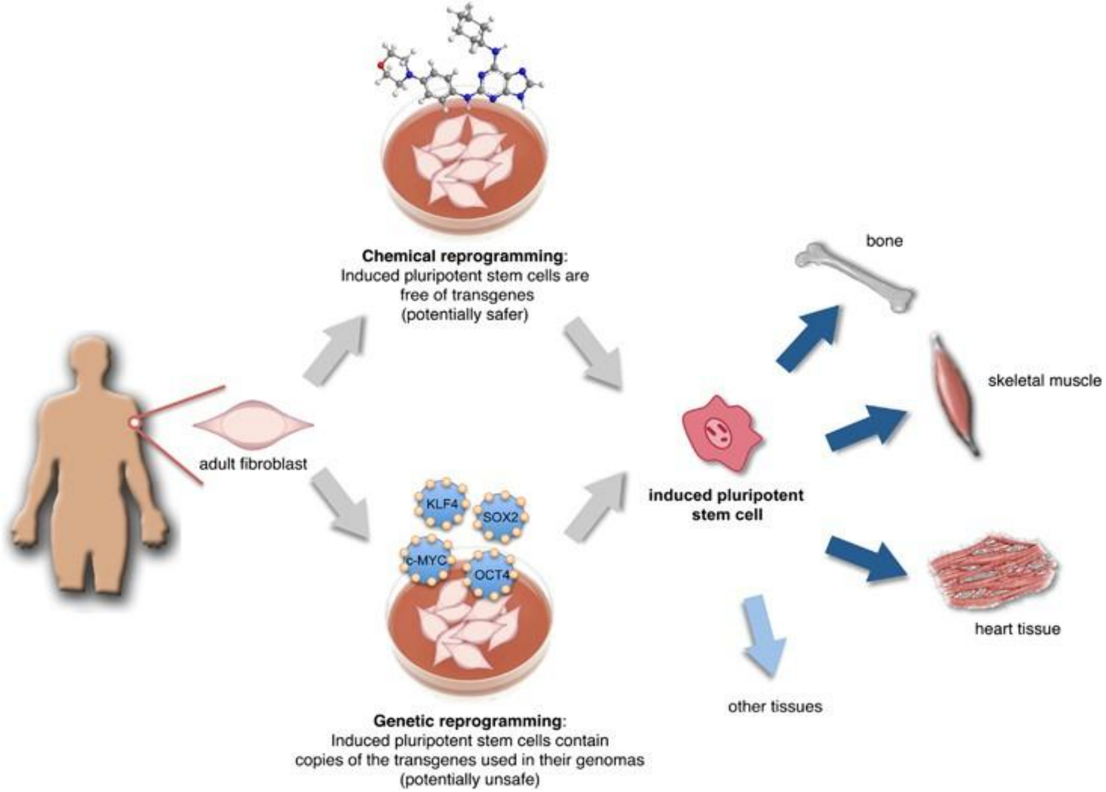
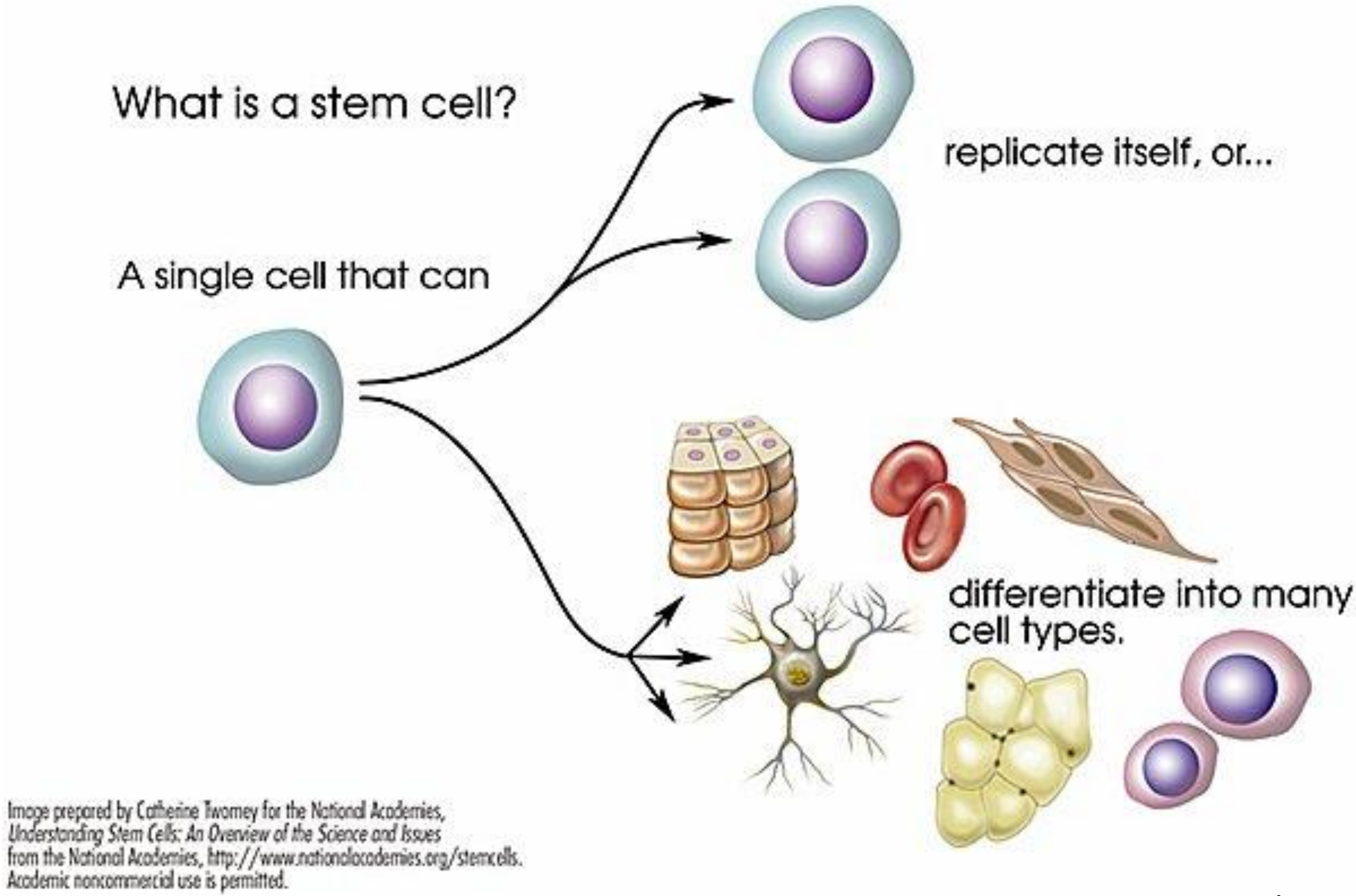
A Directed Transformation of Stem Cells to Neurons by Furan-based Macrocyclic Small Molecules: Validation by RNA Transcriptomic Analysis

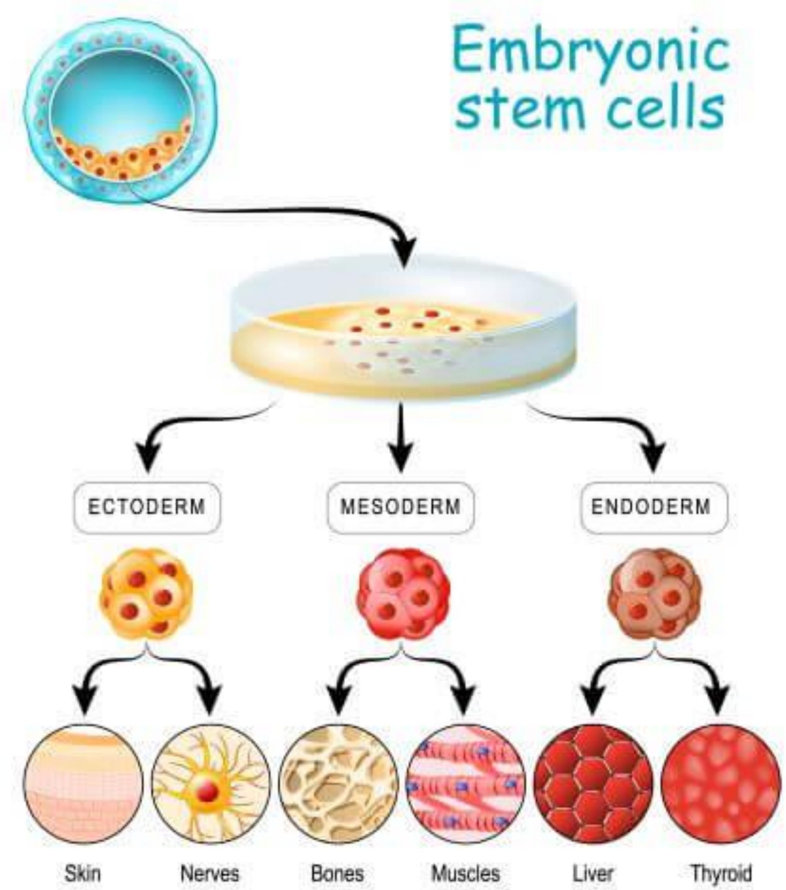
The Implications to Neurological Disorders

Javed Iqbal



August 16, 2025

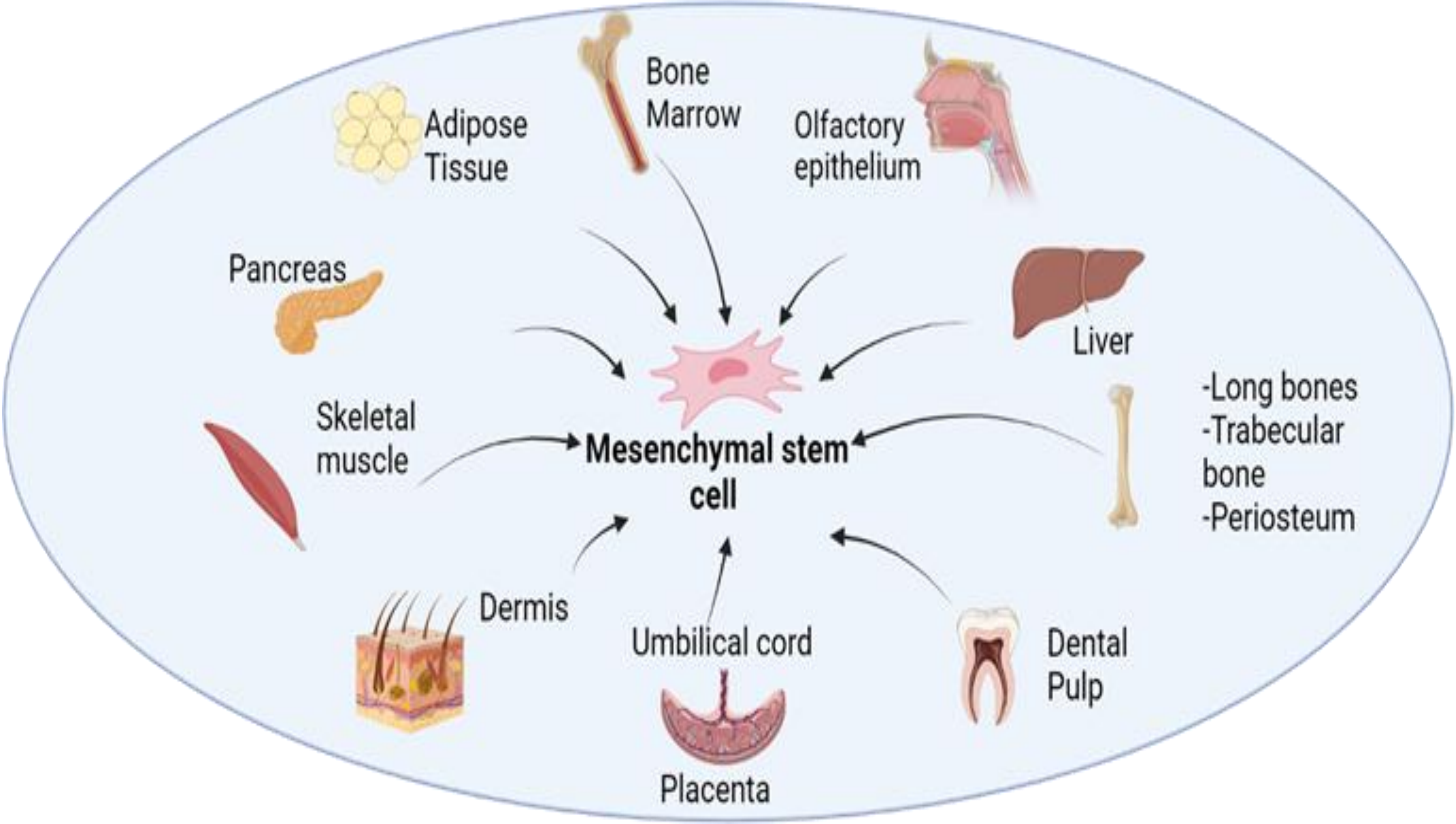

What is a stem cell?
A single cell that can
replicate itself, or...
differentiate into many cell types.
Image prepared by Catherine Twomey for the National Academies,
Understanding Stem Cells: An Overview of the Science and Issues
from the National Academies, http://www.nationalacademies.org/stemcells.
Academic noncommercial use is permitted.



Embryonic stem cells (ESCs) are pluripotent cells derived from the inner cell mass of a blastocyst, a very early stage embryo. They have the unique ability to differentiate into any cell type in the body (pluripotency) and to self-renew, making them valuable for research and potential therapeutic applications.

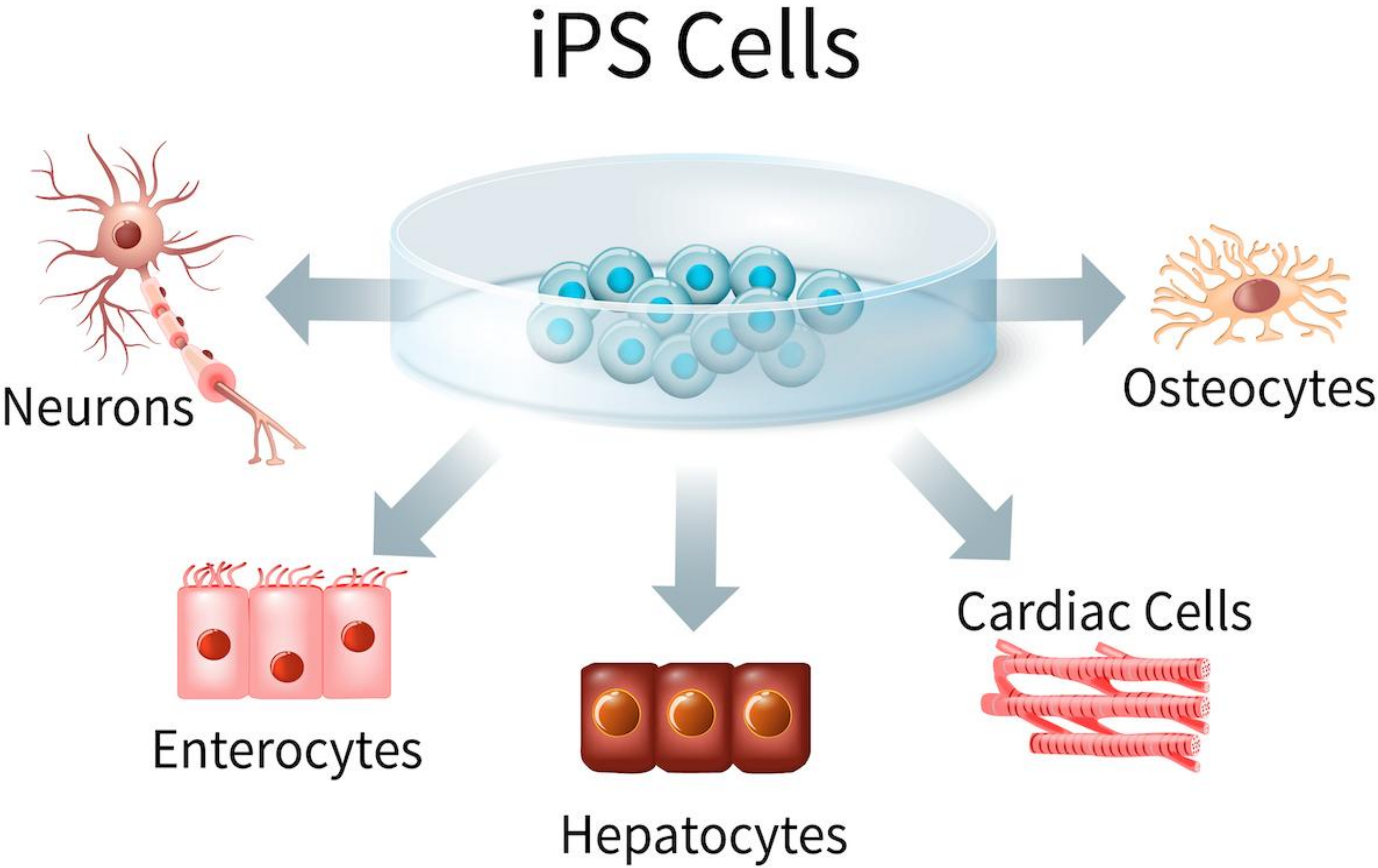
But there are ethical issues…….

Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into many types of specialized cells



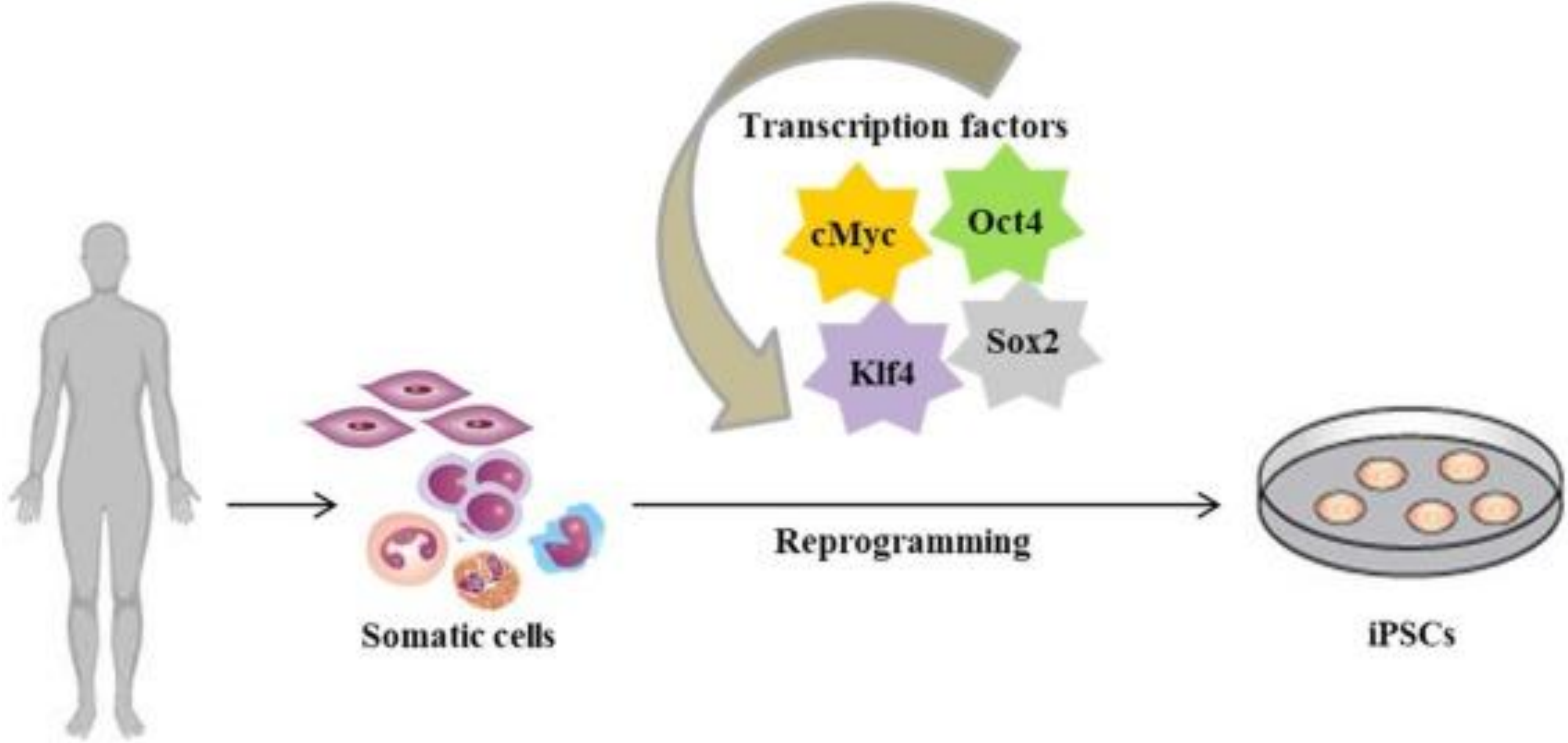
The process of differentiation can be influenced by a variety of substances, including: Transcription factors, Transforming growth factor beta, and Wnt proteins

Induced Pluripotent Cells (iPSC)

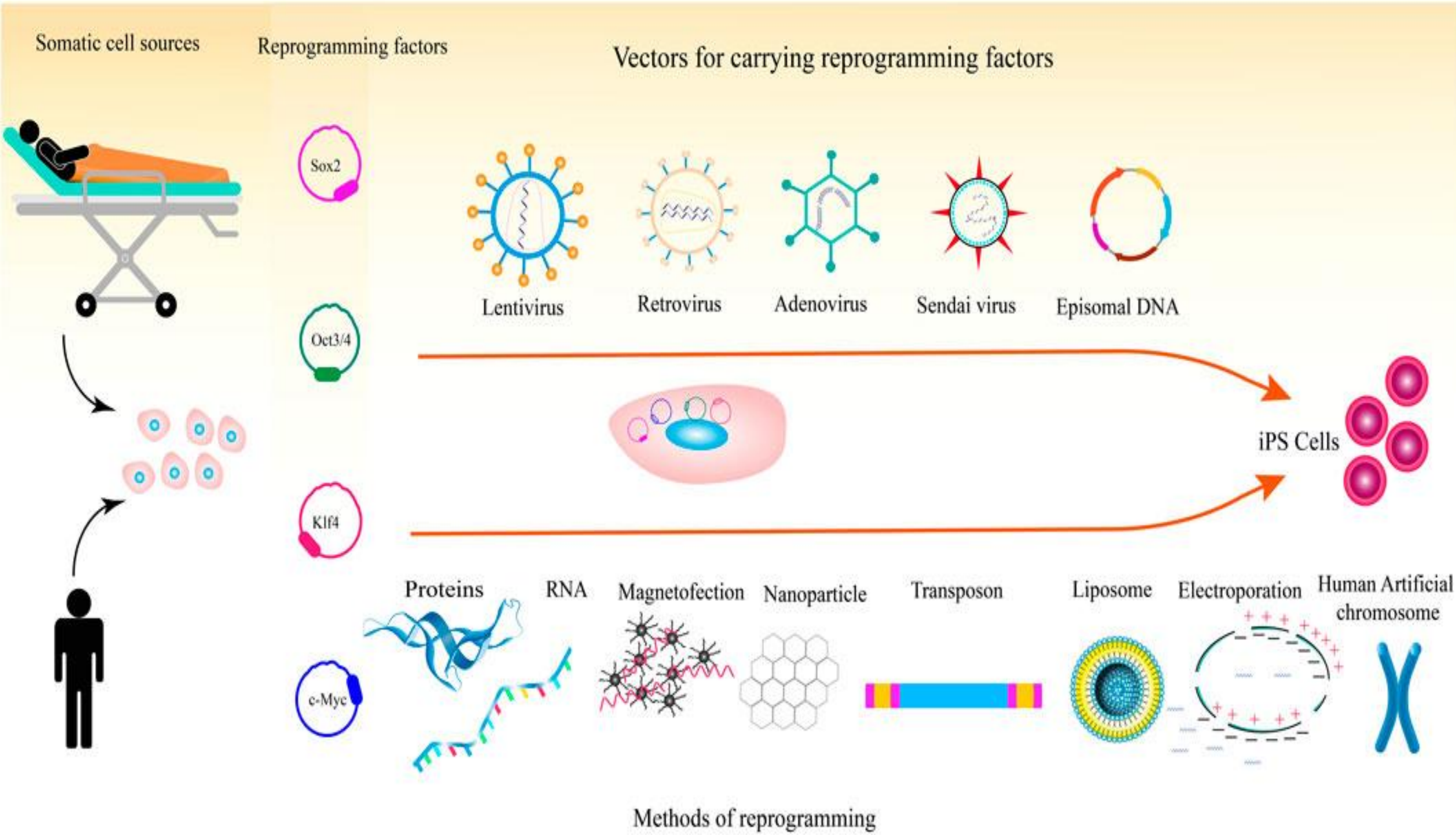


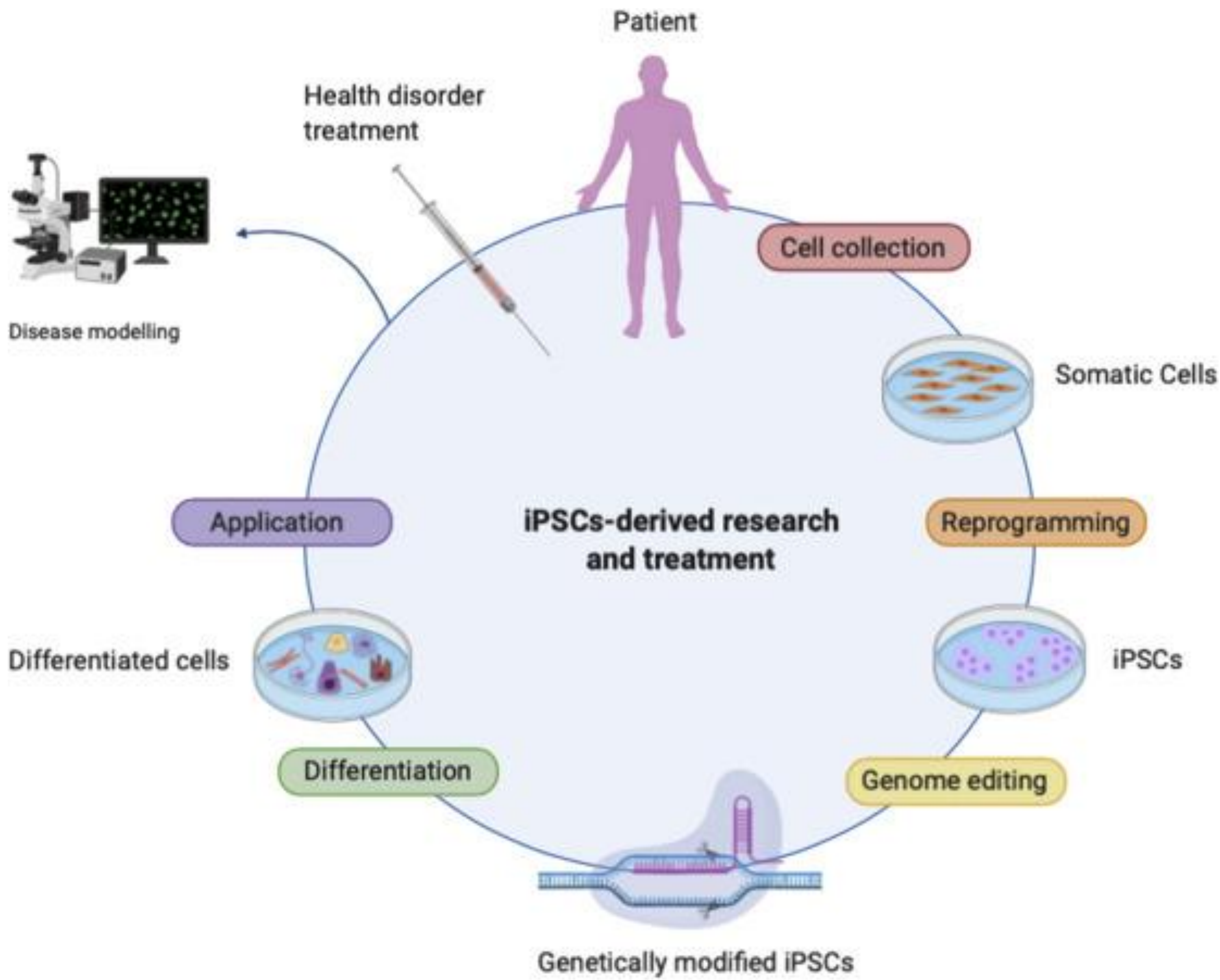
Shinya Yamanaka, winner of the 2012 Nobel Prize in Medicine and Physiology (Yamanaka Factors)

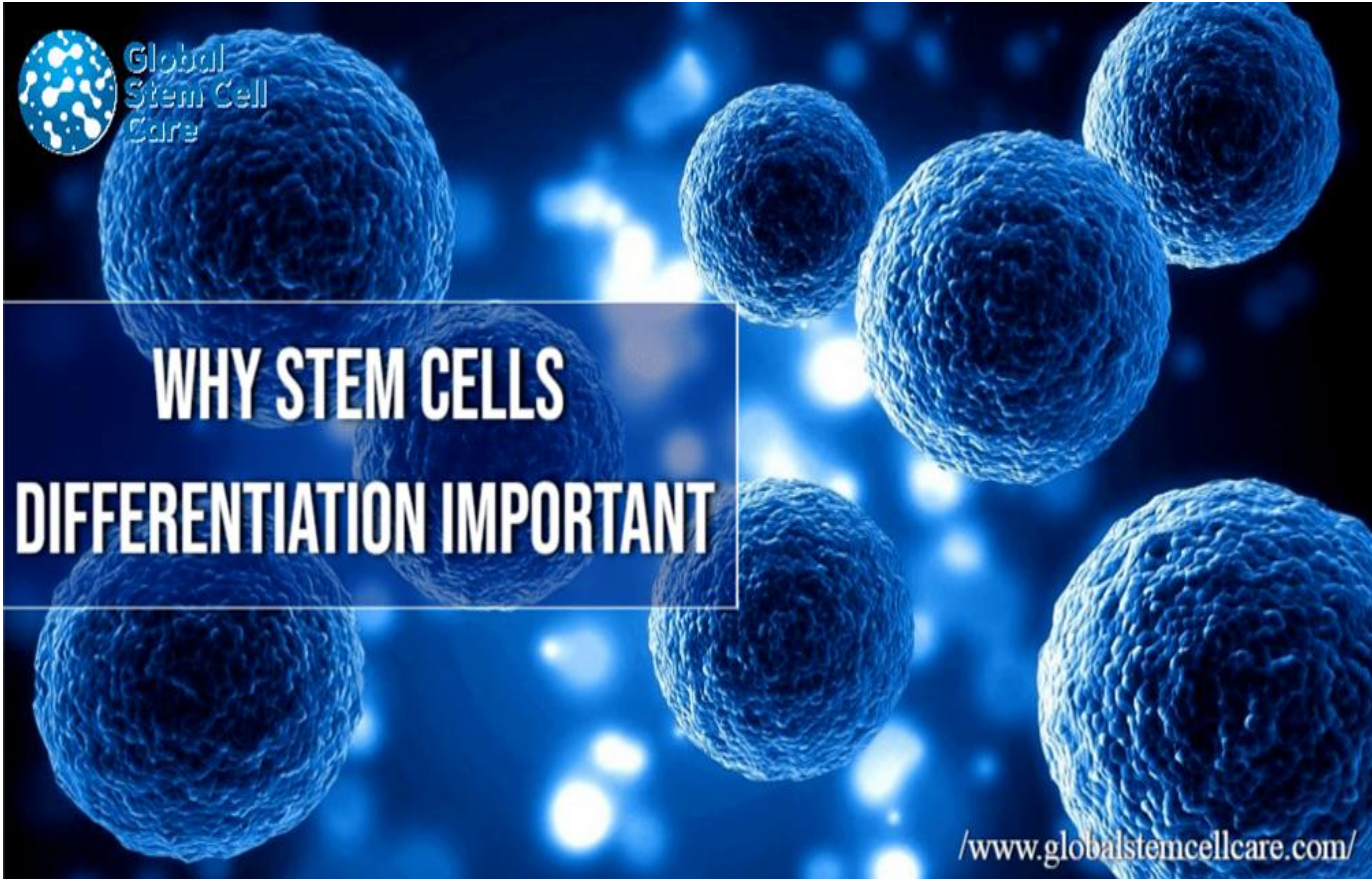
Induced Pluripotent Stem Cells (iPSCs) are formed by 4 Transcription factors from somatic cells



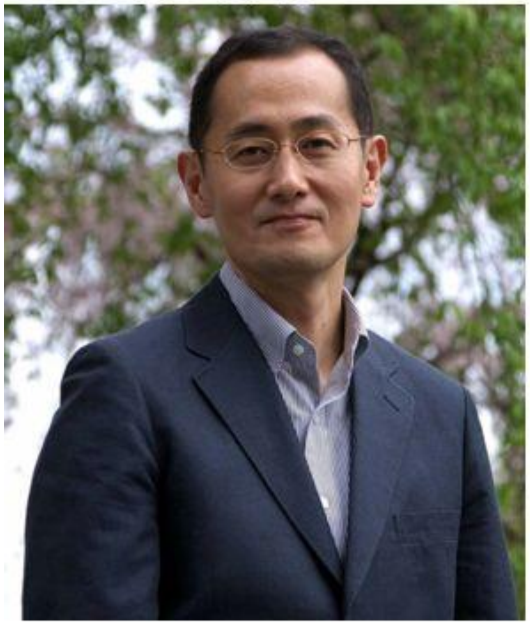
Generation Of iPSC by Yamanaka Factors




Patient
Health disorder
treatment
Disease modelling
Cell collection
Somatic Cells
Reprogramming
iPSCs-derived research
and treatment
Application
iPSCs
Differentiated cells
Genome editing
Differentiation
Genetically modified iPSCs


Global
Stem Cell
Care
WHY STEM CELLS
DIFFERENTIATION IMPORTANT
/www.globalstemcellcare.com/

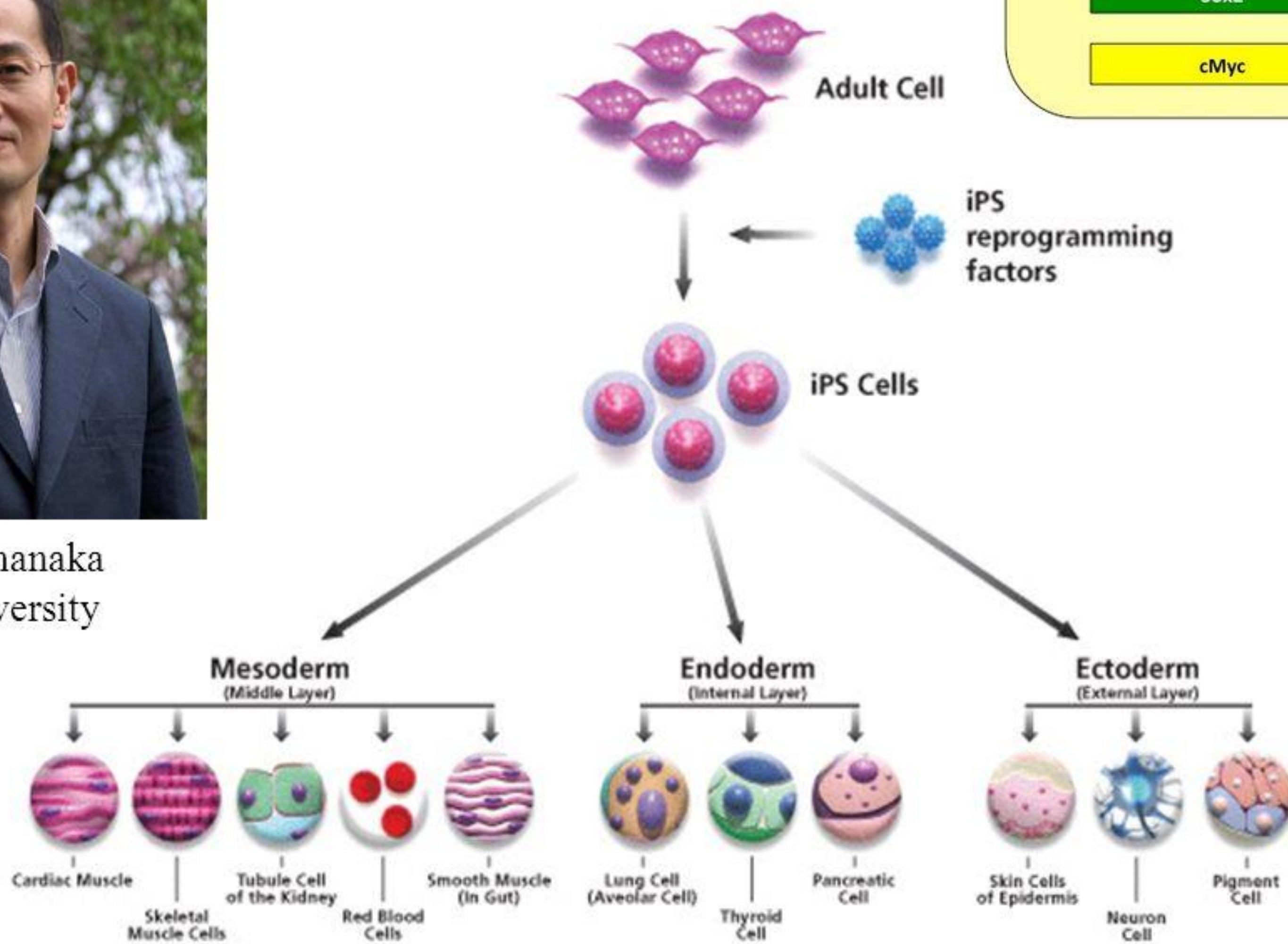
iPSC



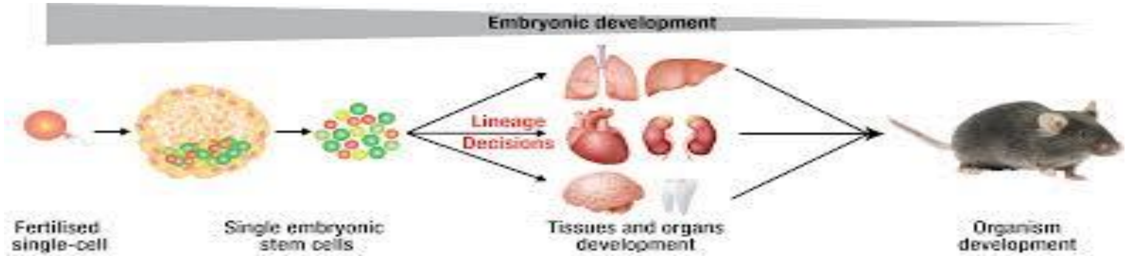
Shinya Yamanaka
Kyoto University

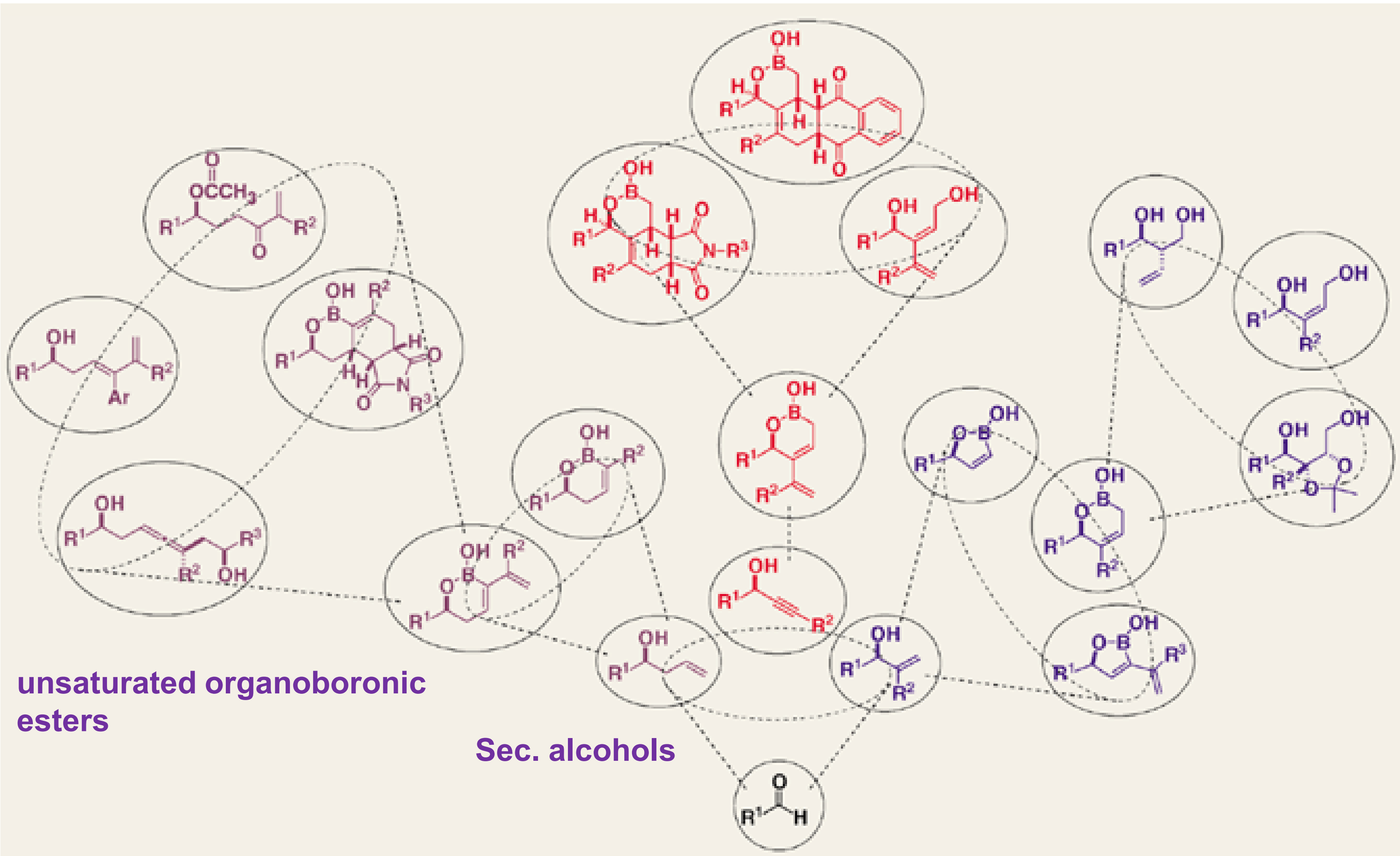
Yamanaka Transcription Factors

- Oct4
- KLF4
- Sox2
- cMyc



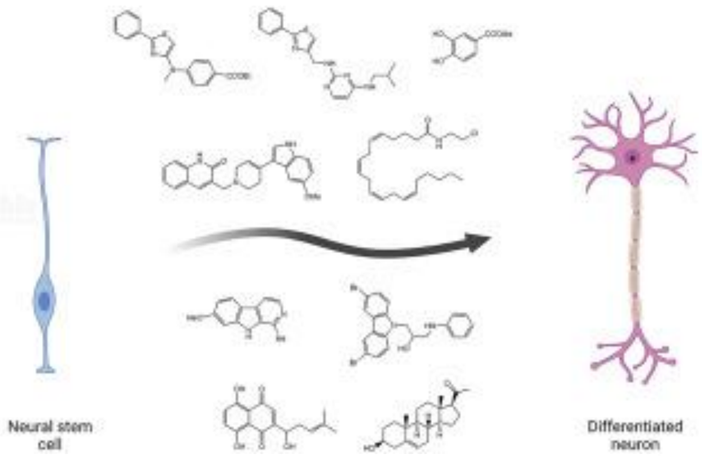
Chemical equivalent of a stem cell-a "pluripotent" organic functionality



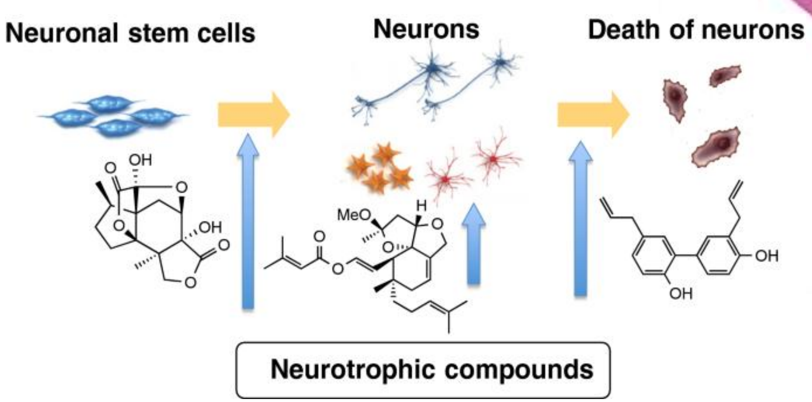


Can Small molecules be used to produce iPS Cells?

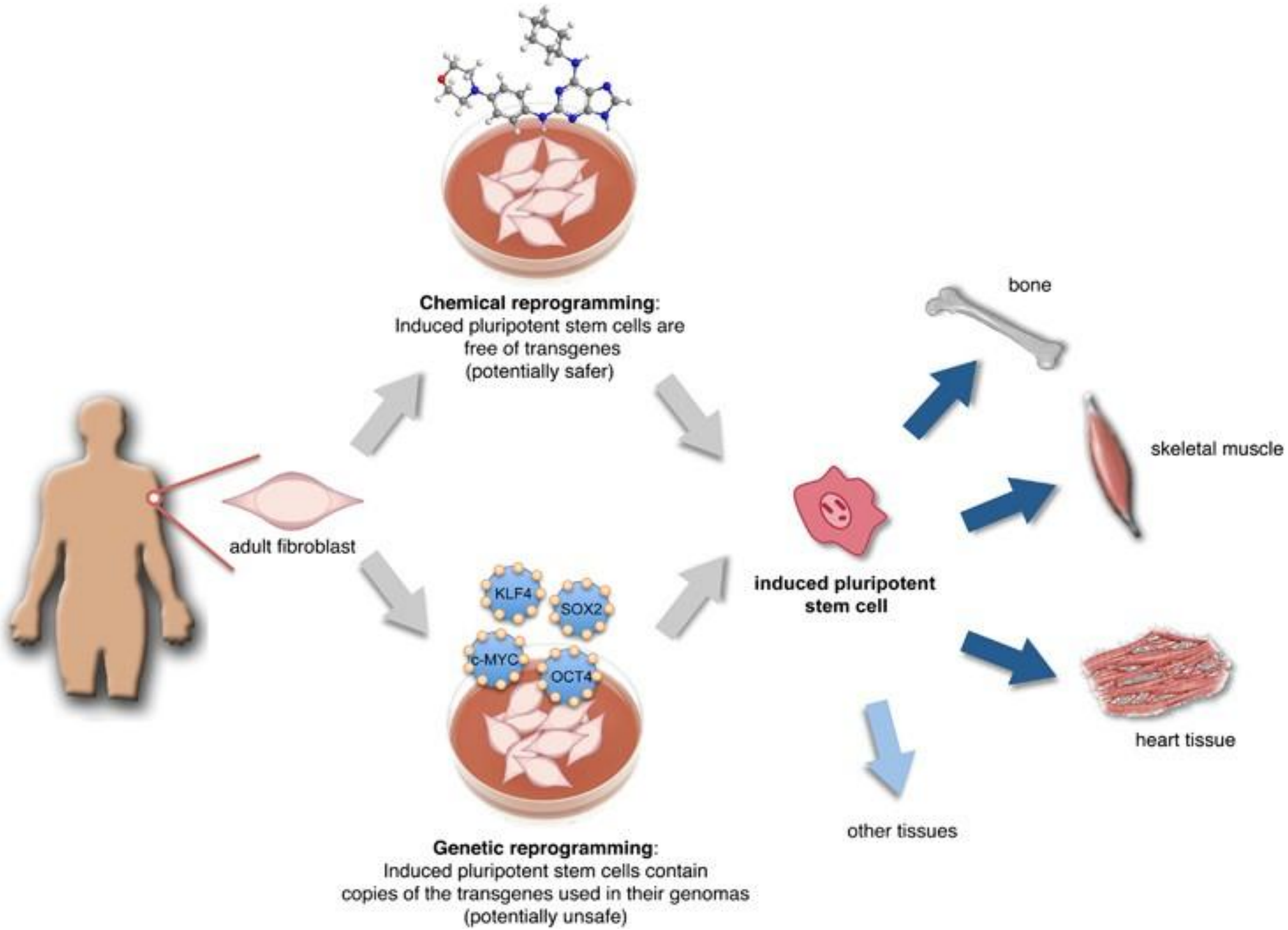
Chemical approaches to studying stem cell biology!


Neural stem cell
Differentiated neuron

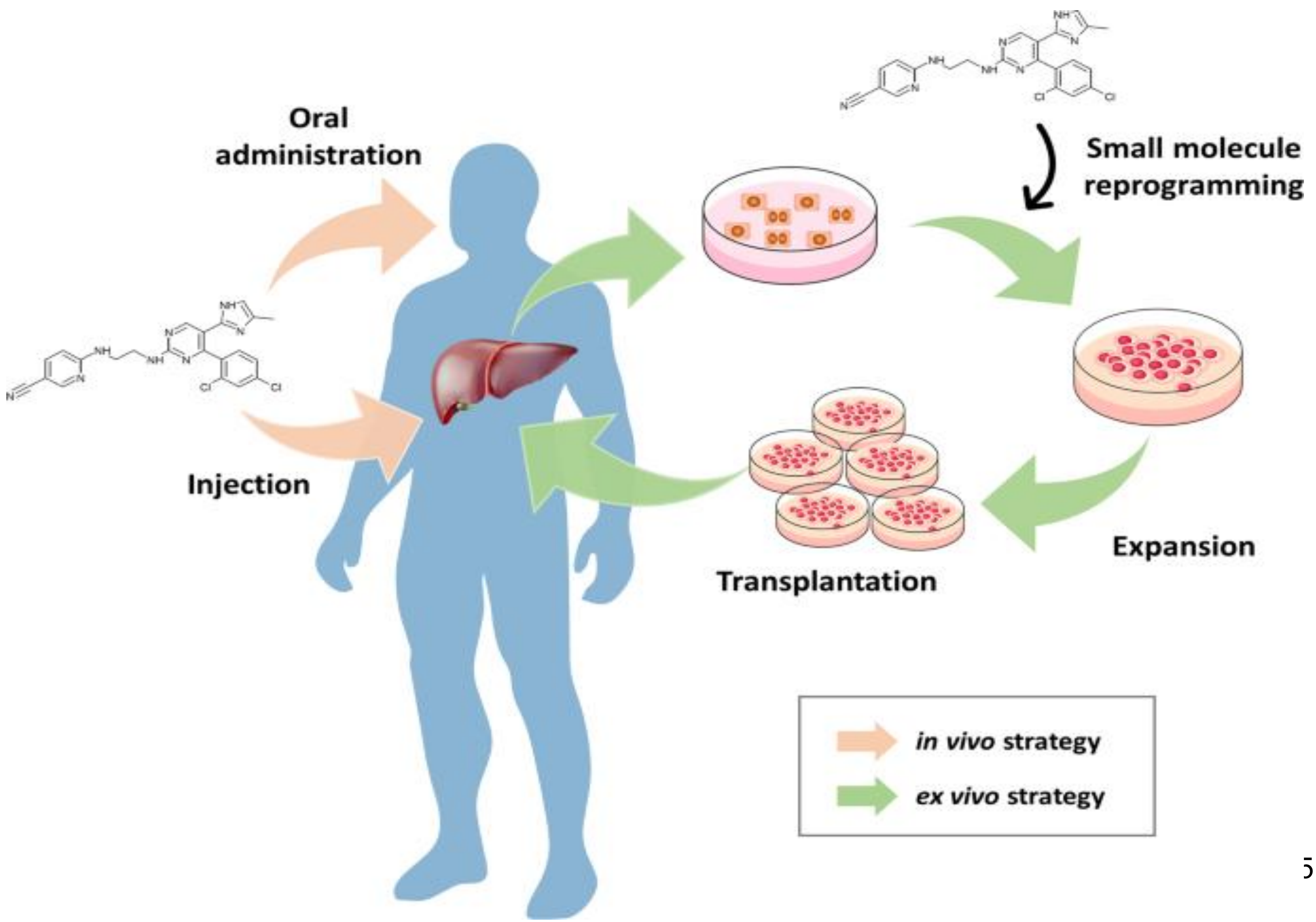

Good Value
Cost effective compared to Viral vectors and Antibody kits
Good Temporal Control
Can be rapidly applied and removed through media exchange
HIGH QUALITY GUARANTEED
High Quality
High purity >98%, with low lot-lot variability
Why use Small Molecules?
Synthetically Synthesized
Animal-free production
Cell-permeable
Permits simple probe delivery
Tunable
Concentration-dependent effects


Neuronal stem cells
Neurons
Death of neurons
Neurotrophic compounds

Small Molecules versus Transcription factors mediated differentiation of Stem Cells



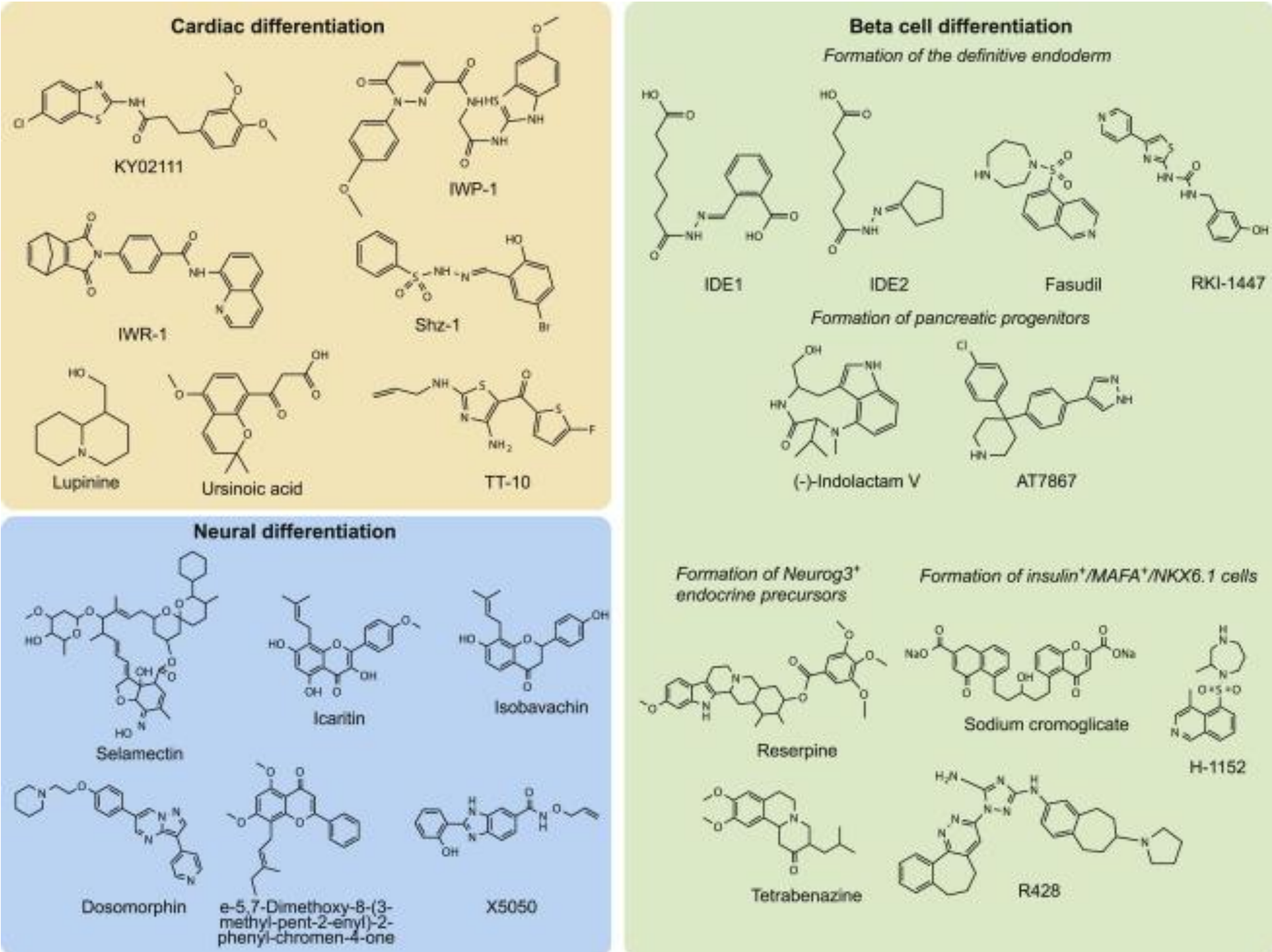
Small-molecule-mediated reprogramming: a silver lining for regenerative medicine



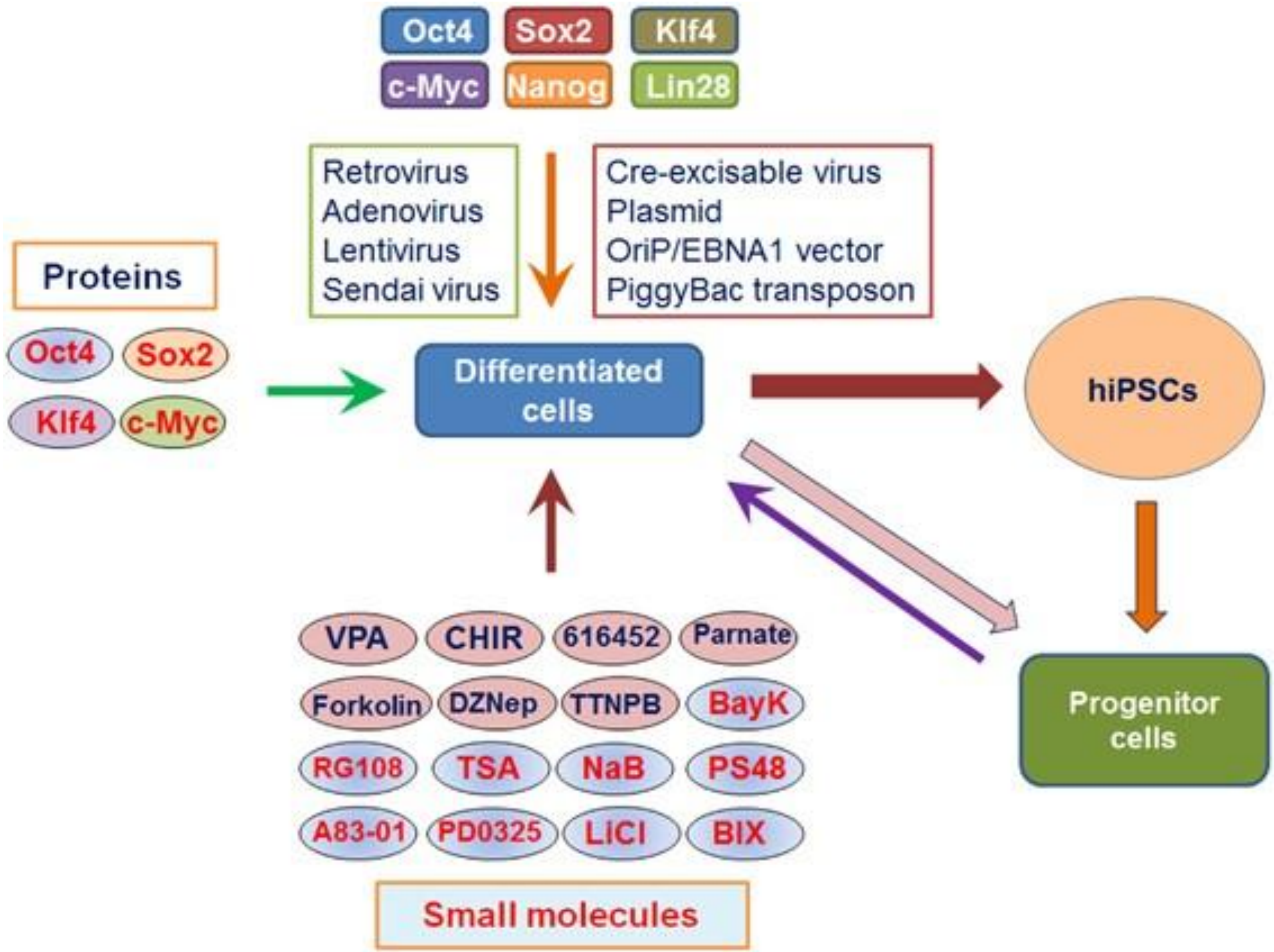
Synthetic small molecules that control stem cell fate

Some Examples of Small Molecules Directed Differentiation of Stem Cells

Structures of small molecules that promote reprogramming to generate iPSCs



Cells can be reprogrammed to iPSCs using viral or non-viral introduction of exogenous TFs or protein transfection



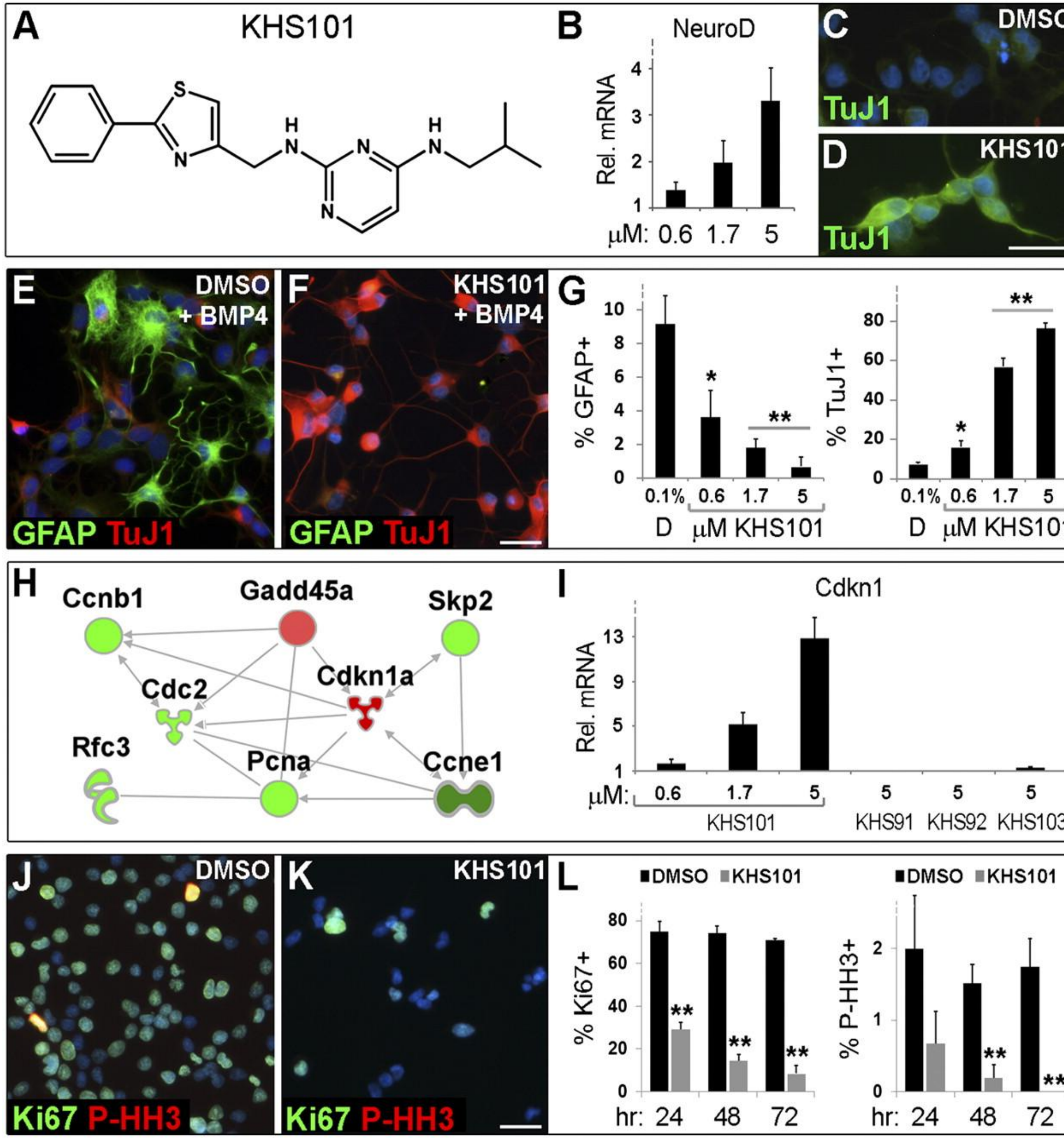
A small molecule accelerates neuronal differentiation in the adult rat

Heiko Wurdak, Shoutian Zhu, Kyung Hoon Min and Peter G. Schultz

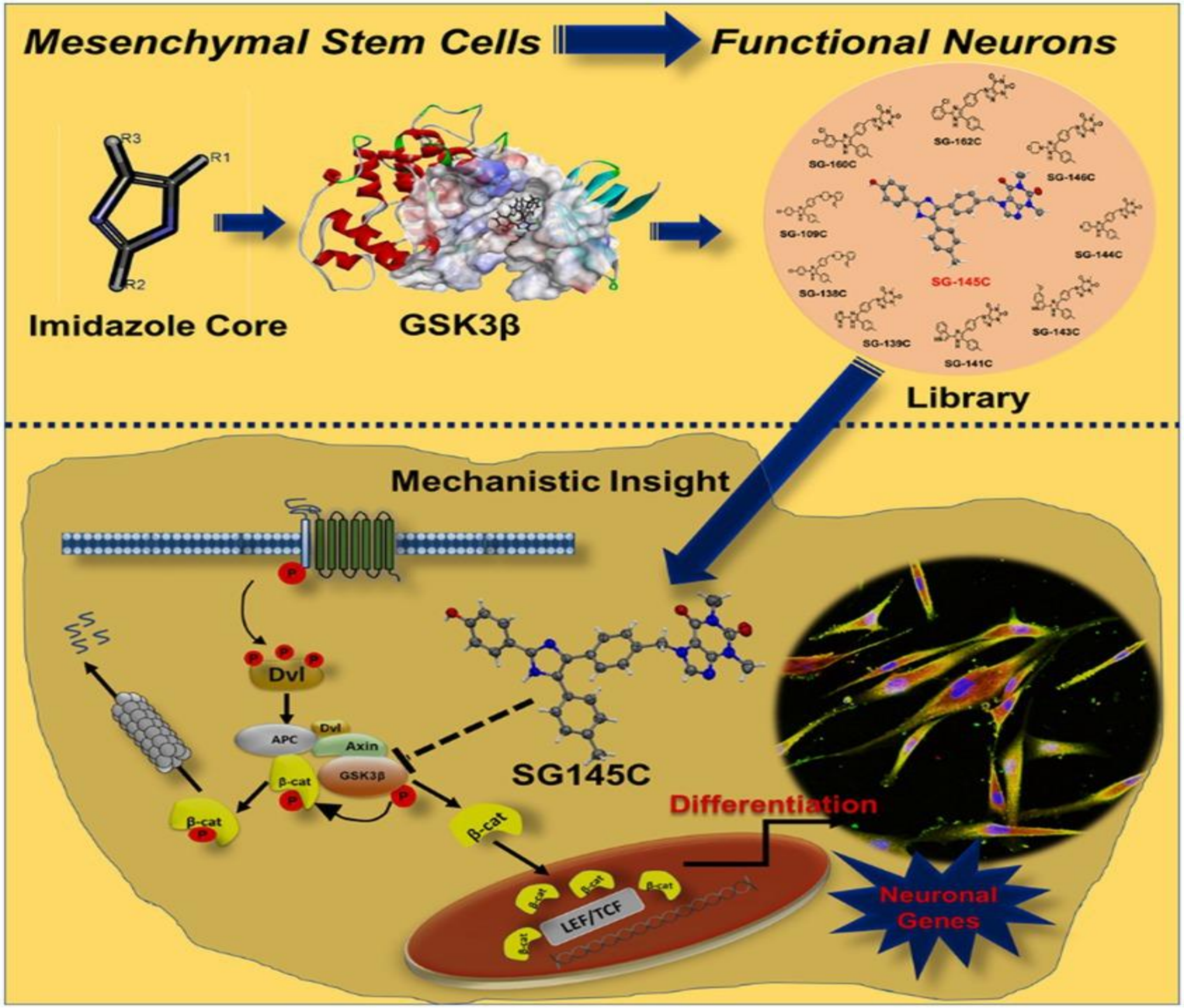
September 7, 2010

107 (38) 16542-16547

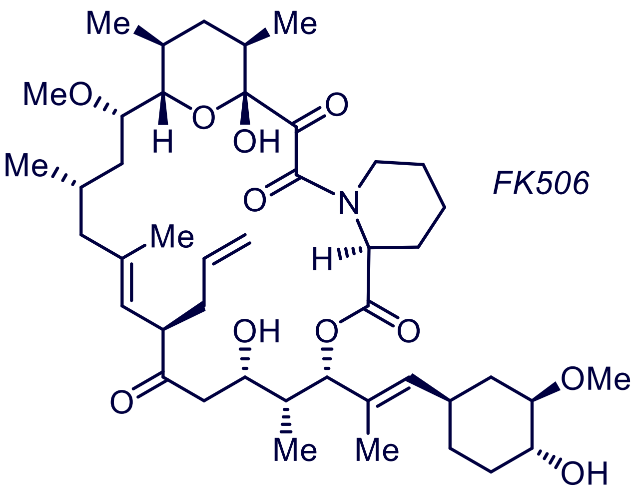
https://doi.org/10.1073/pnas.1010300107



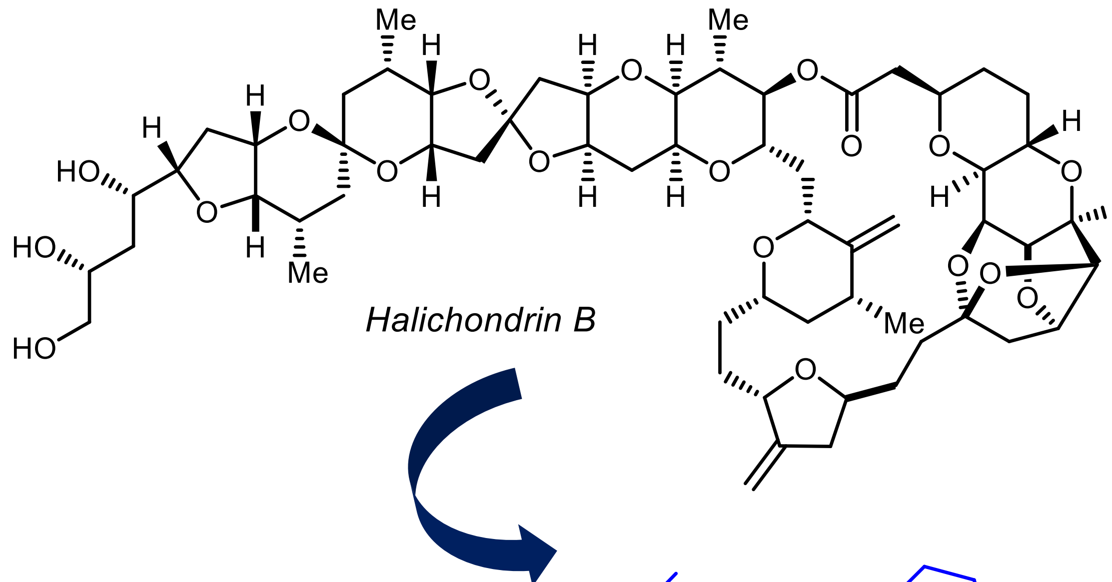
Our Foray into Small molecules induced trans-differentiation of Stem cell to Neuron like cells



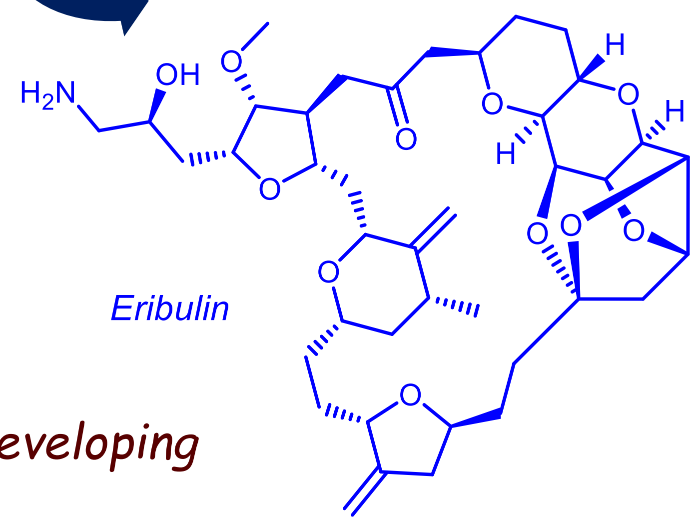
Natural products as a source of inspiration



FK506



Halichondrin B



Eribulin

- present complex 3D architectures
- dense display of stereo-defined groups
- *challenging task in placing them on the drug discovery path!*
- excellent track record as small molecule modulators of protein-protein interactions
- *can serve as a good source of inspiration for developing novel scaffolds in the drug discovery arena!*

Our Chemical Toolbox-based Working Model

Bioactive Natural Products → Natural Product Inspired Sub-structures / Natural Product Fragments as Sub-structures → Exploring Macrocyclic Chemical Space!

- Cyclic compounds
- Large surface area
- Pre-organization
- Less freely rotating bonds
- Enhanced cell permeation

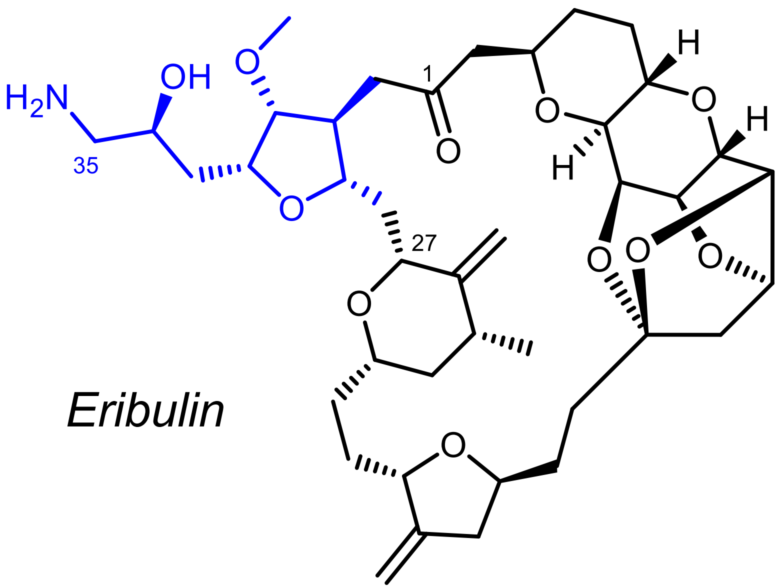
Key features in our design:

- 3D architectures
- sufficient complexity
- stereochemical and skeletal diversity
- synthesis in a reasonable time-scale
- easy to follow-up medicinal chemistry studies

From Arya Research Team:

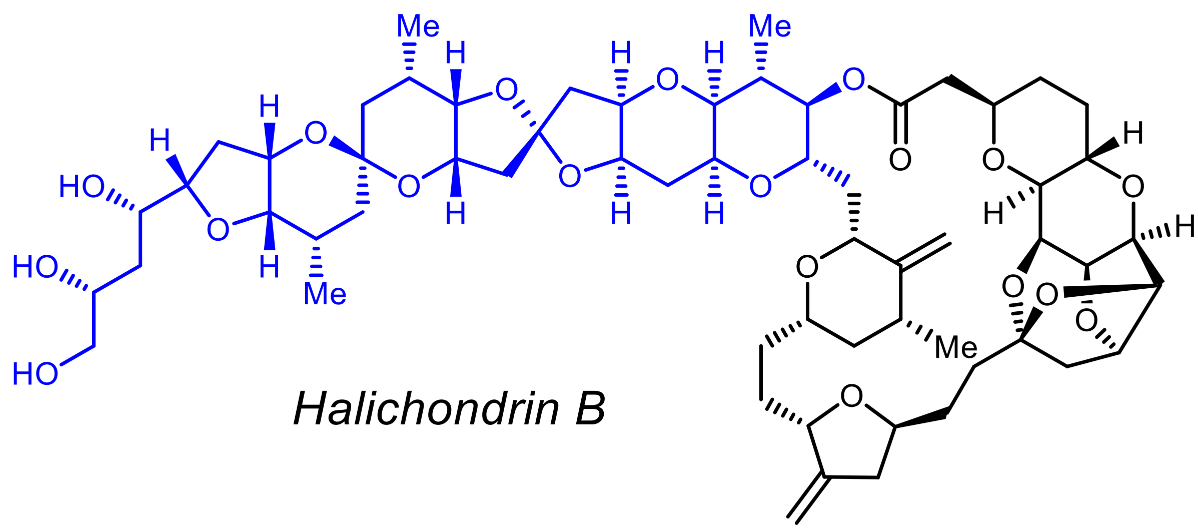
Chem. & Biol. 163 (**2005**)
Curr. Opin. Chem. Biol. 247 (**2005**)
Chem. Rev. 1999 (**2009**)

Introduction



Eribulin

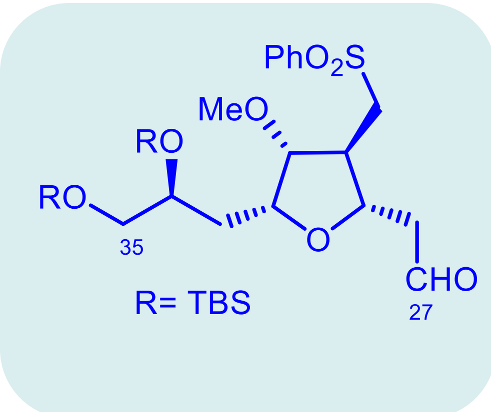
- Anticancer drug approved by FDA (November 15, 2010) and marketed by Eisai
- Synthetic analog of a marine sponge natural product, *halichondrin B*
- Mechanistically, a unique inhibitor of microtubule dynamics



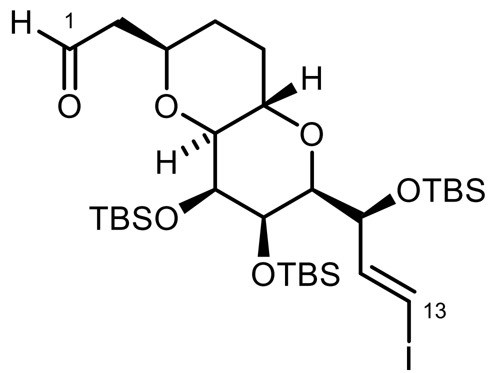
Halichondrin B

Three Key Fragments of Eribulin

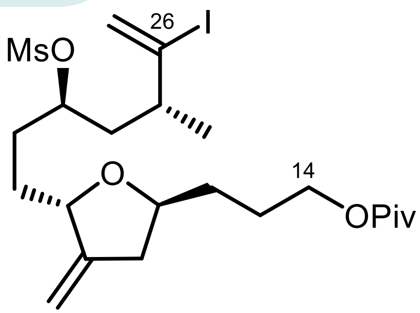
A

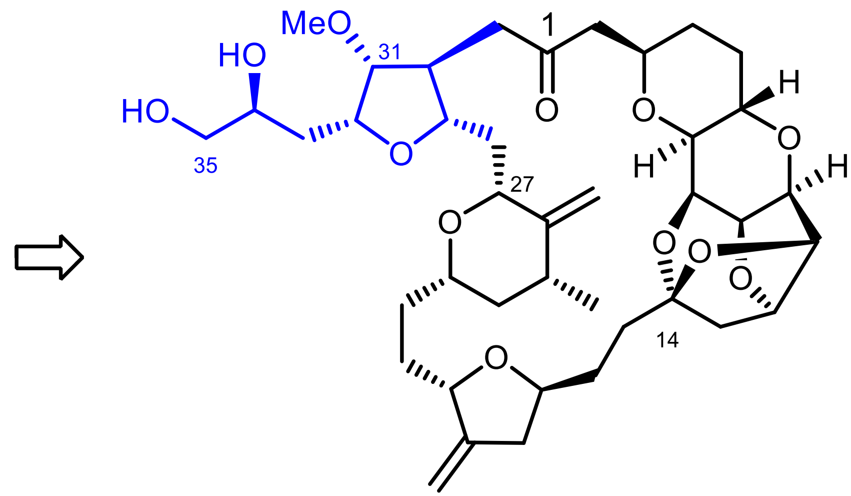


B



C

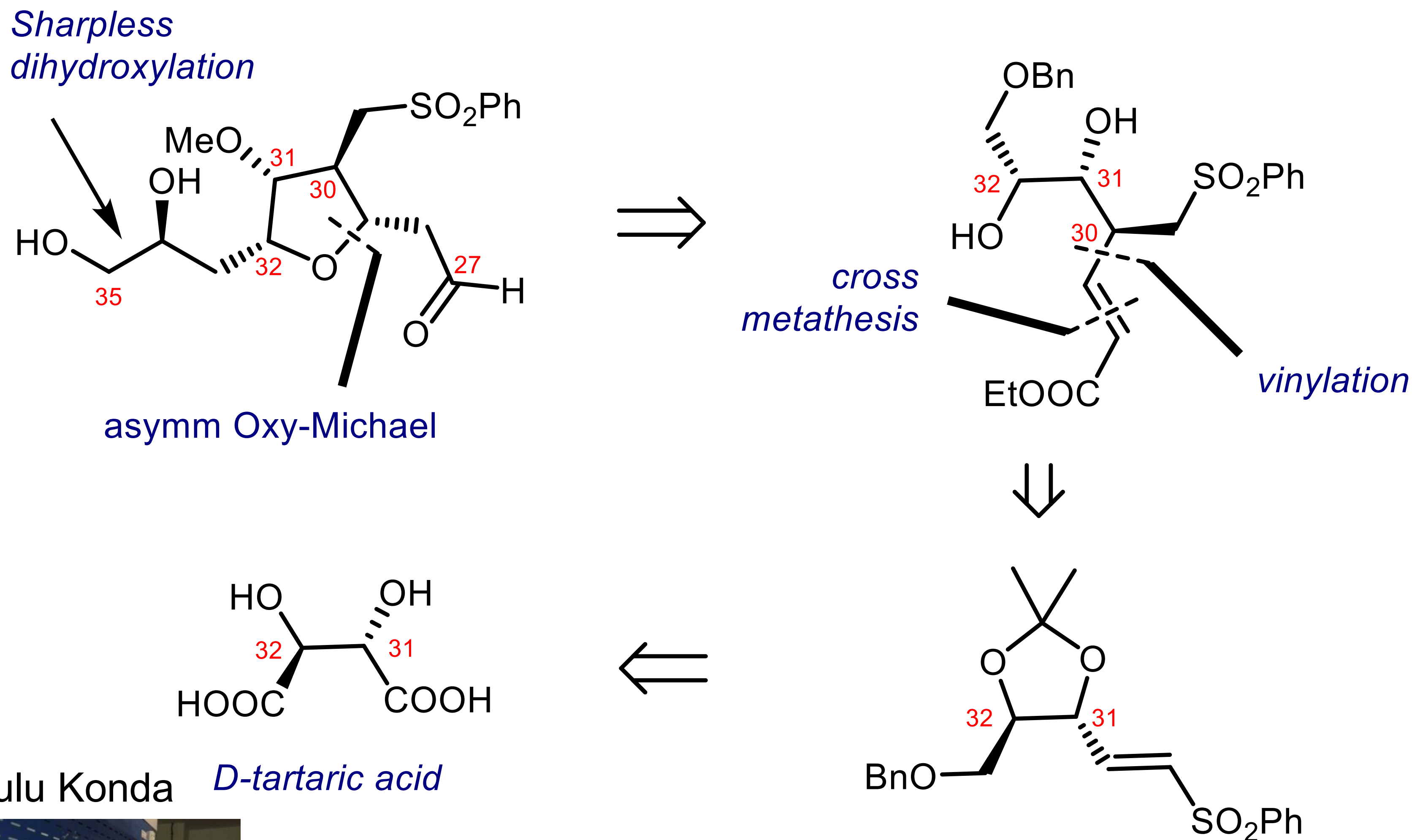




ER-076349

From Kishi's Lab:
Bioorg. Med. Chem. Lett. **2004**, *14*, 5551-5554

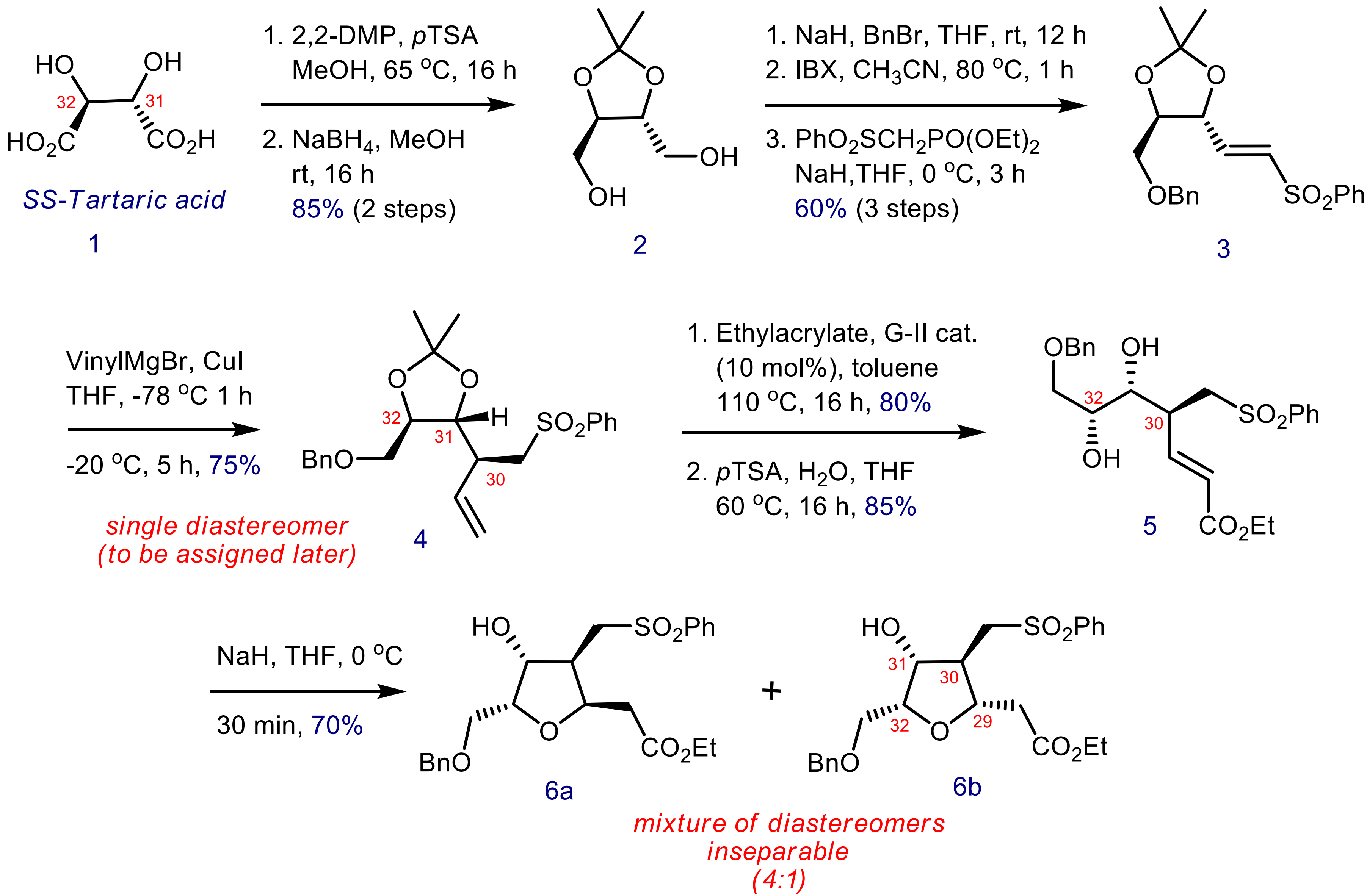
Our Next Generation Approach (Oxy-Michael)



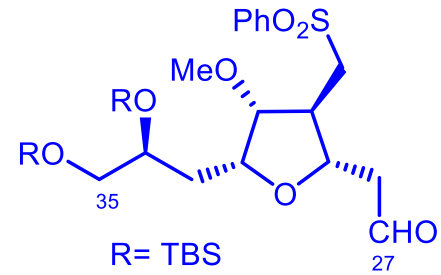
Saidulu Konda

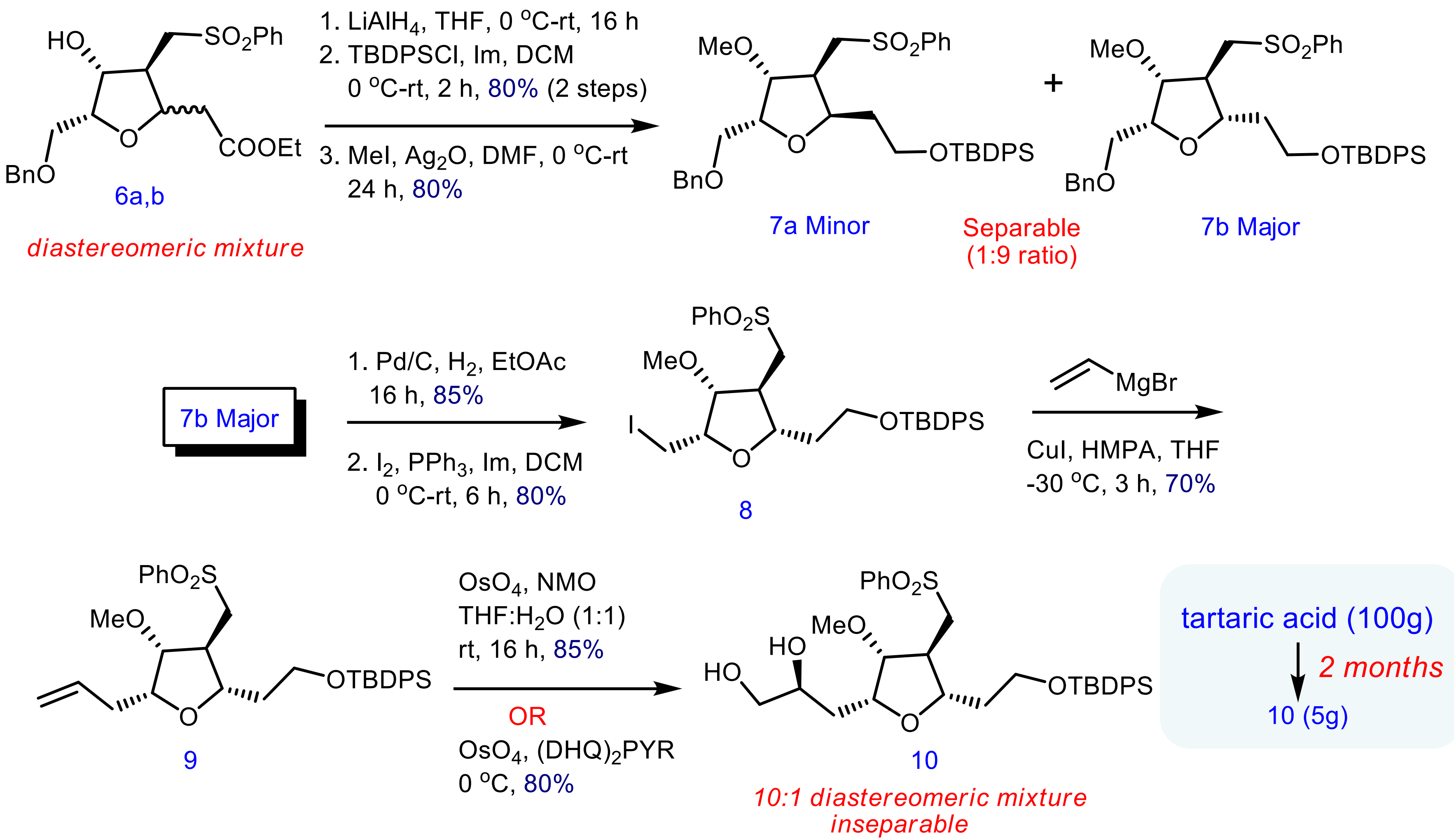
Goal: Develop a Practical and Scalable Method

Our Synthetic Path

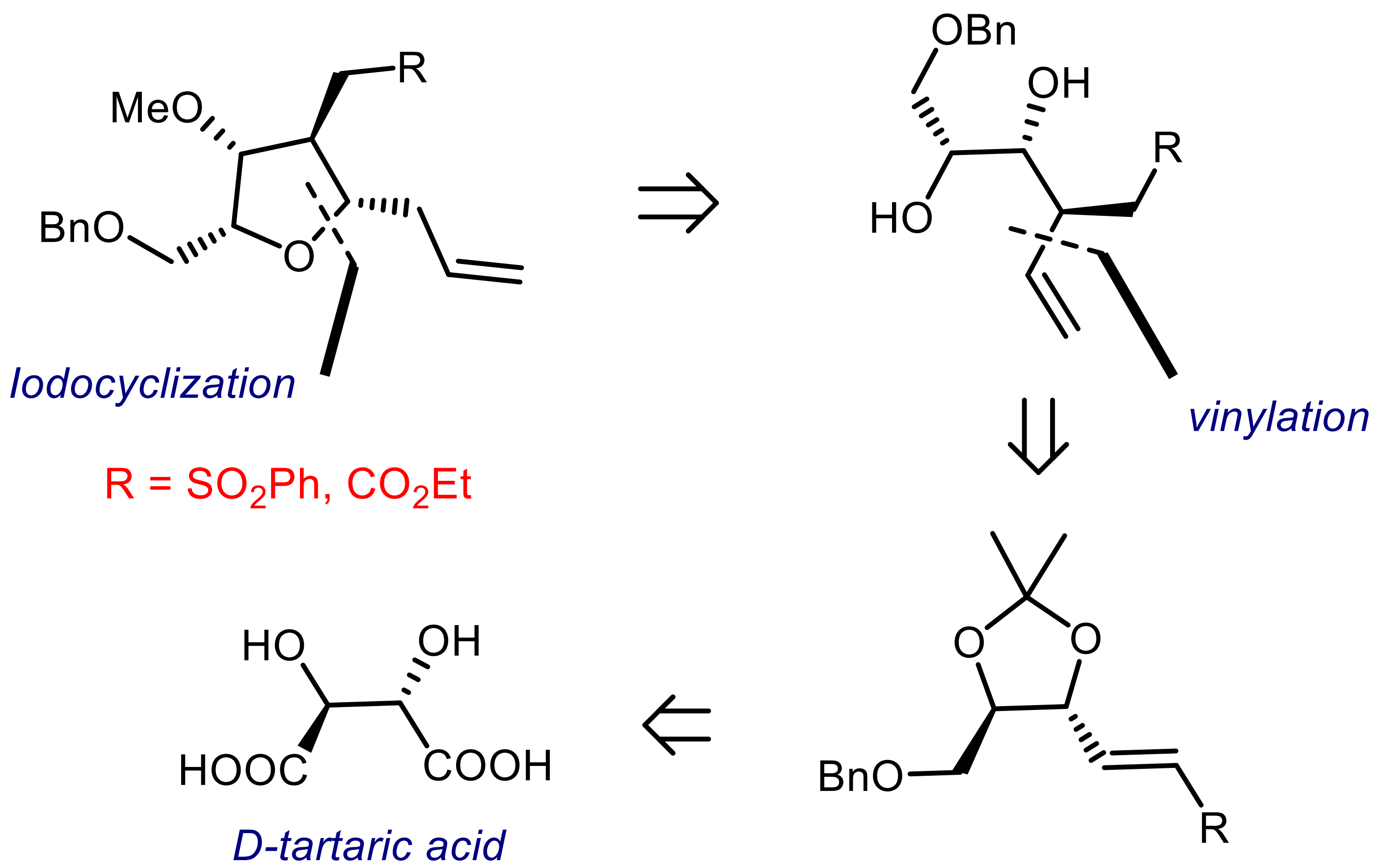


Completion of Fragment A Synthesis



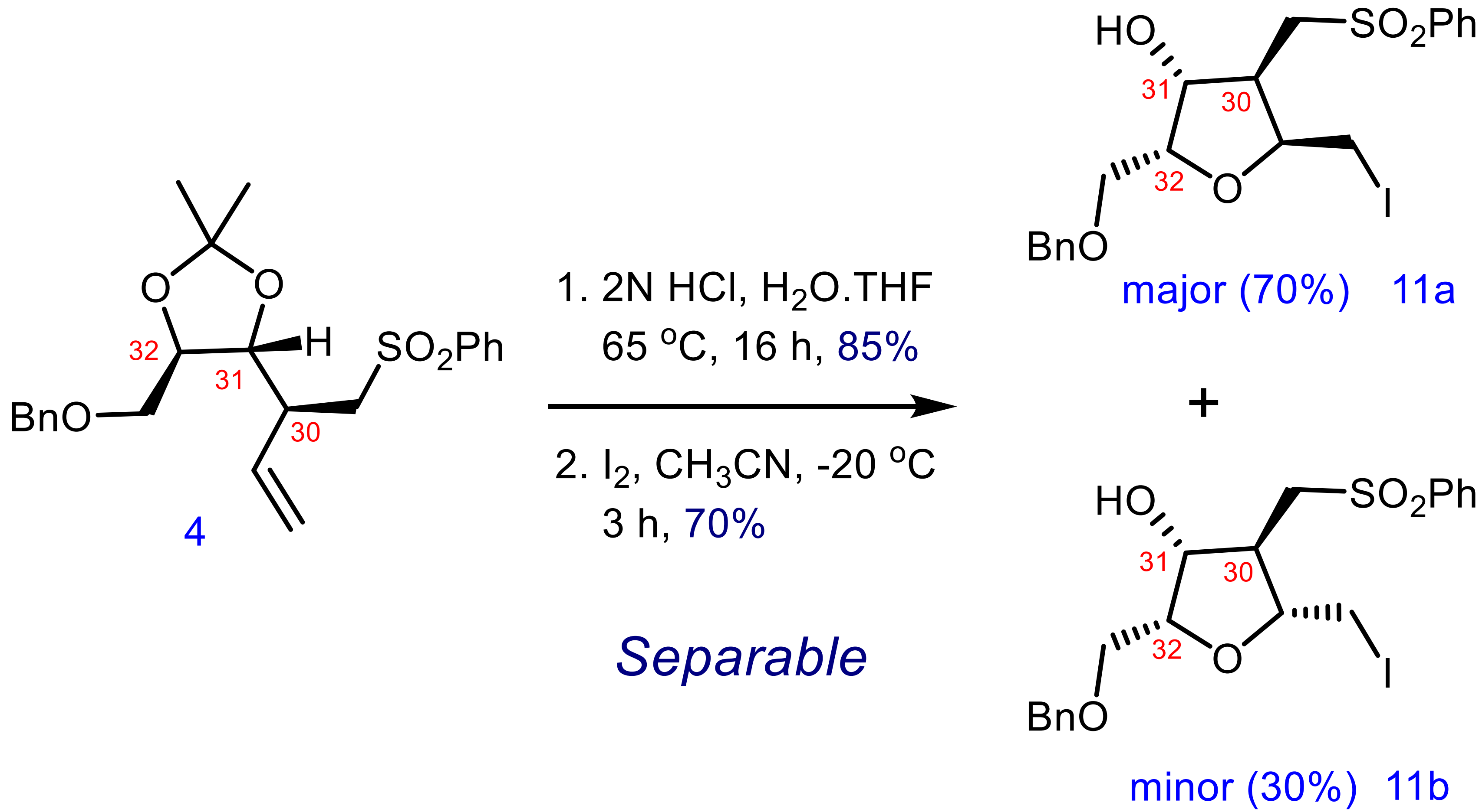


Our Second Approach (Iodocyclization)

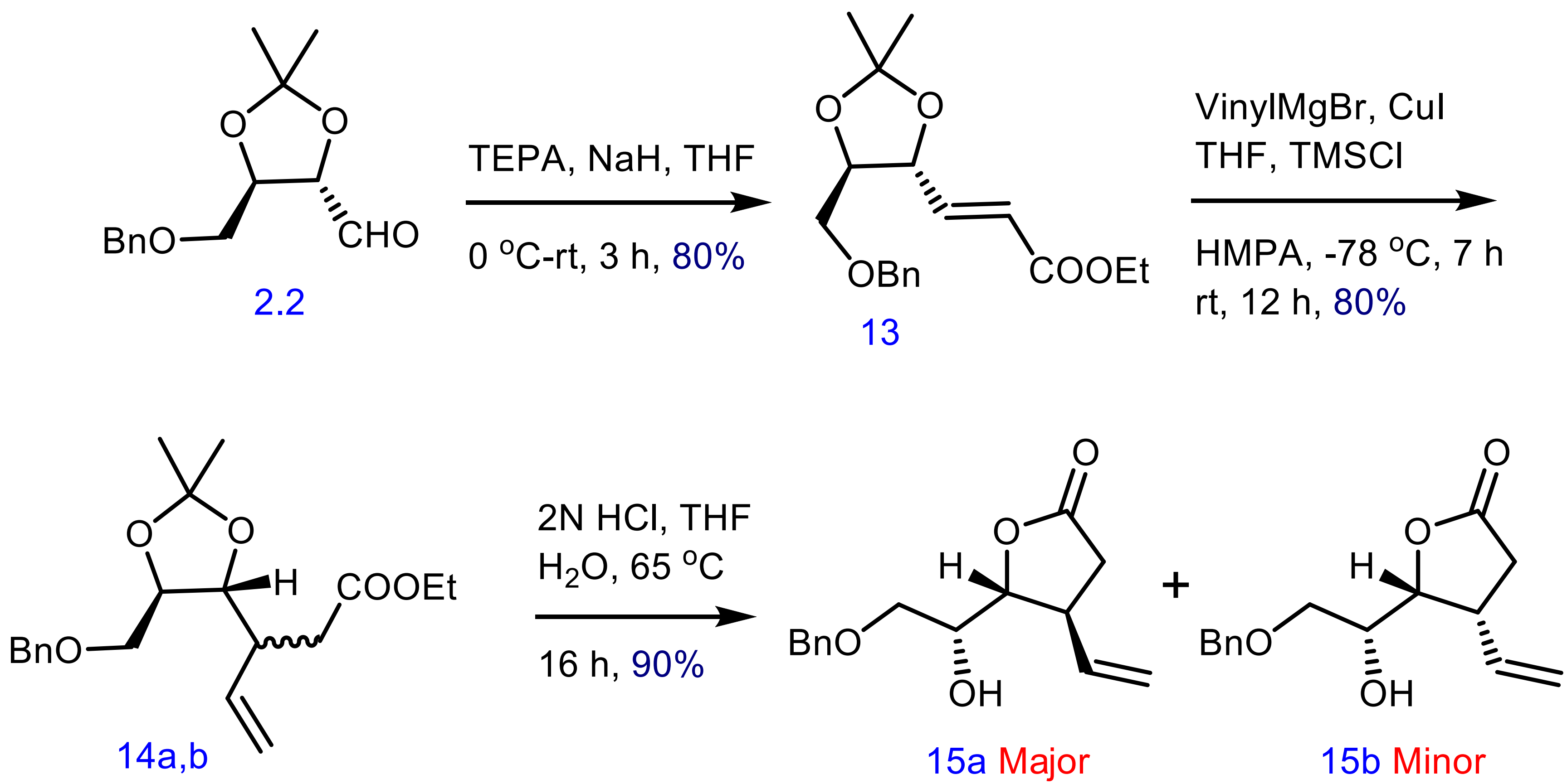


R = SO_2Ph, CO_2Et

Our Synthetic Path

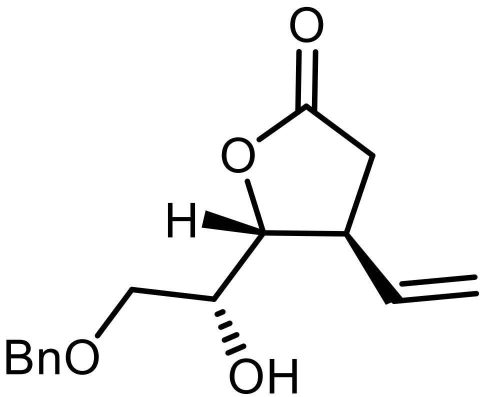


Our Synthesis Path (EWG = -COOEt)



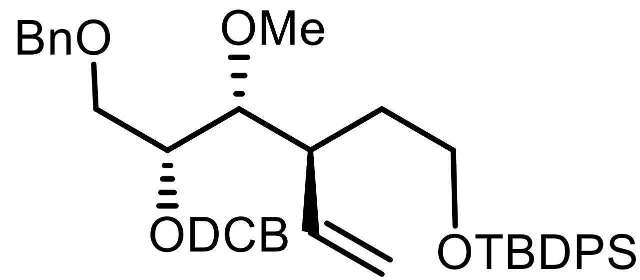
mixture of diastereomers (inseparable, 13:1)

Our Key Iodocyclization



15a

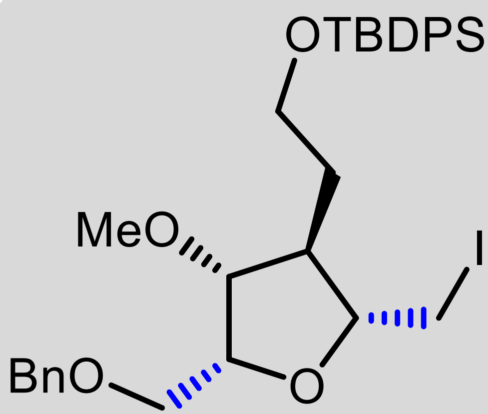
1. Ag_2O, DCBCl, DMF
   TBAI, rt, 16 h, 70%
2. $LiAlH_4$, THF, 0 ^oC-rt
   16 h, 75%
3. TBDPSCl, Im, DCM
   0 ^oC-rt, 3 h, 85%
4. NaH, MeI, DMF, 0 ^oC
   1 h, 80%



16

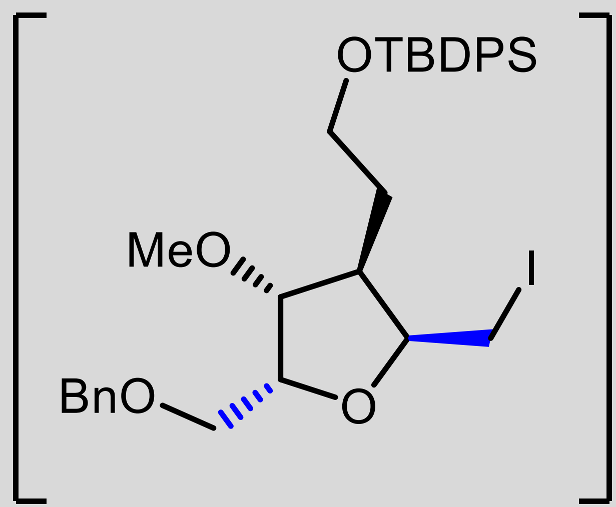
I_2, CH_3CN
-20 ^oC, 3 h

80%



17

Single diastereomer



not observed

Key Features in Our Building Macrocyclic Designs

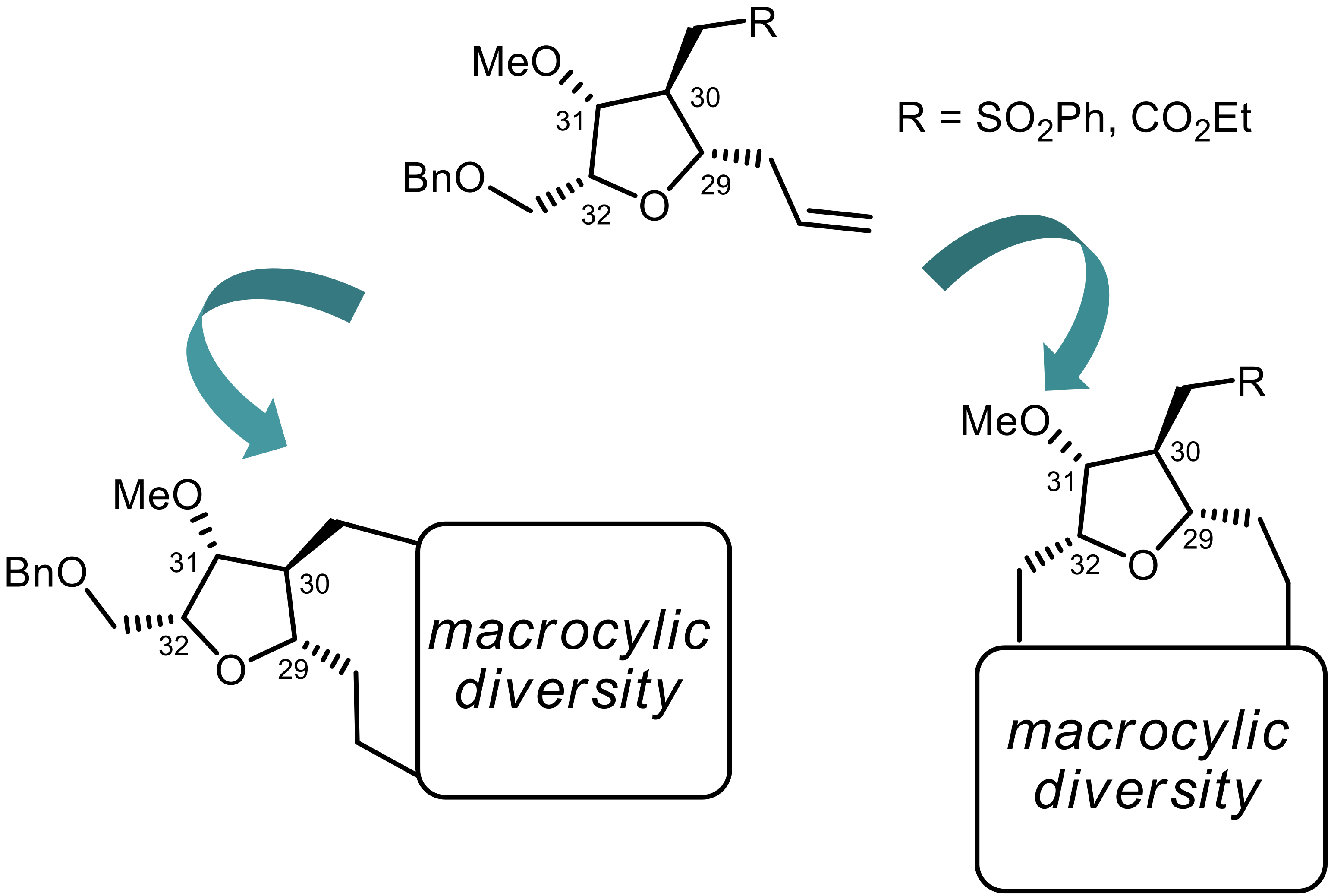
1. Natural product-inspired 3D-architectures

2. Stereochemical and skeletal diversity

3. Practical and scalable synthesis

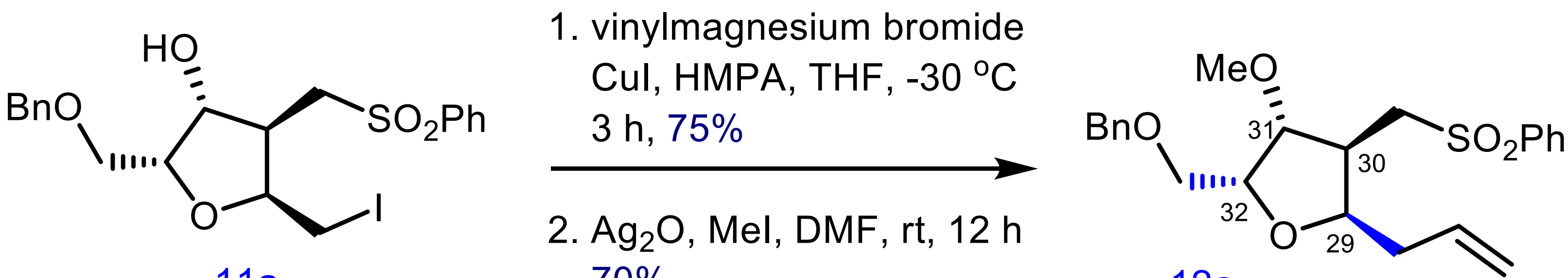
4. Easy structural modifications for undertaking medicinal chemistry projects

Eribulin Fragment-based Macrocyclic Diversity

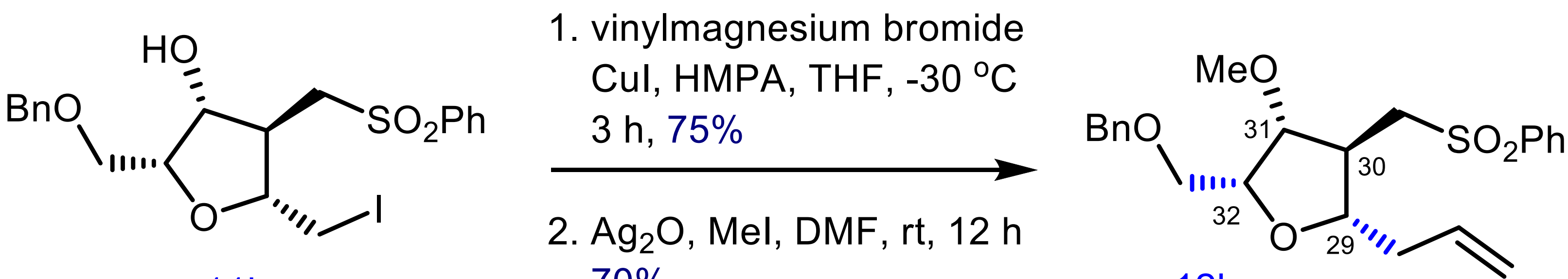


Goal: Develop Modular Approaches to Obtain Different Macrocycles to Explore their Biological Properties

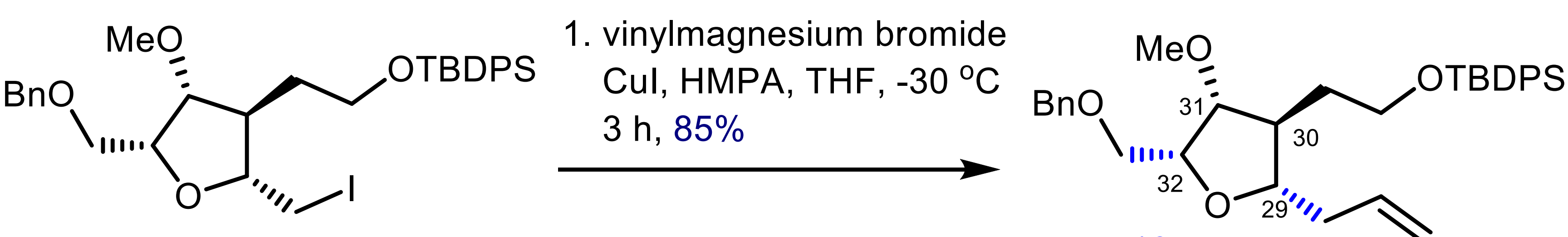
Synthesis of Key Fragments



11a
12a

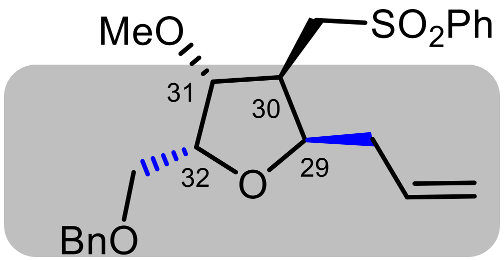


11b
12b



17
18

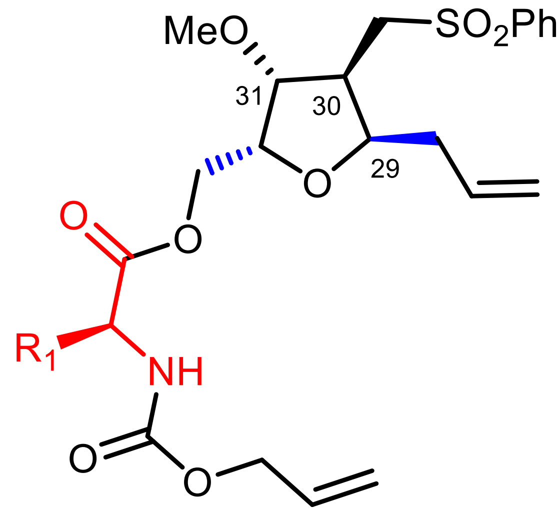
Our Synthetic Path to Building A Macrocyclic Diversity (Approach 1)



1,3-*trans*

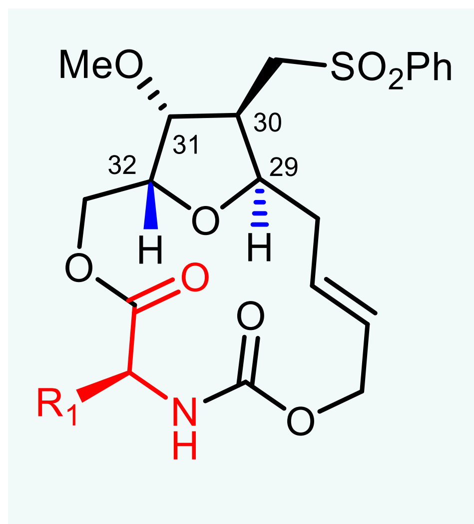
1. $TiCl_4$, DCM, 3 h, 75%

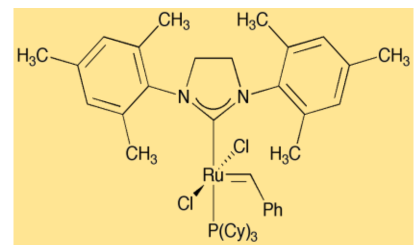
2. EDCI, DMAP, DCM
N-Alloc-AA, rt, 3 h
80-90%



G-II cat.(10 mol%)

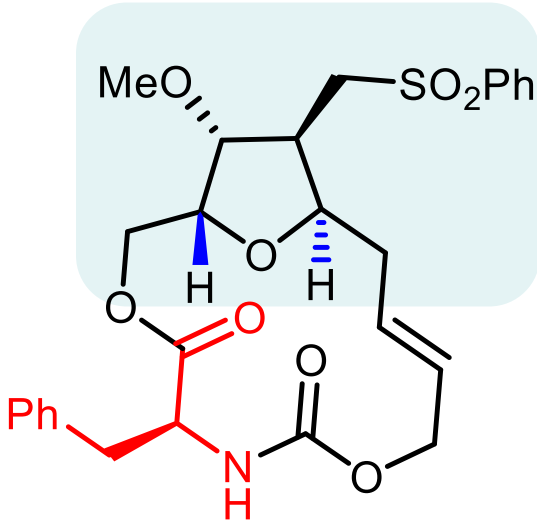
DCM, 40 ^{o}C, 16 h
70-75%

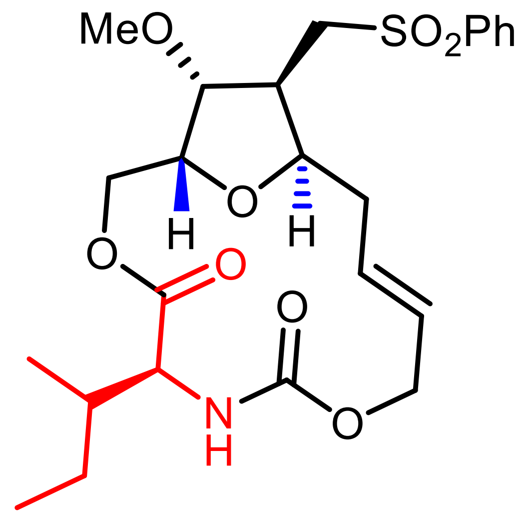


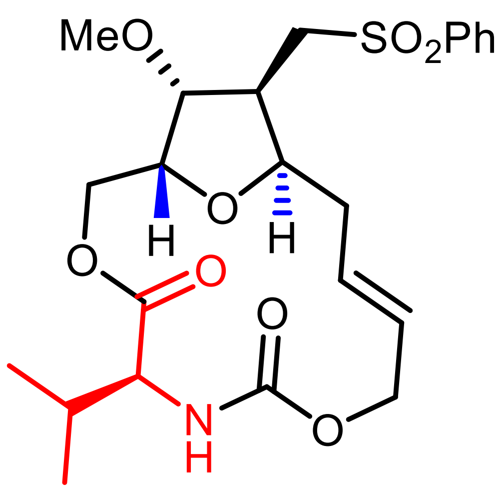


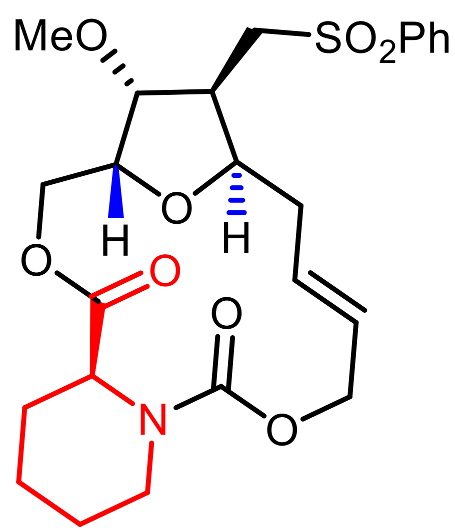
Grubbs 2nd gen. cat.

1,3-*trans* Relationship-based Four Macrocycles

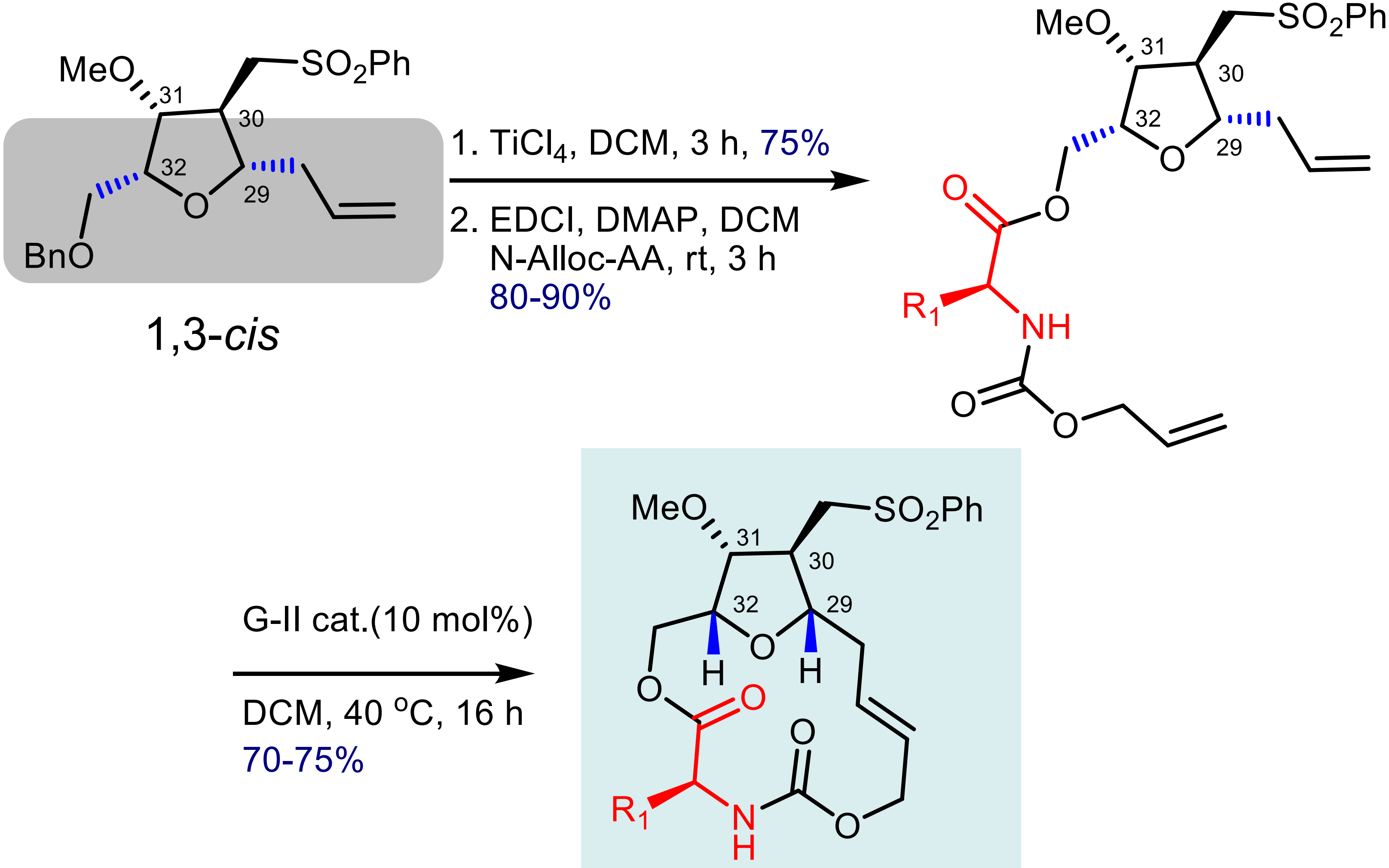




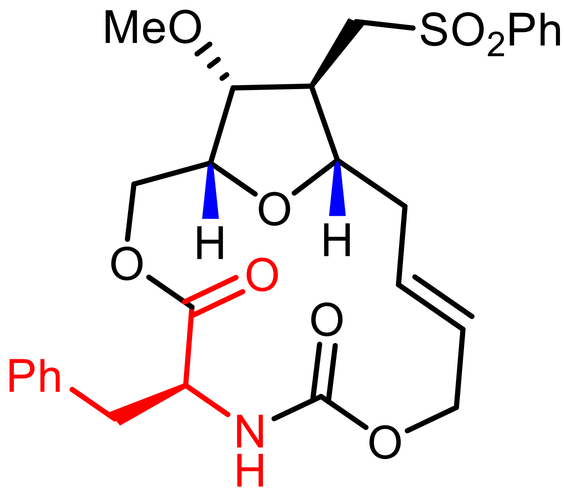


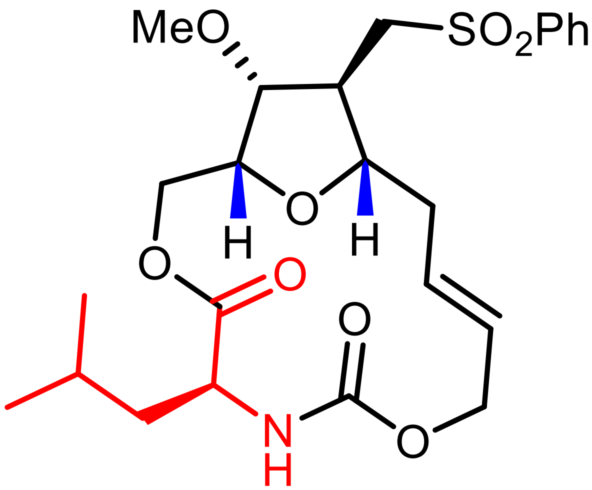


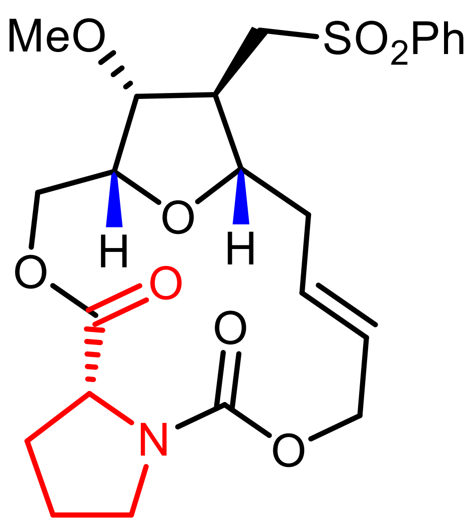
Diastereomeric Macrocyclic Diversity (Approach 2)

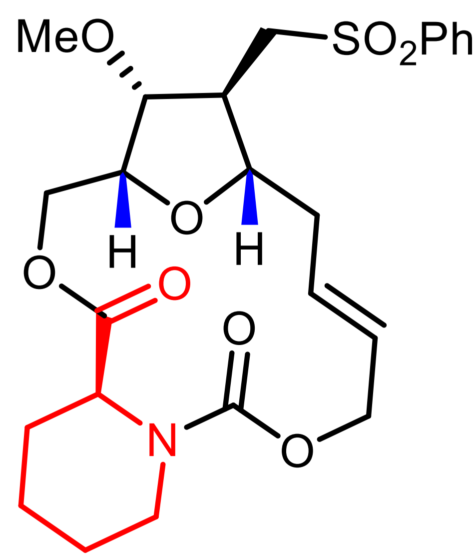


1,3-*cis* Relationship-based Four Macrocycles

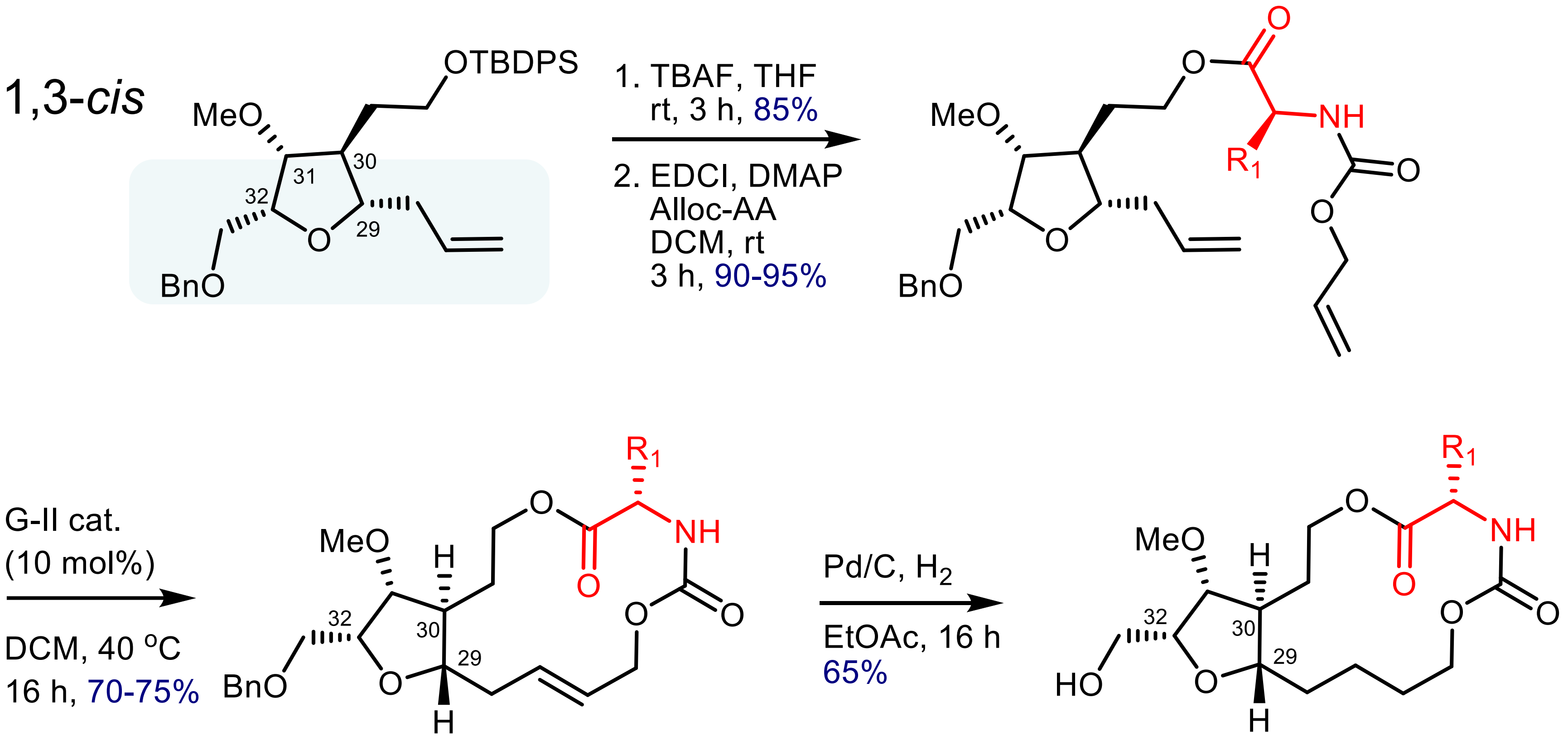




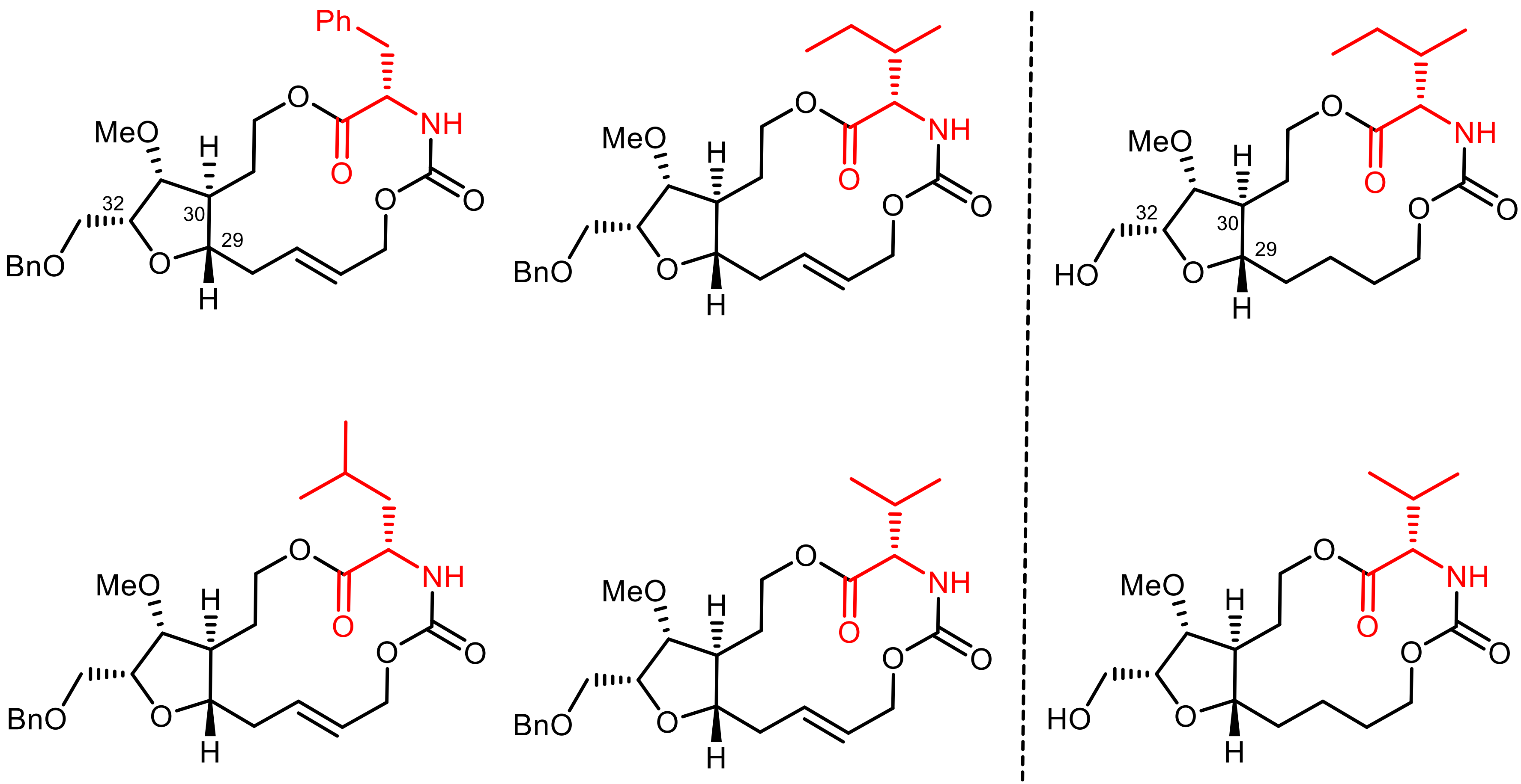




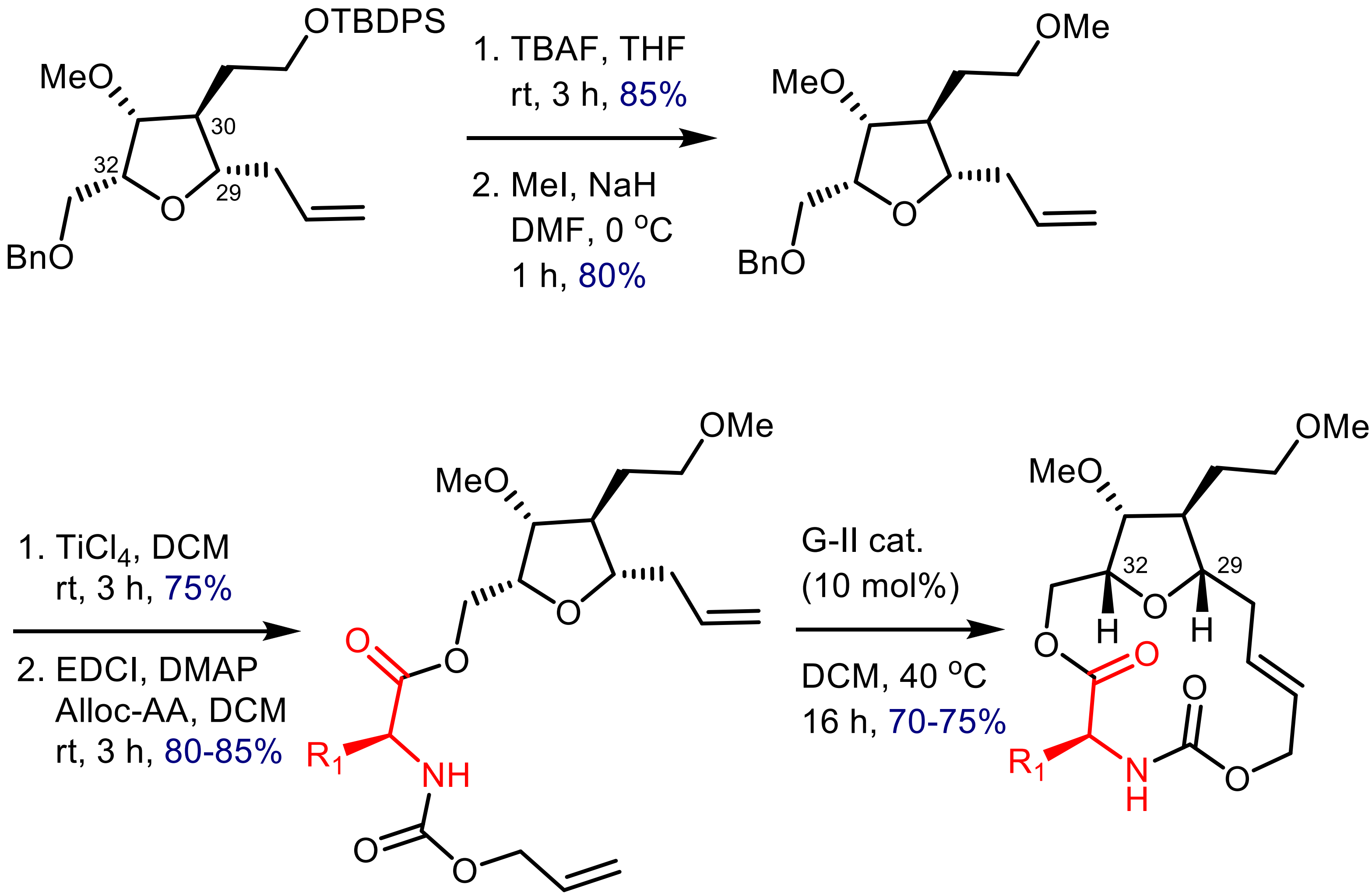
Another Approach to Building the Macrocyclic Diversity



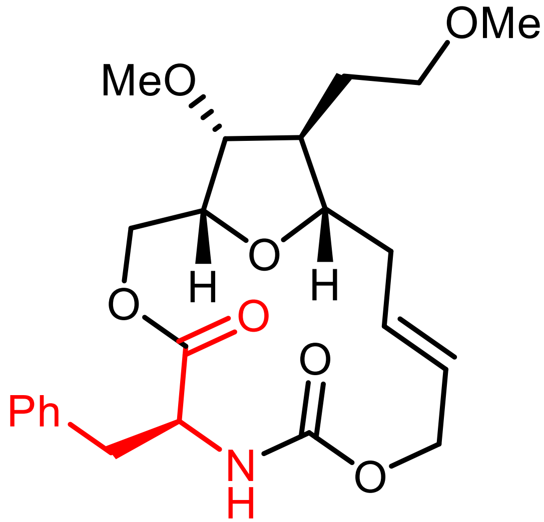
Derivatives Synthesized

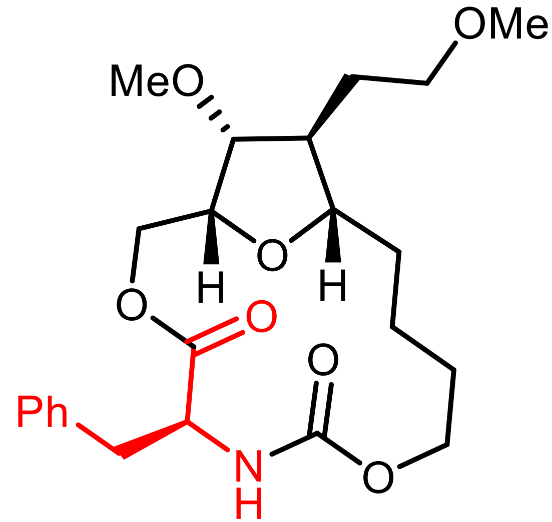


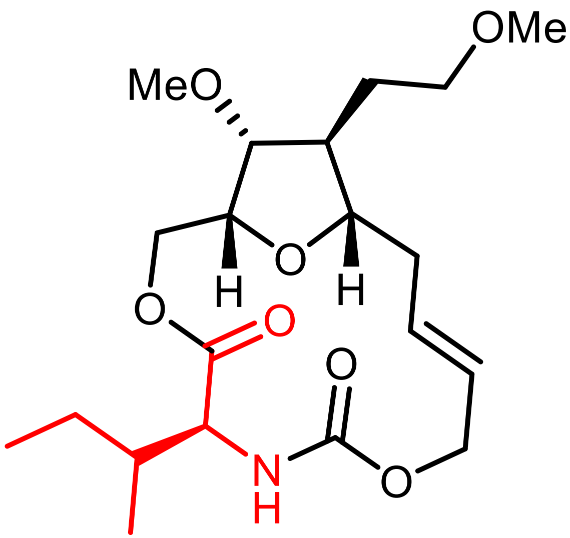
Macrocyclic Diversity using 1,3-*Cis* (C29-C32) Tetrahydrofuran

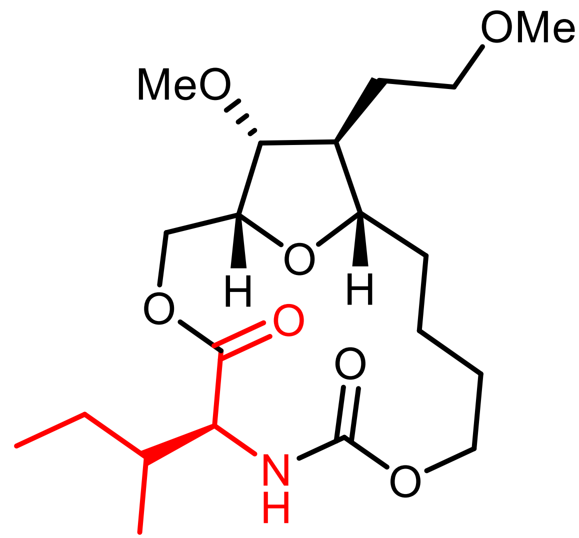


Derivatives

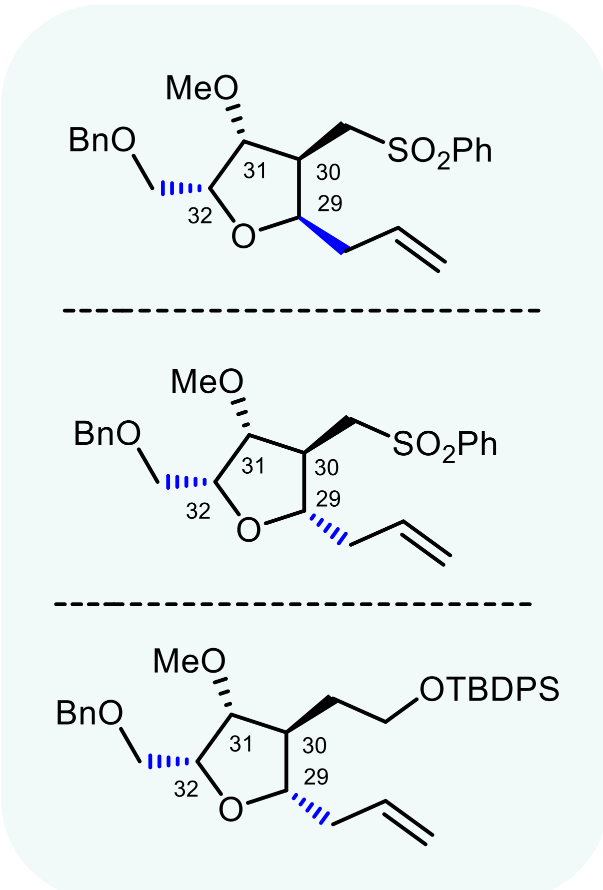


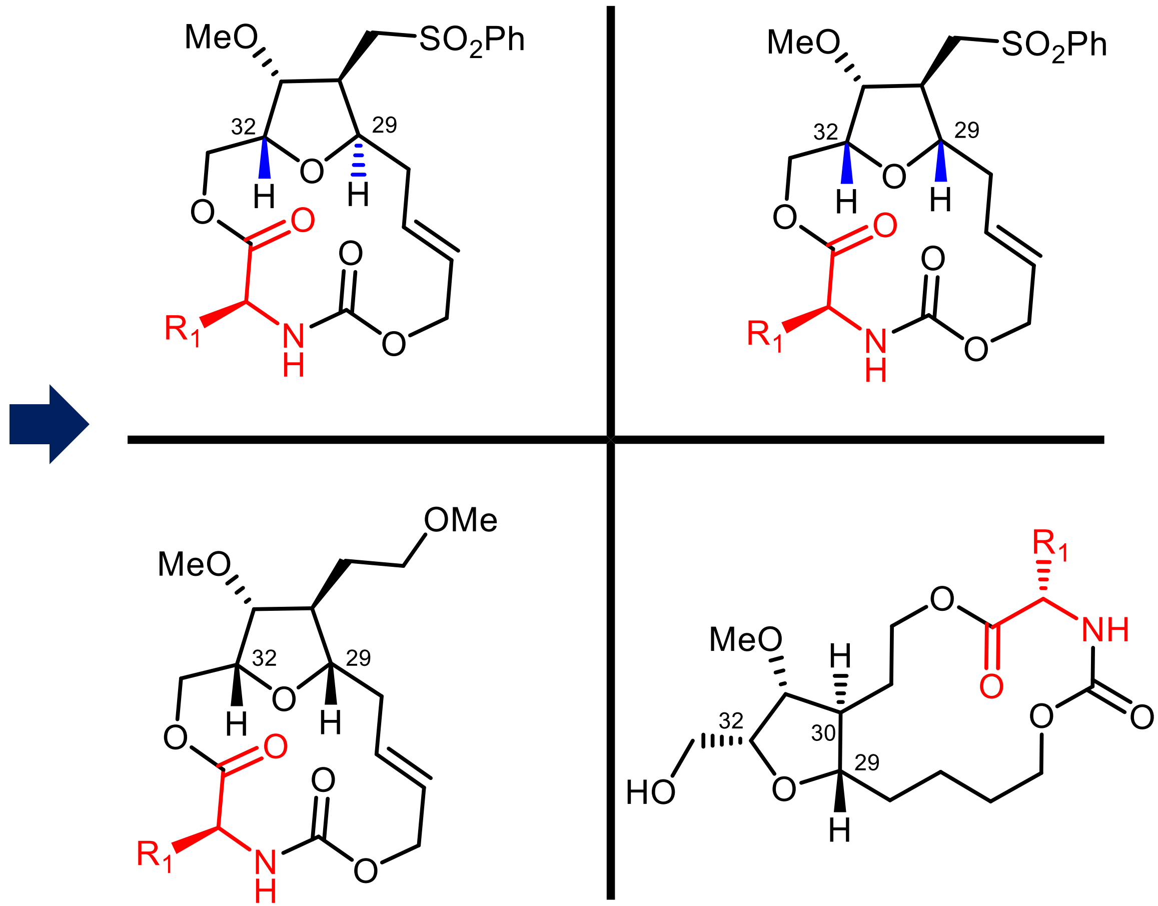






Our Macrocyclic Synthesis Roadmap



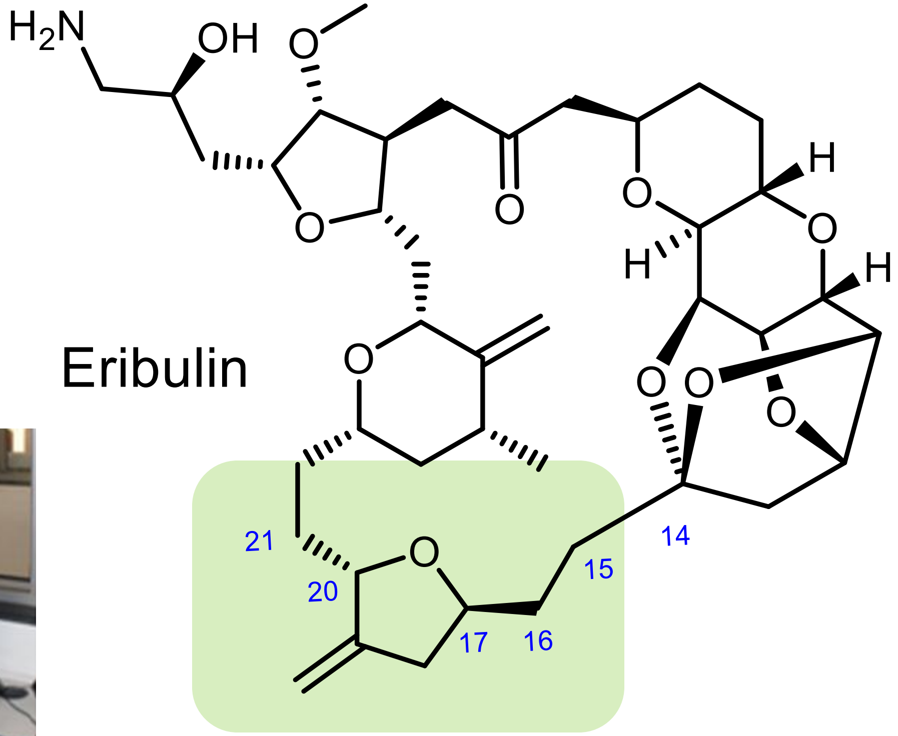


Enantiomerically pure

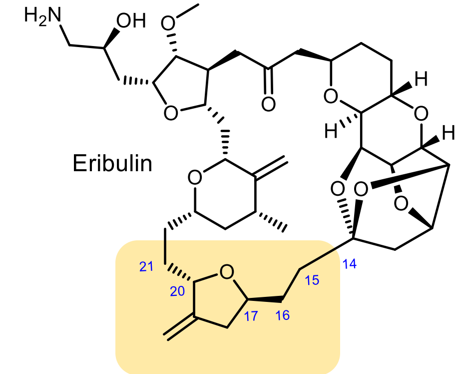
Part 2: Stereoselective Synthesis of C14-C21 Fragment of Eribulin & Derived Macrocyclic Diversity

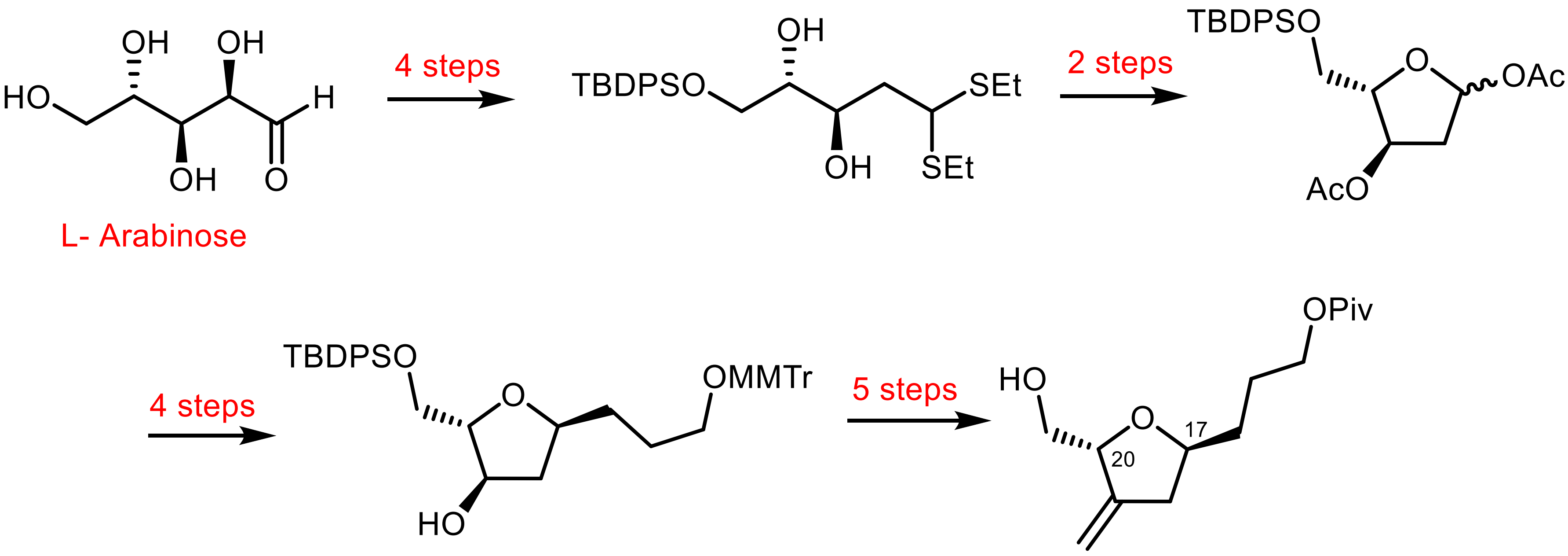
Naveen Kumar

Eribulin



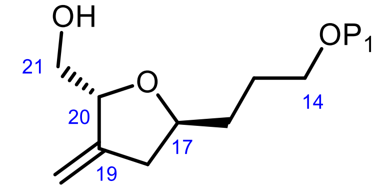
Literature (Kishi Approach)

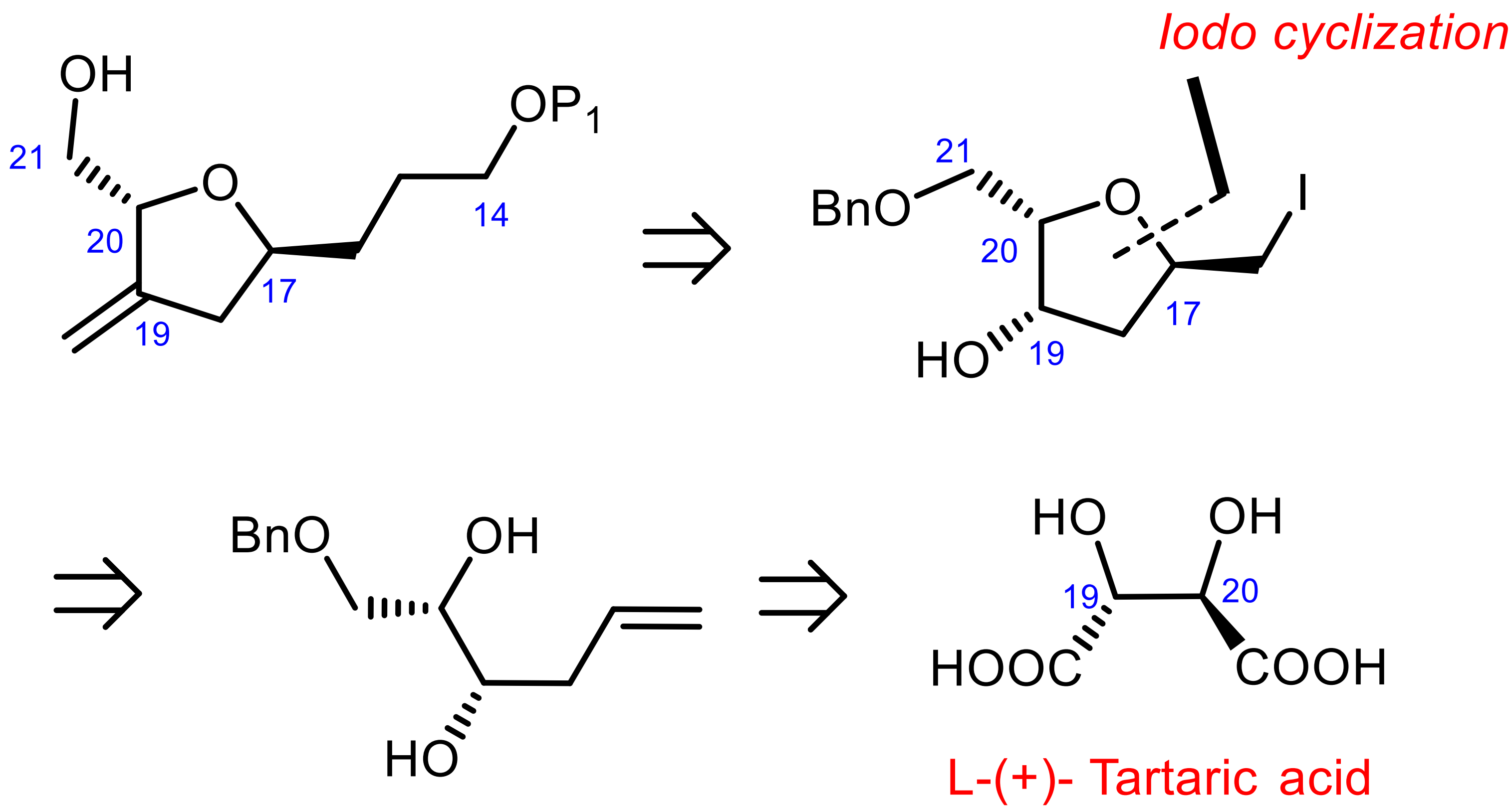


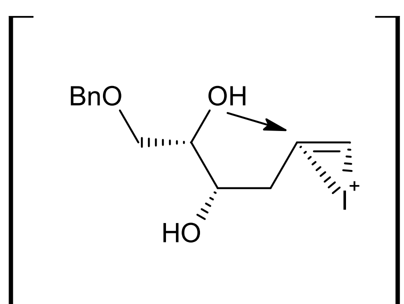


J. Am. Chem. Soc., **1992**, *114*, 3163

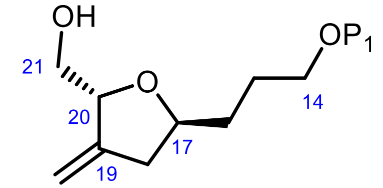
Our Synthetic Planning

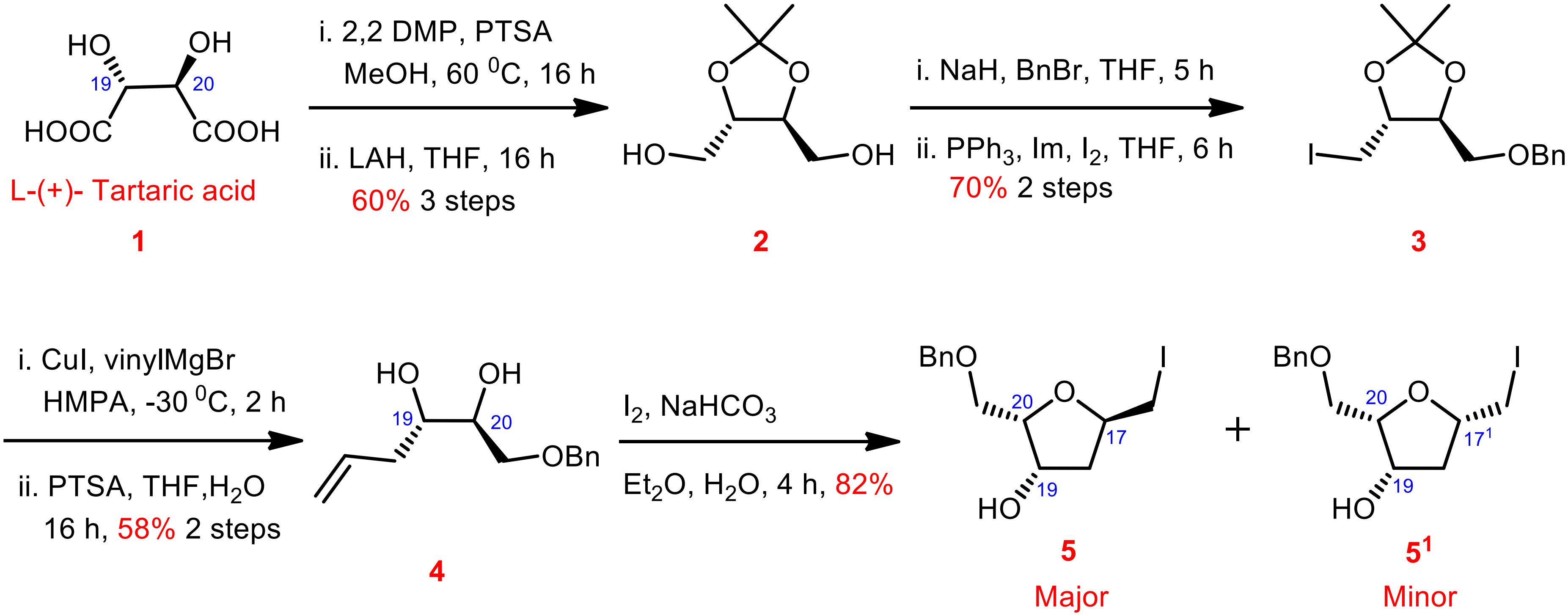






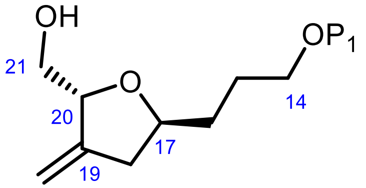
Stereoselective Synthesis of Furan Ring

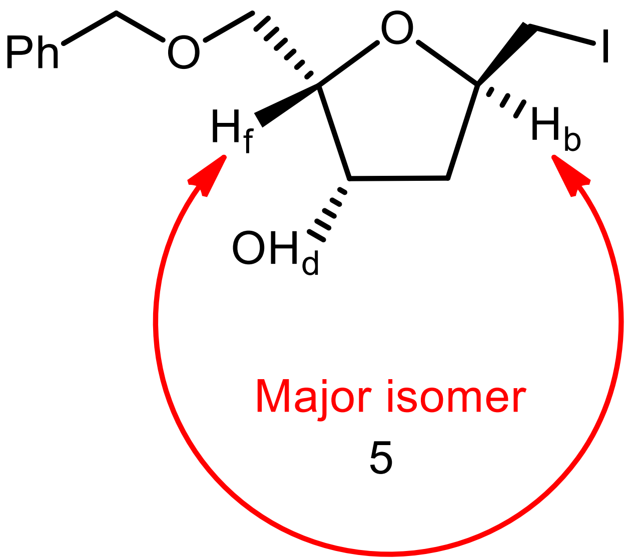




4:1 Separable diastereomers

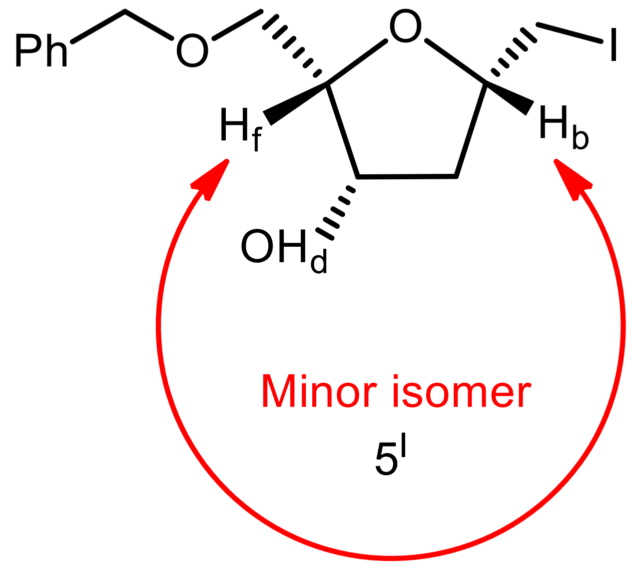
nOe Studies





Major isomer
5

No nOe

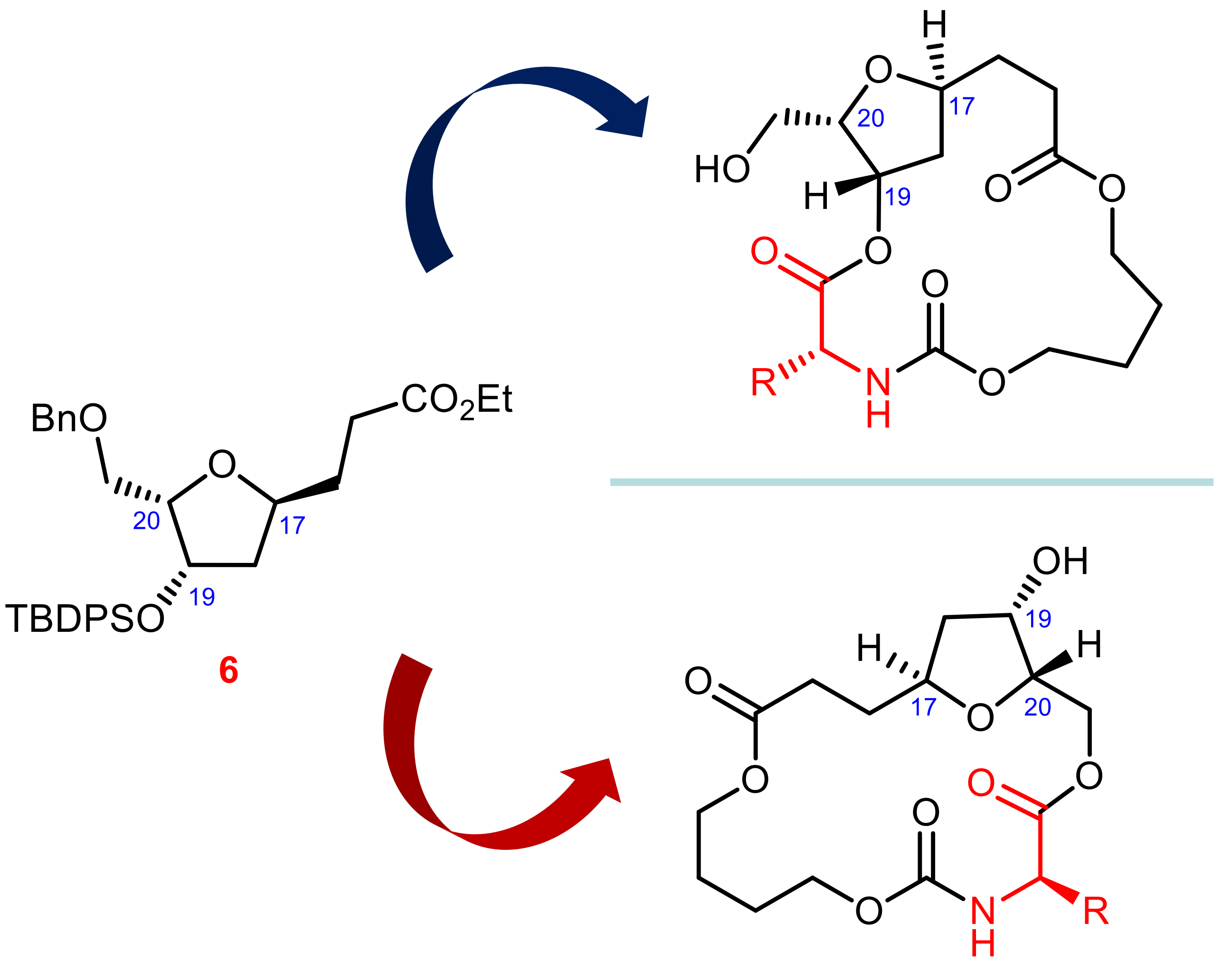


Minor isomer
5I

nOe

Our Plan to Building the Macrocyclic Diversity

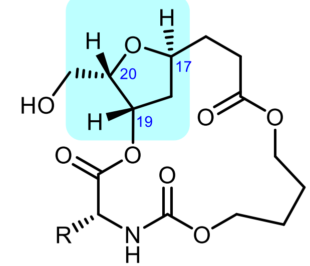
Macrocycle A

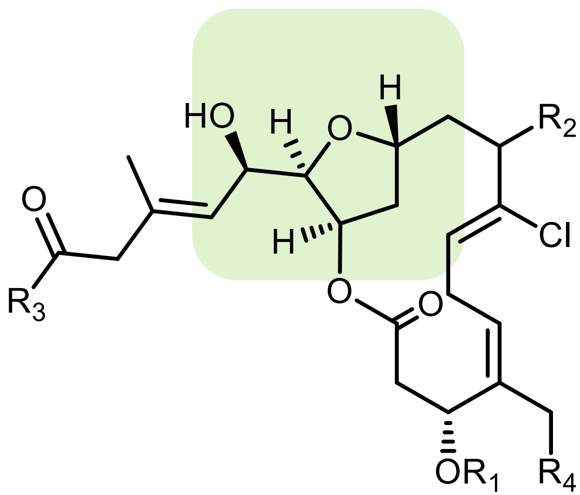


Macrocycle B

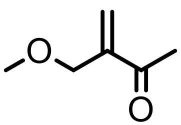
Natural Products with Tetrahydrofuran Ring

Macrocycle A





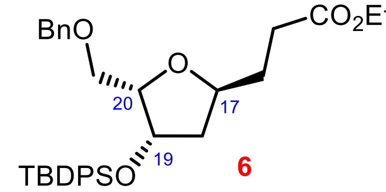
Haterumalides

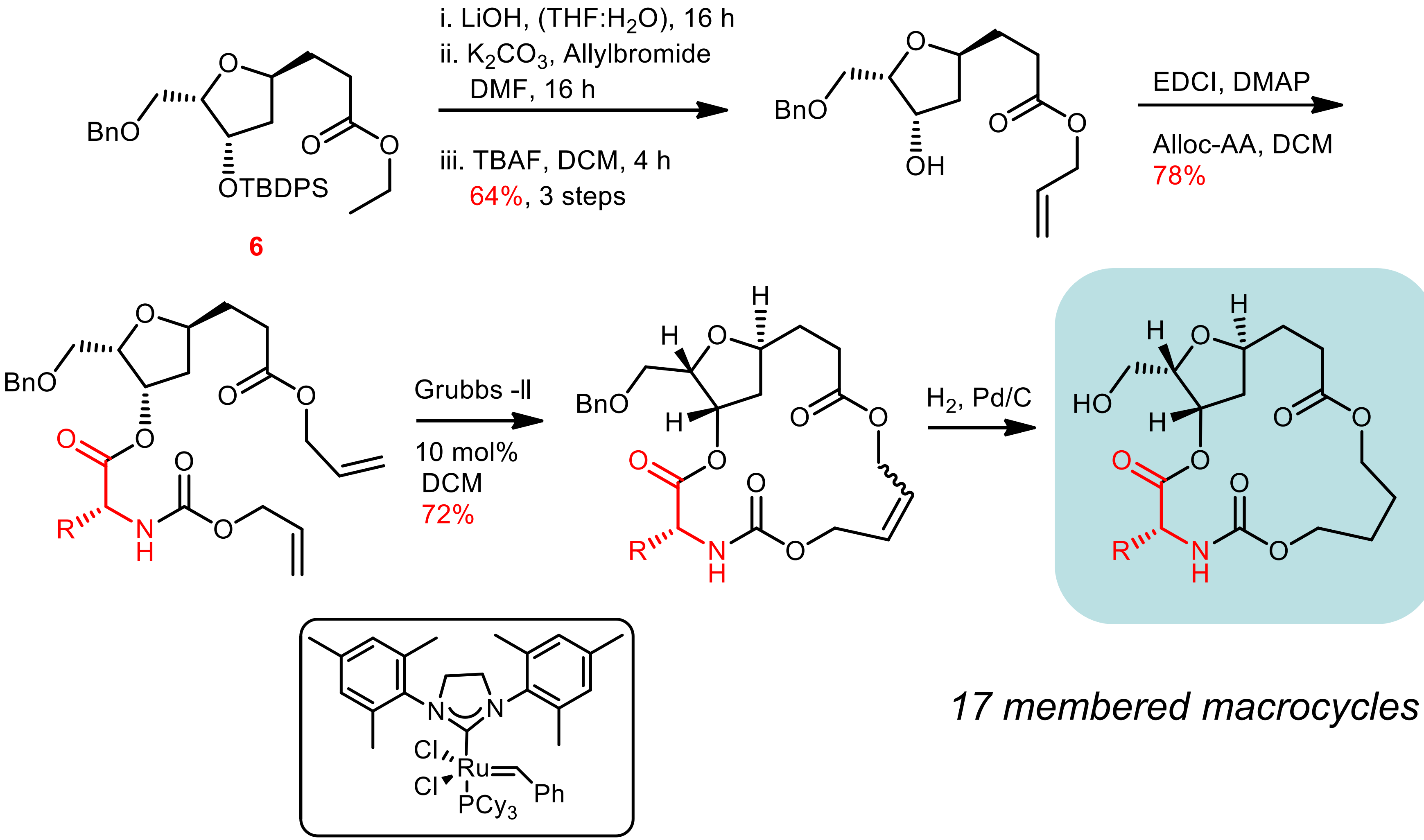
	R_1	R_2	R_3	R_4
Haterumalide NA	Ac	H	OH	H
Haterumalide NB	Ac	H	O-n-Bu	H
Haterumalide NC	Ac	OH	O-n-Bu	H
Haterumalide ND	Ac	OH	OH	H
Haterumalide NE	H	H	OH	H
Haterumalide B	Ac	H		H

- Uemura and coworkers isolated haterumalides in 1999 from the Okinawan sponge *Ircinia* sp.

- Haterumalide NA exhibited cytotoxicity against P388 cells with an IC50 of 0.32 μg/mL and moderate acute toxicity against mice with an LD99 of 0.24 g/kg.

Our Approach to Macrocycle A

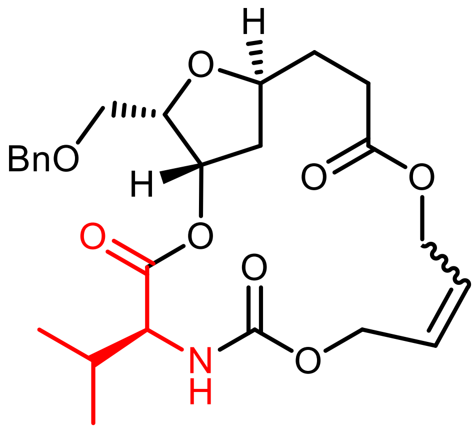


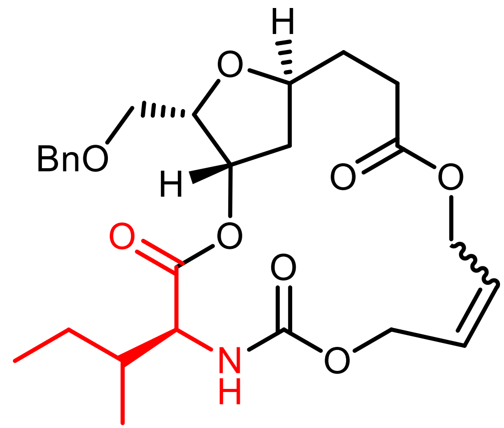


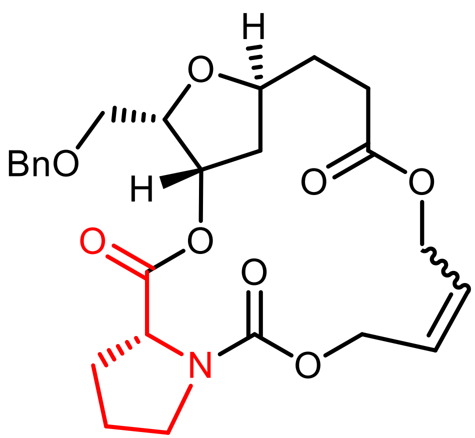
17 membered macrocycles

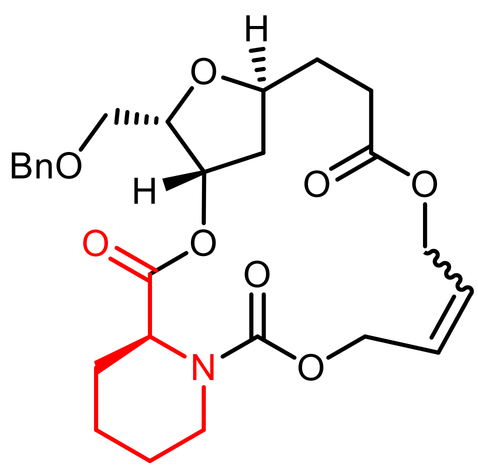
Grubbs 2nd gen. cat.

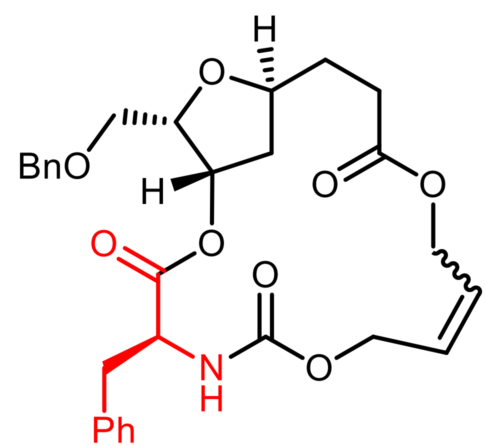
Synthesis of Different Macrocycles Achieved





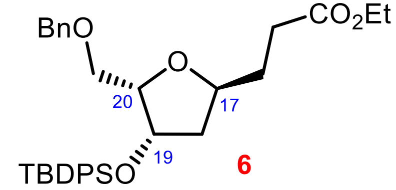




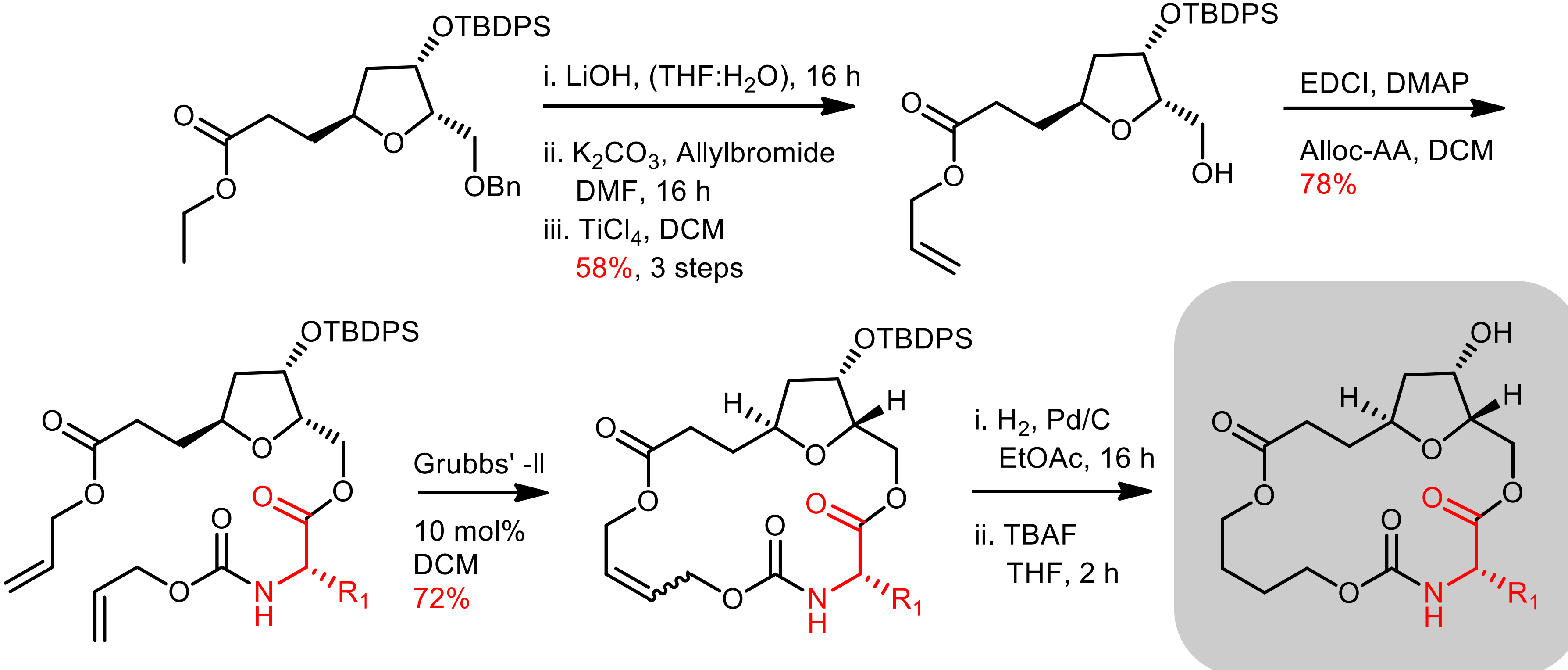


17 membered macrocycles

Our Synthesis Execution for Macrocycle B

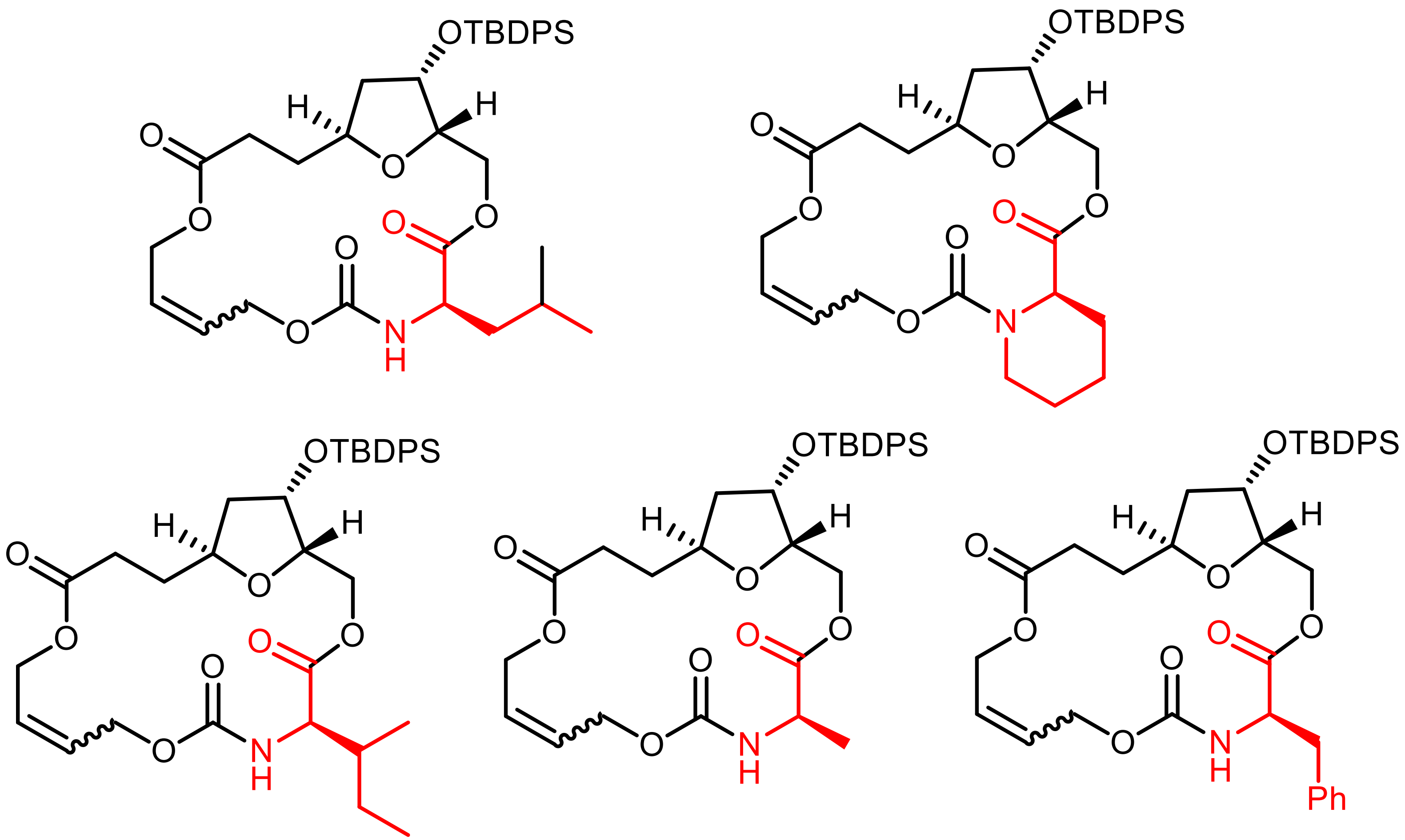


6



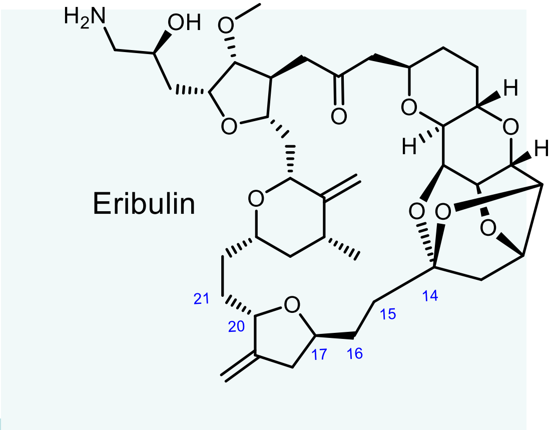
18 membered macrocycles

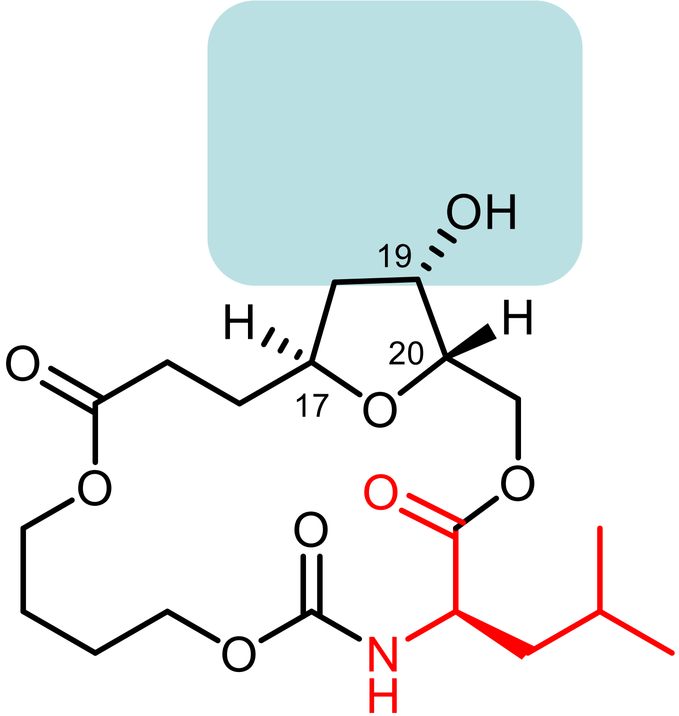
Synthesis of Different Macrocycles Achieved

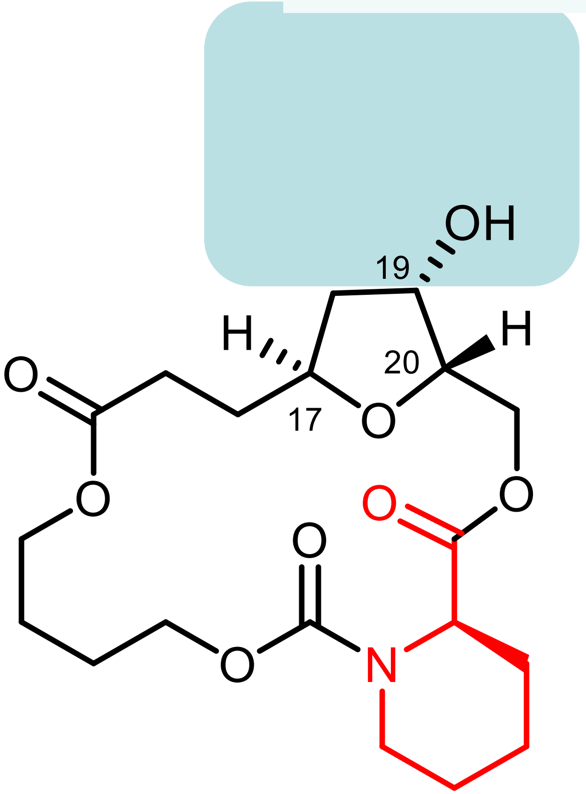


18 membered macrocycles

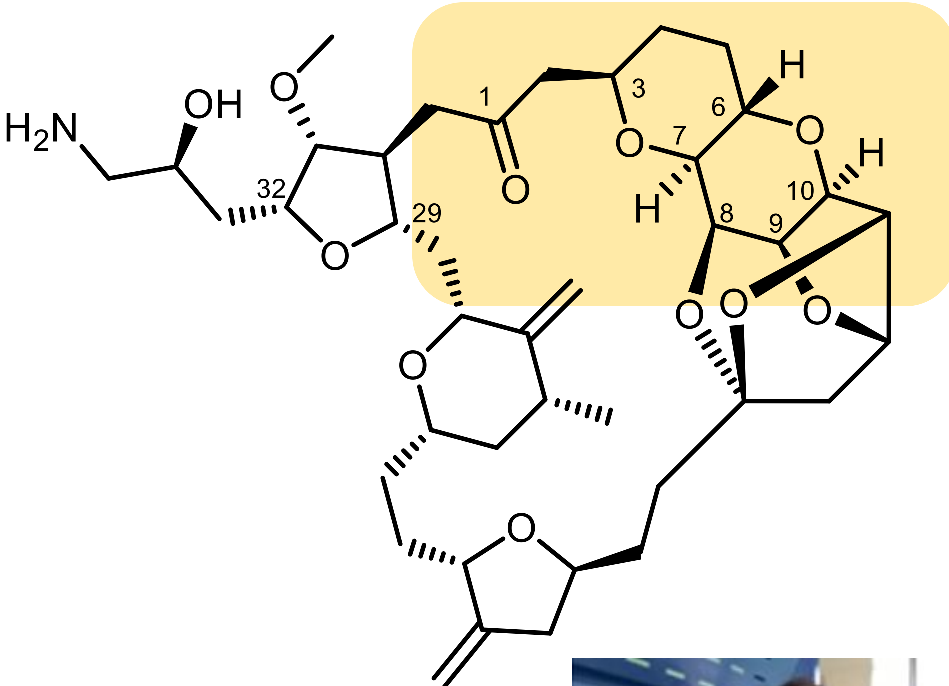
Synthesis of Fully De-protected Macrocycles Achieved



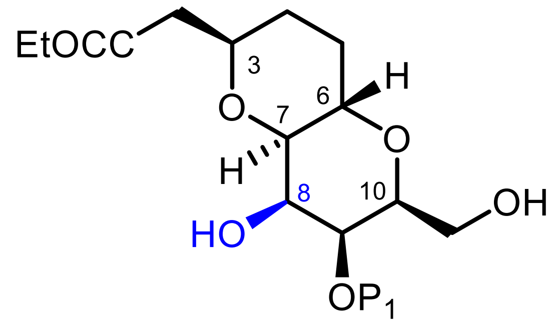




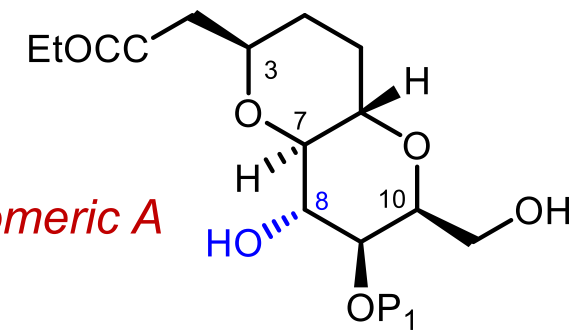
Part 3: Another Eribulin Fragment



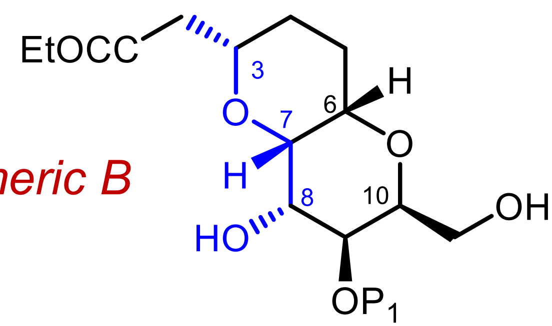
Precise



Isomeric A



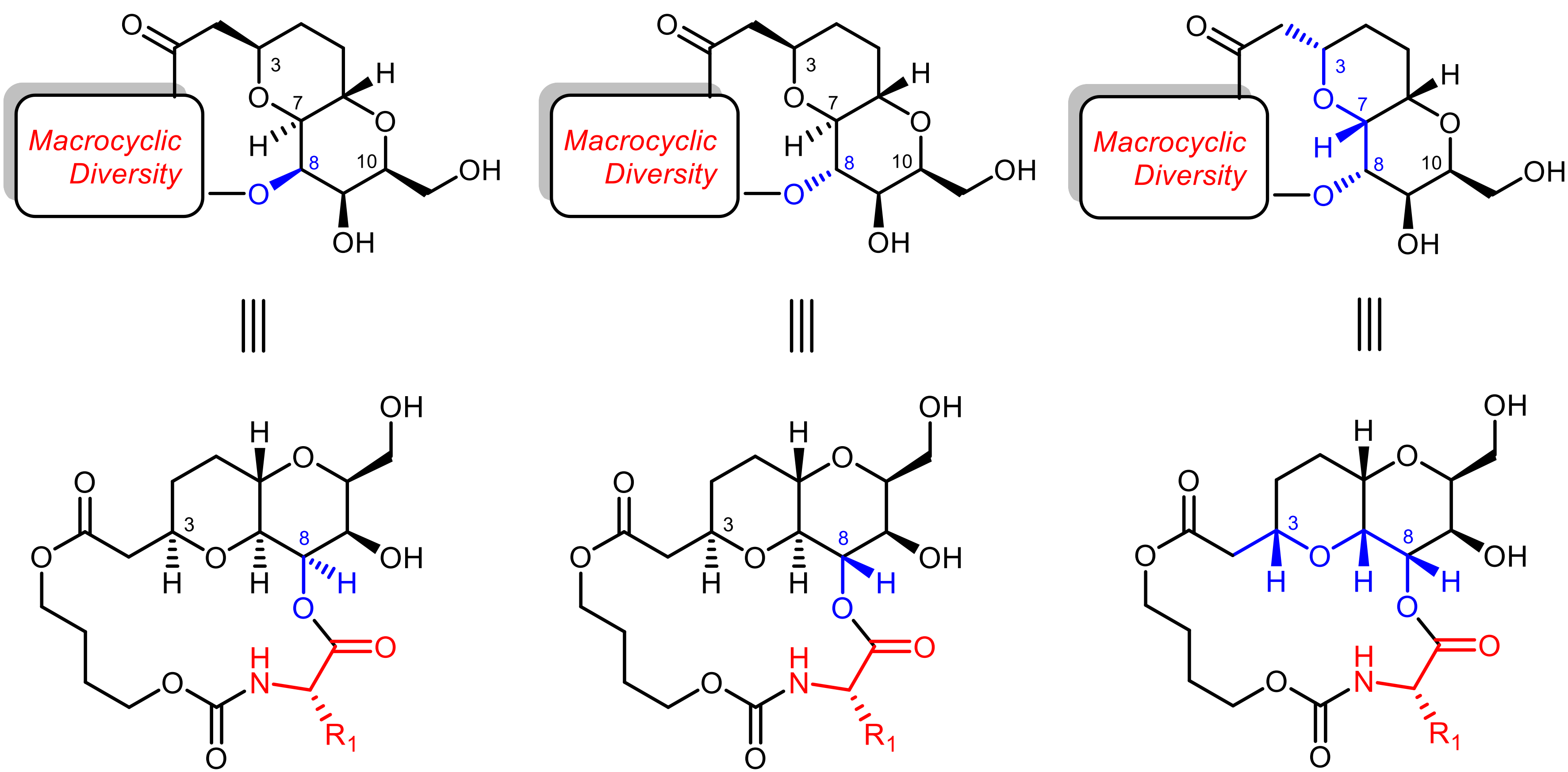
Isomeric B



Mahender Khatravath

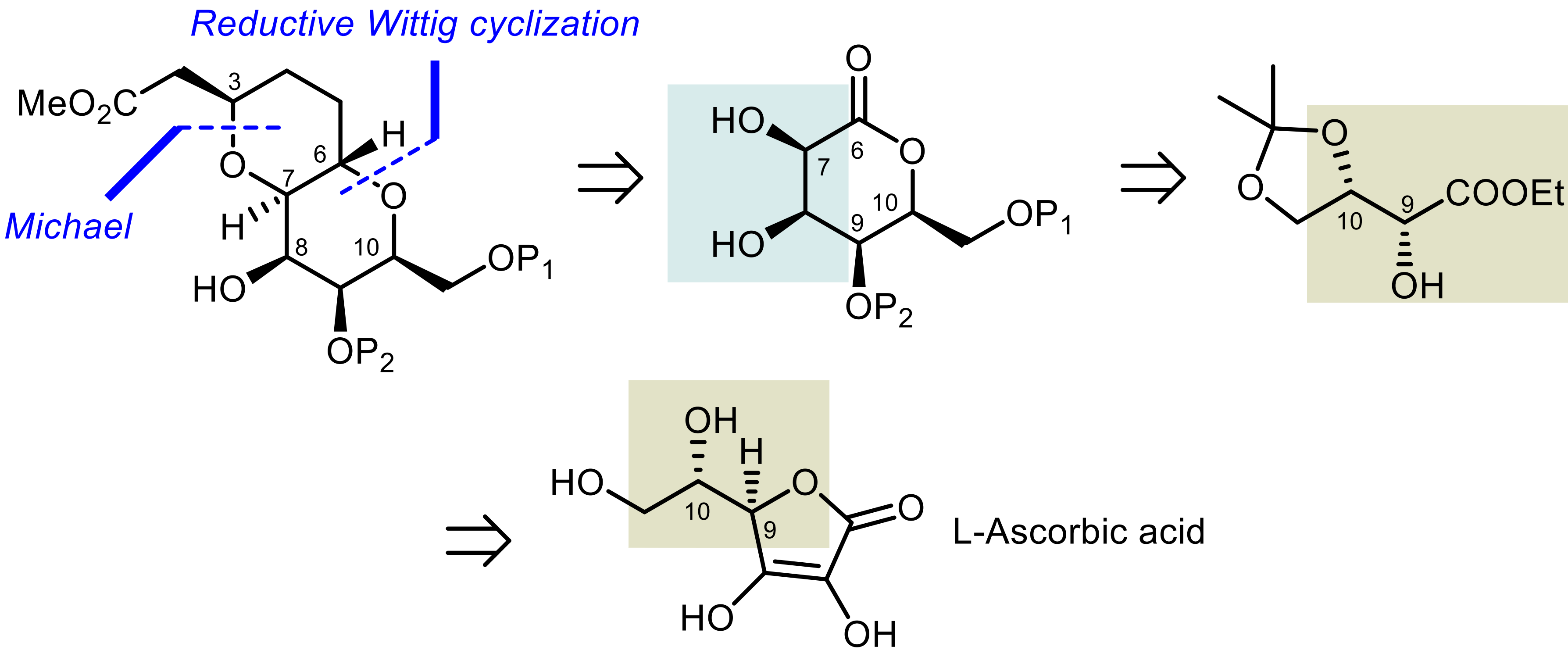
Macrocyclic Diversity Synthesis Plan

Precise | Isomeric A | Isomeric B



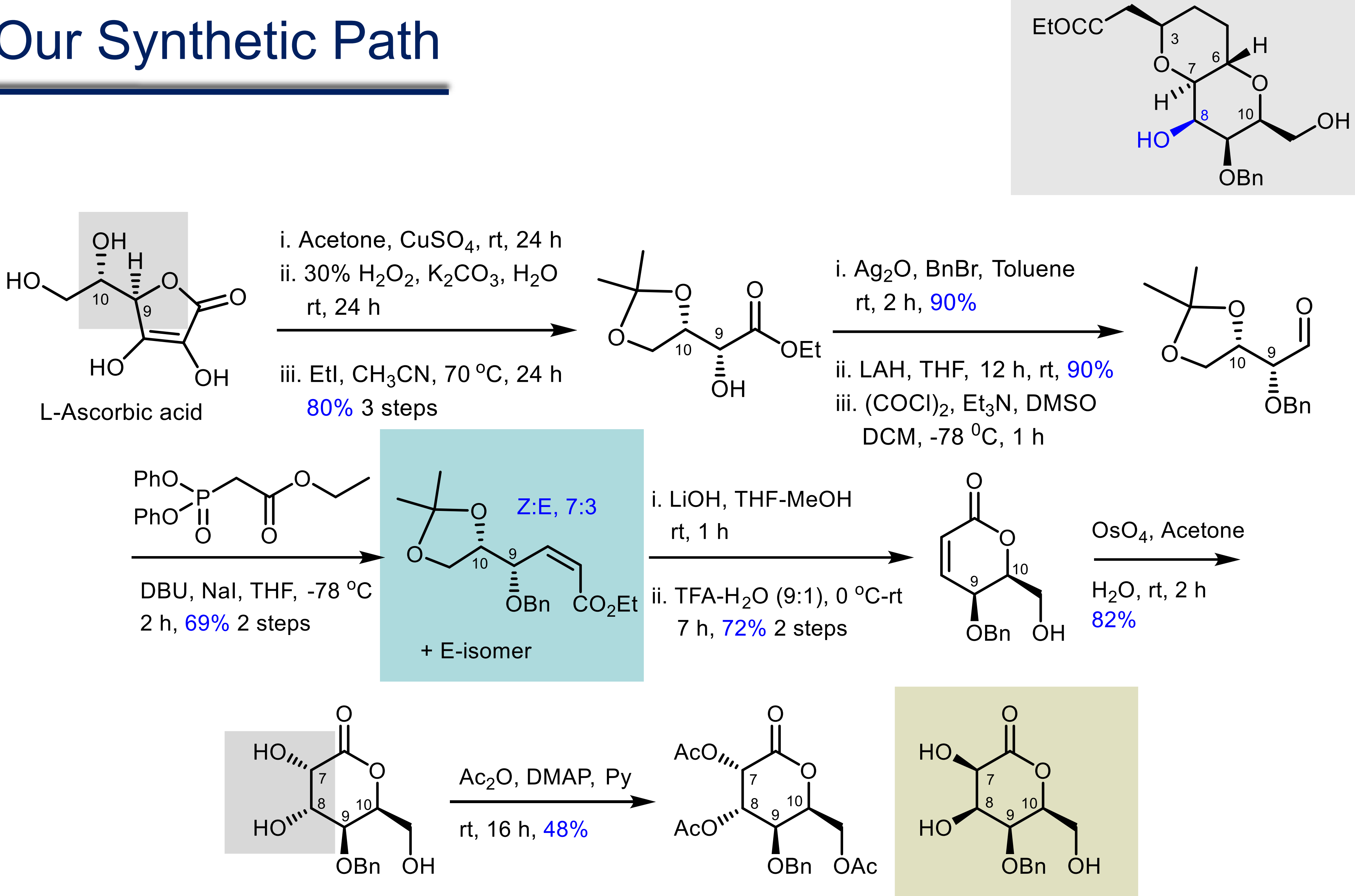
17 Membered Macrocycles

Our Synthetic Planning

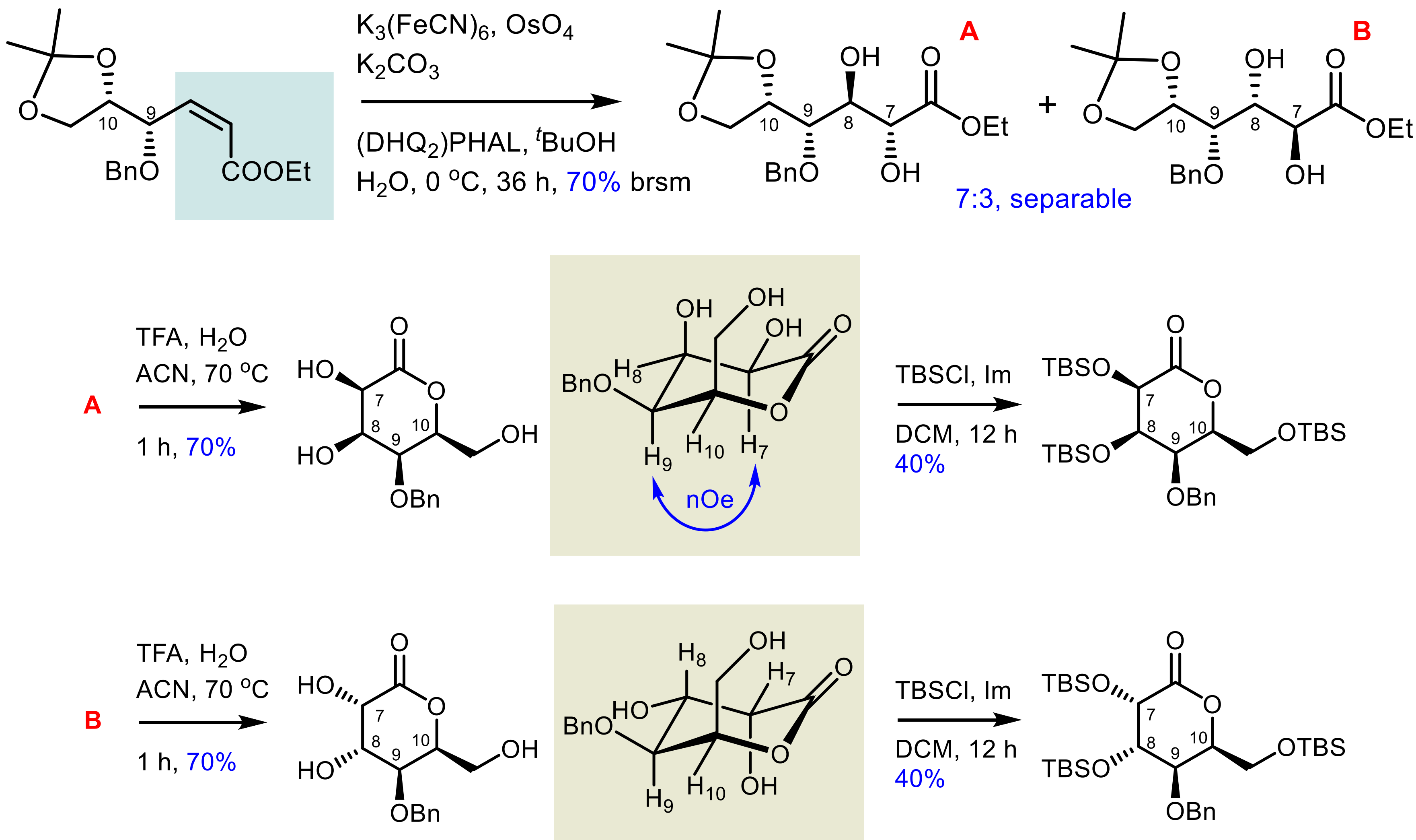


L-Ascorbic acid

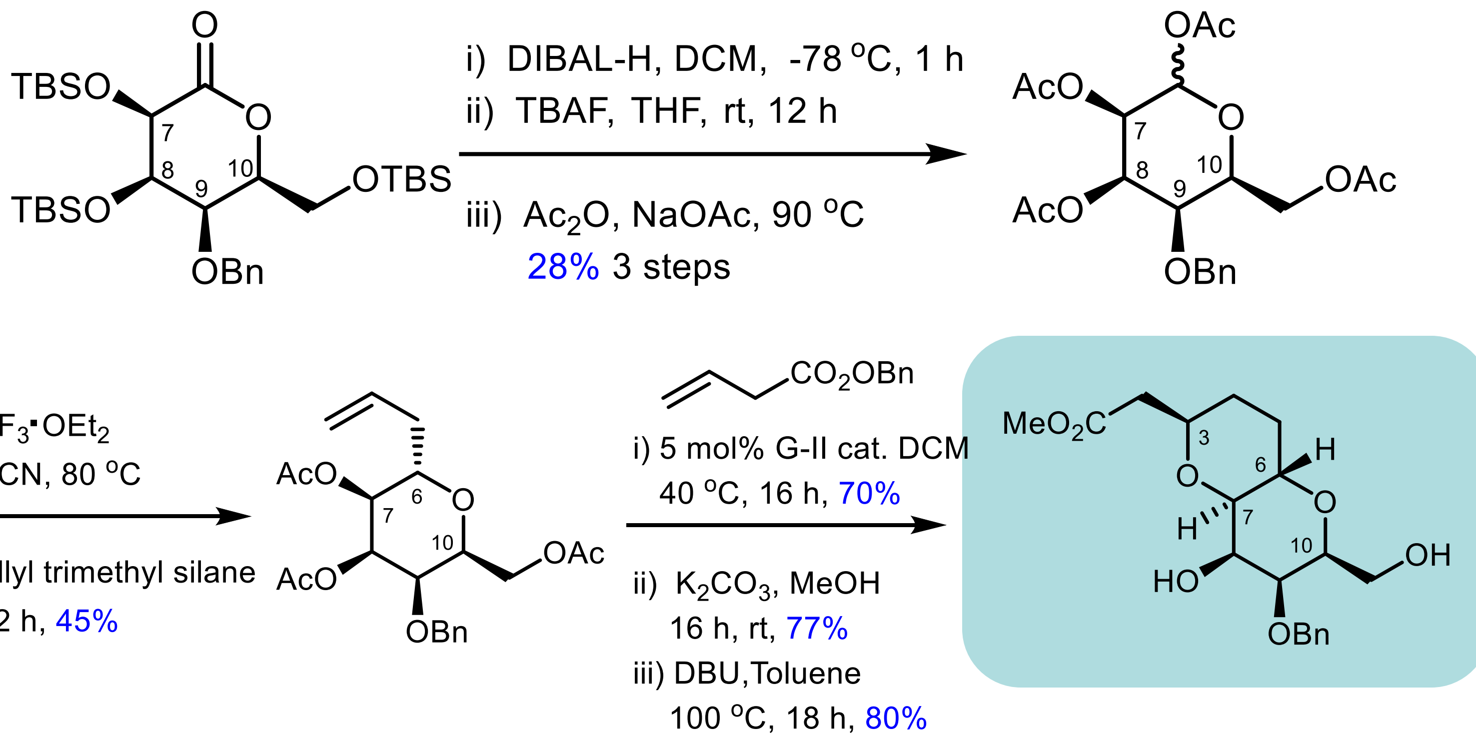
Our Synthetic Path

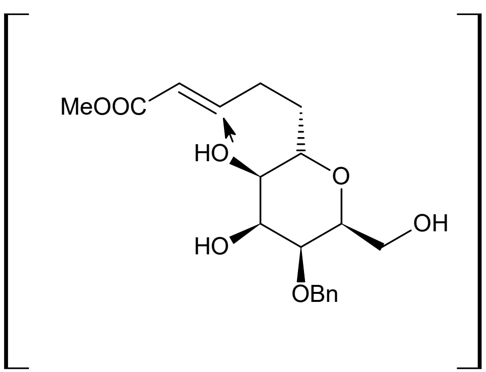


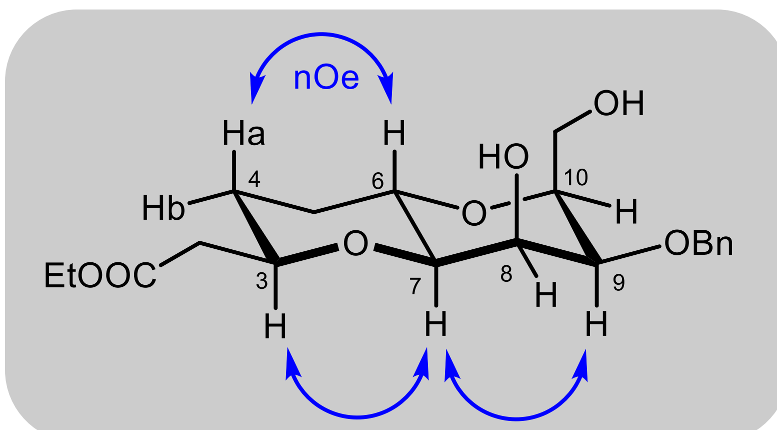
Deviation in Our Approach



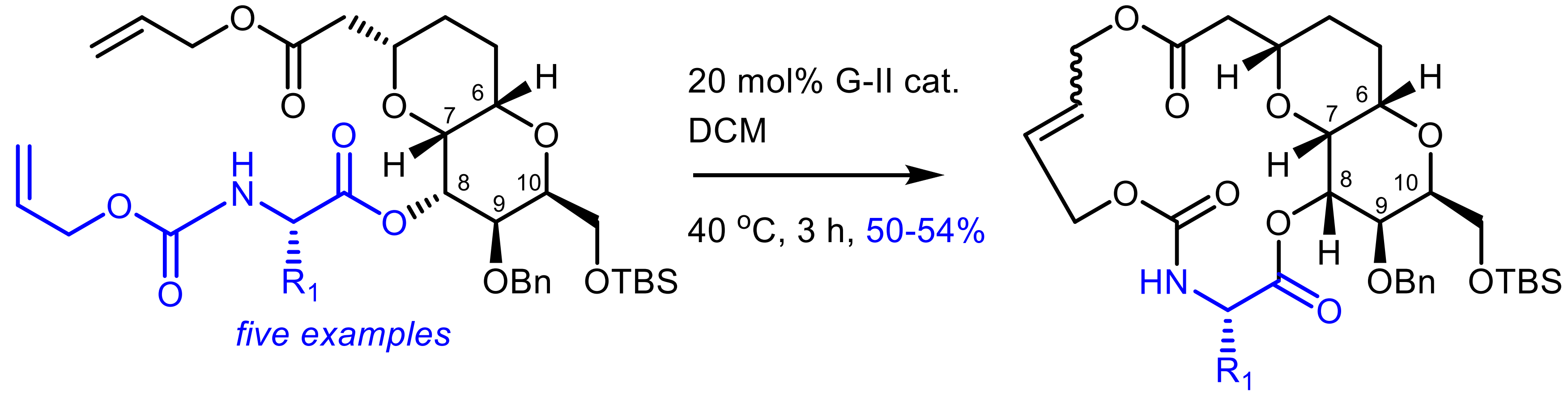
Stereocontrolled Michael!





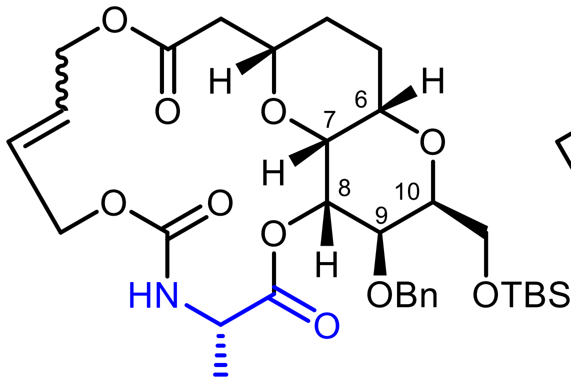


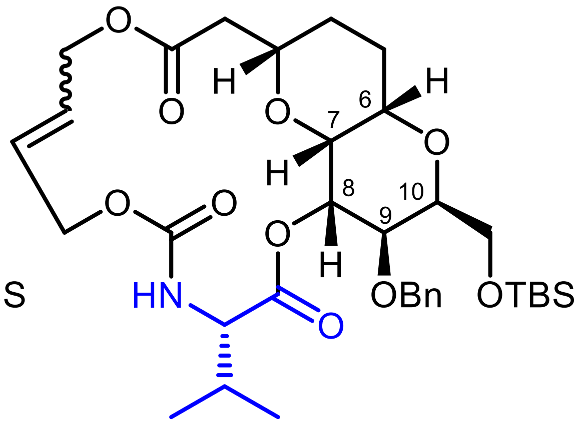
Macrocyclic Diversity- An Example

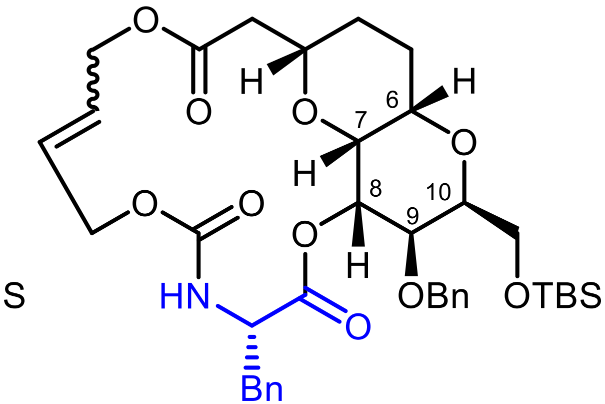


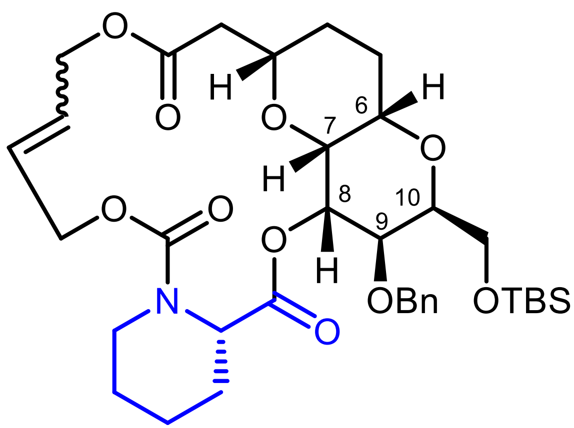
single olefin geometry
- not assigned yet

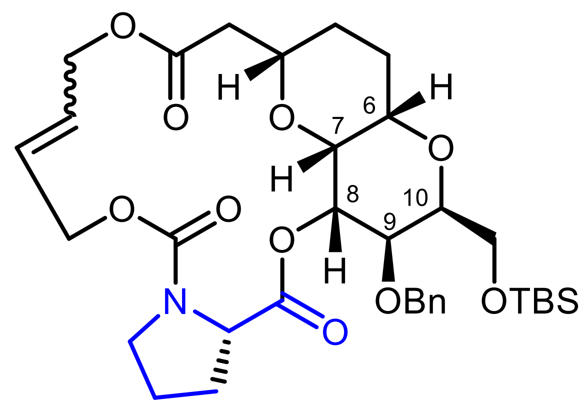
Macrocyclic Diversity- An Example



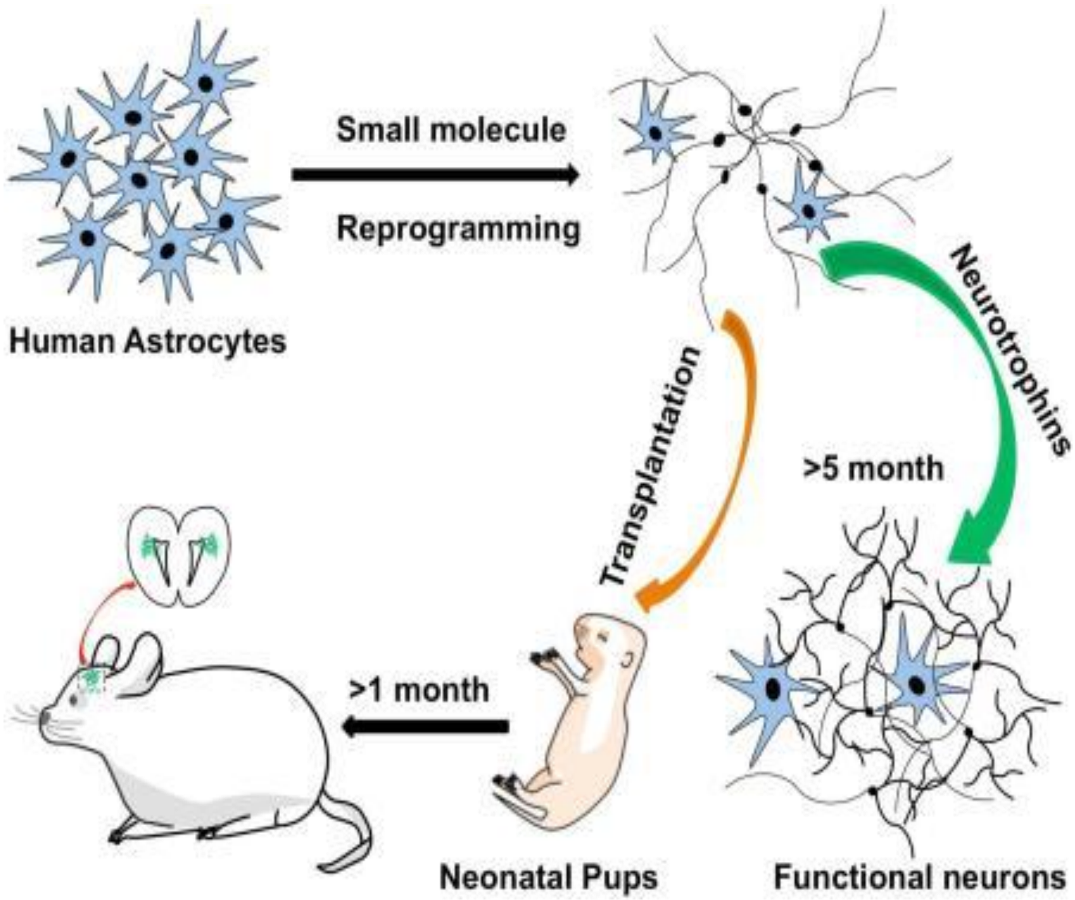


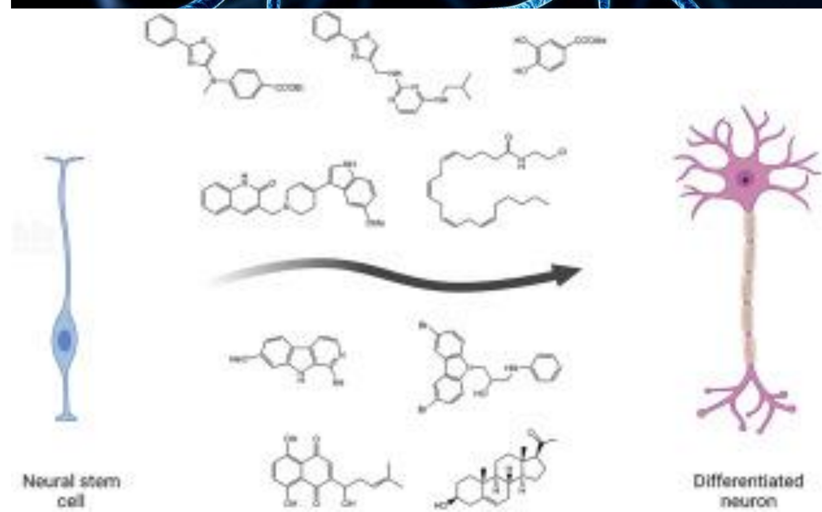




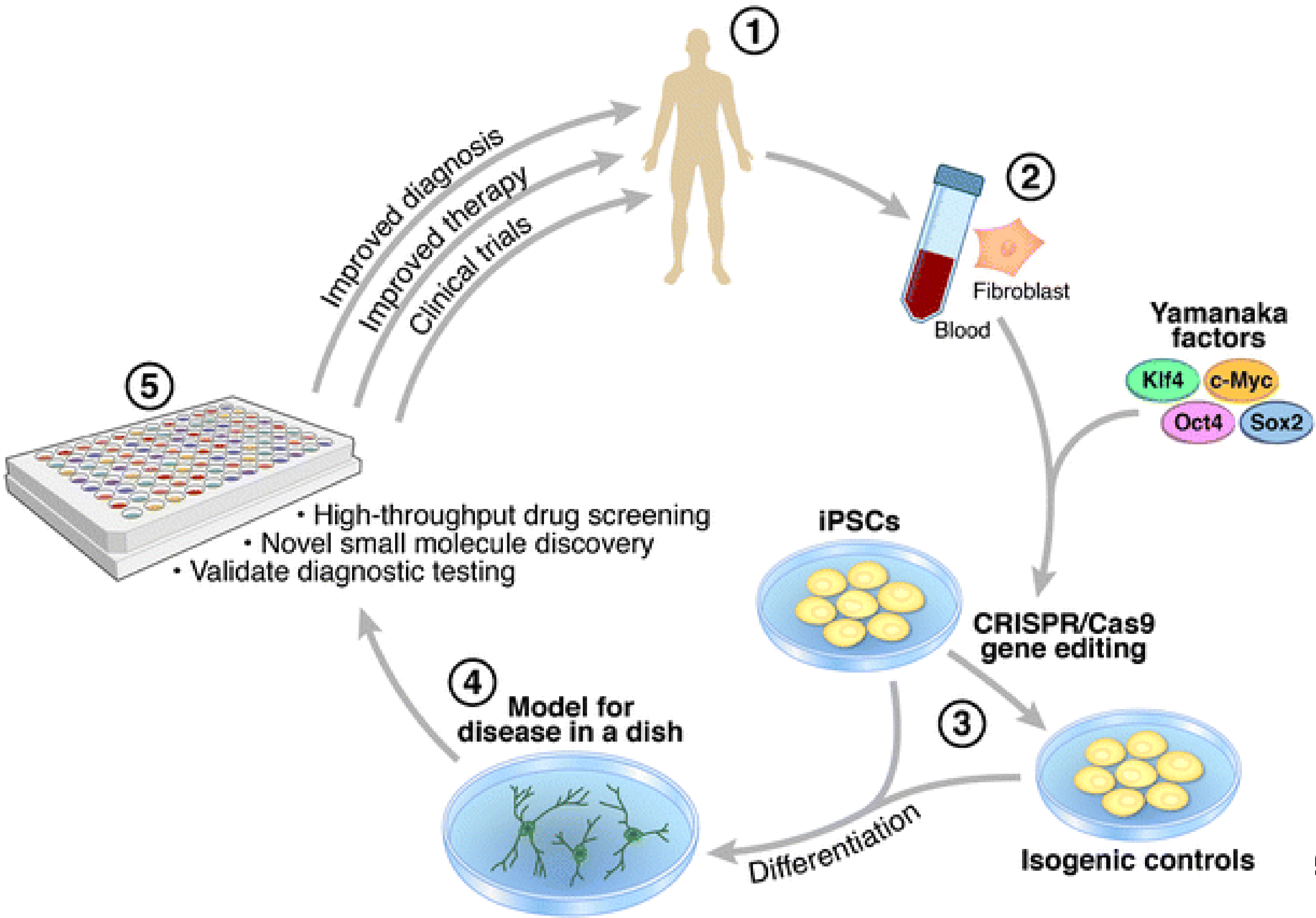


Neural Differentiation with Small Molecules

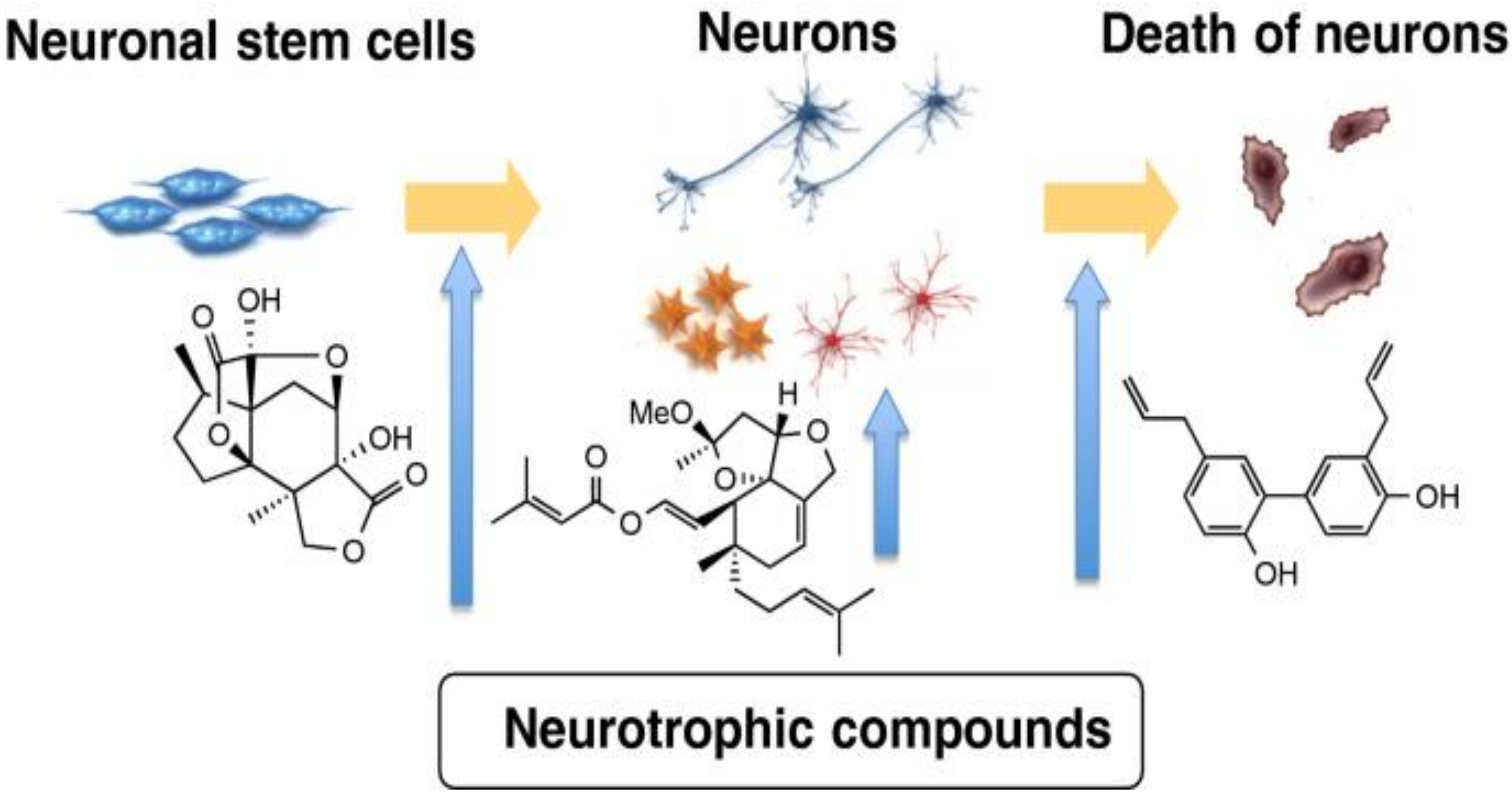




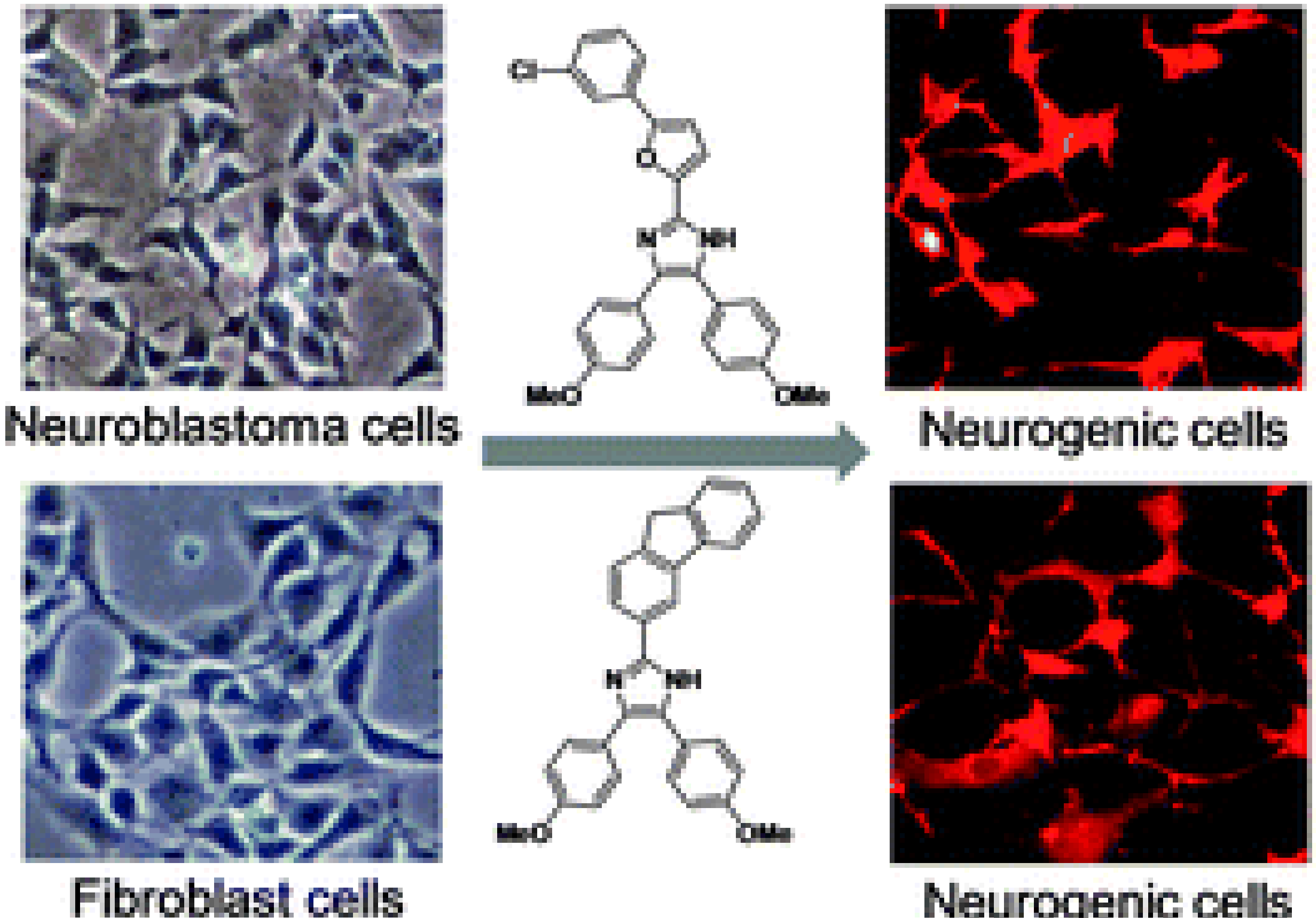
The regenerative repair of neurons in acute injuries and neurodegeneration diseases



Reprogramming cell fates by small molecules

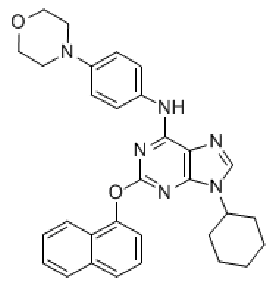


Synthetic small molecules that induce neuronal differentiation in neuroblastoma and fibroblast cells, *Mol. BioSyst.*, 2015,**11**, 2727-2737

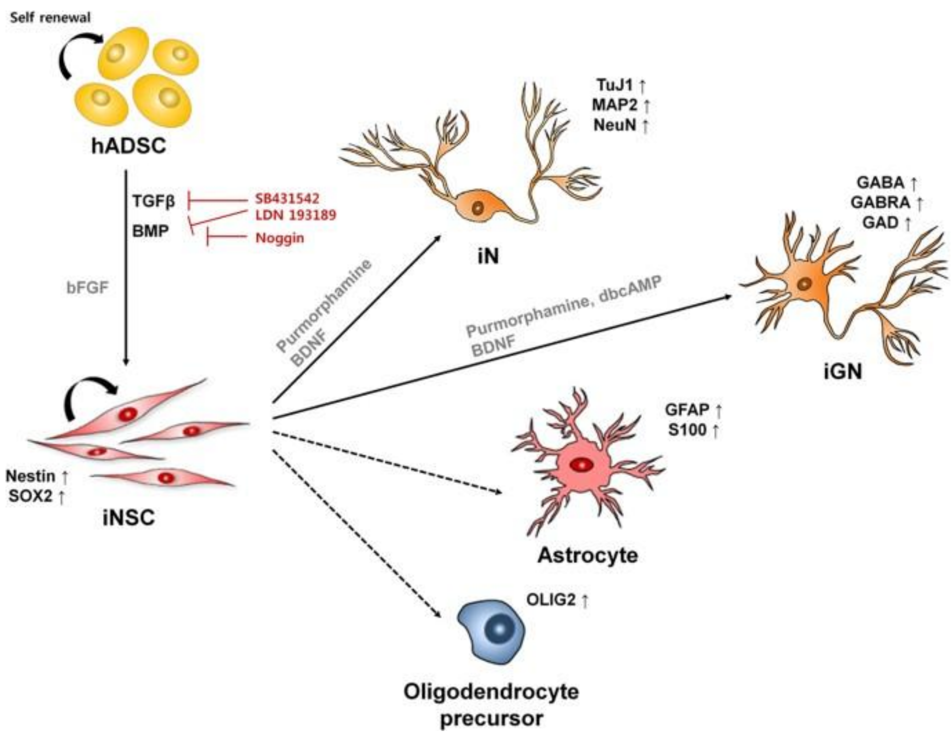


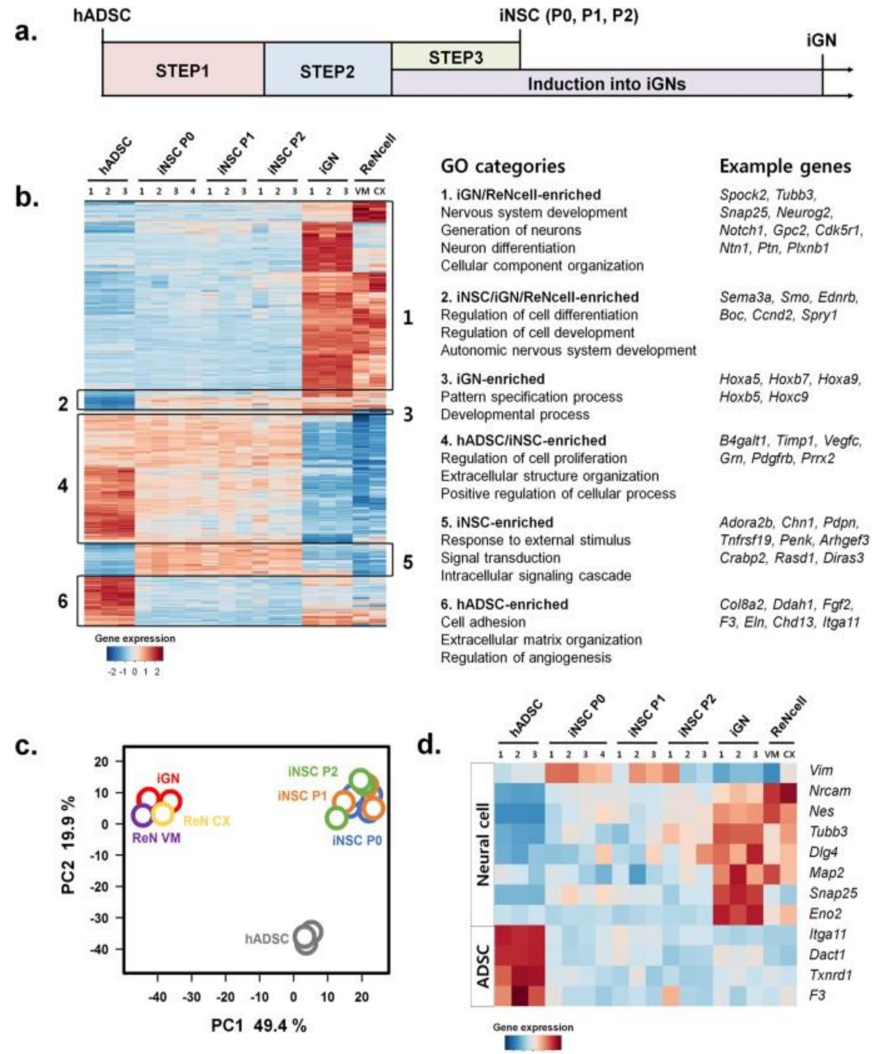
Small molecule-based lineage switch of human adipose-derived stem cells (hADSC) into neural stem cells and functional GABAergic neurons (iGN , iN)

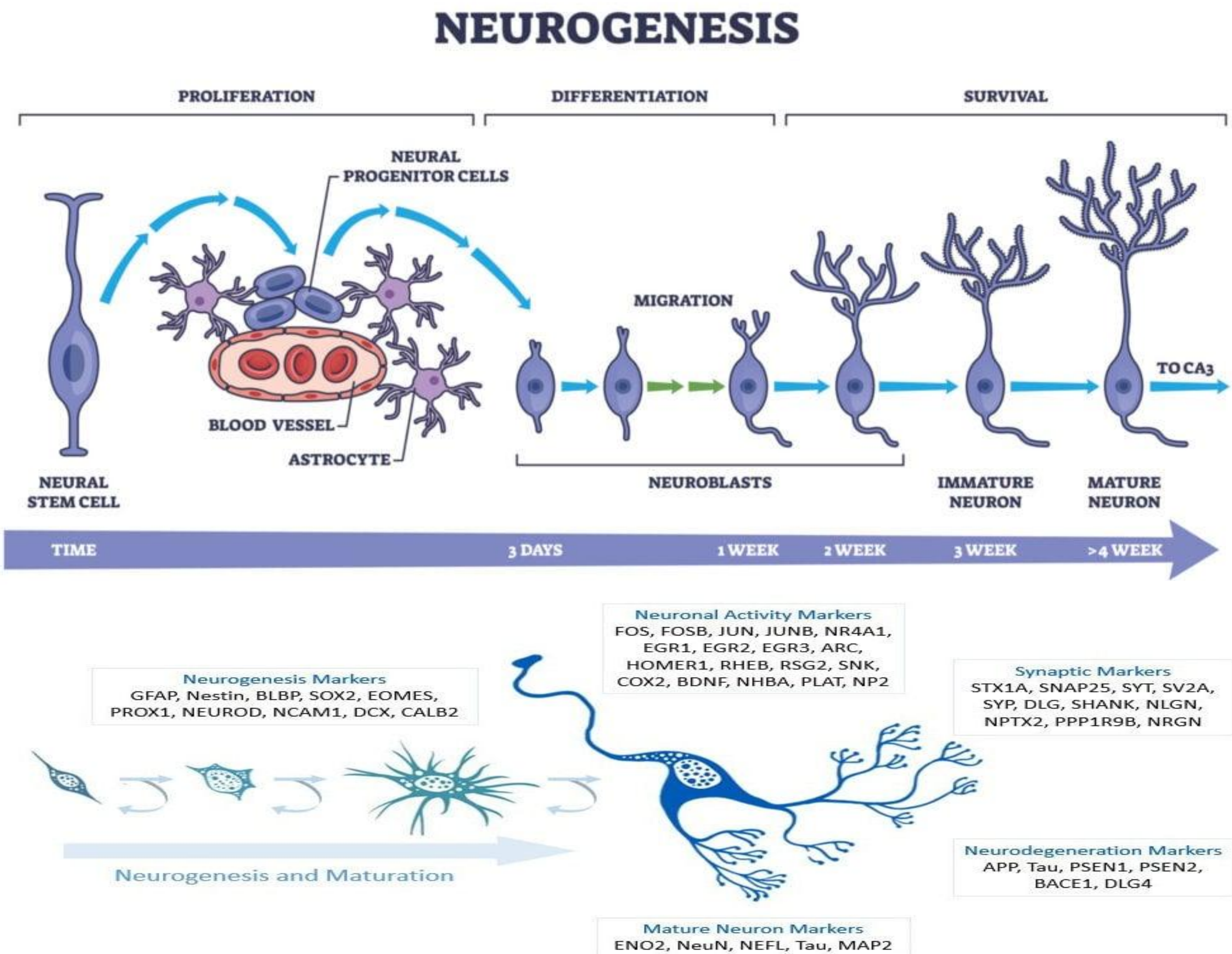
Scientific Reports **volume 7**, Article number: 10166 (2017)



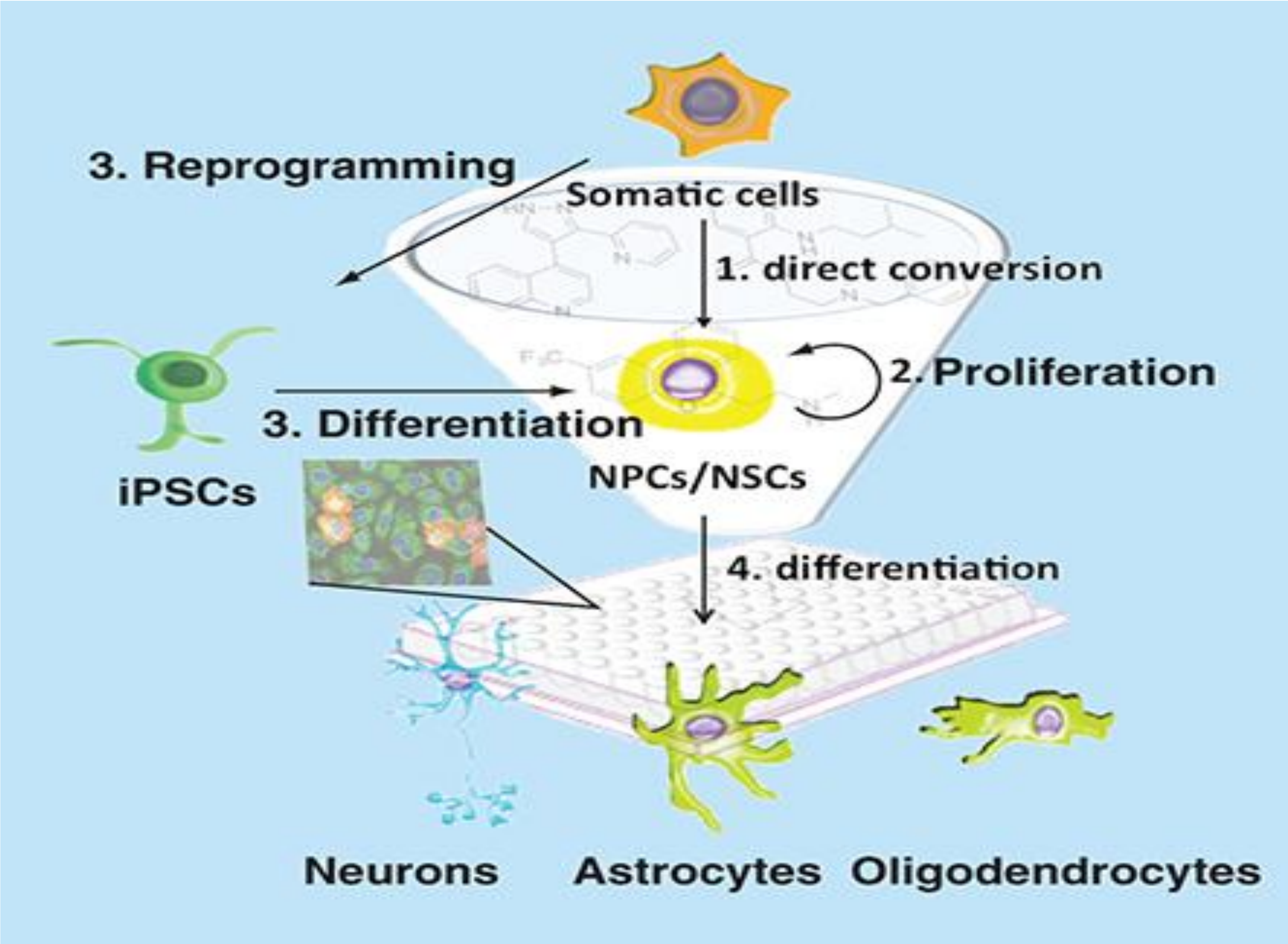
Purmorphamine






NEUROGENESIS
PROLIFERATION
DIFFERENTIATION
SURVIVAL
NEURAL PROGENITOR CELLS
MIGRATION
TO CA3
BLOOD VESSEL
ASTROCYTE
NEURAL STEM CELL
NEUROBLASTS
IMMATURE NEURON
MATURE NEURON
TIME
3 DAYS
1 WEEK
2 WEEK
3 WEEK
>4 WEEK
Neuronal Activity Markers
FOS, FOSB, JUN, JUNB, NR4A1, EGR1, EGR2, EGR3, ARC, HOMER1, RHEB, RSG2, SNK, COX2, BDNF, NHBA, PLAT, NP2
Neurogenesis Markers
GFAP, Nestin, BLBP, SOX2, EOMES, PROX1, NEUROD, NCAM1, DCX, CALB2
Synaptic Markers
STX1A, SNAP25, SYT, SV2A, SYP, DLG, SHANK, NLGN, NPTX2, PPP1R9B, NRGN
Neurodegeneration Markers
APP, Tau, PSEN1, PSEN2, BACE1, DLG4
Neurogenesis and Maturation
Mature Neuron Markers
ENO2, NeuN, NEFL, Tau, MAP2

Our Studies



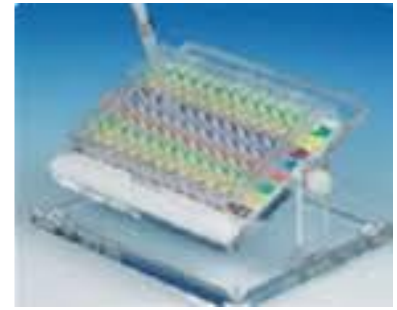
Somatic cells
3. Reprogramming
1. direct conversion
2. Proliferation
iPSCs
3. Differentiation
NPCs/NSCs
4. differentiation
Neurons
Astrocytes
Oligodendrocytes

Chemical Approaches to Trans-differentiation of Human Mesenchymal Stem Cells (from Healthy Donors) to Neurons ; Our Approach

Mesenchymal Stem Cells seeded onto a 60 mm plate

(~3 million cells/well)

The cells are allowed to reach ~80-90% confluency after which corresponding media is added to each well



Medium in wells is changed every 72 hr

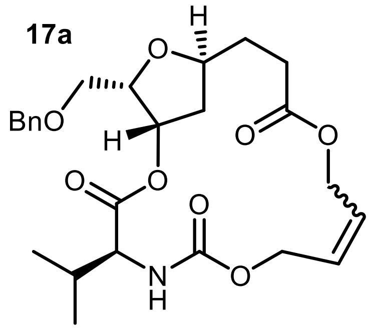
Towards the end of the induction, the cells are scraped and saved for further analysis and characterization

Cells grown in different growth media are used for RT-PCR analysis to determine the expression levels of lineage specific genes

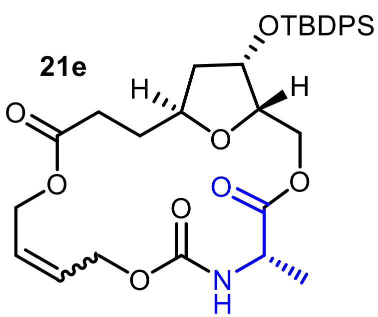
Adopted from Transcell's IP:
PCT/IB2014/000226
WO/2014/ 135950 PCT

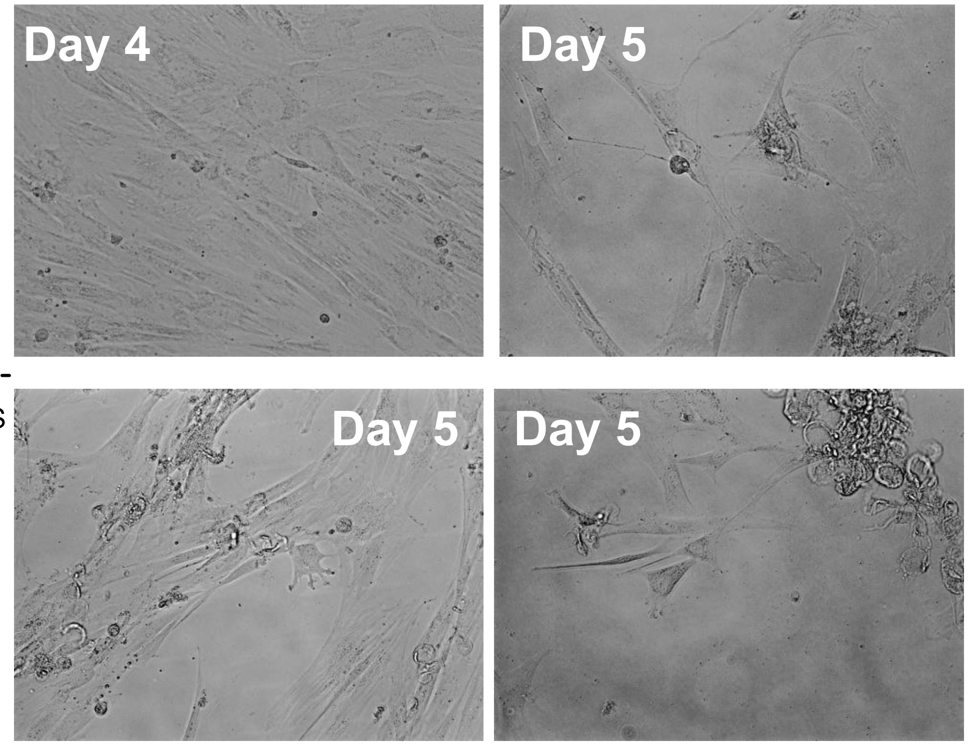
Small Molecule-directed Differentiation of Stem Cells to Neuron-like Cells

17a

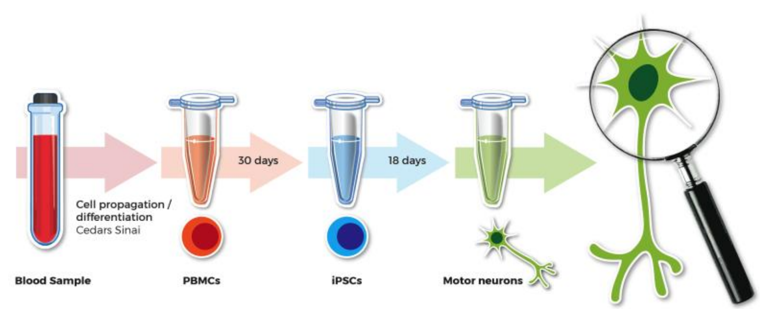


21e





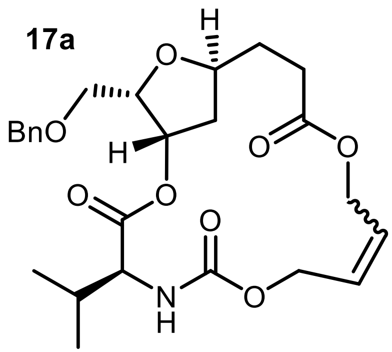
iPS cells are stem cells created from a patient's own cells, such as skin or blood.

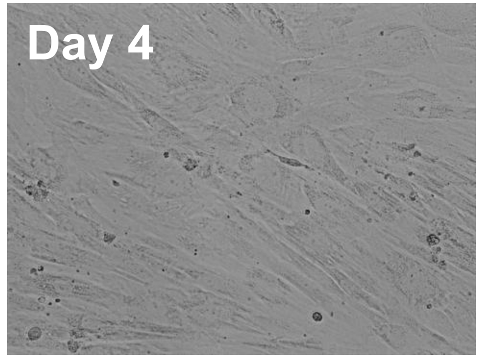


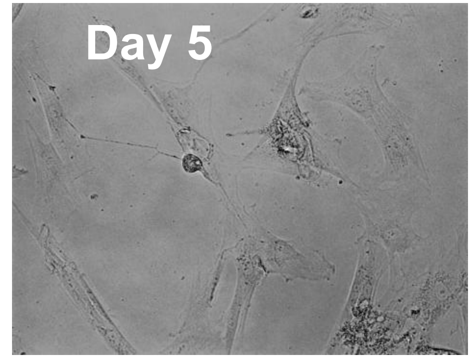
August 16, 2025

Small molecule induced trans-differentiation of human stem cells to neuron-like cells

17a



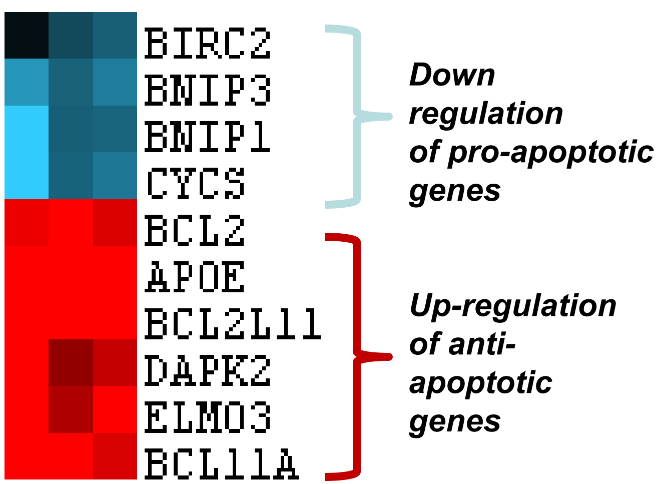


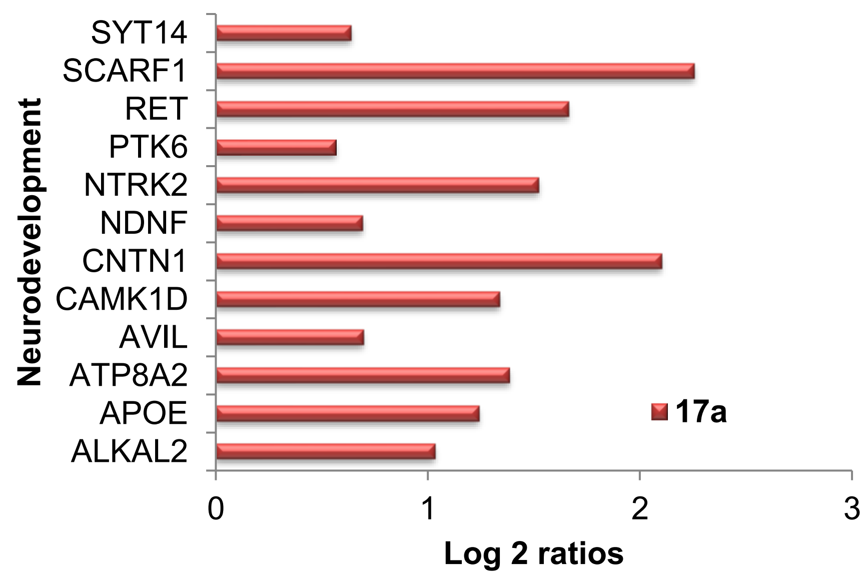


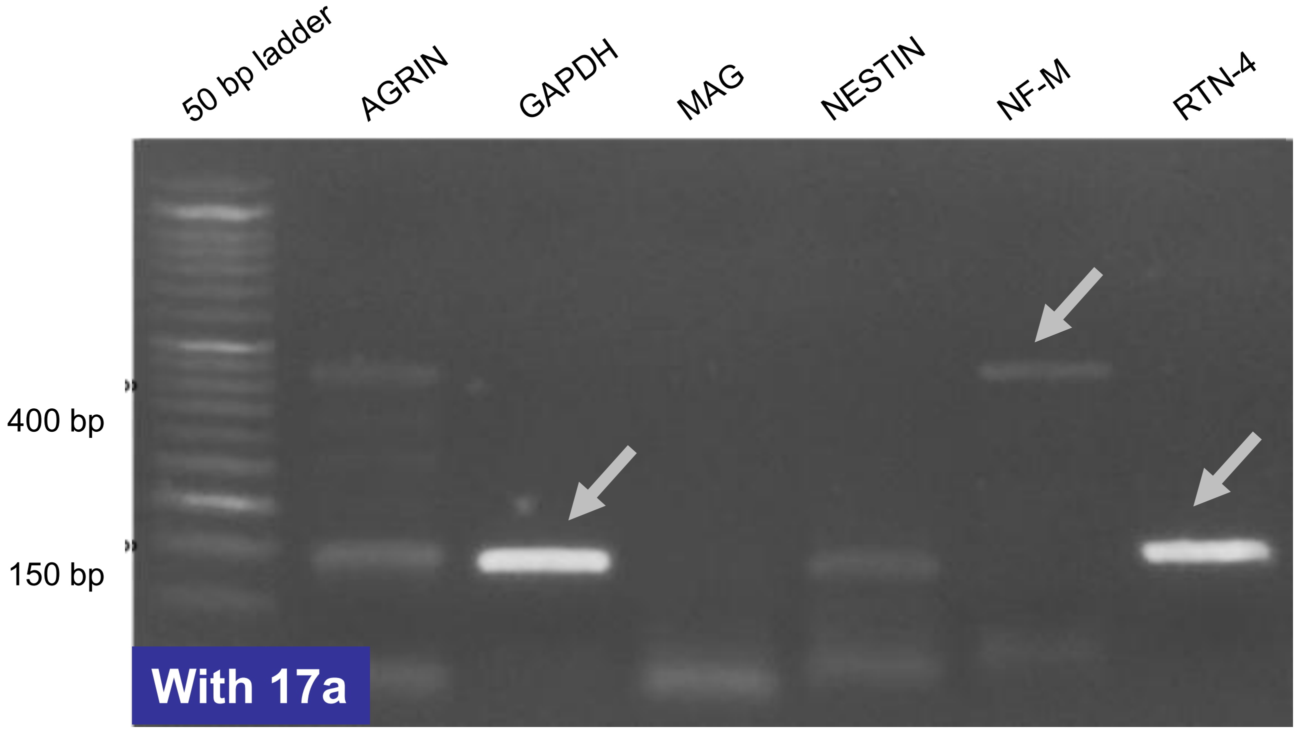
Hierarchical clustering *analysis:*

Selected neuro-development genes up-regulation:

With 17a triplicates



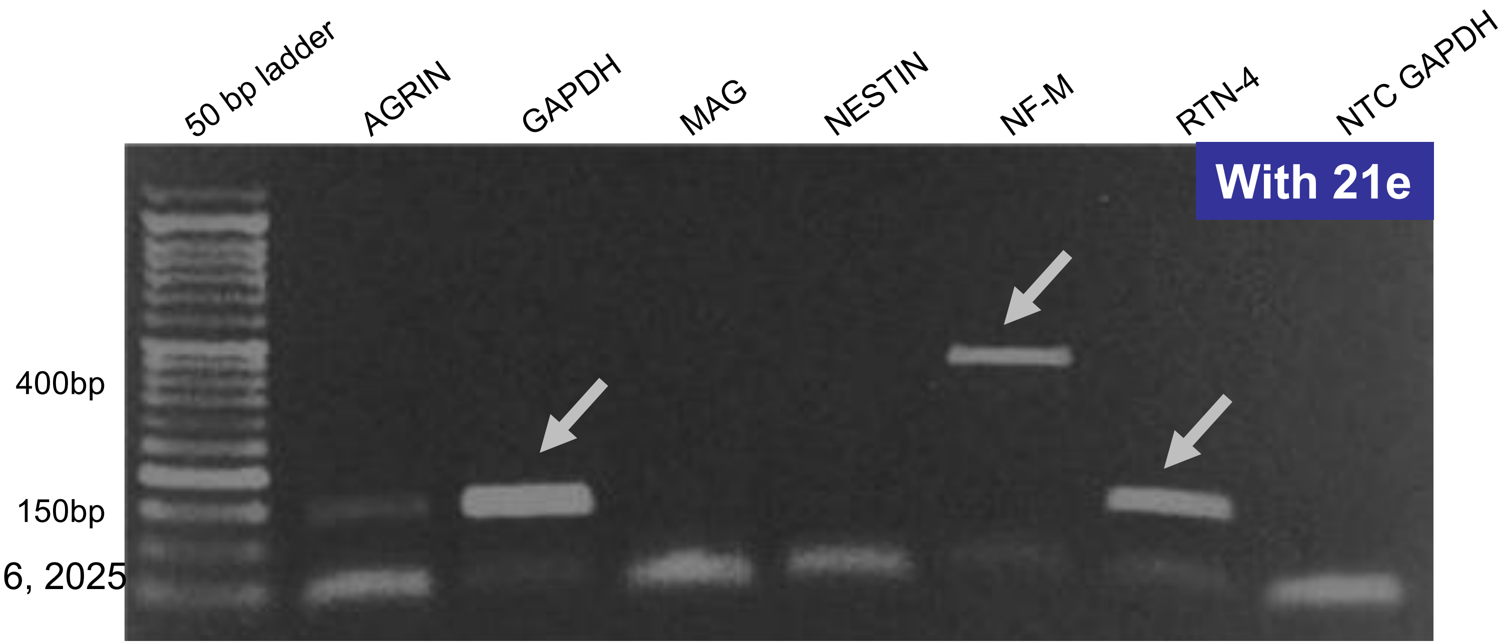




GAPDH mRNA is present in brain

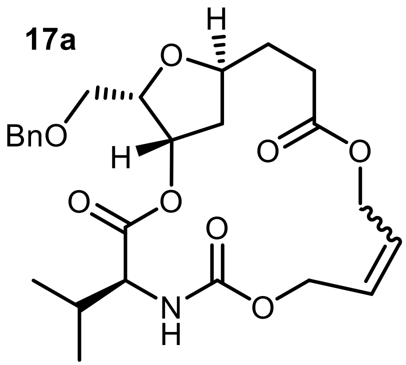
NEFM is a biomarker of neuronal damage

RTN4 expressed during neuronal development by neurons



Next Generation Sequencing - RNA Transcriptomic Studies

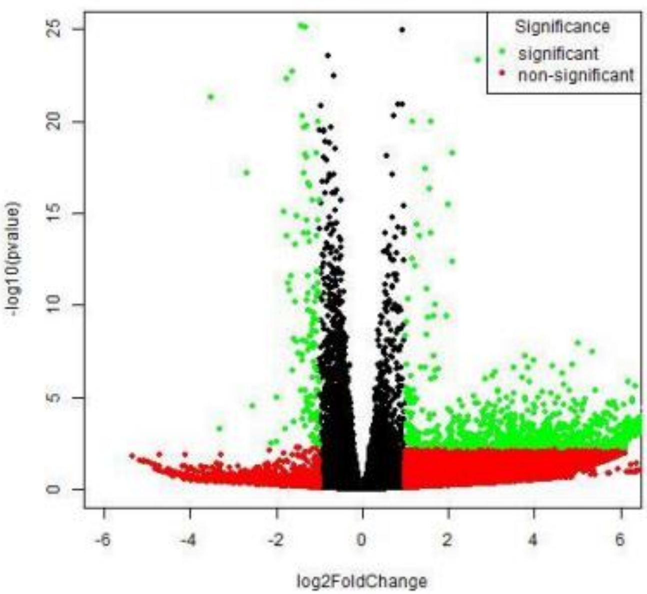
17a

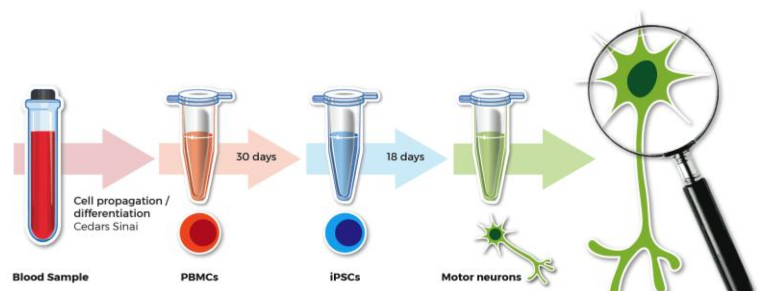


Collaborative work:
- *Arya Group*
- *Transcell Biologics Hyderabad*
- *MedGenome Labs Bangalore*

Control Vs Furan, 17a	Up regulated	Down regulated
Control Vs Furan, 17a	1372	1325

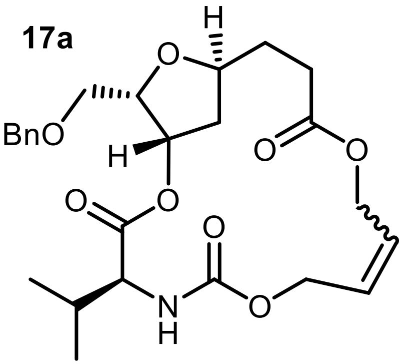
Triplicate studies

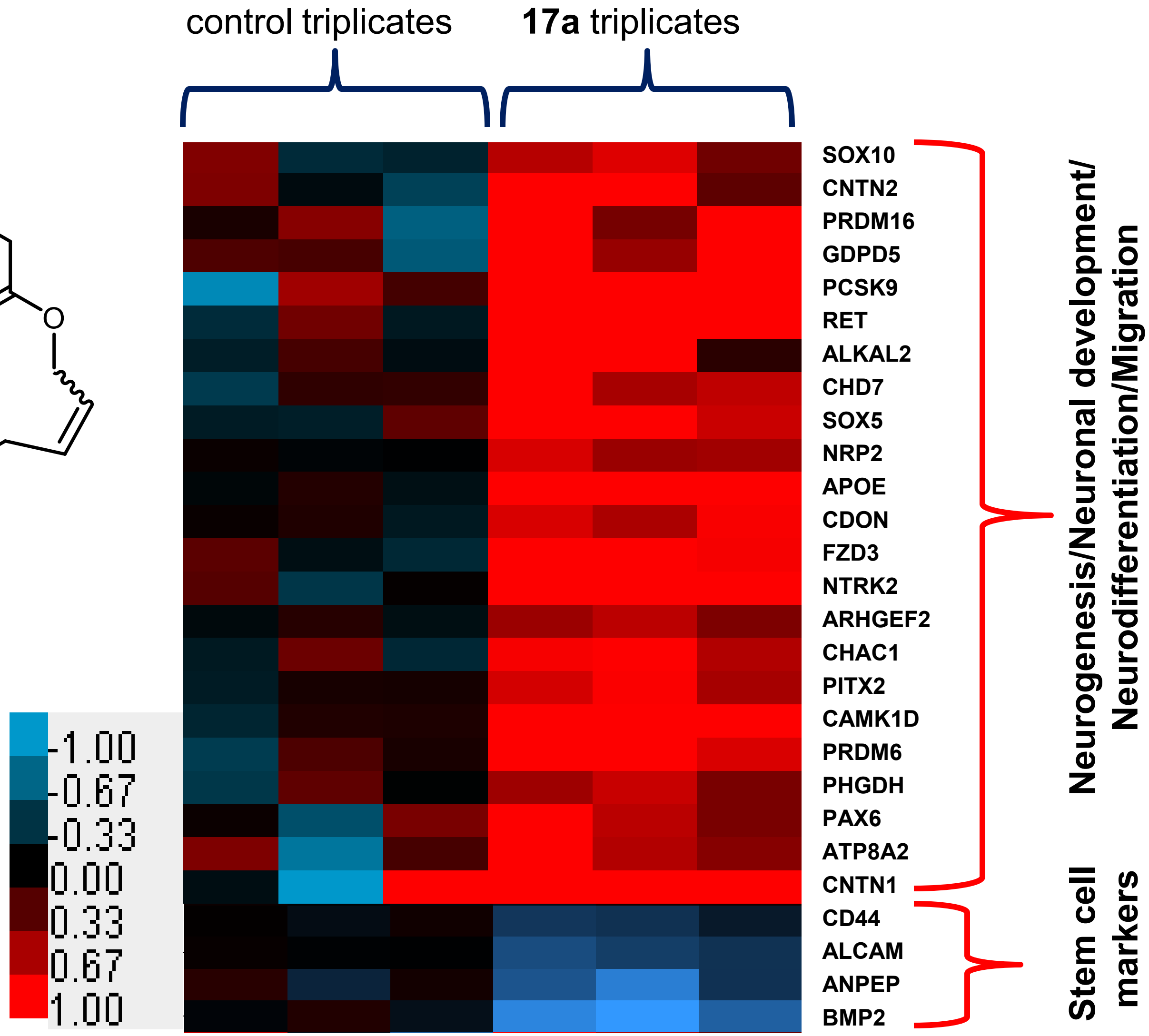




Hierarchical clustering

17a



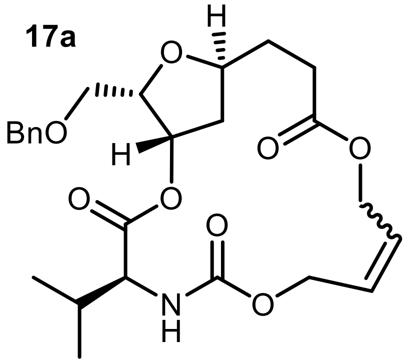


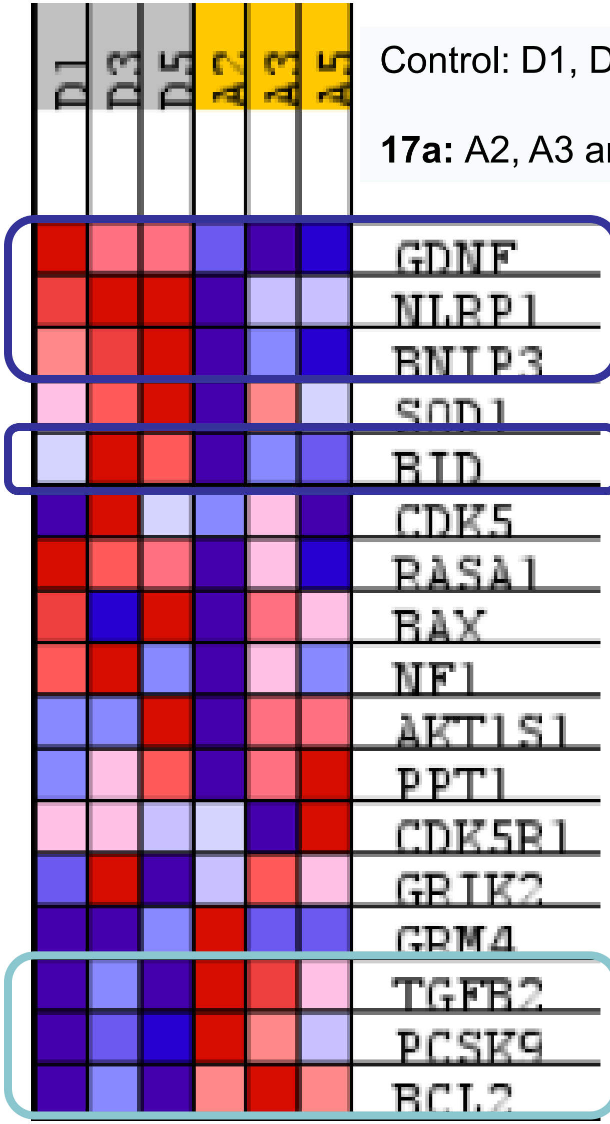
Hierarchial Clustering is visually represented as Dendrogram:
It is a method for grouping objects. The objects within a group are like each other and different from objects in other groups.

Effect on Pro-and Anti-Apoptosis Genes

Triplicate studies

17a

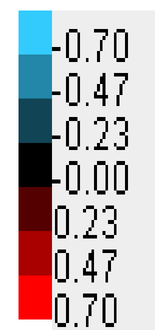




Control: D1, D3 and D5

17a: A2, A3 and A5

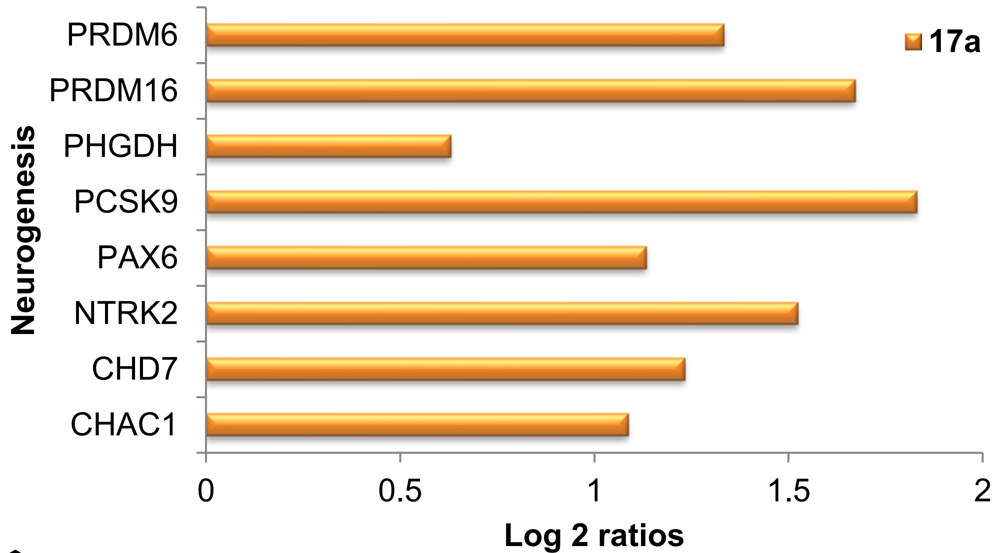
Down regulation of pro-apoptotic genes



Up-regulation of anti-apoptotic genes

Apoptosis mediates the precise and programmed natural death of neurons and is a physiologically important process in neurogenesis during maturation of the CNS.

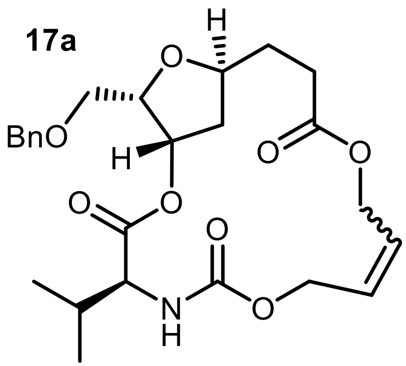
Effect on Neurogenesis and Neurodevelopment

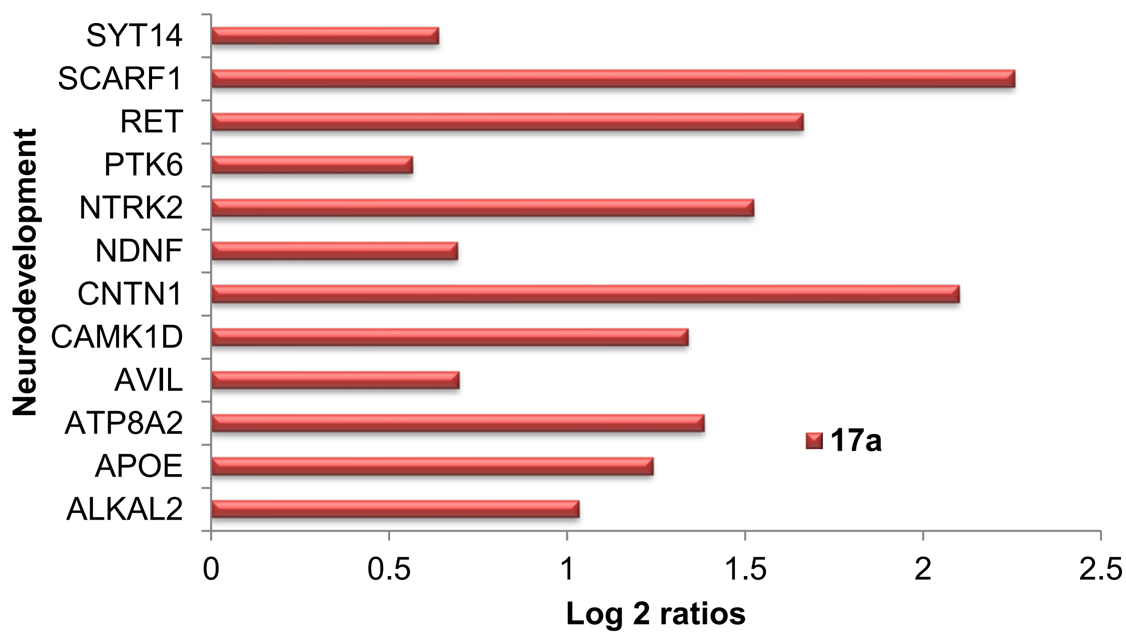


Neurogenesis: new neurons are formed in the brain

Up-regulated genes upon treatment with 17a

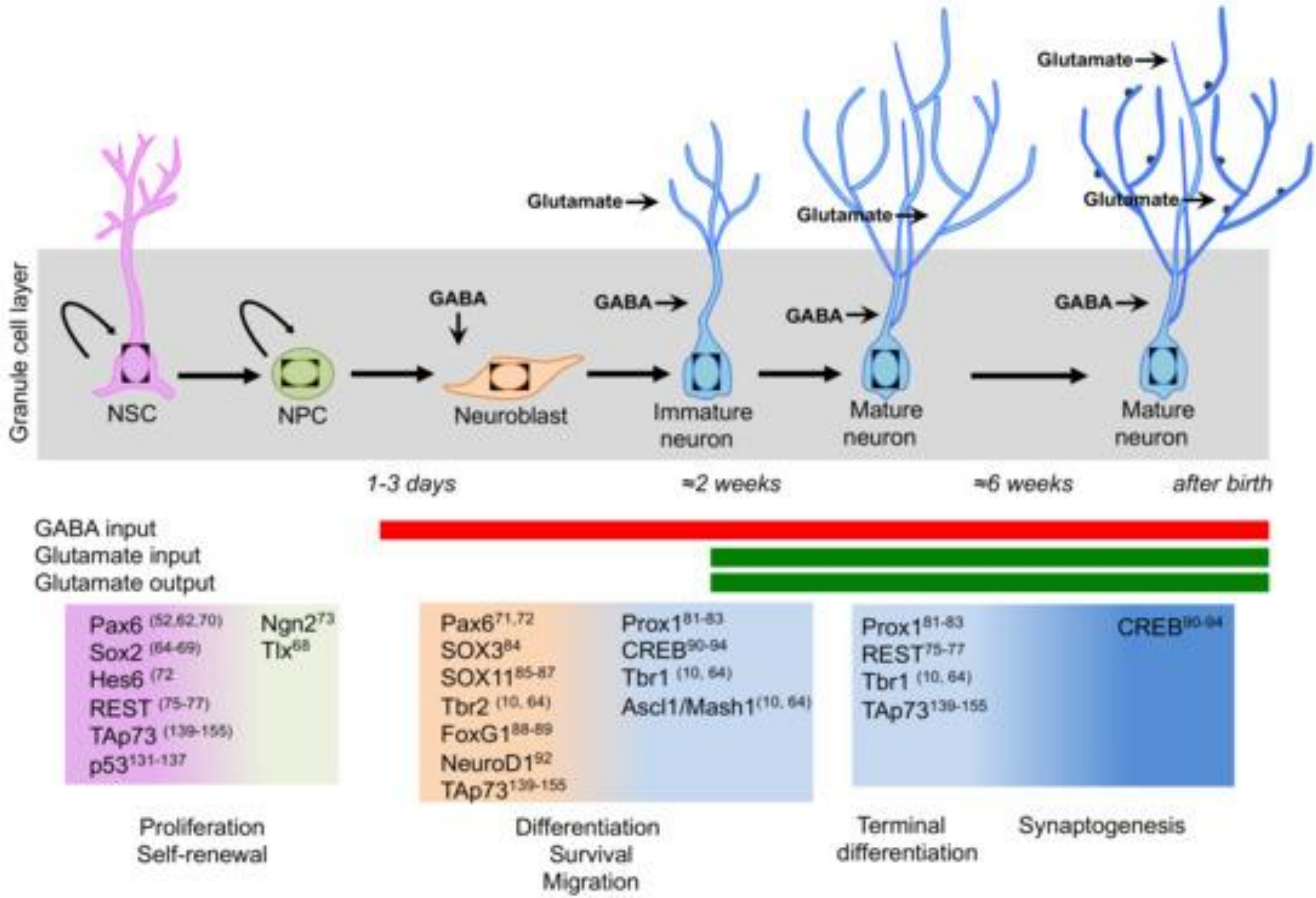
17a





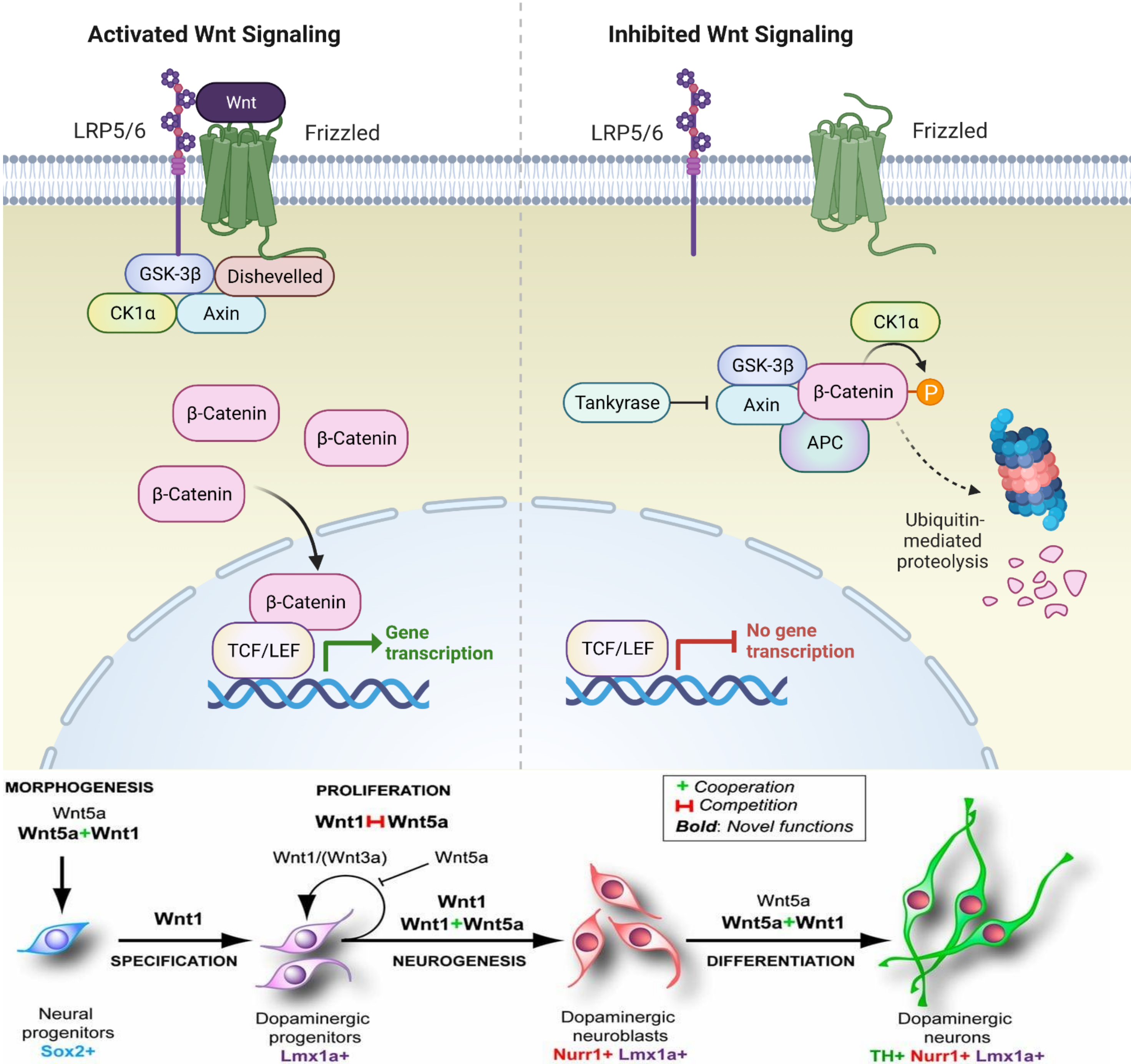
Neurodevelopment: Brain's development of neurological pathways that influence performance or functioning.

Neurogenesis Biomarkers



Mechanism of Neurogenesis: WNT Pathway

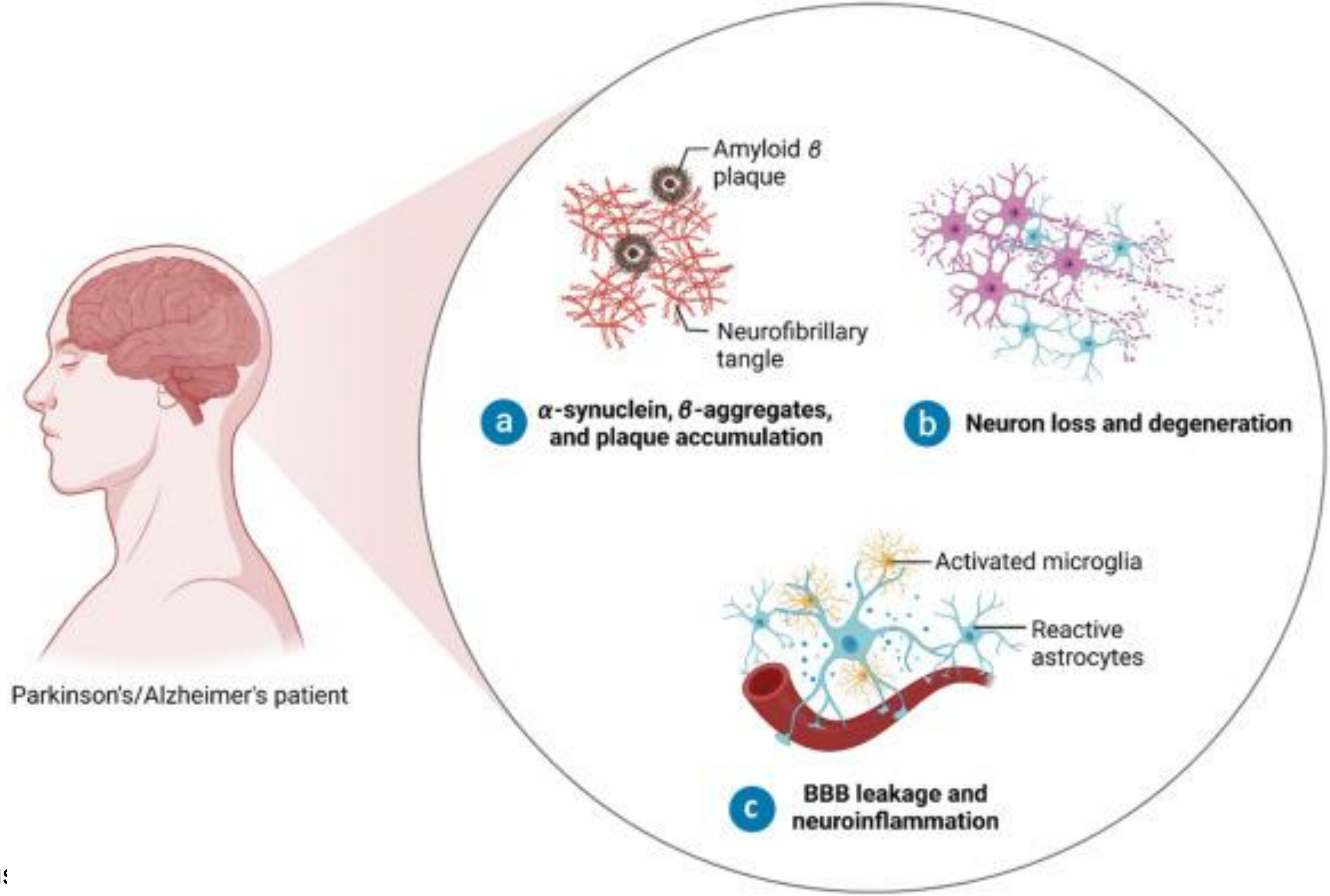
The complex of WNT, LRP, and Frizzled promotes the translocation of β-catenin into the nucleus, resulting in the transcription of β-catenin target genes.

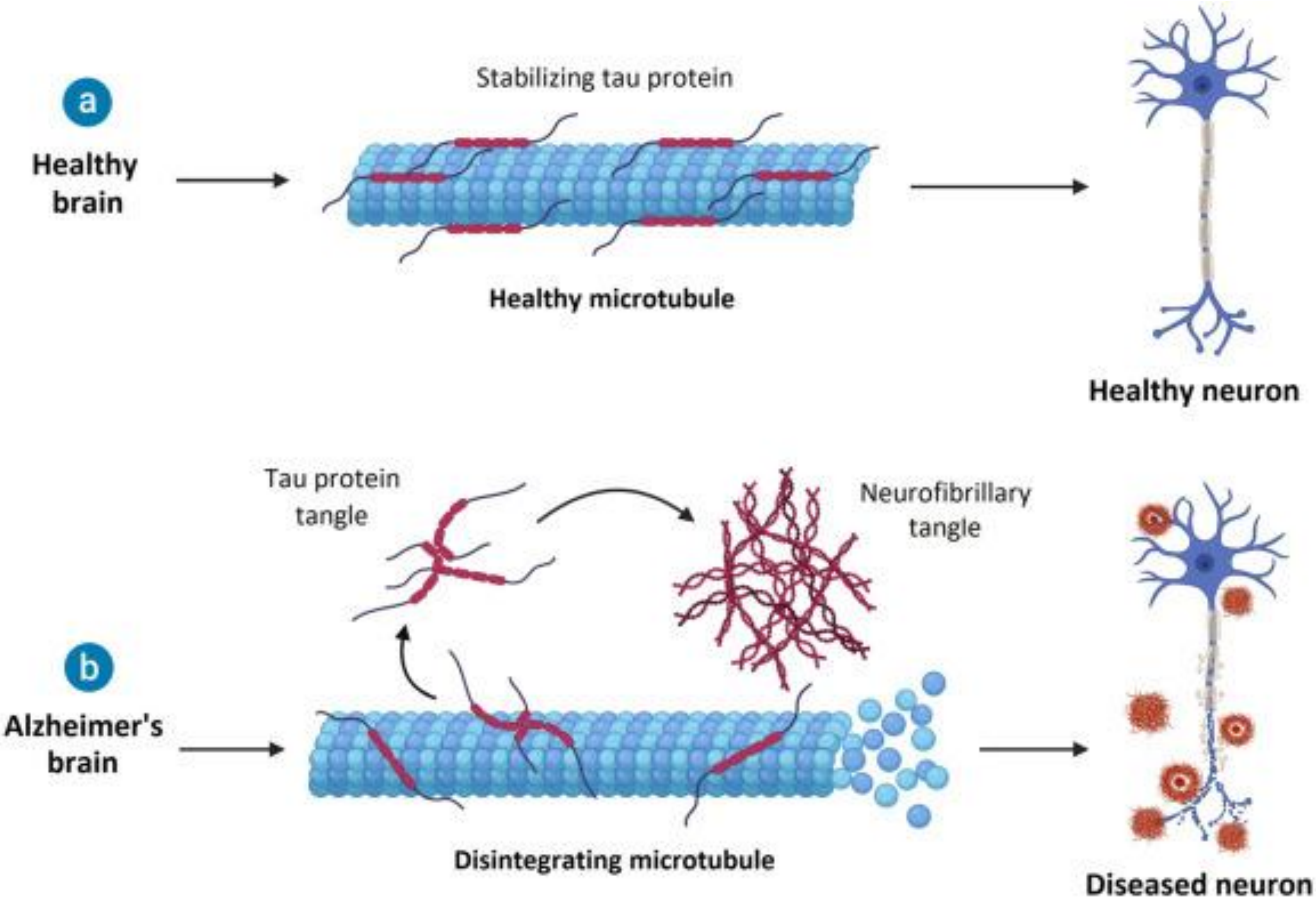


Transcriptome data clearly showed that 17a up-regulated genes related to neurogenesis, axaongenesis, neurodifferentiation, neurodevelopment and neuronal migration.

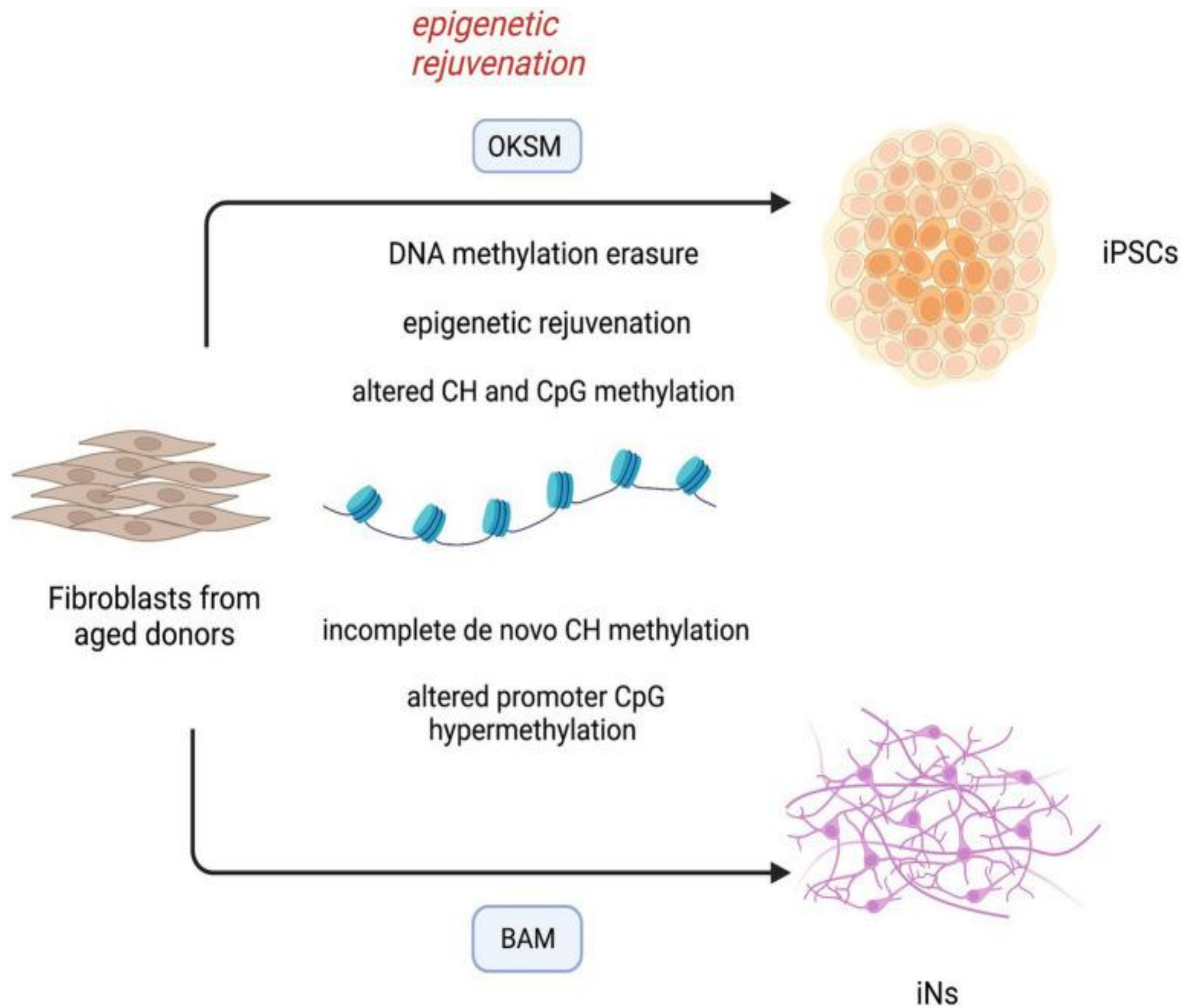
- As shown 17a up-regulated genes NTRK2, CNTN1, NDNF, RET, CAMK1D and ATP8A2 which are known to be involved in neuronal development and differentiation.
- An Increased PCSK9 expression is also known to inhibit the amyloid β peptide (Aβ)-induced neuronal apoptosis by reducing the Aβ generation
- The genes involved in neurogenesis, such as, CHAC1, CHAD7, PAX6, PCSK9, PHGDH, PRDM6 were upregulated.
- Several critical genes (for example, BCl2, TGFB2 and PCSK9) that are responsible for anti-apoptosis were found to be up-regulated

Neuron's role in Neurological disorders (Alzheimer's and Parkinson)




a
Healthy brain
Stabilizing tau protein
Healthy microtubule
Healthy neuron
b
Alzheimer's brain
Tau protein tangle
Neurofibrillary tangle
Disintegrating microtubule
Diseased neuron

DNA methylation landscape in induced-pluripotent stem cells (iPSCs) and directly converted neurons (iNs).



OKSM pathway

Reprogramming of aged fibroblasts to the pluripotent cell (iPSCs)stage is characterized by methylation erasure and reset of epigenetic clock.

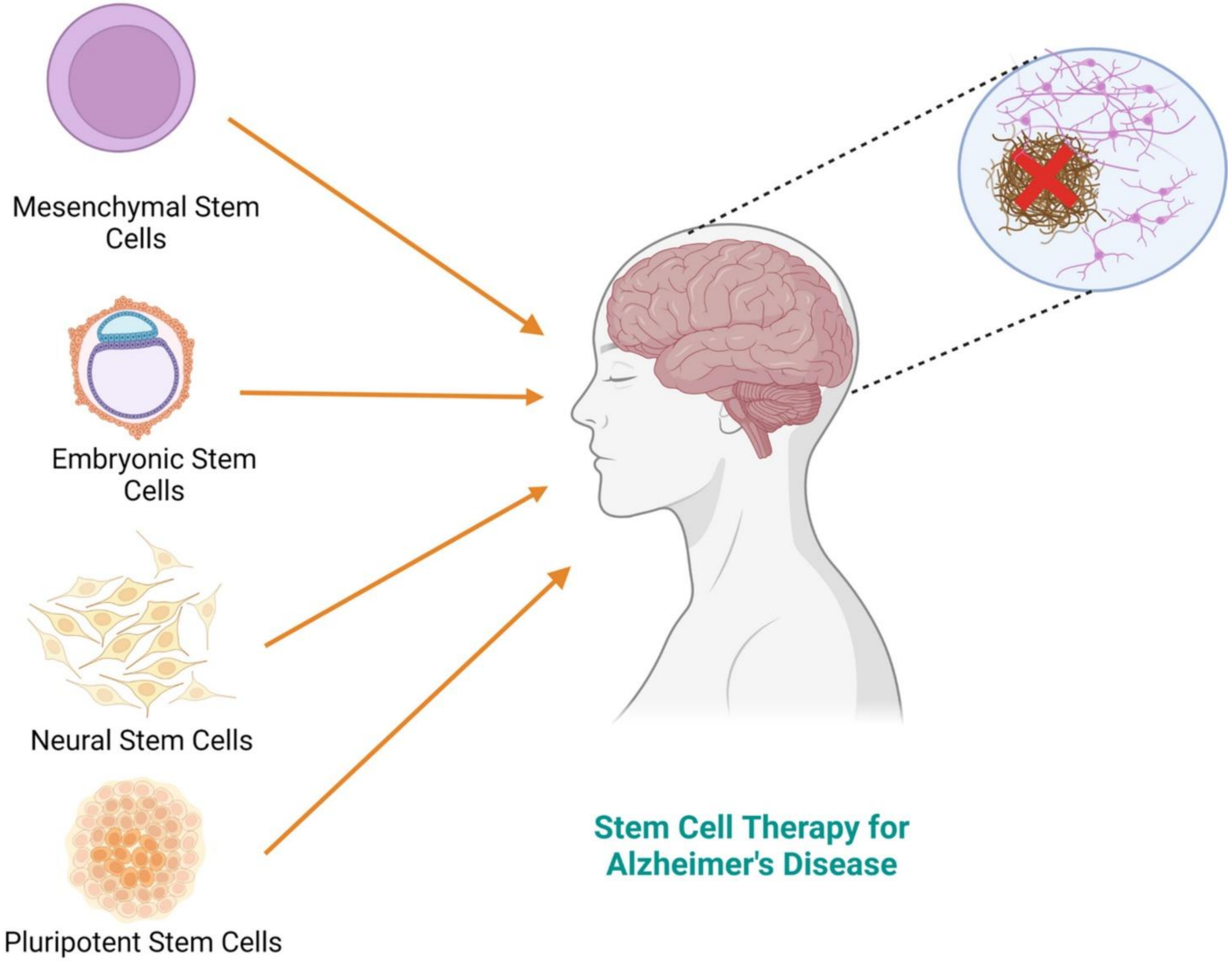
OKSM (OCT4, KLF4, SOX2, C-MYC);

BAM (ASCL1, BRN2, MYT1L).

BAM Pathway

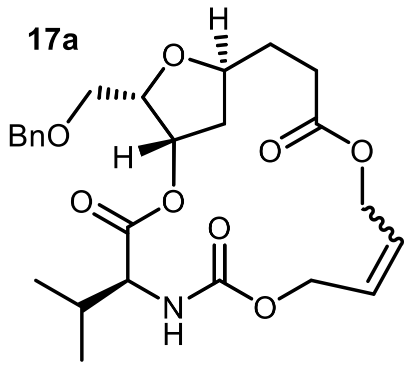
iNs derived from aged fibroblasts bypass the intermediate pluripotent cell stage, but still lack a faithful CH DNA methylation and show an altered CpG hypermethylation pattern at promoter regions.

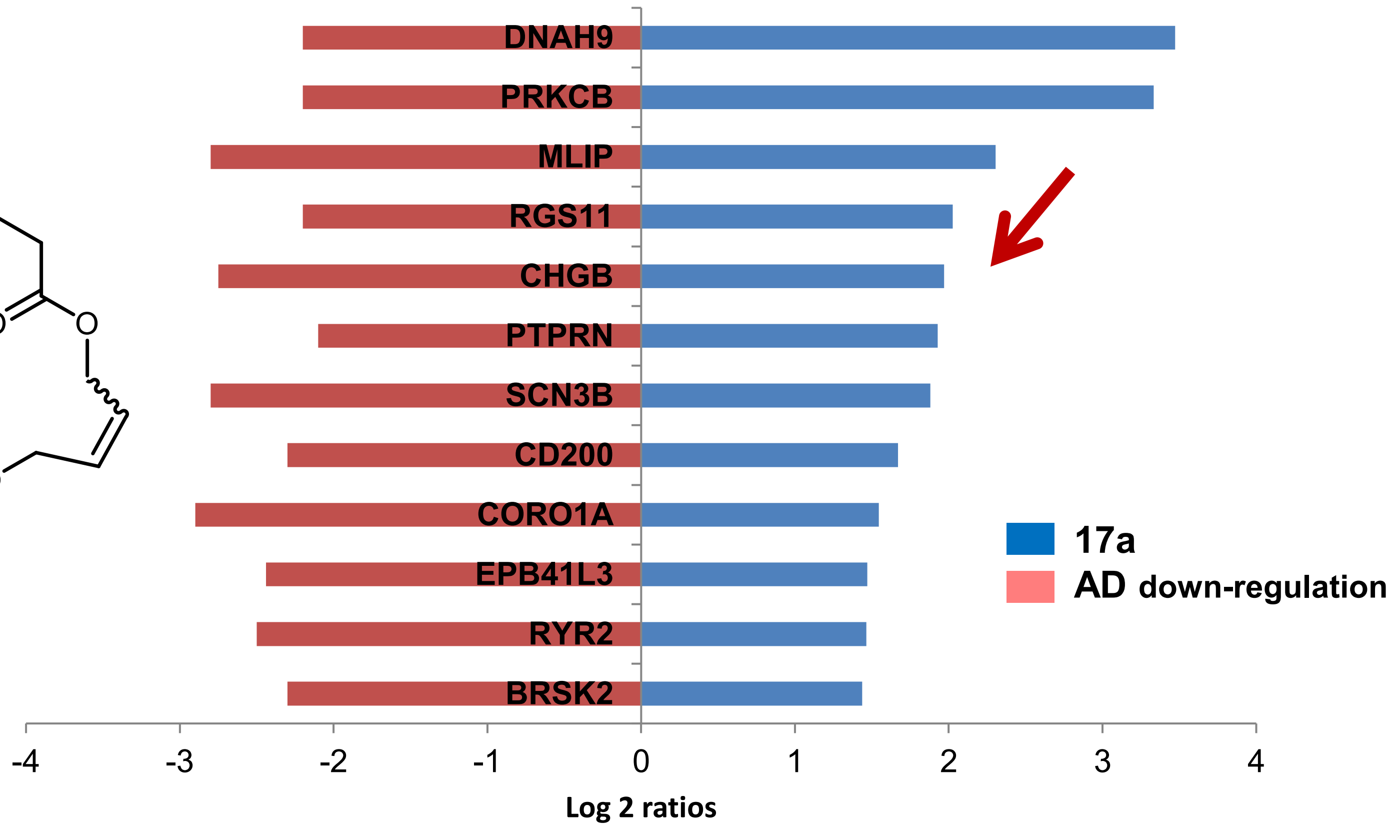
Our Studies on Small Molecule based differentiation of iPSC derived from samples of Alzheimer's and Parkinson patients



Up-regulation of Genes That are known to be Down Regulated in Patients with Alzheimer’s

17a





CHGB gene: Encodes a tyrosine-sulfated secretory protein abundant in neurons

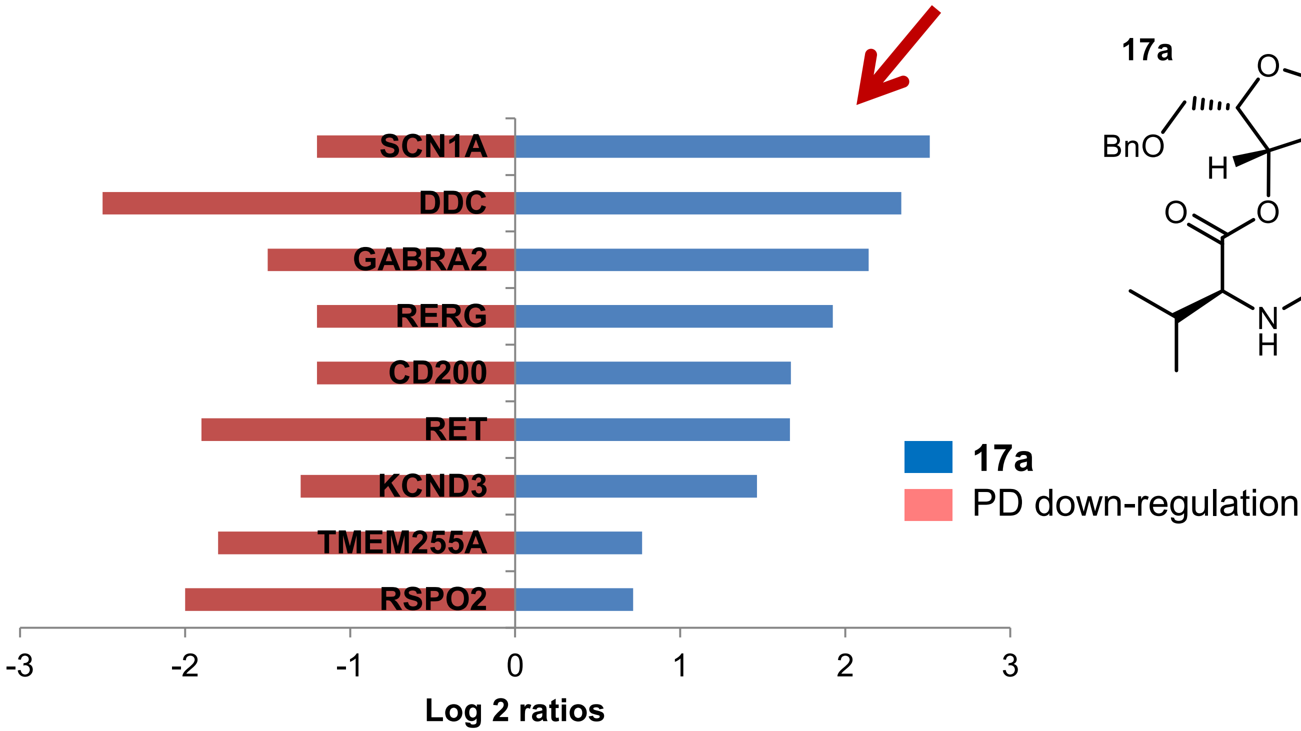
Upon treatment with small molecule, 17a

iPS cells are stem cells created from a patient's own cells, such as skin or blood.

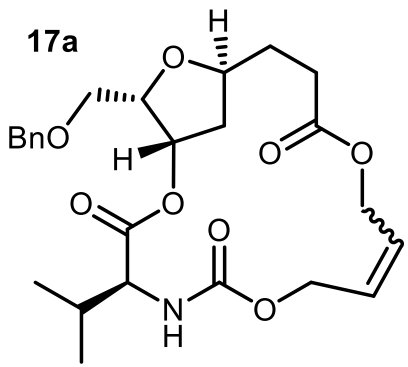
Upregulation of genes in Alzheimer's patient's stem cells during chemical trans-differentiation

- **SCN3B Gene:** Responsible for generation and propagation of action potentials in neurons and muscle
- **CD200 Gene:** negative regulation of neuroinflammatory response and neuron death
- **Coro1A Gene:** negative regulation of neuron apoptotic process
- **EPB41L3 Gene:** neuron projection morphogenesis
- **BRSK2 Gene:** neuron differentiation

Up-regulation of Genes That are known to be Down Regulated in Patients with Parkinson's



17a



SCN1A gene: Provides instructions for making one part of sodium channel NaV 1.1 transmitting signals in neurons found in the brain.

Upon treatment with small molecule, 17a

Upregulation of genes in Parkinson's patient's stem cells during chemical trans-differentiation

- **DDC Gene:** Help in producing dopamine and serotonin neurotransmitter

- **GABRA2 Gene:** receptor in mammalian brain

- **KCND3 Gene:** Voltage gated K channel regulating neurotransmitter release and neuronal exitability

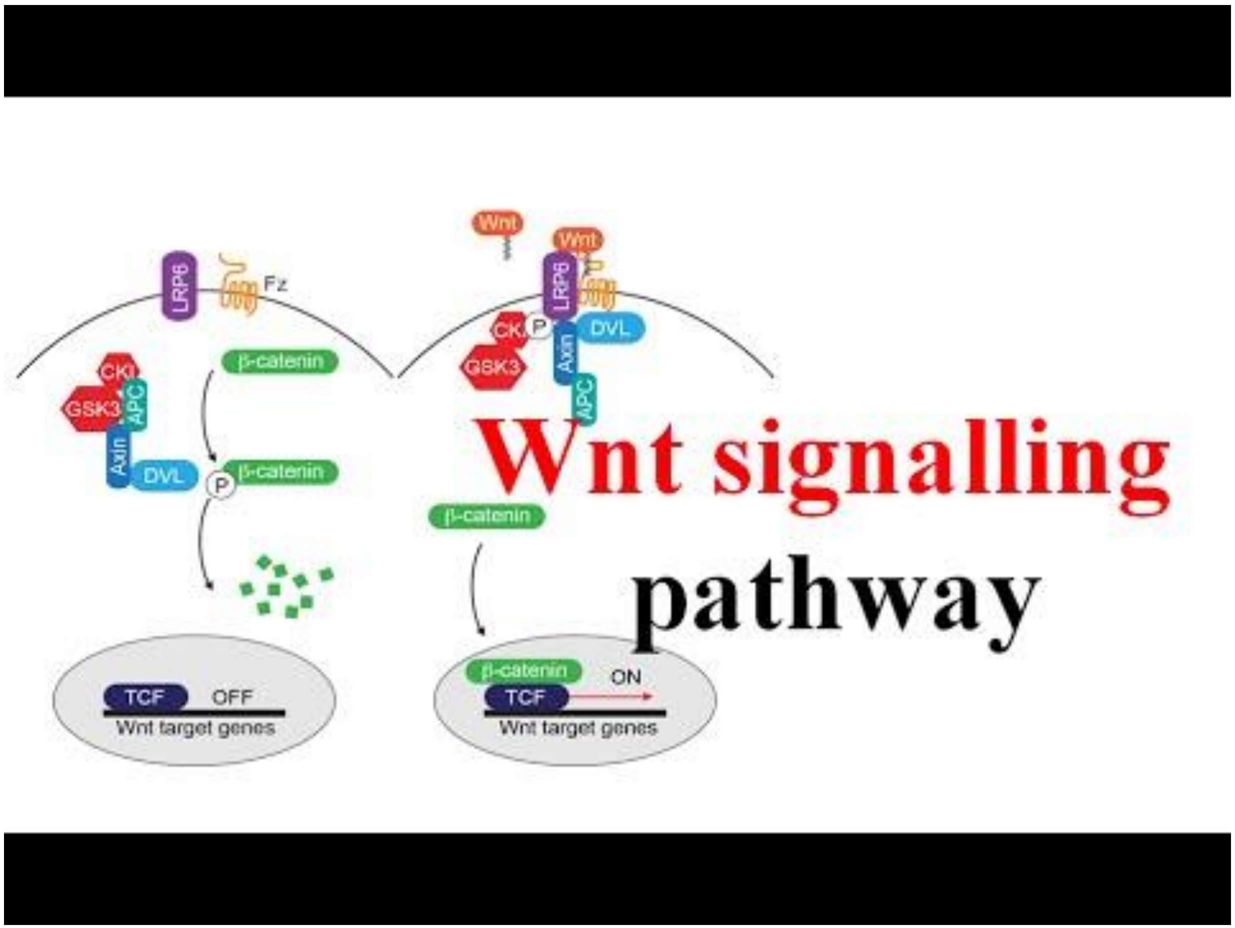
In Conclusion……..

1. Natural products-inspired small molecules are good probes for trans- differentiation of iPSC to Neuron like cells.

2. Cells derived from Alzheimer’s and Parkinson patients undergo small-molecules promoted differentiation of stem cells to neuron like cells.

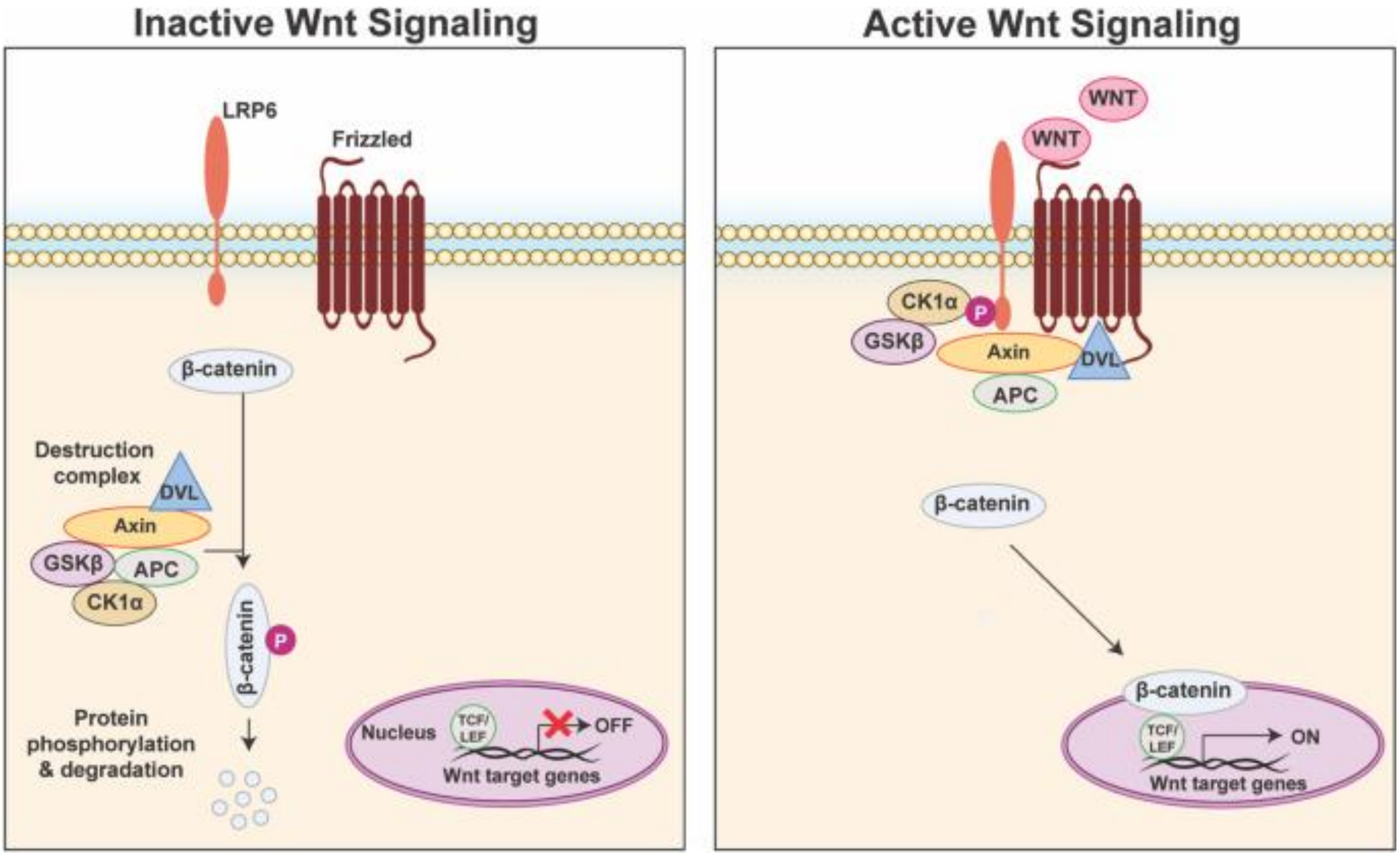
3. These studies promise to be a viable Human iPSC-based therapy.

Role of Furan Macrocycles in modulating Wnt signalling pathway in Cancer stem Cell: A quick look



Activation of Wnt Signaling

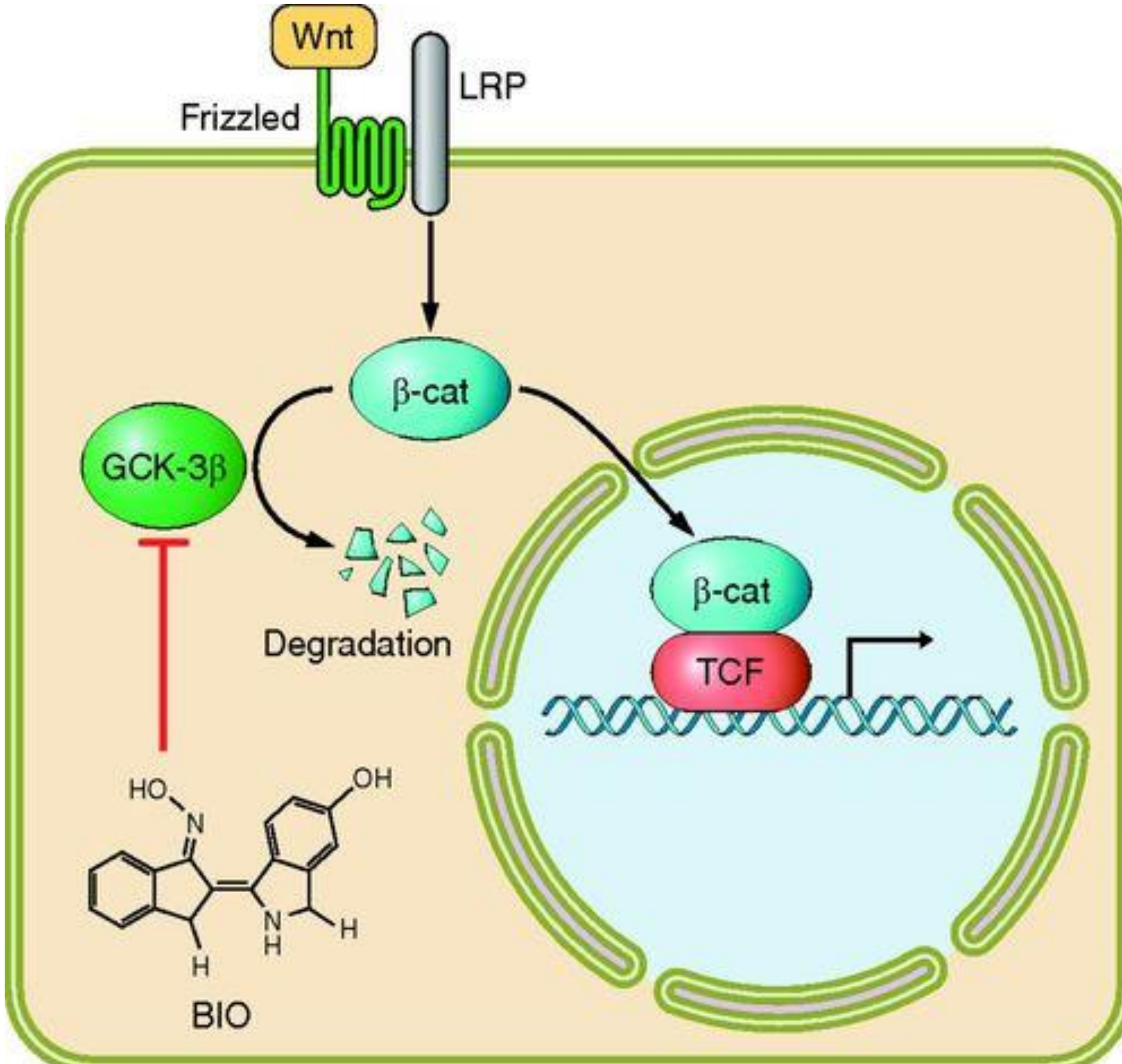
Involvement of the Wnt–β-catenin–GSK3 signaling axis in the formation and maintenance of cancer stem cells (CSC) is responsible for tumor growth in several types of human malignancies



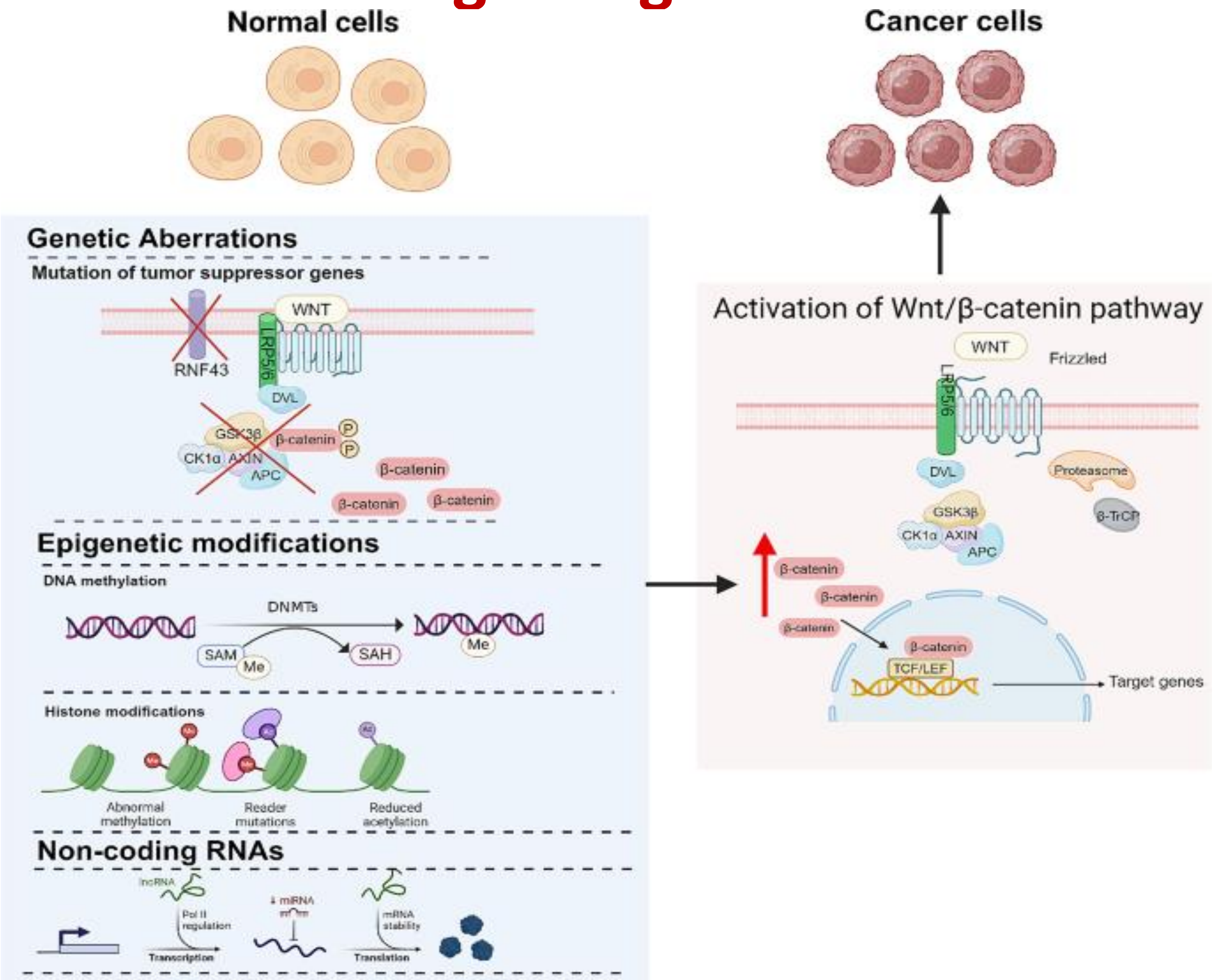
Wnt signaling regulates fate decisions in embryonic stem cells.

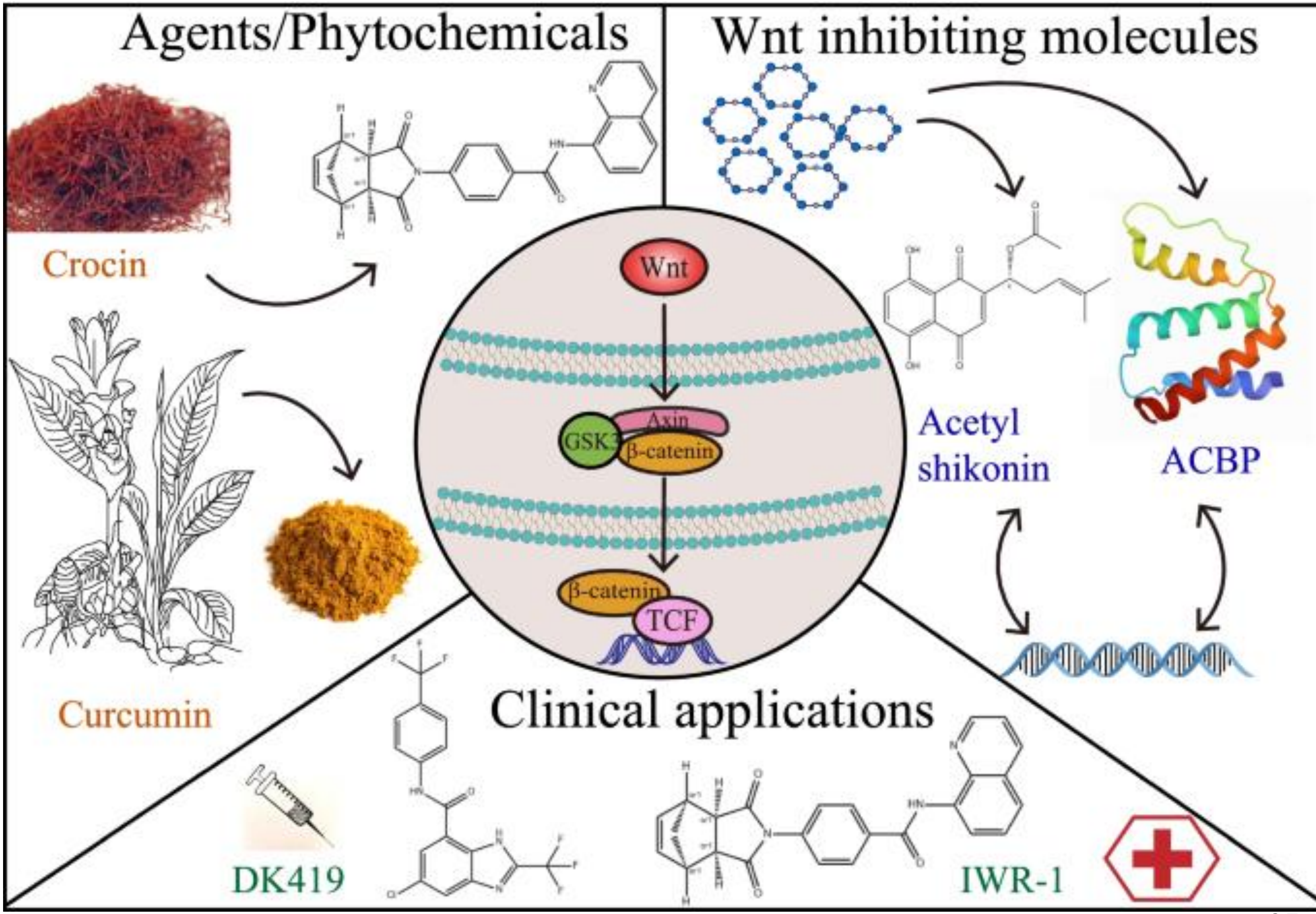
1. GSK3β acts as a tumor suppressor by curbing canonical Wnt–β-catenin signaling.

2. The small molecule (BIO) inhibits GSK3β and thus increases the activity of β-catenin.

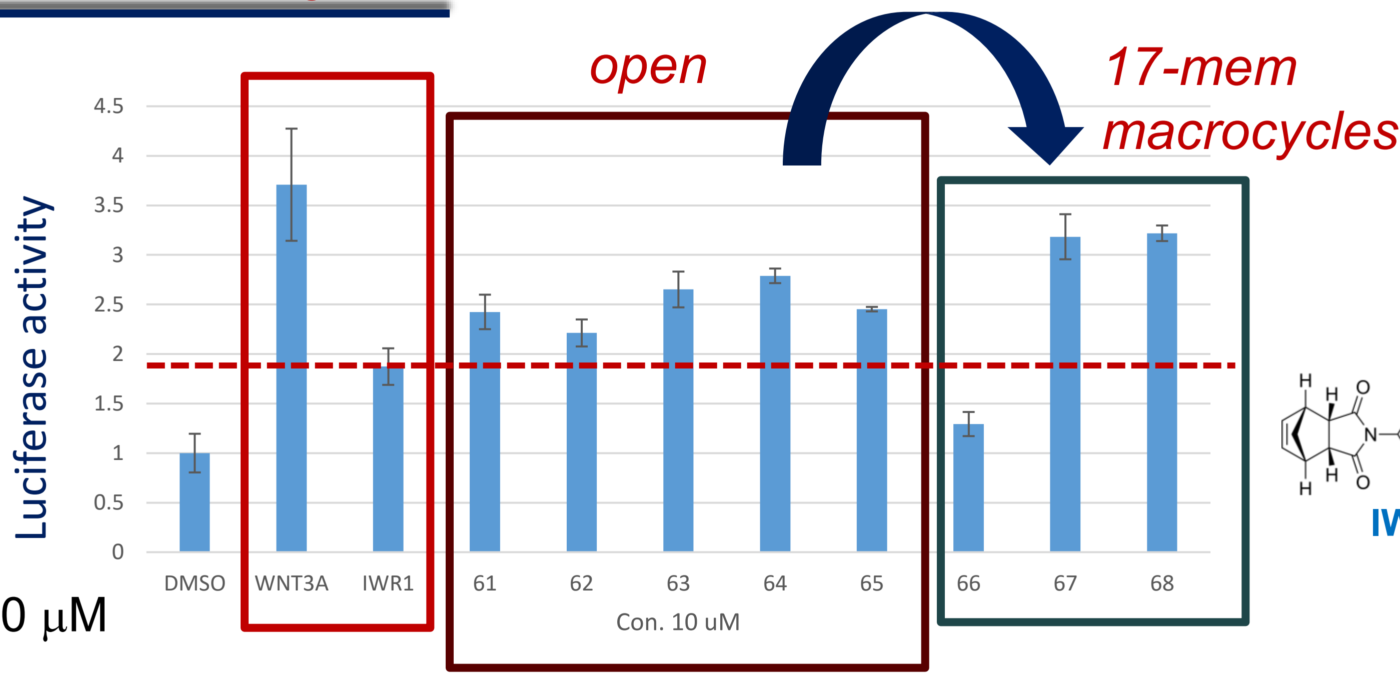


Wnt signaling in cancer

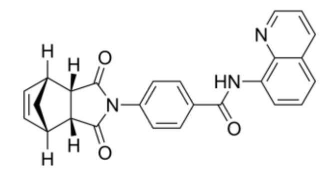



Agents/Phytochemicals
Wnt inhibiting molecules
Crocin
Curcumin
Wnt
Axin
GSK3
β-catenin
β-catenin
TCF
Acetyl
shikonin
ACBP
Clinical applications
DK419
IWR-1

Wnt Luciferase Screening Data



@10 μM

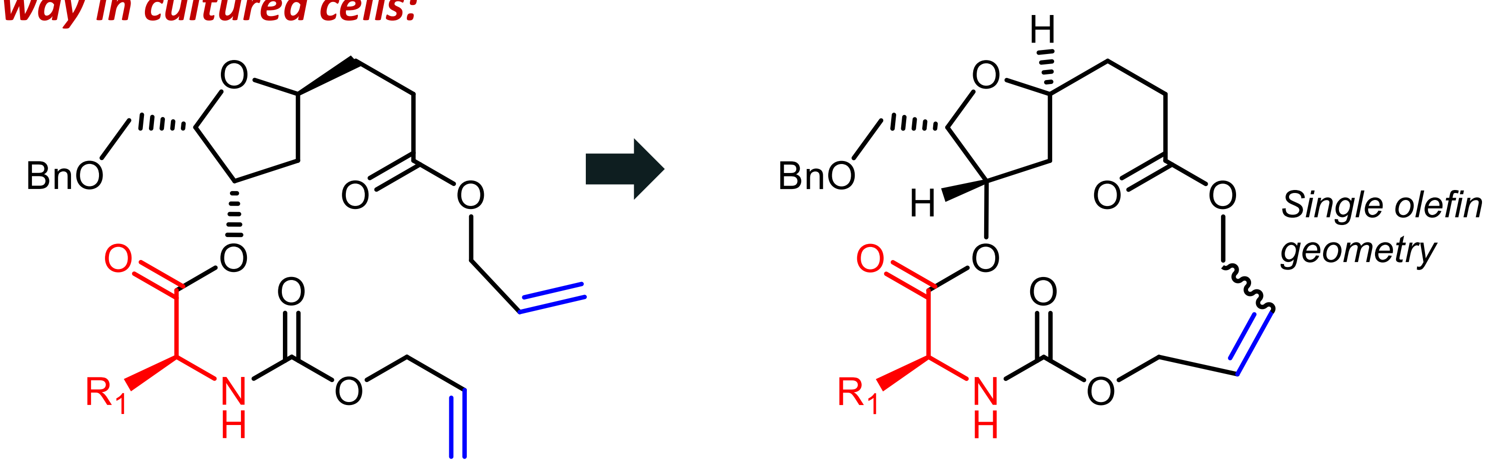


IWR-1

a cell-based test that uses a luciferase gene to measure the activity of the Wnt/β-catenin signaling pathway in cultured cells:

Five Analogs

Family 1 Hits

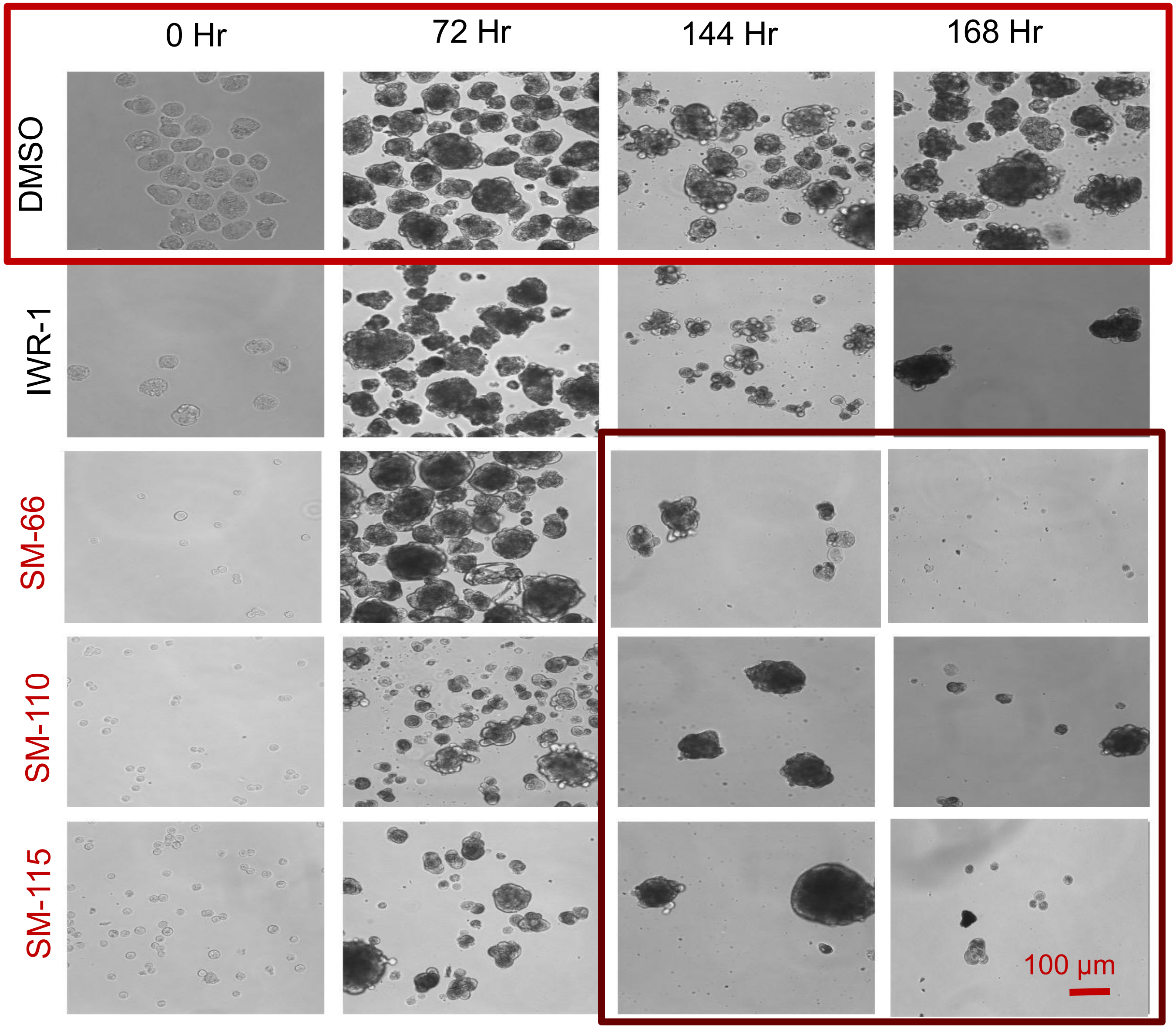


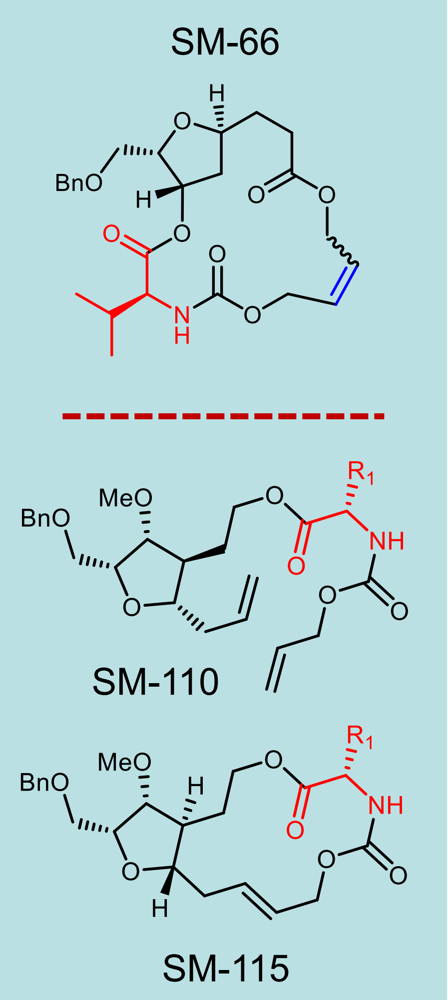
SM-2015-61/62/63/64/65

SM-2015-66/67/68/69/70

Effect on Tumorspheres (G2*) from Patient Buccal Mucosa (Indian Patient 1)

G2 = 2nd generation





In Summary:

The studies involving furan-based macrocyclic molecules clearly highlight the potential therapeutic utility for such inhibitors to mediate Wnt–GSK3–β-catenin axis activity at the expense of Cancer Stem Cells self-renewal and survival.

Sincere Thanks to…

The Arya Group
DRILS Hyderabad

Transcell Biologics
Hyderabad

MedGenome Labs
Bangalore

DR. REDDY'S
INSTITUTE OF LIFE SCIENCES

Karolinska Institutet

McGill

Department of Science & Technology
Ministry of Science & Technology

Department of Biotechnology
Ministry of Science and Technology

MoES/CSIR-CDRI

Science and Technology Park
University of Pune

Thank You