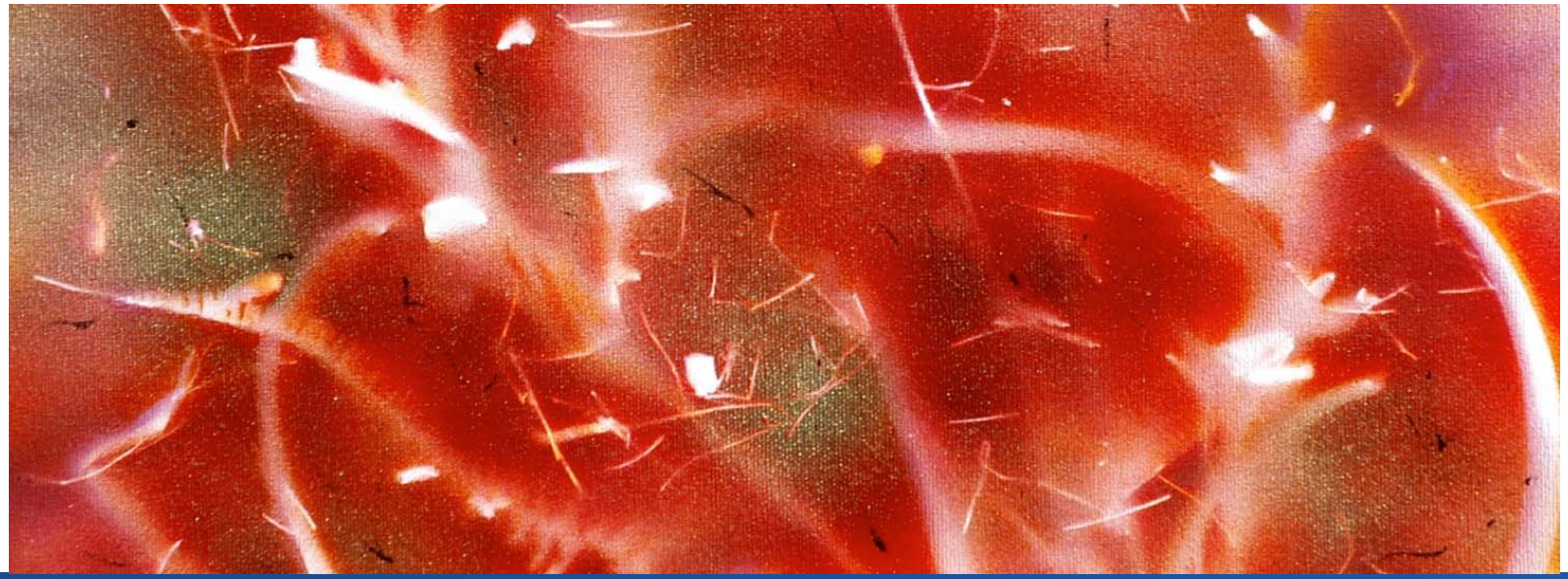


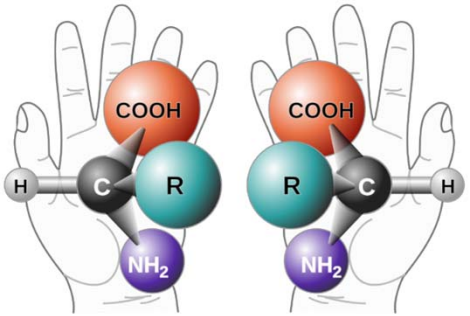
# *Determining the Absolute Configuration of the α-Carbon in JNJ-A (a GPR40 Superagonist) by NMR Spectroscopy*

2019 Eastern Analytical Symposium & Exposition: NMR Spectroscopy Instrumentation and Application
11:10 AM, Nov. 19th, 2019

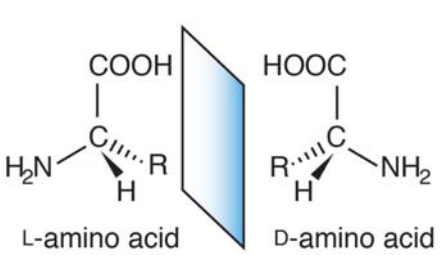
**Ronghui Zhou, Ph.D.**
rzhou11@its.jnj.com
Discovery Chemistry, Janssen R&D
Spring House, PA

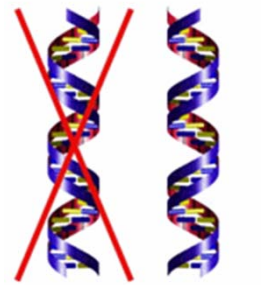
janssen | PHARMACEUTICAL COMPANIES OF Johnson & Johnson

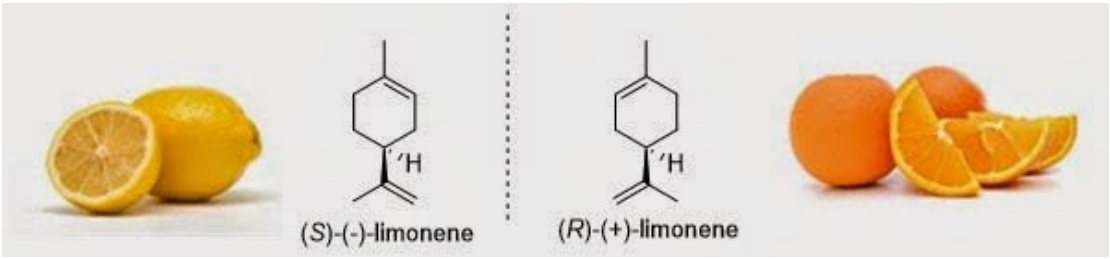
# Chirality

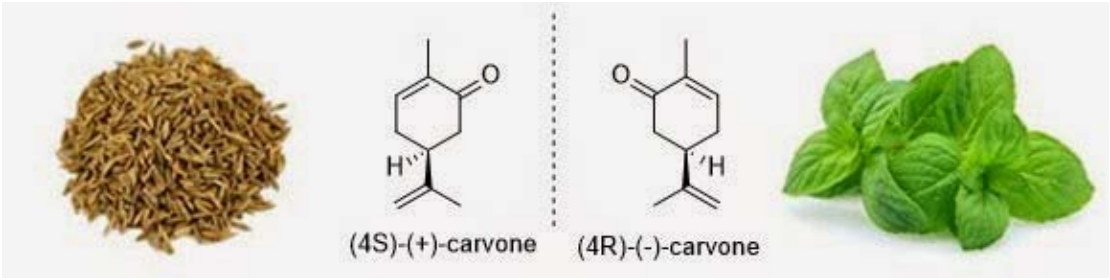



Handedness



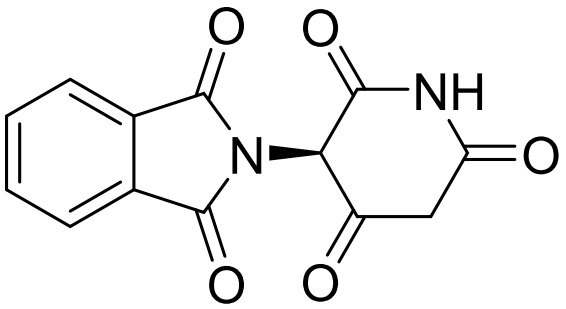





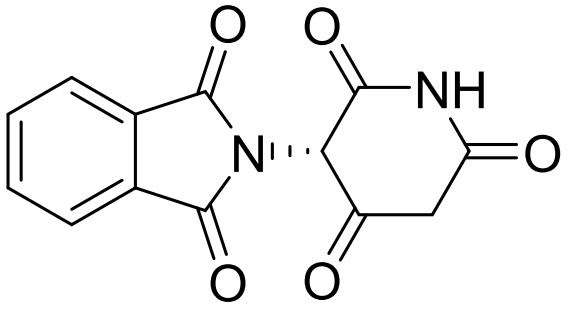



# Chiral Drug: the Thalidomide Tragedy in the 1960s

Thousands of children around the world were born with severe birth defects.



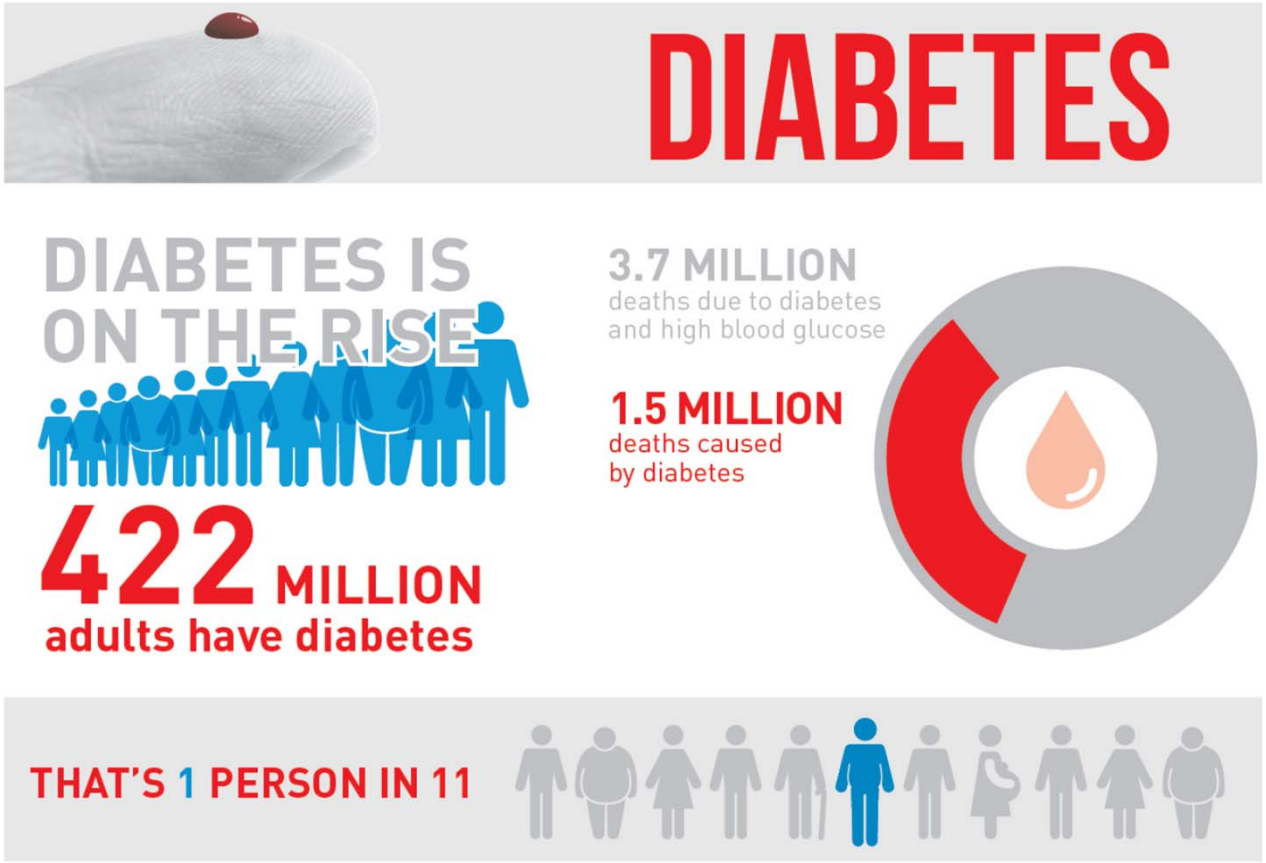

(*R*)-
thalidomide



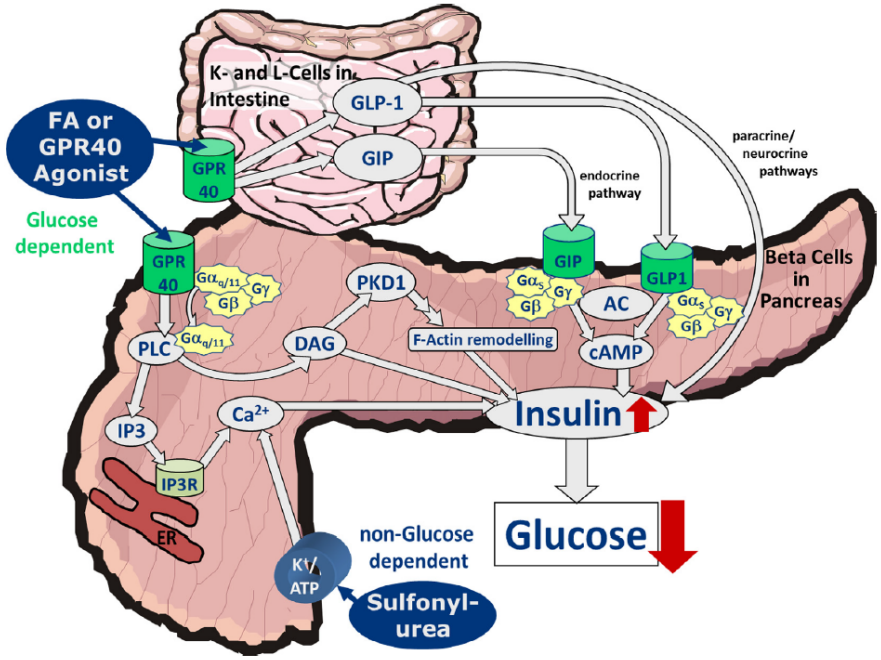

(*S*)-
thalidomide

The *S* enantiomer causes birth defects, while the *R* enantiomer is effective against morning sickness.

# Diabetes is a Global Health Crisis



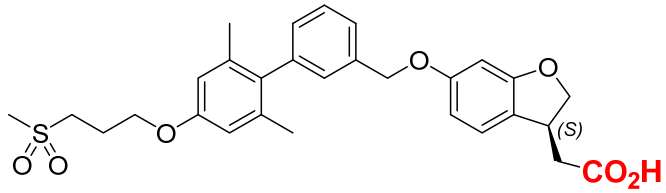
# GPR40 Agonism is a Novel Mechanism for T2D



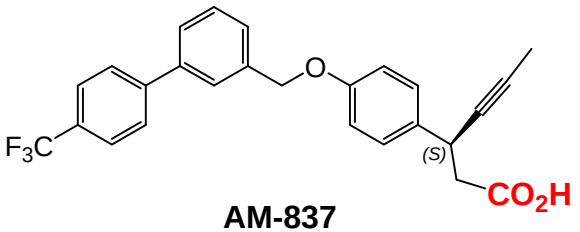

*Defossa and Wagner (2014) Bioorganic and Medicinal Chemistry Letters, 24, 2291-3000*

- A "nutrient sensor" GPCR on the pancreatic beta cell
- Highly expressed in the pancreas, intestine, also human brain
- GPR40 activation induces insulin secretion, in a glucose-dependent manner
- Expected to lower glucose in patients with T2DM with low risk of **hypoglycemia**
- GPR40 agonist has additional benefit of **weight loss**

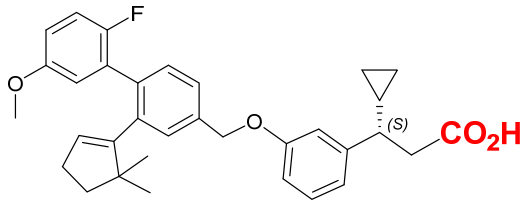
# GPR40 Agonists: Conformationally Constrained Fatty Acid Mimetics



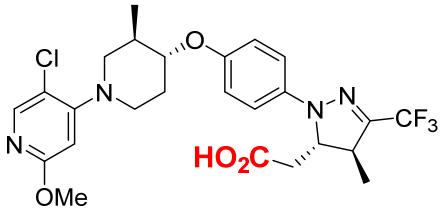
**TAK-875**
**Ph 3, Terminated**
partial agonist



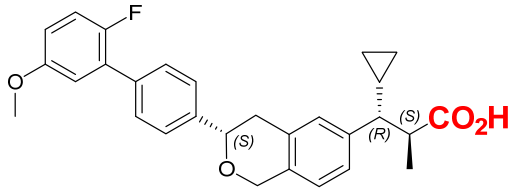
**AM-837**
**Ph 1 Discontinued**
partial agonist



**AM-1638**
full agonist

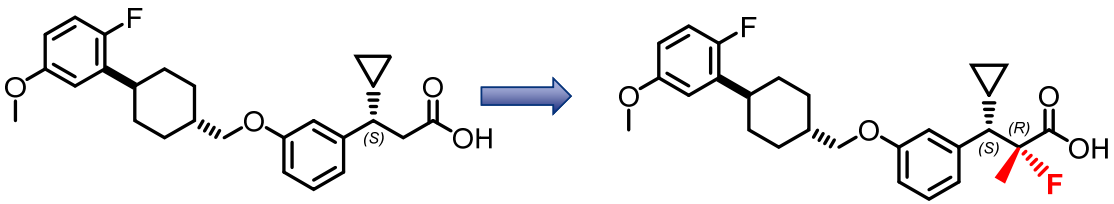


**BMS-986118**
full agonist

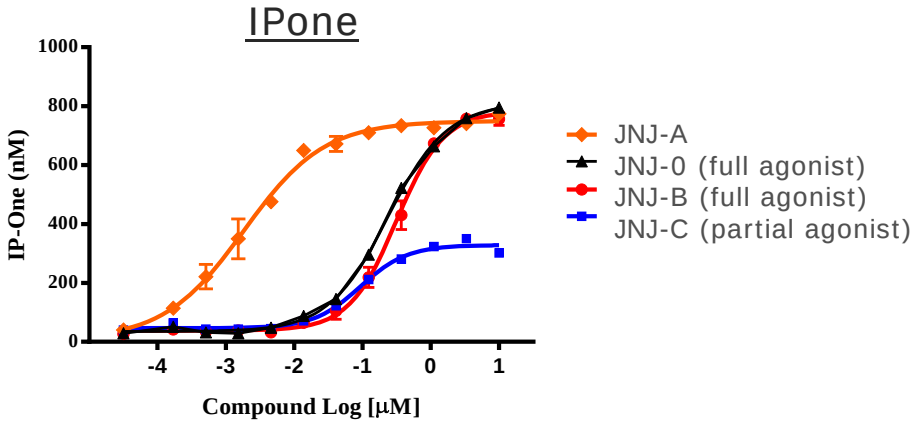


**Merck, WO2016022448**
full agonist

# JNJ-A is a GPR40 Superagonist

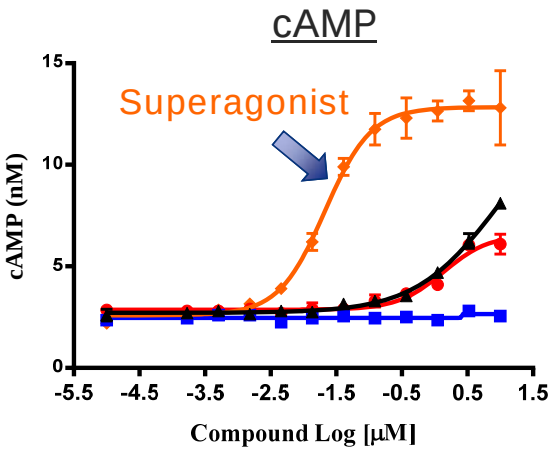



**JNJ-0** **JNJ-A**



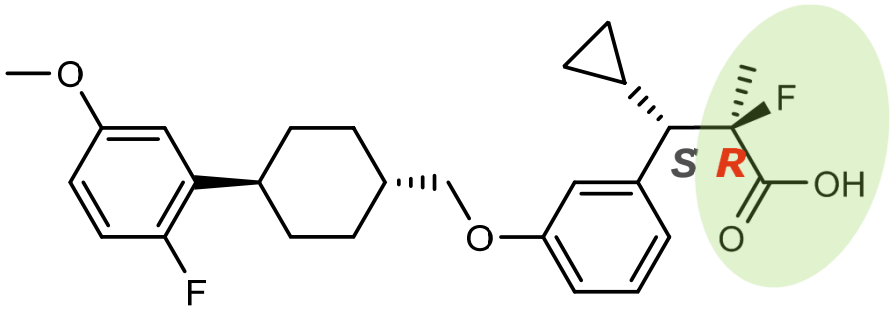

## $EC_{50}$ –Calcium Mobilization (nM) (Mean ± SD)

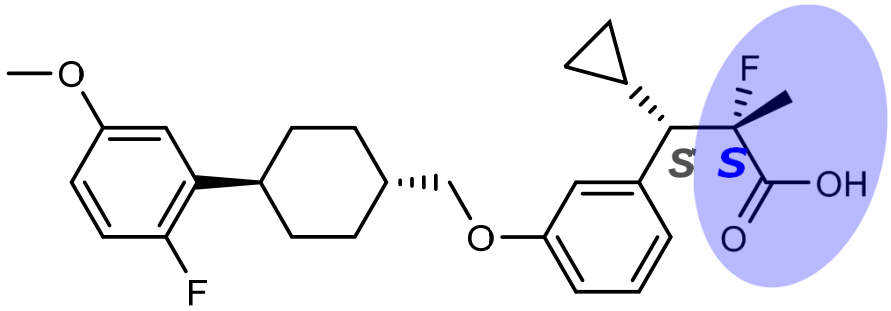
| | hGPR40 (n=) | rGPR40 (n=) |
|---|---|---|
| **JNJ-A** | 0.25 ± 0.13 (6) | 0.34 ± 0.18 (6) |
| **JNJ-0** | 0.94 ± 0.37 (10) | 4.1 ± 2.4 (10) |




*ACS Med. Chem. Lett.* **2019**, *10*, 1, 16-21

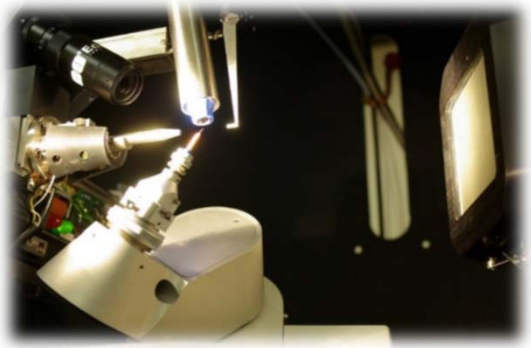
# The Absolute Configuration of α-carbon in JNJ-A




How to determine the absolute configuration of a-carbon: ***R*** or ***S*** ?

# Absolute Configuration: Analytical Techniques



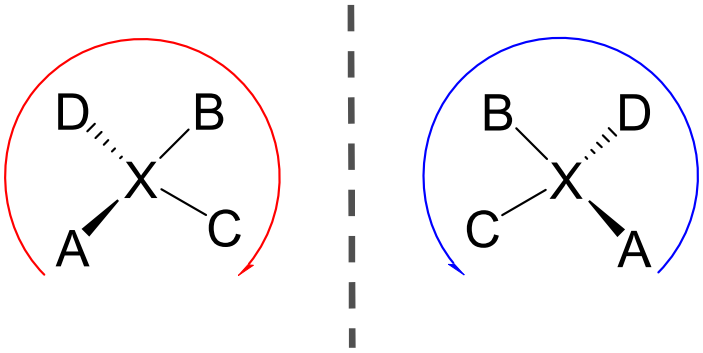
Single-crystal
X-ray Diffraction

Vibrational
Circular Dichroism

Chiral chromatography


NMR

# Absolute Configuration: NMR Methods




the (*R*)/(*S*) notation
Cahn-Ingold-Prelog Rules

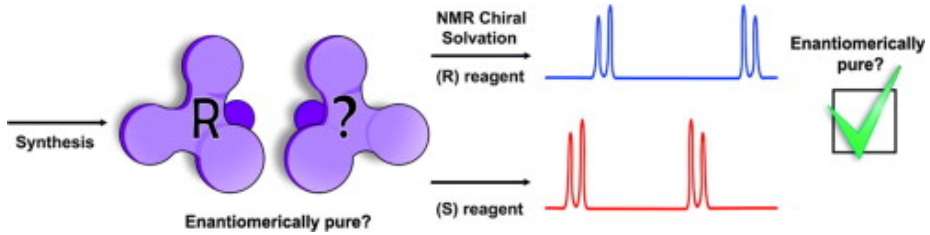
- Chemical Shift
- Coupling Constant
- Nuclear Overhauser Effect
- Computation
- Machine Learning

# Molecular Structure Feature

Single chiral center or remote chiral centers

## Chiral Auxiliary

Chiral derivatizing agent: Mosher's acid
Chiral solvating/shift agent
Alignment medium



# Molecular Structure Feature

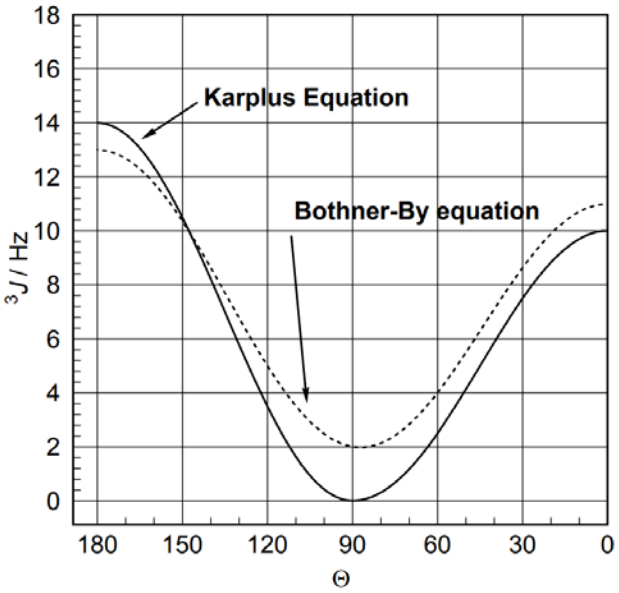
**Scalar Coupling Constant**

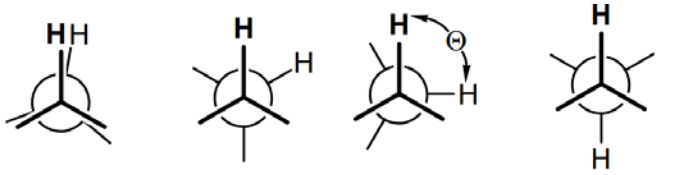
**Nuclear Overhauser Effect**

**Conformational Analysis**

With neighboring predefined chiral center

# Scalar Coupling Constant & the NOE Effect



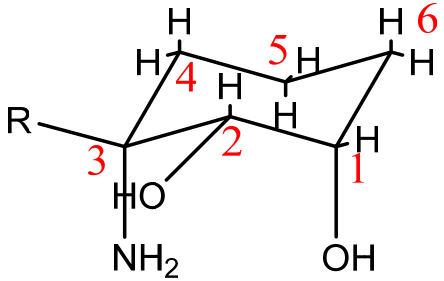

**Karplus Equation**

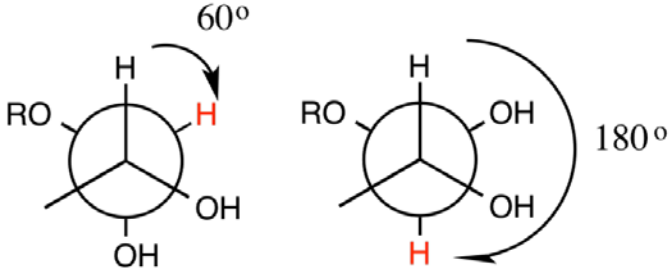
$^3J_{HH} = J_o \cdot \cos^2\Theta - K$

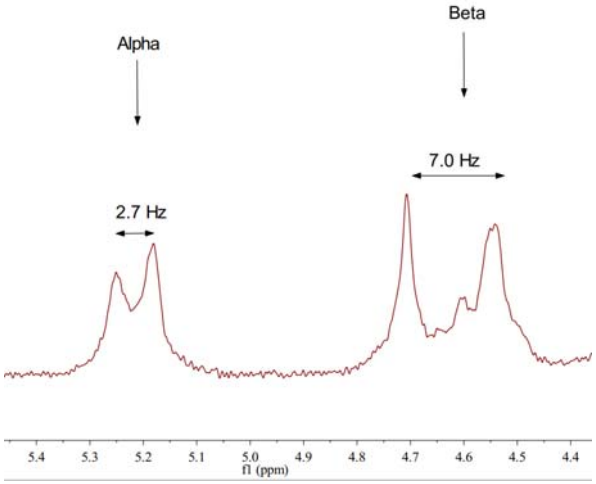
$J_o$ = 14 (90-180°), $J_o$ = 10 (0-90°), K = 0

**Bothner-By equation**

$^3J_{HH} = 7 - \cos\Theta + 5 \cdot \cos 2\Theta$



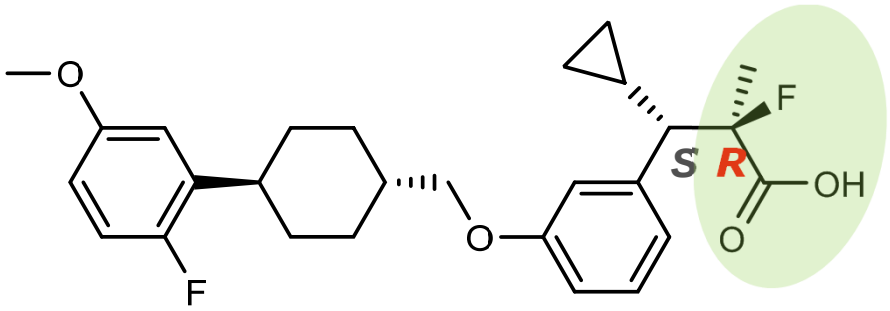



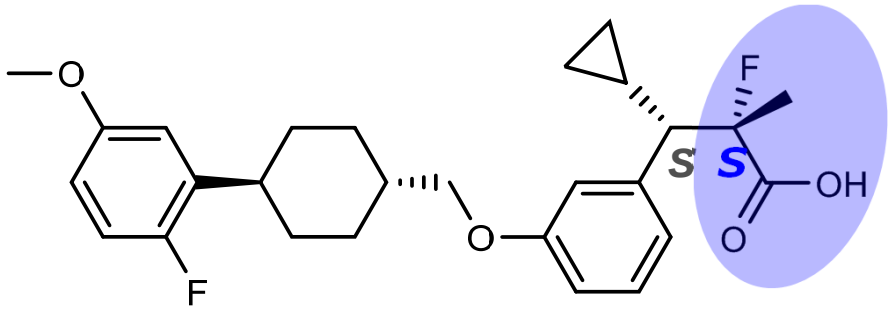




Magritek Application Note 5: Glucose Anomers

# The Absolute Configuration of the α-carbon in JNJ-A

## Acyclic & highly flexible carbon-carbon single bond

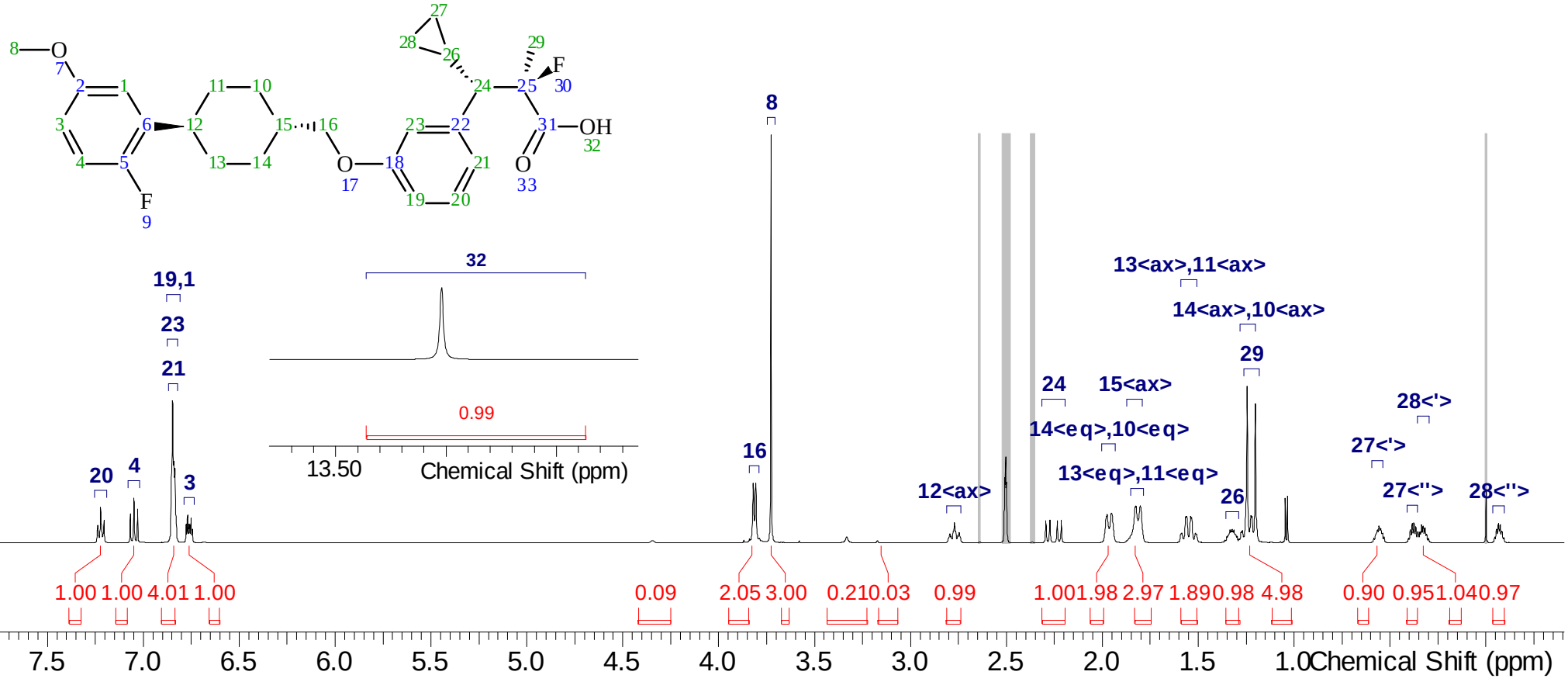


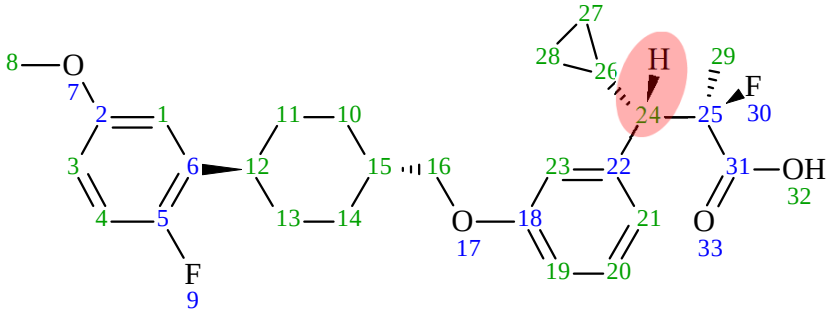

What can we do to determine the absolute configuration of α-carbon: ***R*** or ***S*** ?

Chiral Auxiliary: CDA, CSA, Alignment Medium
Conformational Analysis & Relative Stereochemistry

# 1D $^{1}$H NMR Fully Assigned



# H-F Coupling: Anti-conformer Predominates




Large coupling constant (~30 Hz) between $H_{24}$ and $F_{30}$

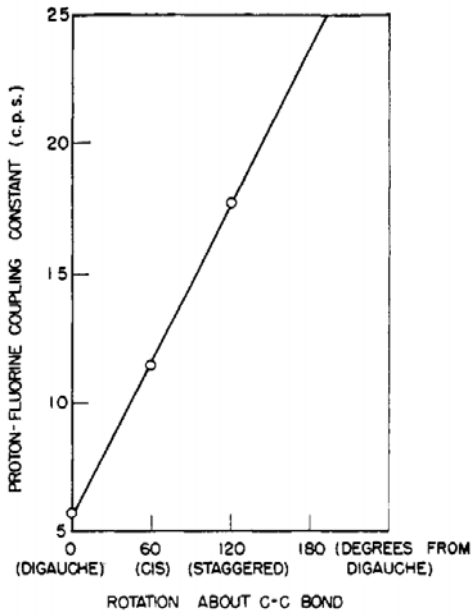
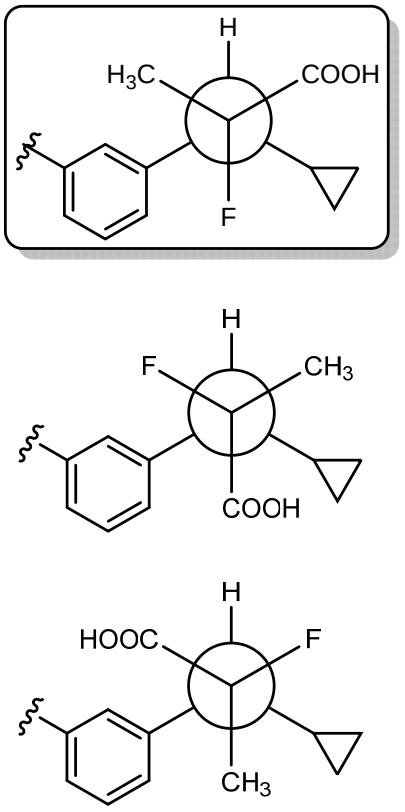


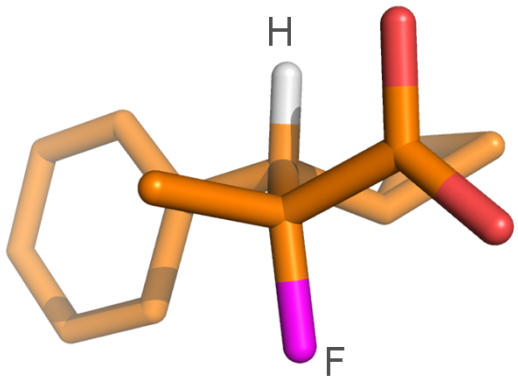

Figure 3. Variation of H-F coupling with dihedral angle

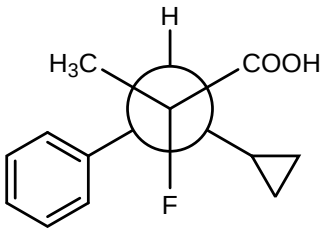
*Anal. Chem.*1965, 37, 3, 403-405



# DFT Calculation: Anti-conformer Predominates

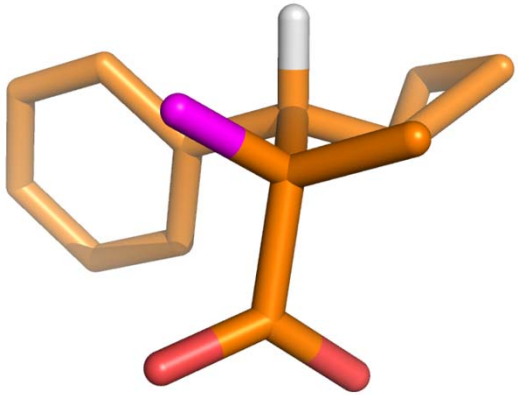
93.1%

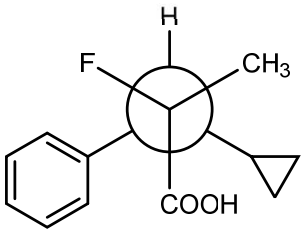



>>

5.5%



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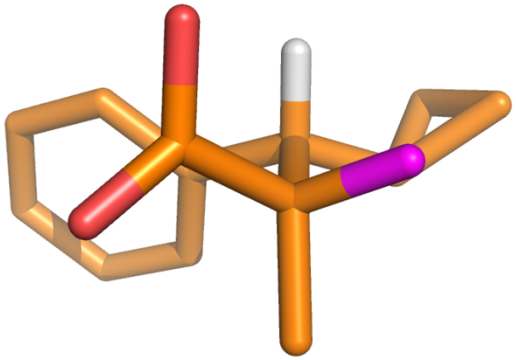
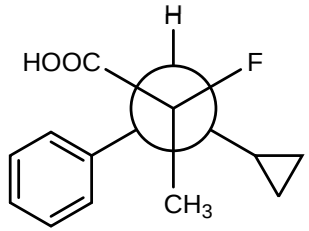
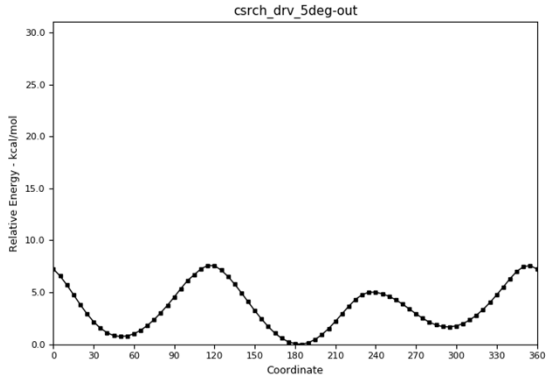
1.3%



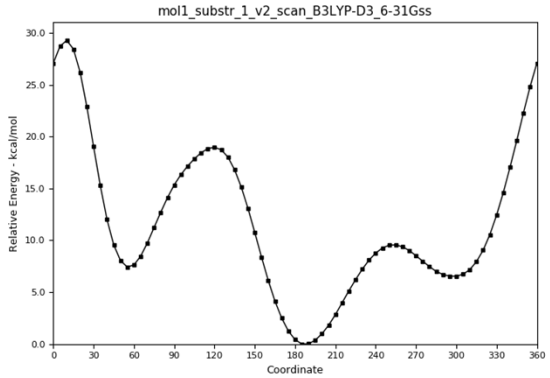



B3LYP-D3/6-31G** optimization of 20 conformers from OPLS3e conformational search
PCM water

# MM / QM Calculation: Torsion / Energy Profiles

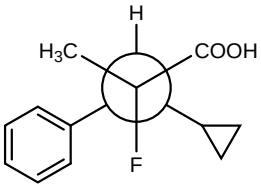


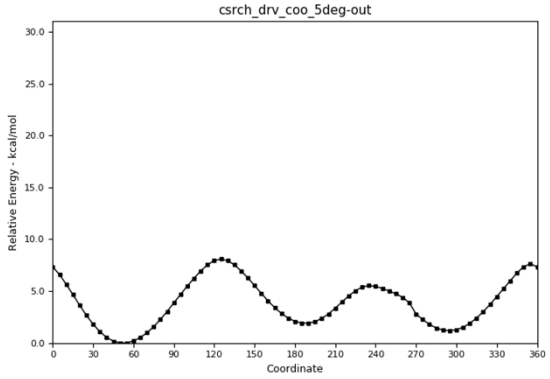

**COOH**



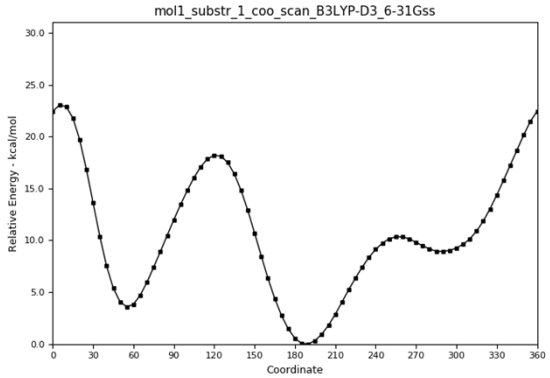

OPLS3e
GB/SA water

DFT
B3LYP-D3
PCM water
(rigid scan)

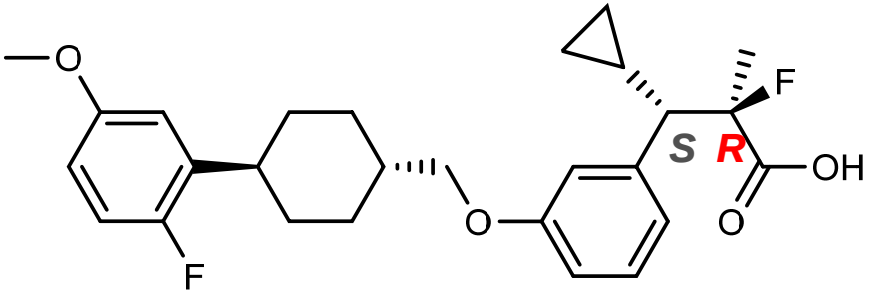



**COO-**

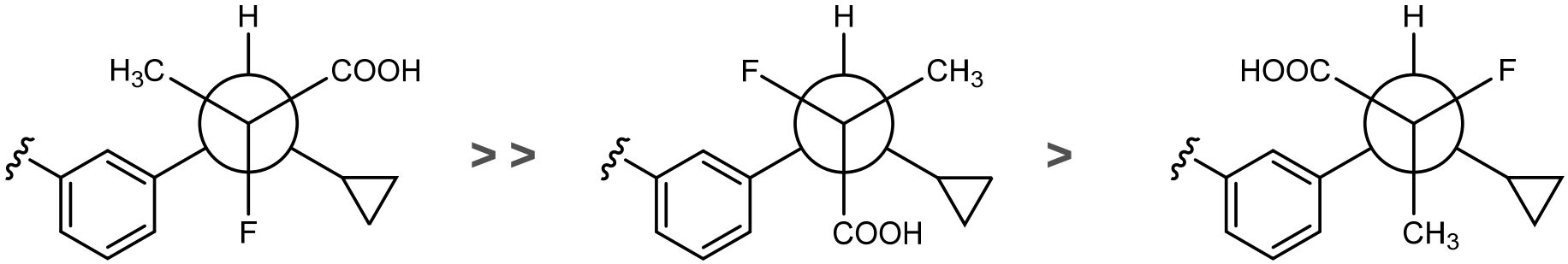


# Why Anti-conformer: Steric Hindrance?

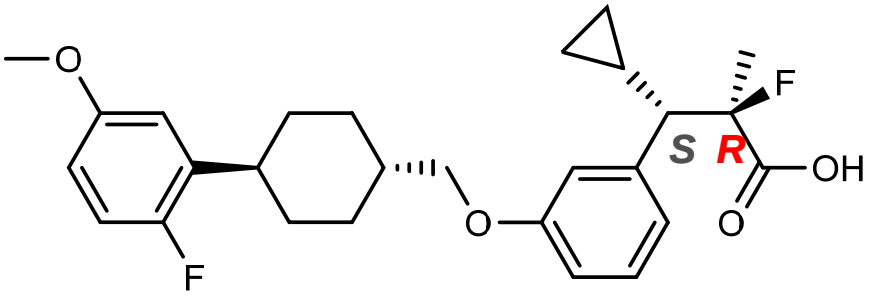


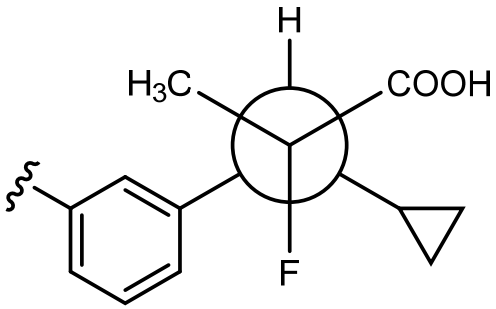

**Relative size (A-value):**

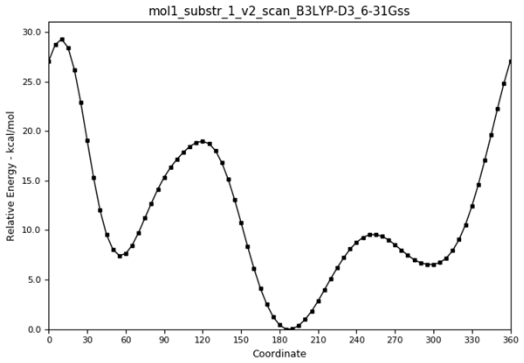
$CH_3$ ~ COOH >> F

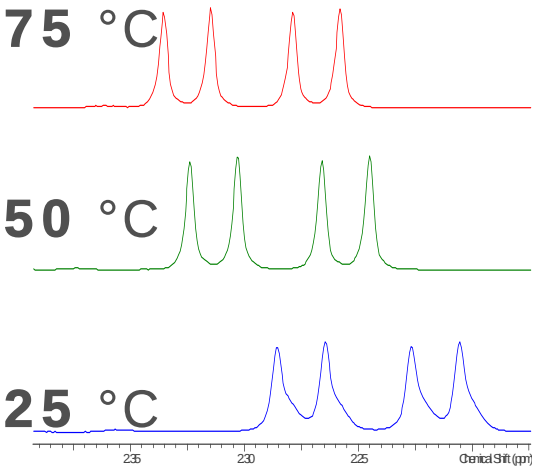


# Steric Hindrance: Energy Barrier?

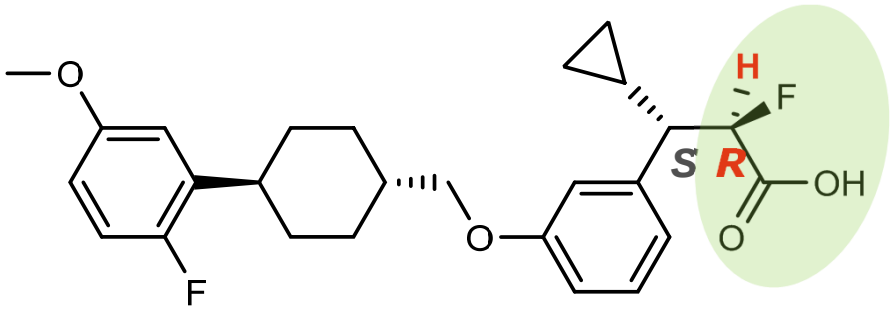


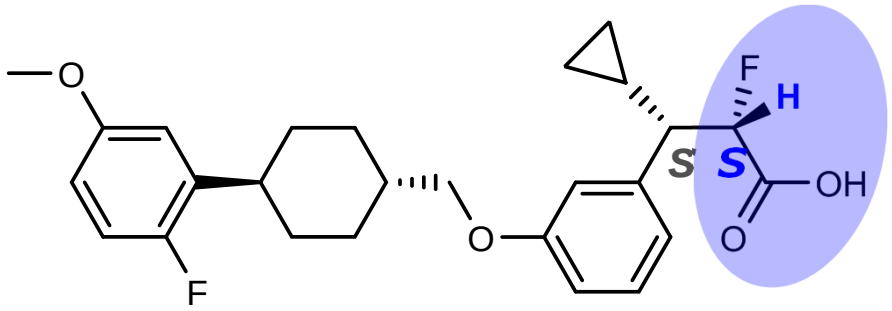



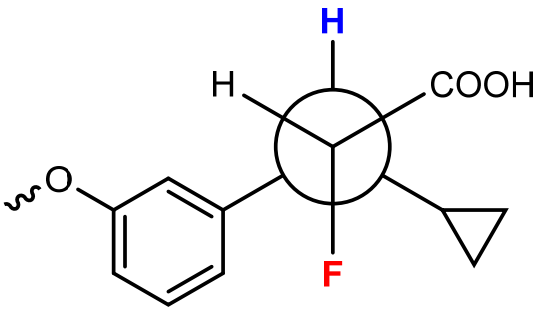
# The Absolute Configuration of the α-carbon in JNJ-B

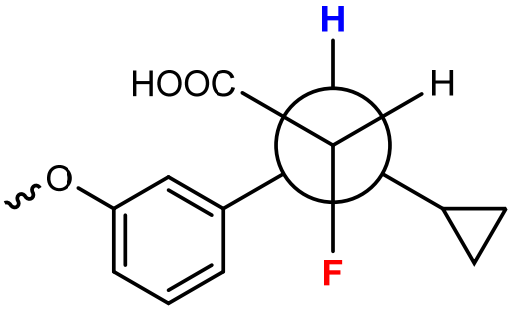


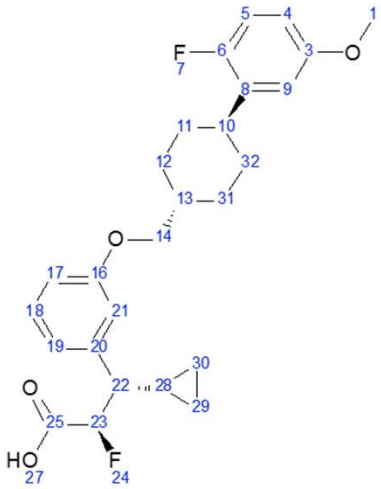



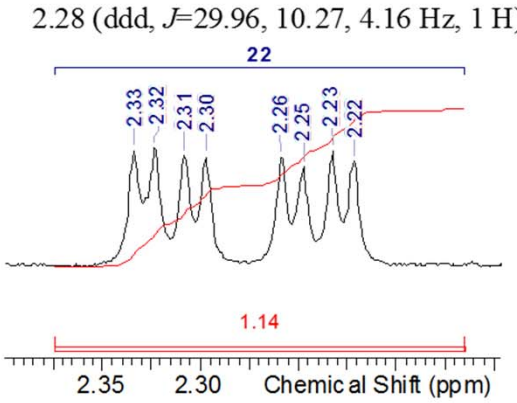

H instead of $CH_3$
How to determine the absolute configuration of α-carbon: ***R*** or ***S*** ?

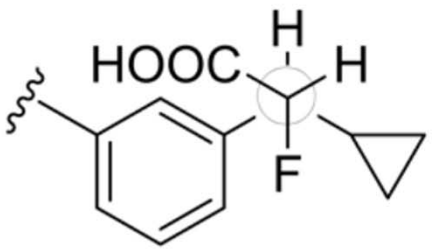
# Anti-conformer: beyond Steric Hindrance



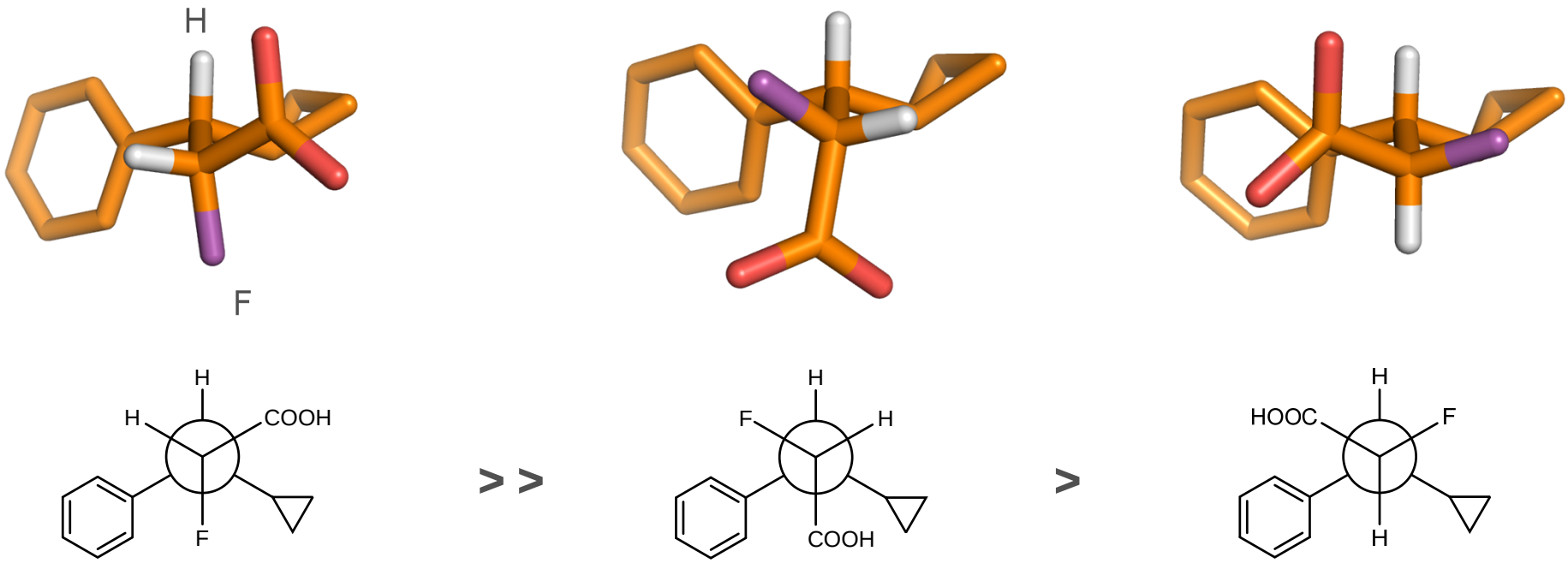



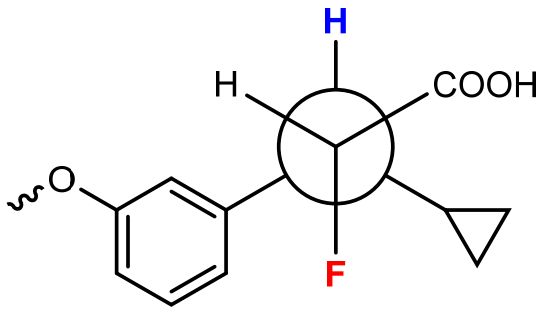
# DFT Calculation: Anti-conformer Predominates

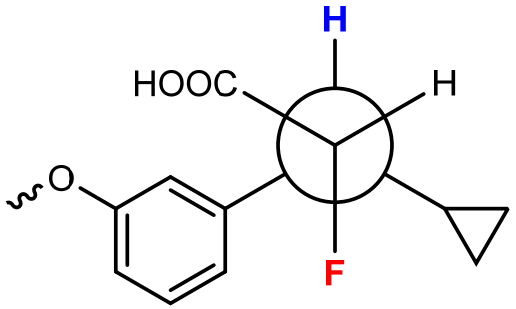
91.7% 7.6% 0.5%




B3LYP-D3/6-31G** optimization of 13 conformers from OPLS3e conformational search
PCM water

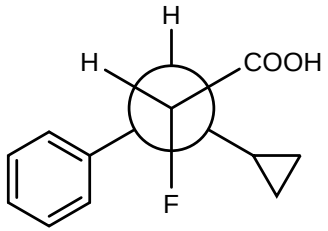
# Anti-conformer: beyond Steric Hindrance




**Further investigation:**

1. What's the driving force for the anti-conformer in this case?
2. What's the role of carboxylic acid?

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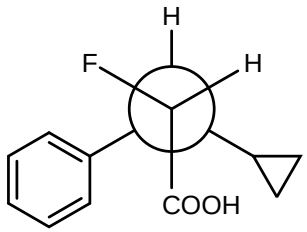


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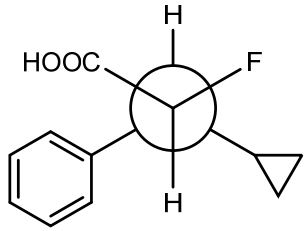
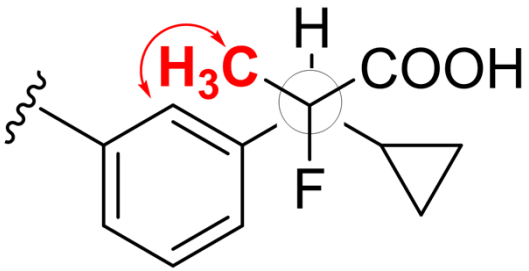
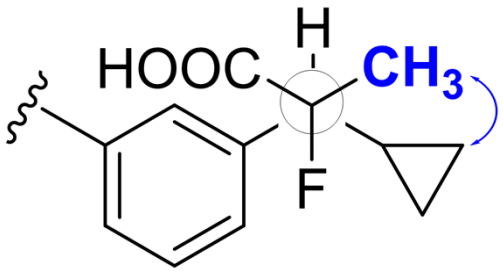


# The Nuclear Overhauser Effect: *R* or *S* at α-carbon

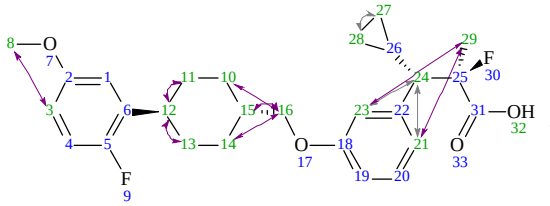


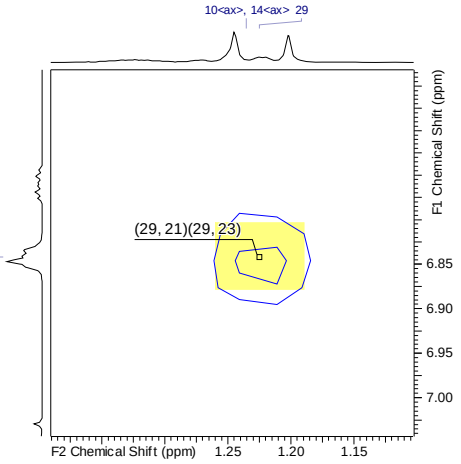

vs

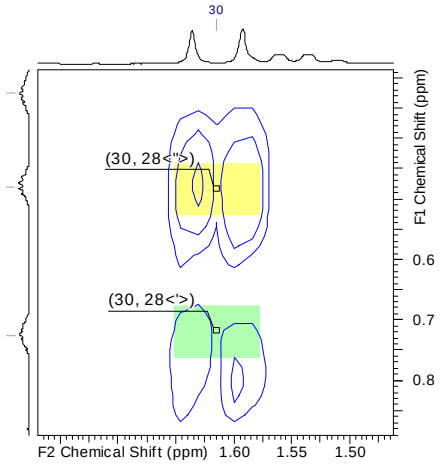


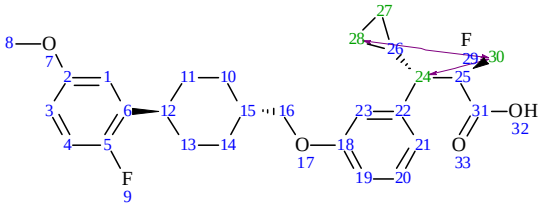

***R*-configuration**

***S*-configuration**

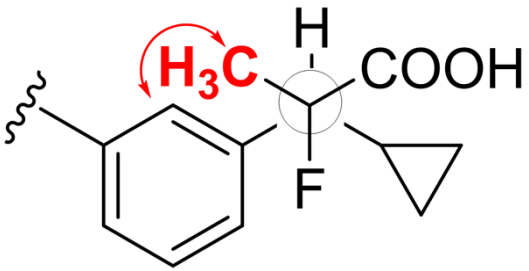




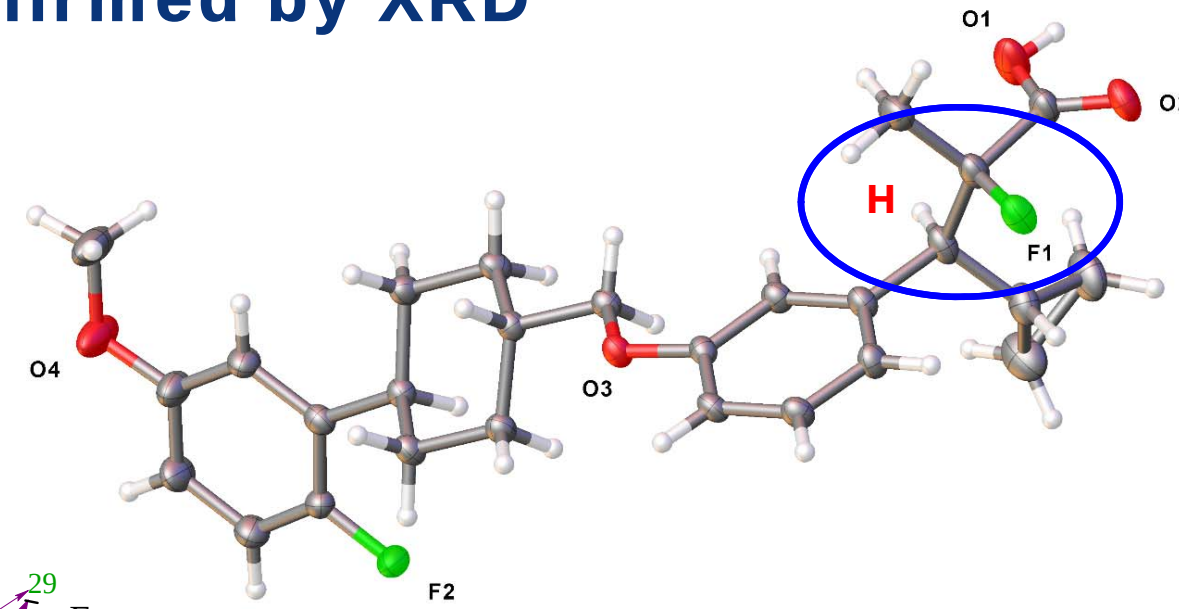



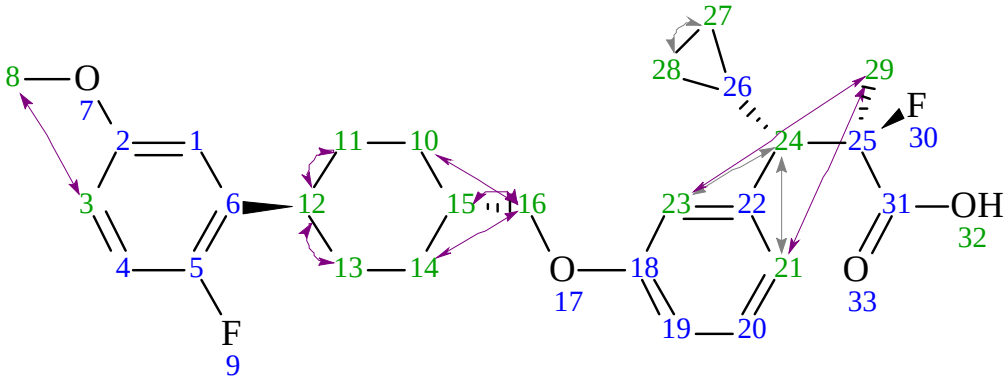



# *R*-configuration Confirmed by XRD




***R***-configuration




XRD Obtained on a Bruker D8 Platinum[135] CCD diffractometer equipped with Cu $K_a$ radiation (l = 1.5478) at UCSD Crystallography Lab.

# Summary

1. The absolute configuration: NMR consistent with XRD
2. The steric hindrance around the α- & β-carbons locks the anti-conformation between the β-proton $H_{24}$ and fluorine
3. Key information: scalar coupling constant and NOE
4. MM/QM: energy profile and population distribution
5. NMR: convenient and reliable alternative to XRD

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