

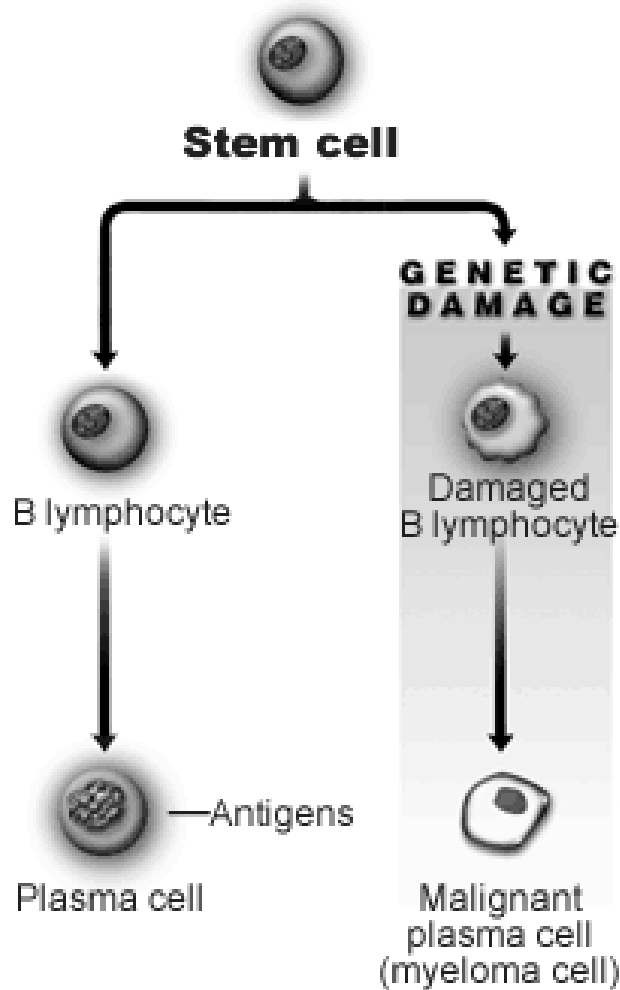
Human Metabolism of a Novel Chemotherapeutic: Illuminating the Mechanisms of P450-Catalyzed Deboronation

J. Scott Daniels

Pharmacokinetics, Dynamics and Metabolism
Pfizer, Inc.
Chesterfield, MO

Multiple Myeloma (MM)

plasma cell malignancy



- 5 cases in 100,000 people

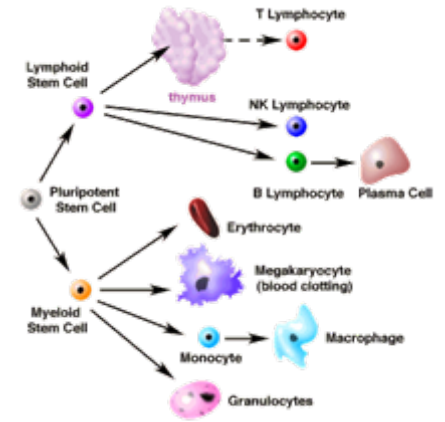
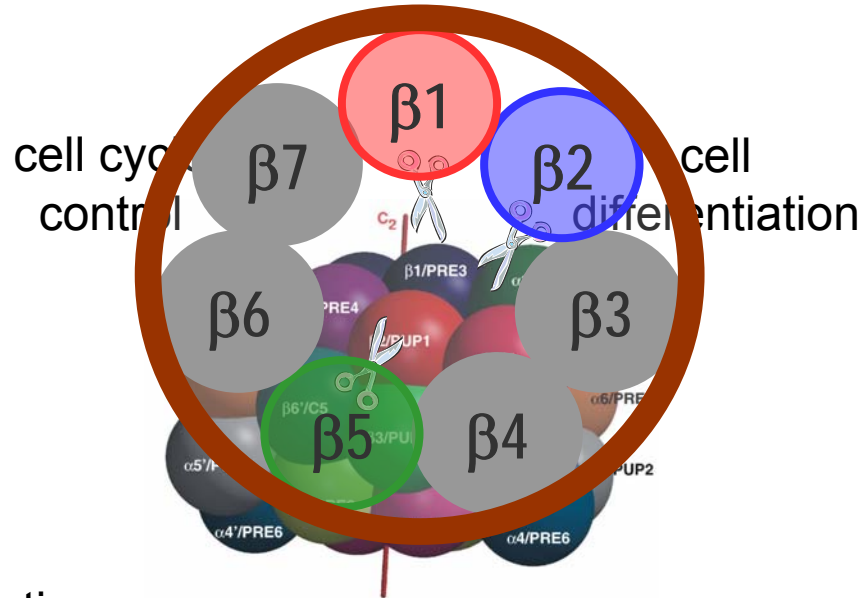
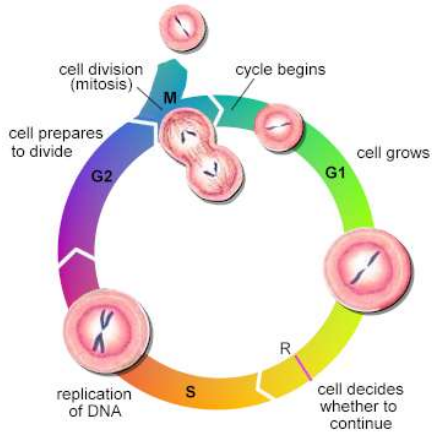
- YR 2005 : 16,000 new cases

- 50,000 with MM in USA

Multiple Myeloma (amplified proteasome activity)

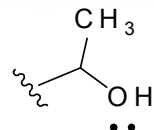
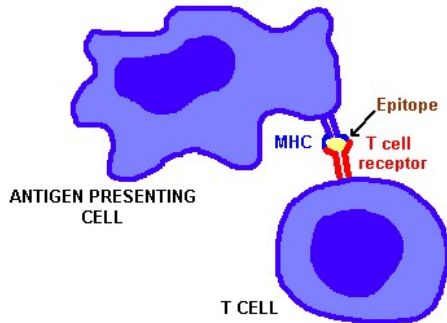
- (1) uncontrolled division
- (2) traffic to bone
- (3) cell adhesion
- (4) ↑ M protein

MM Cells Dependent on 26S Proteasome

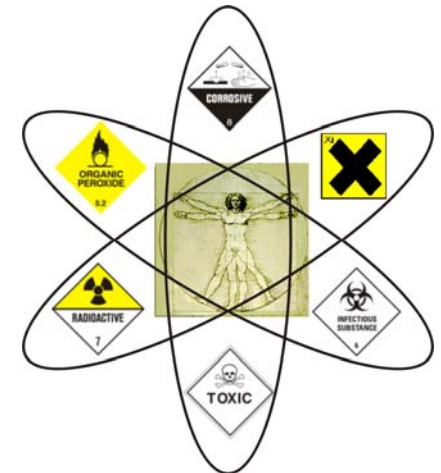


antigen processing

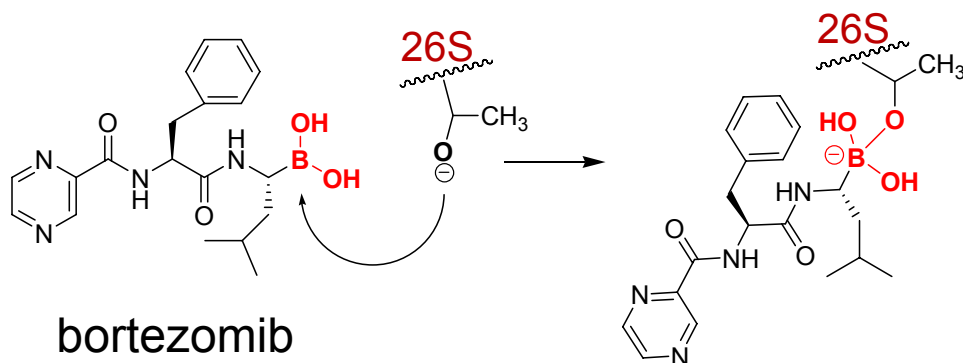
stress response



Threonine protease



Bortezomib : Transition State Analog of 26S



enzyme	K_i (nM)
20S proteasome	0.62
Leuk. elastase	2,300
cathepsin G	630
chymotrypsin	320
thrombin	13,000



- approved in 2003 for treatment of multiple myeloma*
- currently approved for 2nd line treatment
- undergoing multiple clinical trials

Bioorg. Med. Chem. Lett. **1998**, 8, 333-338; *Clin. Cancer. Res.* **2004**, 10, 3954-3964.

*relapsed, refractory

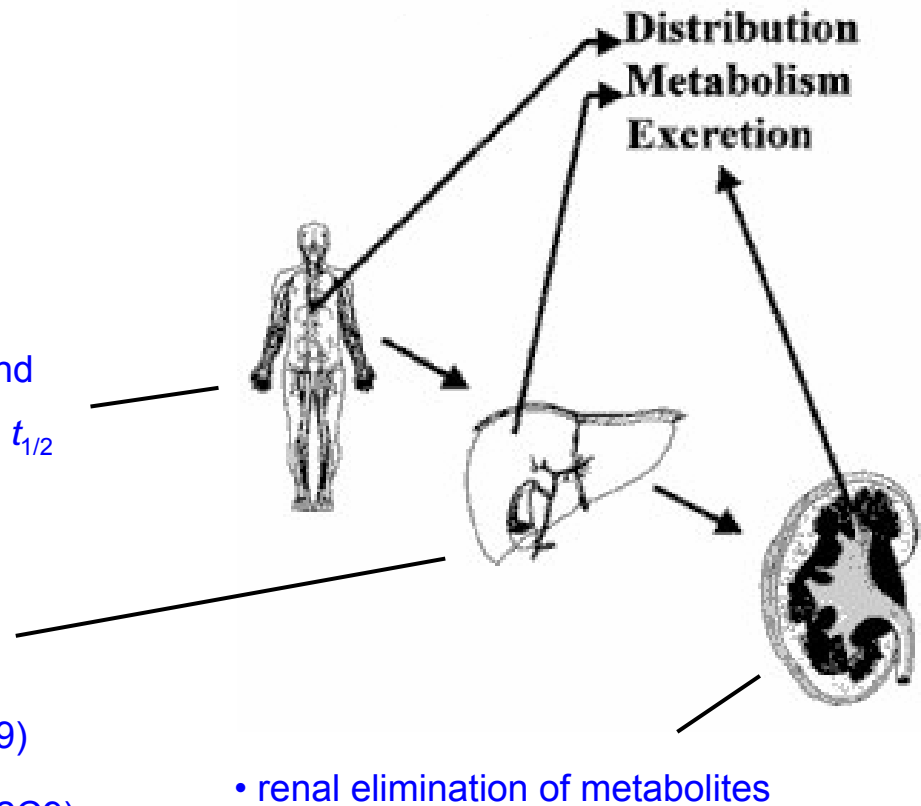
Human Disposition of Bortezomib



for intravenous injection

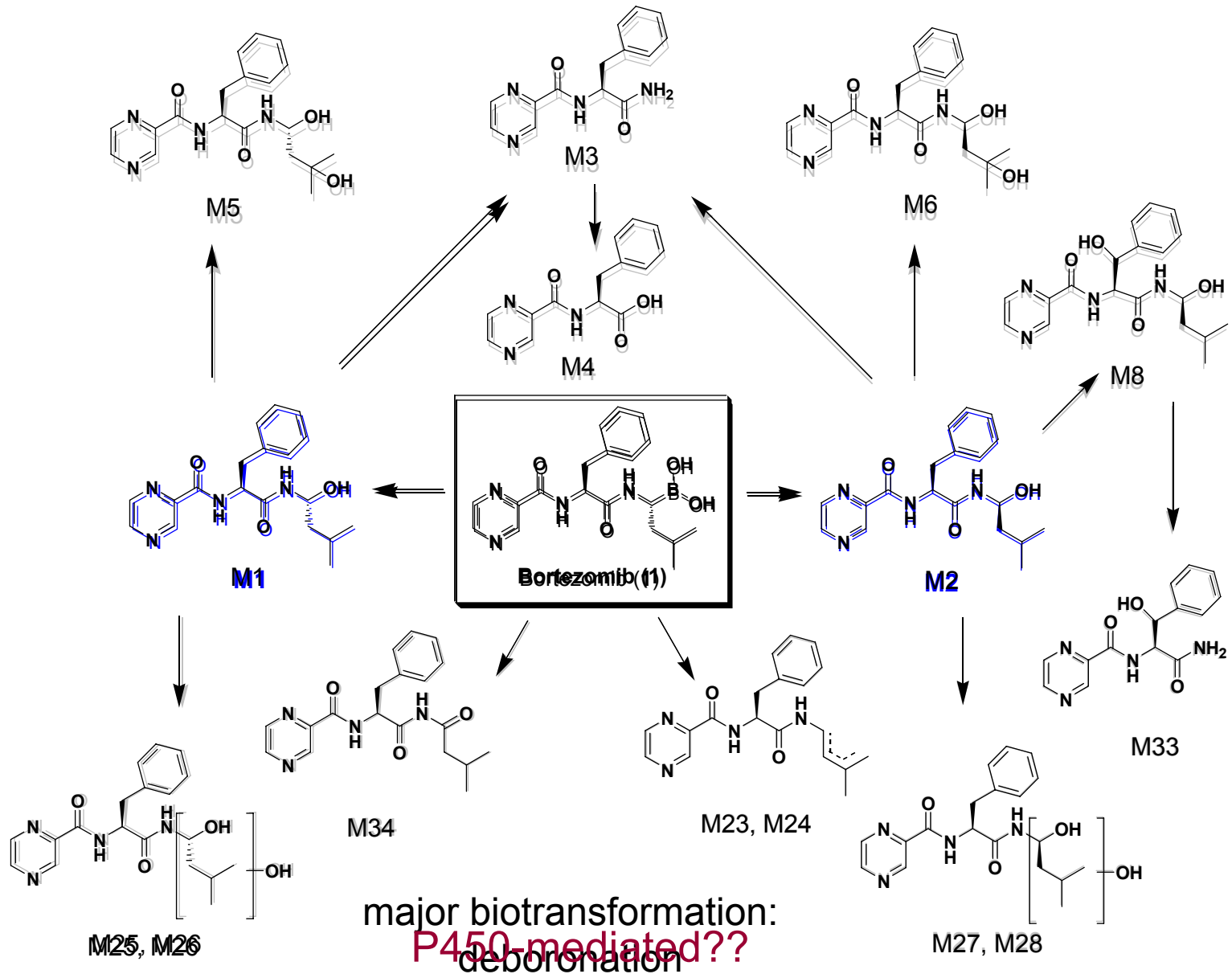
- ~ 83% protein bound
- high V_d ; long elim. $t_{1/2}$

- not subjected to drug efflux (e.g., Pgp)
- does not inhibit efflux in Caco-2 cells
- No obsvd inhibition of P450 (M1/M2-2C19)
- P450 phenotype (3A4>2C19>1A2>2D6>2C9)

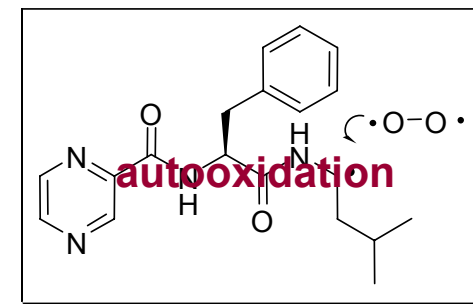
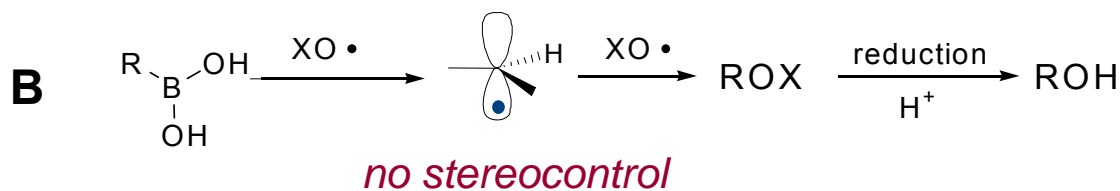
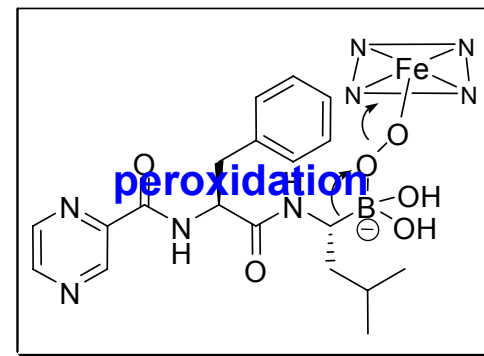
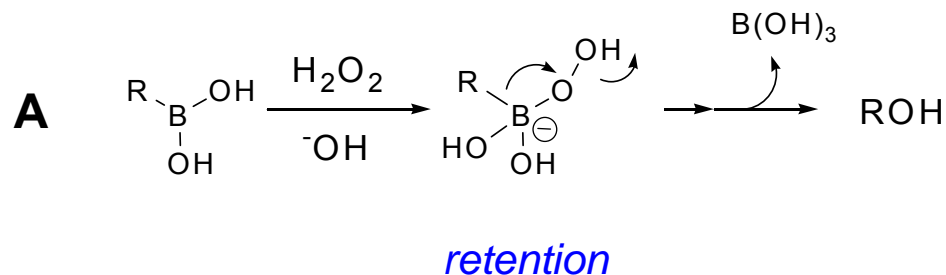


- renal elimination of metabolites

Human Metabolism of Bortezomib



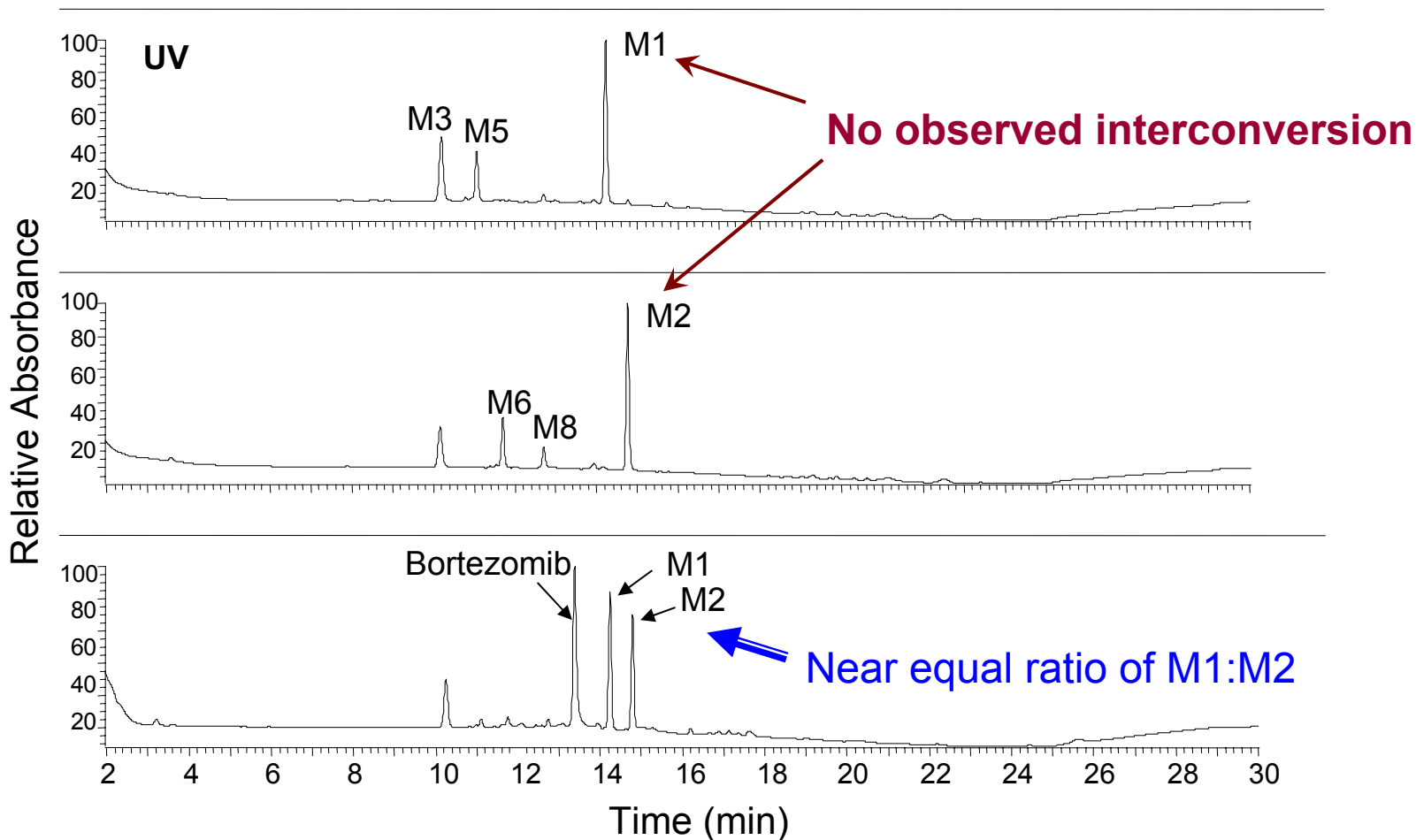
Routes of Borane/Boronate Oxidation (H.C. Brown)



$\text{XO}\cdot = \text{O}_2$ or peroxy radical

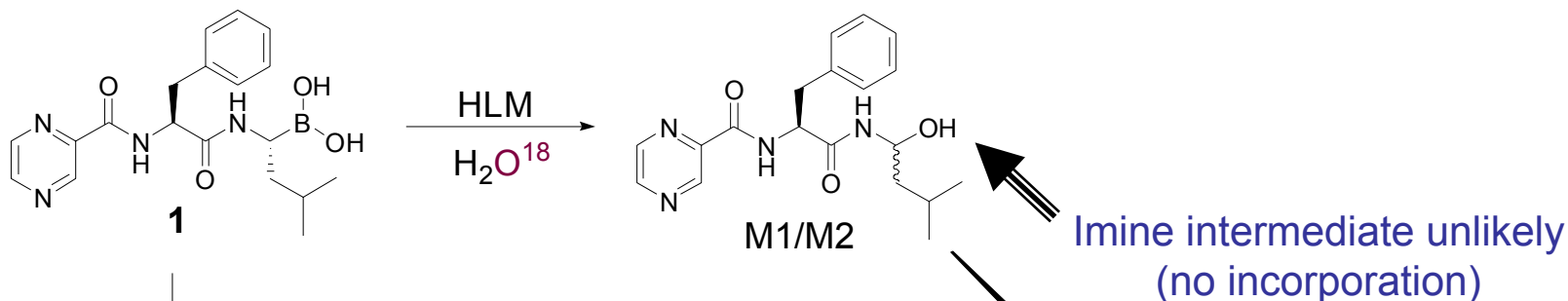
No published accounts of boronic acid metabolism (i.e., P450)

Human Liver Metabolism of Bortezomib

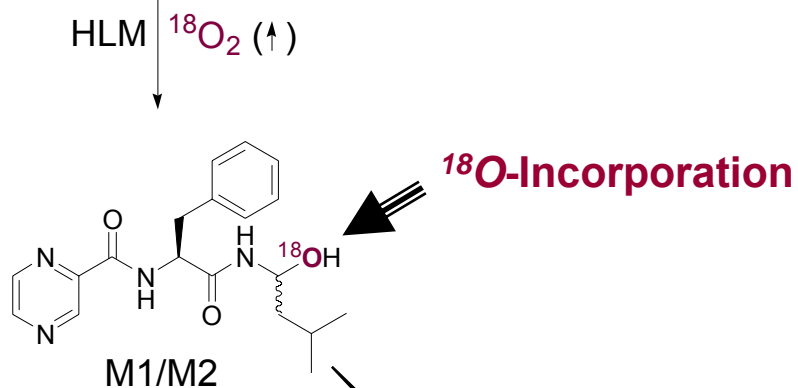


What is the mechanism of this novel P450 reaction?

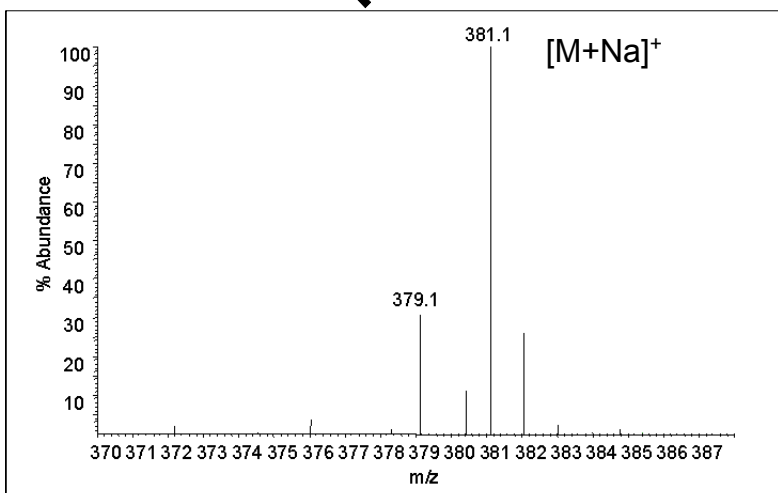
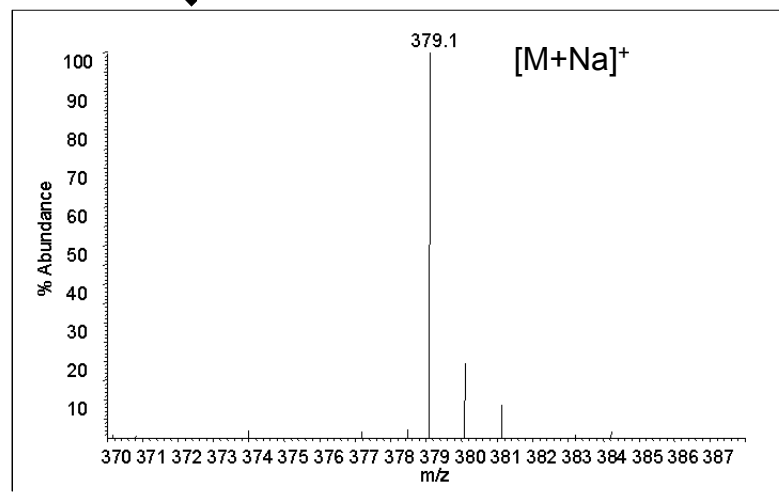
ESI-LC/MS to Detect Isotope (^{18}O) Incorporation



Imine intermediate unlikely (no incorporation)



^{18}O -Incorporation

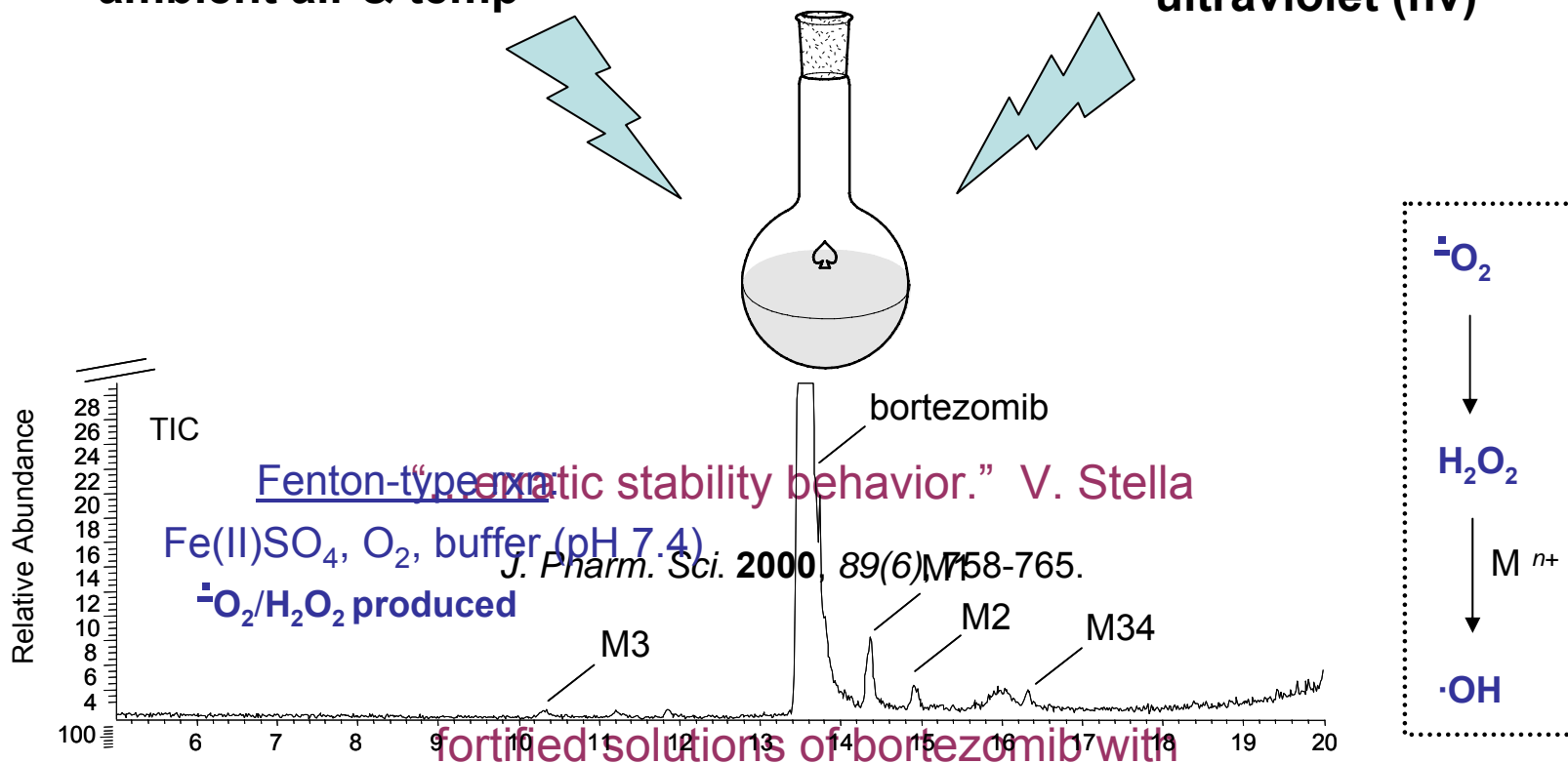


Use of $^{18}\text{O}_2$ resulted in the formation of ^{18}O -labeled M1 and M2.

Stability of Bortezomib in Formulation

ambient air & temp

ultraviolet (hv)

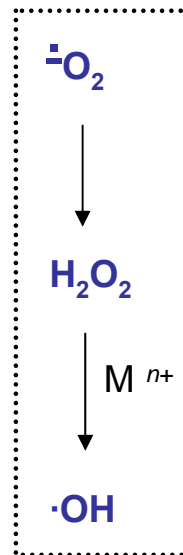


Fenton-type oxidative stability behavior. V. Stella

Fe(II)SO₄, O₂, buffer (pH 7.4)

J. Pharm. Sci. 2000, 89(6), 758-765.

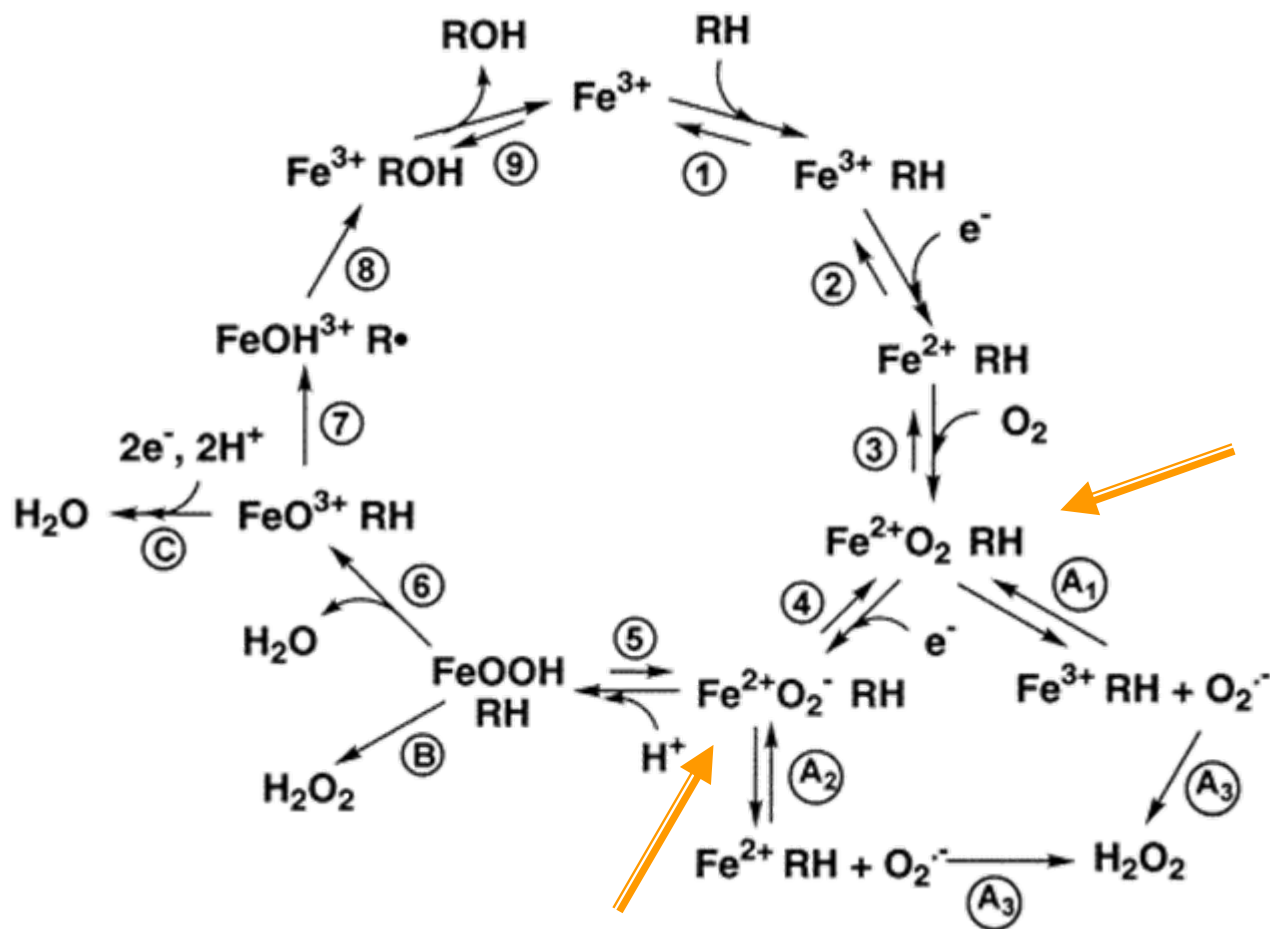
$\cdot\text{O}_2^-/\text{H}_2\text{O}_2$ produced



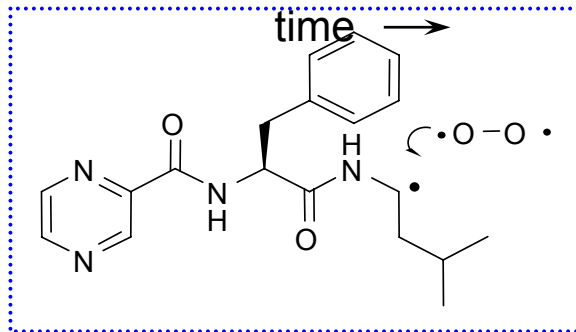
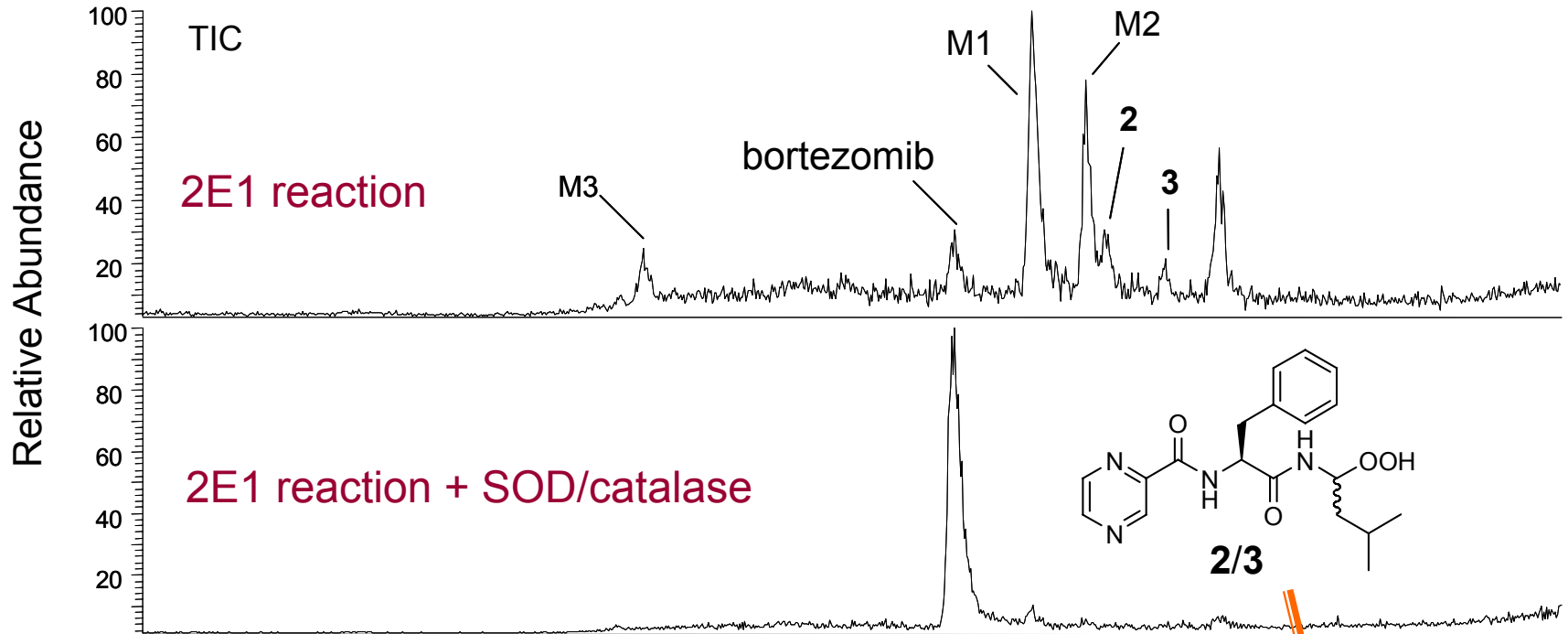
Ascorbate & EDTA

reactive oxygen species mediate deboronation

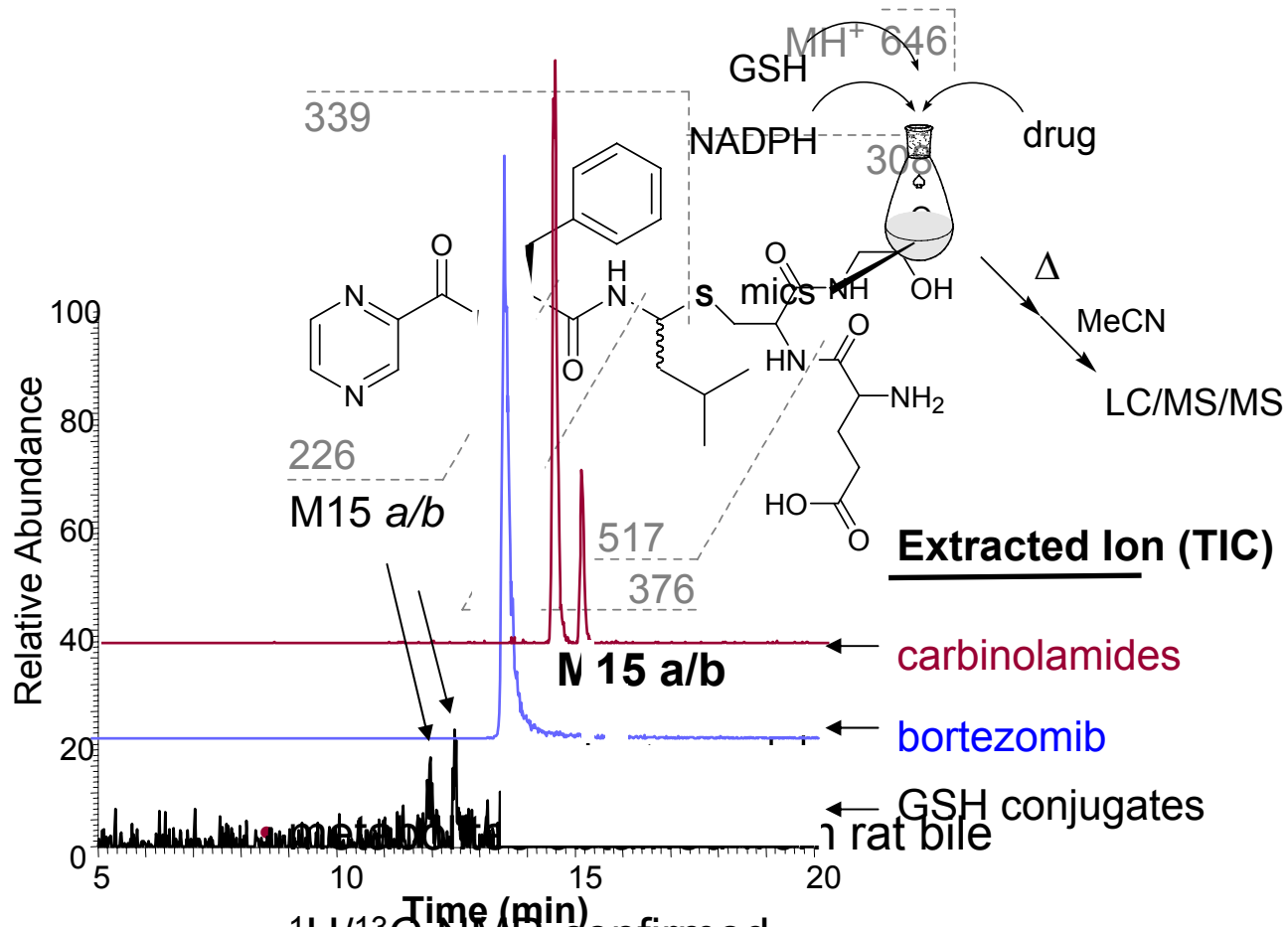
Oxidase Activity of P450: ROS Formation (“uncoupling”)



P450 2E1 Metabolism of Bortezomib “ the leaky CYP ”



Evidence of Radical Intermediate?



- $^1H/^{13}C$ NMR-confirmed

- regiochemistry of carbinolamides

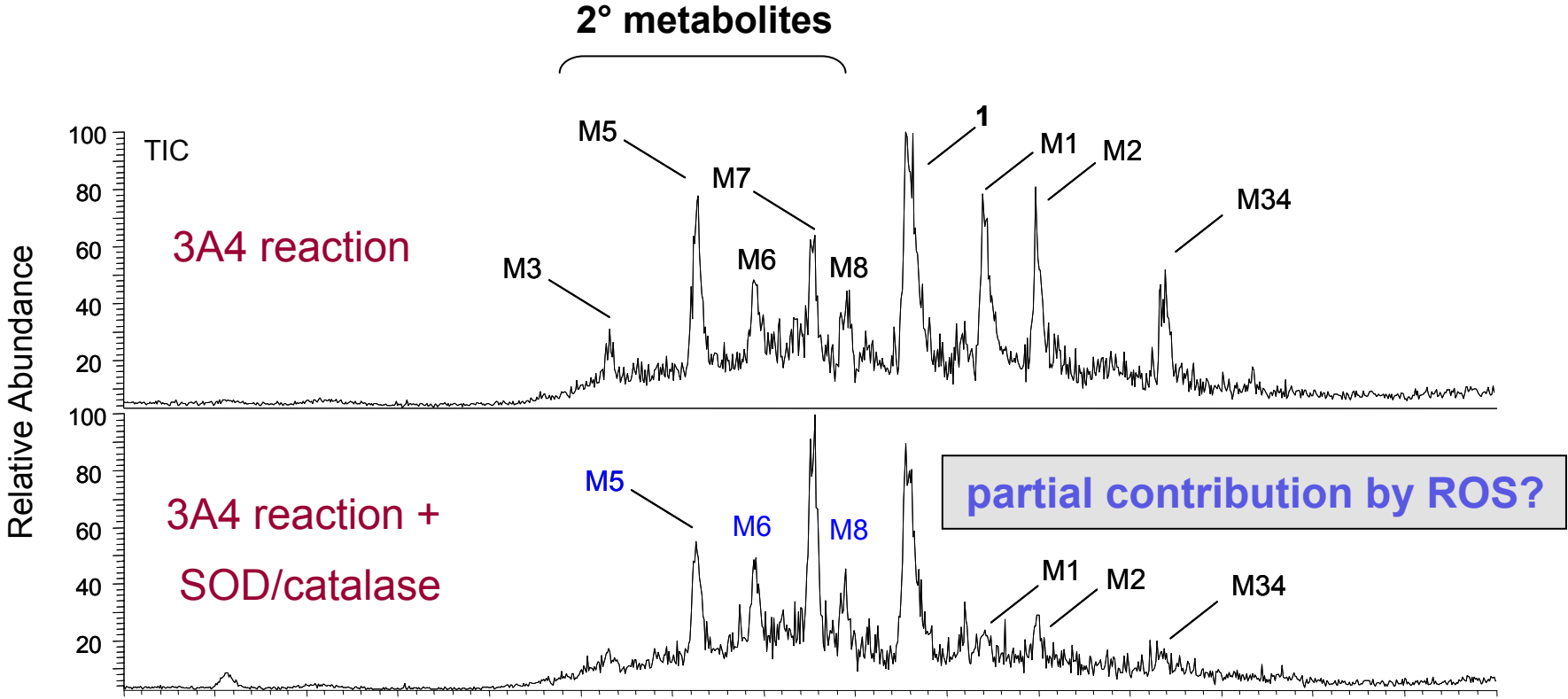
Zen and the art of P450 Catalysis...

“The real purpose of the scientific method is to make sure Nature hasn’t misled you into thinking you know something you don’t actually know. **If you get careless or go romanticising scientific information**, giving it a flourish here or there, **Nature will soon make a fool out of you.**”

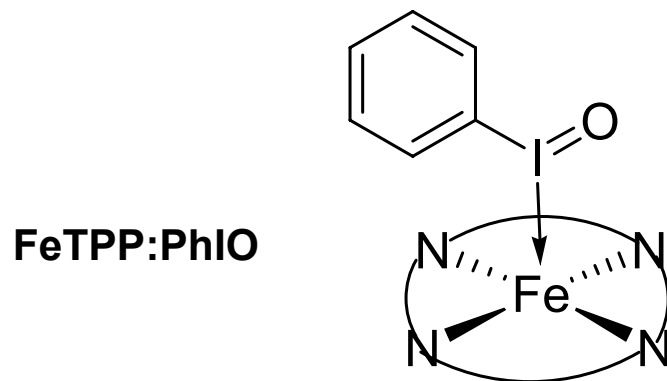
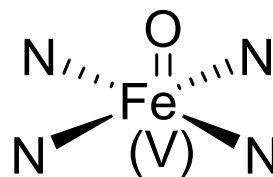
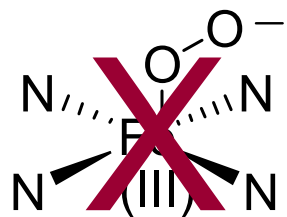
Robert Pirsig

Zen and the Art of Motorcycle Maintenance (Copyright© 1974, Pirsig)

3A4 Metabolism of Bortezomib



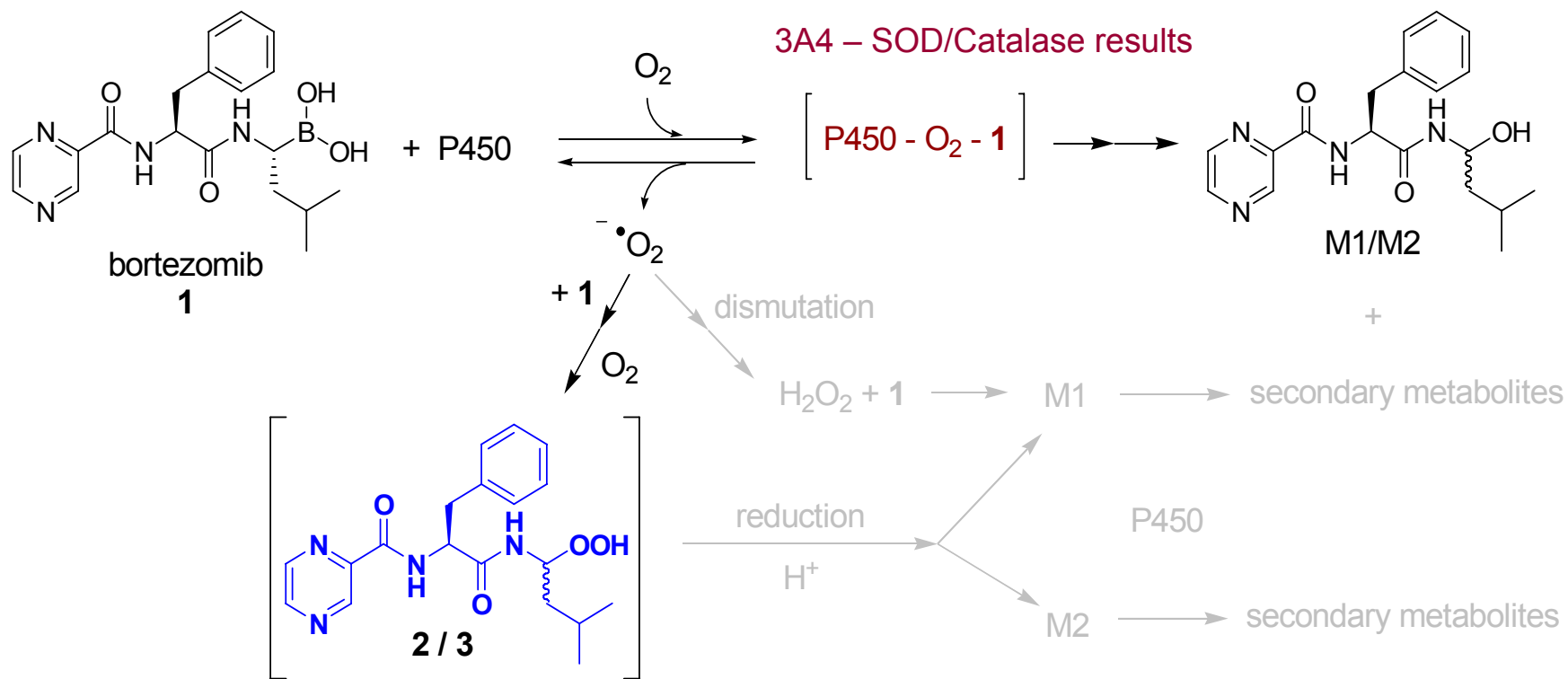
Enzyme-bound oxidants contributing?



6-fold increase in M1:M2
over control (PhIO)

see references within

Summary of this Novel P450 Reaction



peroxycarbinolamides = putative radical

[We can teach you metabolism; we can't teach you chemistry.]

G.T. Miwa

Thank you Gerald.

Sincerely,

A Grateful Chemist

Acknowledgements

Millennium DMPK

- Lawrence Gan (biotransformation)
- Jason Labutti
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- Professor Paul Ortiz de Montellano (*UCSF*)
- Professor Neal Castagnoli Jr. (*Va Tech*)
- VELCADE™ Oncology Team
 - Mark Williamson, Larry Dick and Chris Tsu

email: scott.daniels@pfizer.com