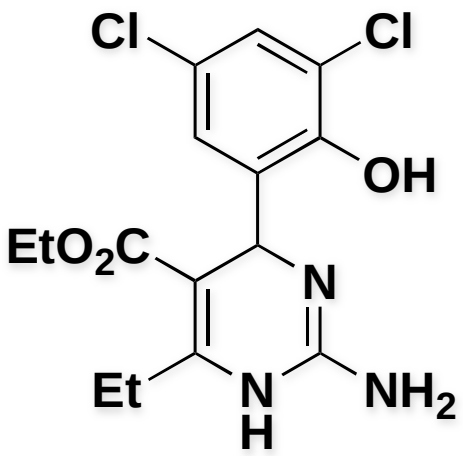


gemmacin

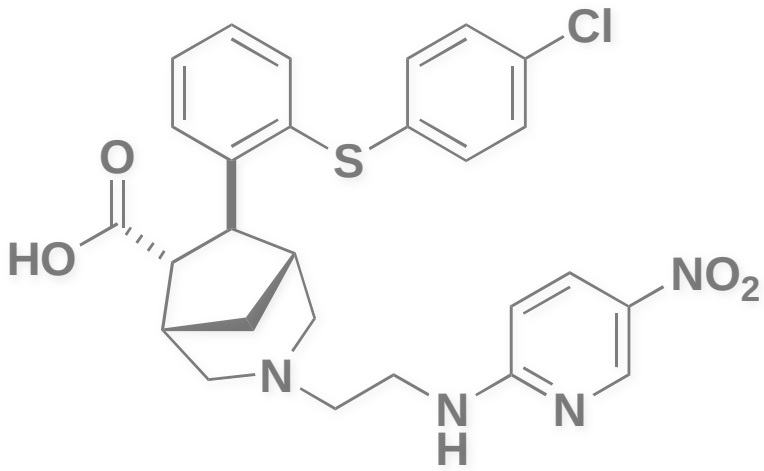
Gemma Thomas, Clare Bryant

Antibacterial Screening

*Emmacin & gemmacin only affect cell growth of **human** epitheleal cells at concentrations above 250 μM.*



emmacin



gemmacin

But what are the mode of actions?

Gemma Thomas, Clare Bryant

Target Identification

Mode of action assays (>20):

ATP synthesis uncoupling

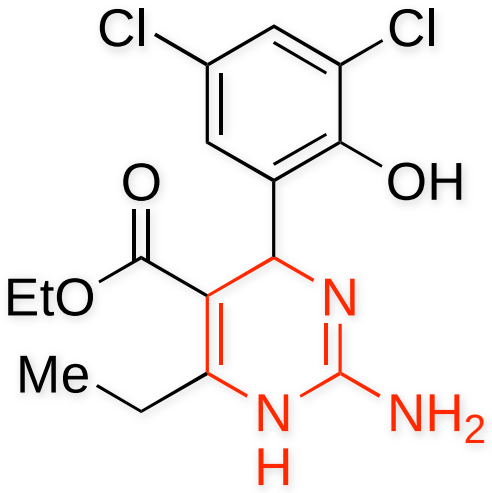
protein synthesis inhibition

generation of reactive oxygen species

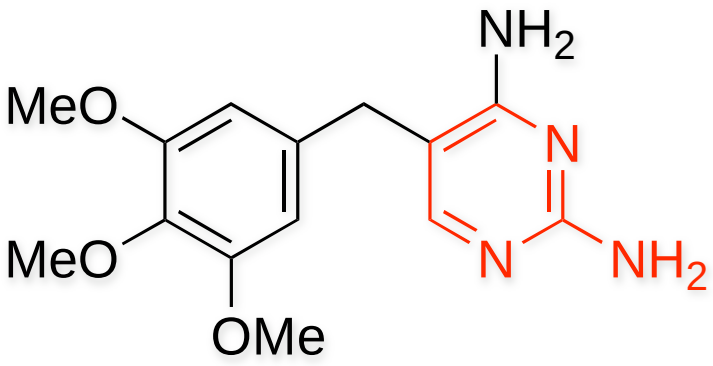
***Emmacin**: non-toxic, selective for bacteria…?*

Chem. Commun. **2008**, 4962-4964

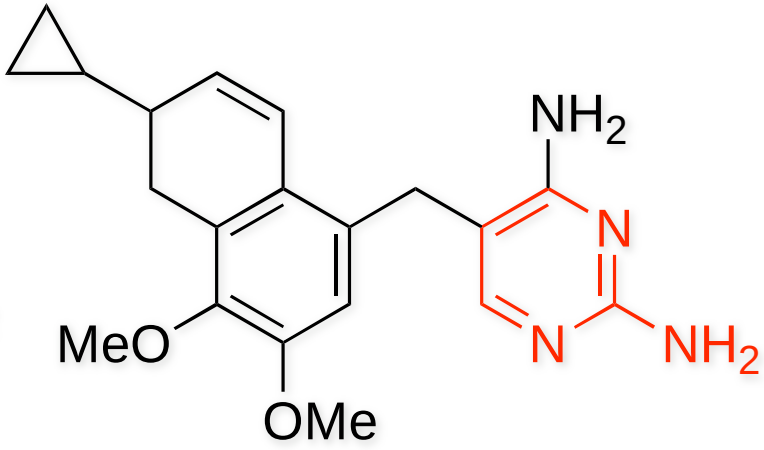
Target Identification



emmacin



trimethoprim

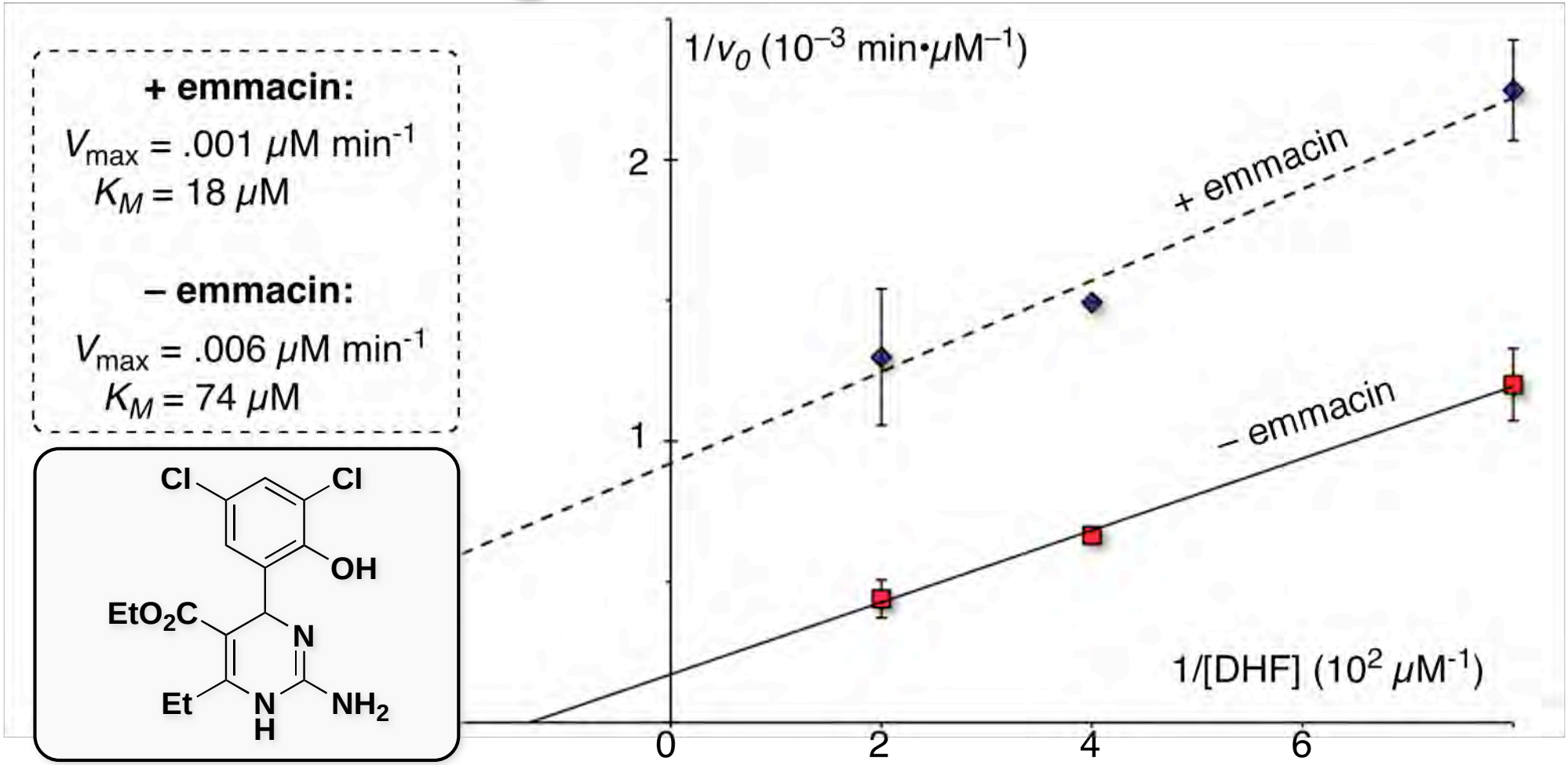


iclaprim

Structural similarities to known bacterial DHFR inhibitors

Chem. Commun. **2008**, 4962-4964

Target Identification



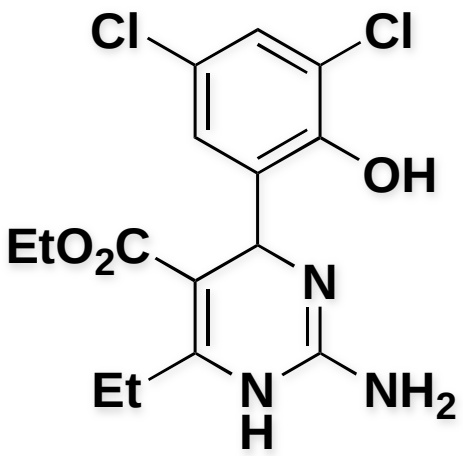
Emmacin *represents a new structural sub-class of bacteria-selective DHFR inhibitors*

Gemma Thomas, Martin Welch

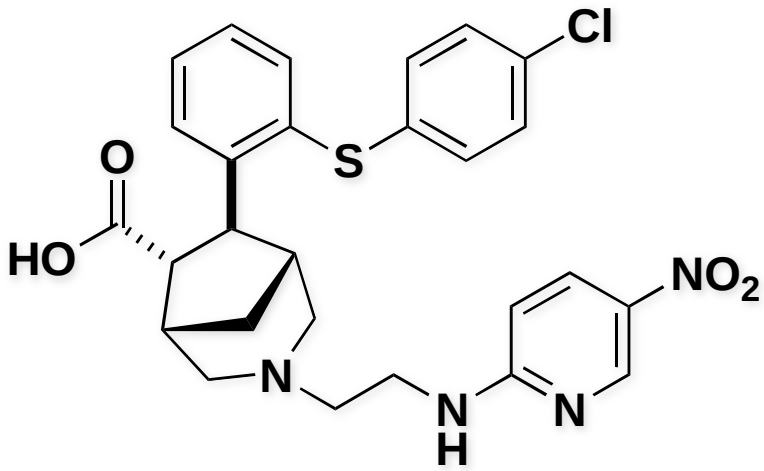
Chem. Commun. **2008**, 4962-4964

Antibacterial Screening

*Emmacin & gemmacin only affect cell growth of **human** epitheleal cells at concentrations above 250 μM.*



emmacin



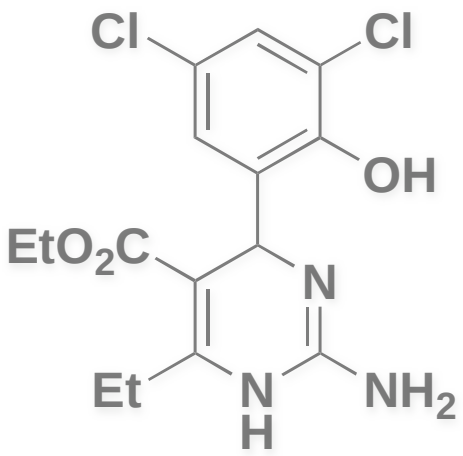
gemmacin

But what are the mode of actions?

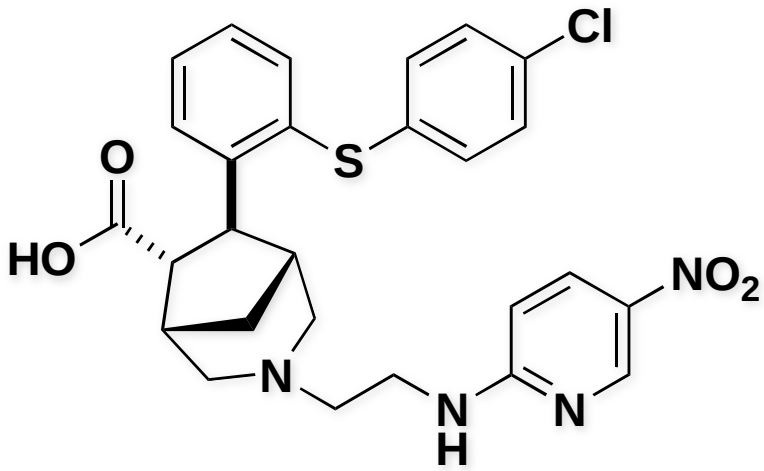
Gemma Thomas, Clare Bryant

Antibacterial Screening

*Emmacin & gemmacin only affect cell growth of **human** epitheleal cells at concentrations above 250 μM.*



emmacin



gemmacin

But what are the mode of actions?

Gemma Thomas, Clare Bryant

Target Identification

Mode of action assays (>20):

ATP synthesis uncoupling

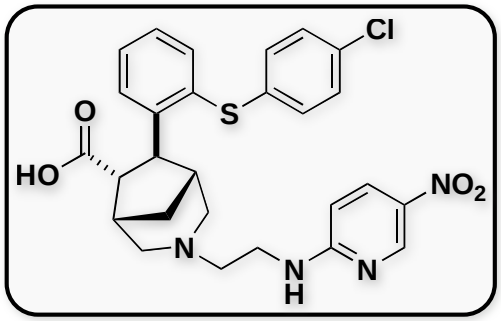
protein synthesis inhibition

generation of reactive oxygen species

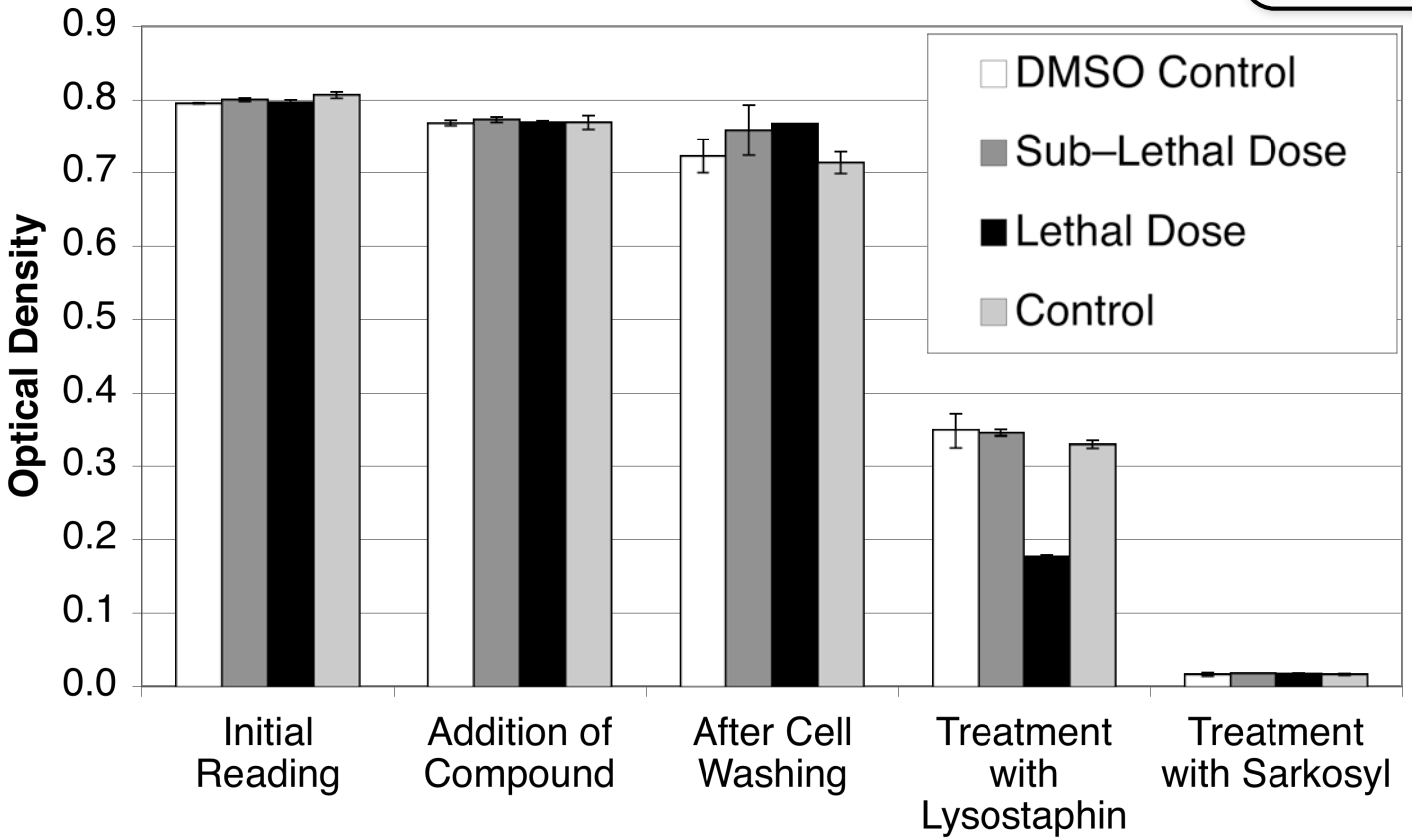
***Gemmacin**: positive for ROS generation*

Angew. Chem. Int. Ed. **2008**, 47, 2808-2812

Target Identification



Membrane disrupter of MRSA.



Lysostaphin cleaves pentaglycine bridges in the cell walls of staphylococci.

Gemma Thomas, Jodi Lindsay

Angew. Chem. Int. Ed. **2008**, 47, 2808-2812

Summary

Diversity-oriented synthesis
(~500 compounds; ~50 scaffolds)

Antibacterial screening
(EMRSA-15 & EMRSA-16)

Discovery of two new antibacterial molecules
(emmacin & (–)-gemmacin)

Mode of action & target identification
(bacteria selective mechanisms)

Chem. Commun. **2006**, 3296-3298; *Chem. Commun.* **2008**, 4962-4964; *Angew. Chem. Int. Ed.* **2008**, 47, 2808-2812

Acknowledgements

Andreas Bender, Martin Welch, Clare Bryant, Jodi Lindsay, Derek Brown, Olivier Loiseleur, Mark Ladlow

EPSRC, BBSRC, MRC, Frances and Augustus Newman Foundation Wellcome Trust, GSK, AstraZeneca, Pfizer, Syngenta, Lilly, UCB

Acknowledgements

Andreas Bender, Martin Welch, Clare Bryant, Jodi Lindsay, Derek Brown, Olivier Loiseleur, Mark Ladlow

EPSRC, BBSRC, MRC, Frances and Augustus Newman Foundation
Wellcome Trust, GSK, AstraZeneca, Pfizer, Syngenta, Lilly, UCB