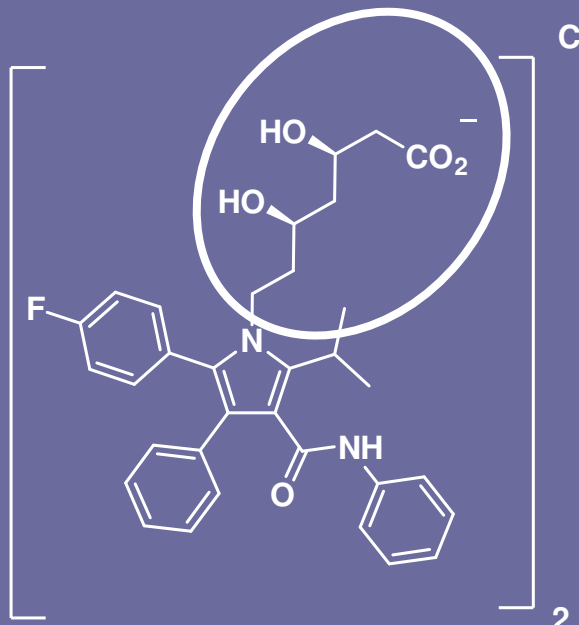
The Story of LIPITOR® - *A Peek into the World of Pharmaceutical Process Chemistry*



Atorvastatin calcium
LIPITOR®

Chemical Synthesis

LIPITOR® – $12 billion/year sales (2005)

Chiral side chain (circled) – 220 ton/year

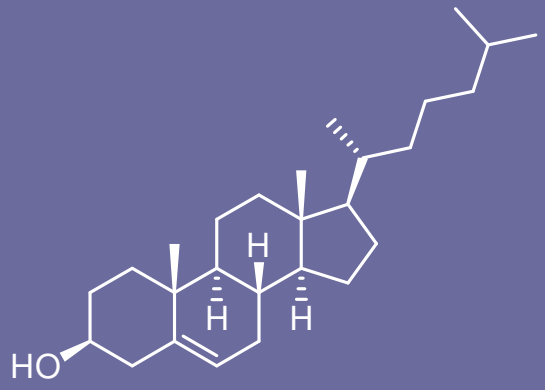
Biocatalysis

Aman Desai

7th Feb. 2007

The Problem – The "Bad" Cholesterol

- Cholesterol – a very important biological molecule.
- Most cholesterol is not dietary, it is synthesized internally.
- Cholesterol is bound to lipoproteins and transported through blood.
- 2 kinds of lipoproteins
  - High Density Lipoprotein (HDL) – "good"
  - Low Density Lipoprotein (LDL) – "bad"



Cholesterol

↓

atherosclerosis

↓

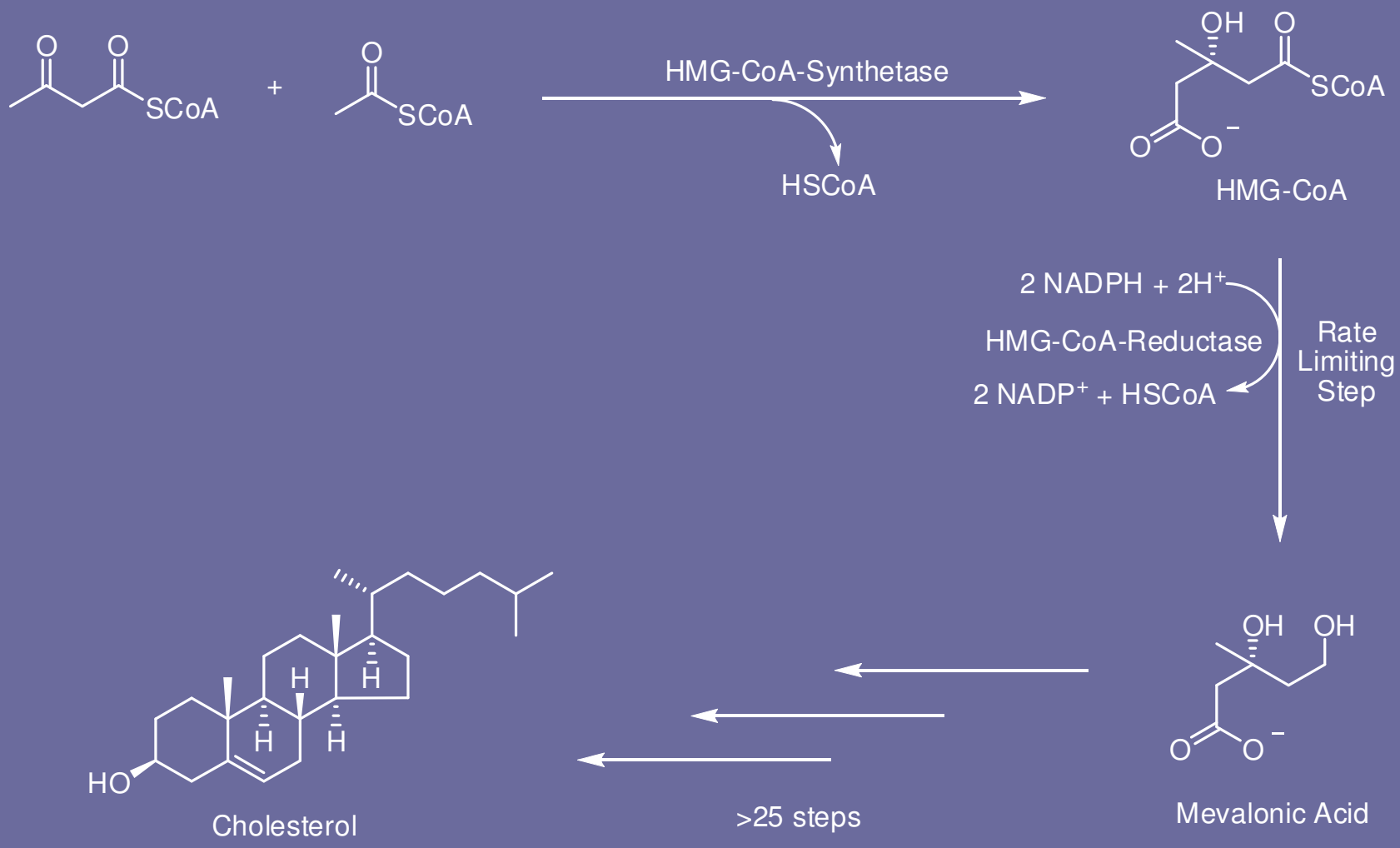
coronary heart disease & other cardiovascular diseases

↓

One of the leading causes of death in the world today!

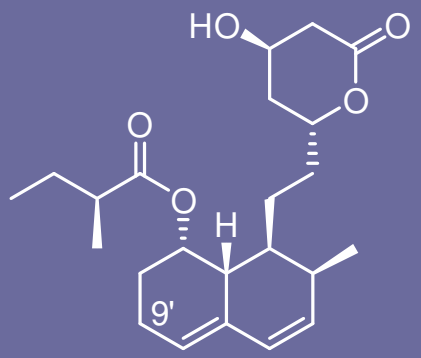
www.wikipedia.org
www.americanheart.org

The Solution – Suppressing Cholesterol Biosynthesis

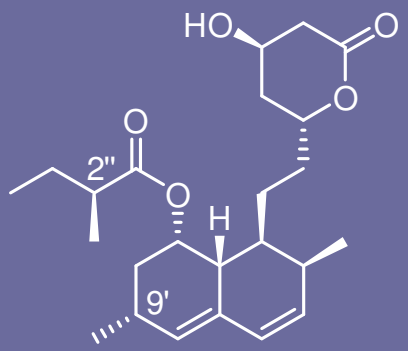


http://www.med.unibs.it/~marchesi/cholest.html

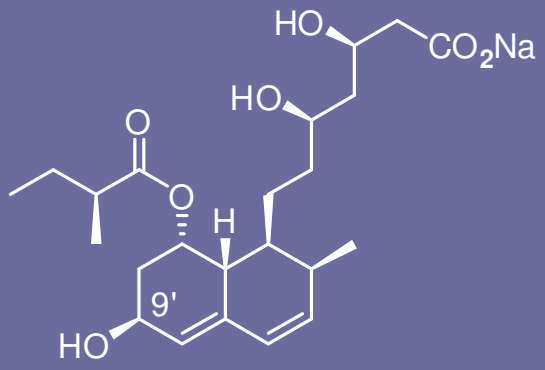
The Solution – Suppressing Cholesterol Biosynthesis



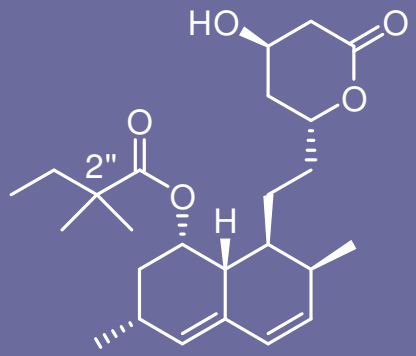
Mevastatin



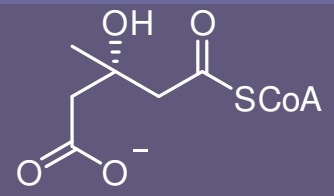
Lovastatin (MEVACOR®)
MERCK



Pravastatin (PRAVACOL®)
BRISTOL - MYERS SQUIBB



Simvastatin (ZOCOR®)
MERCK



HMG-CoA

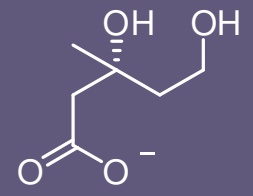
2 NADPH + 2H$^+$

HMG-CoA-Reductase

2 NADP$^+$ + HSCoA

Rate Limiting Step

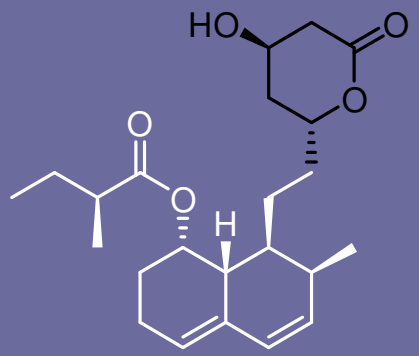
Inhibition



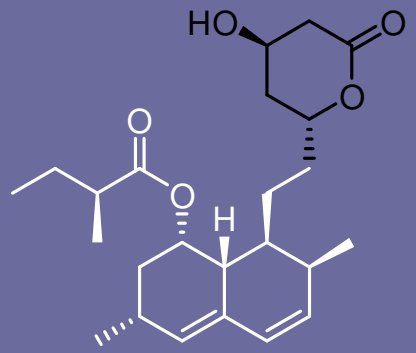
Mevalonic Acid

Endo, A. *J. Lipid Res.* **1992**, *33*, 1569-1582.
Roth, B. D. *Prog. Med. Chem.* **2002**, *40*, 1-22.

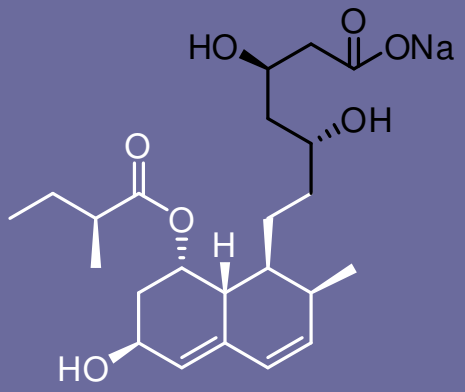
Mechanism of Action of Statin Drugs



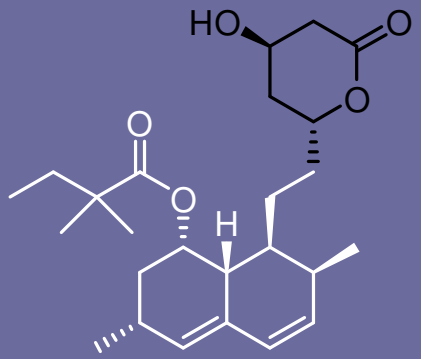
Mevastatin



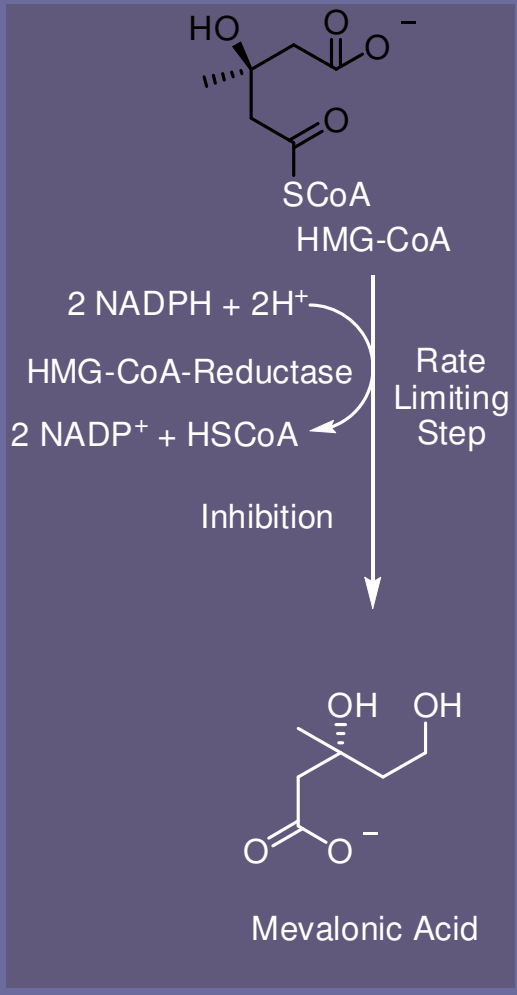
Lovastatin (MEVACOR®)
MERCK



Pravastatin (PRAVACOL®)
BRISTOL - MYERS SQUIBB

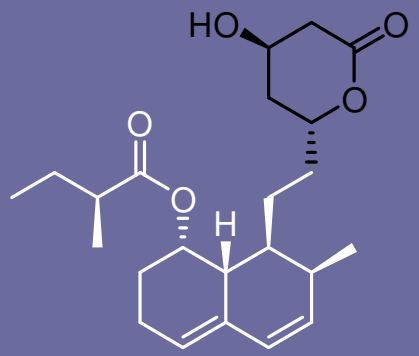


Simvastatin (ZOCOR®)
MERCK

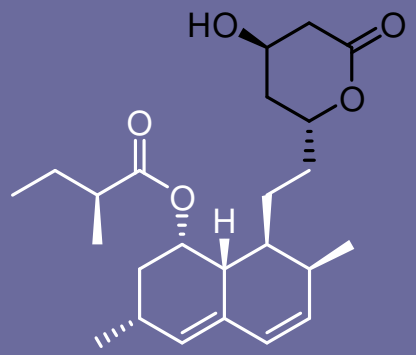


http://www.med.unibs.it/~marchesi/cholest.html

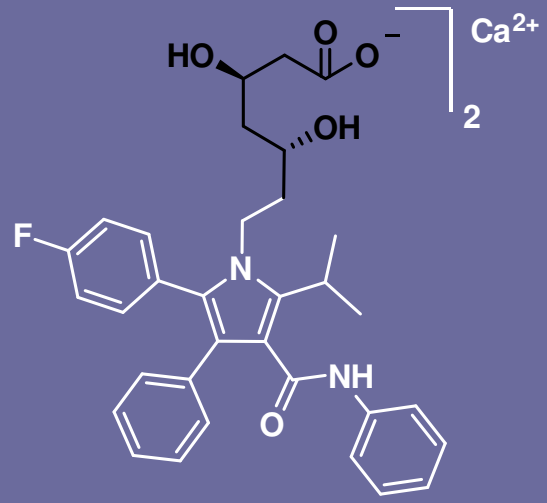
Mechanism of Action of Statin Drugs



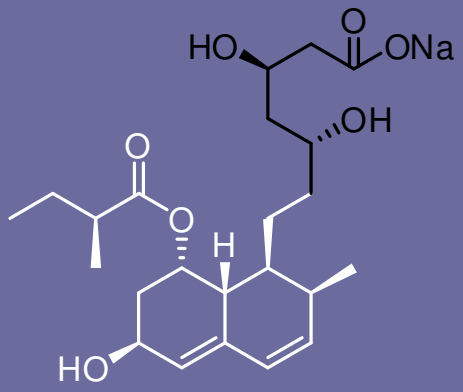
Mevastatin



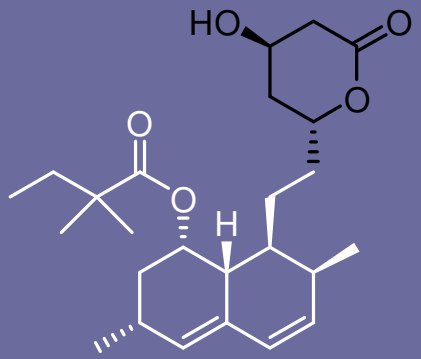
Lovastatin (MEVACOR®)
MERCK



Atorvastatin calcium (LIPITOR®)
PFIZER



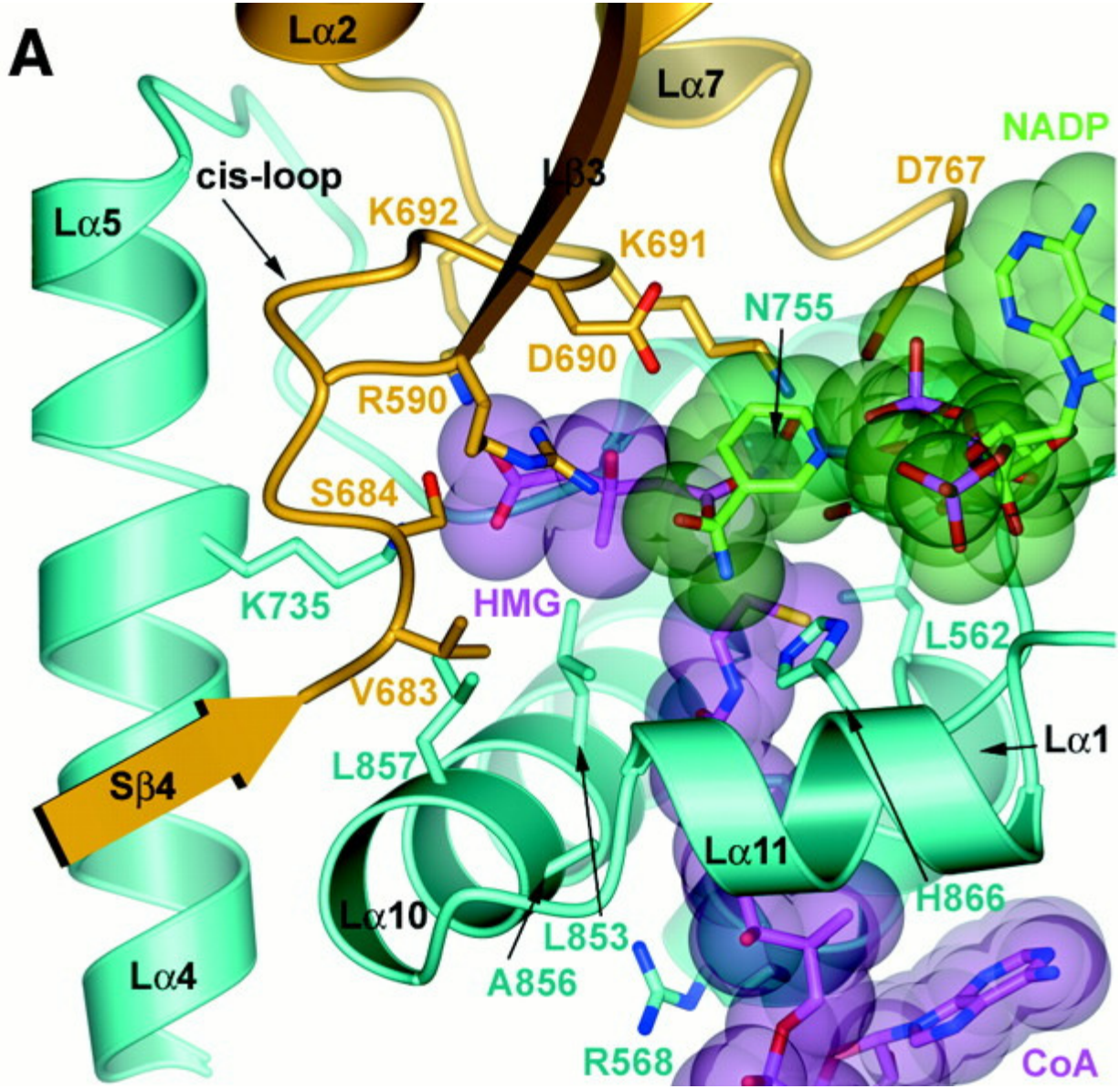
Pravastatin (PRAVACOL®)
BRISTOL - MYERS SQUIBB

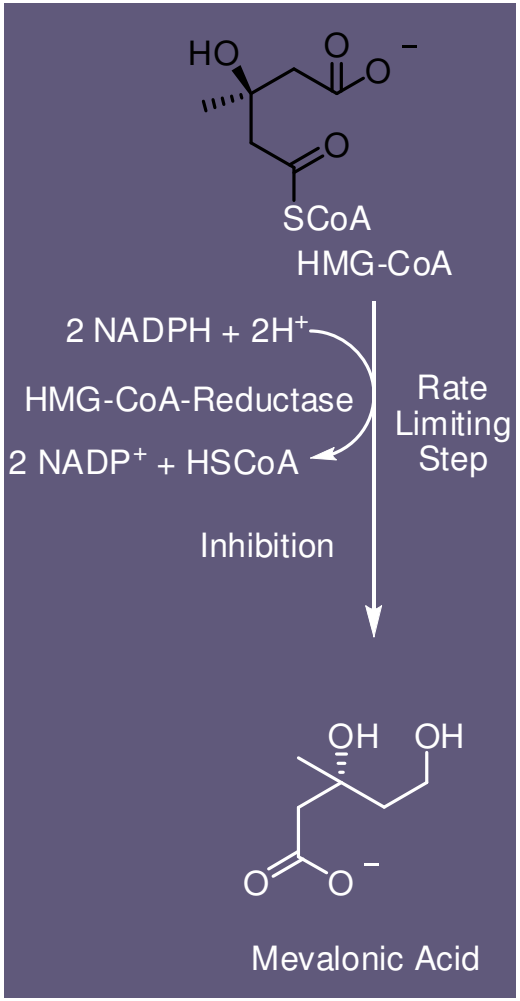


Simvastatin (ZOCOR®)
MERCK

http://www.med.unibs.it/~marchesi/cholest.html

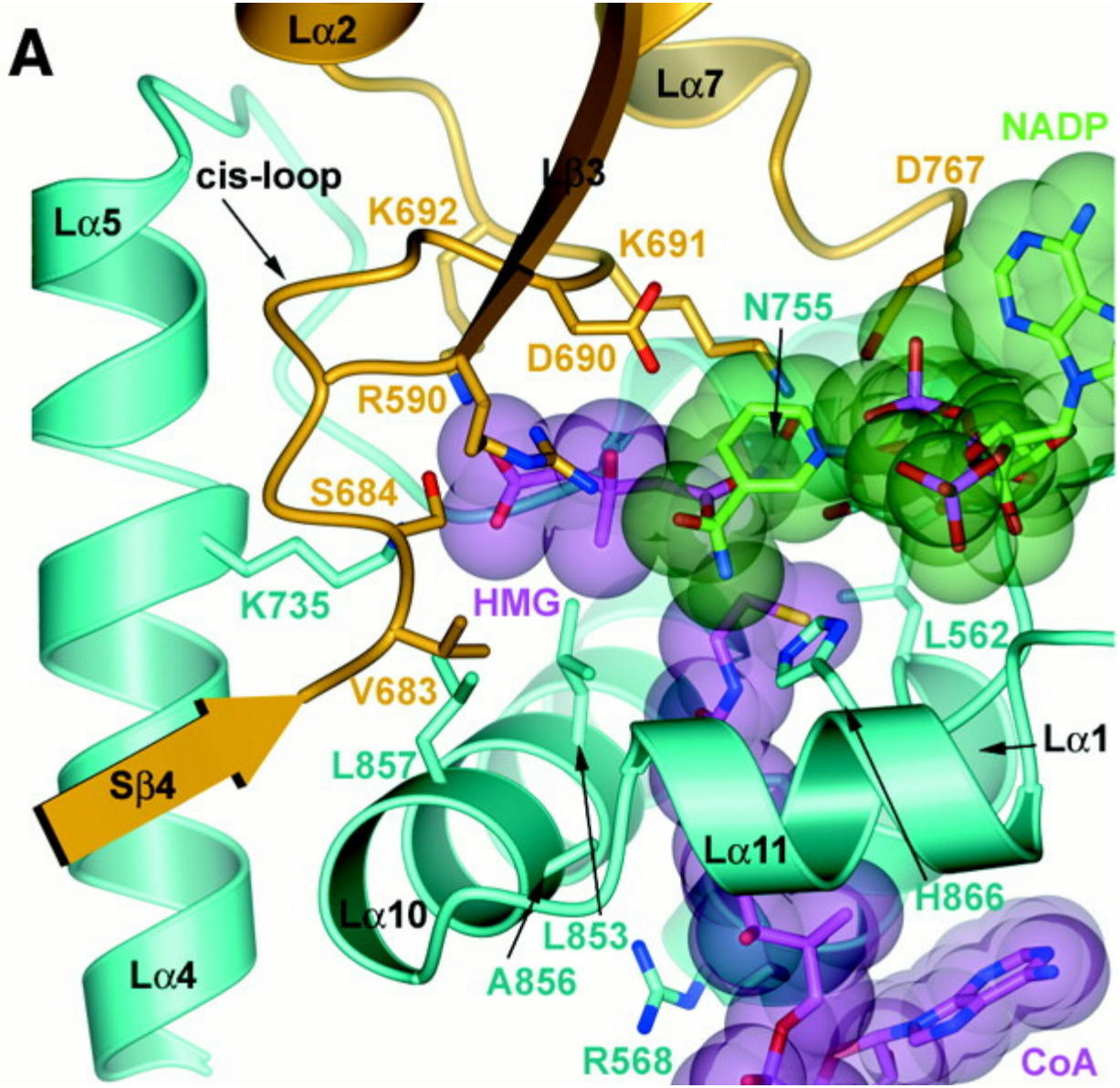
Human HMGR with Natural Substrates

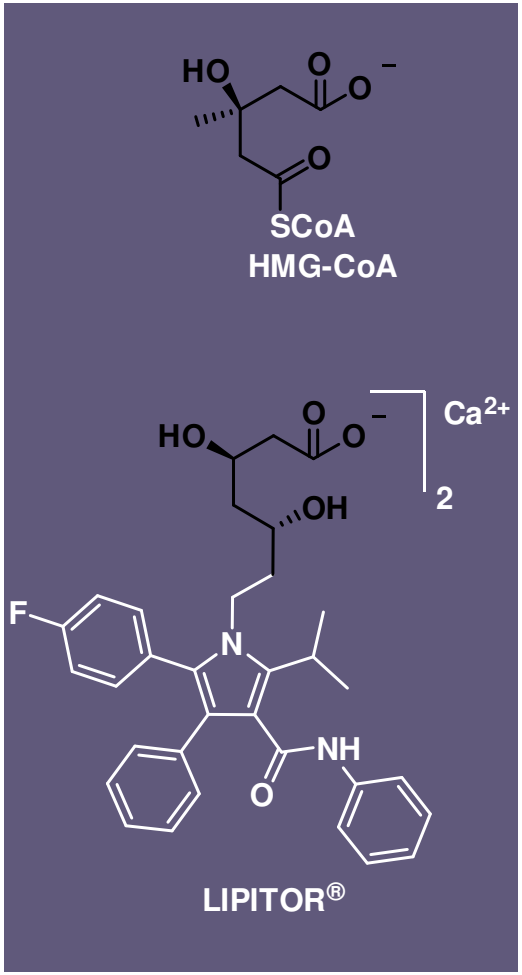




Istvan, E. S.; Deisenhofer, J. *Science* **2001**, *292*, 1160-1164.

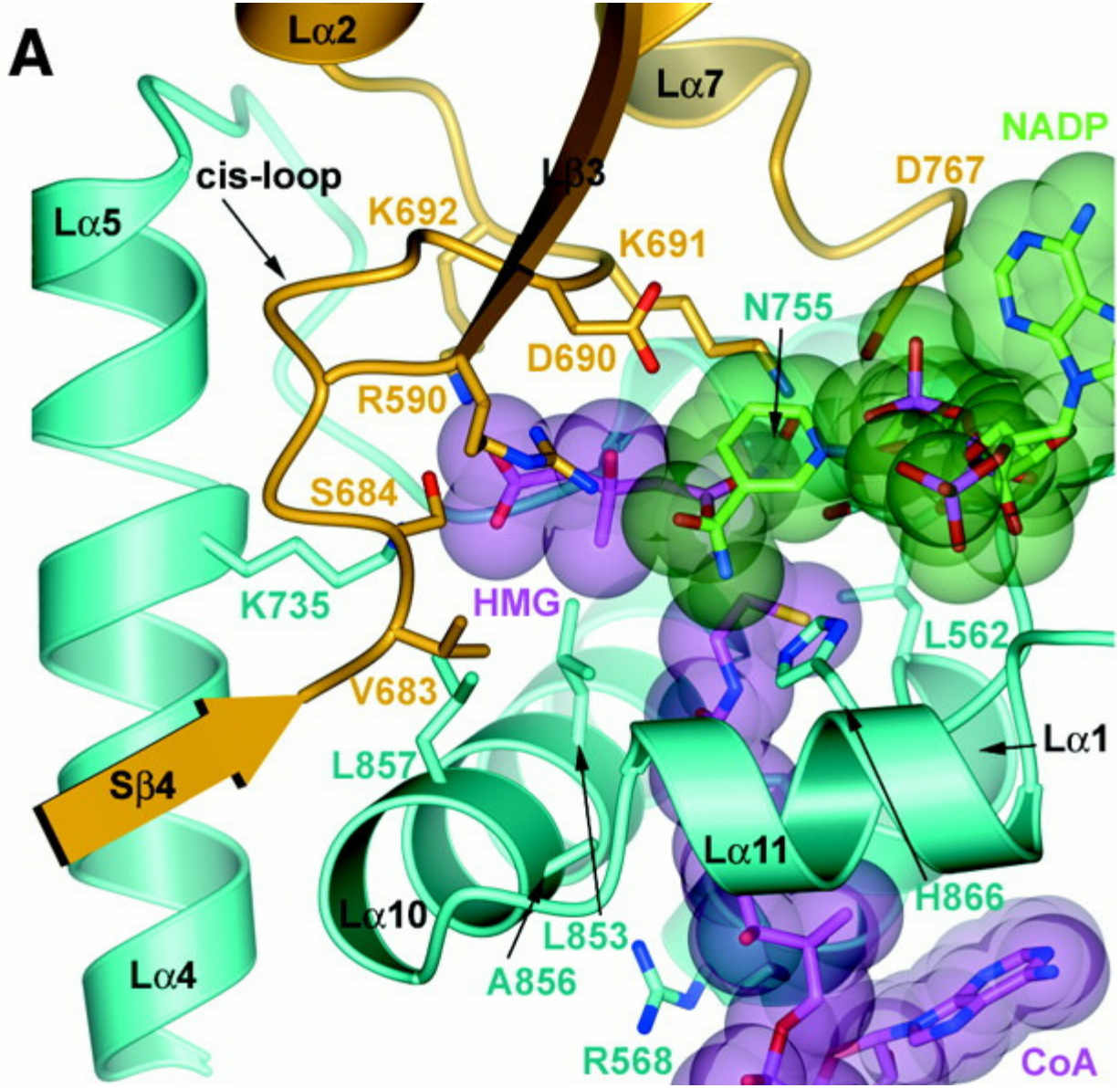
Human HMGR with Natural Substrates

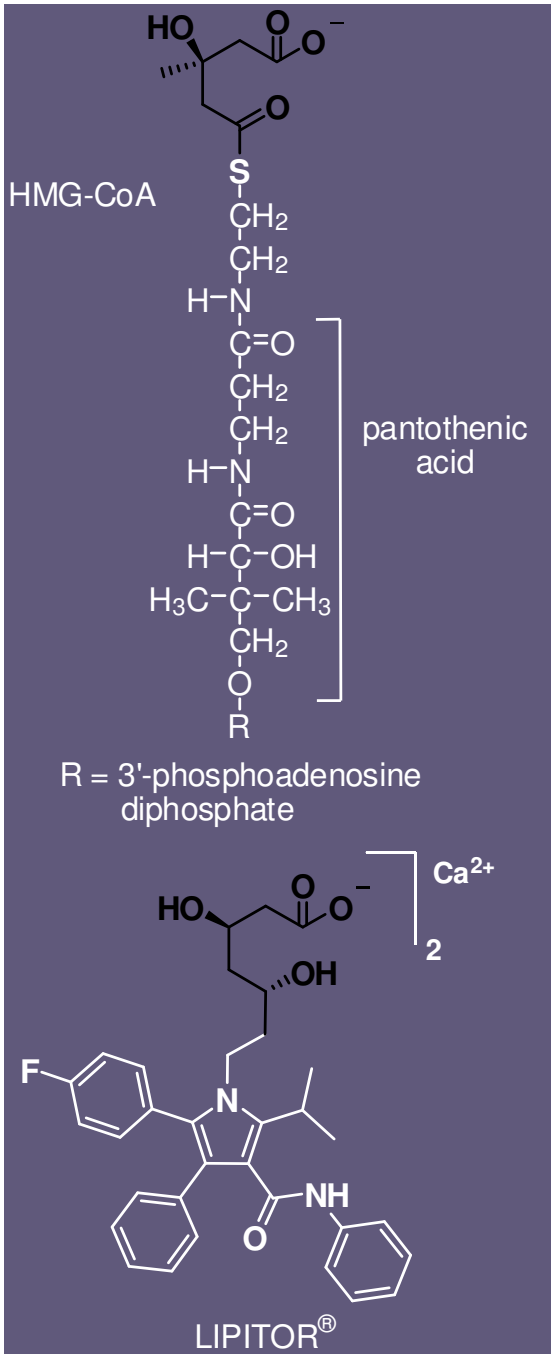




Istvan, E. S.; Deisenhofer, J. *Science* **2001**, *292*, 1160-1164.

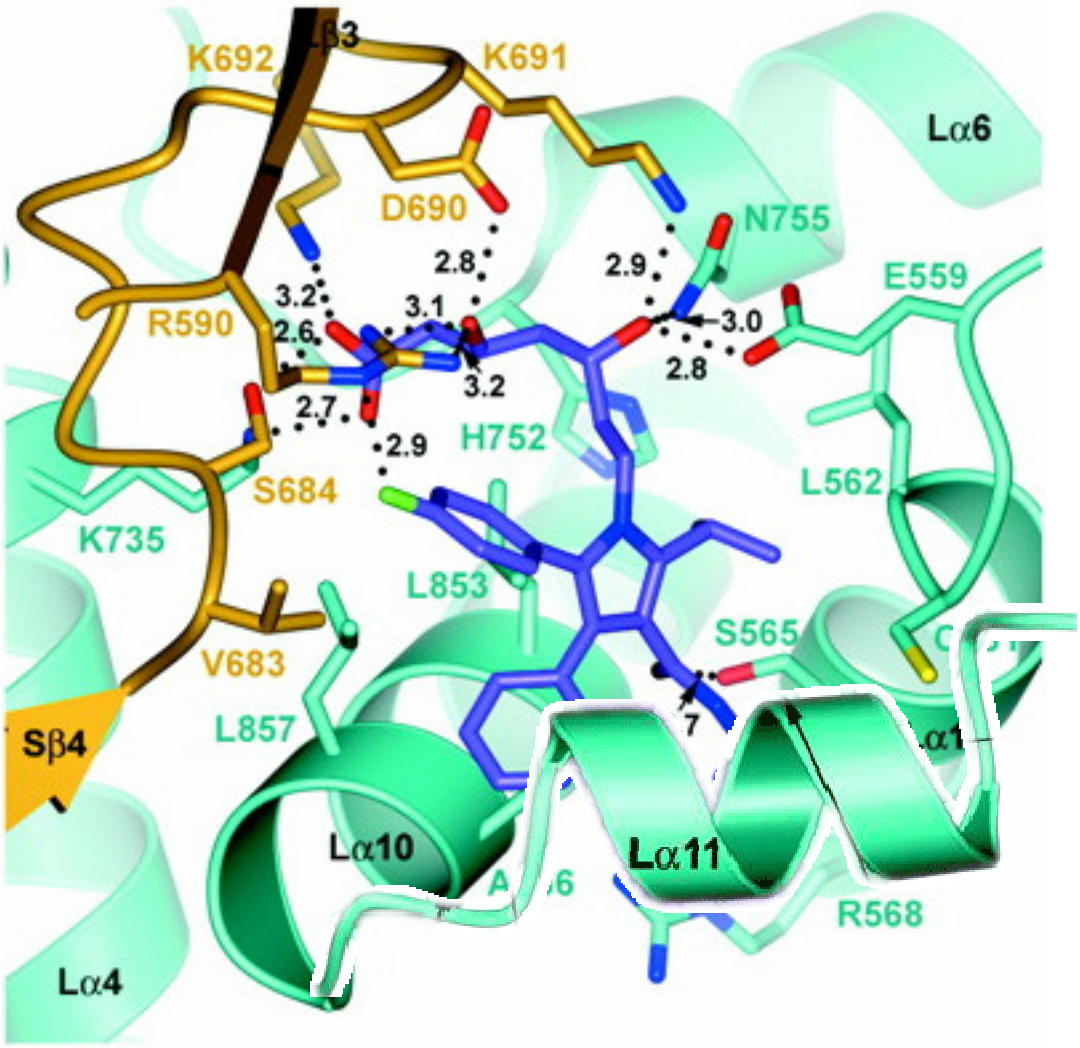
Human HMGR with Natural Substrates

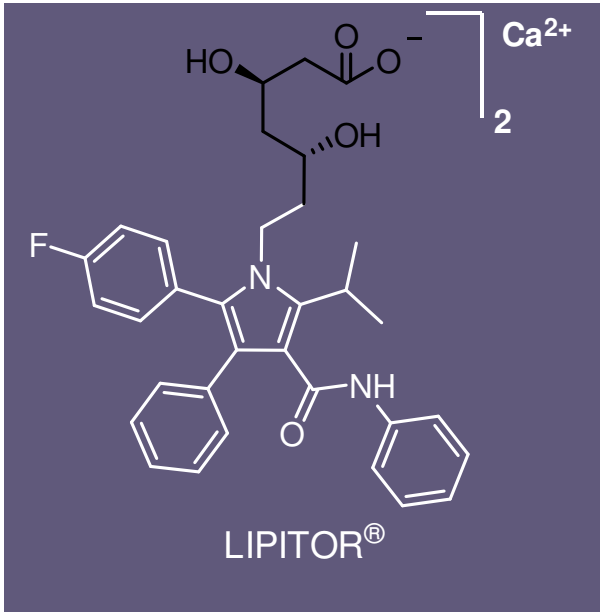




Istvan, E. S.; Deisenhofer, J. *Science* **2001**, *292*, 1160-1164.

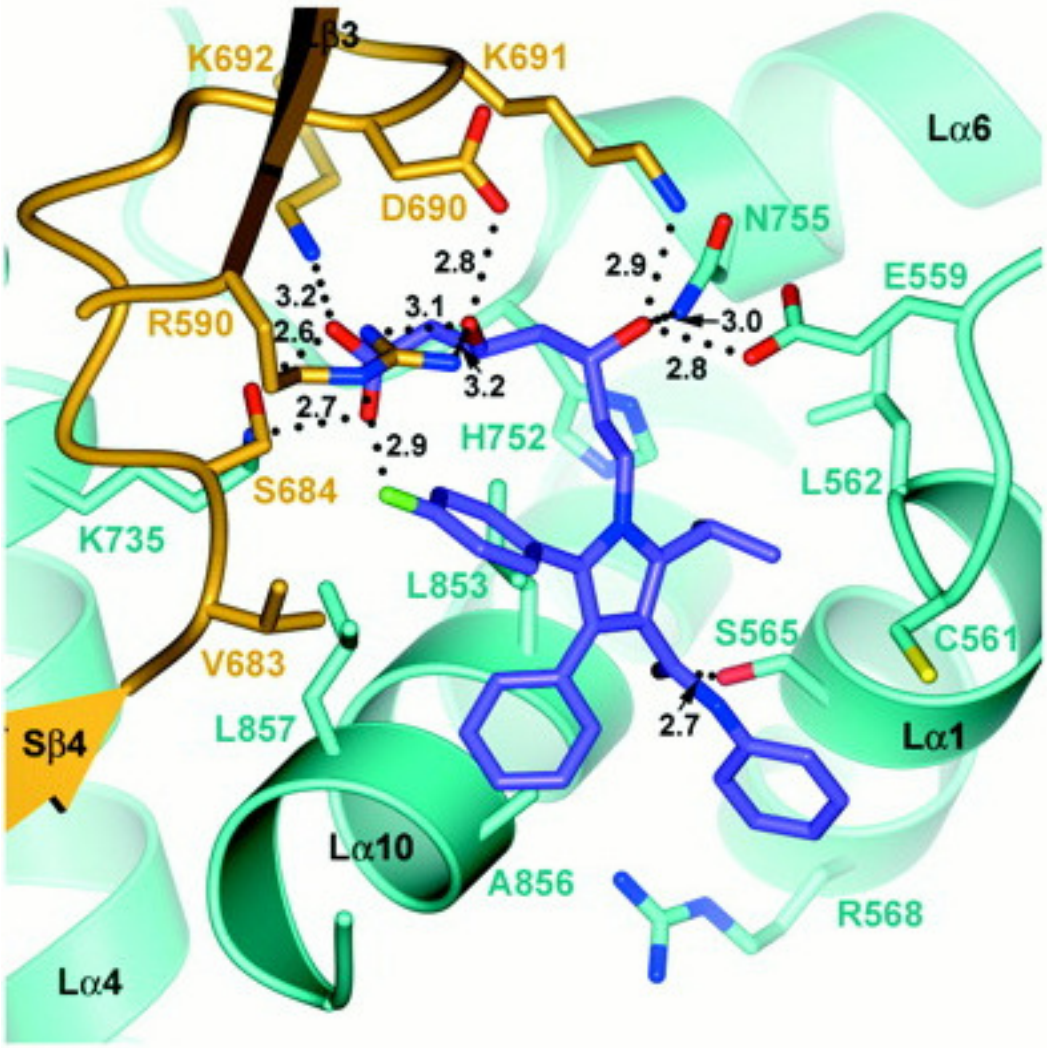
Human HMGR with LIPITOR®

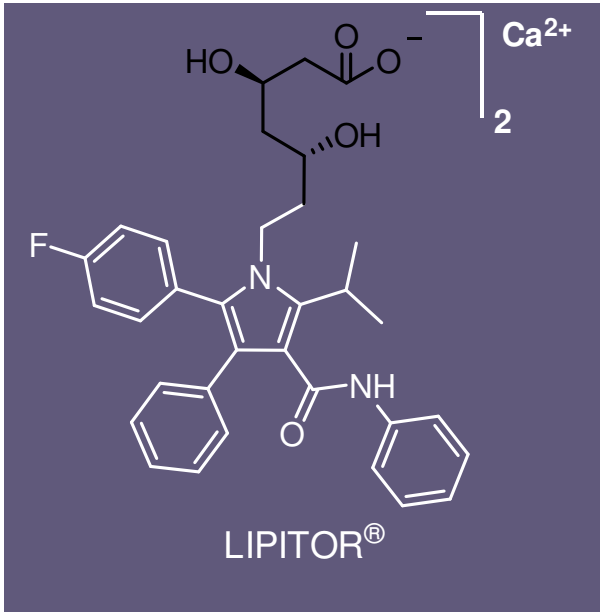




Istvan, E. S.; Deisenhofer, J. *Science* **2001**, *292*, 1160-1164.

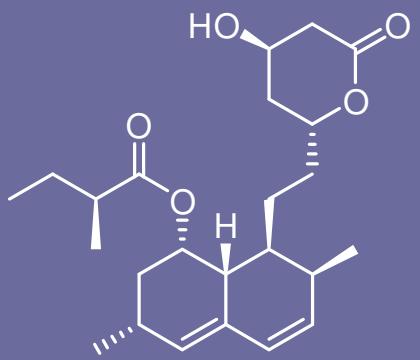
Human HMGR with LIPITOR®



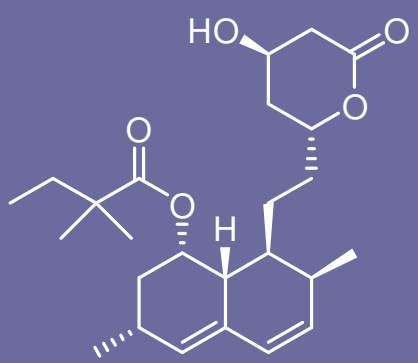


Istvan, E. S.; Deisenhofer, J. *Science* **2001**, *292*, 1160-1164.

Circa 1995 – The Statin Drugs Market



Lovastatin (MEVACOR®)
MERCK

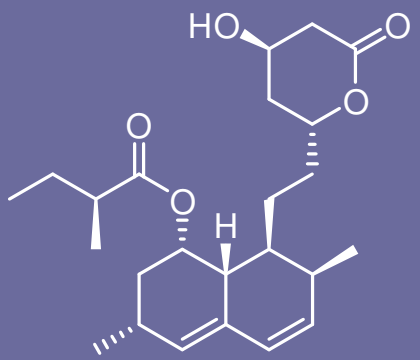


Simvastatin (ZOCOR®)
MERCK

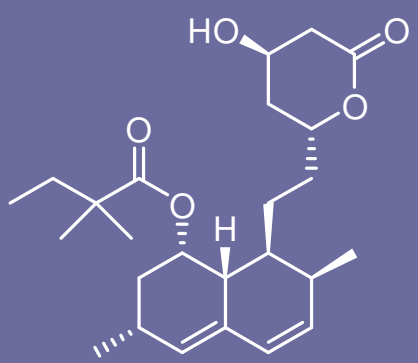
- Merck = cholesterol control
- At 20 mg, ZOCOR® lowered LDL by -29%.

Thayer, A. M. *Chem. Eng. News,* **2006**, *84*, 33, 26-27.
Jones P.; Kafonek, S.; Laurora, I.; Hunninghake, D. *Am. J. Cardiol.* **1998**, *81*, 582-587.

Circa 1995 – The Statin Drugs Market



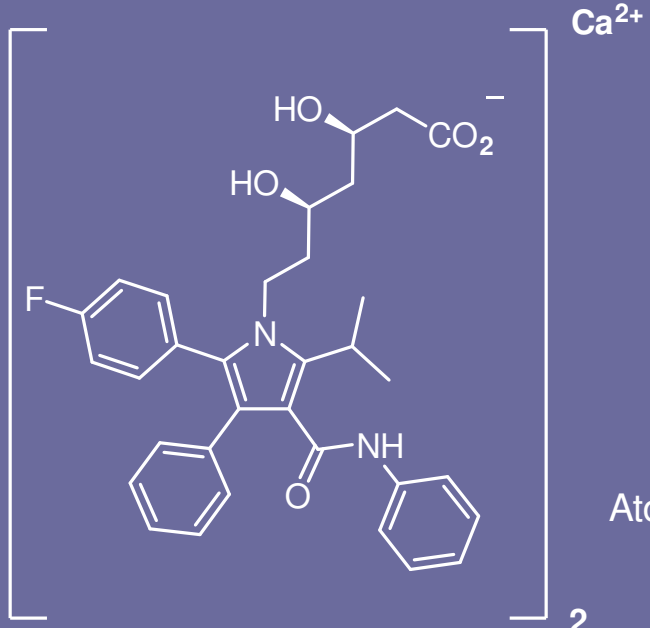
Lovastatin (MEVACOR®)
MERCK



Simvastatin (ZOCOR®)
MERCK

- Merck = cholesterol control.
- At 20 mg, ZOCOR® lowered LDL by -29%.

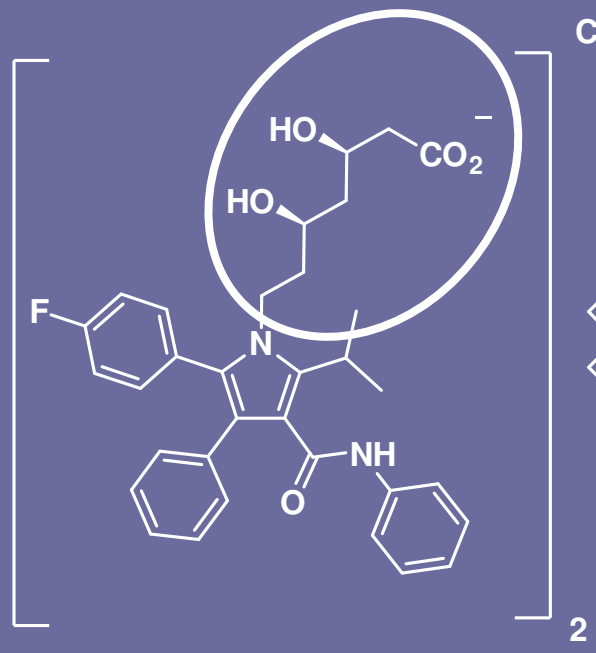
Spring 1997 – Pfizer launches LIPITOR®!



Atorvastatin calcium
LIPITOR®

- At 20 mg, LIPITOR® lowered LDL by -46%.
- 2005 – $12 billion sales, used by over 45 million people.

The Story of LIPITOR® - *a Peek into the World of Pharmaceutical Process Chemistry*



Atorvastatin calcium
LIPITOR®

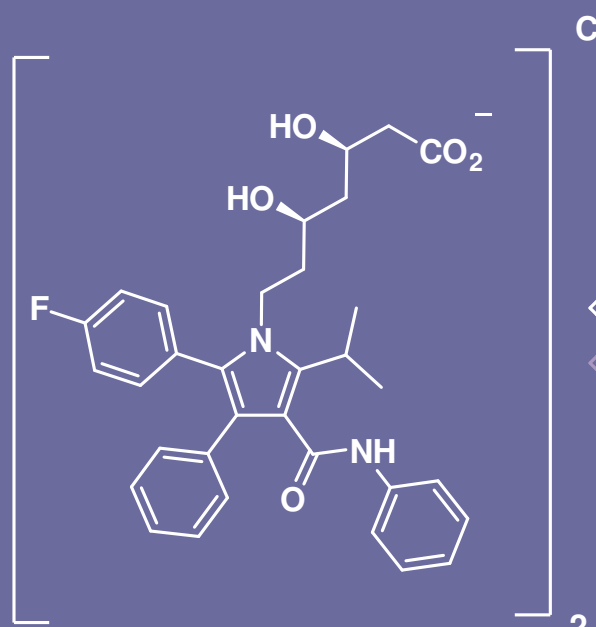
Chemical Synthesis

Biocatalysis

Drug Discovery

Process Development

The Story of LIPITOR® - *a Peek into the World of Pharmaceutical Process Chemistry*



Atorvastatin calcium
LIPITOR®

Chemical Synthesis

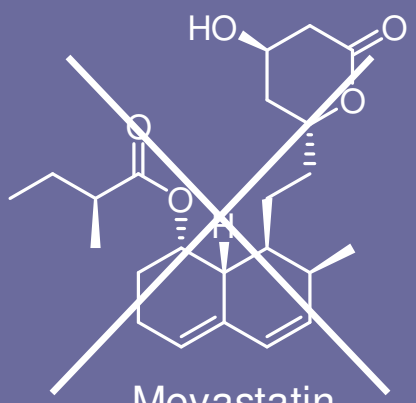
Drug Discovery

Process Development

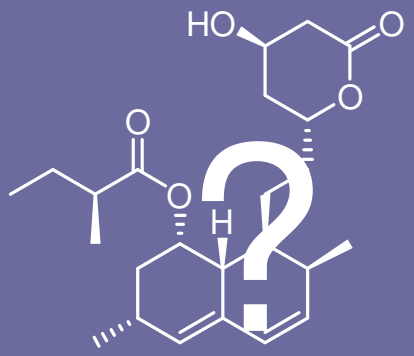
Biocatalysis

The Drug Discovery

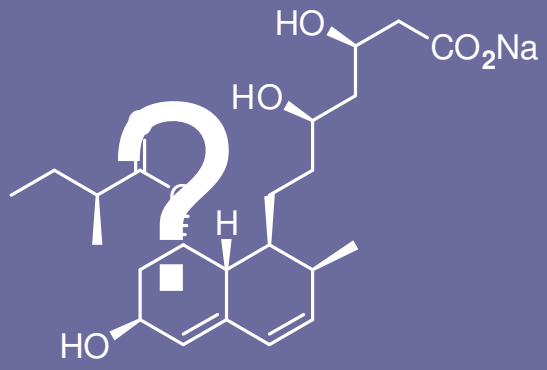
The Decision of the Core Template



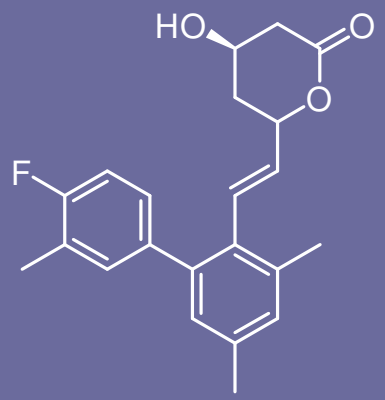
Mevastatin



Lovastatin (MEVACOR®)
MERCK



Pravastatin (PRAVACOL®)
BRISTOL - MYERS SQUIBB

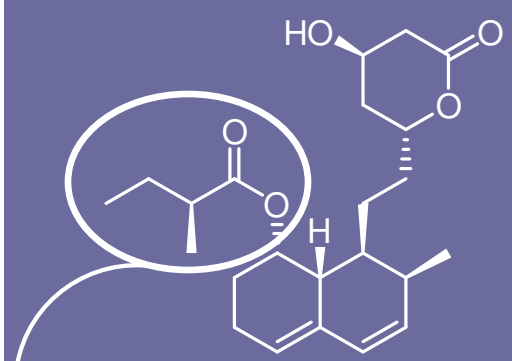


MERCK

Willard, A. K.; Novello, F. C.; Hoffmann, W. F.; Cragoe, E.; E. J. Jr. *USP* 4459422, **1984**.
Fortune, **2003**, January 20.
Roth, B. D. *Prog. Med. Chem.* **2002**, *40*, 1-22.
Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

The Drug Discovery

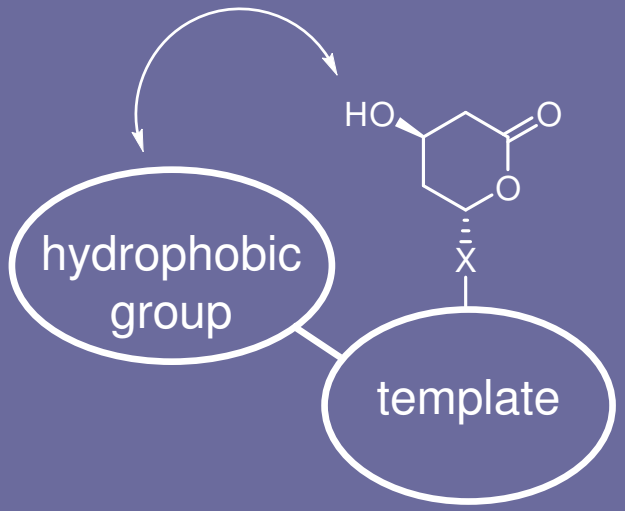
The Decision of the Core Template



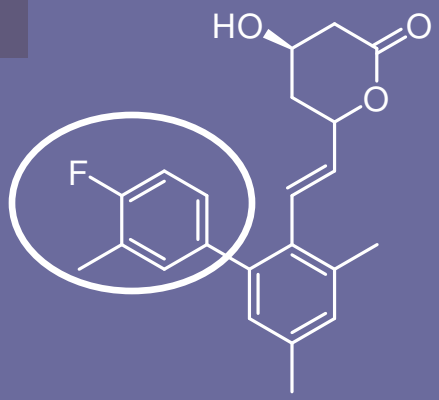
Mevastatin

Hydrolysis – 100-fold loss in potency

correct spatial relationship



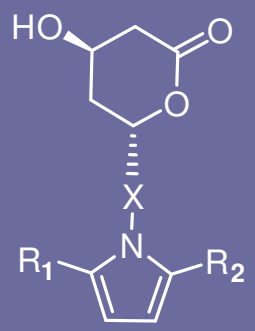
A Potent HMGR Inhibitor



MERCK

Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

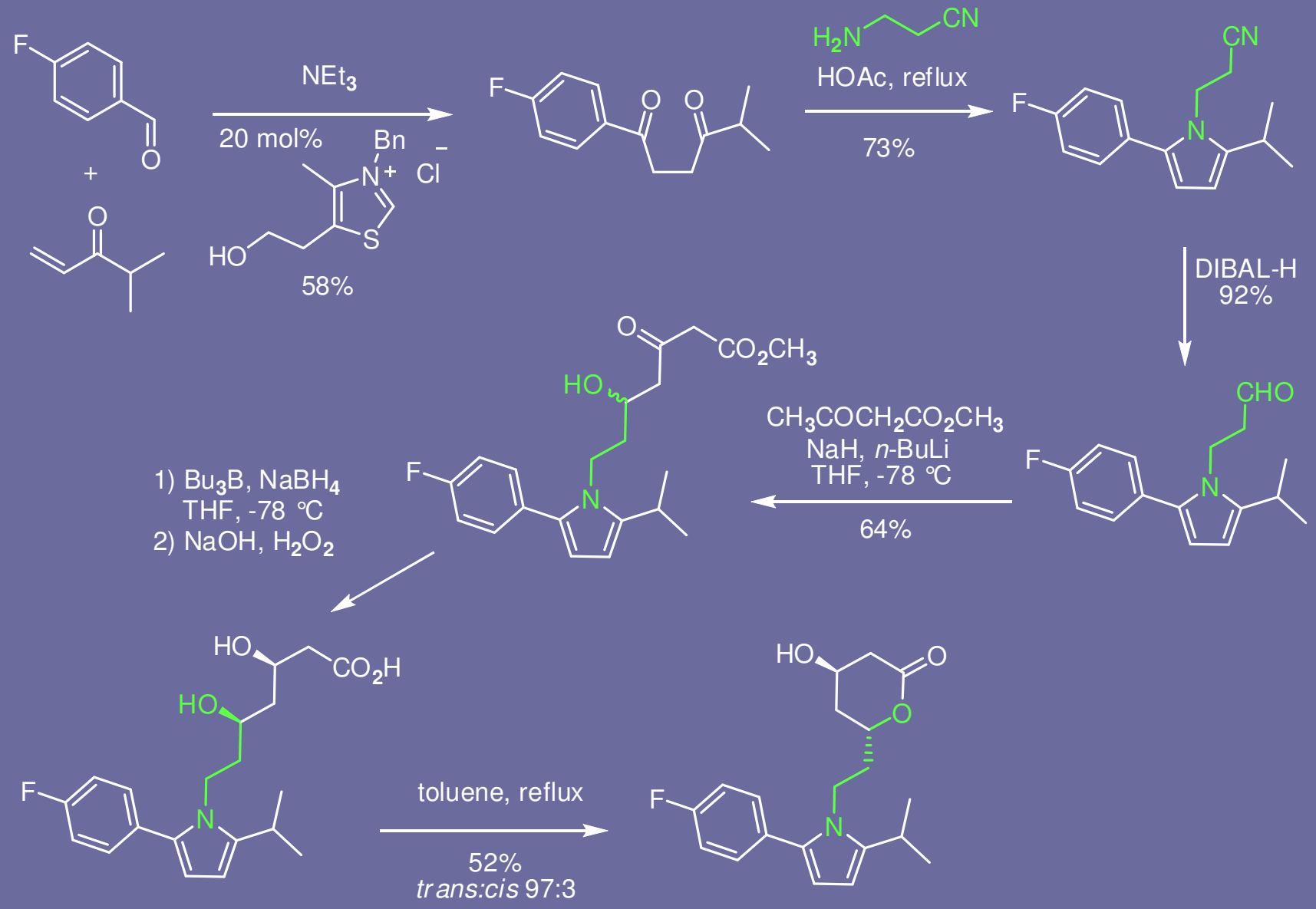
The Pyrrole Template



Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

The Drug Discovery

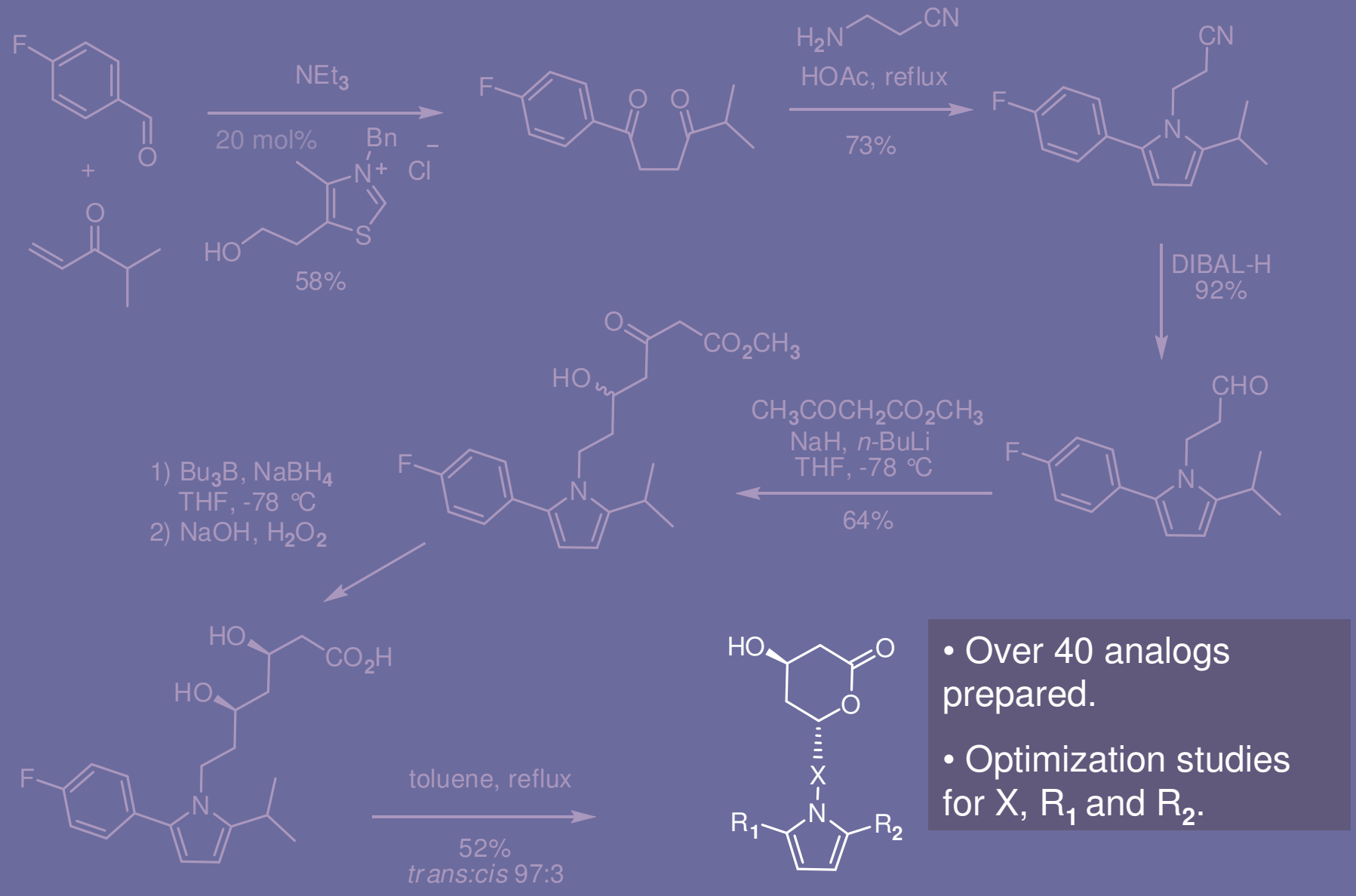
The Pyrrole Template



Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

The Drug Discovery

The Pyrrole Template

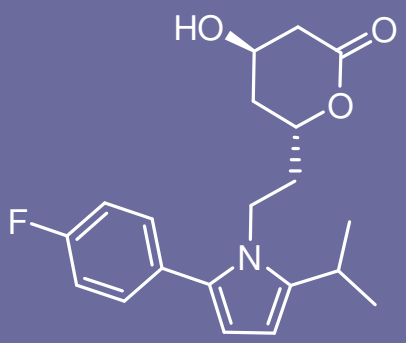


- Over 40 analogs prepared.
- Optimization studies for X, R_1 and R_2.

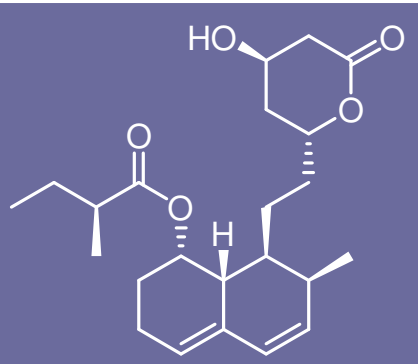
Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

The Drug Discovery

The Need for Additional Functionality



- IC_{50} (analog): 0.40 μM.
- Limit of current synthetic route.



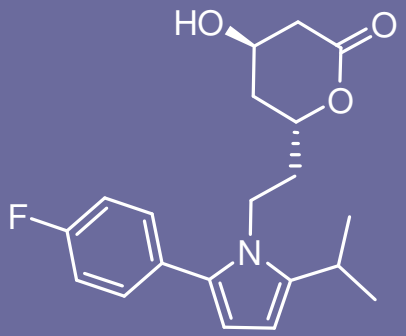
Mevastatin
MERCK

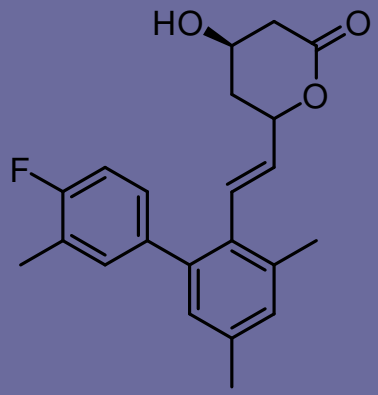
IC_{50} – 0.030 μM.

Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

The Drug Discovery

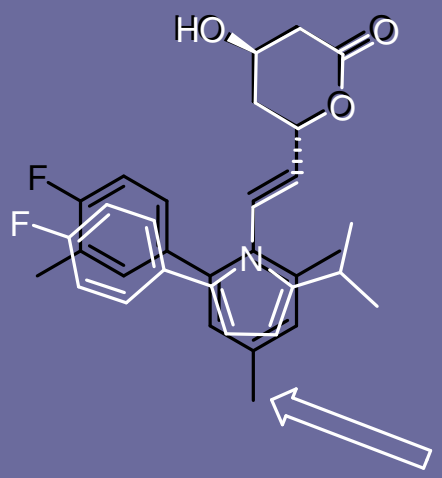
The Need for Additional Functionality





MERCK

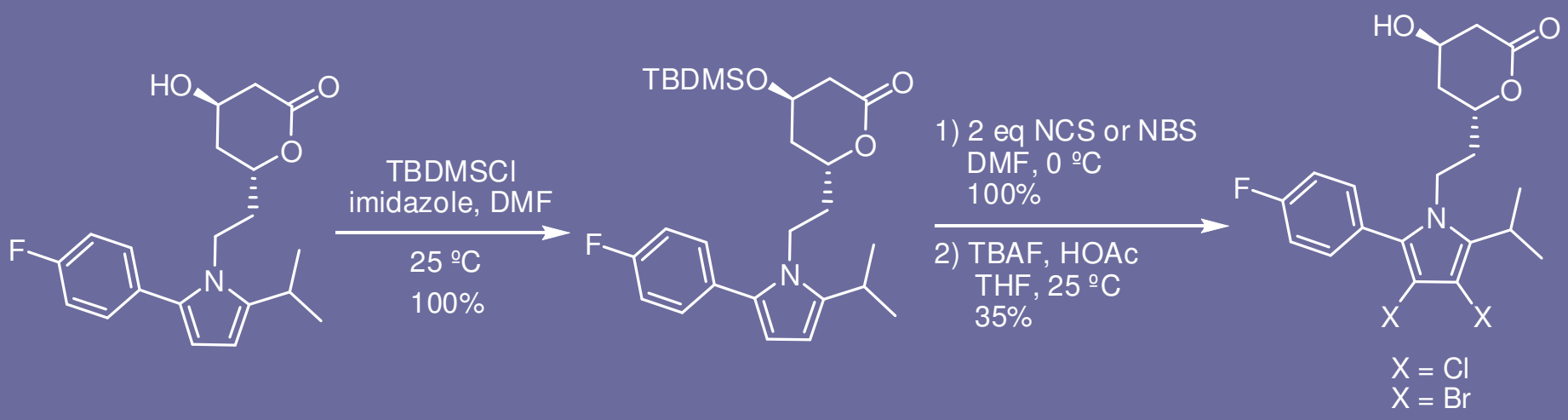
An overlay



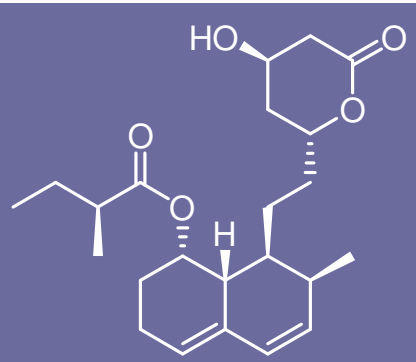
Roth, B. D. *Prog. Med. Chem.* **2002**, *40*, 1-22.

The Drug Discovery

Incorporation of Additional Functionality



X	IC_{50} (μM)
H	0.23
Cl	0.028
Br	0.028



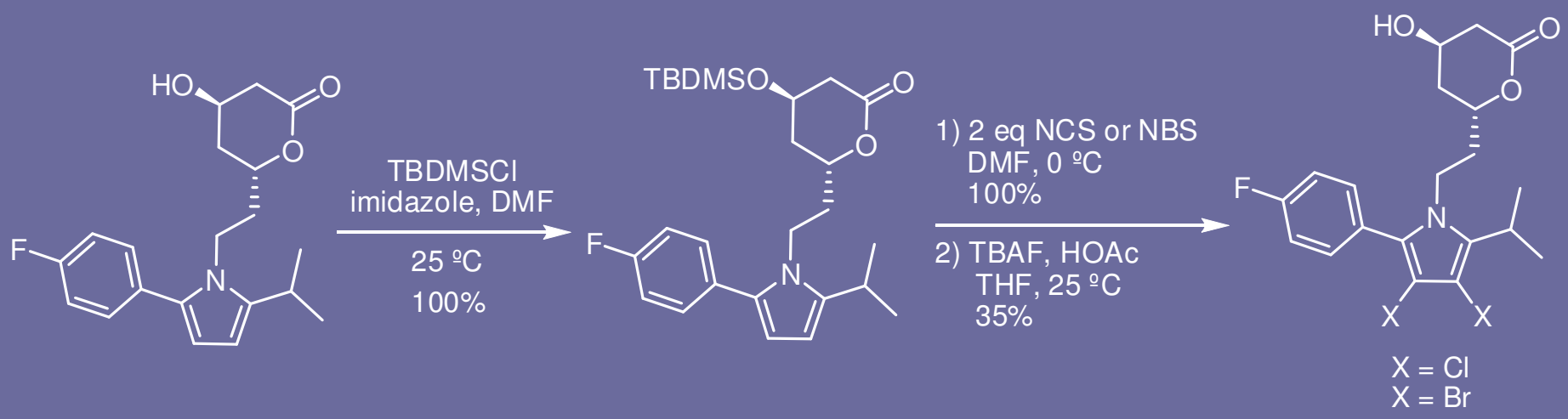
Mevastatin
MERCK

IC_{50} – 0.030 µM.

Roth, B. D. *et al. J. Med. Chem.* **1991**, *34*, 357-366.

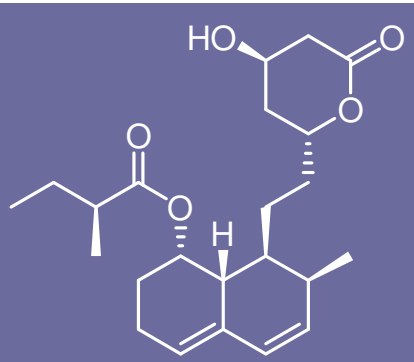
The Drug Discovery

Incorporation of Additional Functionality



Toxicity in early preclinical development!

X	IC_{50} (μM)
H	0.23
Cl	0.028
Br	0.028



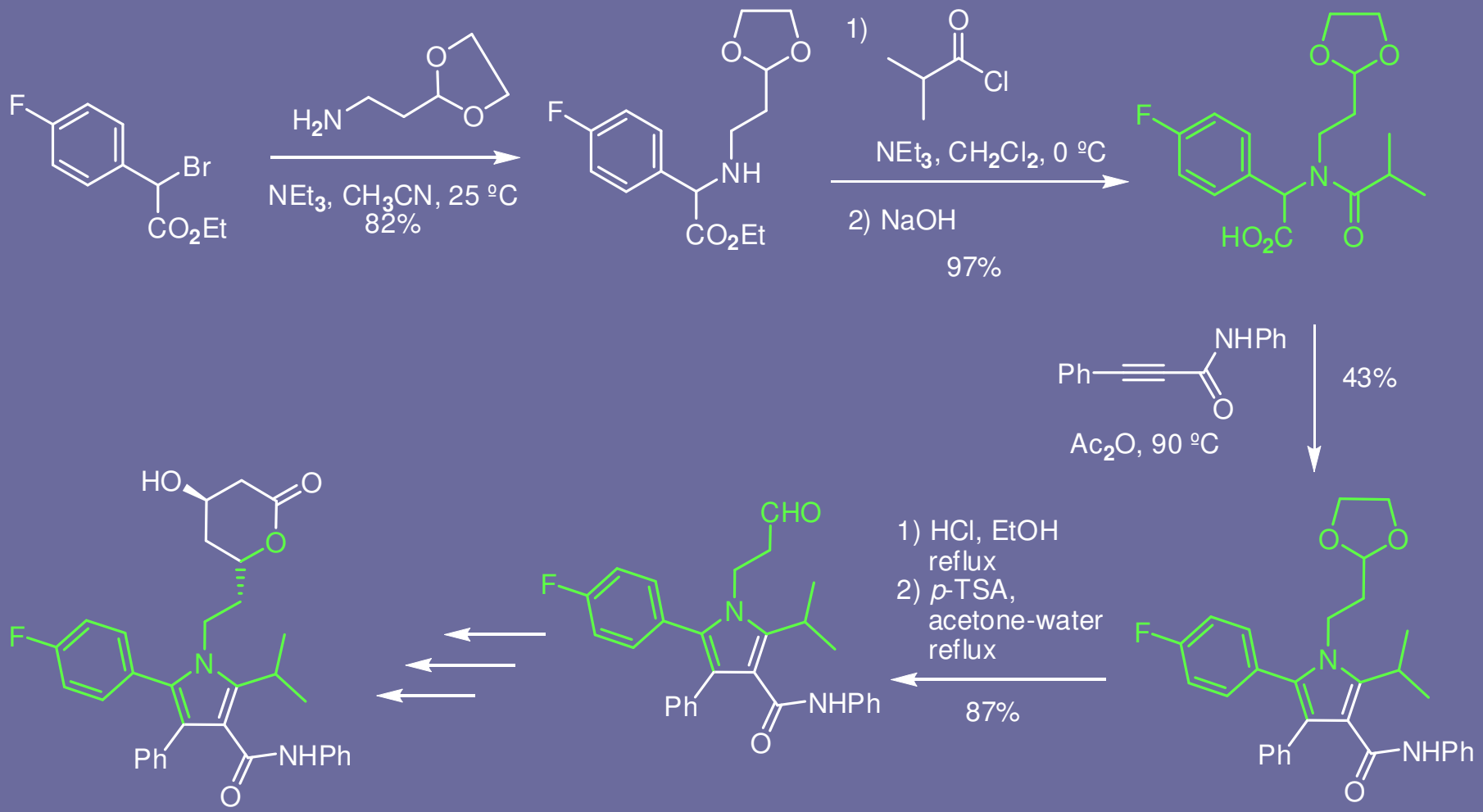
Mevastatin
MERCK

IC_{50} – 0.030 µM.

Roth, B. D. *Prog. Med. Chem.* **2002**, *40*, 1-22.

The Drug Discovery

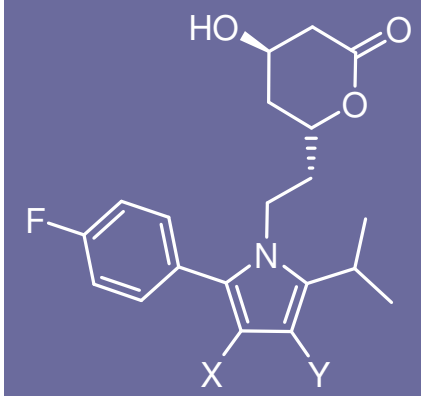
Penta-substituted Pyrroles via [3+2]



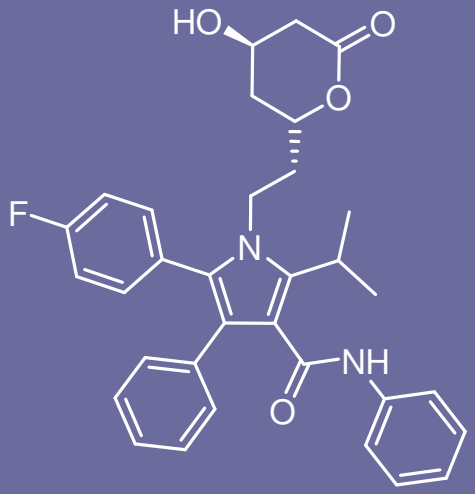
Roth, B. D. *et al. J. Med. Chem.* **1991**, *34*, 357-366.

The Drug Discovery

Penta-substituted Pyrroles via [3+2]

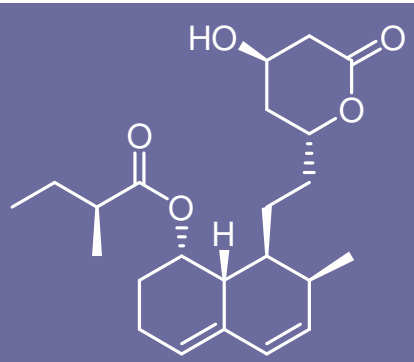


20 analogs tested



(racemic)

IC_{50} – 0.025 µM



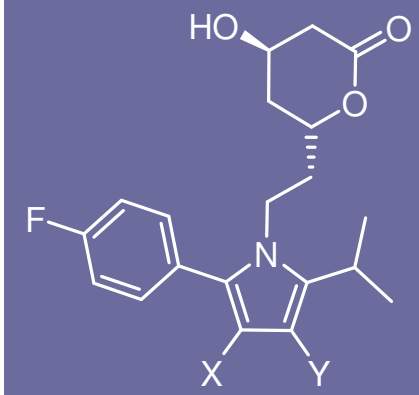
Mevastatin
MERCK

IC_{50} – 0.030 µM.

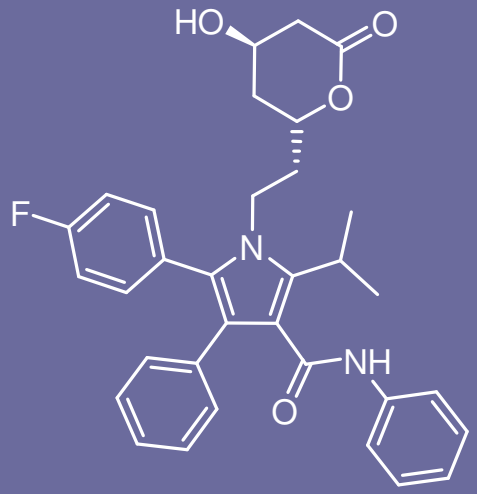
Roth, B. D. *et al. J. Med. Chem.* **1991**, *34*, 357-366.

The Drug Discovery

Penta-substituted Pyrroles via [3+2]



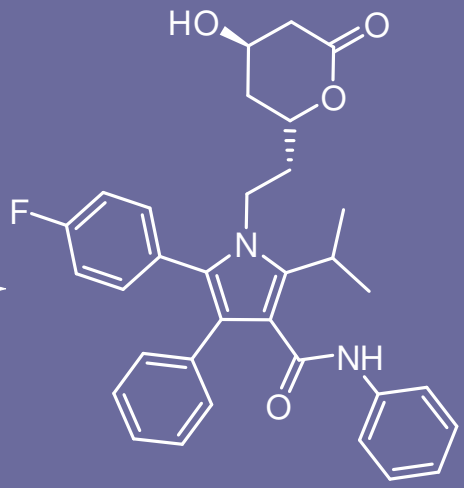
20 analogs tested



(racemic)

IC_{50} – 0.025 µM

resolution

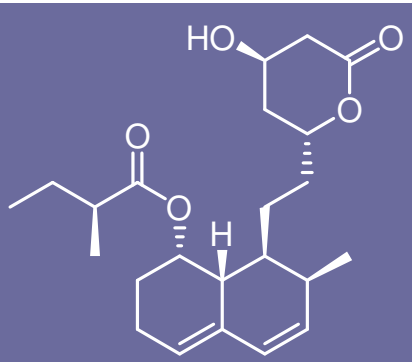


(*R, R*)

IC_{50}(µM)

(*R, R*) – 0.007

(*S, S*) – 0.44



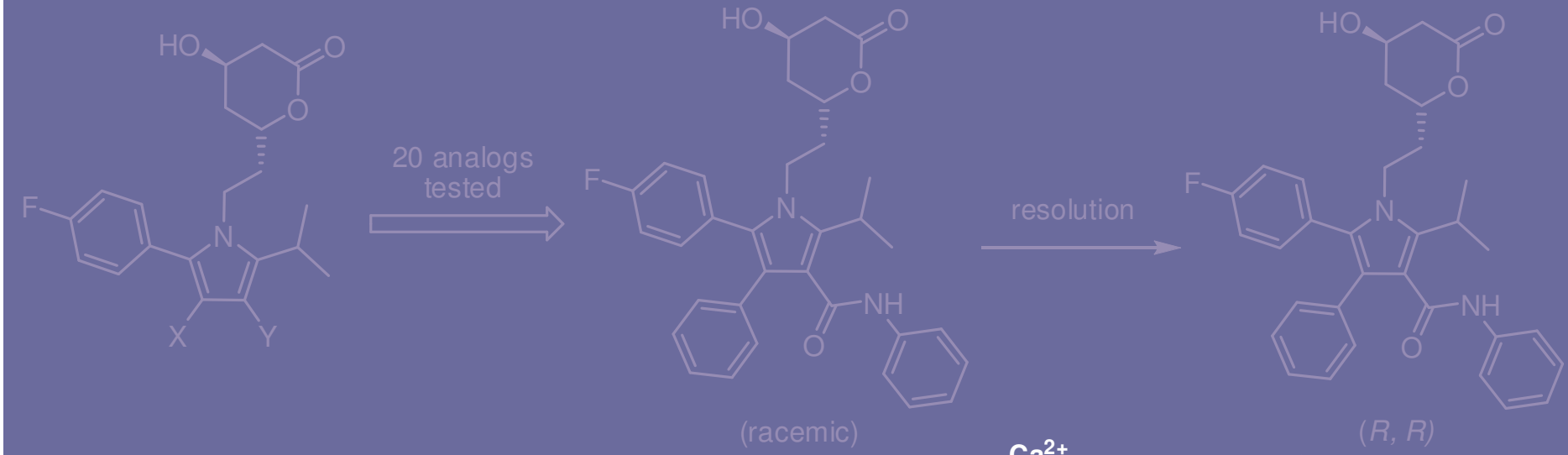
Mevastatin
MERCK

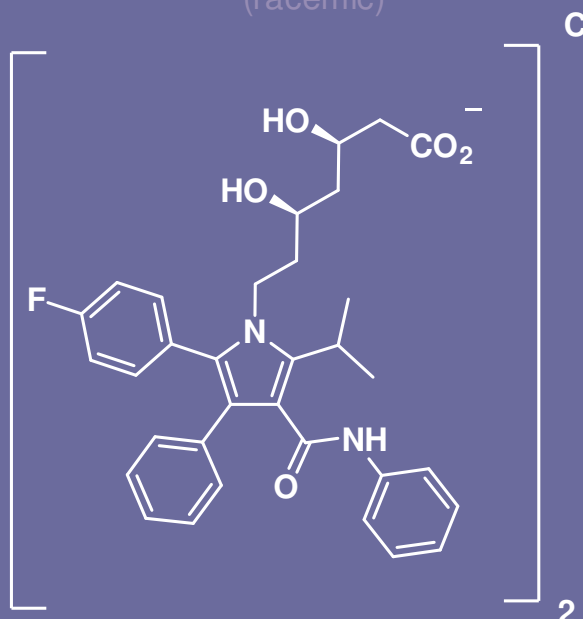
IC_{50} – 0.030 µM.

Roth, B. D. *et al. J. Med. Chem.* **1991**, *34*, 357-366.

The Drug Discovery

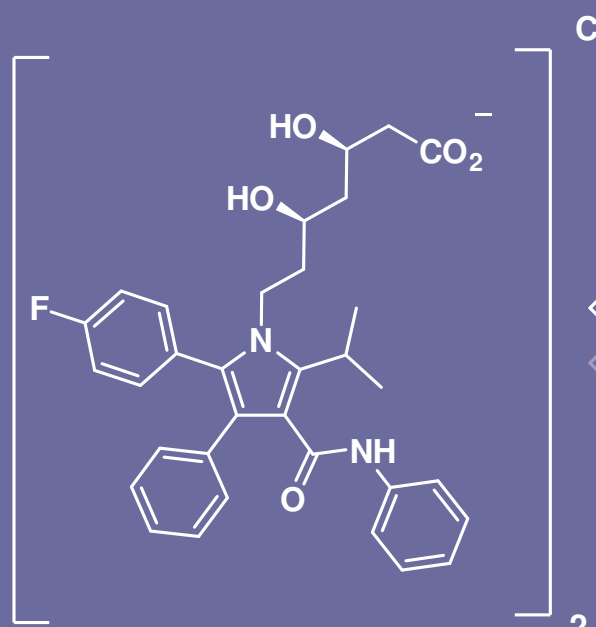
The Birth of LIPITOR®!





Atorvastatin calcium
LIPITOR®

The Story of LIPITOR® - *a Peek into the World of Pharmaceutical Process Chemistry*



Atorvastatin calcium
LIPITOR®

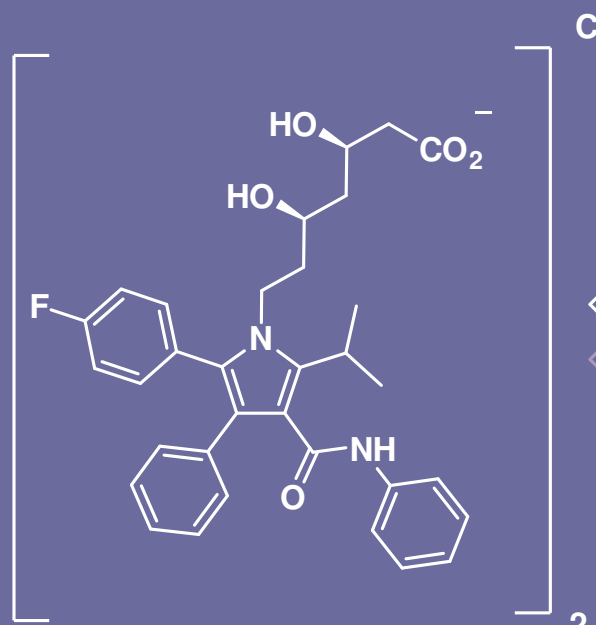
Chemical Synthesis

Drug Discovery

Process Development

Biocatalysis

The Story of LIPITOR® - *a Peek into the World of Pharmaceutical Process Chemistry*



Atorvastatin calcium
LIPITOR®

Chemical Synthesis

Drug Discovery

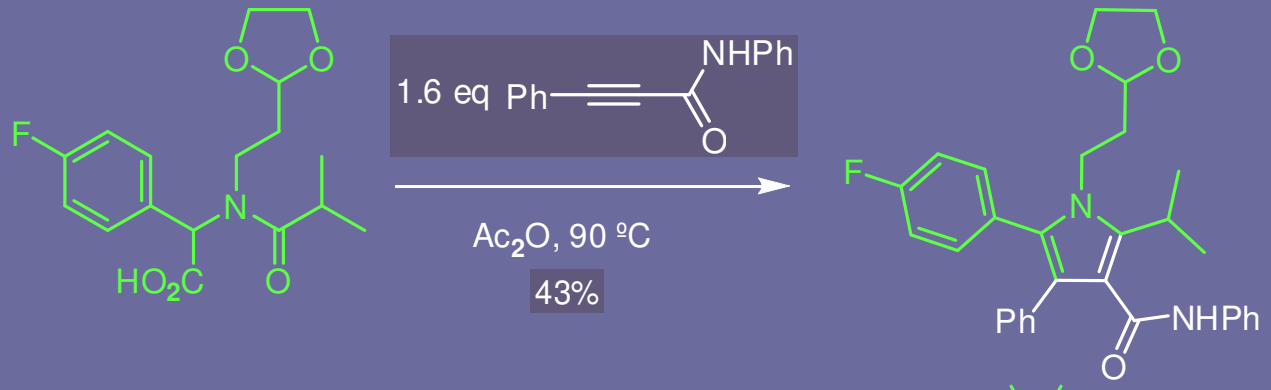
Process Development

Biocatalysis

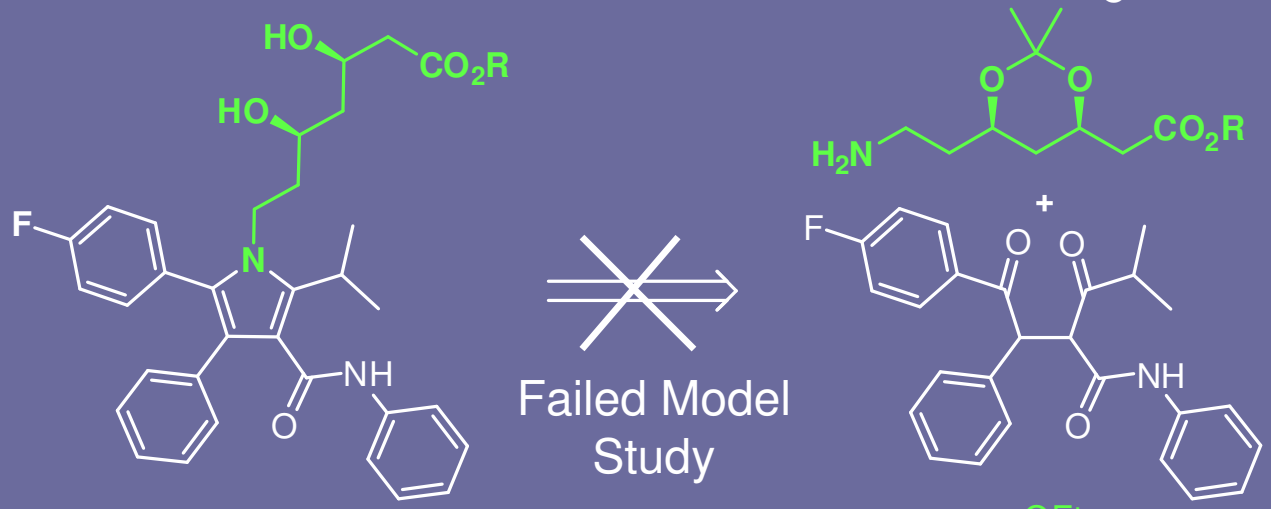
The Process Development

Scale-up Issues and Potential Solutions

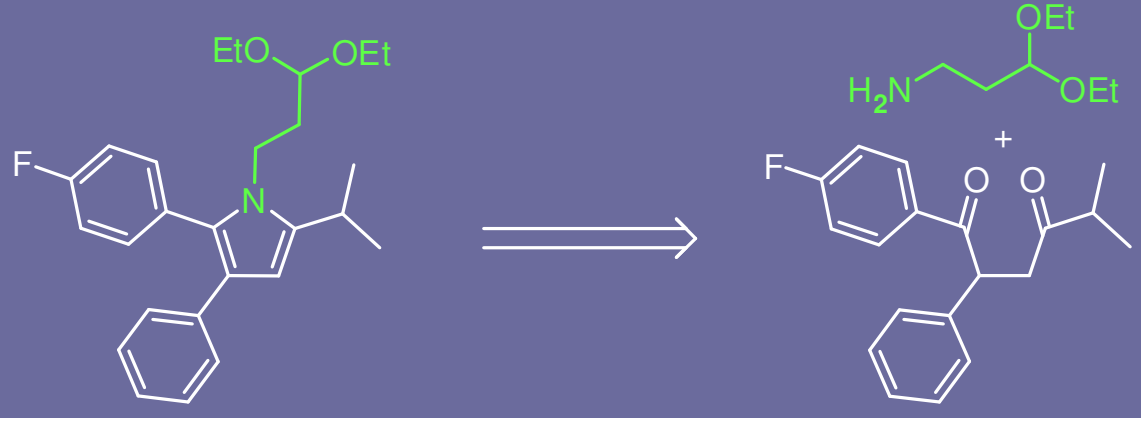
[3+2] Cycloaddition Route



Paal-Knorr Route: Penta-substituted Pyrroles



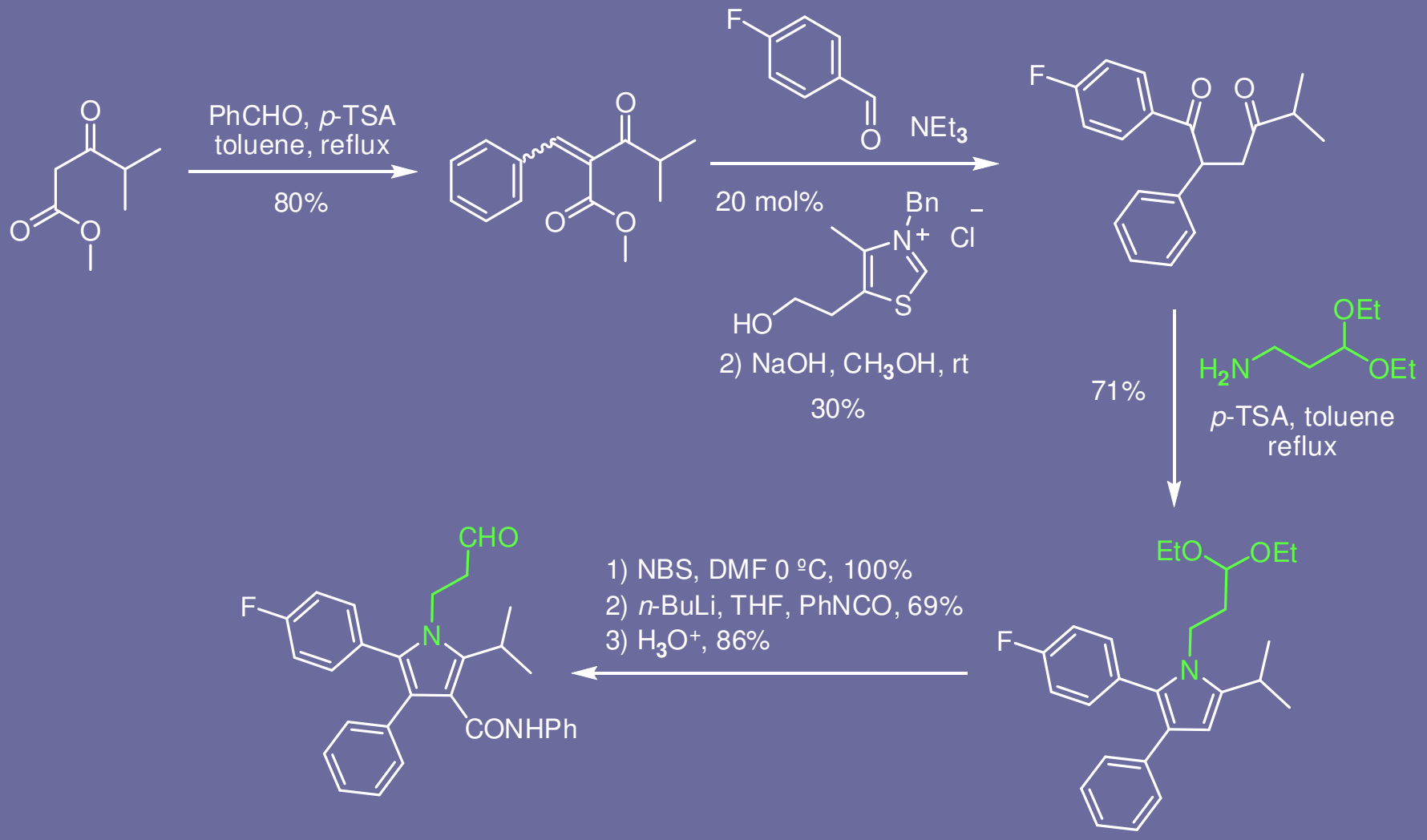
Paal-Knorr Route: Tetra-substituted Pyrroles



Roth, B. D. *Prog. Med. Chem.* **2002**, *40*, 1-22.

The Process Development

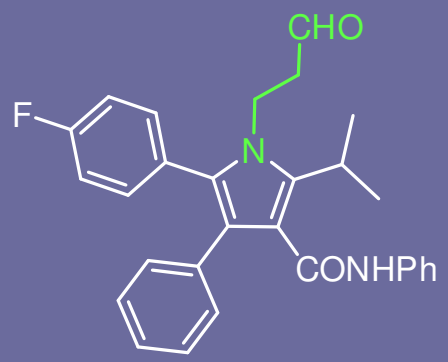
From Tetra- to Penta-substituted Pyrroles



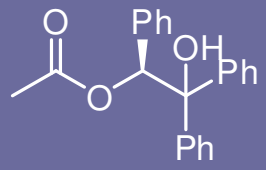
Roth, B. D. *et al. J. Med. Chem.* **1991**, *34*, 357-366.
Roth, B. D. *Prog. Med. Chem.* **2002**, *40*, 1-22.

The Process Development

From Tetra- to Penta-substituted Pyrroles



1)

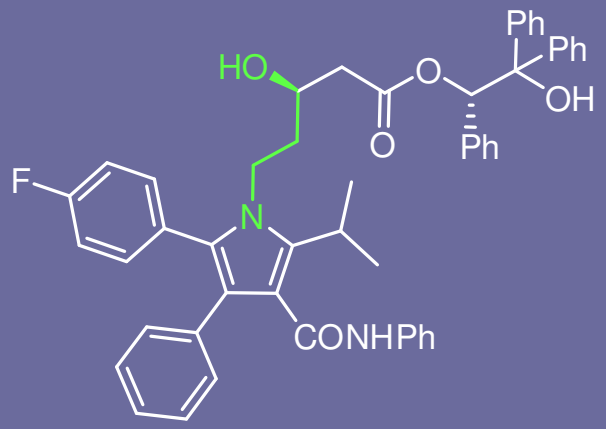


2 eq. LDA, $MgBr_2$ **-78 ºC**

2) Recrystallization **-10 ºC**

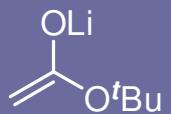
dr 98:2

38%

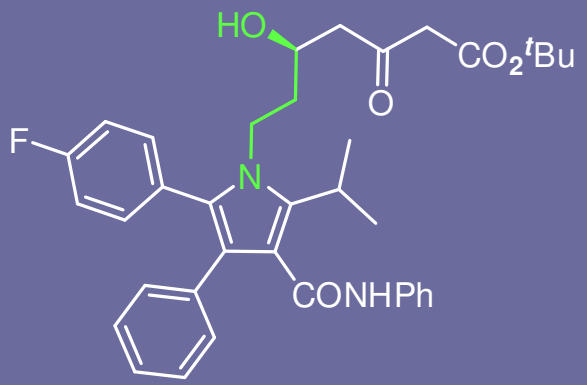


1) $NaOCH_3$ **0 ºC**

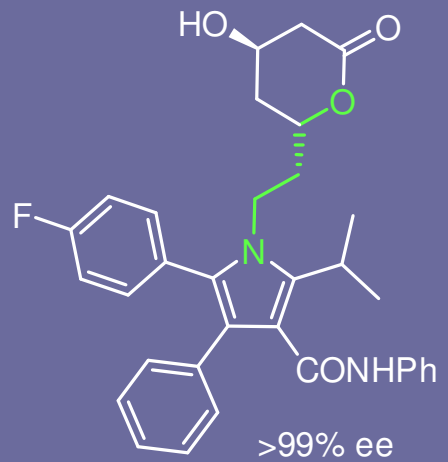
68%

2)  **-70 ºC**

78%



1) Et_3B. $NaBH_4$ **-78 ºC**
2) H_2O_2, NaOH
3) toluene, reflux

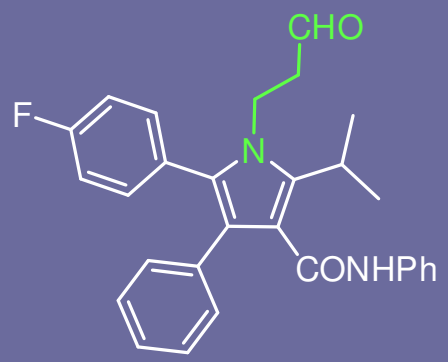


>99% ee

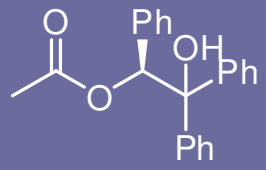
Roth, B. D. *et al. J. Med. Chem.* **1991**, *34*, 357-366.

The Process Development

From Tetra- to Penta-substituted Pyrroles



1)

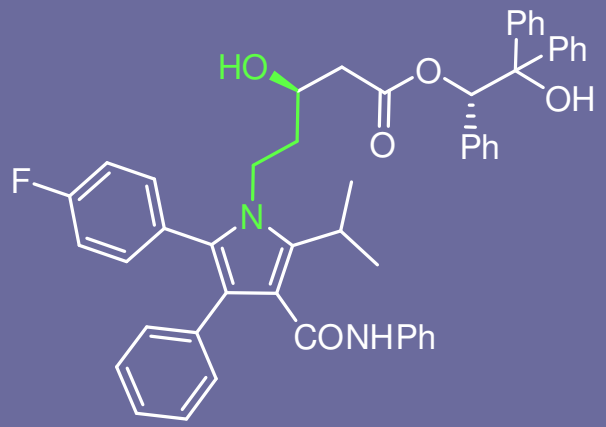


2 eq. LDA, $MgBr_2$ **-78 ºC**

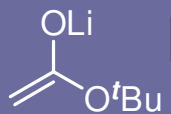
2) Recrystallization **-10 ºC**

dr 98:2

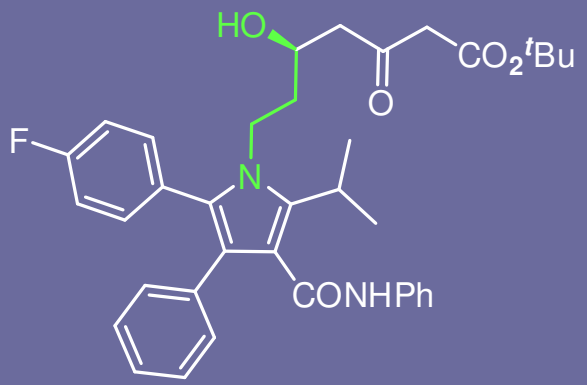
38%



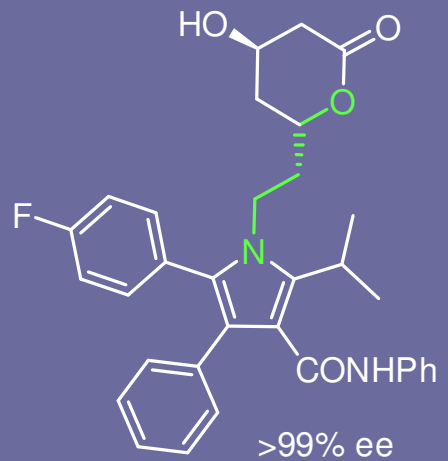
1) $NaOCH_3$ **0 ºC**
68%

2)  **-70 ºC**

78%



1) Et_3B. $NaBH_4$ **-78 ºC**
2) H_2O_2, NaOH
3) toluene, reflux

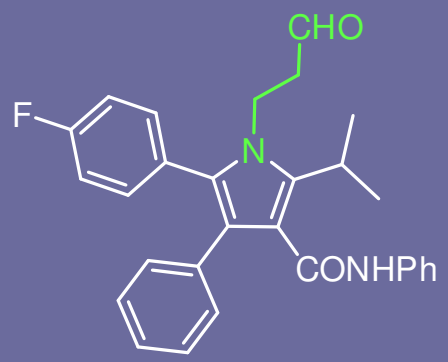


>99% ee

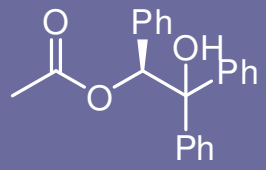
Roth, B. D. *et al. J. Med. Chem.* **1991**, *34*, 357-366.

The Process Development

From Tetra- to Penta-substituted Pyrroles



1)

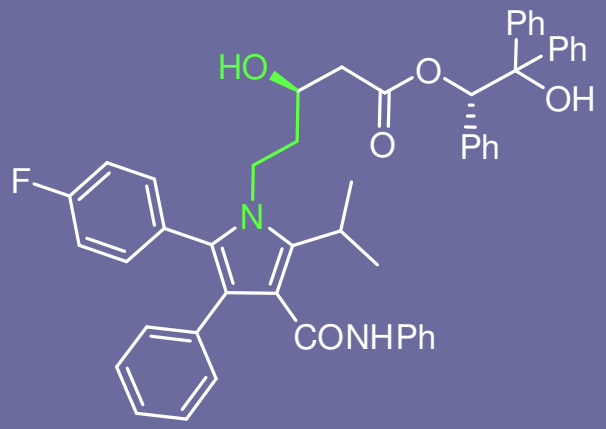


2 eq. LDA, $MgBr_2$ **-78 ºC**

2) Recrystallization **-10 ºC**

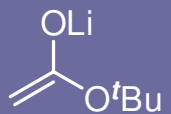
dr 98:2

38%

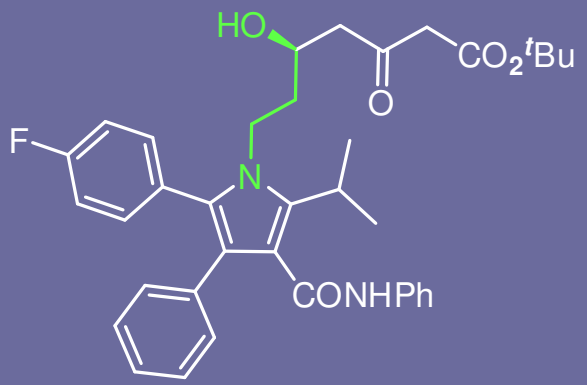


1) $NaOCH_3$ **0 ºC**
68%

2)

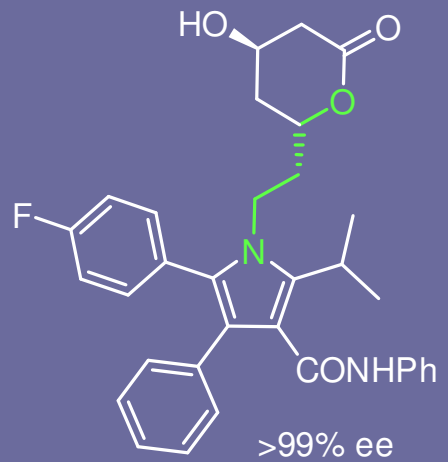


-70 ºC
78%



1) Et_3B. $NaBH_4$ **-78 ºC**
2) H_2O_2, NaOH
3) toluene, reflux

3 columns & 1 crystallization
7% yield



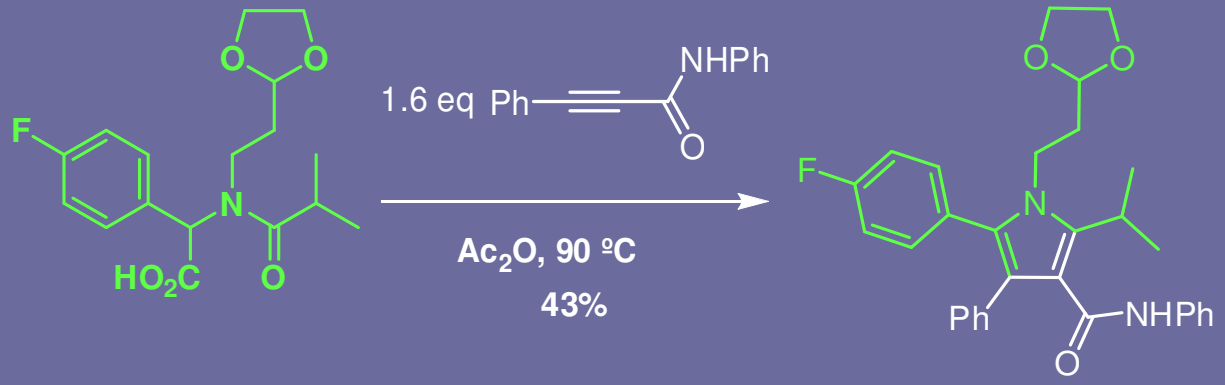
>99% ee

Roth, B. D. *et al. J. Med. Chem.* **1991**, *34*, 357-366.

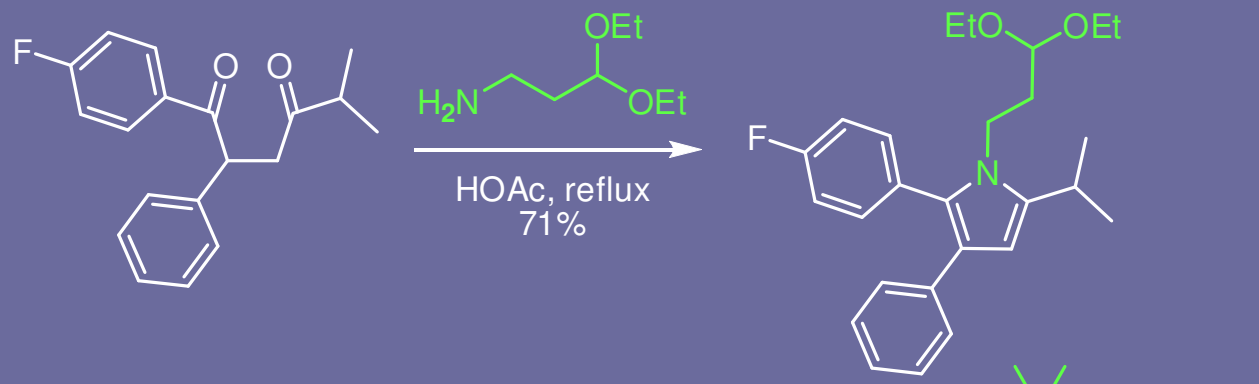
The Process Development

A Recap – The Failures

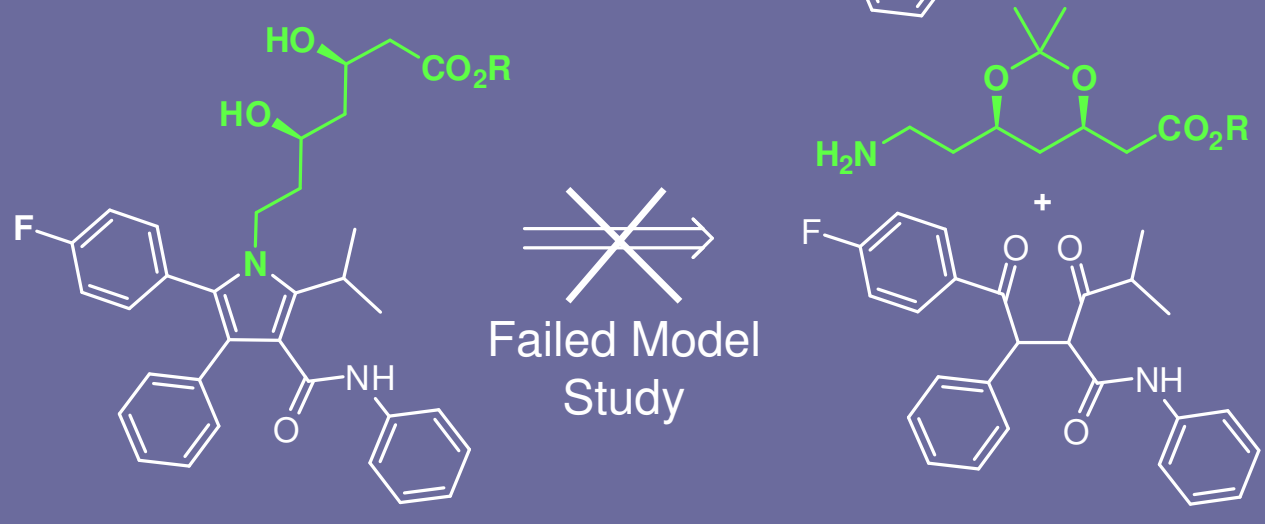
[3+2] Cycloaddition Route



Paal Knorr Route: Tetra-substituted Pyrroles

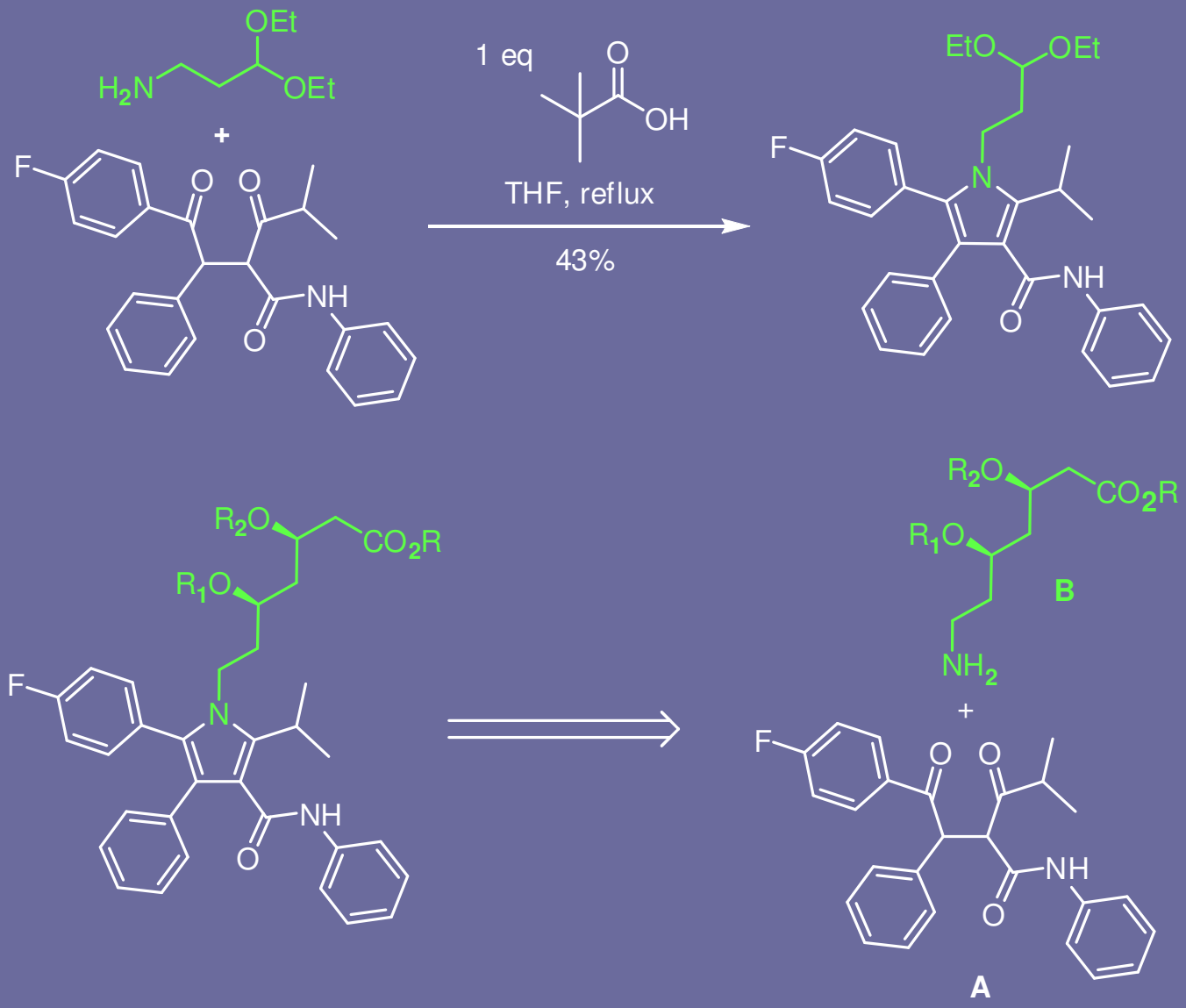


Paal Knorr Route: Penta-substituted Pyrroles



The Process Development

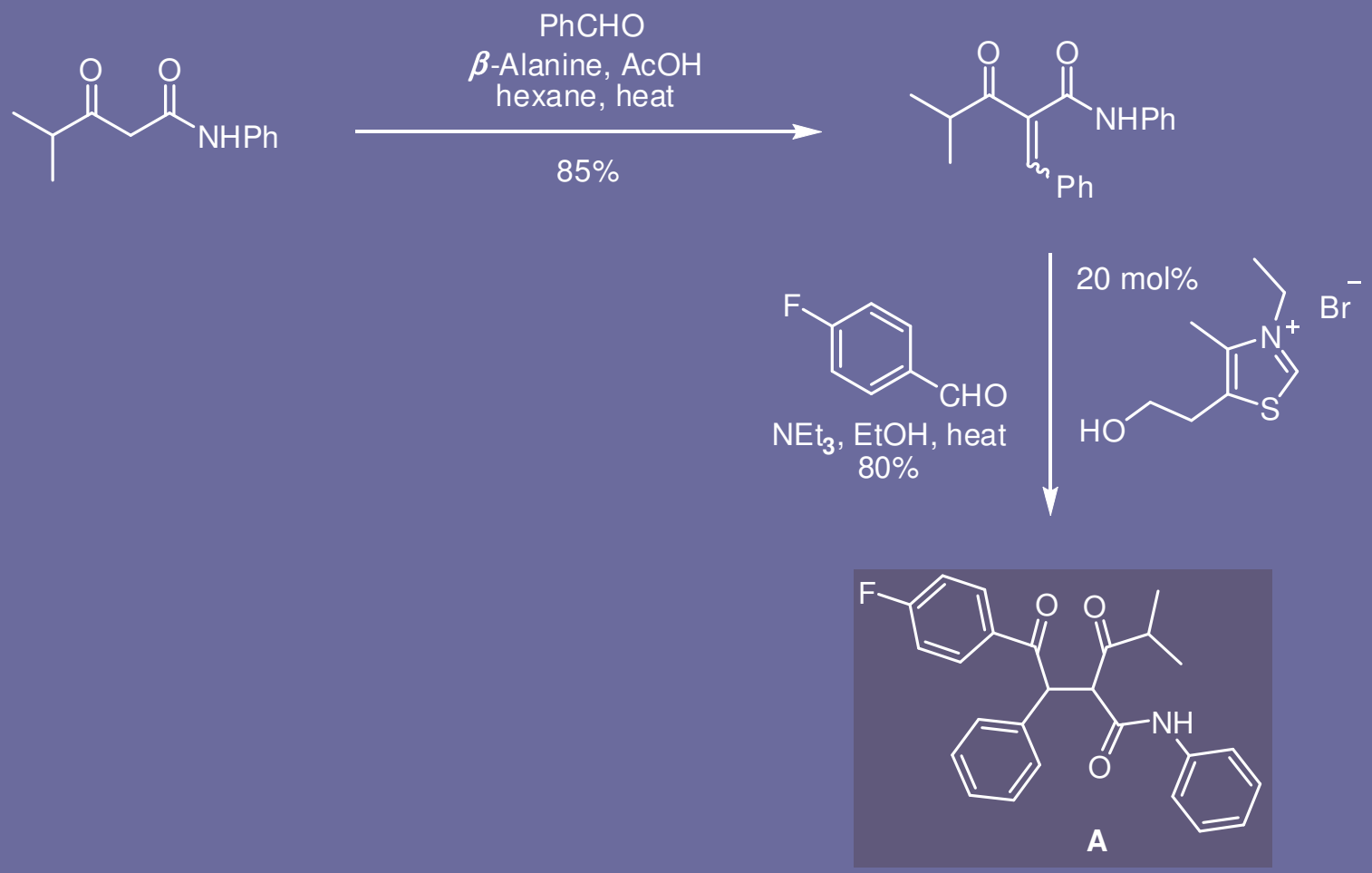
Paal Knorr Route: Penta-substituted Pyrroles



Roth, B. D. *Prog. Med. Chem.* **2002**, *40*, 1-22.

The Process Development

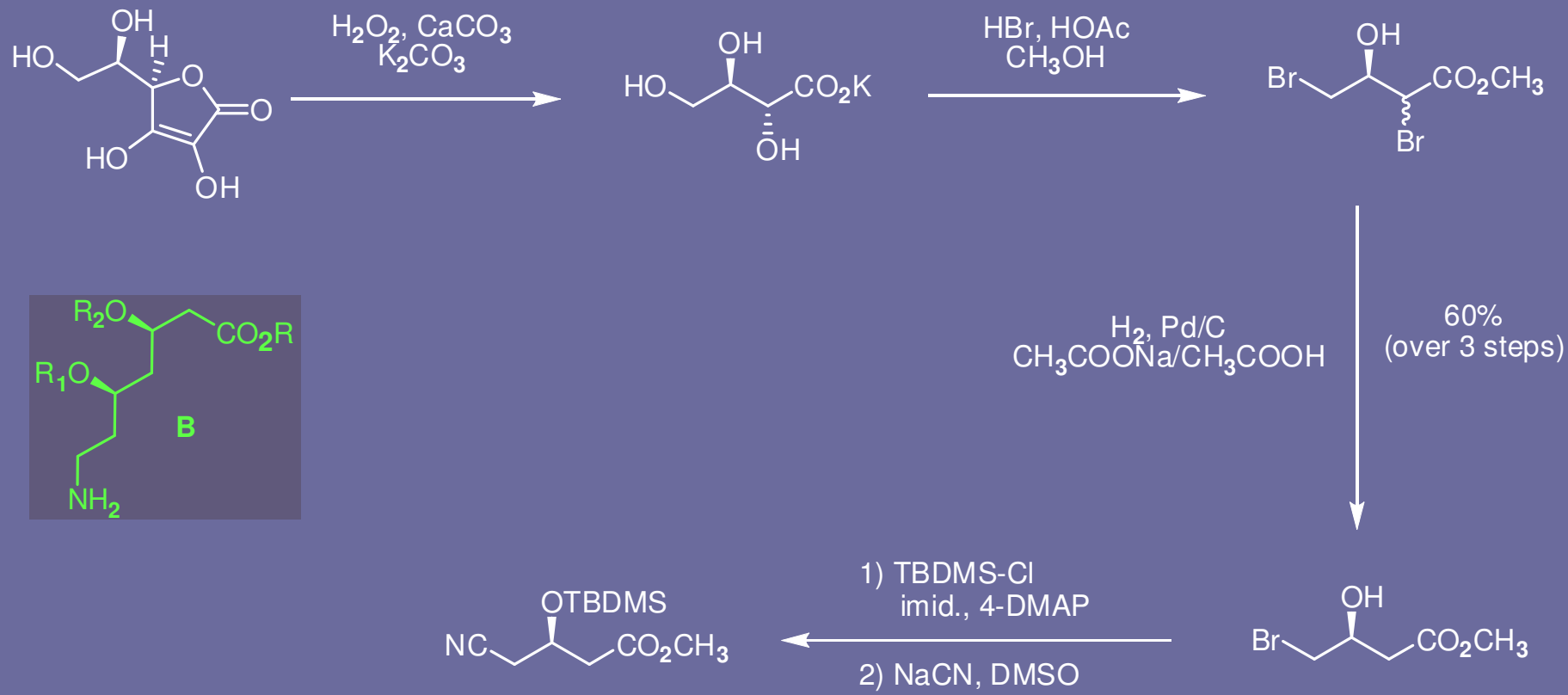
Pfizer's Commercial Route: Fragment **A**



Dr. Bruce D. Roth (VP, Global Research & Development, Pfizer), personal communication.
Baumann, K. L. *et al. Tetrahedron Lett.* **1992**, *33*, 2283-2284.

The Process Development

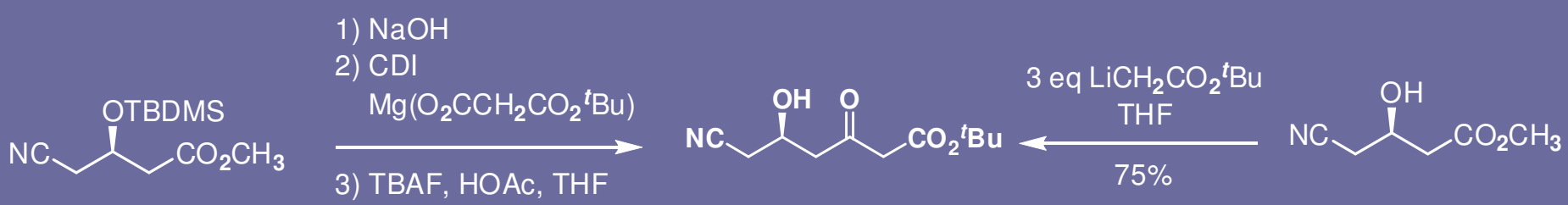
Pfizer's Commercial Route: Fragment B

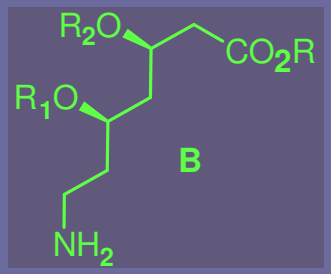


Browser, P. L. *et al. Tetrahedron Lett.* **1992**, *33*, 2279-2282.
Roth, B. D. *Prog. Med. Chem.* **2002**, *40*, 1-22.

The Process Development

Pfizer's Commercial Route: Fragment B

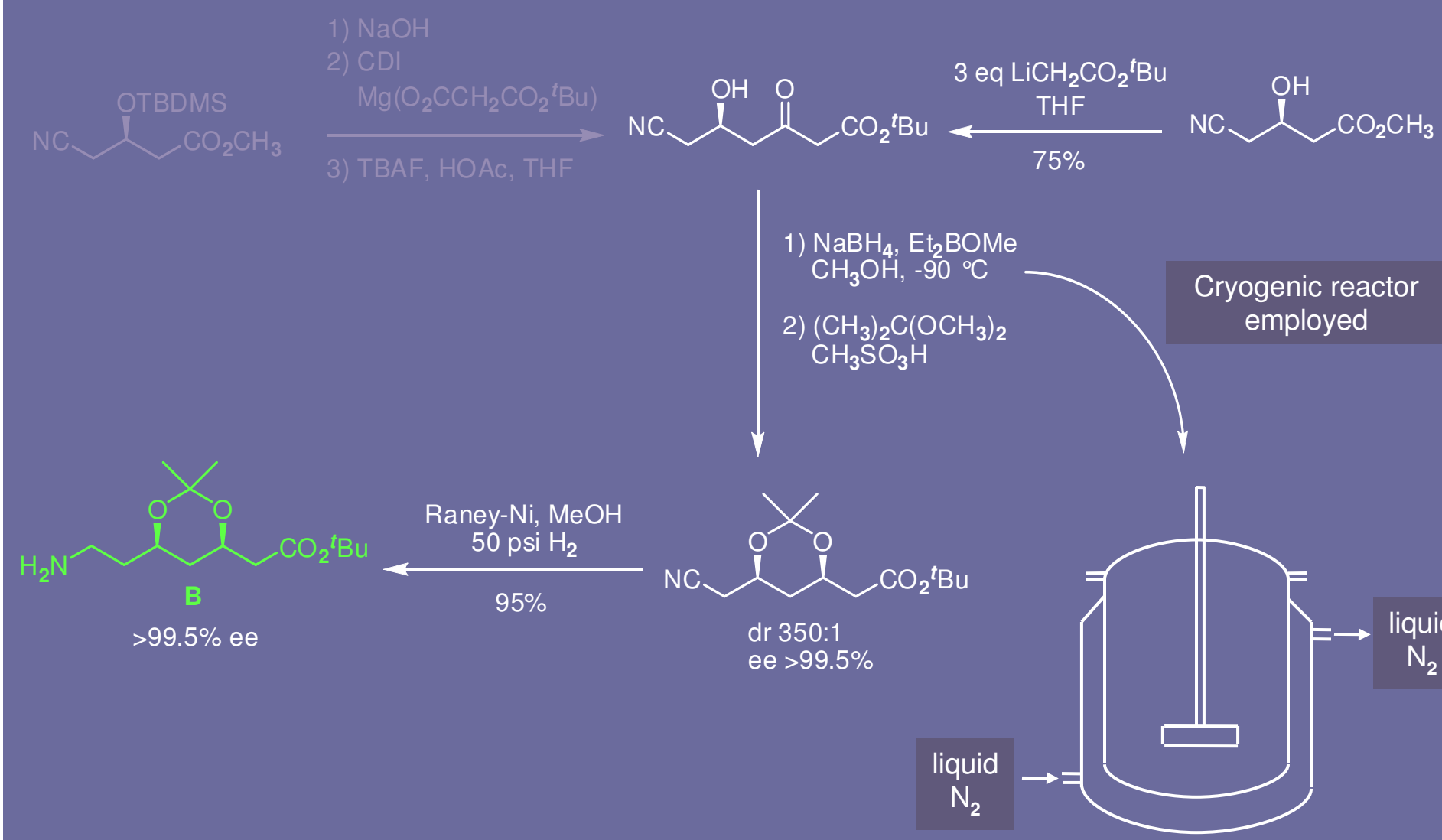




Dr. Donald E. Butler (Former Process Development Leader, Pfizer), personal communication.
Browser, P. L. *et al. Tetrahedron Lett.* **1992**, *33*, 2279-2282.

The Process Development

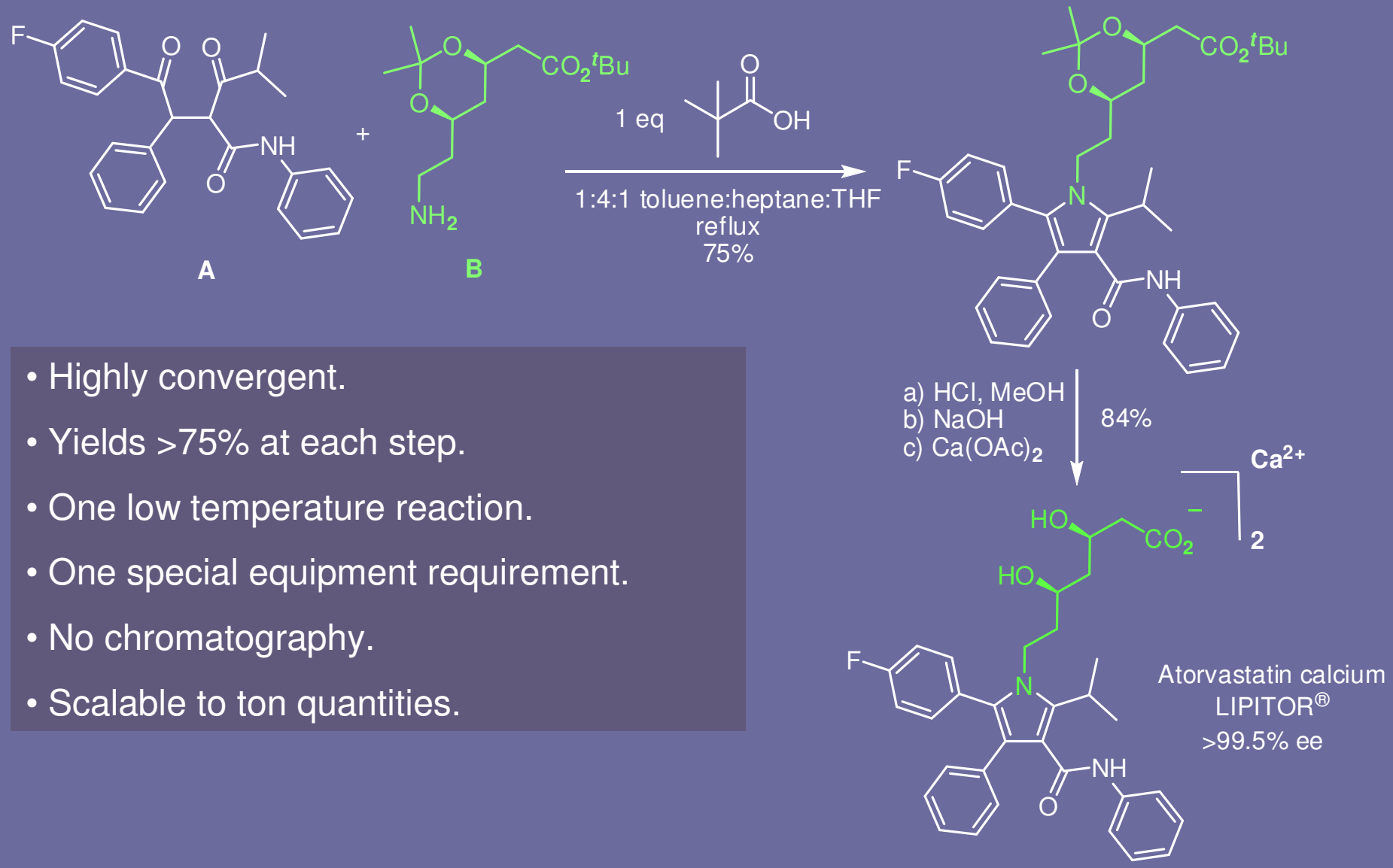
Pfizer's Commercial Route: Fragment B



Dr. Donald E. Butler (Former Process Development Leader, Pfizer), personal communication.
Browser, P. L. *et al. Tetrahedron Lett.* **1992**, *33*, 2279-2282.

The Process Development

Pfizer's Commercial Route



Atorvastatin calcium
LIPITOR®
>99.5% ee

- Highly convergent.
- Yields >75% at each step.
- One low temperature reaction.
- One special equipment requirement.
- No chromatography.
- Scalable to ton quantities.

Baumann, K. L. *et al. Tetrahedron Lett.* **1992**, *33*, 2283-2284.
Dr. Donald E. Butler (Former Process Development Leader, Pfizer), personal communication.

LIPITOR®: Drug tackled, *the struggles remained…*

"The number of factors, internal and external, that had to come together for the drug to be a success really boggles the mind" – Bruce D. Roth

The problems:

- By 1987, three statins in market.
- A decade since the first statin introduced.
- Warner-Lambert was floundering.
- *Come on, how good could it be?*
- On the verge of terminating LIPITOR®.

And the facts now:

- #1 statin in the market.
- Top selling drug in history.
- 2005: $12 billion sales & used by >45 million people.
- *"LIPITOR® is on track to have greater benefit for more people than any other drug in the history of the industry in terms of lives improved and saved."*

- Nobel Laureate Michael Brown

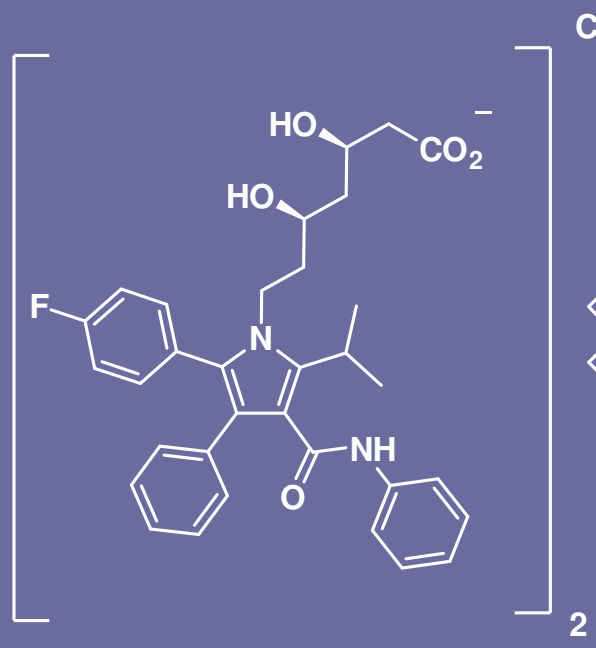
Fortune, **2003**, January 20.

The Story of LIPITOR®

"The story of how Pfizer acquired the rights to an improved statin and turned it into the all-time biggest blockbuster is a tale of hyperaggresive marketing, deft timing, financial power and plain dumb luck!"

Fortune, **2003**, January 20.

The Story of LIPITOR® - *a Peek into the World of Pharmaceutical Process Chemistry*



Atorvastatin calcium
LIPITOR®

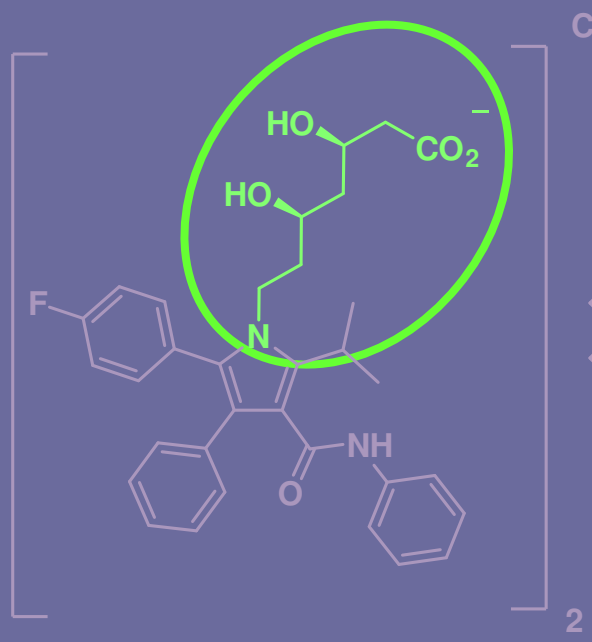
Chemical Synthesis

Biocatalysis

Drug Discovery

Process Development

The Story of LIPITOR® - *a Peek into the World of Pharmaceutical Process Chemistry*



Atorvastatin calcium
LIPITOR®

Chemical Synthesis

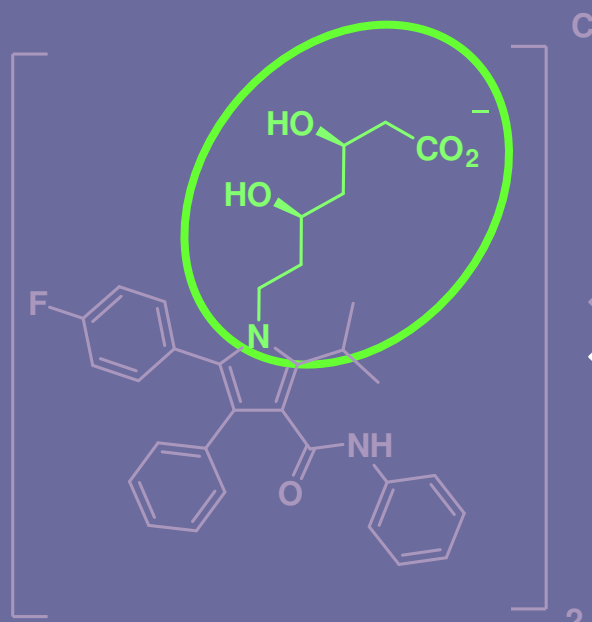
Biocatalysis

220 ton/year market
($500 million)

Drug Discovery

Process Development

The Story of LIPITOR® - *a Peek into the World of Pharmaceutical Process Chemistry*



Atorvastatin calcium
LIPITOR®

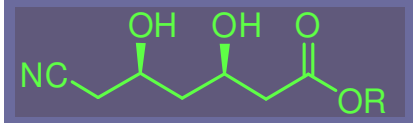
Chemical Synthesis

Drug Discovery

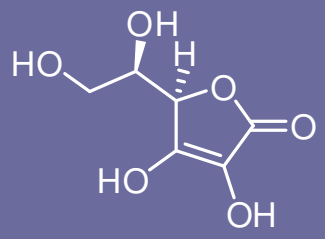
Process Development

220 ton/year market
($500 million)

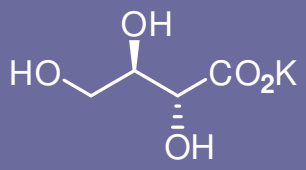
Biocatalysis



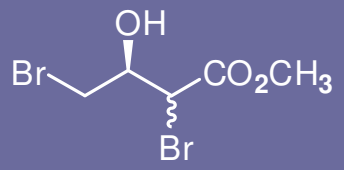
Existing Route & the Need for Improvement



H_2O_2, $CaCO_3$
K_2CO_3

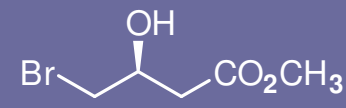


HBr, HOAc
CH_3OH

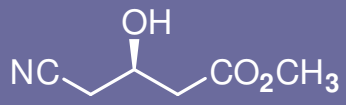


H_2, Pd/C
CH_3COONa/CH_3COOH

60%
(over 3 steps)

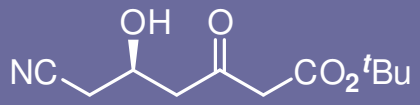


NaCN, DMSO



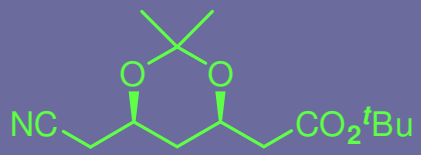
3 eq $LiCH_2CO_2{}^tBu$
THF

75%

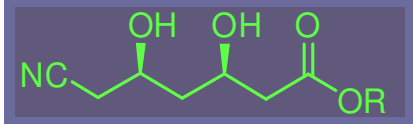


1) $NaBH_4$, Et_2BOMe
CH_3OH, -90 °C

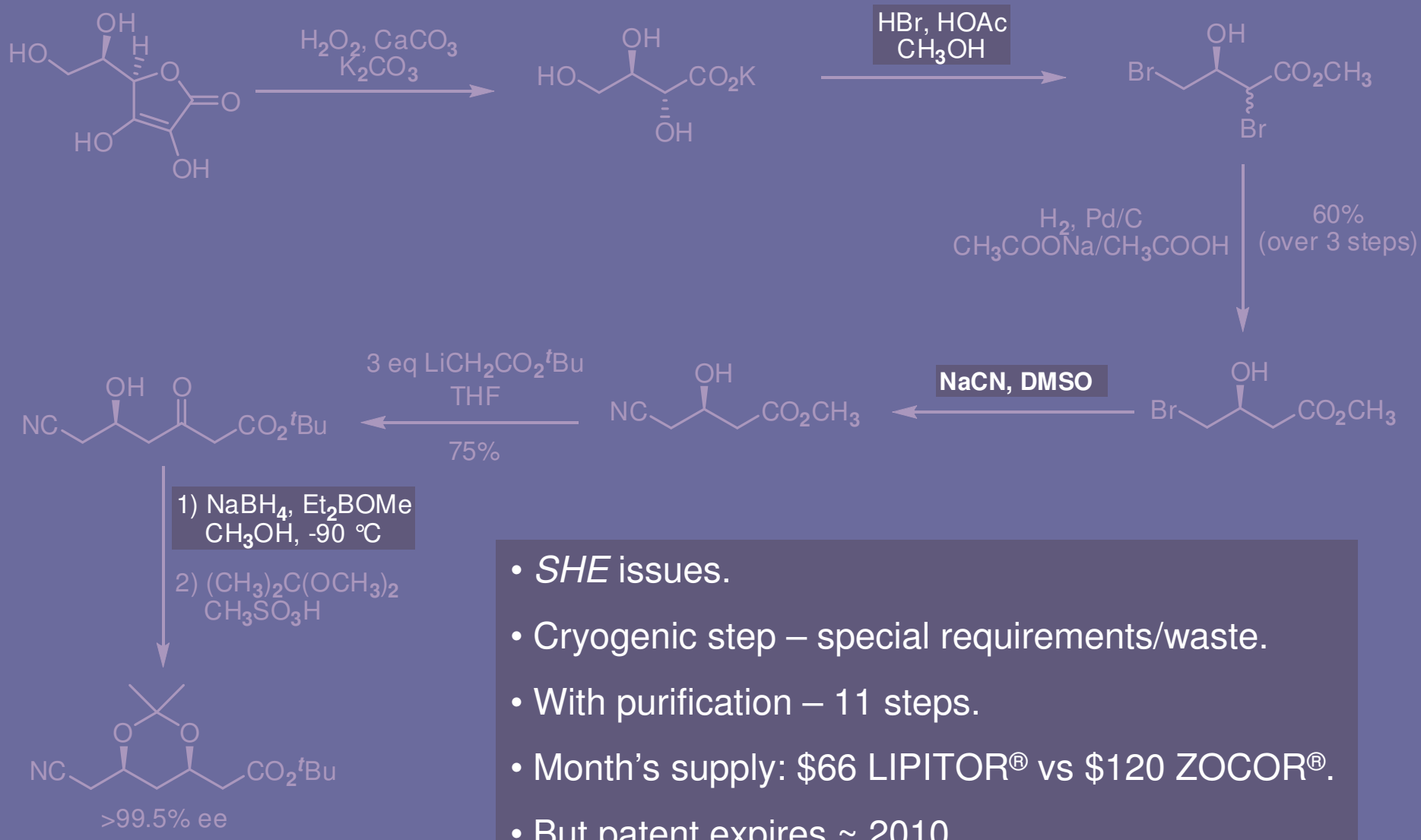
2) $(CH_3)_2C(OCH_3)_2$
CH_3SO_3H



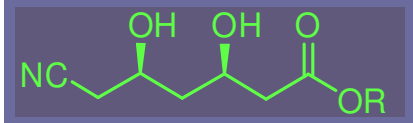
>99.5% ee



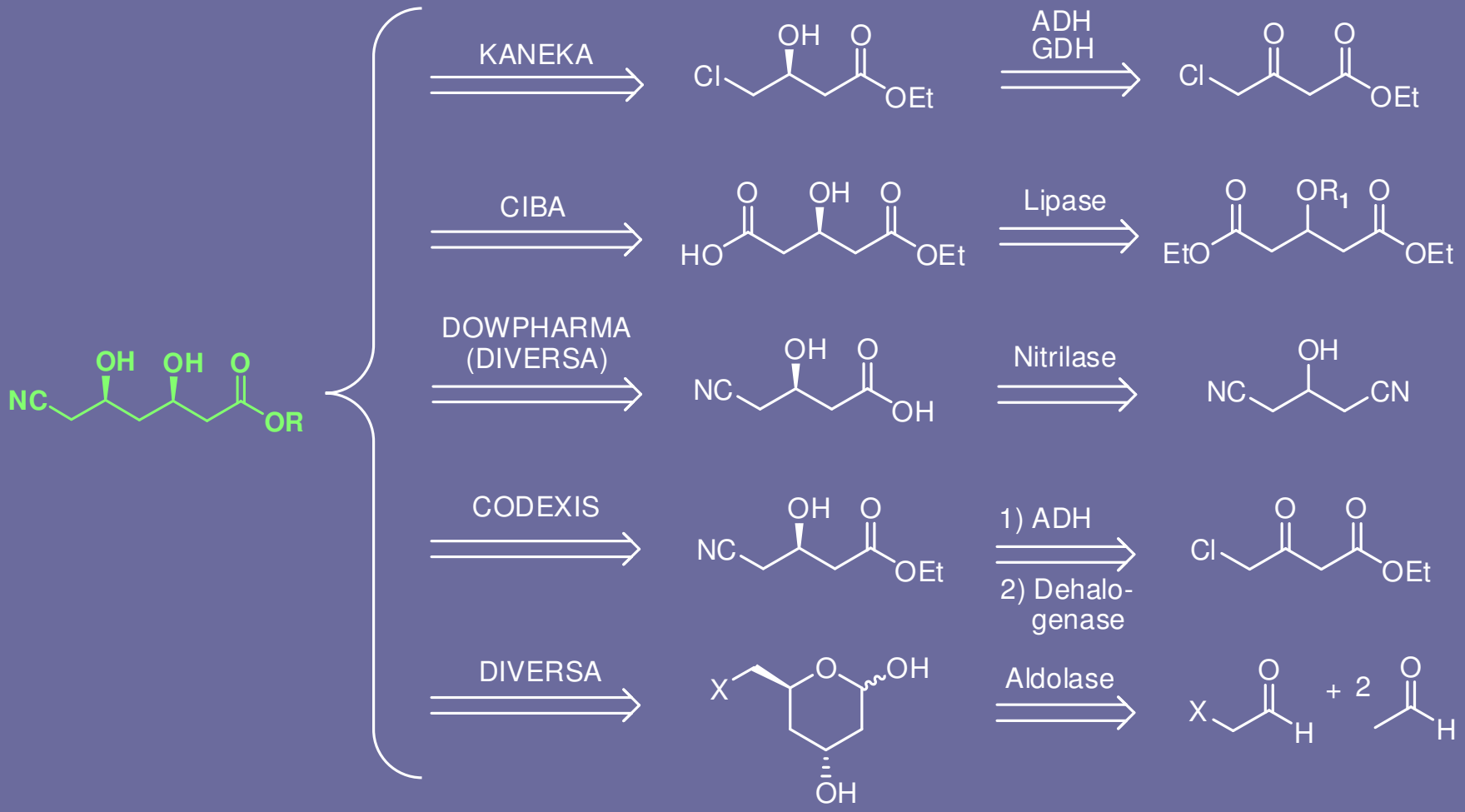
Existing Route & the Need for Improvement



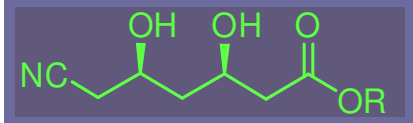
- *SHE* issues.
- Cryogenic step – special requirements/waste.
- With purification – 11 steps.
- Month's supply: $66 LIPITOR® vs $120 ZOCOR®.
- But patent expires ~ 2010.



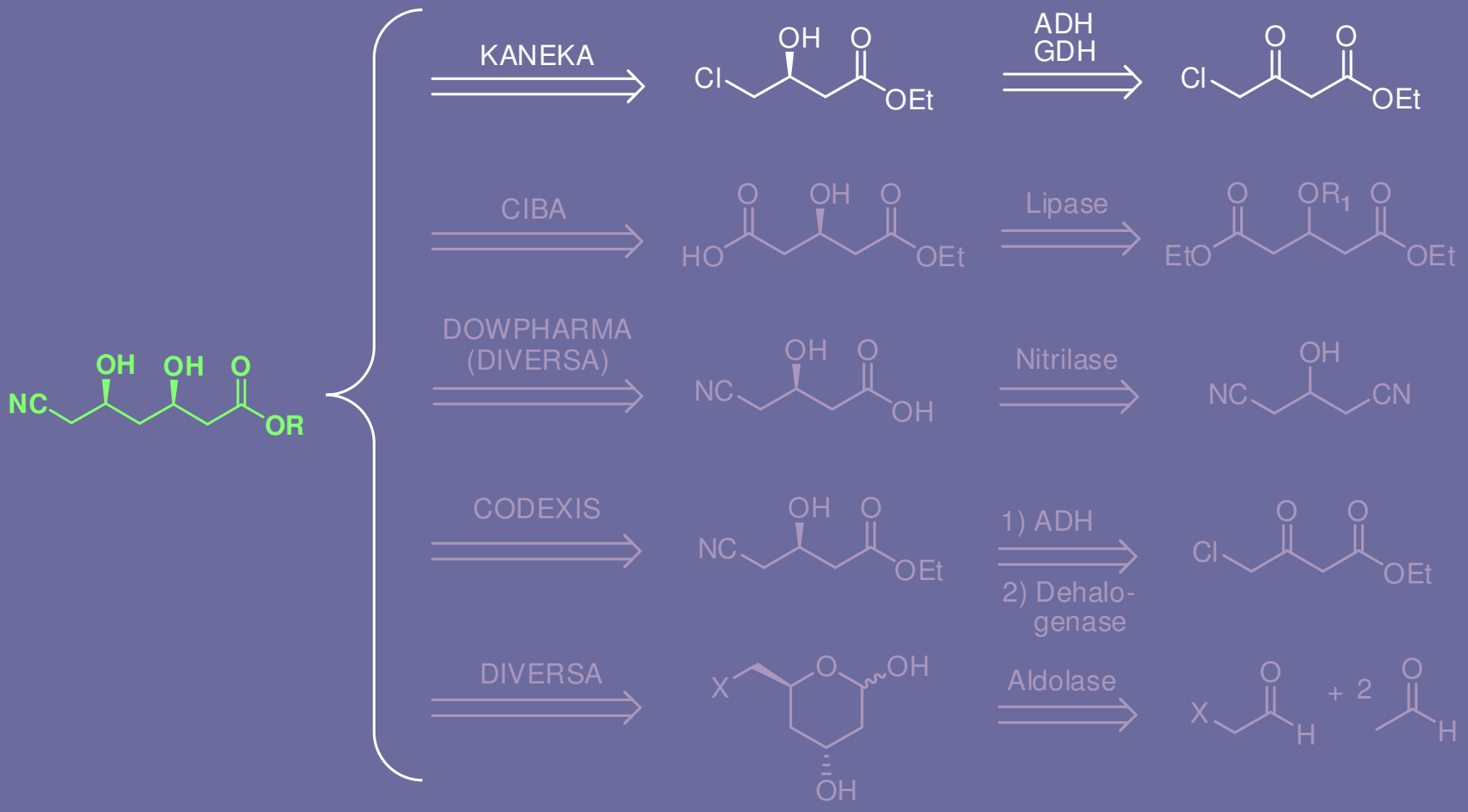
Biocatalytic Routes for the Chiral Side Chain



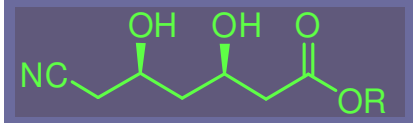
Thayer, A. M. *Chem. Eng. News* **2006**, *84*, (33), 26-27.
Muller, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 362-365.



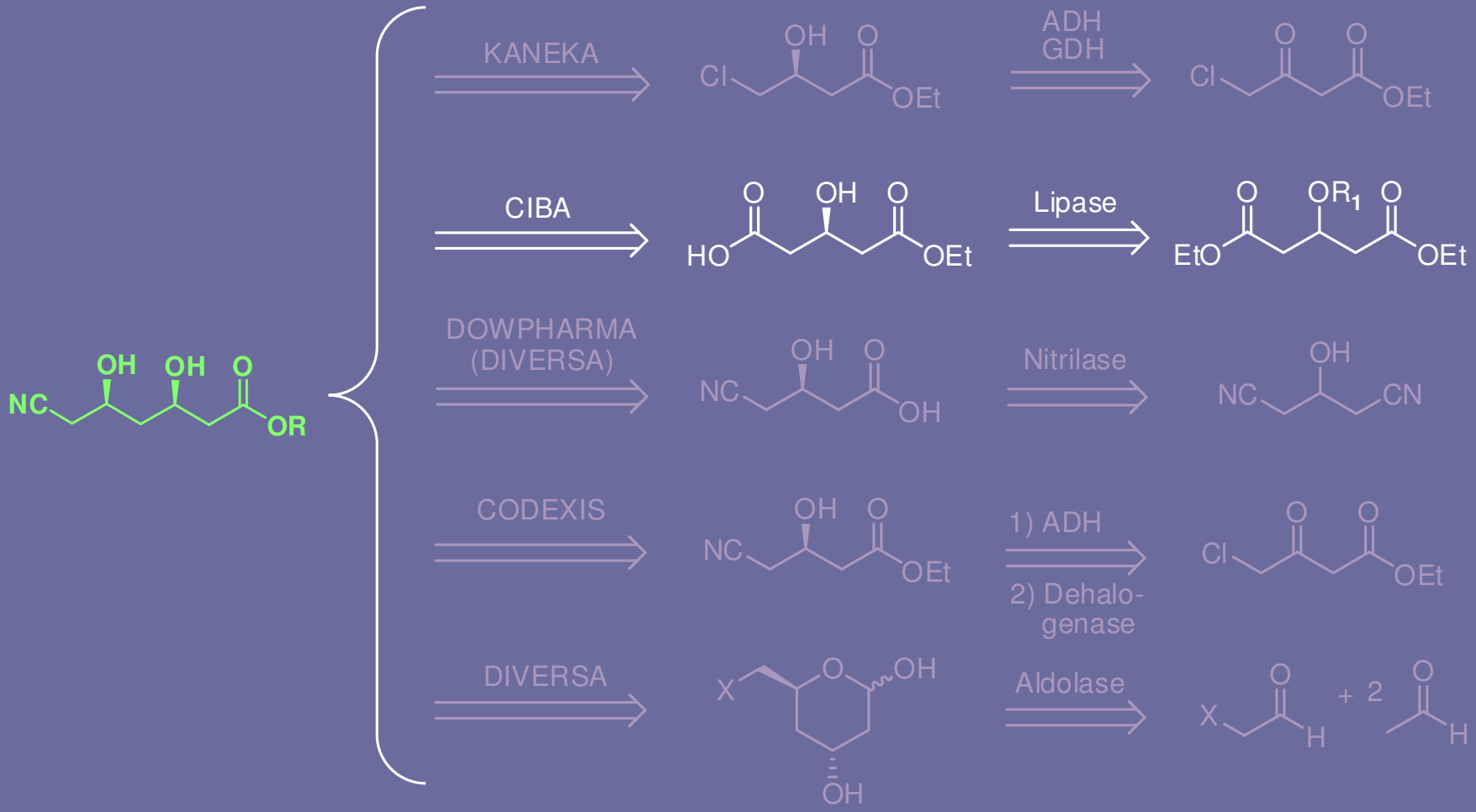
Biocatalytic Routes for the Chiral Side Chain



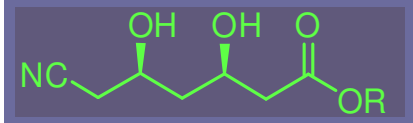
Thayer, A. M. *Chem. Eng. News* **2006**, *84*, (33), 26-27.
Muller, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 362-365.



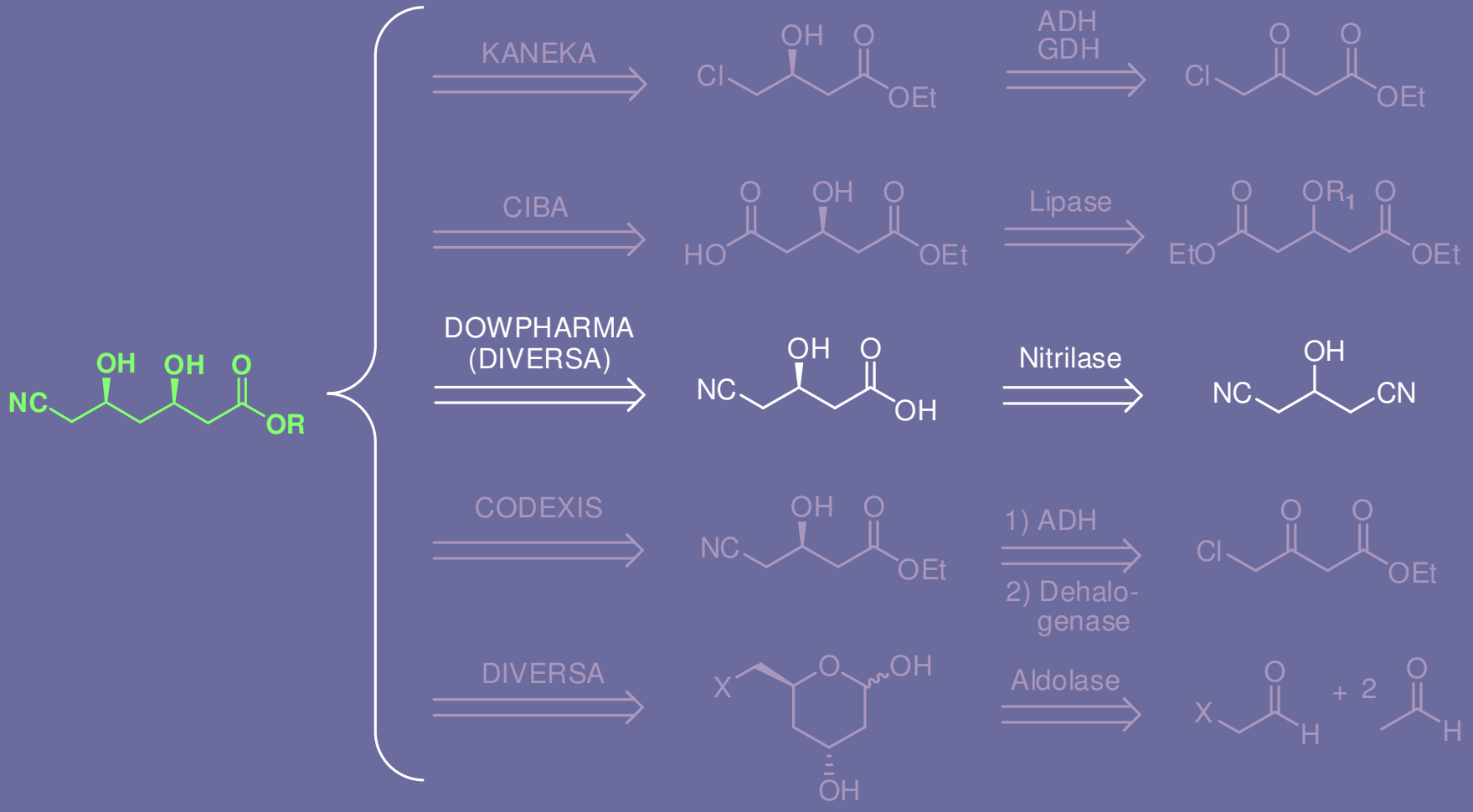
Biocatalytic Routes for the Chiral Side Chain



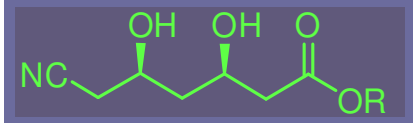
Thayer, A. M. *Chem. Eng. News* **2006**, *84*, (33), 26-27.
Muller, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 362-365.



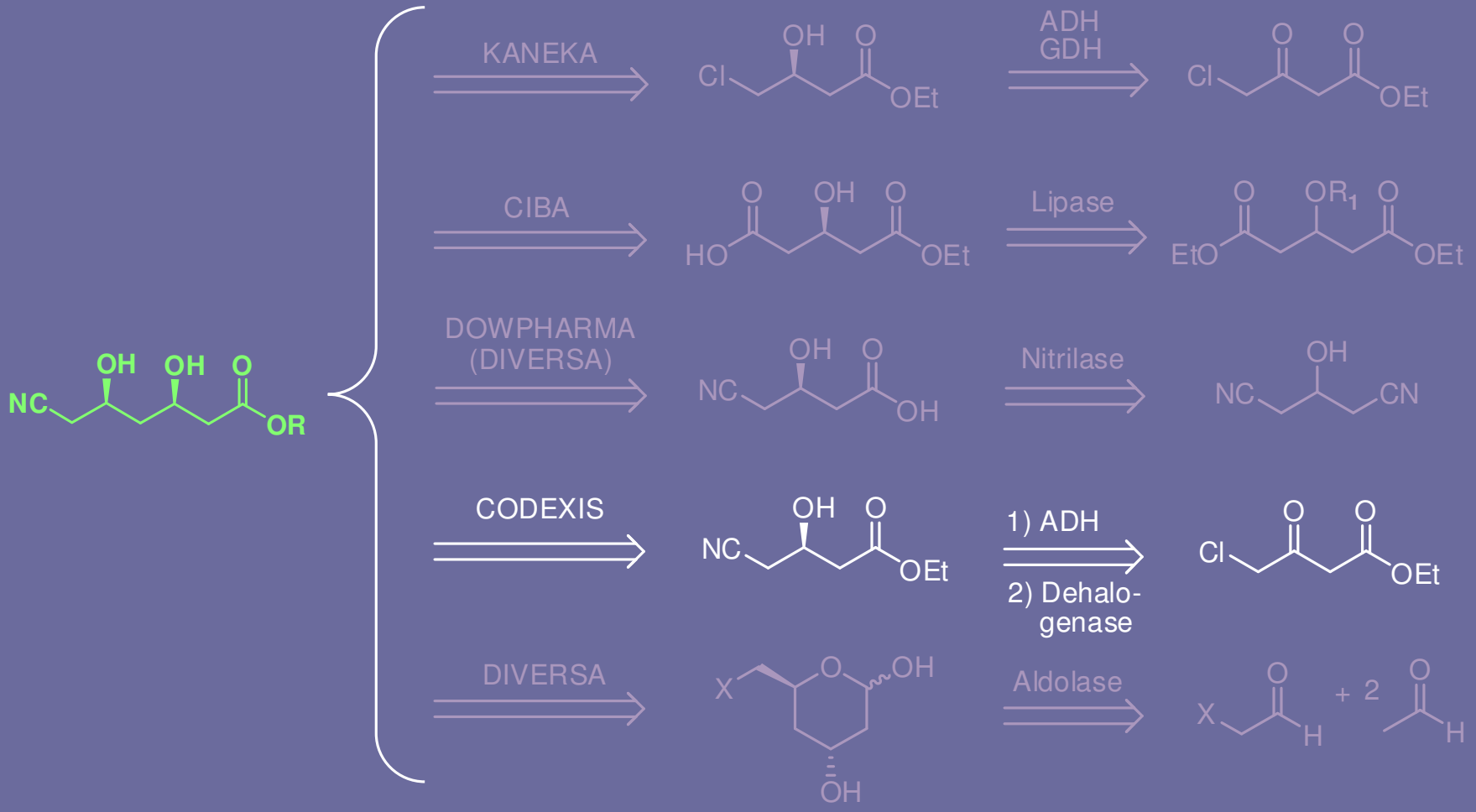
Biocatalytic Routes for the Chiral Side Chain



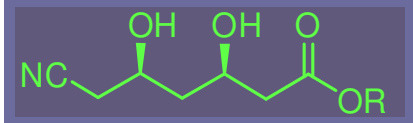
Thayer, A. M. *Chem. Eng. News* **2006**, *84*, (33), 26-27.
Muller, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 362-365.



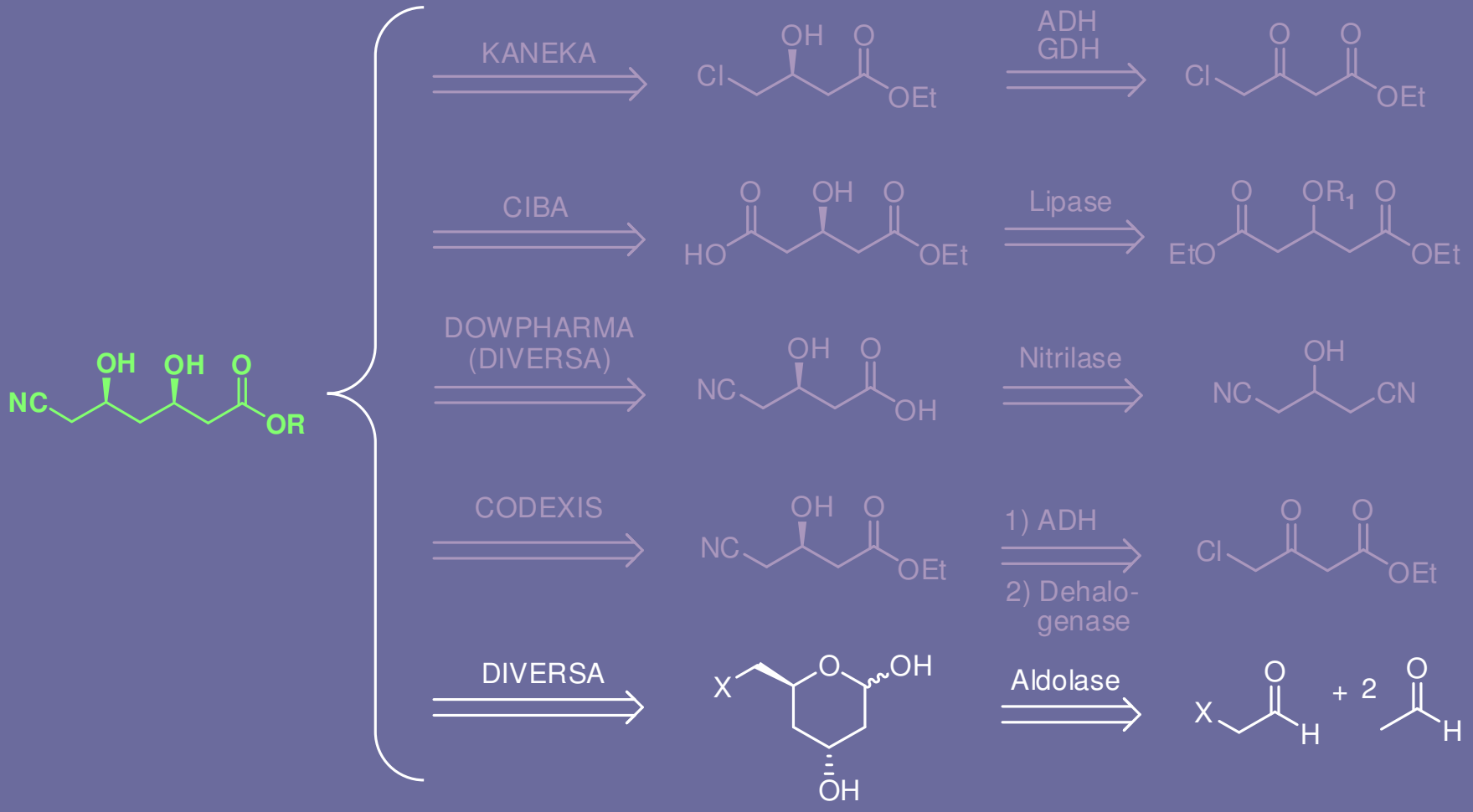
Biocatalytic Routes for the Chiral Side Chain



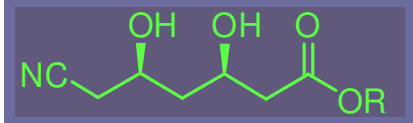
Thayer, A. M. *Chem. Eng. News* **2006**, *84*, (33), 26-27.
Muller, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 362-365.



Biocatalytic Routes for the Chiral Side Chain

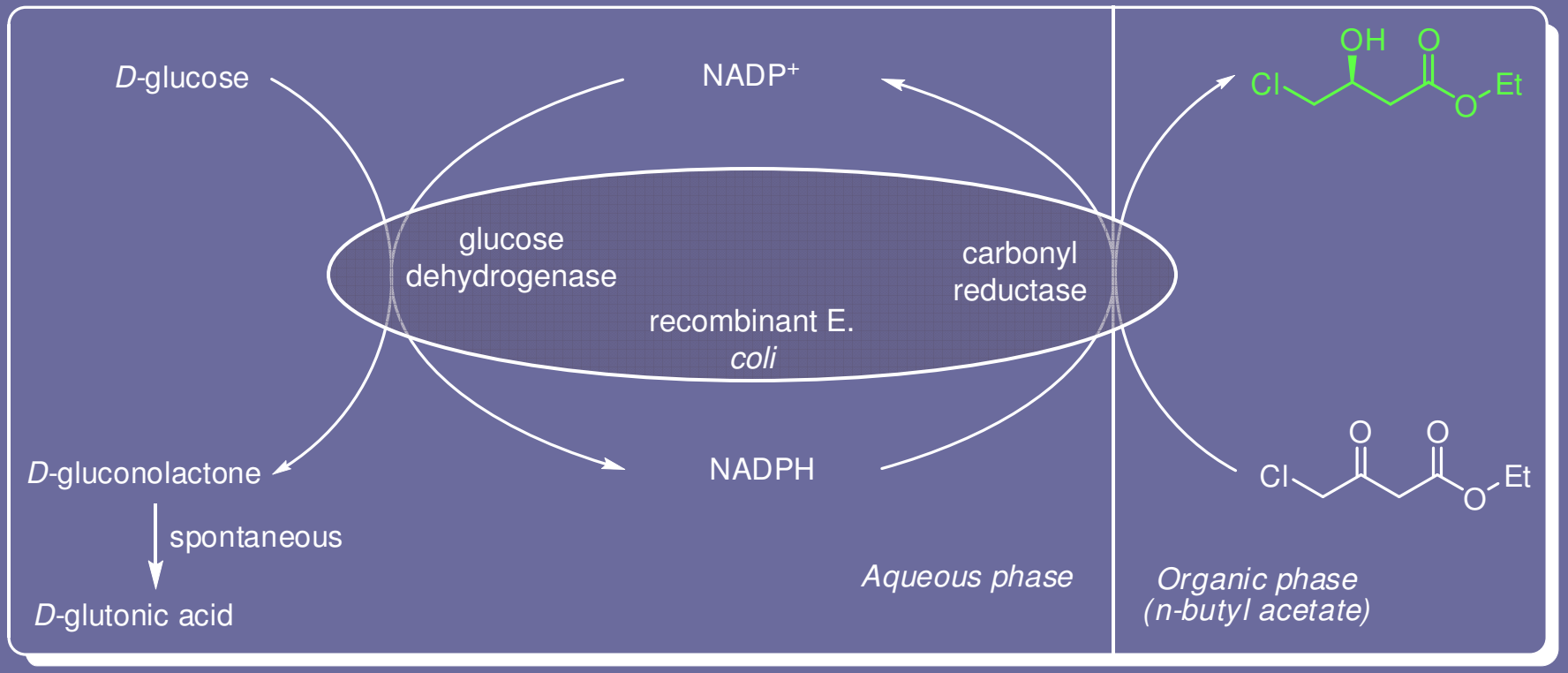


Thayer, A. M. *Chem. Eng. News* **2006**, *84*, (33), 26-27.
Muller, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 362-365.



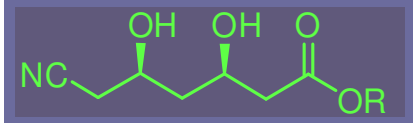
Kaneka's Route

kaneka
株式会社カネカ



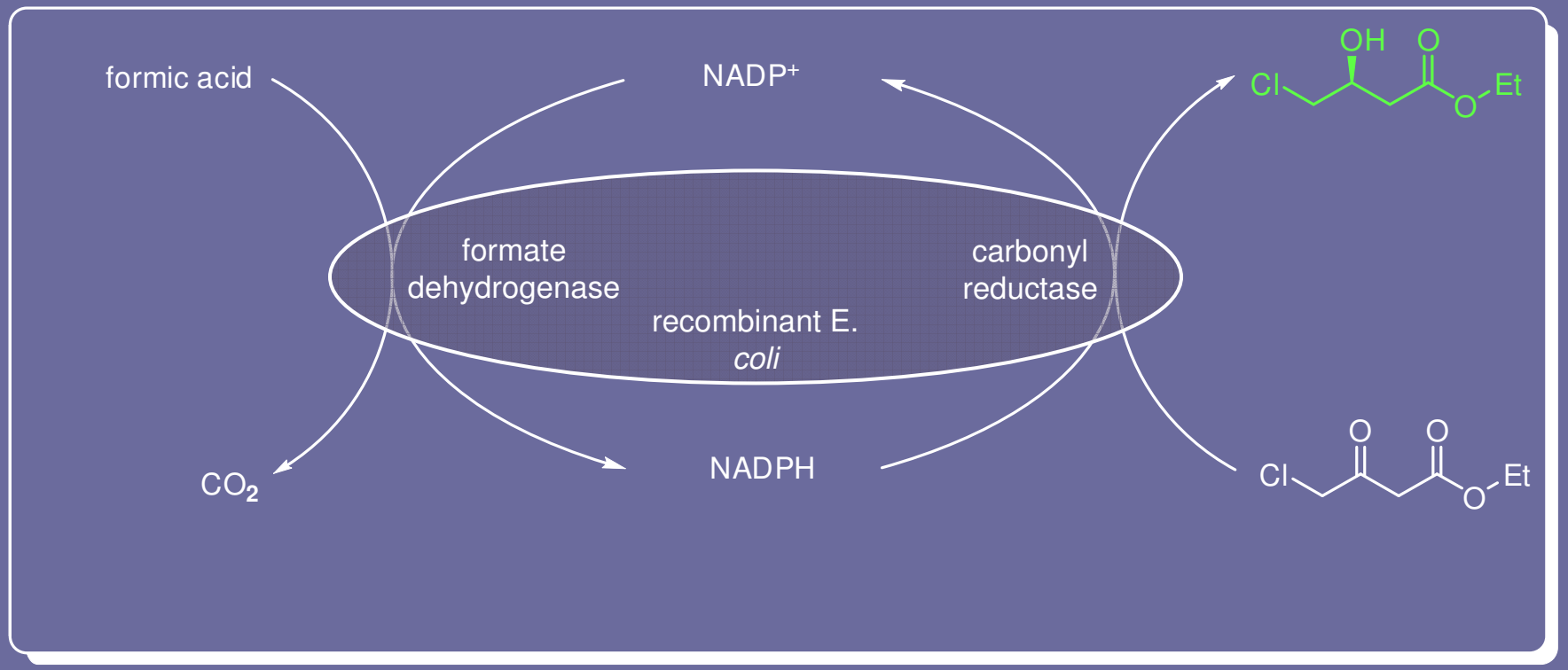
- >99.9% ee, 89% yield.
- Product concentration: 450 g/L.
- NADP+ TON: 16,200 mol/mol.
- Problematic product separation.

Kizaki, N. *et al. Appl. Microbiol. Biotechnol.* **2001**, *55*, 590-595.
Yasohara, Y. *et al. Tetrahedron: Asymmetry* **2001**, *12*, 1713-1718.



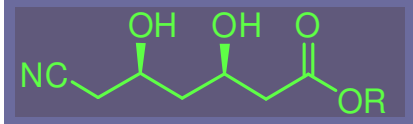
Daicel's Route

DAICEL CHEMICAL INDUSTRIES, LTD.



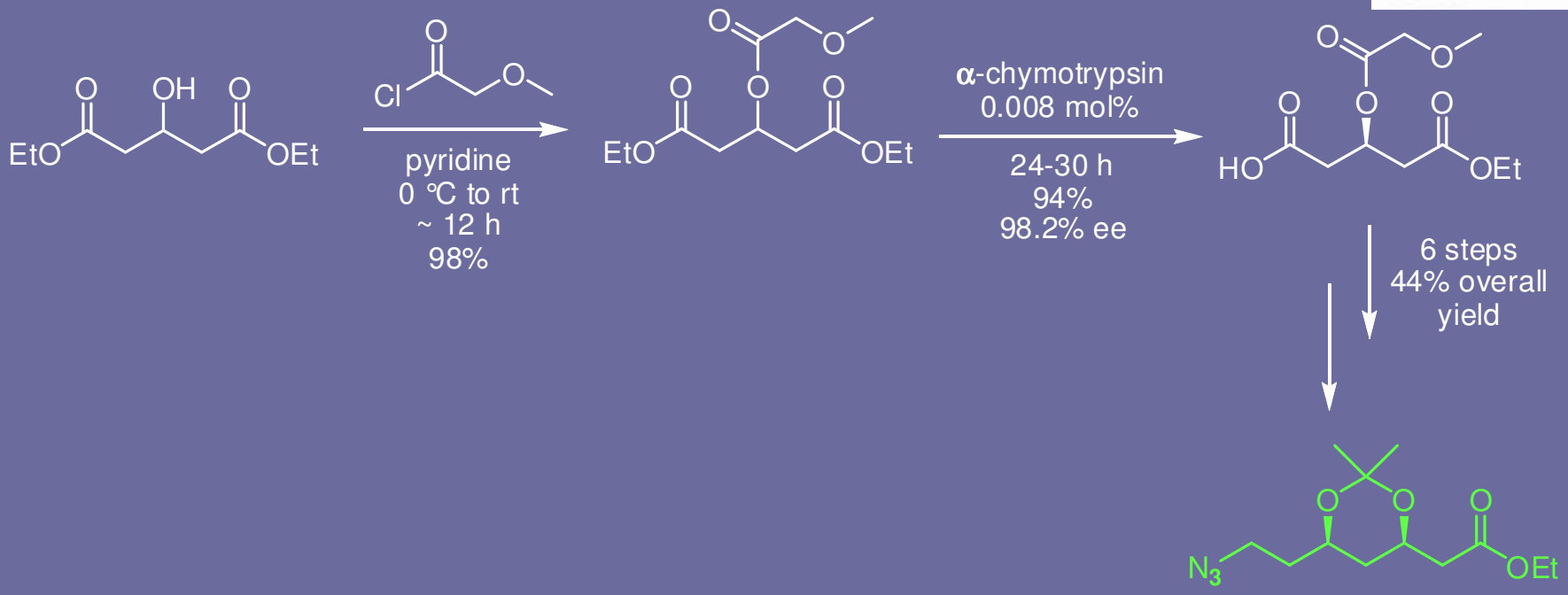
- >99% ee.
- Product concentration: 50 g/L.
- Easy product separation.
- Commercially used: >100 ton/year.

Rouhi, A. M. *Chem. Eng. News* **2004**, *82*, (24), 47-62.



Ciba's Route

Ciba



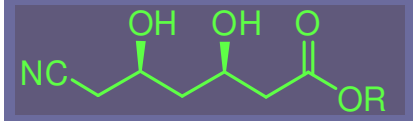
The good things

- 94% yield, 98.2% ee.
- Substrate concentration: 285 g/L.
- Kilogram scale.
- Cheap & robust biocatalyst.

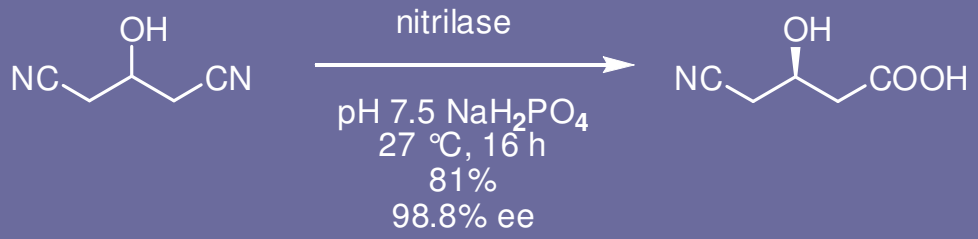
The bad things

- Follow up chemistry – long.
- Low temperature reactions.
- Column chromatography.

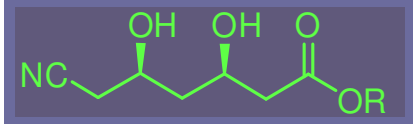
Öhrlein, R.; Baischf, G. *Adv. Synth. Catal.* **2003**, *345*, 713-715.



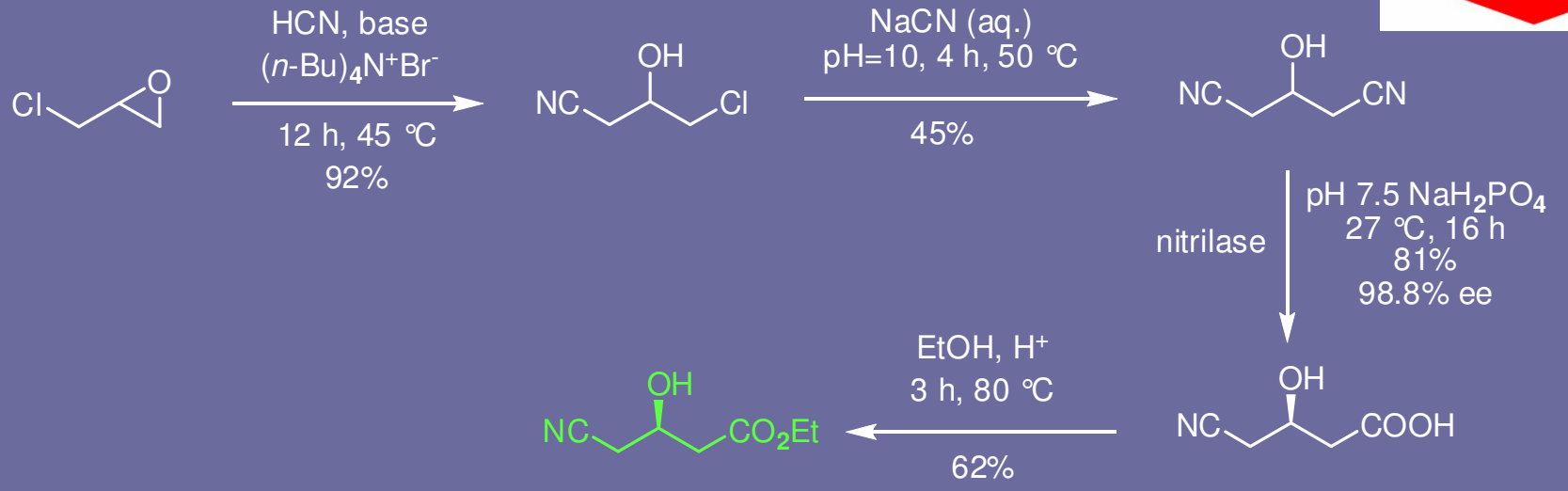
Diversa/Dowpharma's Route



DeSantis, G. *et al. J. Am. Chem. Soc.* **2002**, *124*, 9024-9025.
DeSantis, G. *et al. J. Am. Chem. Soc.* **2003**, *125*, 11476-11477.



Diversa/Dowpharma's Route



The good things

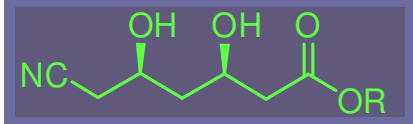
- Cheap starting material.
- Efficient enzymatic step: 3 M [substrate] & 619 g L^{-1} d^{-1}.
- Low cost of catalyst by expression in *Pseudomonas fluorescens* developed by Dow.

Scale-up economics good!

The bad things

- HCN under heated alkaline conditions.
- Special equipment for purification.
- Some low yield steps.

Bergeron, S. *et al. Org. Process Res. Dev.* **2006**, *10*, 661-665.

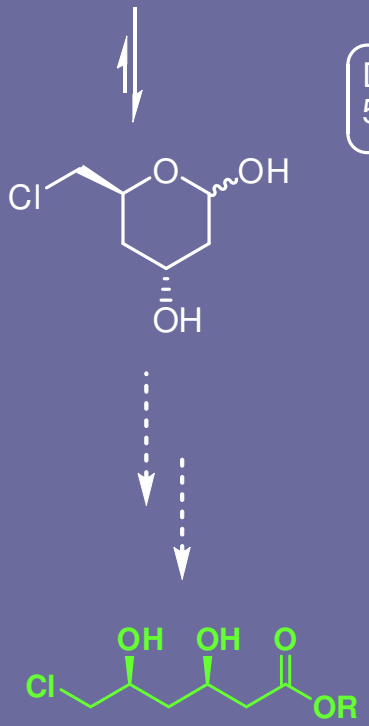


Diversa's Route

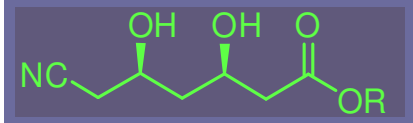
DIVERSA
HARNESSING THE POWER OF ENZYMES

DERA = deoxyribose-5-phosphate aldolase

	Wild Type DERA
Catalyst Load	20% w/w
Product Isolation	Difficult
Reaction Time	6 days
[chloroacetaldehyde]	100 mM
Volumetric Productivity	2 g L^{-1} d^{-1}
ee (de)	Unknown
Practical?	NO



Gijsen, H. J. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 8422-8423.
Wong, C.-H. *et al. J. Am. Chem. Soc.* **1995**, *117*, 3333-3339.

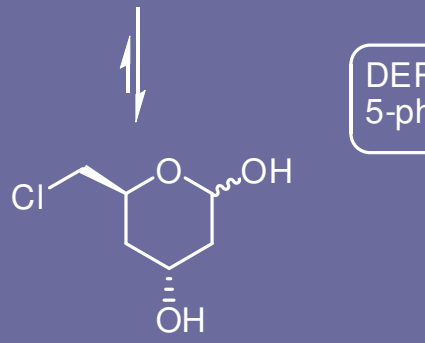


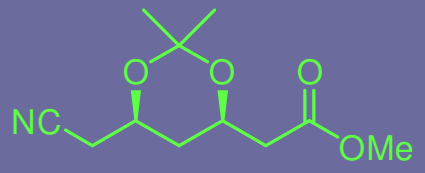
Diversa's Route

DIVERSA
HARNESSING THE POWER OF ENZYMES

DERA = deoxyribose-5-phosphate aldolase

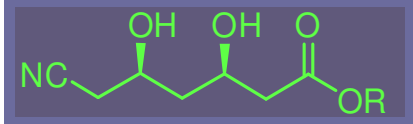
	Wild Type DERA	Improved DERA
Catalyst Load	20% w/w	2% w/w
Product Isolation	Difficult	Simple
Reaction Time	6 days	3 h
[chloroacetaldehyde]	100 mM	Fed-batch process
Volumetric Productivity	2 g L^{-1} d^{-1}	735 g L^{-1} d^{-1}
ee & de	Unknown	≥ 99.9%
Practical?	NO	YES





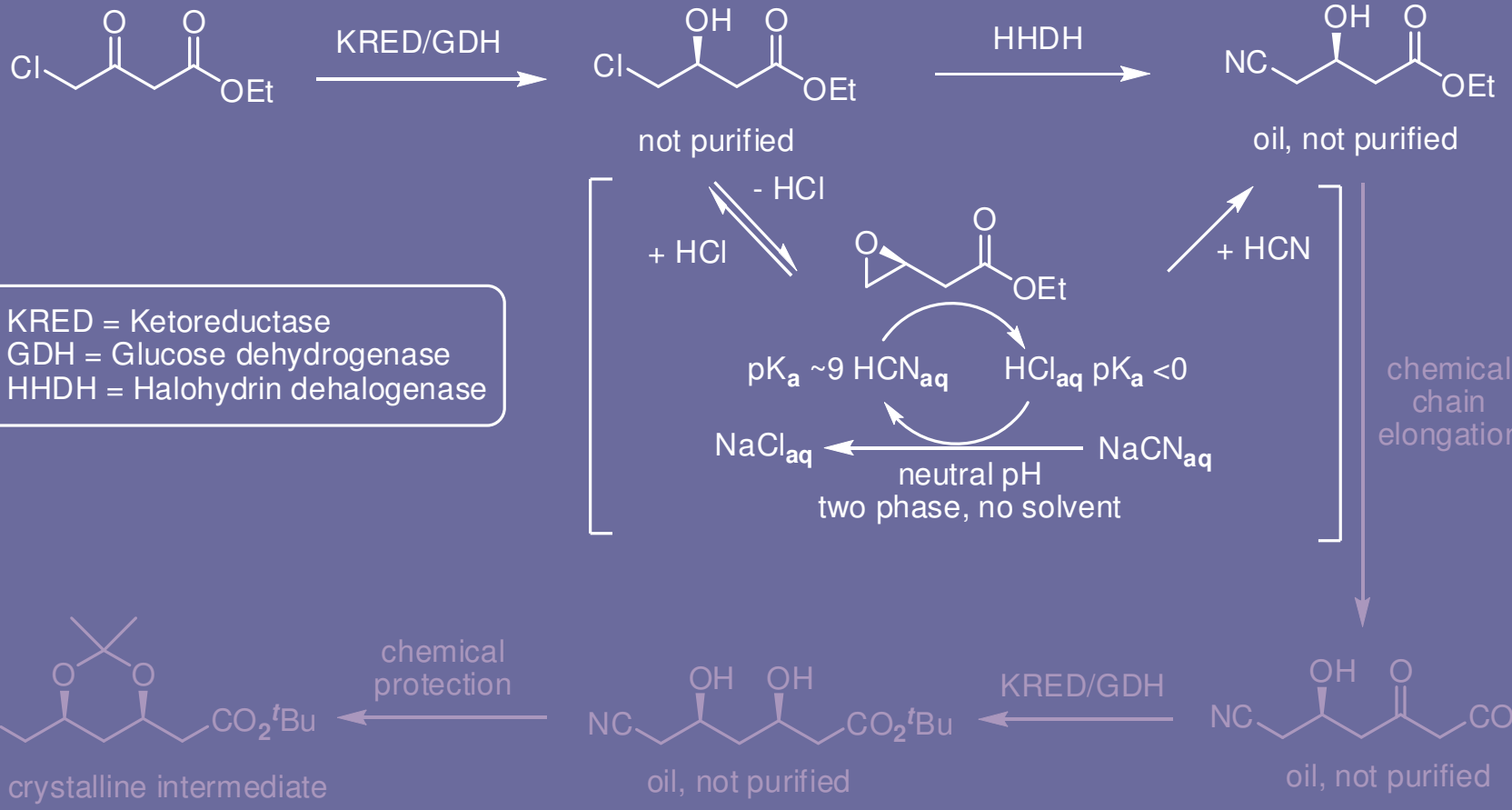
23% yield
3 overall steps

Greenberg, W. A. *et al. Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5788-5793.

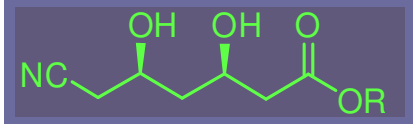


Codexis Route

CODEXIS

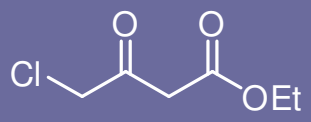


Dr. Peter Seufer-Wasserthal (VP, Head of Codexis Pharma Services), personal communication.

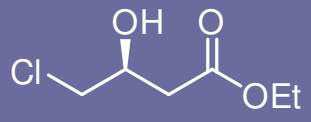


Codexis Route

CODEXIS

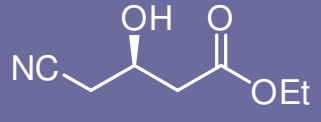


KRED/GDH



not purified

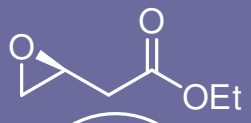
HHDH



oil, not purified

KRED = Ketoreductase
GDH = Glucose dehydrogenase
HHDH = Halohydrin dehalogenase

- HCl
+ HCl



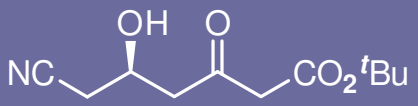
+ HCN

pK_a ~9 HCN_{aq} HCl_{aq} pK_a <0

$NaCl_{aq}$ $NaCN_{aq}$

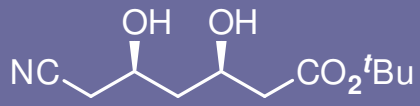
neutral pH
two phase, no solvent

chemical chain elongation



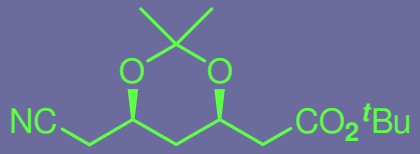
oil, not purified

KRED/GDH



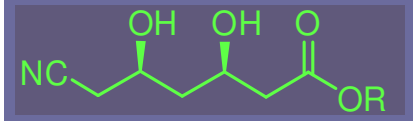
oil, not purified

chemical protection



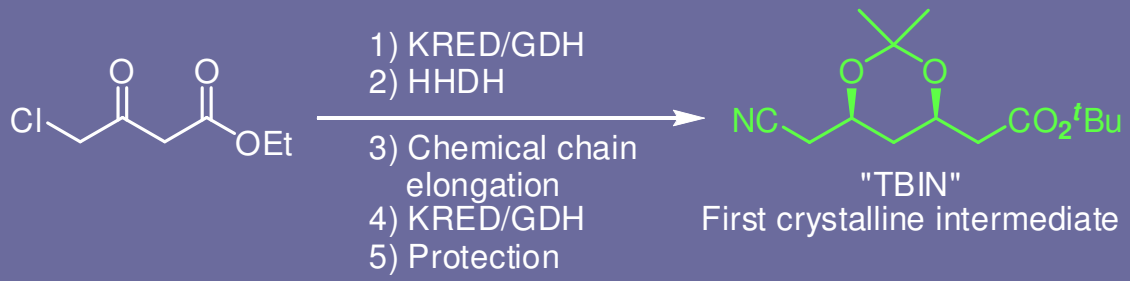
first crystalline intermediate

Dr. Peter Seufer-Wasserthal (VP, Head of Codexis Pharma Services), personal communication.



Codexis Route

CODEXIS



"TBIN"
First crystalline intermediate

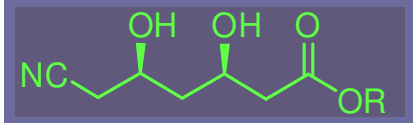
- Excellent selectivities and yields.
- Purification only at the last step – reduced requirements.
- Green and safe.
- Reduced by-products.
- Mild reaction conditions.
- Efficient, scalable and cost effective.
- Commercialized: (1) Arch Pharmalabs, India – Codexis production partner.

  (2) Lonza – a Pfizer supplier.

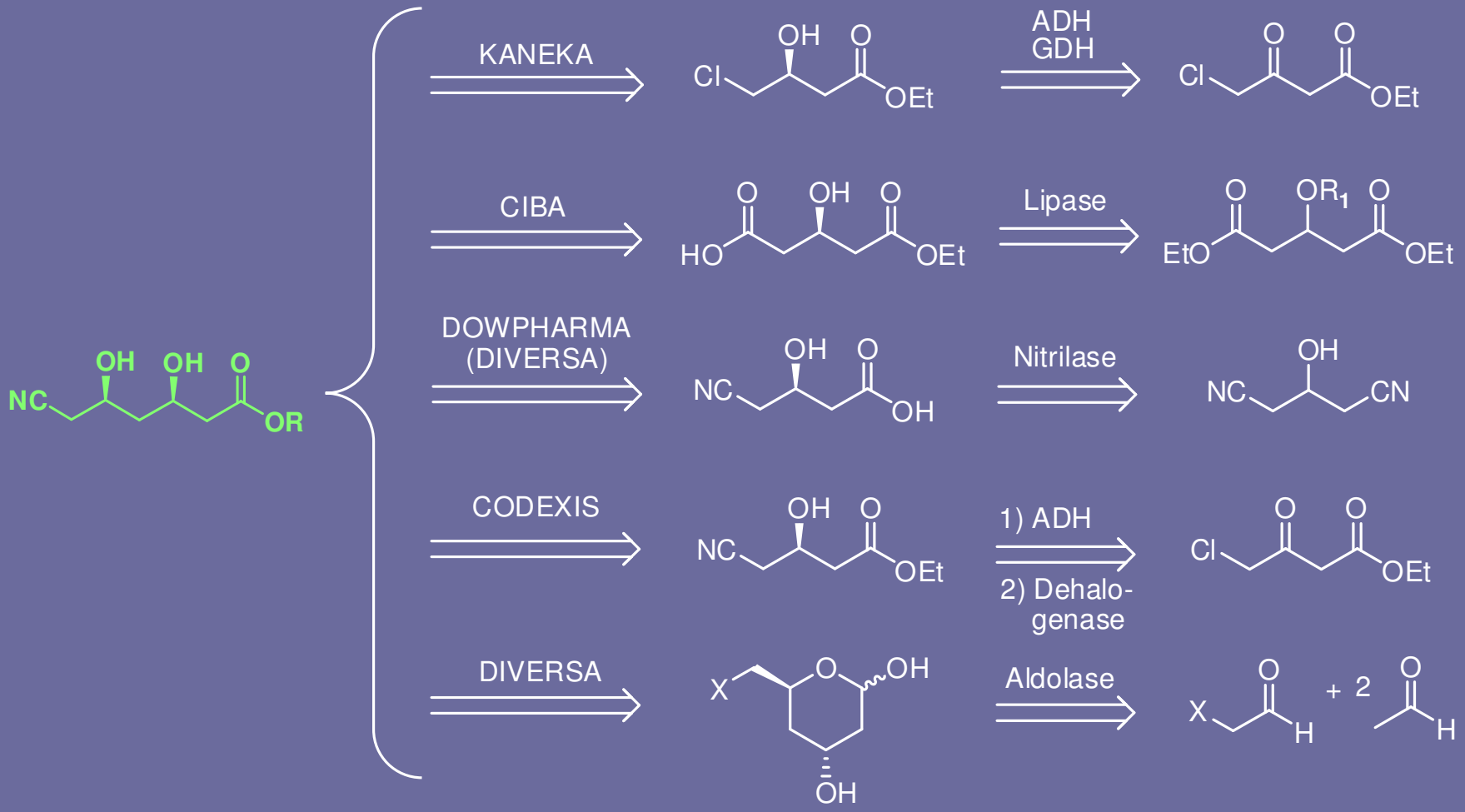
GREEN CHEMISTRY CHALLENGE

Presidential Green Chemistry Challenge Award, 2006

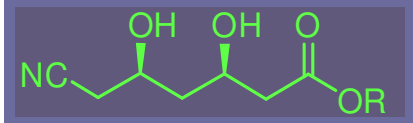
Dr. Peter Seufer-Wasserthal (VP, Head of Codexis Pharma Services), personal communication.



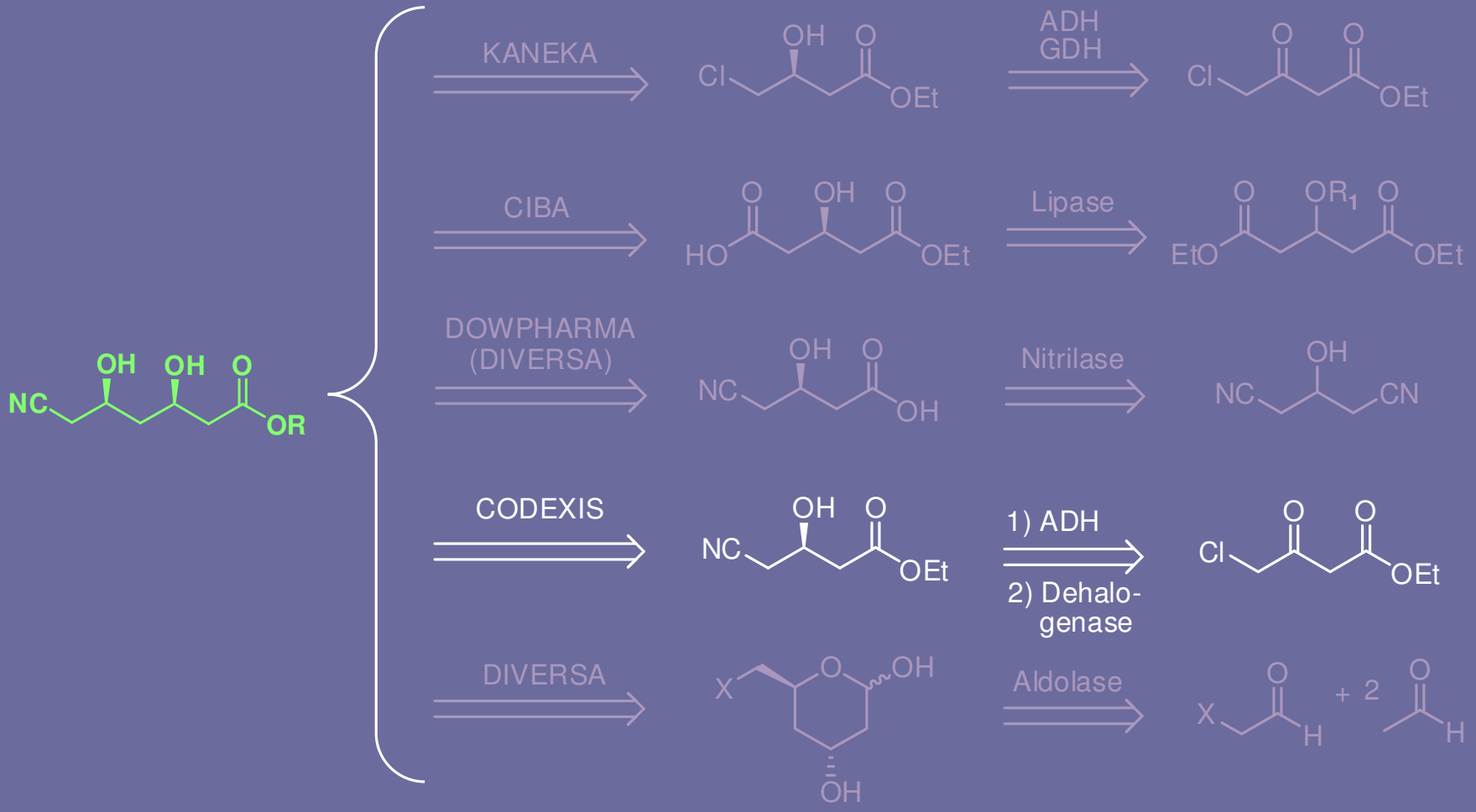
Biocatalytic Routes for the Chiral Side Chain



Thayer, A. M. *Chem. Eng. News* **2006**, *84*, (33), 26-27.
Muller, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 362-365.

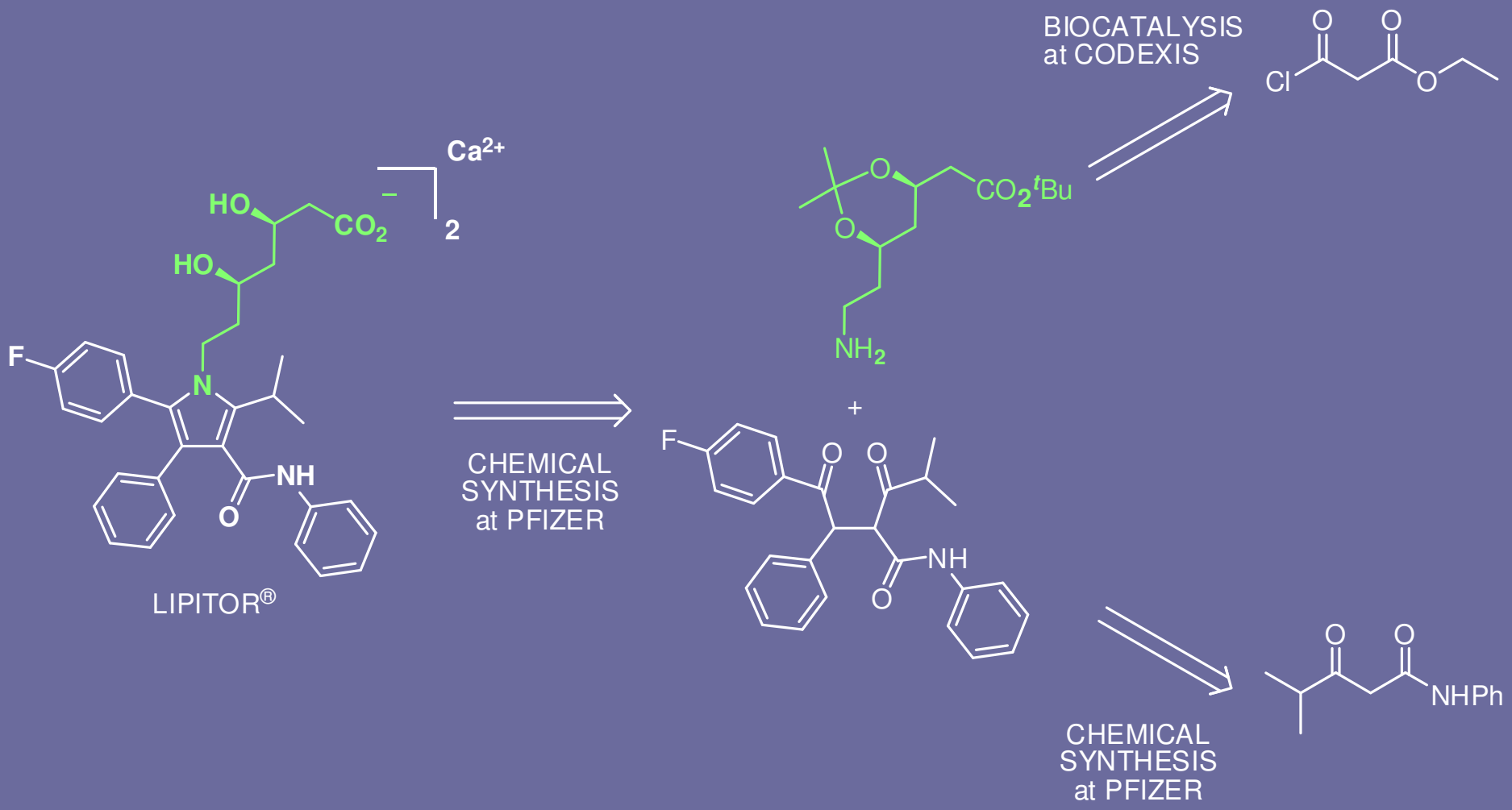


Biocatalytic Routes for the Chiral Side Chain

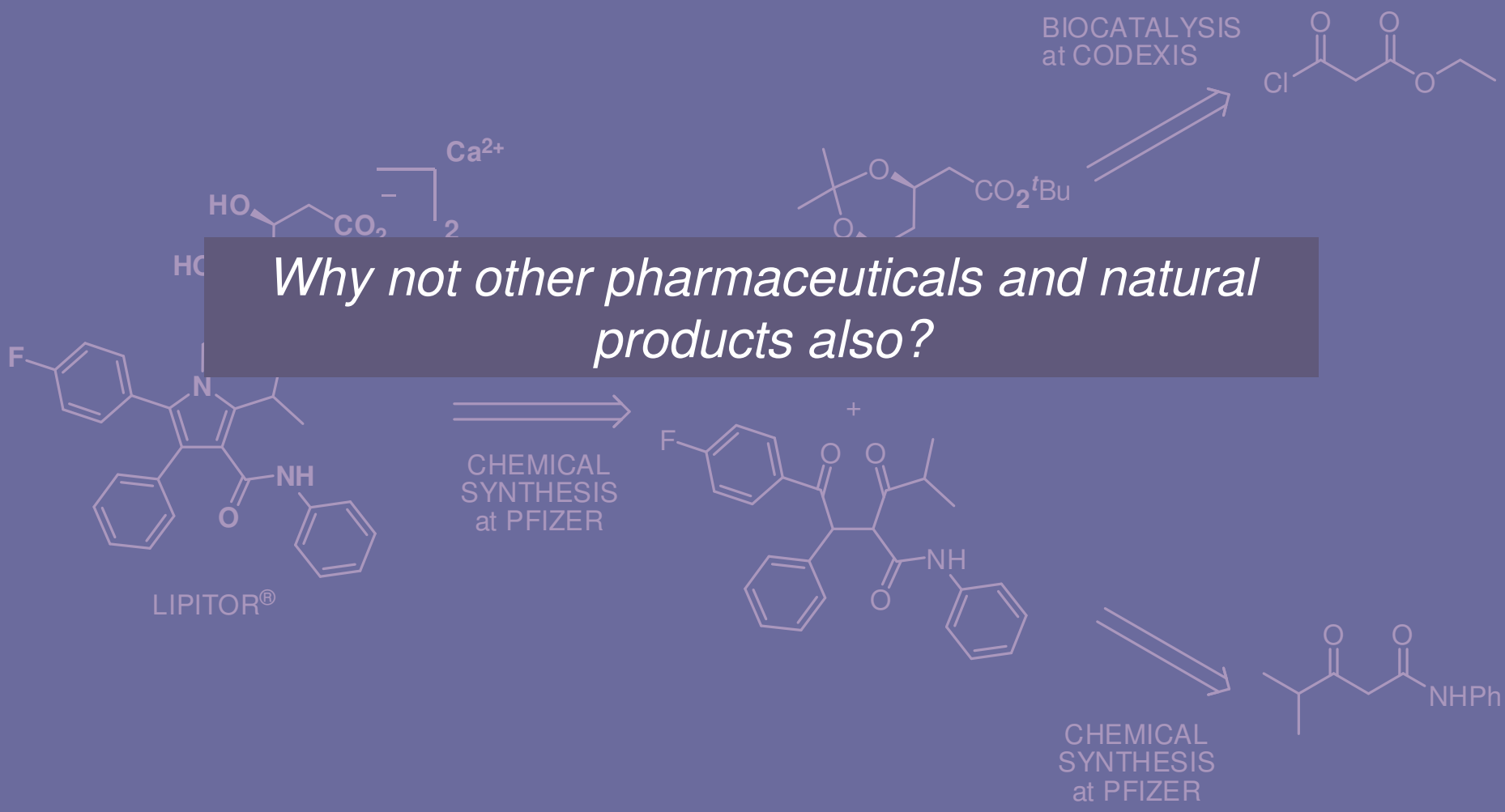


Thayer, A. M. *Chem. Eng. News* **2006**, *84*, (33), 26-27.
Muller, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 362-365.

An Extremely Attractive Chemo-enzymatic Approach



An Extremely Attractive Chemo-enzymatic Approach



Why not other pharmaceuticals and natural products also?

The Take-Home Message

The Story of LIPITOR®: Drug Discovery & Chemical Development

- The world of process chemistry works on the same basic principles as us here in this building.
- But in many respects, it is a vastly different world, driven by an entirely different set of considerations.

The Story of LIPITOR®: Biocatalytic Routes for the Sidechain

- State-of-art enzymatic transformations have reached an extraordinary level, making them valuable and competitive methods for use in the pharmaceutical industry.
- Biocatalysis has been emerging

The Take-Home Message

The Story of LIPITOR®: Drug Discovery & Chemical Development

- The world of process chemistry works on the same basic principles as us here in this building.
- But in many respects, it is a vastly different world, driven by an entirely different set of considerations.

The Story of LIPITOR®: Biocatalytic Routes for the Sidechain

- State-of-art enzymatic transformations have reached an extraordinary level, making them valuable and competitive methods for use in the pharmaceutical industry.
- Biocatalysis has ~~been emerging~~ clearly emerged as a very attractive tool in a synthetic chemist's toolkit.

Acknowledgements

Dr. Wulff

Dr. Walker Dr. Borhan Dr. Maleczka

Dr. Bruce Roth (VP, Global R&D, Pfizer)

Dr. Donald Butler (Former Process Development Leader, Pfizer
Chief Science Advisor, Austin Chemical Company)

Dr. Peter Seufer-Wasserthal (VP, Head of Pharma Services, Codexis)

Dr. Stephen Ritter (Senior Editor, C&EN)

Dr. Christopher Schmid (Lilly) Professor Samir Zard

Dr. Jos Brands (Merck) Dr. Michael Lipton (SPCorp)

Keith, Ding, Cory, Zhenjie, Gang, Chunrui, Alex, Kostas, Dima, Nilanjana, Anil, Munmun, Li, Yong

Janelle

Aman K., Toyin, Luis, Brian and Alli & Dan

Other Beneficial Effects of Statin Drugs

- Promotion of new blood vessel growth.
- Stimulation of bone formation.
- Protection against modification of low density lipoproteins.
- Reduction of clot-forming process so important in plaque formation.
- Improvement in endothelial function.
- Reduction of inflammation in blood vessel tissue.

Istvan, E. S.; Deisenhofer, J. *Science* **2001**, *292*, 1160-1164.
Rosanoff, A.; Seelig, M. S. *Journal of the American College of Nutrition* **2004**, *23*, 501S-505.

Some Rare Adverse Effects of Statin Drugs

• The most common side effects are gas, constipation, stomach pain and heartburn.

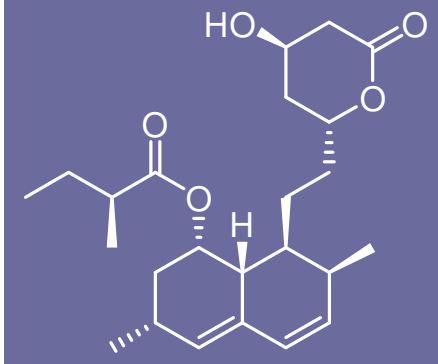
• Rare Myalgia (muscle pain). → possibly life threatening!

• Rare Rhabdomyolosis (skeletal muscle failure – loss of kidney function).

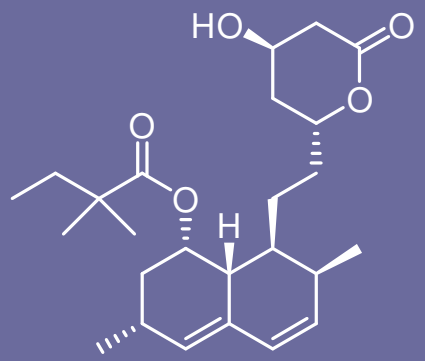
Uncommon side reactions occur mainly when statins are co-prescribed with other interacting drugs.

www.lipitor.com
Rosanoff, A.; Seelig, M. S. *Journal of the American College of Nutrition* **2004**, *23*, 501S-505.

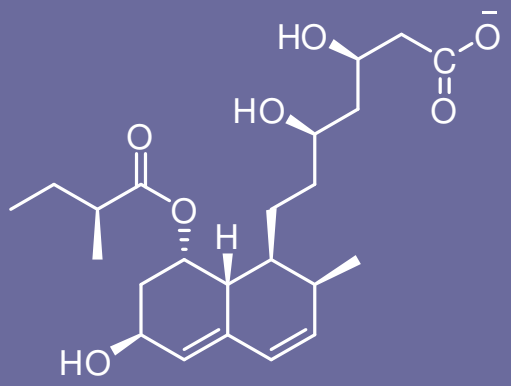
Statin Drugs



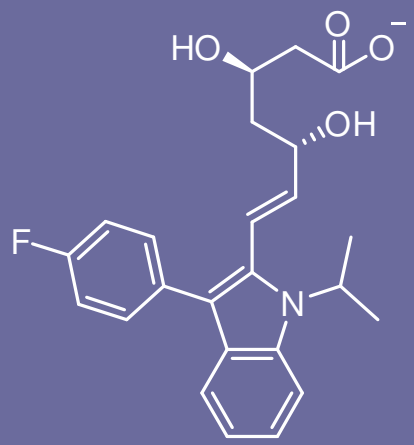
Lovastatin (MEVACOR®)
MERCK



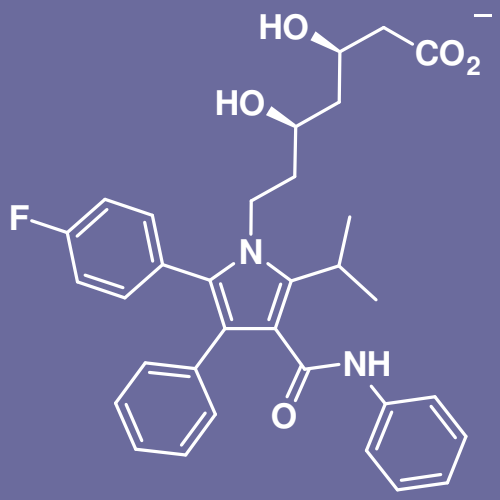
Simvastatin (ZOCOR®)
MERCK



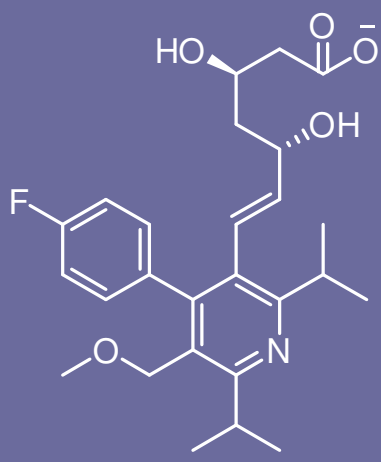
Pravastatin (PRAVACOL®)
BRISTOL - MYERS SQUIBB



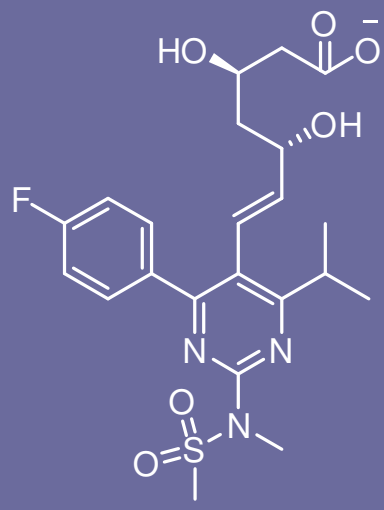
Fluvastatin (LESCOL®)
NOVARTIS



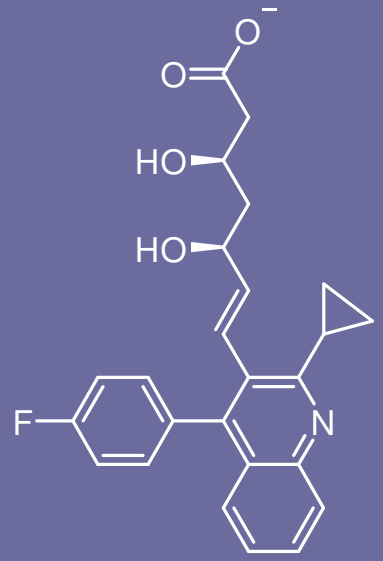
Atorvastatin (LIPITOR®)
PFIZER



Cerivastatin (LIPOBAY®)
BAYCOL
withdrawn in 2001

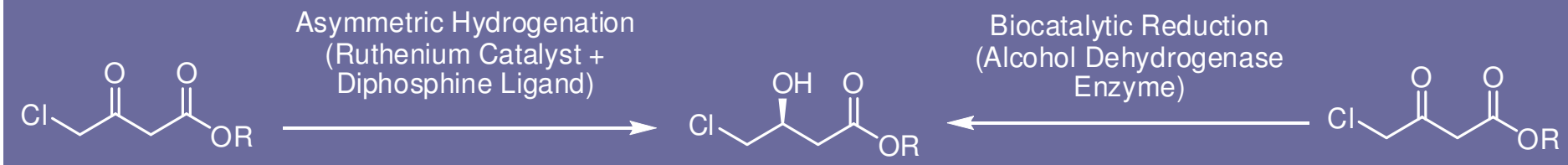


Rosuvastatin (CRESTOR®)
ASTRAZENECA



Pitavastatin (LIVALO®)
KOWA

Chemo-catalytic vs. Bio-catalytic: Wacker Specialty Chemicals



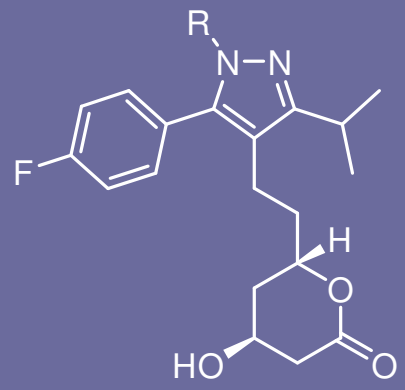
	Chemo-catalysis	Bio-catalysis
%*ee* (yield)	98 (95)	>99.9 (97)
Scale	Multi-ton	Multi-ton
Cost/kg (product)	< $85	< $100
SHE issues	100 °C, MeOH solvent, handling H_2	None – ambient temp. & pressure
Equipment	Standard	Standard
Waste/kg (product)	< 100 g	2 L
Relative Throughput	3	1 (dilute conditions)

Requirement of price & purity will dictate the route.

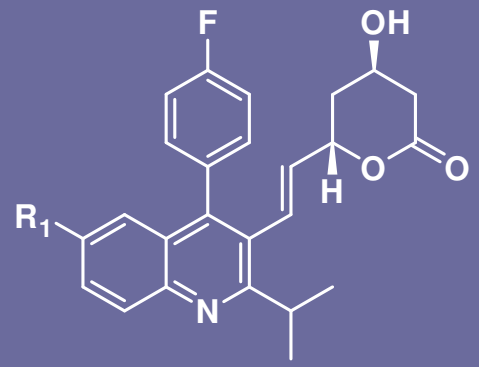
Rouhi, A. M. *Chem. Eng. News* **2004**, *82*, (24), 47-62.

The Drug Discovery

Other Templates Attempted

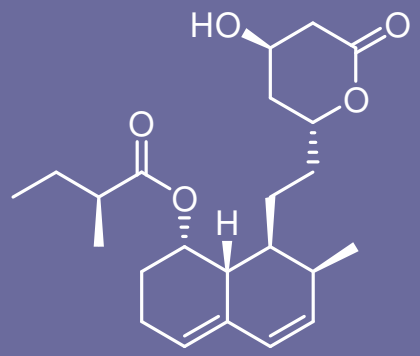


R = Ph (IC_{50} = 0.035 μM)



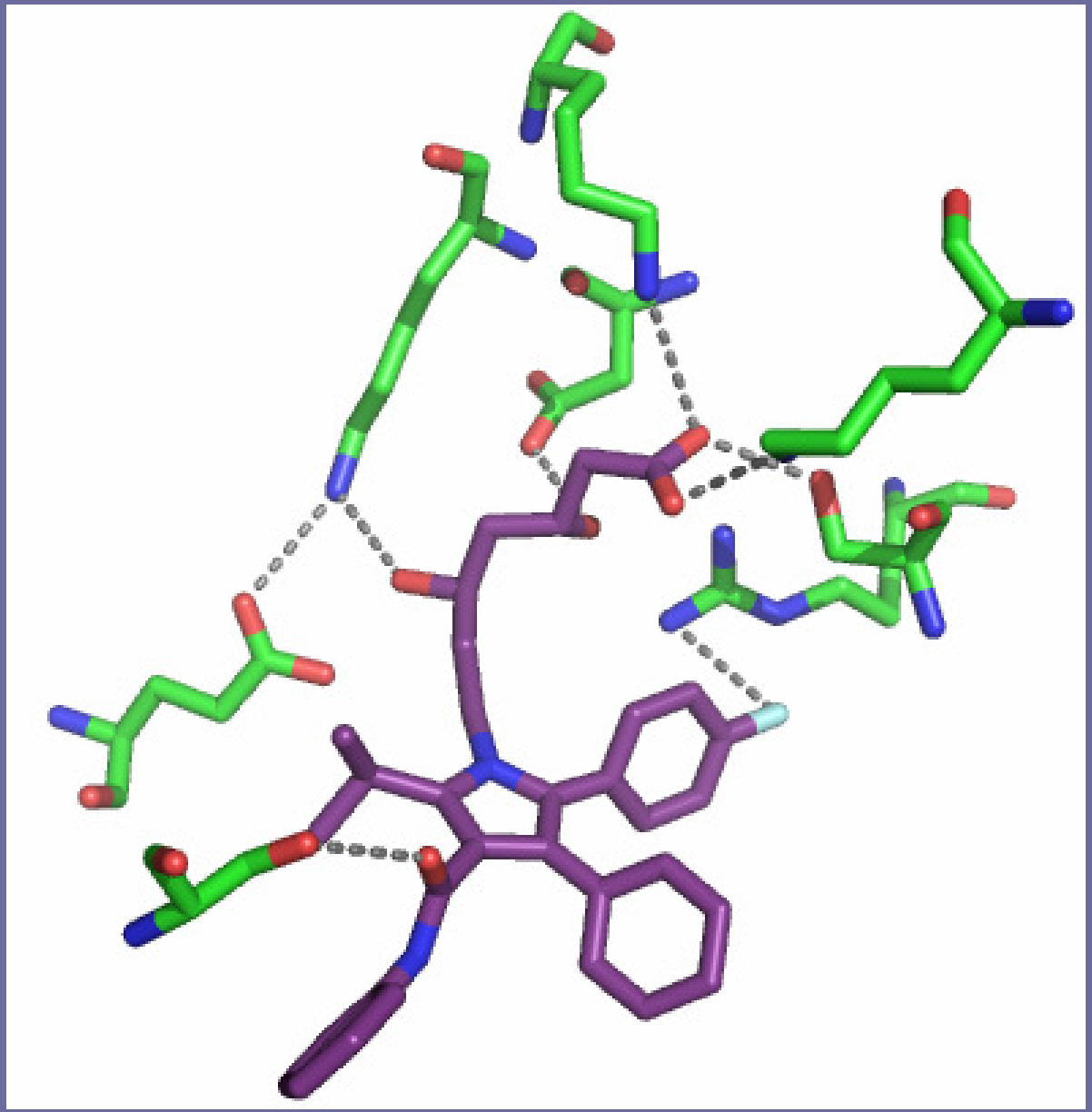
Compound	R_1	IC_{50} (μM)
1	Cl	0.032
2	OCH_3	0.013
3 (*N*-oxide)	F	0.018

mevastatin: IC_{50} = 0.030 μM

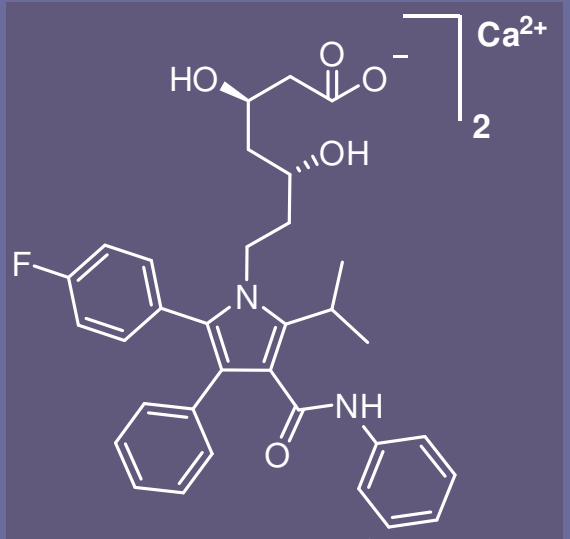


Sliskovic, D. R. *et al. J. Med. Chem.* **1991**, *34*, 367-373.
Sliskovic, D. R. *et al. J. Med. Chem.* **1990**, *33*, 31-38.

Human HMGR with LIPITOR



Istvan, E. S.; Deisenhofer, J. *Science* **2001**, *292*, 1160-1164.

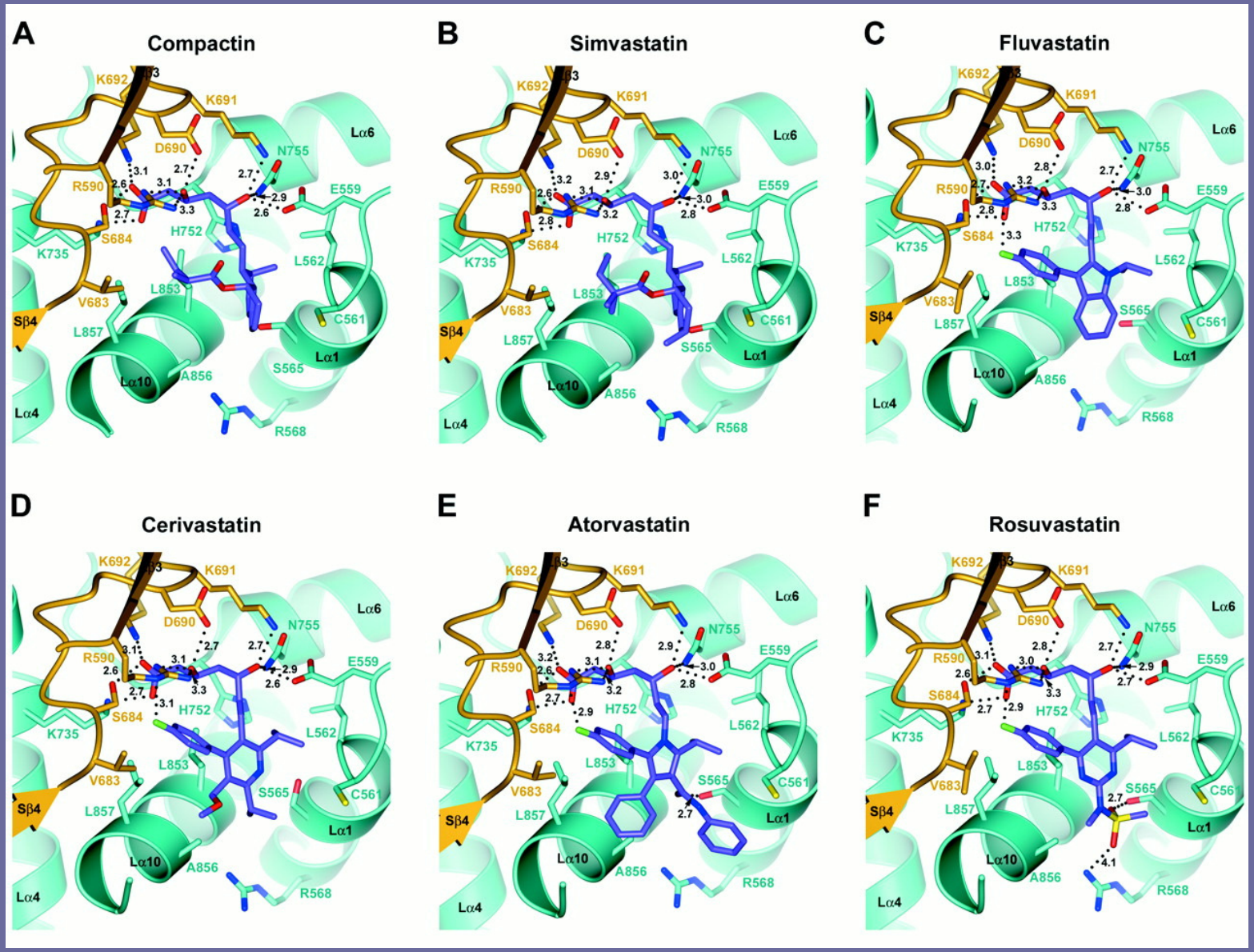


LIPITOR®

The specificity & tight binding achieved by:

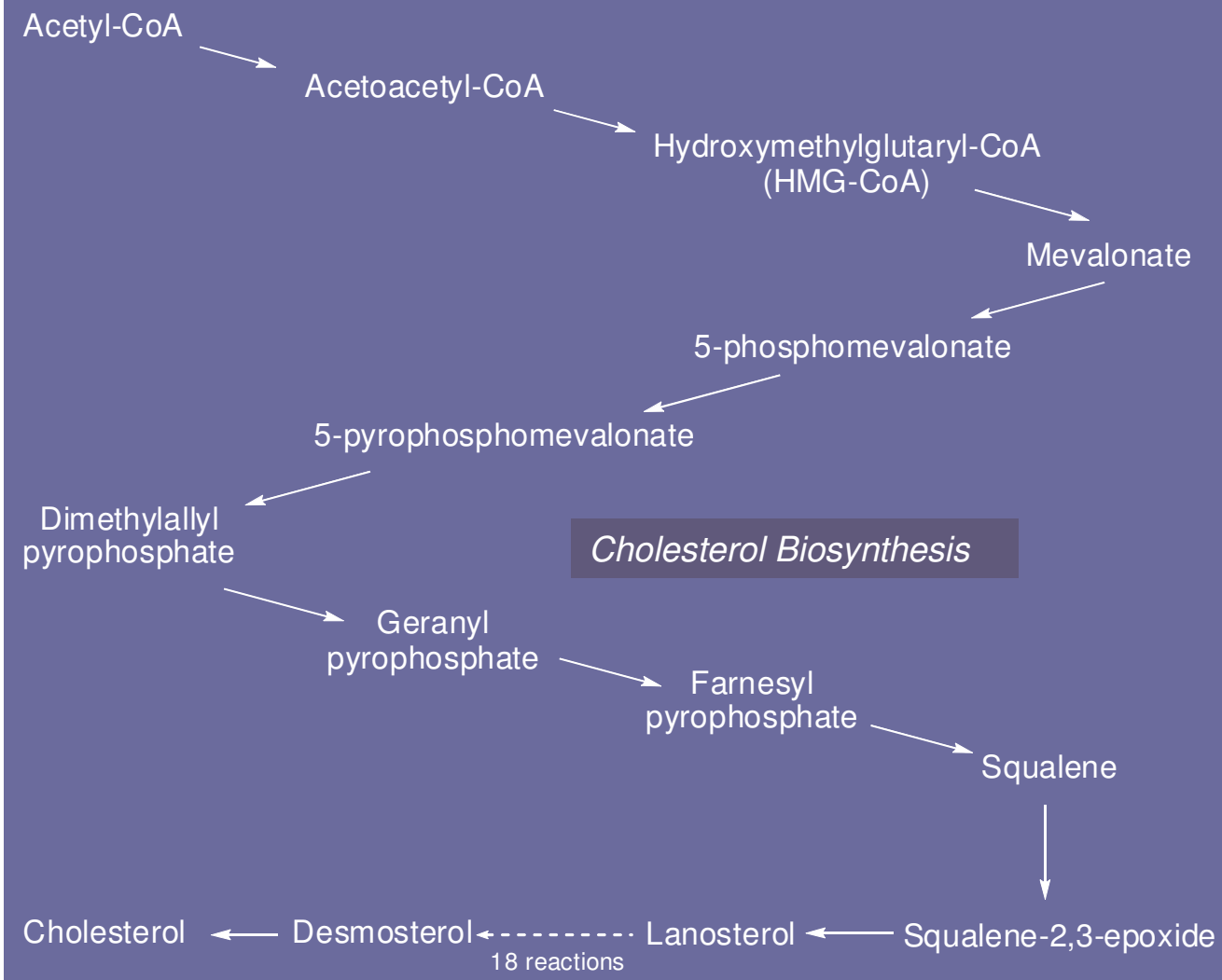
- hydrogen bonds
- ionic interactions
- polar interactions

Human HMGR with Statin Drugs



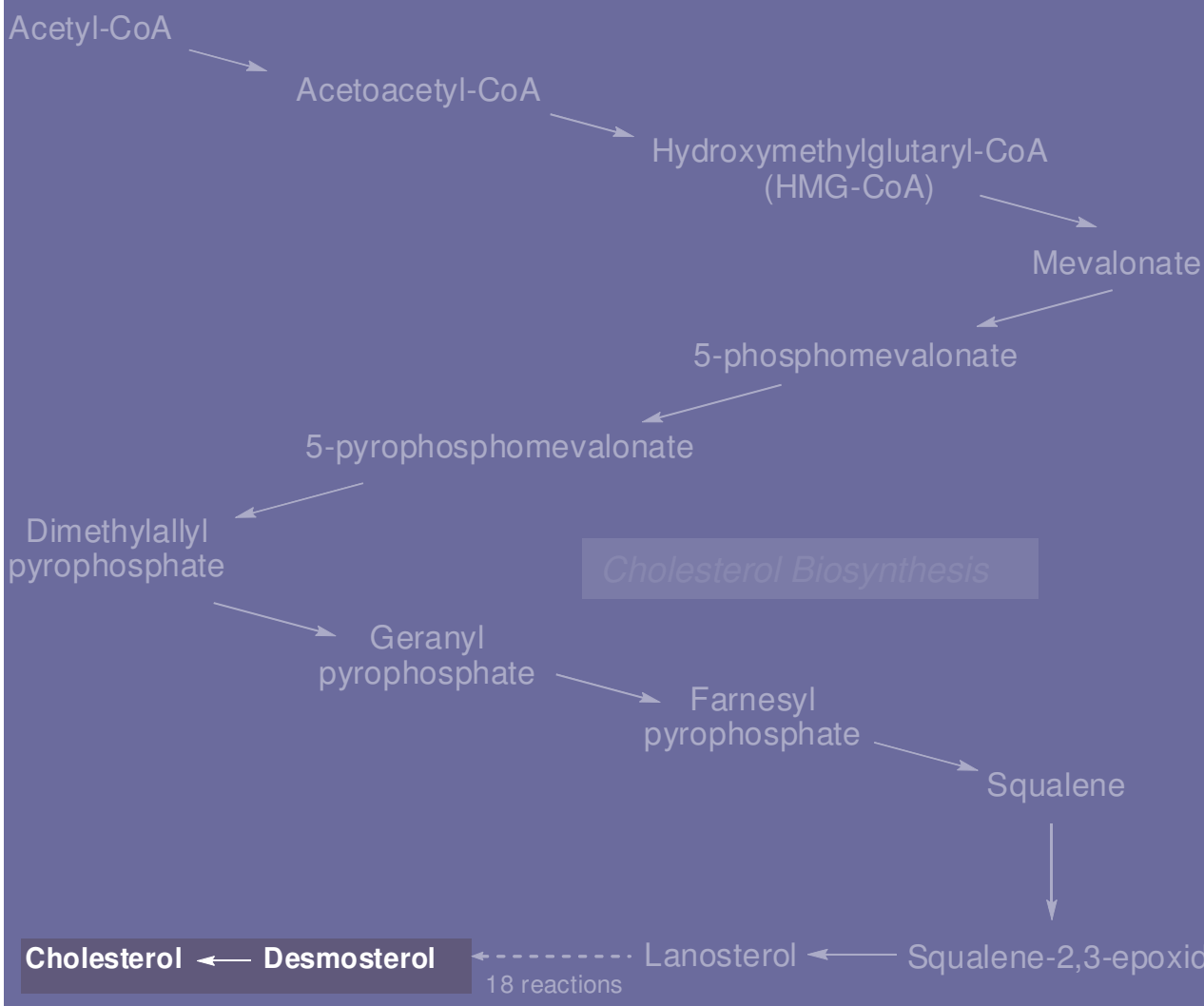
Istvan, E. S.; Deisenhofer, J. *Science* **2001**, *292*, 1160-1164.

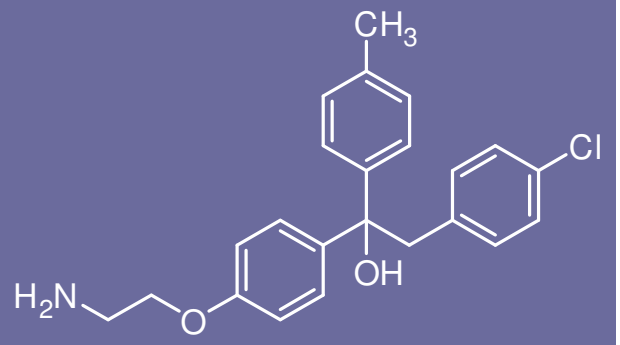
The solution – *suppressing cholesterol biosynthesis*



www.cellml.org

The solution – *suppressing cholesterol biosynthesis*





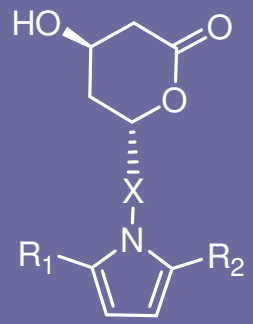
Triparanol (MER-29)

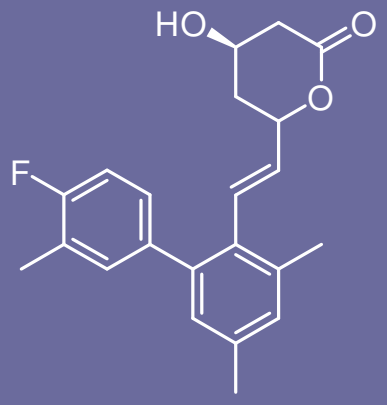
Severe side effects – failed!

Steinberg, D.; Avigan, J. *J. Biol. Chem.* **1960**, *235*, 3127-3129.

The Drug Discovery

Structure-Activity Relationship Studies



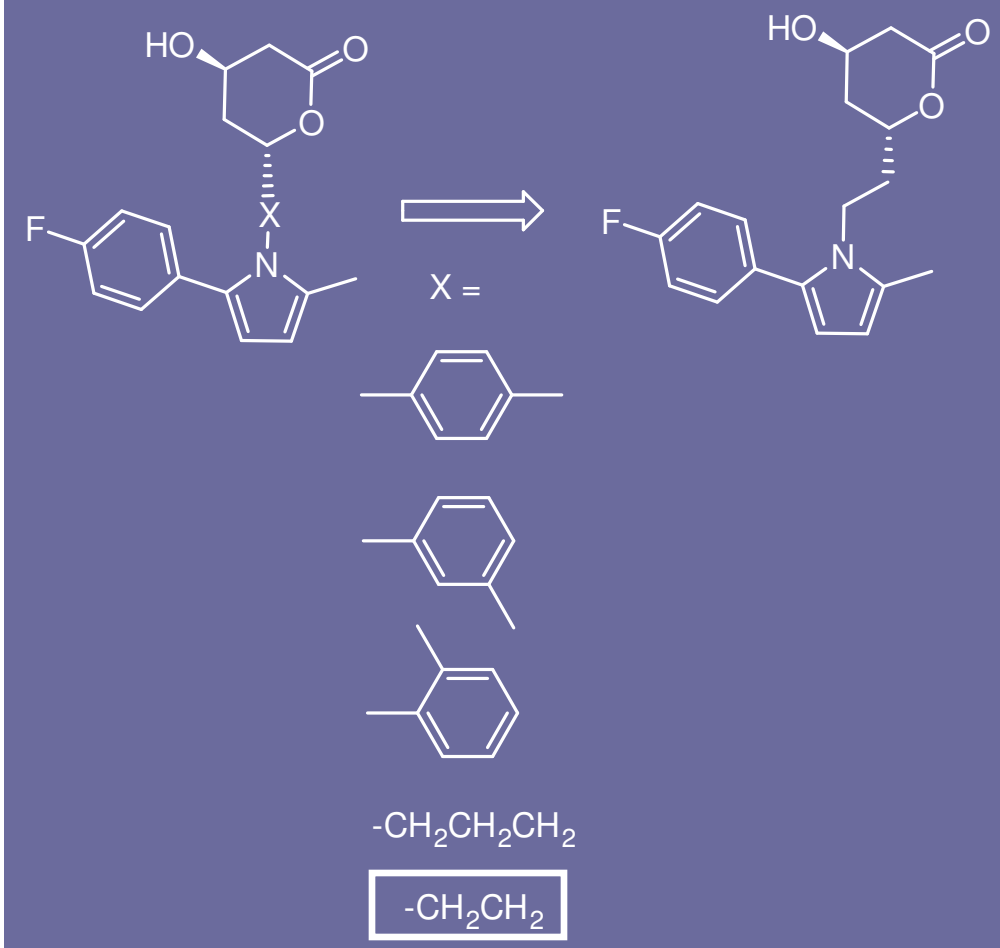


1
MERCK

Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

The Drug Discovery

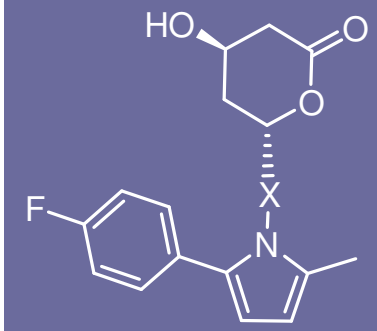
Structure-Activity Relationship Studies

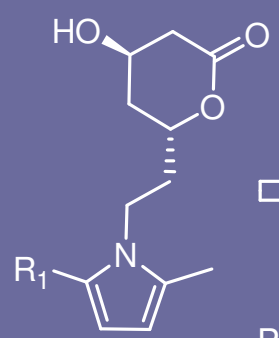


Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

The Drug Discovery

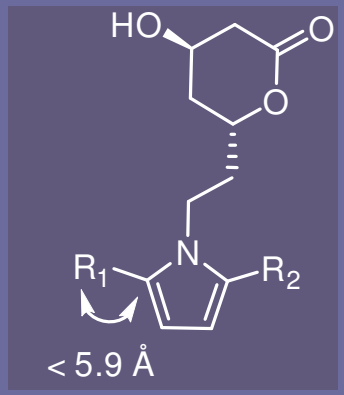
Structure-Activity Relationship Studies

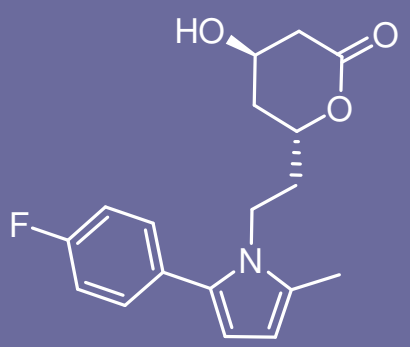




R_1 =

Ph	2-naphthyl
4-PhC_6H_4	1-naphthyl
4-$MeOC_6H_4$	cyclohexyl
4-ClC_6H_4	
4-HOC_6H_4	
4-FC_6H_4	
3-$F_3CC_6H_4$	
3-$MeOC_6H_4$	
3-HOC_6H_4	
2-$MeOC_6H_4$	
2-HOC_6H_4	

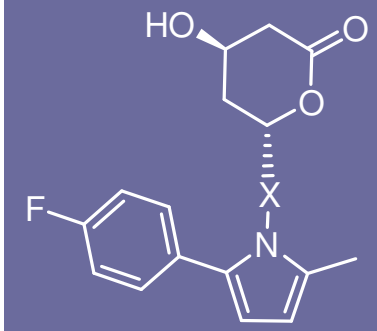


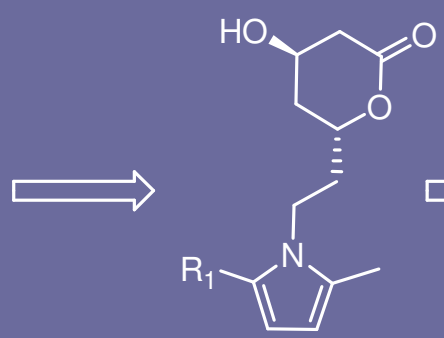


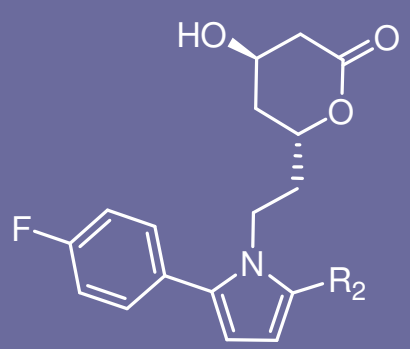
Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

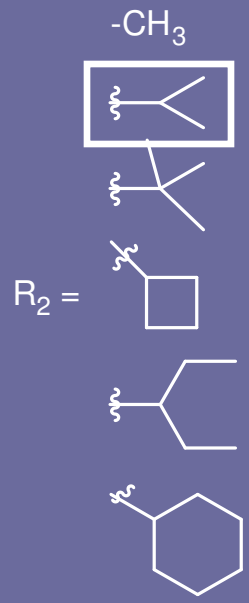
The Drug Discovery

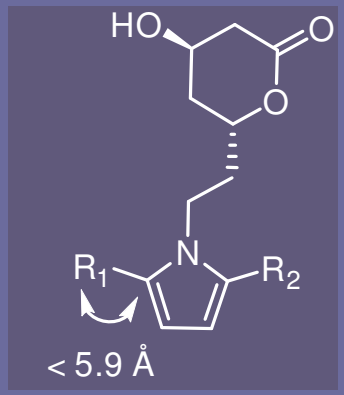
Structure-Activity Relationship Studies

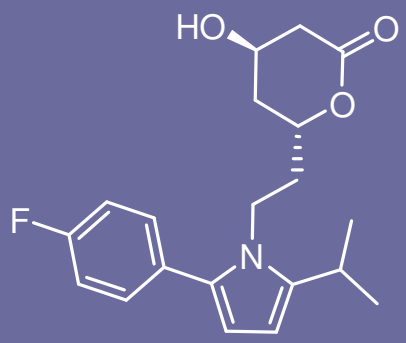








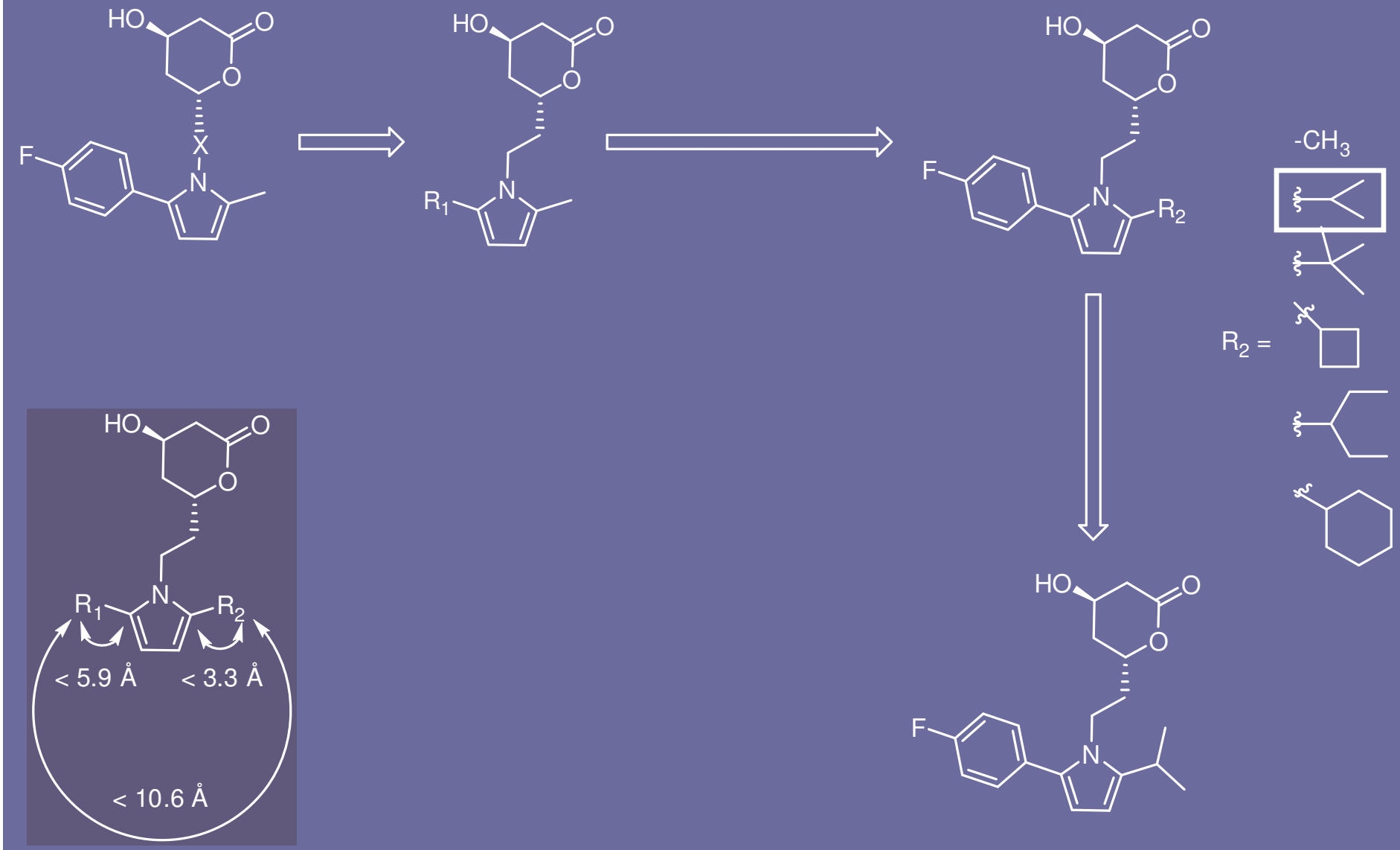




Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

The Drug Discovery

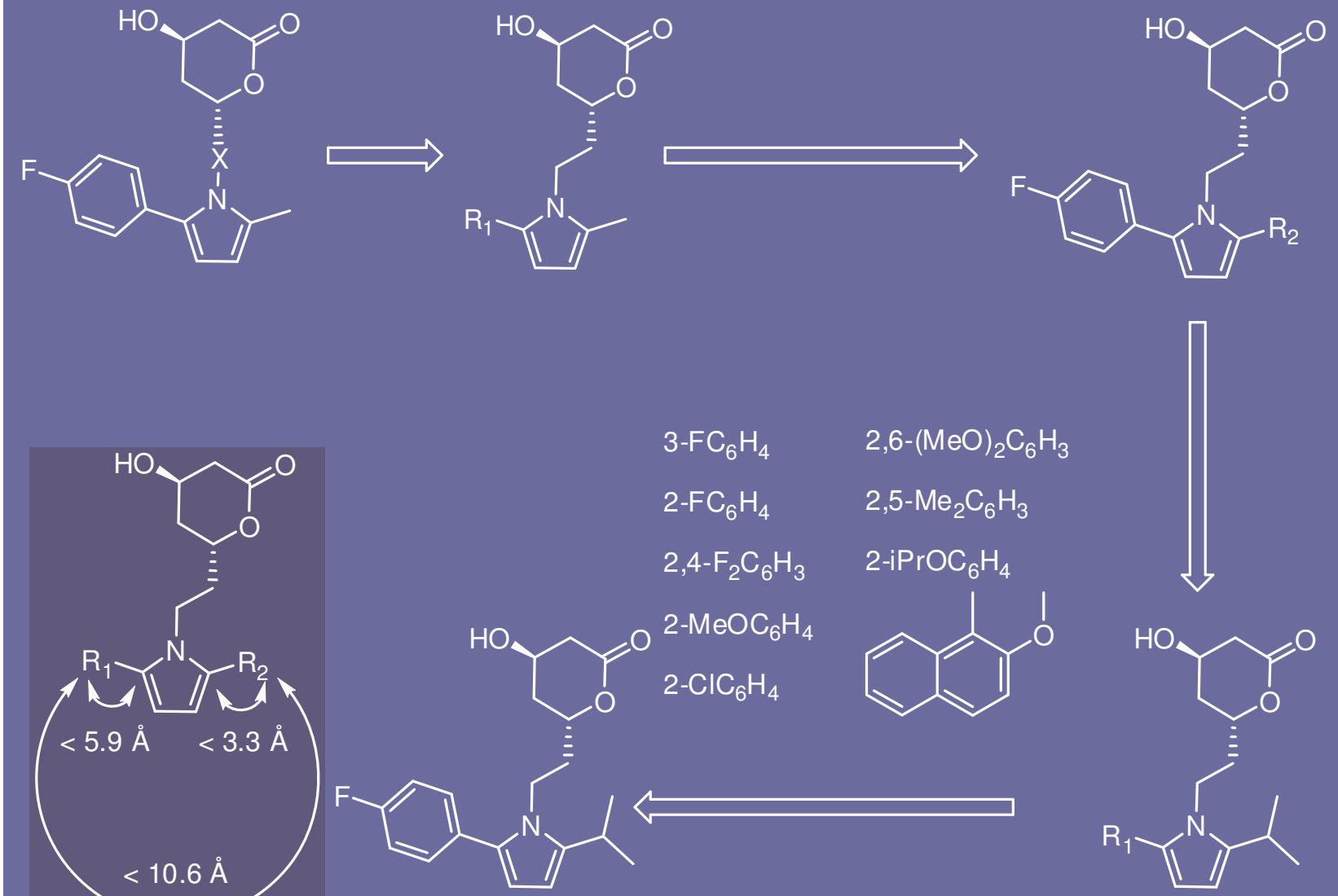
Structure-Activity Relationship Studies



Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

The Drug Discovery

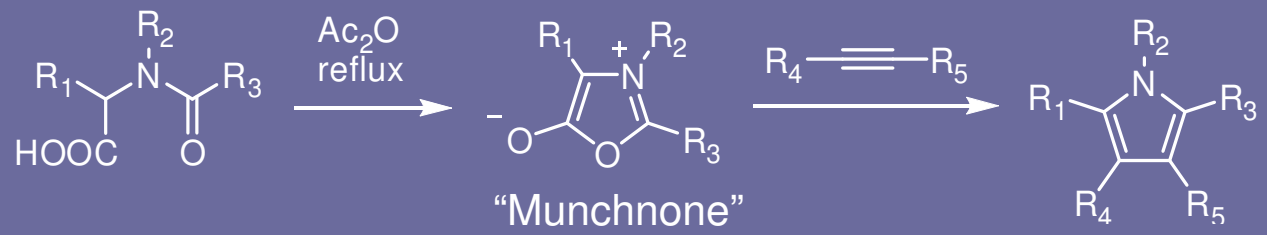
Structure-Activity Relationship Studies



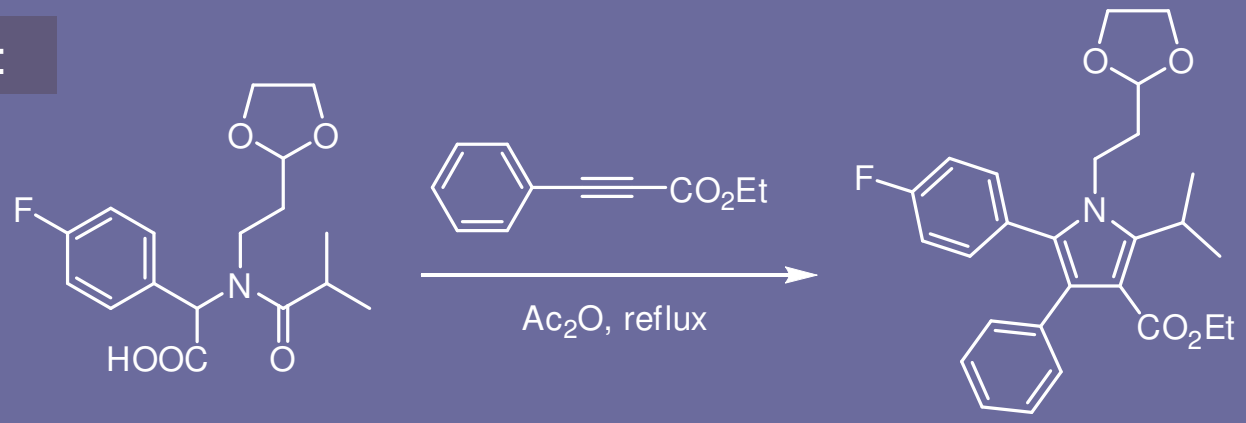
Roth, B. D. *et al. J. Med. Chem.* **1990**, *33*, 21-31.

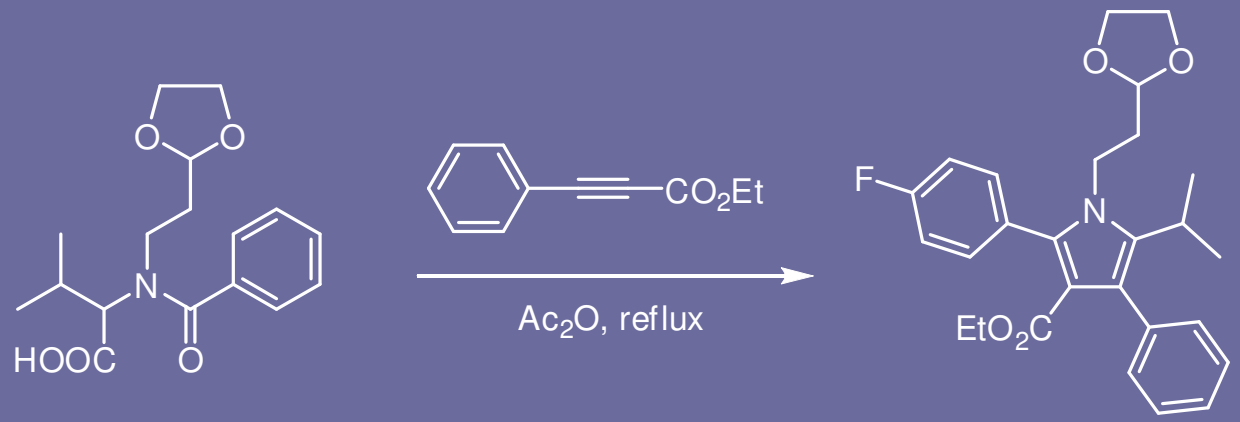
The Drug Discovery

Pentasubstituted Pyrroles via [3+2]

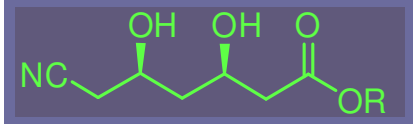


Regiocontrol:

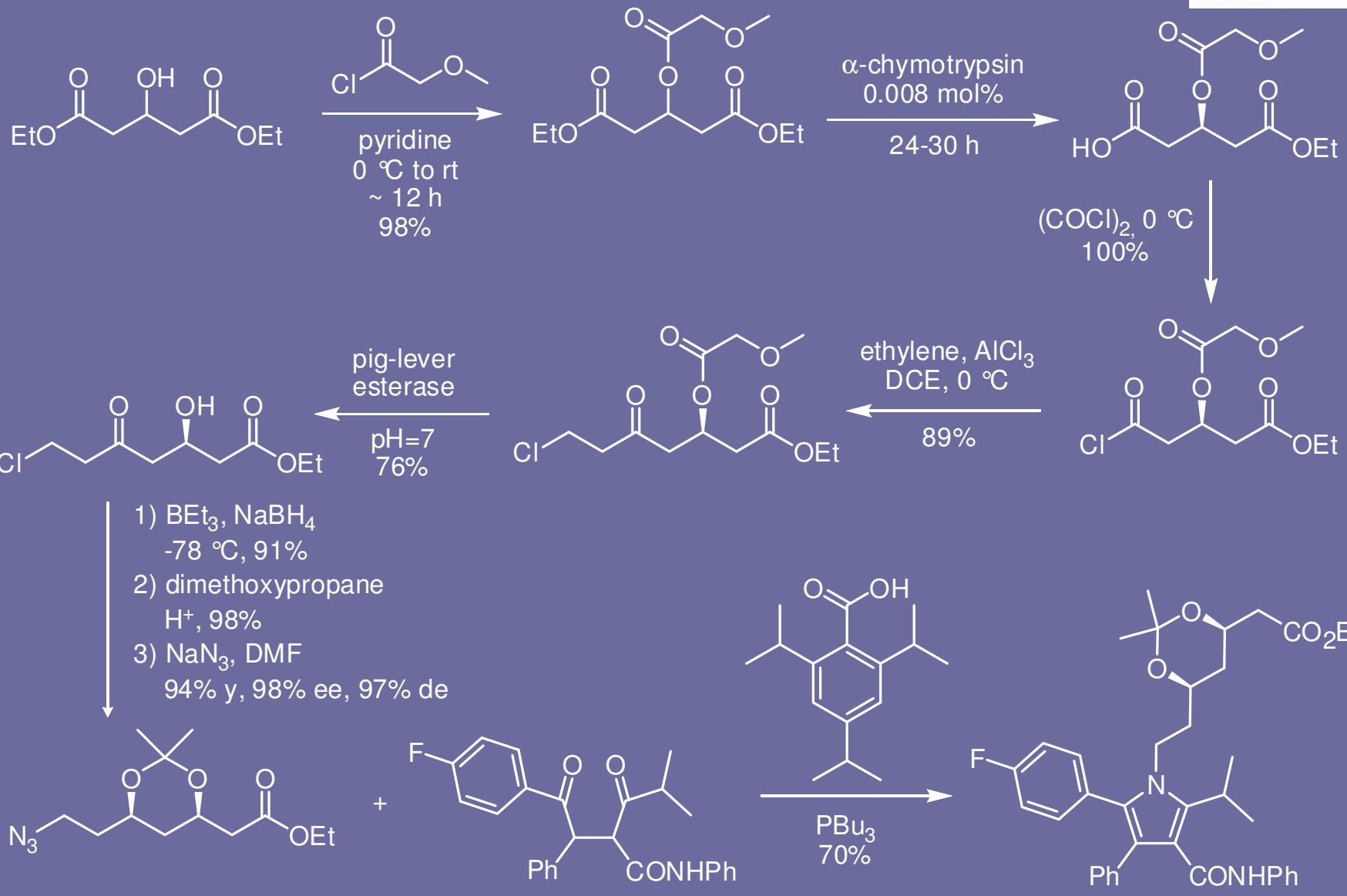




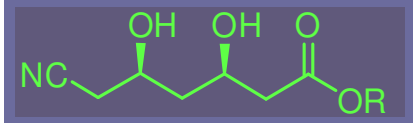
Roth, B. D. *et al. J. Med. Chem.* **1991**, *34*, 357-366.



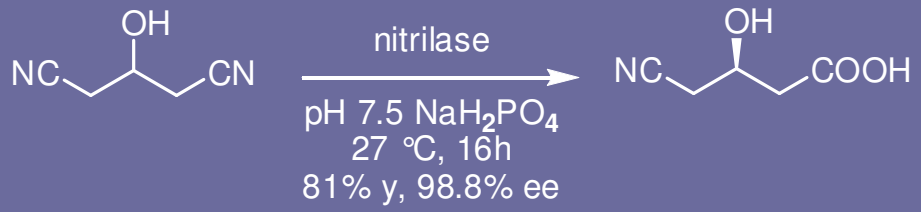
Ciba's Route



Öhrlein, R.; Baischf, G. *Adv. Synth. Catal.* **2003**, *345*, 713-715.



Diversa/Dowpharma's Route



Screen genomic libraries

↓

Most effective WT nitrilase

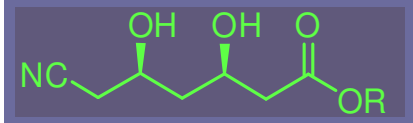
↓

0.24 M [substrate] – 98% y, 95% ee

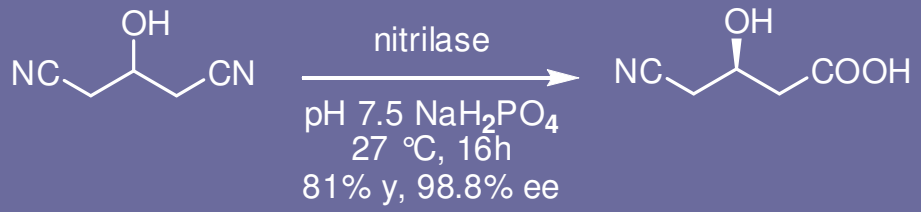
↓

3 M [substrate] – 88% ee

DeSantis, G. *et al. J. Am. Chem. Soc.* **2002**, *124*, 9024-9025.
DeSantis, G. *et al. J. Am. Chem. Soc.* **2003**, *125*, 11476-11477.



Diversa/Dowpharma's Route



Screen genomic libraries

↓

Most effective WT nitrilase → Genetical Engineering gave another library

↓ ↓

0.24 M [substrate] – 98% y, 95% ee

↓

3 M [substrate] – 88% ee

Best mutant

↓

2.25 M [substrate] – 98% ee in 15 h

DeSantis, G. *et al. J. Am. Chem. Soc.* **2002**, *124*, 9024-9025.
DeSantis, G. *et al. J. Am. Chem. Soc.* **2003**, *125*, 11476-11477.

Diversa's GSSM™: Gene Site Saturation Mutagenesis

1. Target Protein for Improvement

Identify protein for optimization through extensive screening of gene libraries.

2. Change Amino Acid(s)

Evolve the gene encoding the protein by systematically changing each amino acid in the sequence to every other possible amino acid.

3. Complete Variant Library

A new gene variant library is born containing genes with every single site variation in sequence.

4. Select Variants

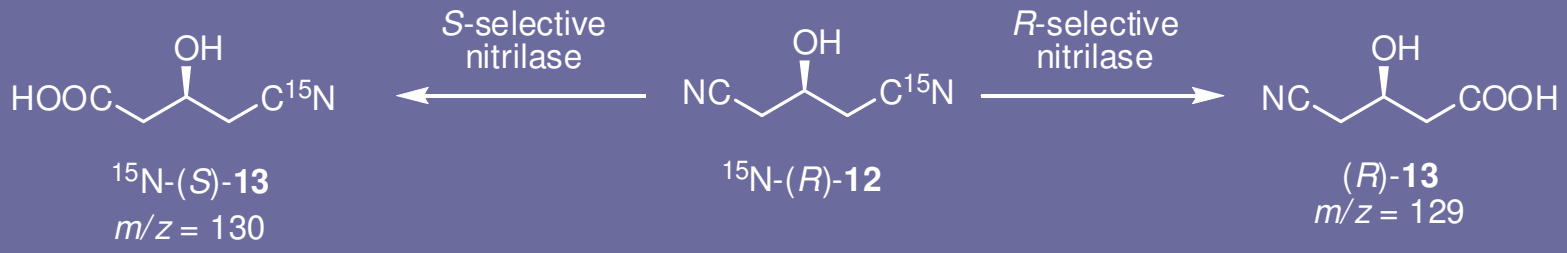
Through screening, identify the variants that demonstrate improved characteristics.

5. Combine Mutations

Tests all potential combinations of the single amino acid changes that demonstrated improved characteristics and select the optimal combination.

6. Generate Optimized Protein

Diversa's High Throughput Screen for Improved Selectivity

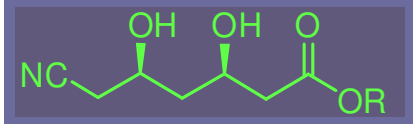


^{15}N-(*S*)-**13**
m/z = 130

^{15}N-(*R*)-**12**

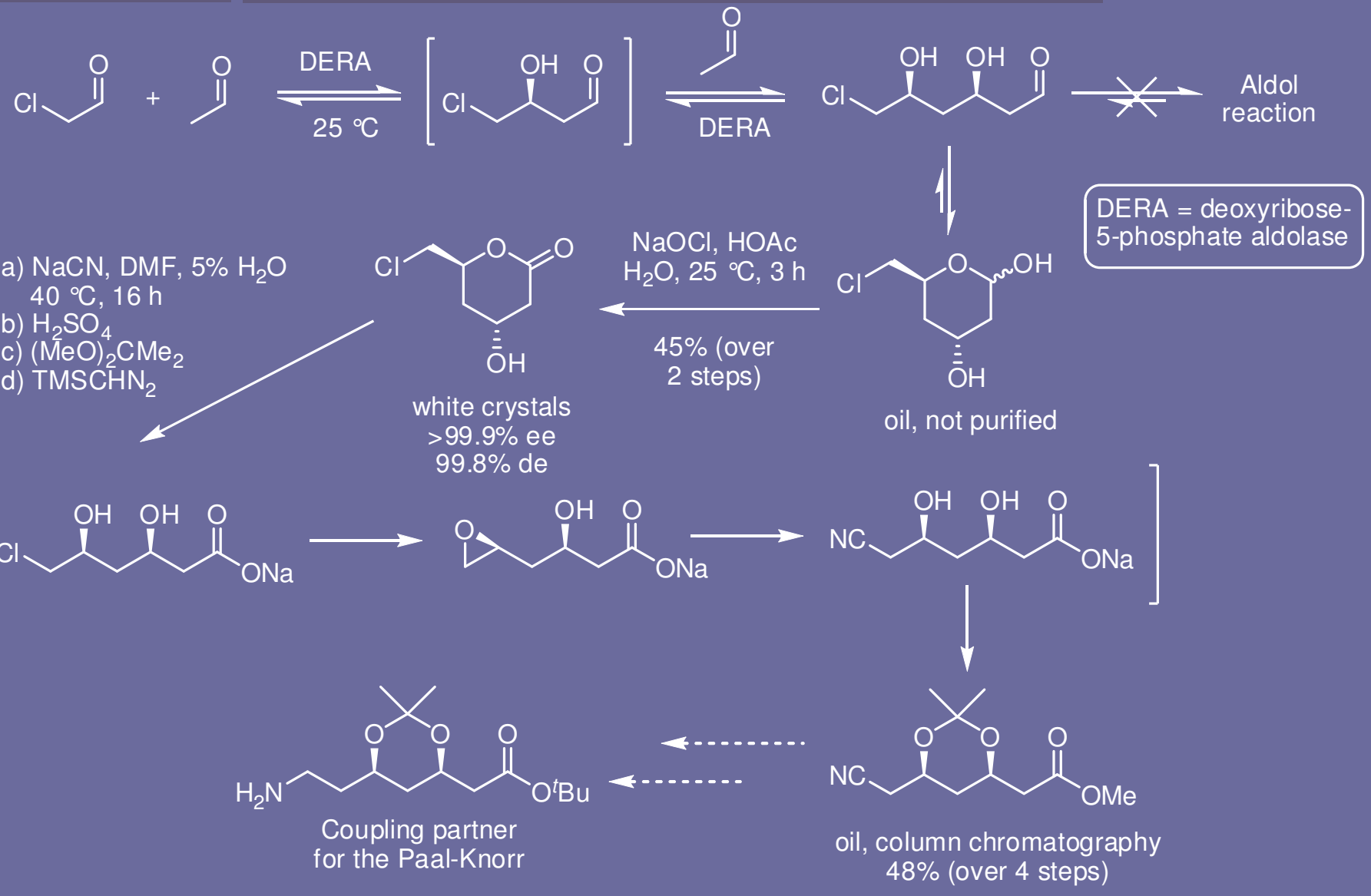
(*R*)-**13**
m/z = 129

DeSantis, G. *et al. J. Am. Chem. Soc.* **2003**, *125*, 11476-11477.



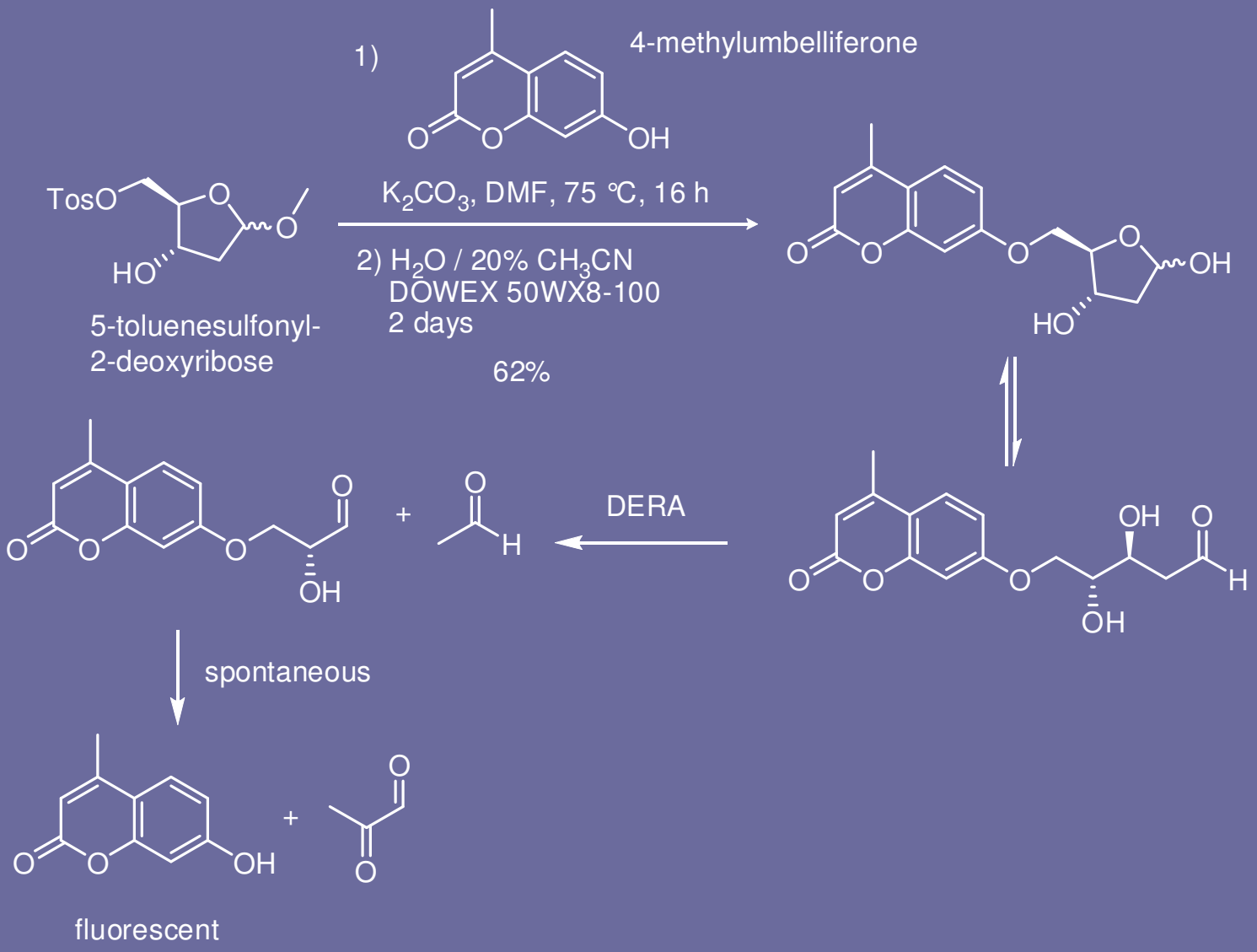
Diversa's Route

DIVERSA
HARNESSING THE POWER OF ENZYMES

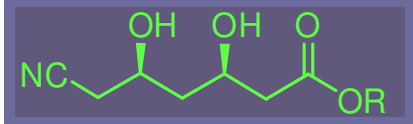


Greenberg, W. A. *et al. Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5788-5793.

Diversa's Fluorogenic Activity Based Screen

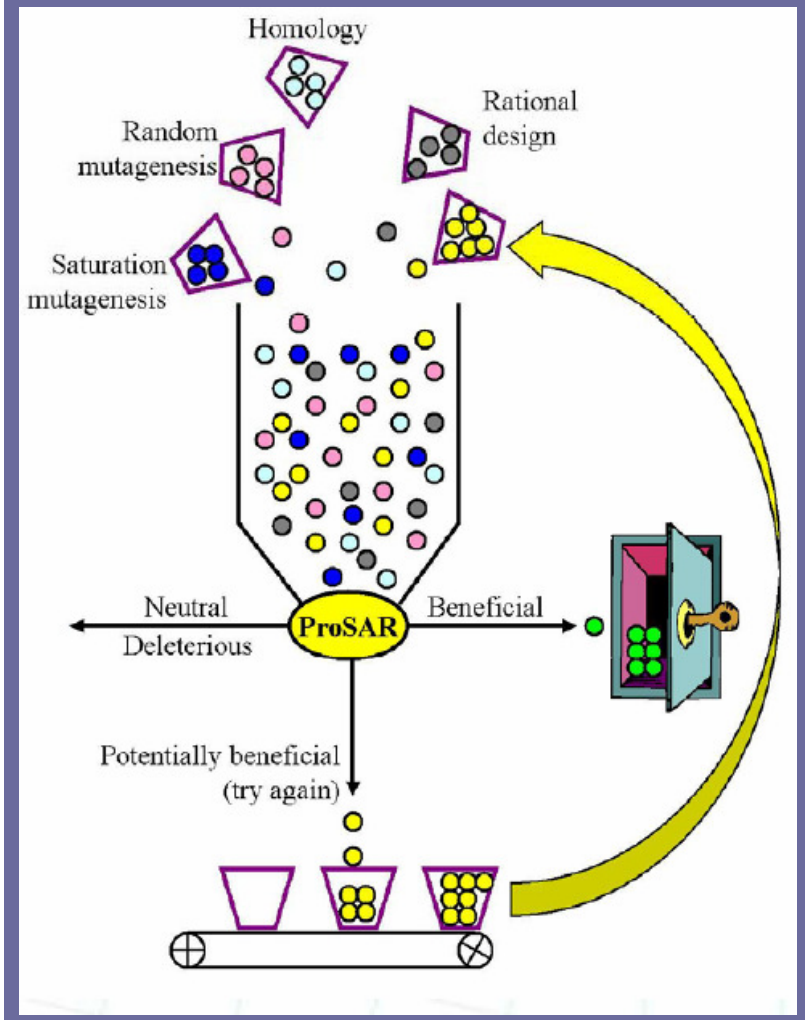


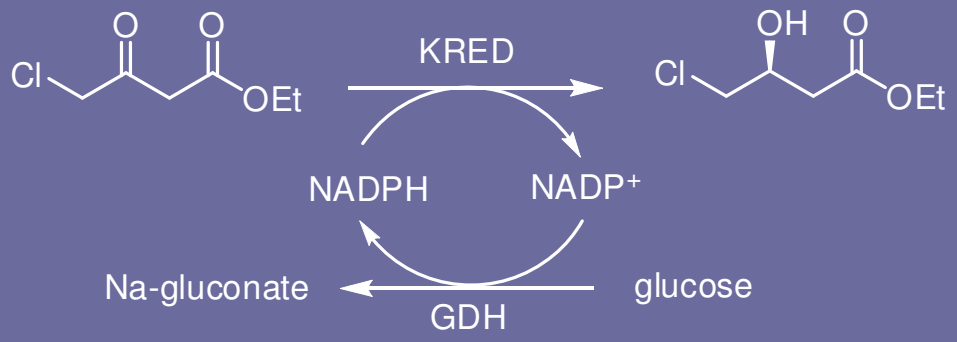
Greenberg, W. A. *et al. Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5788-5793.



Codexis Biocatalyst Improvement

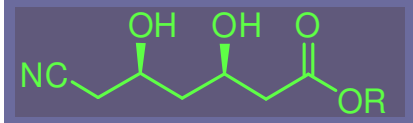
CODEXIS



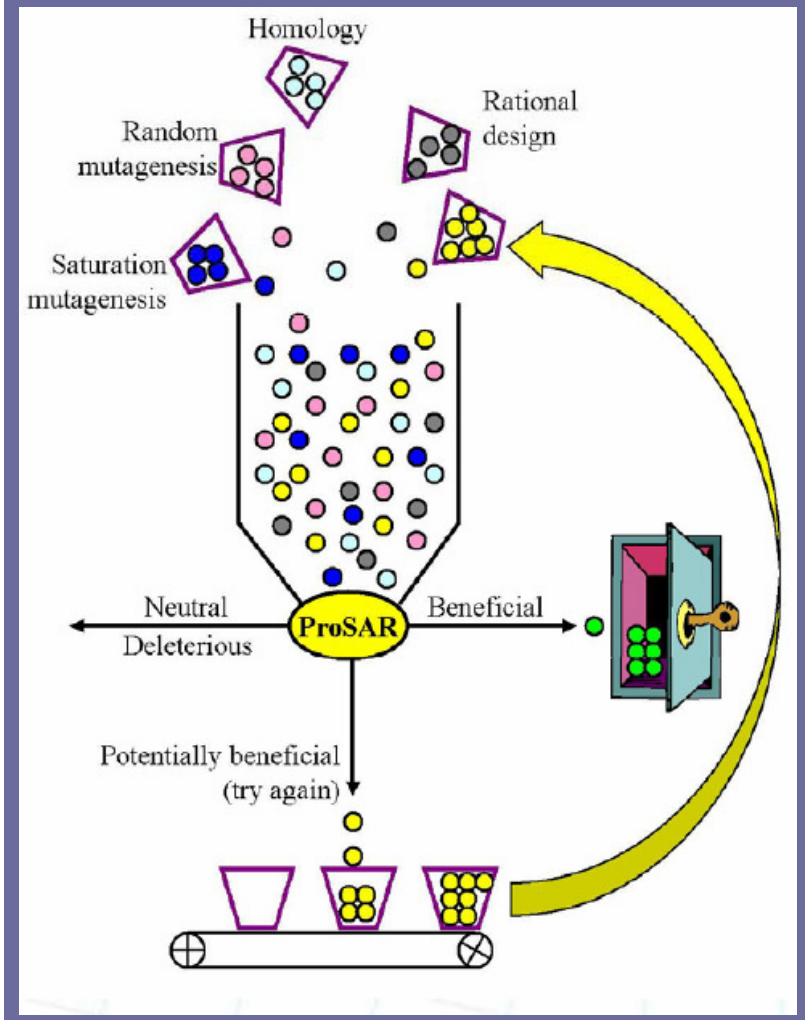


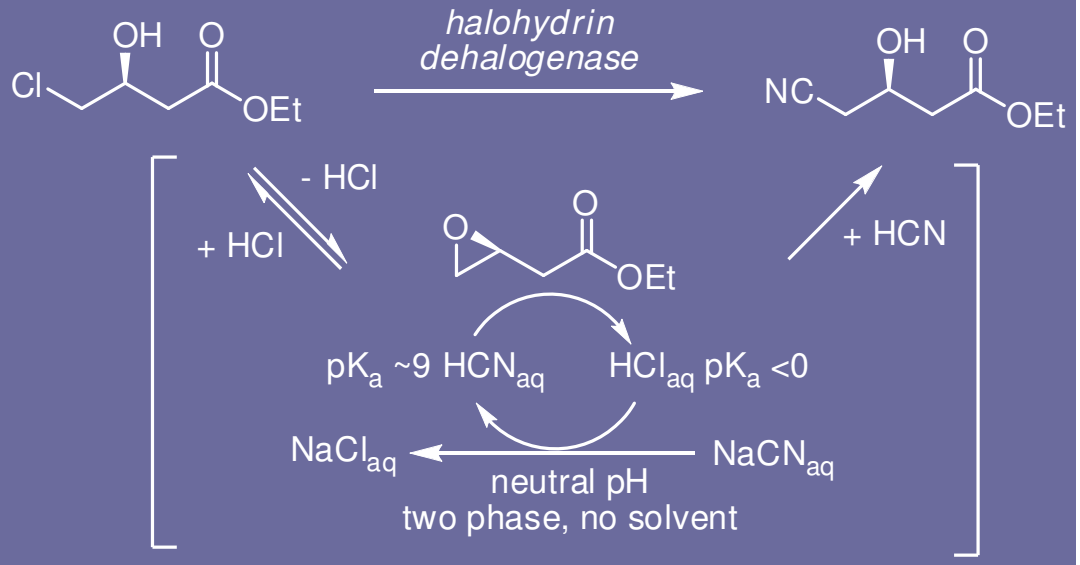
Parameter	Initial	Final
Substrate loading	80 g/L	180 g/L
Reaction time	24 h	8 h
Enzyme loading	10 g/L	0.7 g/L
Isolated yield	~80%	97%
Phase separation time	>1 h	~ 1 min
Volumetric productivity	80 g/L.day	540 g/L.day

Dr. Peter Seufer-Wasserthal (VP, Head of Codexis Pharma Services), personal communication.



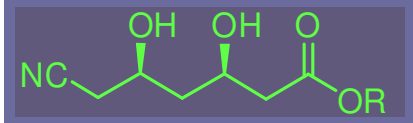
Codexis Biocatalyst Improvement





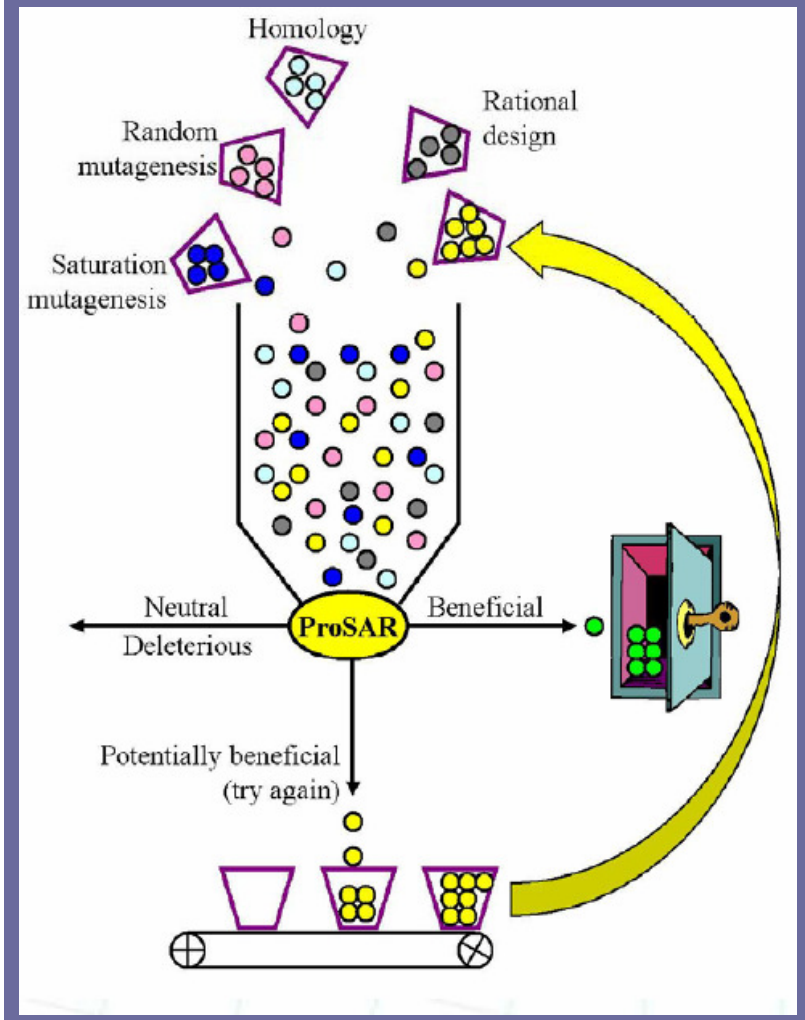
Parameter	Initial	Final
Substrate loading	20 g/L	140 g/L
Reaction time	72 h	5 h
Enzyme loading	130 g/L	1.2 g/L
Isolated yield	~60%	92%

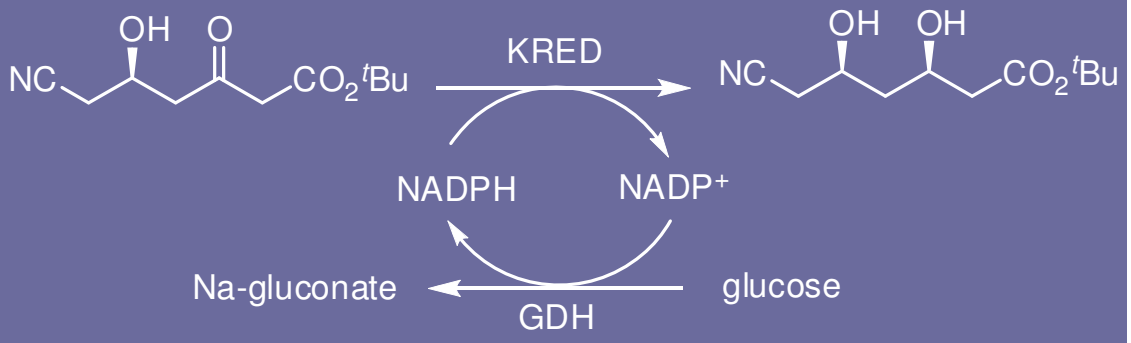
Dr. Peter Seufer-Wasserthal (VP, Head of Codexis Pharma Services), personal communication.



Codexis Biocatalyst Improvement

CODEXIS

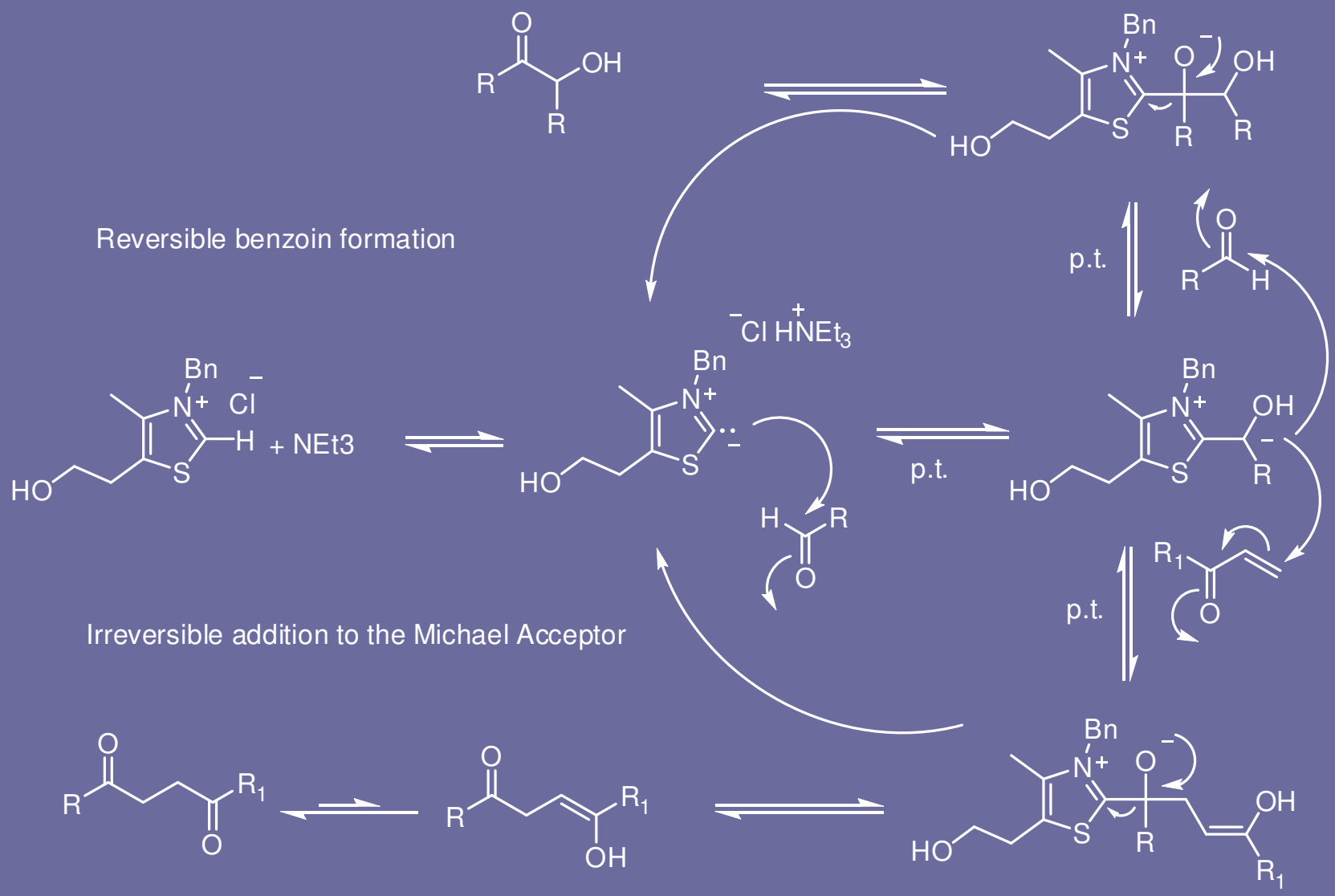




Parameter	Initial	Final
Substrate loading	120 g/L	300 g/L
Reaction time	65 h	22 h
Conversion	87%	99.3%
Diastereomeric excess	100%	100%
Work-up	difficult	facile

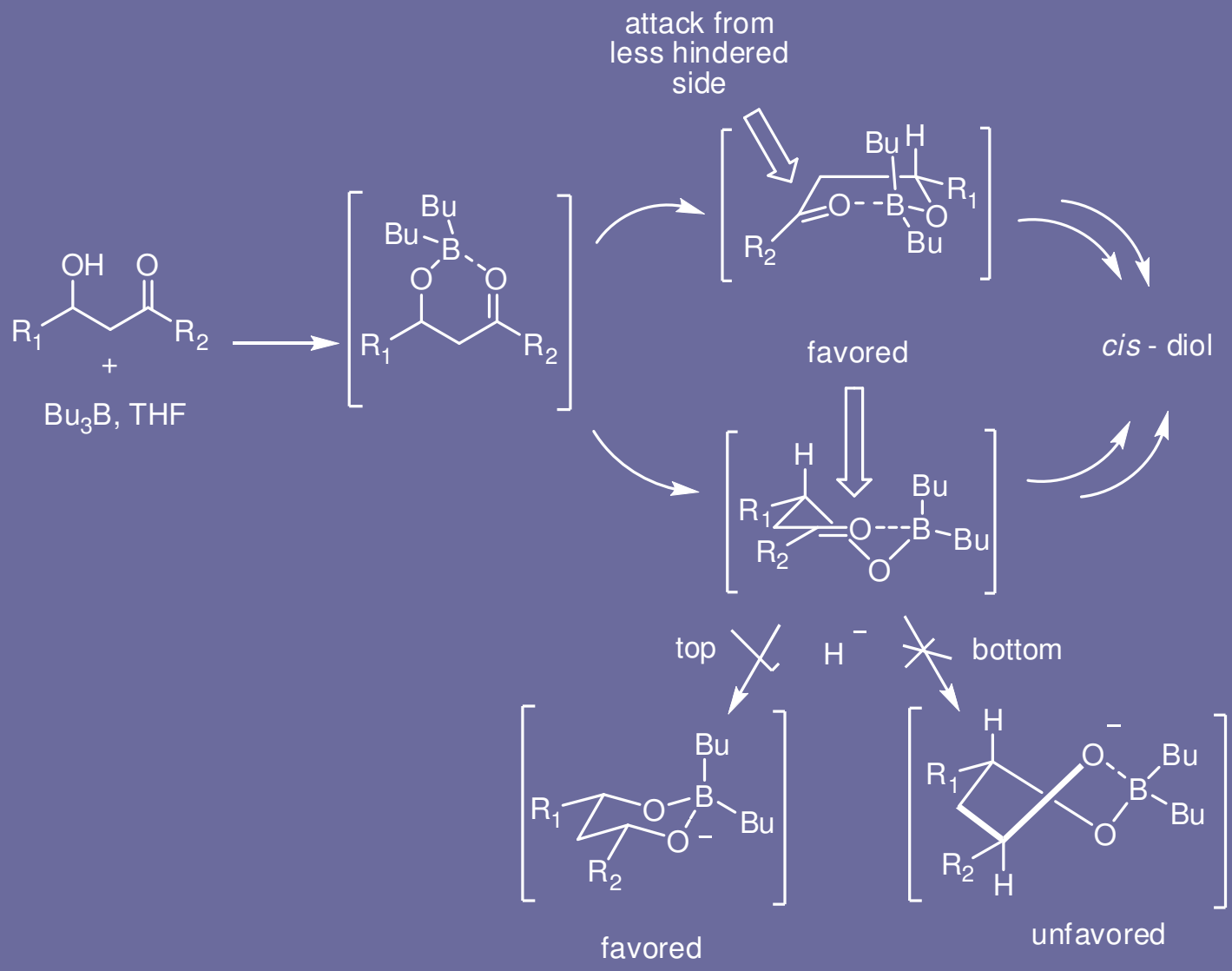
Dr. Peter Seufer-Wasserthal (VP, Head of Codexis Pharma Services), personal communication.

Stetter Reaction Mechanism



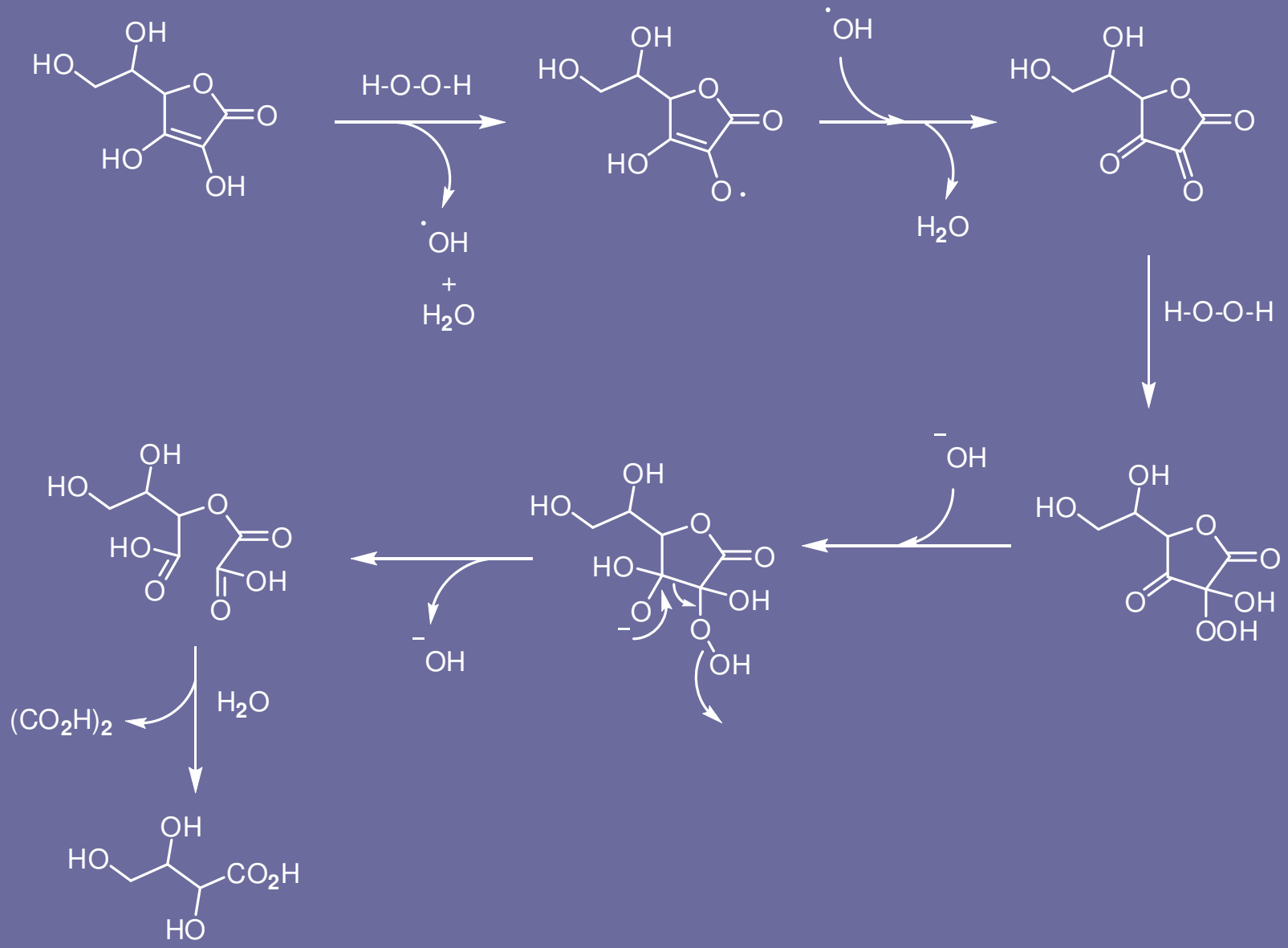
Hermann, S. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 639-647.

Syn-selective reduction of β-hydroxy ketones

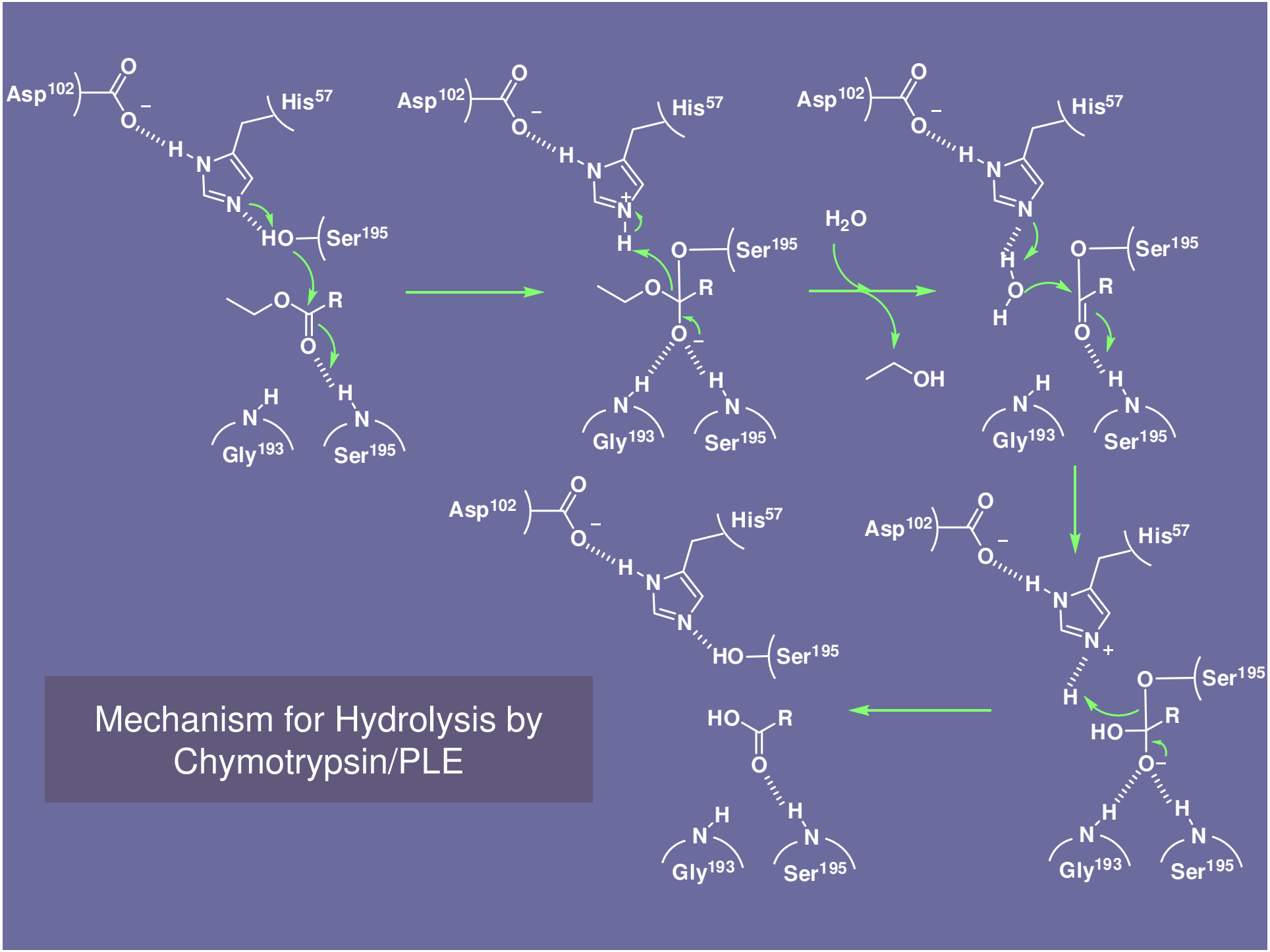


Narasaka, K.; Pai, H. C. *Chem. Lett.* **1980**, 1415-1418.

Mechanism for H_2O_2 Oxidation of Ascorbic Acid

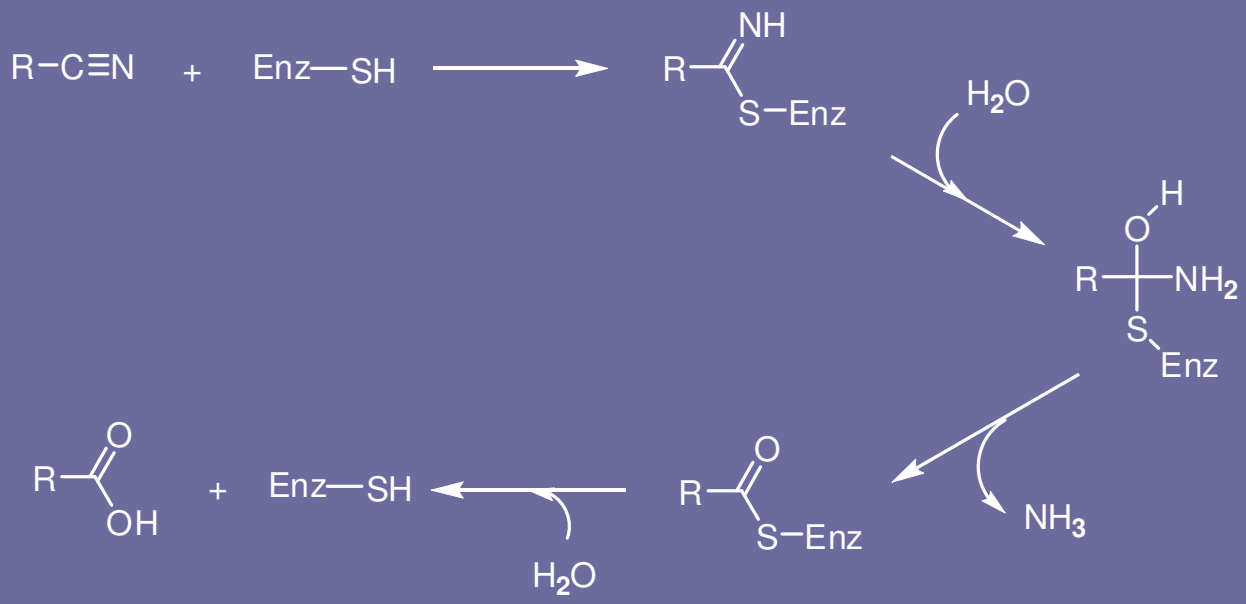


Isbell, H. S.; Frush, H. L. *Carbohyd. Res.* **1979**, *72*, 301-304.

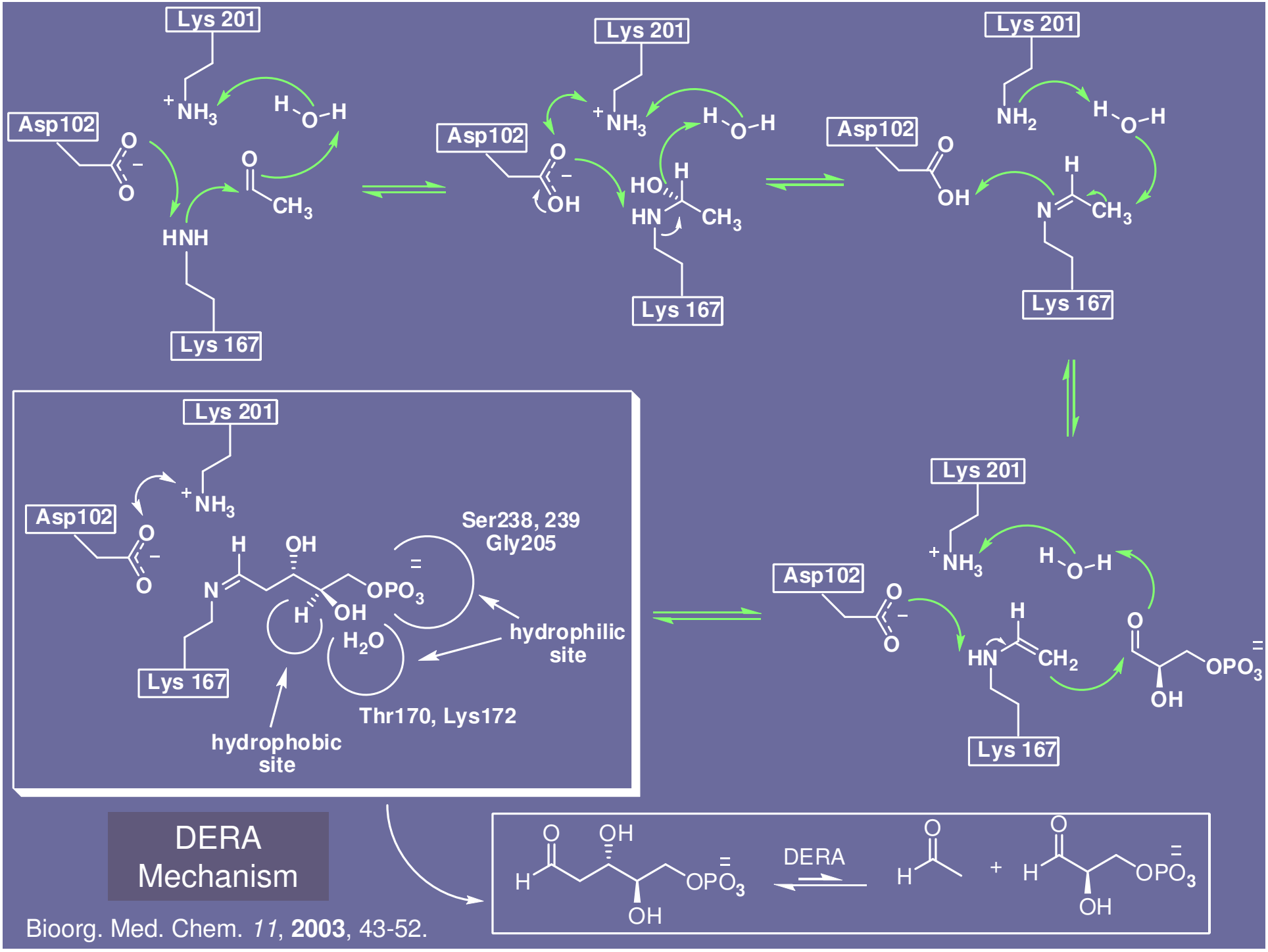


Mechanism for Hydrolysis by Chymotrypsin/PLE

Mechanism for Hydrolysis by Nitrilase



Faber, K. *Biotransformations in Organic Chemistry*, Springer-Verlag, **2004**.



Bioorg. Med. Chem. *11*, **2003**, 43-52.