Chemistry 304B **Lecture notes 36--The last lecture** **Fall, 1999**

Final Exam: Thursday, May 13th Review sessions (tentative) May 12 at 2 pm in rm 324 Frick
May 11 at 2 pm in rm 120 Frick

Watch your email. We will do the course evaluations at the Final exam

Catalytic Antibodies:

I. Organic chemists can synthesize a wide range of organic molecules; no limit in principle

Problems:

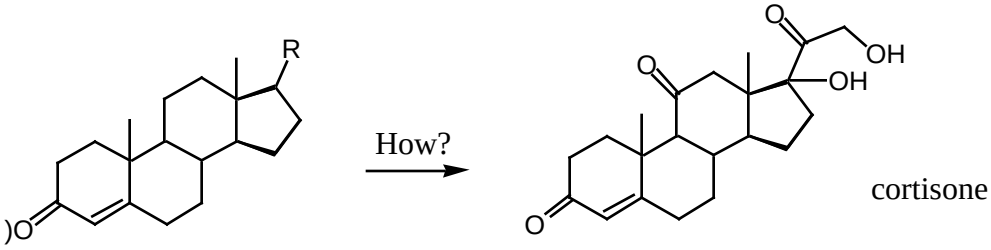
A. Selectivity

1. Functional group compatibility--form new bonds selectively at certain carbons without interference by other functional groups
2. Stereo-selectivity
   produce E or Z double bond selectively
   produce one enantiomer or diastereoisomer selectively
3. Regio-selectivity--selective reaction at one end of a functional group with two or sites of similar reactivity. E.g., orientation in addition to a double bond.

B. Efficiency--yields, scale, byproducts

II. Nature provides ENZYMES to catalyze organic reactions Selective, efficient

Problem: Can only do the reaction needed for Nature.



cortisone

Impossible in conventional organic chemistry

III. Do-it-yourself enzymes: Define a needed reaction and find an protein to catalyze it. Not necessarily a "natural" enzyme.

Monkey/typewriter approach: Make an infinite (or at least large) number of polypeptides. One of them, by chance, should be a good catalyst for a given reaction.

Improve the odds: Directed synthesis: find a way to make a large set of polypeptides which can associate with substrates in a way to favor the desired reaction. Then find the best one, and develop it.

What kind of binding do we want?

An enzyme makes a reaction faster by lowering the energy of the transition state: recall chymotrypsin

Enzyme catalysis can be very effective by binding to the **transition state** for a reaction,
more than to the reactants or the products.

Problem:

1. Create a transition state for a reaction
2. Find a protein to bind to it.

How did Nature do it? Evolution, with a time cycle of thousands of years. Too slow.

But there is one mechanism for generation and rapid "evolution" in the properties of proteins:
The Immune System

A foreign molecule (or part of a larger structure)

Antigen: recognized by the immune system and a protein is created to bind to it--the antibody

Mechanism: the genes coding for antibodies are rapidly shuffled during the immune response and 10^6-10^8 distinct protein structures are created. Those which bind well to the antigen are produced in quantity and also refined in structure by a second iteration of antibody generation. A collection of proteins are generated in quantity which bind tightly and in different ways to the antigen. The antibody-antigen complex is dealt with by the further processes in the immune response.

Proposal: create a "transition state" for a reaction (a molecule, the antigen), feed it to a mouse, and raise antibodies. These antibodies should then bind to the substrates and favor formation of the transition state; they work as enzyme-like catalysts for the reaction. Isolate the most effective antibody, add the substrates for the reaction, and assay the rate enhancement (catalysis) due to the effect of the antibody binding.

Problem: When an organism generates antibodies, a large collection (mixture) is produced. Not easy to separate on large scale.

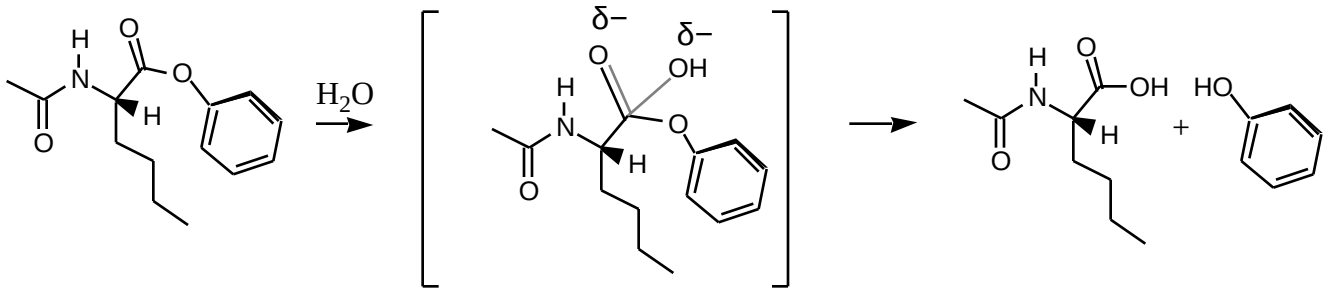
New technology: 1975: hybridoma technology. Create "immortal" spleen cells, which can grow in cell culture and produce one antibody type for each cell. Analytical techniques to do "cell sorting", and then grow homogeneous cell type, which make a single antibody. Eventually can produce multi-gram quantities of homogeneous.protein, "monoclonnal" antibodies.

Now: Feed "transition state" antigen to mouse, allow antibody response, sort the spleen cells for those generating antibodies with good affinity for the antigen. Culture those cells and harvest a small collection of pure antibodies.

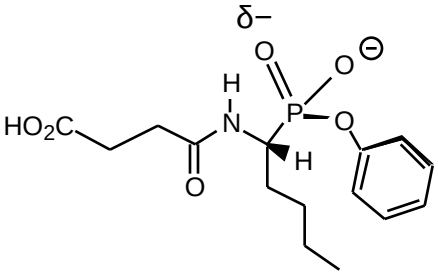
Case studies:

A. Ester hydrolysis

partial charges, long bonds



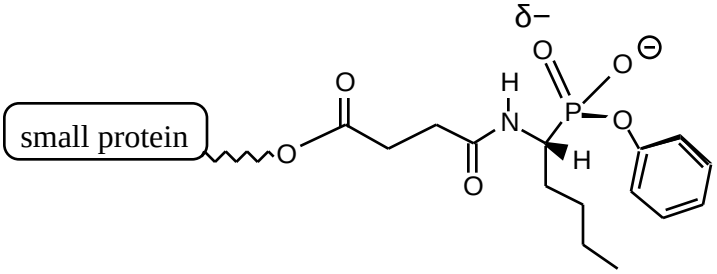
Transition state analog:



a phosphonate derivative

charge, partial charge, long bonds

Attach to a convenient antigenic protein to engage the immune system:



a phosphonate derivative

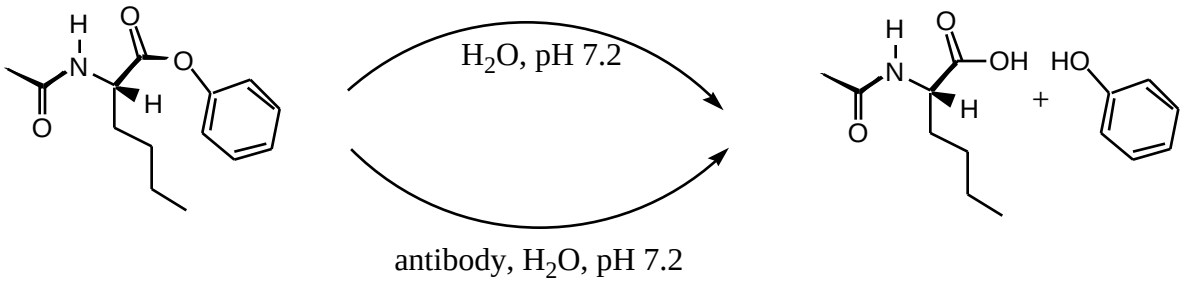
charge, partial charge, long bonds

This is the antigen: inject in a mouse, harvest the spleen cells and evaluate the antibodies being produced. Some bind to the protein, some to the desired molecule portion

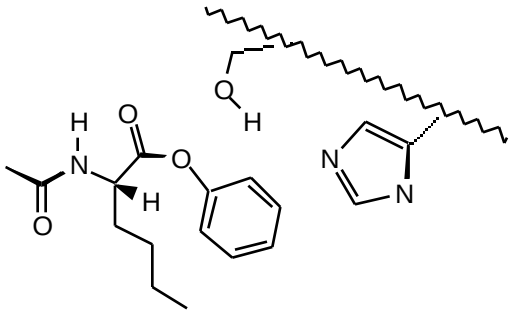
Grow those cells which produce antibody which binds strongly to the transition state analog (not protein)

Evaluate each in turn for catalytic activity:

In this case, one antibody (17E8) was particularly effective $k_{cat}/k_{uncat} = 2.2 \times 10^4$



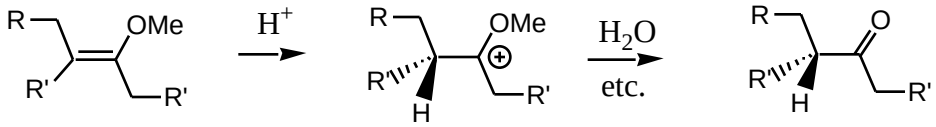
The antibody can be crystallized while bound to the antigen, and the detailed structure determined by X-ray crystallography:



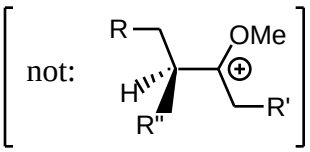
histidine/serine side chains
(just like regular acyl cleavage enzymes)

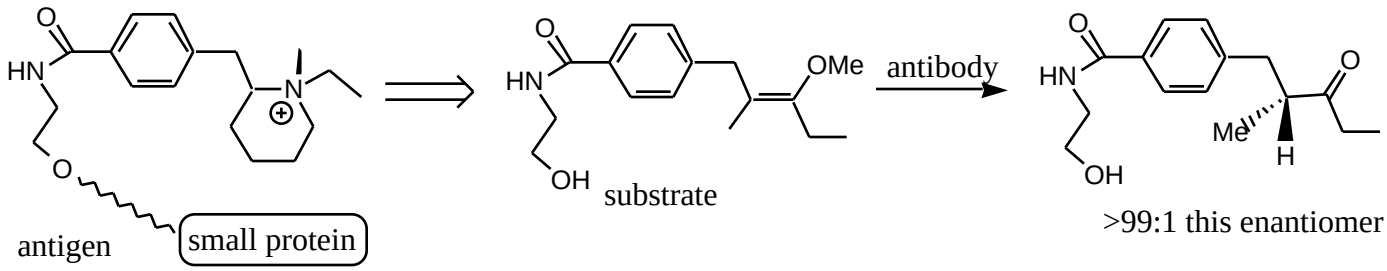
B. Asymmetric protonation

Desired:



not:

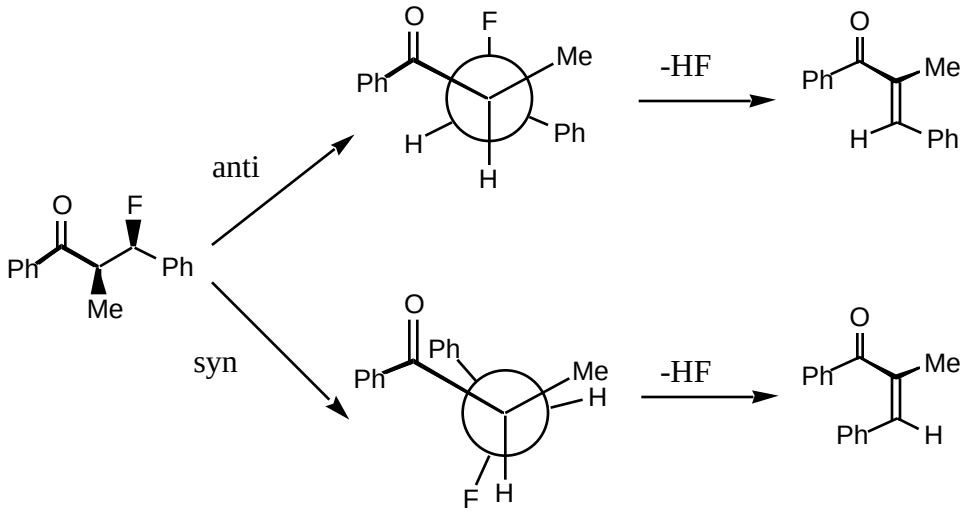




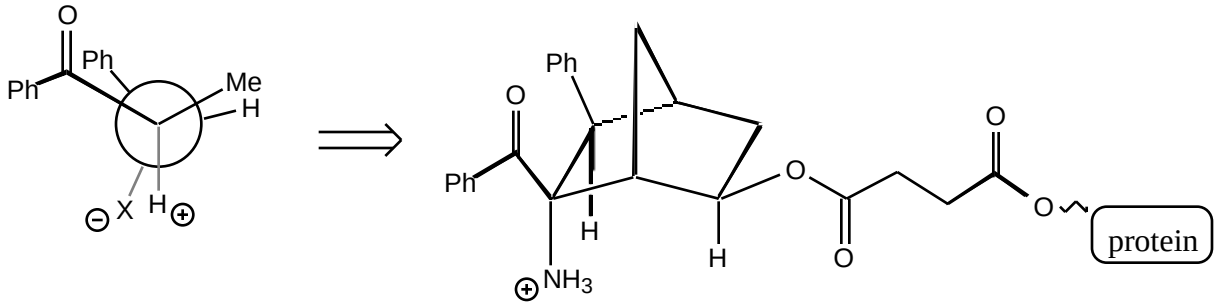
Quite general catalyst for protonation of substrates (e.g., think about acid catalyzed epoxide ring opening)

C. Catalyzing the "wrong" selectivity

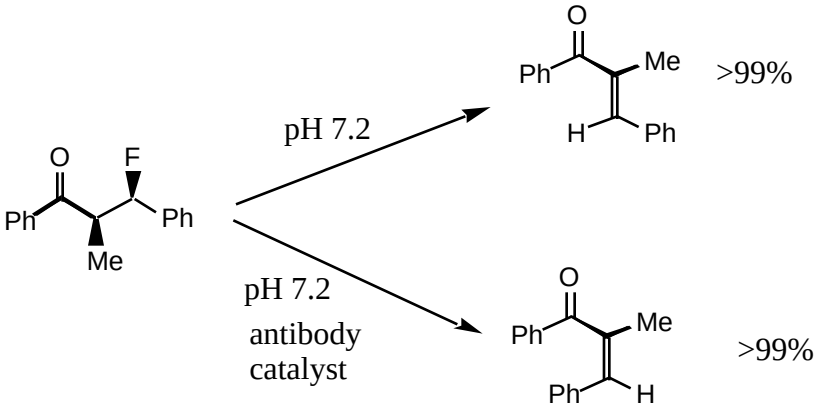
If two competing transition states, in principle the antibody catalyst can be generated for the less favorable TS, and make it the favored process under catalysis: E2 elimination



Design transition state mimic for SYN transition state:



Hook onto antigenic protein, raise antibodies, select best binders, culture to produce large amounts, test:



At least 5 kcal/mol extra stabilization for the syn TS

That's it for Orgo! Best wishes on the final and I hope you find Orgo coming back to you in the future.

Check your email from time-to-time.