Organic Synthesis Supported by Antibody Catalysis

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new tools

Abstract: Catalytic antibody research has provided a remarkable number of catalyzed reactions since it was begun thirteen years ago. This paper introduces a few antibody-catalyzed reactions that may be of particular interest to the organic synthetic chemist. It also gives a short introduction into the production of hapten-binding monoclonal antibodies and modern improvements for catalyst discovery.

Key words: biocatalysis, catalytic antibodies, aldol reactions, pericyclic reactions, cationic olefin cyclizations, kinetic racemate resolution

1 Introduction

This contribution is aimed at providing the chemist with a brief chronological survey of some of the synthetically more useful transformations catalyzed so far by antibodies. It is also meant to give a means to evaluate the practical utility of the presented catalytic antibodies. For an exhaustive compilation of the abzyme literature until 1996, the reader is referred to a review article by Thomas.[1] Recent surveys give a balanced profile over the entire field,[2,3] or discuss the stereoselectivies induced by antibodies[4] and the physicochemical conclusions derived from the complete body of work in the field[5].

The analogy between enzyme activity and antibody affinity was first pointed out by Pauling in 1946.[6] Twenty-three years passed until Jencks postulated that antibodies generated against an organic molecule resembling the transition state of a given reaction should catalyze this process.[7] The enzyme-antibody analogy was picked up again in 1975 when Raso and Stollar tried to generate a polyclonal antibody mixture against a quasi-transition-state analogue that would exhibit catalytic activity for Schiff-base formation as part of a reaction analogous to pyridoxal-assisted amino acid metabolism.[8] Indeed, they observed binding of both substrates in this bi-molecular reaction, but no catalysis. With the introduction of the hybridoma technology in 1976[9] that allows for the large-scale production of monoclonal antibodies of the G class (IgG, see glossary), the stage was set for discovery of catalysis in such preparations by Tramontano et al.[10] and Pollack et al.[11] in 1986. Monoclonal antibodies are by definition "pure chemicals", as are for instance low-molecular-weight transition-metal catalysts. They exhibit a defined three-dimensional structure with such minor flexibilities, as also observed with induced-fit phenomena in enzyme catalysis.[12,13]

2 Immunization

To produce monoclonal antibodies with predetermined specificity for a small organic molecule – the hapten – one needs to prepare an antigen that triggers a satisfactory immune response.[14] Small molecules are not generally immunogenic. Thus, they need to be displayed on a carrier protein as a surface epitope to be recognized by the immune system. Two different proteins are commonly used, keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). A hapten design always needs to include a linker moiety for conjugation to lysine or cystein residues on the protein surface. Only a sufficient spacer length ensures full recognition by the immune system. The resulting hapten-KLH conjugate is solely used for immunization because its particularly foreign nature towards the mouse immune system results in high immunogenicity. The hapten-BSA conjugate is instead preferred in the enzyme-linked immunosorbent assay (ELISA) for two reasons:1) A carrier protein different from the one used for immunization needs to be employed in affinity tests like ELISA to prevent selection of antibodies or hybridoma cells that have solely carrier-protein affinity; 2) BSA is a much more robust protein, showing less tendency to precipitate, and also surviving both storage at -20 °C and repeated thawing.

The immunization is carried out on a time course of approximately six to ten weeks. During this time, two different mechanisms enable the immune system to develop affinities in the order of -6 to -15 kcal/mol ($K_D = 10^{-4}$ to 10^{-10} M) to the presented antigen. First, genetic recombination of the germ-line genes V, D and J (acquired by inheritance) lead to a large number of gene products, the germ-line antibodies, that are already selected for affinity by the immune system (see glossary for special terms). To further diversify and increase the pool of different antibodies for an improvement in affinity, the recombined genes are now subjected to somatic mutation (“affinity maturation”), thereby increasing the chances for selecting high-affinity binders. After about ten weeks, the result of this refinement of the immune response can be harvested. A representative procedure consists of the following:

The immunized mouse is sacrificed to isolate the B (*b*one-marrow) cells in the spleen that carry the complete immune history. Thus, a vast diversity of labile antibody-producing B cells are obtained that are now fused as part of the standard hybridoma procedure with myeloma cells (a cancer cell line) under the influence of polyethylene glycol to give immortal hybridoma cells. They will be plated into fifteen 96-well plates, where most of them will already be monoclonal (the well contains only genetically identical cells). However, some will not live and others will shed extra chromosomes to stabilize. A typical fusion will thus yield from 150 to 1200 hybridomas. Not all of them will be hapten-specific antibody-secreting cells, so they need to be screened for antigen-binding by ELISA. This will yield from one to ten percent (15 - 120) positive clones with the desired specificity. It is common to select only the five to twenty-five best-binding clones for large-scale production of antibodies and subsequent testing for catalysis.

Recently, *in vitro* immunization was presented as a promising variation for the above procedure.[15] In only three days at 37 °C, isolated B cells of ten day-old mice develop specificity for the co-incubated non-conjugated hapten. Subsequent fusion with a myeloma cell line leads to hybridoma cells that are processed as described above. Catalytic antibodies with carbonate-hydrolysis and glycosidase activity have been secured through this novel procedure.[15,16] However, while this technology has apparent advantages for labs that do not have animal facilities, many antigens do not trigger B cells to develop specificity and, in addition, another class of immunoglobulins (IgM) is obtained that generally exhibits lower affinity (10^{-5} - 10^{-6} M) than IgG.

By nature the immune system is chiral. As a consequence, both antipodes of a racemic hapten displayed on a carrier protein will be considered separate surface epitopes that result in the formation of independent antibodies.[17] Notably, their binding sites do not have an enantiomeric relationship; in fact these immunoglobulins are likely to have completely different genetic plans. However, if the generation of an antibody with a predetermined *R* or *S* selectivity is desired, then the use of a hapten displaying the corresponding absolute configuration is useful.[18]

3 Monoclonal Antibody Production

Initially, the selected clones consist of only a few identical cells. These cells constantly divide and can be cultured to raise enough antibody for catalysis testing. Growing large amounts of antibody can be done *in vivo* through the ascites method (see glossary) or *in vitro* through the cell-culture method. The former produces antibodies in large amounts and high concentration and used to be generally superior to the culture method. However, recent market developments are making the latter method more and more competitive by increasing antibody yield while significantly decreasing costs and production time through the use of specially designed cell compartments. In addition, lab animal ethics would suggest preferring the culture method.[19,20] The ascites fluid obtained from mice contains approximately 3-8 mg per milliliter of monoclonal antibody while the supernatant of cultured hybridomas delivers about 0.5-2 mg per milliliter at the current state of the art. The advantages and disadvantages of both methods are currently almost balanced. Depending on one's schedule, one would prefer the ascites method, when small amounts of monoclonal antibody (<100 mg) are needed quickly. Conversely, one would culture the hybridoma clone to obtain large amounts of protein (200 mg or more, where the method becomes increasingly more cost effective) that is initially more pure and needs only two of the purification steps below.

After having grown enough antibody, two to three purification steps are generally applied to obtain highly pure monoclonal antibodies. 1) salt precipitation with sodium ammonium sulfate ("SAS cut", only for initial purification of ascites), 2) ion exchange chromatography on DEAE-sepharose, and 3) a.) affinity chromatography on a column that contains an immobilized receptor ("Protein G") binding strongly to IgG or b.) ion exchange chromatography

Biographical Sketches

Jens Hasserodt was born in Bonn, Germany in 1965. He graduated from the Department of Chemistry, University of Heidelberg in 1990 and received his Ph.D. degree at the same department in 1993 under the supervision of Professor Dr. W. Sundermeyer. He spent two years as a postdoctoral fellow in the group of Profs. R. A. Lerner and K. D. Janda at The Scripps Research Institute before being promoted to Senior Research Associate. Effective January 1998 he was appointed Assistant Professor at the Department of Chemistry at the same Institute. His research interests are directed at the productive interplay of Molecular Biology and Synthetic Chemistry in utilizing and expanding Nature's combinatorial mechanisms for discovery of novel activity.

("Mono Q"). Steps 2 and 3a. will be reversed when purifying culture supernatants, because the comparatively large sample volume obtained by this method does not impair the success of the affinity chromatography. Afterward, the purified monoclonal antibody, either derived from ascites or culture supernatants, only needs to be concentrated by centrifugation in ultrafiltration devices to adjust the concentration to the desired level for carrying out chemical tests.

As has become apparent in sections 2 and 3, production of *anti-hapten* antibodies requires considerable financial and personnel commitment by the laboratory that is interested in generating catalytic antibodies for new synthetic applications. Although the procedure is applied routinely worldwide, a good deal of precaution has to be taken to assure the successful isolation of hapten-specific antibodies. Many organic chemistry labs have thus opted for collaboration with labs that are traditionally involved in hybridoma work. Alternatively, many companies offer such services, too.

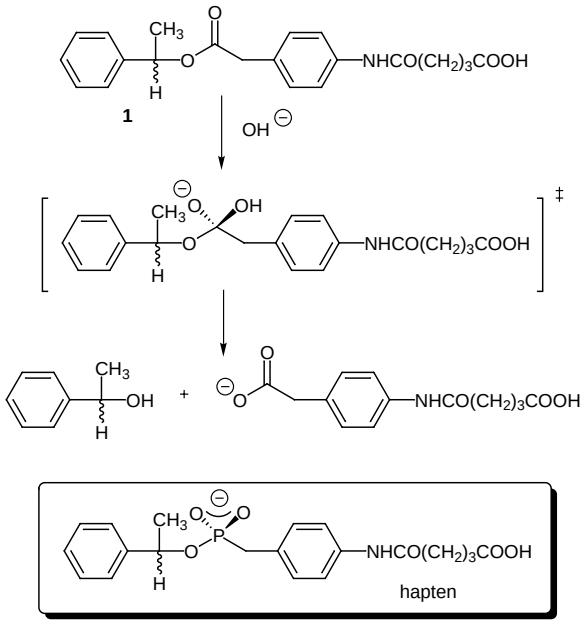
Whether *catalytic* antibodies can be identified from these collections of antibody-producing clones is a completely different matter. It depends on the ingenuity of the chemist in designing a hapten that is most congruent with transition state theory and physical requirements of the antibody-binding site. Additionally, the selection technique employed is of great importance.

The following section provides some examples where these requirements were met resulting in novel biocatalysts for organic transformations.

4 Representative Examples in Antibody-Catalyzed Organic Reactions

Ester Cleavage and Transesterification

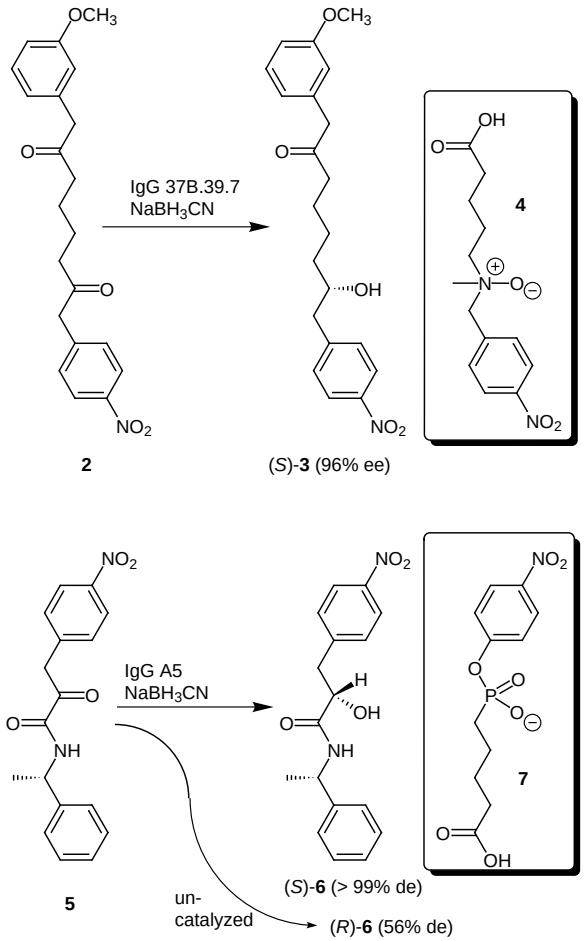
An early example (1989) of acyl-transferase/esterase antibodies already emphasized the potential of abzymes for use as reagents in synthesis.[21] Natural counterparts, the lipases, have been successfully used as transesterification catalysts for stereoselective acylation and kinetic resolution of alcohols.[22] Antibodies PCP2H6 and PCP21H3[#] displayed *(R)* and *(S)* enantiospecificity for an unactivated chiral ester substrate **1**, respectively (Scheme 1). These early abzymes exhibited fairly strict substrate specificity compared to their enzymic cousins. They have been proven to operate through an acyl intermediate in analogy to natural serine proteases.[23-25] Various evidence for "covalent catalysis" has also been accumulated for other hydrolytic antibody catalysts (immunoglobulins 20G9[26], 4A1[27]) and for a duo of genetically related aldolase antibodies (38C2 and 33F12) in analogy to natural class I aldolase enzymes (see below). The latter antibodies were shown to operate by Schiff base formation involving the amino group from a lysine residue. Covalent catalysis is strongly connected to the concept of Effective Molarity (EM) that relates to assisting functionalities within the protein's active site, particularly their concentration at, or proximity to, vital parts of the activated complex.[28] Such functionalities (e.g. an imidazole from histidine, a phenol from tyrosine, a serine hydroxyl group, or lysine amino group) may form a covalent bond to a reaction intermediate.



Scheme 1

Hydride Reduction of Carbonyl Compounds

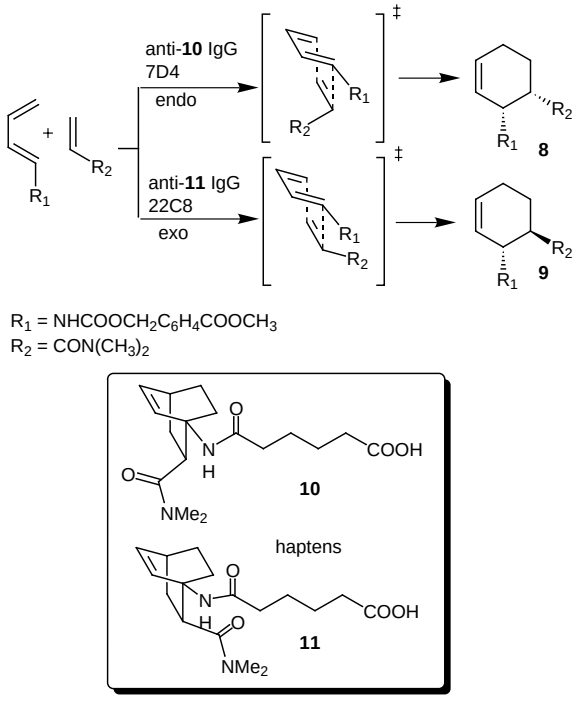
Reductions have been catalyzed, too. Two independent reports from the same laboratory (1992 and 1993) described two antibodies that utilized sodium cyanoborohydrate for reduction (Scheme 2). Antibody 37B.39.3 reduced diketone **2** to the hydroxyketone **3** regio- and enantioselectively.[29] It was elicited against the nitrobenzyl aminoxide hapten **4**. The slight deviation in nature and location of the aromatic substituents in substrate **2** was sufficient for 37B.39.7 to distinguish the two carbonyl groups, thus affording product **3** in 95.5% yield and 96% ee. Antibody A5, elicited against nitrophenyl phosphonate **7**, catalyzed the reduction of alpha-keto amide **5** to the hydroxyamide **6** with sodium cyanoborohydrate diastereoselectively.[30] The reduction of alpha-*(S)*-(-)substrate **5** in the absence of any catalyst resulted in formation of the 2*R*-configurated hydroxyamide **6** with a diastereomeric excess of 56%. Antibody A5 completely rerouted this reaction, forming the 2*S*-configurated **6** in >99% de. Both the *N*-oxide and the phosphonate hapten (**4**, **7**) can be interpreted as true transition-state analogues mimicking the developing negative charge on the carbonyl oxygen of the substrate during hydride attack. The catalysts exhibited good to modest rate accelerations (10^6 and 2.8×10^2, respectively) and did not appear to be significantly harmed by the reductive agent.



Scheme 2

Diels-Alder Cycloadditions

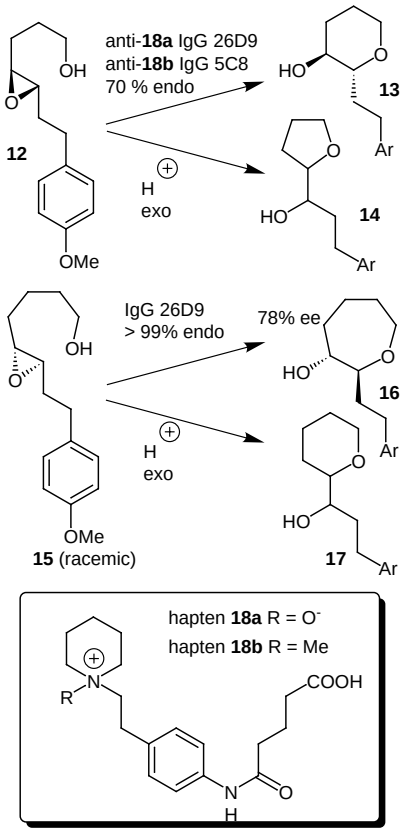
In 1993, an antibody has been elicited that altered the outcome of a Diels-Alder reaction (Scheme 3). The monosubstituted diene and dienophile may approach each other in four different orientations along the reaction coordinate. Both *endo* and *exo* orientations give rise to two chiral cycloaddition products **8** and **9**.[31] Antibody 22C8 was raised against hapten **11** that mimics the *exo* approach of both reactants while representing the expected boat configuration of the transition state. In the absence of any catalyst, the *endo* orientation is favored over the *exo* either in refluxing toluene (66:34) or in aqueous buffer at 37 °C (85:15). This *endo* preference can be attributed to secondary orbital interactions. The energy barriers for the favored *endo* and disfavored *exo* approaches differ by 1.54 kcal/mol, and thus the activation energy for the disfavored *exo* process falls easily within the binding energy deliverable by an antibody (up to 20 kcal/mol). Conclusively, this reaction is ideally suited for antibody catalysis because it is under kinetic control yielding mostly the thermodynamically higher-energy *endo* product. Indeed, antibody 22C8 rerouted this reaction, forming the *exo* product **9** exclusively and with high enantioselectivity (>97%). This result demonstrated the ability of the antibody to act as an entropy trap and distinguish between both possible orientations in the transition state. Immunization with hapten **10** that mimicked the *endo* approach also resulted in an antibody catalyst. And, as designed, this antibody (7D4) accelerated formation of the favored *endo* product. While the utility of these catalysts to the organic chemist is obvious, the reported low rate acceleration calls for some refinement of hapten design or of antibody 22C8 itself. In fact, to outrun the background reaction, showing a reversed isomer distribution, 27 mol-% of catalyst 22C8 had to be used. This makes such a catalytic process interesting only from a theoretical, mechanistic point-of-view. Product inhibition is a particular concern when attempting to raise antibodies with a hapten that resembles a very late, product-like transition state, as is the case for the Diels-Alder reaction. An interesting early accomplishment that overcame this problem involved tetrachlorothiophene-*S*,*S*-dioxide as diene. The resulting cycloadduct spontaneously extruded sulfur dioxide thereby completely loosing any congruency with the antibody-inducing antigen.[32]



Scheme 3

Ring Closure Reactions by Nucleophilic Epoxide Opening

To date, many antibodies (like the Diels-Alderase 22C8) have been generated that catalyze the formation of products along *disfavored* reaction pathways. A groundbreaking result was the characterization of antibody 26D9 in 1993 followed by antibody 5C8 in 1999. Anti-**18a** IgG

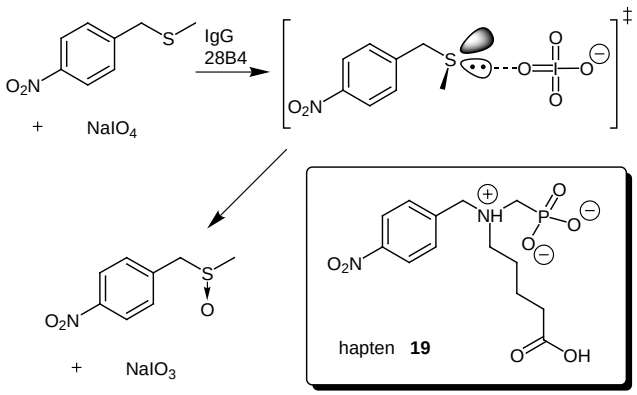


Scheme 4

26C9 and anti-**18b** IgG 5C8 accept substrate **12** to produce tetrahydropyran **13** (Scheme 4).[33,34] This result clearly violates Baldwin's rules of ring formation processes that predict formation of **14** via *exo* addition, as is observed in the absence of antibody.[35] In addition, 26D9 was proven to also reroute transformation of **15**, forming *endo*-product oxepane **16** instead of *exo*-product tetrahydropyran **17**.[36] Conventional syntheses of oxepane systems do not usually start from epoxy alcohols, but use more difficult yet higher yielding routes instead.[37] Antibody 26D9 is stereospecific, transforming only the (*S*,*S*)-enantiomer of substrate **12** to (2*R*,3*S*)-**13** [33] and the (*R*,*R*)-enantiomer of **15** to (2*S*,3*R*)-**16**. 5C8 was found to transform both antipodes of **12** but with different product distributions.[34] Only the (*S*,*S*)-enantiomer is converted by 5C8 to the disfavored product **13** whereas (*R*,*R*)-**12** almost exclusively yields **14** that is also the product of the background reaction. From a stereochemical viewpoint, 26D9 is therefore the more useful catalyst since racemic starting material can be introduced. A structure determination of antibody 5C8 revealed the presence of an aspartate and a histidine at decisive loci, thus favoring a mechanism involving general acid/base catalysis.[34] The 26D9-catalyzed reaction has been automated for large-scale production under biphasic conditions. Hence, portions of starting material were repeatedly introduced into an apparatus containing a reactor that allowed for intermediate removal of the product-containing organic phase before being charged with the next portion. This non-continuous procedure significantly reduced product inhibition, a phenomenon frequently seen in antibody catalysis.[38]

Sulfoxidation

A report from 1994 announced the first example of an antibody-catalyzed oxidation involving an inorganic reagent. Immunization with an alpha-ammonium methanephosphonic acid hapten **19** gave antibody 28B4 which accelerated the bimolecular oxidation of a methionine analogue to its sulfoxide in the presence of sodium periodate (Scheme 5).[39] A rate improvement of 2.2×10^5 over background was observed which makes 28B4 as efficient as natural flavin-dependent monooxygenases and microsomal *P*-450 isozymes. Importantly, the oxidative reagent did not harm the protein catalyst. While the nitro-substituent needed to be present in every substrate for efficient antibody recognition, variation of the aliphatic sulfur substituent was remarkably tolerated by 28B4. The crystal structure obtained for this antibody and its complex with the hapten belongs to the best resolved to date (2.2 Å, 1.9 Å, respectively).[40] The structural details revealed low recognition of the aliphatic linker functionality in hapten **19**, which was in accord with the observed flexibility in accepted aliphatic sulfur substituents. This also appeared to be a reason for the observed modest enantioselectivity of 28B4 (16% ee for chiral sulfoxide formation). The generation of 28B4 is an important improvement in a field that calls for contributions towards highly catalytic, as well as enantioselective sulfoxidation.[41]

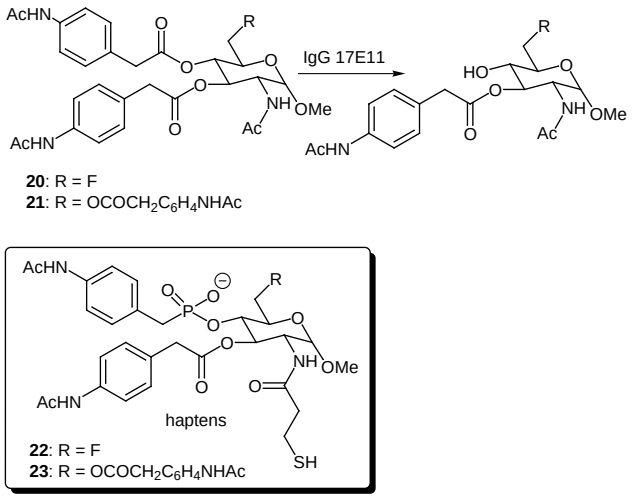


Scheme 5

Regioselective Carbohydrate Deprotection by an Antibody Esterase

Creation of a catalyst that discriminates between chemically identical functional groups is a desirable target. In 1994 an antibody (17E11) has been generated that selectively and efficiently deprotected one particular hydroxyl functionality in carbohydrate **20** (Scheme 6). For induc-

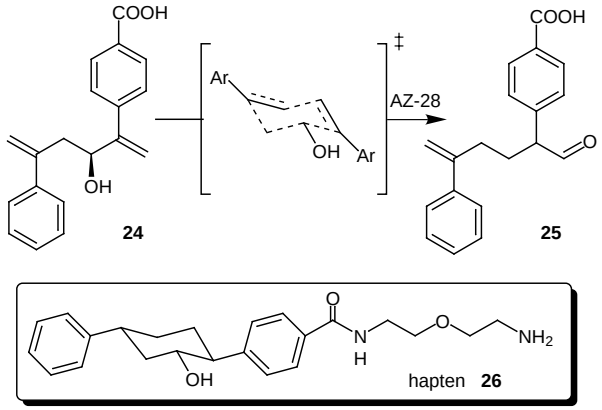
tion of this catalyst, the classical approach for mimicking the transition state of ester hydrolysis was chosen – incorporation of a phosphonate at position C-4. The hapten design was further based on calculations that estimated the total solvent accessible area of the two-ester moiety. Thus it was assumed that the size of the two ester groups would be enough to occupy the antigen-binding site. Conclusively, the combined surface area around C-2, C-6 and the glycosyl moiety should have been solvent accessible and thus allow any obtained catalyst to bind to a variety of substrates. While 17E11 neither discriminated between different configurations at the anomeric center nor between protected or unprotected C-2, the antibody showed reduced activity when charged with fully protected substrate **21**. The tailored specificity for substrates containing no bulky substituent at C-6 reduced the antibody's potential scope. Among the two possible strategies to overcome this problem – immunization with a modified hapten carrying a bulky C-6 substituent or alteration of 17E11 by mutagenesis – the authors chose the latter. To this end they created a combinatorial, phage-displayed library (see section 6) of 17E11 by random mutagenesis of part of the antibody-binding site and selected mutant "115" by affinity to altered hapten **23**.[42] Mutant 17E11-115 indeed catalyzed hydrolysis at C-4 in substrate **21** with 12-fold higher activity over the parent antibody 17E11.



Scheme 6

An Oxy-Cope Rearrangement

Using cyclic structures to mimic highly-organized transition states of electrocyclic reactions is particularly fruitful. The transition state of an oxy-Cope rearrangement has been predicted to involve a cyclic chair conformation. In 1994, immunization with hapten **26**, that displayed these characteristics, resulted in a germ-line antibody (see glossary) that catalyzed transformation of allylic alcohol **24** to aldehyde **25** (Scheme 7).[43] The observed rate enhancement approached that of a related pericyclic reaction catalyzed by the enzyme chorismate mutase.[44] Affinity maturation (see glossary) of this antibody gave AZ-28 that had six amino acid substitutions, one of which resulted in a decrease in catalytic rate.[45,46] This underlines the importance of the notion that one needs to select for catalysis in contrast to the immune system, which refines its antibodies solely on the basis of affinity. Nevertheless, the immune system's affinity maturation has been shown in other cases to be very beneficial if the hapten, as designed by the chemist, has a particular congruency with the transition state.[47]

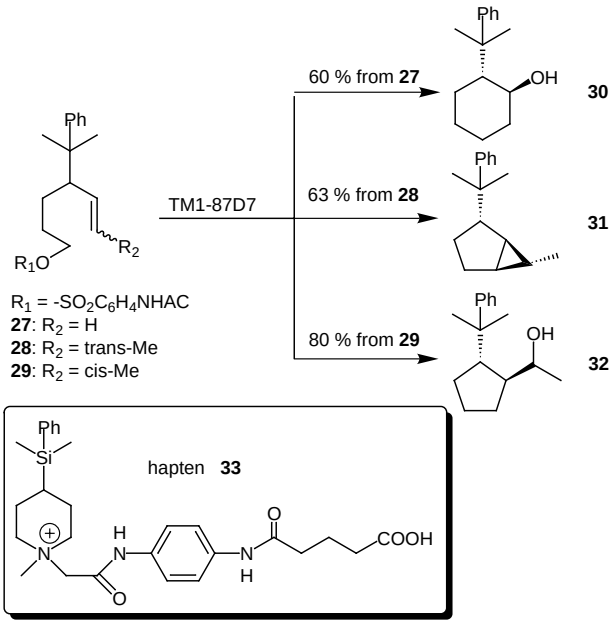


Scheme 7

Cationic Cyclization

Electrophilic ring closure reactions are the basis of diversity seen in the natural products class of the terpenoids. The biosynthesis of cyclic terpenoids starts from substrates that contain a polyisoprenoidal hydrocarbon portion and a leaving group for initial carbocation formation. Nature employs either pyrophosphate or epoxide moieties. In 1994 and 1996, catalytic antibodies have been elicited by use of conceptually different hapten designs that catalyze the simplest form of this cyclization reaction, formation of a monocycle.[48,49] In both cases, an arylsulfonate was chosen as the leaving group for ease of assay and contribution to substrate recognition by the antibody. The earlier investigation yielded an antibody (TM1-87D7) that was raised against hapten **33** to accomplish six-membered ring closure (Scheme 8). Indeed, **30** was formed from substrate **27**, but surprisingly, subtle changes in substrate structure led to remarkable changes in product outcome, as has been reported later.[50] The *trans*-olefin **28** was transformed to a bicyclo[3.1.0] system **31**, exclusively. This product is not observed in the uncatalyzed reaction. Changing to a *cis*-olefinic substrate (**29**) yielded a five-membered ring system (**32**). The rate acceleration by TM1-87D7 was within an order of magnitude of those of natural enzymes catalyzing similar processes.[50] Unfortu-

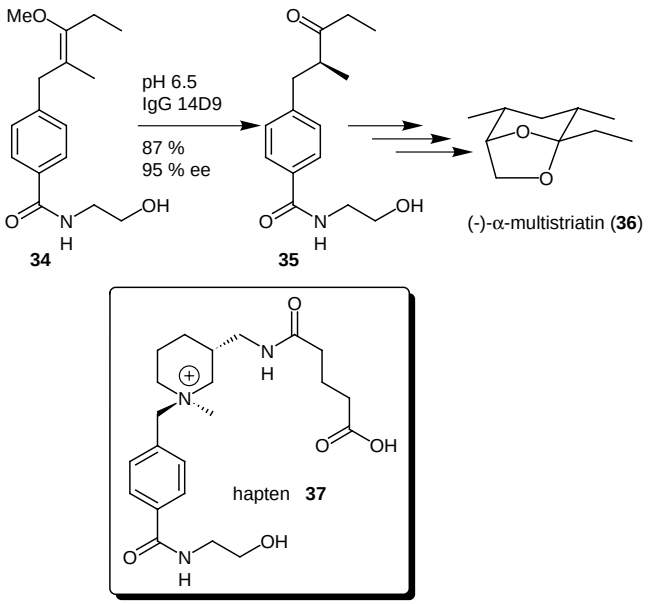
nately, no determination of enantiomeric excess of the products has been reported.



Scheme 8

The Synthesis of the Pheromone Multistriatin with the Crucial Assistance of an Enolase Antibody

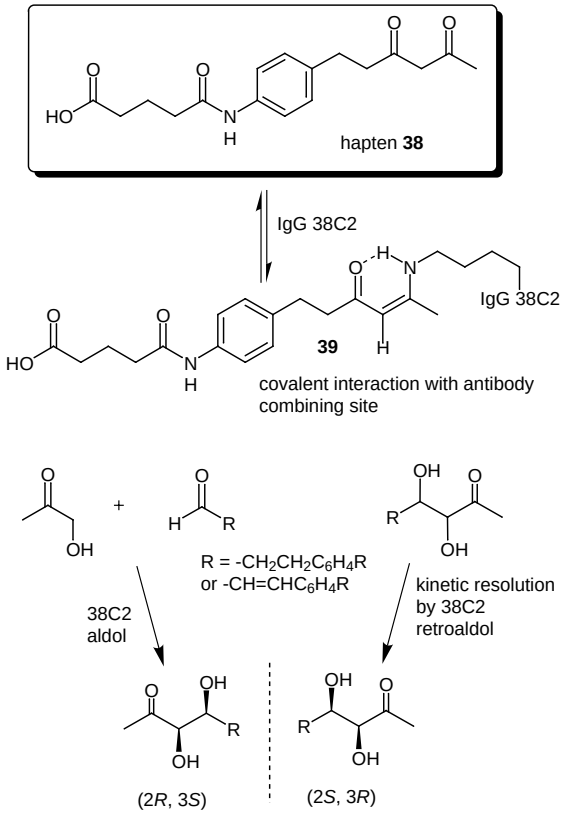
In 1995, an antibody that efficiently catalyzes enantioselective enol ether cleavage has been employed in the total synthesis of (-)-α-multistriatin (**36**) (Scheme 9).[51] While earlier reports announced first automation- or gram-scale experiments with catalytic antibodies[38,52], this is the first time that an antibody was put to work on the testing ground of natural products synthesis. Exposing *Z*-configurated enol ether **34** to antibody 14D9 at pH 6.5 yielded optically active ketone **35** in 87% yield (relative to converted starting material) and 95% ee. The ammonium center in hapten **37** elicited an acidic residue within 14D9 that results in enantiofacial protonation of a variety of enol ethers that, in return, collapse to *S*-configurated ketones selectively.[52,53] Five reaction cycles with a total of 900 mg substrate were carried out to yield 225 mg of ketone. A subsequent synthetic sequence of twelve steps led to the desired aggregation pheromone that was as active in field experiments as the naturally occurring compound.



Scheme 9

Aldol- and Retroaldol Reactions with the Potential for Kinetic Racemate Resolution.

Also in 1995, a conceptually novel method called *reactive immunization*[54] led to the discovery of two of the synthetically most useful antibodies to date.[55] To generate an aldolase antibody that operates with a mechanism analogous to natural class I aldolase enzymes, a hapten **38** containing a beta-diketone moiety was chosen (Scheme 10). Reactive immunogen **38** was meant to form an enamine **39** with any available lysine residue in the antibody-binding site during immunization. In particular, it was hoped that the covalent interaction thus formed would lastingly stimulate any B cell carrying an immunoglobulin



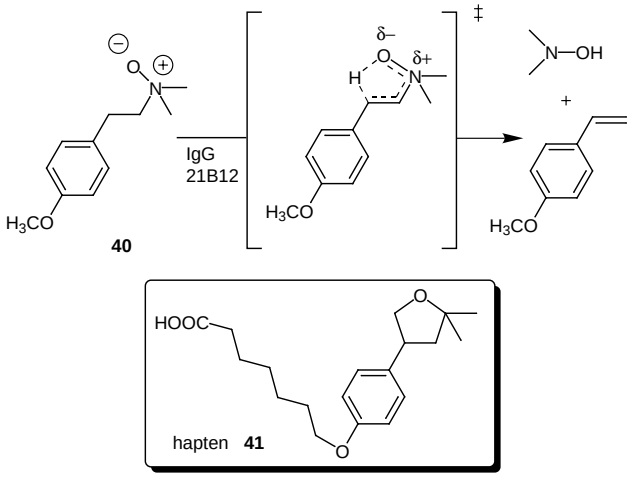
Scheme 10

with a lysine on its surface during the affinity maturation process (see glossary). As a crystal structure revealed[56], antibody 33F12 (and its genetic relative 38C2) indeed carry a reactive lysine that exhibits a perturbed pK_a due to an otherwise predominantly hydrophobic environment. This results in efficient Schiff-base formation with a variety of carbonyl-containing substrates.

38C2 turned out to be a surprisingly versatile antibody aldolase that catalyzes aldol as well as retroaldol reactions with high enantioselectivity. Particularly, hydroxyacetone as donor was determined to yield consistently useful reaction rates with a large number of aldehyde acceptors.[57] But the abzyme accepts a remarkable selection of other donors, too. Kinetic resolution of racemic aldols with this abzyme has recently been extended to those adducts carrying tertiary hydroxyls.[58] A study using a Hammett correlation indicated that the mechanism employed by 38C2 depends on the electronic nature of the aldol substrate. Electron-rich substrates undergo retroaldol reaction through a positively charged transition state. As one proceeds to substrates carrying more electron-withdrawing substituents, catalytic rates decrease.[59]

Cope Reaction and Medium Effects Involved

In 1996, an antibody-catalyzed sigmatropic rearrangement of an aminoxide (a Cope reaction) was reported (Scheme 11).[60] Antibody 21B12 was raised against a 2,2'-dimethyltetrahydrofuran hapten **41,** designed to mimic the conformationally restricted transition state. In addition, the authors anticipated the reduced dipole moment in the hapten relative to the substrate **40** to induce a low dielectric environment in the antibody-binding site. This should accommodate the charge-dispersed transition state better than the ground state of the substrate. Indeed, 21B12 accelerated the elimination of hydroxylamine in aqueous media 1000-fold. Interestingly, performance of the *uncatalyzed* elimination reaction in aprotic solvents like dioxane enhanced the rate significantly, pointing at the importance of medium effects in this elimination reaction. The determination of thermodynamic parameters revealed that 21B12 mainly catalyzed the reaction by lowering the enthalpy of activation, likely through the dissociation and expulsion of highly ordered solvent molecules from the substrate. Medium effects were also determined to be a major contributor to catalytic efficiency in some other antibody-catalyzed reactions [61-64] (for a critical view on this subject, see References[65]).

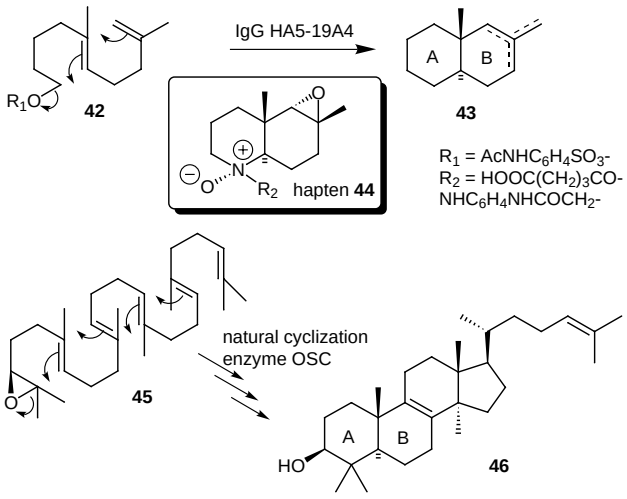


Scheme 11

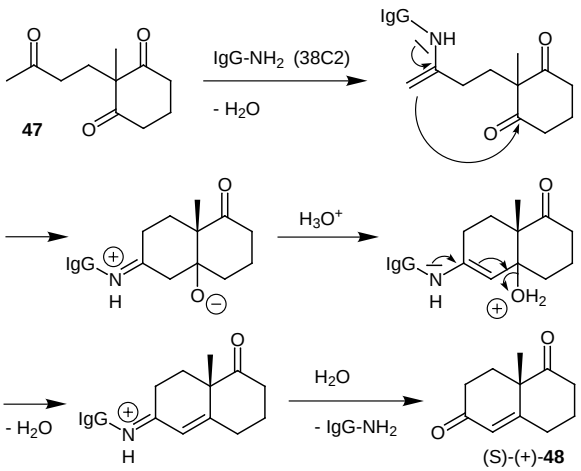
Terpenoid-like Cascade Cyclization

A rich history of *domino* reactions can be found in the chemical literature and among them particularly biomimetic polyene cyclizations.[66] In 1997, an attempt to accomplish an antibody-catalyzed polyene tandem cyclization was successful.[67] This project was inspired by one of nature's most complex enzymic transformations – the biochemical synthesis of lanosterol (**46**) from 2,3-*(S)*-oxidosqualene (**45**), carried out by a single enzyme, oxidosqualene cyclase (OSC), (Scheme 12).[68] A bicyclic bridge-methylated decahydroquinoline *N*-oxide **44** was chosen as hapten to mimic formation of rings A and B of the steroid nucleus from substrate **42**. The various steps of polyene cyclization can be classified into initiation, propagation and termination. Initiation consists of heterolytic cleavage of the bond connecting leaving group and C1 and establishment of the first incipient carbocation. Propagation is the elongation of the reaction cascade that results in ring closure through double bond addition. Finally, termination is caused by saturation of the final carbocation either by nucleophile addition or proton elimination. Initiation is addressed in the racemic hapten design **44** by incorporation of an *N*-oxide that was meant to elicit a counter-charged or -polarized amino acid matrix capable of promoting leaving group departure.[69] Propagation is reflected in **44** through the conformational characteristics of a bicyclic template with a chair/pseudo-chair conformation. This was designed to result in a complementary amino acid side-chain matrix that enforces the necessary chair-chair fold of the hydrocarbon chain in **42** for the cascade to proceed. Termination was hoped to result in alcohol formation by introducing an epoxide moiety in the necessary alpha position of hapten **44** for concerted addition of the internal carbocation and a water molecule to the terminal double bond. Indeed, a catalyst (HA5-19A4) was discovered that produced a bridge-methylated decalene system not seen in the absence of catalyst.[67] As an X-ray structure determination of the Fab fragment (see glossary) of HA5-19A4 revealed, the *N*-oxide moiety of hapten **44** elicited a glutamine residue in a position where a hydrogen bond stabilizes the developing negative charge on the aryl sulfonate leaving group.[70] The rest of the active site is mostly lined with hydrophobic aromatic side chains that are likely responsible for carbocation stabilization through electrostatic attraction to their quadrupole moment.[71] Thus, this antibody's strategy converged with that of natural enzymes catalyzing similar cationic processes. Termination resulted in formation of

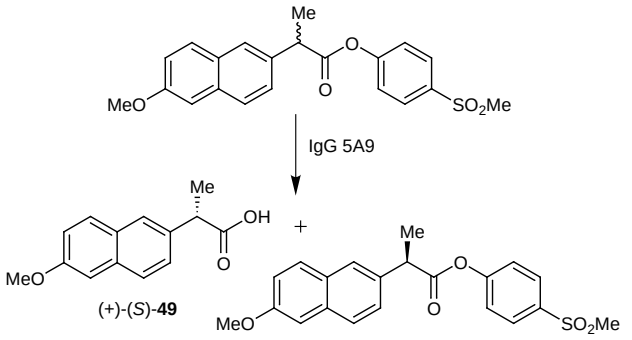
three regioisomeric decalenes (**43**) with ee's from 53% to 80%. Evidently, the hapten design didn't elicit any polar pocket at the termination site to accommodate a water molecule for tertiary alcohol formation. Thus, no decalols were detected in the antibody-catalyzed reaction.[67] Recent research was directed at antibodies that will also trigger and control multi-ring cyclizations starting from an epoxide moiety as seen in sterol biosynthesis.[72]

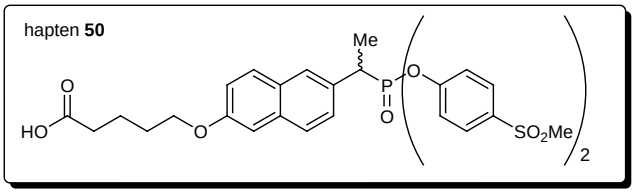


Scheme 12



Scheme 13





Scheme 14

Wieland-Miescher Ketone Formation

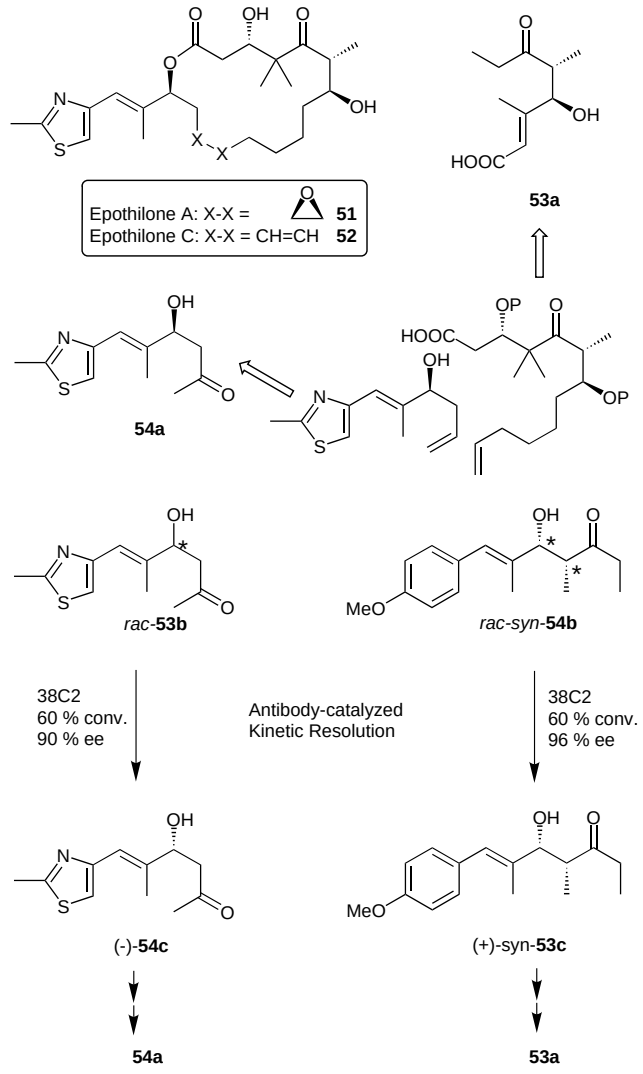
Also in 1997, another reaction related to steroid chemistry has been catalyzed by an antibody. Aldolase antibody 38C2, generated by reactive immunization (see above), accelerated the Robinson annulation enantioselectively, yielding the Wieland-Miescher ketone **48** that occupies a key role in the total synthesis of steroids (Scheme 13, the mechanism shown has been proposed by the authors).[73] The annulation consists of two steps, the Michael addition to **47** and its intramolecular aldol condensation. Surprisingly, the aldolase antibody also weakly accelerated the Michael addition. The catalyst was programmed to form an enamine with the carbonyl group in the prochiral substrate **47** and thus greatly enhanced the rate of aldol condensation to form the MW ketone **48** in >95% ee. This contrasts ee's obtained with optically active proline-induced annulation in organic solvents as seen in conventional synthesis (ca. 70% ee).[74]

Enantiospecific Naproxen Ester Cleavage

Since its inception, the field also aimed at the potential of enantioselective industrial drug production by catalytic antibodies. One attempt in 1998 involved a reactive immunogen (hapten **50**) that elicited a panel of antibodies for naproxen ester cleavage (Scheme 14).[75] Notably, these antibodies displayed high catalytic proficiencies. One of them, 5A9, combined high activity with a 90% ee after 26% conversion for the production of (+)-*(S)*-naproxen acid **49** that is industrially produced also. The resolution of racemic esters by the abzyme resulted in a maximum of 90% ee, a good performance compared to many artificial enantioselective catalysts. However, the particular desire to accomplish complete kinetic resolution with this abzyme as a valuable alternative to diastereomeric crystallization (as is currently used in industry) was not satisfied. The resolution of racemic esters did not exceed 90% ee for the naproxen acid because the poorer substrate enantiomer with the (-)-*(R)* configuration binds more tightly and inhibits the antibody-catalyzed hydrolysis. Since a racemic reactive immunogen was used to generate this antibody, the authors hope to minimize recognition of the unwanted substrate enantiomer by utilizing a homochiral hapten in the future.[#2]

The Synthesis of Epothilones with the Crucial Assistance of an Aldolase Antibody

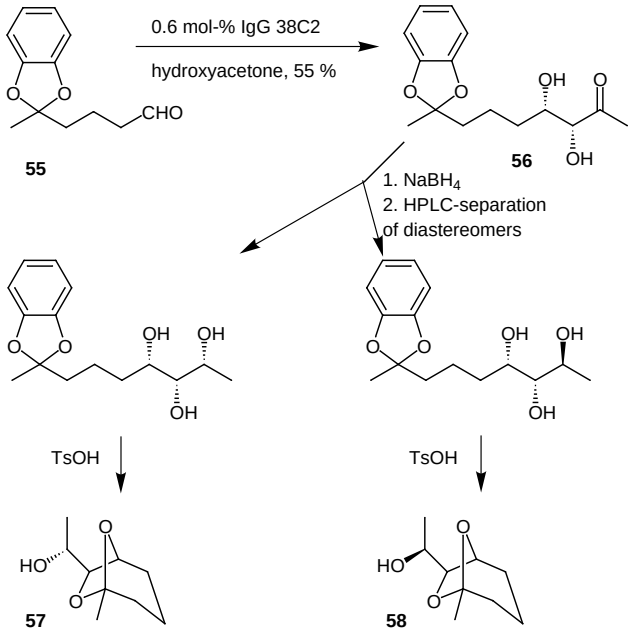
A new height in antibody-supported total synthesis has been reached only recently (1998). Epothilones A (**51**) and C (**52**) were totally synthesized by taking advantage of the efficient kinetic resolution of aldol adducts with antibody aldolase 38C2 (scheme 15).[76] Key intermediates **53c** and **54c** were obtained in high enantiomeric excesses via antibody catalysis.[77] The aromatic moiety in **53b** and **54b** was required, as programmed by the hapten (Scheme 10), to achieve high molecular recognition and thereby the best stereodifferentiation during kinetic resolution. Hence, while the racemic substrate **53b** carried the thiazol unit found in the target compounds, an anisyl moiety had to be incorporated into the racemic substrate **54b**. Consequently, the methoxy-substituted aromatic ring in (+)-*syn*-**53c** was removed in a later step by exhaustive degradation with $RuCl_3/NaIO_4$ to the corresponding carboxylic acid **53a**. A sequence of synthetic steps previously published by Schinzer, et al. completed the synthesis.[78] This is the most demanding synthetic target until now that has been synthesized with the decisive assistance of an antibody catalyst. Three stereocenters have been defined in antibody-catalyzed steps. The following synthetic examples demonstrate that this retroaldolase antibody 38C2 can also be successfully applied to enantioselective aldol-addition reactions.



Scheme 15

Synthesis of Brevicomins by an Aldolase Antibody

A number of representatives from a class of pheromones, the brevicomins, was totally synthesized using aldolase antibody 38C2 (Scheme 16).[79] The following data will give the reader an idea of the scale, concentrations and conditions of the antibody-catalyzed key transformation in this straightforward synthesis. When aldehyde **55** in CH_3CN (1 mL, 100 mM) and 1mL of hydroxyacetone is added to a buffer solution of antibody 38C2 (18 mL), and the reaction is stirred for 36 hours, product **56** can be obtained in 55% isolated yield and 98% ee along with the anti-diastereomer (ratio 4:1). A catalytic rate of 0.65 min^{-1} was observed, which is a strong performance compared to average catalytic antibodies in the literature (Table). Subsequent non-selective reduction, separation of the diastereomers, and deprotection resulted in ketalization to the desired target compounds **57** and **58**. Comparison of enantiomeric excess obtained by the antibody (>99% ee) with that of the Sharpless asymmetric dihydroxylation (89% ee) underscores the utility of this proteinogenic catalyst and the potential of antibody catalysis in general.

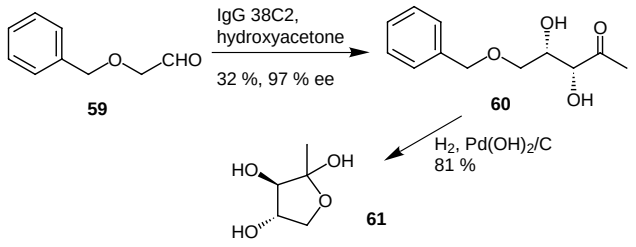


Scheme 16

Synthesis of 1-Deoxy-L-Xylulose by an Aldolase Antibody

Another natural-product synthesis has been shortened by antibody catalysis. A two-step synthesis of 1-deoxy-L-xylulose **61** was made possible with antibody 38C2 (Scheme 17).[80] 38C2 is the only known catalyst that can accelerate

the aldol addition of unprotected hydroxyacetone to an aldehyde. Dihydroxyketone **60** (97% ee) was isolated in 32% yield with 97% ee, when this reaction was performed with a catalyst loading of only 0.04 mol-% and terminated after 48 hours (56% conversion of aldehyde **59**). Simple deprotection of the benzyl protecting group furnishes the target compound. The authors envision an easy access to isotopically labeled 1-deoxy-xyluloses for biological studies of this important carbohydrate that is part of the vitamin B_1 and B_6 biosynthesis, and part of an alternate non-mevalonate terpenoid biosynthetic pathway.



Scheme 17

5 Practical Utility of Antibody Catalysts

While examples in the previous chapter serve to give an impression of the variety of organic synthetic reactions that can be catalyzed by abzymes, they do not per se show the reader whether these catalysts can be applied to daily routine laboratory synthesis. The Table sums up the kinetic performance of each of the immunoglobulins presented in this review.

Catalytic antibodies, like normal enzymes, generally follow Michaelis-Menten kinetics, meaning they reach a maximum rate of conversion where the substrate concentration saturates the active site. They can be characterized by their turnover number "k_{cat}" (see glossary), their Michaelis constant K_m (see glossary) and the resulting kinetic efficiency k_{cat}/K_m. Where applicable, the background rate for the same reaction in the absence of antibody catalyst, k_{un}, is also given for comparison. Not all entries represent the best catalytic efficiencies achieved by these catalysts with their best substrates, since only those were included that are of particular significance to the synthetic operation presented.

Table

entry	catalytic antibody[a]	k_{cat} (min^{-1})	k_{un} (min^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1} min^{-1}$)	$t_{1/2}$ [b] (h)	ref.
1	esterase 2H6	4.6	5.5×10^{-5}	4000	1.15×10^{3}	1.0	21
2	esterase 21H3	0.09	5.6×10^{-5}	334	2.69×10^{2}	9.3	21
3	oxidored.(hydride) 37B.39	0.097	1.1×10^{-3} M^{-1}	52	1.87×10^{3}	84	29
4	oxidored. (Hydride) A5	0.104	3.6×10^{-4}	1240	84	14	30
5	*exo*-Diels-Alderase 7D4	0.0034	7.2×10^{-4} M^{-1}	960 (diene) 1700 (dienoph.)	3.54	260	31
6	*endo*-Diels-Alderase 22C8	0.0032	1.8×10^{-4} M^{-1}	700 (diene) 7500 (dienoph.)	4.57	260	31
7	hydroxy-THP synth. 26D9	0.91	undetectable	356	2.56×10^{3}	0.92	33
8	oxepane synth. 26D9	0.89	undetectable	196	4.54×10^{3}	0.94	34
9	thioether oxidoreductase 28B4	492	51 M^{-1}	43 (substrate) 252 ($NaIO_4$)	11.4×10^{6}	1.7×10^{-3}	39
10	esterase 17E11-115	0.22	9.8×10^{-5}	130	1.69×10^{3}	3.8	42
11	oxy-Cope isomerase AZ-28	0.026	4.9×10^{-6}	74	351	32	43
12	cyclopropanase 87D7	0.021	undetectable	102	206	40	50
13	enolase (multistriat.) 14D9	0.36	5.5×10^{-6}	230	1.57×10^{3}	2.3	51
14	Cope-eliminase 21B12	0.000023	2.7×10^{-8}	235	9.79×10^{-2}	3.6×10^{4}	60
15	tandem cyclase 19A4	0.021	undetectable	320	65.6	40	66
16	WMketone synthase 38C2	0.086	2.4×10^{-8}	2340	36.8	1877	73
17	naproxen esterase 5A9	2.3	4.2×10^{-5}	11.5	2.00×10^{5}	0.36	75
18	retroaldolase(epothil.) 38C2	1.4	8.2×10^{-8}	270	5.19×10^{3}	0.59	76
19	xylulose synthase 38C2	0.0067	2.3×10^{-7} M^{-1}	17	3.94×10^{2}	124	80
20	intra-amidase 14-10	0.0093	4.7×10^{-7}	1400	6.64	173	86
21	inter-amidase 14-10	0.000133		370 (substrate) 136 (phenol)	0.36	6.3×10^{3}	87

[a] in order of appearance in the text. [b] $t_{1/2}$ (h) for conversion of [S] = 1 mM and [IgG] = 10 μM; for $K_m < 1$ mM → $t_{1/2}$ = 0.5 mM/($k_{cat} \times 10$ μM × 60); for $K_m > 1$ mM → $t_{1/2} = 0.69K_m/(k_{cat} \times 10$ μM × 60); equations from Mader and Bartlett[81].

To get a better impression of the actual timeframe of antibody-catalyzed processes, Mader et al. introduced a very useful estimation by determining the half-lives of substrate consumption at a given abzyme concentration.[81] The parameter values in that review article were also applied to the entries in the table to allow direct comparison. At 10 µM active site concentration (which corresponds to 5 µM bivalent IgG), a 100 mL reaction requires 75 mg protein (IgG). Under these conditions, the half-lives reveal that only half of the presented examples would encourage an attempt to apply them to synthetic problems in routine lab work, particularly entries 1, 2, 4, 7-10, 13, 17 and 18. In most of these cases, the reactions are carried out in aqueous solution with the assistance of up to 10% water-miscible cosolvents like DMSO, DMF or acetone to assure substrate and product solvation. Products can then be isolated by dialysis, extraction, or e.g. ion-exchange chromatography while preserving the precious biocatalyst. In some studies, biphasic conditions were successfully applied when strong agitation ensured efficient mixing of the phases. This can greatly simplify work-up but may over time deteriorate the antibody.[82]

It should be noted that, where antibodies catalyze product formation enantioselectively, the background reaction usually gives racemic material. Here, the degree of rate acceleration becomes particularly significant to achieve enantiomeric excesses that are not diluted by the background reaction. Thus, for slow antibodies the protein concentration necessary for satisfactory results may be prohibitively high. A related problem is posed where the reaction is rerouted by the catalyst to form a product not observed in the background reaction. Even though stereopurity of the disfavored products may be high, a strong background reaction leading to other products may deprive the antibody of valuable substrate and has to be countered again with high concentrations of catalyst. This emphasizes the importance of decent rate accelerations before a catalyst should be considered practical.

From an enzymological viewpoint, direct comparison of background rate with catalytic rate may not be feasible in some cases, because both processes pass through different transition states, for instance in the case of acyl-transfer antibodies. Here, a covalent interaction with the abzyme characterizes the catalyzed reaction whereas the background process involves hydroxide attack on the substrate. Yet to evaluate a novel catalyst for purely synthetic purposes, such considerations are of less importance and acceleration of product formation should be the primary focus.

6 Current Developments Towards Improved Catalyst – Discovery Techniques

In order to increase chances for abzyme discovery, improvements have been made on the three major fronts in abzyme generation: A. Hapten design and immunization; B. Enlargement of pool size of hapten-specific antibodies (diversity) for screening; C. Selection.

A. While there are natural limits to our ability to precisely match the transition state with one stable molecular structure, strategies have been successful that aim to install particular functional groups at decisive positions within the antibody-binding site. *Reactive immunization* has already been introduced in the previous chapter. Another strategy that focusses on *bait-and-switch*-designed haptens is viewed as a principal alternative to the design of transition-state analogs.[83] *Bait-and-switch* haptens usually contain strongly charged or polarized functional groups that act as a "bait" to the immune system. Counter-charged or -polarized residues in the obtained binding sites that bind these hapten features are the primary target of such an approach. When the obtained antibodies are reacted with the substrate (one "switches" from the inducing antigen to the substrate), these amino-acid residues are expected to play an important role in catalysis.

Another strategy was termed *heterologous immunization* for its consecutive use of two related hapten structures in the immunization of a single animal.[84] They carry different functional groups that simulate stereoelectronic features of the transition state which cannot be easily incorporated into a single hapten simultaneously. The aim of this study was amide hydrolysis that is of particular interest in regard to the identification of catalysts for selective peptide cleavage. Sequential immunization with both haptens did indeed yield an antibody ("14-10"), which exhibits superior performance over other antibodies generated against each hapten *alone* (> 5-fold improvement). IgG 14-10 showed a rate enhancement over background of 2×10^4. Only an *activated* amide bond in *p*-nitroanilide substrates was the subject in this study. The generation of de-novo designed catalysts or abzymes for cleavage of *un*activated amide bonds remains an elusive task because of their exceptional stability. However, *heterologous immunization* has a potential for application to other reactions where the transition state cannot be mimicked satisfactorily by a single hapten structure.

B. On the second front, procedures have been developed that increase the pool of possible candidates from the approximately 50-120 hybridoma clones mentioned earlier. Either the fusion result (up to 2000 hybridoma clones) was directly screened for catalysis without prior selection of clones by ELISA,[85,86] or no fusion was carried out at all, thus eliminating an undesired selection process based on other factors than catalysis or hapten affinity. Instead, libraries of antibodies (or functional antibody fragments) have been created from the spleen cells that encompass $10^8 - 10^9$ members.[87] The demonstration that antigen-binding fragments of antibodies (Fab, Fv) could be expressed in bacteria was crucial for this new technology.[88,89] The successful library member, once isolated, can only be grown in any desired amounts if the genetic information for its construction is covalently linked to it. This is achieved by employing the phage-display technology[90,91] that incorporates the antibody genes into the phage genome (phage are viruses that infect bacteria). Once phage assembly is complete at the end of a bacterial

infection, hundreds of identical phage particles are released into the media, carrying the genetic information for the specific antibody that is covalently linked to their surface. The phage-display library now contains the entire immune response in the form of antibody heavy- and light chains that have been randomly recombined to form functional antibody-binding sites. Here, the selection process is started:

Library members are present in such low abundance that a special enrichment procedure for hapten affinity had to be developed, generally referred to as "panning".[87] To this end, the initial collection of clones is incubated with immobilized hapten on a plate and the non-specific members are washed off. The obtained smaller collection of positive clones will still contain a fraction of non-specific binders so that the procedure has to be repeated until only strongly-binding individuals remain. As pointed out above, these can be grown to obtain sufficient amounts for catalysis-testing using the "attached" genetic information.

Further diversifying methods include the polymerase chain reaction (PCR) using an error-prone polymerase[92] or PCR using oligonucleotides with randomized sequences. These techniques can be used to introduce point mutations in libraries with predetermined hapten specificity derived from a *complete* immune response. They can also be applied to prepare a variant library from a *single* catalytic antibody. Candidates with improved or altered characteristics were selected from such a phage-display library by affinity screening[42] (see 17E11-115 and table, entry 10). However, a similar phage-display study involving another hydrolytic antibody only yielded an improved catalyst when the selection criterion was changed from affinity to catalysis.[93]

Recently, others developed a method that involves "chopping" of existing biocatalyst genes (or any functional protein for that matter) and randomly recombining the fragments thereafter.[94,95]

C. On the third front, selection techniques are further shifted into the direction of catalysis rather than affinity. The ELISA assay for clonal selection has thus been modified by immobilizing the substrate rather than the antigen (catELISA).[96] More sensitivity is needed to make up for increased library sizes resulting from more advanced diversifying methodologies. This can for instance be achieved through the use of chromogenic or fluorogenic assays,[97,98] through ESI mass spectrometry,[99] or through the selective amplification of catalytic events. Here, studies have been published that either rely on PCR[100] or on phage growth.[101,102] Also, a method was described based on fluorescence correlation spectroscopy.[103] It allows for fast detection with a sensitivity for concentrations of less than 10^{-15} M for any candidate labeled with a specific fluorescent tag.

Finally, selection schemes can be imagined for a wide range of reactions of metabolic processes where the successful clone that produces a catalytic antibody will have a growth advantage or will even be the only survivor. Such a strategy has already been applied to the identification of a yeast clone that relies on expression of a catalytic antibody with chorismate-mutase activity, essential for phenylalanine and tyrosine biosynthesis.[104] A similar tactic was reported for the selection of orotate-decarboxylating catalysts from an antibody library expressed in E.coli.[105]

7 Conclusion

The field of catalytic antibodies has made significant advances over the past 13 years, giving us new insights into how proteins achieve catalysis and what mechanisms they employ. It also provided us with a few catalysts that either rival natural enzymes in rate acceleration,[39,44,50,106,107] or extend their scope[40,57] or even reroute chemical reactions that have been hitherto difficult to redirect by other means.[31,33,50] Nonetheless, it has become evident in this account that future improvements in catalytic antibody generation would be most welcome to manage more complex reaction pathways. Multiple catalytic residues in the binding site or "arrangements" like catalytic triades (e.g. as seen in natural serine proteases) may be difficult or impossible to elicit with only one hapten design or even by way of heterologous immunization. This becomes clear when considering that the antibody is selected by the immune system only for hapten affinity and develops only so many tools of molecular recognition as to obtain the necessary dissociation constant (10^{-4} to 10^{-10} M). Thus, it may become unnecessary for the immune system to recognize all features of the hapten design.

While the frontier of achieving antibody rate accelerations exhibited by enzymes is advancing continously (k_{cat} = 81.4 min^{-1} and k_{cat}/k_{un} = 2.3 x 10^8 for a novel retro-aldolase antibody[108]), the majority of antibodies fall short of the catalytic rates of their natural counterparts (albeit achieved through millions of years of evolution). Intrinsic difficulties in designing stable structures that really match the features of the transition state, fundamental limitations in the biological process that generates antibodies, and the difficulty to elicit antibodies that effectively differentiate ground state from transition state (or substrates/products from hapten) have been named as likely causes.[81] However, catalytic antibodies have been called the "currently most successful enzyme mimics".[28] Their application in complex synthetic schemes has been demonstrated.[51,76] In comparison, attempts to generate wholly synthetic catalytic peptides have typically resulted in compounds with weak structural integrity and poor catalytic activity, although two notable advances have been reported.[109,110] The fundamental difference between this de-novo-design approach and that of inducing nature (the immune system) to produce artificial enzymes has been pointed out.[109,111]

In light of the above considerations, the most attractive feature may well be the combinatorial characteristics of the antibody system. The opportunities for improvement and expansion through its natural modular design in geno-

type and phenotype make it ideally fit for generation of diversity and subsequent selection. To generally select novel activity, nature moves within the vast structure space of biological molecules in a Darwinian, post-synthesis style.[112,113] Synthetic chemists traditionally select their targets on the basis of certain structural criteria in a pre-synthesis fashion. Immunization with a hapten, that has been rationally selected by pre-synthesis design and synthesized by the chemist, results in an enormous pool of antibodies with predetermined specificity. To obtain candidates with the necessary refinements in active site architecture and function, one now has to select a second time for an altered criterion – catalysis. Thus, the potential of the technology of catalytic antibodies lies in its combination of both strategies, pre-synthesis and post-synthesis selection.

Acknowledgement

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Glossary

affinity maturation – a process the immune system employs to refine antigen affinity of its germ-line antibodies; involves point mutation and selection

ascites – the fluid that develops in the abdomen of laboratory mice when being injected the hybridoma clone; contains large amounts of comparatively pure monoclonal antibody constantly secreted by the rapidly dividing hybridoma cells.

clone – generally all cells (or multicell organisms) containing identical genetic information belong to the same clone. Specifically, a hybridoma clone is obtained by dilution procedures until a single cell per container is obtained, its antibody-secreting descendants (through cell division) are genetically identical and therefore also belong to the same *clone*.

DEAE-sepharose – a bead-formed cellulose-based ion exchanger carrying *di*e*thyl*a*mino*e*thyl* groups

ELISA – a semi-quantitative detection method for antibodies or antibody-producing cells based on antigen affinity: the immobilized hapten is reacted with the sample to be tested for positive antibodies. The degree of colorization achieved with a helper system then corresponds to the density of antibodies bound on the surface.

epitope – the group (generally composed of several amino acid residues) that is recognized by an antibody on the surface of an antigen

Fab – two identical fragments (with one antigen-binding site each) are obtained when treating an IgG molecule with the protease papain, mass: 50,000.

Fv – antigen-binding *f*ragment that consist of the *v*ariable region of heavy and light chain of the IgG molecule only, a good candidate for bacterial expression (production) because of reduced size, mass: 25,000.

germ-line antibody – protein consisting of precisely the amino acid sequences as are encoded in the individual's inherited immunoglobulin genes V, D, J

IgG – *I*mmuno*g*lobulin *G*, class of immunoglobulins predominantly found in blood serum, carries two antigen-binding sites, mass: 150,000.

IgM – *I*mmuno*g*lobulin *M*, class of immunoglobulins observed in the blood serum during early stages of the immune response, generally less antigen-specific, pentameric immunoglobulin, mass: nearly 950,000.

immunogen = generally "antigen", more specifically "hapten".

k_{cat} – the turnover number (or catalytic constant) represents the maximum number of substrate molecules converted to products per active site per unit time.

K_m – the *Michaelis* constant is, in a simplified sense, an apparent overall dissociation constant of all enzyme-bound substrates.

reactive immunization – utilizes haptens that have a balanced reactivity so that they aren't deactivated too quickly during immunization before being recognized by the immune system; they are expected to form covalent bonds to specific residues within antibody-binding sites on the surface of B cells

References and Notes

[#] Names given to monoclonal antibodies are derived from the well number in which the clone was discovered, e.g. "21H3". The name may also include the abbreviation for the inducing antigen, e.g. "PCP".

[#2] Note added in proof:
An extension of the work on enantiospecific Naproxen ester cleavage has just been published (Datta, A; Wentworth, P., Jr.; Shaw, J. P.; Simeonov, A.; Janda, K. D. *J. Am. Chem. Soc.* **1999**, web release date of *asap* article: Oct 23, 1999): Immunization with a transition-state analogue hapten instead of a reactive immunogen resulted in the generation of three antibodies that combine excellent rate enhancements ($k_{cat}/k_{un} \approx 1 \times 10^6$) with the desired ee's of >98% for the kinetic resolution depicted in Scheme 14. However, their performance suffers from some inhibition by the phenolic product. At roughly 20% conversion, from 28% to 56% activity is retained in these catalytic antibodies."

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