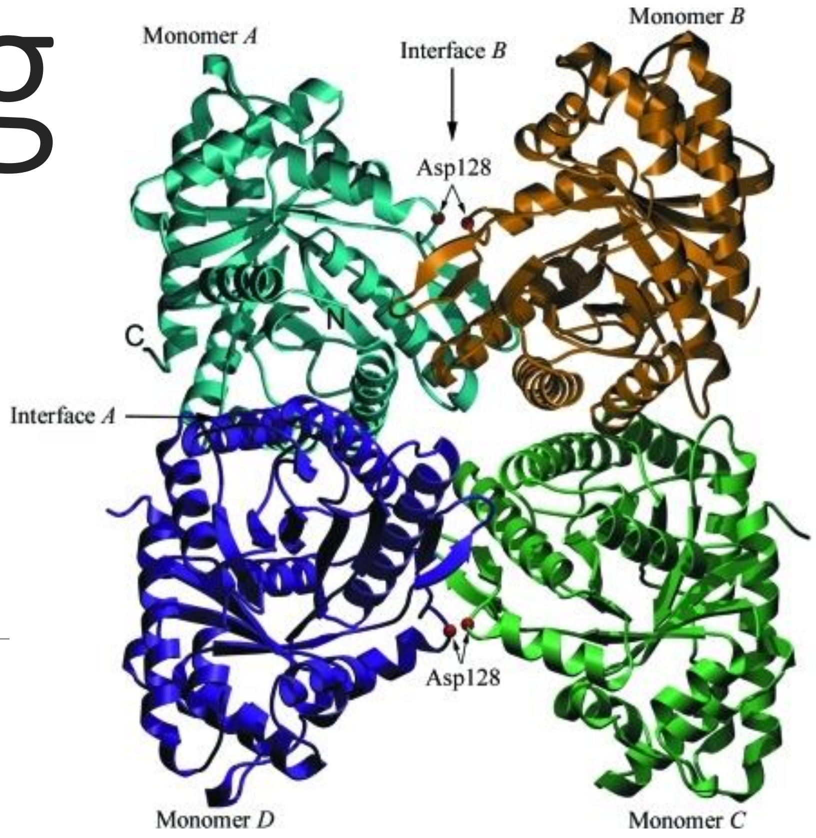
C-C Bond Forming Lyases in Organic Synthesis

RACHEL WHITTAKER

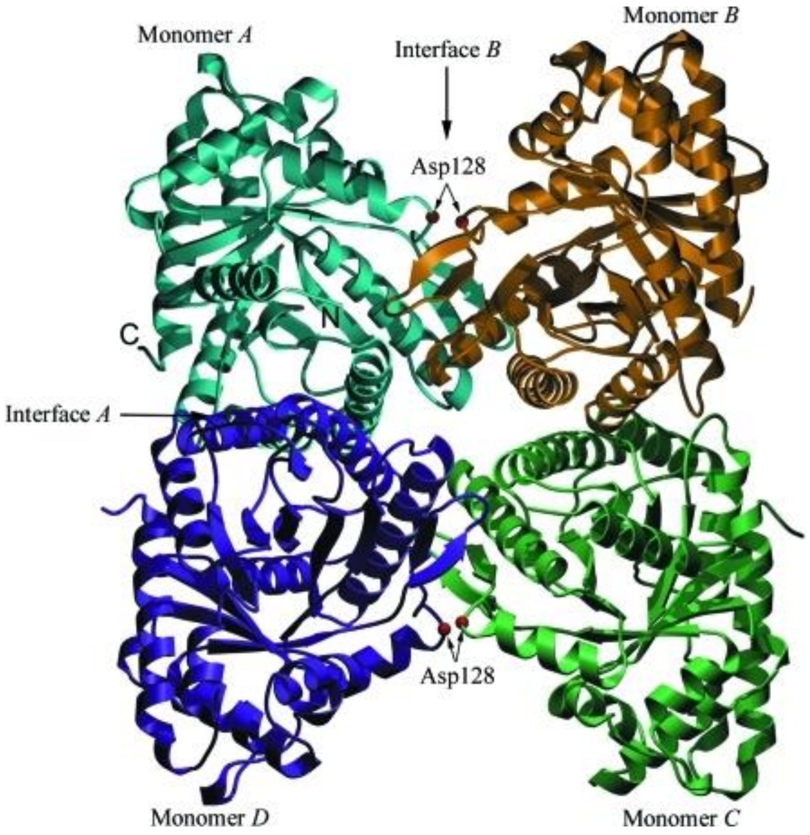
MARCH 4, 2015



Brovetto, M.; Gamenara, D.; Mendez, P. S.; Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

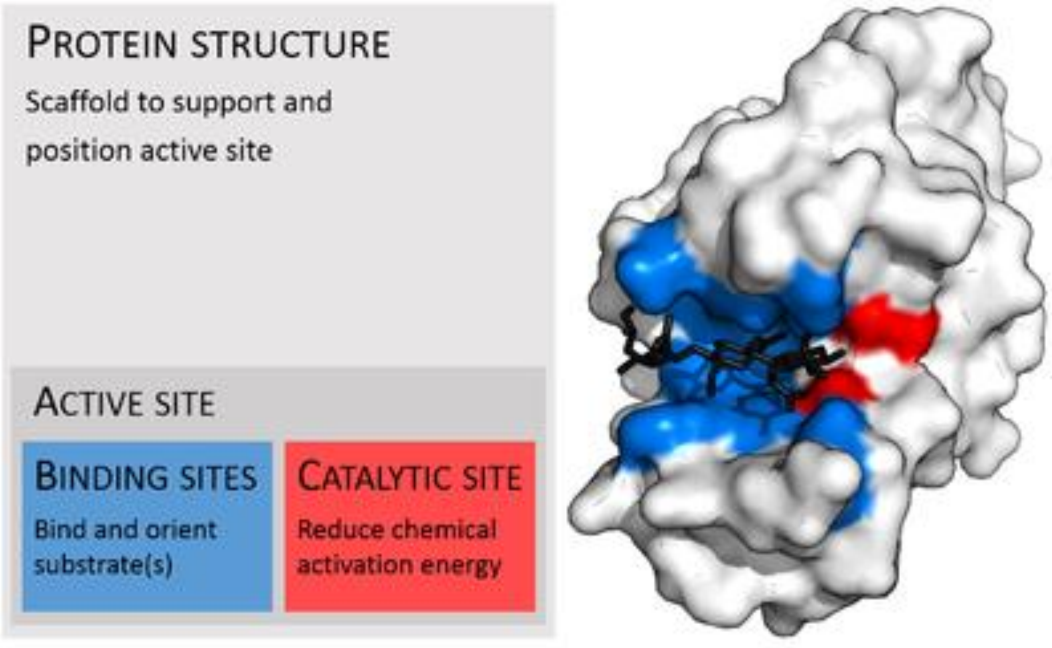
Overview

- Brief Refresher on Enzymes
- Enzymatic Aldol Reactions
- Acyloin Condensation
- Cyanohydrin Formation
- Enzymatic Friedel-Crafts
- “Diels-Alderases”
- Future Directions



Enzymes 101:

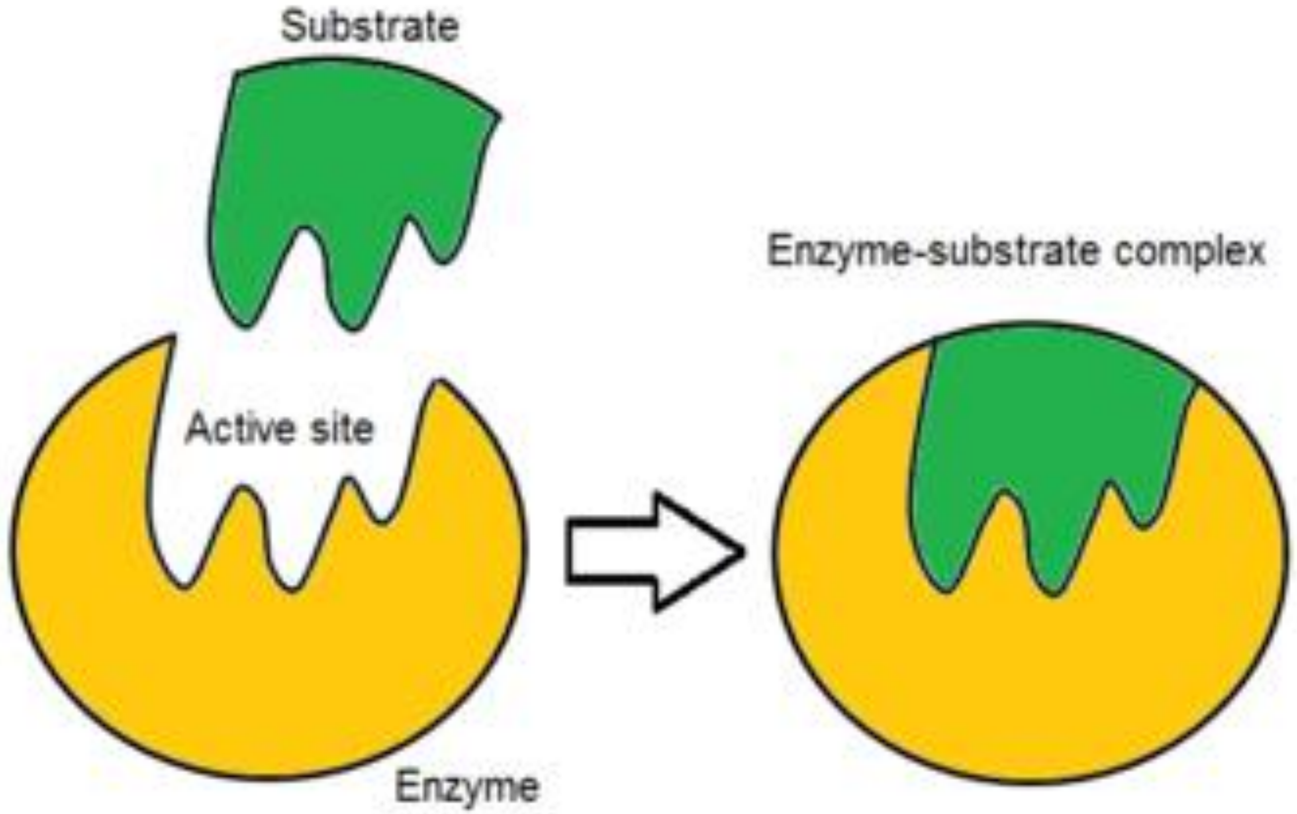
- Enzymes are proteins (usually) that catalyze chemical reactions
- Cells need enzymes in order to perform necessary metabolic reactions at rates fast enough for life to be maintained
- Reactions occur at the active site, where amino acid residues can bind the substrate and catalysis can occur
  - In some cases, the enzyme instead will bind a cofactor that serves as the actual catalyst
- Reactions and substrates are generally very specific
  - Due to steric and electronic factors in the active site



http://en.wikipedia.org/wiki/Enzyme

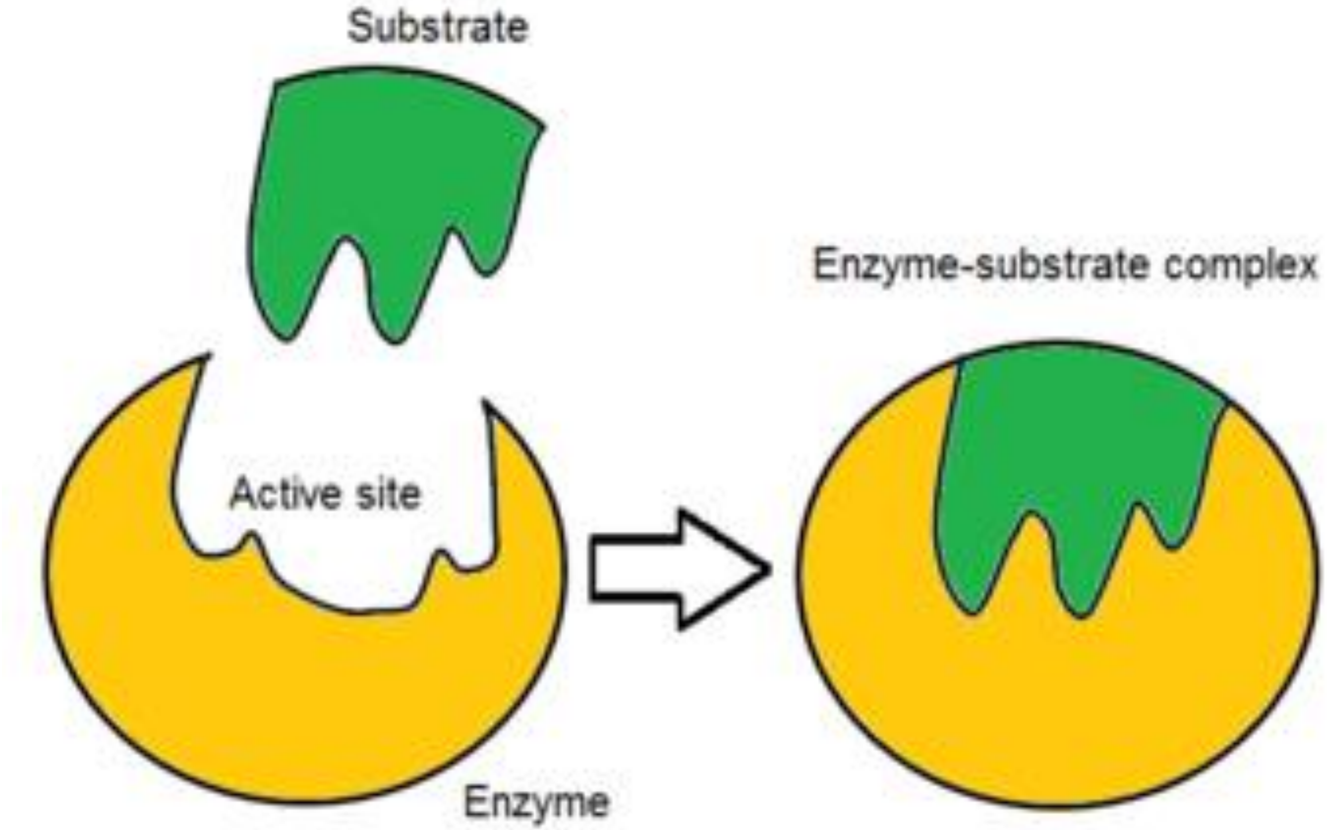
Lock and Key Model

- Most basic model of specificity posited by Emil Fisher in 1894



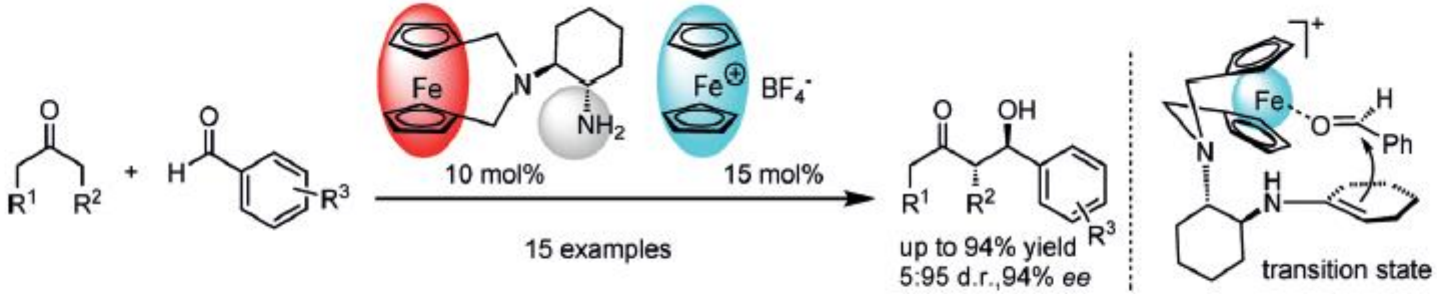
Induced Fit Model

- Enzyme is flexible and substrate helps determine the actual shape



What Can Enzymes Do For Us?

- Do the work for us!
- Get highly chemo-, regio-, diastereo-, and enantioselective reactions
- Very mild conditions (neutral, aqueous) and protecting group, chiral auxillary, etc free!
- Non-enzymatic methods can be costly, tedious, or may still be underdeveloped



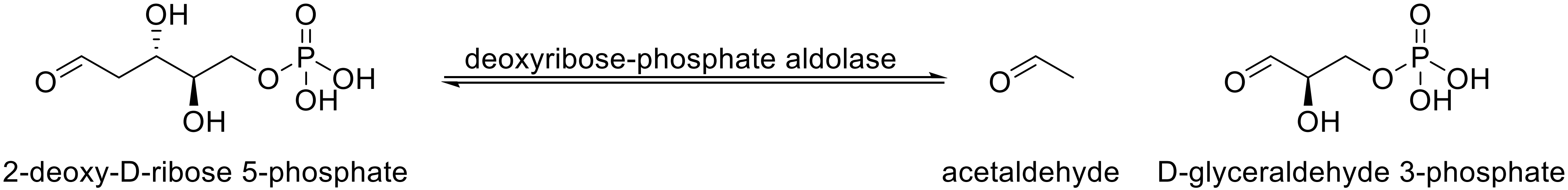
Wu, Y. *Angew. Chem., Int. Ed.* ASAP.

How To Control Enzymes?

- Enzymes in nature generally have one set of substrates and one set of product(s)
- How can we harness them for general reactivity for synthetic purposes?
  - Screen substrates for promiscuous activity in the enzyme (Maybe unnatural ones can fit)
    - Donor specific
    - Acceptor specific
  - Mutate enzyme (Change amino acids to allow for your substrate)
    - Directed evolution

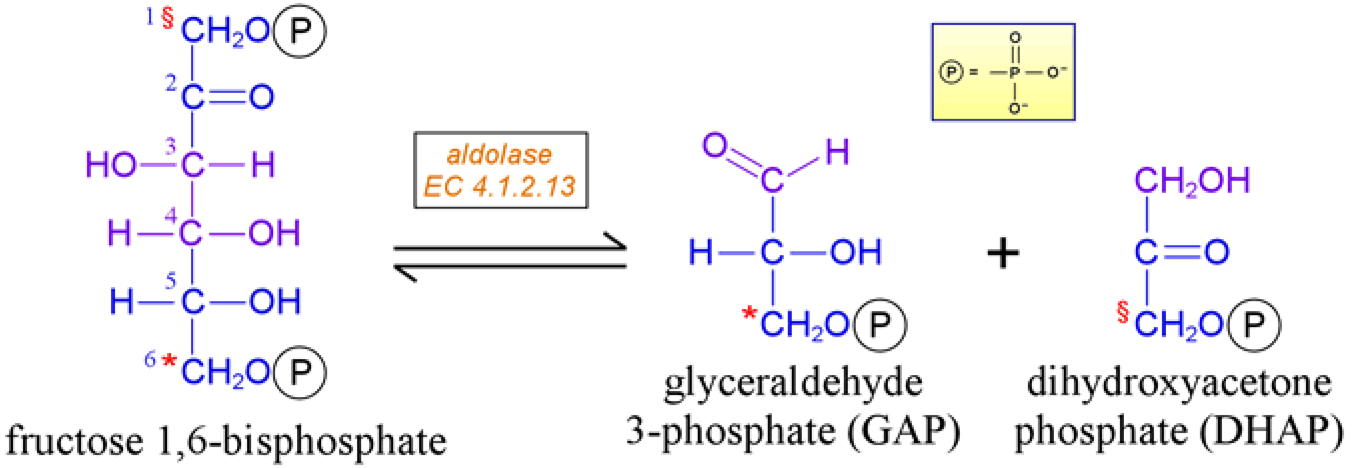
Lyases

- A subclass of enzyme that catalyze bond construction or cleavage through mechanisms other than hydrolysis or redox reactions
- C-C, C-N, C-O or C-S bonds may be cleaved, but we will focus on C-C bonds
- Unique from other enzymes in that they require one substrate for the reaction in the forward direction, but two for the reverse reaction



Enzymatic Aldol Reactions

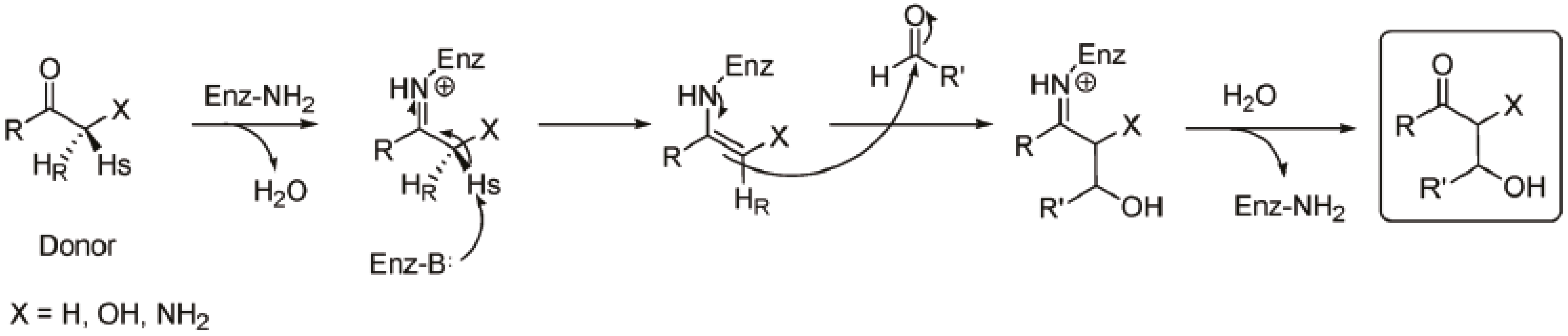
- Critical reaction not just for organic synthesis, but from a metabolic standpoint
  - The most important biochemical process for the production of naturally occurring carbohydrates
  - Also found naturally in the biosynthetic pathways of keto acids and amino acids
- Over 40 naturally occurring aldolases known
  - Most catalyze the stereoselective aldol addition of a ketone to an aldehyde



Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

Type I Aldolase Mechanism

- Key Feature: Schiff-base Intermediate



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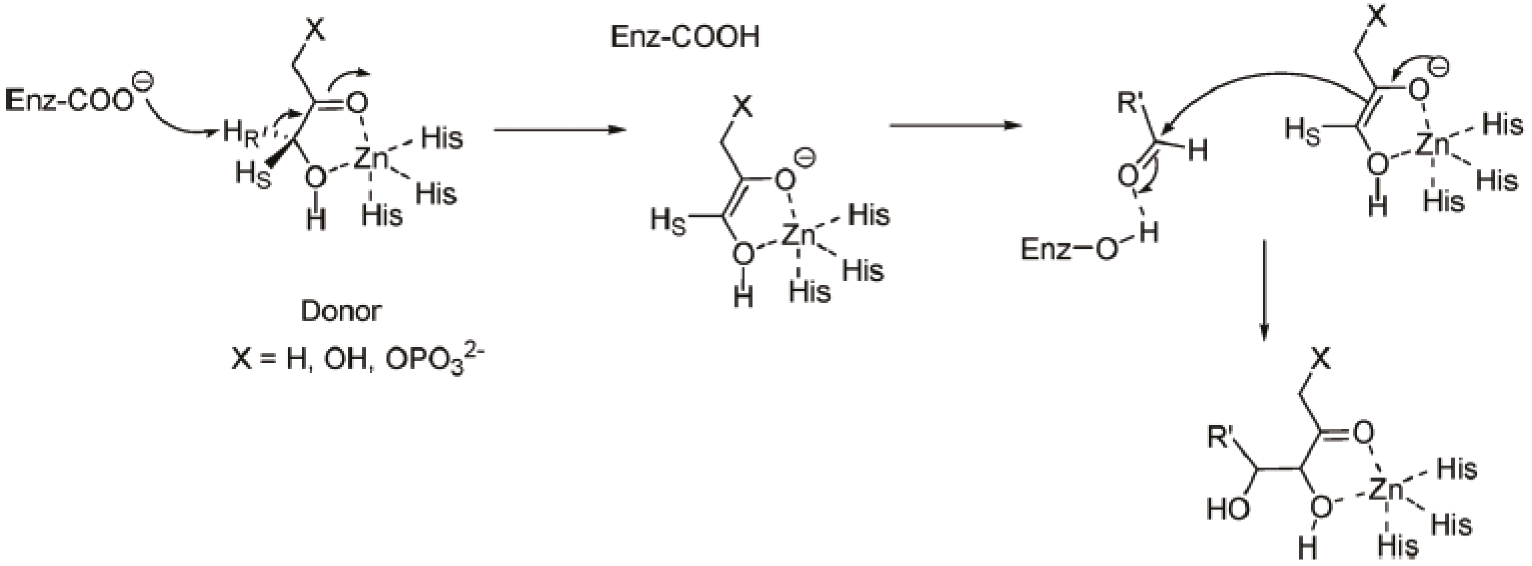
Type II Aldolase Mechanism

- Key Feature: Has a Zn^{2+} cofactor in the active site



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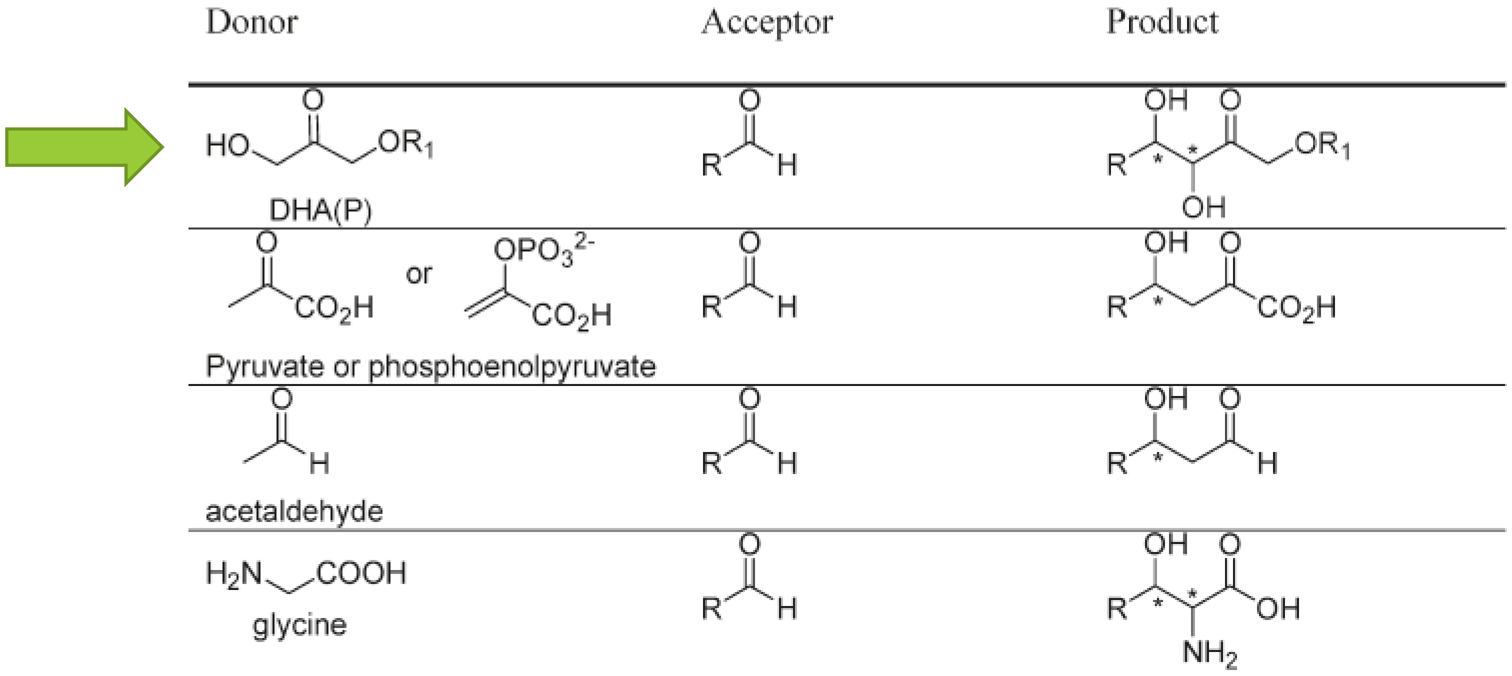
4 Functional Main Groups of Aldolases

- Based on donor (Acceptor is generic aldehyde)



R_1:$PO_3^{=}$ or H *: new chiral carbon atom

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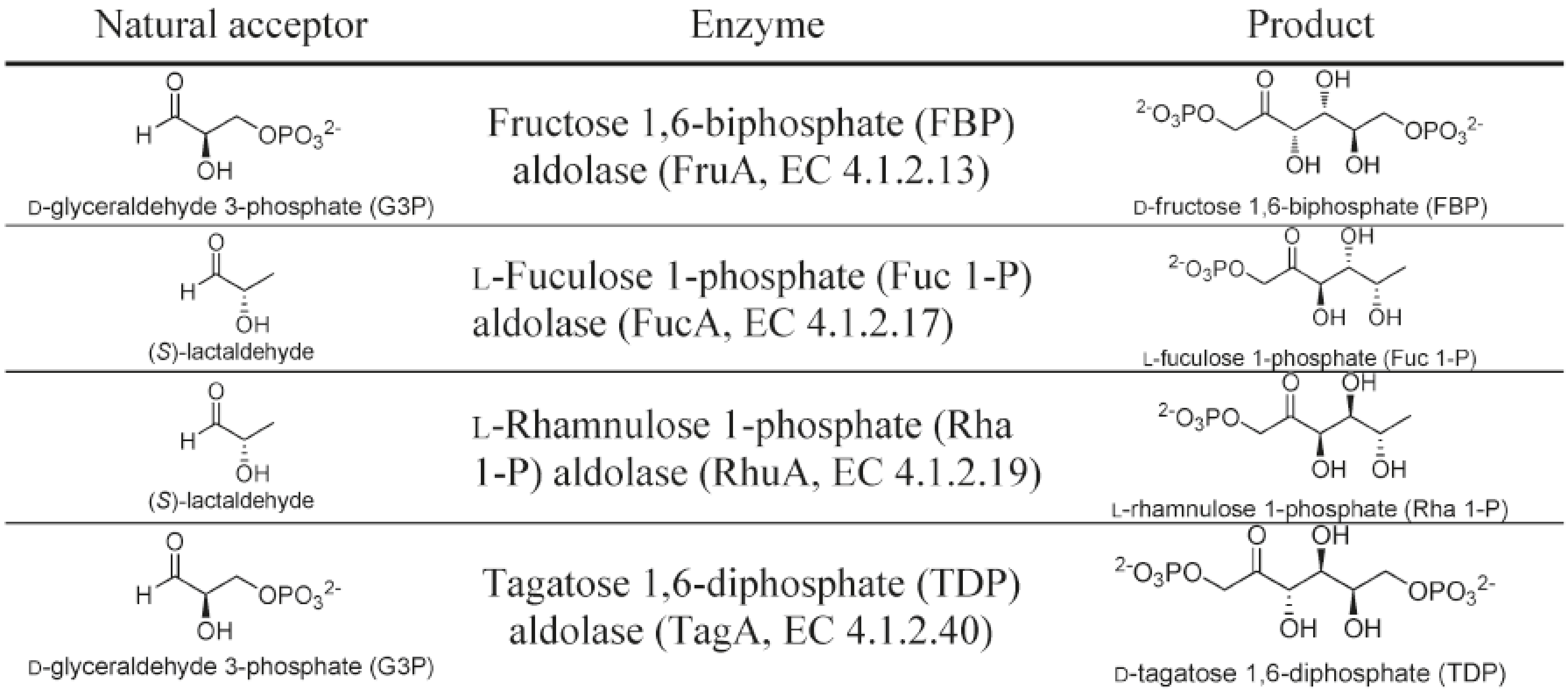
DHAP Dependent Aldolases



Natural acceptor	Enzyme	Product
D-glyceraldehyde 3-phosphate (G3P)	Fructose 1,6-biphosphate (FBP) aldolase (FruA, EC 4.1.2.13)	D-fructose 1,6-biphosphate (FBP)
(*S*)-lactaldehyde	L-Fuculose 1-phosphate (Fuc 1-P) aldolase (FucA, EC 4.1.2.17)	L-fuculose 1-phosphate (Fuc 1-P)
(*S*)-lactaldehyde	L-Rhamnulose 1-phosphate (Rha 1-P) aldolase (RhuA, EC 4.1.2.19)	L-rhamnulose 1-phosphate (Rha 1-P)
D-glyceraldehyde 3-phosphate (G3P)	Tagatose 1,6-diphosphate (TDP) aldolase (TagA, EC 4.1.2.40)	D-tagatose 1,6-diphosphate (TDP)

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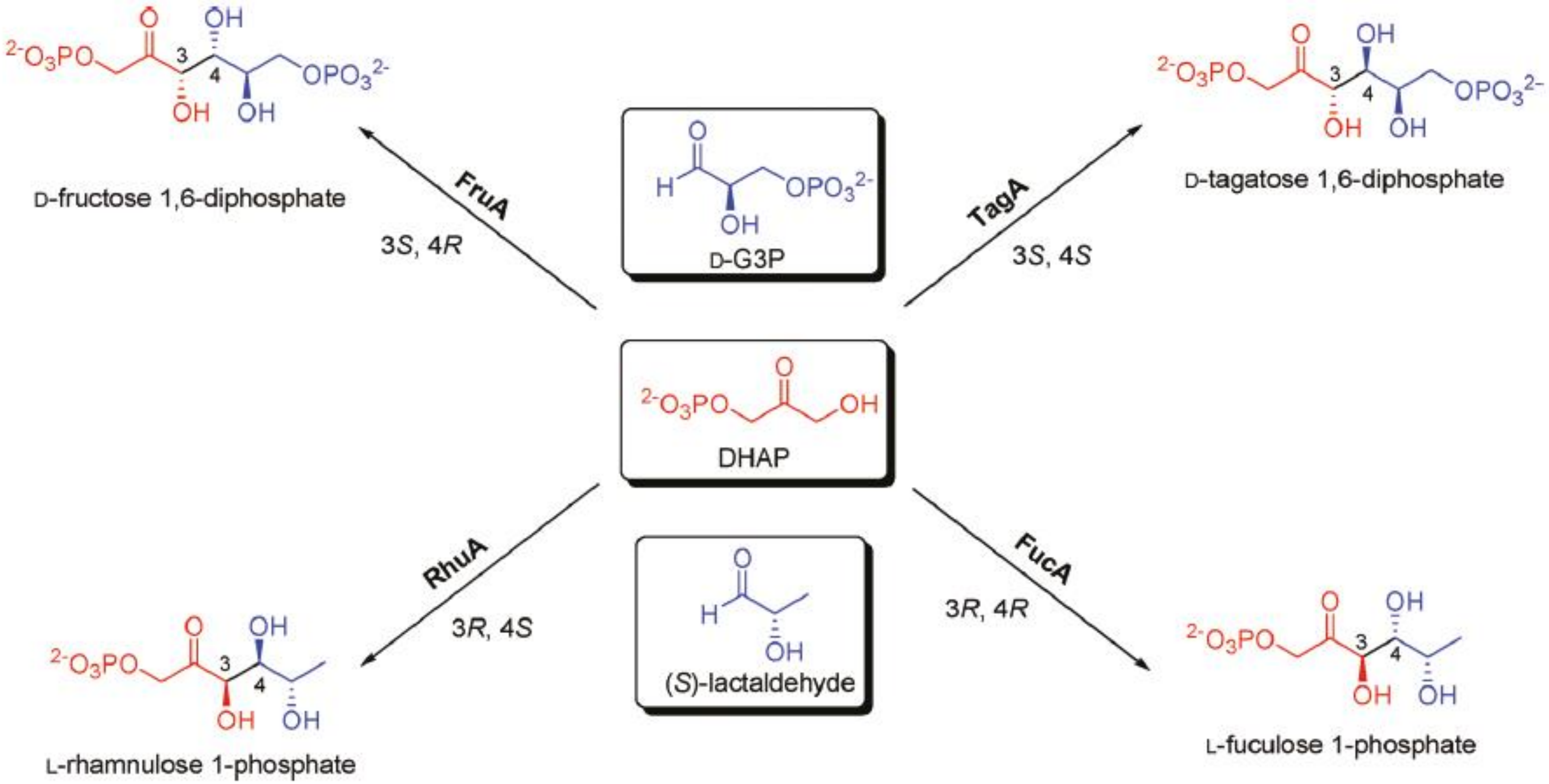
DHAP-Dependent Aldolase Stereochemical Complementarity



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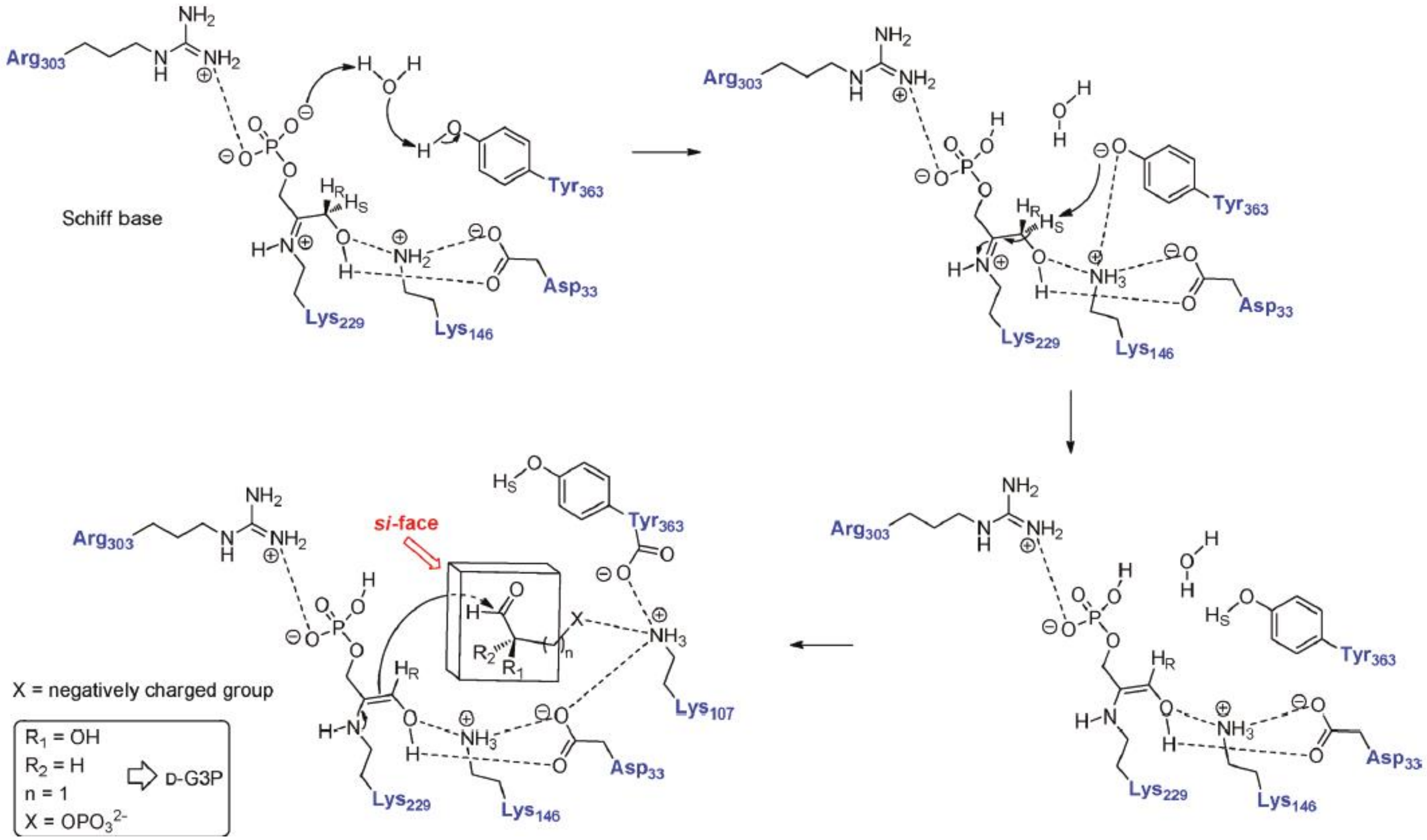
Origin of Stereospecificity



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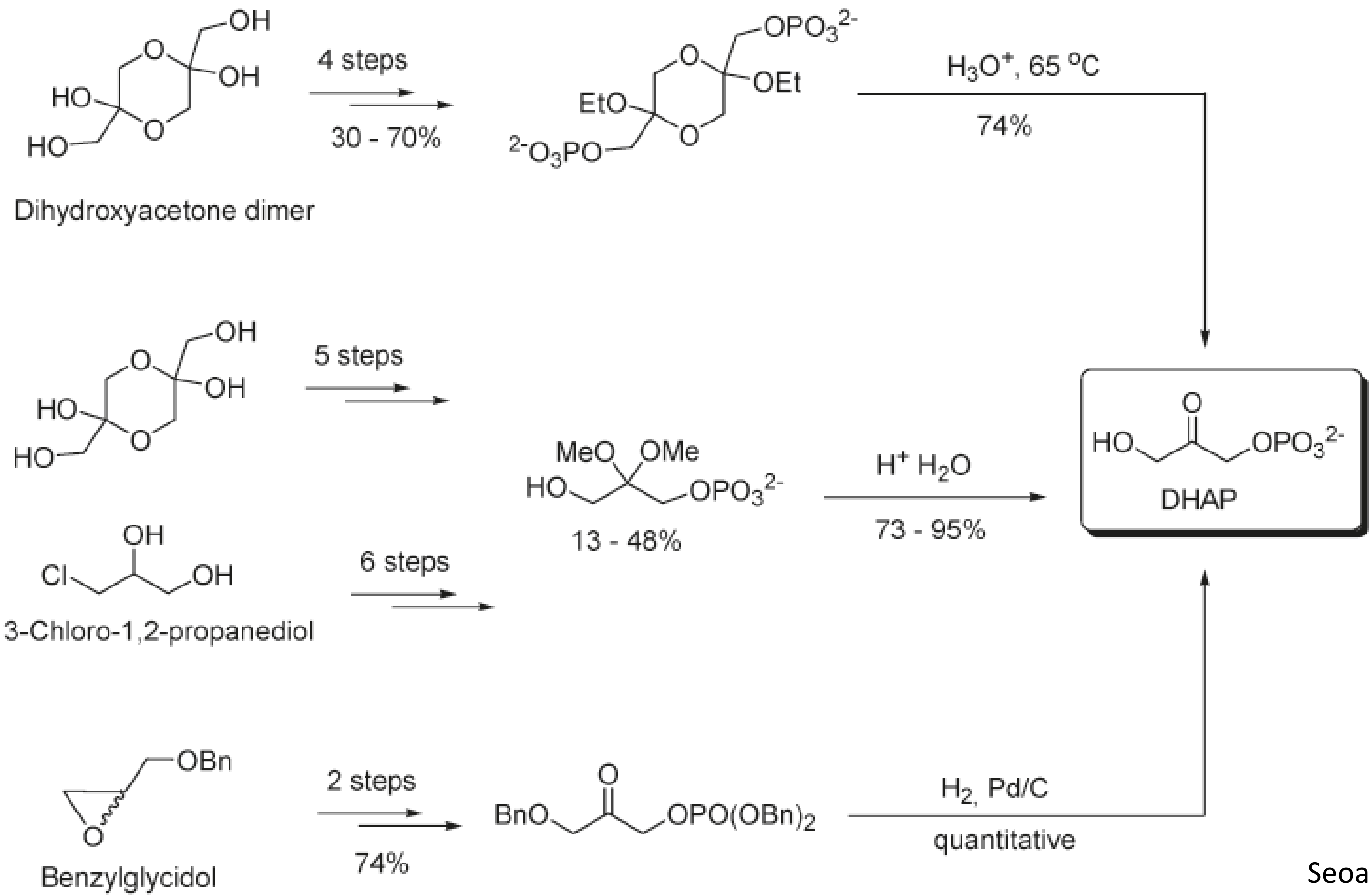
DHAP is the Worst



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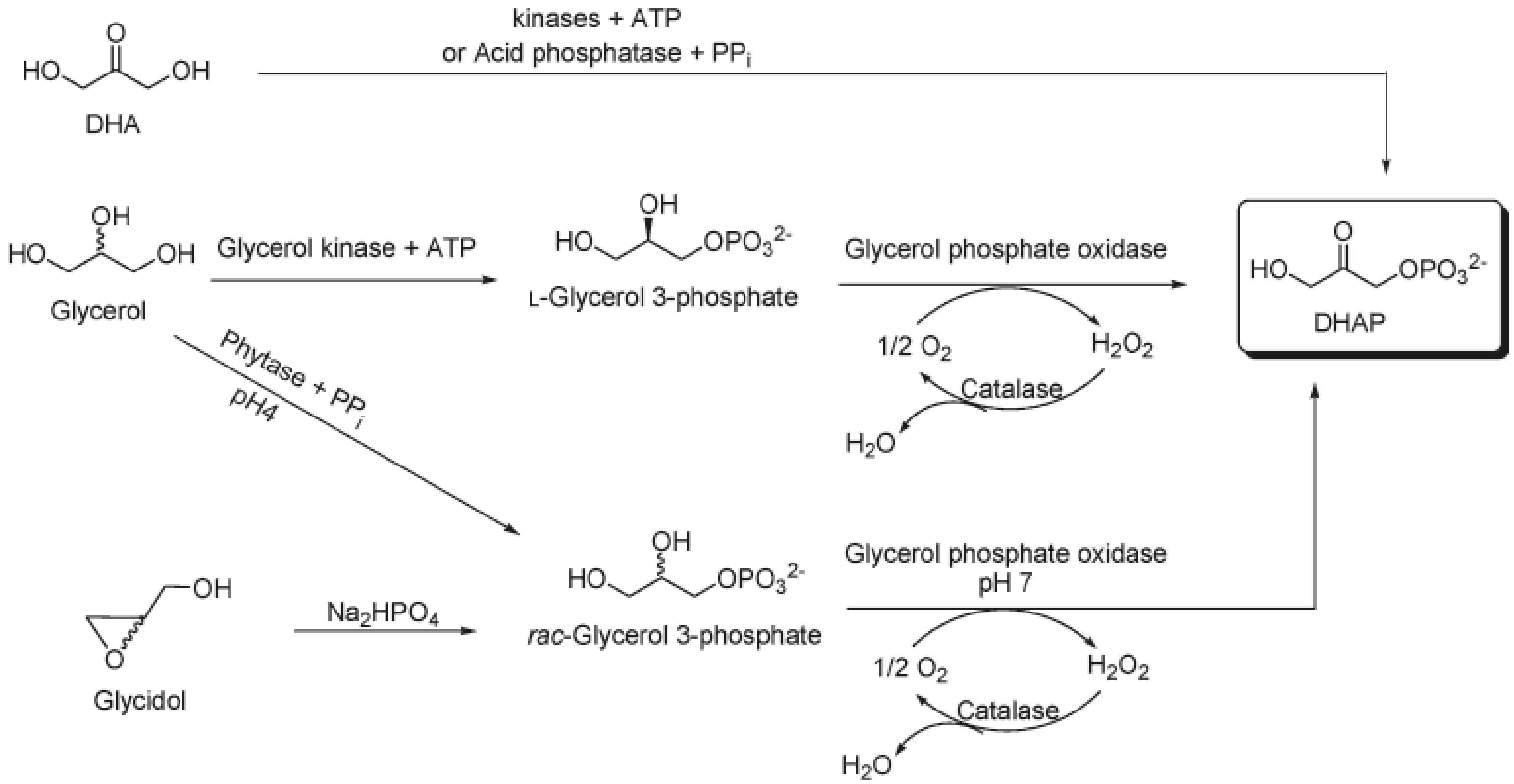

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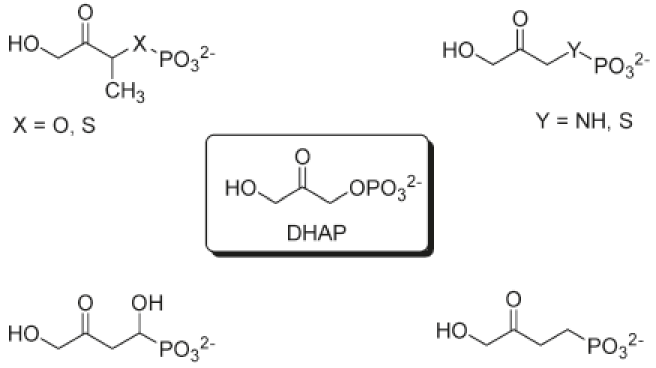
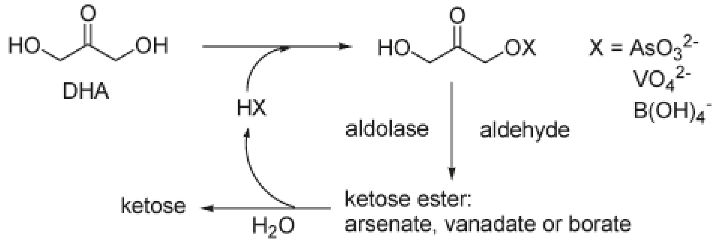
Specificity of Donor

- Only a few analogs of DHAP are substrates and activity drops to ~10% of DHAP



- Changing the counterion can be tolerated in some cases, however



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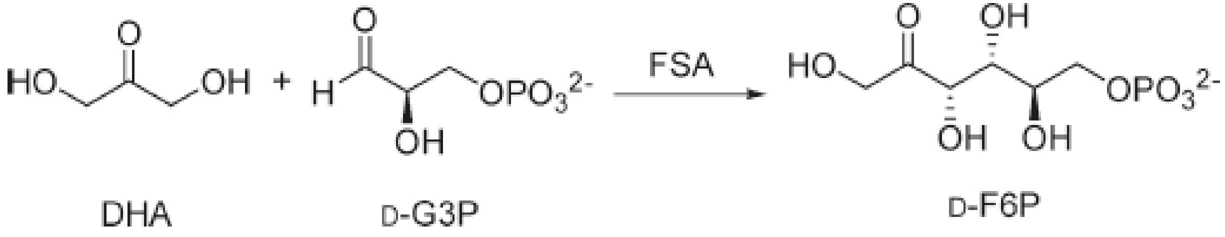
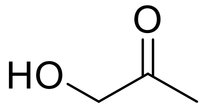
Fructose 6-Phosphate Aldolase (FSA)

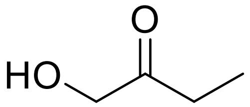
- New aldolase isolated from *E. coli* that directly uses DHA as the donor substrate



- Can also use monohydroxy acetone and 1-hydroxyl-2-butanone as donors with no loss in activity



monohydroxyacetone



1-hydroxy-2-butanone

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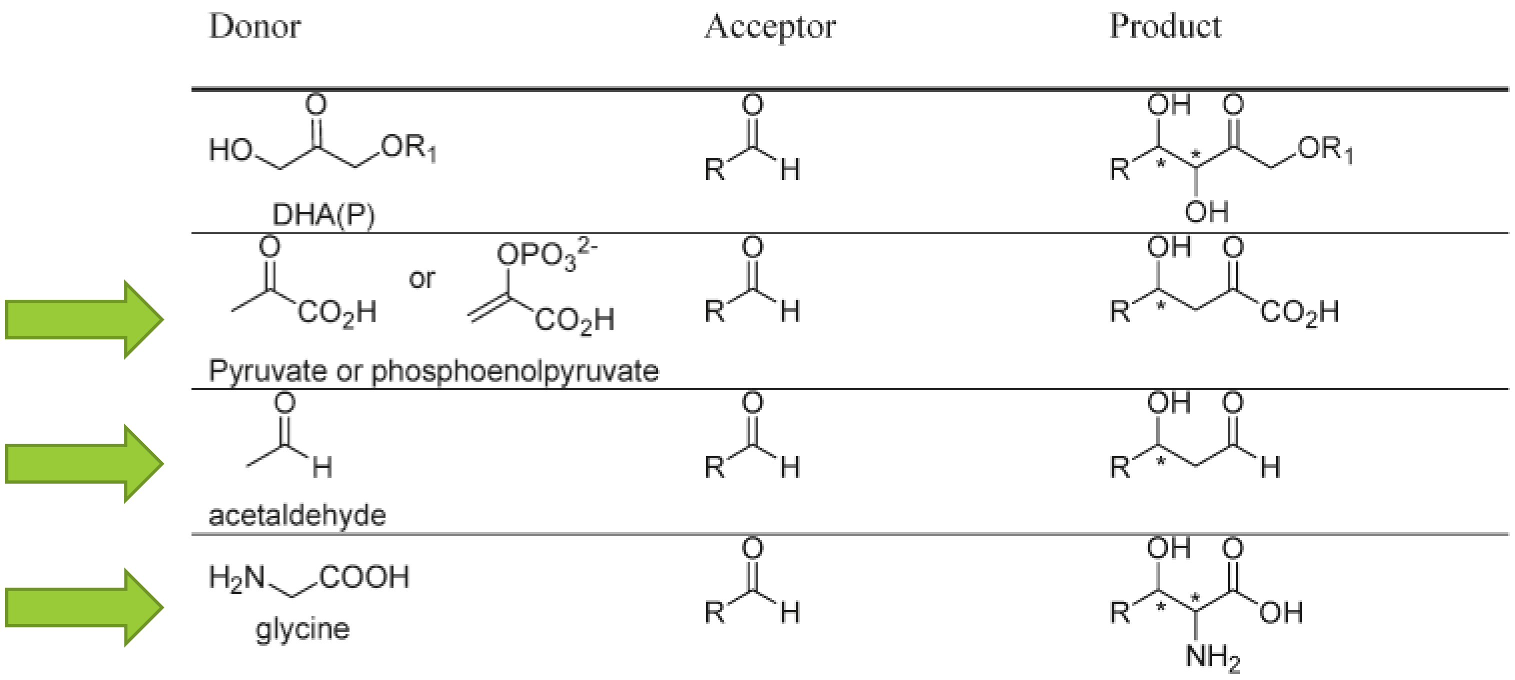
4 Functional Main Groups of Aldolases

- Based on donor (Acceptor is generic aldehyde)



R_1:$PO_3^=$ or H *: new chiral carbon atom

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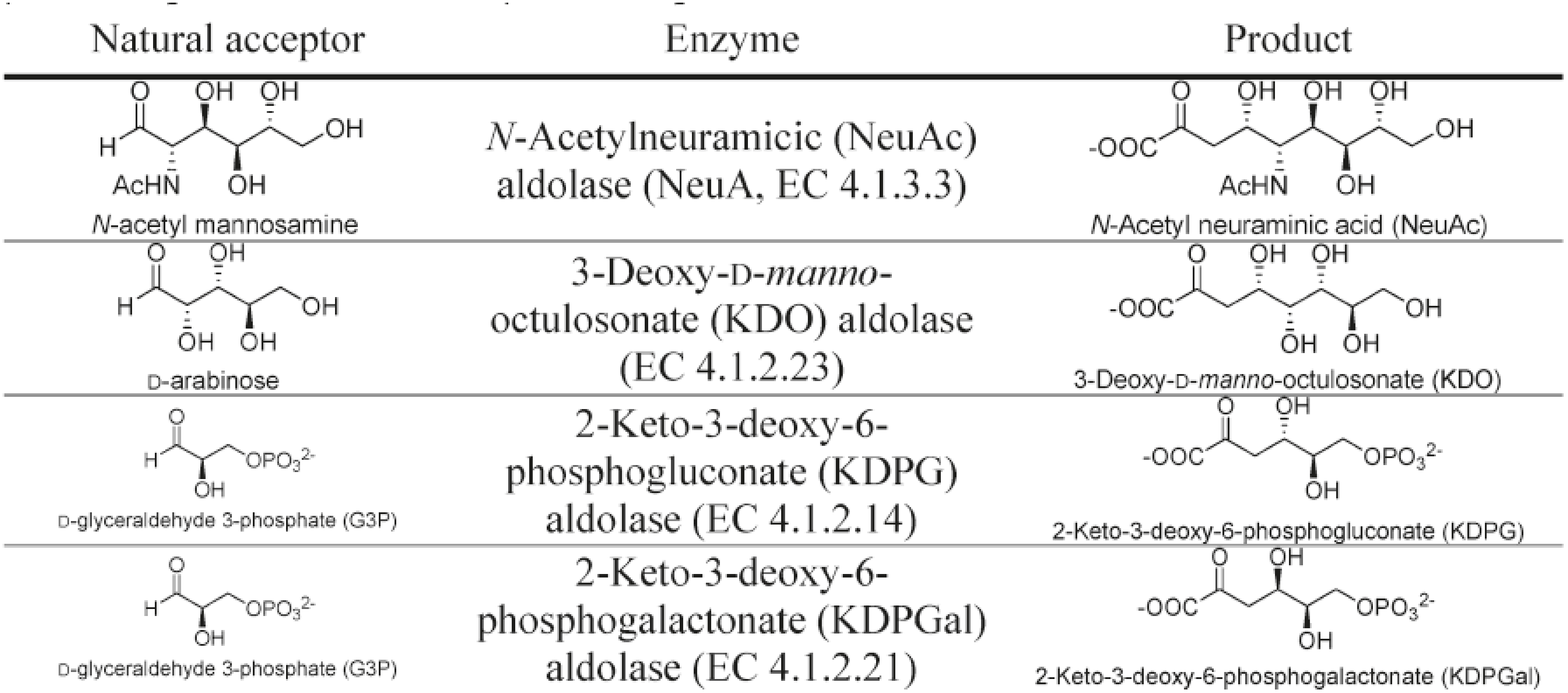
Pyruvate-Dependent Aldolases



Natural acceptor	Enzyme	Product
N-acetyl mannosamine	*N*-Acetylneuramicic (NeuAc) aldolase (NeuA, EC 4.1.3.3)	*N*-Acetyl neuraminic acid (NeuAc)
D-arabinose	3-Deoxy-D-*manno*-octulosonate (KDO) aldolase (EC 4.1.2.23)	3-Deoxy-D-*manno*-octulosonate (KDO)
D-glyceraldehyde 3-phosphate (G3P)	2-Keto-3-deoxy-6-phosphogluconate (KDPG) aldolase (EC 4.1.2.14)	2-Keto-3-deoxy-6-phosphogluconate (KDPG)
D-glyceraldehyde 3-phosphate (G3P)	2-Keto-3-deoxy-6-phosphogalactonate (KDPGal) aldolase (EC 4.1.2.21)	2-Keto-3-deoxy-6-phosphogalactonate (KDPGal)

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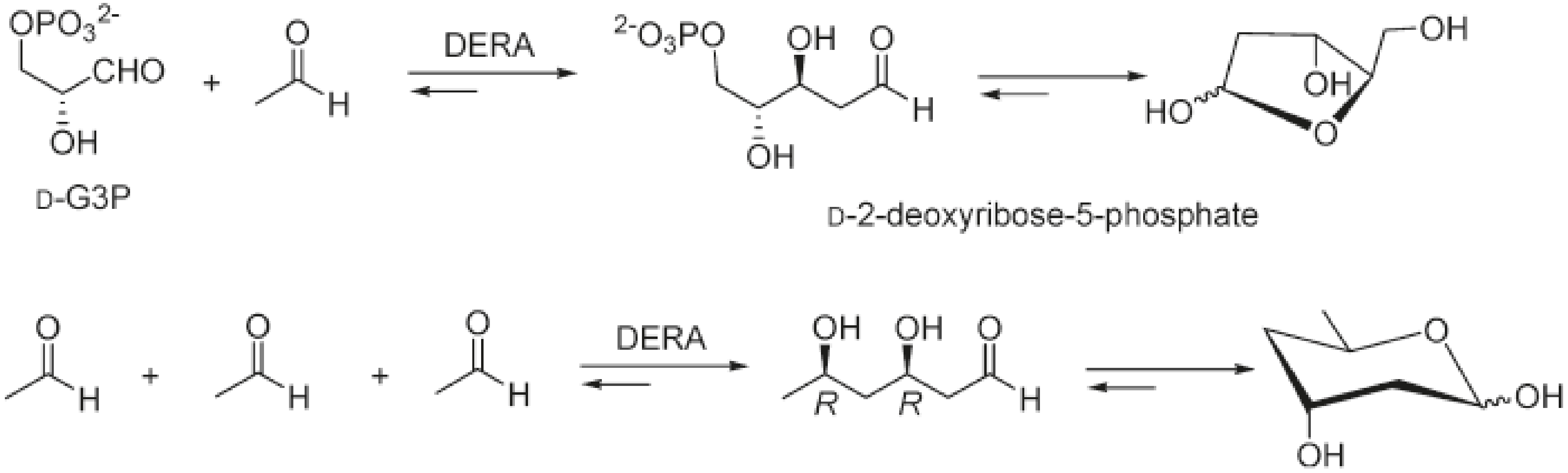
Acetaldehyde-Dependent Aldolases

- 2-deoxyribose-5-phosphate aldolase (DERA) is the only one that is known
- Rare, because both donor and acceptor are aldehydes- can thus do sequential aldols



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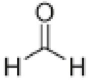
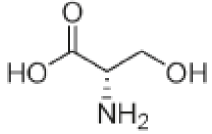
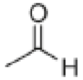
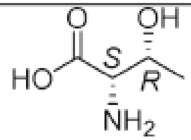
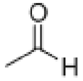
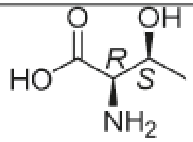
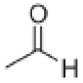
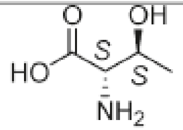
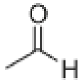
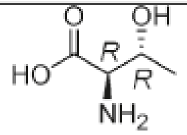
Glycine-Dependent Aldolases

Natural acceptor	Enzyme	Product
 formaldehyde	Serine hydroxymethyltransferase (SHMT) (EC 2.1.2.1)	 L-Serine
 acetaldehyde	L-Threonine (Thr) aldolase (ThrA, EC 4.1.2.5)	 L-Threonine
 acetaldehyde	D-Threonine (Thr) aldolase (ThrA, EC 4.1.2.42)	 D-Threonine
 acetaldehyde	L-*allo*-Threonine (Thr) aldolase (ThrA, EC 4.1.2.6)	 L-*allo*-Threonine
 acetaldehyde	D-*allo*-Threonine (Thr) aldolase (ThrA)	 D-*allo*-Threonine

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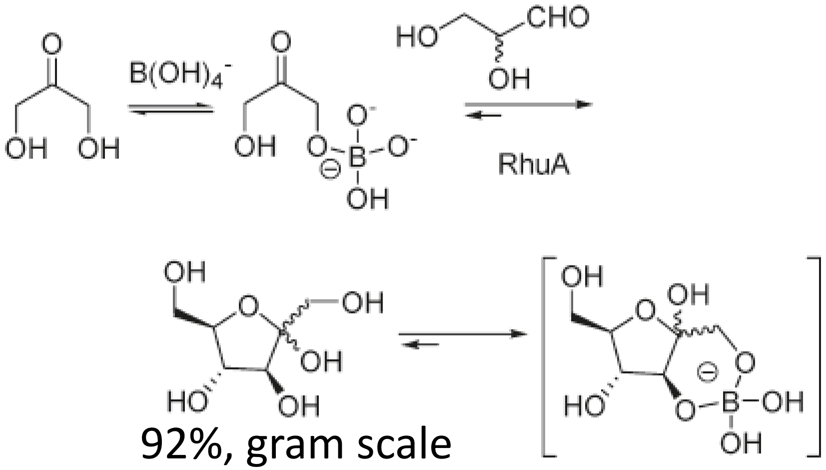
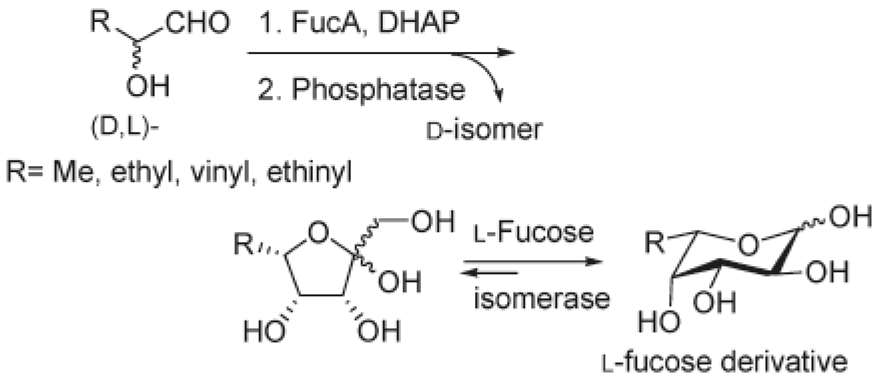
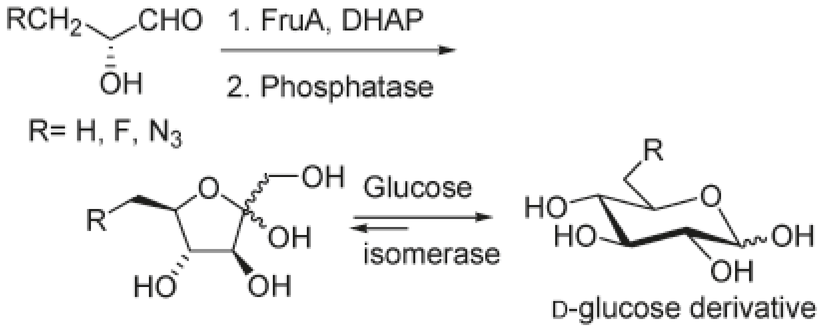
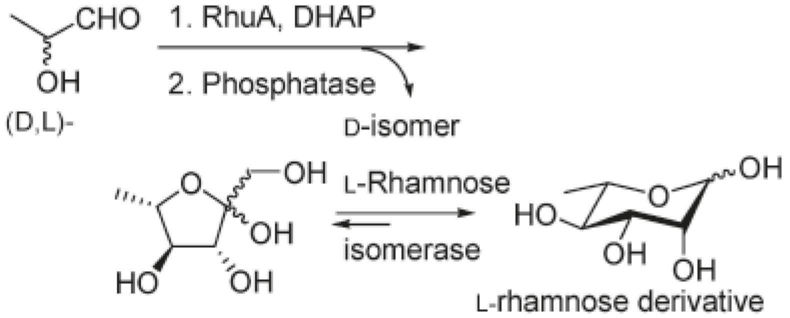
Monosaccharide Synthesis



92%, gram scale







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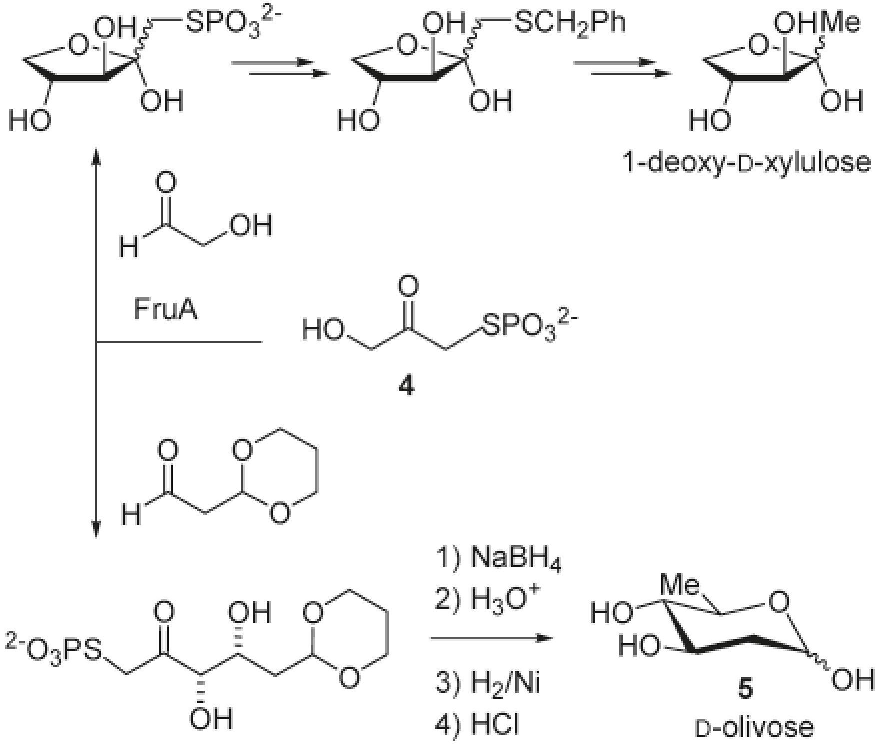
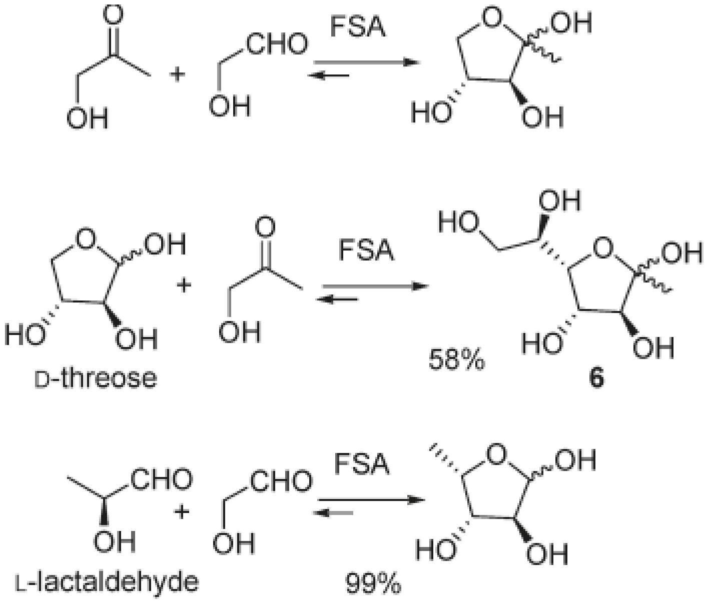
Deoxysugar Synthesis





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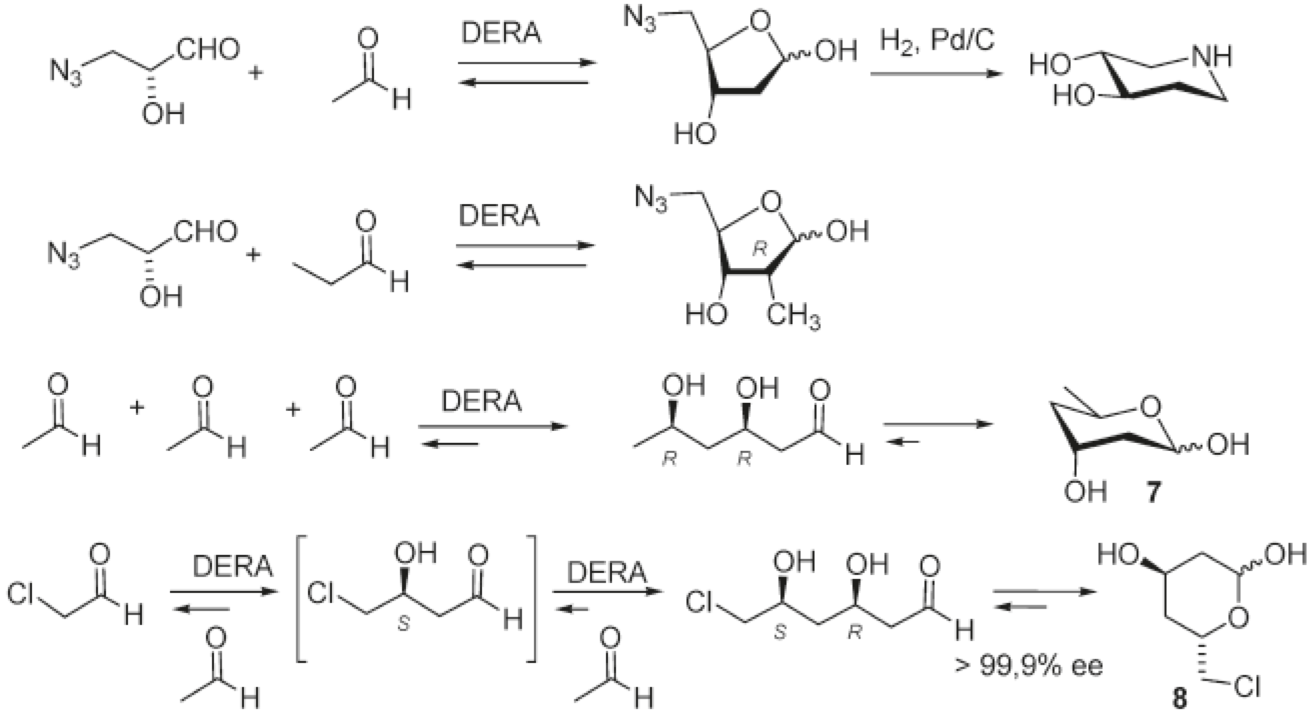

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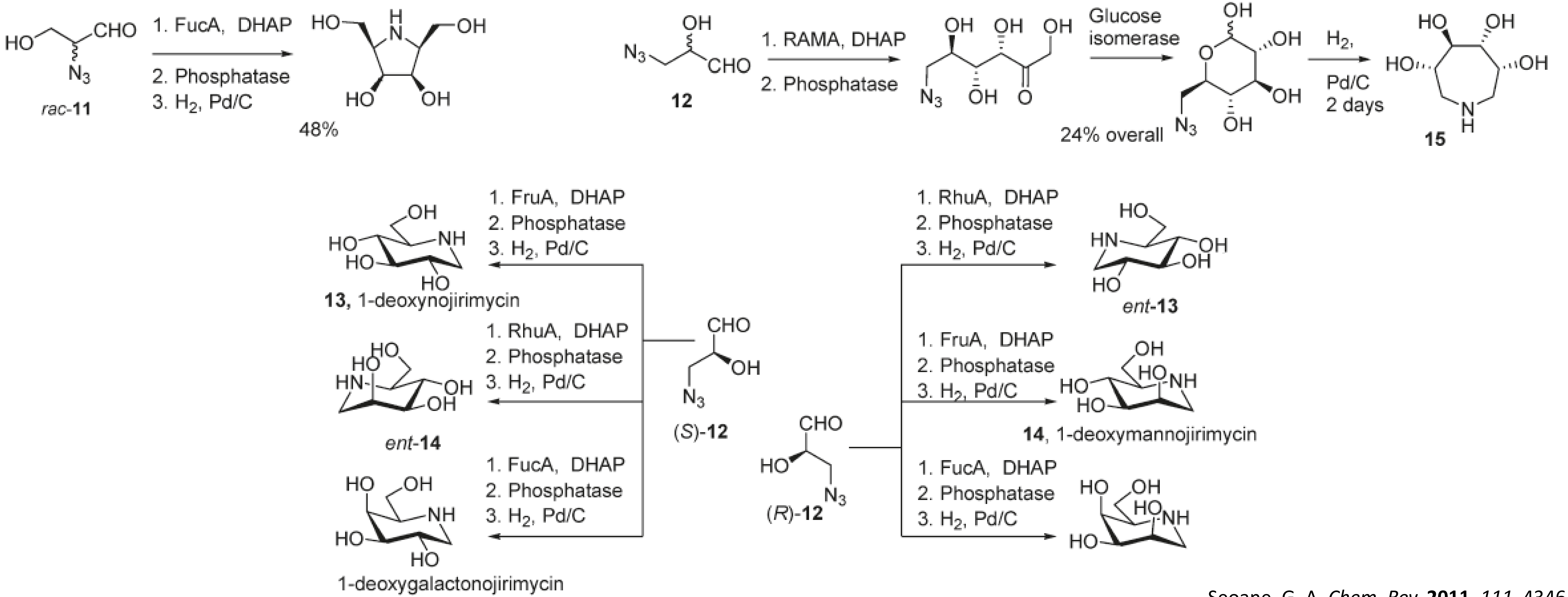
Iminosugar Synthesis



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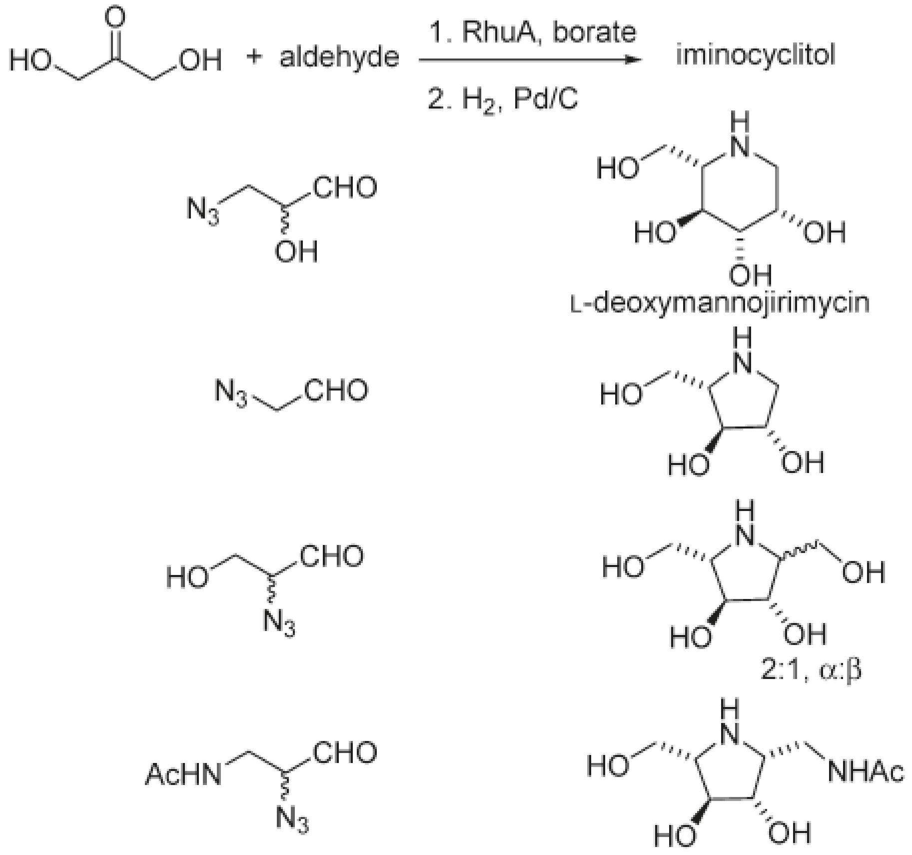
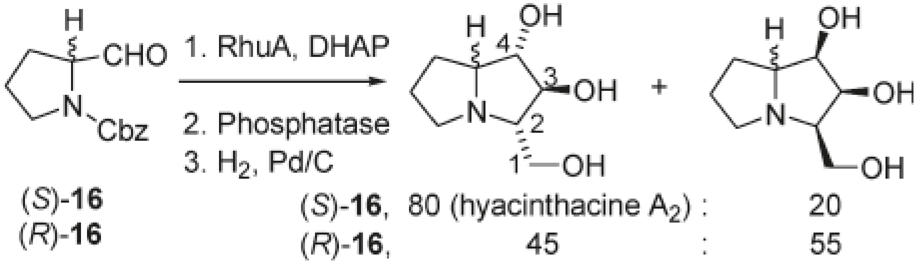
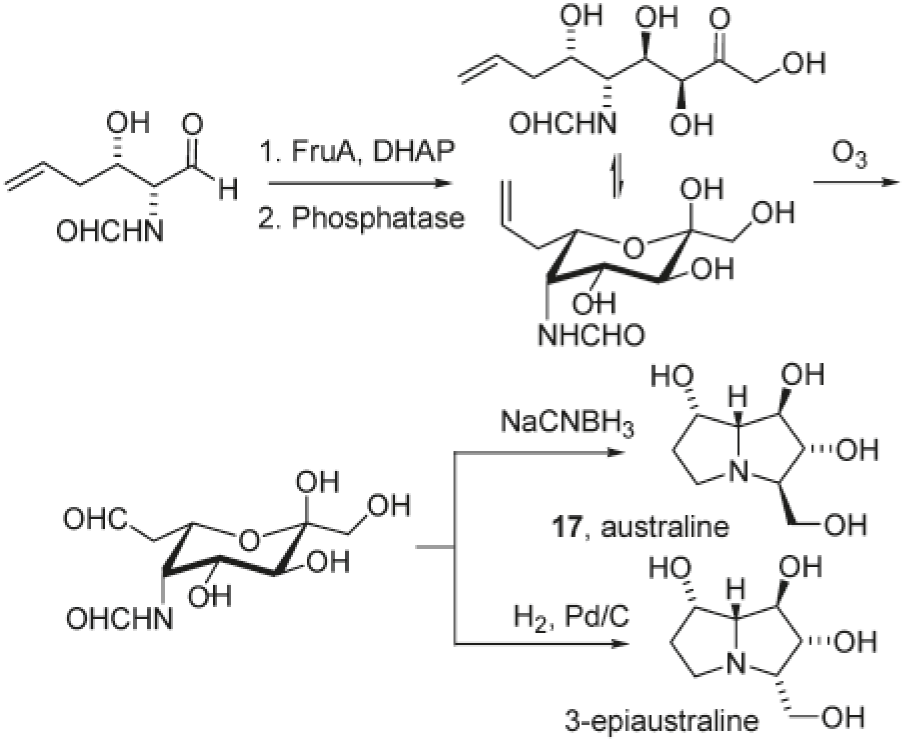
Iminosugar Synthesis







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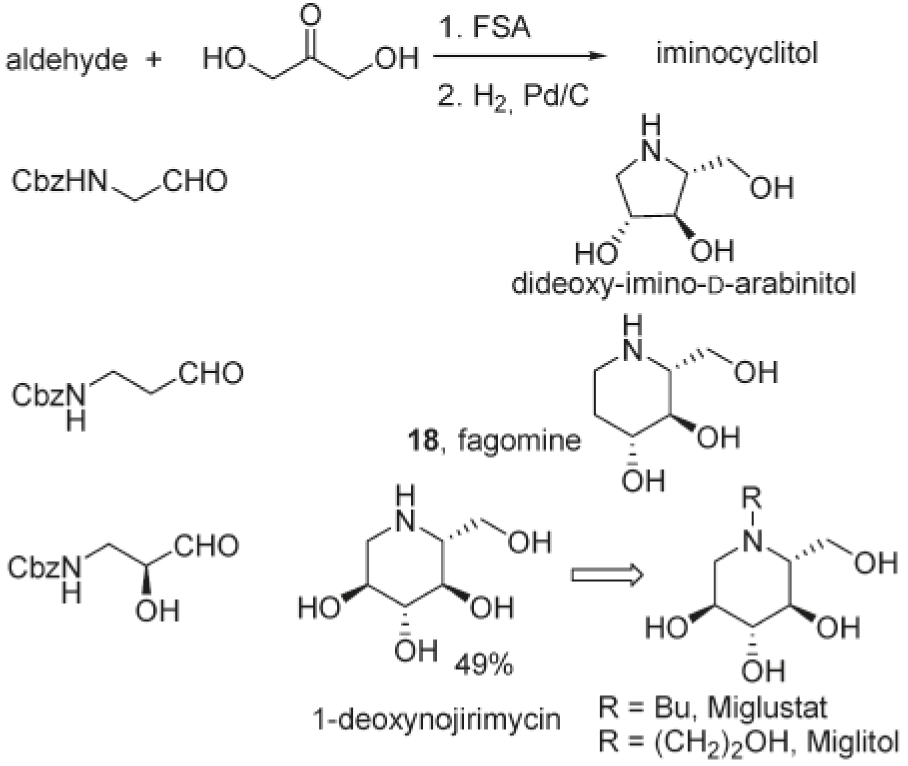

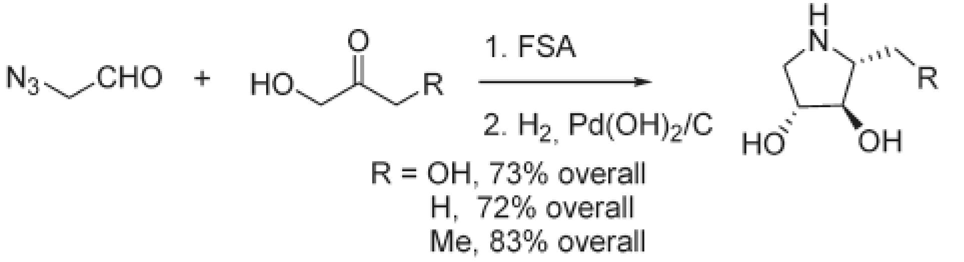

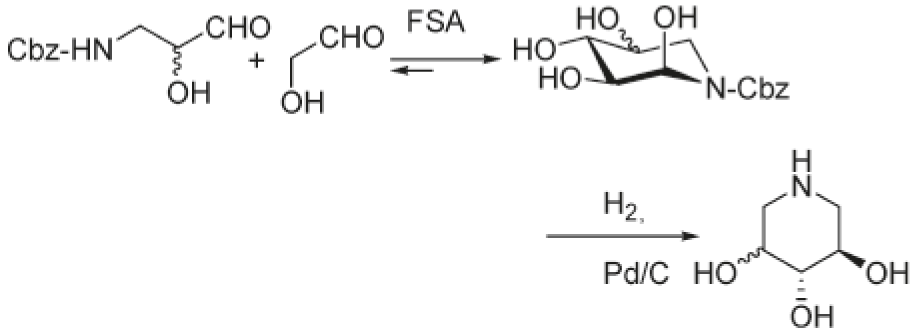

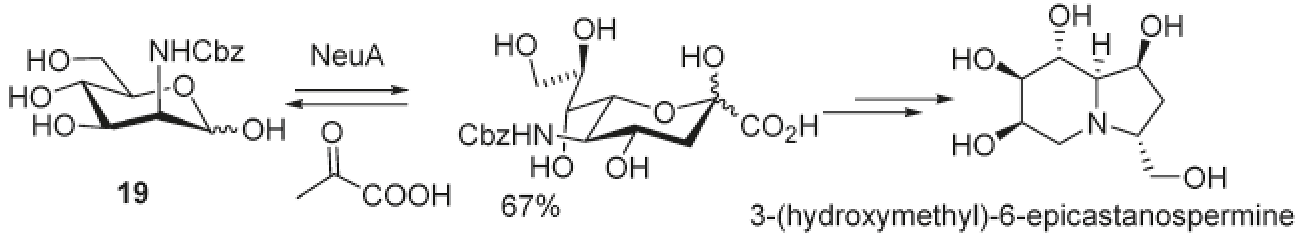

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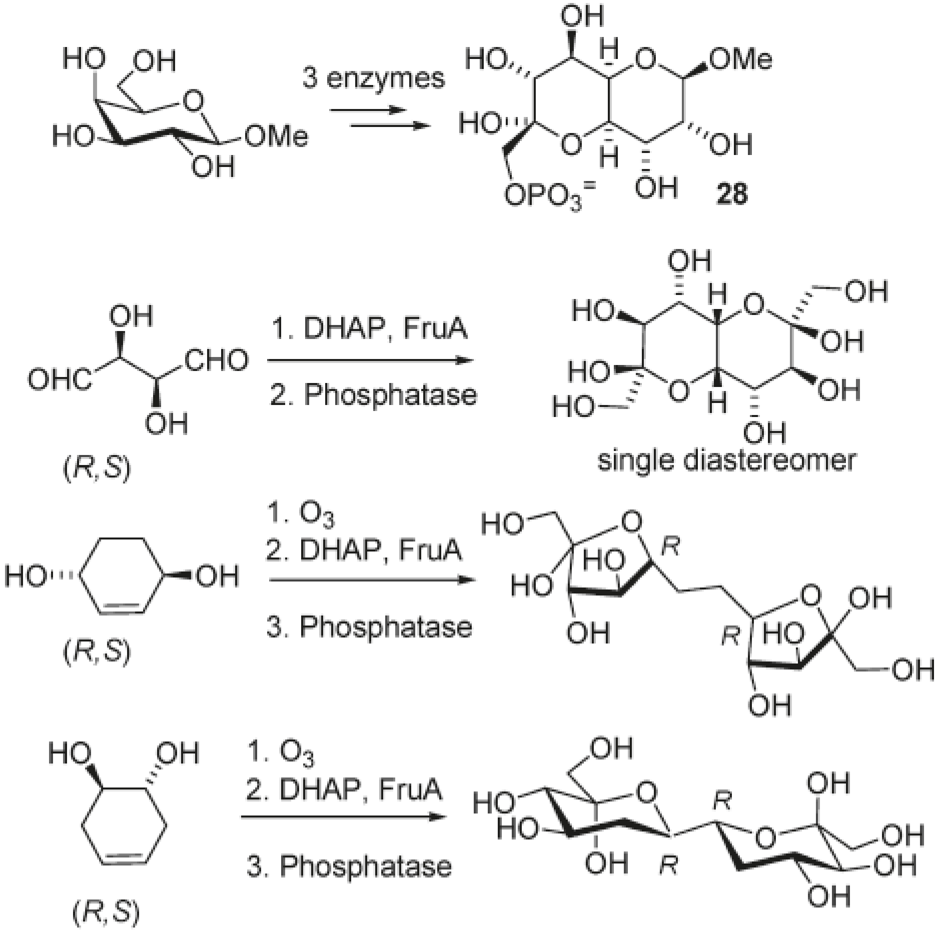
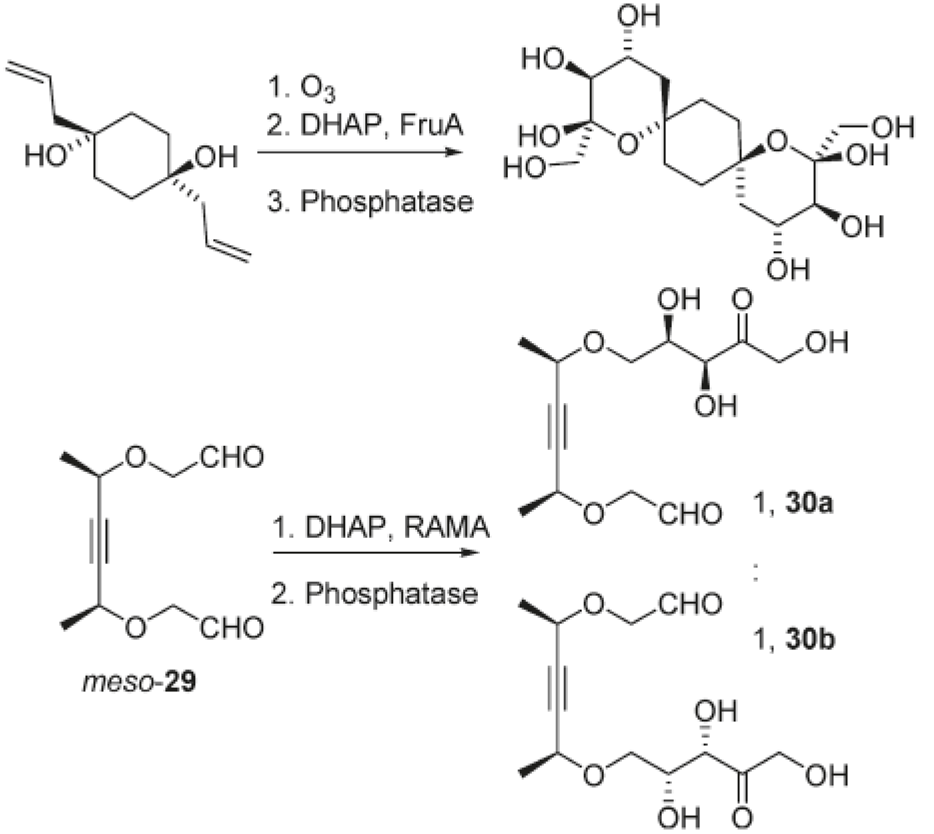
Higher Carbon Sugars and Analogs





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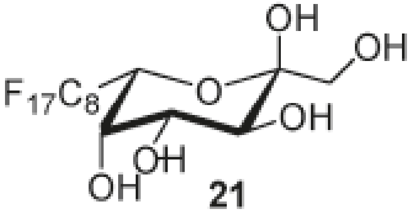
Higher Carbon Sugars and Analogs

- Prepared using DHAP aldolases



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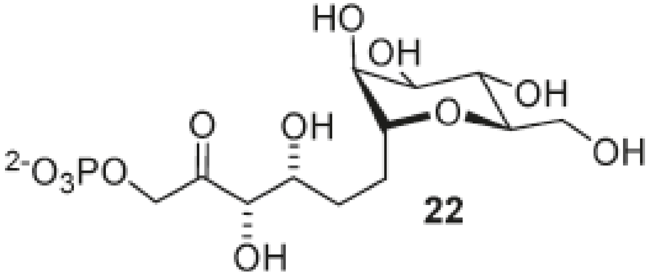

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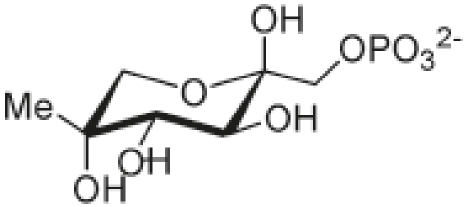

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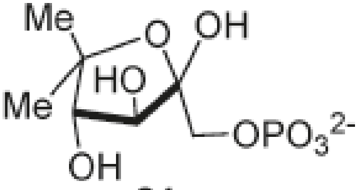

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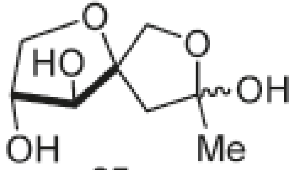

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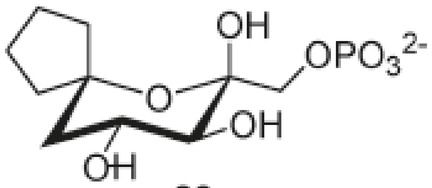

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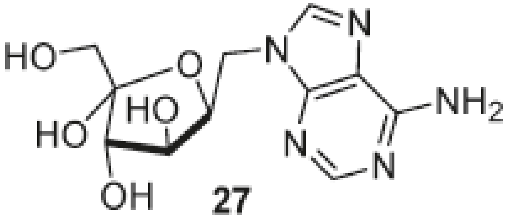

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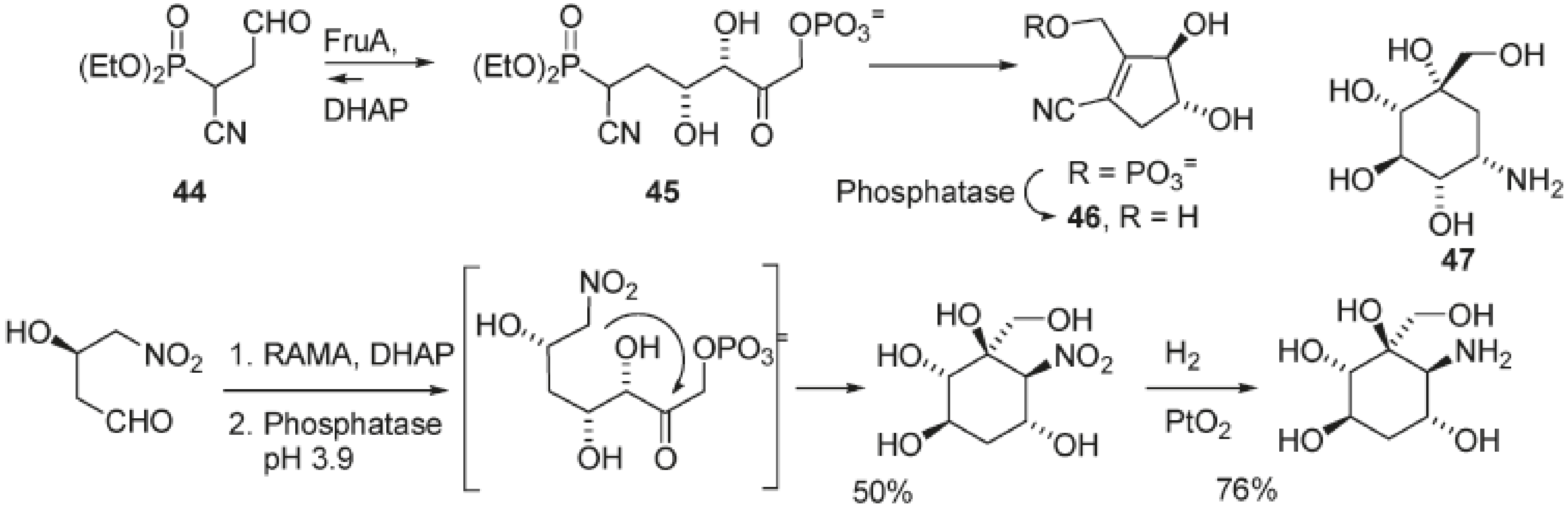
Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

Carbocycle Synthesis



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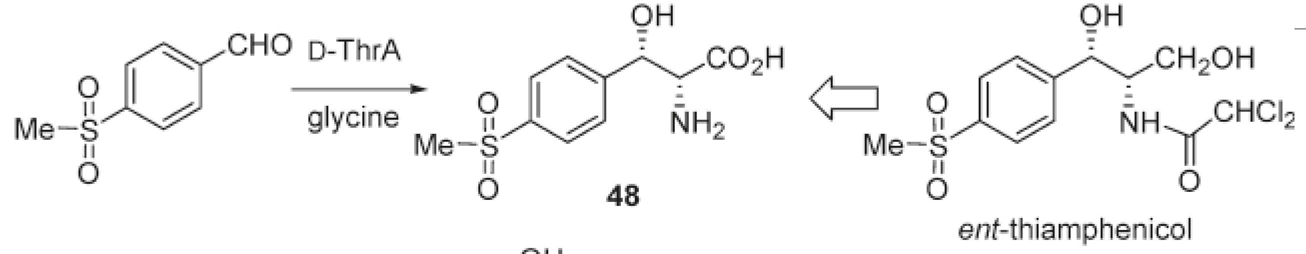
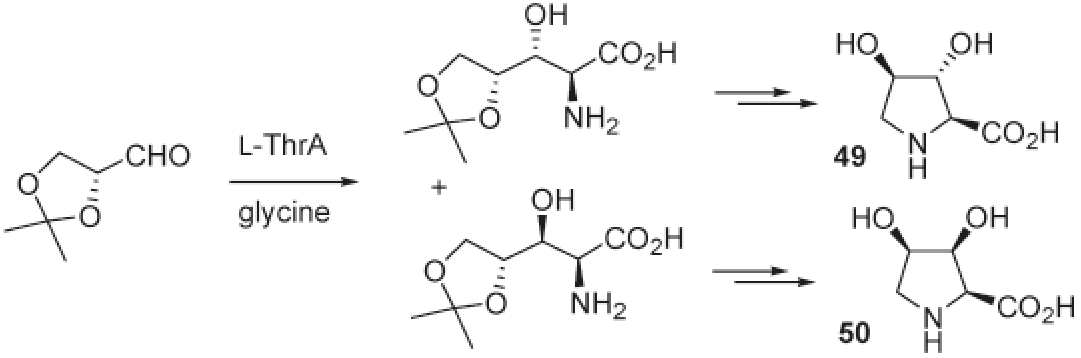
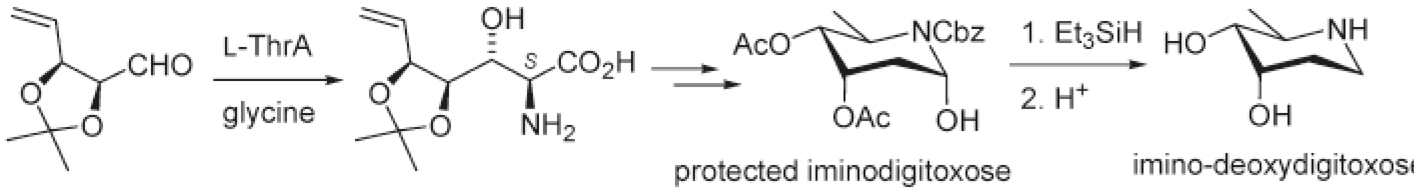
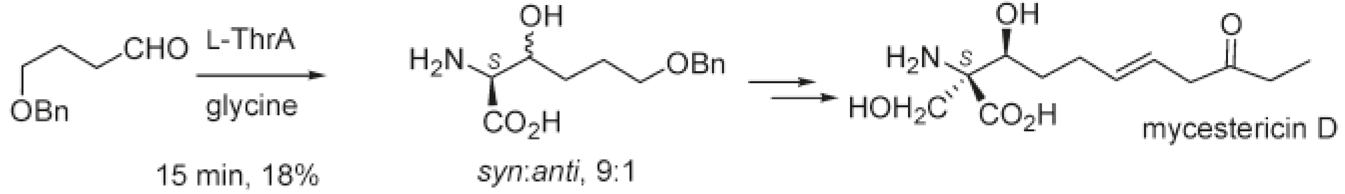
Amino Alcohol/Acid Synthesis









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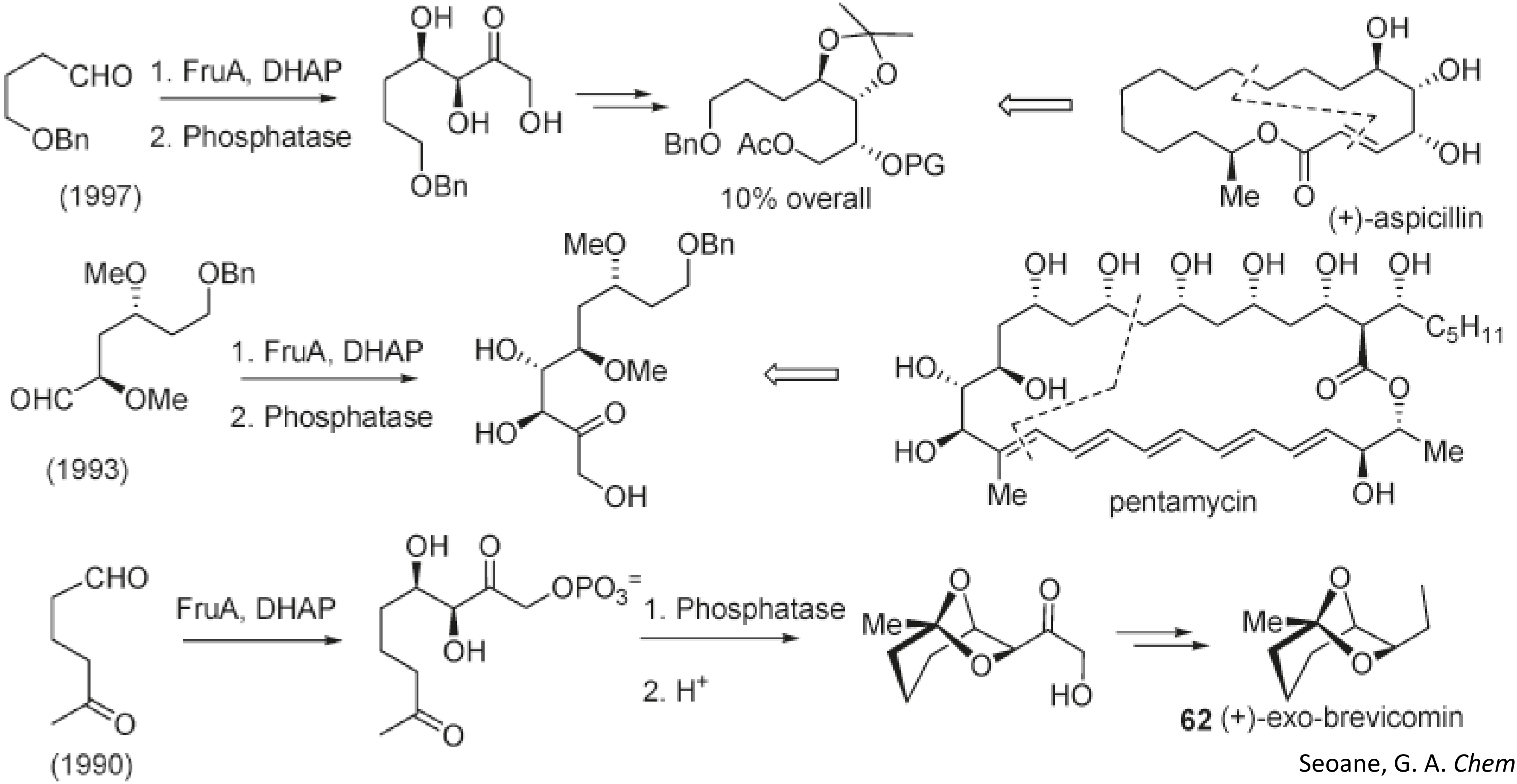
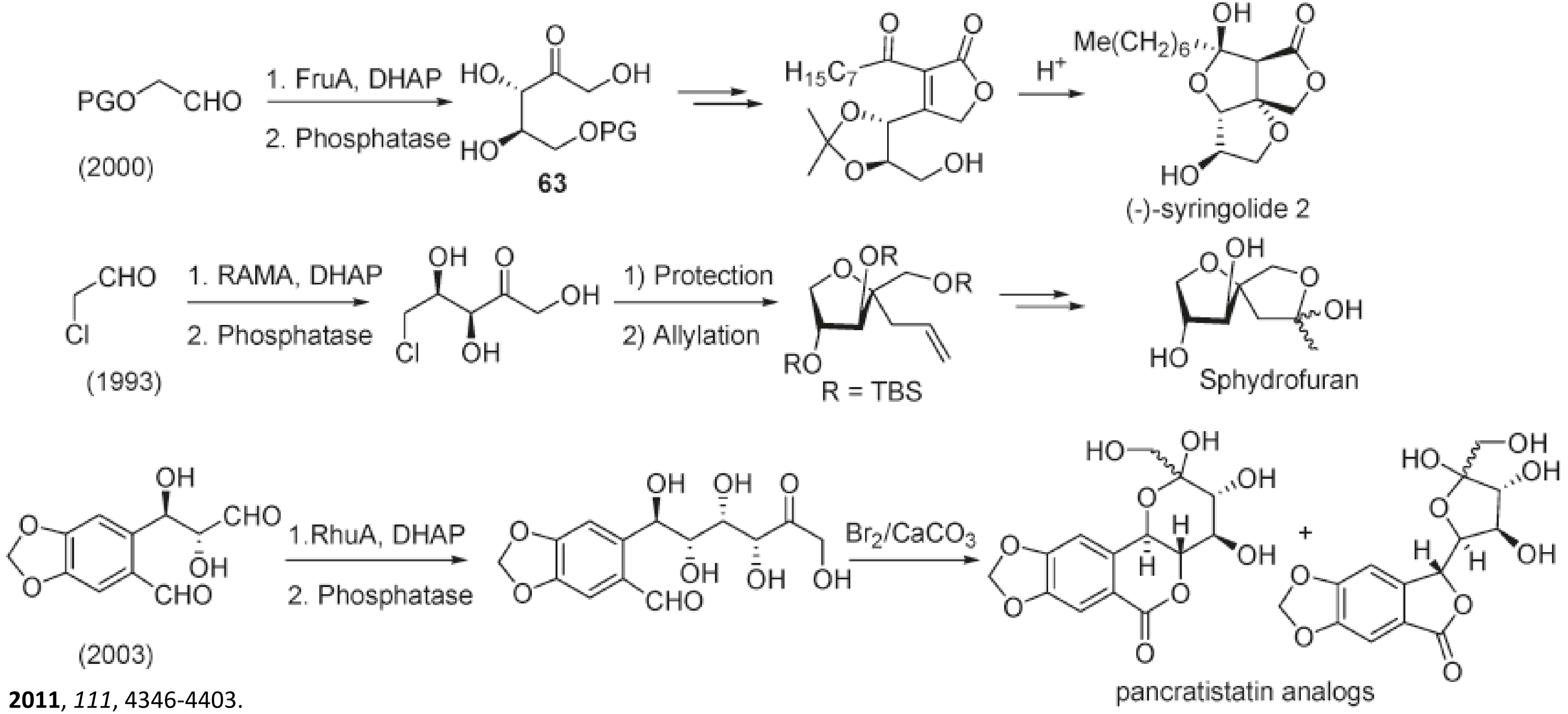
DHAP-Dependent Aldolases in Natural Product Synthesis



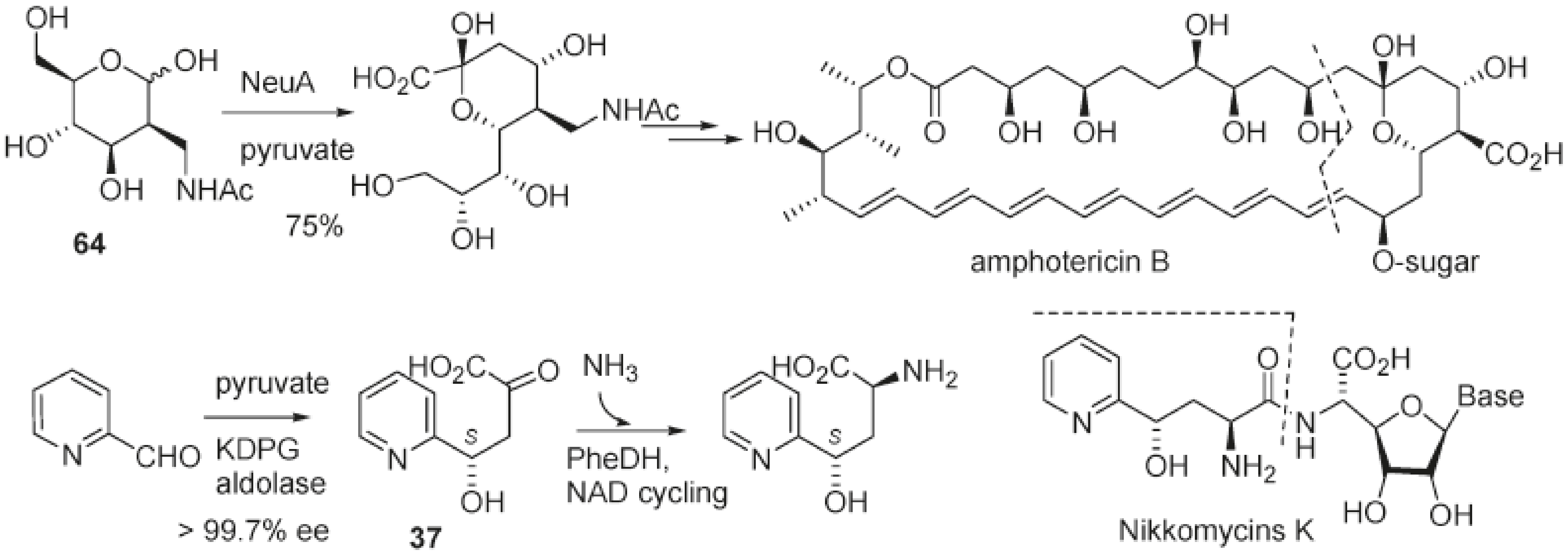
Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

DHAP-Dependent Aldolases in Natural Product Synthesis



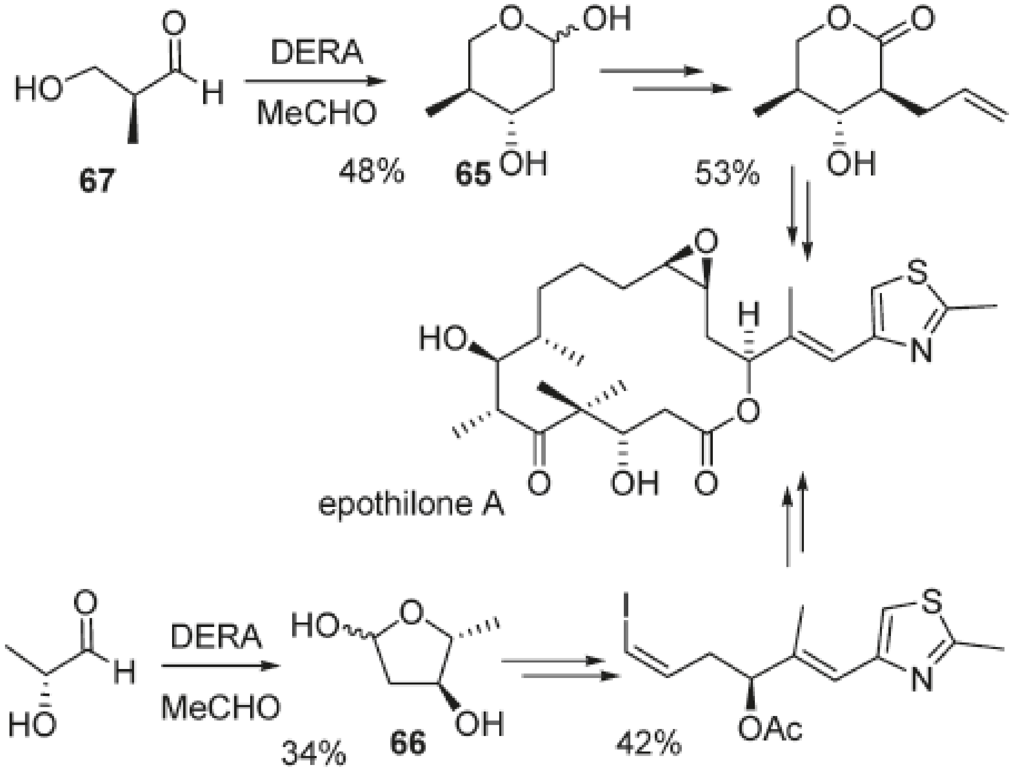
Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

Pyruvate-Dependent Aldolases in Natural Product Synthesis



Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

DERA Catalysis in Natural Product Synthesis



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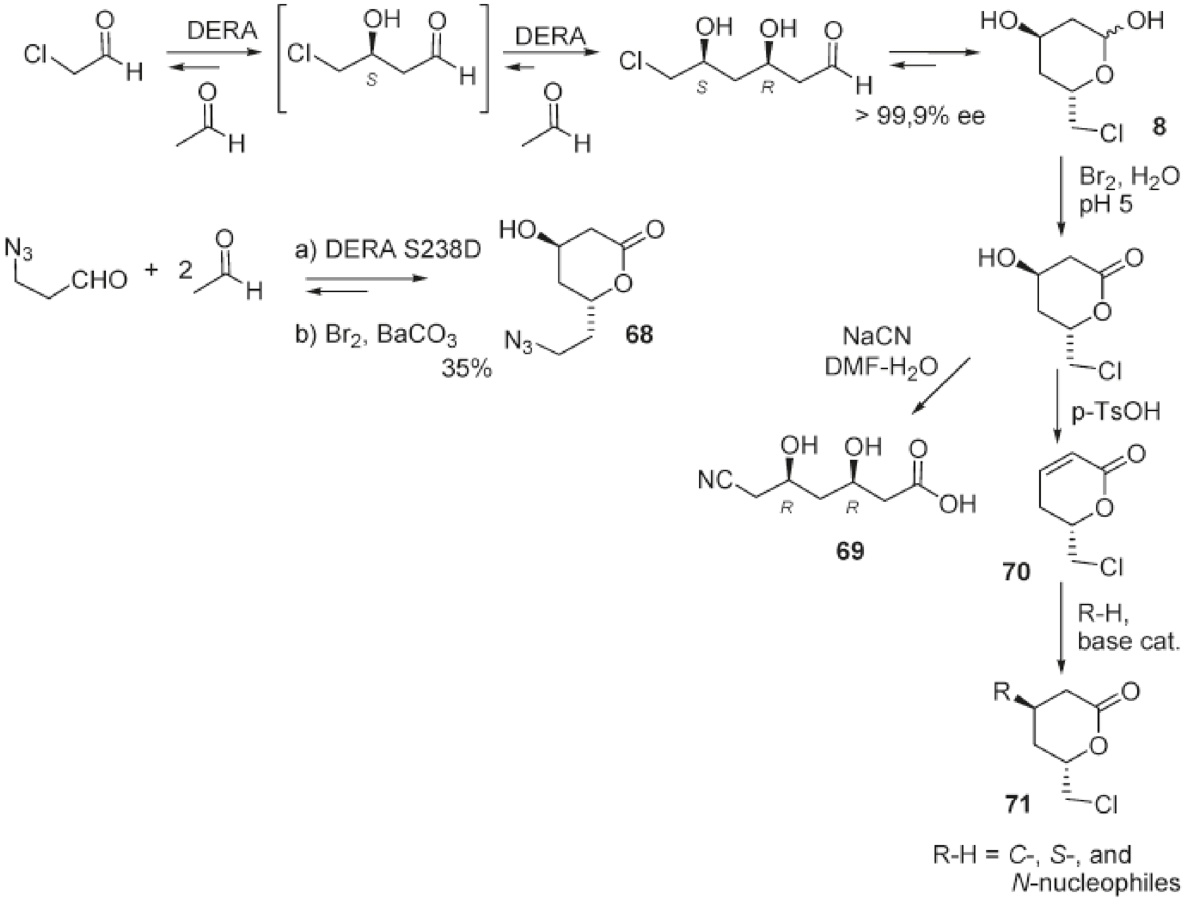
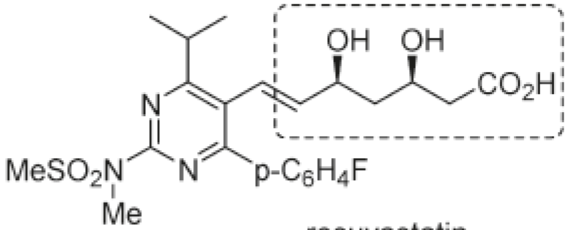
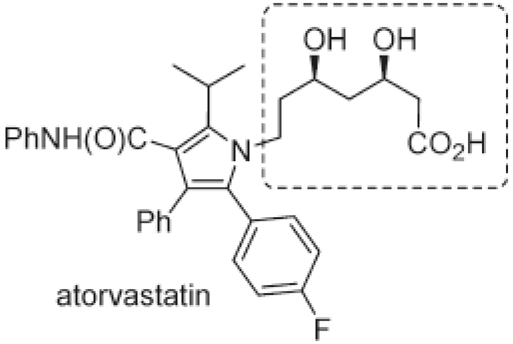
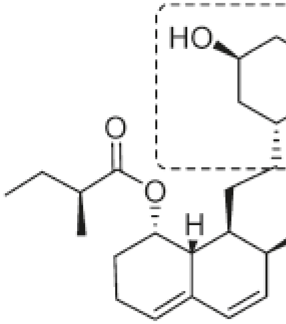
DERA Catalysis in the Pharmaceutical Industry




rosuvastatin


atorvastatin


compactin

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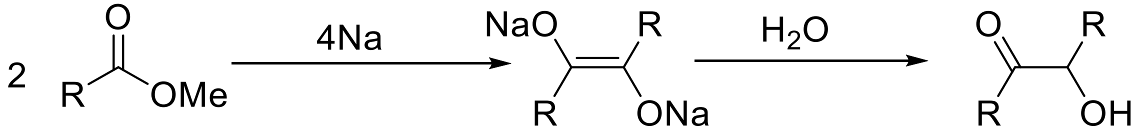
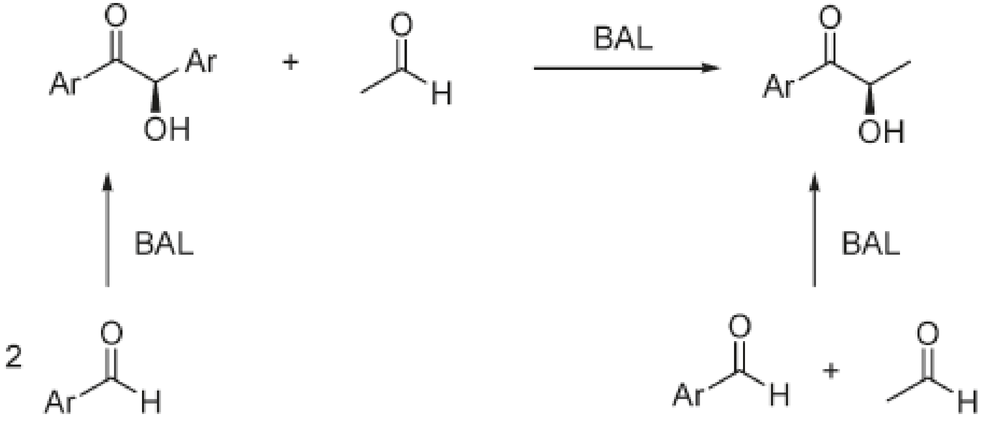
Acyloin Condensation

- Traditional acyloin condensation

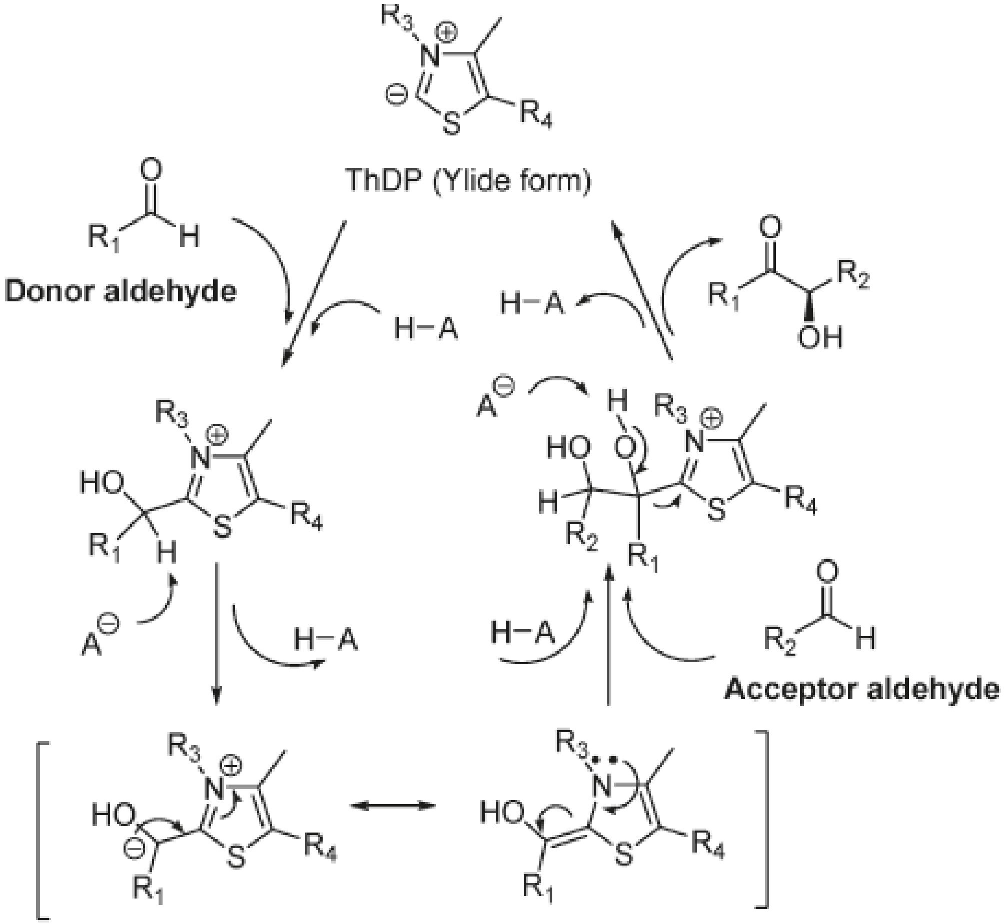


- Enzymatically controlled formation



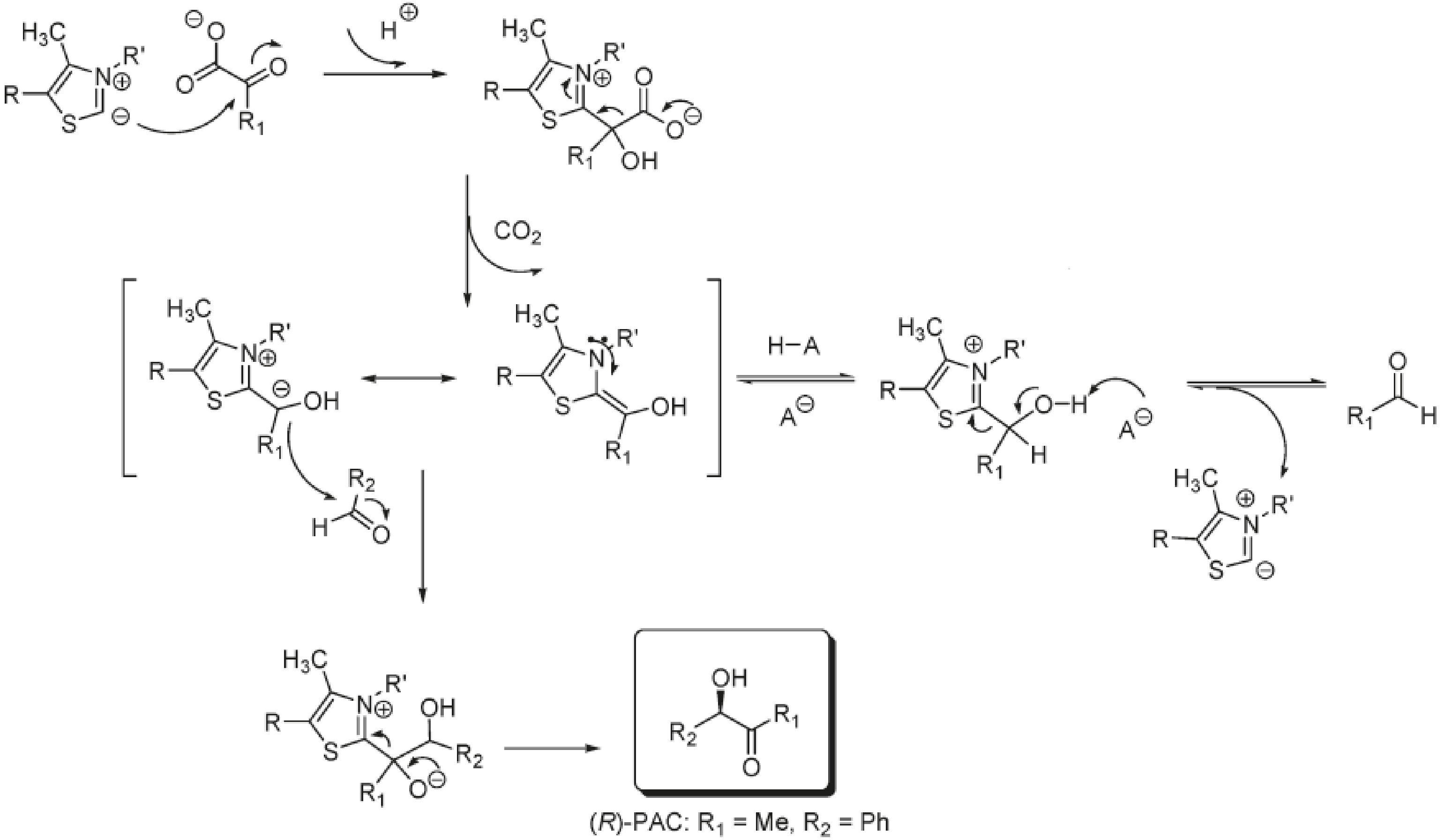
Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

ThDP-Dependent Lyase Mechanism



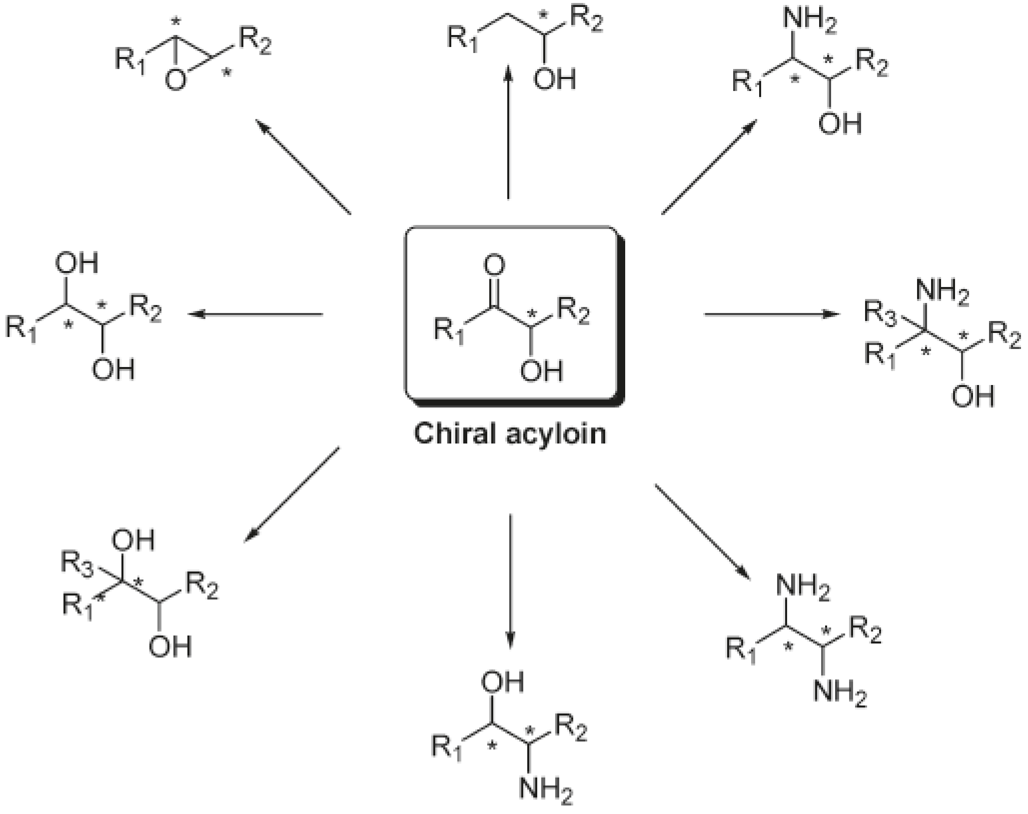
Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

PDC Catalysis Mechanism



Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

Chiral Acyloin Applications



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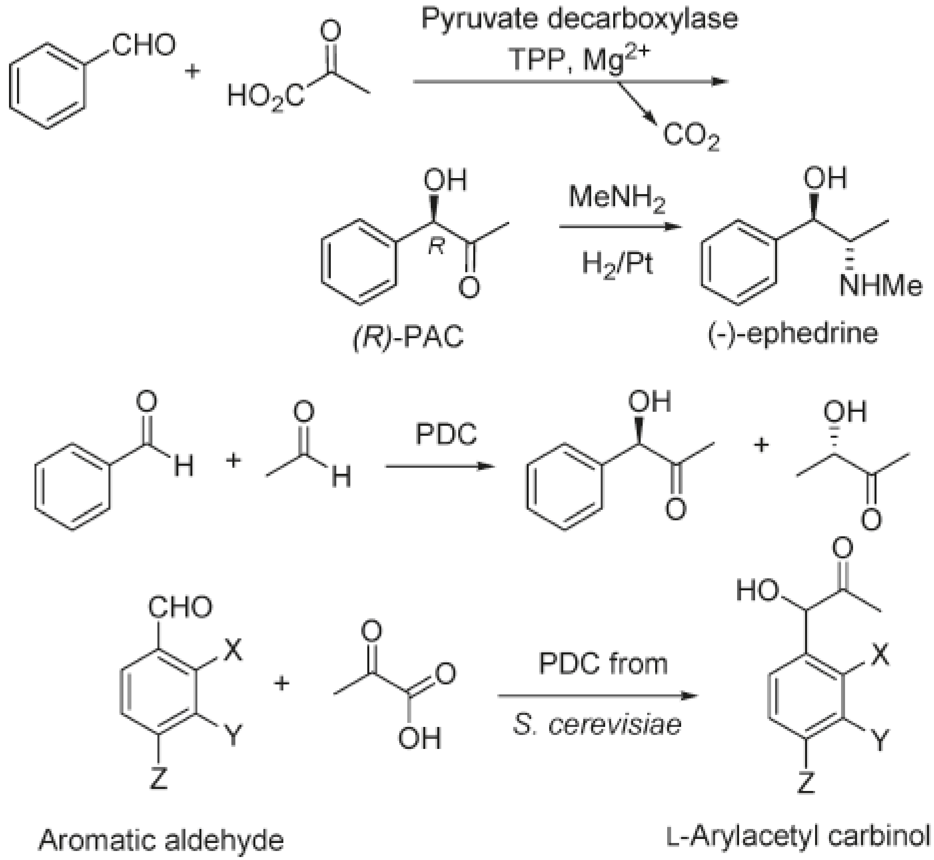
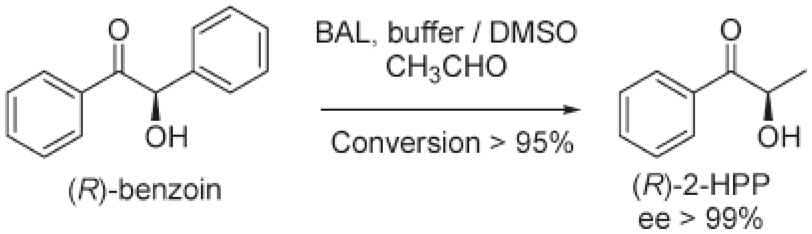
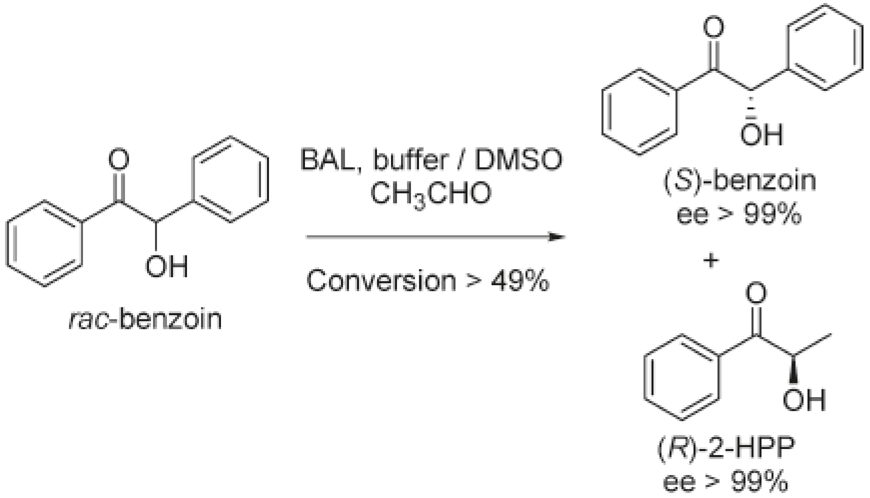
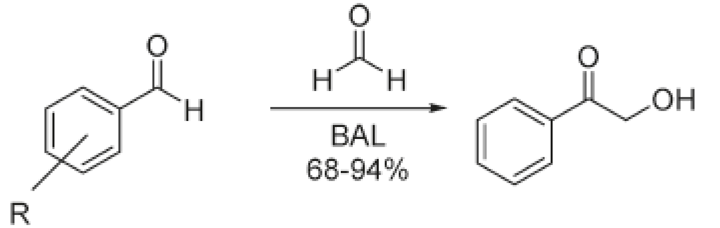
First performed in the 1920's!



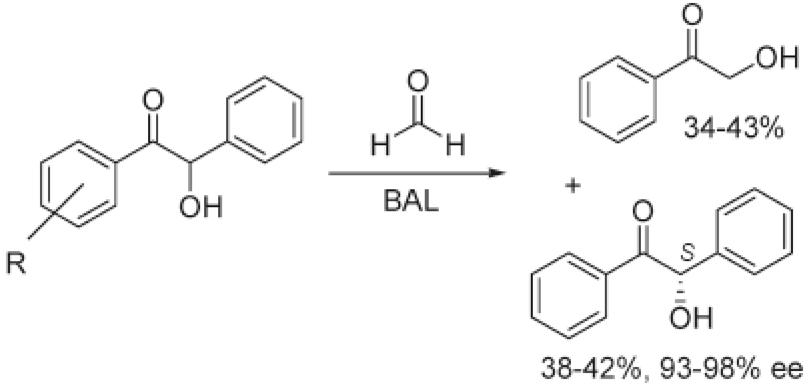
Kinetic Resolution with Benzaldehyde Lyase







R = H, *p*-Me, *o*, *m*, *p*-MeO, *m*-Br, *o*, *p*-Cl, *p*-OH, *o*-furanyl

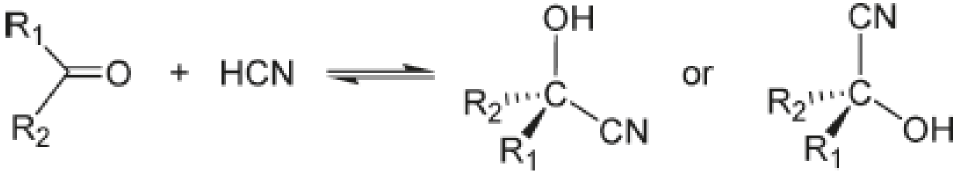


R = H, *p*-Cl, *o*, *p*-MeO, *p*-Me, *o*-furanyl

Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

Cyanohydrin Formation

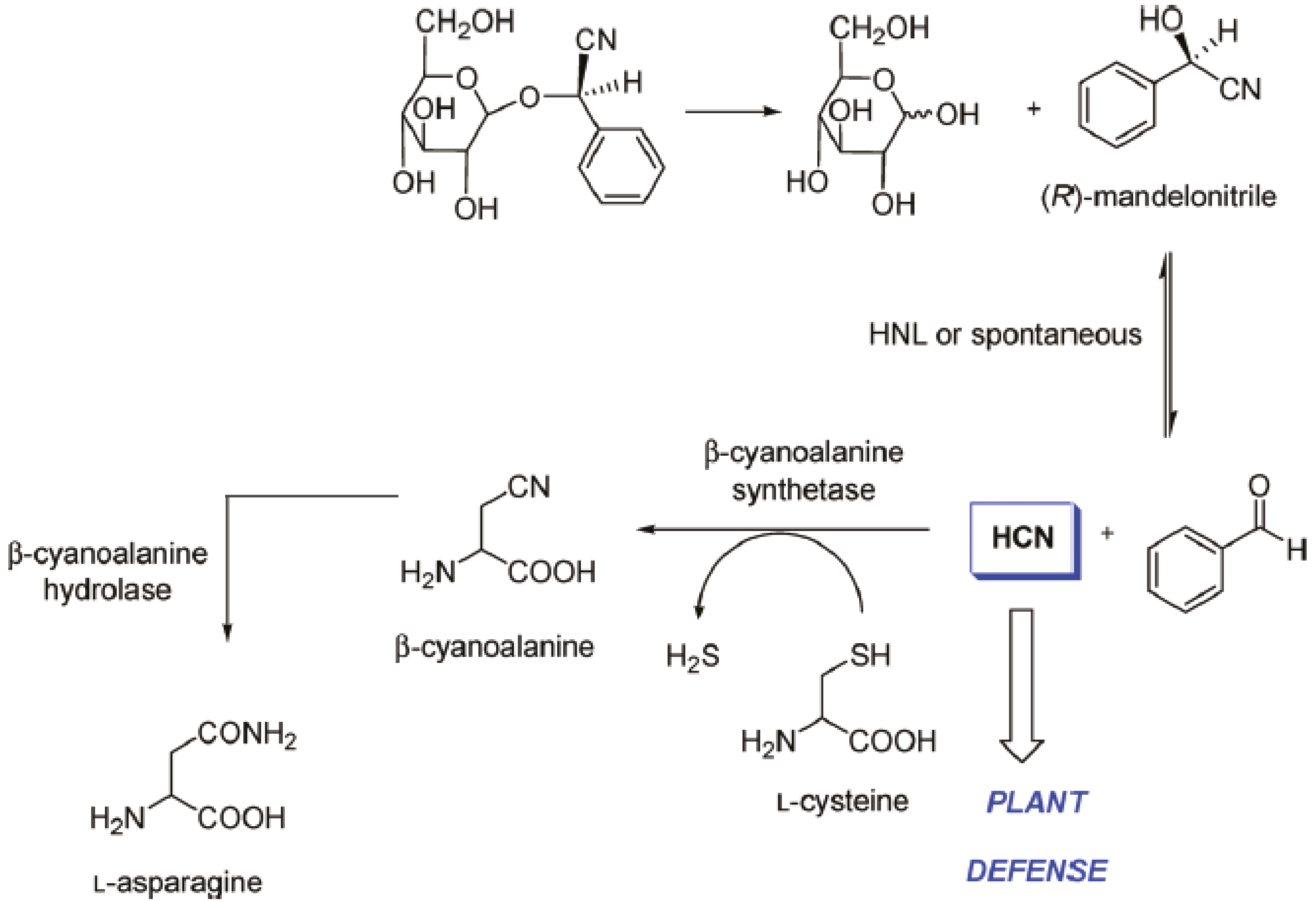
- Hydroxynitrile lyases (HNLs) cleave cyanohydrins *in vivo* but can be used in synthesis due to their reversible nature



- Reversibility allows for enantioselective condensation of HCN with aldehydes or ketones to give (*R)* or (*S*)-α-hydroxyl nitriles

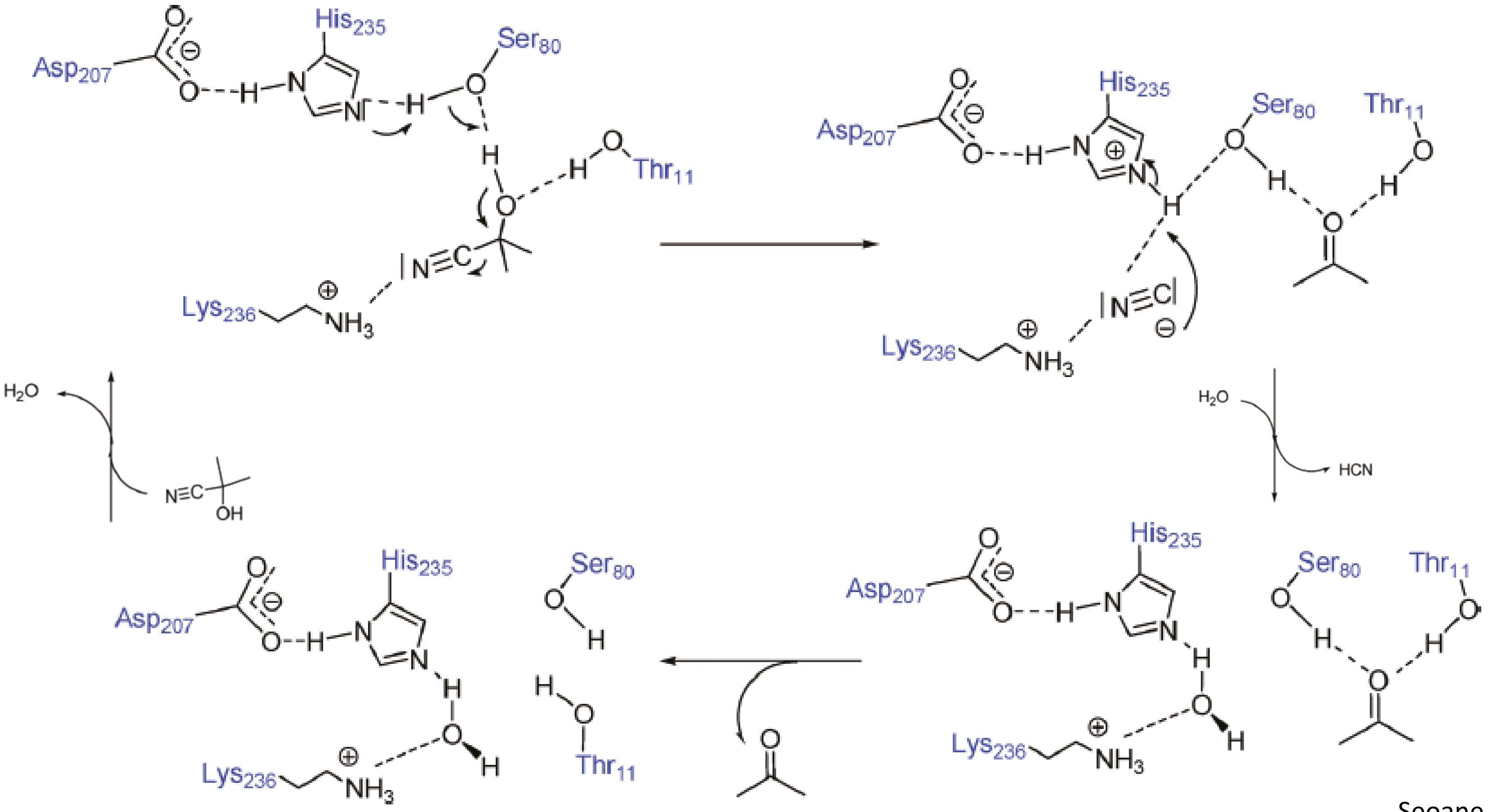
Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

Uses of Cyanohydrins in Nature



Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

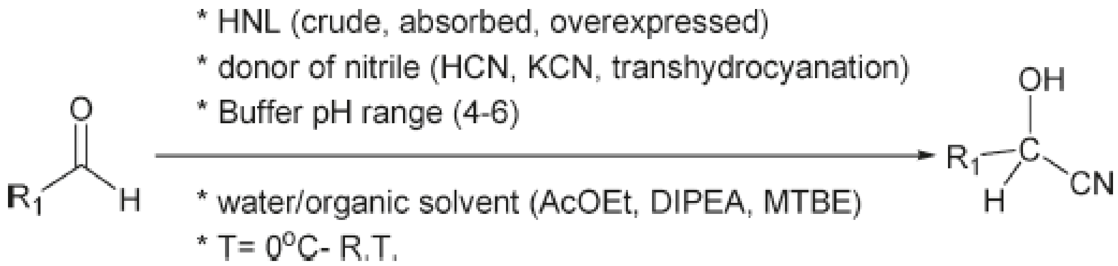
Hb-HNL Catalysis Directed for Cyanohydrin Cleavage



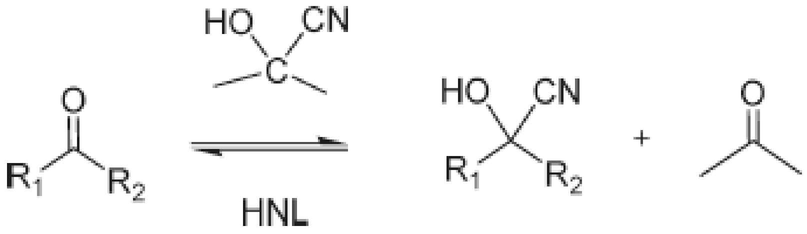
Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

Do We Need to Use HCN?

- Reaction parameters must be considered



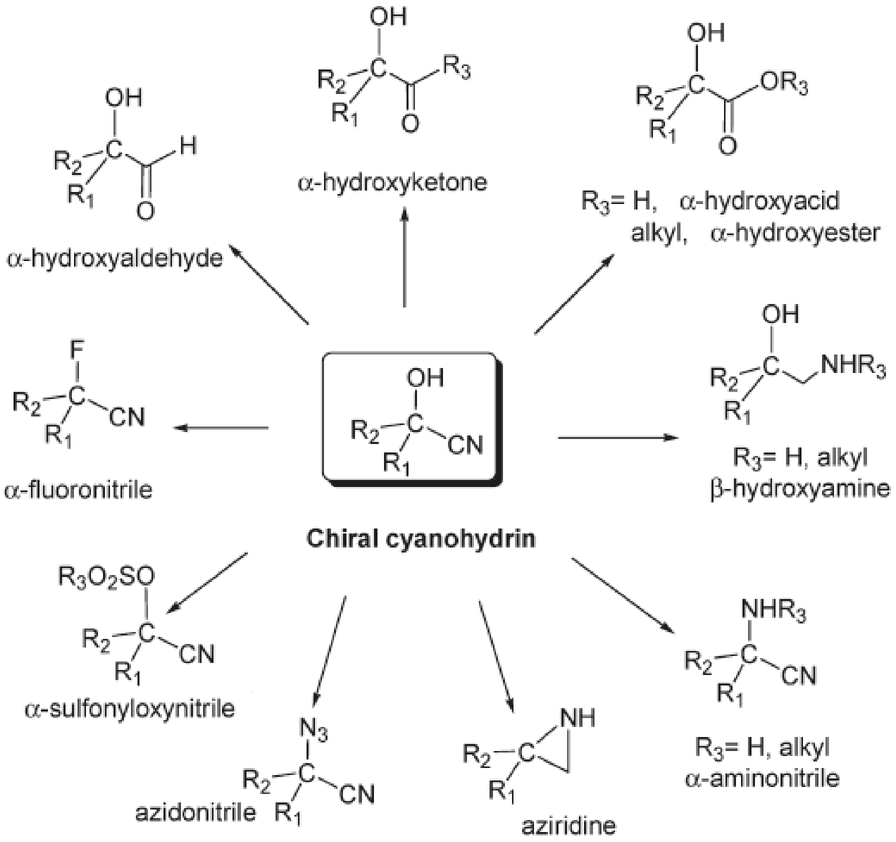
- Transhydrocyanation is possible



Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

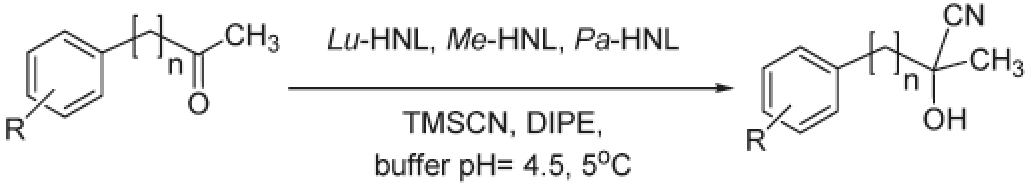
Synthetic Applications

- First activity detected in almonds by Friedrich Wohler in 1837



Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

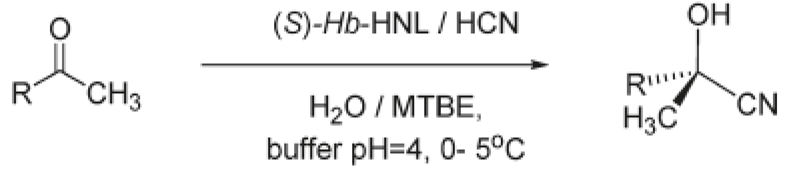
Synthetic Applications



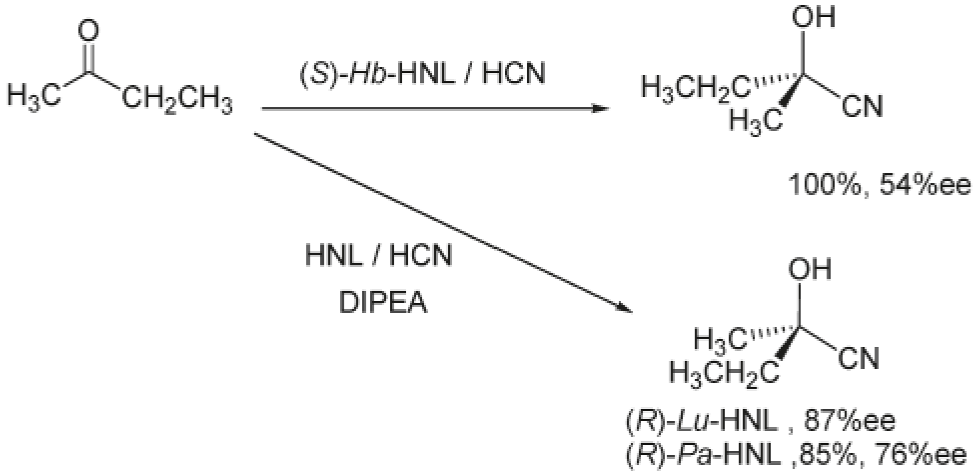
n=0, R= H

n=1, R= X (2,3,4-position); 3-CH_3 ;CH_3O (3,4-position)

n=2 R= H ; 4-OH ; 4-CH_3O

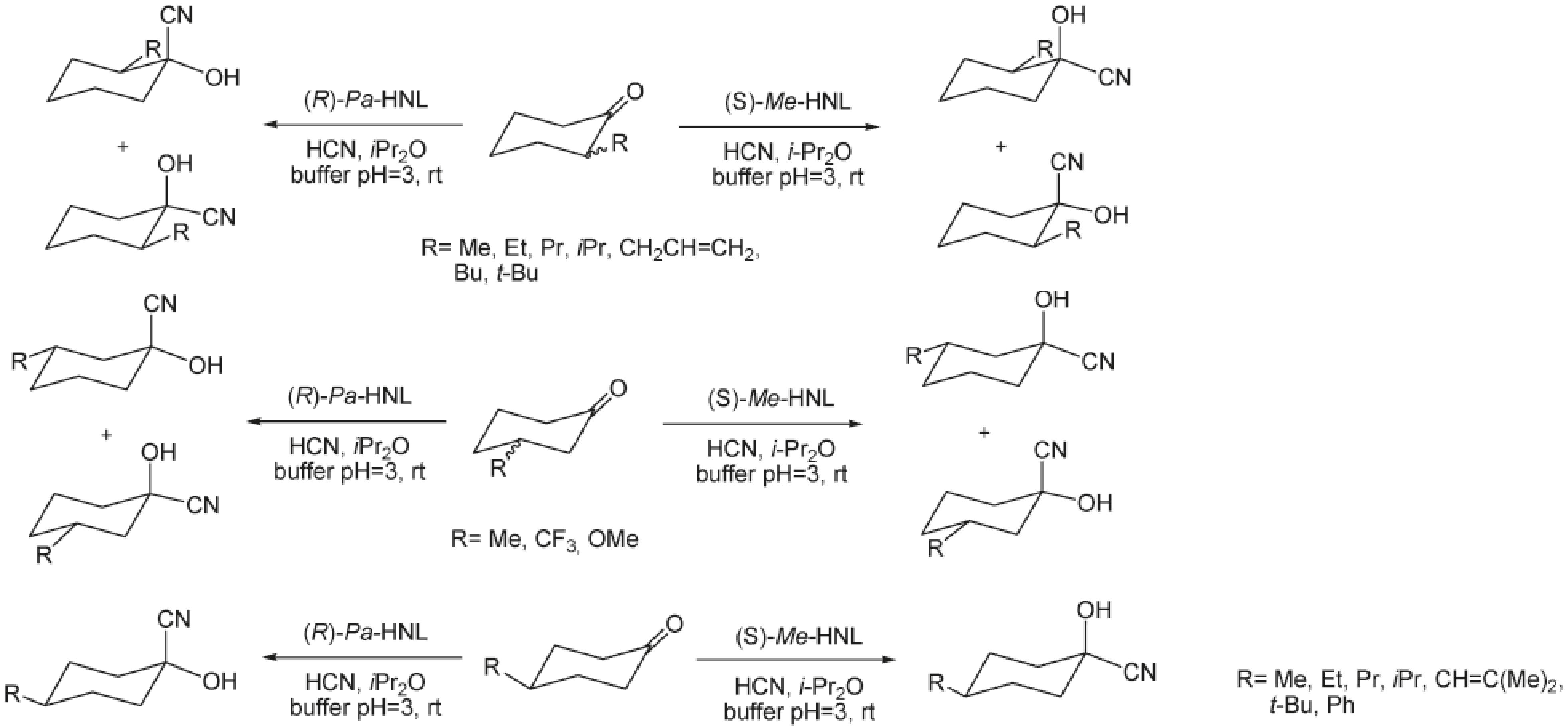


R= Pr (99%, 74%ee); *i*Pr (99%,98%ee); *i*Bu (86%, 99%ee),
Bu (59%, 99%ee); Ph (40%, 99%ee)



Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

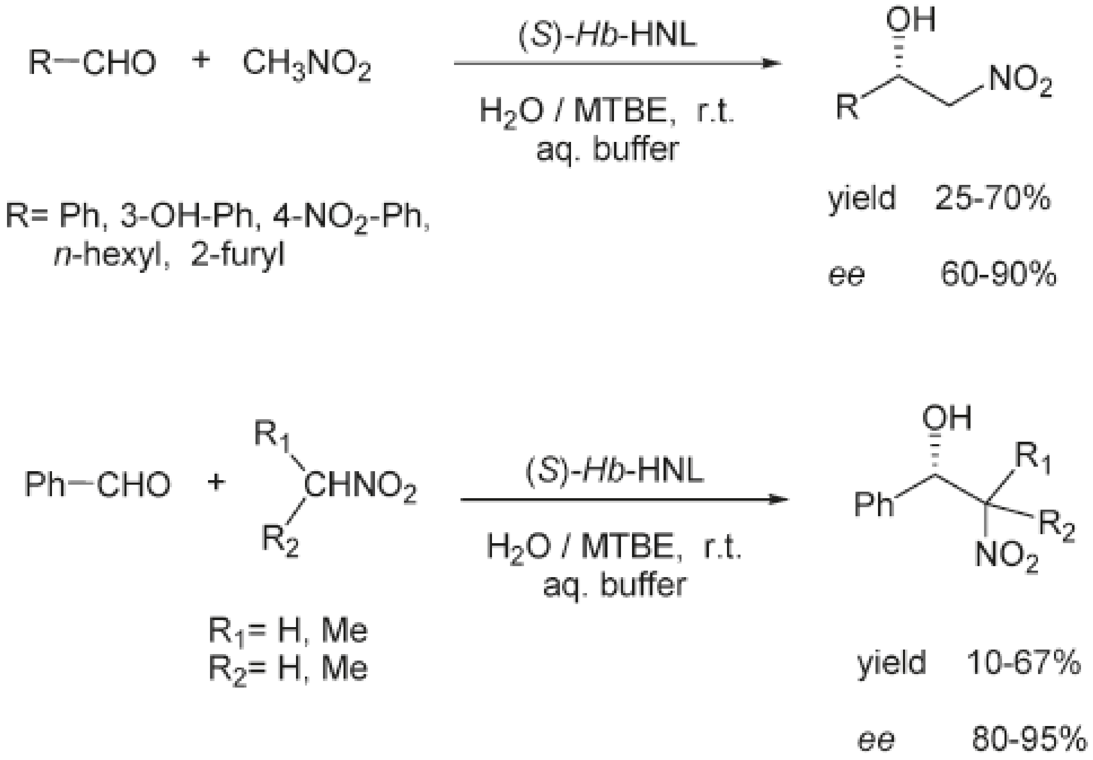
Synthetic Applications



Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

What About Other Nucleophiles?

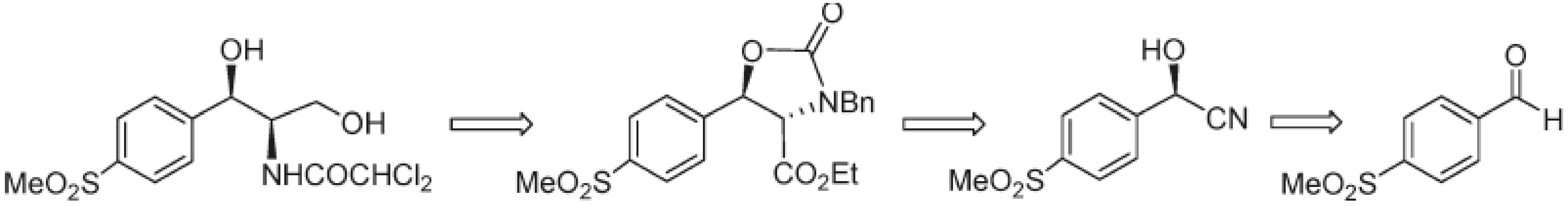
- Purkarthofer and coworkers discovered HNL’s can also catalyze the Henry reaction



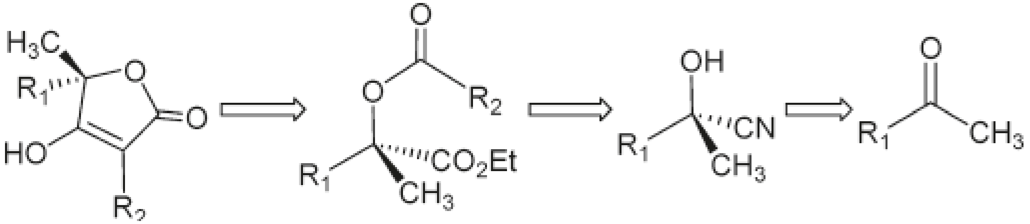
Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

Natural Product Synthesis

- Thiamphenicol synthesized with enantioselective hydrocyanation reaction catalyzed by (*R*)-HNL from *Badamu*



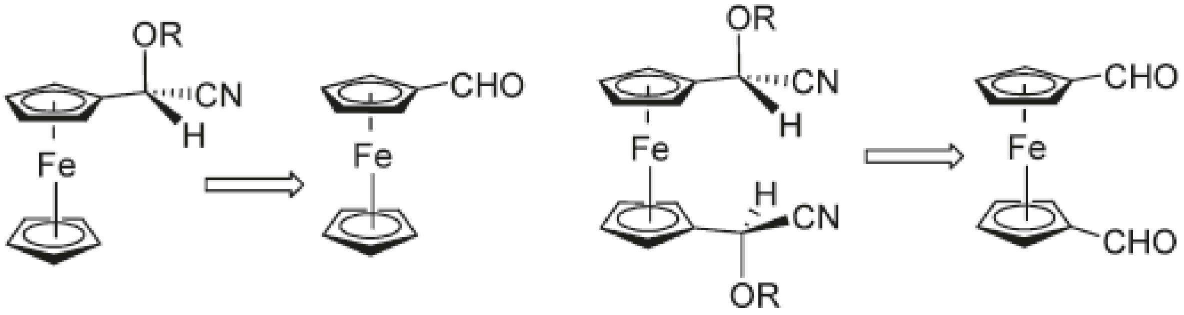
- Various tetronic acids synthesized enantioselectively through hydrocyanation via *Me*HNL



Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

One Last Example

- Interestingly, *Hb*HNL was found to catalyze hydrocyanation of ferrocenes

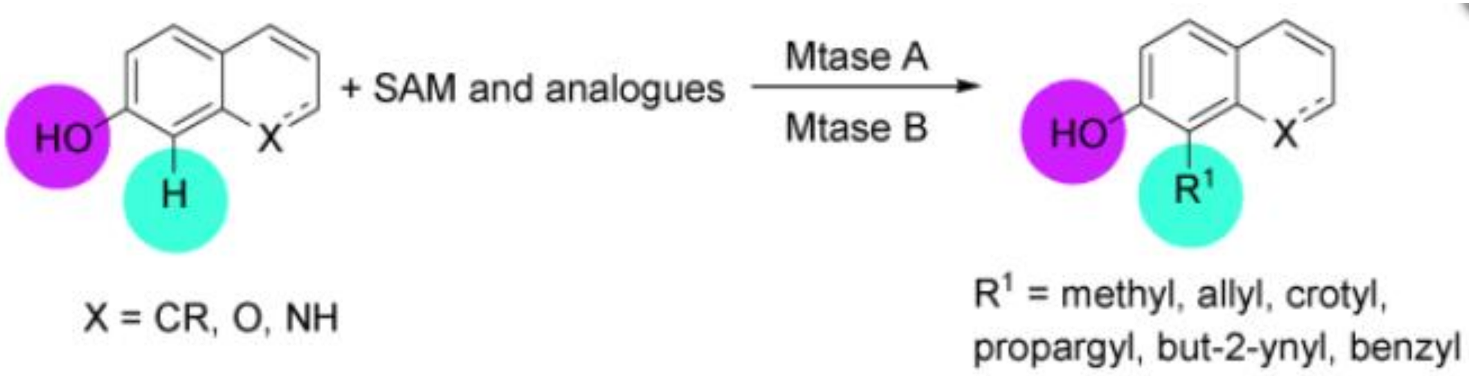


- Resulting ferrocenyl-oxazolidinones used chiral auxiliaries for asymmetric alkylations and aldol reactions

Seoane, G. A. *Chem. Rev.* **2011**, *111*, 4346-4403.

Biocatalytic Friedel-Craft’s

- Methylation of aromatic rings in nature can be performed by methyltransferases (Mtases), with the help of the cofactor *S*-adenosyl-L-methionine (SAM)



Gruber-Khadjawi, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 9456.

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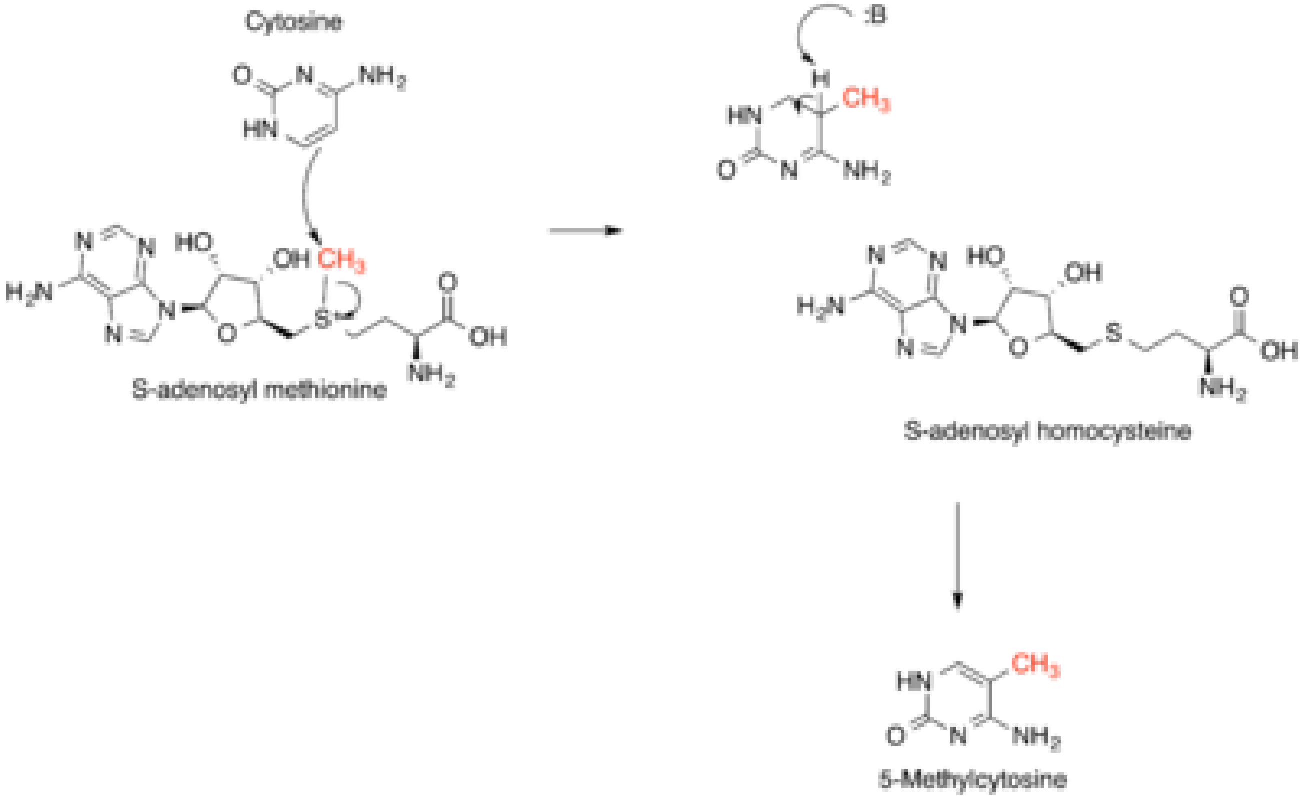
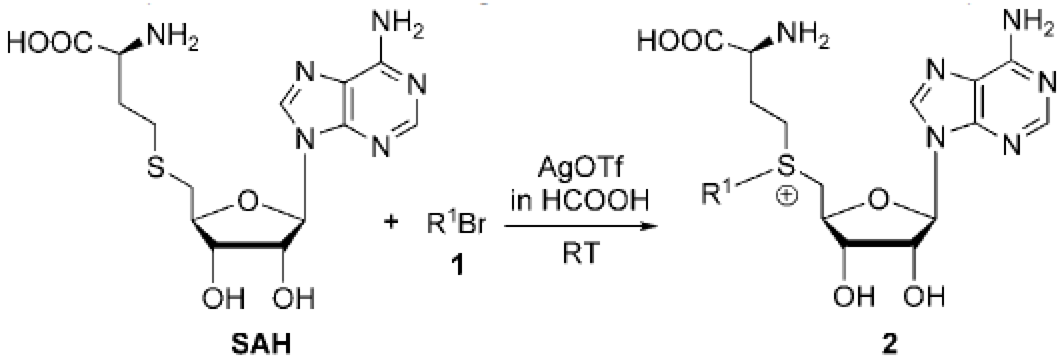
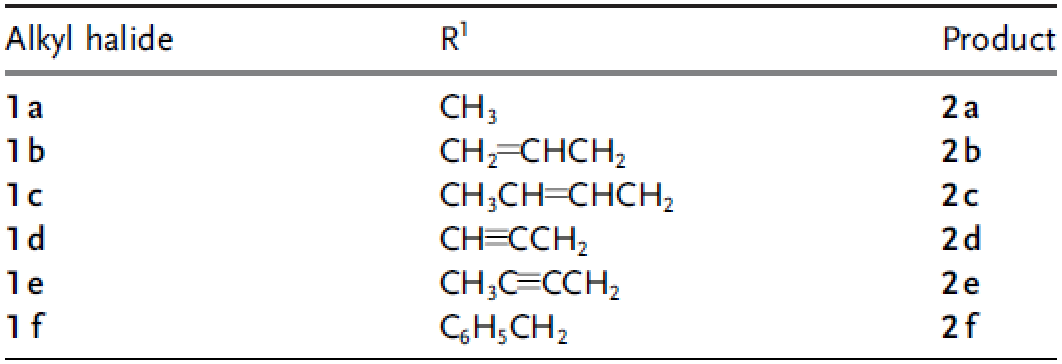


Gruber-Khadjawi, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 9456.

Unnatural Cofactors

- Switching from SAM to SAR's can allow for alkylation with other R groups



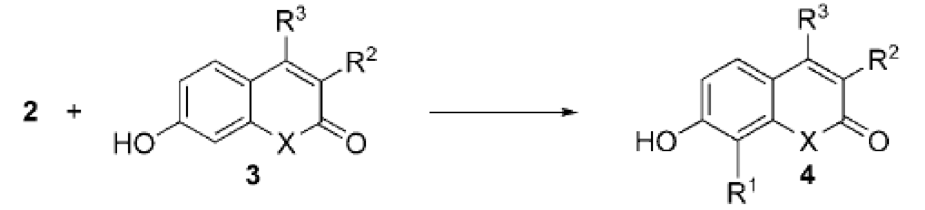


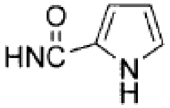
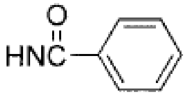
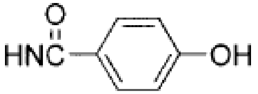
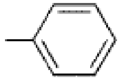
Alkyl halide	R^1	Product
1a	CH_3	**2a**
1b	$CH_2{=}CHCH_2$	**2b**
1c	$CH_3CH{=}CHCH_2$	**2c**
1d	$CH{\equiv}CCH_2$	**2d**
1e	$CH_3C{\equiv}CCH_2$	**2e**
1f	$C_6H_5CH_2$	**2f**

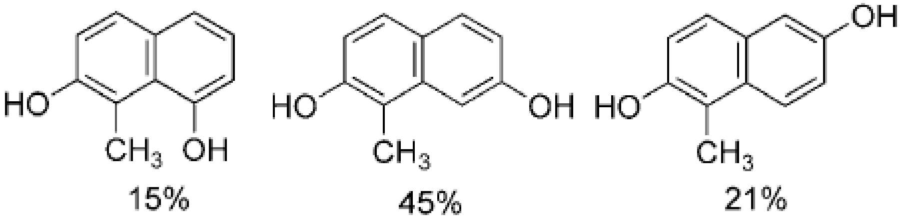
Gruber-Khadjawi, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 9456.

Unnatural Cofactors

- Switching from SAM to SAR's can allow for alkylation with other R groups



	Substrate			Product
	X	R^2	R^3	R^1=CH$_3$
3 a	O		OH	**4 a**
3 b	O		OH	**4 b**
3 c	O		OH	**4 c**
3 d	O		OH	**4 d**
3 e	O	CH_2COOH	CH_3	**4 e**
3 f	NH	H	CH_3	**4 f**

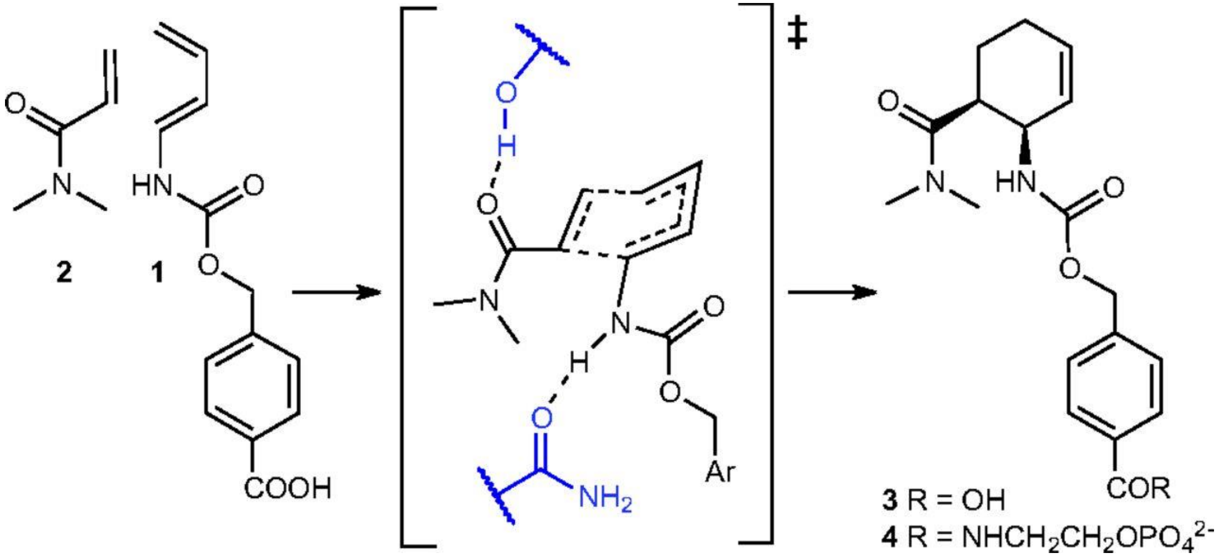


Other napthyl substrates could also be tolerated by Mtase and SAM

Gruber-Khadjawi, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 9456.

The Legendary “Diels-Alderase”

- Scientists have investigated whether an enzyme could catalyze a [4+2] cycloaddition for many years, due to cyclohexene ring existence in natural products
- How might this work?
  - 1) Not involved in actual catalysis-just a chiral template
  - 2) Enzyme lowers ΔG
    - Entropy
    - Enthalpy
    - Both



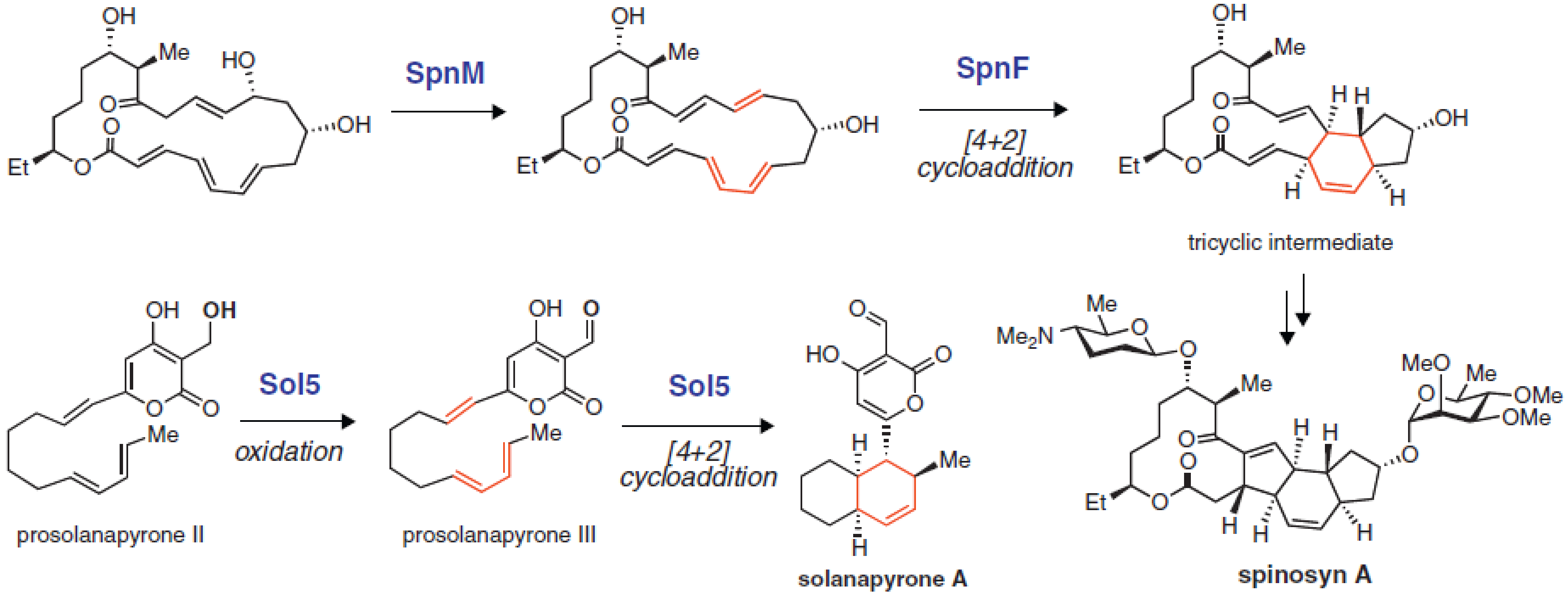
3 R = OH

4 R = $NHCH_2CH_2OPO_4^{2-}$

Liu, H-W. *Curr. Opinion Chem. Bio.* **2012**, *16*, 124.

The Legendary “Diels-Alderase”

- Though much research has been done, no natural examples have been proven yet



tricyclic intermediate

prosolanapyrone II

prosolanapyrone III

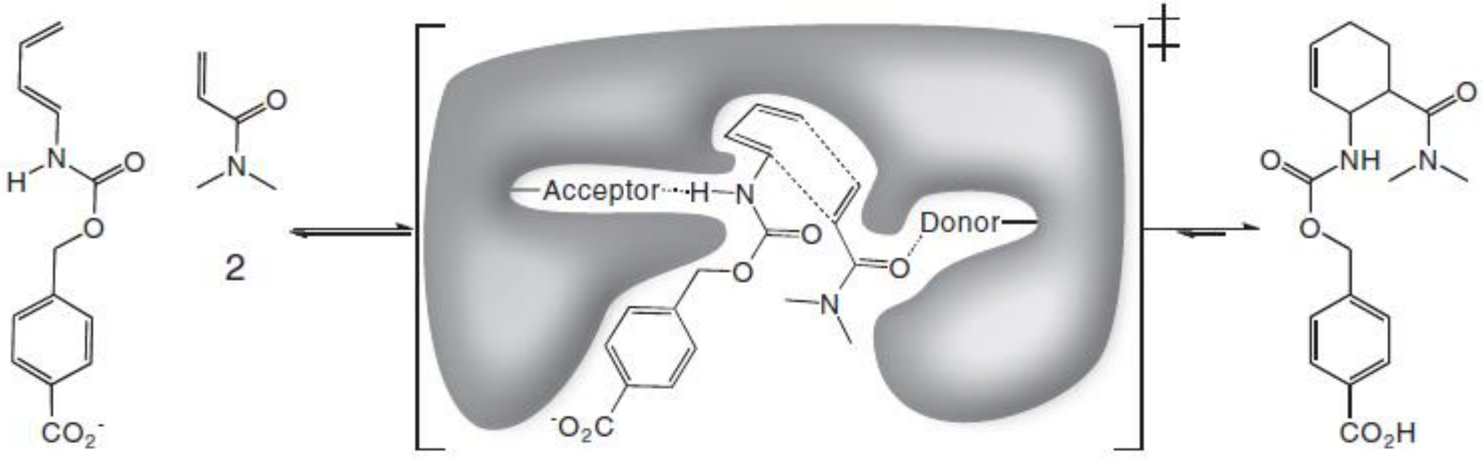
solanapyrone A

spinosyn A

Liu, H-W. *Curr. Opinion Chem. Bio.* **2012**, *16*, 124.

The Legendary “Diels-Alderase”

- A non-natural example, through computational analysis, has been created

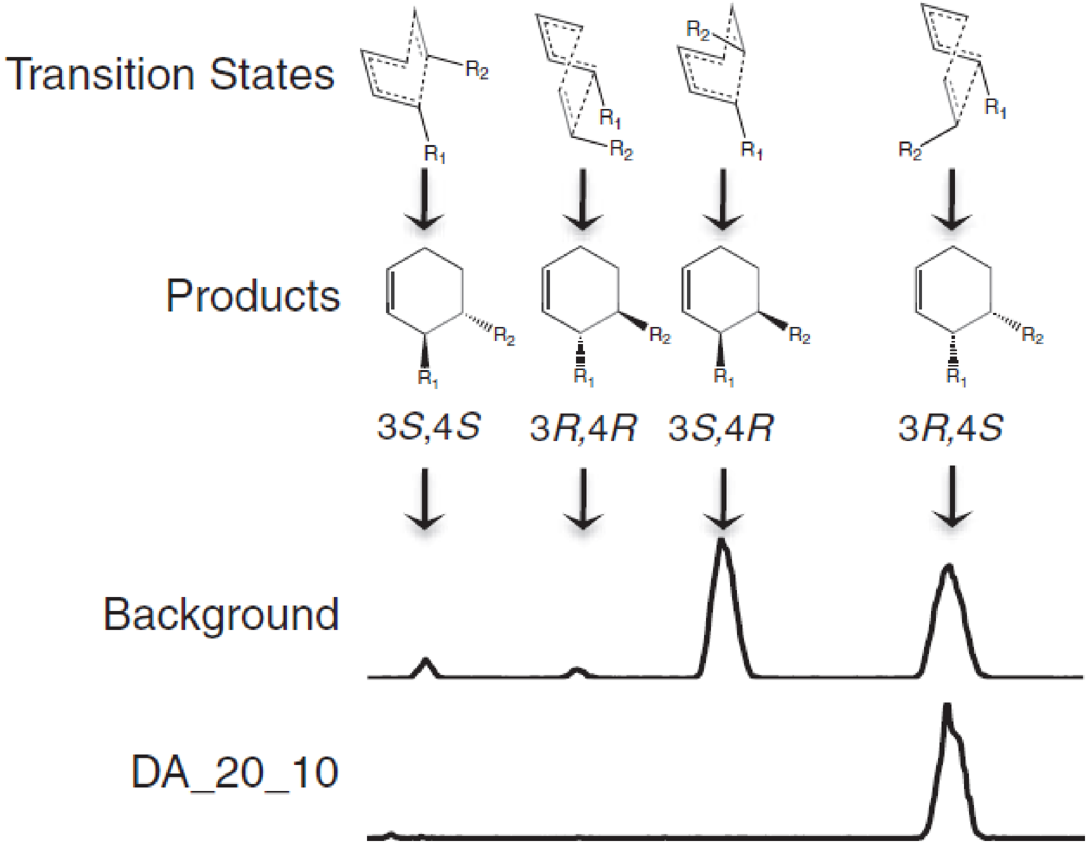


1 2 3 4

Stoddard, B.L., Houk, K.N., Michael, F.E., Baker, D. *Science* **2010**, *329*, 309.

The Legendary "Diels-Alderase"

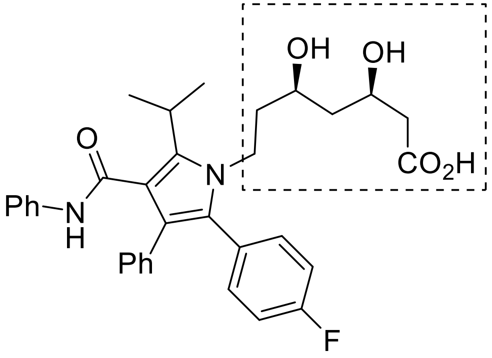
- A non-natural example, through computational analysis, has been created



Stoddard, B.L., Houk, K.N., Michael, F.E., Baker, D.
Science **2010**, *329*, 309.

Conclusions and Future Directions

- Enzymes are slowly growing in popularity as more is known about their mechanisms
- They are attractive to use due to ‘greenness’ of conditions, PG-free, and high degrees of stereoselectivity.
- In the future, if limitations can be overcome, enzymes will most likely be used more and more in organic synthesis and industry

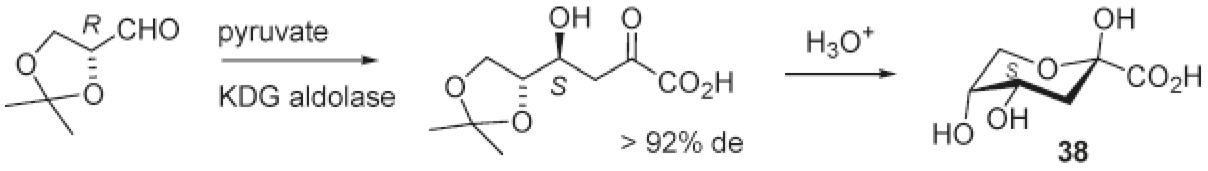


Atorvastatin (Lipitor)

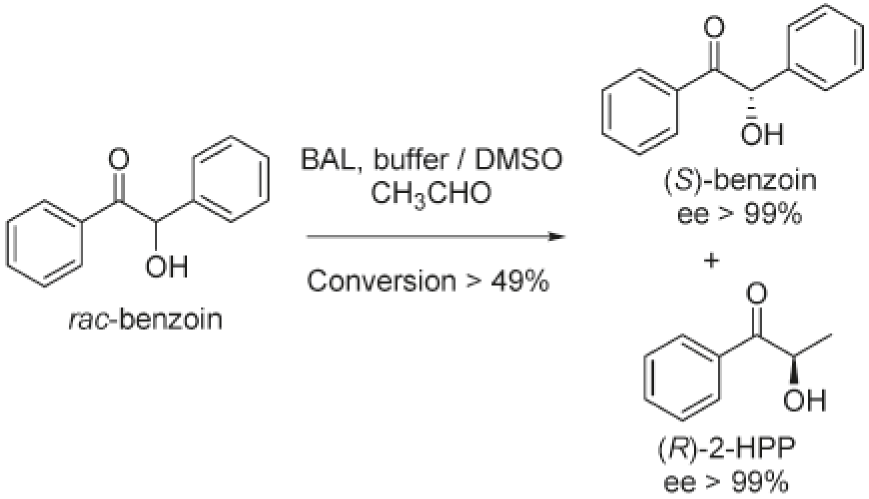
Thanks! Questions/Comments?

Question 1:

- Predict the products:
  - A)

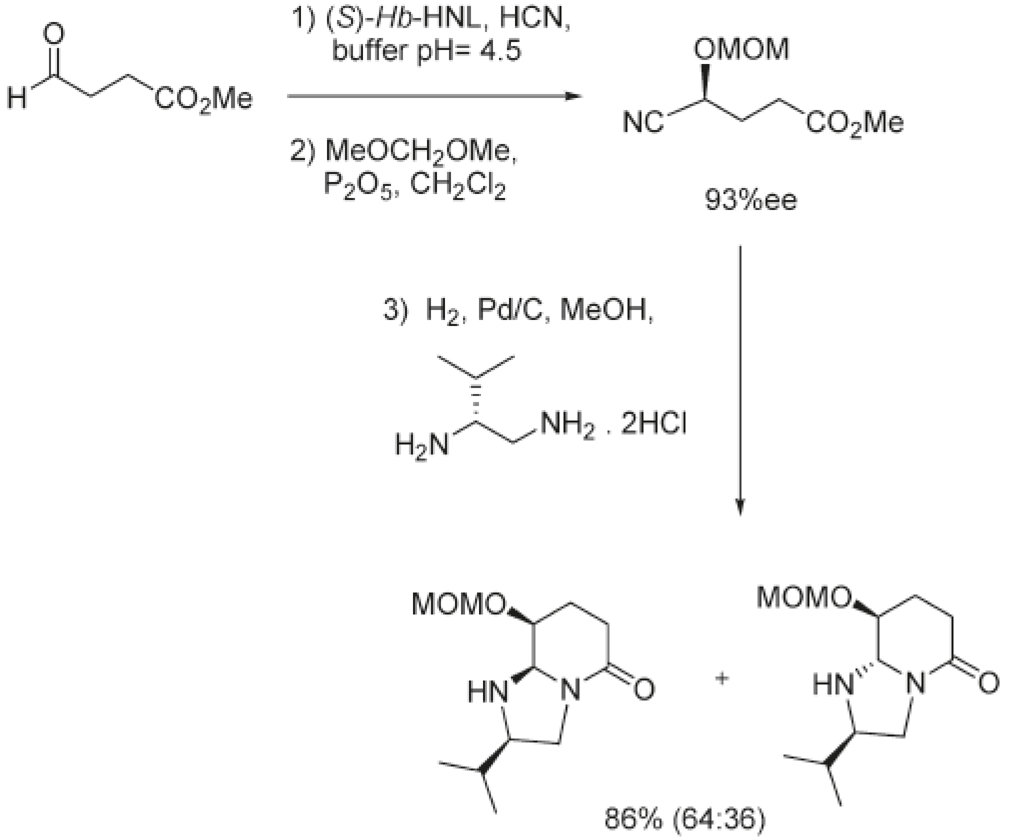


  - B)



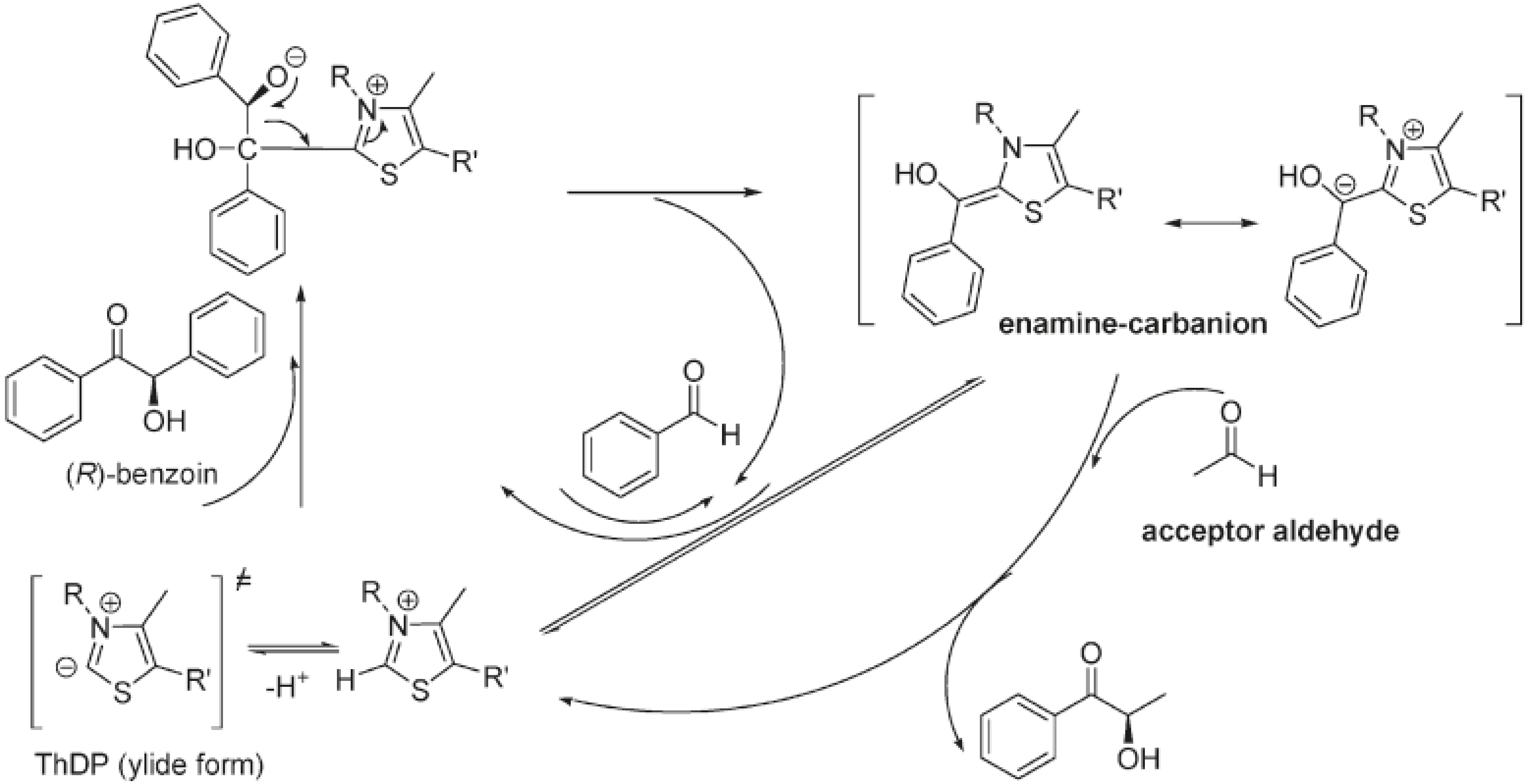
Question 1:

- Predict the products:
  - C)



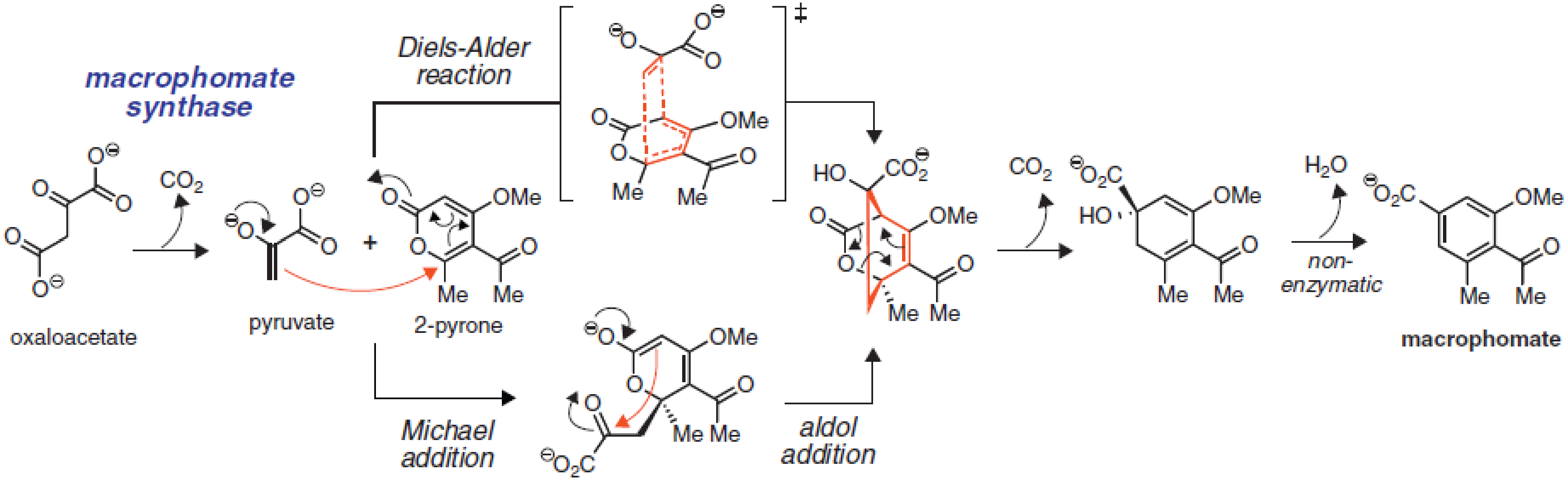
Question 2:

- Propose a mechanism for carboligation reaction



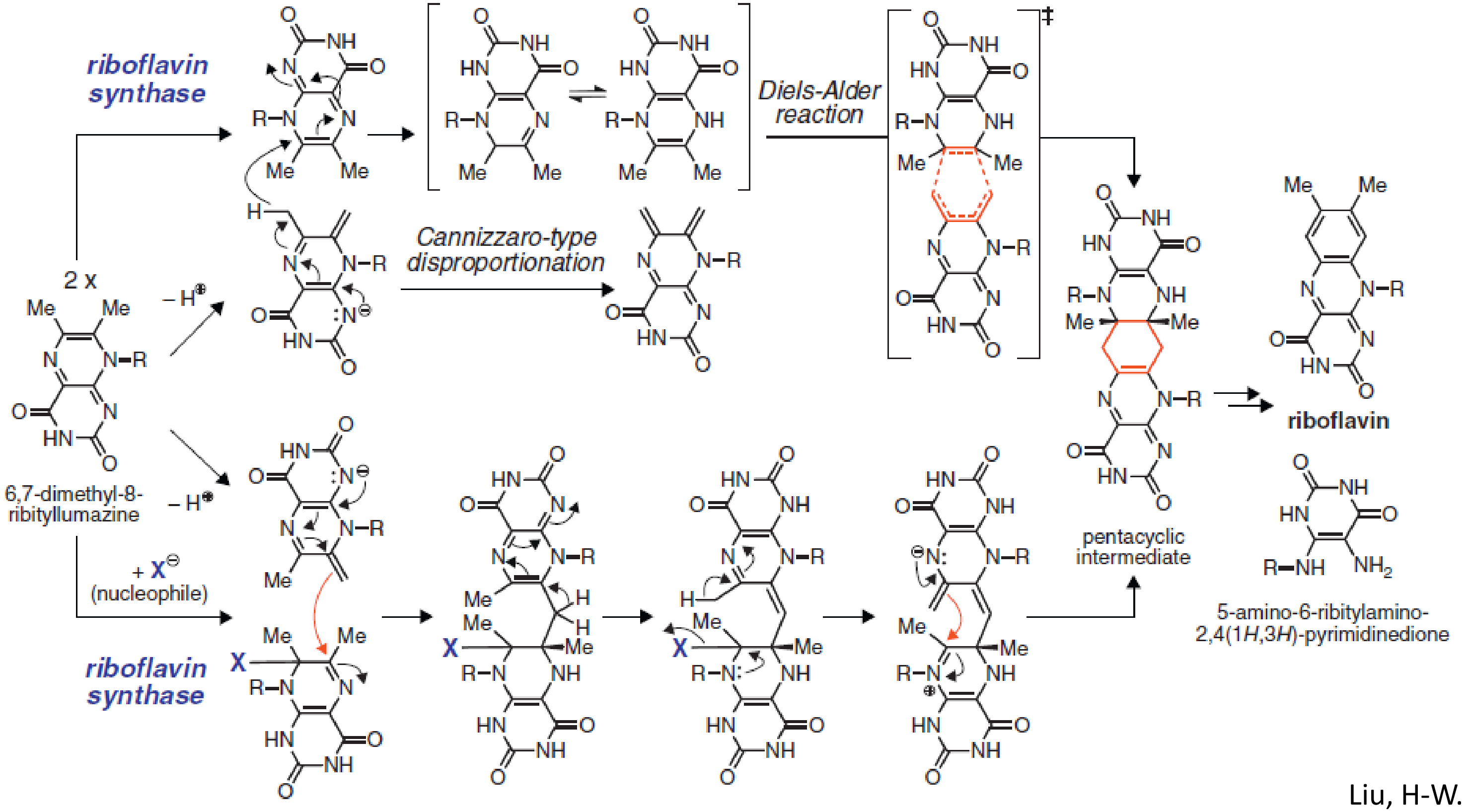
Question 3:

- Show non-Diels-Alder mechanism for formation of macrophomic acid from oxaloacetate and 2-pyrone



The Legendary “Diels-Alderase”

- Though much research has been done, no natural examples have been proven yet



Liu, H-W. *Curr. Opinion Chem. Bio.* **2012**, *16*, 124.