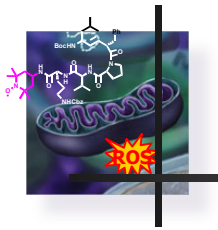
University of Pittsburgh

# Targeting Mitochondria: An Emerging Therapeutic Area?

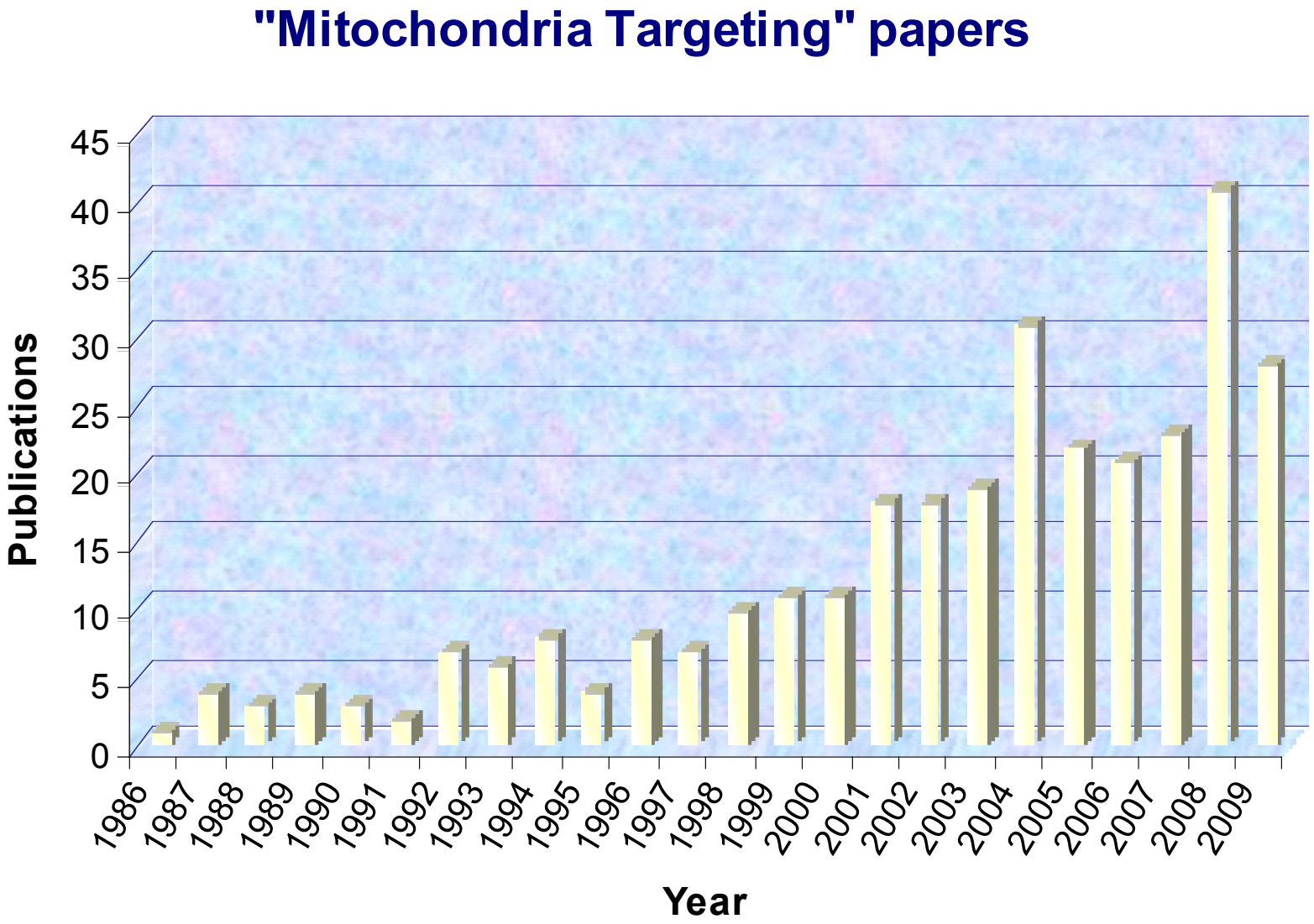

**Marie-Céline Frantz, Ph.D.**

Wipf Group Research Topic Seminar

May 8, 2010



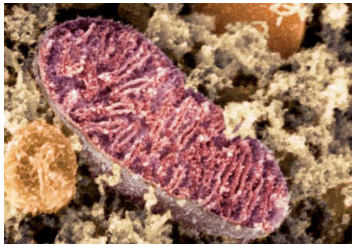

# Mitochondria Topic: Statistics

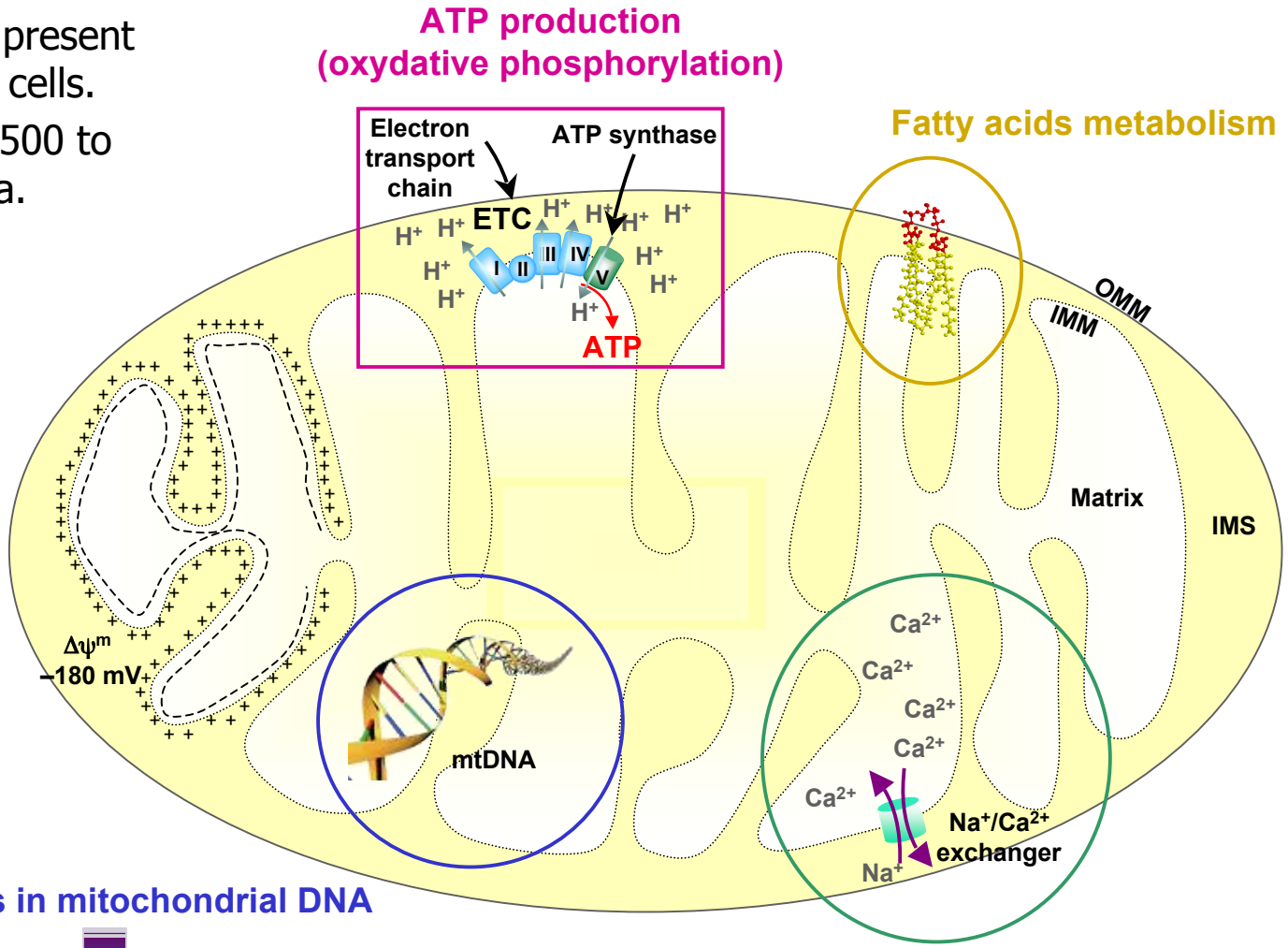



Source: SciFinder 2010

# The Mitochondrion: "Power Plant of the Cell"

- Discreet organelle present in most eucaryotic cells.
- Each cell contains 500 to 2 000 mitochondria.



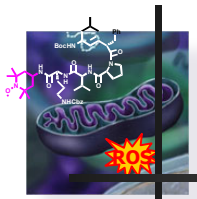



**Mutations in mitochondrial DNA**

**Mitochondrial genetic diseases**

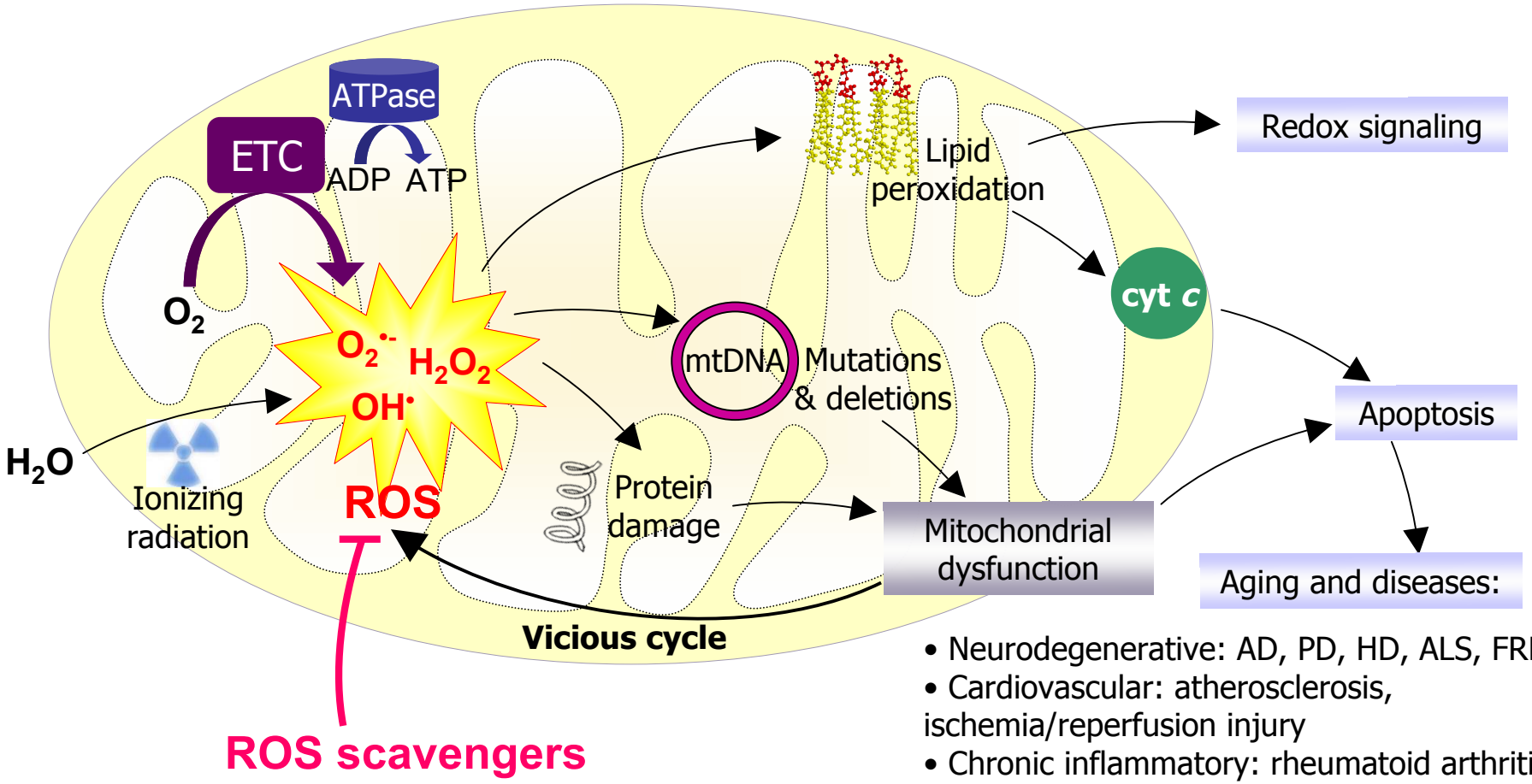
**Calcium homeostasis**

McBride, H. M.; Neuspiel, M.; Wasiak, S. *Curr. Biol.* **2006**, *16*, R551.



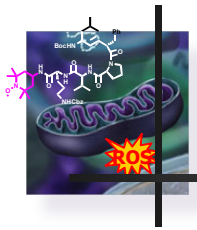

# Mitochondria & Oxidative Stress

- "Free radical theory of aging" (Harman, 1956): link between aging and ROS.
- ~0.2% of cellular $O_2$ converted into ROS, 90% of ROS generated in mitochondria.




- Neurodegenerative: AD, PD, HD, ALS, FRDA
- Cardiovascular: atherosclerosis, ischemia/reperfusion injury
- Chronic inflammatory: rheumatoid arthritis
- Metabolic: diabetes, obesity
- Hyperproliferative: cancers

Balaban, R. S. *et al. Cell* **2005**, *120*, 483.
Mattson, M. P.; Kroemer, G. *Tr. Mol. Med.* **2003**, *9*, 196.
Kagan, V. E. *et al. Free Rad. Biol. Med.* **2009**, *46*, 1439.




# Redox modulation by antioxidants

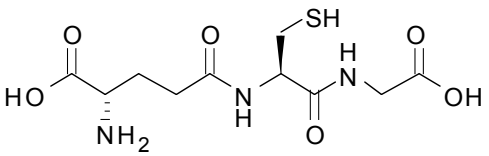
- Redox enzymatic processes:
  - Superoxide dismutase (SOD)
  - Superoxide reductase (SOR)
  - Catalase
  - Peroxiredoxin (Prx)
  - Gluthatione peroxidase (GP)
  - Thioredoxin/Thioredoxin reductase (Trx/TrxR)

$$O_2^{\bullet -} \xrightarrow{SOD} H_2O_2 \xrightarrow{catalase} H_2O + O_2$$

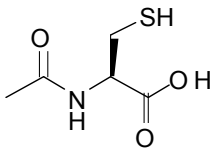
$$Trx(SH)_2 + H_2O_2 \xrightarrow{Prx} TrxS_2 + 2\,H_2O$$

$$2\,GSH + H_2O_2 \xrightarrow{GP} GSSG + 2\,H_2O$$

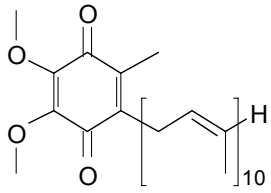
- Antioxidant molecules:



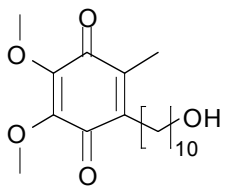

GSH (gluthatione)



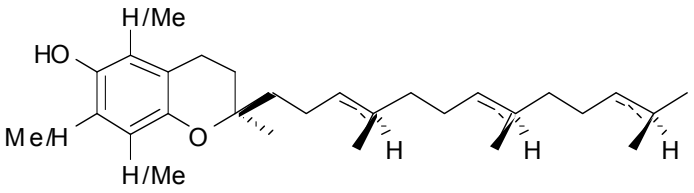

NAC
(*N*-acetyl-L-cysteine)



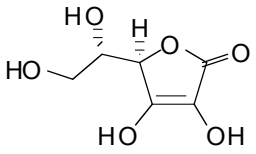

$CoQ_{10}$



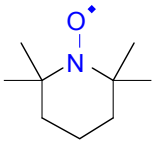

Idebenone




Vitamin E

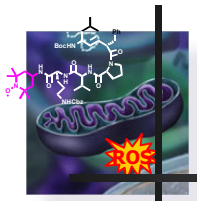



Vitamin C



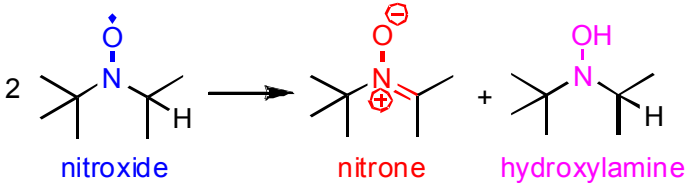

nitroxyl radicals
ex: TEMPO

Balaban, R. S. *et al. Cell* **2005**, *120*, 483. Frantz, M.-C.; Wipf, P. *Environ. Mol. Mutagen.* **2010**, *ASAP*.

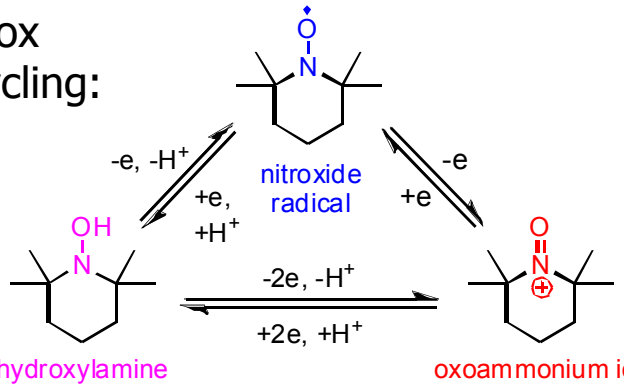


# Nitroxyl Radicals

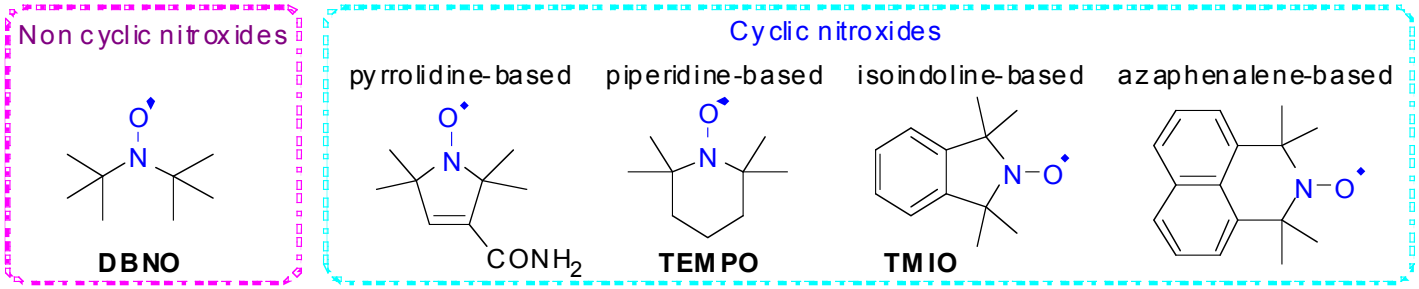
- Nitroxides with α-H are unstable due to dismutation forming a nitrone:



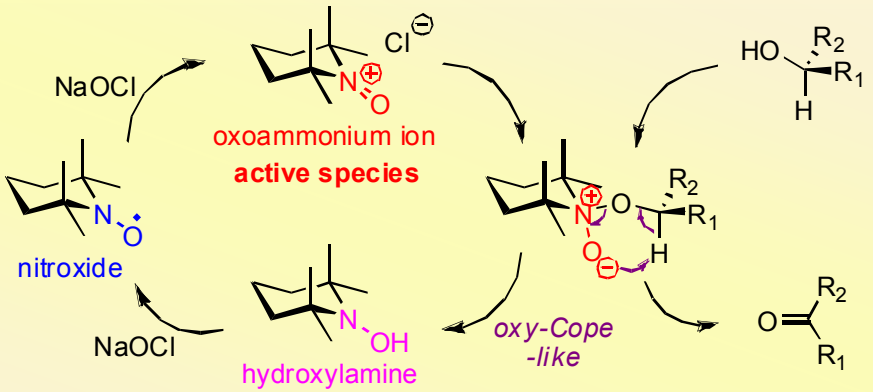

- Redox recycling:




- Known stable hindered nitroxides:




**TEMPO** widely used as a catalyst for alcohol oxidation:

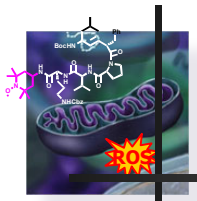



**Isoindoline nitroxides:**

- Excellent thermal and chemical stability.
- Resistant to ring opening reactions which are decomposition pathway for 5/6-membered ring nitroxides.

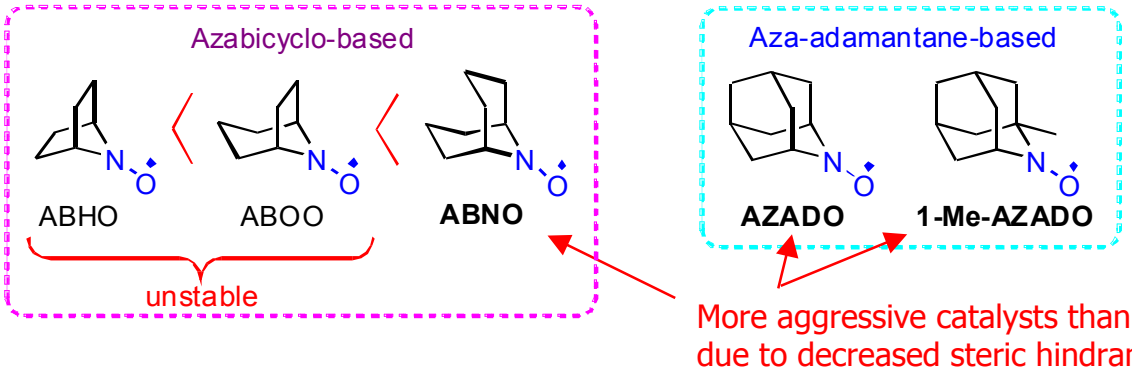
Bottle, S. E. *et al. J. Org. Chem.* **2008**, *73*, 6763. Iwabuchi, Y. *J. Synth. Org. Chem. Jpn* **2008**, *66*, 1076.

Bottle, S. E. *et al. J. Chem. Soc., Perkin Trans. 2* **1999**, 65.



# Nitroxyl Radicals

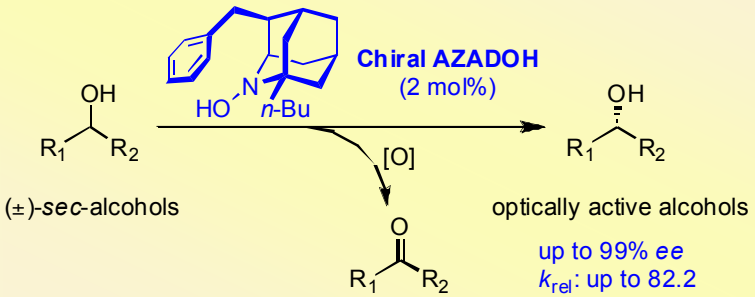
- Stable unhindered Bredt's rule protected cyclic nitroxides:



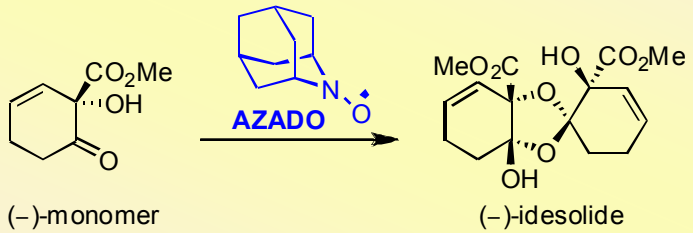

**Bredt's rule (empiric):**
"A double bond cannot be placed at the bridgehead of a bridged ring system, unless the rings are large enough."

- First synthesized by Dupeyre and Rassat (ABNO: 1966; AZADO: 1978).
- Developed as oxidation catalysts by Iwabuchi *et al.* since 2006.
  - High efficiency for oxidation of sterically hindered secondary alcohols.
  - Catalytic efficiency: **AZADO < 1-Me-AZADO < ABNO**.

- Oxidative kinetic resolution of *sec*-alcohols:

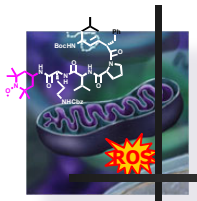



- AZADO-catalyzed dimerization as the key step in the first total synthesis of (–)-idesolide:



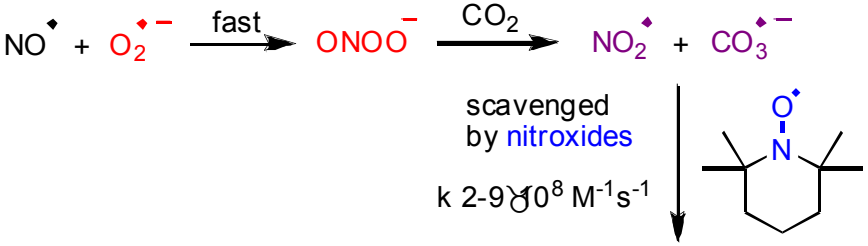


Fawcett, F. S. *Chem. Rev.* **1950**, *47*, 219. Dupeyre, R.-M.; Rassat, A. *J. Am. Chem. Soc.* **1966**, *88*, 3180. Dupeyre, R.-M.; Rassat, A. *Tetrahedron* **1978**, *34*, 1901. Iwabuchi, Y. *J. et al. J. Am. Chem. Soc.* **2006**, *128*, 8412. Iwabuchi, Y. *J. Synth. Org. Chem. Jpn* **2008**, *66*, 1076. Iwabuchi, Y. *et al. J. Org. Chem.* **2009**, *74*, 4619. Iwabuchi, Y. *et al. Org. Lett.* **2009**, *11*, 1829. Iwabuchi, Y. *et al. Org. Lett.* **2010**, *12*, 980.

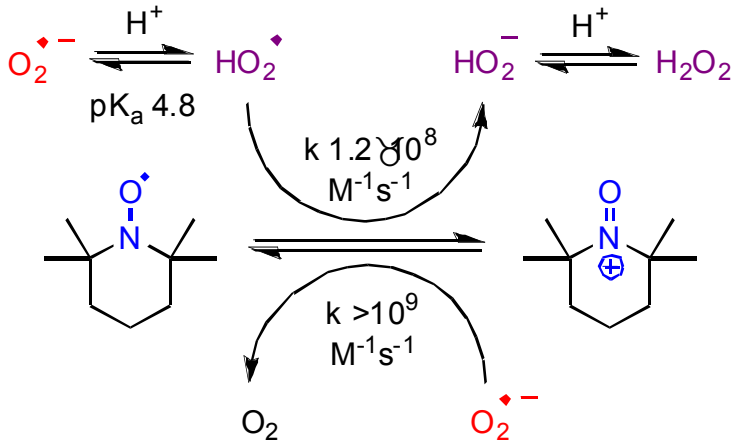



# Cyclic nitroxides: ROS scavengers & SOD mimics

- Free radical scavengers
  - Inhibition of lipid peroxidation
  - Very efficient at quenching products of peroxinitrite reactions with $CO_2$:



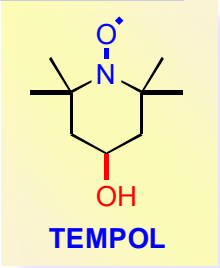

- Superoxide dismutase (SOD) mimetic activity:



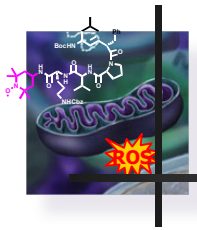

Goldstein, S. *et al. J. Phys. Chem. A* **2006**, *110*, 3679. Soule, B. P. *et al. Free Rad. Biol. Med.* **2007**, *42*, 1632.

## Therapeutic applications: TEMPOL, the first nitroxide in clinical trials

- Phase I completed for treatment of alopecia in radiation oncology.
- Limitations:
  - High mM concentrations required => adverse side effects.
  - Partly due to poor cellular partitioning.

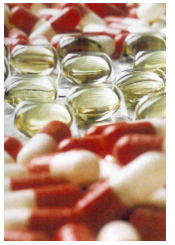



Hahn, S. M. *et al. Int. J. Radiat. Oncol. Biol. Phys.* **1998**, *42*, 839. Soule, B. P. *et al. Antioxid. Redox Signal.* **2007**, *9*, 1731.



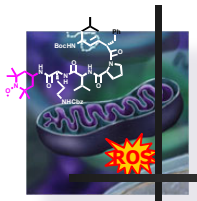

# Objective: Targeting Mitochondria

- Goals
  - To selectively deliver **nitroxides** into **mitochondria** to control ROS overproduction.
  - To develop a **small molecule drug candidate** active as a targeted **antioxidant**, able to pass cell membranes and to accumulate into mitochondria.
  - To propose a **new therapeutic strategy** for the treatment of:
    - Aging and age-related degenerative diseases
    - Ischemia-reperfusion injury
    - Cancers
    - Radiation injury: CMCR program (Center for Medical Countermeasures Against Radiation)
      - Identify and develop small molecule radiation protectors and mitigators
      - Easy access and administration route (f. ex.: skin patch)
      - Use in the event of large-scale radiological or nuclear emergency



- Collaborations at Pitt

  - Prof. Valerian E. Kagan (Dpt Environmental & Occupational Health): apoptosis (*in vitro* cellular assay)
  - Mitchell P. Finck (Dpts Critical Care Medicine and Surgery): rat model of hemorrhagic shock
  - Dr. Laura J. Niedernhofer (Dpt Microbiology & Molecular Genetics): murine model of accelerated aging
  - CMCR program: Joel S. Greenberger, Dr. Michael W. Epperly (Dpt Radiation Oncology): radioprotection, cancer radiotherapy (cellular and mouse models)



# Mitochondria Targeting: Strategies

**Peptides alterning aromatic/basic residues**

SS peptides

**Tethering to peptide antibiotic**

GS-based nitroxide antioxidants

**Tethering to mitochondrial targeting sequences**

Porphyrin-MTS chimera

**Vesicle-based transporter**

Encapsulation into liposomes

**Delocalized lipophilic cations**

TPP-based

Choline-based

Cationic agents

TOXICITY ! Membrane depolarization

D-(KLAKLAK)$_2$ amphipathic α-helical peptides

**Membrane-disrupting peptides**

ETC

ROS

ATP

OMM

IMM

Matrix

IMS

Fusion with OMM

Δψ$^m$ −180 mV

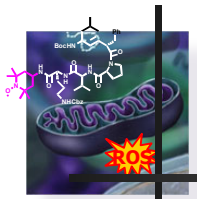
Membranes disruption

Na$^+$/Ca$^{2+}$ exchanger

Benzothiazepines

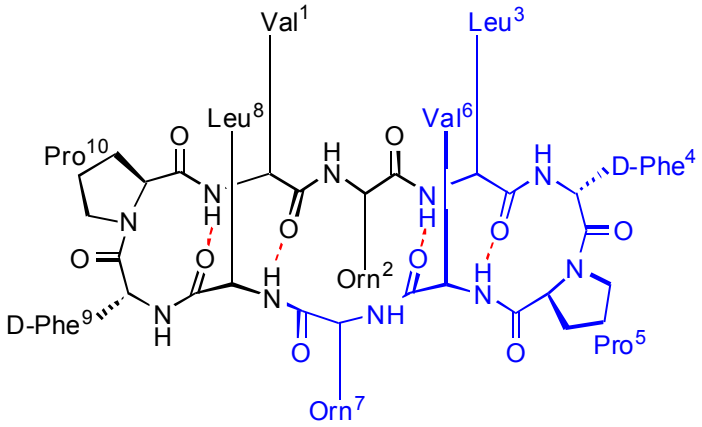
**Mitochondrial proteins targeting**

Frantz, M.-C.; Wipf, P. *Environ. Mol. Mutagen.* **2010**, *ASAP*.



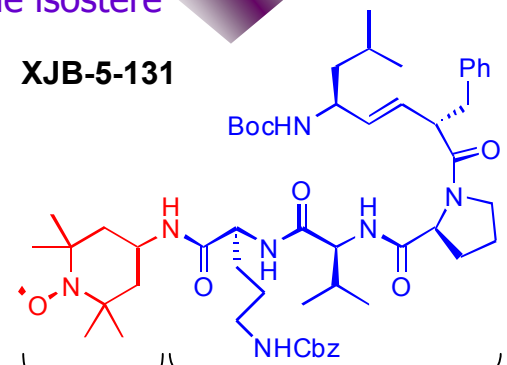

# Inverse Design of Natural Product Chimeras

- Gramicidin S (GS): scaffold for subcellular-targeting probes



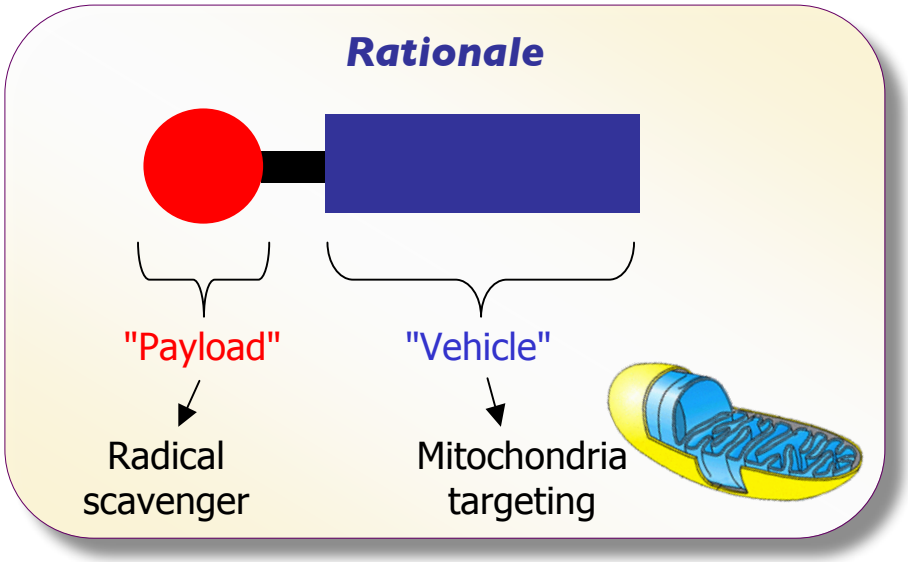

- β-turn targeting sequence
- (*E*)-alkene isostere

**XJB-5-131**




**"Payload" TEMPO** **"Vehicle" Hemi-GS**

- Structure:
  - Amphipathic antiparallel β-sheet
  - 2 type II' β-turns (*D*Phe-Pro)
- Mode of action:
  - Interaction with *microbial membrane lipids*
  - Dissipation of the chemiosmotic potential
  - Inhibition of respiratory enzymes

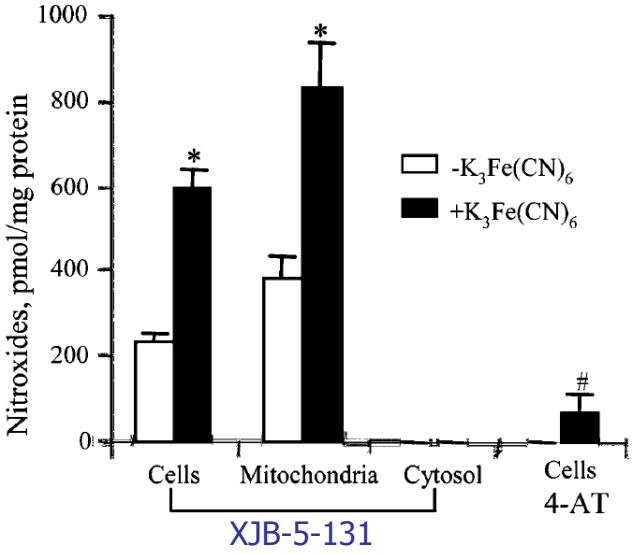


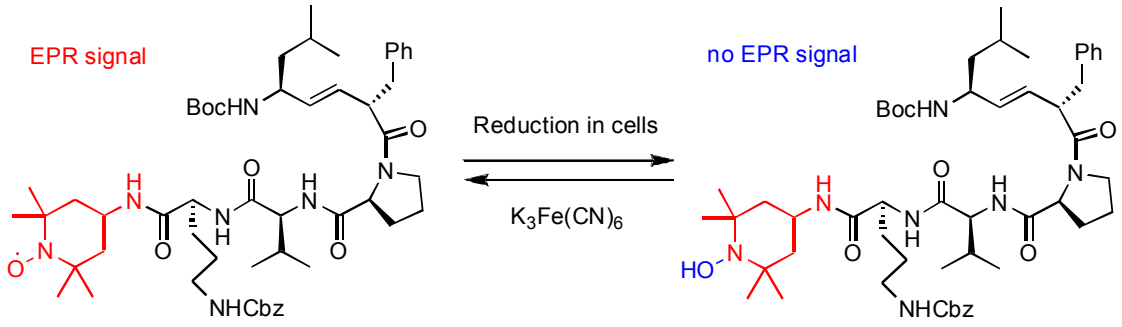

Prenner, E. J. *et al. Biochim. Biophys. Acta* **1999**, *1462*, 201. Wipf, P. *et al. Acc. Chem. Res.* **2008**, *41*, 87.

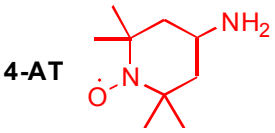
# XJB series: *In Vitro* Biological Studies

- XJB-5-131: Mitochondrial enrichement

**EPR-based analysis of the integration and reduction of nitroxides in cells.**

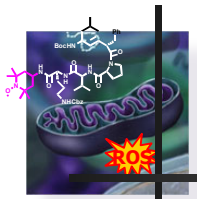



- ***Untargeted 4-AT does not effectively partition into either cells or mitochondria.***
- ***XJB-5-131 concentrates in mitochondria, where it is reduced.***

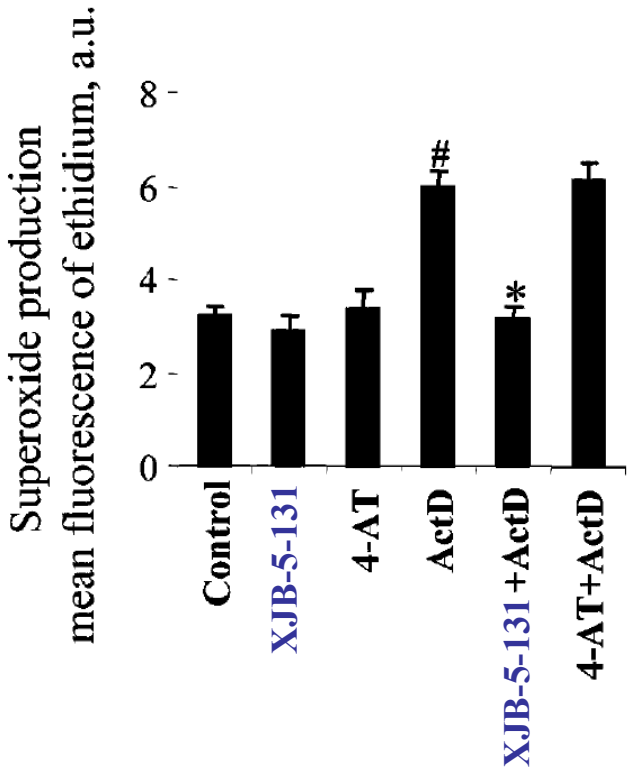
Wipf, P.; Kagan, V. E. *et al. J. Am. Chem. Soc.* **2005**, *127*, 12460.



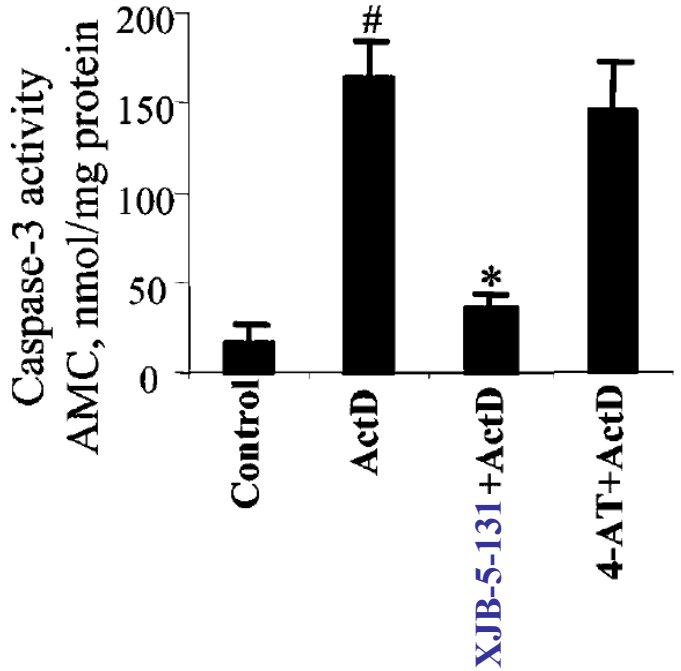

# XJB series: *In Vitro* Biological Studies

- XJB-5-131: Anti-apoptotic activity

**Intracellular superoxide generation triggered by actinomycin D (ActD).**




**ActD-induced caspase-3 activation (marker of apoptosis)**

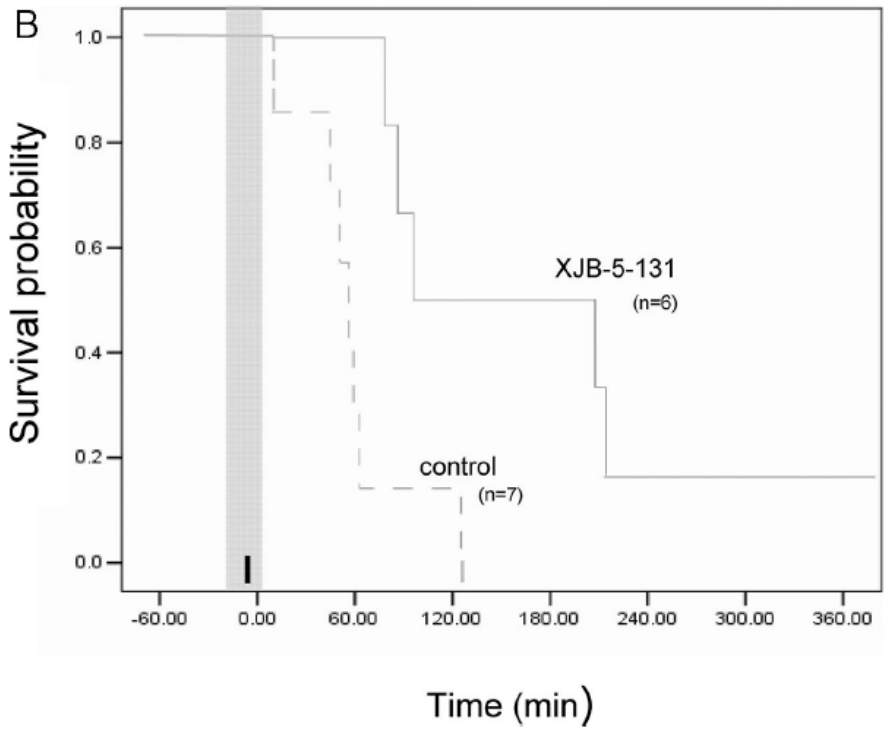



***XJB-5-131 inhibits ActD-induced superoxide production and protects cells against ActD-induced apoptosis.***

Wipf, P.; Kagan, V. E. *et al. J. Am. Chem. Soc.* **2005**, *127*, 12460.

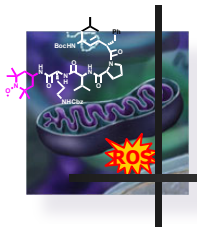
# XJB series: *In Vivo* Biological Studies

- Rat model of lethal hemorrhagic shock
  - Hemorrhagic shock => cellular hypoxia => mitochondria ROS leakage


***XJB-5-131 prolongs survival of rats with lethal hemorrhagic shock.***

Fink, M. P. *et al. Ann. Surg.* **2007**, *245*, 305.




# XJB series: *In Vivo* Biological Studies

*Laura Niedernhofer,*
*Dpt Microbiology & Molecular Genetics*

- Mouse model of accelerated aging

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

Observation → Images taken (15 weeks) → Euthanization / Analyses (18 weeks)

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

## Overall appearance

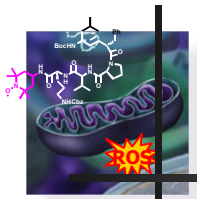
**XJB**

**Aging score: 87%**

**oil**

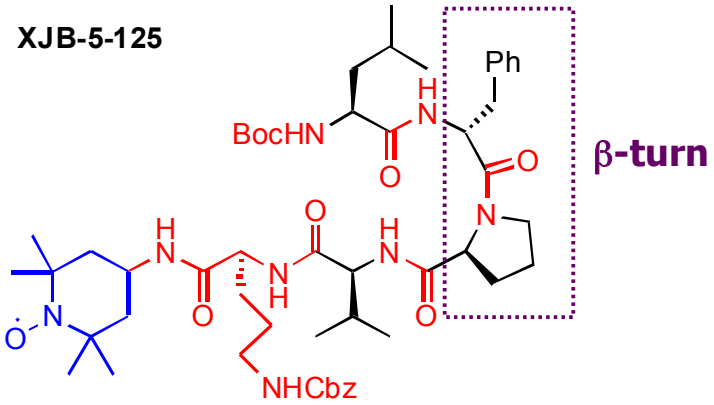
**Aging score: 8%**

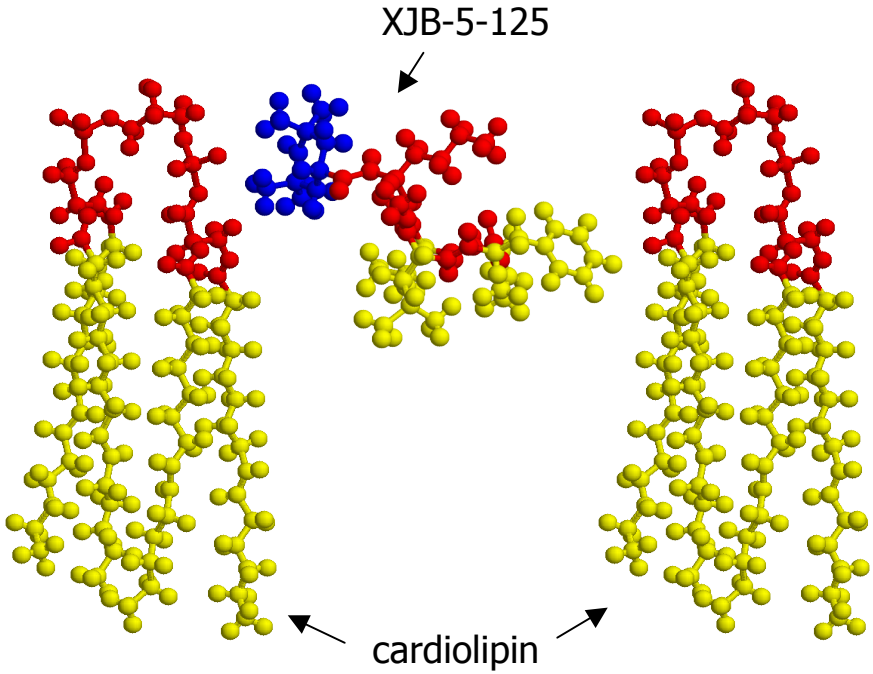
- ***Evidence in favor of the oxidative stress theory.***
- ***XJB-5-131 delays the onset of age-related degeneration.***



# XJB series: Rationale for its Activity?

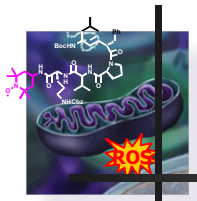
- Monte-Carlo simulation within lipid membrane:



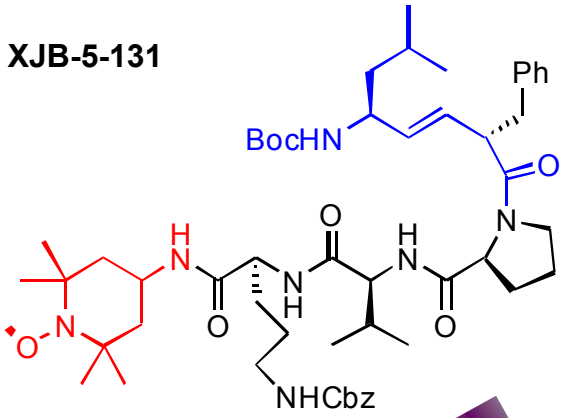

- Hypothesis:
  - Positioning of the nitroxide at the polar/nonpolar interface of the lipid membrane essential for activity, to allow successful competition with $O_2$ for electrons from ETC.
  - Accomplished by the intact β-turn motif of the targeting peptide sequence.

Kagan, V. E. *et al. J. Pharmacol. Exp. Ther.* **2007**, *320*, 1050.




# Small Molecule Design

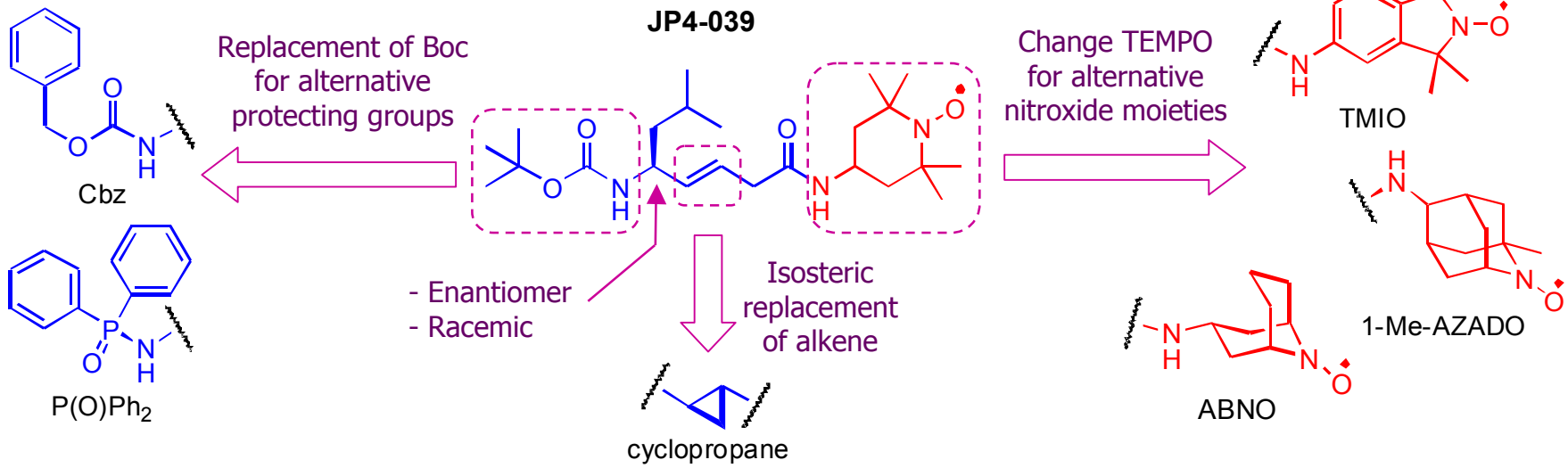
**XJB-5-131**

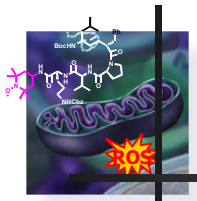



- Broad therapeutic potential, but
- Limited use of peptides as drugs:
  - High molecular weight
  - Poor oral absorption
  - Metabolic instability
  - Immunogenicity…

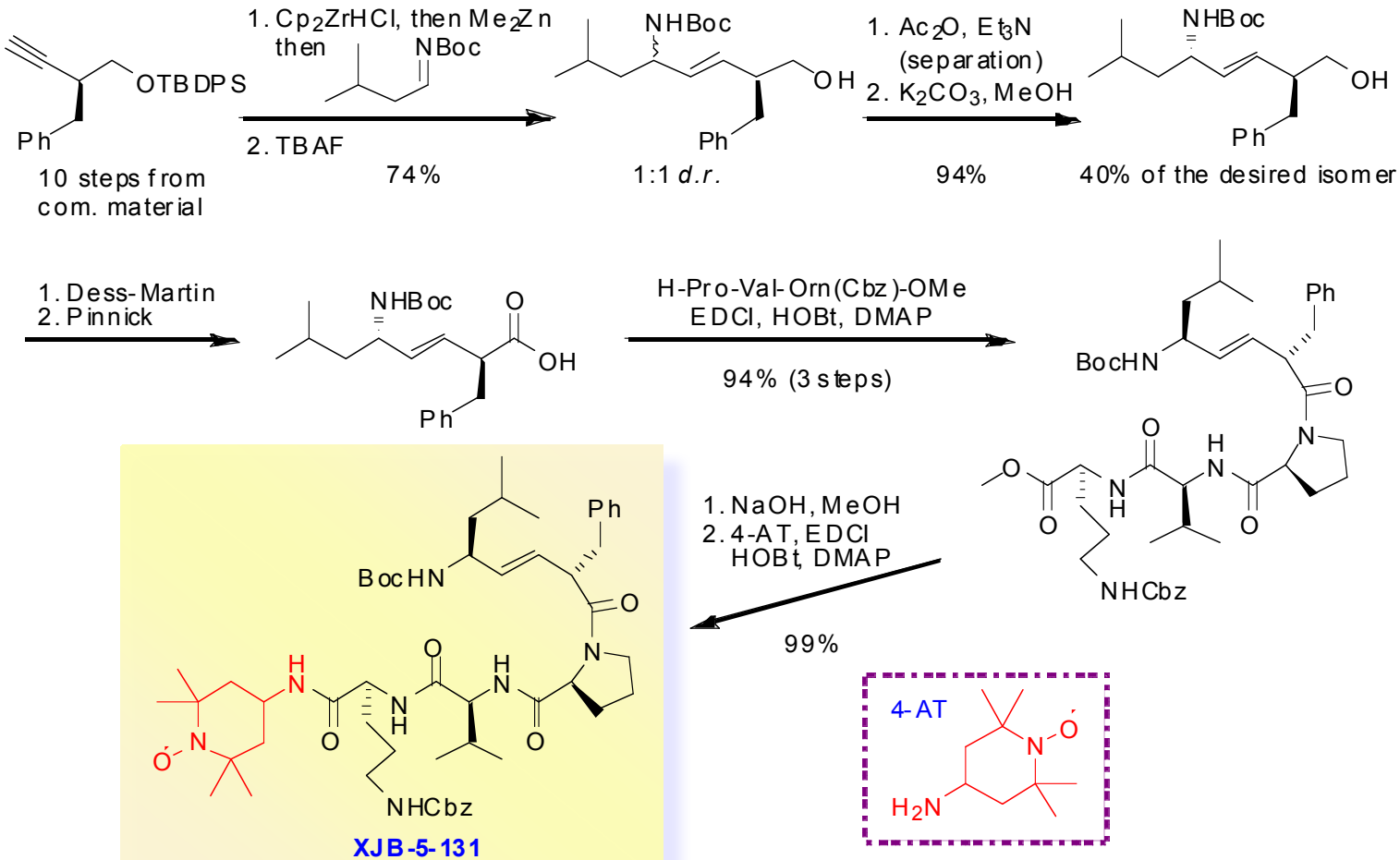
- Shortened peptide isostere sequence
- Further isosteric replacements to improve activity and drug-like properties

**JP4-039**

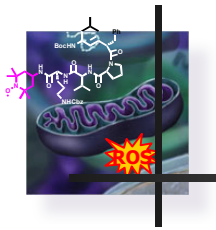




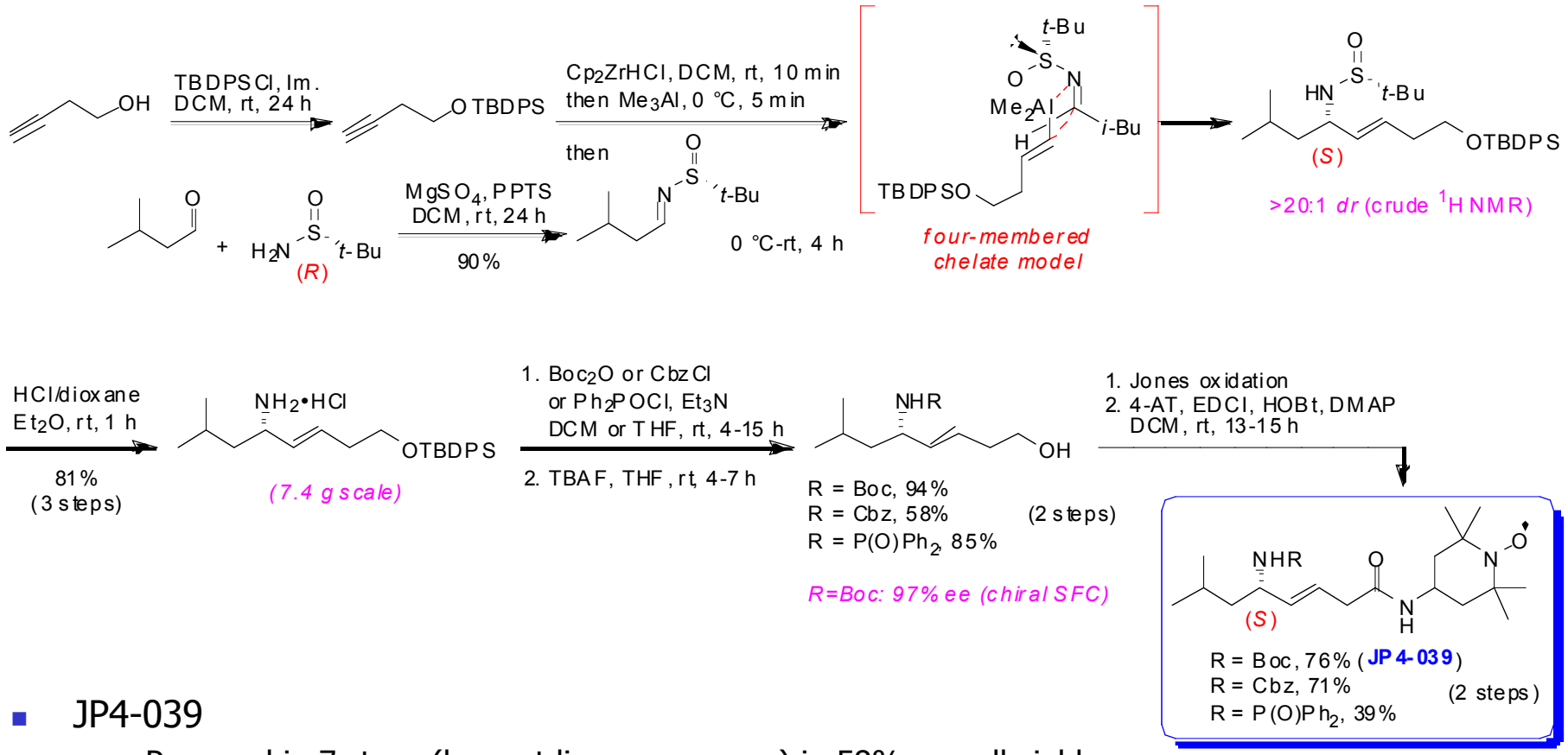
# First Generation Synthesis of the XJB Series

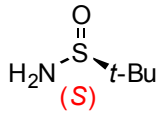



Wipf, P.; Kagan, V. E. *et al. J. Am. Chem. Soc.* **2005**, *127*, 12460.



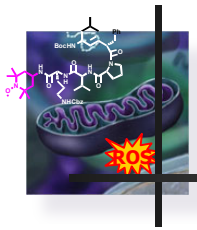
# Asymmetric Synthesis of Allylic Amines: Application to the Synthesis of JP4-039 and analogs



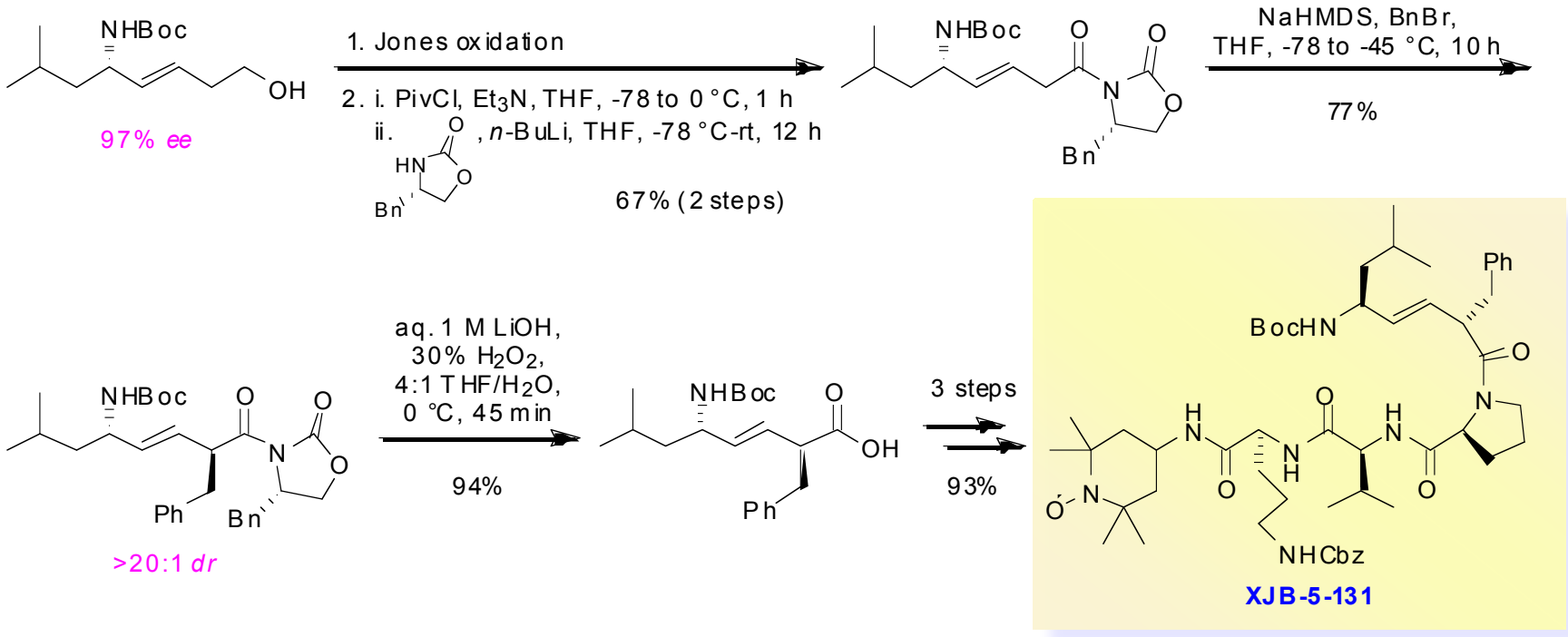

- JP4-039
  - Prepared in 7 steps (longest linear sequence) in 58% overall yield.
  - Scale-up by Asymchem: 157 g batch (99% purity, 100% *ee*) in 22% overall yield.
  - (*R*)-Enantiomer also prepared from 


ASYMCHEM

Pierce, J. G. University of Pittsburgh, Pittsburgh, 2008.
Wipf, P; Pierce, J. G. *Org. Lett.* **2006**, *8*, 3375.

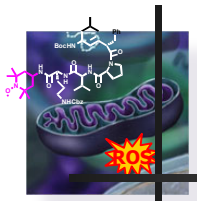


# Second Generation Synthesis of the XJB Series



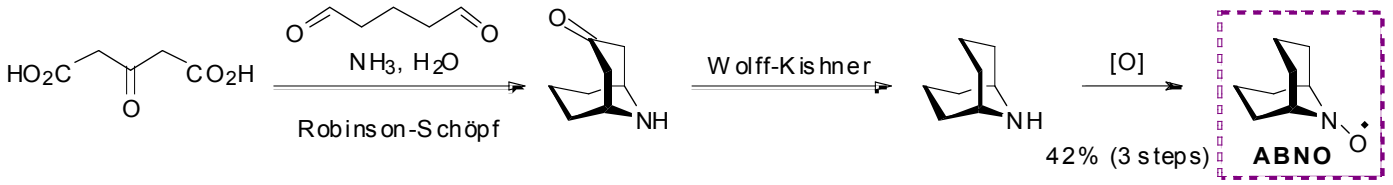

- XJB-5-131
  - First generation: 19 steps (1 separation of diastereomers), 7.5% overall yield
  - Second generation (optimized): 12 steps, 34% overall yield, 2 g batch prepared

Wipf, P.; Xiao, J.; Stephenson, C. R. J. *Chimia* **2009**, *63*, 764.



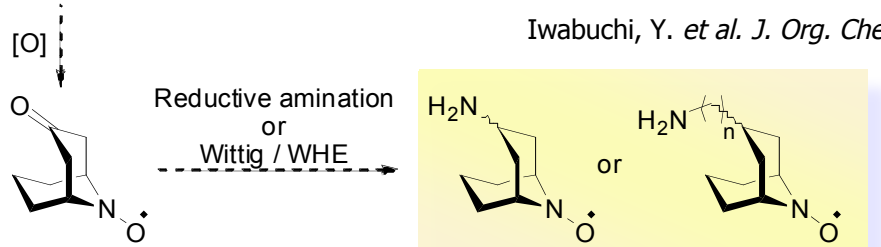
# 3-Amino-9-Azabicyclo[3.3.1]nonane *N*-oxyl

- Gram-scale synthesis of ABNO by Iwabuchi et al.:



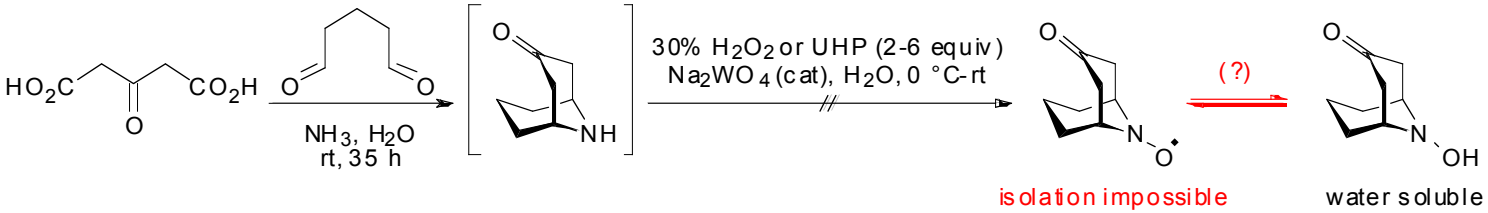

Iwabuchi, Y. *et al. J. Org. Chem.* **2009**, *74*, 4619.

- Idea:

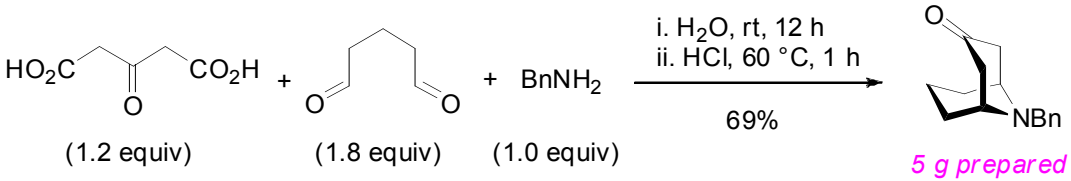



Dupeyre, R.-M.; Rassat, A. *J. Am. Chem. Soc.* **1966**, *88*, 3180.

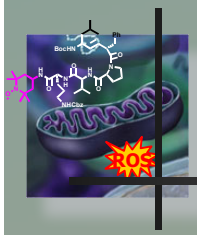
- Application:




- Modified route (optimized conditions):




Mach, R. H. *et al. J. Med. Chem.* **1993**, *36*, 3707.

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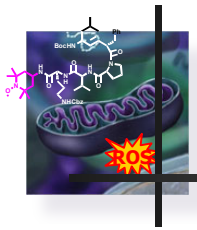
- MS & NMR facilities
- Wipf group members past & present




# The Myth of Eternal Youth