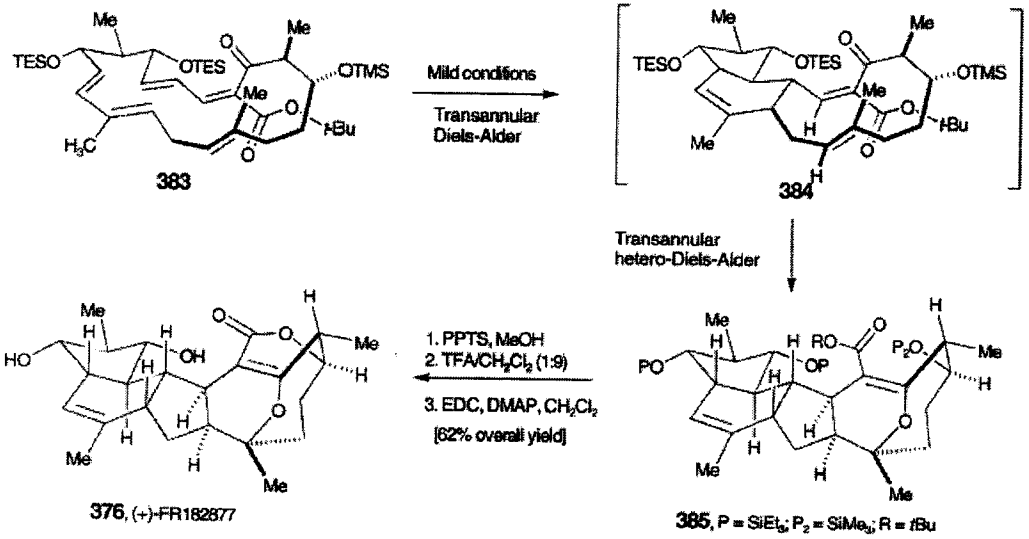
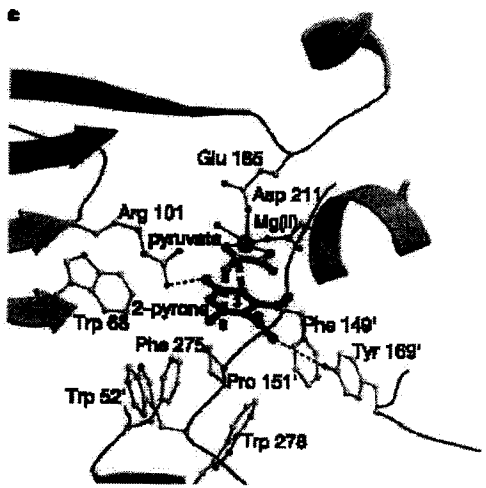
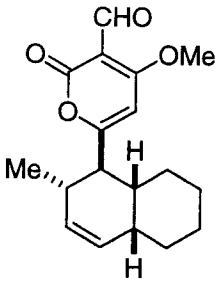


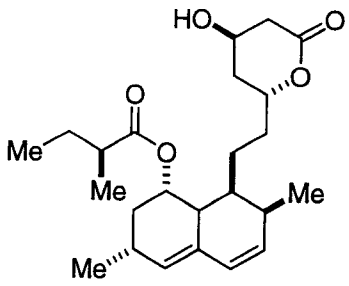


Biosynthetic Diels-Alder Reactions: Do Diels-Alderases Exist?

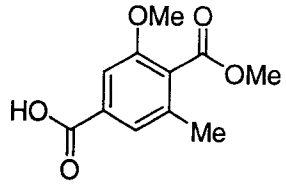
Steve Tymonko
9/30/03



Solanapyrone A



Lovastatin



Macrophomic Acid

Why Diels-Alder?

Several hundred biosyntheses have been proposed to include Diels-Alder reactions

Despite hundreds of known enzymatic reactions, no enzyme catalyzed cycloadditions are known

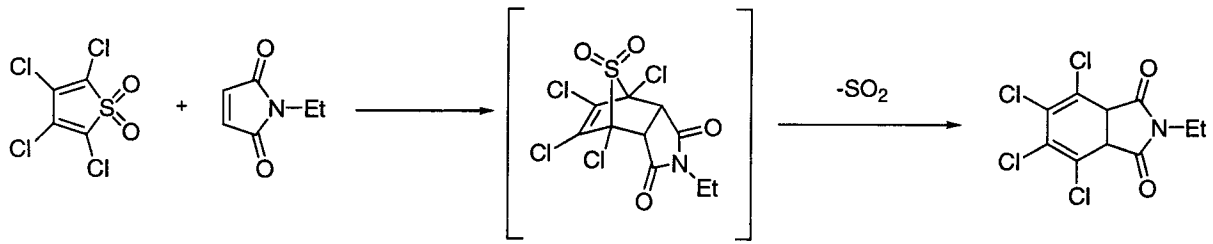
Enzymes catalysis usually due to stabilization of transition state
Diels-Alder products closely resemble their transition states
-Product inhibition of the enzyme is expected to dominate

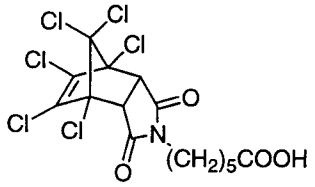
Challenges for Diels-Alderase identification
- Product inhibition must be minimal
- Identification of enzyme-catalysis must be distinguished from thermal cyclization
  - Unexpected stereochemistry of cyclized products
  - Kinetic data

Catalytic Antibodies

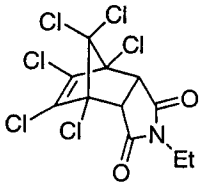
Basics for generating catalytic antibodies-

1) Hapten covalently bound to protein known to give immune response
2) Immune response intiated, spleen cells fused with myeloma cells
3) cells incubated and screened for activity





1

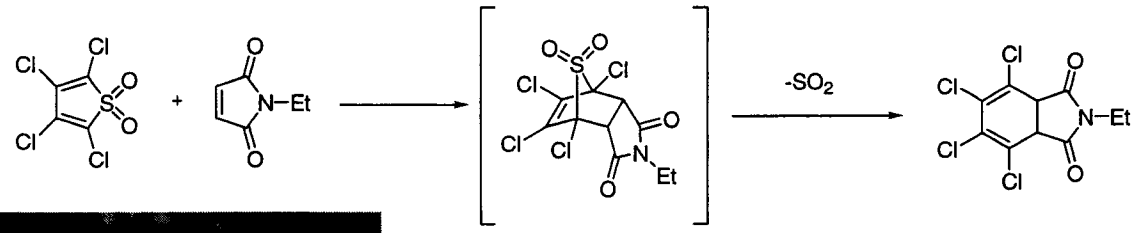


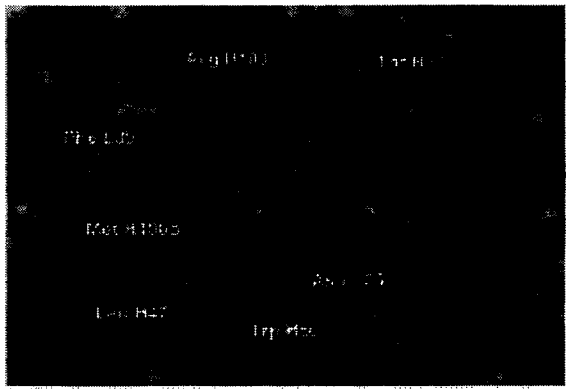
2

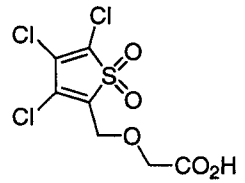
Generated antibodies to hapten **1**
SO_2 extrusion prevents product inhibition
Compound **2** completely inhibits reaction
even with 10^3 excess maleimide

Hilvert, D. et. Al. *J. Am. Chem. Soc.* **1989**, *111*, 9261

Catalytic Antibodies







3

Crystal structure shows hapten bound in tight hydrophobic pocket

H-bond from Asn H35 to maleimide carbonyl stabilizes TS

Kinetics studies conducted with **3**

k_{cat} = 13.0 min^{-1}
k_{uncat} = 0.013 M^{-1} min^{-1}
k_{cat}/k_{uncat} = 1000 M effective molarity

Catalyzed	Uncatalyzed
$\Delta H^{\ddagger}$ = 11.3 kcal/mol	$\Delta H^{\ddagger}$ = 15.5 kcal/mol
$\Delta S^{\ddagger}$ = -22.1 cal K^{-1} mol^{-1}	$\Delta S^{\ddagger}$ = -21.5 cal K^{-1} mol^{-1}

Antibody acts by lowering enthalpy of activation

Hilvert, D. et. Al. *Science.* **1999**, *268*, 2345

Catalytic Antibodies

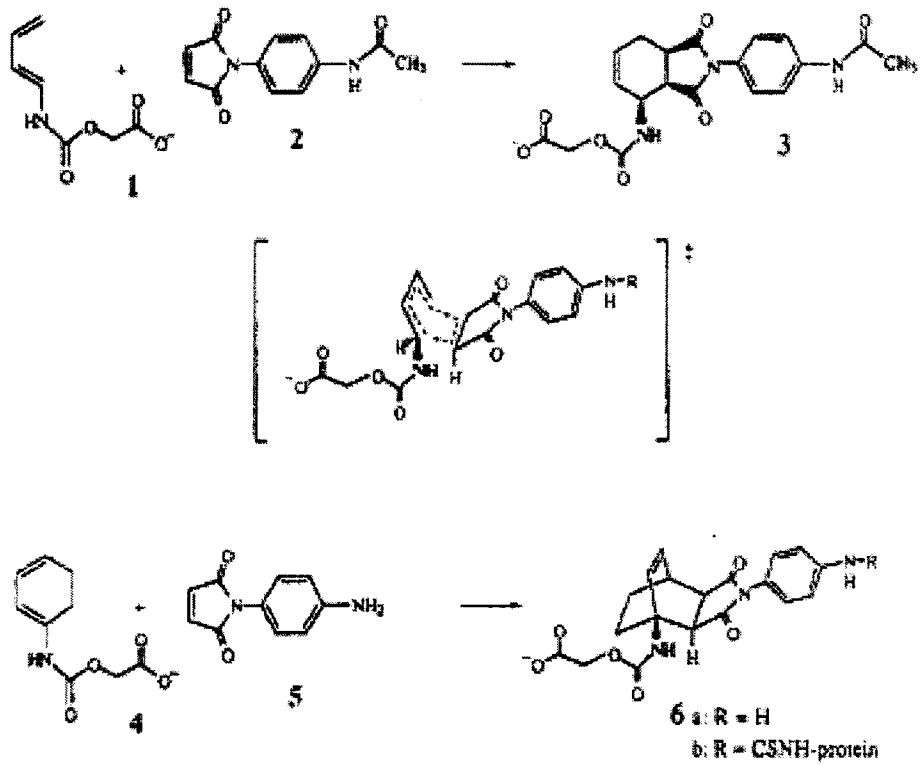
Proposed ~~chair~~ boat-like TS would prevent product inhibition

Used hapten **6** to generate antibodies

Antibody A11 was found to catalyze the reaction

Kinetics- k_{cat}= 0.67 s^{-1}
k_{cat}/K_m= 900 $M^{-1}s^{-1}$
k_{uncat}= 1.9 $M^{-1}s^{-1}$
k_{cat}/k_{uncat} = 2.83 M

Much less efficient catalyst



Schultz, P.; Braisted, A. *J. Am. Chem. Soc.* **1990**, *112*, 7430

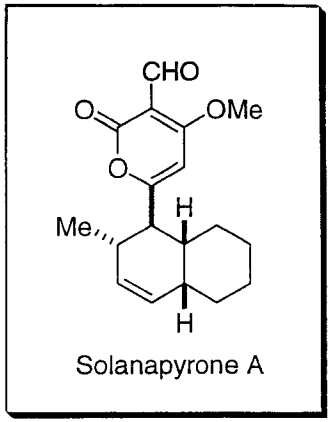
Catalytic Antibodies

Crystal structure displays 2 key H-bonds
Carbamate carbonyl to Trp^{H50}
Succinimide to Asn^{H35}
π–stacking from maleimide to Trp^{H50}

Propose that H-bonding makes dienophile more reactive and tight binding serves as entropy trap

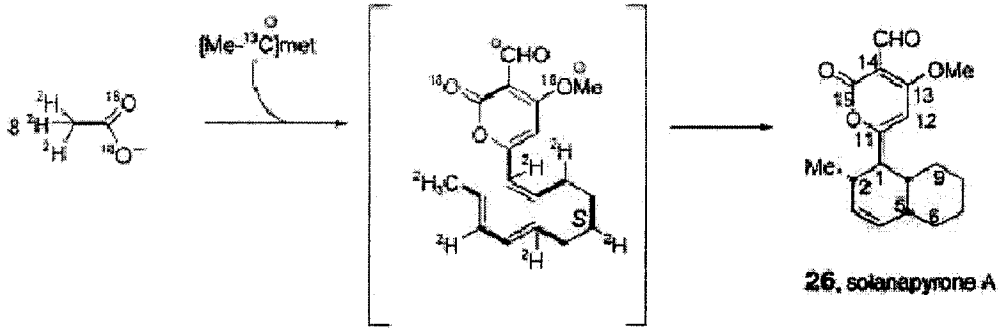
Schultz, P. et. Al. *Science.* **1998**, *279*, 1929

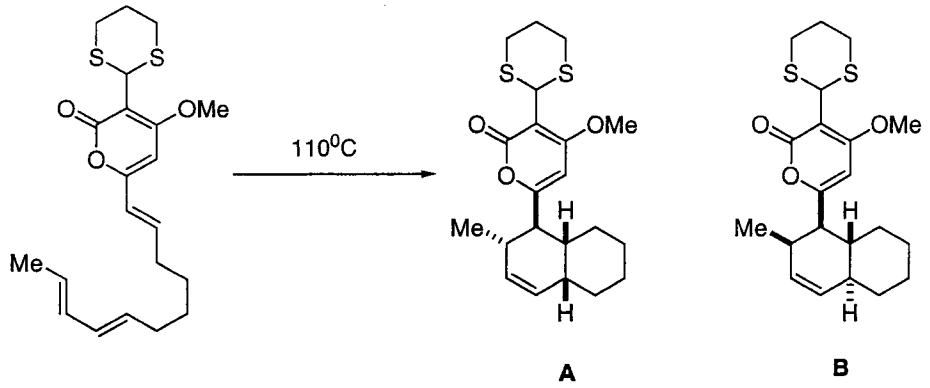
Solanapyrone: Initial Evidence



Solanapyrone A

- Solanapyrone A isolated from *Alternaria solani*
- Principle toxin of potato blight

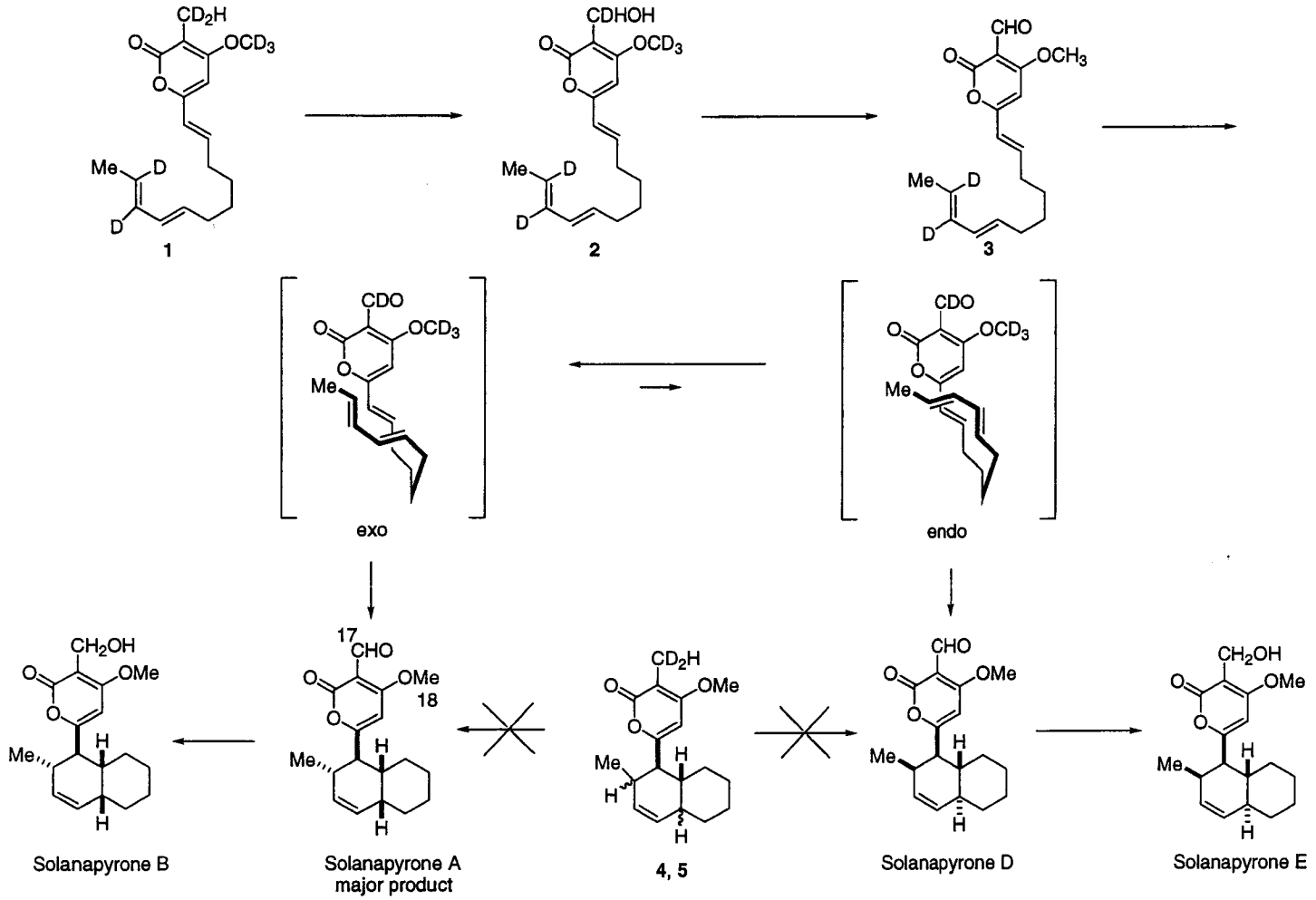




Solvent	A: B
$CHCl_3$	1: 2.5
C_6H_6	1: 2.0
H_2O	1: 7.0

Oikawa, H. et. Al. *J. Chem. Soc. Chem. Commun.* **1989**, 1282
Ichihara, A. et. Al. *Tetrahedron Lett.* **1987**, 1175

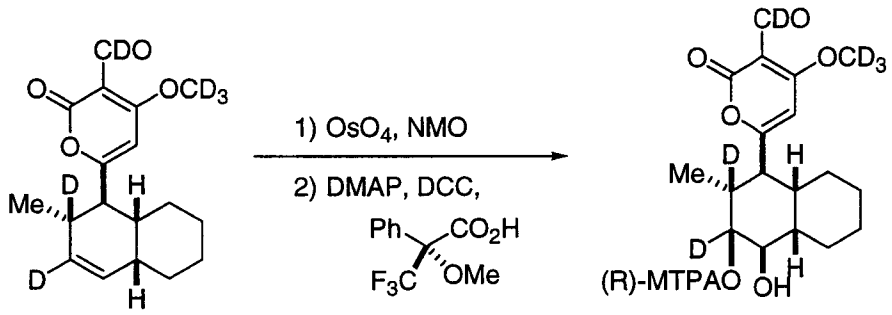
Solanapyrone: Labeling Studies



Feeding of **1**, **2**, and **3** resulted in labeled Solanapyrone A, no label was detected from **4** or **5**
^{2}H NMR of major product from **1** showed C^{17}:C^{18} deuterium ratio of 1:4.3
5:1 mixture of Solanapyrone B and E showed 1:1 ratio in ^{2}H NMR

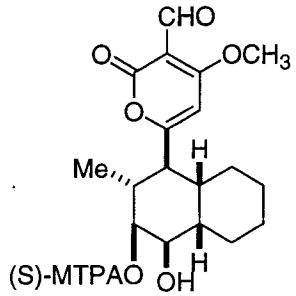
Oikawa, H. et. Al. *JACS*, **1994**, 3605
Oikawa, H. et. Al. *J. Chem. Soc. Perkin Trans I*, **1999**, 1225

Labeling Studies



6

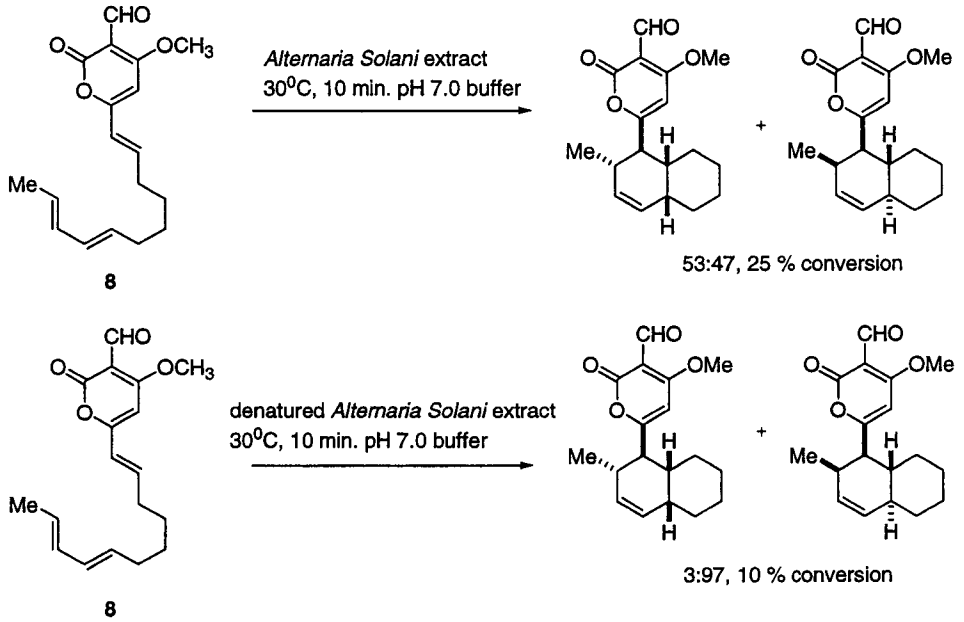
- Combined derivative **6** with diastereomer **7**, separated and took ^{2}H NMR
- Deuterium observed in **6** but not in **7**
- Concluded reaction must be enzymatic to give enantiopure Solanapyrone



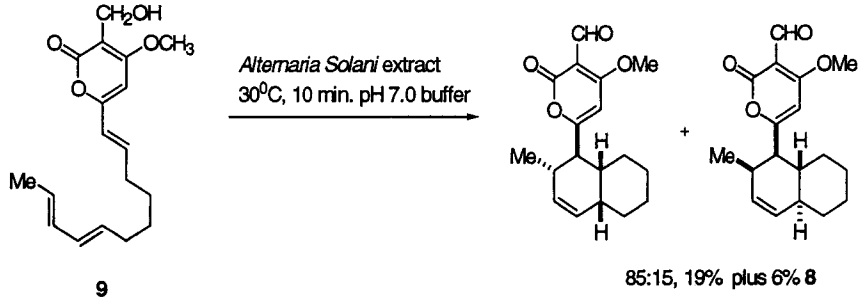
7

Oikawa, H. et. Al. *J. Chem. Soc. Perkin Trans I*, **1999**, 1225

Solanapyrone: Final Evidence



Calculate 87:13 selectivity of crude enzyme based on background reaction

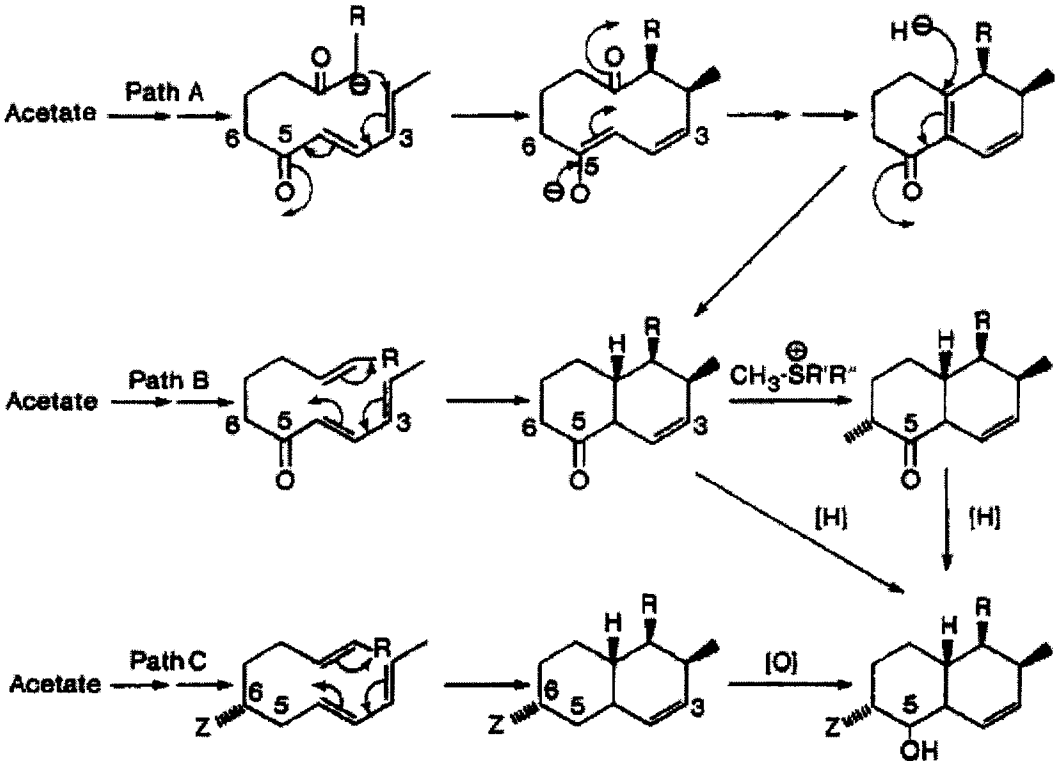


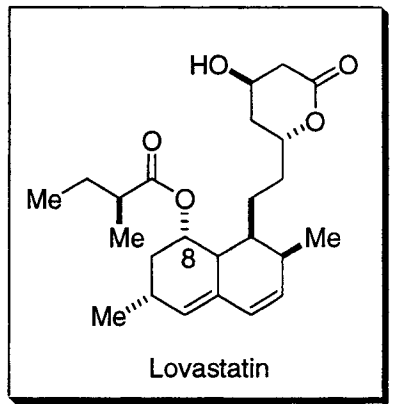
No Solanapyrone B was formed from **9** under Ar atmosphere.

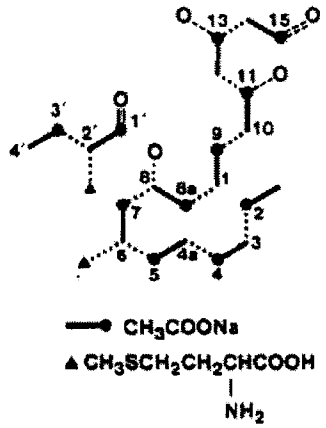
Oikawa, H. et. Al. *J. Chem. Soc. Chem. Commun*, **1995**, 1321

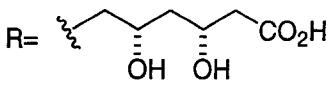
Lovastatin

- Also known as Mevinolin, marketed as Mevacor®
- Lowers cholesterol by blocking mevalonate synthesis
- Feeding studies demonstrate polyketide connectivity
  - Oxygen at C8 from O_2, all others from acetate
- Initial Proposed Biosynthesis





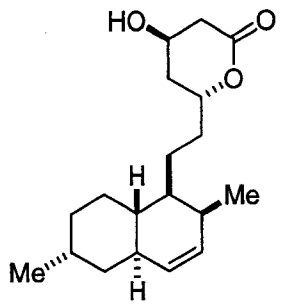




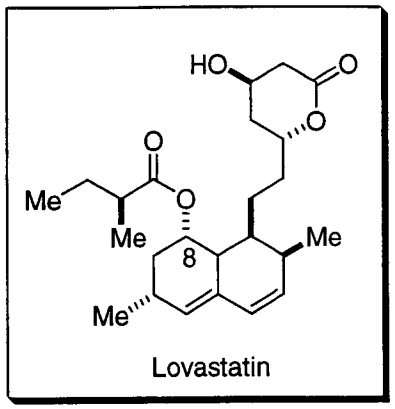
Vederas, J. et. Al. *JACS*, **1983**, 3334
Vederas, J. et. Al. *JACS*, **1985**, 3694

Lovastatin: Revised Proposal

- **10** was isolated and demonstrated to be an intermediate in Lovastatin synthesis



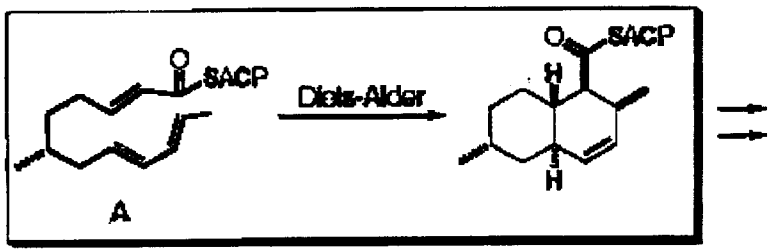
10

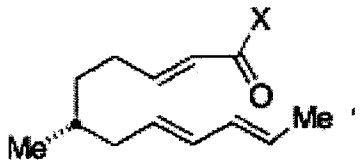


Lovastatin

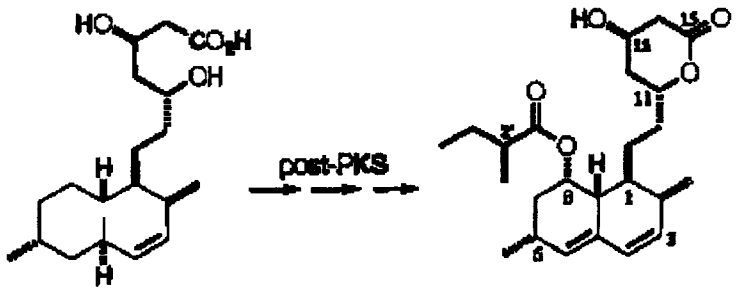
- Proposed enzymatic Diels-Alder during polyketide synthesis

- Conducted model study for cyclization of **A**



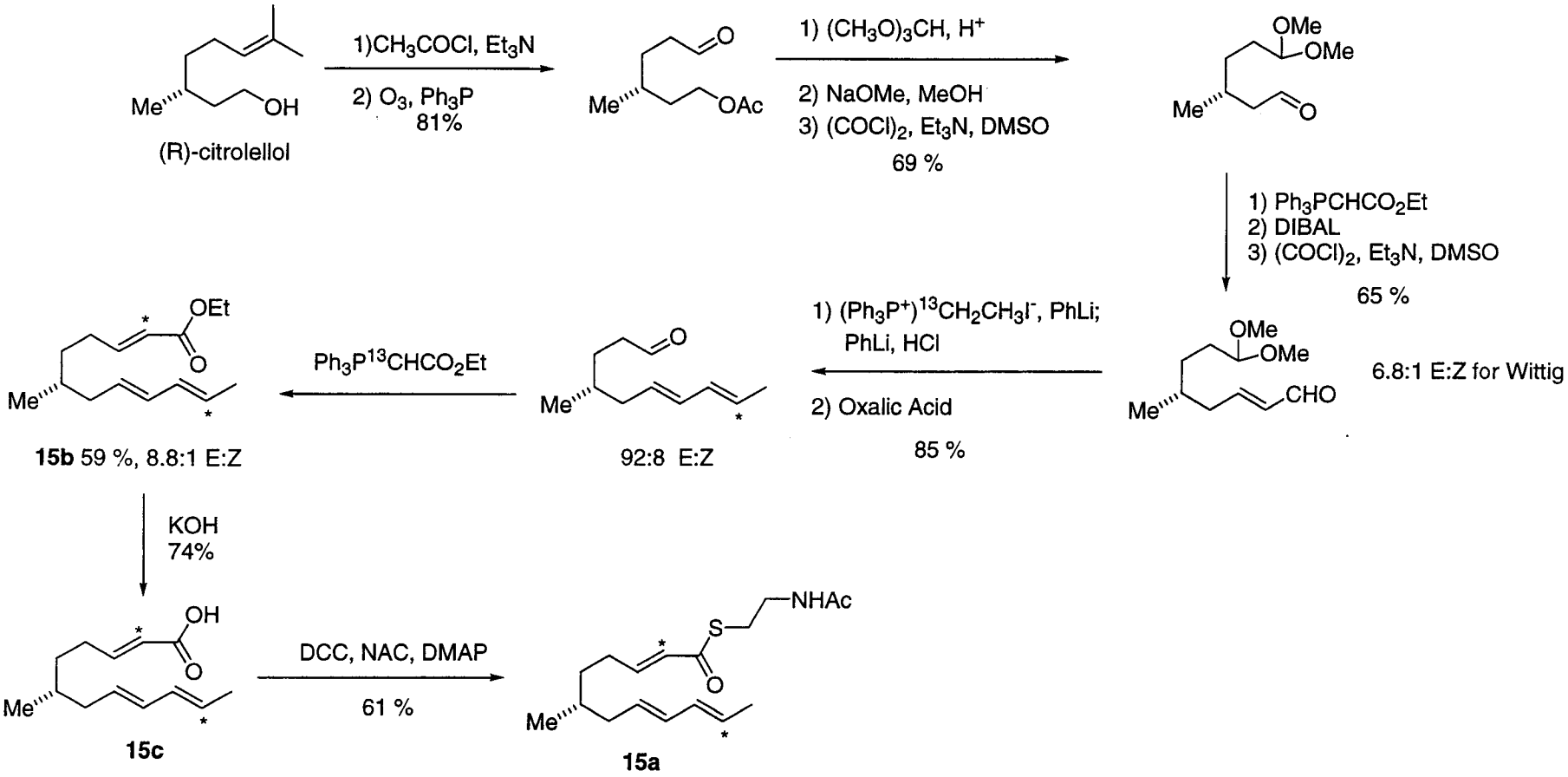


15a, X= $S(CH_2)_2NHAc$
15b, X= OEt
15c, X= OH



Vederas, J. et. Al. *JOC*, **1996**, 2613

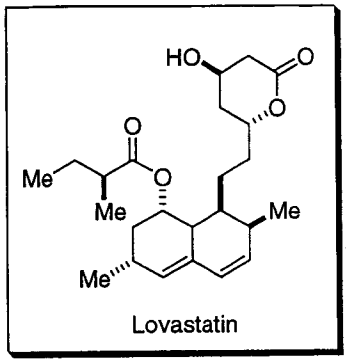
Synthesis of Hexaketide Analogs

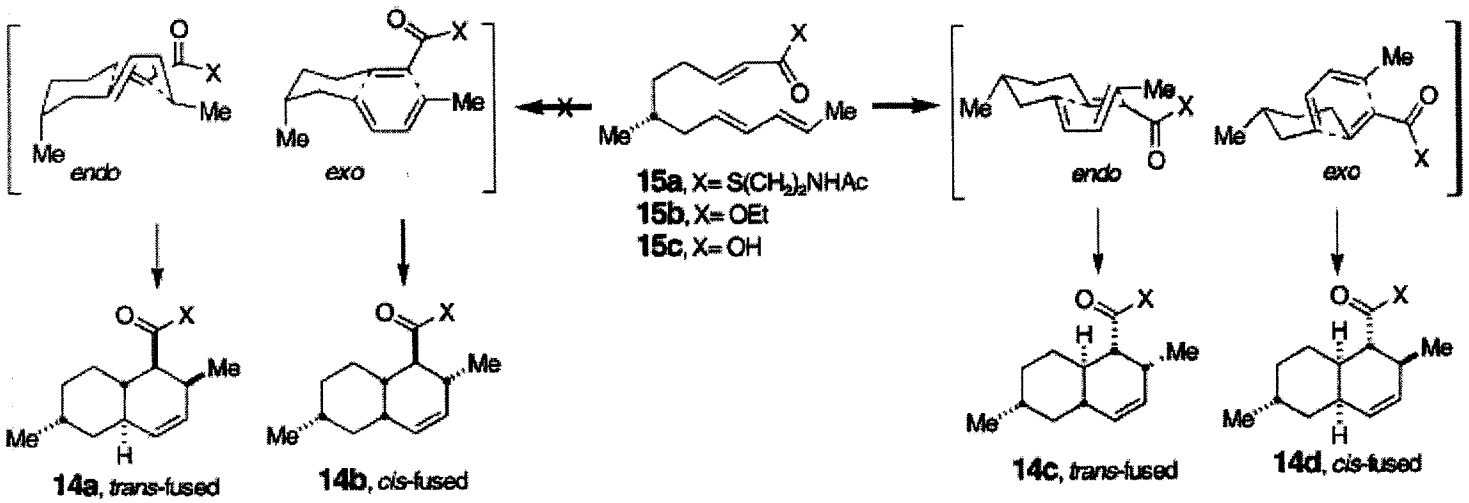


Small quantity of **15a** spontaneously cyclized (10 day half-life at rt.)

Vederas, J. et. Al. *JOC*, **1996**, 2613

Model Study



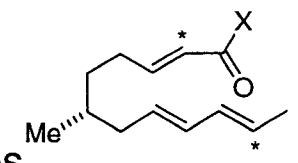


Compound	Conditions	Yield	Products
15a	160^{0}C, 4d	81%	1:1 **14c:14d**
15b	160^{0}C, 4d	72 %	1:1 **14c:14d**
15c	160^{0}C, 4d	83 %	1:1 **14c:14d**
15a	0.9 eq. $EtAlCl_2$, rt. 3h	80 %	19:1 **14c:14d**
15b	0.9 eq. $EtAlCl_2$, rt. 3h	58 %	9:1 **14c:14d**

14a was not observed under any conditions

Vederas, J. et. Al. *JOC*, **1996**, 2613

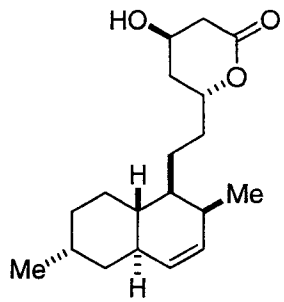
Biological Studies



15a X= $S(CH_2)_2NHAc$
15b X= OEt
15c X= OH

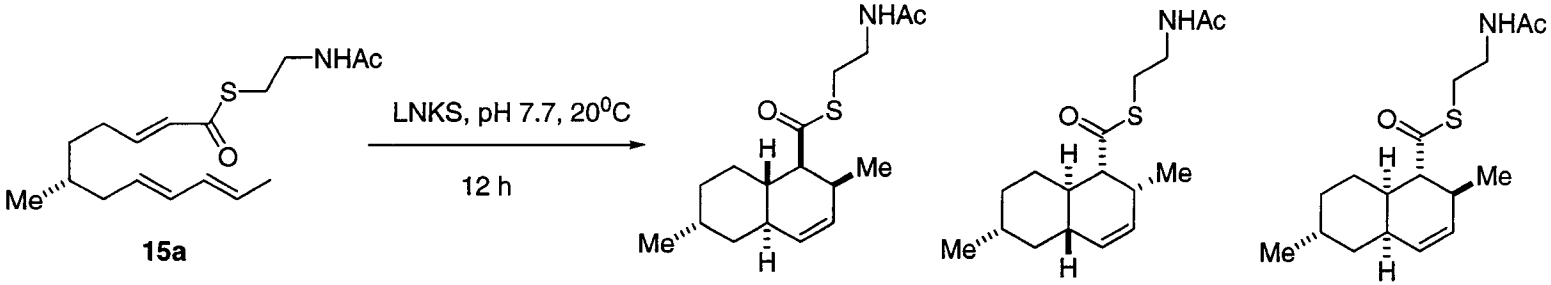
- Labeled **15** was not incorporated into the natural product in feeding studies
  - **15** could not be isolated from the fermentation mixture

- Isolated and purified LNKS (Lovastatin nonaketide synthase)
  LNKS with protein *lovC* produces Dihydromonacolin L **10**



10

Determine Diels-Alder must take place with covalently bound analog of **15a**
LNKS is the first example of a purified Diels-Alderase

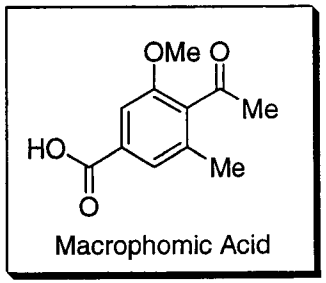


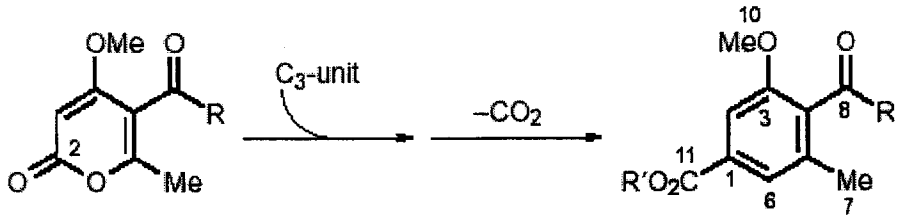
15a → **14a** : **14c** : **14d**

	1	:	15	: 15
denatured LNKS	0	:	1	: 1

Vederas, J. et. Al. *JOC*, **1996**, 2613
Vederas, J. et. Al. *JACS*, **2000**, 11519

Macrophomic Acid

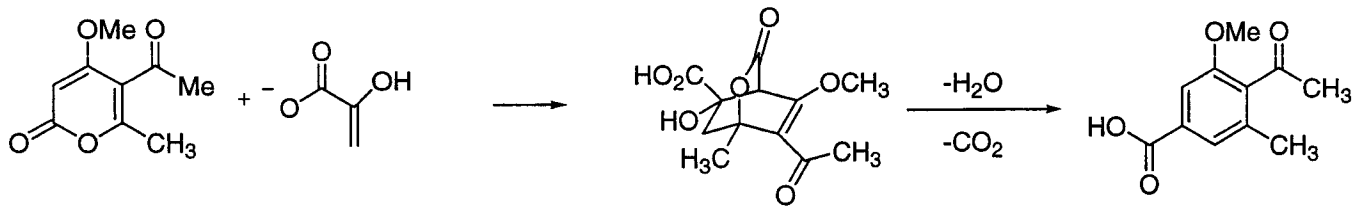


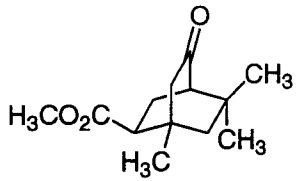


1 R = CH=CHMe
3 R = Me

2 R = CH=CHMe, R′ = H
4 R = Me, R′ = H
5 R, R′ = Me

Feeding experiments with [^{13}C]-acetate demonstrate all but C^{1}, C^{6}, and C^{11} derived from acetate
Pyranone 1 isolated from fermentation mixture is converted to Macrophomic Acid in whole cells
[^{13}C]-glycerol feeding results in incorporation into C^{1}, C^{6}, and C^{11}

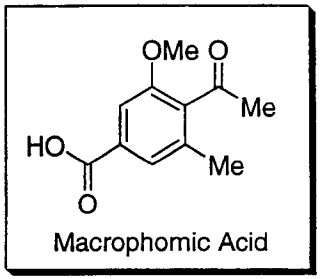




gave 23 % inhibition

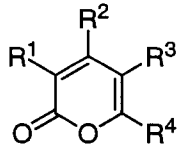
Yamamoto, Y. et. Al. *Chem. Pharm. Bull.* **1985**, *33*, 5141
Oikawa, H. et. Al. *Chem. Commun.* **1997**, 97

Purified Enzyme

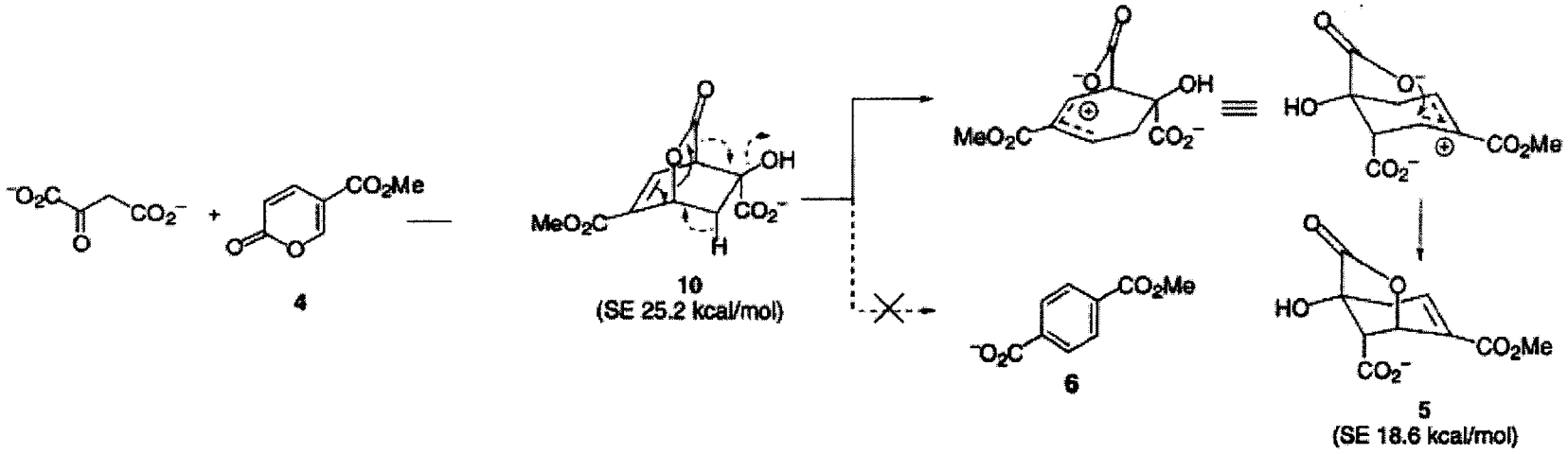


Macrophomic Acid

Macrophomate Synthase (MPS) was successfully purified
40 kDa enzyme, dimer, Mg^{2+} required
Only oxalacetate and pyruvate are react with pyranones in the presence of MPS

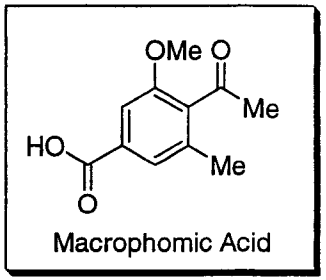
Tested 40 pyranone analogs 

R^1 must be H, R^3 must be $COCH_3$ or CO_2R
R^2 = OEt, OBn, Cl are reactive, OTBDPS, OAc give no reaction
R^4 = Me reacts, H gives aberrant product

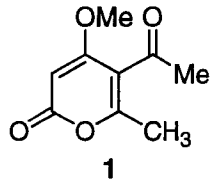


Oikawa, H. et. Al. *Tetrahedron Lett.* **1999**, *40*, 6183
Oikawa, H. et. Al. *Tetrahedron Lett.* **2000**, *41*, 1443
Oikawa, H. et. Al. *Biosci. Biotechnol. Biochem.* **2000**, *3*, 530

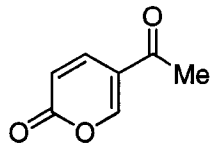
Kinetics



Reaction	k_{cat}
1.Macrophomate formation	
a. Oxalacetate + **1**	0.60 s^{-1}
b. Pyruvate + **1**	0.027 s^{-1}
2. Decarboxylation of Oxalacetate	16.3 s^{-1}
3. Adduct Formation	
a. **2**	15.4 s^{-1}
b. **3**	5.9 s^{-1}



1



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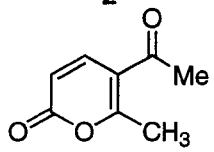

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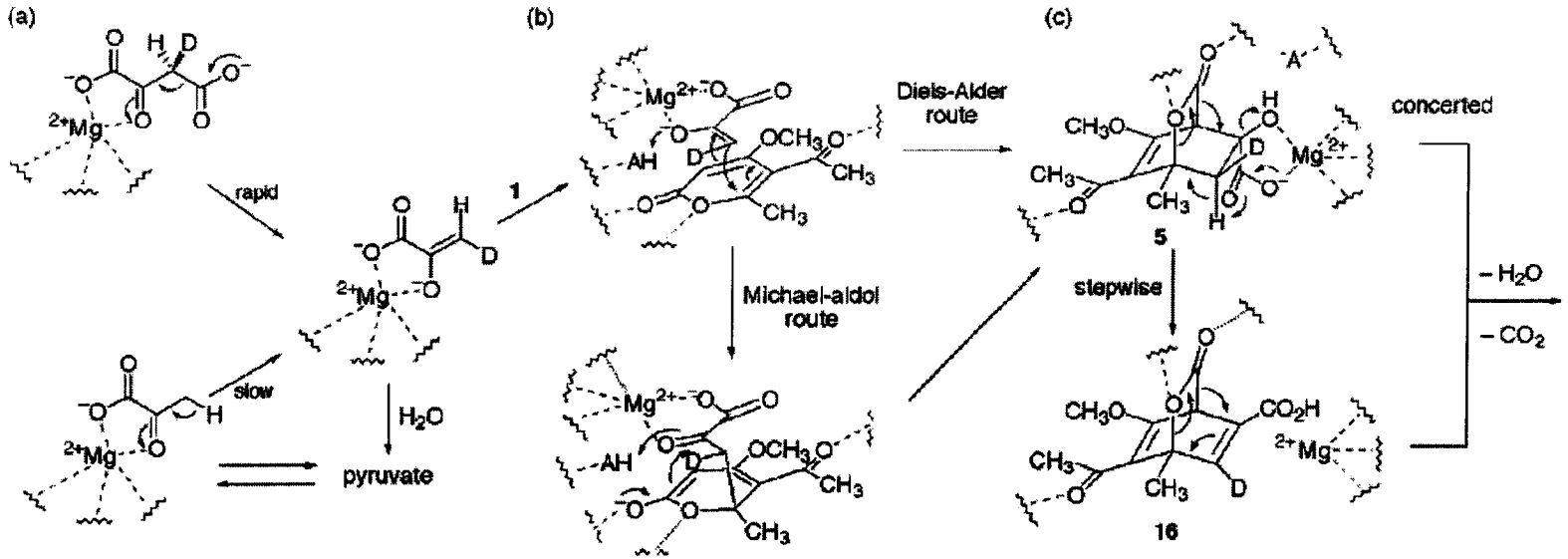
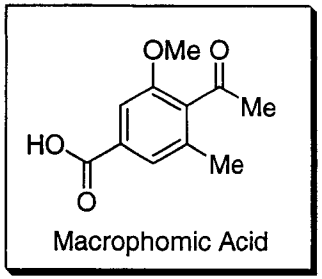
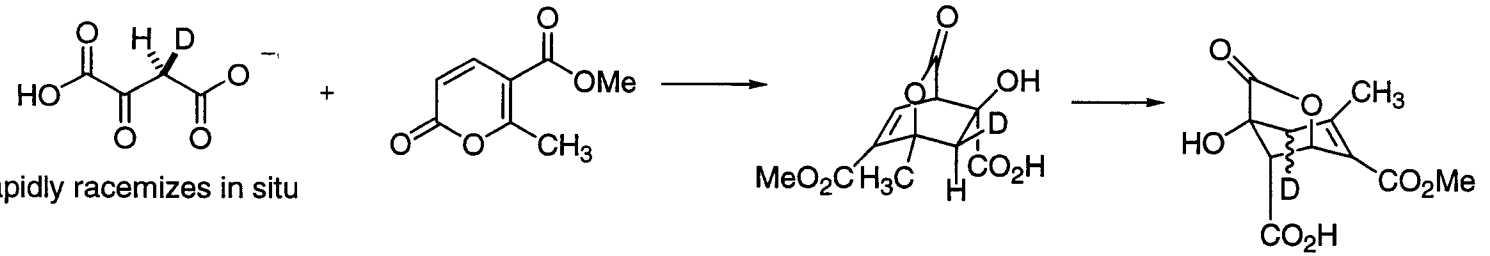


Labeling Study



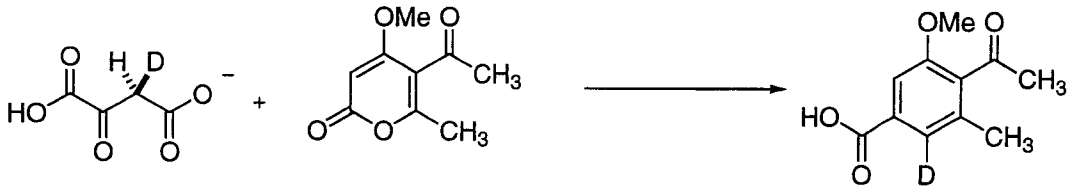
Macrophomic Acid



rapidly racemizes in situ

31% D in pro-R position
13% D in pro-S position

Authors conclude that 66% of label is lost, 26% derived from racemization, and 18% from the reaction of specifically labeled material (110% !)

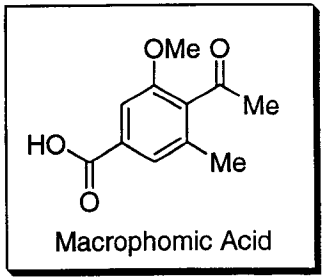


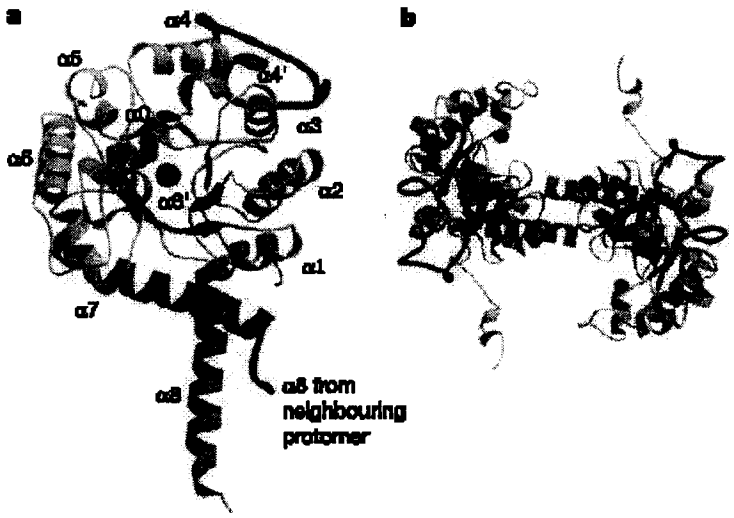
43 % deuterium incorporation

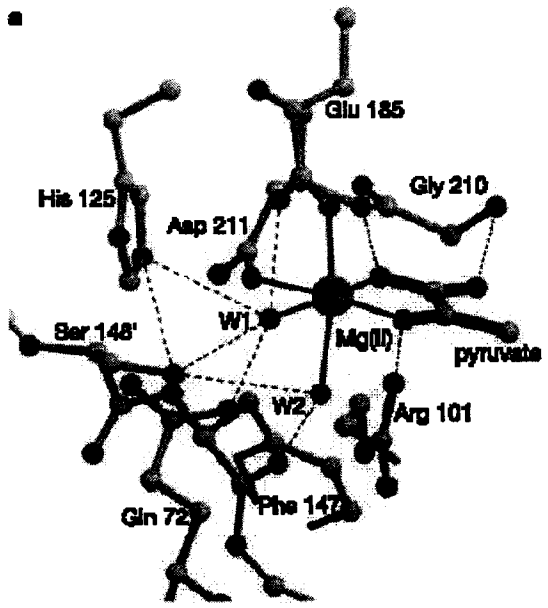
Based on deuterium incorporation, propose anti-elimination to form macrophomic acid

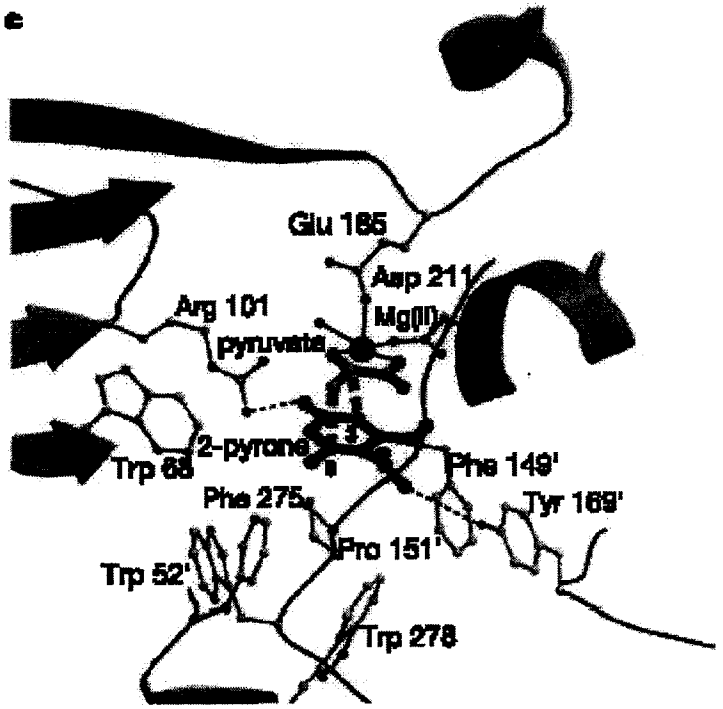
Oikawa, H. et. Al. *J. Biol. Chem.* **2000**, *275*, 38393

MPS Crystal Structure





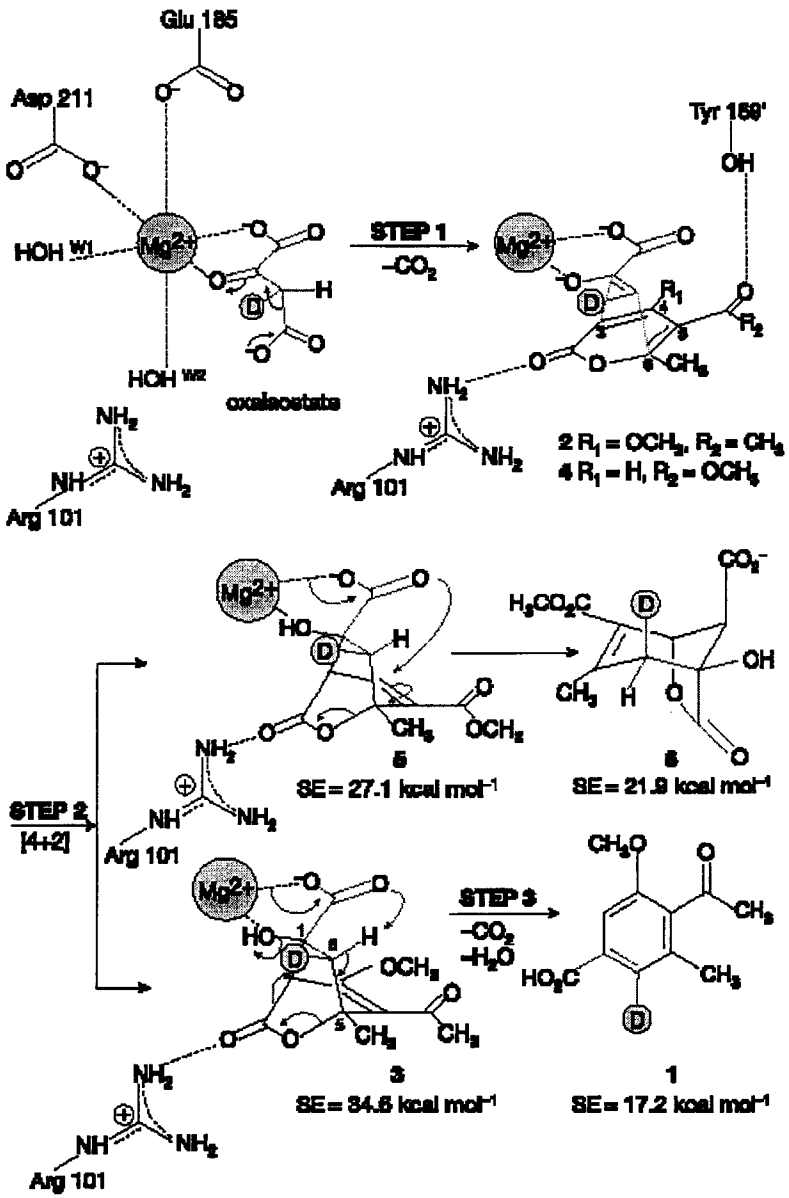


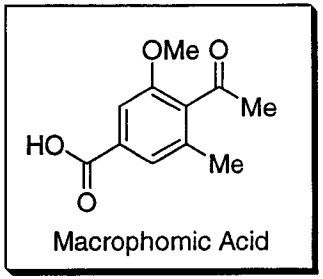


Mutations at Arg 101 and Tyr 169' result in loss or reactivity
Model also explains aberrant products and lost reactivity in Pyranone derivatives

Oikawa et. Al. *Nature*, **2003**, *422*, 185

Final Mechanism





Macrophomic Acid

Oikawa et. Al. *Nature*, **2003**, *422*, 185

Conclusions

Catalytic antibodies demonstrate the viability of Diels-Alderases

Solanapyrone and Lovastatin provide circumstantial evidence for the existence of Diels-Alderases

The isolation and crystal structure of Macrophomate Synthase give solid evidence that biosynthetic Diels-Alder reactions can be enzyme catalyzed

Can a Diels-Alderase be used preparatively to prepare otherwise difficult or inaccessible substrates?