Targeting Mitochondria

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SCI/RSC Symposium on Advances in Synthesis and Medicinal Chemistry

BioPark, Welwyn Garden City, UK

May 1, 2012

- A Collaborative Approach to Drug Discovery
- Chemical Diversity & Peptide Bond Isosteres
- Targeting Mitochondria
  - General Strategies
  - Gramicidin S Based Nitroxide Targeting Agents
  - Biological Analyses of XJB-5-131 & JP4-039

Opportunities & Challenges in Academic Drug Discovery

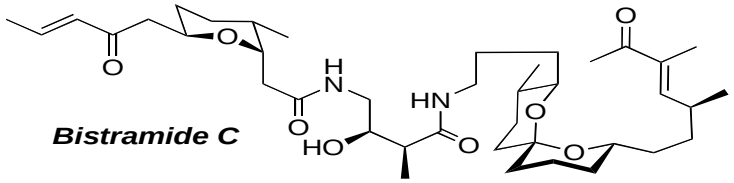
- The pharmaceutical industry is strongly market-driven and under cost pressures.
- More fundamental drug discovery research is shifting to Universities and independent institutes.
- Curiosity-driven research might lead to innovative new targets and novel lead structures, but also end up pursuing non-druggable targets and unselective leads.
- HTS-driven hit identification is costly and inefficient for small academic groups; a paradigm shift away from HTS might be necessary.
- It is difficult to assemble pre-INDA project teams in academia (synthetic, biological, analytical, pharmacokinetics, toxicological, regulatory, legal...).
- Most projects will have to be handed off to companies pre-INDA or at Phase I. This raises IP and licensing challenges.

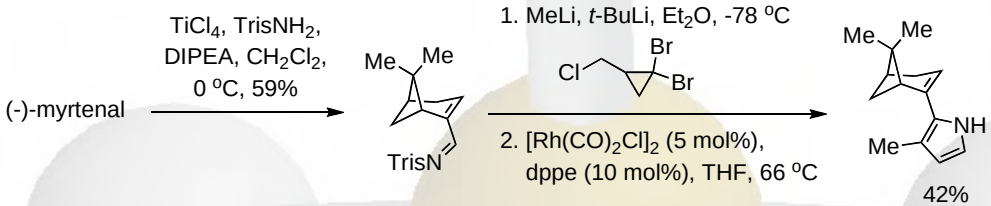
Overview of Our Medicinal Chemistry Projects

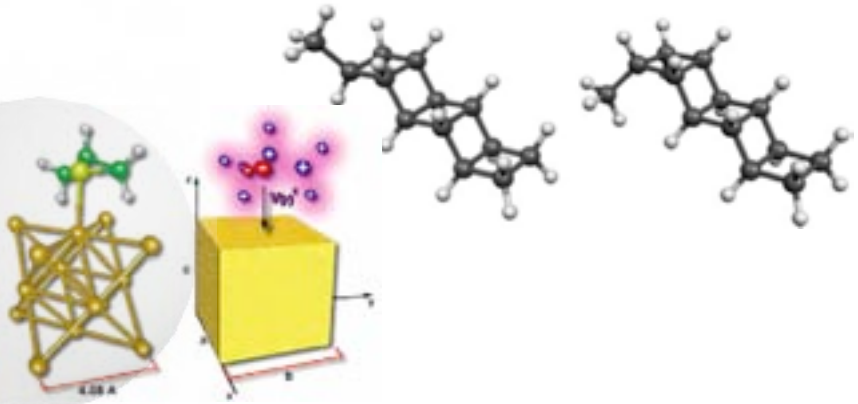
- CDC25 Phosphatases*: Preclinical/Early Stage
- Tubulin Disruptors*: Preclinical/Early Stage
- PI-3 Kinase*: Clinical/Phase II
- Hsp70*: Preclinical/Early Stage
- BoNT/A LC Inhibitors: Preclinical/Early Stage
- Natural Killer T-Cells/Immunology: Preclinical/Early Stage
- Neurodegeneration & Radiation: Preclinical/Under Development
- Malaria: Preclinical/Early Stage
- PKD*: Preclinical/Early Stage
- Ca-Channel Agonists: Preclinical/Early Stage
- STAT-3*: Preclinical/Early Stage

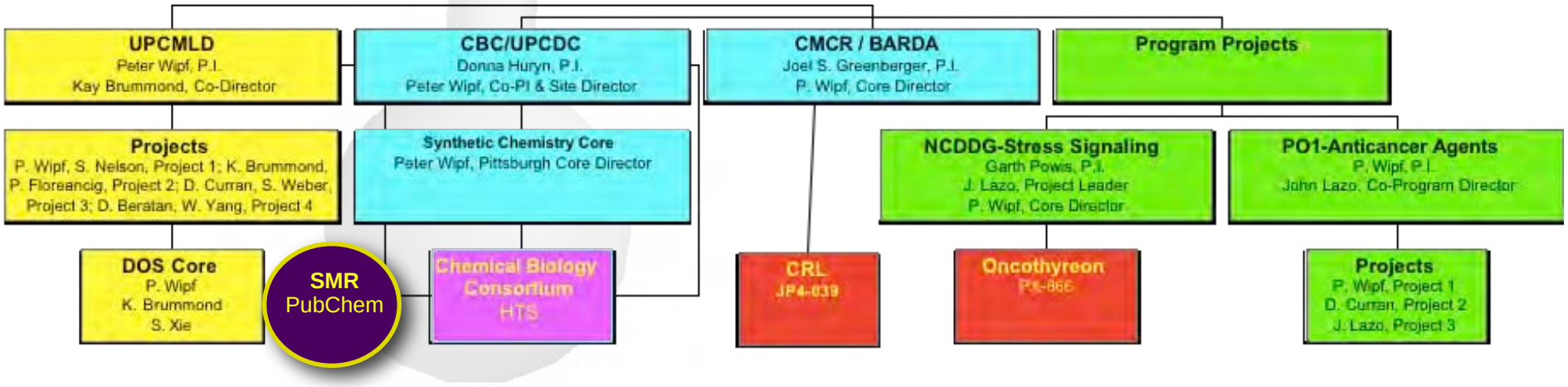
A Highly Integrated, Collaborative Academic Drug Discovery Model

- **Natural Product Total Synthesis**
- **Organometallic Methodology**
- **Chiroptical Analyses & Computation**
- **Medicinal Chemistry**



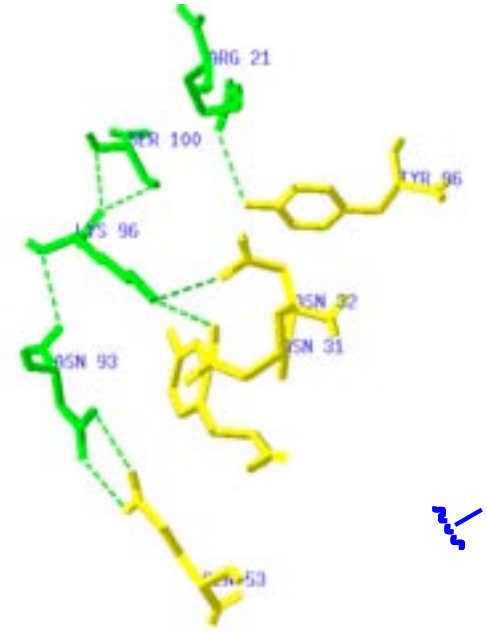


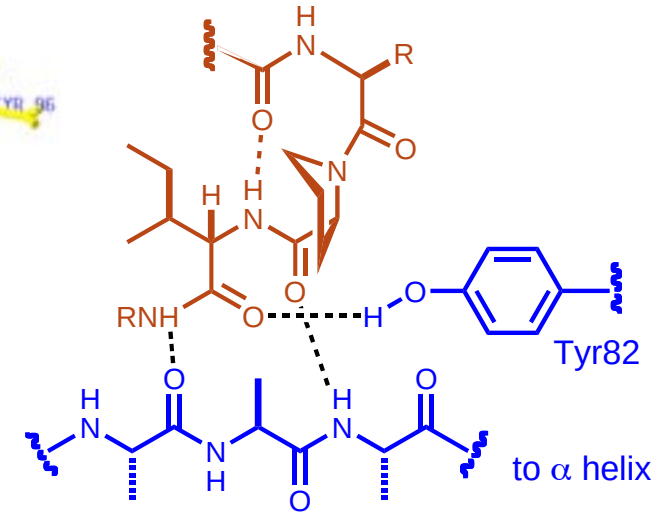




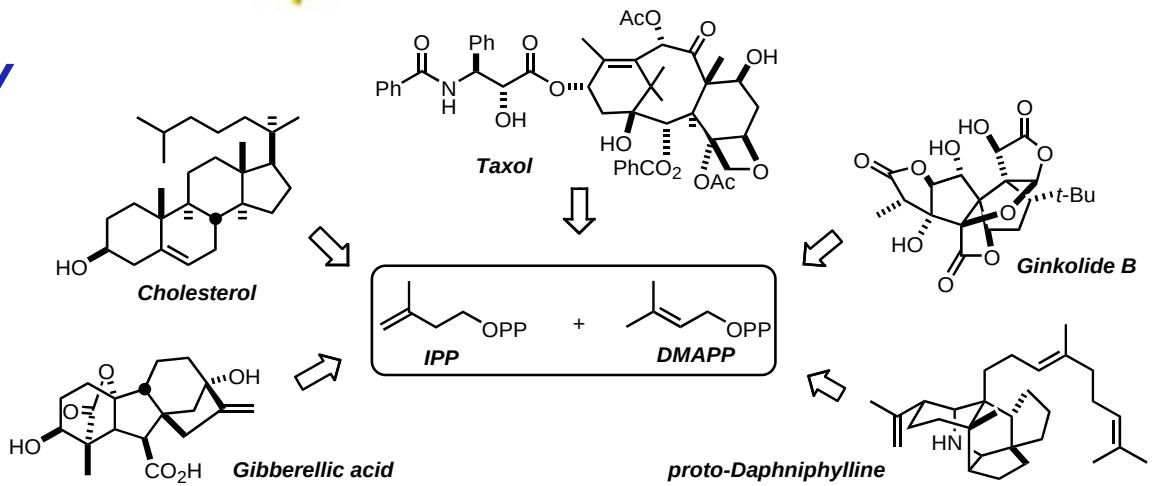
Fundamental Chemical Diversity Elements

- *Side Chain Diversity*

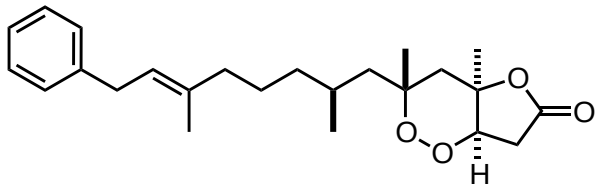




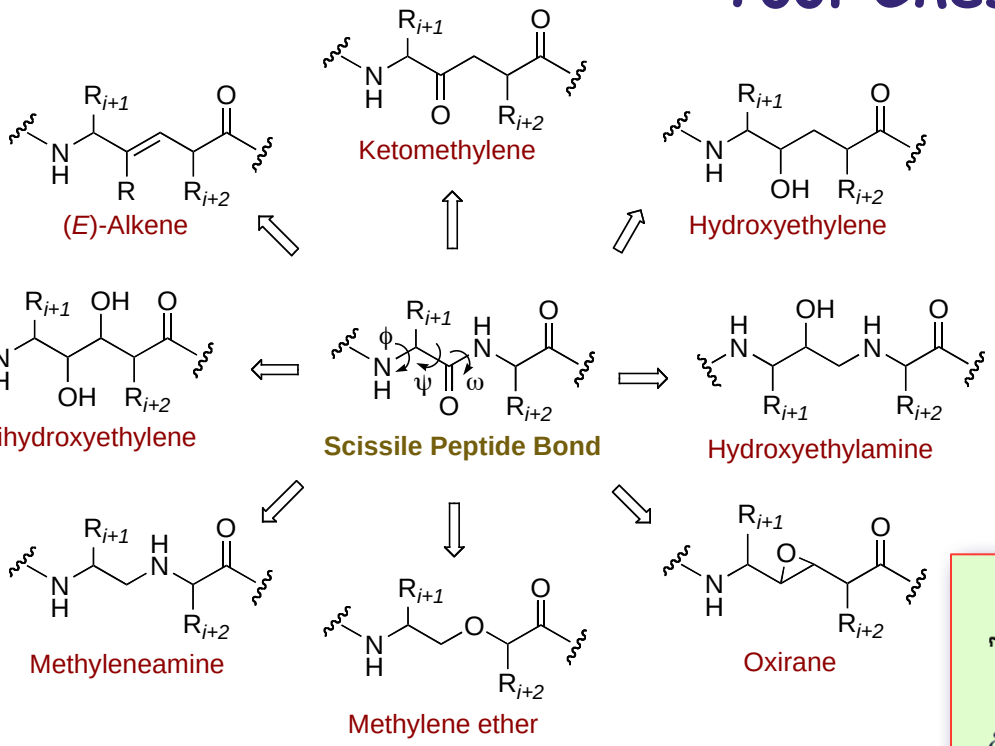
- *Scaffold Diversity*

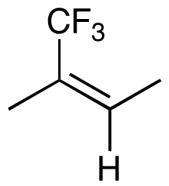


- *Functional Group Diversity*

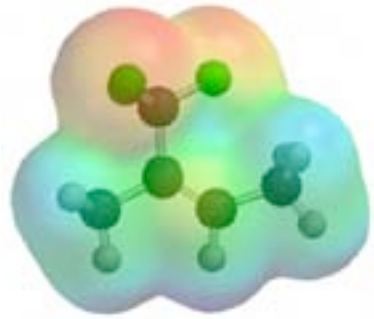


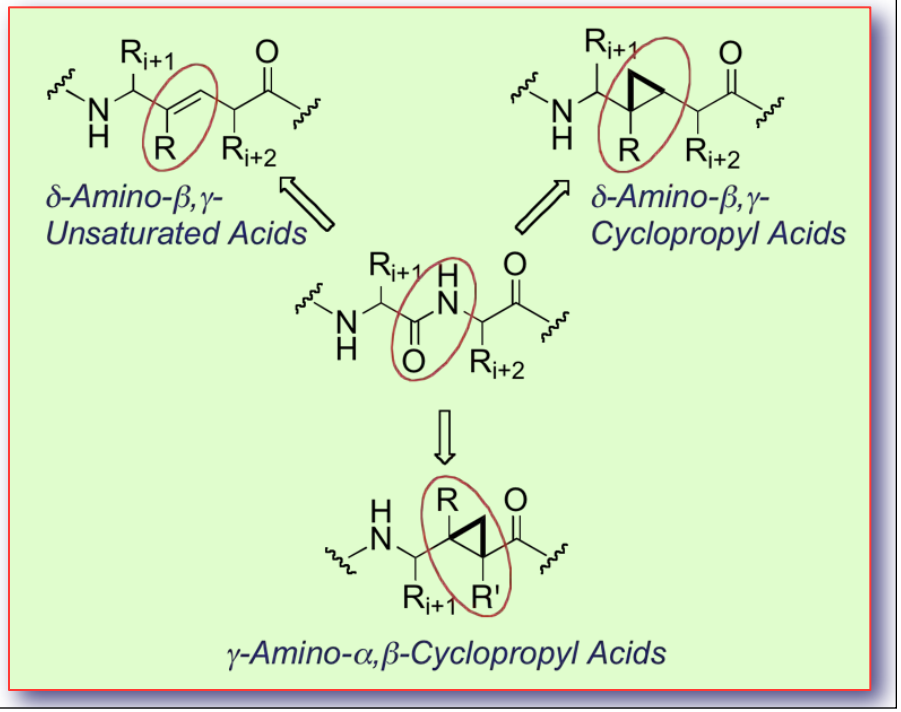
Combining Side Chain & Scaffold Diversity with the Golden Tool Chest



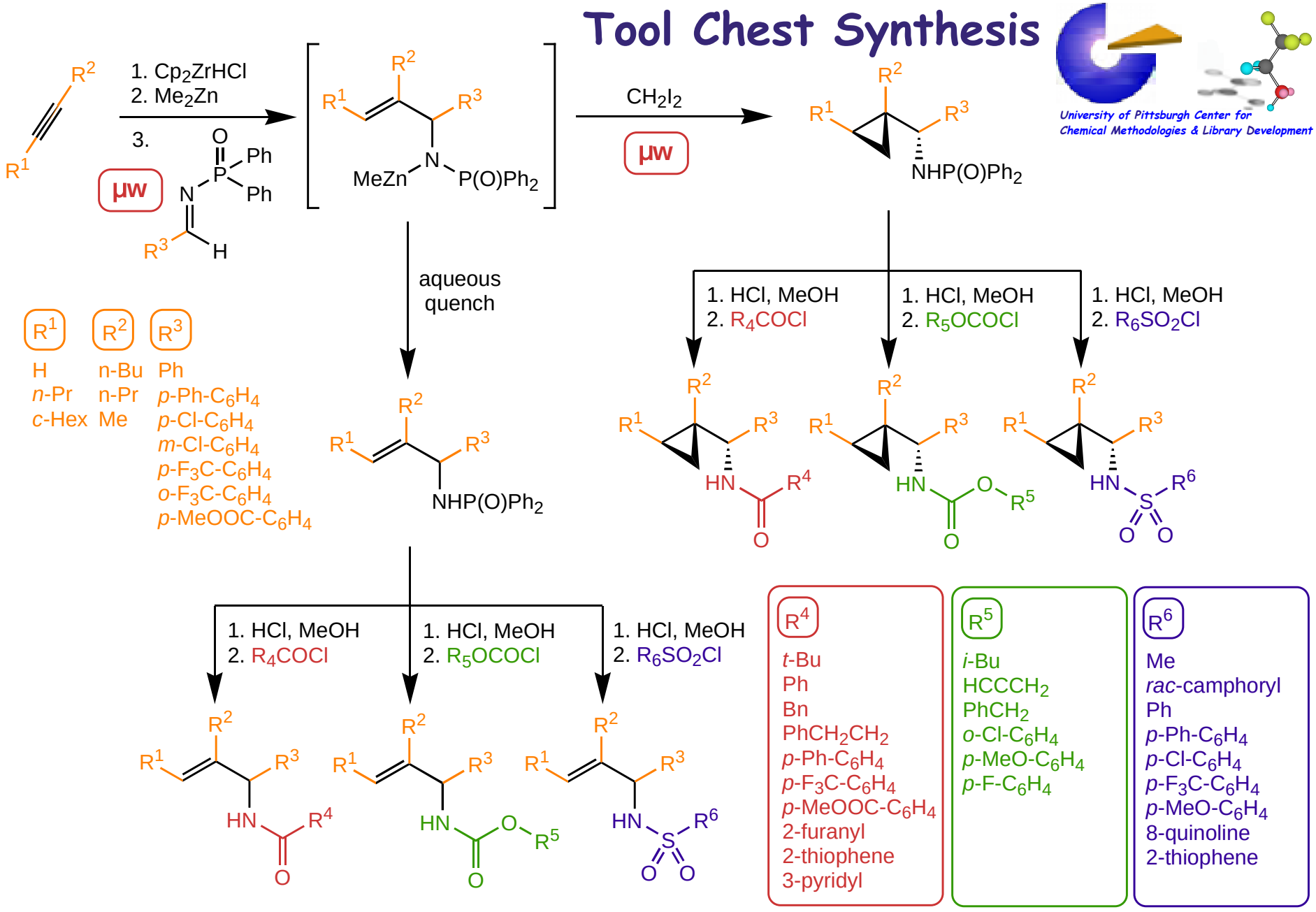


μ = 2.3 D





Tool Chest Synthesis

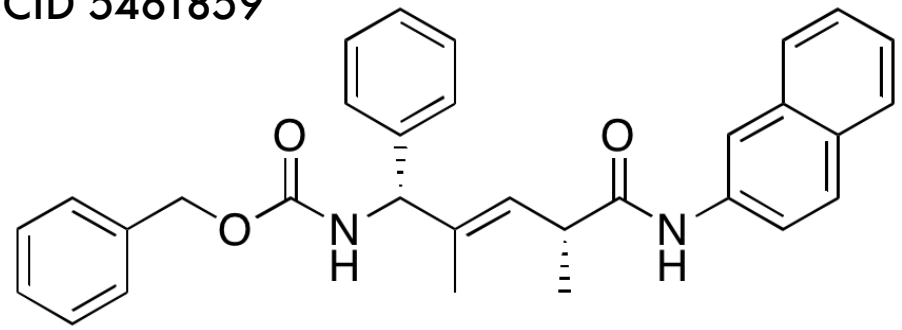


University of Pittsburgh Center for Chemical Methodologies & Library Development

Wipf, P., Coleman, C. M., Janjic, J., Iyer, P. S., Fodor, M. F., Shafer, Y. A., Stephenson, C. R. J., Kendall, C., Day, B. W. *J. Comb. Chem*. **2005**, *7*, 322-330.

Does "Backbone Inversion" Lead to Biological Diversity?

CID 5461859



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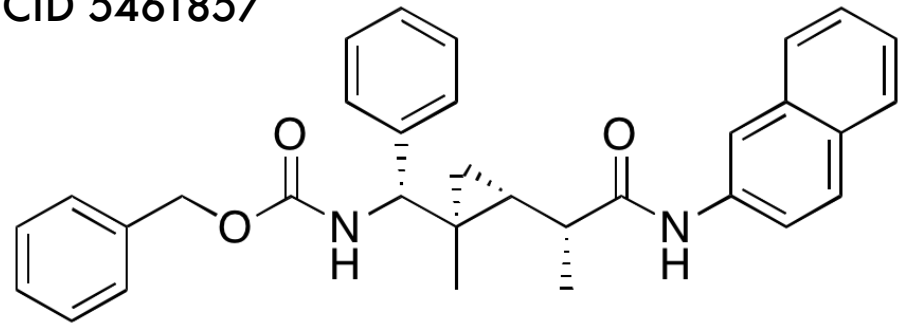

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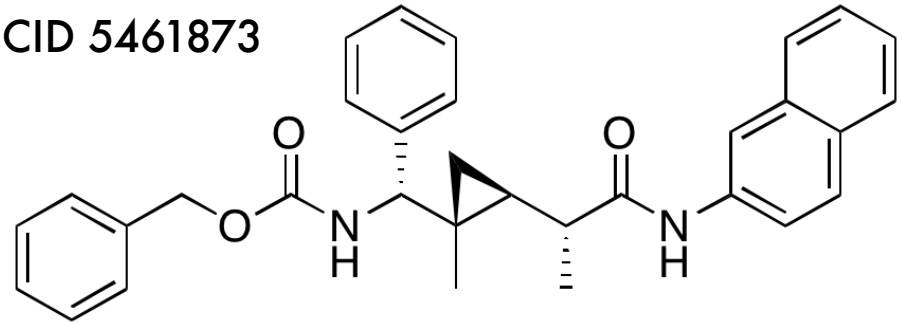

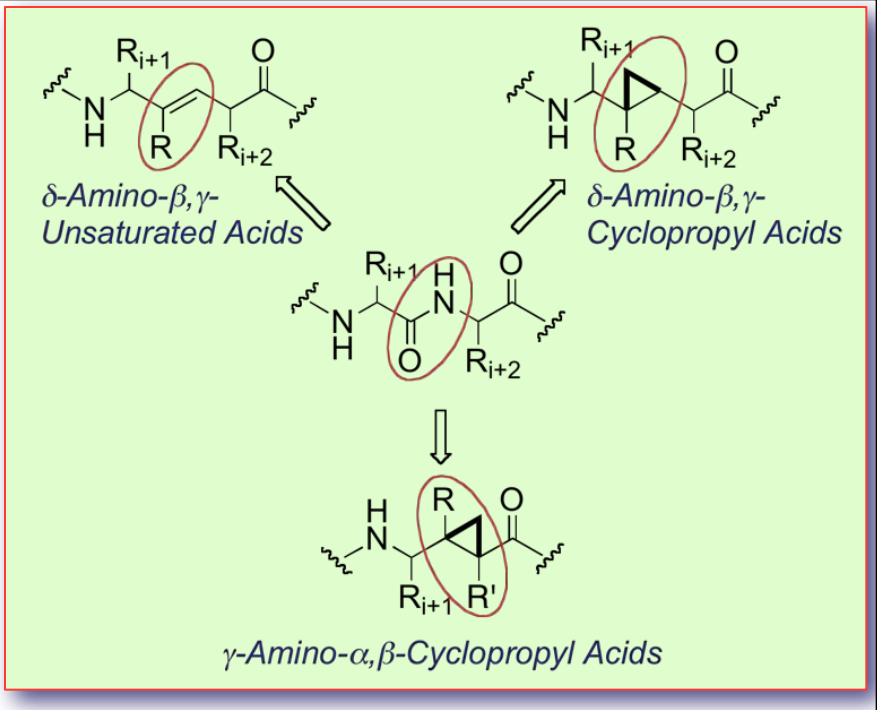

PubChem BioAssay

Does "Backbone Inversion" Lead to Biological Diversity?

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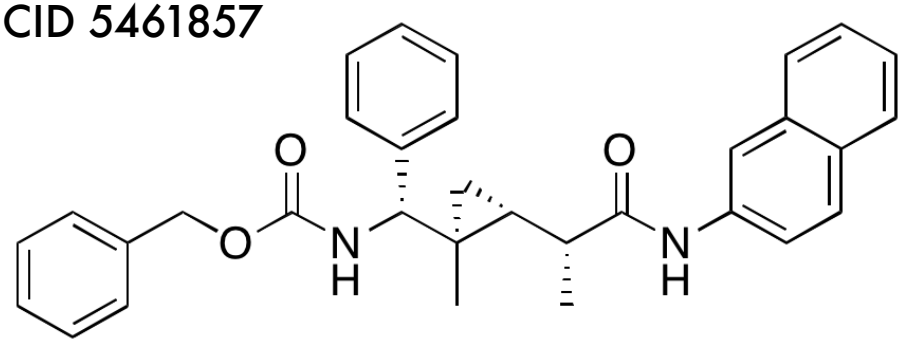

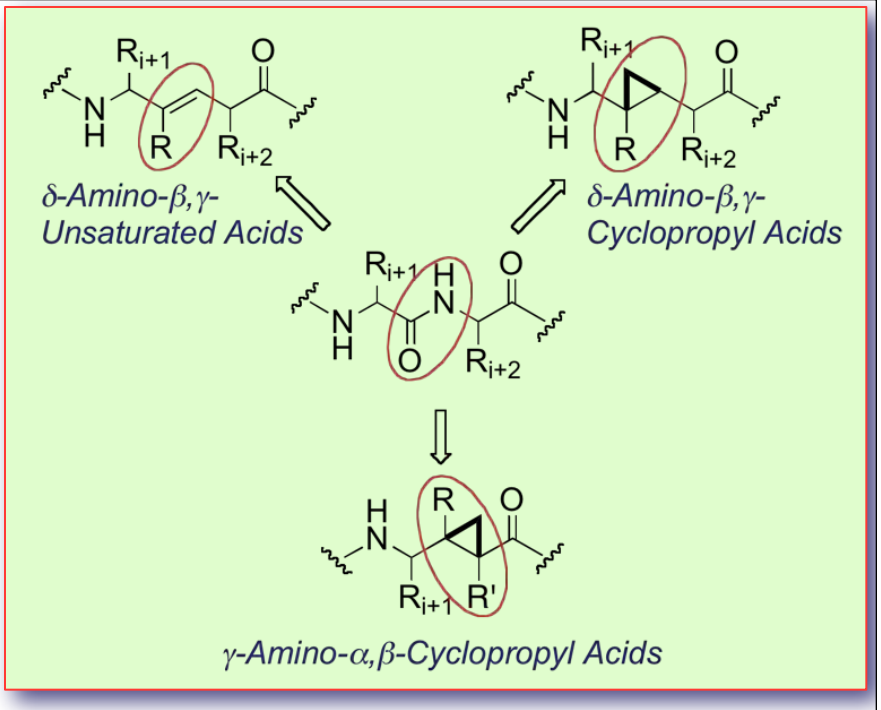

Tested in BioAssays: All: 530 Active: 12 Inactive: 518

- Assay for Inhibitors and Substrates of Cytochrome P450 2C19, 3A4, 2C9, 1A2
- Assay for Delayed Death Inhibitors of Malarial Parasite Plastid
- Assay for Non-Nucleoside Inhibitors of Measles Virus RNA-Dependent RNA Polymerase Complex
- Assay for Inhibitors of AmpC Beta-Lactamase
- Assay for Inhibitors of Shiga Toxin
- Assay for Sphingosine 1-phosphate Receptor Type 3 Antagonists

Does "Backbone Inversion" Lead to Biological Diversity?

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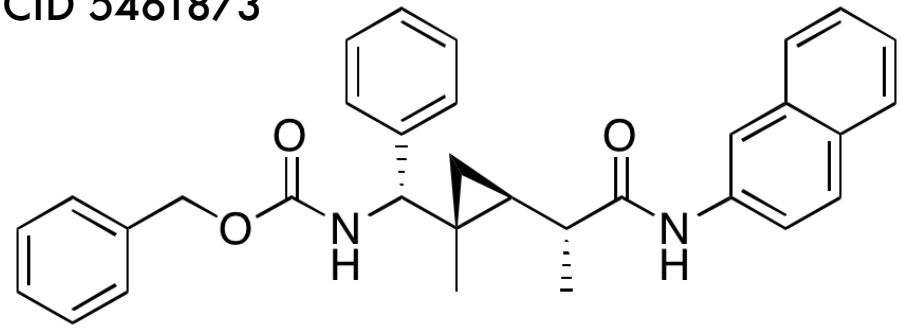

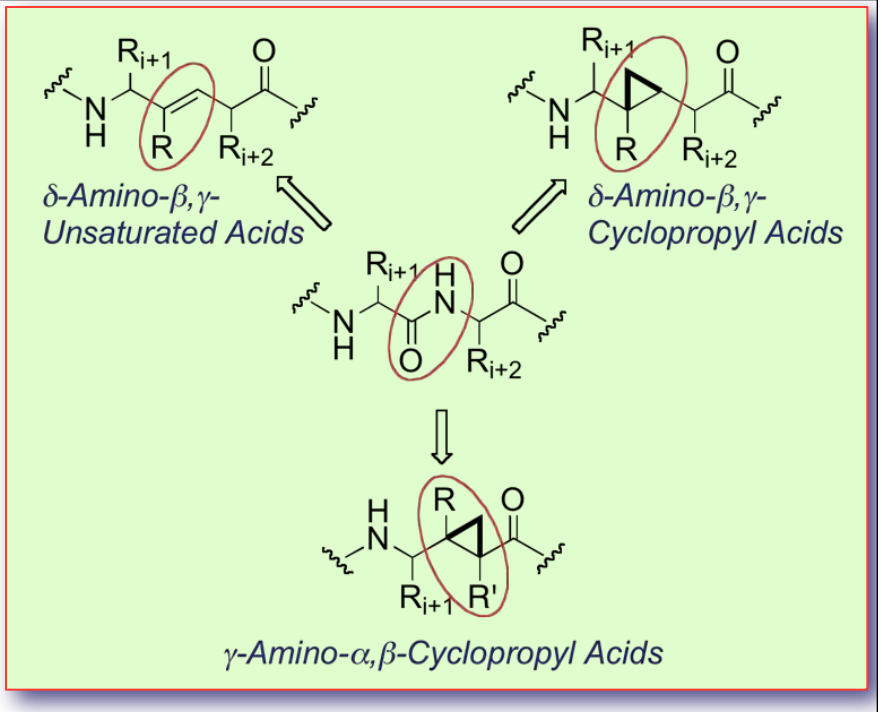

Tested in BioAssays: All: 412 Active: 4 Inactive: 400

- Assay for Inhibitors and Substrates of Cytochrome P450 Subtypes
- Activators of Mouse Intestinal Alkaline Phosphatase

Mitochondria as Therapeutic Targets

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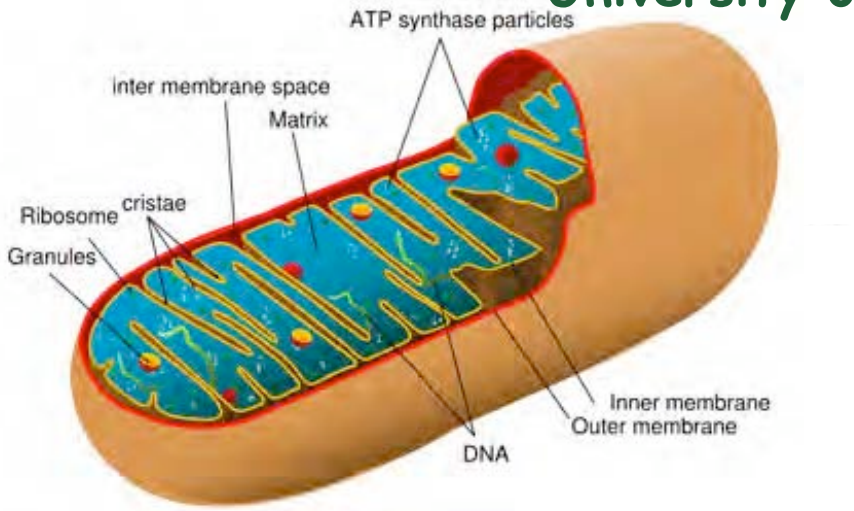
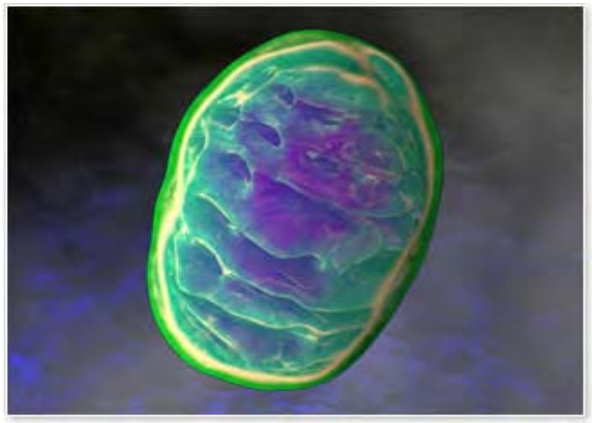
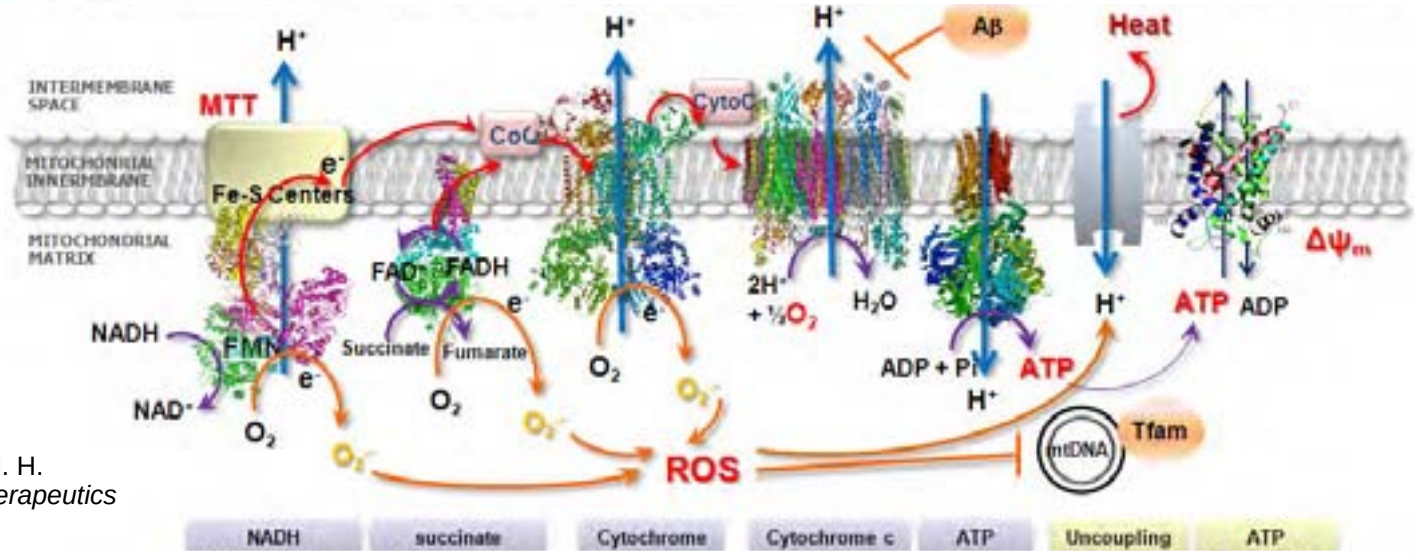
& Center for Medical Countermeasures against Radiation (CMCR)
University of Pittsburgh

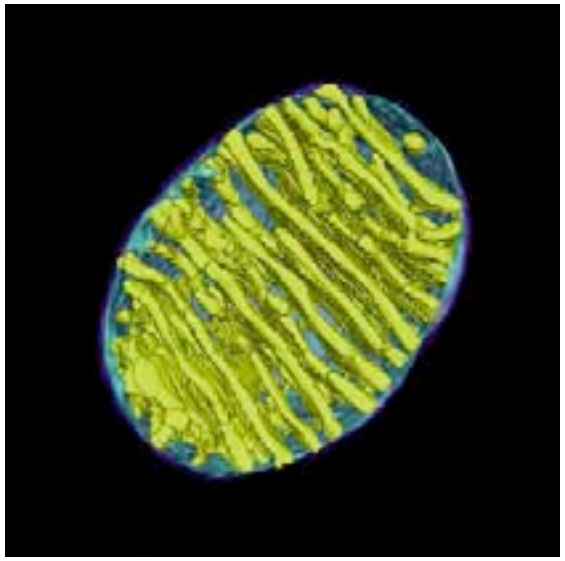


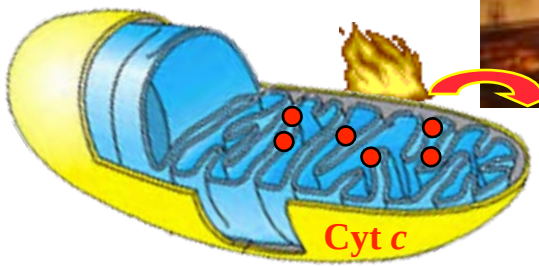




Pak, Y. K.; Jeong, J. H.
Biomolecules & Therapeutics
2010, *18*, 235-245.

Inverse Design: Targeting Mitochondria





ROS: $O_2^{-\bullet}$, $HO^{\bullet}$, $ONOO^{-\bullet}$

Can we design small organic molecules that pass cell membranes, localize in mitochondria, and scavenge ROS?

- In 1956, Harman proposed the "free radical theory" of aging and associated degenerative diseases
- Ca. 0.2% of cellular O_2 converts into ROS, and 90% of ROS are generated in Mitochondria as a consequence of oxidative phosphorylation
- Escaping ROS damage many intracellular targets in vicious cycle
- Radiation damage induces formation of ROS
- Atherosclerosis, Alzheimer's disease, cancer, neuronal death including ischemic stroke, acute and chronic degenerative cardiac myocyte death, are among the consequences of ROS leakage

Targeting Mitochondria

SS peptides

GS-based nitroxide antioxidants

Porphyrin-MTS chimera

Vesicle-based transporter

Cationic agents

Fusion with OMM

Membrane disruption

D-(KLAKLAK)$_2$ peptides

Sulfonylureas

Benzothiazepines

Benzodiazepines

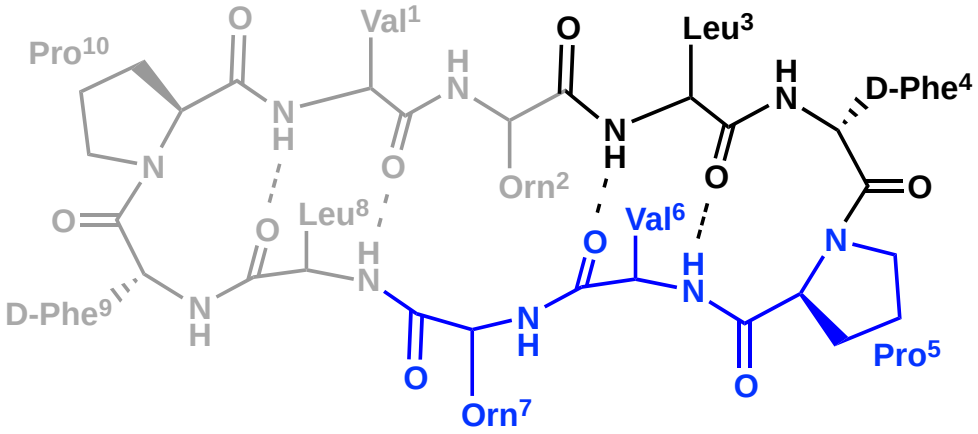
Frantz, M.-C.; Wipf, P.W. *Environ. Mol. Mutagenesis*, **2010**, *51*, 462-75.

Inverse Design: Targeting Mitochondria

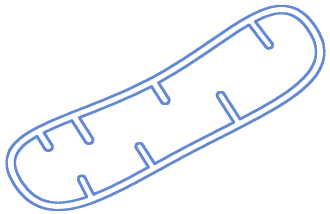
Gramicidin S as Scaffold for Subcellular-Targeting Probes

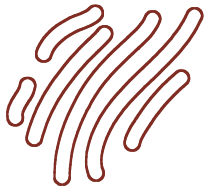
Pro–Val–Orn–Leu–D-Phe
| |
D-Phe–Leu–Orn–Val–Pro

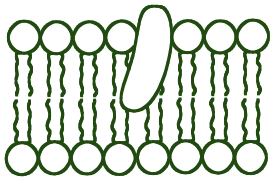
- Mode of action includes interaction with ***microbial membrane lipids***, dissipation of the chemiosmotic potential, and inhibition of respiratory enzymes.
- Amphipathic antiparallel β-sheet; two type II′ β-turns.

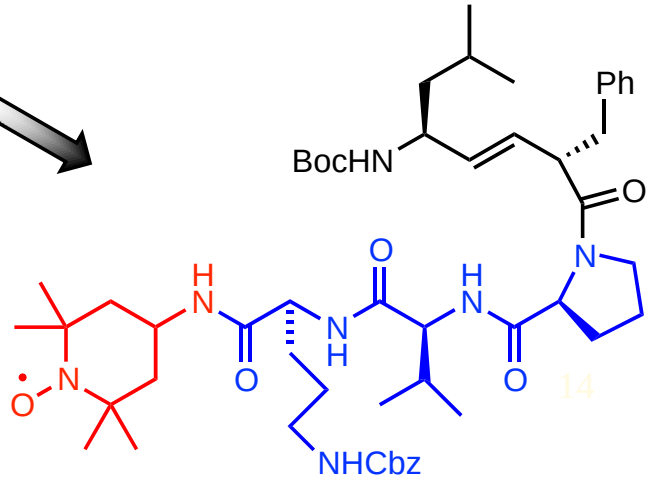


Gramicidin S



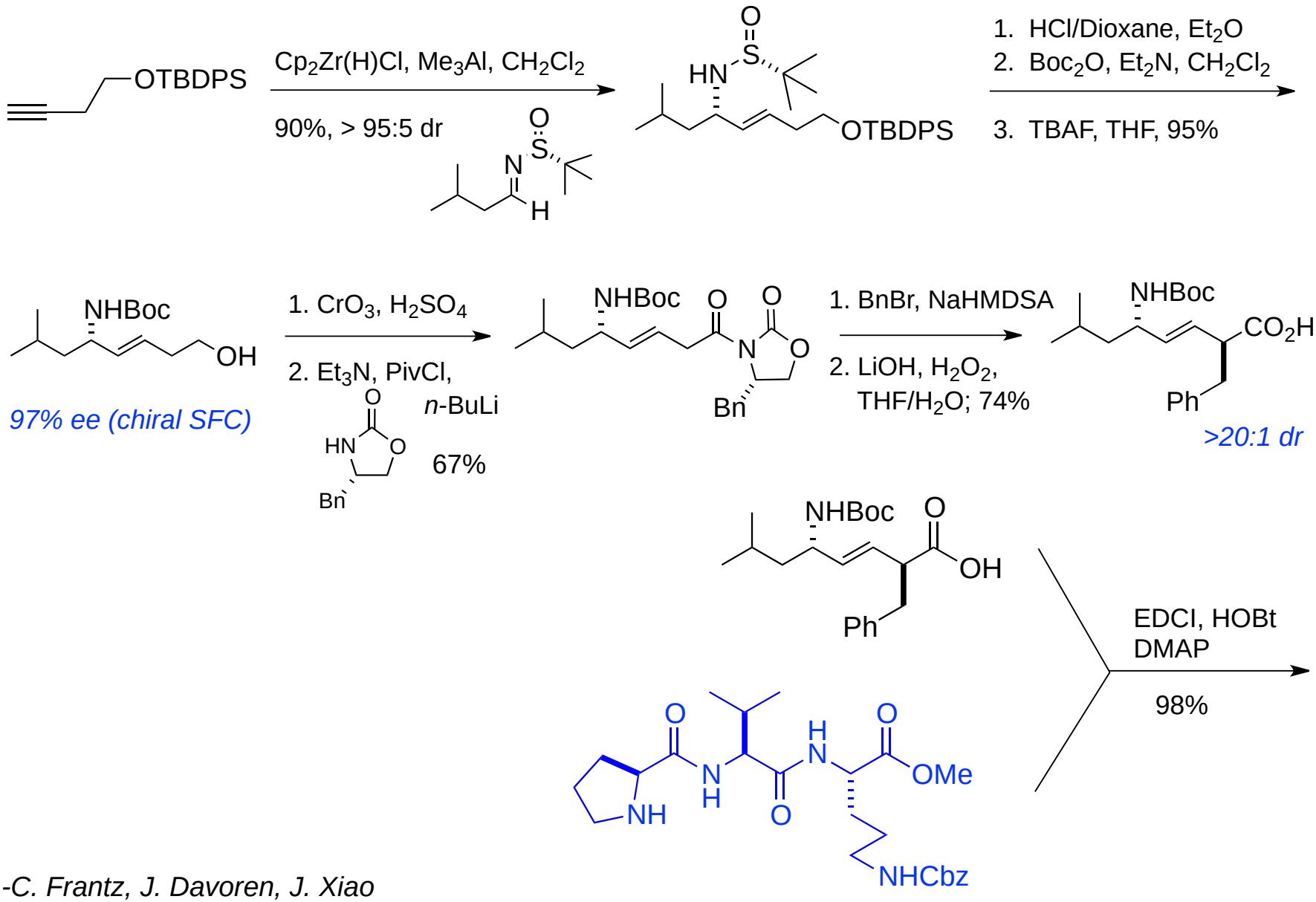






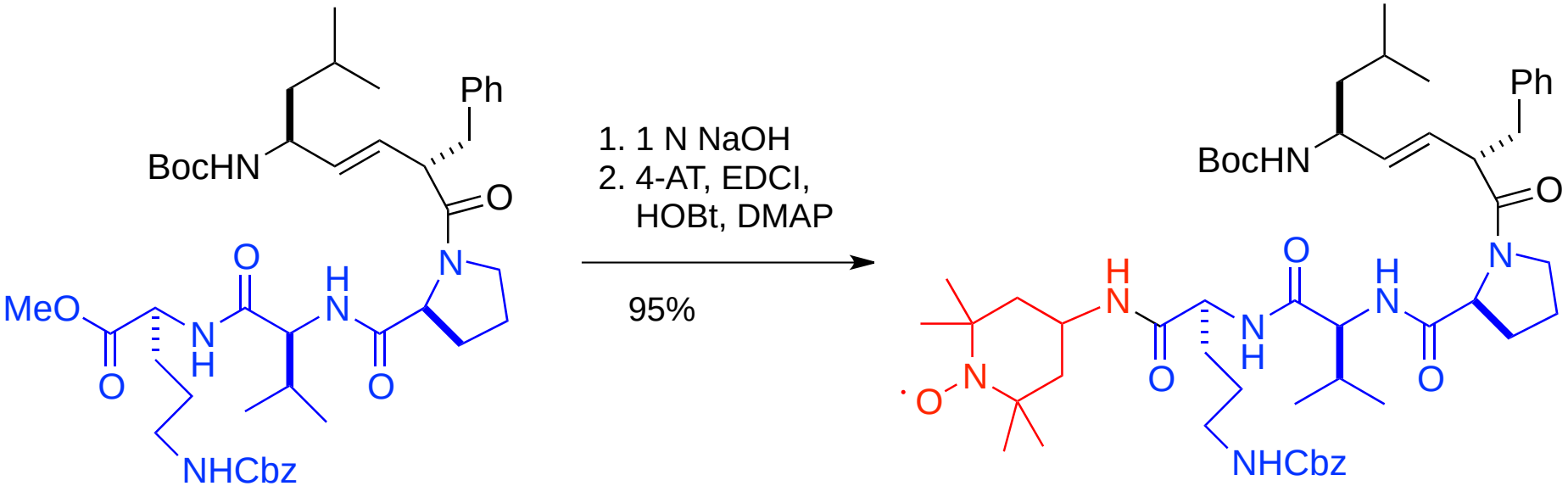
XJB-5-131

Synthesis of XJB-5-131 for Targeting Mitochondria



M.-C. Frantz, J. Davoren, J. Xiao

Synthesis of XJB-5-131 for Targeting Mitochondria

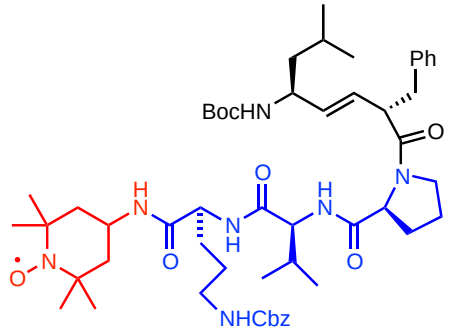


XJB-5-131

12 steps, 34% overall yield

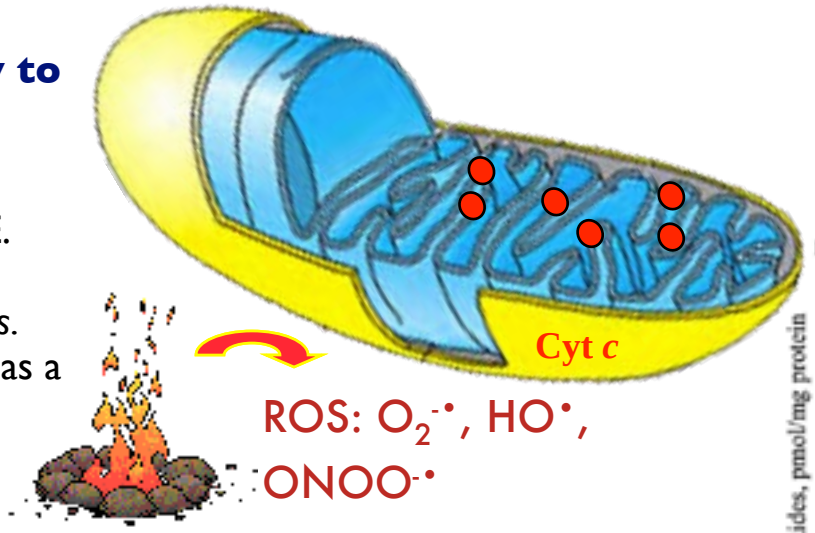
M.-C. Frantz, J. Davoren, J. Xiao

Functional Assays of Mitochondria-Targeting Nitroxides



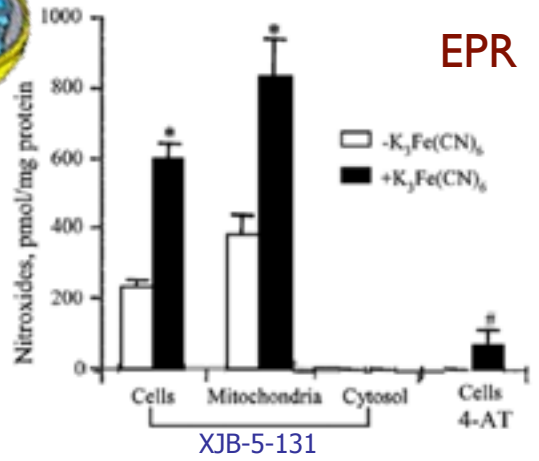
XJB-5-131

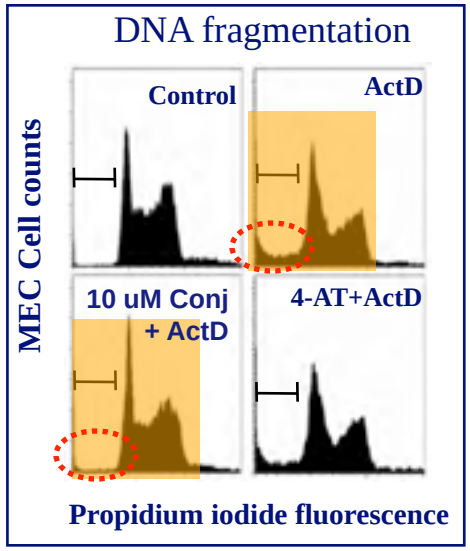
ROS Scavenger Delivery to Mitochondria

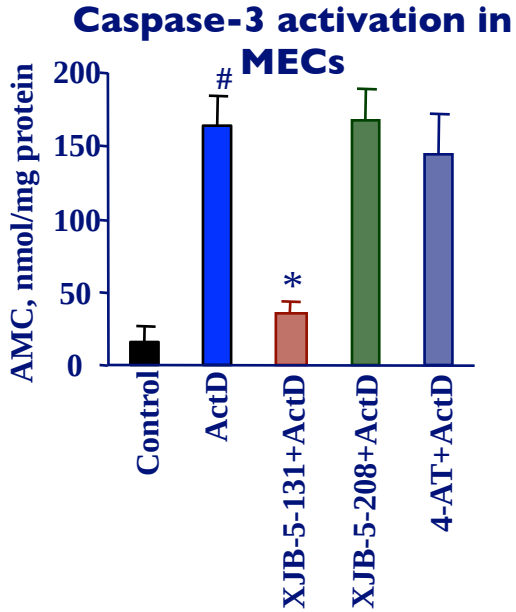


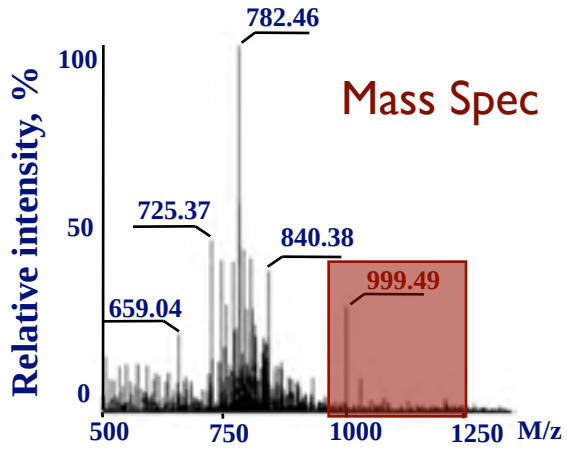
Wipf, P.; Fink, M. P.; Kagan, V. E. Frantz, M.C. et al.; "Targeting Mitochondria." *Acc. Chem. Res.* **2008**, *41*, 87. "Mitochondria as a target in treatment." *Environ. Mol. Mut.* **2010**, *51*, 462.

Presence in Mitochondria

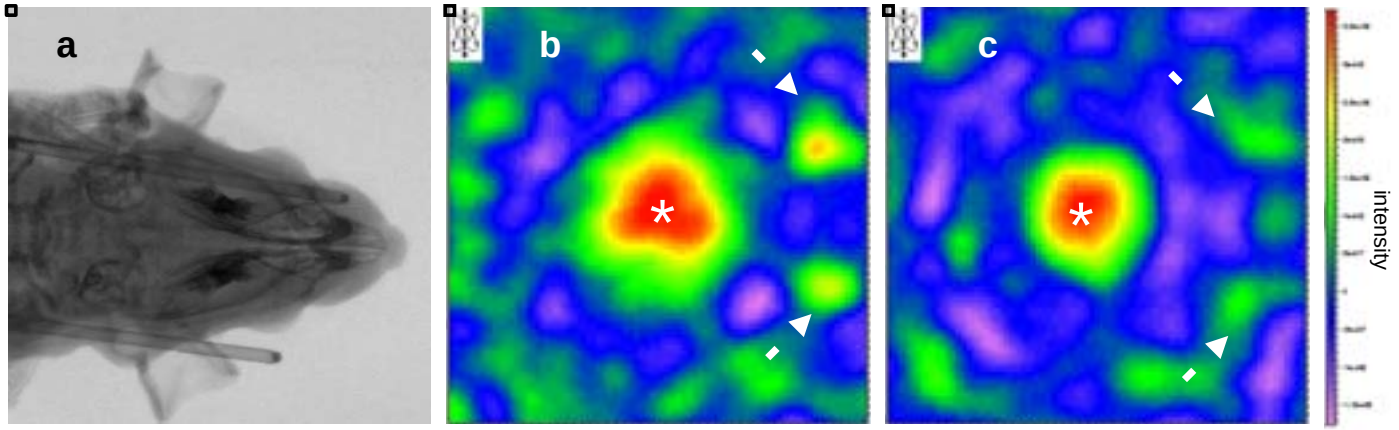








XJB-5-131 Partitions into the Brain



Computerized tomography (CT) and L-Band *in vivo* EPR imaging showed a time- and dose-dependent distribution of XJB-5-131 in naïve rat brain after intraperitoneal injection.

XJB-5-131 also shows promising efficacy in improving neurocognitive outcome after traumatic brain injury in rats.

Ji, J.; Wipf, P.; Tyurin, V. A.; Alexander, H.; Kochanek, P. M.; Kagan, V. E.; Bayır, H. *J. Neurotrauma* **2009**, *26*, A62.

Aging Symptoms Delayed in Ercc1 $^{-/\Delta}$ Mice

- Mouse model of accelerated aging

age 0 — Tri-weekly treatment with XJB-5-131 or oil — **15 weeks** — **18 weeks**

Observation → Images taken → Euthanization Analyses

Symptoms associated with premature aging
Age-at-onset
↓
Aging score

Overall appearance

XJB

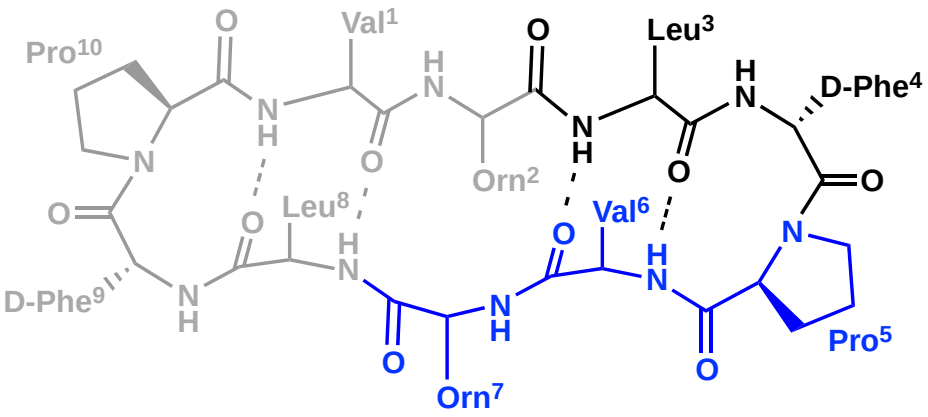
Aging score: 87%

oil

Aging score: 8%

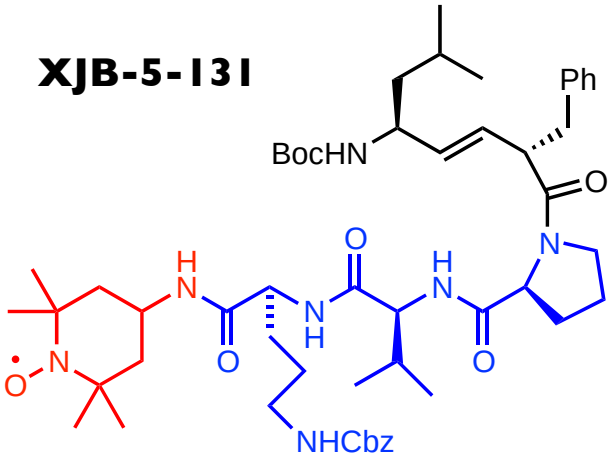
- ***Evidence in favor of the oxidative stress theory.***
- ***XJB-5-131 delays the onset of age-related degeneration.***

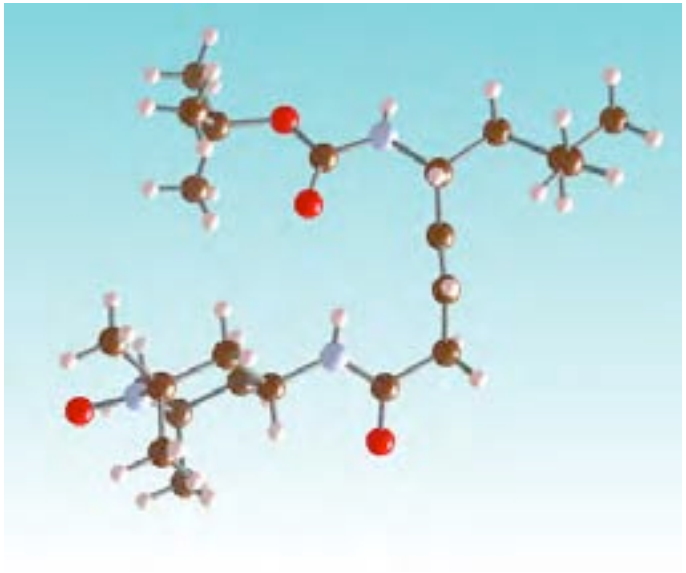
JP4-039 Design

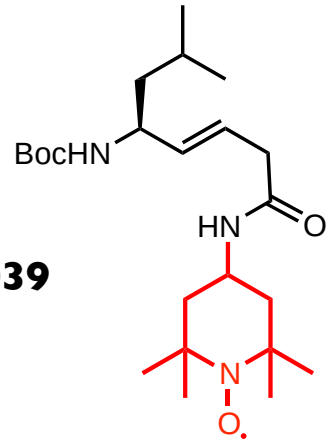


Gramicidin S

XJB-5-131

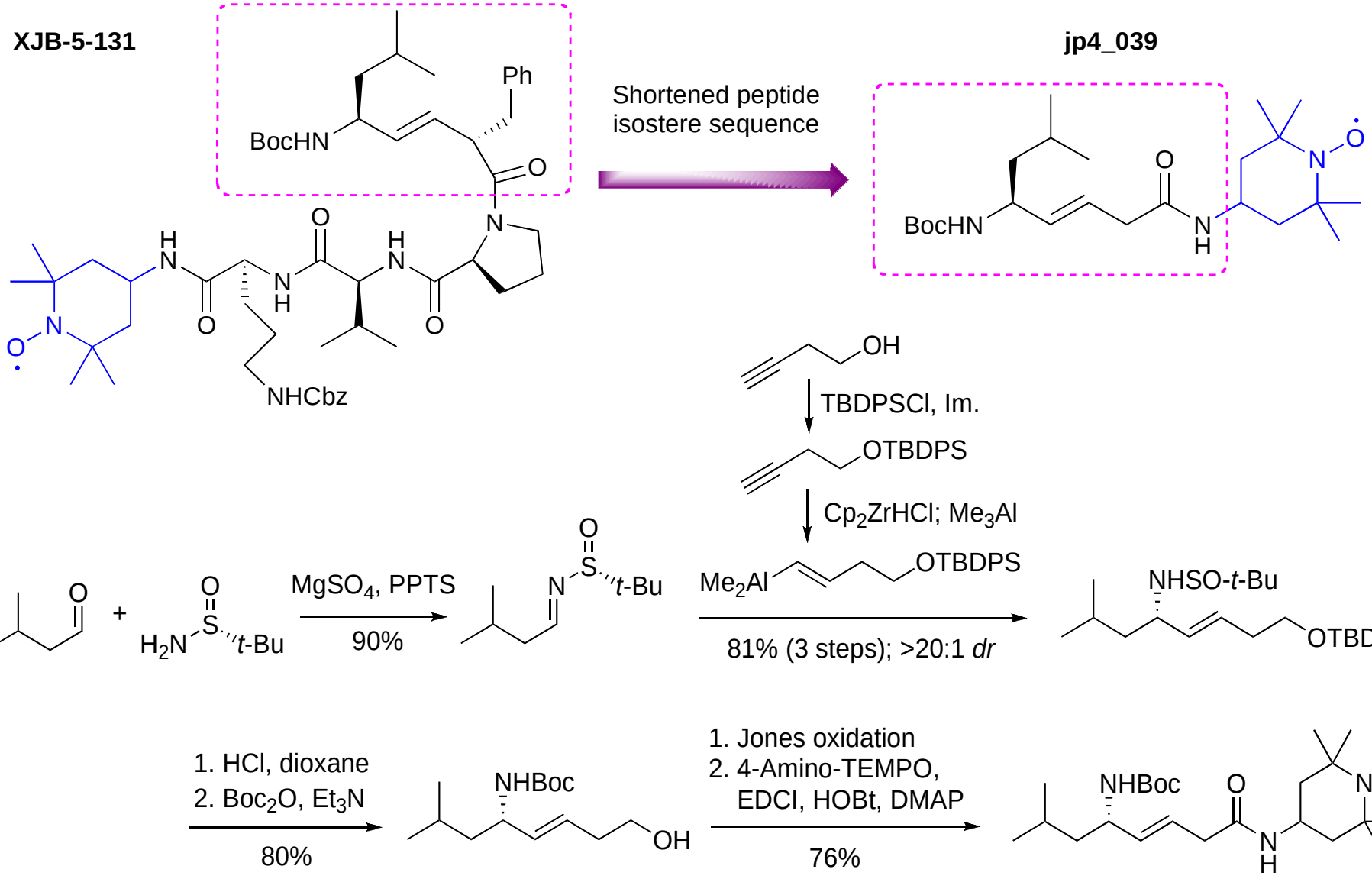






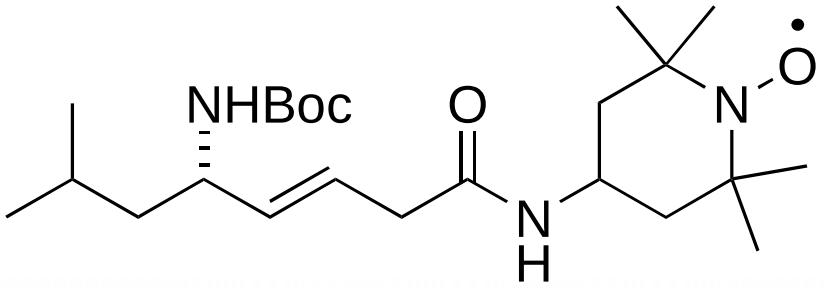
JP4-039

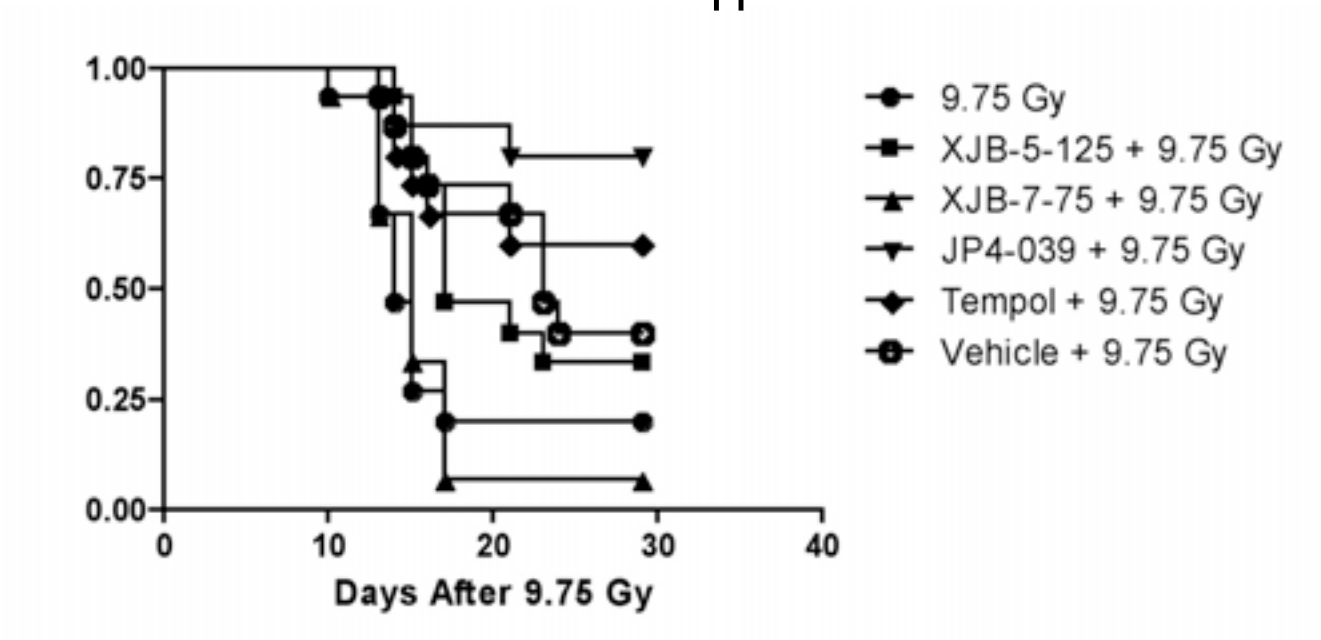
JP4-039 Synthesis



J. Davoren, J. Xiao

JP4-039 is an Effective Radiation Protector in Mice

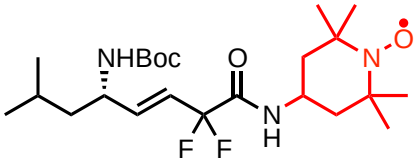




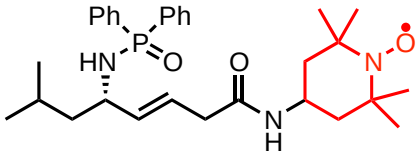
Mice were injected with 1 mg/kg of agent before irradiation with 9.75 Gy.

J. Pierce, M. Epperly, J. Greenberger

JP4-039 Chemical Library



MCF324-02a



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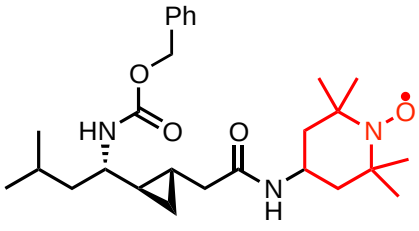

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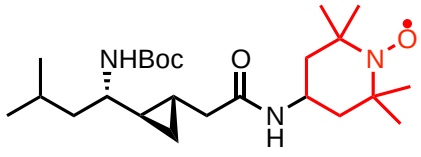

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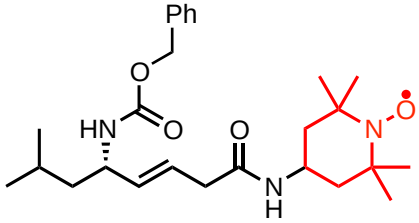

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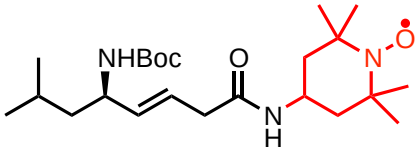
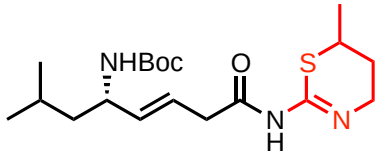
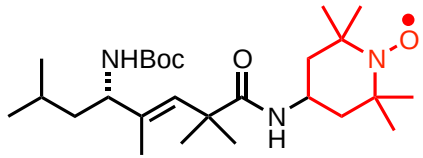


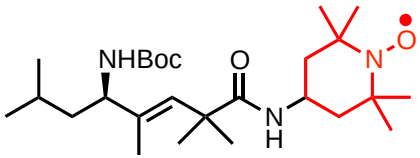
MCF237-18 (*epi*-jp4_039)



MCF201-89



GCD-0249-023a



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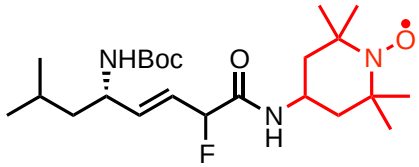
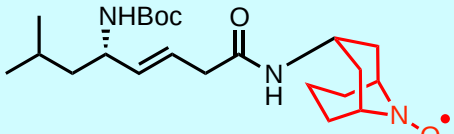
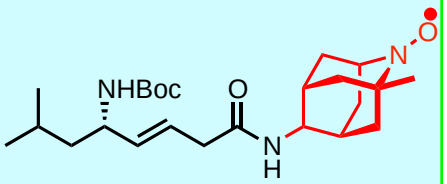


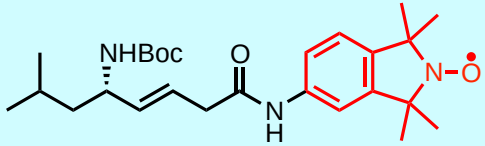
monofluoro analog



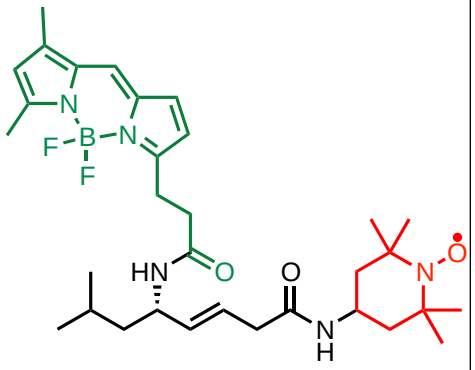
MCF262-89 (ABNO analog)



MCF292-29 (Me-AZADO analog)

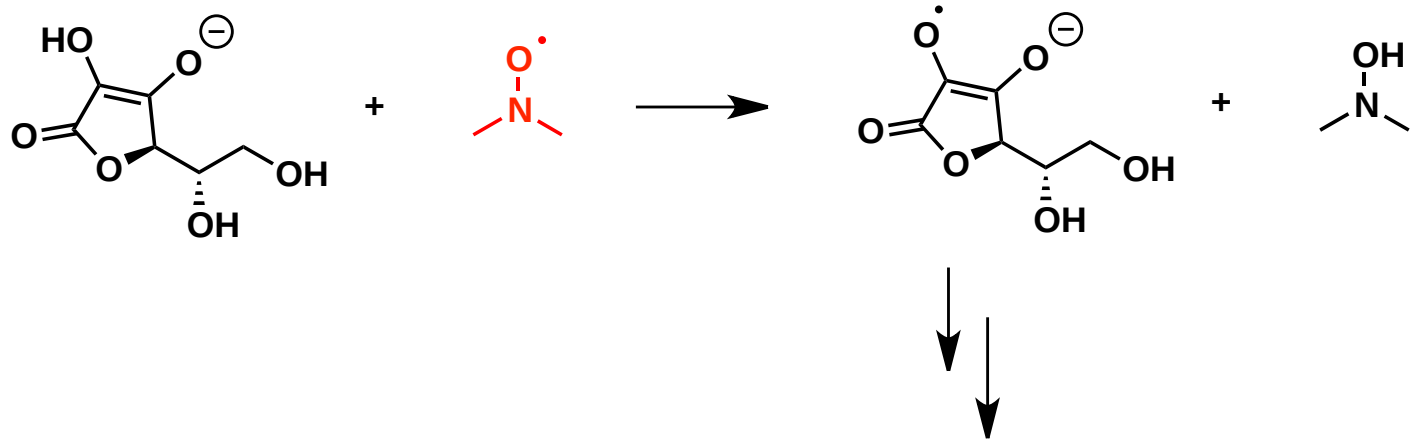


MCF179-54 (TMIO analog)



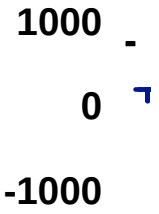
EMF338-008

Ascorbic Acid Electron Scavenging ESR Assay



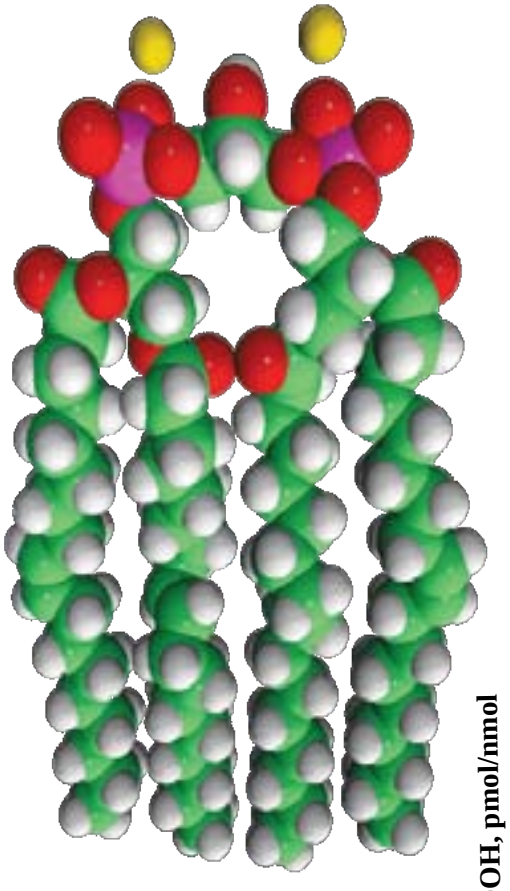
30μM TEMPO+DTPA 150μM
+33μL PBS+35μL DMSO

0.5μM Tempo+DTPA 150μM
+280μM Ascorbate 33μL PBS
+35μL DMSO
0.5 min

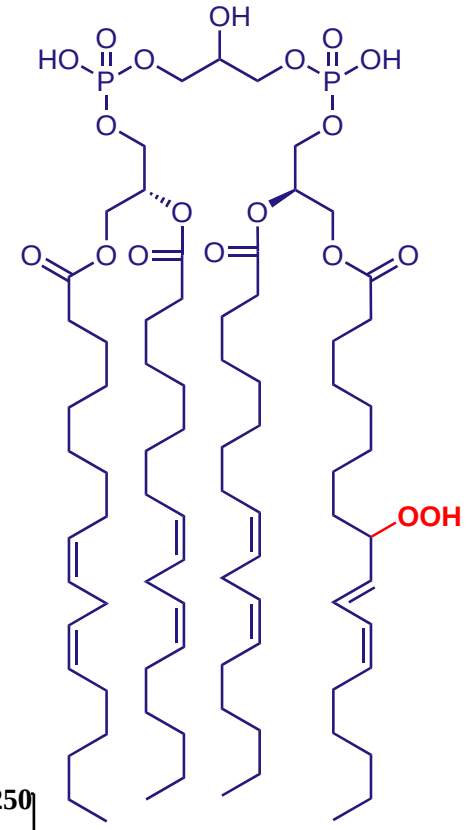


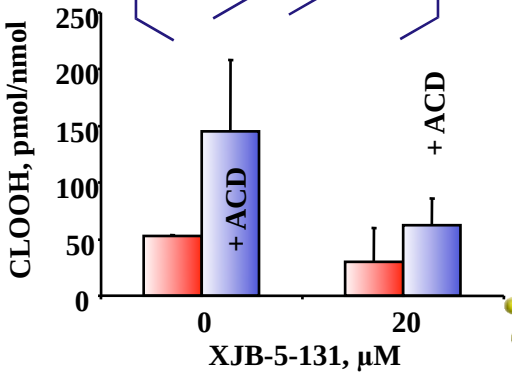
V. Kagan; V. Tyurin

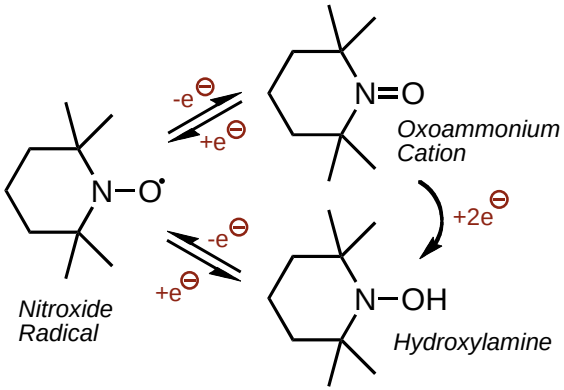
Active Hemigramcidin S - Tempo Conjugates Prevent Cardiolipin Peroxidation

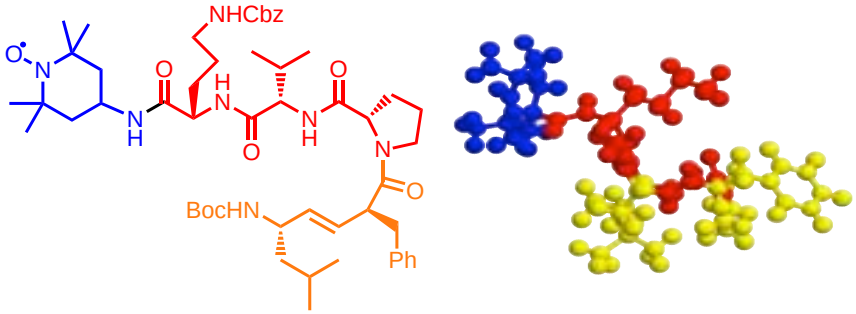


Cardiolipin

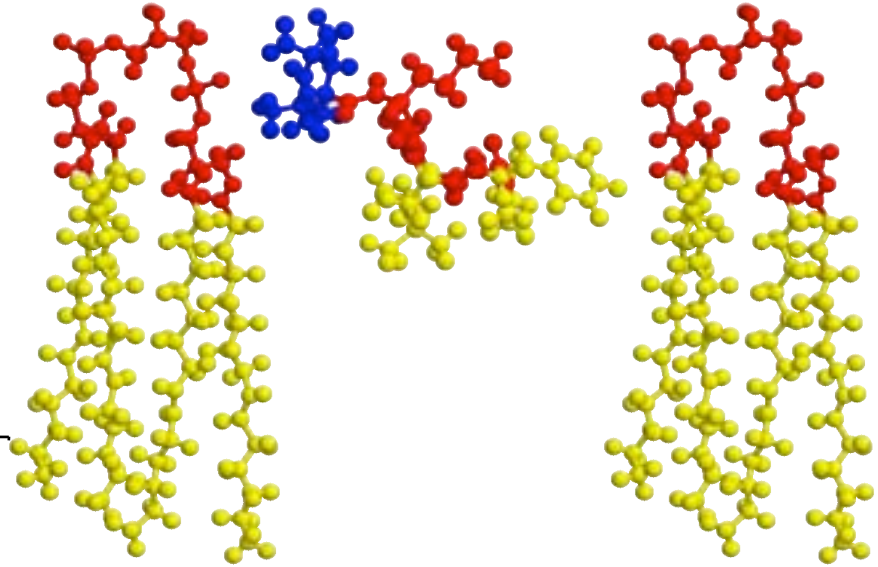








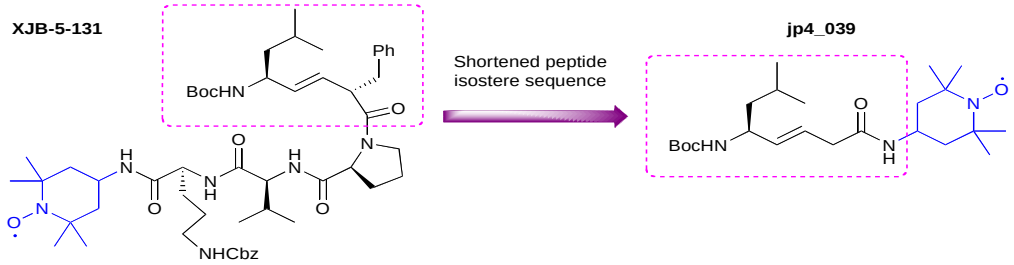
Active peptide –TEMPO conjugate



J. Pharmacol. Exp. Ther. **2007**, *320*, 1050; *Am. J. Physiol.* **2010**, *299*, L73.

Conclusion and Future Work

- Designed effective mitochondrial targeting agents



- Synthesized a library of GS-derived alkene peptide isosteres with a range of radical and electron scavenging abilities

- Demonstrated effectiveness in:
  - Radiation protection and mitigation in cells and mice
  - Treatment of traumatic brain injury
  - Rescue of senescence in oxidatively damaged cells
  - Prevention of cardiolipin peroxidation
  - Effectiveness in Ercc1 and HD150KI mouse models

- Utilize BODIPY-labeled analogs for visualization in mitochondria

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