Antibodies as Designer Enzymes

Frontiers of Chemistry

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Wipf Group

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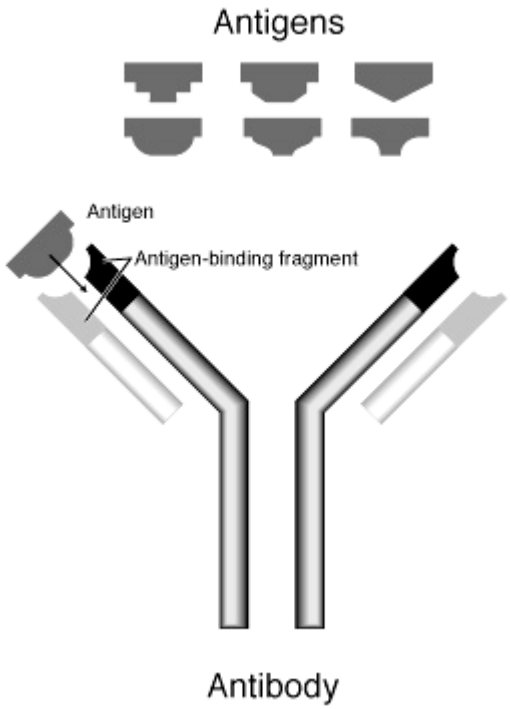
Outline

1. Introduction
2. Hydrolytic Antibodies
   a. Transition state analogue
   b. Bait-and-switch
   c. Heterologous immunization
   d. Reactive immunization
   e. In Vitro evolution
3. Other Transformations Catalyzed by Antibodies
4. Conclusion and Future Work

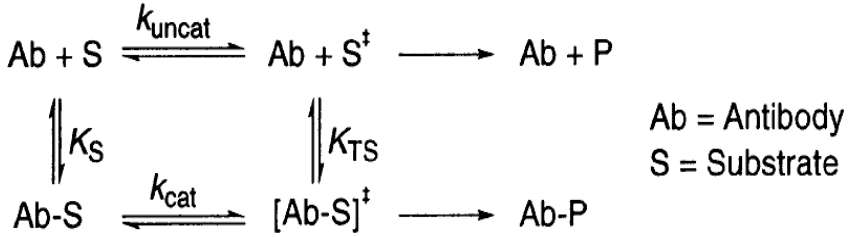
Antibody

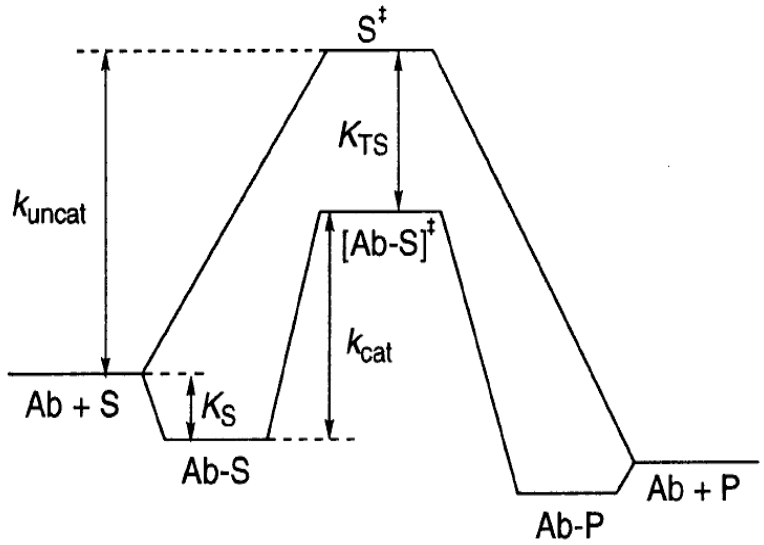
- “An antibody or immunoglobulin is a large Y-shaped protein used by the immune system to identify and neutralize foreign objects like bacteria and viruses. Each antibody recognizes a specific antigen unique to its target.”



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

Catalytic Antibodies (Abzymes): The Original Concept





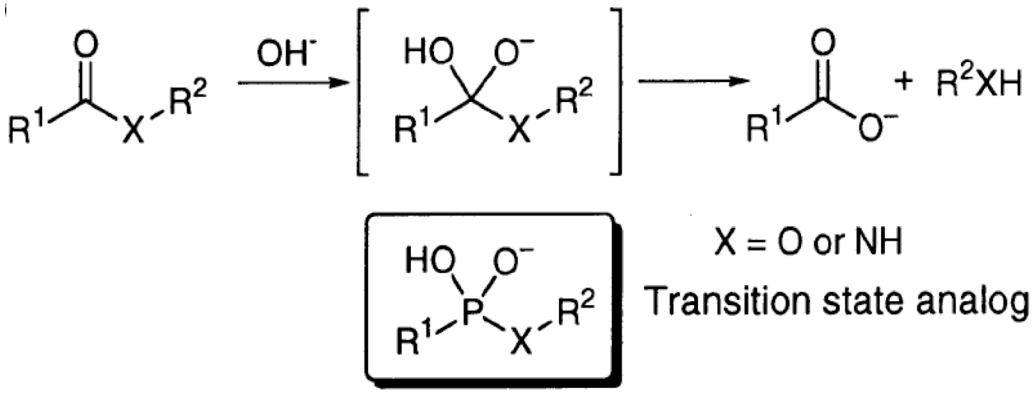
The Principle: Proteins can catalyze a chemical reaction by selective binding to transition state.

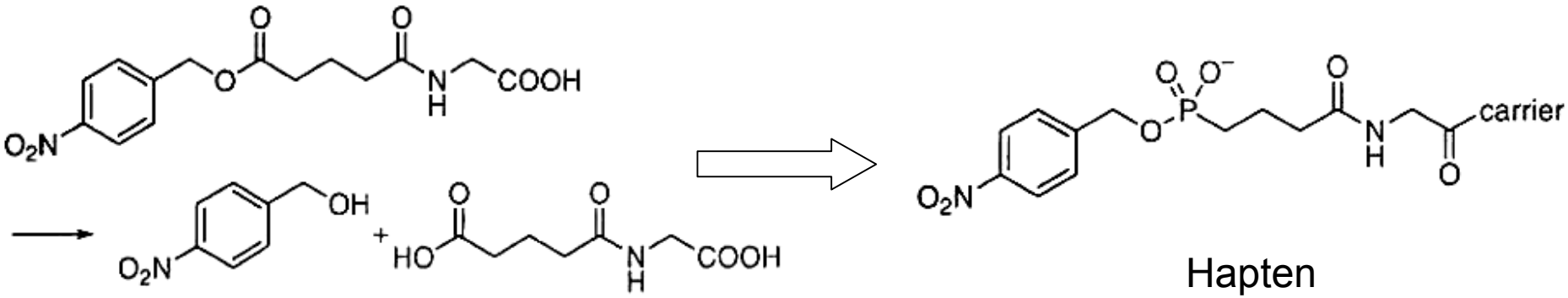
The Idea: Immunizing an animal with a transition state analog ("hapten") could form antibodies that bind to the transition state and catalyze a given reaction.

Tanaka, F. *Chem. Rev.* **2002,** *102,* 4885

Design and Synthesis of A Hapten

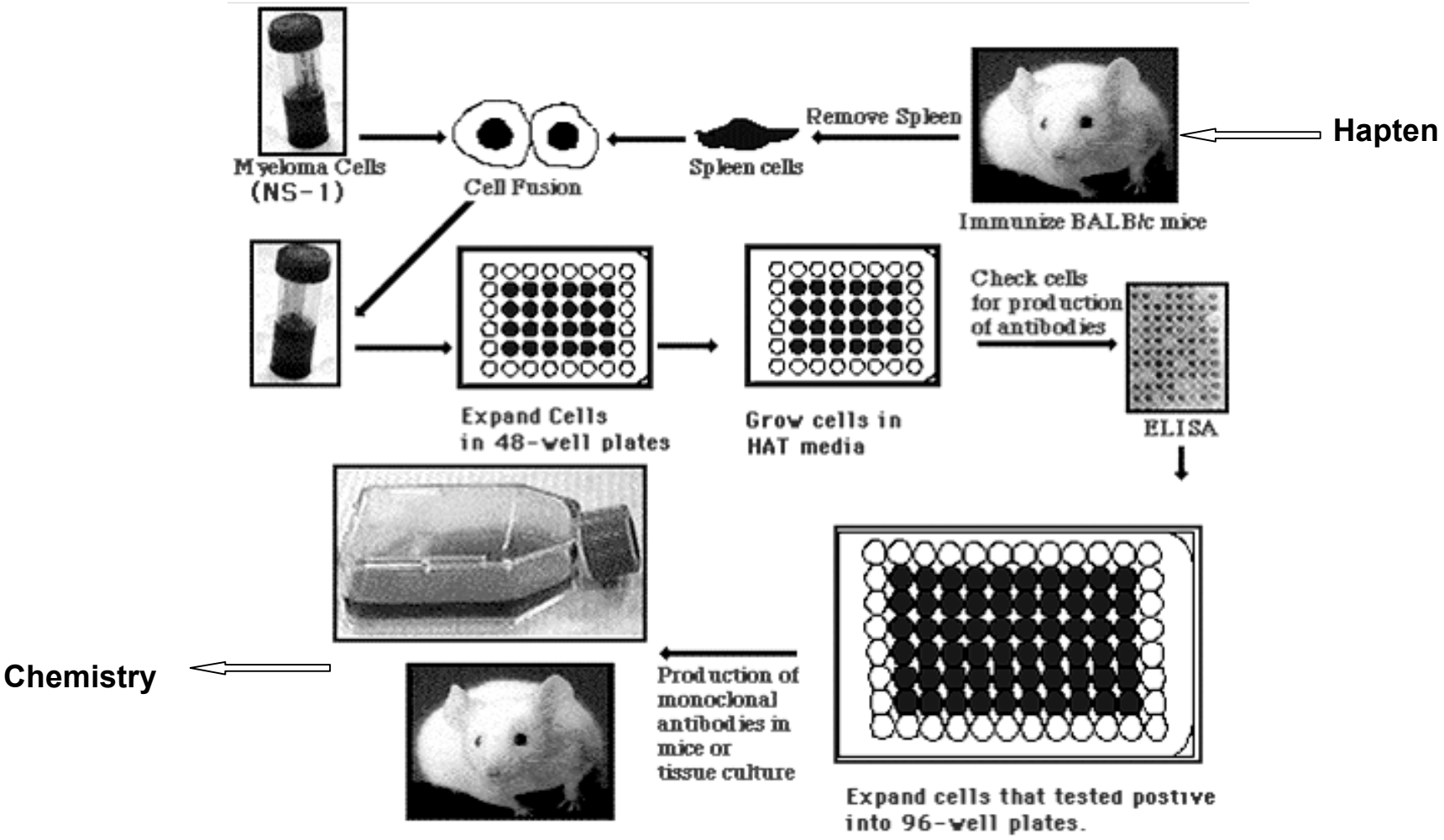
Ester hydrolysis:





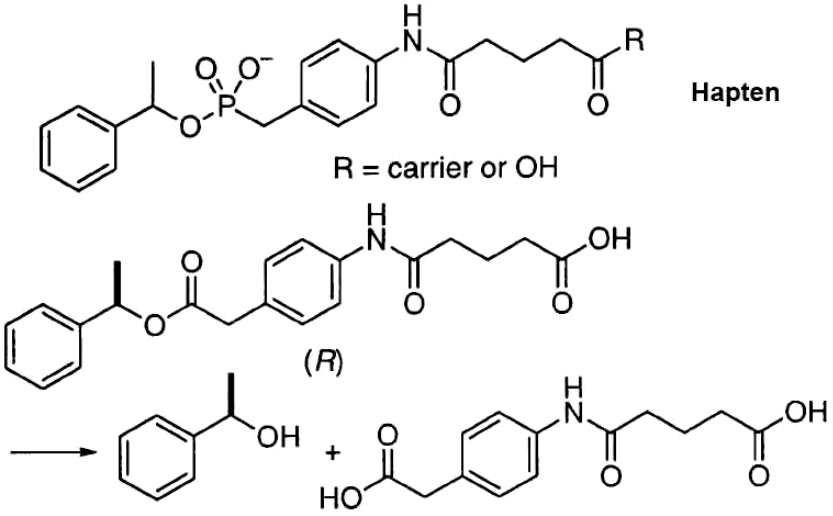
Charbonnier, J.-B.; Golinelli-Pimpaneau, B.; Giant, B.; Tawfik, D. S.; Chap, R.; Schindler, D. G.; Kim, S.-H.; Green, B. S.; Eshhar, Z.; Knossow, M. *Science* **1997**, *275*, 1140.

Preparation of Catalytic Antibodies



www.chembio.uoguelph.ca/educmat/chm455/woo-total.ppt

Hydrolytic Antibodies: Transition State Analogue (TSA) Strategy -Ester Hydrolysis

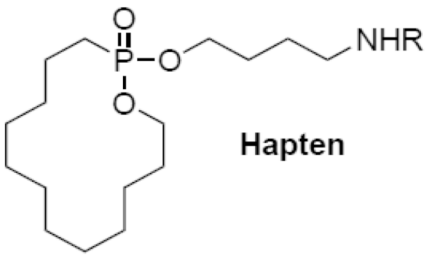


Name of catalytic Ab	k_{cat} [k_{cat}/k_{uncat}]
2H6	4.6 min^{-1} for (*R*) [8.3 x 10^4] at pH 9.0
21H3	0.09 min^{-1} for (*S*) [1619] at pH 9.0

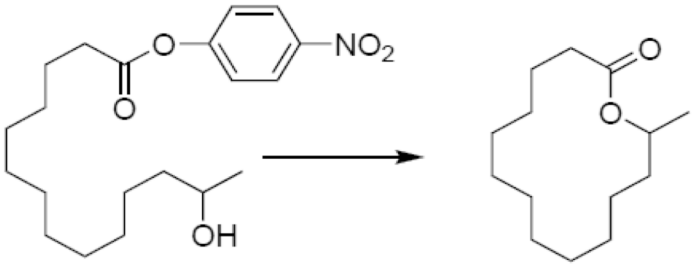
- An enatioselective hydrolysis was realized from a racemic hapten.

Janda, K. D.; Benkovic, S. J.; Lerner, R. A. *Science* **1989**, *244*, 437

Hydrolytic Antibodies: Macrolactonization



R = carrier

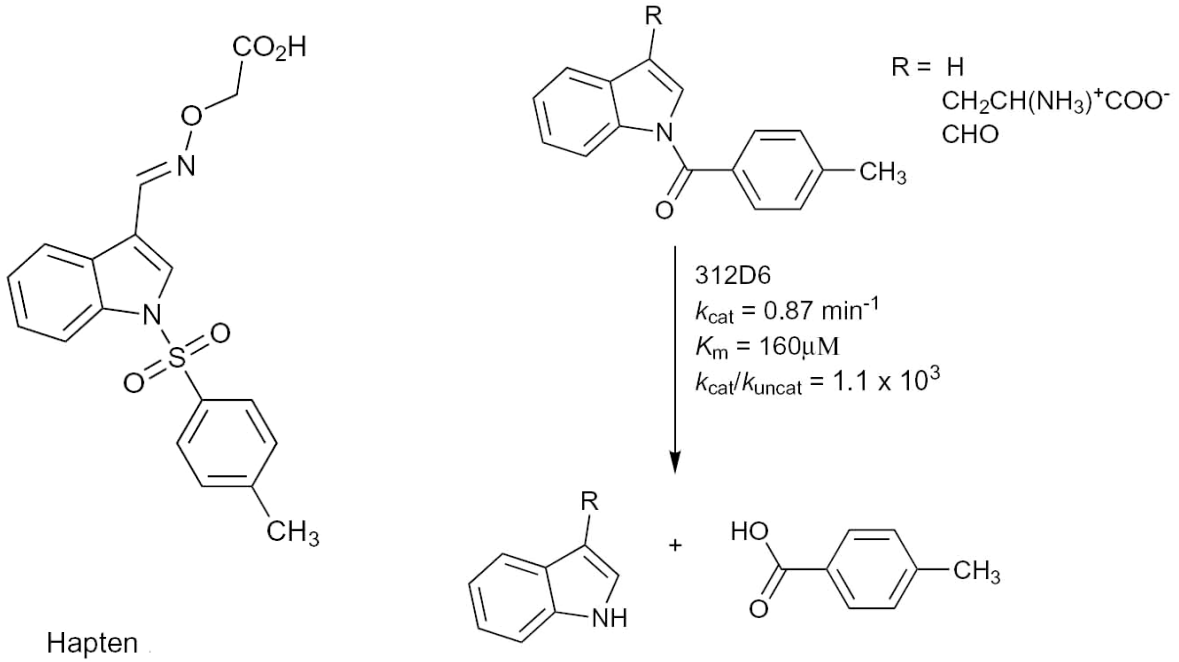


Name of catalytic Ab	k_{cat}
F123	0.01 min^{-1} at pH = 7.4

- Antibody F123 catalyzed an intramolecular transesterification of the corresponding hydroxy ester to give a 14-membered ring lactone.

Pungente, M. D.; Weiler, L. and Ziltener H. J. *Can. J. Chem*. **2002,** *80*, 1643

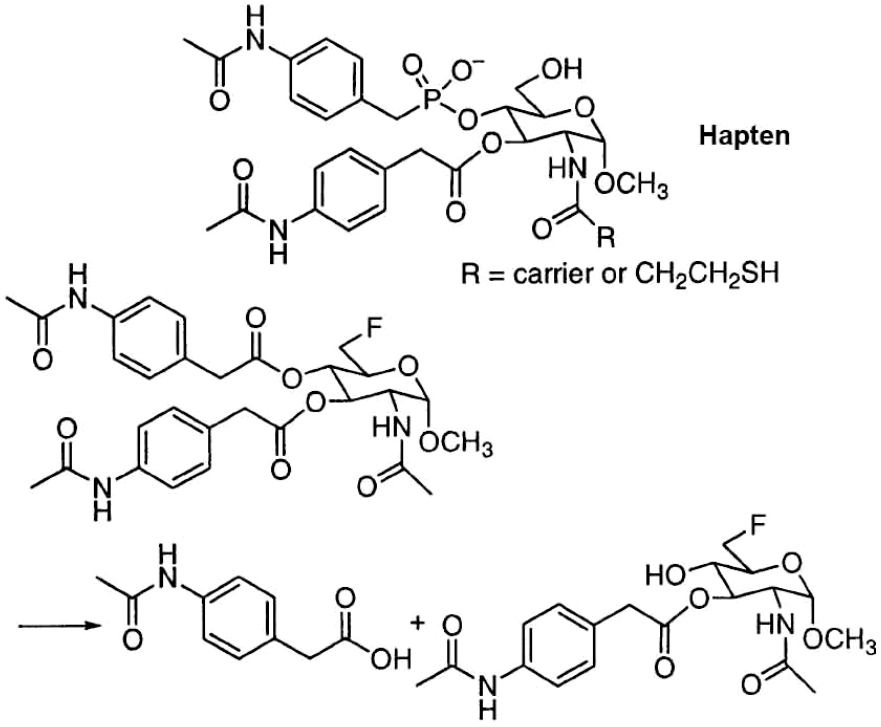
Hydrolytic Antibodies: Transition State Analogue (TSA) Strategy -Amide Hydrolysis



- The sulfonamide in the hapten mimics the tetrahedral intermediate as well as the distorted conformation of a amide bond.

Aggarwal, R.;Benedetti, F.;Berti, F.;Buchini, S.; Colombatti, A.;Dinon, F.;Galasso, V.;Norbedo, S. *Chem. Eur. J.* **2003**, *9*, 3132.

Hydrolytic Antibodies: Regioselective Deprotection

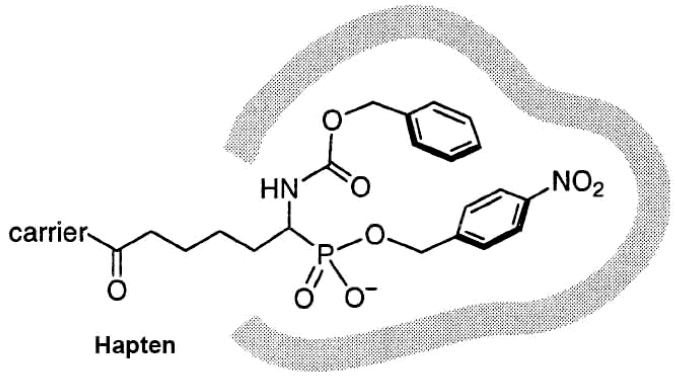


Name of catalytic Ab	k_{cat} [k_{cat}/k_{uncat}]
17E11	0.182 min^{-1} [2730] at pH 8.2

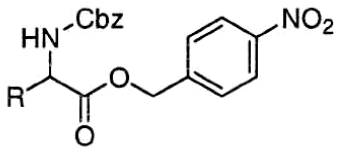
- Antibody 17E11 catalyzed the regioselective deprotection of ester group to afford the 4-OH and 3-OH products in a ratio of 20:1.

Iwabuchi, Y.; Miyashita, H.; Tanimura, R.; Kinoshita, K.; Kikuchi, M.; Fujii, I. *J. Am. Chem. Soc.* **1994**, *116*, 771

Hydrolytic Antibodies: Relaxing Substrate Specificity



Hapten



Subatrate

Name of catalytic Ab	k_{cat}
7G12	0.028 min^{-1} for R = CH_3
	0.037 min^{-1} for R = $(CH_3)_2CHCH_2$
	0.028 min^{-1} for R = $CH_3(CH_2)_3$

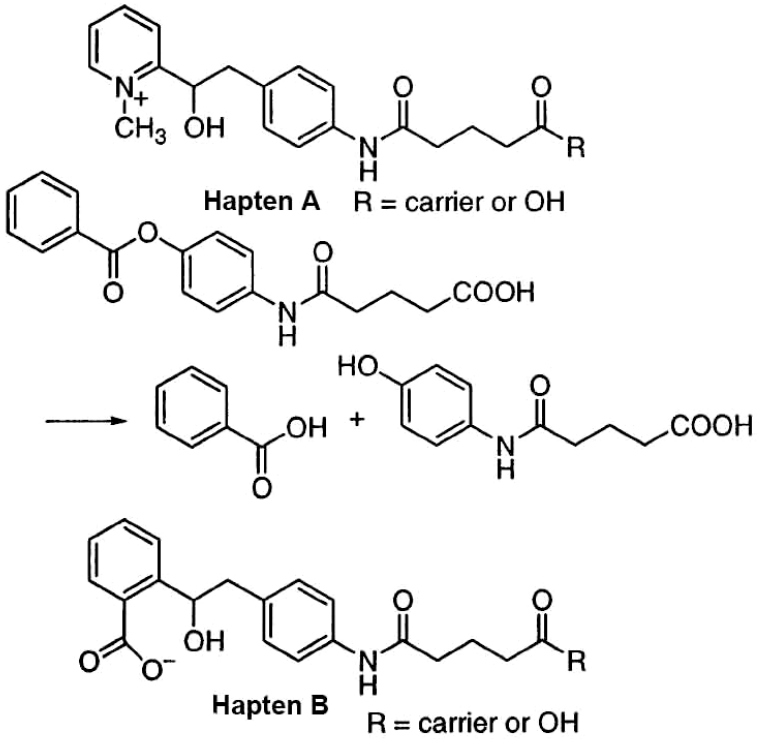
- The hapten's sidechain was excluded from the binding pocket, thus relaxing its specificity.

Tanaka, F.; Kinoshita, K.; Tanimura, R.; Fujii, I. *J. Am. Chem. Soc.* **1996**, *118*, 2332.

TSA Hydrolytic Antibodies: Strength And Weakness

- Strength
  - Catalytic efficiency (k_{cat}/k_{uncat}) ranges from 10^2 to 10^5.
  - Simple and stable haptens are used.
- Weakness
  - A significant fraction of hapten binders failed to exhibit activity.
  - Their activity are still not as good as natural enzymes (k_{cat}/k_{uncat} ~ 10^6 to 10^8).
  - Possible causes:
    - Product inhibition
    - Inability to faithfully mimic TS (fractional bonds orders, distorted bond angles, charge distributions, *etc.*)
    - Lack of covalent interactions between antibodies and TS
    - Lack of metals and other cofactors
    - Differences in time scales for the evolution of natural enzymes and abzymes: millions of years versus months

Hydrolytic Antibodies: Bait-and-Switch Strategy

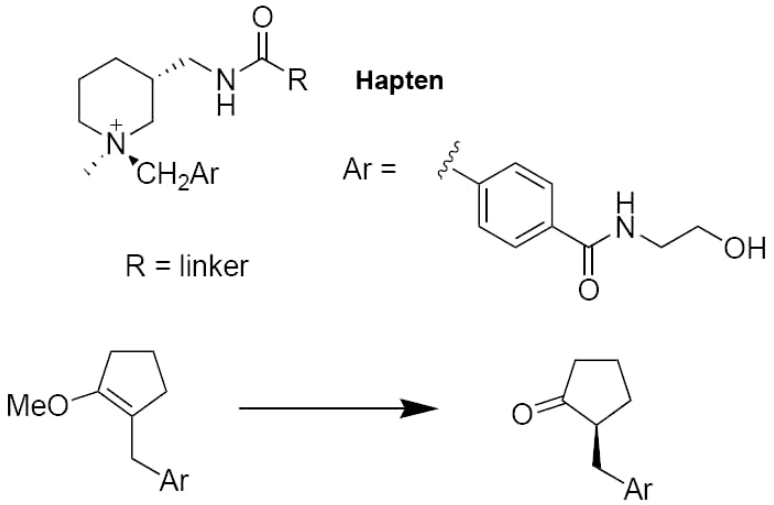


Hapten	Name of catalytic Ab	k_{cat}
A	30C6	0.005 min^{-1} at pH 7.2
B	27A6	0.01 min^{-1} at pH 8.5

- Charges on the haptens induce complementary charged residues in the active sites
- The induced charges in antibodies function as general acid/base or nucleophilic catalysts

Janda, K. D.; Weinhouse, M. I.; Schloeder, D. M.; Lerner, R. A.; Benkobic, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 1274.

Hydrolytic Antibodies: Bait-and-Switch Strategy

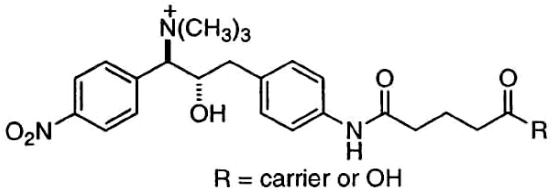


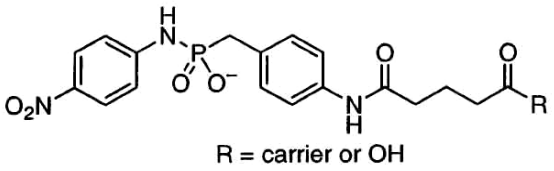
Name of catalytic Ab	k_{cat} [k_{cat}/k_{uncat}]
14D9	0.4 s^{-1} [10^4] at pH 6.0

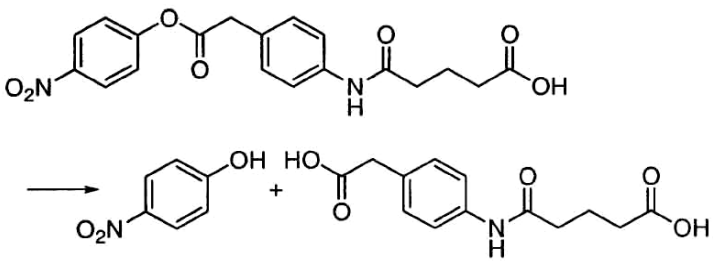
- The hapten was originally designed for glycosidic bond hydrolysis.
- Antibody 14D9 was later found to catalyze enatioselective hydrolysis of the shown enol ether.
- Proposed mechanism included a carboxylic acid in the active site induced by the postitive charge on the hapten (Bait-and-switch).
- The ketone was obtained in 62% yield and 86% ee on a half gram scale.
- 14D9 was recovered after each run and retained 95% of its activity.

Remond, J. L.; Reber, J. L.; Lerner, R. A. *Angew. Chem. Int. Ed.* **1994**, *33*, 475

Hydrolytic Antibodies: Heterologous Immunization





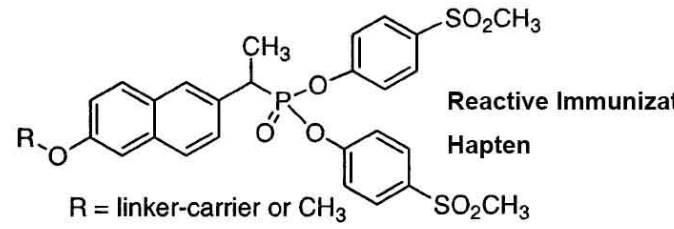


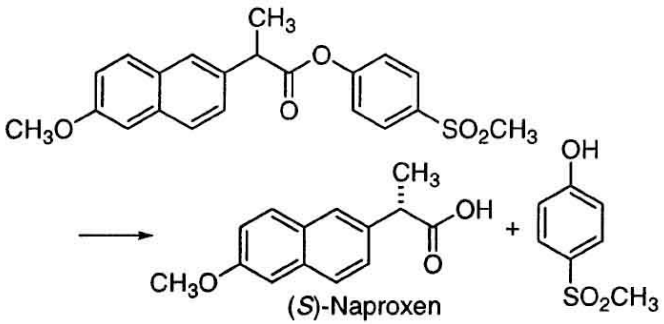
Hapten	Name of catalytic Ab	k_{cat} [k_{cat}/k_{uncat}]
A+B (heterologous immunization)	H5H2-42	12.5 min^{-1} [68000] at pH 7.0
A (homologous immunization)	H5-25	0.79 min^{-1} [2700] at pH 7.0
B (homologous immunization)	H2-23	3.2 min^{-1} [11000] at pH 7.0

- An animal was injected with two different haptens (A+B).
- An antibody capable of binding both haptens was produced. It provided higher activity than homologous immunization.

Suga, H.; Ersoy, O.; Williams, S. F.; Tsumuraya, T.; Margolies, M. N.; Sinskey, A. J.; Masamune, S. *J. Am. Chem. Soc.* **1994**, *116*, 6025.

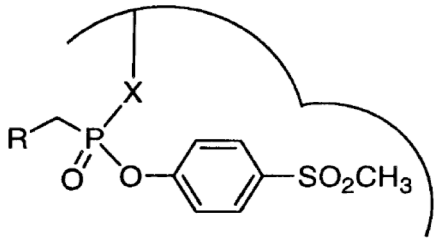
Hydrolytic Antibodies: Reactive Immunization Strategy





Hapten	Name of catalytic Ab	k_{cat} [k_{cat}/k_{uncat}]
RI	15G12	28 min^{-1} for (*S*)- [6.6 x 10^5] 0.23 min^{-1} for (*R*)- at pH 8.0
TSA	6G6	81 min^{-1} for (*S*)- [1.9 x 10^6] at pH 8.0

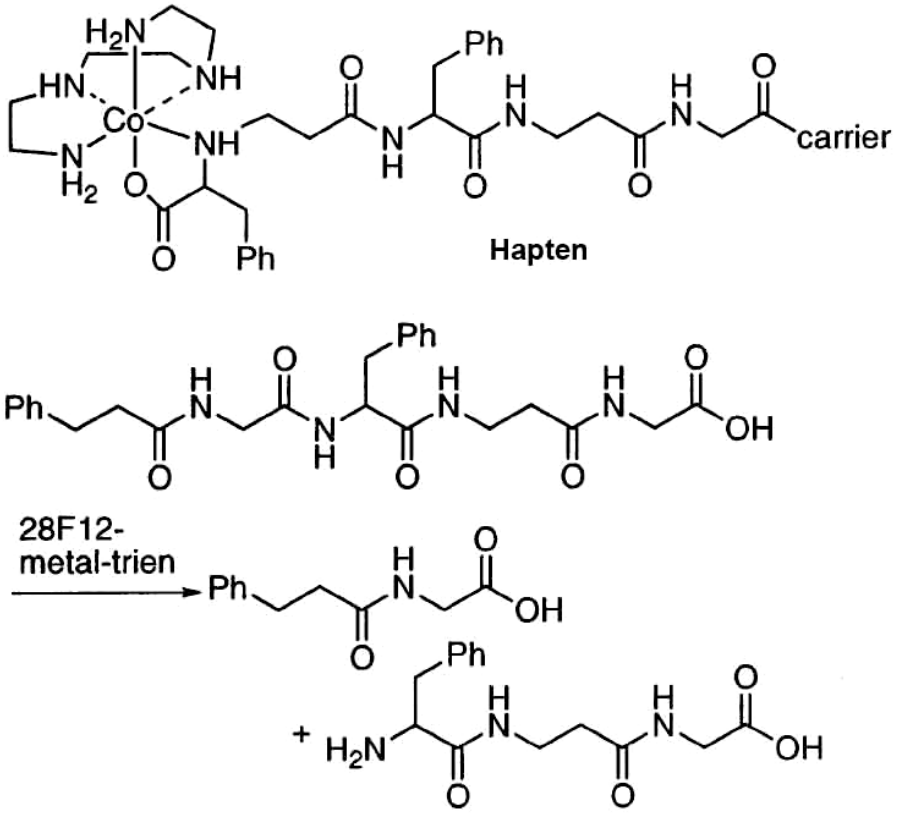
- The reactive hapten was covalently trapped by a nucleophilic residue in the antibody combining site during immunization.
- The trapped monoester functioned as a transition state analogue.
- No improvement in catalytic efficiency (k_{cat}/k_{uncat}) was observed as compared to the TSA approach.



Wirsching, P.; Ashley, J. A.; Lo, C.-H. L.; Janda, K. D.; Lerner, R. A. *Science* **1995**, *270*, 1775-1782

Lo, C.-H. L.; Wentworth, P., Jr.; Jung, K. W.; Yoon, J.; Ashley, J. A.; Janda, K. D. *J. Am. Chem. Soc.* **1997**, *119*, 10251

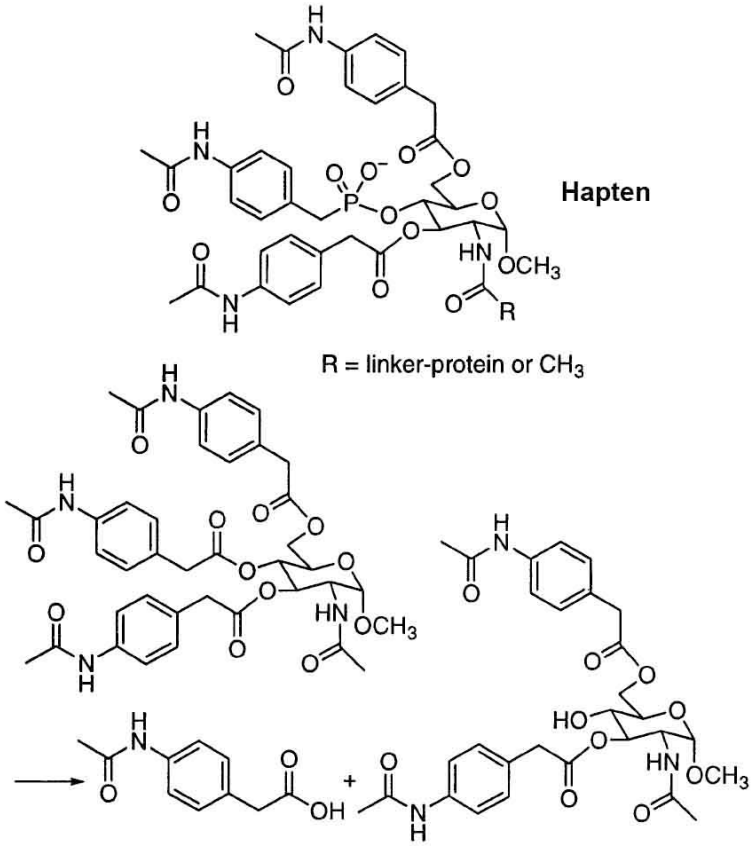
Hydrolytic Antibodies: Cofactor Strategy



- The hapten contained a Co-triethylenetetramine (trien) moiety.
- The catalytic antibody functioned as a protease in the presence of Metal-trien.
- Active metals: Zn(II), Ga(III), In(III), Fe(III), Cu(II), NiII), Lu(II), Mn(II), and Mg(II).
- Proposed mechanism:

Iverson, B. L.; Lerner, R. A. *Science* **1989**, *243*, 1184

Hydrolytic Antibodies: in Vitro Evolution

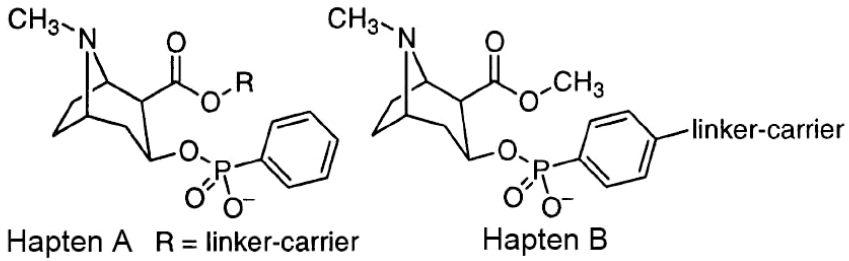


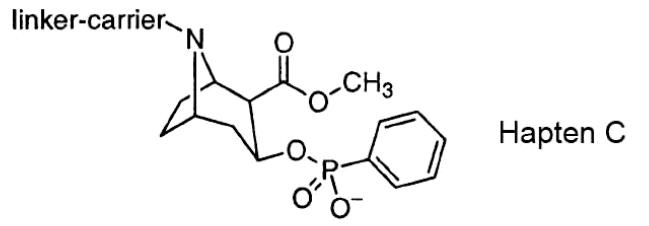
Name of catalytic Ab	k_{cat} [k_{cat}/k_{uncat}]
Wild type17E11	0.018 min^{-1} [184] at pH 8.0
mutant 115	0.22 min^{-1} [2248] at pH 8.0

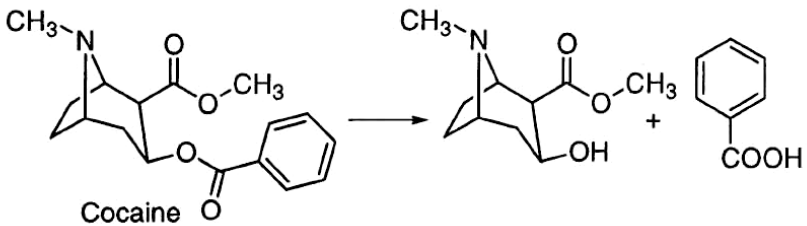
- The hapten-derived antibody 17E11 catalyzed the hydrolysis of protected glucosamine with low activity.
- Molecular modeling suggested steric congestion at C-6.
- A library of 6 mutants were prepared and screened.
- Mutant 115 provided higer activity.

Fujii, I.; Fukuyama, S.; Iwabuchi, Y.; Tanimura, R. *Nat. Biotechnol.* **1998**, *16*, 463.

Hydrolytic Antibodies: Drug Degradation





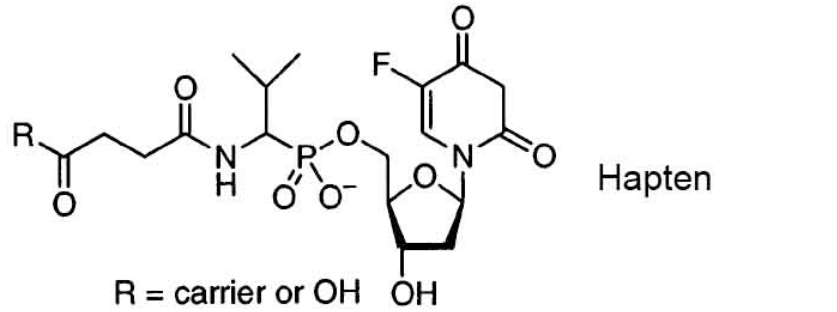


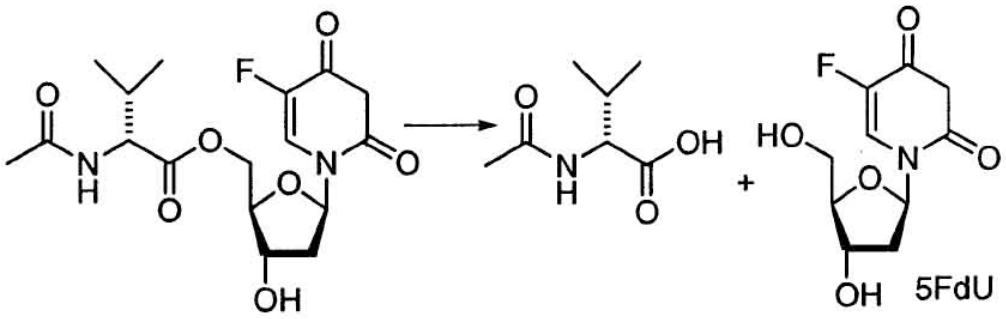
Hapten	Name of catalytic Ab	k_{cat} [k_{cat}/k_{uncat}]
A	15A10	2.3 min^{-1} [2.3 x 10^4] at pH 7.8
A	3B9	0.11 min^{-1} [1100] at pH 7.8

- An antibody derived from TSA hapten A was capable of hydrolyzing cocain.

Yang, G.; Arakawa-Uramoto, A.; Wang, X.; Gawinowicz, M. A.; Zhao, K.; Landry, D. W. *J. Am. Chem. Soc.* **1996**, *118*, 5881.
Matushita, M.; Hoffman, T. Z.; Ashley, J. A.; Zhou, B.; Wirsching, P.; Janda, K. D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 87.

Hydrolytic Antibodies: Prodrug Activation



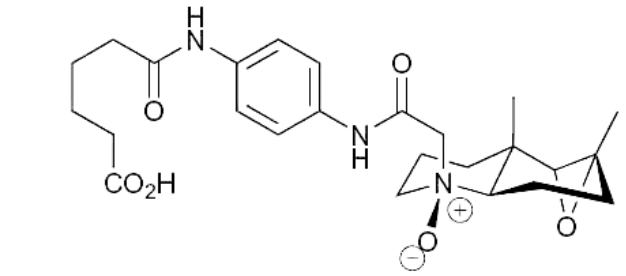


Name of catalytic Ab	k_{cat} [k_{cat}/k_{uncat}]
49.AG.659.12	0.03 min^{-1} [968] at pH 8.0

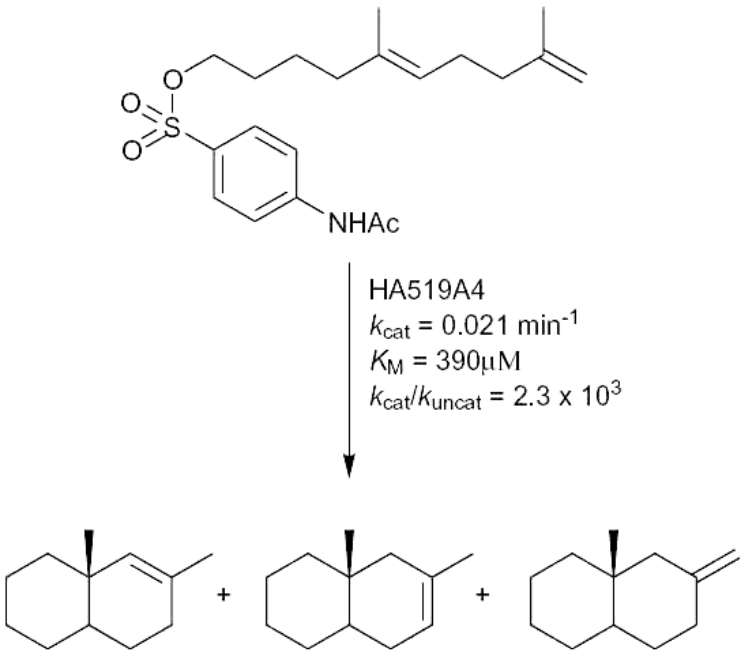
- An antibody was capable of recognizing a prodrug and hydrolyzing it to the active 5-FDU.

Cambell, D. A.; Gong, B.; Kochersperger, L. M.; Yonkovich, S.; Gallop, M. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1994**, *116*, 2165.

Antibody-catalyzed Cationic Cyclization



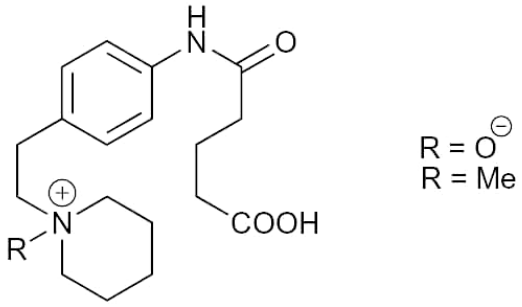
Hapten



- An X-ray of the hapten and antibody complex suggested the active site forces the substrate into a productive chair-chair conformation.

Hasserodt, J.;Janda, K. D.;Lerner, R. A. *J. Am. Chem. Soc*. **1997**, *119*, 5993

Antibody-catalyzed Disfavored Ring Closure

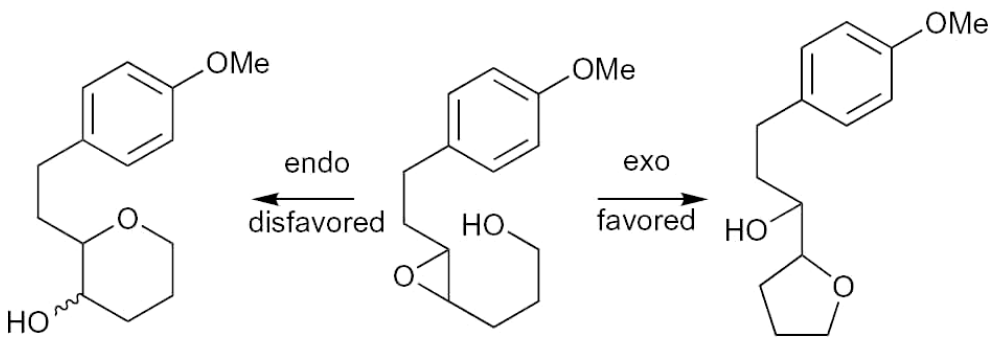


Hapten for immunization

R = O⊖
R = Me

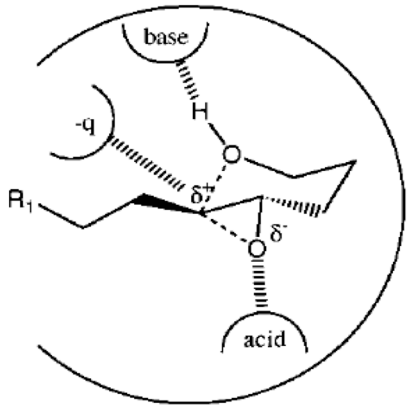
29D9
k_{cat} = 0.91 min^{-1}
K_M = 356 μM

5C8
k_{cat} = 1.7 min^{-1}
K_M = 595 μM



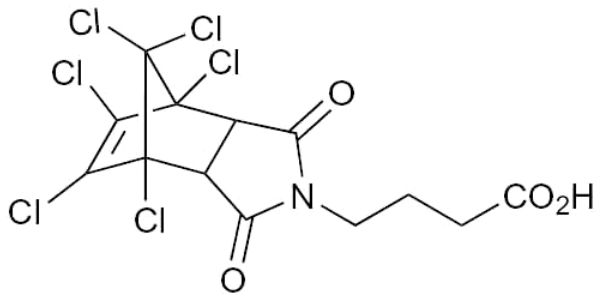
(Racemic)

- Antibodies 29D9 and 5C8 catalyzed the 6-endo cyclization exclusively.
- The reaction catalyzed by 5C8 was enatioselective.
- An X-ray of the hapten and 5C8 complex suggested general acid-base catalysis.
- Proposed mechanism:



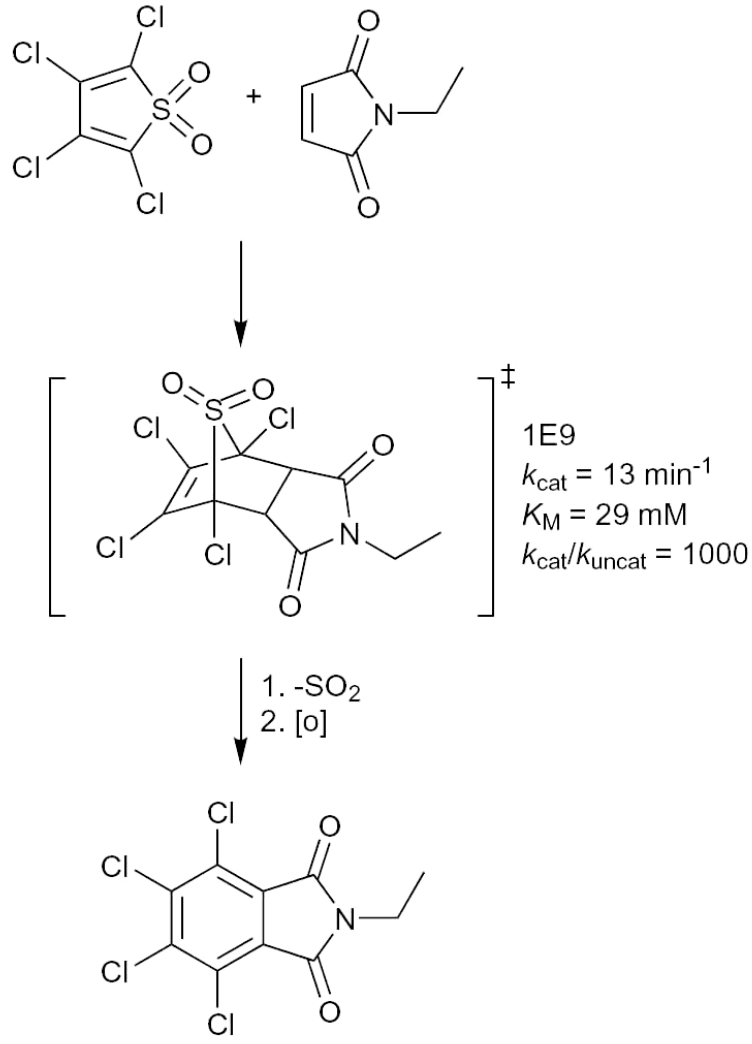
Janda, K. D.;Shevlin, C. G.;Lerner, R. A. *Science* **1993**, *259*, 490
Gruber, K.;Zhou, B.;Houk, K. N.;Lerner, R. A.; Shevlin, C. G.;Wilson, I. A. *Biochemistry* **1999**, *38*, 7062

Antibody-catalyzed Diels-Alder Reaction



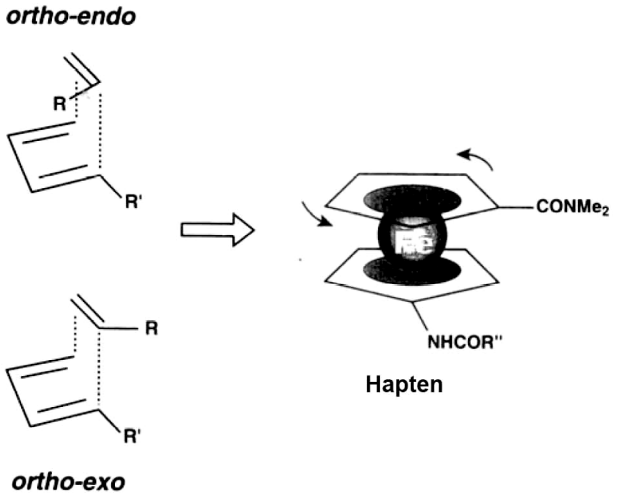
Hapten

- The Diels-Alder reaction is an ideal target for antibody catalysis due to its highly organized TS.
- In this case, product inhibition was not observed as it is structurally dissimilar to the hapten.

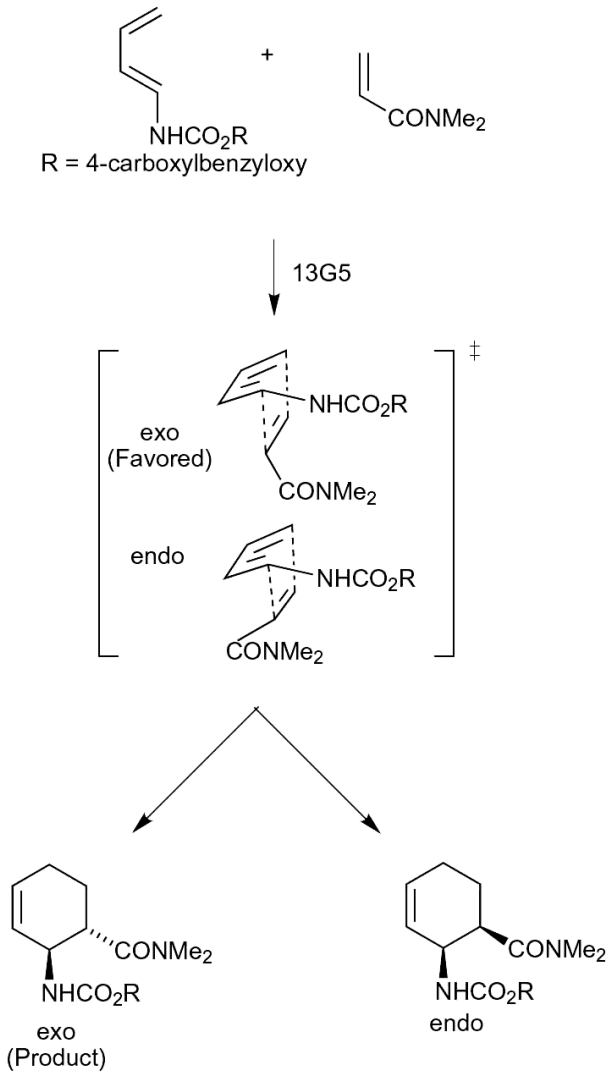


Hilvert, D.;Hill, K. W.;Nared, K. D.;Auditor, M. T. M.
J. Am. Chem. Soc. **1989**, *111*, 9261.

Antibody-catalyzed Diels-Alder Reaction



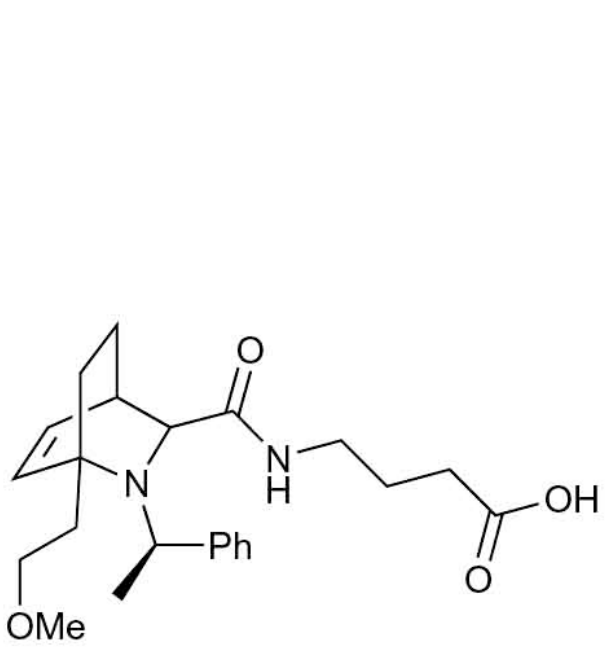
- A cyclopentadienyl iron complex was used as a hapten.
- Antibody 13G5 catalyzes the Diels-Alder reaction with high regio-, diastereo- and enatio-selectivity.
- An X-ray of the antibody and hapten complex suggested Lewis acid catalysis



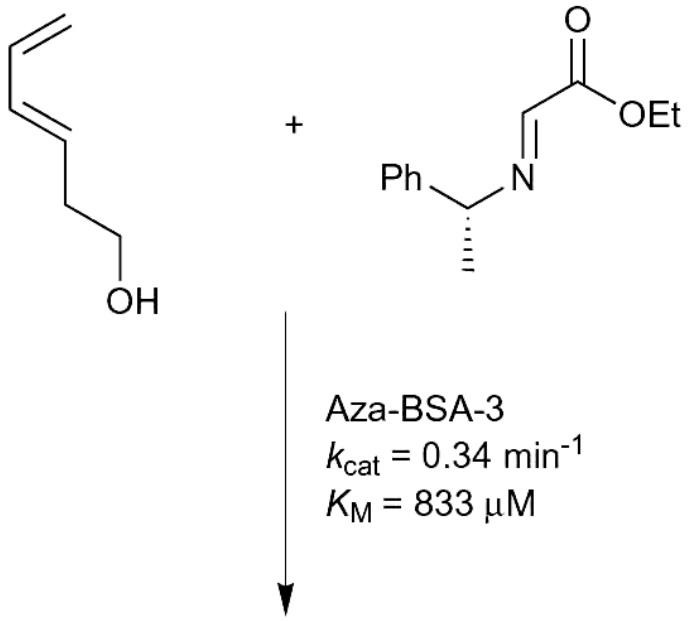
Ylikauhaluoma, J. T.;Ashley, J. A.;Lo, C. H.;Tucker,L.; Wolfe, M. M.; Janda, K. D. *J. Am. Chem. Soc*. **1995**, *117*, 7041.

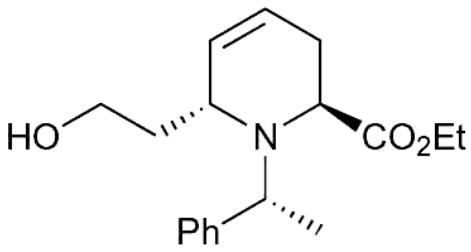
34. Heine, A.;Stura, E. A.;Yli-Kauhaluoma, J. T.;Gao, C. S.;Deng, Q. L.;Beno, B. R.;Houk, K. N.;Janda, K. D.; Wilson, I. A. *Science* **1998**, *279*, 1934.

Antibody-catalyzed Aza-Diels-Alder Reaction



Hapten

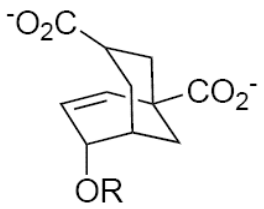




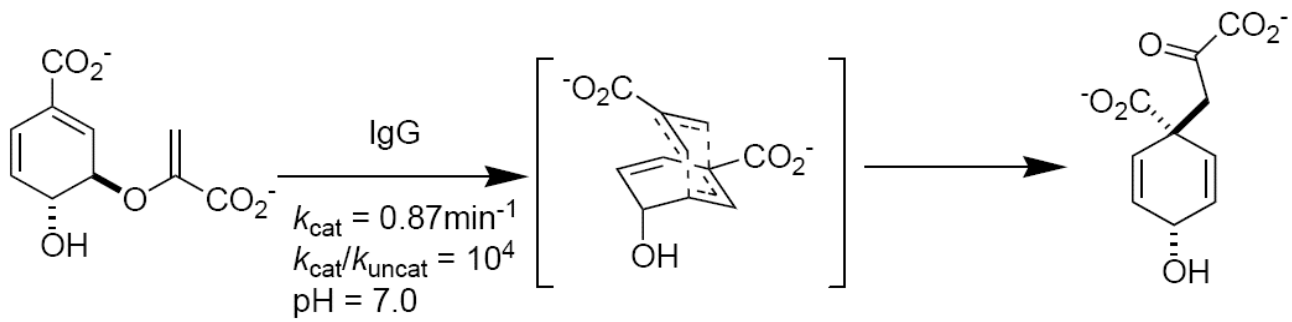
exo

Shi, Z. D.;Yang, B. H.;Wu, Y. L.;Pan, Y. J.; Ji, Y. Y.; Yeh, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2321.

Antibody-catalyzed Claisen Rearrangement



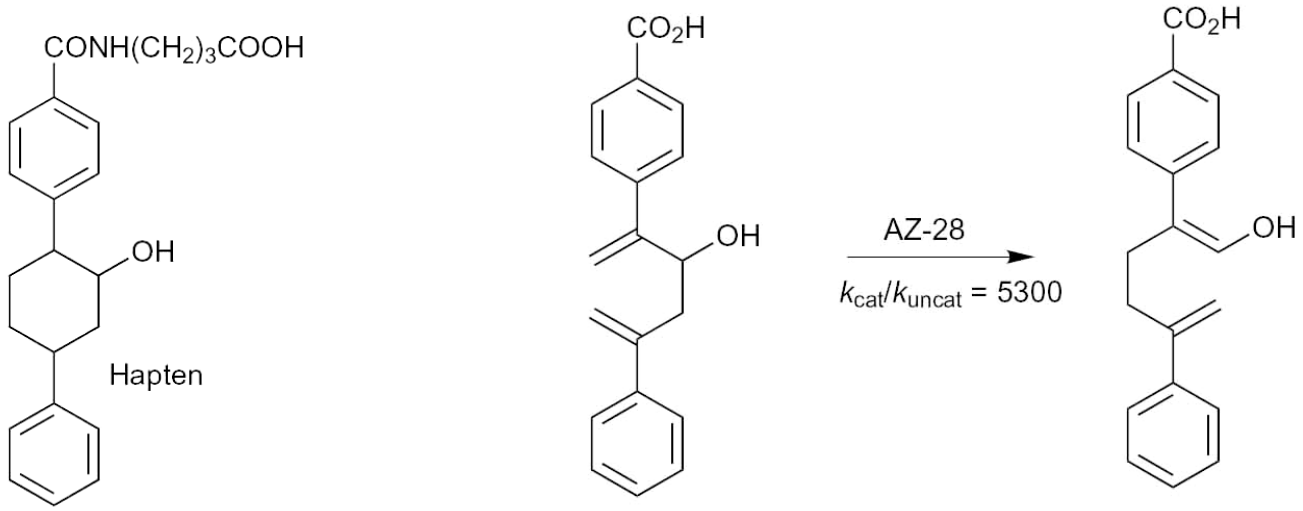
R = H: chorismate mutase inhibitor
R = Linker: Hapten



- The hapten was based on known chorismate mutase inhibitor (mechanism-based inhibitor strategy).
- The antibody's catalytic efficiency (10^4) is lower than the natural enzyme ($3x10^6$).

Jackson, D. Y.; Jacobs, J. W.; Sugasawara, R.; Reich, S. H.; Bartlett, P. A. and Schultzg, P. G. *J. Am. Chem. SOC*. **1988**, *110*, 4841-4842

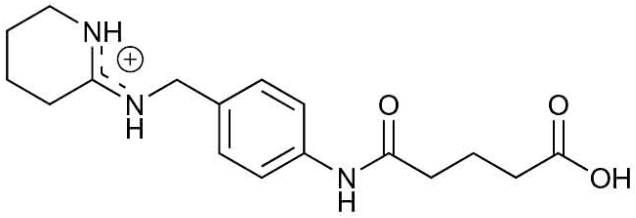
Antibody-catalyzed oxy-Cope Reaction



- The hapten was designed to orient the substrate into its productive chair conformation.

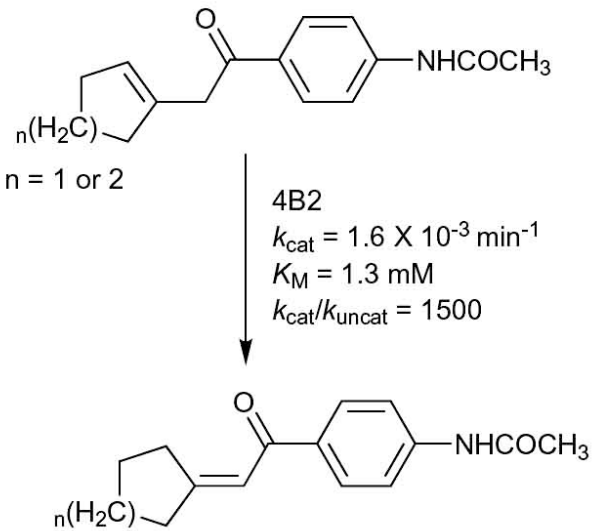
Ulrich, H. D.;Mundroff, E.;Santarsiero, B. D.;Driggers, E. M.;Stevens, R. C.;Schultz, P. G. *Nature* **1997**, *389*, 271.

Antibody-catalyzed Double-bond Isomerization



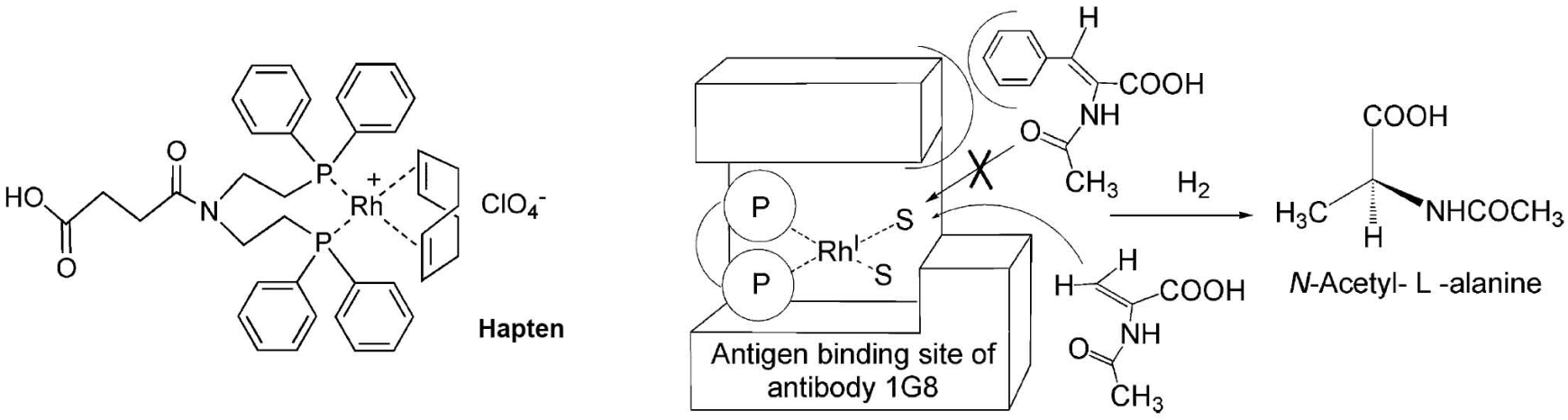
Hapten

- The hapten induced an acidic residue in the active site (Bait-and-switch strategy).
- A dienol intermediate was proposed.



Goncalves, O.;Dintinger, T.;Lebreton, J.;Blanchard, D.;Tellier, C. *Biochem. J.* **2000**, *346*, 691.

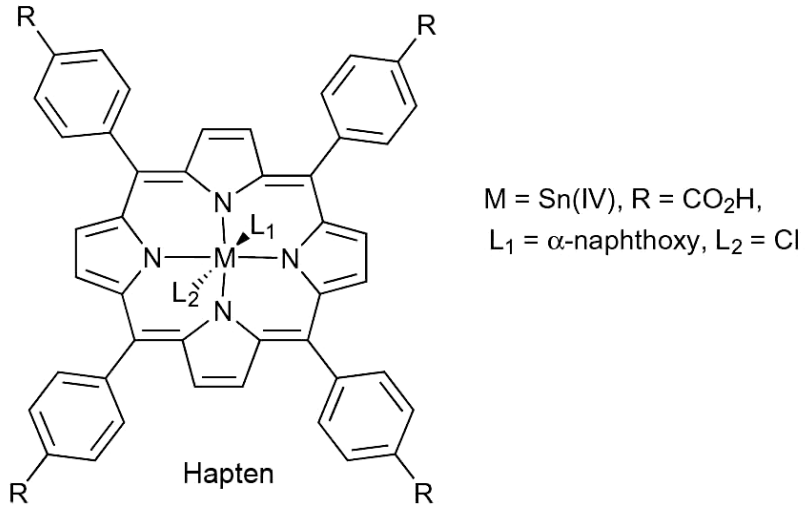
Antibody-catalyzed Asymmetric Hydrogenation



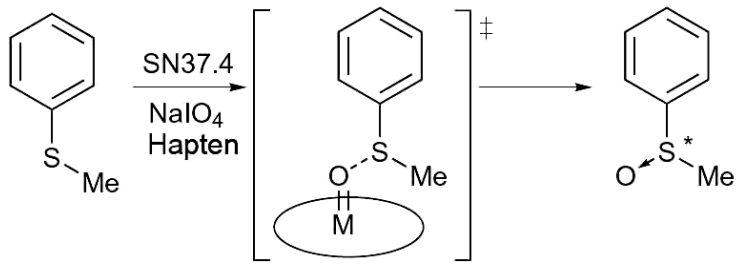
- The hapten was also used as a cofactor for the antibody.
- Yield was low (23%) but enatioselectivity was high (99% ee).

Yamaguchi, H.; Hirano, T.; Kiminami, H.; Taura, D. and Harada, A. *Org. Biomol. Chem.*, **2006**, *4*, 3571–3573

Antibody-catalyzed Oxidation



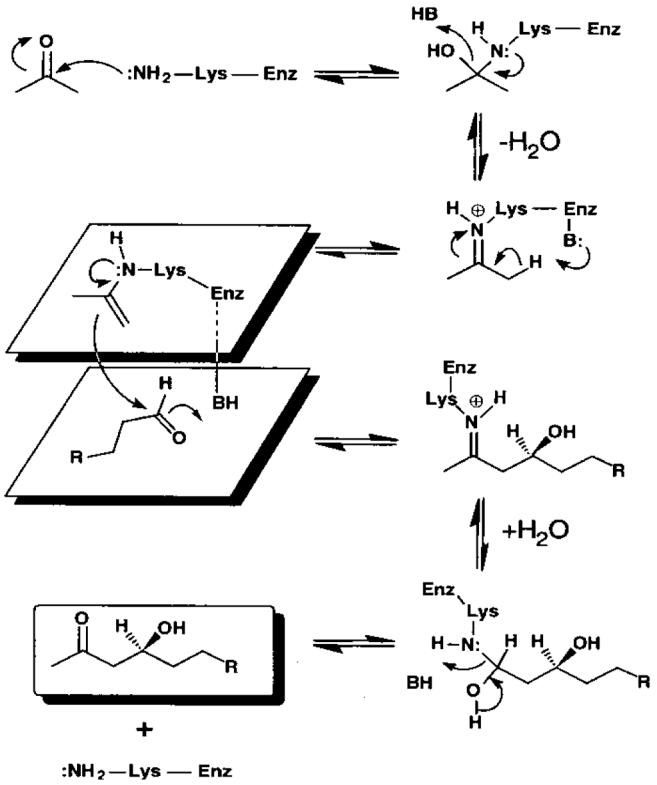
M = Sn(IV), R = CO_2H,
L_1 = α-naphthoxy, L_2 = Cl

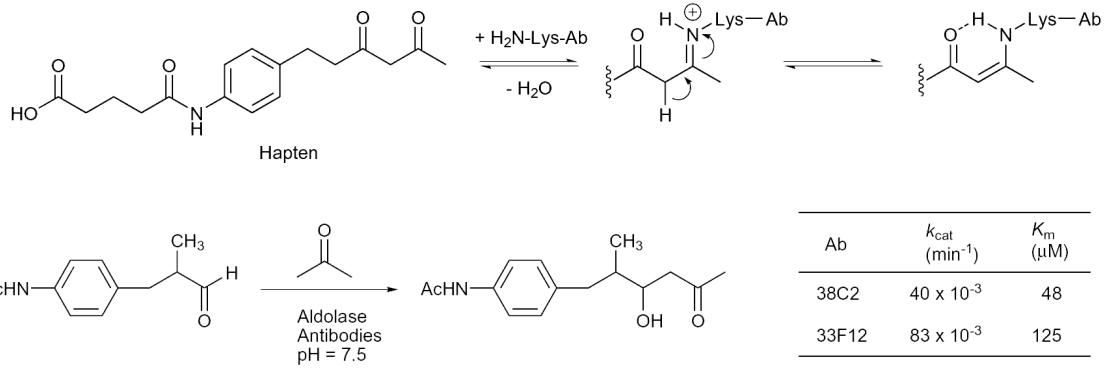


- A Sn(IV)-prophyrin complex was used to elicit the antibody.
- Antibody SN37.4 together with the hapten catalyzed an enatioselective oxidation of aromatic sulfides to sulfoxides.

Nimri, S.;Keinan, E. *J. Am. Chem. Soc*. **1999**, *121*, 8978.

Antibody-catalyzed Aldol Reaction



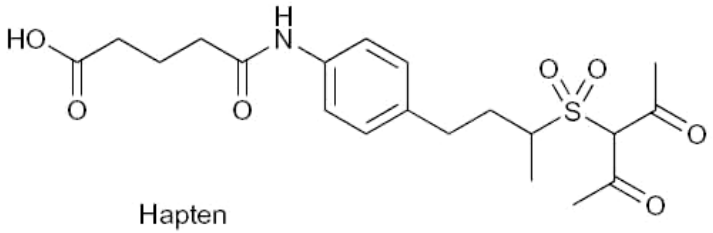


Ab	k_{cat} (min^{-1})	K_m (μM)
38C2	40×10^{-3}	48
33F12	83×10^{-3}	125

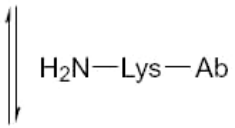
- The hapten trapped a lysine side chain in the antibody, forming an enaminone, thus mimicking the natural class I aldolase mechanism (reactive immunization).

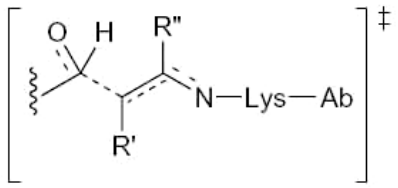
Wagner, J.;Lerner, R. A.;Barbas, C. F. *Science* **1995**, *270*, 1797. 70.
Barbas, C. F.;Heine, A.;Zhong, G. F.; Hoffmann, T.; Gramatikova, S.;Bjornestedt, R.; List, B.;Anderson, J.;
Stura, E. A.;Wilson, I. A.;Lerner, R. A. *Science* **1997**, *278*, 2085.

Antibody-catalyzed Aldol Reaction



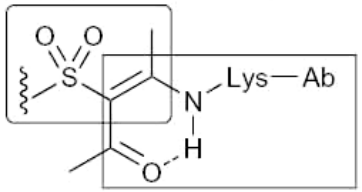
Hapten



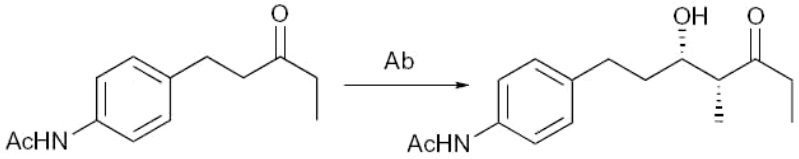


Transition state

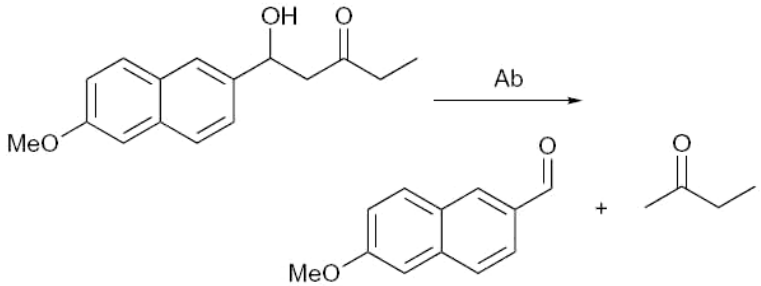
Transition state analog



Reactive immunization



Ab	% *de*	% *ee*
93F3	90 (*syn*-α-isomer)	90
38C2	62 (*anti*-isomer)	59

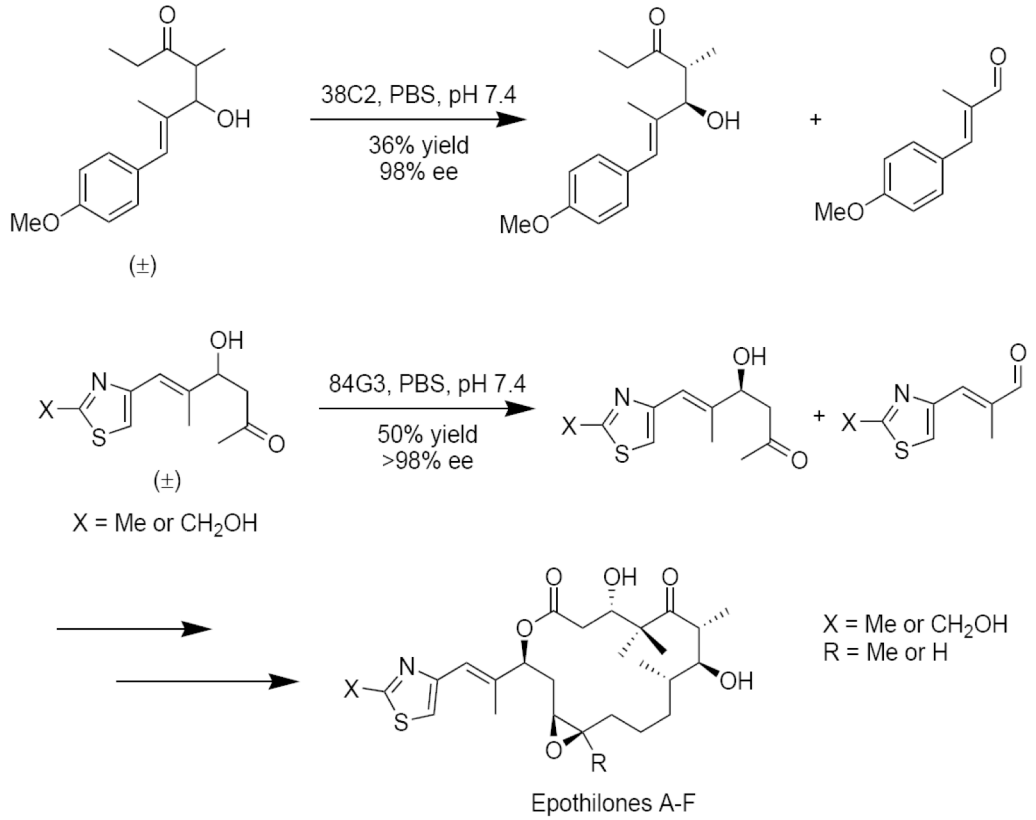


Ab	k_{cat}/k_{uncat}
93F3	4.9×10^7
84G3	5.4×10^7
38C2	5.8×10^4

- The hapten design combined the reactive immunization and TSA strategy

Zhong, G. F.;Lerner, R. A.;Barbas, C. F. *Angew. Chem., Int. Ed*. **1999**, *38*, 3738.

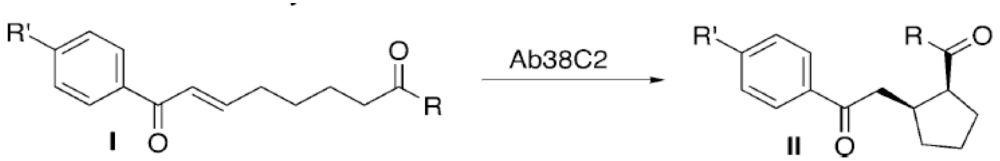
Antibody-catalyzed Aldol Reaction: Application to The Total Synthesis of Epothilones A-F



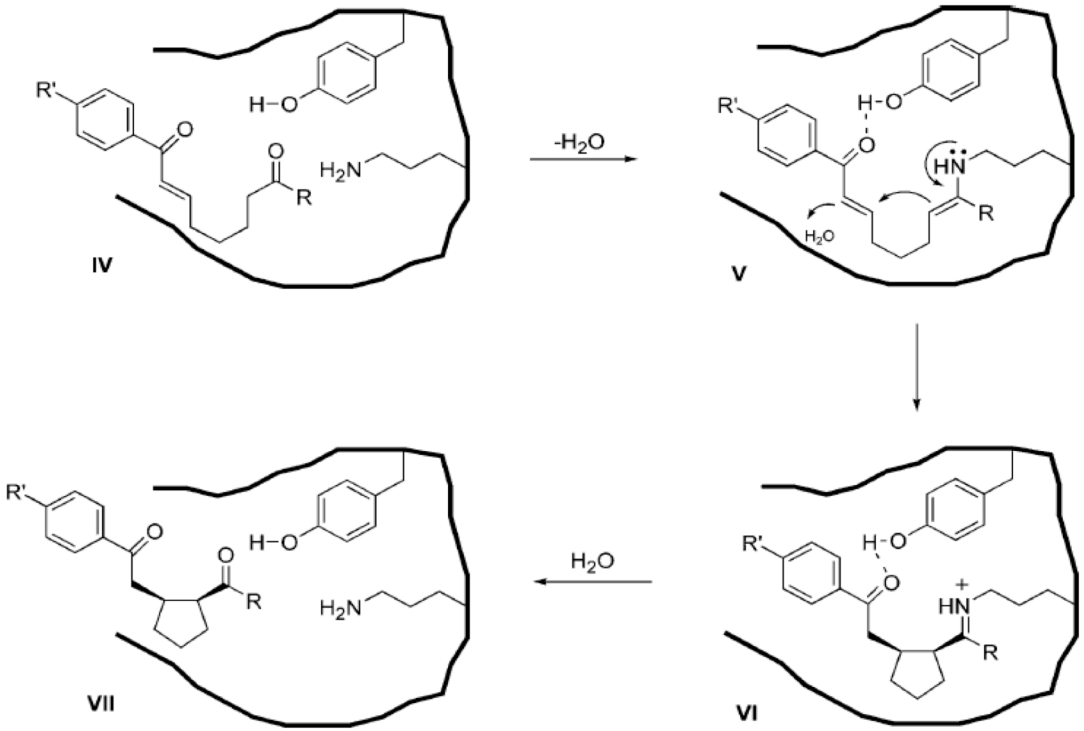
- Aldolase antibodies 38C2 and 84G3 catalyzed enantioselective retro-aldol reactions to resolve racemic subtrates.
- Reactions were performed at multigram scale.

Sinha, S. C.;Sun, J.;Miller, G. P.; Wartmann, M.; Lerner, R. A. *Chem. Eur. J.* **2001**, *7,* 1691.

Antibody-catalyzed Intramolecular Michael Addition



Proposed mechanism:



- The aldolase antibody 38C2 also catalyzed intramolecular Michael addition of aldehydes and ketones.
- Thermodynamically disfavored *cis* products were formed preferetially.
- *Cis*-*trans* ratios of 3:1 to 90:1, ee of 88% to 98% were achieved depending on R'.

Weinstain,R.; Lerner, R. A.; Barbas, III, C. F. and Shabat, D. *J. AM. CHEM. SOC*. **2005**, *127*, 13104-13105

Conclusion

- Catalytic antibodies can be raised against designed haptens, providing artificial enzymes with predefined active site that are capable of catalyzing desired reactions with good efficiency and high selectivity. These transformations often have no natural enzyme counterpart for them.
- The inherent diversity in immune response further expanded the scope of antibody catalysis.
- Although catalytic antibodies may never find large-scale industry applications, it may evolve into a powerful tool for synthetic chemists to realize recalcitrant transformations.
- Catalytic antibodies may also function as drug or drug helper.

Future Work

- Substrate scope
- Catalytic antibody manipulation and evaluation
- Catalysis in organic solvents
- Further increasing its activity
  - Using real transition states as haptens
  - Cofactors