Innovative Biotechnology Companies

and their Academic Origins

Jack Terrett

MacMillan Group Meeting

November 20th, 2013

The Future of Therapeutics

- Highlighting four diverse biotechnology companies

- All four featured companies are based on discoveries and innovations in academic labs

Tetralogic Pharmaceuticals - Yigong Shi

Peptidream - Hiroaki Suga

Scifluor Life Sciences - Tobias Ritter

Tetraphase Pharmaceuticals - Andy Myers

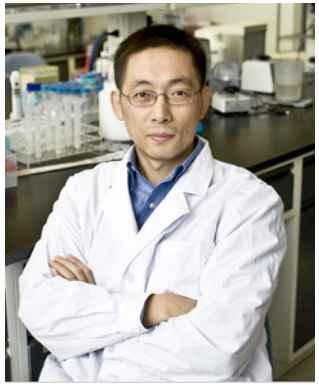
- Each company is focusing on therapeutic development in totally distinct ways

  - Three small molecule approaches , one peptide-based therapeutic approach

How should drug discovery be accomplished in the 21st century?

How do individual companies stand out and become successful?

TetraLogic Pharmaceuticals

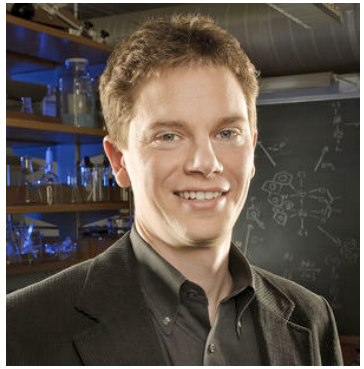


PeptiDream

PEPTIDREAM INC.

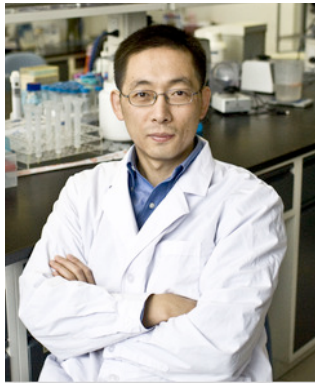
INNOVATIVE PEPTIDE THERAPEUTICS

SciFluor Life Sciences



TETRAPHASE PHARMACEUTICALS

TetraLogic Pharmaceuticals



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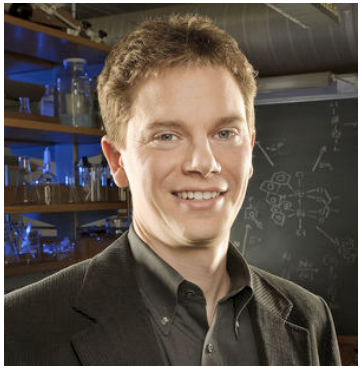


TETRAPHASE PHARMACEUTICALS

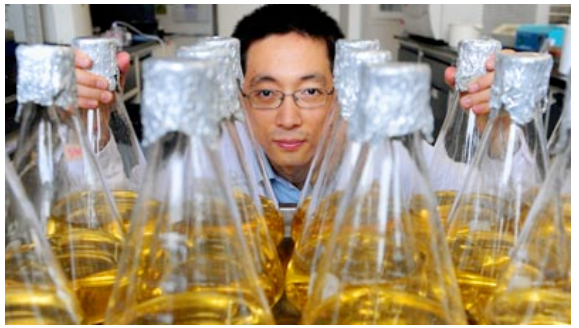
Tetralogic Pharmaceuticals

- Pennsylvania-based pharmaceutical company

- Founded in 2003 by Yigong Shi

- Small molecule Smac mimetics for targeting apoptosis of cancer cells

- About Yigong Shi



- 1989-1994: Ph.D. with Jeremy Berg (Johns Hopkins)

- 1994-1997: Postdoctoral work with Nikola Pavletich (Sloan-Kettering)

- 1998-2001: Assistant Professor, Princeton University (Department of Molecular Biology)

- 2001-2003: Associate Professor, Princeton University

- 2003-2008: Professor, Princeton University

- 2008-present: Professor, Tsinghua University

Featured in NYT article: "Fighting Trend, China is Luring Scientists Home" (Jan. 7, 2010)

Cell Apoptosis

- Insufficient programmed cell death has implications in many diseases, notably cancer
- As such, targeting apoptosis pathways is therapeutically attractive
- Many biological factors are involved in cell death

  - Importantly, caspases are produced in cells as active proteases in cell degradation
  - IAPs (inhibitors of apoptosis proteins) bind caspases, preventing cell death
  - The BIR domain (baculoviral IAP repeat) directly inhibits caspase enzymatic activity
  - Smac (second mitochondria-derived activator of caspases) inhibits BIR, allowing release of caspases

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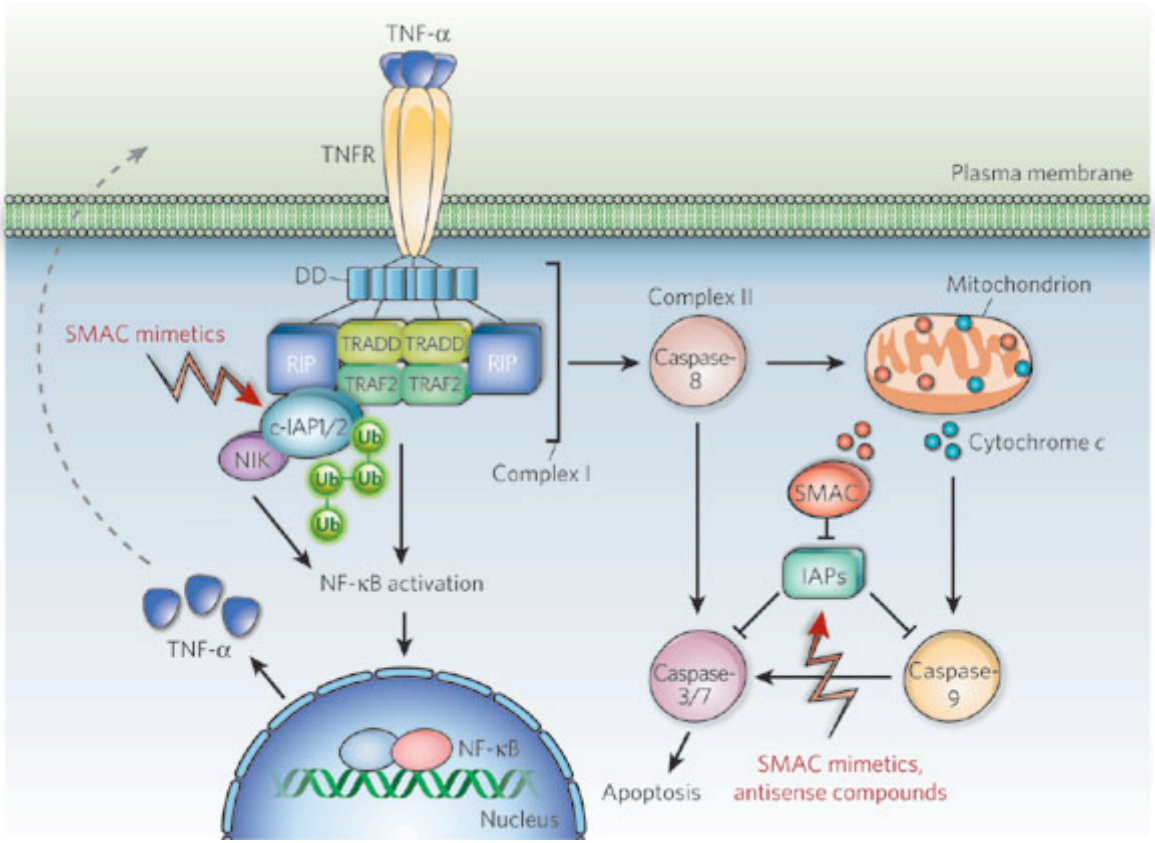
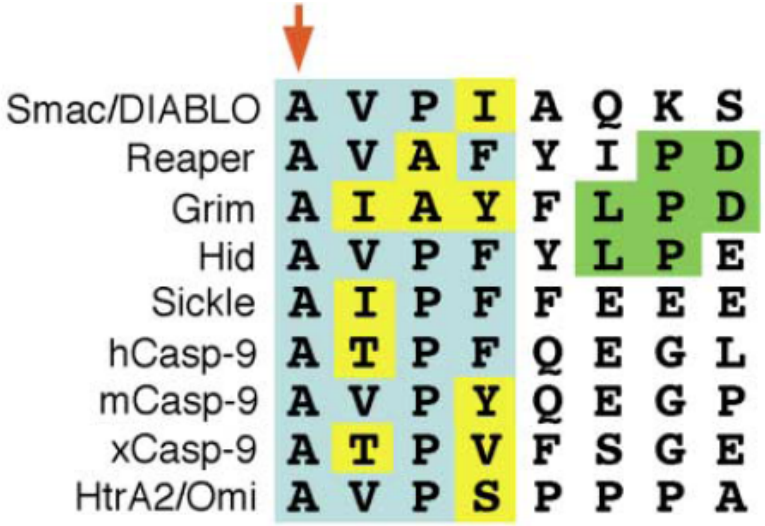


Hoeller, D.; Dikic, I. *Nature* **2009**, *458*, 438.

Cell Apoptosis

- Smac inhibits xIAP and degrades cIAP, releasing caspases (9,3,7)
- In tumour cells, IAPs are over-expressed and Smac levels are low
- Shi's seminal studies into structure of Smac and BIR binding pocket revealed common motif

  - The *N*-terminus of Smac/DIABLO homologues are highly conserved (mammals and *Drosophila*)
  - BIR domain binds substrate predominantly of *N*-terminal four peptide sequence

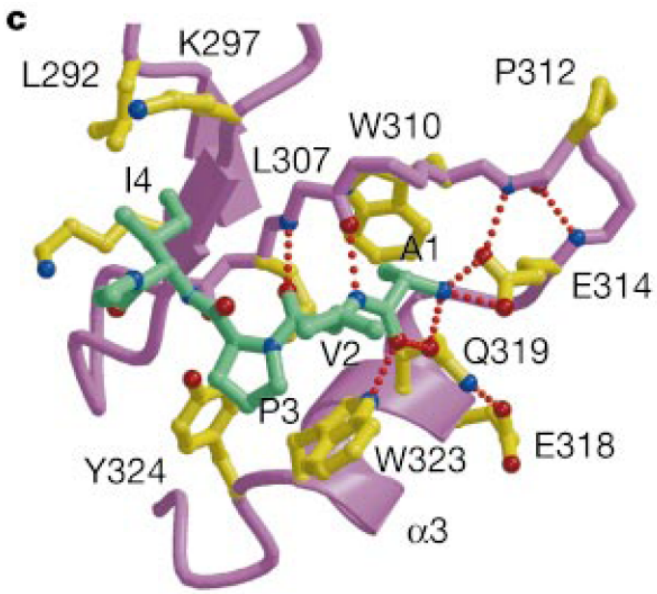


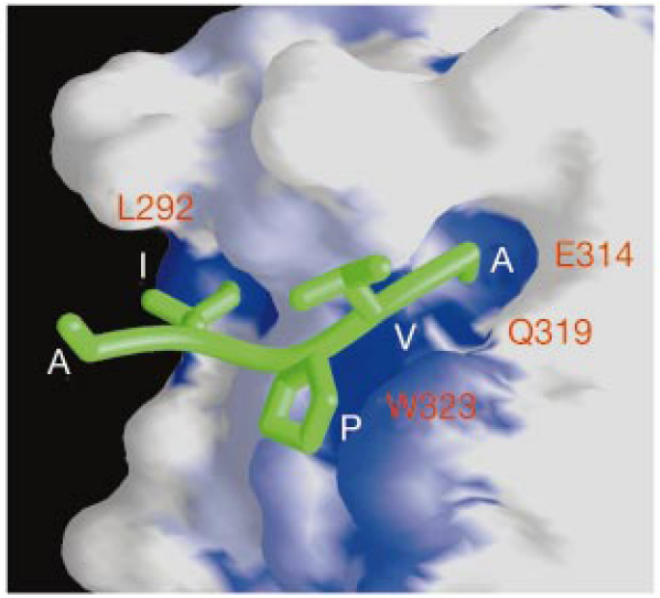
Shi, Y. *Cell Death Differ.* **2002**, *9*, 93.

Shi, Y. *Mol. Cell* **2002**, *9*, 459.

Smac Activation of Caspases

- Tetrapeptide motif on Smac binds BIR domain (Ala-Val-Pro-Ile)
- Alanine residue sits in hydrophobic pocket, H-bonds to neighbouring xIAP residues
- Single point mutation of Smac AVPI motif results in loss of binding affinity



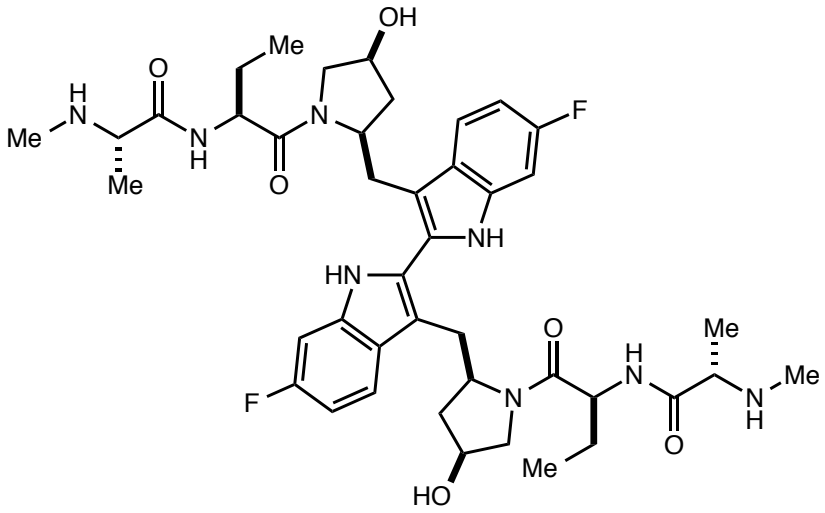


Chai, J.; Du, C.; Wu, J. -W.; Kyin, S.; Wang, X.; Shi, Y. *Nature*, **2000**, *406*, 855.

Wu, G.; Chai, J.; Suber, T. L.; Wu, J. -W.; Du, C.; Wang, X.; Shi, Y. *Nature* **2000**, *408*, 1008.

Tetralogic Develops Smac Mimetic

- Recognition of tetrapeptide binding unit of Smac lends potential to therapeutics
- Peptidomimetic drugs containing AVPI-type motif should funtion as effective IAP binder
- Tetralogic developed lead candidate drug, **birinapant**

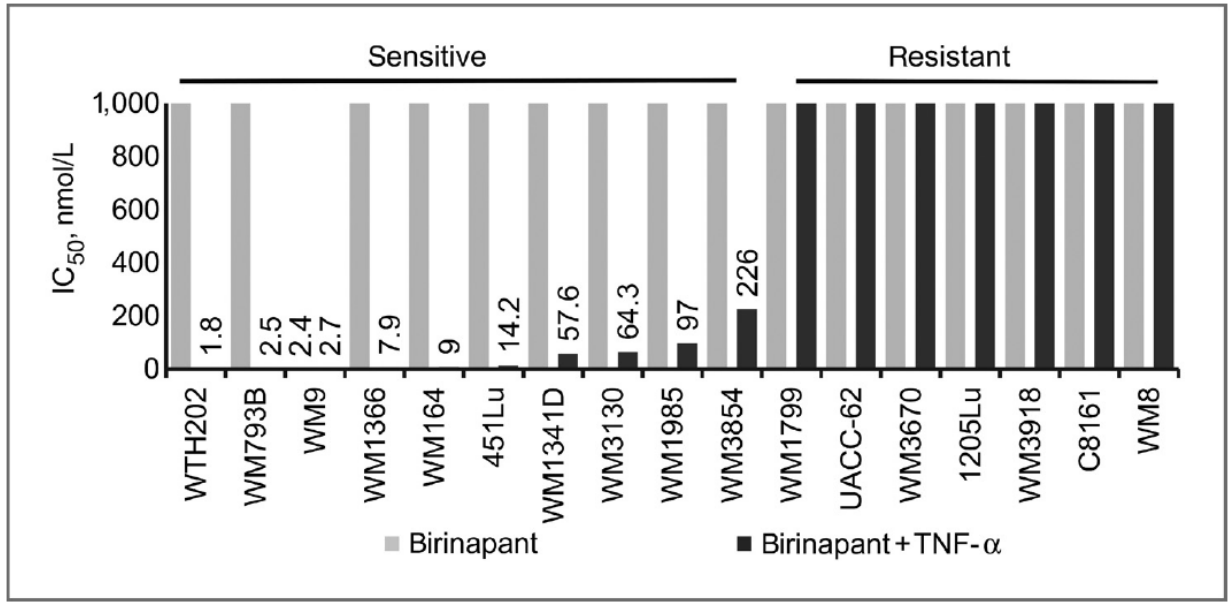


birinapant

Krepler et al. *Clin. Cancer Res.* **2013**, *19(7)*, 1784.

Tetralogic Develops Smac Mimetic

- Birinapant was studied as single agent and combination therapy with TNF-α
- Birinapant degrades $cIAP_1$ and $cIAP_2$ allowing TNF-α to signal apoptosis (via caspase-8)
- Cotreatment is highly effective against a range of melanoma cell lines

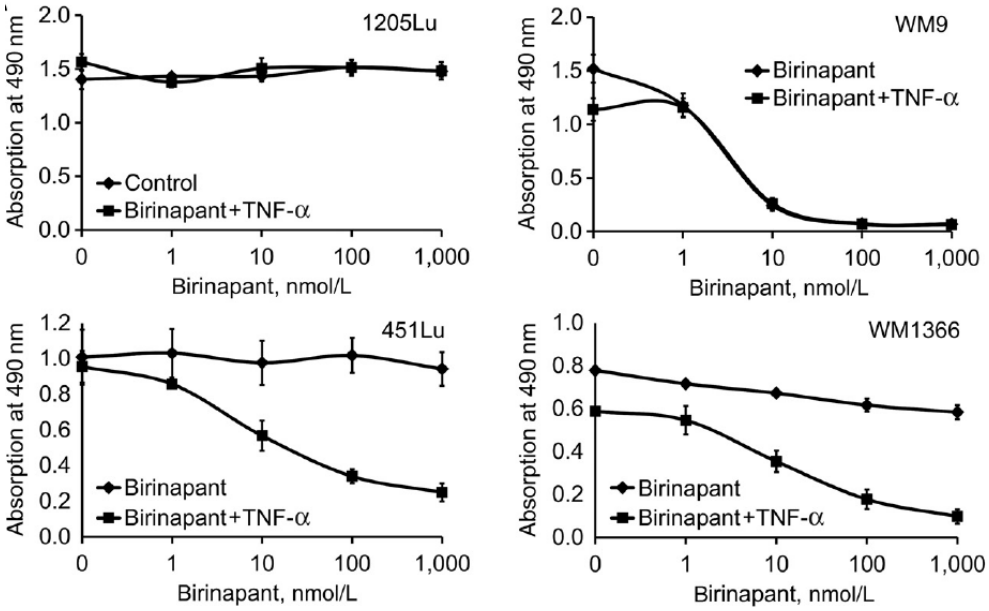


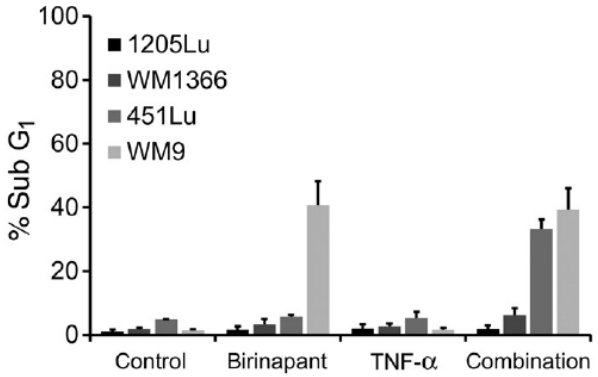
Birinapant (1, 10, 100, 1000 nmol/L), TNF-α (1 ng/mL)

Krepler et al. *Clin. Cancer Res.* **2013**, *19(7)*, 1784.

Tetralogic Develops Smac Mimetic

- Four cancer cell lines were further analyzed: WM9, WM1366, 451Lu, 1205Lu
- Absorption at 490nm directly proportional to number of living cells (MTS assay)
- Increase in sub-G_1 fractions is indicative of apoptosis





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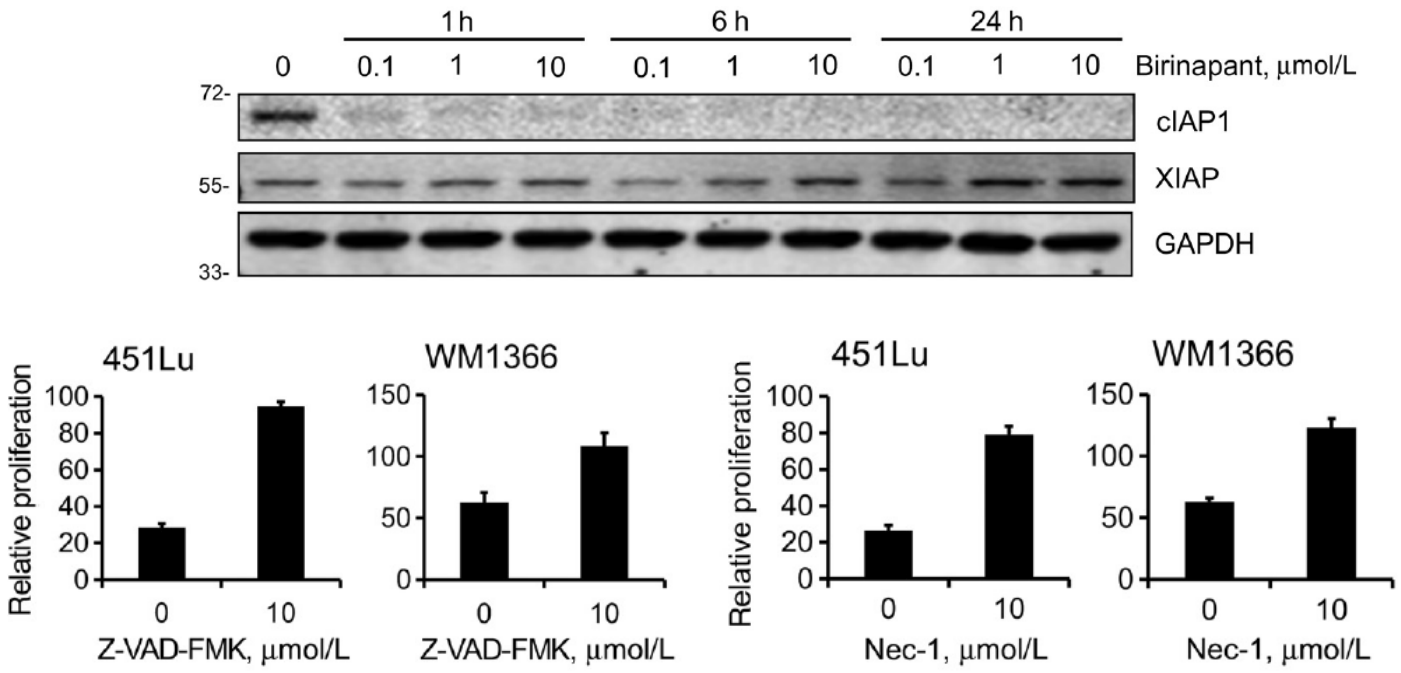
Tetralogic Develops Smac Mimetic

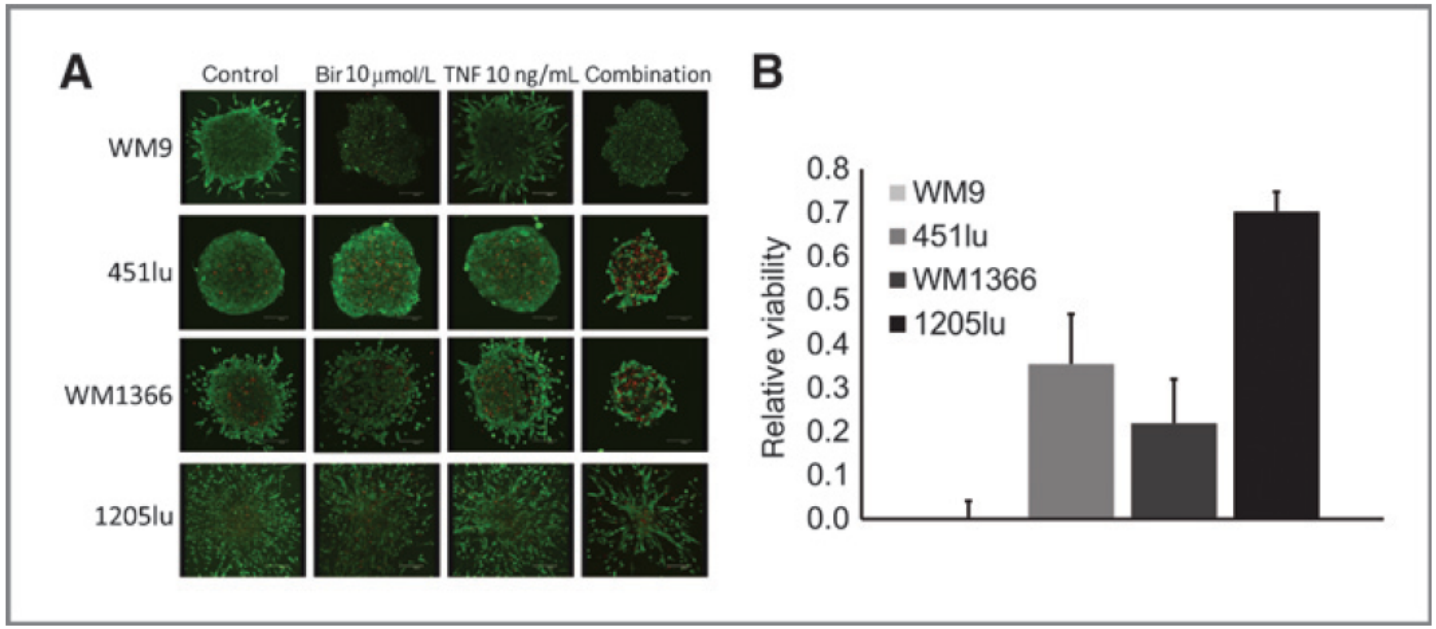
- Birinapant shows cIAP1 protein degradation at 100 nmol/L after 1 hour (XIAP unaffected)
- To determine apoptosis is caspase dependent, Z-VAD-FMK was added (caspase inhibitor)
- Necrostatin-1 (RIP1 kinase inhibitor) also reversed effect of birinapant/TNF-α



Krepler et al. *Clin. Cancer Res.* **2013**, *19(7)*, 1784.

Tetralogic Develops Smac Mimetic

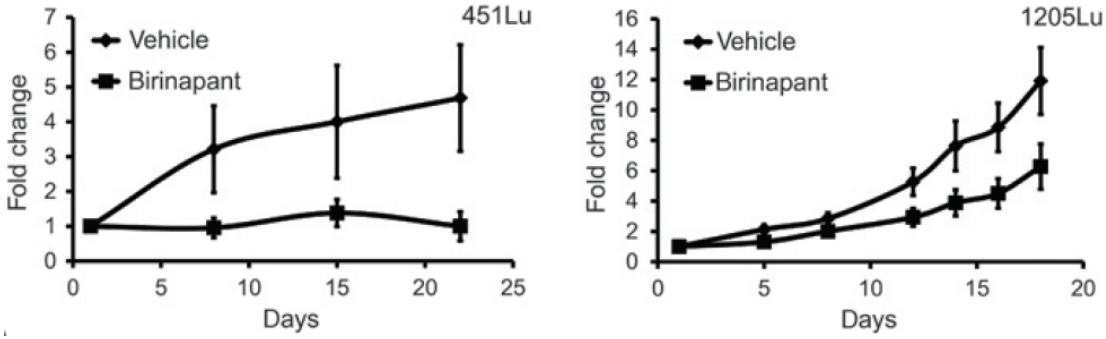
- Cells grown in 3D spheroid cultures, more similar to *in vivo* environments
- Similar effects as in previous *in vitro* studies for each cell line



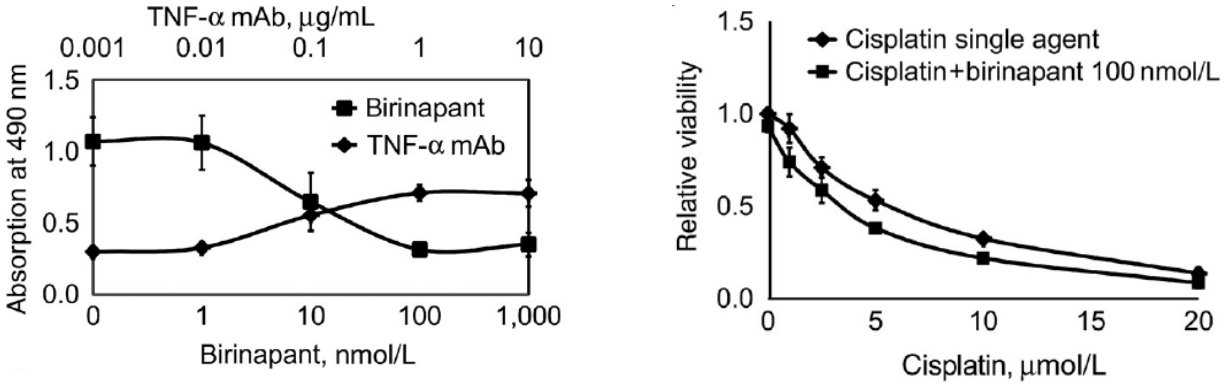
Krepler et al. *Clin. Cancer Res.* **2013**, *19(7)*, 1784.

Tetralogic Develops Smac Mimetic

- *In vivo* studies show birinapant is effective against 451Lu and slows tumor growth in 1205Lu



- Addition of TNF-α antibodies to WM9 culture shows dependence on endogenous TNF-α
- Birinapant in combination with cisplatin improves antitumor activity (451Lu, WM1366)



Krepler et al. *Clin. Cancer Res.* **2013**, *19(7)*, 1784.

Tetralogic Moves Birinapant to Clinical Trials

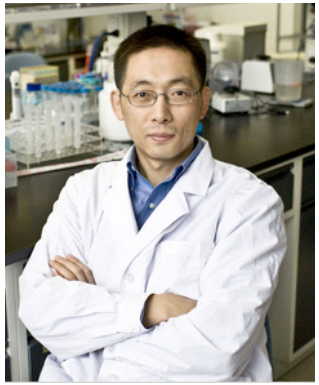
- Birinapant entered Phase I and II clinical trials for a variety of targets:
  - Colorectal cancer (combination with irinotecan)
  - Ovarian cancer (single agent, and combination with conatumumab)
  - AML/ALL (single agent)
  - Myelodysplastic syndrome (MDS) (combination with azacitidine)
  - Hepatitis B (preclinical trials)
- Tetralogic has library of over 3000 Smac mimetics

TetraLogic Pharmaceuticals

- Continuing to develop peptidomimetic therapeutics for both oncology and non-oncology indications
  - Both as single agent and combination therapies
- Investors include:
  - Nextech Invest, Clarus Ventures, HealthCare Ventures, Quaker BioVentures, Novitas Capital, Hatteras Venture Partners, Pfizer Ventures, Latterell Venture Partners, The Vertical Group, Amgen Ventures, Kammerer Associates

Having spent $77+ million developing birinapant, Tetralogic plans to raise **$90-$100 million** in upcoming IPO (rumoured to offer 6.4 million shares at $13-$15)

TetraLogic Pharmaceuticals



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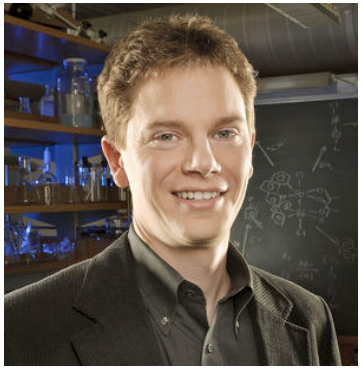


TETRAPHASE PHARMACEUTICALS

Peptidream

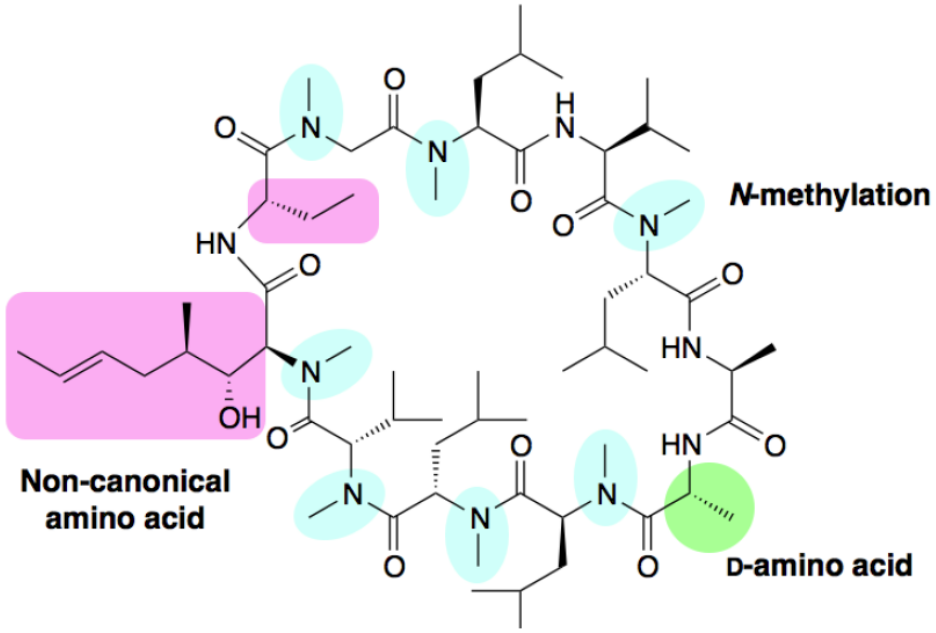
- Tokyo-based pharmaceutical company
- Founded in July 2006 by Hiroaki Suga
- Novel peptide therapeutics using proprietary Peptide Discovery Platform System (PDPS)
- About Hiroaki Suga

  - 1989-1994: Ph.D. with Satoru Masamune (MIT)
  - 1994-1997: Postdoctoral work with Jack Szostak (Harvard Medical School)
  - 1997-2003: Assistant and Associate Professor at SUNY Buffalo
  - 2003-present: Professor at University of Tokyo

Peptidream

PEPTIDREAM INC.
INNOVATIVE PEPTIDE THERAPEUTICS

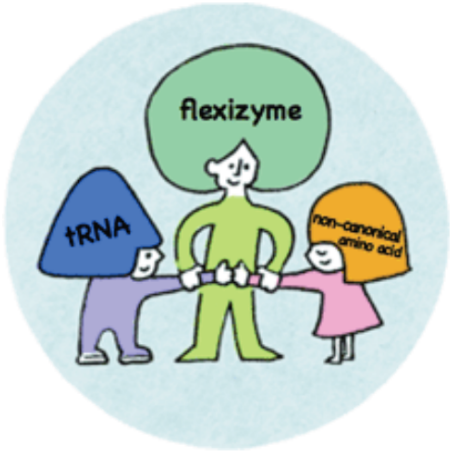
- Non-standard peptides are appealing therapeutic class
  - Very few systematic methods to synthesize and develop as drugs
- Non-traditional peptides may include:
  - Non-canonical sidechains
  - D-amino acids
  - *N*-methyl modification
- Macrocyclization and *N*-methylation improve membrane permeability and bioavailability



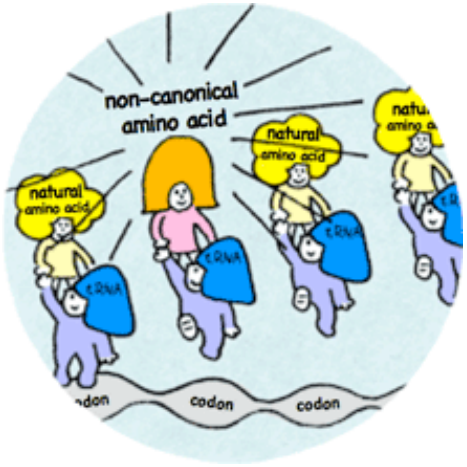
cyclosporin A

Research in the Suga Lab

Artificial Ribozymes



Genetic Code Reprogramming

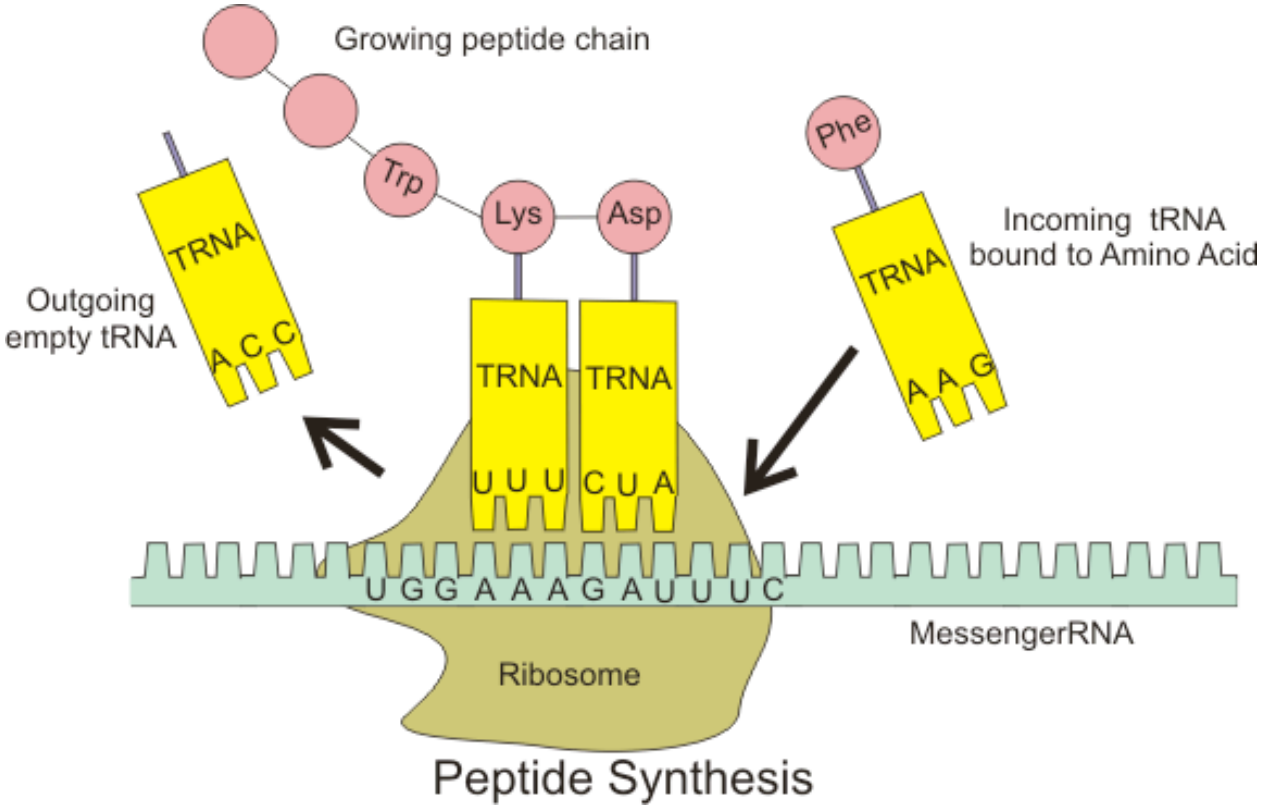


Ribosomal Synthesis of Non-Standard Peptides

Non-Standard Peptide Probes

A Brief Overview of Translation

Ribsomal Peptide Synthesis (Translation)



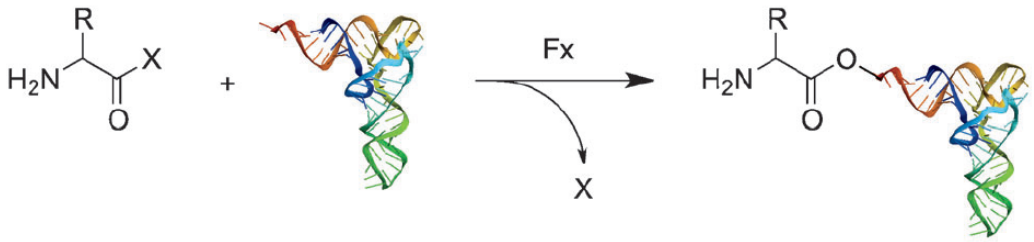
Genome British Columbia, www.genomebc.ca/education/articles/translation

Flexizyme Technology

- aminoacyl-tRNA synthetases (ARSs) catalyze ligation of amino acids to their respective tRNA
- Recombinant ARSs can ligate non-canonical AAs to tRNA, but substrate promiscuity is low

A new approach is necessary!

- **Ribozymes** - an RNA capable of enzymatic processes
- Flexizymes = highly promiscuous aminoacylating ribozyme ARSs



Passioura, T.; Suga, H. *Chem. Eur. J.* **2013**, *19*, 6530.

Ito, K.; Passioura, T.; Suga, H. *Molecules* **2013**, *18*, 3502.

Flexizyme Technology

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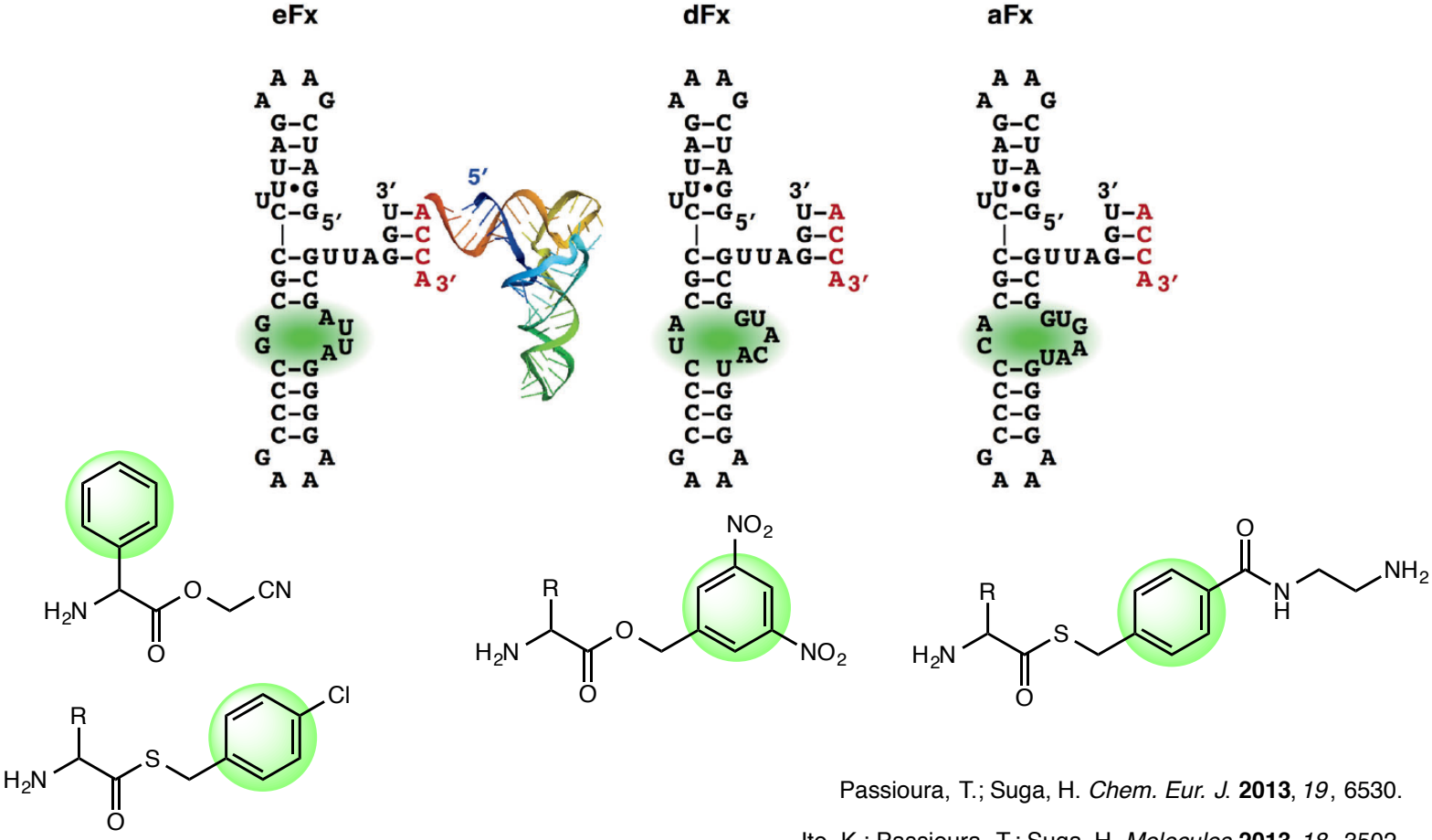

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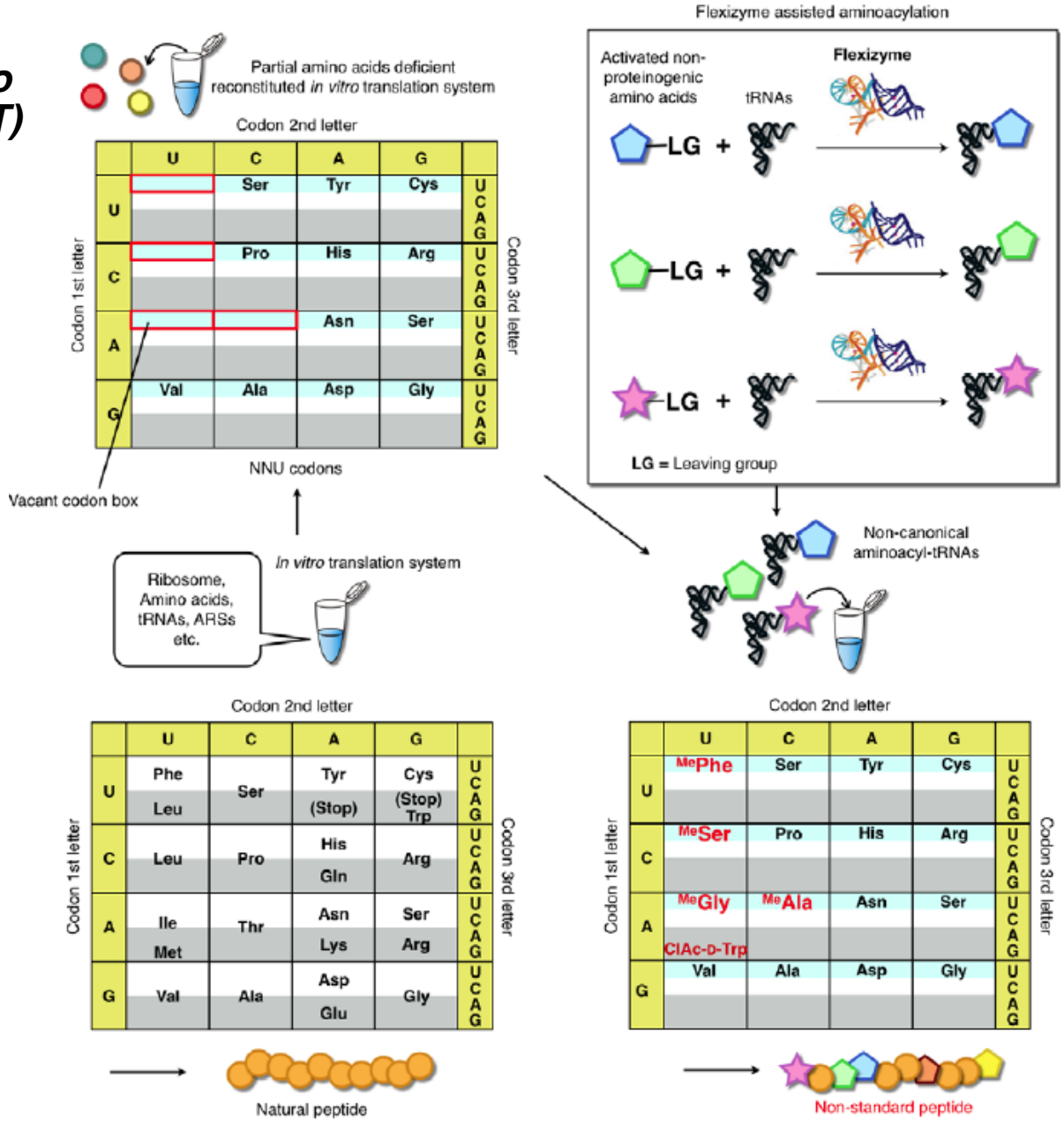
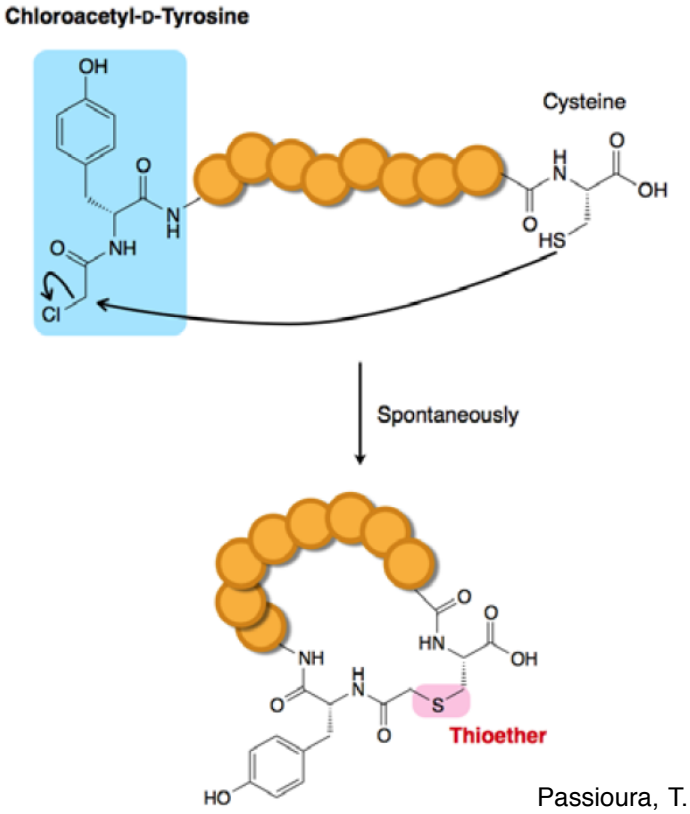
Flexible in vitro Translation (FIT) System



Methods for Peptide Macrocyclization

- Replacing *N*-terminus (initiator codon) with *N*-2-chloroacetyl amino acid
- Intramolecular thioether bond formation with downstream cysteine
- Spontaneous cyclization (may even occur within ribosome)



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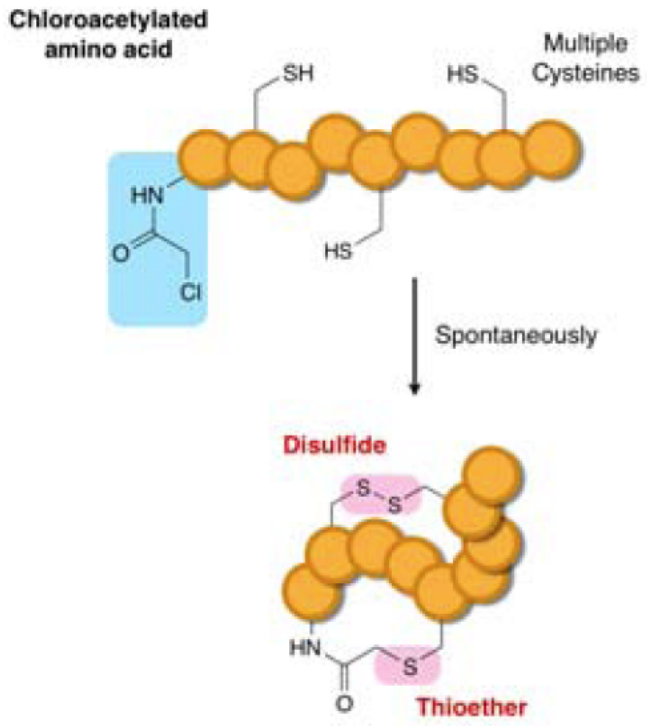
Methods for Peptide Macrocyclization

- Bicyclic peptide macrocycles can be synthesized
- Multiple cysteine residues allow thioether and disulphide bond formations



Iwasaki, K.; Goto, Y.; Katoh, T.; Suga, H. *Org. Biomol. Chem.* **2012**, *10*, 5783.

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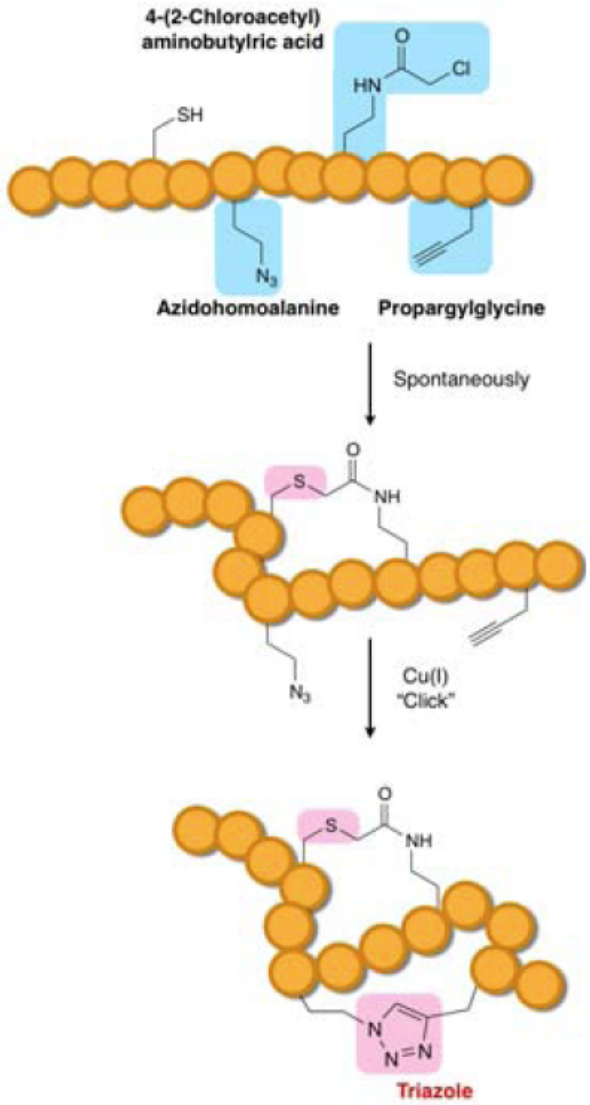
Methods for Peptide Macrocyclization

- Bicyclic peptide macrocycles can be synthesized
- Other functional handles can be manipulated using post-translational reactions

Cu-catalyzed alkyne-azide cyclization



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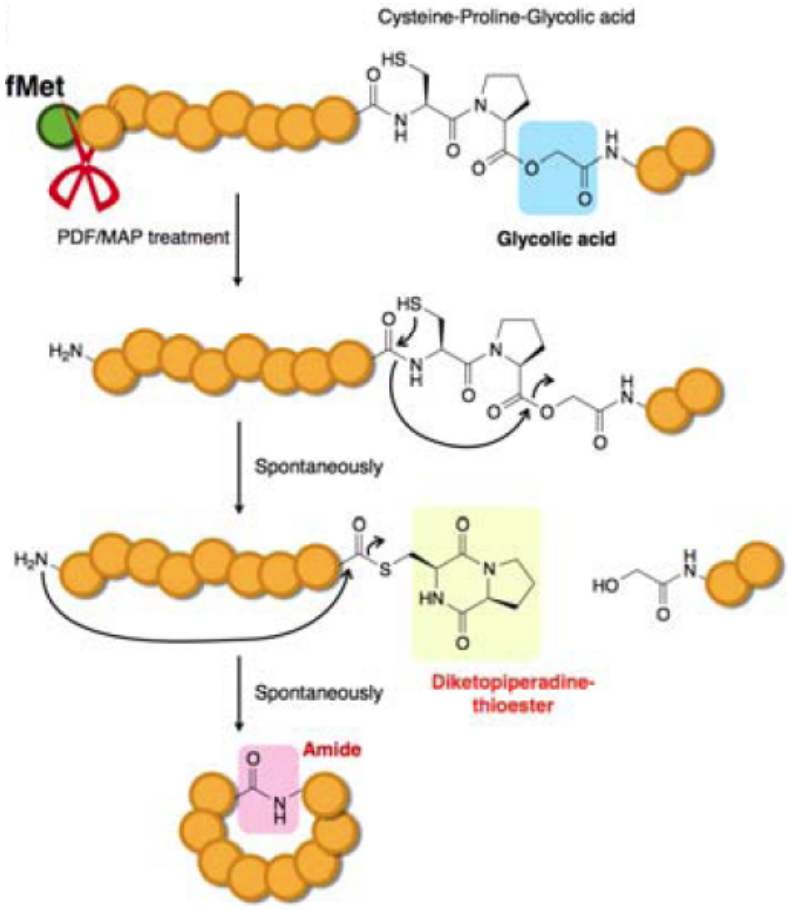
Methods for Peptide Macrocyclization

- Macrocycles with peptide bond can be synthesized
- FIT system must contain peptide deformylase (PDF) and methionine aminopeptidase (MAP)
- Cys-Pro-glycolic acid motif cyclizes to dkp-thioester
- Enzymatic removal of fMet liberates free NH_2



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RaPID System

- RaPID = Random non-standard Peptides Integrated Discovery system
- Highly efficient system for building up peptide library with high selectivity for a target protein
- The concept involves ligating mRNA strand to its corresponding peptide, observing binding affinity of peptide to protein, then over expressing RNA/peptide that selectively binds
- Overall, combination of FIT system and modified mRNA display

  - selection of bioactive non-standard peptides

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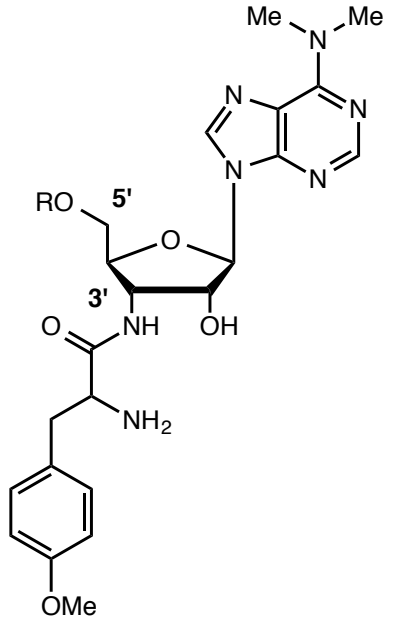
RaPID System

How does RaPID work?

Start with a library of diverse mRNA

- Typical sequence: AUG-random sequence (5-15 codons)-UGU-$(GGC\text{-}AGC)_3$-UAG
- AUG = start codon, UGU = cysteine, UAG = stop codon
- G rich section designed to anneal to DNA in puromycin linker

T4 RNA ligase links all mRNA strands to puromycin-DNA oligonucleotide



puromycin

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RaPID System

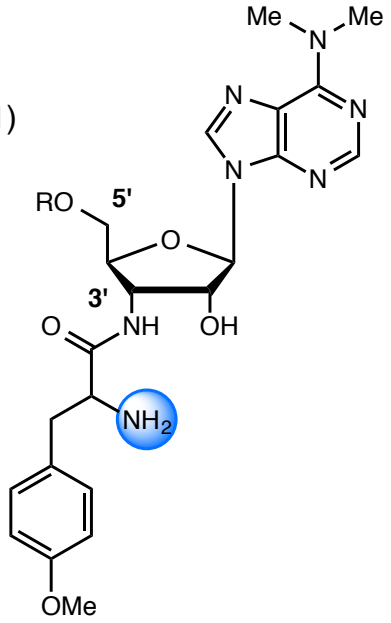
■ How does RaPID work?

■ FIT system results in translation of mRNA chain by ribosome

- Standard and non-standard amino acids incorporated
- Initiation codon reprogrammed to ClAc-amino acid
- Downstream cysteine cyclizes with N-terminal ClAc group
- At stop codon, ribosome stalls due to lack of Release Factor 1 (RF1)

■ α-amino group on puromycin linker is ligated to C-terminus of growing peptide chain by ribosome

- Forms the RNA-peptide adduct



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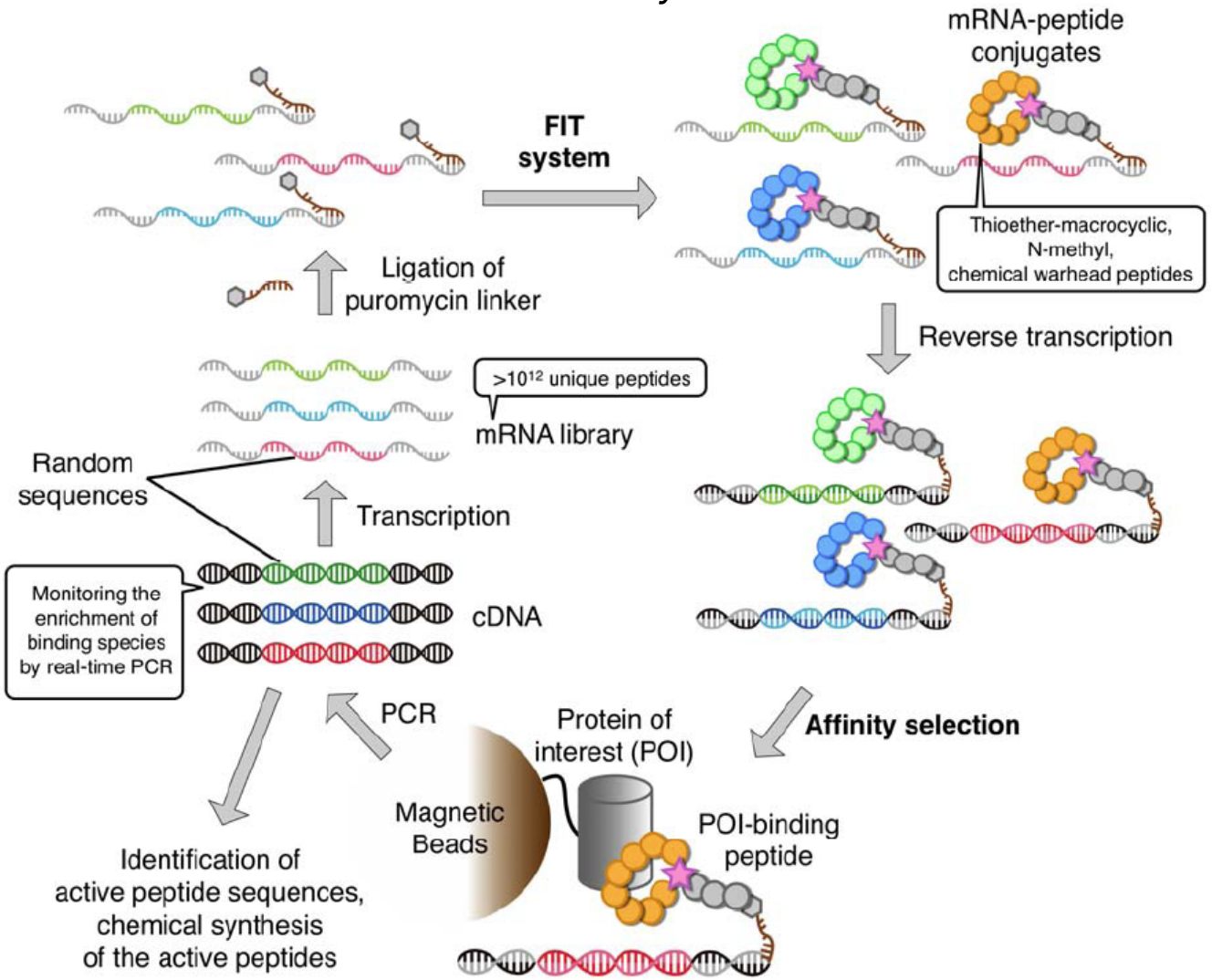

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RaPID System

- The RaPID cycle is repeated several times to enrich the mRNA pool
- One round of selection and enrichment is completed in < 1 day
- Once complete, the enriched pools are subjected to DNA sequencing
  - Binding is confirmed by resubjecting to target protein
  - Each peptide is then chemically synthesized for further studies of binding affinity and bioactivity

The diversity of non-canonical peptide residues is essentially infinite.

RaPID presents a very fast method for peptide therapeutic development!

Passioura, T.; Suga, H. *Chem. Eur. J.* **2013**, *19*, 6530.

Ito, K.; Passioura, T.; Suga, H. *Molecules* **2013**, *18*, 3502.

RaPID System in Drug Discovery

- Suga and Peptidream have applied the RaPID system towards novel peptide therapeutics
- Proof of concept: **discovery of Akt2 inhibitor**

- Akt kinase family play critical roles in signal transduction pathways

  - Akt1 and Akt2 indicated as potential oncogenes - over-expression suppresses cell apoptosis
  - Akt3 least understood - activation of growth factors in brain
  - Akt2 involved in insulin receptor signal transduction - possible target for diabetes treatment

- Appealing target for therapeutics, but difficult isoform-selectivity has hampered efforts

Hayashi, Y.; Morimoto, J.; Suga, H. *ACS Chem. Biol.* **2012**, *7*, 607.

RaPID: Discovery of Akt2 Inhibitor

■ Four classes of Akt2 inhibitors:

1) Bind to ATP-binding site
2) Bind to pleckstrin homology (PH) domain
3) Bind to an allosteric site
4) Bind to active site (peptide-binding domain)

■ Use of RaPID display system with macrocyclic peptides:

- ClAc^{L}Y or ClAc^{D}Y employed as the initator amino acid

- Random AA sequence composed of 4-12 units, of standard amino acids

- End sequence with cysteine (for thioether bond formation) and puromycin linker

■ Six rounds of RaPID to generate highly enriched mRNA pool against Akt2

Hayashi, Y.; Morimoto, J.; Suga, H. *ACS Chem. Biol.* **2012**, *7*, 607.

RaPID: Discovery of Akt2 Inhibitor

- From ^{L}Y- and ^{D}Y- pools, the best peptide binders were DNA sequenced
- Pakti-L_1 and Pakti-D_1 were the most abundant sequences
- Inhibitory effects were determined by in vitro kinase assays

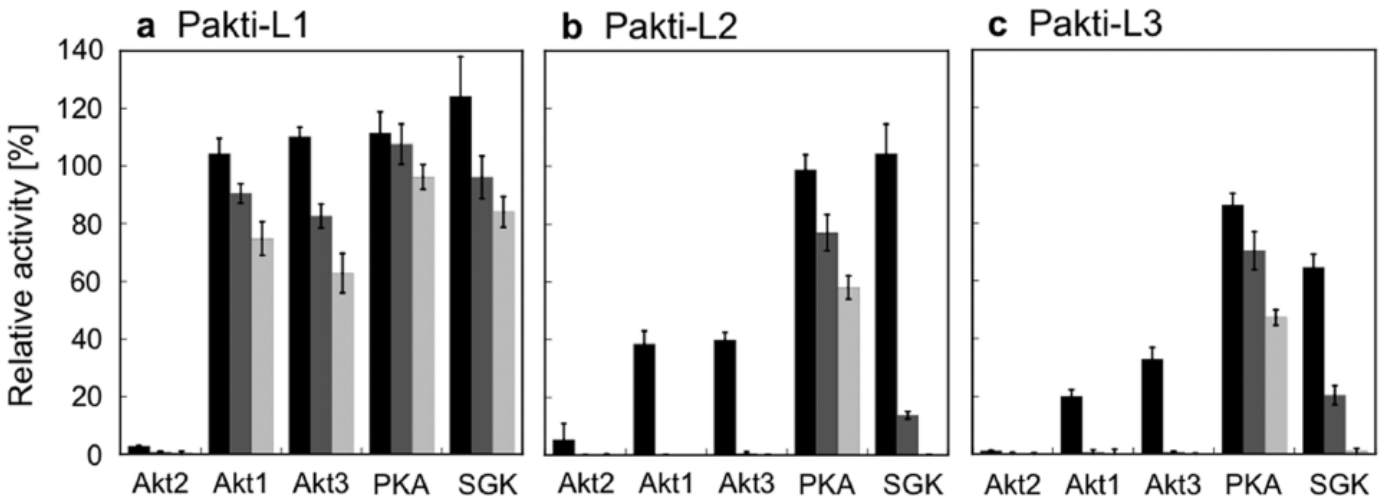
Peptide	Sequence	Frequency	IC_{50} [nM]		
			Akt2	Akt1	Akt3
Pakti-L1	Ac-^{L}YILVRNRLLRVDCG-NH_2	28/37	110	>25,000	4,200
Pakti-L2	Ac-^{L}YWILITWPLVRRKCG-NH_2	2/37	120	~1,000[a]	~1,000[a]
Pakti-L3	Ac-^{L}YWIVLTWPIVTRRCG-NH_2	2/37	92	~1,000[a]	~1,000[a]
Pakti-L4	Ac-^{L}YTYWFVSMICG-NH_2	1/37	inactive	N.D.	N.D.
Pakti-L5	Ac-^{L}YIRRPWVPIMYLGCG-NH_2	3/37	active	N.D.	N.D.
Pakti-L6	Ac-^{L}YILVRNRPLRVDCG-NH_2	1/37	active	N.D.	N.D.
Pakti-D1	Ac-^{D}YAVRILGHYLQVGCG-NH_2	35/37	active	N.D.	N.D.
Pakti-D2	Ac-^{D}YLSRRHGLLFLIRCG-NH_2	1/37	inactive	N.D.	N.D.
Pakti-D3	Ac-^{D}YLSREFNLLFLVRCG-NH_2	1/37	active	N.D.	N.D.

Hayashi, Y.; Morimoto, J.; Suga, H. *ACS Chem. Biol.* **2012**, *7*, 607.

RaPID: Discovery of Akt2 Inhibitor

- Pakti-L_1,L_2,L_3 showed best inhibitory effect against Akt kinases

- Pakti-L_1 showed tremendous isoform-selectivity for Akt2 (over Akt1 and Akt3)



PKA = Protein kinase A, SGK = serum- and glucocorticoid-regulated protein kinase)

Black, dark grey, light grey correspond to 1, 5, and 10 μM peptide concentrations.

Hayashi, Y.; Morimoto, J.; Suga, H. *ACS Chem. Biol.* **2012**, *7*, 607.

RaPID: Discovery of Akt2 Inhibitor

■ Remarkably, RaPID system delivered effective inhibitors of Akt2

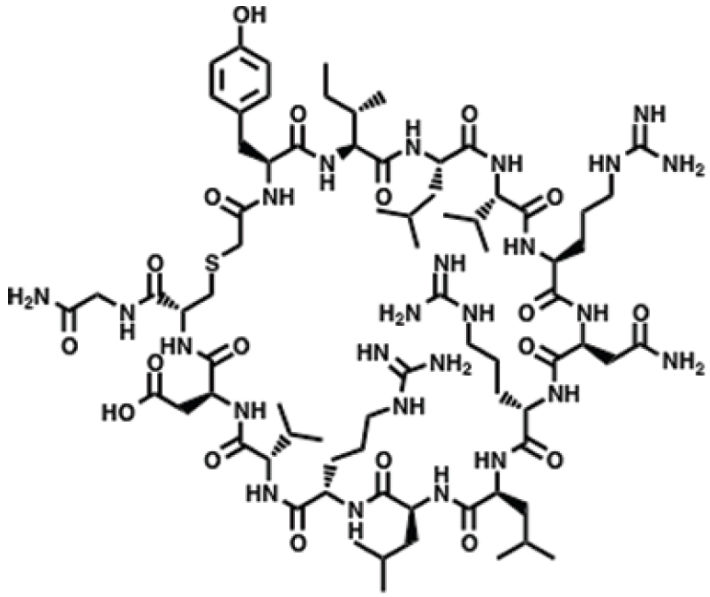
- Display system only examines binding affinity, but corresponds well to inhibition

- Interestingly, ^{L}Y- and ^{D}Y-peptides had very difficult sequences indicating different structural orientation

■ Pakti-L_1 mode of binding to Akt2 is unknown

- Possibly interacts with substrate-binding domain or allosteric site

■ Pakti-L_1 exhibitied unprecedented levels of Akt2-isoform selectivity and potency



Pakti-L_1
Akt2-selective inhibitor

Hayashi, Y.; Morimoto, J.; Suga, H. *ACS Chem. Biol.* **2012**, *7*, 607.

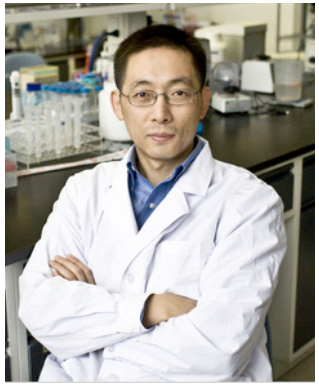
Peptidream

- Peptidream currently applying Suga technology to novel therapeutic discovery
- Peptide Discovery Translation System (PDTS) and Peptide Discovery Display System (PDDS)
- Numerous multi-target discovery deals signed:

  - Jul. 2007 and Oct. 2009 - MedImmune/Astra Zeneca
  - Aug. 2010 - Novartis
  - Oct. 2010 - Amgen
  - Nov. 2010 - BMS
  - Dec. 2010 - Pfizer
  - Dec. 2010 - Mitsubishi-Tanabe Pharma
  - Jul. 2012 - Daiichi Sankyo
  - Sep. 2012 - GSK
  - Apr. 2013 - Ipsen

Novel non-standard macrocyclic peptides show promise as potent and selective therapeutics!

TetraLogic Pharmaceuticals

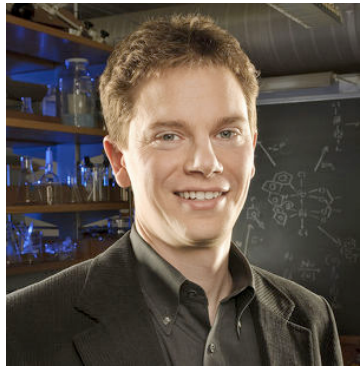


PeptiDream

PEPTIDREAM INC.

INNOVATIVE PEPTIDE THERAPEUTICS

SciFluor Life Sciences



TETRAPHASE PHARMACEUTICALS

SciFluor Life Sciences

- Launched in February 2011
- Founded by Tobias Ritter (Harvard)
- Initial $5 million investment by Allied Minds

SciFluor
Life Sciences

- Late-stage fluorination of therapeutics (Fluoropeutics)

  - Fluorination of already known compounds with established biological targets:
    with the goal of improving potency, metabolic stability, binding affinity, bioavailability, and blood-brain barrier penetration

  - "De-risked" candidates, due to precedent of parent compound in pre-clinical/clinical trials

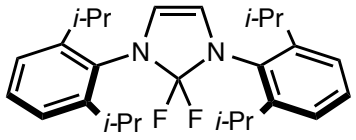
- Employing Ritter technology for ^{18}F PET tracers

SciFluor Life Sciences

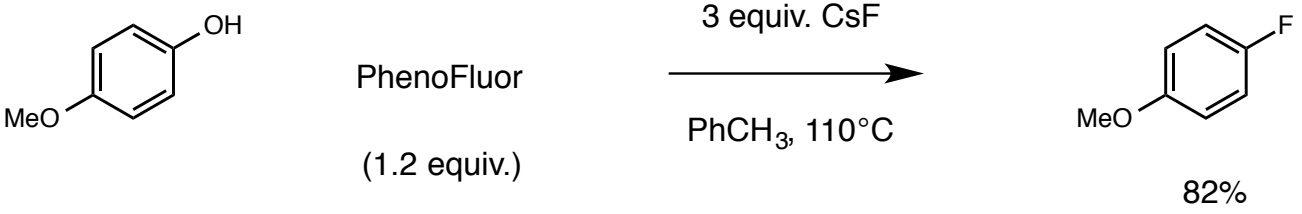
■ SciFluor employs "PhenoFluor" for late-stage fluorination

- Novel deoxyfluorinating reagent discovered by Ritter and coworkers

- Marketed by SciFluor through Sigma-Alrich and Strem



PhenoFluor

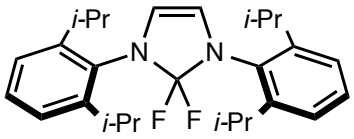


Other commercially available deoxyfluorinating agents (DAST, DEOXYFLUOR, Xtalflour) gave <1% yield

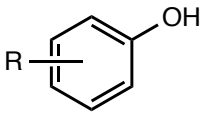
Tang, P.; Wang, W.; Ritter, T. *J. Am. Chem. Soc.* **2011**, *133*, 11482.

PhenoFluor™

■ Deoxyfluorination of phenols



PhenoFluor

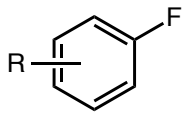


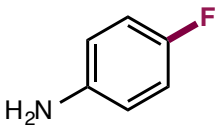
PhenoFluor

(1.2 equiv.)

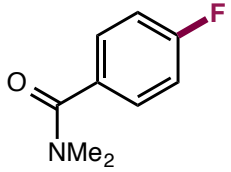
3 equiv. CsF

$PhCH_3$, 110°C

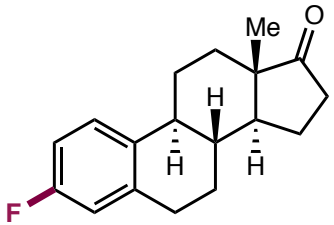




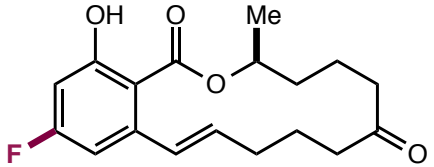
75%



91%



90%



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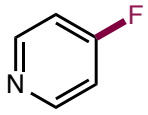

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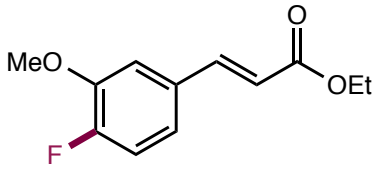
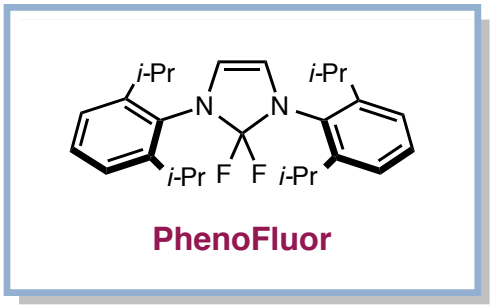


88%

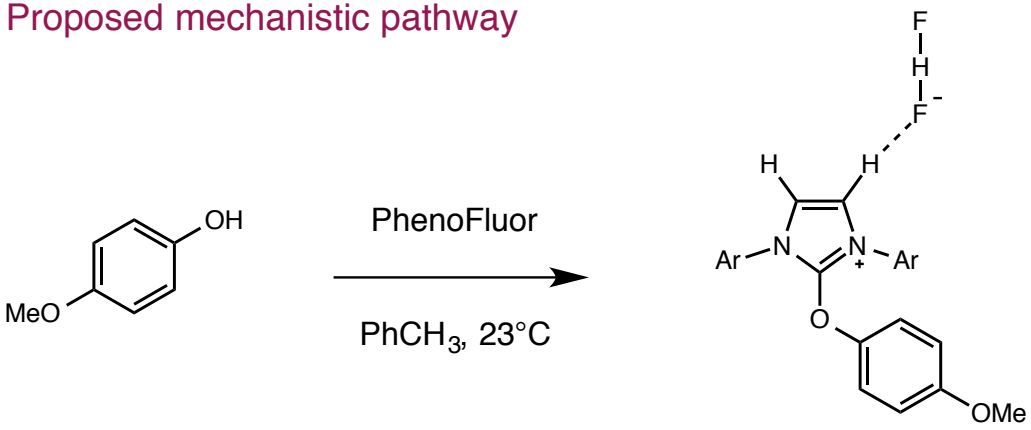
Tang, P.; Wang, W.; Ritter, T. *J. Am. Chem. Soc.* **2011**, *133*, 11482.

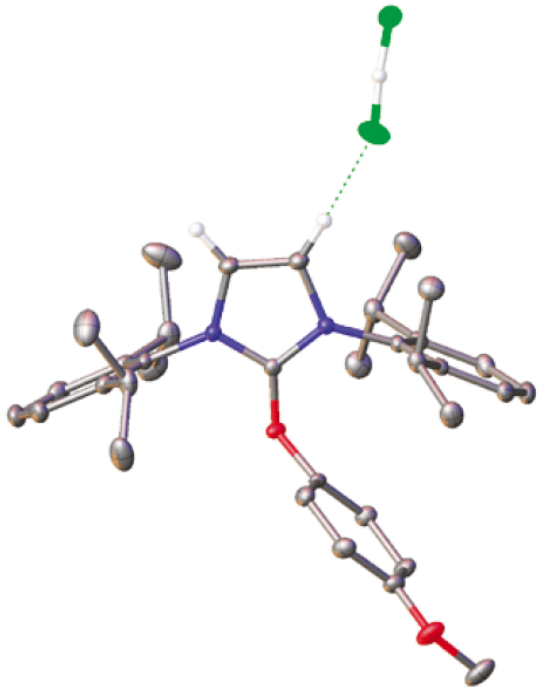
PhenoFluor™



PhenoFluor

■ Proposed mechanistic pathway

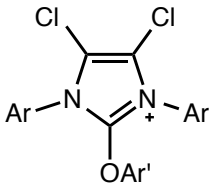


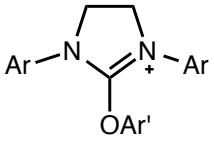


H-bonding interaction facilitates fluorination

a) makes the uronium a better leaving group
b) brings fluoride in closer proximity to *ipso*-carbon

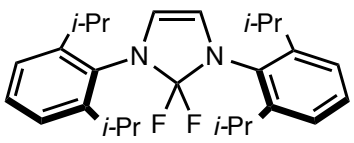
Control experiments: no H-bonding, no reaction





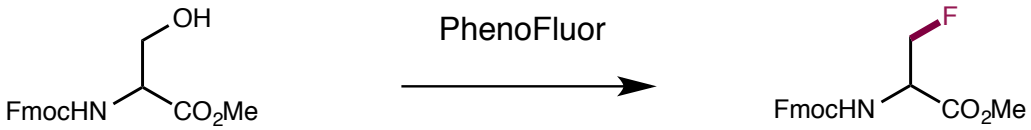
Tang, P.; Wang, W.; Ritter, T. *J. Am. Chem. Soc.* **2011**, *133*, 11482.

PhenoFluor™



PhenoFluor

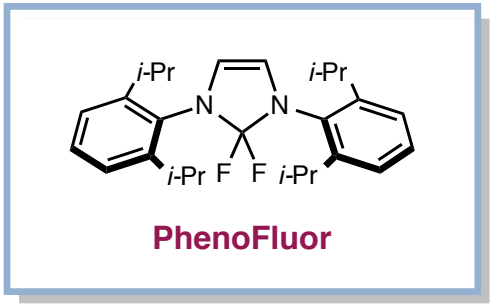
■ Deoxyfluorination of aliphatic alcohols



Byproducts observed with conventional fluorination reagents:

Sladojevich, F.; Arlow, S. I.; Tang, P.; Ritter, T. *J. Am. Chem. Soc.* **2013**, *135*, 2470.

PhenoFluor™



PhenoFluor

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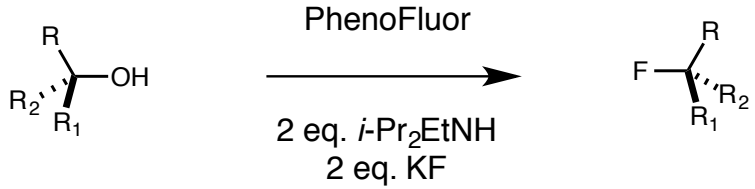
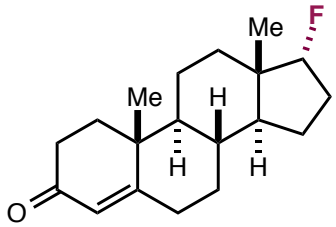


inversion of stereochemistry



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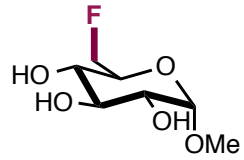

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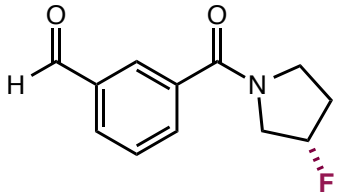

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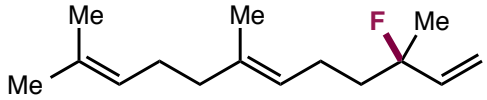
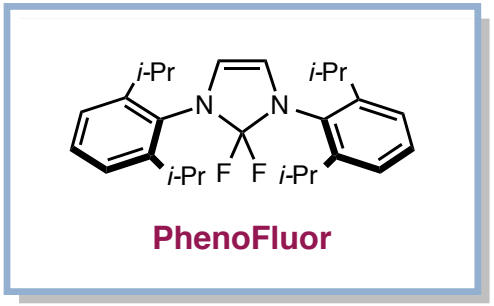


53%

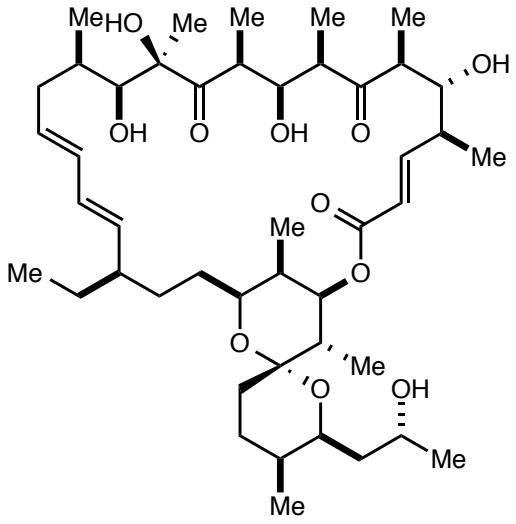
Sladojevich, F.; Arlow, S. I.; Tang, P.; Ritter, T. *J. Am. Chem. Soc.* **2013**, *135*, 2470.

PhenoFluor™



PhenoFluor

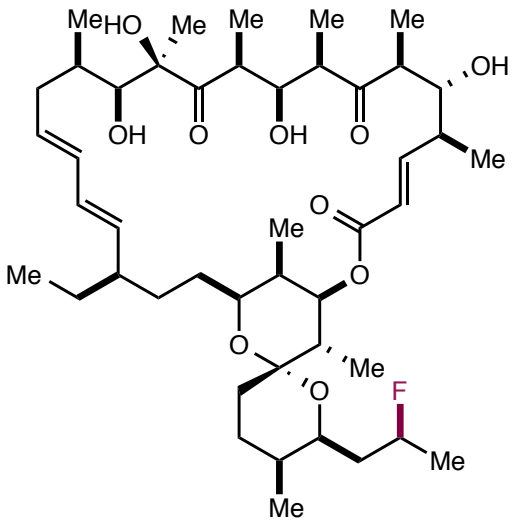
■ Deoxyfluorination of aliphatic alcohols



oligomycin A

PhenoFluor

2 eq. *i*-Pr_2EtNH
2 eq. KF



71% yield

Sladojevich, F.; Arlow, S. I.; Tang, P.; Ritter, T. *J. Am. Chem. Soc.* **2013**, *135*, 2470.

PhenoFluor™

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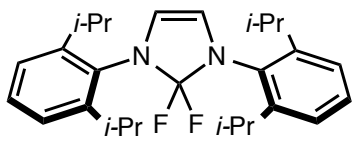


PhenoFluor

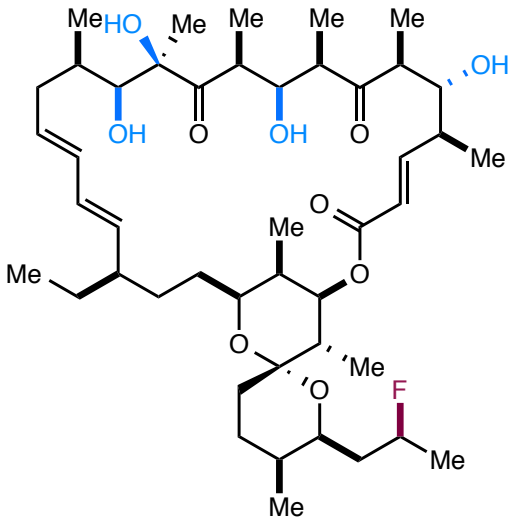
PhenoFluor has excellent chemoselectivity

a) 1° alcohols fluorinated selectively over 2° and 3°

b) β,β'-dibranched 2° alcohols react significantly slower (unless allylic)

c) 3° alcohols do not react (unless allylic)

d) hydoxyl groups involved in H-bonding do not react

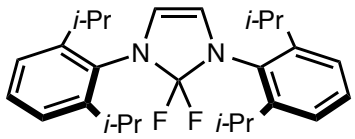


71% yield

Sladojevich, F.; Arlow, S. I.; Tang, P.; Ritter, T. *J. Am. Chem. Soc.* **2013**, *135*, 2470.

PhenoFluor™

■ PhenoFluor is a versatile tool for SciFluor's late-stage fluorination approach



PhenoFluor

Advantages

a) Air-stable reagent, operationally simple (non-explosive)

b) Excellent selectivity (predictable)

c) Functional group tolerant

d) Avoids byproducts (elimination of H_2O), yields single isomer

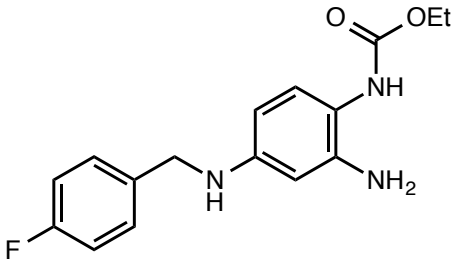
Disadvantage: stoichiometric waste, not ideal for scale-up

Tang, P.; Wang, W.; Ritter, T. *J. Am. Chem. Soc.* **2011**, *133*, 11482.

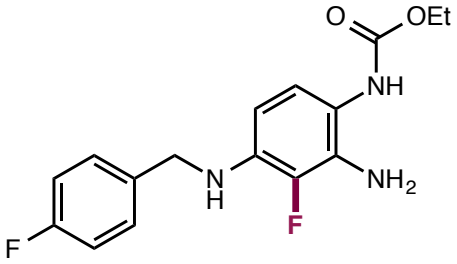
Sladojevich, F.; Arlow, S. I.; Tang, P.; Ritter, T. *J. Am. Chem. Soc.* **2013**, *135*, 2470.

SciFluor's Potassium Channel Opener: SF0034

■ SciFluor have identified a potent therapeutic for the treatment of partial-onset seizure



Ezogabine (Valeant/GSK)



Fluoropeutic SF0034

- Ezogabine was the first potassium channel (KCNQ2/3) opener for epilepsy treatment (approved June 2011)

- Binds to voltage-gated K^+ channel, opening it, and allowing repolarization of the neuron

- Stops the high levels of neuronal action potential burst firing - associated with seizure onset

Furuya, T.; Edwards, D. S.; Duggan, M; Askew, B. C. *International Epilepsy Conference* (2013).

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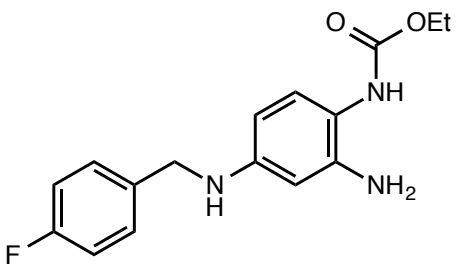
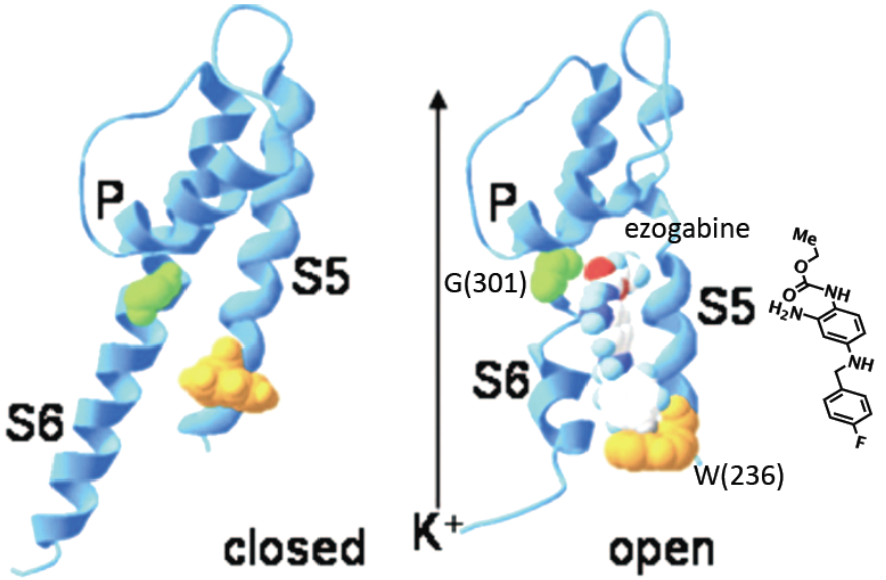


Ezogabine (Valeant/GSK)



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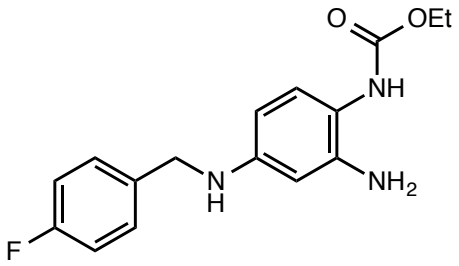
Furuya, T.; Edwards, D. S.; Duggan, M; Askew, B. C. *International Epilepsy Conference* (2013).

SciFluor's Potassium Channel Opener: SF0034

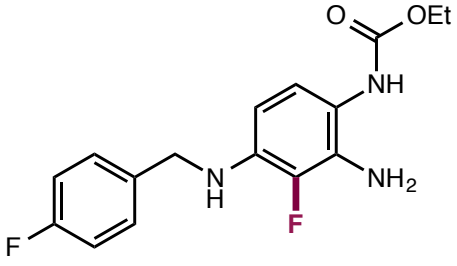
■ Selectivity in activating KCNQ2/3 over KCNQ4 is essential

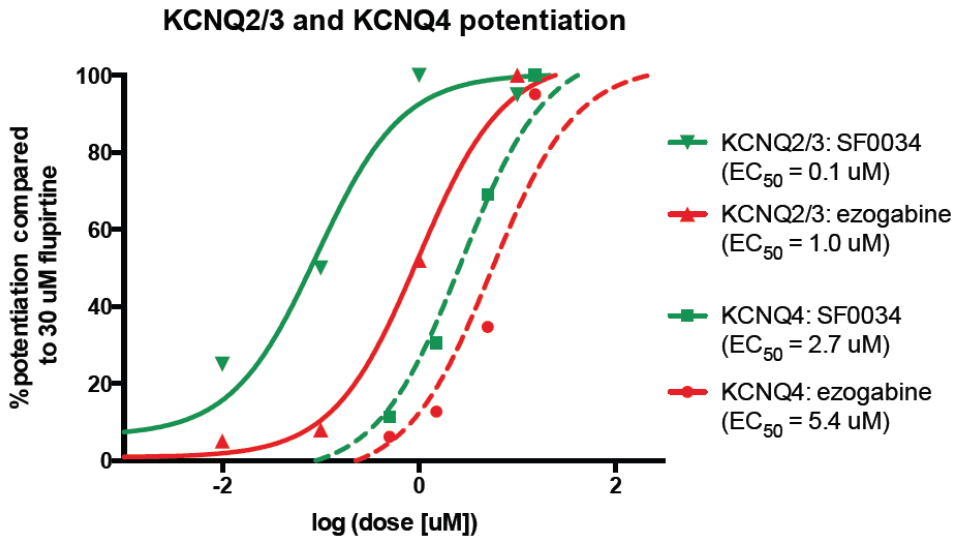
- KCNQ4 activation results in a urinary retention side effect

Ezogabine



SF0034





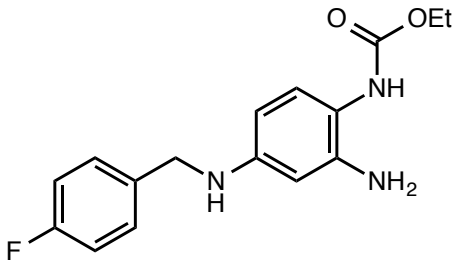
Furuya, T.; Edwards, D. S.; Duggan, M; Askew, B. C. *International Epilepsy Conference* (2013).

SciFluor's Potassium Channel Opener: SF0034

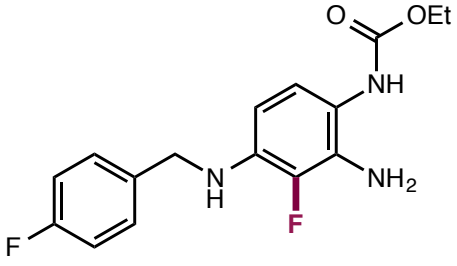
■ Selectivity in activating KCNQ2/3 over KCNQ4 is essential

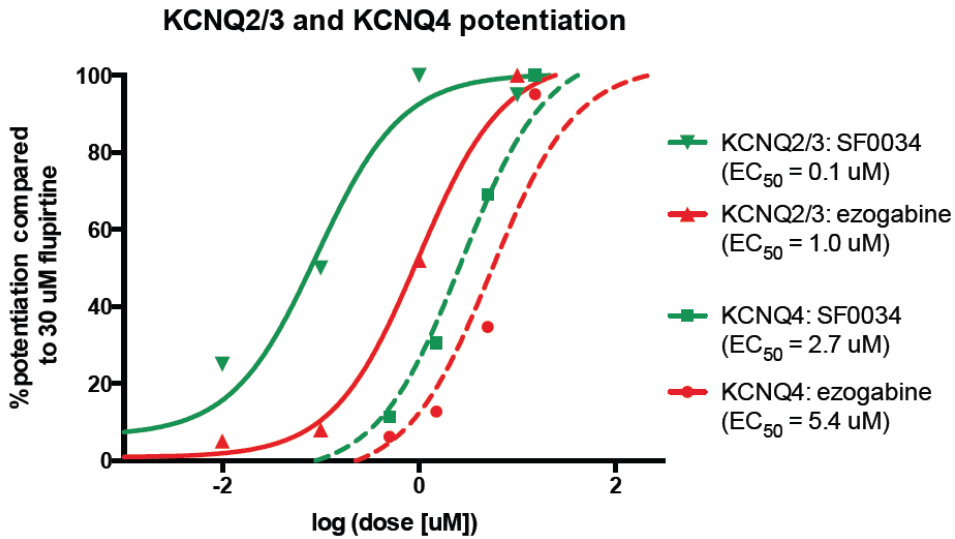
- KCNQ4 activation results in a urinary retention side effect

Ezogabine



SF0034





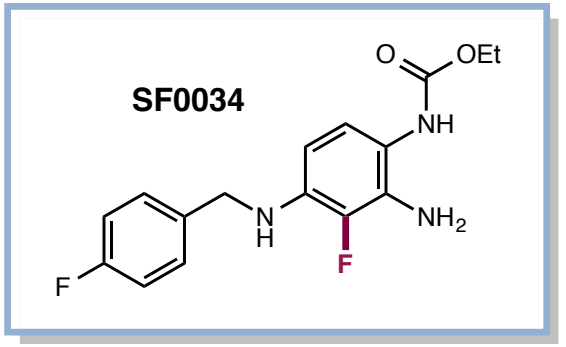
SF0034 is **10 times** more potent and **5 times** more selective than ezogabine

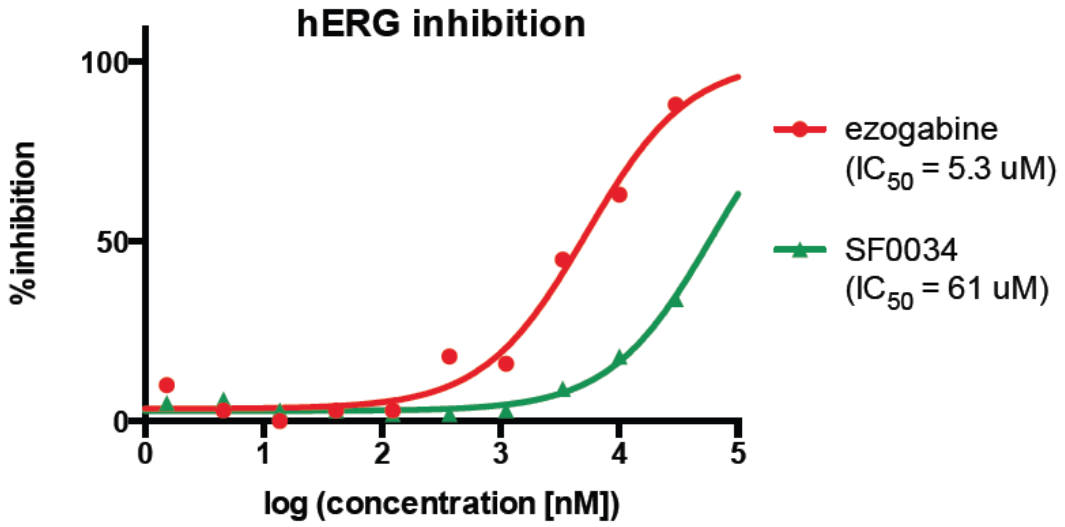
Furuya, T.; Edwards, D. S.; Duggan, M; Askew, B. C. *International Epilepsy Conference* (2013).

SciFluor's Potassium Channel Opener: SF0034

■ SF0034 shows no mutagenicity and reduced hERG inhibition

- Ames test on SF0034 showed no mutagenicity or cytotoxicity



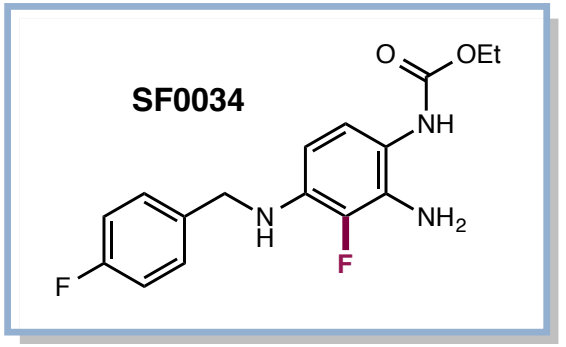


SF0034 has >**10 times higher** IC_{50} values for hERG inhibition than ezogabine

Furuya, T.; Edwards, D. S.; Duggan, M; Askew, B. C. *International Epilepsy Conference* (2013).

SciFluor's Potassium Channel Opener: SF0034

■ SF0034 *in vitro* and *in vivo* data



	T_{max} (hr)		C_{max} (ng/mL)		AUC (hr × ng/mL)		PPB (human)	PPB (mouse)	Human Hepatocyte Clearance (mL/min/g)	CYP inhibition @ 10 μM		
	mouse	rat	mouse	rat	mouse	rats				3A4	2C9	2D6
Ezogabine	0.25	1.0	3044	1458	10084	5757	80%	84%	0.84	18%	0%	2%
SF0034	0.25	0.67	2651	497	6046	2684	89%	90%	1.20	18%	15%	3%

	MES ED_{50} (therapeutic index)	scMET ED_{50} (therapeutic index)	6 Hz ED_{50} (therapeutic index)
Ezogabine	14 mg/kg (4.8)	43 mg/kg (1.6)	11 mg/kg (6.1)
SF0034	**6.6 mg/kg (9.1)**	**27 mg/kg (2.3)**	**12 mg/kg (5.0)**

$$TI = \frac{TD_{50}}{ED_{50}}$$

Furuya, T.; Edwards, D. S.; Duggan, M; Askew, B. C. *International Epilepsy Conference* (2013).

SciFluor Life Sciences Pipeline

- SF0034 has demonstrated improved potency and selectivity to ezogabine

  - Overall more favourable phacological profile, including reduced side effect profiles

  - SciFluor seeking industry partner to develop SF0034 as next-generation anti-epileptic drug

- Efforts using fluoropeutics to target cardiovascular disease, infectious disease, CNS, and oncology are currently ongoing

"Precedented drugs" vs. "Me-Too drugs" (Xconomy, Feb. 2013)

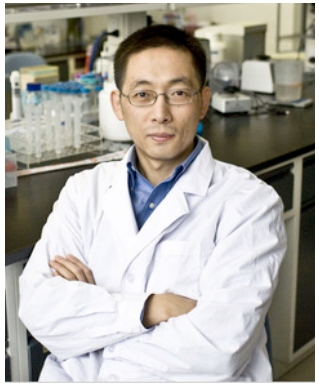
SciFluor Life Sciences

"The most fruitful basis for the discovery of a new drug is to start with an old drug."

"A competitor can patent new molecules based on a rival's older drugs. But the competitor must make changes to the original drug that are truly novel, and that would not have been obvious innovation routes for the creators of the original drug."

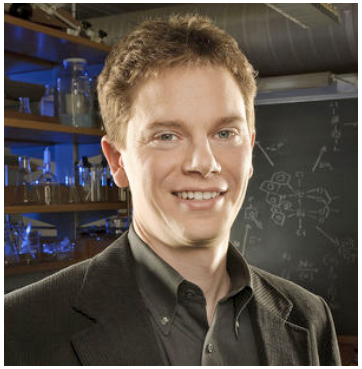
Arthur Hiller, Former CEO, SciFluor Life Sciences

TetraLogic Pharmaceuticals



PeptiDream PEPTIDREAM INC.
INNOVATIVE PEPTIDE THERAPEUTICS

SciFluor Life Sciences



TETRAPHASE PHARMACEUTICALS

Tetraphase Pharamaceuticals

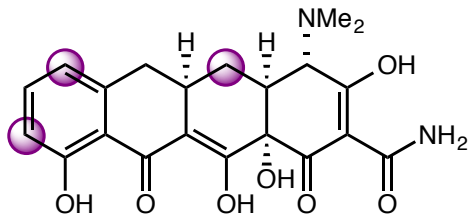
- Founded in 2006
- Watertown, MA

TETRAPHASE PHARMACEUTICALS

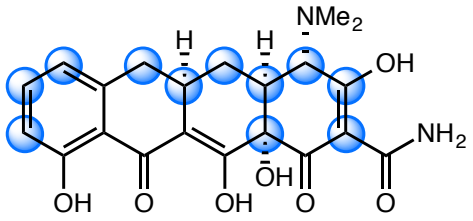
- Based on Andrew Myers' tetracycline synthetic efforts (Harvard University)

Tetraphase's mission is to bring novel tetracycine antibiotics to market to target multidrug resistant (MDR) infections

The Myers/Tetraphase approach to tetracycline synthesis is convergent and allows rapid diversification:



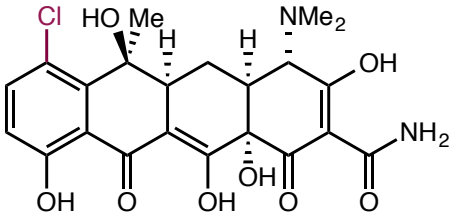
Conventional methods of modification



Tetraphase technology

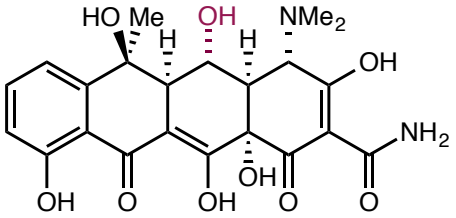
A History of Tetracyclines

- First tetracycline antiobiotic isolated in 1948 - Benjamin Duggar, Lederle Laboratories



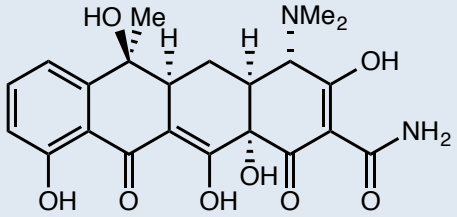
aureomycin (chlorotetracycline)

- In 1950, Pfizer isolated terramycin



terramycin (oxytetracycline)

- In 1953, tetracycline was first prepared by Lloyd Conover at Pfizer
  - later determined to be a natural product



tetracycline

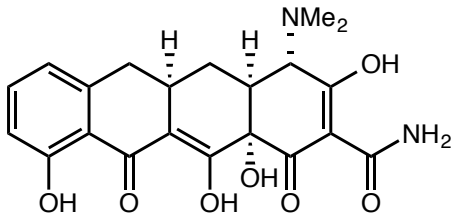
Total Syntheses of Tetracyclines

■ Numerous syntheses of tetracycline and analogues

- Woodward, Shemyakin, Muxfeldt, Stork, Tatsuta

- All syntheses apply a "left to right" approach (D ring to A ring)

■ Total synthesis of tetracycline analogue accomplished by Woodward in 1968



6-deoxy-6-demethyltetracycline

25 steps, 0.002% yield

"the originial effort of Woodward has survived as the basic strategy
for the total synthesis of this series and at greater than 25 steps
is clearly not to be considered as practical....."

Woodward et al. *J. Am. Chem. Soc.* **1968**, *90*, 439.

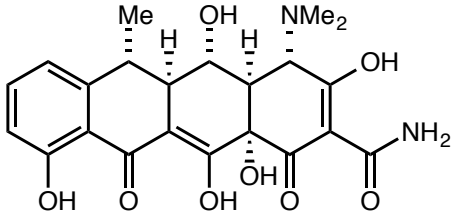
Podlogar, B. L.; Ohemeng, K. A.; Barrett, J. F. *Expert Opin. Ther. Patents* **2003**, *13*, 467.

History of Tetracycline Antibiotics

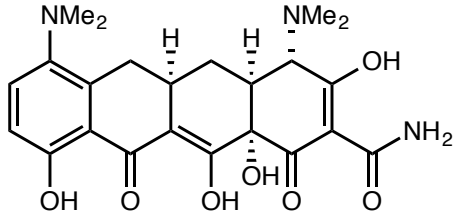
- Aureomycin, terramycin, and tetramycin identified as powerful antibiotics

- Three major tetracycline antiobiotics over the last 50 years

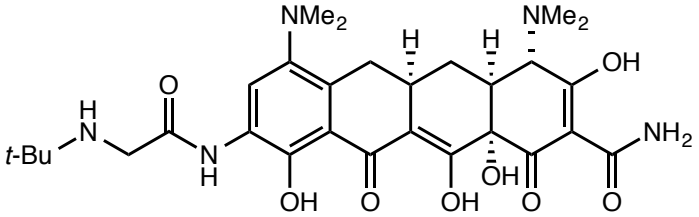
- Doxycycline (Pfizer 1967)



- Minocycline (Lederle 1972)

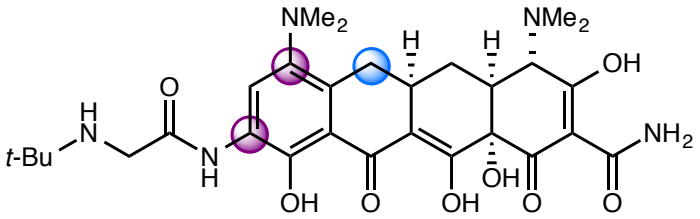


- Tigecycline (Wyeth 2005)



History of Tetracycline Antibiotics

- Tigecycline



- Removal of C6-hydroxyl group improved metabolic stability, retention of antibacterial activity

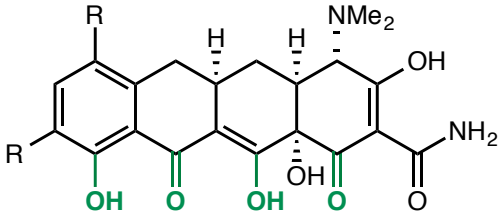
- Derivatization only possible at C7,C9 positions (electrophilic aromatic substitution)

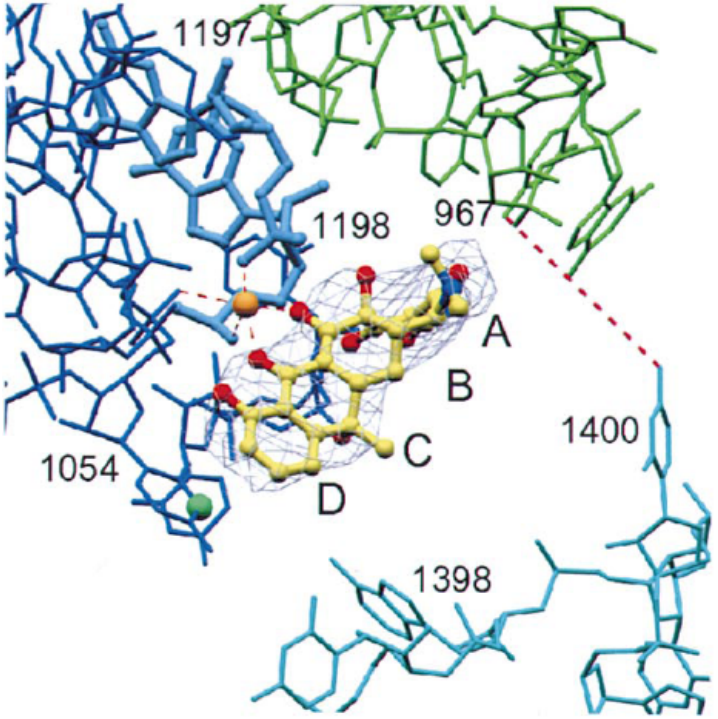
All FDA approved tetracycline antibiotics are made exclusively
via **fermentation** or **semi-synthesis**

Given the D to A ring synthetic approaches, variation on the D ring
is challenging given current methods

History of Tetracycline Antibiotics

- Ribosomal binding



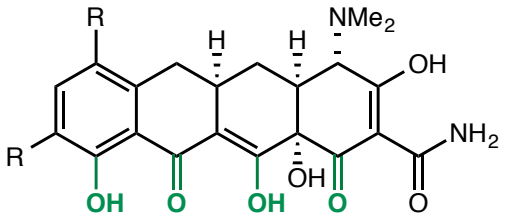


- Tetracyclines bind to the 30S subunit of the bacterial ribosome through hydrophilic groups
- Blocks the accetor site for aminoacylated tRNA
- Prevents binding of amino acid to ribosome
  - inhibits protein synthesis

Brodersen, D. E.; Clemons, W. M.; Carter, A. P.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V. *Cell* **2000**, *103*, 1143.

History of Tetracycline Antibiotics

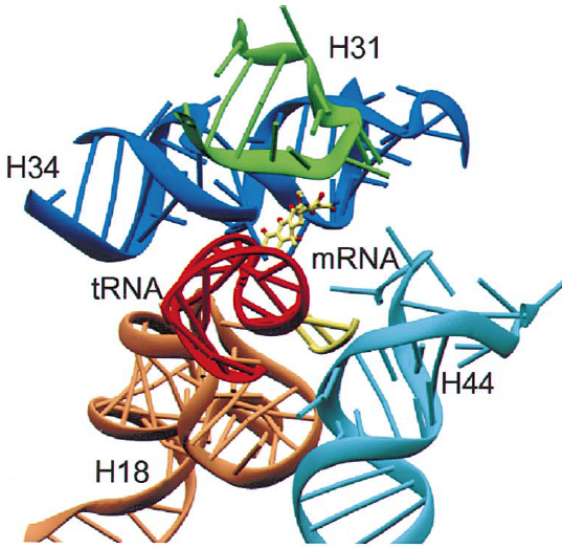
■ Ribosomal binding

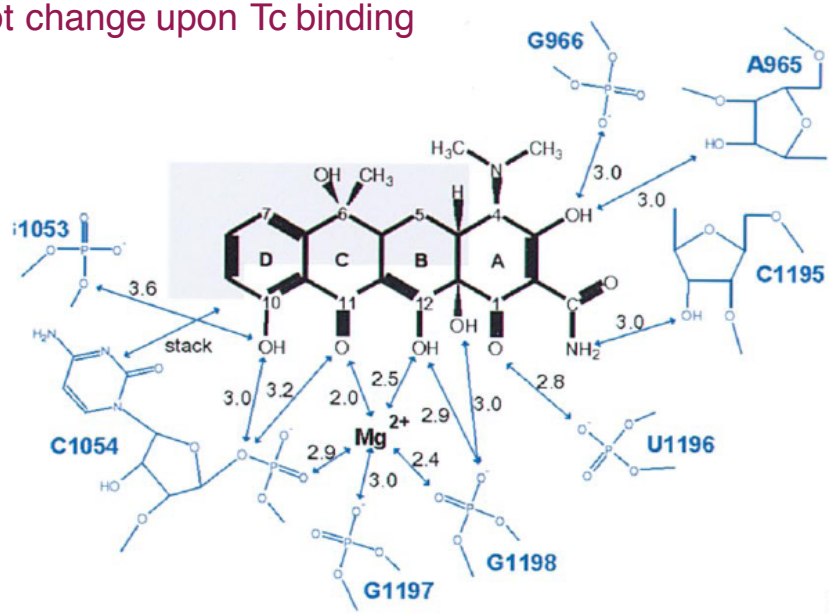


"Tc"

■ Within the 30S subunit, tetracyclines bind to the major grove of 16S RNA

- predominantly H-bonding with phosphate backbone
- Mg^{2+} present in ribosome and helps binding
- interestingly, the conformation of RNA does not change upon Tc binding

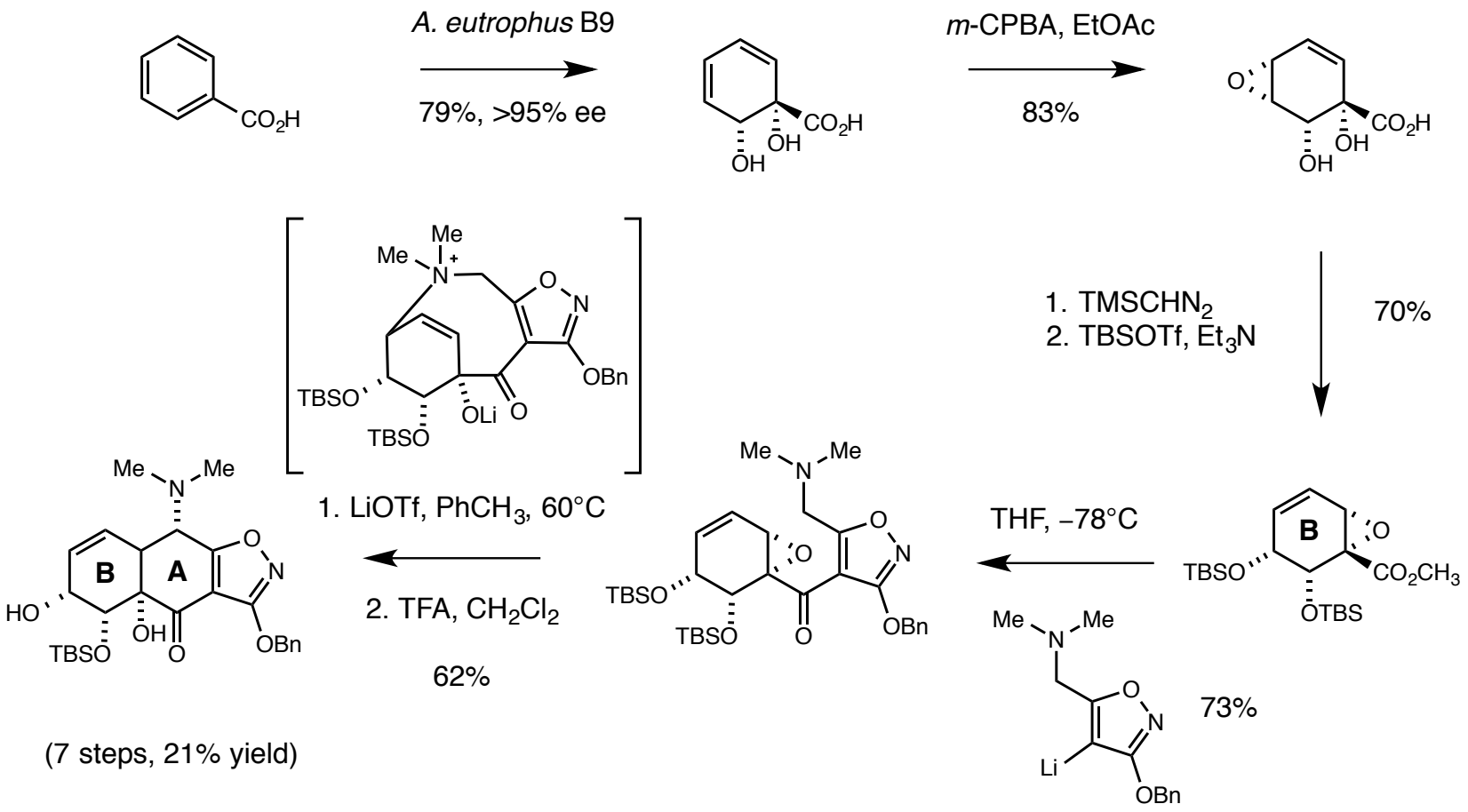




Brodersen, D. E.; Clemons, W. M.; Carter, A. P.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V. *Cell* **2000**, *103*, 1143.

The Myers Synthesis

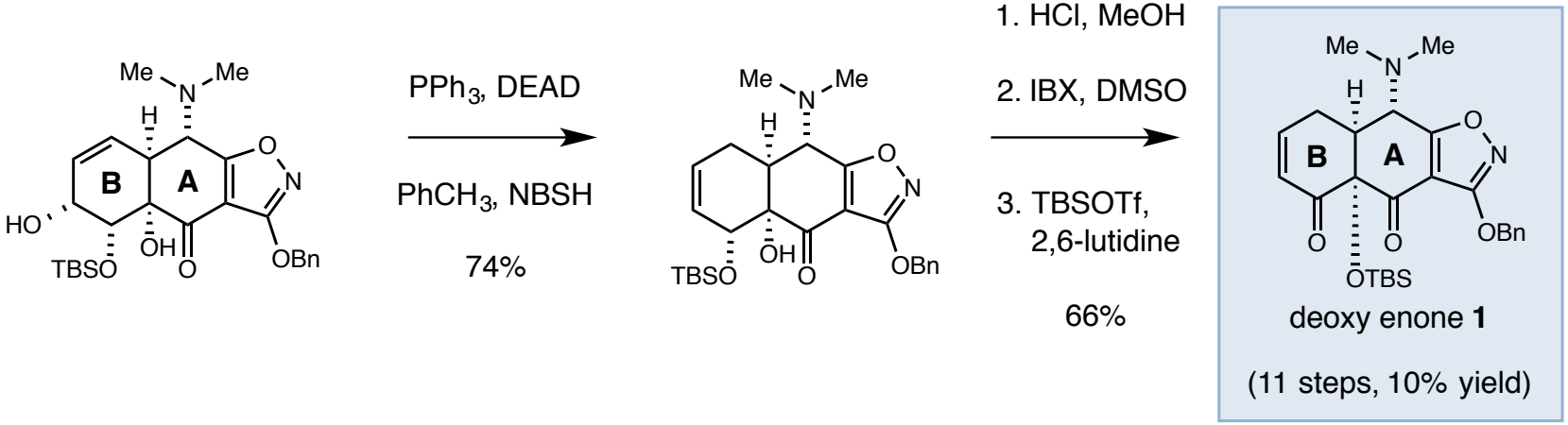
■ A,B ring synthesis



Charest, M. G.; Lerner, C. D.; Brubaker, J. D.; Siegel, D. R.; Myers, A. G. *Science* **2005**, *308*, 395.

The Myers Synthesis

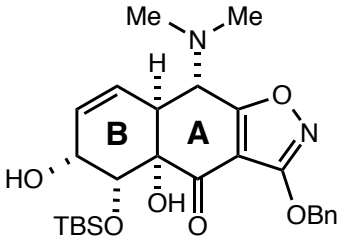
■ A,B ring synthesis - deoxy enone **1**



Charest, M. G.; Lerner, C. D.; Brubaker, J. D.; Siegel, D. R.; Myers, A. G. *Science* **2005**, *308*, 395.

The Myers Synthesis

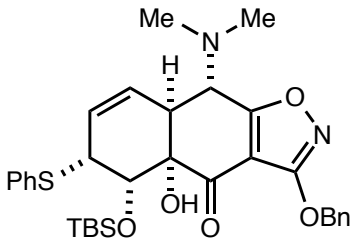
■ A,B ring synthesis - oxy enone **2**



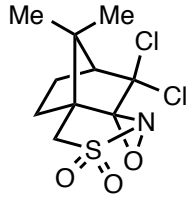
1. CBr_4, PPh_3

2. PhSH, Et_3N

87%

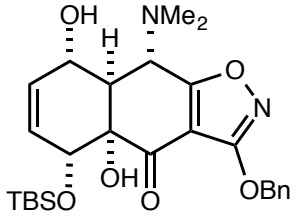


1.

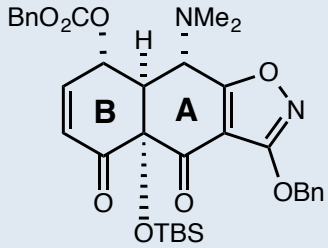


2. $P(OMe)_3$, MeOH, 70°C

76%



1. BnO_2CCl, DMAP
2. TBAF, HOAc
3. IBX, DMSO
4. TBSOTf, Et_3N



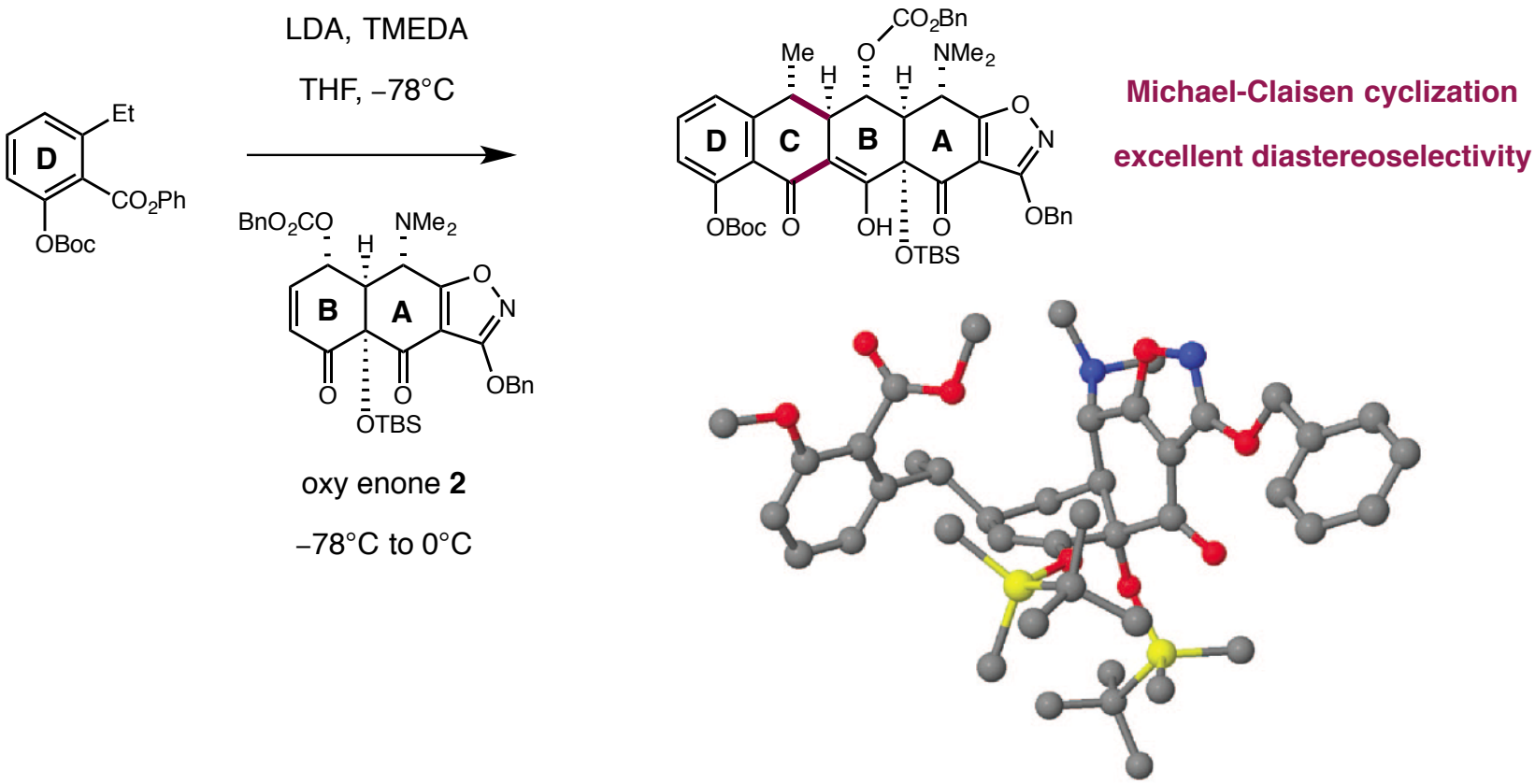
oxy enone **2**

(15 steps, 12% yield)

Charest, M. G.; Lerner, C. D.; Brubaker, J. D.; Siegel, D. R.; Myers, A. G. *Science* **2005**, *308*, 395.

The Myers Synthesis

■ Key step forms C ring, resulting in ABCD architecture



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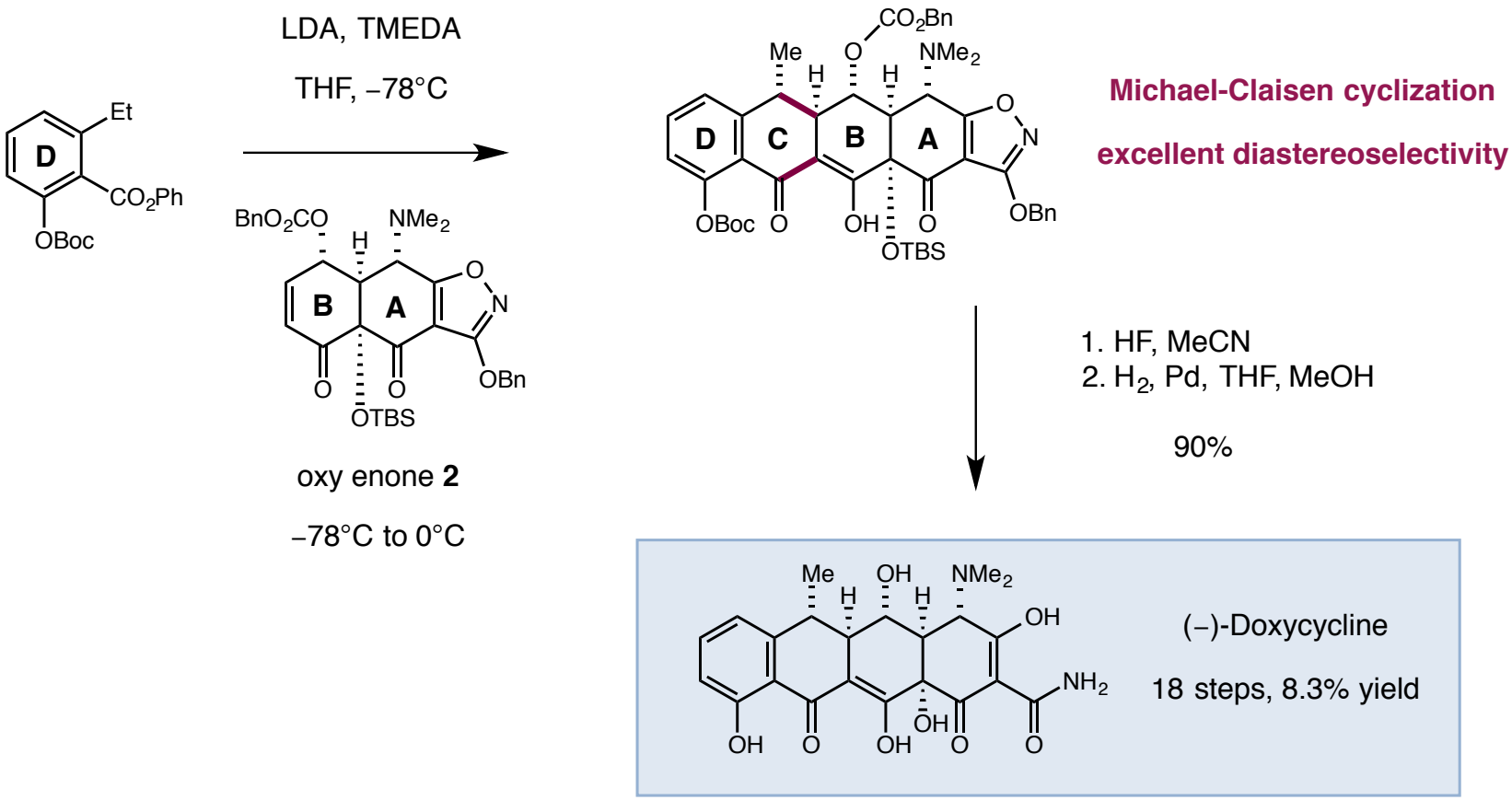

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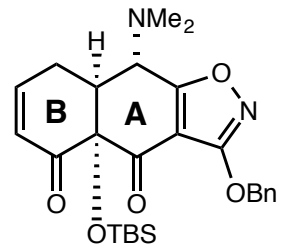
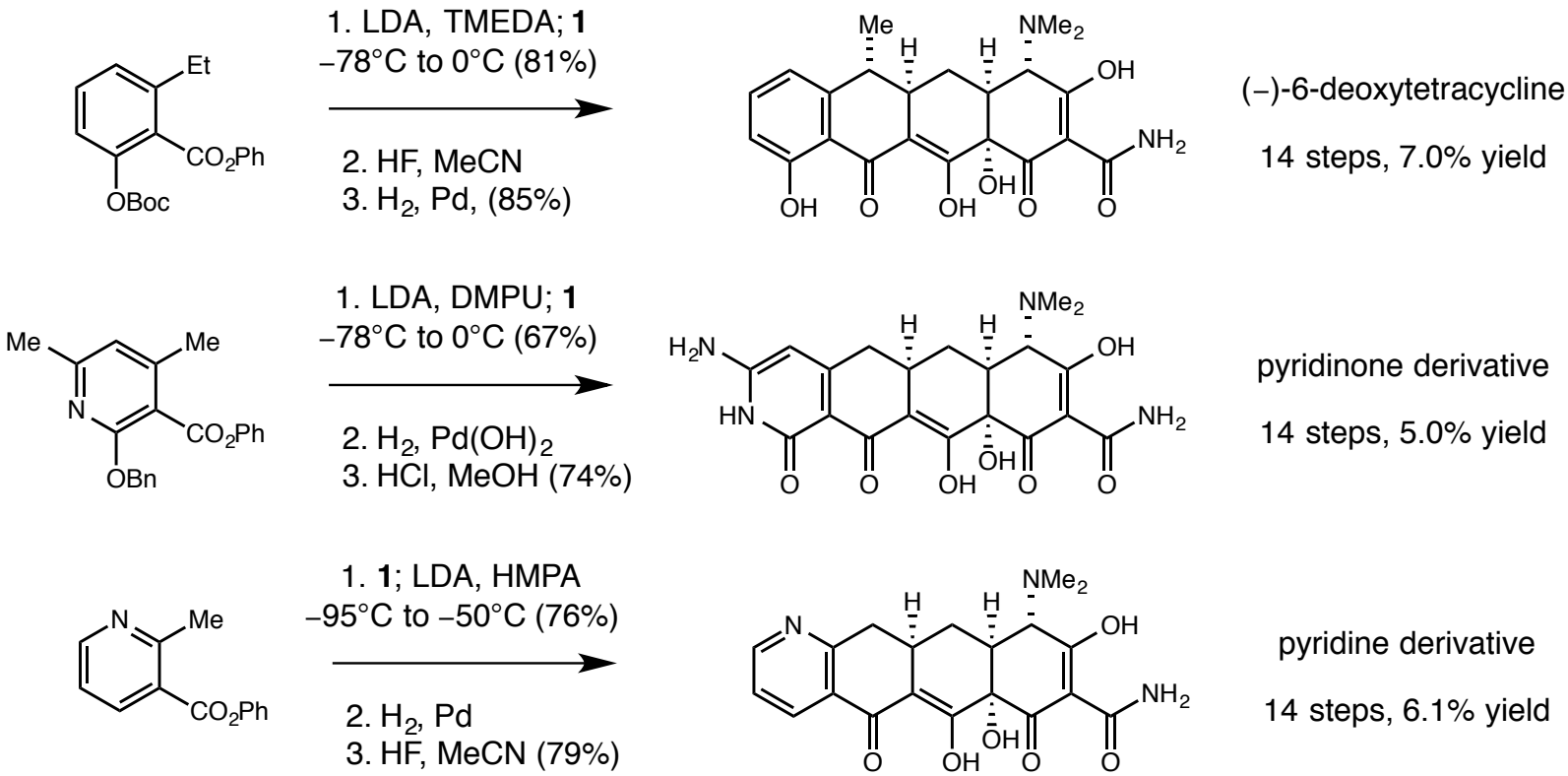
The Myers Synthesis

■ Late-stage C-ring construction allows rapid diversification

1 =





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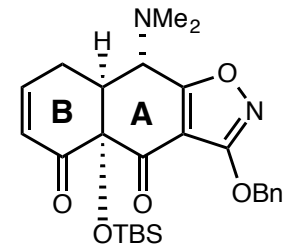
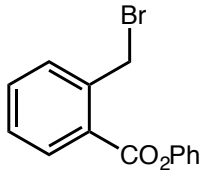
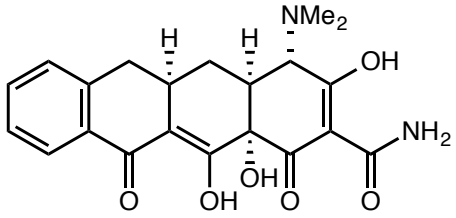




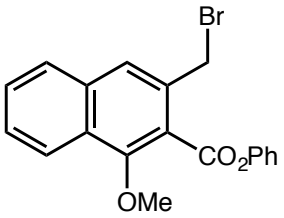
1. **1**; *n*-BuLi
–100°C to –70°C (81%)

2. HF, MeCN
3. H_2, Pd, (83%)



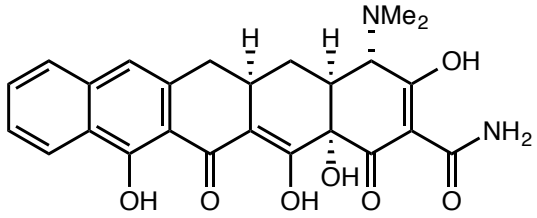
10-deoxysancycline

14 steps, 6.8% yield



1. **1**; *n*-BuLi
–100°C to 0°C (75%)

2. HF, MeCN
3. H_2, Pd
4. BBr_3, CH_2Cl_2
–78 to 23°C (74%)



pentacycline derivative

15 steps, 5.6% yield

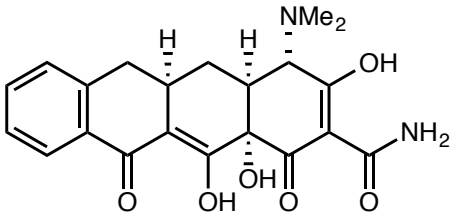
Charest, M. G.; Lerner, C. D.; Brubaker, J. D.; Siegel, D. R.; Myers, A. G. *Science* **2005**, *308*, 395.

The Myers Synthesis

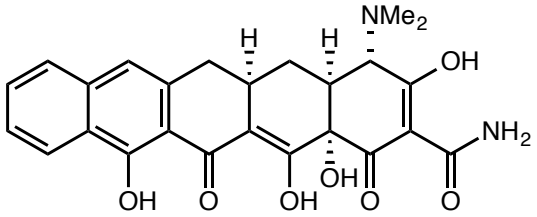
■ The pentacycline derivative showed promising antibacterial activities

Bacterial Strains Tested				
Gram-Positive Organisms				
S. aureus ATCC 29213	S. epidermidis ACH-0016	S. haemolyticus ACH-0013	E. faecalis ATCC 700802	S. aureus ATCC 700699
Gram-Negative Organisms				
P. aeruginosa ATCC 27853	K. pneumoniae ATCC 13883	E. coli ATCC 25922	E. coli ACH-0095	E. coli pBR322

(–)-Tetracycline



pentacycline derivative



MIC (μg/mL)					
	1	1	8	1	>64
	32	32	1	>64	>64

1	0.5	1	1	1
>64	>64	>64	>64	>64

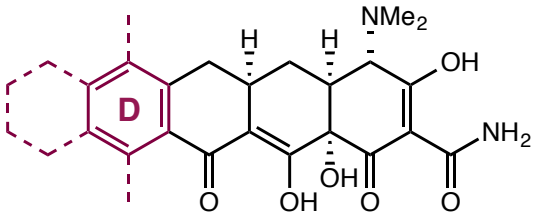
Charest, M. G.; Lerner, C. D.; Brubaker, J. D.; Siegel, D. R.; Myers, A. G. *Science* **2005**, *308*, 395.

Tetraphase Starts Searching for Leads

■ Tetraphase begins investigating various classes of tetracycline analogues

- Pentacyclines
- 8-Azatetracyclines
- Fluorocyclines

■ Focus on D ring manipulation to overcome tetracycline-resistance



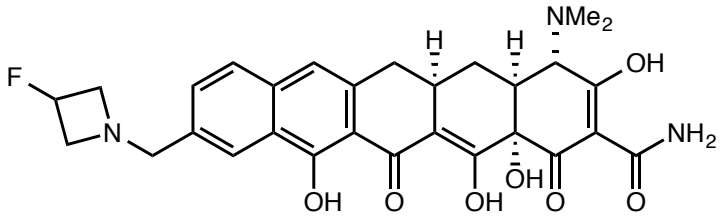
Two primary tetracycline-resistance mechanisms:

1) active transport via efflux pumps (*tet*A–*tet*D, *tet*K–*tet*L)

2) ribosomal protection (*tet*M–*tet*O)

Tetraphase Starts Searching for Leads

■ Pentacyclines deliver potential candidates showing strong in vitro and in vivo data



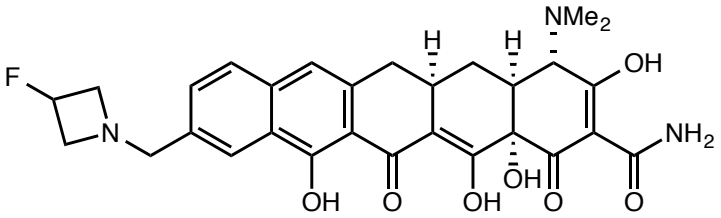
In vitro MIC data

		MIC (μg/mL)[a]												
		SA101	SA161[b]	SA158[c]	EF103	EF159[c]	SP106	SP160[c]	EC107	EC155[c]	AB110	PA111	ECl108	KP109	KP153[c]
		29213	MRSA, tetM	tetK	29212	tetM	49619	tetM	25922	tetA	19606	27853	13047	13883	tetA
19a-17	3-F-azetidinomethyl	0.5	2	0.5	0.5	2	0.125	0.25	2	8	1	>32	8	8	16
minocycline		0.0625	8	0.0313	1	16	≤0.0156	2	0.5	8	0.0625	16	2	1	8
tigecycline		0.0625	0.125	0.0625	0.0313	0.0625	0.0156	0.0156	0.0313	0.5	0.25	8	0.25	0.125	1

Sun, C.; Hunt, D. K.; Clark, R. B.; Lofland, D.; O'Brien, W. J.; Plamondon, L.; Xiao, X. -Y. *J. Med. Chem.* **2011**, *54*, 3704.

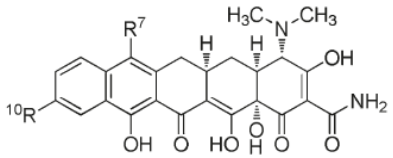
Tetraphase Starts Searching for Leads

■ Pentacyclines deliver potential candidates showing strong in vitro and in vivo data



"19a-17"

In vivo phamacokinetic profile

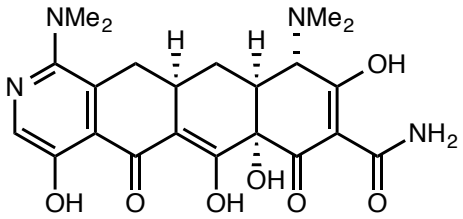
			PK (IV)[a]					%F[a]	MIC[b]	PD_{50}[c]	
			C_{max}	$AUC_{\infty obs}$	Cl	V_z	$T_{1/2}$		SA100	PO	IV
Compound	**R^7**	**R^{10}**	ng/mL	ng*hr/mL	mL/min/kg	L/kg	hr		µg/mL	mg/kg	mg/kg
19a-17	H	3-F-azetidinomethyl	814	3457	4.82	1.4	3.35	18	1	12.2 (3.6-20.8)	0.36 (0.17-0.55)
tetracycline			583	802	20.5	3.68	4.5	12	0.25	8.1 (0.25-16)	0.35 (0.34-0.37)
tigecycline			428	1052	15.5	6.12	4.6	1.1	0.0625	ND	0.35 (0.24-0.47)

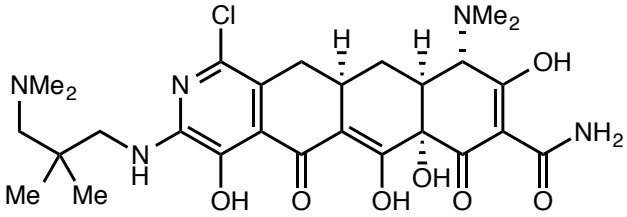
Sun, C.; Hunt, D. K.; Clark, R. B.; Lofland, D.; O'Brien, W. J.; Plamondon, L.; Xiao, X. -Y. *J. Med. Chem.* **2011**, *54*, 3704.

Tetraphase Starts Searching for Leads

■ 8-Azatetracyclines showed promise in overcoming tetracycline-resistance

"20f"





"24l"

In vitro MIC data

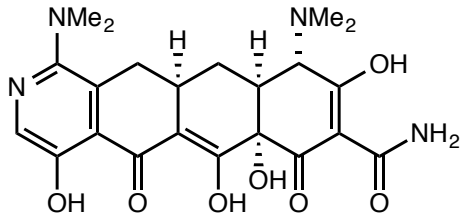
		MIC (μg/mL)							
		S. aureus			*S. pneumoniae*		*E. coli*		*K. pneumoniae*
compd	R_1	wild type[a]	*tet*(M)[b]	*tet*(K)[c]	wild type[d]	*tet*(M)[c]	wild type[e]	*tet*(A)[c]	wild type[f]
20f	$(CH_3)_2N$-	0.031	16	2	0.063	8	0.125	>32	0.25
24l		0.5	2	0.125	0.016	0.125	0.5	8	2
tetracycline		1	>32	32	0.25	32	2	>32	4
minocycline		0.125	16	0.25	<0.016	8	0.5	8	1

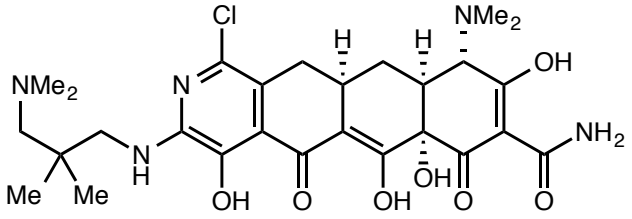
Clark, R. B.; He, M.; Fyfe, C.; Lofland, D.; O'Brien, W. J.; Plamondon, L.; Sutcliffe, J. A.; Xiao, X. -Y. *J. Med. Chem.* **2011**, *54*, 1511.

Tetraphase Starts Searching for Leads

■ 8-Azatetracyclines showed promise in overcoming tetracycline-resistance

"20f"





"24l"

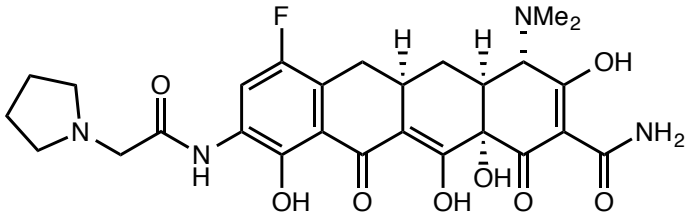
In vivo mouse septicemia model

	S. aureus			*E. coli*		
compd	MIC (μg/mL)	PD_{50} (mg/kg)	95% C.I.	MIC (μg/mL)	PD_{50} (mg/kg)	95% CI
20f	0.031	<0.30[b]		0.13	4.3	4.1−4.6
24l	0.5	0.36	0.36−0.56	0.5	17	4.1−30
tetracycline	0.25	0.35	0.34−0.37	1	17	7.3−27
tigecycline	0.063	0.35	0.24−0.47	0.13	2.1	1.8−2.4

Clark, R. B.; He, M.; Fyfe, C.; Lofland, D.; O'Brien, W. J.; Plamondon, L.; Sutcliffe, J. A.; Xiao, X. -Y. *J. Med. Chem.* **2011**, *54*, 1511.

Tetraphase Starts Searching for Leads

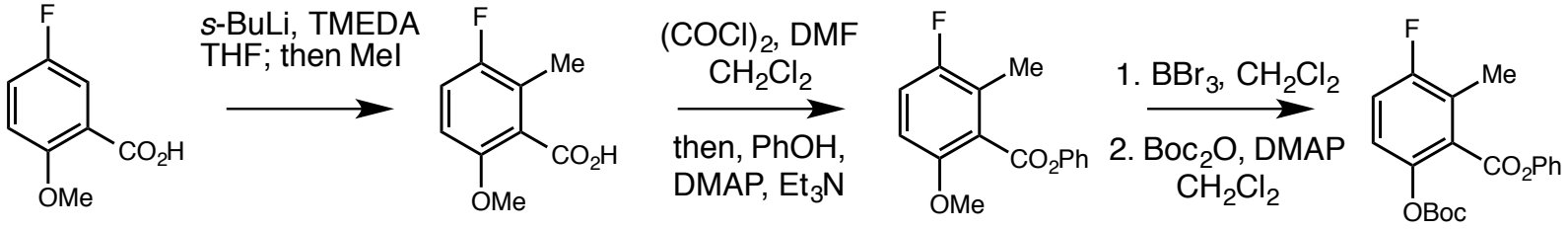
■ 7-Fluoro-9-pyrrolidinoacetamido-6-demethyl-6-deoxytetracycline shows best potency yet



"17j"

TP-434

■ Synthesis



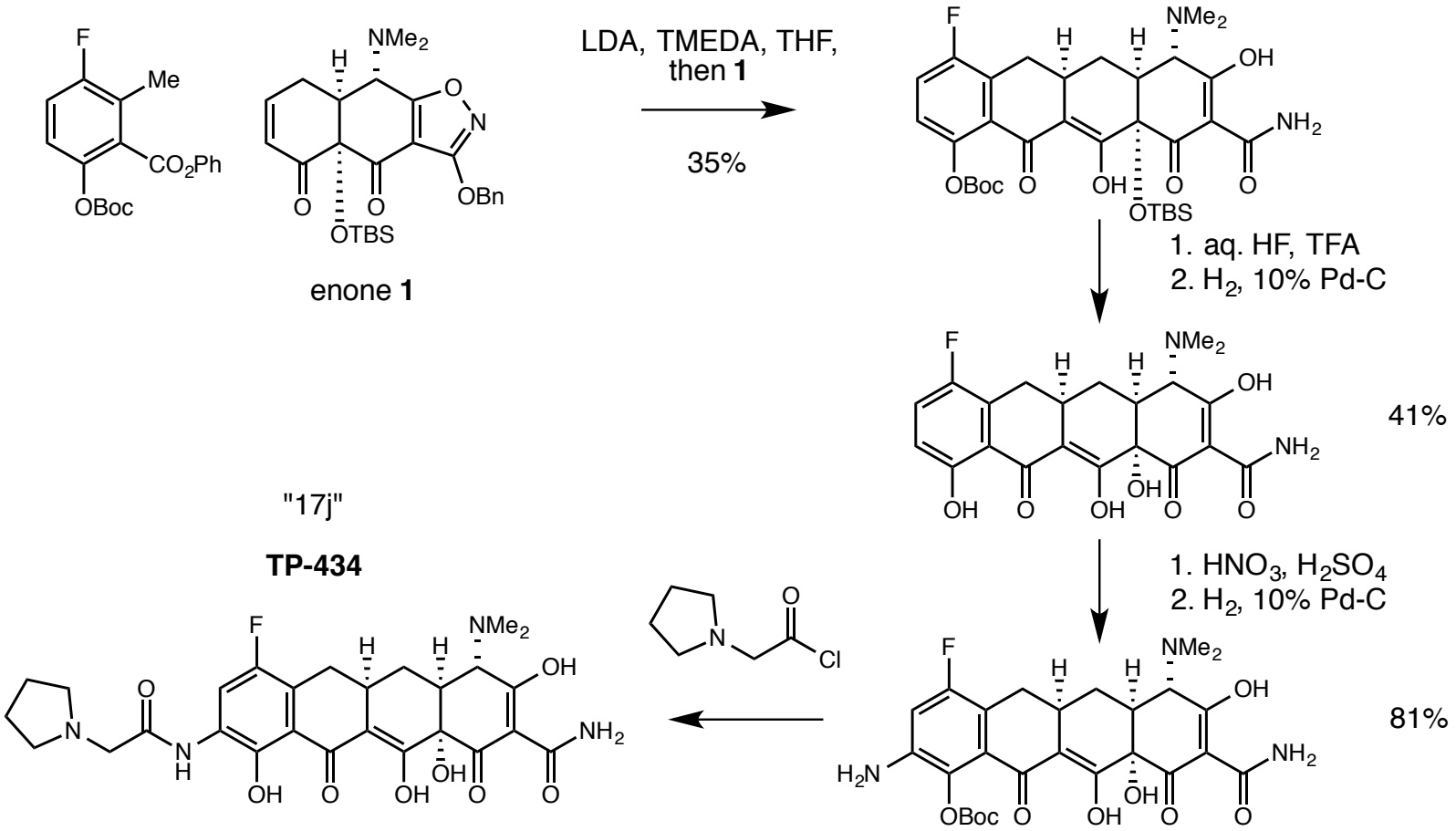
52% over 2 steps

75% over 2 steps

Xiao, X. -Y.; Hunt, D. K.; Zhou, J.; Clark, R. B.; Dunwoody, N.; Fyfe, C.; Grossman, T. H.; O'Brien, W. J.; Plamondon, L.; Ronn, M.; Sun, C.; Zhang, W. -Y.; Sutcliffe, J. A.; . *J. Med. Chem.* **2012**, *55*, 597.

Tetraphase Starts Searching for Leads

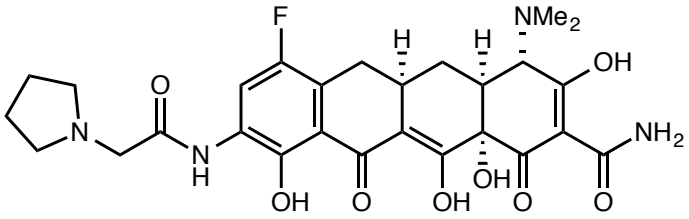
■ Rapid synthesis of fluorocycline analogue



Xiao, X. -Y.; Hunt, D. K.; Zhou, J.; Clark, R. B.; Dunwoody, N.; Fyfe, C.; Grossman, T. H.; O'Brien, W. J.; Plamondon, L.; Ronn, M.; Sun, C.; Zhang, W. -Y.; Sutcliffe, J. A.; . *J. Med. Chem.* **2012**, *55*, 597.

Tetraphase Starts Searching for Leads

■ TP-434 shows best efficacy of all Tetraphase compound library



"17j"

TP-434

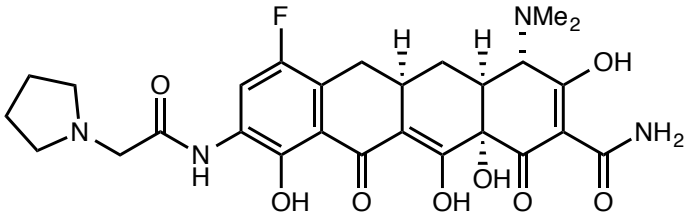
In Vitro Antibacterial Activity

Compound	RR'N-	MIC (μg/mL)[a]													
		SA101	SA161[b]	SA158[c]	EF103	EF159[c]	SP106	SP160[c]	EC107	EC155[c]	AB110	PA111	EC108	KP109	KP153[c]
		29213	MRSA, *tet* (M)	*tet* (K)	29212	*tet* (M)	49619	*tet* (M)	25922	*tet* (A)	19606	27853	13047	13883	*tet* (A)
17j		0.0156	0.0156	0.0156	0.0156	0.0156	0.0156	0.0156	0.0156	1	0.0312	8	0.125	0.125	0.5
Tetracycline		0.125	64	32	16	64	0.25	32	1	>64	1	16	1	2	>64
Tigecycline		0.0625	0.125	0.125	0.0625	0.0625	0.0156	0.0156	0.125	1	0.5	16	0.25	0.25	1

Xiao, X. -Y.; Hunt, D. K.; Zhou, J.; Clark, R. B.; Dunwoody, N.; Fyfe, C.; Grossman, T. H.; O'Brien, W. J.; Plamondon, L.; Ronn, M.; Sun, C.; Zhang, W. -Y.; Sutcliffe, J. A.; . *J. Med. Chem.* **2012**, *55*, 597.

Tetraphase Starts Searching for Leads

■ TP-434 shows best efficacy of all Tetraphase compound library



"17j"

TP-434

In Vivo Activity

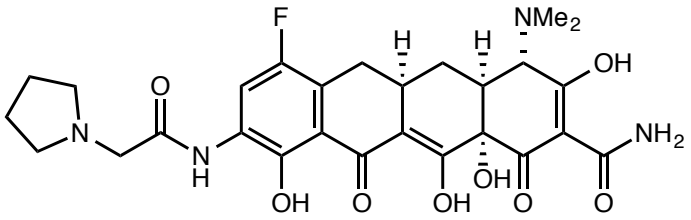
model	strain		17j	tigecycline	vancomycin
murine septicemia	EC133 *tet* (B)	MIC (μg/mL)	0.125	0.125	NT
		PD_{50} (mg/kg)	1.3	3.5	NT
neutropenic thigh	SA191 *tet* (M) (MRSA)	MIC (μg/mL)	0.25	0.25	1
		dose at 1 log reduction (mg/kg)	0.6	3	0.75
		dose at 3 log reduction (mg/kg)	3	17.3	10

compd	dosage route (mg/kg)	CLs (L/h/kg)	V_z (L/kg)	$T1/2$ (L/kg)	C_{max} (hr)	AUC_{last}	% F (%)
17j	IV (1)	0.564	3.2	4.0	0.812	1.766	
	PO (10)			6.9	0.045	0.295	1.7
tetracycline	IV (1)	0.542	1.2	4.6	2.664	3.083	
	PO (10)			5.2	0.791	4.536	14.9
tigecycline	IV (1)	0.929	6.12	4.6	0.428	1.052	
	PO (10)			3.98	0.0278	0.107	1.0

Xiao, X. -Y.; Hunt, D. K.; Zhou, J.; Clark, R. B.; Dunwoody, N.; Fyfe, C.; Grossman, T. H.; O'Brien, W. J.; Plamondon, L.; Ronn, M.; Sun, C.; Zhang, W. -Y.; Sutcliffe, J. A.; . *J. Med. Chem.* **2012**, *55*, 597.

"Eravacycline" Moves Forward

■ TP-434 renamed "eravacycline" and is moved onto clinical trials



TP-434 = eravacycline

■ Tetraphase timeline:

Feb. 2012: Biomedical Advanced Research and Development Authority (BARDA) award Tetraphase with $67 million contract for development of eravacycline

Jul. 2013: Eravacycline designated a Qualified Infectious Disease Product (QIDP) by FDA

Sept. 2013: Eravacycline entered Phase 3 clinical trials (for cIAI and cUTI)

TP-834 and TP-271 currently in preclinical development

■ Mar. 2013 - Tetraphase initial public offering on NASDAQ (10,714,286 shares at $7.00 each)

- Currently trading between $10-$12 per share


TetraLogic
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PEPTIDREAM INC.
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TETRAPHASE
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