

5

Catalytic Antibodies in Natural Products Synthesis

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5.1

Introduction

Natural product synthesis is the centerpiece of organic chemistry because it has always been the ultimate testing ground for new concepts and new synthetic methods. In fact, much of what we know about organic reaction mechanisms has come from attempts to carry out selective chemical transformations on the journey to the construction of these often very complex naturally occurring molecules. In spite of the tremendous successes in the fields of organic synthesis and synthetic methodology [1], there is a continual search for methods to improve organic synthesis and provide practical routes to drugs, natural products, and other important chemicals.

Enzymes and other biocatalysts have been attracting ever-increasing attention as useful tools in organic synthesis, mainly because of their high selectivity, specificity, and phenomenal rate acceleration. Enzymes may offer solutions to many synthetic problems that are difficult or even impossible to solve efficiently by other chemical means [2]. Biocatalysis has been successfully used not only in small-scale synthesis but also in the pharmaceutical industry for the preparation of enantiomerically pure intermediates and products [3]. Furthermore, recently developed biological tools, such as directed evolution of enzymes [4, 5, 6], phage display selection methods [7, 8], techniques of molecular genetics [9], as well as traditional screening methods [10], have increased the scope of opportunities of using enzymes in organic synthesis. Yet, for many chemical transformations there is no known natural enzyme, and in many cases the relevant enzyme is either difficult to isolate from its natural source or is too unstable for synthetic applications. Moreover, the high specificity and narrow substrate range of most enzymes are major drawbacks for general application in organic synthesis, where catalysts that are more promiscuous with respect to their range of substrates are desirable. These limitations have led to increased search, particularly over the last two decades, for new biocatalysts. Undoubtedly, of the various strategies to generate new biocatalysts, antibody catalysis has been the most successful and practical approach.

Challenging the immune system with stable analogs of the transition state of a given reaction has proven to be a useful approach to achieve monoclonal antibodies

that catalyze the reaction. Since the demonstration of this idea by Lerner [11] and Schultz [12], its implementation coupled with versatile screening assays has yielded an abundance of catalytic antibodies for more than 100 reactions. Most of these reactions represent useful transformations for organic synthesis [13], including pericyclic processes, group transfer reactions, additions and eliminations, oxidations and reductions, aldol condensations, and miscellaneous cofactor-dependent transformations. In many cases, the selectivities of these catalysts rival those of natural enzymes, and transformations that could not be achieved efficiently or selectively via more traditional chemical methods were shown to be possible via antibody catalysis [14, 15].

One of the main goals of the field of antibody catalysis [11, 12, 16] has been the achievement of custom catalysts for synthetic schemes in order to open routes that are otherwise inaccessible or cumbersome. In this way, one hopes to improve the overall yield and allow practical construction of synthetic drugs and important natural products. In general, in addition to being highly chemoselective, most antibody-catalyzed reactions are diastereoselective, enantioselective [17], and regioselective [18], even reversal of chemoselectivity having been observed [19]. The relevance of these catalysts to synthetic organic chemistry has been demonstrated by the possibility of running reactions with gram scale quantities [20].

Since many organic synthetic methods require the manipulation of highly reactive, water-sensitive intermediates, the handling of such intermediates along with catalytic antibodies is an important issue for synthetic applications. It has been shown that such chemistry is accessible using catalytic antibodies in aqueous media [21]. Antibody 14D9 catalyzed the protonation of a prochiral enol ether with complete enantioselectivity to form a highly water-sensitive oxocarbenium ion intermediate. In the hydrophobic environment of the antibody active site, this short-lived intermediate reacts intramolecularly with a neighboring hydroxyl group to form an enantiomerically pure ketal. This reaction is normally not possible in aqueous medium because the oxocarbenium ion is trapped by a water molecule to give the corresponding ketone. The ability to promote reaction pathways that are normally disfavored or have low probability represents one of the greatest advantages of antibody catalysis [14].

This review will not survey all the useful transformations catalyzed so far by antibodies, as other chapters in this book adequately cover these reactions. This chapter covers mainly work that resulted in either total synthesis or formal total synthesis of a natural product. It is important to emphasize that the key point in all of these examples is not simply that one can make specific target molecules, such as multistriatin, epothilones, brevicomins, etc., or even that the methods used are now the best available to synthesize the compounds, but rather that catalytic antibodies perform competitively in the important testing ground of natural product synthesis.

5.2

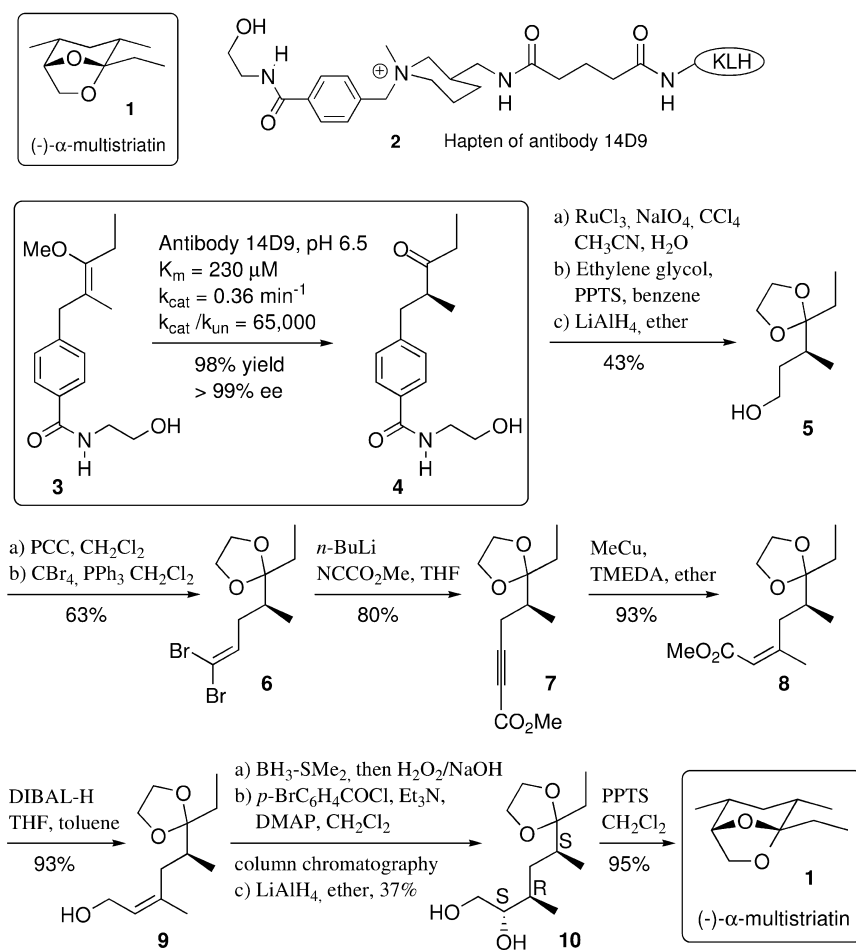
Total Synthesis of α -Multistriatin via Antibody-Catalyzed Asymmetric Protonolysis of an Enol Ether

The compound (–)- α -multistriatin, **1**, is one of the three essential components of the aggregation pheromone of the European elm bark beetle, *Scolytus multistriatus* (Marsham), the principal vector of Dutch elm disease in Europe and North America [22]. The severe devastation of the elm population in Northeastern USA has resulted in extensive studies of the synthesis and field utilization of **1** [23]. Since the discovery of this pheromone by Silverstein in 1975 [24], it has been the subject of numerous synthetic efforts [25]. Field experiments showed that the inactive, (+)-enantiomer of **1** inhibits the biological activity of the naturally occurring, (–)-enantiomer [26]. Therefore, the high enantiomeric purity is a crucial issue in the synthesis of this pheromone. The enantioselective synthesis of **1** by Sinha and Keinan represents the first example of a natural product total synthesis that involves antibody catalysis [27].

The retrosynthetic analysis of **1** took advantage of the opportunity to obtain α -branched ketones with high enantiomeric purity via antibody 14D9-catalyzed enantioselective protonolysis of the appropriate enol ether [28]. The ability to carry out this reaction under mild conditions was of particular importance because high enantiomeric purity of a tertiary carbon center adjacent to a carbonyl function is difficult to generate and retain. Antibody 14D9 has already been proven to be an effective catalyst for the hydrolysis of a variety of oxygen functions, including acetals [29], ketals [30], epoxides [17], and enol ethers [28, 31]. Since all these reactions are characterized by a positively charged transition state, it is conceivable that antibody 14D9, which was elicited against the positively charged piperidinium cation **2**, catalyzes the reactions by stabilizing the positive charge that is created along the reaction coordinate [29]. Indeed, mechanistic studies with this antibody had suggested that an ionizable side chain in the active site combined with hydrophobic interactions directly participate in transition-state stabilization to achieve its catalysis [28].

Thus, substrate **3** (Scheme 5.1) was chosen for the antibody-catalyzed preparation of the desired α -branched ketone, **4**, the key asymmetric building block of the entire synthesis [27]. The Z enol ether **3** together with its E stereoisomer, along with two other regioisomers, were prepared from 3-pentanone and methyl 4-bromomethylbenzoate in a four-step sequence of chemical transformations followed by chromatographic separation. In acidic, aqueous media, all isomers are hydrolyzed to the racemic ketone **4**. Antibody 14D9 catalyzed this reaction under mildly acidic conditions, with hydrolysis of the Z enol ether, **3**, being much more effective ($K_m = 230 \mu\text{M}$, $k_{\text{cat}} = 0.36 \text{ min}^{-1}$ at pH 6.5, $k_{\text{cat}}/k_{\text{uncat}} = 65\ 000$) than that of the E isomer ($K_m = 310 \mu\text{M}$, $k_{\text{cat}} = 0.044 \text{ min}^{-1}$ at pH 6.0 ($k_{\text{cat}}/k_{\text{uncat}} = 5000$). These observations enabled a mixture of all four isomeric enol ethers to be used in the antibody-catalyzed hydrolysis, resulting in complete, selective consumption of **3**, and leaving the other three isomers essentially intact. Ketone **4** was thus obtained with configuration (S) in greater than 99% *ee*.

Another practical advantage of this process originated from the fact that there is no known enzyme or other biological component which can catalyze the enol



Scheme 5.1

ether hydrolysis. Consequently, there was no need to use a purified antibody to catalyze this reaction. Usually, catalytic antibodies that are produced from murine hybridoma cell lines are obtained from ascites fluid followed by ammonium sulfate (SAS) precipitation, anion exchange, and affinity chromatography with immobilized protein G [32]. Efficient catalysis was thus achieved with a partially purified antibody 14D9, which was precipitated from the ascites fluid by SAS. Catalytic activity of this crude antibody was completely inhibited by the hapten, **2**, thereby ruling out any non-specific catalysis by other components in the crude SAS-fraction.

The antibody-catalyzed reaction was carried out on a preparative scale using very simple organic-laboratory equipment. In each catalytic cycle, a solution of the enol ether **3** (180 mg, 0.65 mmol) in DMF (1 mL) was added to a solution of a crude SAS fraction of antibody 14D9 (22.5 mL containing 225 mg protein, 0.0015 mmol) in

bis-tris buffer (50 mM, pH 6.5), and the mixture was stirred at 24 °C. Progress of the reaction could be observed visually, as the starting mixture was a turbid white (because of lower solubility of the starting material relative to that of the product) and became clear as the reaction reached completion. The reaction was transferred into a cellulose dialysis bag and dialyzed into the same buffer. The recovered antibody solution was taken to the next catalytic cycle with a fresh solution of **3**. Only minor deterioration of catalytic activity could be observed over five cycles of the reaction.

The crucial antibody-catalyzed step was followed by several chemical steps to complete the synthesis of **1** (Scheme 5.1). Ketone **4** was treated with RuCl₃ and sodium periodate. The resultant keto-acid was then converted to a ketal-ester by reaction with ethylene glycol and catalytic amounts of pyridinium *p*-toluenesulfonate in benzene. Reduction of the resultant carboxylic ester with LiAlH₄ in ether afforded alcohol **5**, which was oxidized with pyridinium chlorochromate in dichloromethane to the corresponding aldehyde. The latter was treated with a solution of triphenylphosphine and carbon tetrabromide in dichloromethane to give the dibromoalkene **6**. Treatment of **6** with *n*-butyllithium and methyl cyanofornate in hexane-THF produced the substituted methyl propargylate **7**. Reaction of the latter with methylcopper afforded geometrically pure (*Z*) α,β-unsaturated ester **8**. Reduction of this ester with diisobutylaluminum hydride in toluene-THF afforded the corresponding allylic alcohol, **9**, with retention of the (*Z*) geometry. Treatment of **9** with a solution of borane-dimethyl sulfide complex in THF followed by oxidation with basic hydrogen peroxide produced a 70:30 mixture of two diastereomeric products, **10**, which had the desired 2*S*,3*R*,5*S* configuration, and its 2*R*,3*S*,5*S*-diastereomer, respectively. The chromatographically purified diol **10** was treated with catalytic amounts of pyridinium *p*-toluenesulfonate in dichloromethane followed by kugelrohr distillation at 110 °C to produce (–)-α-multistriatin, **1**, in the form of a colorless oil. The synthetic pheromone **1** has been checked in field experiments and found to be as active as the naturally occurring compound in attracting the European elm bark beetles into traps loaded with a mixture of **1** with (–)-α-cubebene (a host-produced component) and (–)-4-methylheptan-3-ol [27].

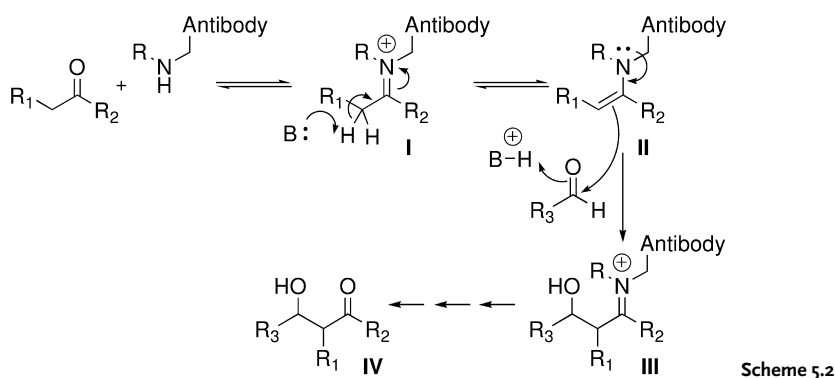
To summarize this achievement, all four asymmetric centers of the target molecule had originated from the chirality that was accomplished in the antibody catalyzed step, i.e., enantioselective protonolysis of an enol ether. That specific step is a unique example of a chemical transformation that is difficult to achieve either by an available synthetic methodology or via catalysis with a known enzyme.

5.3

Total Synthesis of Epothilones using Aldolase Antibodies

The total synthesis of many epothilone derivatives by Sinha and coworker represents the most advanced example of natural product synthesis assisted by antibody catalysis [33]. Several aldolase antibodies were employed to catalyze both aldol and retroaldol reactions in order to achieve the required enantioselectivity in the synthesis of various epothilone derivatives.

An active site nucleophilic lysine residue with a highly perturbed pK_a is an essential element of the catalytic machinery that is available to both the natural type I aldolase enzymes and the aldolase antibodies elicited by reactive immunization [34, 35]. Unlike normal immunization, in reactive immunization [36], chemically reactive haptens are used as immunogens, so that a chemical reaction occurs in the binding site of an antibody during its induction. This strategy creates antibodies with a broad substrate scope, which is the result of the fact that the chemical event is covalent and occurs early in the process of the antibody binding site refinement. After formation of a covalent bond between the hapten and antibody, further improvement of the binding site via non-covalent bonds cannot meaningfully increase the binding energy, and thus mutations are no longer selectable [37]. Thus, reactive immunizations often yield antibody binding sites into which an efficient chemical mechanism has been installed but are not otherwise highly refined. Such catalysts can be expected to be both efficient and promiscuous in both the aldol and retroaldol reactions [38]. Chapter 11 of this book comprehensively covers these issues [39].

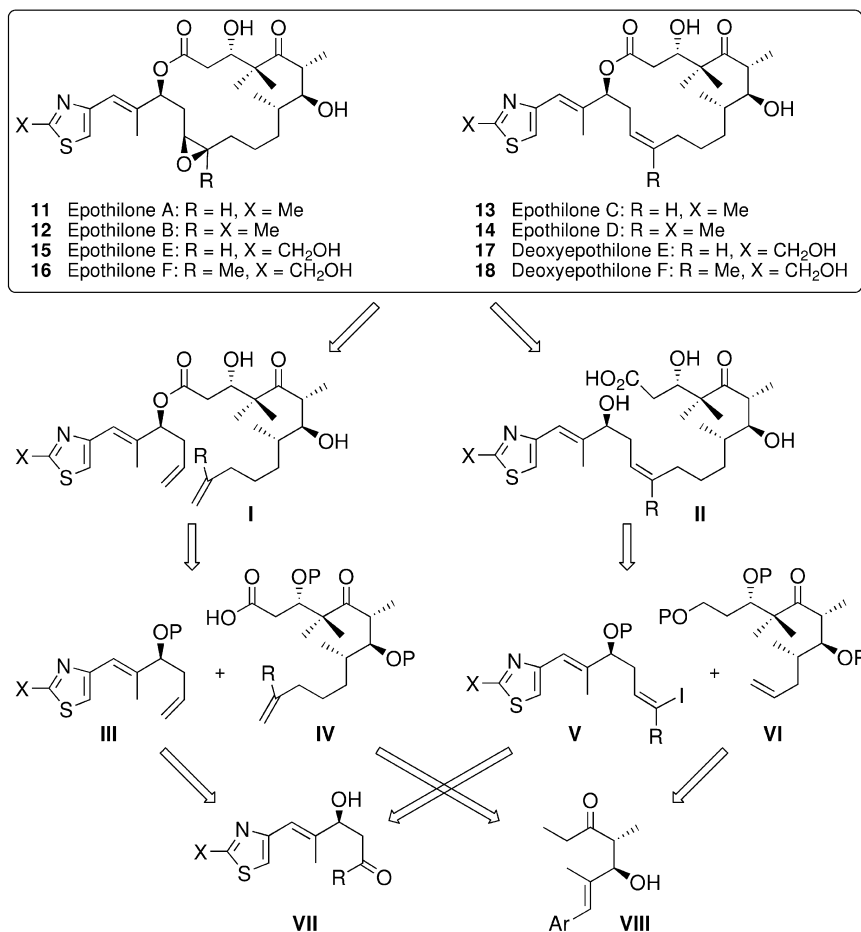


Scheme 5.2

In the aldolase antibodies, such as 38C2 and 33F12 [34], 24H6 [35] and 84G3, 85H6 and 93F3 [40], which were generated against β -diketone haptens, a hydrophobic microenvironment accounts for tuning the pK_a of the ϵ -amino group of this lysine residue [41]. This nucleophilic lysine reacts with ketones and aldehydes to form an imine (or iminium ion, I) and enamine (II) intermediates (Scheme 5.2). These intermediates may lead to many carbonyl transformations, such as carbonyl condensation reactions, alkylation, decarboxylation, etc. The aldol reaction [42], for example, involves a nucleophilic addition of the enamine intermediate, II (Scheme 5.2), to a carbonyl acceptor, e.g., an aldehyde, to form the iminium intermediate III and eventually produce the aldol product IV. The synthetic advantages of these aldolase antibodies, which share both the characteristics of the natural class I aldolases and broad substrate scope, have been demonstrated by various examples [43, 44].

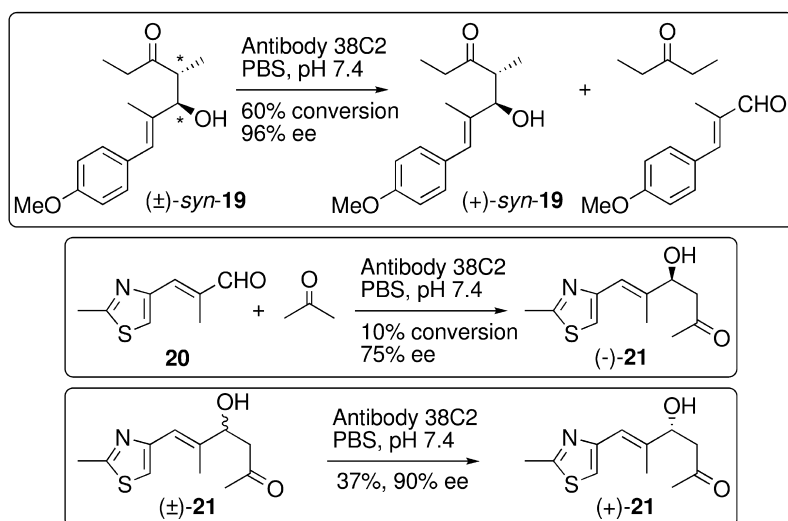
Epothilones are molecules of current interest because of their medical promise and synthetic challenges [45, 33]. Epothilone A (11) and epothilone B (12) are powerful cytotoxic agents isolated from myxobacteria (*Sorangium cellulosum* strain 90). They possess a taxol-like mode of action, functioning through stabilization of cellular microtubules, and exhibit cytotoxicity even in taxol-resistant cell lines. Epothilone B has

been reported to be about 3400 times more active than taxol against the resistant human leukemic cell line CCRF-CEM/VBL in cell-culture cytotoxicity studies. Desoxy precursors of **11** and **12**, epothilones C (**13**) and D (**14**) also possess comparable biological properties, particularly the tubulin polymerization activity. Besides epothilones A–E, many analogs of these compounds have been synthesized and studied for their effects on tubulin polymerization *in vitro* and *in vivo*.



Scheme 5.3

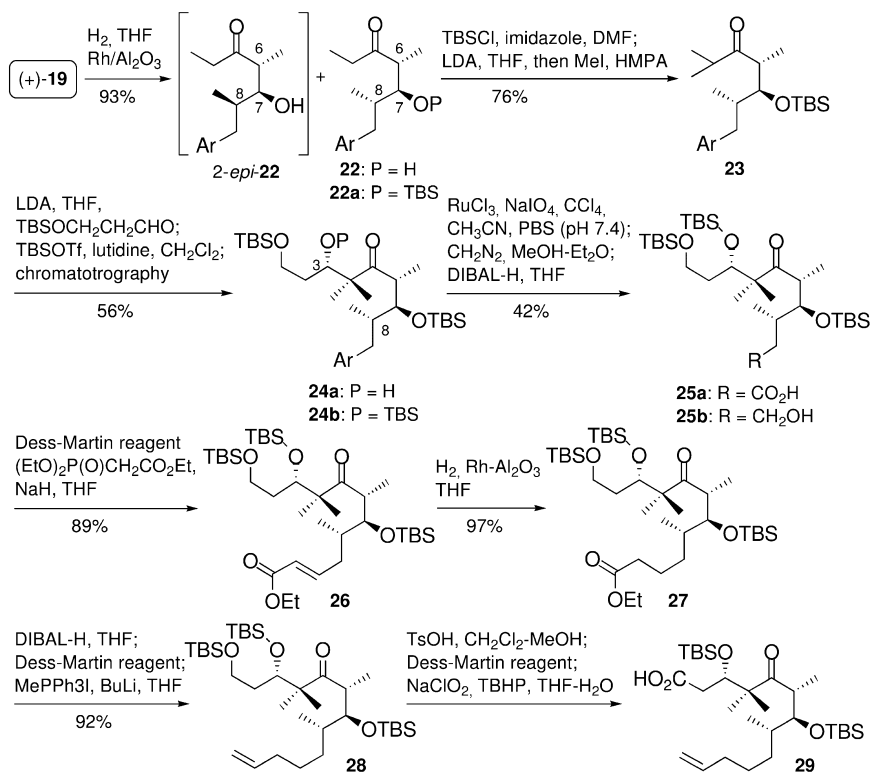
Retrosynthetic analysis of the epothilone skeleton suggested that compounds **11–18** could be obtained either from **I** via metathesis or from **II** via macrolactonization (Scheme 5.3). Each of the intermediates, **I** and **II**, could be constructed from two major building blocks, **I** from **III** and **IV**, and **II** from **V** and **VI**. Both intermediates **III** and **V** could be obtained from a common aldehyde **VII**. Similarly, both intermediates **IV** and **VI** could be obtained from the common aldehyde **VIII**.



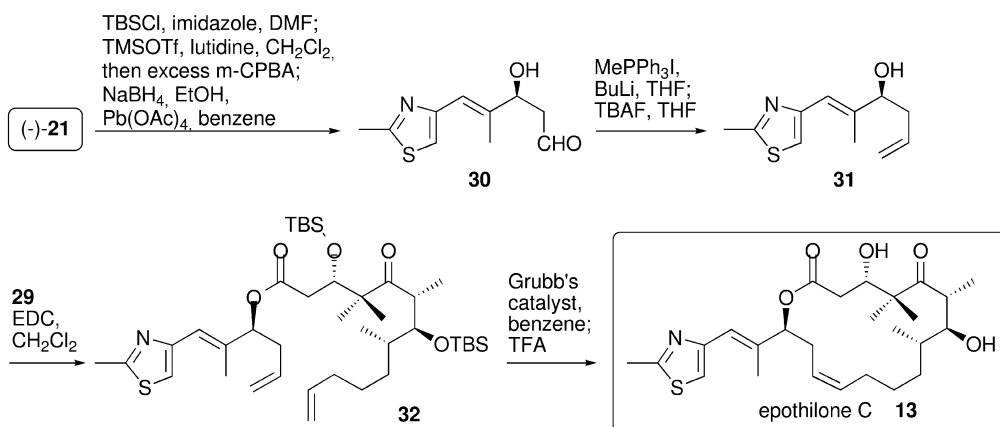
Scheme 5.4

Preliminary investigations have indicated that both enantiomers of each of the required aldol precursors, **VII** and **VIII**, could be accessible by antibody 38C2-catalyzed aldol and retro-aldol reactions [46]. Indeed, aldol $(\pm)\text{-syn-19}$ underwent a retro-aldol reaction to produce 4-methoxy- α -methylcinnamaldehyde and pentan-3-one (Scheme 5.4). At pH 5.4 (PBS) with 0.125 mol% of 38C2 at 60% conversion, the recovered unreacted aldol, $(+)\text{-syn-19}$, was obtained with 96% *ee*. An important point regarding this step is that the reaction could be carried out on gram scale. Typically, 0.75 g of compound $(\pm)\text{-syn-19}$ was resolved to afford enantiomerically pure $(+)\text{-syn-19}$ (0.3 g). Similarly, as expected on the basis of previous results [34, 38], antibody 38C2 also catalyzed the aldol condensation between aldehyde **20** and acetone to produce aldol $(-)\text{-21}$ with 75% *ee* (Scheme 5.4). The reaction was carried out with 0.06 mol% catalyst and was interrupted at 10% conversion and 51% yield. At higher conversions the retro-aldol reaction became more significant, leading to diminished enantiomeric purity of **21**. An alternative approach to $(-)\text{-21}$ used enantioselective 38C2-catalyzed retro-aldol reaction with $(\pm)\text{-21}$ to produce $(+)\text{-21}$ followed by inversion of the hydroxyl configuration by the Mitsunobu reaction to give $(-)\text{-21}$ (Scheme 5.4).

With the enantiomerically enriched building blocks $(+)\text{-syn-19}$ and $(-)\text{-21}$ in hand, synthesis of the naturally occurring epothilone **A** (**11**) was achieved via its desoxy precursor epothilone **C** (**13**) using either the metathesis (Schemes 5 and 6) or the macrolactonization approach. The use of building block $(+)\text{-syn-19}$ secured the two stereogenic centers at C-6 and C-7 that allowed for the transfer of chirality to the remaining centers. Hydrogenation of $(+)\text{-syn-19}$ afforded an easily separable 1:1 mixture of compounds **22** and *epi-22*. The free alcohol in **22** was protected in the form of a TBS ether, **22a**, before undergoing monomethylation to give **23**. Aldol reaction with 3-*tert*-butyldimethylsilyloxy propanal afforded a mixture of diastereomeric aldol products, with the desired stereoisomer, **24a**, being the major product. The latter was



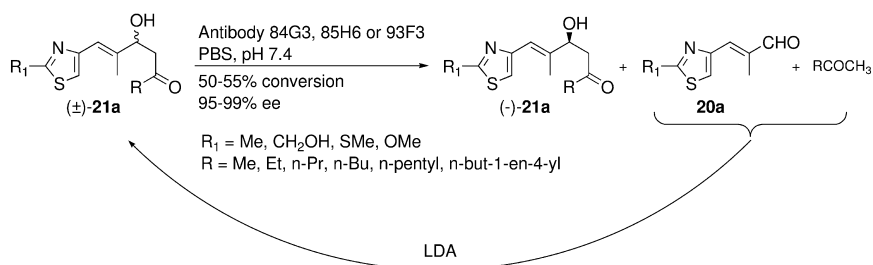
Scheme 5.5



Scheme 5.6

silylated to give **24b**. Exhaustive degradation of the aromatic ring with $\text{RuCl}_3\text{-NaIO}_4$ afforded a carboxylic acid, **25a**, which was transformed to the corresponding alcohol **25b**. The latter was oxidized to the corresponding aldehyde, which was subjected to a Wittig olefination reaction to produce an α,β -unsaturated ester, **26**. Hydrogenation of the alkene gave the saturated ester **27**. Reduction of the ester to a primary alcohol and oxidation with Dess-Martin reagent afforded the corresponding aldehyde, which was reacted with methyltriphenylphosphorane to produce a terminal alkene, **28**. Deprotection of the primary alcohol followed by oxidation afforded the carboxylic acid **29**.

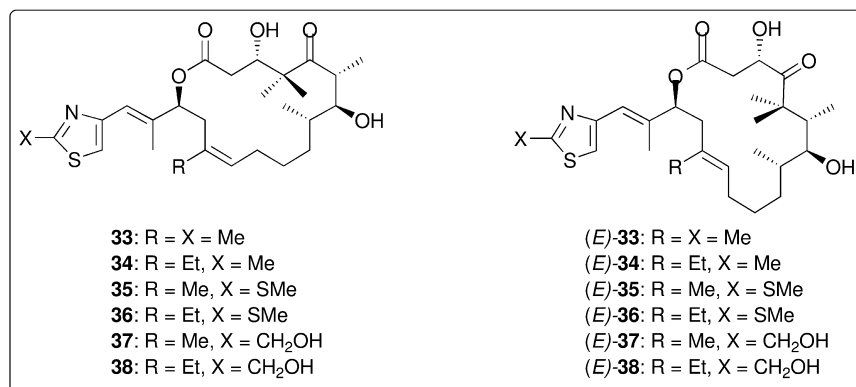
The other aldol building block, (-)-**21**, was first protected in the form of a TBS ether (Scheme 5.6). The latter was converted to the trimethylsilyl enol ether, which was then reacted with *m*-CPBA to produce the primary hydroxyketone. Reduction of the ketone to the vicinal diol followed by cleavage with $\text{Pb}(\text{OAc})_4$ gave aldehyde **30**. The latter was subjected to a Wittig reaction with methylphosphorane followed by hydrolysis of the TBS ether to yield alcohol **31**. Esterification of the carboxylic acid **29** with alcohol **31** afforded ester **32**, which was converted to a macrocyclic lactone (a 3:2 mixture of the *cis:trans* isomers) via metathesis reaction with Grubb's catalyst. Deprotection of the alcohols using TFA, followed by chromatographic separation, afforded epothilone C (**13**). Epoxidation of **13** with methyl(trifluoromethyl)dioxirane afforded epothilone A (**11**). An alternative approach to compound **13** employed the macrolactonization strategy, using precursor (-)-**21** [33].



Scheme 5.7

The above-described syntheses were based on the aldol products (+)*syn*-**19** and (-)-**21**. However, the antibody 38C2-catalyzed aldol reaction afforded compound (-)-**21** in modest enantiomeric purity (75% *ee*) at 10% conversion, and these yields decreased even further as the reaction progressed as a result of retro-aldol reaction. This problem was satisfactorily solved by using the more recently discovered antibodies, 84G3, 85H6, and 93F3 [40]. These aldolase antibodies exhibited antipodal reactivities in comparison with 38C2, allowing for the resolution of compound (\pm)-**21a**, in very high enantiomeric purity (Scheme 5.7). These aldol derivatives were practically resolved in multigram (> 16 g) quantities and essentially enantiomerically pure form using 0.003, 0.005, and 0.0004 mol % of antibodies 84G3, 85H6 and 93F3, respectively [47, 48]. Since these reactions and their work-up did not require harsh conditions, it was possible to recycle the thiazol aldehydes (**20**) for the synthesis of the racemic aldol substrate, (\pm)-**21a**, using LDA (Scheme 5.7).

With these chiral building blocks in hand, a sequence of chemical steps previously published by Schinzer et al. allowed the completion of the total synthesis of the above-mentioned epothilones, **11**, **12**, **15**, and **16**, via their desoxyepothilones, **13**, **14**, **17**, and **18** (Scheme 5.3). Recently, preliminary results were published in the synthesis of 13-alkyl analogs of epothilones A–E and fluoroepothilones using the same strategy [47, 49]. Other naturally occurring epothilones as well as many non-natural analogs, such as **33–38** (Scheme 5.8), were synthesized from the key building blocks, including (+)-**19** and (–)-**21** and similar precursors, all generated via antibody-catalyzed aldol and retroaldol reactions.



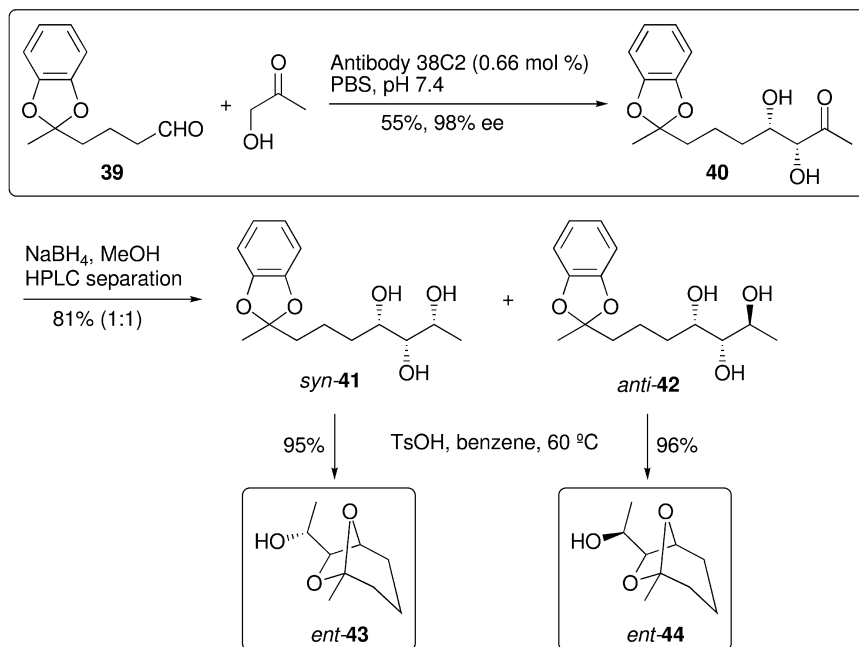
Scheme 5.8

In summary, the two antibody-catalyzed steps have created the required chirality in the total syntheses of epothilones A–F and their analogs. Three stereogenic centers were thus incorporated into the epothilone skeleton.

5.4

Total Synthesis of Brevicomins using Aldolase Antibody 38C2

The brevicomins are derivatives of 6,8,-dioxabicyclo[3.2.1]octanes, which are known pheromones of a variety of bark beetle species [50]. Extensive outbreaks of bark beetles may result in the destruction of millions of trees per year, causing great ecological and economic damage [51]. Aldolase antibody 38C2 was successfully used by Barbas and coworkers for the enantioselective total syntheses of several brevicomins [52]. The key steps in these syntheses were achieved via either an antibody-catalyzed aldol addition or retroaldol reaction. For example, 38C2-catalyzes aldol reactions between hydroxyacetone as donor, and various aldol aldehyde and ketone acceptors afforded α,β -dihydroxyketones with an $\alpha(2R,3S)$ configuration in high regio- and stereoselectivity [37]. The usefulness of this reaction was demonstrated by the short syntheses of hydroxybrevicomins *ent*-**43** and *ent*-**44** in very high enantiomeric purity (Scheme 5.9). The synthesis was carried out by reacting aldehyde **39** and hydroxyacetone on a prepar-

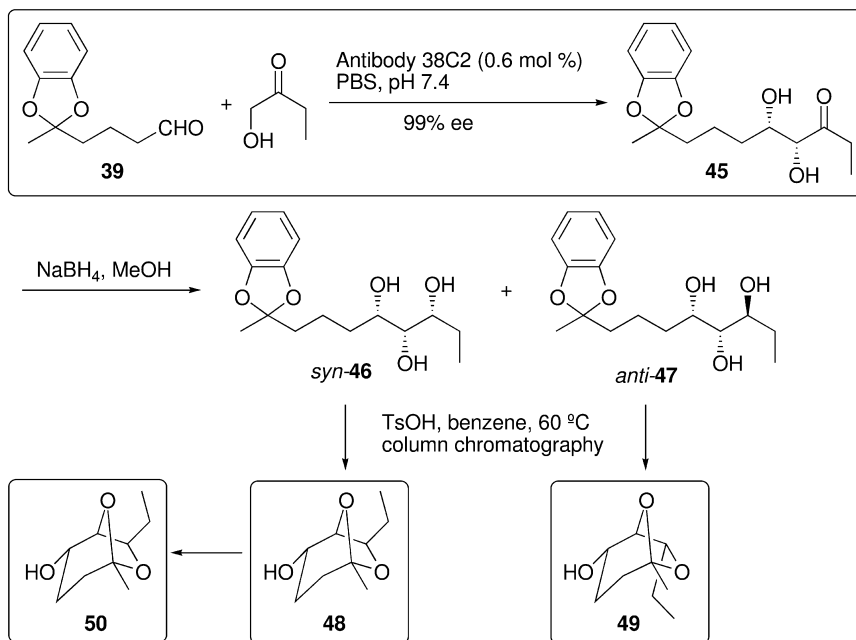


Scheme 5.9

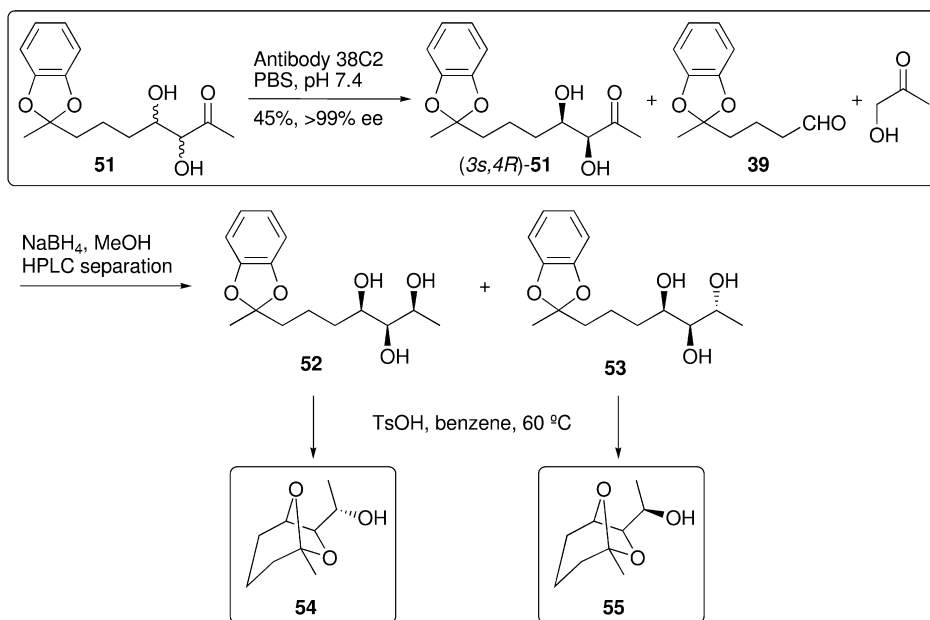
ative scale in the presence of antibody 38C2 (0.66 mol %) to give diol **40** in 55% yield and 98% *ee*, along with the *anti*-diastereomer (4:1). Subsequent non-selective reduction with NaBH_4 followed by separation of diastereomers afforded triols *syn-41* and *syn-42*. Hydrolysis of the ketals and *in situ* ketalization produced the desired brevicomins *ent-43* and *ent-44*, respectively.

Antibody 38-catalyzed aldol reaction between aldehyde **39** and 1-hydroxybutan-2-one yielded the aldol product **45** in 99% *ee* (Scheme 5.10). As described above, reduction and separation of diastereomers produced triols *syn-46* and *syn-47*. Deprotection and intramolecular ketalization afforded brevicomins **48** and **49**, respectively. Brevicomin **48** can be easily converted to brevicomin **50** by reductive dehydroxylation, as described previously [53].

Since the aldolase antibodies are able to catalyze both the aldol addition and the retro-aldol reaction, these catalysts are useful in the kinetic resolution of aldol products [46]. A single antibody catalyst can therefore be used for the preparation of both aldol enantiomers. This concept was applied in the synthesis of hydroxybrevicomins **43** and **44**. Antibody 38C2 catalyzed the retro-aldol reaction of the racemic mixture of **51** to produce (3*S*,4*R*)-**51** in 99% *ee* after 55% conversion of racemate (Scheme 5.11). Using the same procedures described in Scheme 5.9, diol (3*S*,4*R*)-**51** can be converted to the natural products **43** and **44**. These examples demonstrated that catalytic antibodies could be used to decrease the total number of synthetic steps and to increase enantioselectivity of natural product synthesis.



Scheme 5.10



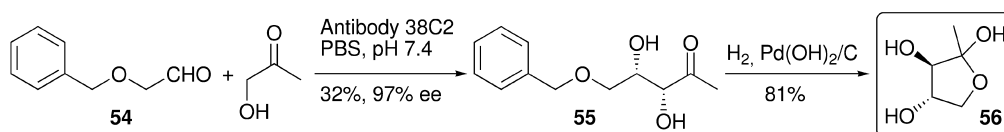
Scheme 5.11

5.5

Synthesis of 1-Deoxy-L-Xylose using 38C2 Antibody

1-Deoxy-L-xylose (**56**), which was isolated from *Streptomyces hygroscopicus*, is a key intermediate in the biosynthesis of thiamin (vitamin B₁) [54] and of pyridoxal (vitamin B₆) [55], as well as an alternative non-mevalonate biosynthetic precursor of terpenoid building blocks. Using 38C2, the total synthesis of this natural product by Shabat et al. was achieved via a two-step synthesis, which is considered to be the shortest synthesis known to date for this molecule [56].

While all the natural aldolases use hydroxyacetone in its phosphate-protected form, aldolase 38C2 antibody is known to use unprotected hydroxyacetone in the aldol addition reaction. As shown in Scheme 5.12, the key step of the antibody 38C2-catalyzed synthesis of **56** is an aldol reaction between unprotected hydroxyacetone and aldehyde **54**. Using only 0.04 mol% of 38C2 afforded dihydroxyketone **55** in 32% yield and 97% ee after 56% conversion of aldehyde **54**. Hydrogenolysis of the benzyl protecting group produced the target product, **56** in 81% yield.



Scheme 5.12

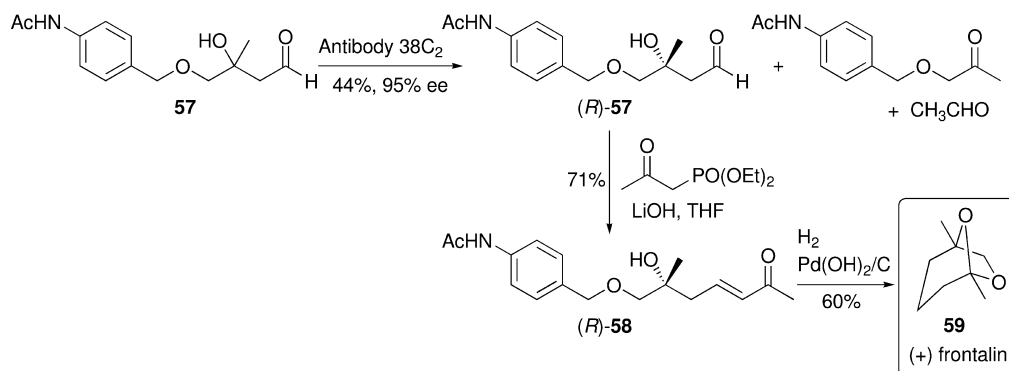
5.6

Synthesis of (+)-Frontalin and Mevalonolactone via Resolution of Tertiary Aldols with 38C2

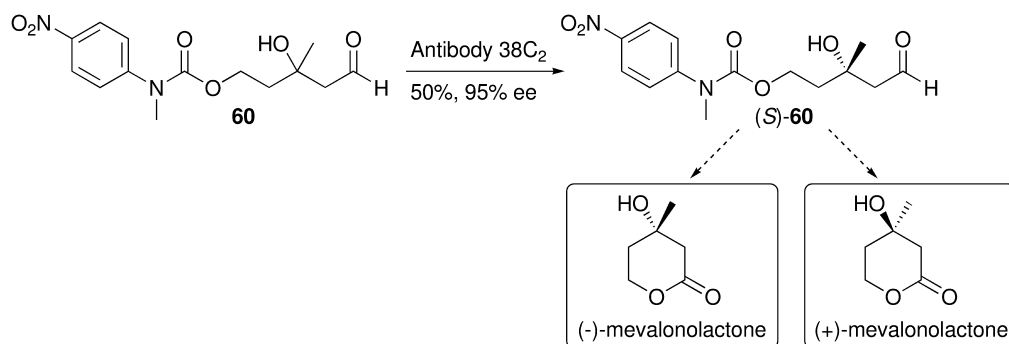
Both chemical and enzymatic approaches have been applied almost exclusively to the synthesis of secondary β -hydroxy carbonyl compounds (secondary aldols). General methods, either chemical or enzymatic, for the preparation of enantiomerically enriched tertiary aldols have not been developed. Tertiary aldols, which contain a heteroatom-substituted quaternary carbon stereocenter, constitute a challenge in synthetic chemistry. This is particularly true when this problem is approached through aldol chemistry. Interestingly, although tertiary aldols represent a common structural motif in many bioactive natural products, natural enzymes cannot be applied to this problem because no known natural aldolase catalyzes the synthesis of tertiary aldols.

Antibody 38C2 was found by List et al. to be a practical and highly enantioselective catalyst for the formation of tertiary aldols, accepting a broad variety of substrates [57]. This antibody catalyzes the retro-aldol reaction of various tertiary aldols, thus providing a rapid entry to highly enantiomerically enriched tertiary aldols (typically >95%) via kinetic resolutions. The utility of this approach has been demonstrated in the synthesis of (+)-frontalin (**59**), a sex pheromone, which was found in several beetle species [58] as well as in the temporal gland secretion of the male Asian elephant [59]. The

enantioselective synthesis of (+)-frontalin via kinetic resolution with 38C2 is shown in Scheme 5.13. Racemic aldol **57** (50 mg) was resolved with antibody 38C2 to give aldol product (*R*)-**57** (22 mg, 44%) in 95% *ee*. LiOH-mediated Horner-Wadsworth-Emmons reaction of aldehyde (*R*)-**57** with diethyl (2-oxopropyl)phosphonate afforded enone **58**, which was subjected to Pd-catalyzed hydrogenolysis and spontaneous cyclization to produce (+)-frontalin (**59**).



Scheme 5.13



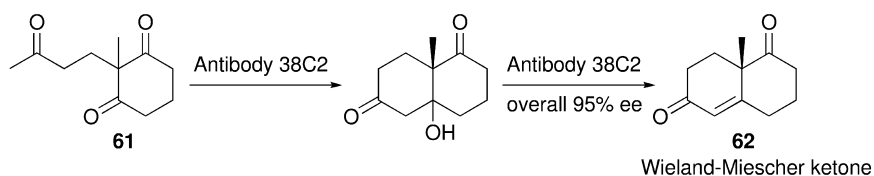
Scheme 5.14

Similarly, the formal synthesis of (–)- and (+)-mevalonolactone (Scheme 5.14) was achieved by the kinetic resolution of the racemic tertiary aldol **60**. The enantiomerically pure (*S*)-**60** could be converted to (–)-mevalonolactone by oxidation, basic hydrolysis, and lactonization. It has already been shown that (*S*)-**60** can also be used for the synthesis of (+)-mevalonolactone [60]. Since there are many other natural products containing the tertiary aldol moiety, such as saframycin H [61], the above-described examples represent a general approach to the synthesis of such compounds [57].

5.7

Wielaned-Miescher Ketone via 38C2-Catalyzed Robinson Annulation

The remarkable substrate scope of antibody 38C2 allows it to catalyze a broad variety of enantioselective aldol-type reactions, including the Robinson annulation (Scheme 5.15) [62]. Zhong et al. reported that antibody 38C2 catalyzes not only the intramolecular cyclization of triketone **61**, but also the following dehydration of the resultant aldol product (**62**) with >95% *ee*. Aldol **62**, known as Wielaned-Miescher ketone, represents a useful precursor in the total synthesis of various steroid derivatives.

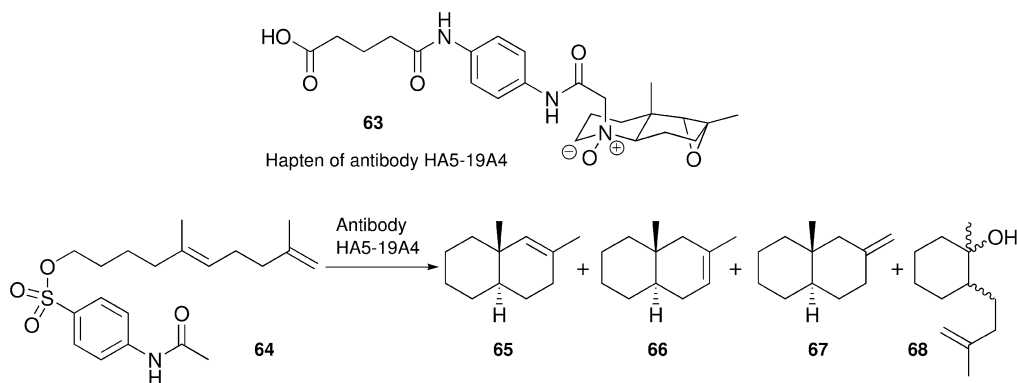


Scheme 5.15

5.8

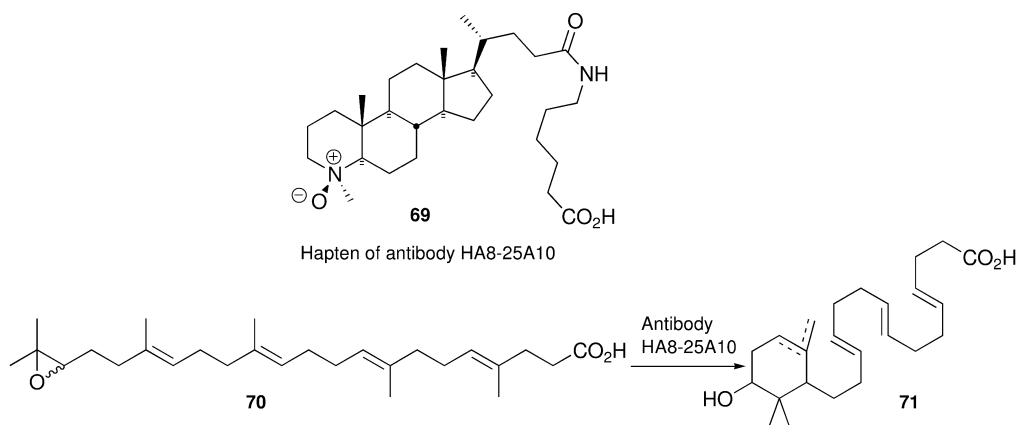
Formation of Steroid A and B Rings via Cationic Cyclization

In efforts to mimic the natural enzyme oxidosqualene cyclase, several catalytic antibodies were elicited against charged transition state analogs. For example, Hasserodt et al. employed the bicyclic bridge-methylated decahydroquinoline *N*-oxide **63** as a hapten to elicit antibody HA5-19A4, which catalyzed the cationic cyclization of the dienol sulfonate **64** to produce the closely related decalin systems, **65–67**, with an average enantiomeric excess of 53% (Scheme 5.16), which represent rings A and B of the steroid nucleus [63]. An extension of this work from the same group presented



Scheme 5.16

cationic cyclization that focused on substrates analogous to those seen in triterpene biosynthesis. Three antibodies, 15D6, 20C7, and 25A10, which have been elicited against a 4-aza-steroid aminoxide hapten, **69**, initiated the cationic cyclization of an oxidosqualene derivative and catalyzed the formation of compound **71** from polyene **70** at neutral pH (Scheme 5.17). The latter represents ring A of the lanosterol nucleus [64].



Scheme 5.17

5.9

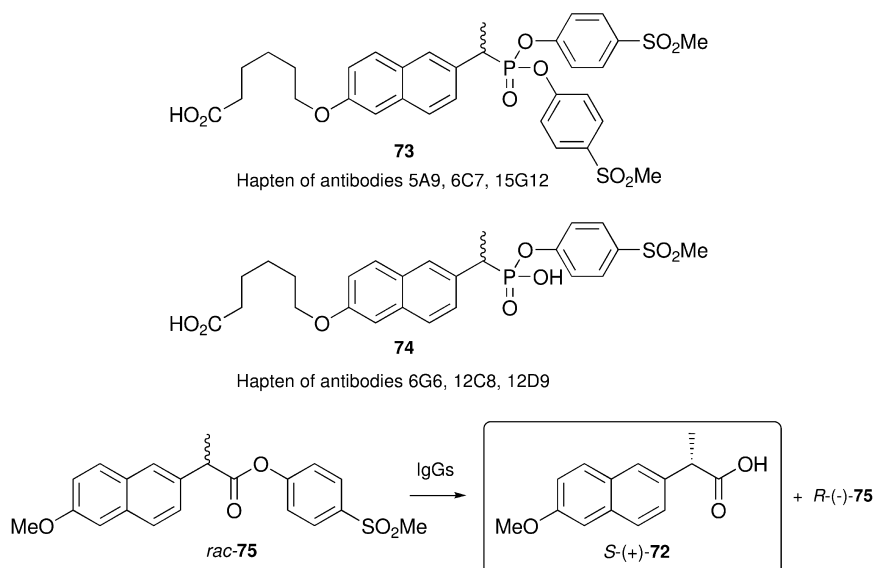
Synthesis of Naproxen via Antibody-Catalyzed Ester Hydrolysis

Considerable efforts have been devoted to improving the preparation of the enantiomerically pure *S*-(+)-naproxen, **72**, a widely prescribed non-steroidal *anti*-inflammatory drug (NSAID), which has been made industrially through diastereomeric crystallization. Antibodies 5A9, 6C7, and 15G12, which were elicited against the phosphonate diester **73** in a reactive immunization approach, hydrolyze *S*-(+)-naproxen *p*-methylsulfonylphenyl ester **75** with rate enhancement as high as 6.6×10^5 and a kinetic resolution of *rac*-**75**, leading to *S*-(+)-**72** in 90% *ee* for 35% conversion (Scheme 5.18) [65]. Improved catalysts were obtained by employment of phosphonate monoester **74**, leading to a library of catalysts, 6G6, 12C8, and 12D9, with excellent turnover numbers, $k_{\text{cat}}/k_{\text{uncat}}$ as high as 1.9×10^6 and a useful kinetic resolution of *rac*-**75**, generating *S*-(+)-**72** in >98% *ee* with up to 50% conversion [66].

5.10

Conclusions

The synthesis of natural products remains the ultimate testing ground for new concepts in organic chemistry. One of the main goals of the field of antibody catalysis



Scheme 5.18

has been to learn how to design catalysts to improve the overall yield of existing synthetic routes, thus allowing practical construction of more totally synthetic drugs and other important natural products. The examples of natural products synthesis covered by this chapter, including (–)- α -multistriatin, epothilones, brevicomins, 1-deoxy-L-xylose, (+)-frontalin, the formal synthesis of (–)- and (+)-mevalonolactone, partial synthesis of the steroid skeleton, and naproxen, testify to the important role catalytic antibodies may play in asymmetric synthesis. The remarkable ability of these biocatalysts to control the rate, stereo-, regio-, chemo-, and enantioselectivity of many reactions, including highly disfavored chemical processes and sometimes even those with high substrates promiscuity, will undoubtedly place these agents in a central position on the map of total synthesis of pharmaceuticals, fine chemicals, and complex natural products.

To reach a wider use of catalytic antibodies in synthesis, an obvious need is the reduction of costs. This may be achieved through less expensive production and/or facile means of catalyst recovery. Much work has been done to reduce the cost of antibody production, including over-expression in bacteria, plants, seeds, and algae [67]. Production in seeds is particularly attractive because it may afford a highly concentrated source of stable protein that is easily stored in large quantities. Thus, the seed becomes a dry, well-protected storage device for the catalyst. A number of methods have been reported for immobilization and recovery of antibodies to allow operation in continuous-flow reactors. These methods include attachment to insoluble solid supports or soluble polymer, or entrapment in sol-gel matrices.

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