

Development of a Multi-kilogram
Synthetic Route to IPI-926, A Novel
Hedgehog Pathway Antagonist for the
Treatment of Malignant Diseases.

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B. C. Austad; J. Adams; M. L. Behnke; A. C. Castro; M. J. Campbell; D. Genov; J. Ferguson; M. A. Foley; M. J. Grogan; L. Grenier; A. Hague; J. C. Helble; B. Lane; A. Lescarbeau; P. Lo; C. Lory; D. Mann; S. J. Nair; S. Peluso; J. R. Porter; M. R. Tremblay; L. Yu; A. B. Charette

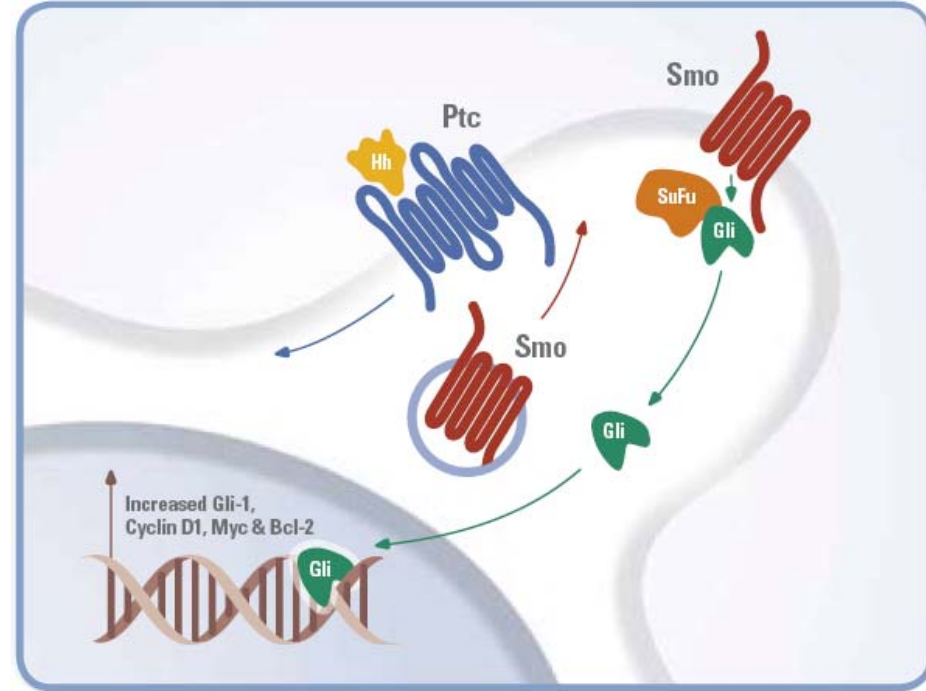
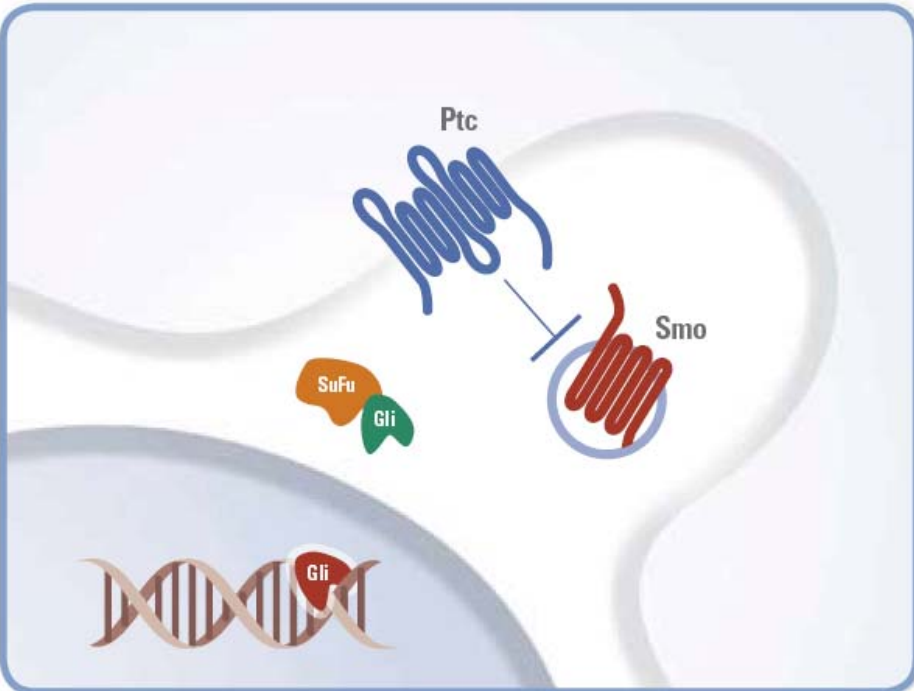
Disclosure

- I am an employee and stockholder of Infinity Pharmaceuticals, Inc.

Overview

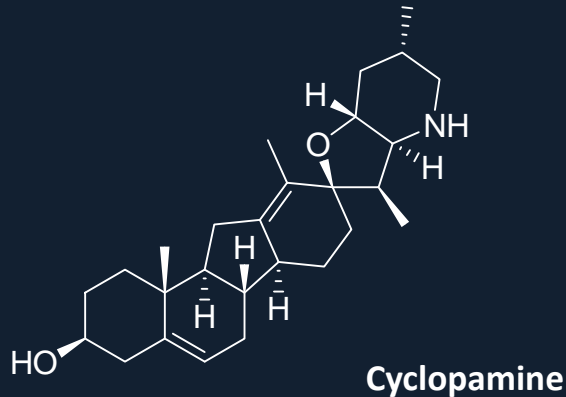
- Introduction to Hedgehog pathway
- IPI-926 discovery
- Optimization D-ring expansion chemistry

Activation of Hedgehog Pathway Signaling



Gorlin's Syndrome: BCC, Medulloblastoma
Pancreatic, Ovarian, Prostate, Colorectal, SCLC
CML , CLL, ALL, MM, SCLC, Chondrosarcoma

Chemistry Meets Hedgehog Genetics



- First isolated from *Veratrum californicum*: Keeler RF. *Phytochemistry*. **1968**;7:303.
- Structure elucidation: Keeler RF. *Phytochemistry*. **1969**;8:223.



Chemistry

Cyclopamine results in cyclopic animals

- Keeler RF, Binns W. *Teratology*. **1968**;1:5.

Genetics

Mutation of hedgehog pathway results in cyclopic animals

- Chiang et al, *Nature*. **1996**; 383: 407.
- Roessler et al, *Nat. Genet.* **1996**; 14: 357
- Belloni et al. . *Nat. Genet.* **1996**; 14: 353

Cooper MK, et al., *Science*. **1998**;280:1603.

Cyclopamine is Readily Available from Wild Harvest

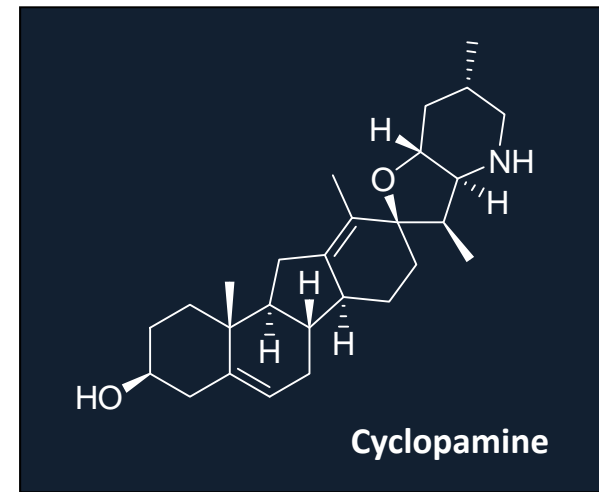


Veratrum californicum

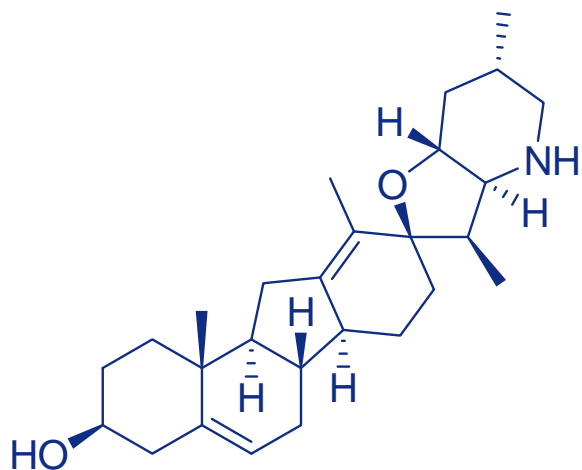
Harvest/Dry/Mill



Extract/Purify/Isolate

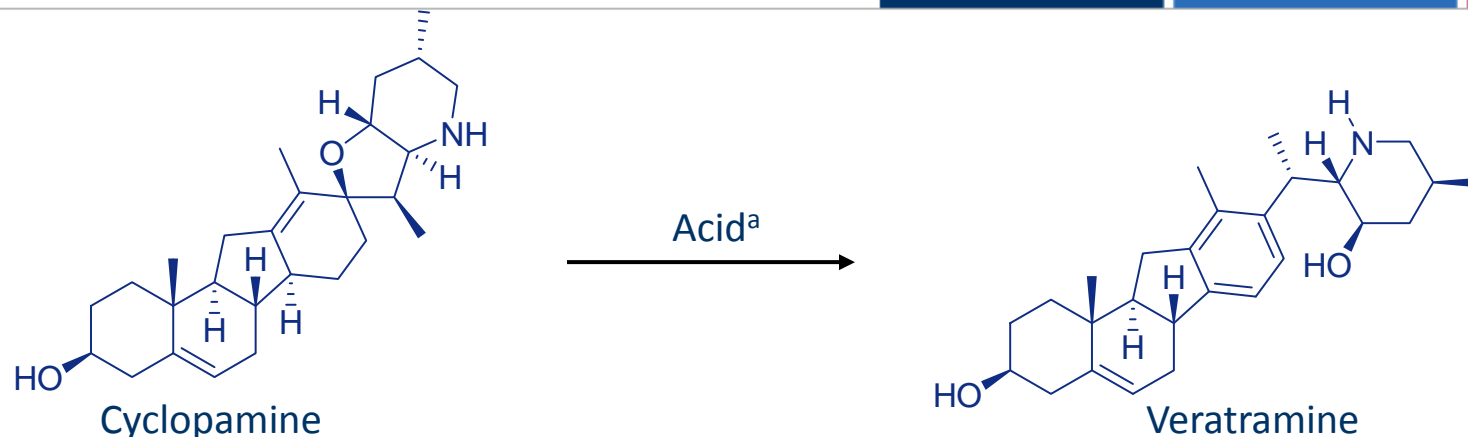


Cyclopamine: Starting Point for an Oral Hh Antagonist?



- **Poor pharmaceutical properties**
 - Solubility (<10 ug/ml in pH 7)
 - Chemical stability (low at pH 1.9)
- **Hh Pathway Potency**
 - Cellular IC₅₀ ~ 300 nM

Cyclopamine is Acid Sensitive



~40% degradation of cyclopamine after 60 min at 37°C in simulated gastric fluid (pH 1.9)^e

- **Not a Hedgehog pathway antagonist**
- **Known off-target activities;**
 - Blocks sodium channel^b
 - 5HT agonist in presynaptic neuron^c
 - Cause hemolysis of human RBC^d

^aKeeler *Phytochemistry*, **1968**, 7, 303.

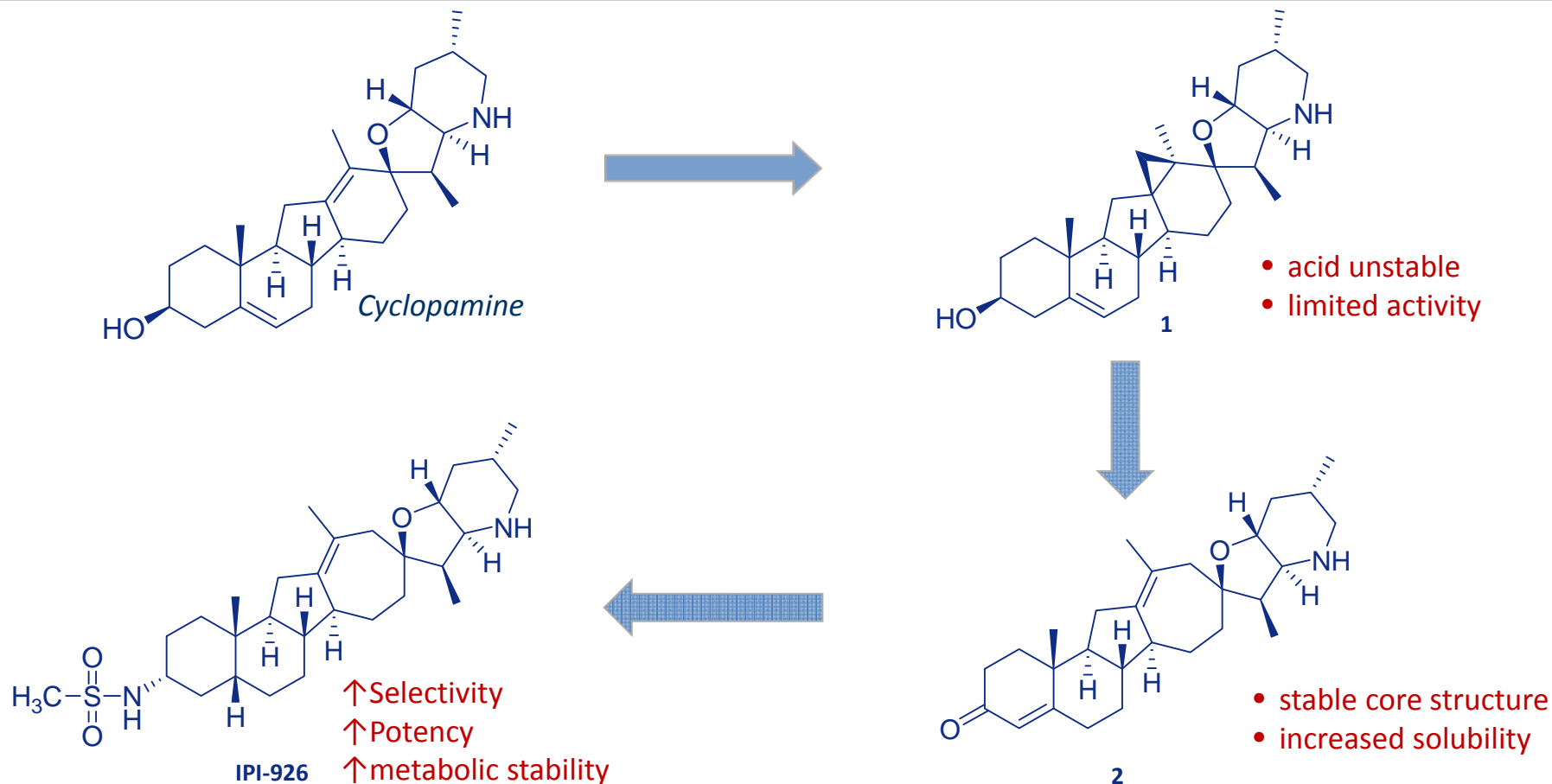
^bHonerjager *Rev. Physiol. Biochem. Pharmacol.*, **1982**, 92, 1.

^cNagata et al. *Japan J. Pharmacol.*, **1991**, 55, 129.

^dBadria et al., *Pharmazie*, **1995**, 50, 421.

^eTremblay MR, Nevalainen M, Nair SJ et al. *J Med Chem.* **2008**;51:6646.

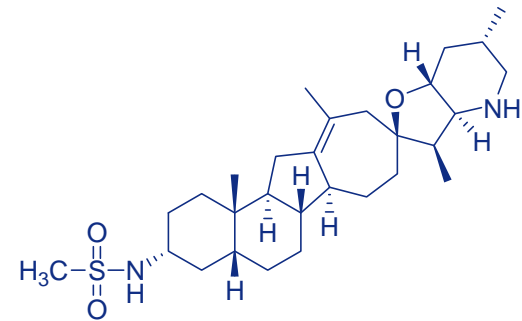
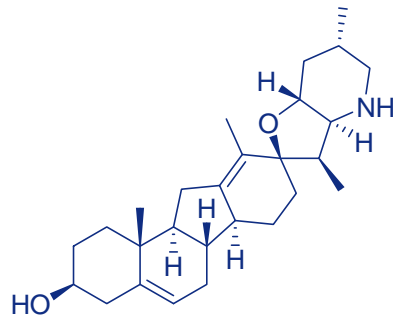
Discovery of Potent D-homo Cyclopamine Analogs



Tremblay MR, Nevalainen M, Nair SJ, et al. *J Med Chem.* **2008**;51:6646.

Tremblay MR, Lescarbeau A, Grogan M, et al. *J. Med. Chem.*, **2009**;52:4400.

Development of the Expanded D-Ring Series

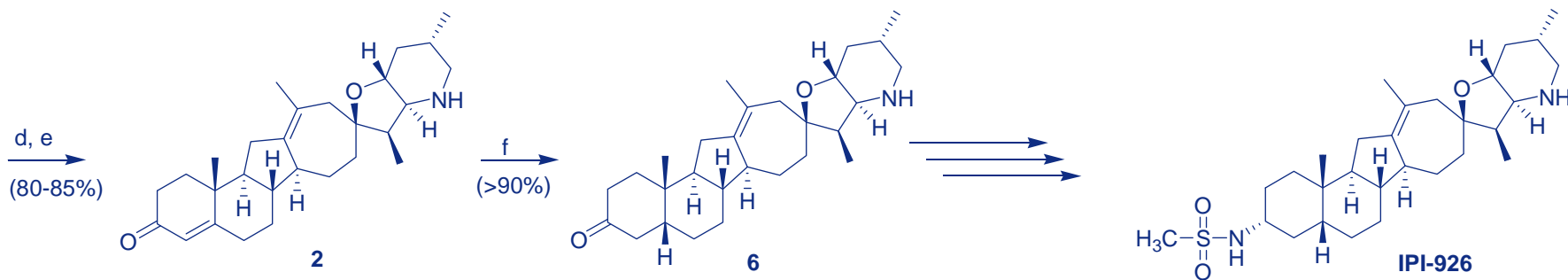
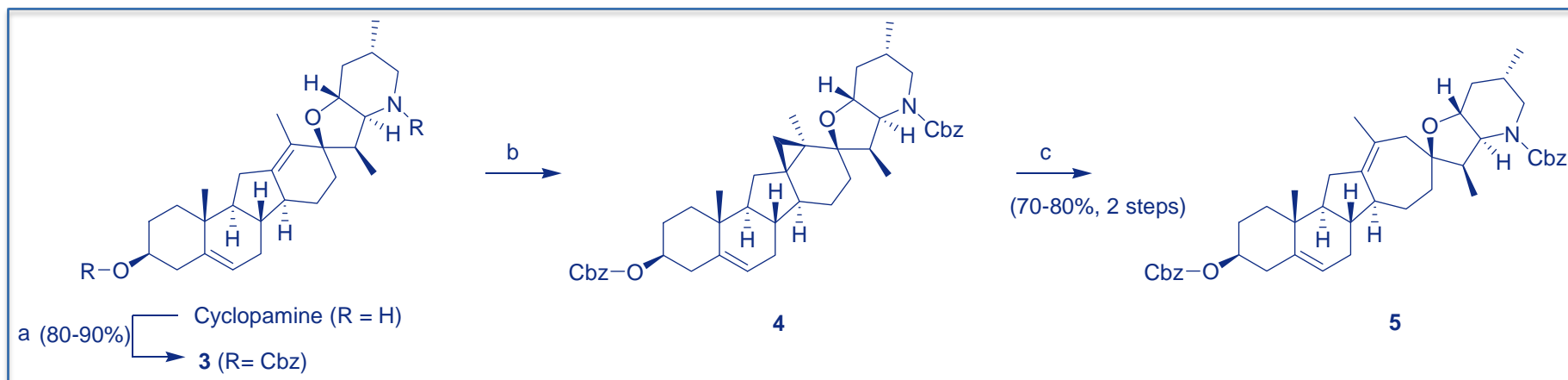


		Cyclopamine	IPI-926**
Potency	C3H10 (EC ₅₀)	300-400 nM	7-15 nM
	Smo binding (IC ₅₀)	114 nM	1-2 nM
Mouse PK	Bioavailability (PO)	33%*	~100%
	Half-life	4 h*	10.5 h
	Vol. of distribution	-	11 L/kg
Other		Poor stability at pH < 2. Low water solubility (5 µg/mL @ pH 7) Limited efficacy in xenograft models	>> Improved PK and PD Efficacious in multiple xenograft models.

*From Lipinski et al. (2008): Species: Female C57BL/6J mice at 12–16 weeks of age.

** Tremblay MR, Lescarbeau A, Grogan M, et al. *J. Med. Chem.*, **2009**, 52 (14), 4400

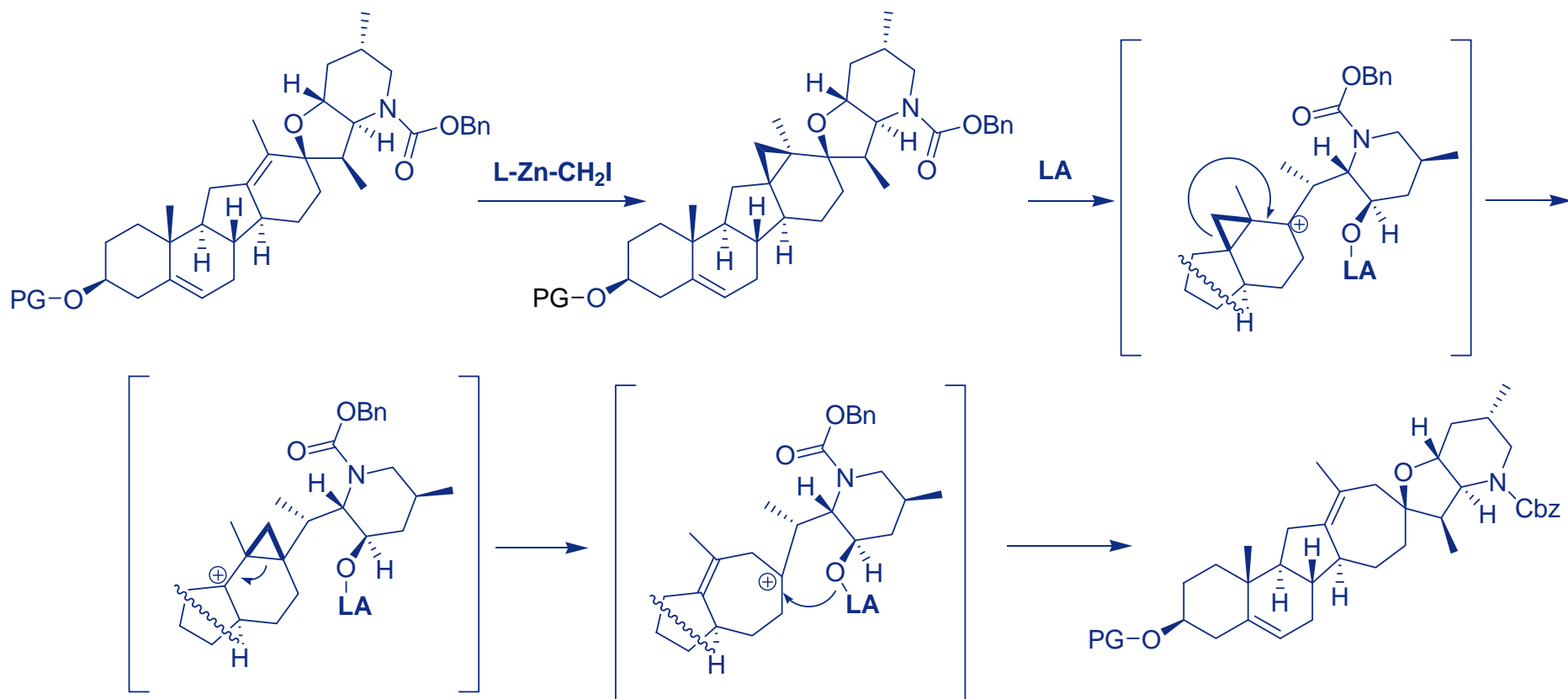
Semi-synthesis of IPI-926



Reagents and Conditions

(a) Cbz-OBt, Et₃N, DMAP, EtOAc, 40 °C; (b) Et₂Zn, (ArO)₂P(O)OH, CH₂I₂, DCM, 27 °C; (c) MsOH, -45 °C; (d) H₂, Pd/C, toluene/IPA; (e) Al(Ot-Bu)₃, toluene/MEK, 75 °C; (f) H₂, Pd/C, 3-Picoline

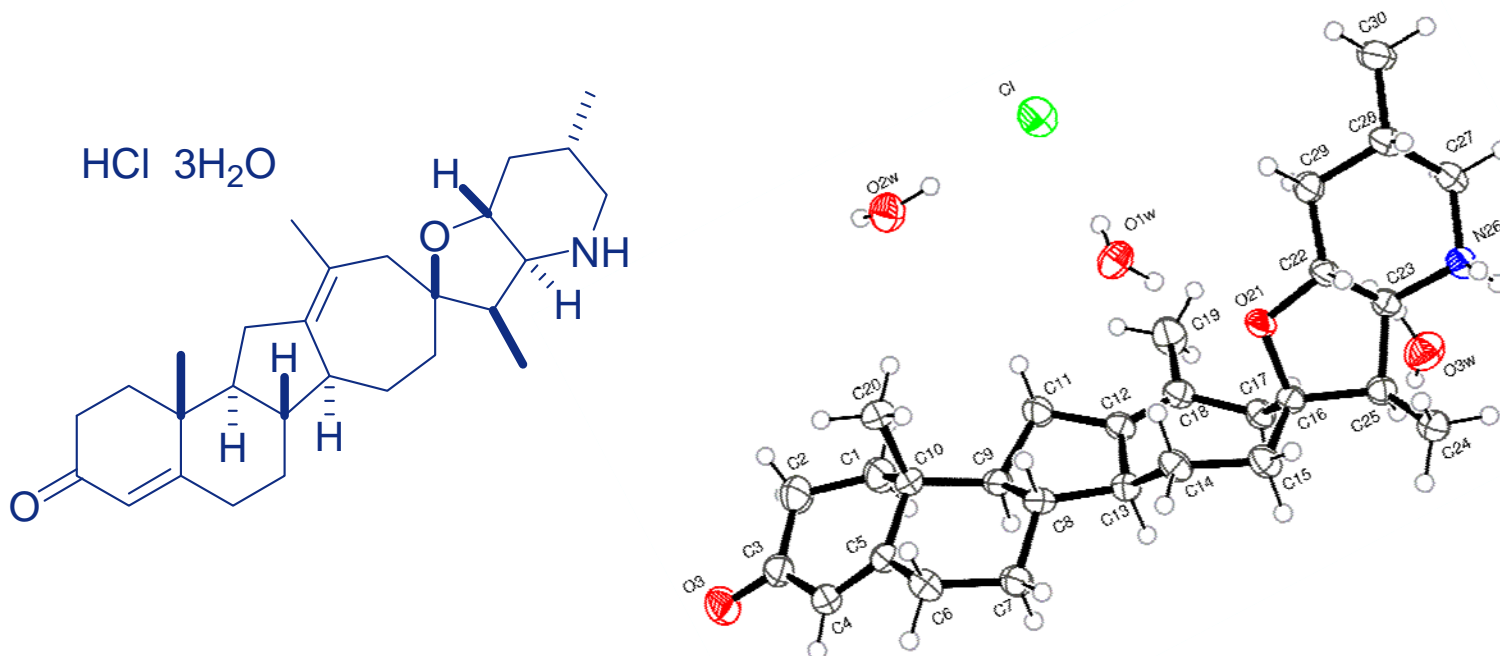
Proposed Ring Expansion Mechanism



Tremblay MR, Nevalainen M, Nair SJ et al. *J. Med. Chem.*, **2008**, 51, 6646.

Hayasaka, K; et al. *Tetrahedron Lett.*, **1985**, 26, 873.

X-ray Crystal structure of Key Intermediate



Tremblay MR, Nevalainen M, Nair SJ et al. *J Med Chem.*, **2008**, *51*, 6646

Challenges with Cyclopropanation Reactions

- Solvent Limitations
- Reagent decomposition
- Work up challenges

- Reports of explosions
 - EtZnCH₂I
 - Zn(CH₂I)₂

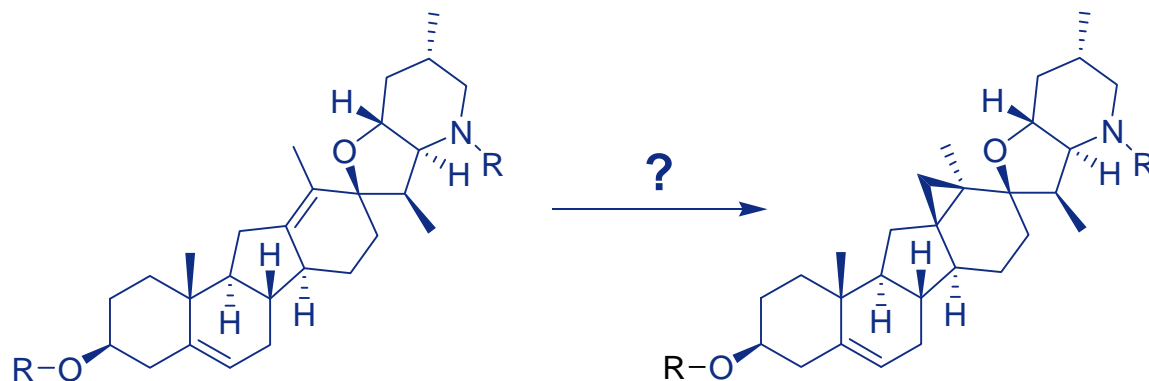


a) Charette, A.B. et al. *J. Org. Chem.*, **1995**, *60*, 1081

b) Charette, A.B. *Chemical & Engineering News* (6 Feb 1995) Vol. 73, No. 6, pp. 2.

c) American Industrial Hygiene Association: Laboratory Safety Incidents

Optimization Goals

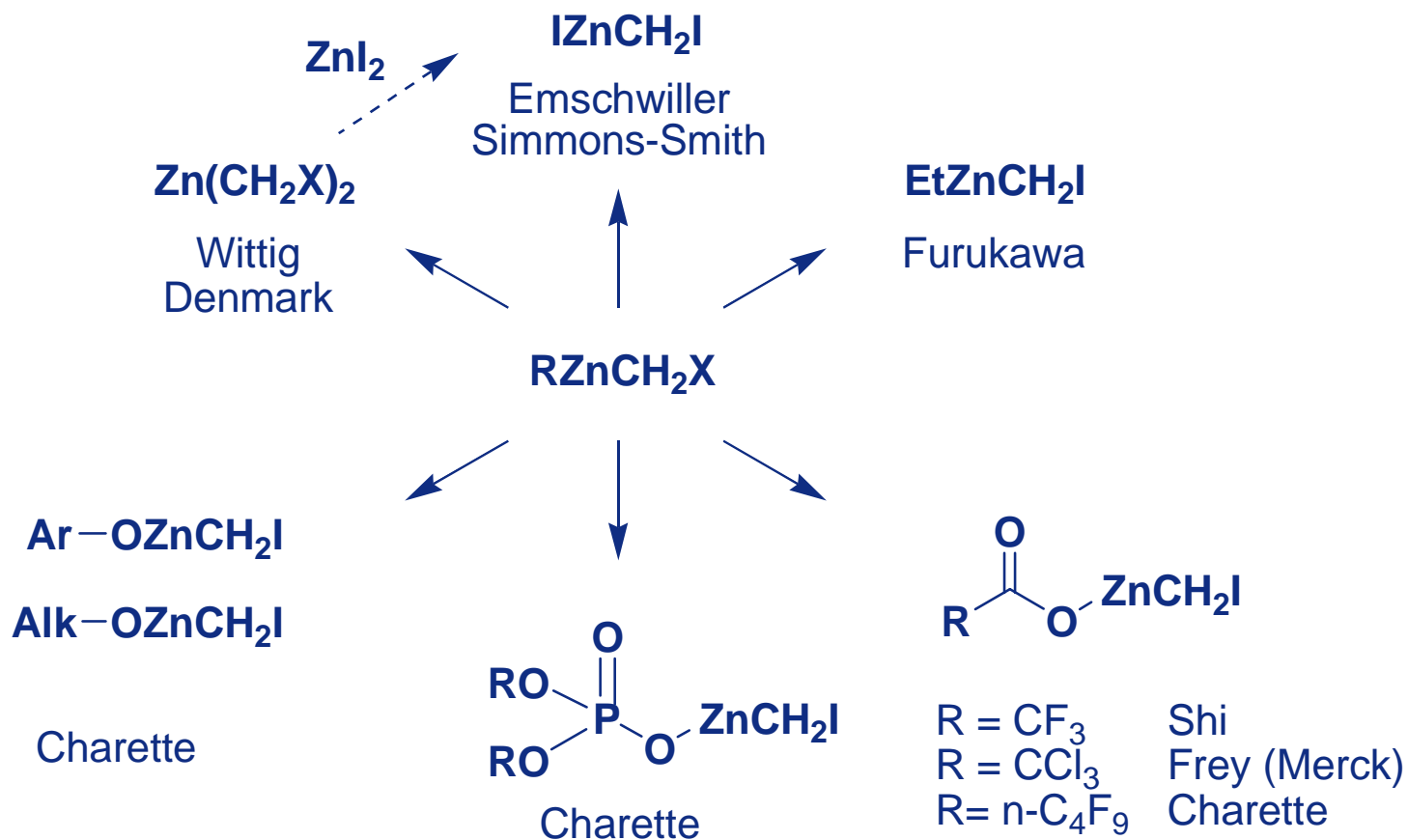


- Robust/reproducible results
- Safe, scalable process
- Homogeneous reaction system
- Balanced reactivity/selectivity
- Byproducts removable with workup

Reagent Screening

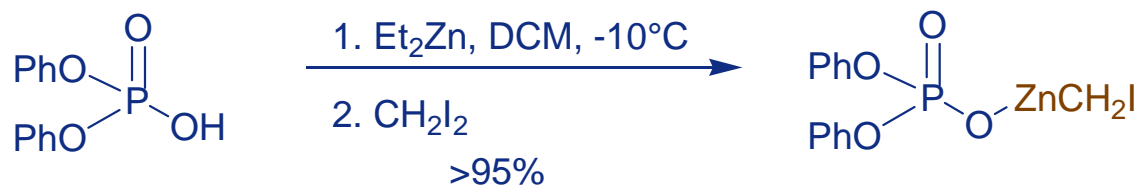
- Zn carbenoids
 - Zn(Cu)/CH₂I₂ – Simmons, et al. (1973)
 - Et₂Zn/CH₂I₂ – Furukawa, et al. (1968)
 - Et₂Zn/I-CH₂-Cl – Denmark, et al. (1997)
 - Et₂Zn/CH₂I₂/DME – Charette; Denmark
 - Et₂Zn/TFA/CH₂I₂ – Shi, et al. (1998)
 - Et₂Zn/TCA/CH₂I₂ – Frey, et al. (2003)
- Al carbenoids
 - (Me₃Al or iBu₃Al)/CH₂I₂ – Yamamoto, et al. (1985)
- In carbenoids
 - In /CH₂I₂ – Sain, et. Al. (2005)
- Transition metal diazoalkanes decomposition
 - CH₂N₂/Pd(II) , Cu(II), and Ru(II) – Tomilov (1990), Pincock (1964)
- Haloform derived carbenes
 - NaOH / CHCl₃ – Kraus (1972)

Useful Modifications of the Simmons-Smith



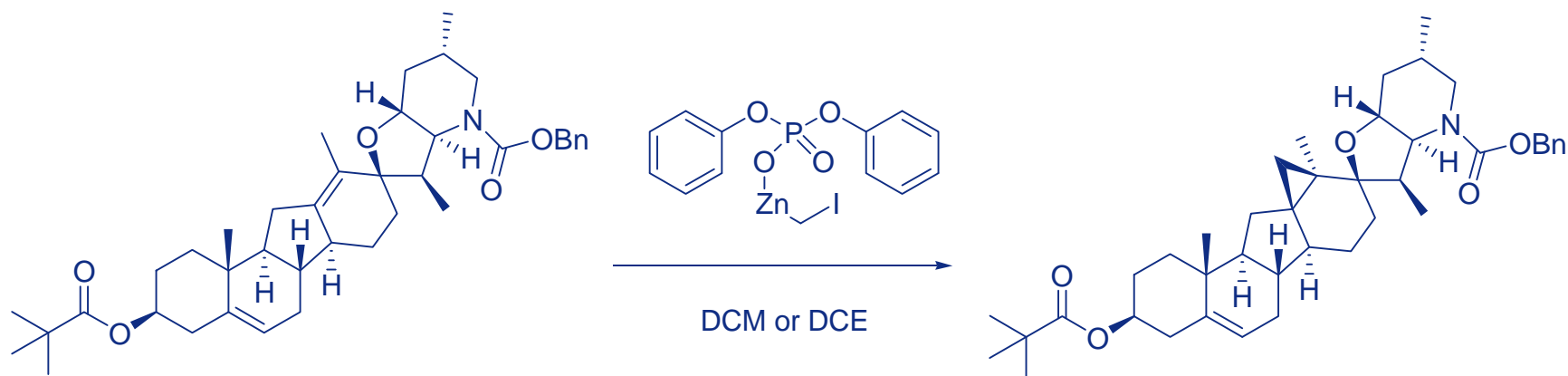
Charette, A.B. et al. *J. Org. Chem.*, **2010**, 75, 1244

Initial Results with Bisaryl Phosphate Zn Carbenoids



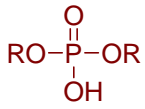

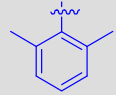
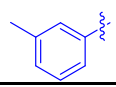
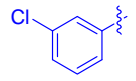
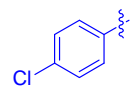
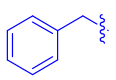
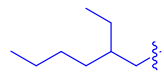
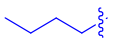
^1H NMR: 1.3 ppm (bs)
 ^{13}C NMR: -25.4 ppm

Charette, A.B., et al. *J. Am. Chem. Soc.*, **2005**, 127 (36),12440

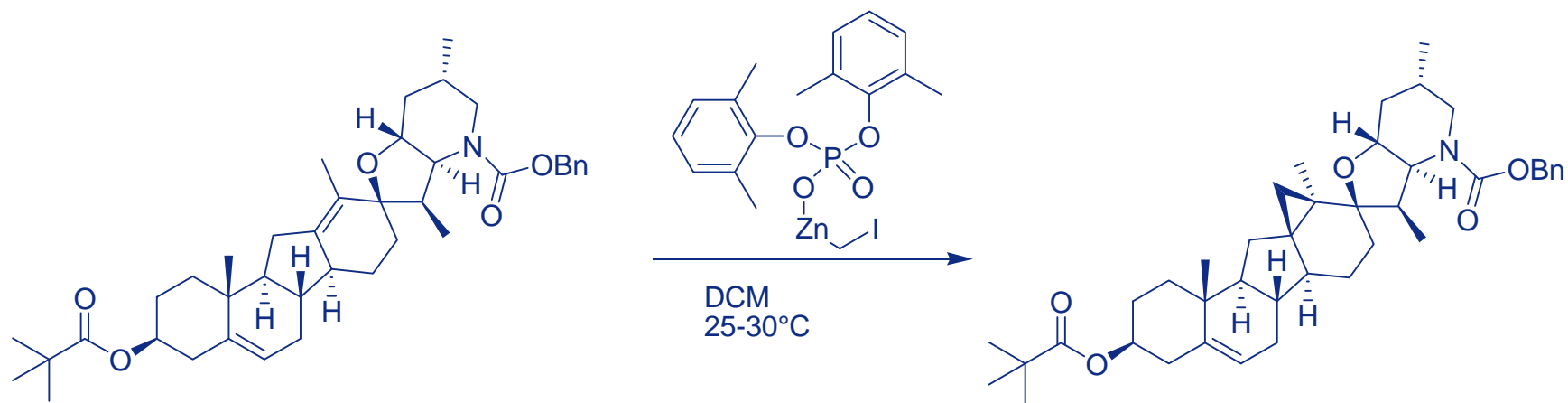


- Good selectivity
- Low solubility of DPPA zinc carbenoid

Further Development of Phosphate Ligand

	Physical state	Solubility (DCM)	Solubility in basic aqueous phase	Carbenoid Solubility (DCM)	Reactivity
	Solid	Medium	Yes	Low	High
	Solid	High	Yes	High	High
	Oil	High	Yes	High	High
	Oil	High	Emulsion	High	ND
	Solid	High	Emulsion	Low	ND
	Solid	High	Low	High	Medium
	Liquid	High	Emulsion	High	Low
	Liquid	High	Emulsion	High	Very Low

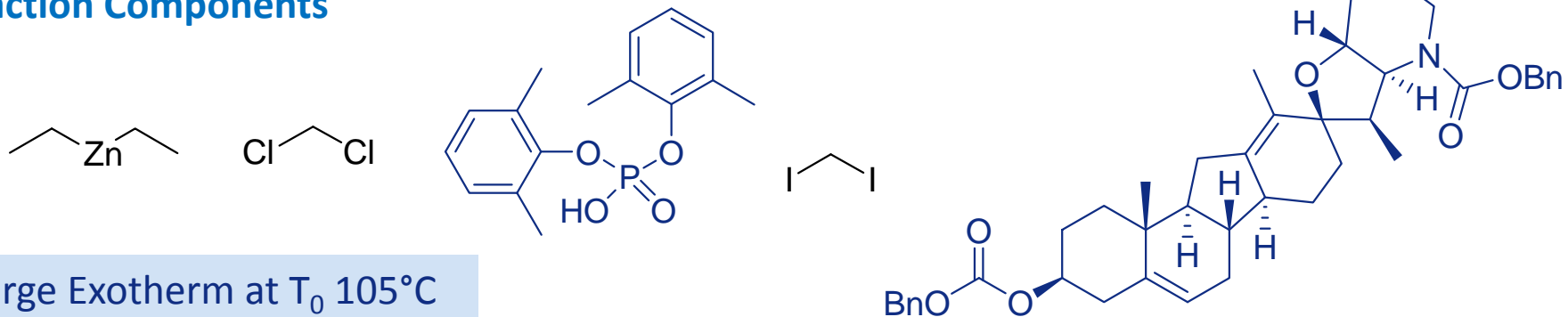
Optimization of Cyclopropanation Conditions



- Established preferred stoichiometry of all the reagents
- Established preferred temperatures and addition rates
- Established protocols for reagent and SM quality
 - *Residual Solvents*
 - *Reagent specifications*
 - *Moisture and Oxygen control*

Differential Scanning Calorimetry

Reaction Components



Large Exotherm at T_0 105°C

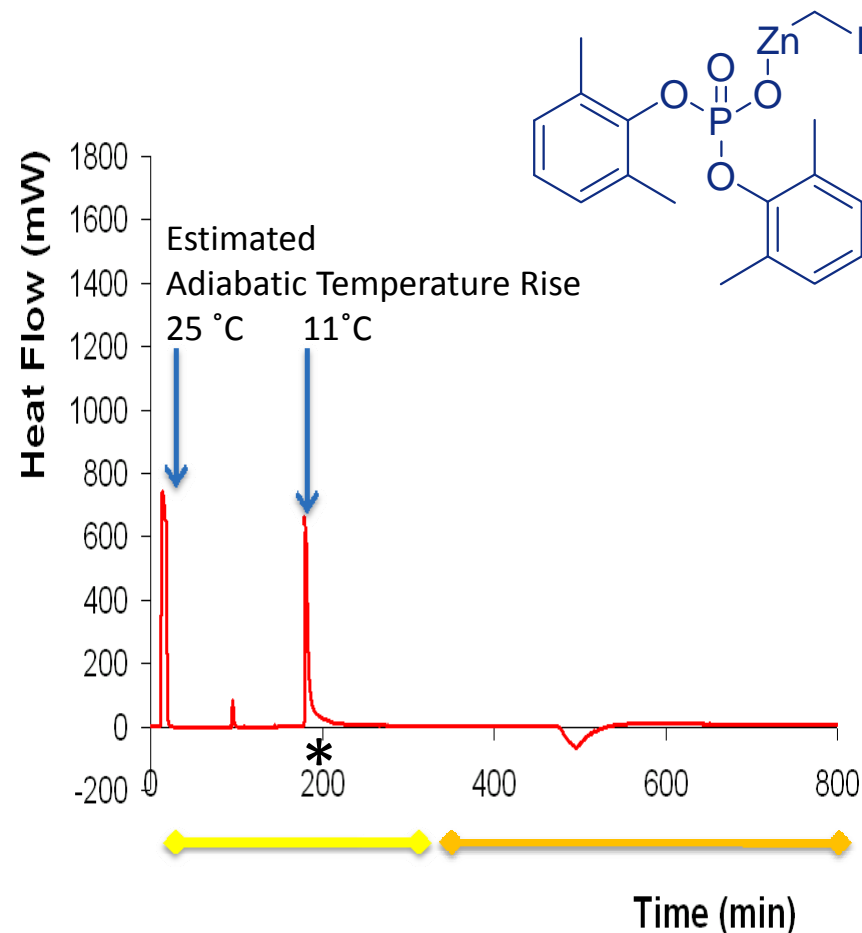
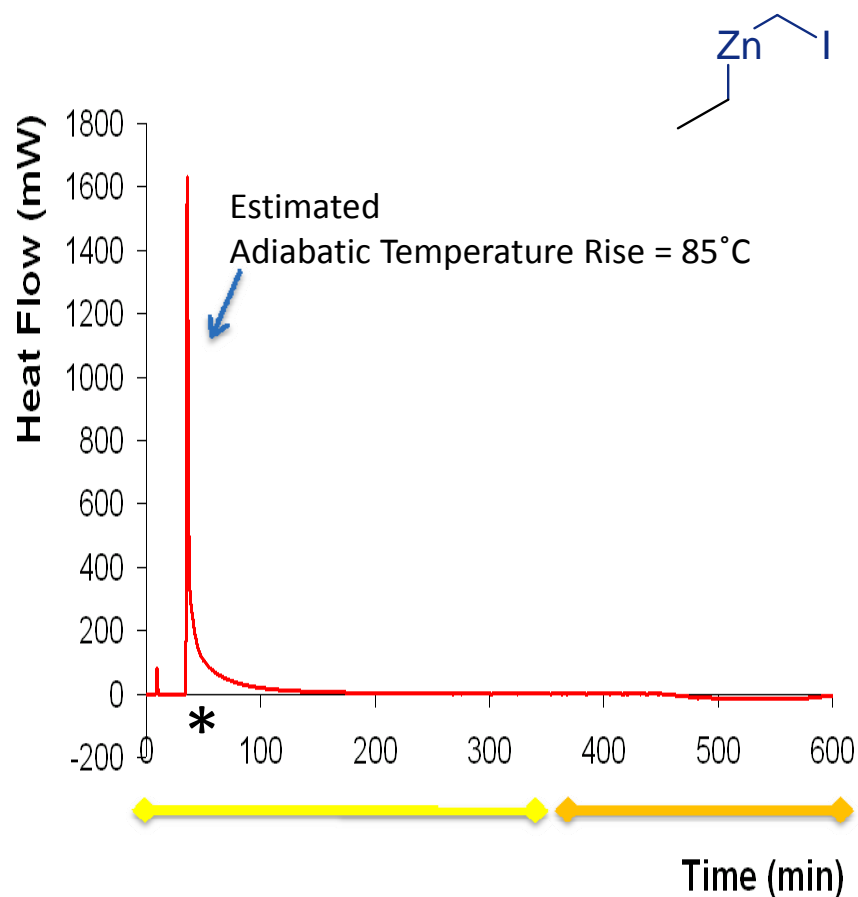
No Exotherm up to 160°C

Modest Exotherm below 120 °C

No Exotherm up to 160 °C

- *Diethylzinc solution in DCM should be handled at 0-10 °C*
- *Reaction is safe to handle at 30 °C*

Reaction Calorimetry: Comparative Profile



Reagent preparation
(0°C)

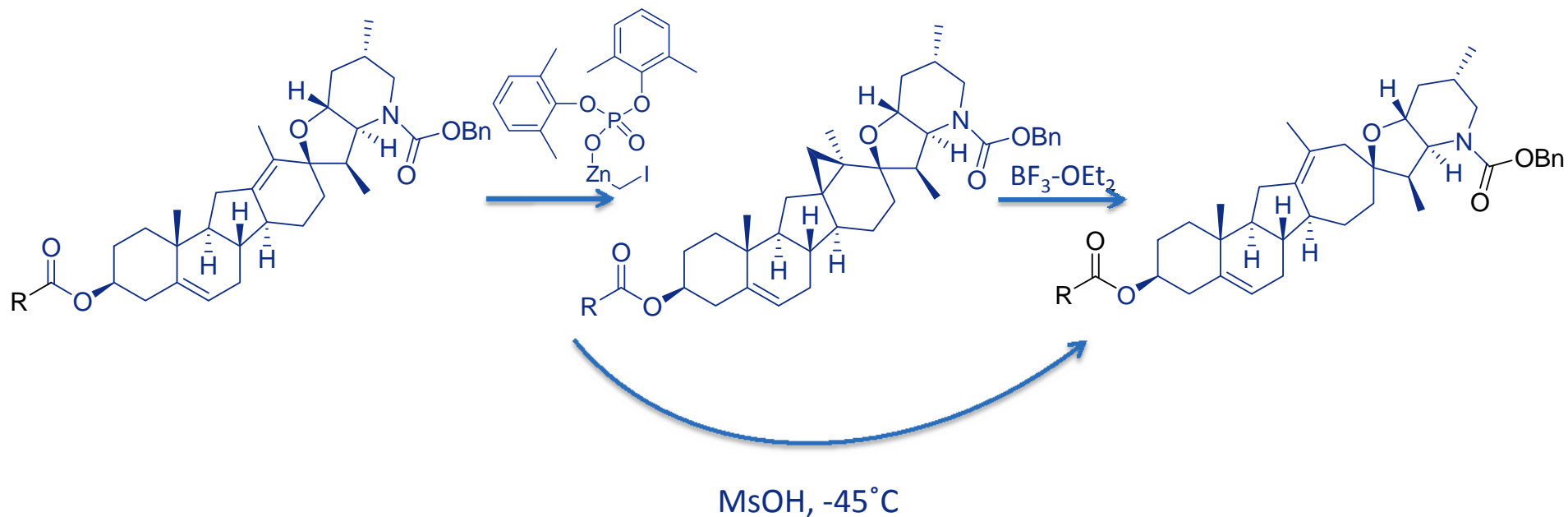


Addition of CH_2I_2
(0°C)



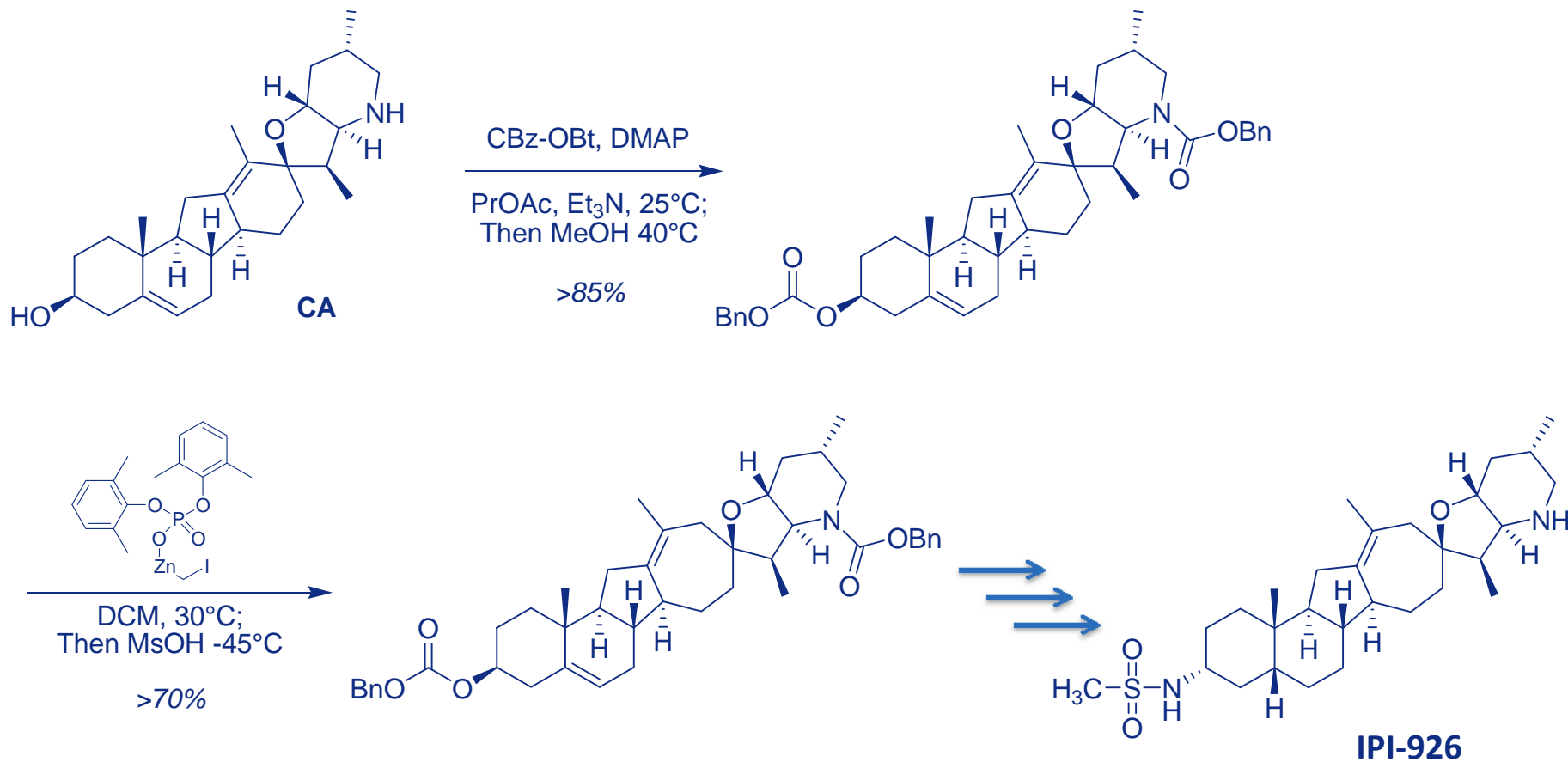
Cyclopropanation reaction
(30°C)

One Pot Expansion of the D-ring



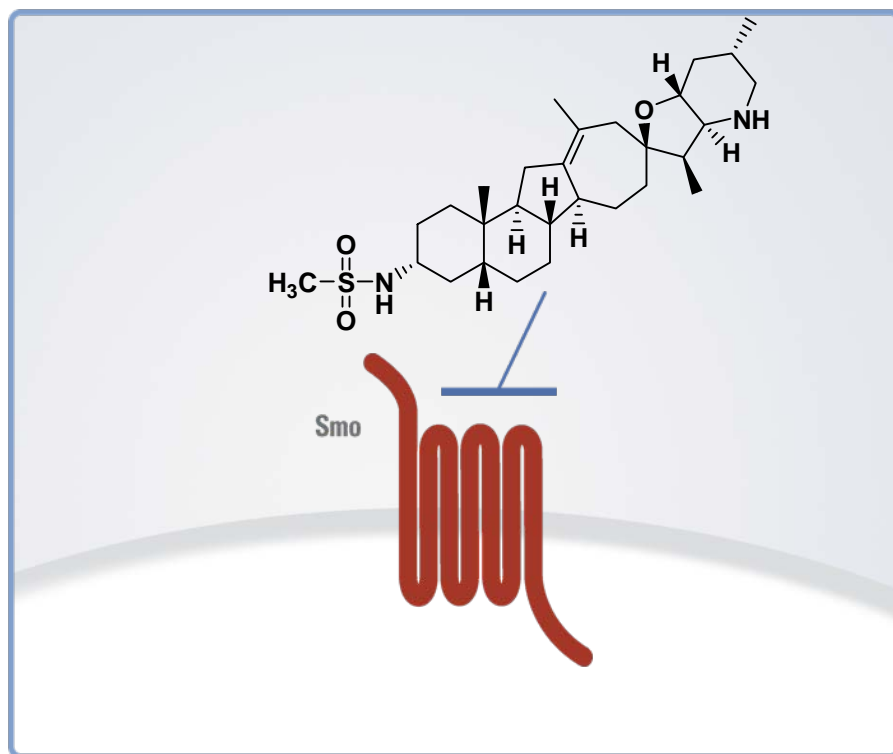
- RC1 and Hastelloy testing complete
- Established by-product waste streams
 - Phosphate removed in aqueous wash and can be recycled
- Product can be crystallized after solvent exchange

Larger Scale Demonstration



➤ Pilot plant: 12-25 kg CA

IPI-926 Targets the Hedgehog Pathway by Inhibiting Smo



Potency	
C3H10 (EC ₅₀)	7-15 nM
Smo binding (IC ₅₀)	1-2 nM

Clinical studies	
Dosing regimen	Oral, once daily, continuous
T _{1/2}	20-40 hrs after a single dose
Ongoing clinical studies	Phase 1 in solid tumors* Phase 2 in metastatic pancreatic cancer

**In an ongoing Phase 1 study, IPI-926 has been well tolerated and has demonstrated clinical activity in patients with basal cell carcinoma*

Evidence of Clinical Activity in Patients with BCC

Patient A

Baseline



6 Mos.



C. Rudin, G. J. Weiss, A. Chang, A. Savage, R. Ross, A. Jimeno – Data presented at ESMO , 2010

IPI-926 Next Steps

- **2011 AACR Presentations**

- **Sunday April 3 1-3 pm** *Hedgehog Signaling Pathway: Biology and Therapeutics*
“Direct targeting of tumor cells with Smoothened inhibitor IPI-926” –Margaret Read
- **Tuesday April 5 1-5 pm** *Late-Breaking Research: Experimental and Molecular Therapeutics 2*
“Direct targeting of the Hedgehog pathway in primary chondrosarcoma xenografts with the Smoothened inhibitor IPI-926” – Veronica Campbell

- **Clinical trials**

- *A Phase 1b/2 Study Evaluating IPI-926 in Combination with Gemcitabine in Patients with Metastatic Pancreatic Cancer*
- *A Phase 2, Placebo-Controlled Study Evaluating the Efficacy and Safety of IPI-926 in Patients with Metastatic or Locally Advanced (Unresectable) Chondrosarcoma*
- *A Phase 1 study of IPI-926 in patients with advanced and/or metastatic solid tumor malignancies*
- *Additional trials planned in 2011*

Summary

- Developed effective homogeneous CP reagent, including...
 - *Optimized phosphate and reactions conditions*
 - *Developed the one pot CP-RE procedure*
- Demonstrated the D-ring expansion @ >25 kg
- Enabled Multi-kilograms synthesis of IPI-926
- Ongoing preparations for further scale up

Acknowledgements

Collaborators

- Prof. André B. Charette, U. Montréal
- US Forest Service

Infinity

- Hedgehog Team

Ph1 Investigators

- Charles Rudin, JHU
- Glen Weiss, TGEN
- Antonio Jimeno, U Colo
- Ann Chang, Tony Oro, Stanford
- Scott Gettinger, Yale
- William Miller, McGill
- Bernhardt Eigl, TBCC

Patients and their caregivers

Purdue Pharmaceuticals

Mundipharma