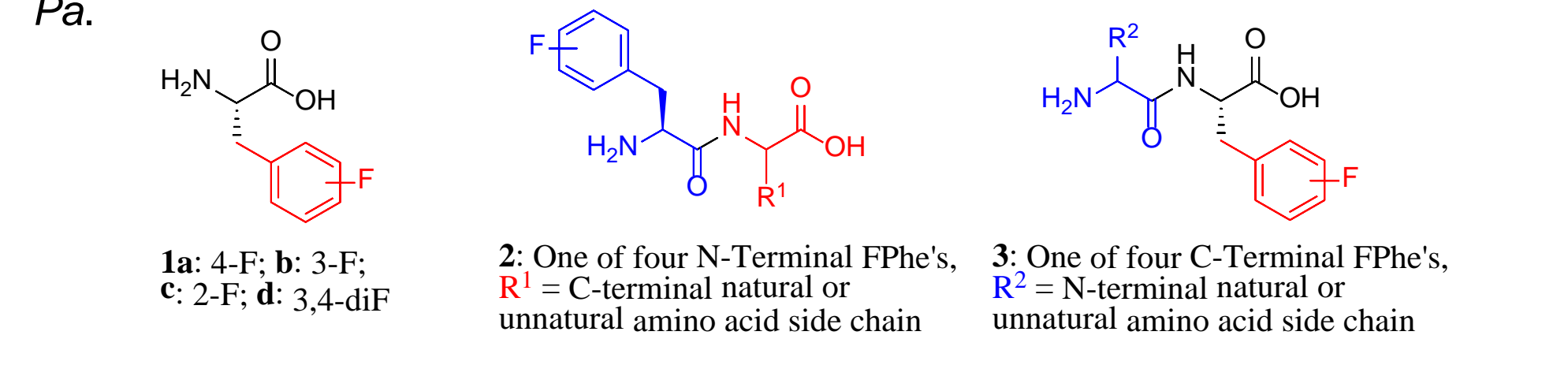


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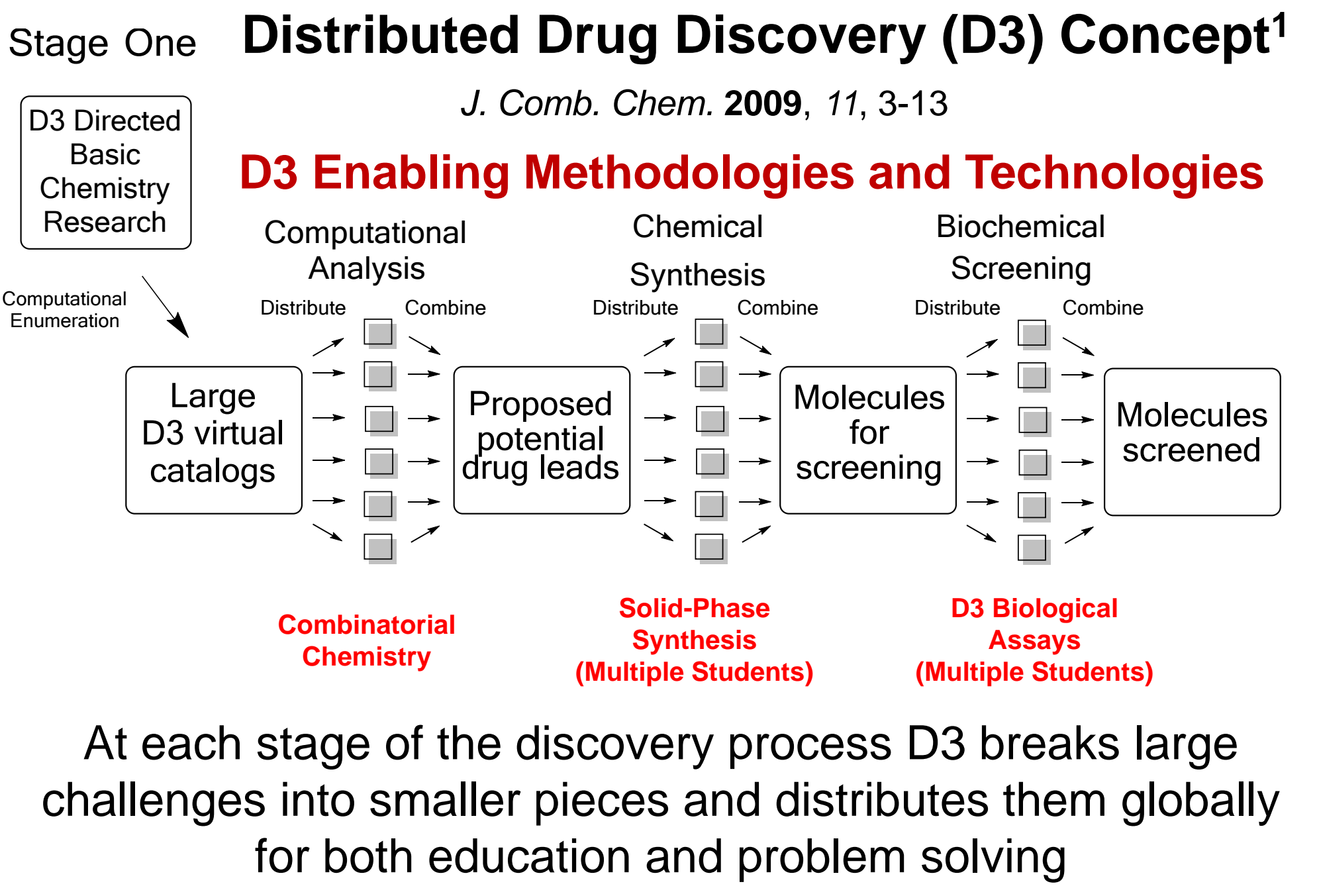
^aIndiana University Purdue University Indianapolis (IUPUI), ^bColorado College, ^cSanta Clara University, ^dUniversidad Nacional Autónoma de México, ^eUniversity of Puerto Rico, ^fInnoventyx, ^gGoshen College, ^hUniversity of Indianapolis

Abstract

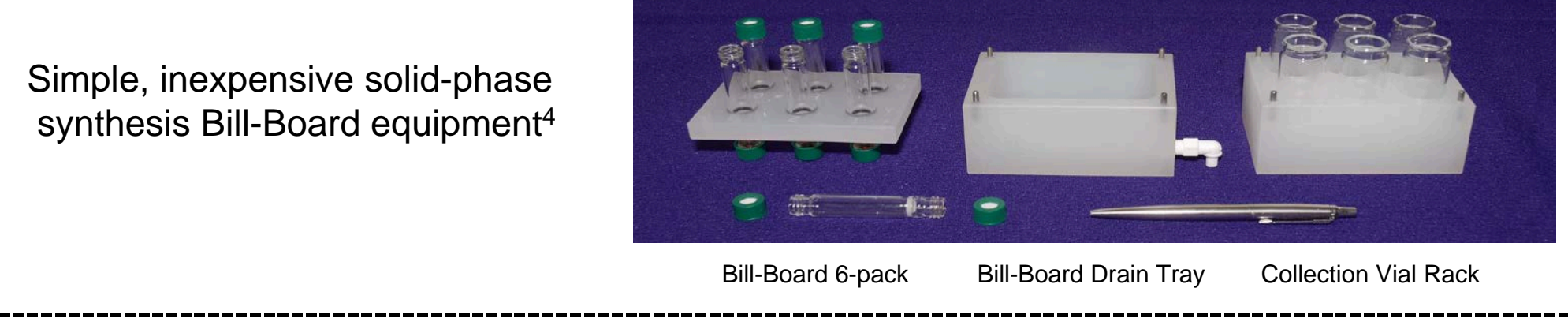
Through Distributed Drug Discovery (D3)¹ the unnatural amino acid (S)-4F-phenylalanine (**1a**: -4F) was rediscovered as a potent inhibitor of *Pseudomonas aeruginosa* (Pa) growth. Three close analogs (**1b**: -F; **1c**: 2-F; and **1d**: 3,4-diF) were also made and found to be active. Subsequently it was shown that unnatural dipeptides of generic structure **2** and **3** were active, presumably as prodrugs of **1**. The D3 program, which teaches solid-phase organic and peptide synthesis to students at global schools,^{2,3} targeted an 80 compound subset of **2** for distributed synthesis. In this subset, the C-terminal residue is one of 20 natural amino acids and the N-terminal residue is either (S)-4F, 3-F, 2-F, or 3,4-diF-phenylalanine. A complementary biology laboratory was developed at IUPUI to explore the activity of these compounds against Pa. We report the multi-school distributed synthesis and testing of members of this targeted 80 compound subset and the resulting discovery of many unnatural dipeptides **2** active against Pa.



The D3 Vision



Equipment Used: Simple Solid-Phase Synthesis Apparatus



D-Tyrosine analogs: Candidates for D3 *Pseudomonas aeruginosa* (Pa) antibiotic research?

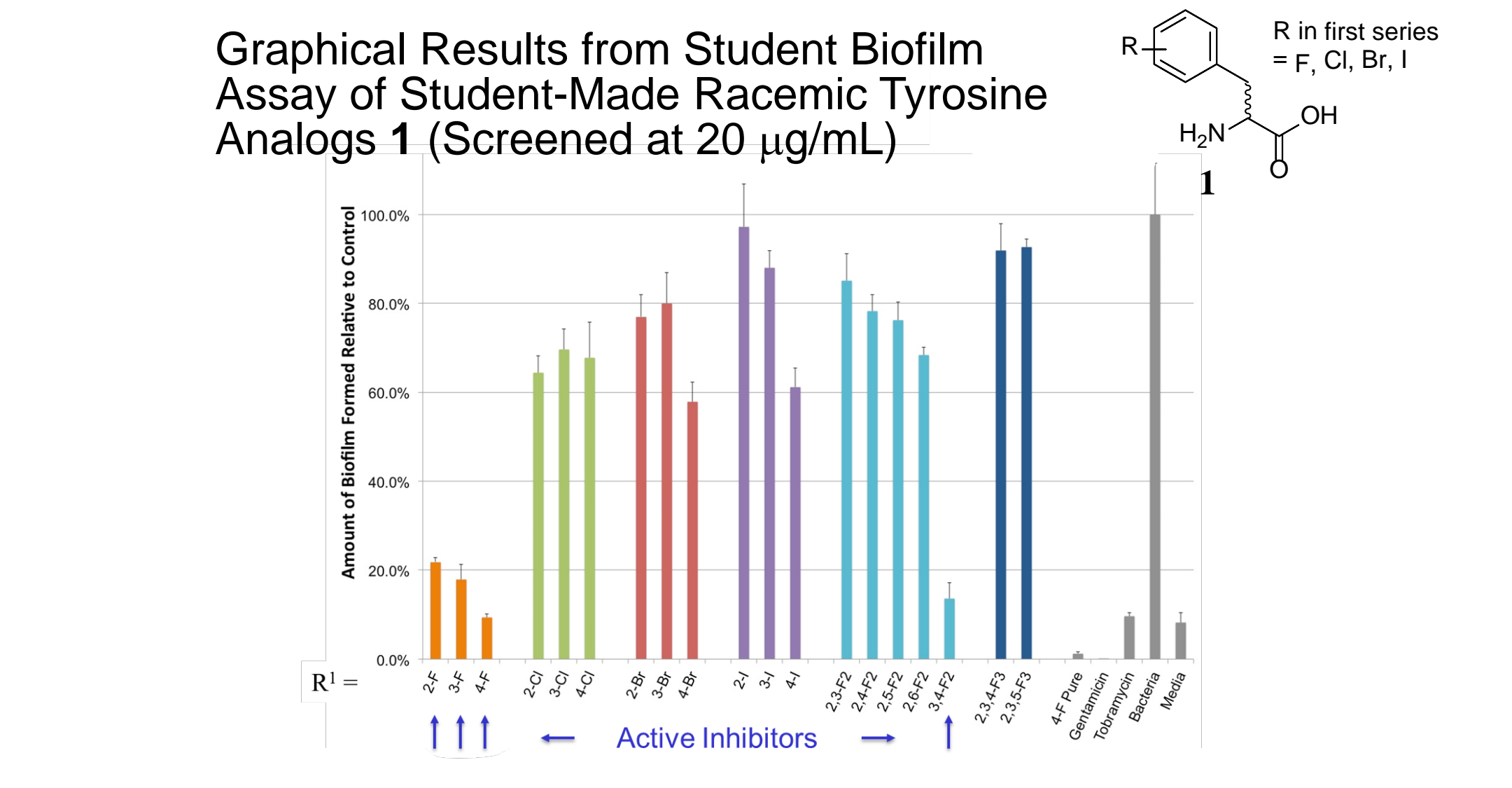
Target: Analogs of Unnatural enantiomer D-Tyrosine

D-Amino Acids Trigger Biofilm Disassembly: Kolodkin-Gal I, Romero D, Cao S, Clardy J, Kolter R, Losick R, *Science* **328**, 627 (2010). (Results not reproduced: Kao et al. *Laryngoscope Investigative Otolaryngology* **2**: February 2017, p. 4-9)

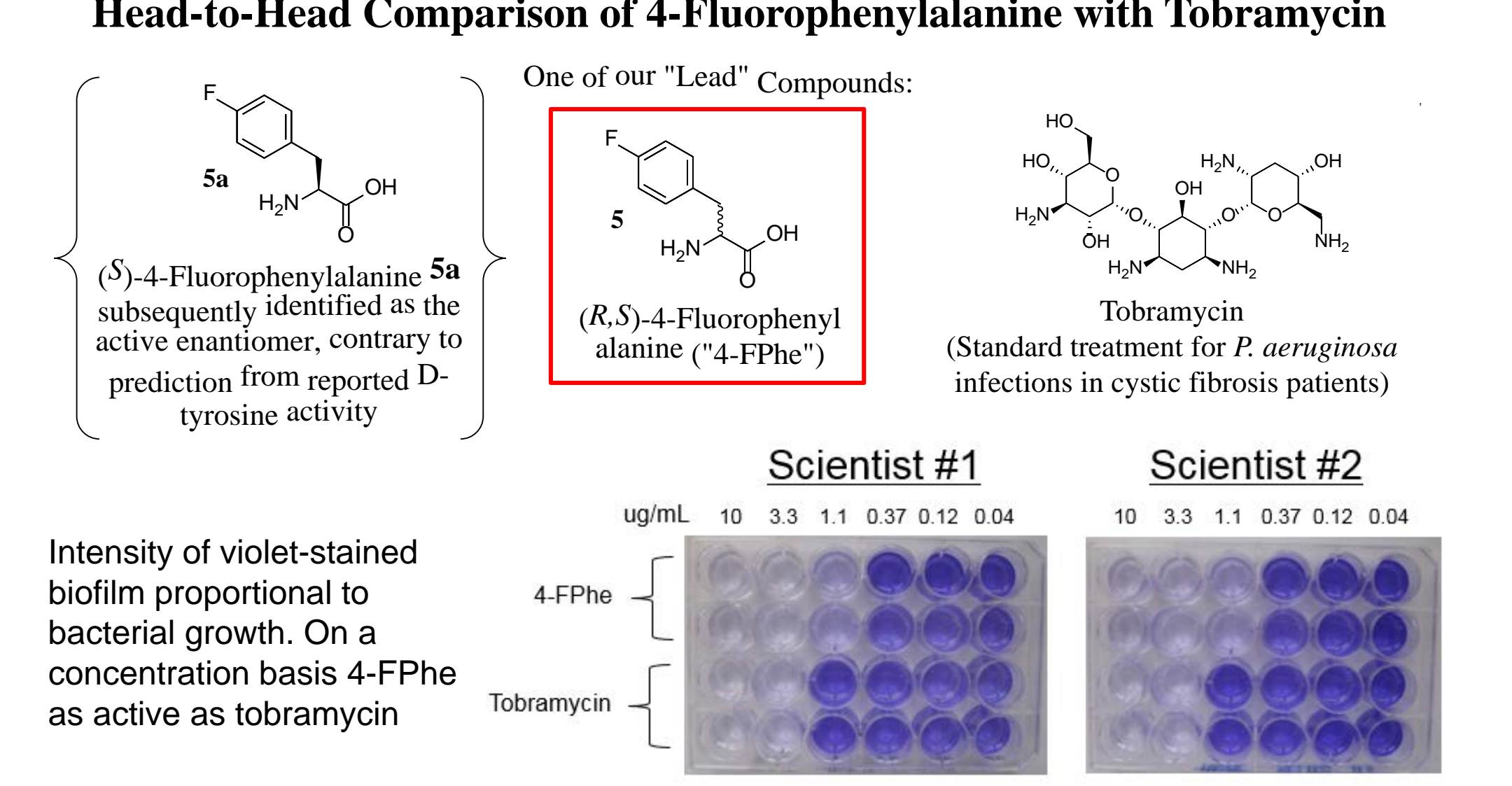
Possible drug relevance: D-tyrosine reported to modulate biofilm formation by *Pseudomonas aeruginosa*

1, Generic version of racemic tyrosine analogs accessible by O'Donnell/Scott unnatural amino acid SPS (*J Am Chem Soc* **1996**, *118*, 6070-1)

Identification of FPhe Pa inhibitors



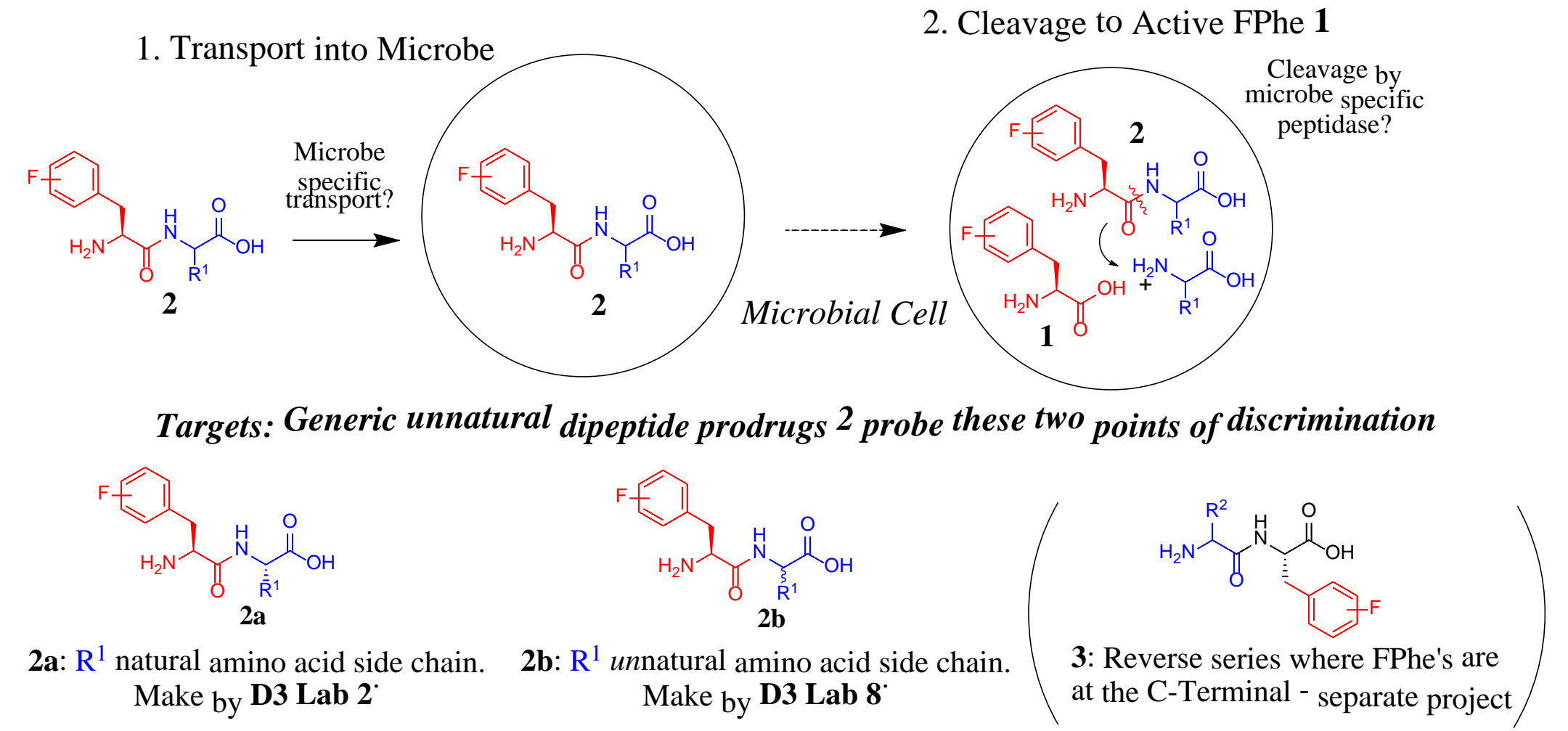
4-FPhe Hit Follow Up



Exciting, but 4-Fluorophenylalanine Antimicrobial Activity Known in 1949!
 "In Vitro Effects of Metabolite Displacers on *Pseudomonas Aeruginosa*" (G.J. Martin and J.N. Moss, *Amer. J. Pharm and the Sciences Supporting Public Health*, **1949**, *121*, 169-172)
 Other literature noted mammalian toxicity: How to minimize – Prodrugs?

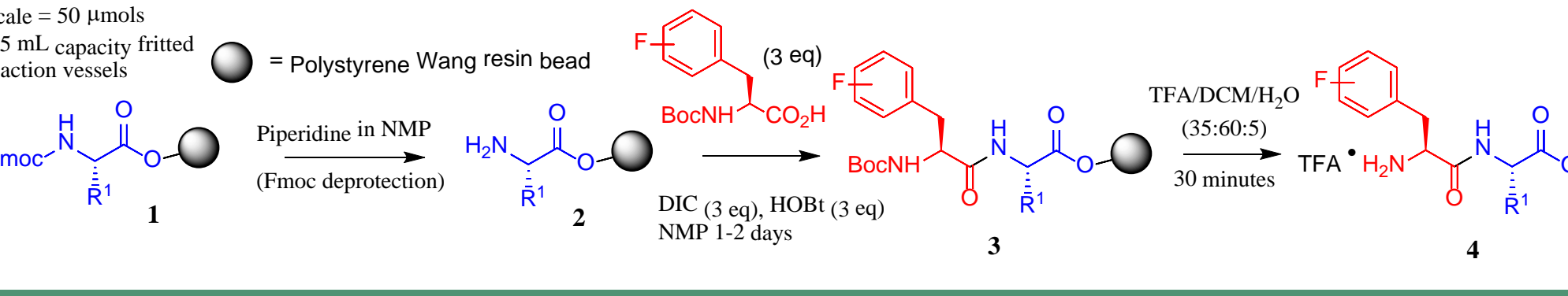
D3 Prodrugs of FPhe's

Unnatural Dipeptide Prodrugs **2** or **3**: Possible Points of Selective Toxicity for Microbes versus Mammalian Cells

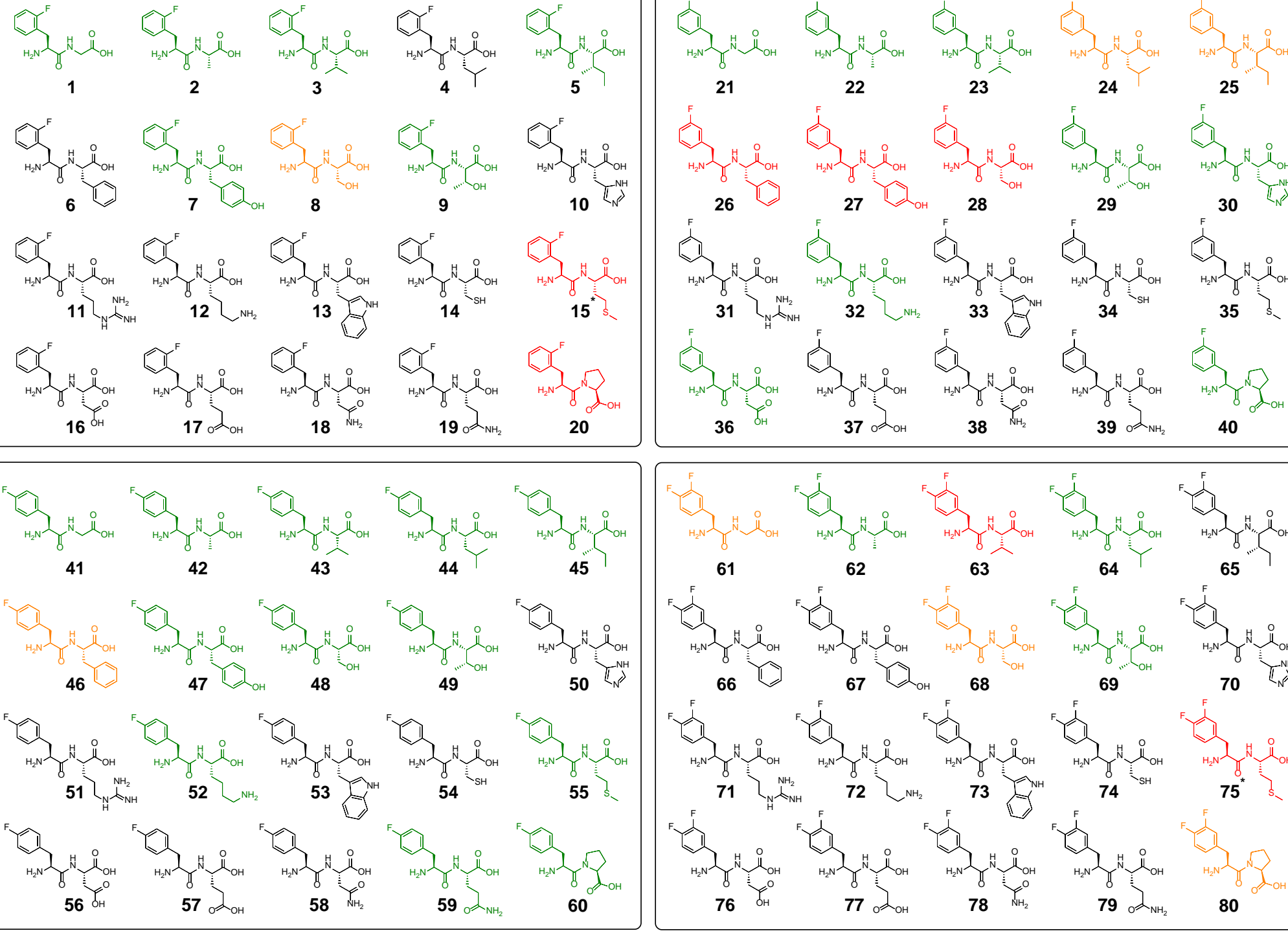


Goal: Distributed Synthesis/Testing of 80 Compounds **2a** (four FPhe's x 20 AAs)

D3 Unnatural Dipeptide Lab 2



80 Compound **2a** Target Quadrants with Test Results



Summary and Conclusions

Summary and Conclusions:

- D3 Synthesis and testing of subset of 80 target prodrugs identifies many potent Pa inhibitors
- Activity likely a function of documented transport⁵ of dipeptides into Pa with subsequent enzymatic cleavage
- Most active quadrant has N-terminal 4-FPhe, consistent with activity of 4-FPhe itself
- Data (not shown) on unnatural FPhe N-terminal dipeptides with *unnatural C-terminal* AA's shows critical nature of this residue - many close analogs are inactive (e.g. 4-FPhe-D-Ala, 4-FPhe-L-Pip, 4-FPhe-L-Phg are inactive at 10 µg/mL)

Important Qualifications: There is no indication these molecules directly inhibit biofilm growth – instead they inhibit biofilms by inhibiting bacterial growth. Also, activity of FPhe's is only seen in the absence of Phe in the media (Phe antagonizes activity - literature indicates FPhe's are antimetabolites acting through Phe pathways)

Challenges: 1) Coordinating multiple sites 2) Collecting and curating compounds – physically and electronically 3) Synthesizing compounds with problematic amino acids

References and Acknowledgements

References:

- J. Comb. Chem.* **2009**, *11*, 1-13; 14–33
- J. Chem. Educ.* **2015**, *92*, 819–826
- J. Chem. Educ.* **2019**, *96*, 1731-1737
- Bill-Board from Chemglass Life Sciences CG-1869
- PLoS One.* 2014; 9(10): e111311. doi: 10.1371

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Analytical Support: Lilly Research Laboratories

Contact email: wscott@iupui.edu

Student Researchers: Multiple chemistry and biology students too numerous (>2000) to list individually

Best So Far

From set made and tested most active compound is **42**. Estimated IC₅₀ = 0.5 µg/mL

Other D3 Goals

• Emphasize Replication

Project Cmpd#	School	Lot #	TFA salt?	10 ug/mL	5 ug/mL	1 ug/mL	0.5 ug/mL
42	IUPUI	Ave C344 SP19	Yes	0%			
42	IUPUI	F18-T01-B1	Yes	2%		37%	
42	CC	CH-6-A1	No		2%	9%	42%
42	CC	BW-6-A1	No		2%	4%	22%
42	Uindy	UI-S19-T1-A1	Yes	1%		3%	
42	Uindy	UI-S19-T2-A1	Yes	4%		46%	
42	Uindy	UI-S19-T3-A1	Yes	5%		53%	
42	Uindy	UI-S19-T4-A1	Yes	4%		28%	
42	Uindy	UI-S19-T5-A1	Yes	-1%		14%	
42	Uindy	UI-S19-T6-A1	Yes	2%		80%	

e.g. Compound **42** (4-FPhe-Ala) made by multiple researchers and each lot tested by multiple biologists

• Teach Chemistry
 Students learn solid-phase organic or peptide synthesis through making unnatural dipeptides **2a** in regular organic synthesis teaching labs or while doing independent research

• Teach Biology
 Students in regular microbiology lab learn about bacterial growth while testing new compounds as potential antibiotics. Independent student researchers study compound biological activity in more detail

• Teach Drug Discovery
 Students learn the collaborative interdisciplinary research process critical to drug discovery. They participate in drug discovery for under-researched diseases, in this project the search for drugs to treat *P. aeruginosa* infections

• Collaborate Globally
 D3 seeks to harness the power of globally distributed research to expand resources while uniting scientists around a common humanitarian challenge. Outside the continental United States this project added Puerto Rico and Mexico to the D3 collaborative network.