Discovery of a Novel Notch Inhibitor

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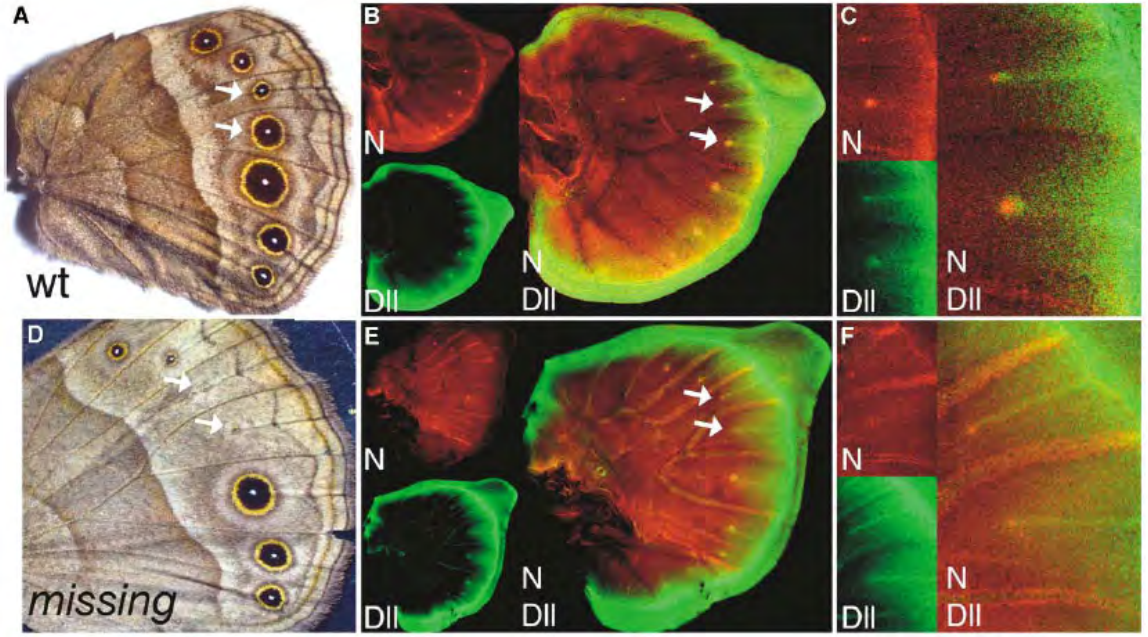
Presentation Outline

- The discovery of a novel Notch inhibitor is highlighted.
- Background biology and target rationale shared.
- A first generation inhibitor will be summarized and a concise SAR described.
- The *in vitro* notch inhibition, summary physiochemical properties, and some preclinical animal PK included.
- Two synthetic routes, a crystal structure of the novel inhibitor, and an unusual atropisomerism discussed.

Summary Background of Notch

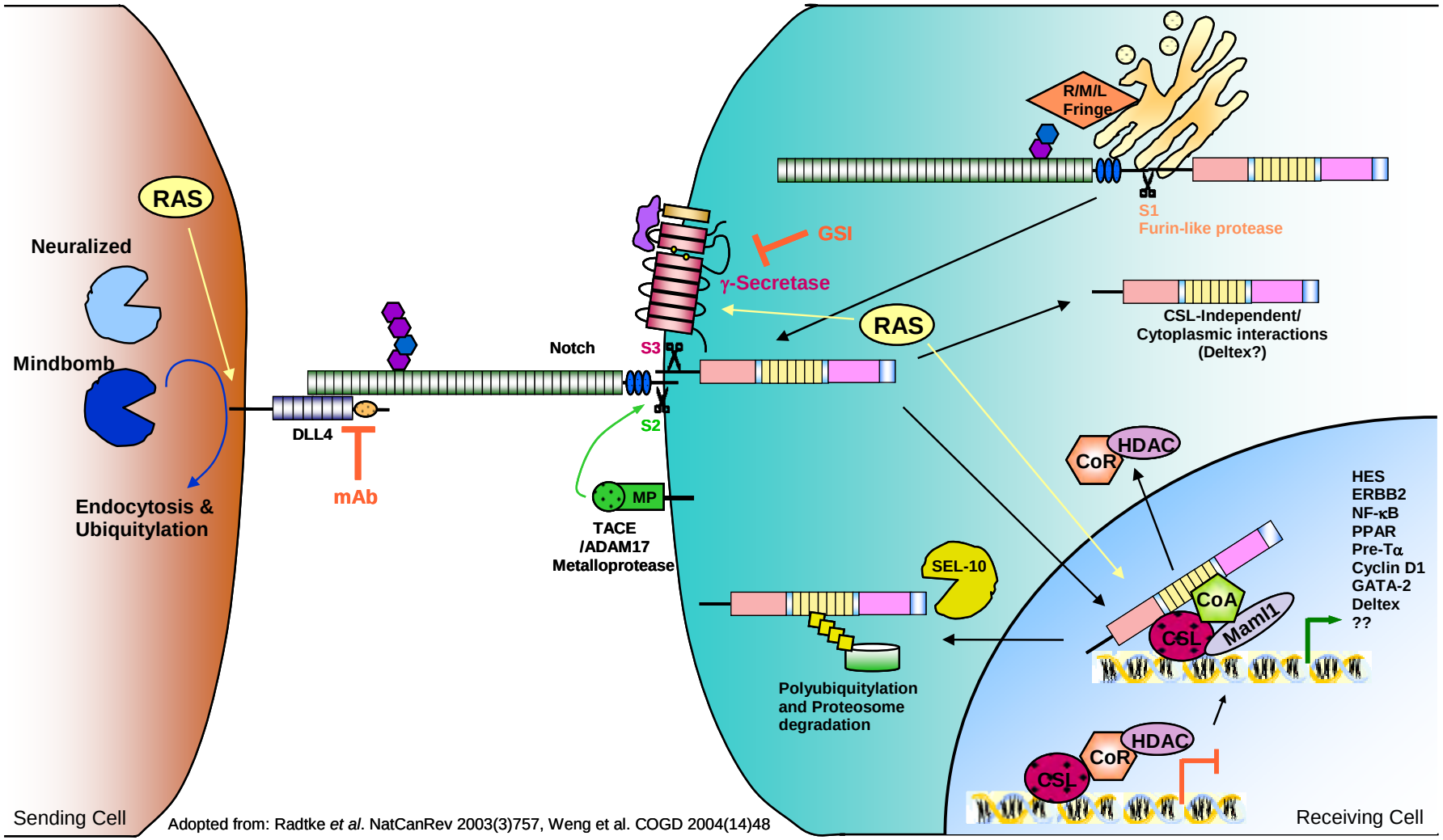
- Notch was first discovered in the early 1900's as a developmental pathway in Drosophila and later found to play central roles in cell fate decisions and in cancer.
- Notch signaling is an evolutionary conserved pathway that plays an integral role in the development and tissue homeostasis in mammals.
- There are four known Notch receptors found in rodents and humans, termed Notch 1 to Notch 4.
- The protease complex γ-secretase cleaves the Notch Intracellular Cleavage Domain (NICD) from the cell surface, which translocates to the nucleus to form a transcription factor complex.
- NICD is the active form of the protein.



In wild-type *B. anynana hindwings (A), N and Dll were detected in mid-fifth instar wing discs in focal patterns associated with all eyespots* (B), including those affected by the *missing mutant (C). The missing mutant of B. anynana shows a dramatic reduction in the size of two* specific eyespots (arrows) on the ventral hindwing of the adult (D). Antibody stains for N and Dll in wing discs from *missing larvae demonstrate* a corresponding reduction of both N and Dll in the developing foci of these two eyespots (E and F), but not in the unaffected eyespots (E).

Butterfly Wing Pattern Evolution Is Associated with Changes in a Notch/Distal-less Temporal Pattern Formation Process
Reed, R. D.; Serfas, M. S. *Current Biology*, 2004*, 14*, 1159–1166.

Notch Signaling Schematic



Adopted from: Radtke *et al*. NatCanRev 2003(3)757, Weng et al. COGD 2004(14)48

Scheme adopted from: Radtke, F.; Raj, K. *Nature Reviews Cancer* 2003, 3, 757-76. and Weng, Andrew P.; Aster, Jon C. *Current Opinion in Genetics & Development,* 2004, *14(1),* 48-54.

Oncology Notch Inhibitor Hypothesis

Scientific Rationale:

• Notch signaling plays a critical role in stem cell function and during embryonic and post-natal development that is recapitulated in different forms of cancer.

• Inappropriate Notch pathway activity observed in a variety of tumors due to:
- •Receptor amplification
- •Receptor mutations
- •Ligand over-expression

•May lead to enhanced/sustained Notch function, triggering increased tumor cell proliferation/survival and tumor formation.

• γ-secretase cleaves a number of type-I membrane proteins including APP, Notch receptors, and Erb-B4 and regulates their signaling.

Hypothesis:

A small molecule Notch Inhibitor will suppress tumor growth and thus provide a therapeutic benefit to cancer patients.

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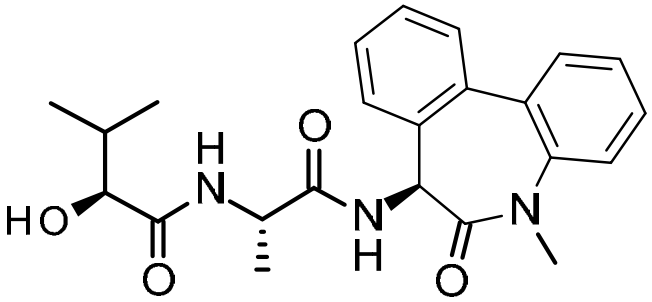
Overview of First Generation Lilly Notch Inhibitor



LY900009

Notch 1 (IC_{50}) = 0.27 nM

MW = 410

cLogP (Chemaxon) = 1.7

PSA (Novartis) = 99

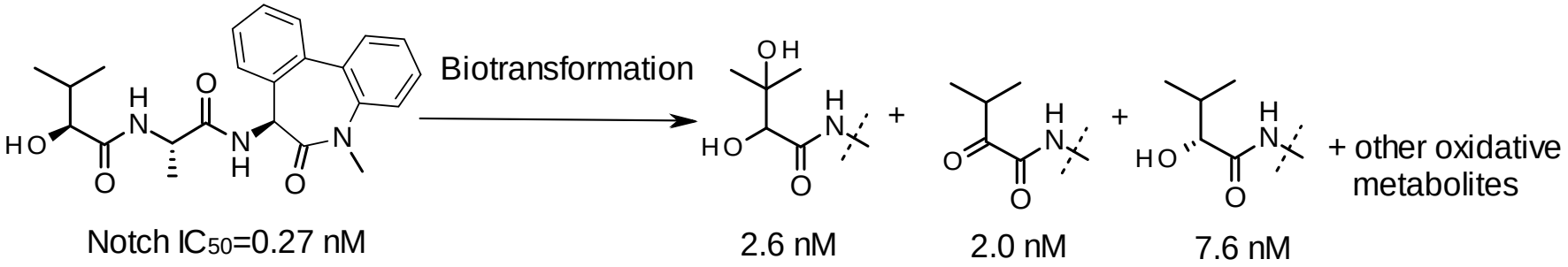
Equilibrium Solubility:

pH2 = 0.06, pH6 = 0.06 , pH7.4 = 0.06 mg/mL

Surrogate SP Metabolism:

m= 58%, r=41%, d=23%, h=27%

CYP (@10uM) 2C9 = 73%



Notch IC_{50}=0.27 nM

2.6 nM

2.0 nM

7.6 nM

• Based on MetID studies in mouse, rat, dog, and human liver microsomes and hepatocytes, as well as mice, rats, and dogs *in vivo*, the biotransformation of LY900009 occurs primarily through oxidation of the aliphatic tail (HVA residue).

• *In vitro* studies indicated involvement of CYP3A4 in metabolism of LY900009.

Notch Inhibitor Project Objectives

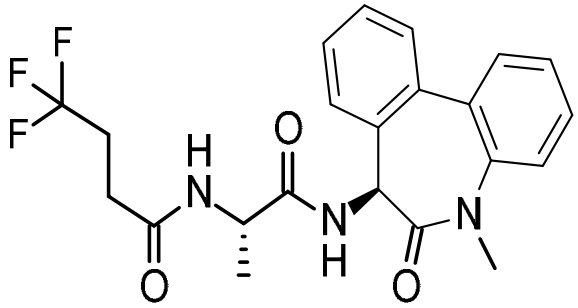
- Retain exquisite intrinsic *in vitro* Notch potency achieved with the first generation inhibitor.

- Alleviate developmental complexities associated with the HVA residue of LY900009
  - Multiple *in vitro* active metabolites are formed *in vivo* across species
  - Multiple diastereomeric metabolites identified
  - Compound primarily metabolized by CYP3A4

- Do not introduce additional toxicology.

- With these properties, we should be able to achieve predictable and consistent PK to allow for a tightly controlled dosing regimen.

Summary of Inhibitor #1



Inhibitor #1

Notch 1 (IC_{50}) = 0.31 nM

MW = 433

cLogP (Chemaxon) = 2.4

PSA (Novartis) = 79

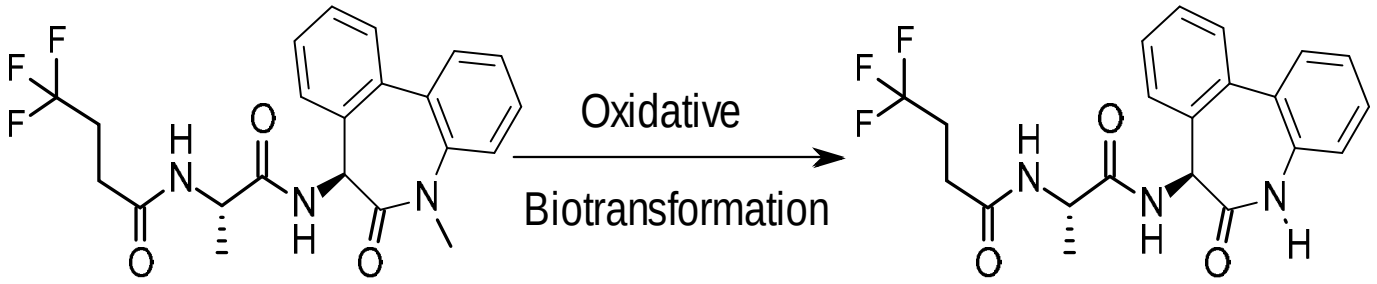
Equilibrium Solubility:

pH2 = 0.20, pH6 = 0.22 , pH7.4 = 0.02 mg/mL

Surrogate SP Metabolism:

m= 15%, r=11%, d=12%, h=0%

CYP (@10uM) 3A4, 2D6, 2C9 = 10, 0, 9%

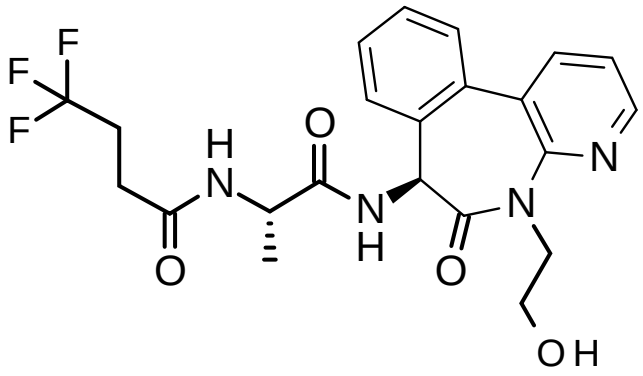


Inhibitor #1

Lactam N-Dealkylation
Notch 1 IC_{50} = 0.22 nM

- *In vitro* active oxidative metabolite observed.
- No α-oxidation on N-terminal tail noted.

Overview of the Novel Notch Inhibitor LY3039478



LY3039478

Notch 1 (IC_{50}) = 0.41 nM

MW = 464

cLogP (Chemaxon) = 1.1

PSA (Novartis) = 112

Equilibrium Solubility:

pH2 = 0.89, pH6 = 0.88 , pH7.4 = 0.88 mg/mL

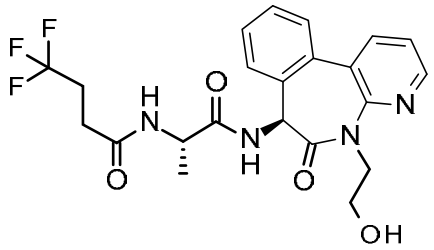
Surrogate SP Metabolism:

m= 8%, r=0%, d=5%, h=15%

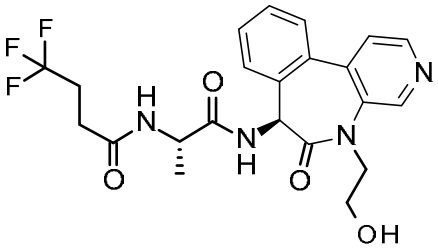
CYP (@10uM) 3A4, 2D6, 2C9 = 0, 0, 0%

- Mouse PK CL=41 mL/min/kg, VDss = 3.8 L/kg, %F =65%
- Rat PK CL=98 mL/min/kg, VDss = 4.9 L/kg, %F =65%
- Dog PK CL=3.8 mL/min/kg, VDss = 1.4 L/kg, %F =67%
- No oxidative metabolites observed in MetID studies.
- Solubility notably improved.
- Overall excellent profile supported progression into advanced preclinical *in vivo* studies.

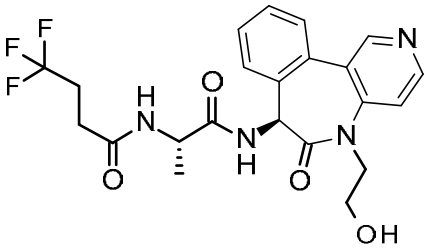
Additional Pyridyl Notch Inhibitors Investigated



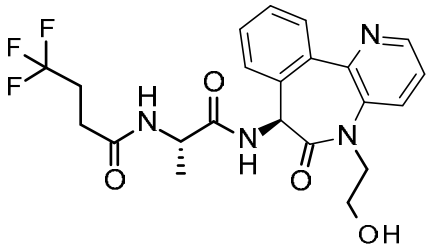
LY3039478



Inhibitor #2



Inhibitor #3

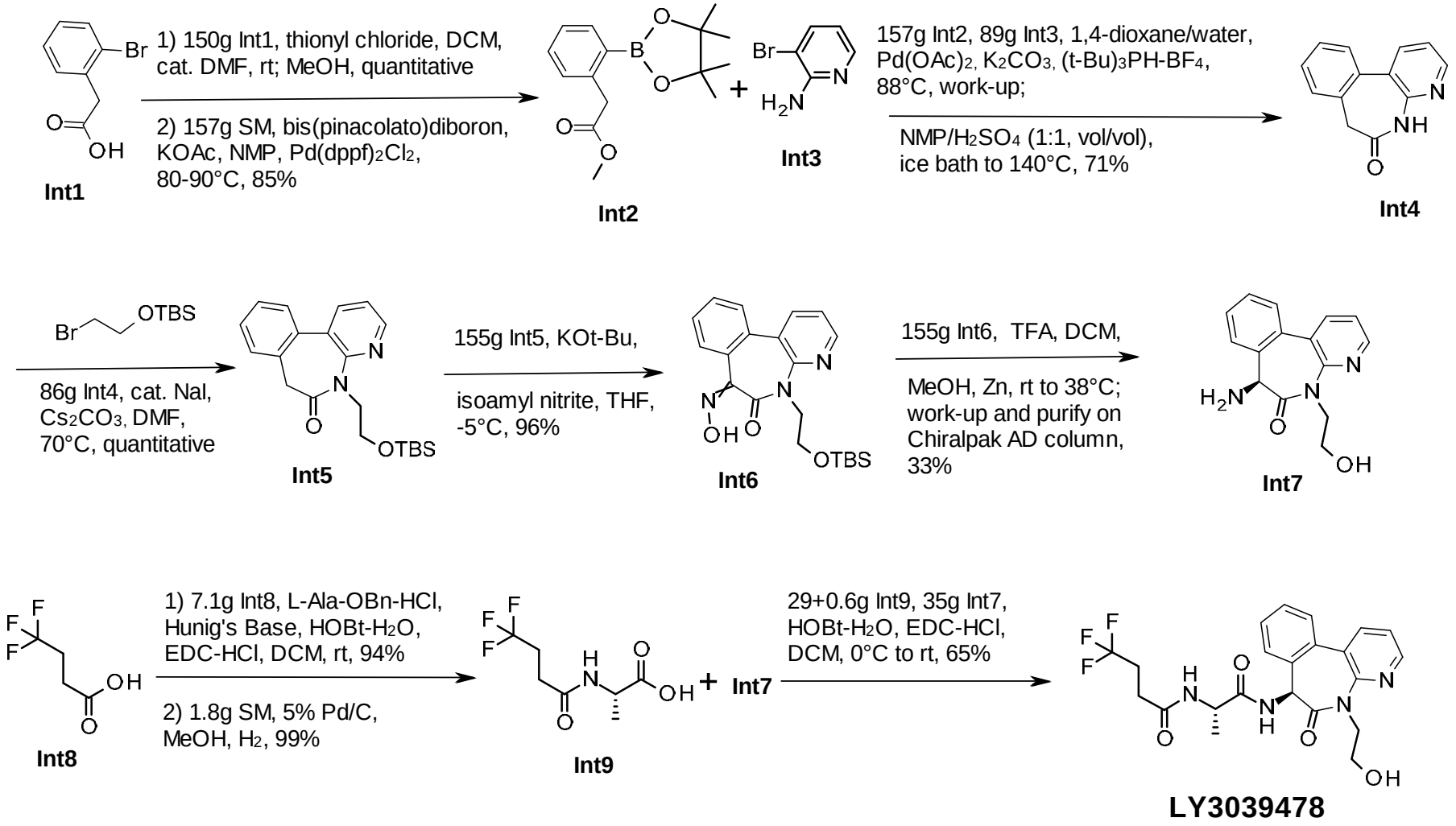


Inhibitor#4

	LY3039478	Inhibitor#2	Inhibitor#3	Inhibitor#4
In vitro Notch IC_{50}	0.41 nM	2.8 nM	2.8 nM	3.6nM
Surrogate Met Stab: mouse	8%	25%	18%	3%
rat	0%	25%	9%	10%
dog	5%	23%	11%	8%
human	15%	16%	8%	8%

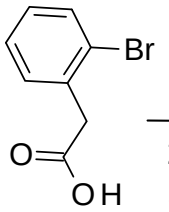
- LY3039478 displays the best overall profile and is unique within the SAR investigated.
- Additional data planned for upcoming presentations will further demonstrate the superiority of LY3039478.

Synthetic Route A to LY3039478



- WO 2013/016081

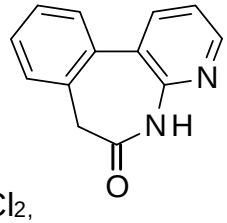
Synthetic Route B to LY3039478 – H_2O



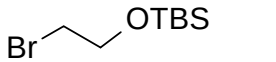
Int1

1) 500g Int1, MeOH, conc. H_2SO_4, 20-30°C to 60-65°C, 97%

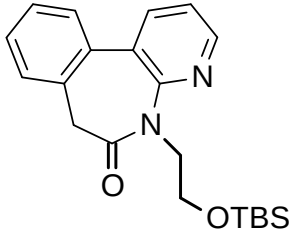
2) 1 kg SM, bis(pinacolato)diboron, KOAc, 1,4-dioxane, NMP, $Pd(dppf)_2Cl_2$, rt to 80-90°C; 2-amino-3-bromopyridine, aq. K_3PO_4, $Pd(dppf)_2Cl_2$, 15-25°C to 80-90°C, 68%



Int4

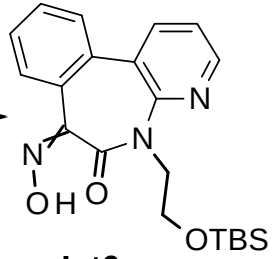


34g Int4, Cs_2CO_3, MeCN, 70-80°C, 92%



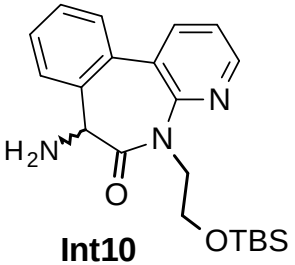
Int5

30g Int5, KOt-Bu, isoamyl nitrite, Tol, -10to0°C, 73%



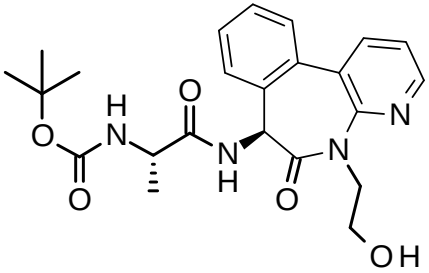
Int6

206g Int6, Raney nickel, H_2 (87psi), 60-65°C, 93%



Int10

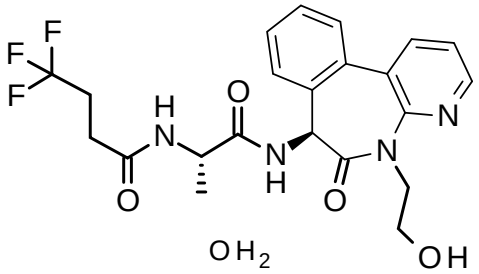
166g Int10, L-Boc-Alanine, Et_3N, HOBt-H_2O, EDC, DCM, 20-30°C, workup; EtOAc, TBAF-$3H_2O$, 20-30°C, 85%de, 100%ee, 51%



Int11

1) 54g Int11, 4 M aq. HCl, MeCN, 20-30°C, filtration work-up, 99%de, 100%ee, 51%

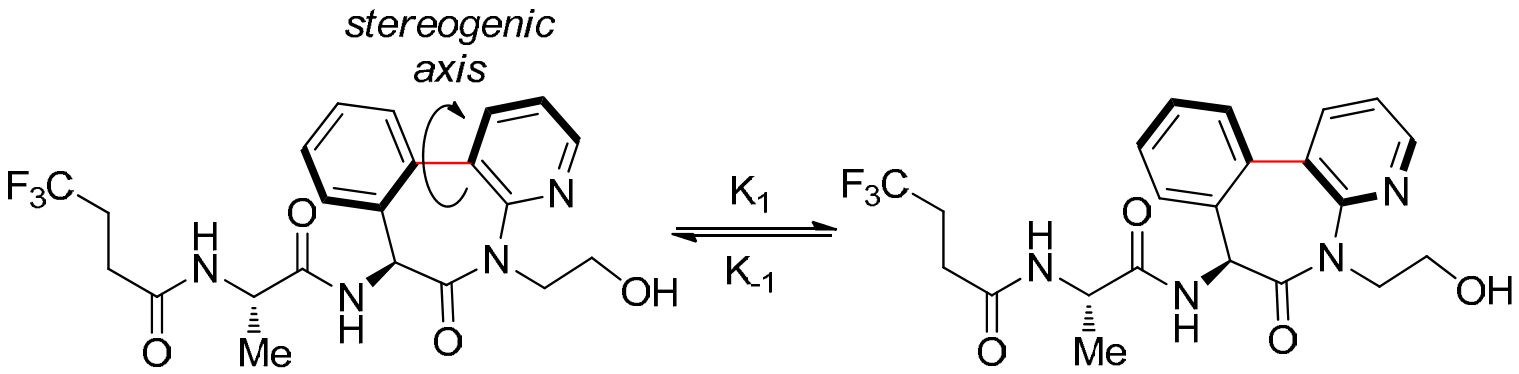
2) 45g SM, Hunig's Base, 4,4,4-trifluorobutyric acid, HOBt-H_2O, EDC-HCl, DMF, 0°C to rt, filtration work-up, 99.6%de, 100%ee, 65%



LY3039478 - H_2O

- WO 2013/016081

LY3039478 Displays Interesting Atropisomer Phenomena

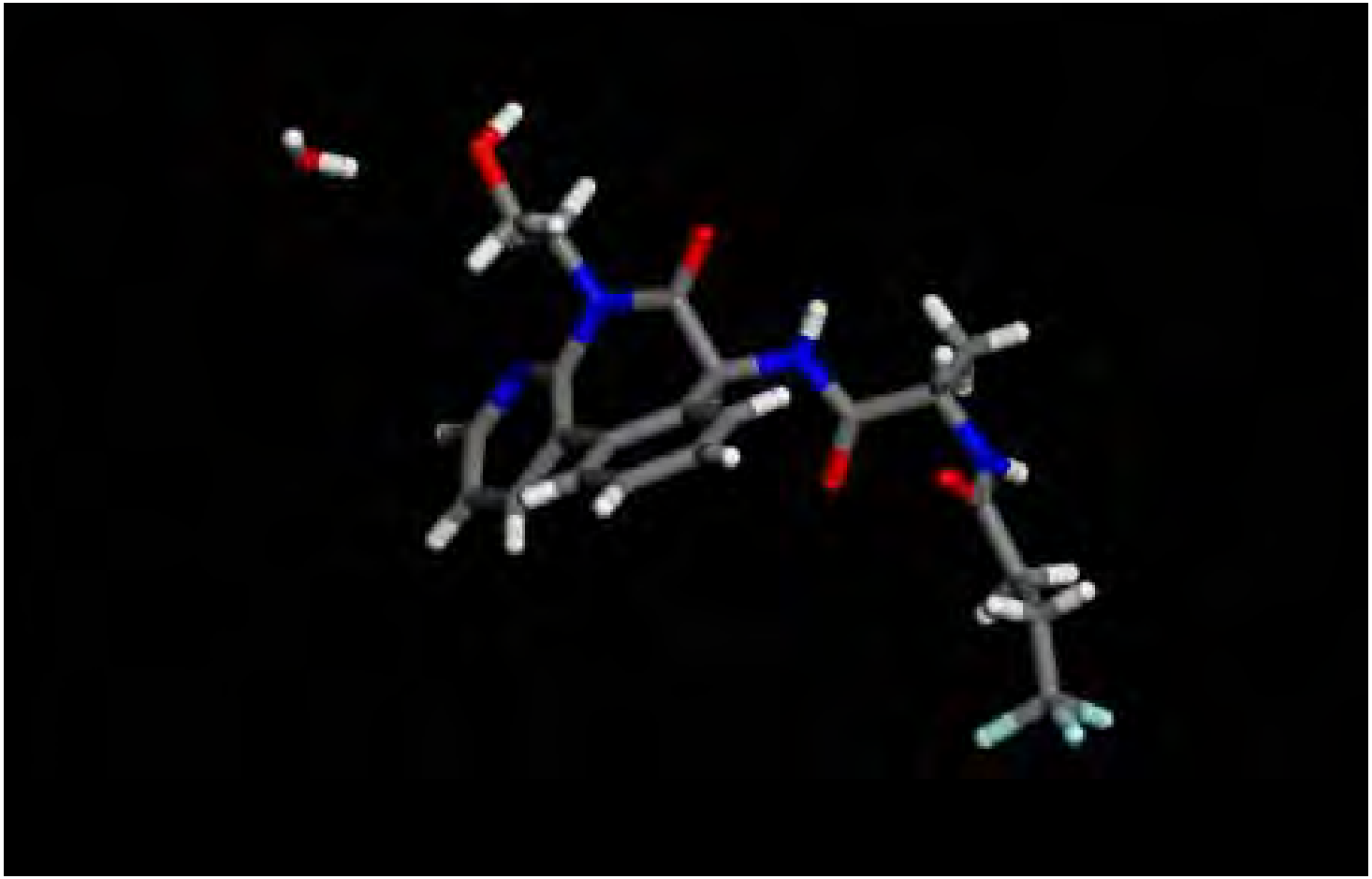


- The average LY3039478 major/minor rotational isomer ratio ranged between 14 and 28 in plasma.
- The ratio was consistent between species (rat, dog, and human).
- The ratio was consistent over time (one month) and plasma storage temperature (room temperature, 4 °C, -20 °C, -70 °C).
- The crystalline monohydrate form of LY3039478 consists of a single rotational isomer and is chemically and physically stable for at least 14 days under accelerated stability test conditions.

Recent Literature Citations:

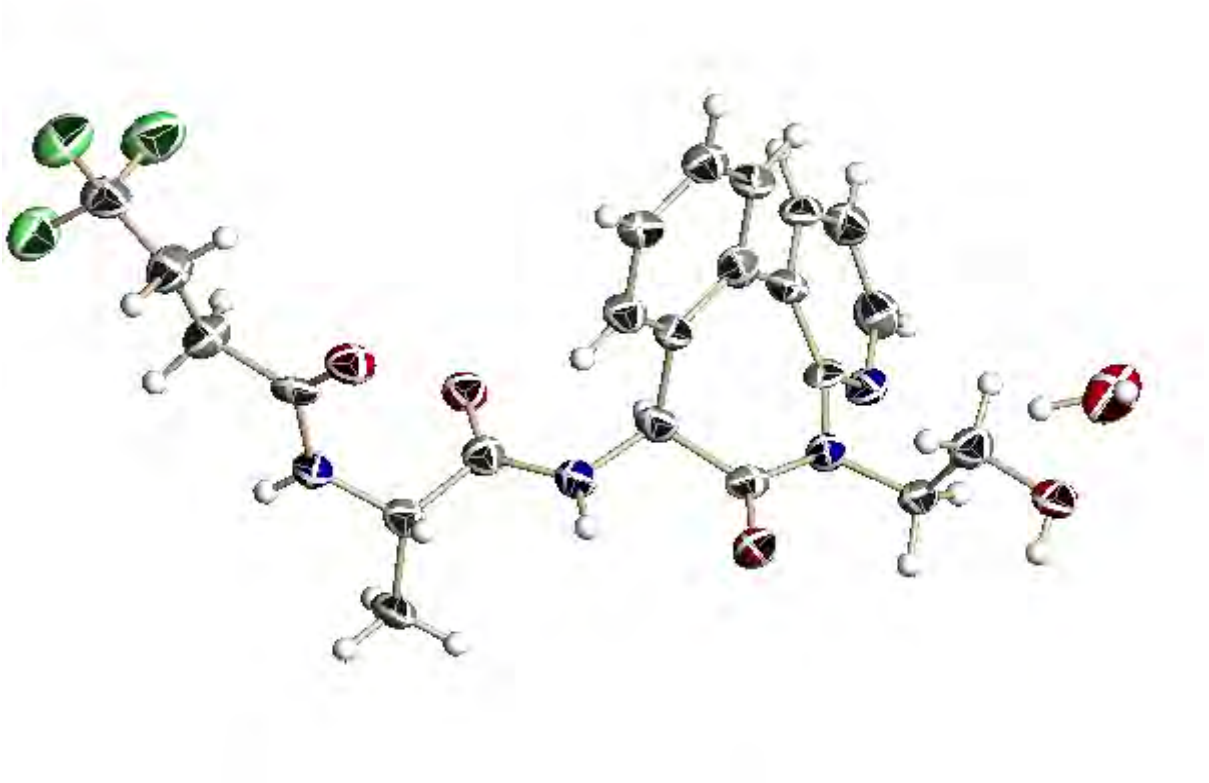
Tabata, H.; Akiba, K.; Lee, S.; Takahashi, H.; Natsugari, H. *Org. Lett*. **2008**, *10*, 4871–4874.
Cole, K. P.; Mitchell, D.; Carr, M. A.; Stout, J. R.; Belvo, M. D. *Tetrahedron: Asymmetry* **2009**, *20*, 1262-1266.

X-Ray Crystal Structure of LY3039478-H_2O

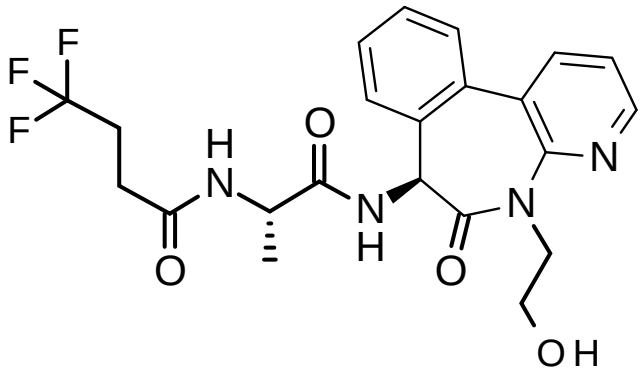


• Assigned structure and stereochemistry consistent with x-ray crystal data, with a mono-hydrate noted.

Alternative View of X-Ray Crystal Structure of LY3039478-H_2O



Review of the Novel Notch Inhibitor LY3039478



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- No oxidative metabolites observed in MetID studies.
- Solubility notably improved.
- Overall excellent profile supported progression into advanced preclinical *in vivo* studies.

Conclusions

A highly potent Notch Inhibitor was discovered which addresses many of the issues identified with our first generation inhibitor. The novel and unique Notch Inhibitor, LY3039478, has progressed into clinical development. Additional disclosures from this project are planned.

Personal Acknowledgements

Jing Bao, Mark Bender, Julia Clay, Oscar De Frutos, Tom Engler, Kelly Furness, Phil Hipskind, Carlos Mateos, Brian Mathes, Dustin Mergott, Bharvin Patel, Jared Piper, Jon Reel, Greg Stephenson, Fei Tian, Paloma Vidal, Zhongyi Wang, and Maciej Zamek-Gliszczynski