Advances in Antibody Catalysis of Cycloaddition Reactions

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Abstract: Pericyclic cycloaddition reactions are among the most useful transformations available to the synthetic organic chemist. All are capable of simultaneously establishing multiple stereogenic centers with high levels of selectivity. Although many powerful chemical catalysts for these reactions exist, biological catalysts for these reactions are rare, and in most cases, non-existent. With the advent of catalytic antibody technology, proteins have been developed that are capable of not only catalyzing the Diels–Alder reaction, but also hetero-Diels–Alder, retro-Diels–Alder, and 1,3-dipolar cycloaddition reactions. This review will summarize the key developments in the use of catalytic antibodies as catalysts for these powerful cycloaddition reactions.

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Keywords: catalytic antibodies; cycloaddition; Diels–Alder reaction; 1,3-dipolar cycloaddition

1 Introduction

Pericyclic cycloaddition reactions are a diverse array of ring-forming reactions that have provided organic chemists with an arsenal of tools with which to synthesize a wide variety of complex molecules. Indeed, the importance of these reactions has been underscored, most notably in the synthesis of natural products, however, fully characterized enzymes that catalyze pericyclic cycloaddition reactions are virtually unknown.[1,2,3] Until recently, Nature has only played witness to the development of catalysts for cycloaddition reactions. But, with the discovery of catalytic antibodies, or abzymes, Nature attained a prominent role in the development of efficient catalysts for pericyclic cycloaddition reactions.

Over the last fifteen years antibody catalysis has traversed many different paths.[4,5] Its inception in 1986[6,7] was based on catalysis of acyl transfer processes, but, quickly it was recognized that for antibodies to have significant impact within the organic community, chemical reactions that are disfavored or ones in which there are no enzyme counterpart would have to be investigated.[8−12]

Bimolecular processes and, in particular, cycloaddition reactions have large entropic barriers, with activation entropies typically in the range of -30 to -40 cal K^{-1} mol^{-1}.[13] Surmounting unfavorable processes such as these seems formidable, however, it was thought that antibodies could overcome such energy barriers by serving as "entropic traps." In this scenario the antibody's role would be: 1) To sequester
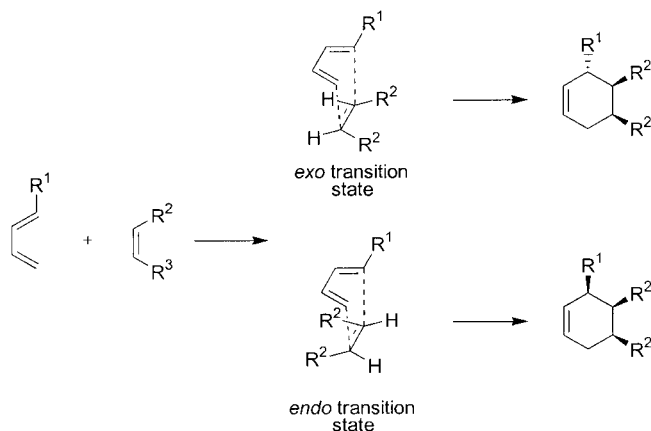

Figure 1. A representative Diels–Alder reaction showing the diastereomeric transition states and corresponding products.

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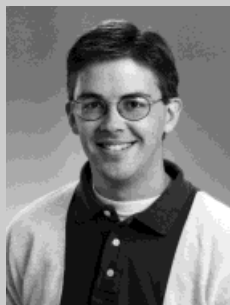
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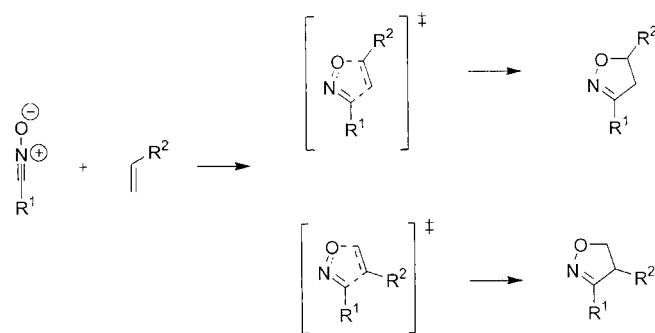

Figure 2. A representative 1,3-dipolar cycloaddition reaction showing the putative transitions states leading to the 5- and 4-acylisoxazoline products.

the two reactants in the antibody combining site. 2) Correctly align in spatial proximity the reactants in its cleft. 3) Release the product being formed such that turnover can be achieved. To accomplish such feats haptens must be designed that will elicit antibodies that can bind to the transition state(s) and ultimately reduce its energy of activation for the reaction being probed. Thus, hapten design can be a daunting task, but fortunately, the mechanistic details of many cycloaddition reactions are well understood (Figure 1 and 2), thus greatly simplifying hapten design and the identification of antibody catalysts. This review article will summarize the major discoveries in the field of antibody-catalyzed cycloaddition reactions, bringing together the concepts behind the design of transition-state analogues and the kinetic properties of the discovered antibodies elicited by immunization with these analogues. Whenever possible, additional information garnered from the crystal structure of the discovered catalytic antibodies will be discussed to rationalize the biological activities of these proteins.

2 Diels–Alder Cycloadditions

An early demonstration by Hilvert and coworkers of an antibody-catalyzed cycloaddition process was in the Diels–Alder reaction between tetrachlorothiophene dioxide (**1**) and *N*-ethylmaleimide (**2**) (Scheme 1).[14,15] The Diels–Alder adduct of this reaction is an unstable tricyclic intermediate that extrudes SO_2 to give the dihydrophthalimide derivative **3** as the product. The hexachloronorbornene hapten **4** was designed as a stable analogue of the tricyclic Diels–Alder product. Since this tricyclic structure is not observed in the final product, this approach is unique in that it allows catalysis of the Diels–Alder reaction without significant product inhibition. Unfortunately, the high reactivity of **1** with the lysine residues required modification of the antibody's surface to avoid the degradation of **1**. With regards to catalysis, the benefit of using antibody 1E9 over the un-
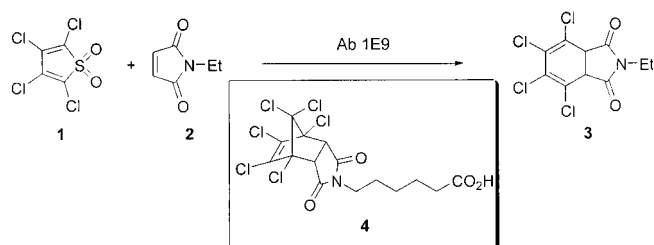
Scheme 1. An initial approach for the production of a Diels–Alderase catalytic antibody.

catalyzed reaction was calculated based on effective molarity. Effective molarity is a measure of the concentration of both substrates that would be needed in the uncatalyzed reaction to achieve the same rate as seen in the antibody ternary complex. Initially the effective molarity calculated for this Diels–Alder reaction was on the order of 10^2 M. However, an accurate measurement of this number was hampered due to the low aqueous solubility of **1**. Later studies using dienes with greater water solubility showed that the actual effective molarity for this catalytic antibody was actually on the order of 10^3 M.[16]

To complement these kinetic studies with structural information, Wilson's group compared the gene encoding the heavy and the light chains of 1E9 with a closely related and structurally characterized anti-progesterone antibody.[17] They suggested that shape complementarity, through van der Waals interactions, was likely to be the dominant factor in facilitating effective pre-organization of the substrates. Later, this assumption was confirmed by the determination of the crystal structure obtained from the complex of the Fab fragment of 1E9 and stable transition state analogue **4**.[16] More than 120 van der Waals contacts were identified between the hapten and antibody including contacts with the peptide backbone and side chains of aliphatic and aromatic residues. The indole

ring of one tryptophan residue (Trp[H50]) was involved in a π-stacking interaction with the succinimide group of **4**, which plays the role of the maleimide substrate. Additionally, a hydrogen bond was discovered between one of the succinimide carbonyl groups and an asparagine residue (Asn[H35]). Because the reaction between **1** and **2** involves two electron-deficient species, the reaction is accelerated by making the dienophile either more electron-deficient (as is the case with a hydrogen bond) or by making it more electron-rich. It was then suggested that the tight fit between 1E9 and the transition state is responsible for binding, but that the increased electrostatic complementarity of the antibody for the transition state is responsible for catalysis.[18] Analysis of the entropy and enthalpy of activation for the catalyzed and uncatalyzed reactions showed that 1E9 does not serve as a classical entropic trap, and in fact, the entropy of activation in the catalyzed reaction was comparable to that of the uncatalyzed reaction. Instead, the rate acceleration of this catalytic antibody is entirely derived from a lowering of the enthalpy of activation.[16]

Concurrent to the work of Hilvert, Schultz reported an antibody-catalyzed [4+2] cycloaddition between acyclic diene **5** and *N*-phenylmaleimide (**6**) (Scheme 2).[19] The strategy for the design of this transition-state analogue was based on the bicyclooctene hapten **8**. This molecule was proposed to be a mimic of the necessary locked boat conformation of the *endo* transition state due to the ethano bridge. It was shown that antibody 39-A11 catalyzed the formation of only one of the two possible structural isomers of the bicyclic adduct **7**, but the low effective molarity (0.35 M) was a major drawback of this catalyst.

The three-dimensional structure of the 39-A11 Fab fragment complexed with the hapten was determined and investigated.[20] From this structure it was gleaned that the "dienophile-like" succimido portion of the hapten **8** is tightly packed against the antibody
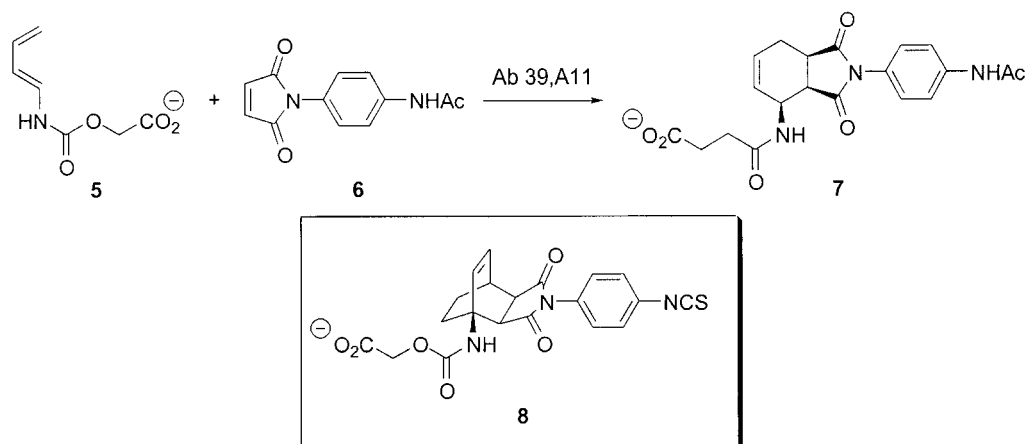

Scheme 2. Conformationally-restricted boat approach (**8**) for the production of an antibody which catalyzes the Diels–Alder reaction between **5** and **6**.

whereas the "diene-like" portion is only loosely packed against residues of the light chain. The structure revealed 89 van der Waals contacts and two hydrogen bonds. As was found in 1E9 (*vide supra*), the indole ring of Trp[H50] in 39-A11 was discovered to bind the succimido group through a π-stacking interaction. The same tryptophan is also involved in a hydrogen bond through a molecule of water with the carbamate of **8**. The structure further revealed that the carbonyl of the succinimido *anti* to the carbamate of **8** is hydrogen-bonded to the side-chain amide group of an asparagine residue (Asn[H35]). This interaction should render the olefin more electron deficient, and consequently, a more reactive dienophile. Although not verified, it was proposed that this hydrogen bond could also allow for the production of the disfavored Diels–Alder regioisomer in the instance of an asymmetric dienophile containing one electron-withdrawing group. In this particular system, the regiochemical output of the reaction is inconsequential because of the symmetry of the dienophile. Regardless of this element of symmetry, the energy of the dienophile's LUMO becomes closer to the diene's HOMO because of this hydrogen bond, thus accelerating the reaction. Antibody 39-A11 has also been modified by site-directed mutagenesis to improve the packing interactions with the diene, affording a 10-fold increase in catalytic rate.[21]

Both Hilvert and Schultz designed haptens that were based on transition state theory. Thus, each hapten differed significantly from the substrate and product. In stark contrast, Suckling reported an approach in which the hapten had a very product-like nature (Scheme 3).[22]

Some features of the obtained antibody from Suckling's approach raised questions whether it catalyzed the [4+2] cycloaddition or simply the hydrolysis of 1-acetoxybutadiene, a process that should accelerate the formation of the Diels–Alder adduct by generating a more reactive diene. Indeed, the major product resulting from the H11-catalyzed reaction between diene **9** and dienophile **10** was Diels–Alder adduct **12**
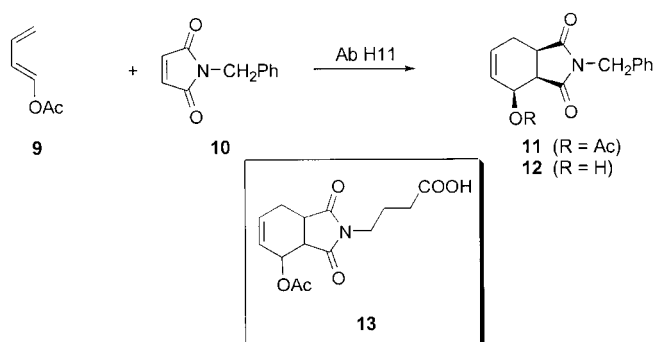

Scheme 3. Antibody H11 generated from hapten **13** that catalyzes the Diels–Alder reaction between **9** and **10**.

bearing an alcohol instead of the expected acetyl ester **11**. Moreover, although Diels–Alder reactions between neutral substrates are expected to be independent of pH, the rate of adduct formation was considerably increased as the pH was raised to 9. Also, the cycloaddition was not inhibited by a relatively high concentration of product (0.45 mM), suggesting that the reaction does not occur in the induced antibody combining site. Surprisingly, methoxybutadiene is not a substrate for H11 even though it is less sterically demanding and intrinsically more reactive than 1-acetoxybutadiene **9**.[23] After a significant number of experiments that consisted of the kinetic characterization of the antibody, it was shown that this protein has catalytic activity mainly through its ability to serve as an esterase, that is, H11 catalyzes the hydrolysis of the acetyl ester, and the Diels–Alder reaction then proceeds at a background rate.[24,25] The stereochemical course of the cycloaddition was shown to produce significant enantiomeric excess, showing that the substrates are, at least partially, captured within the antibody active site during the cycloaddition.

From these examples, it is clear that antibodies can reduce the activation energy barrier and facilitate [4+2] cycloaddition reactions. Antibodies, having intrinsically specific and chiral binding pockets, should also be ideal catalysts for completely controlling the stereochemical outcome of the Diels–Alder reaction. Moreover, it should also enable disfavored chemical processes, such as the production of the *exo* adduct in a [4+2] cycloaddition.

In our laboratories, we generated a pair of antibodies where one catalyzes the formation of the favored *endo* isomer and the other the disfavored *exo* Diels–Alder product.[26] The hapten strategy, illustrated in Scheme 4, features a boat-shaped cyclohexene ring (**18** and **19**) as a mimic for the transition states for either the *endo* or *exo* approach of the dienophile. The stereochemical relationship between the two amido substituents on the bicyclo[2.2.2]octene was essential in order to allow the generation of antibodies possessing complementary structures. Indeed, two antibodies (7D4 and 22C8) were isolated that accelerate the formation of the *endo*- (5-fold) and *exo*-adducts (18-fold), respectively. Gratifyingly, both 7D4 and 22C8 were found to catalyze the formation of their adducts in greater than 98% enantiomeric excess. In comparison, the uncatalyzed reaction gives a racemic mixture of 85:15 *endo*:*exo* products.

While the preceding catalytic antibody reports (*vide supra*) demonstrated the use of highly ordered transition state analogues for the Diels–Alder reaction, another hapten design strategy was introduced by our group. Conformationally unrestricted, ferrocene-based haptens **20** and **21** were synthesized for the eli-
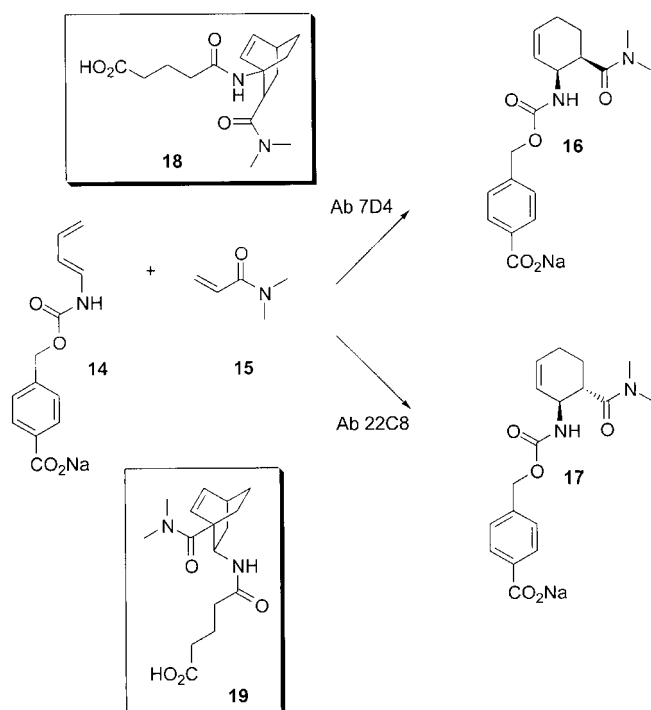
Scheme 4. Haptens **18** and **19** and the chemical scheme for the use of antibodies that control the *exo* and *endo* pathways of the Diels–Alder reaction.

citation of antibodies that would recognize the transition state of a Diels–Alder reaction (Scheme 5).[27] The decision to utilize ferrocene complexes as haptens was fourfold in nature. First, these haptens have two delocalized π-electron ring systems stacked upon each other with an inter-ring distance of approximately 3.3 Å.[28,29] This type of network was anticipated to elicit antibodies with combining sites that could guide the diene and dienophile into a reactive, ternary complex. Second, the rotational freedom allowed by this system provides an opportunity to mimic diastereomeric transition states. The low energy barrier for cyclopentadienyl rotation about the common axis emulates the freedom of translation of substrates and their possible diastereomeric transition states, and allows for the potential to generate antibodies for different diastereomeric products from a single hapten. Third, and most critical to the success of this approach was the ability of the immune system to freeze out a conformer that resembled the desired Diels–Alder transition state. Fourth, the hydrophobic character inherent to the cyclopentadienyl system should both be highly immunogenic, as well as capable of inducing a hydrophobic pocket in the antibody, an important feature needed for the stabilization of the Diels–Alder transition state and improved reaction rates.

This approach successfully allowed the isolation and the characterization of two distinct antibodies (4D5 and 13G5) that catalyze the Diels–Alder reaction

with high enantio- and diastereoselectivity and have effective molarities comparable with those of antibodies obtained using the constrained bicyclo[2.2.2]octene system (Ab7D4 and Ab22C8, *vide supra*). Structurally, the only difference between the haptens **20** and **21** is the linker that was used to attach the hapten to the carrier protein. Consequently, the observed enantio- and diastereoselectivity are due to the ability of the immune system to create a vast library of antibodies from which a catalyst for the desired process can be selected.
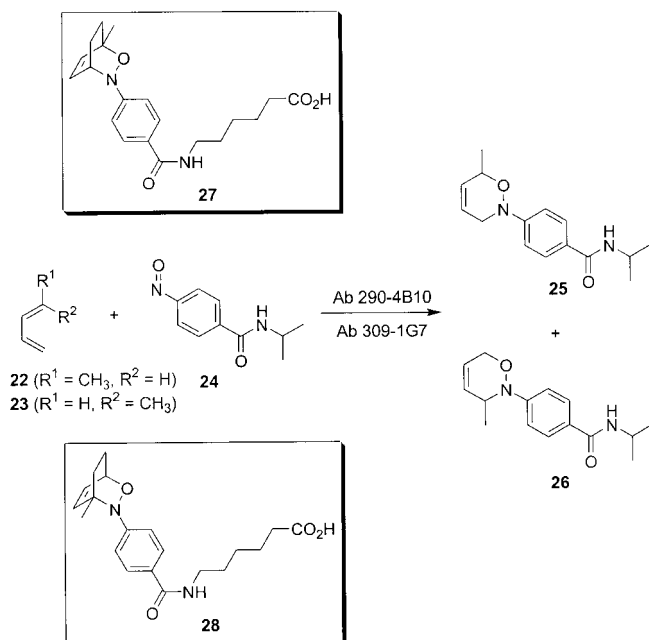
The structure of the Fab fragment of 13G5 in complex with a ferrocene-based molecule related to the compound **21** was determined by X-ray crystallography.[30] A total of 45 van der Waals contacts were identified between the antibody and transition state analogue. Three key antibody residues appear to be responsible for the observed catalysis and product distribution. Two hydrogen bonds, involving Asn[L91] and Asp[H50], are particularly important for the orientation of the diene by binding to its carboxylate group. A tyrosine (Tyr[L36]) acts as a Lewis acid by activating the carbonyl oxygen of the dienophile. This hydrogen-bonding scheme restricts the ferrocene ring rotation of the hapten and effectively freezes out the desired conformer, and it is this network that is believed to lead to both the rate acceleration and pronounced stereoselectivity.
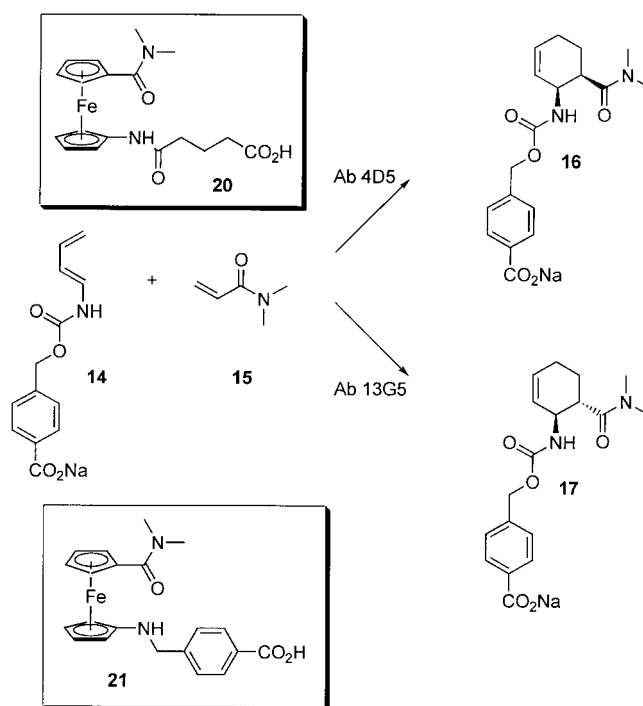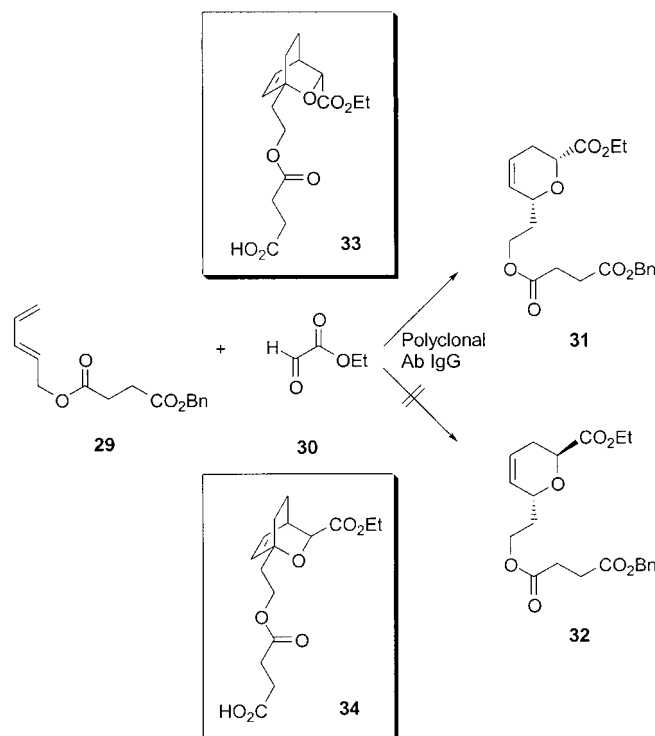

Scheme 5. General approach for the production of antibodies elicited against a conformationally unrestricted hapten.

3 Hetero–Diels–Alder Cycloadditions

An example of an antibody-catalyzed hetero-Diels–Alder reaction was reported in 1995 by Pandit.[51] In this study, the cycloaddition of unsymmetrical *trans*-diene **22** and aryl nitroso derivative **24** was investigated (Scheme 6). The hapten design (compounds **27** and **28**) was based on the bicyclo[2.2.2]octene system developed for the Diels–Alder reaction (Schemes 2 and 4). Significant rate enhancement was observed for two antibodies, namely 290–4B10 (effective molarity = 277 M) and 309–1G7 (effective molarity = 1205 M). Although both the uncatalyzed reaction and the 290–4B10-catalyzed reaction give the same regioisomeric ratio of 58:42 (**25:26**), 309–1G7 causes a shift of this ratio, slightly favoring compound **26** (47:53; **25:26**). In a subsequent study,[32] the authors uncovered the catalytic proficiency of 309–1G7 in the presence of *cis*-diene **23**. This antibody displayed better catalytic activity toward *cis*-diene **23** (effective molarity = 2618 M) than that initially observed for *trans*-diene **22** (effective molarity = 1205 M). Moreover, the uncatalyzed reaction of **23** and **24** gives compounds **25** and **26** in a nearly equimolar regioisomeric ratio of 48:52, respectively, but the presence of 309–1G7 in the reaction results in a product distribution of 32:68 (**25:26**). It is noteworthy that almost exclusive formation of **26** was observed when the entire reaction takes place within the active site (i.e., in the presence of a stoichiometric amount of antibody). Under these conditions, an enantiomeric excess of 82% was obtained, after correction for the background reac-


Scheme 7. Polyclonal antibody approach for catalysts that control the regiochemical outcome of a [4+2] hetero-Diels–Alder reaction.

tion. These results led to the interpretation that the transition state involving the compounds **23** and **24** is better mimicked by hapten **28**. The authors proposed that the methyl group and the ethano bridge in **28** presumably induced a hydrophobic pocket in the antibody that can stabilize the *pseudo*-axial position in the boat-like transition state.[32,33]

The hetero-Diels–Alder reaction between the diene **29** and ethyl glyoxylate (**30**) is a nice example of an attempt to use catalytic antibodies to improve the efficiency of a key reaction in organic synthesis (Scheme 7). Wu and coworkers reported the production of polyclonal antibodies that possess the ability to catalyze the exclusive formation of the 3,4-dihydro-pyran derivative **31**.[34] Polyclonal antibodies were raised against the *endo*-like hapten **33**, however, these antibodies only showed moderate rate enhancement ($K_M = 96$ µM; $V_{max} = 0.98$ µM/min) but, in the absence of the antibodies, no cycloaddition adduct could be detected when the reaction was performed at 37 °C. Although the *exo*-like hapten **34** was synthesized, further studies using this hapten for immunization were not reported.

Scheme 6. Approach for the production of antibodies that catalyze the hetero-Diels–Alder reaction of dienes **22** and **23** with dienophile **24**.

4 Retro–Diels–Alder Cycloadditions

In addition to their role in organic synthesis, antibody-catalyzed reactions have also been studied in
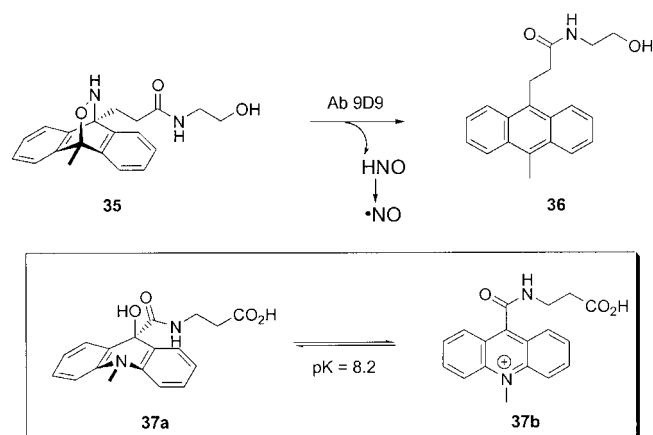
Scheme 8. Equilibrium seen for hapten **37** used in the production of a nitroxyl synthase antibody that catalyzes the retro-Diels–Alder reaction of compound **35**.

binds to the neutral pseudo-base form **37a** much more tightly than to the acidic acridinium form **37b**, suggesting that the non-planar **37a** is a better transition state analogue for the cycloreversion. This was also in agreement with the fact that no product inhibition was observed in the presence of the planar form. In the light of these results, it was proposed that catalysis was mediated by interactions between the two phenyl rings and the antibody. In a subsequent study, the efficiency of 9D9 was further improved by one order of magnitude by immunizing with related analogues of **37a** that converted the hydroxy group to either a hydrogen or nitrile substituent.[56]

5 1,3-Dipolar Cycloadditions

The 1,3-dipolar cycloaddition reactions are of great utility in organic synthesis, particularly for the generation of heterocyclic molecules. While these reactions have been known for more than one hundred years, control of regio-, diastereo-, and enantioselectivity remains a very challenging task. Although methods exist for performing asymmetric 1,3-dipolar cycloaddition reactions using chiral chemical reagents and catalysts,[57] to date there is no known naturally occurring biocatalyst for this reaction.

Among the known 1,3-dipolar cycloaddition reactions, the formation of an isoxazoline ring from an olefin and a nitrile oxide has been shown to be particularly useful synthetically. It is stable toward a number of reagents such that transformations can be carried out on pendant groups. However, the reductive cleavage of isoxazolines by lithium aluminum hydride or Raney nickel opens up a selection of branched-chain derivatives.[58] Catalytic asymmetric
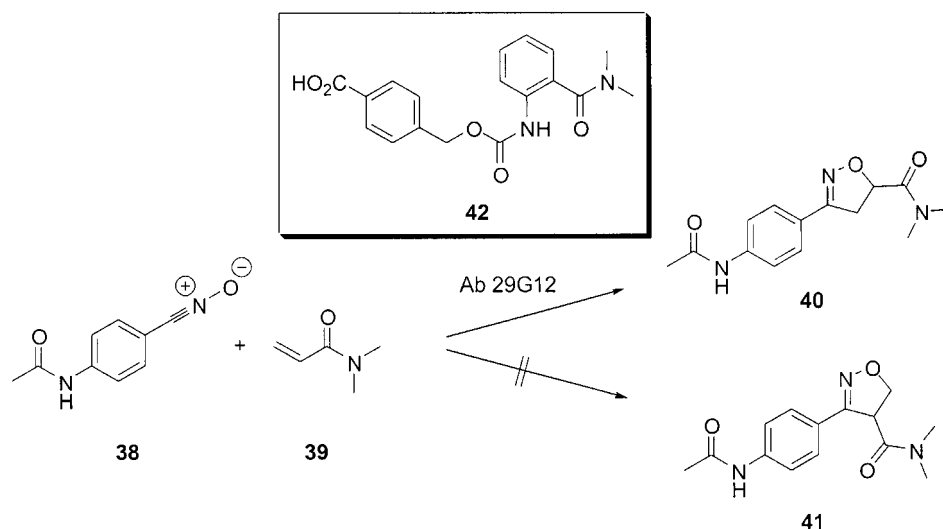
the context of prodrug activation. Reymond, Lerner and coworkers have developed a particularly clever approach based on an antibody that catalyzes a retro-Diels–Alder reaction producing the highly potent molecule, nitric oxide from inert prodrug **35** (Scheme 8).[55] Under physiological conditions, the transition state analogue is in equilibrium between its neutral form **37a** and acridinium form **37b**. Consequently, the hapten presents two different orientations of the phenyl rings, either flat (product-like) or puckered boat-form, which is believed to be closer to the actual transition state of the retro-Diels–Alder reaction. Among three antibodies resulting from immunization with **37(a/b)**, 9D9 was shown to chemoselectively catalyze the formation of anthracene **36**, without observed product inhibition up to 60 µM of compound **35**. The catalysis is independent of pH, showing that no general acid/base or ionizable group is involved in the mechanism. It was found that 9D9

Scheme 9. Hapten **42** and the use of an antibody that catalyzes the biomolecular asymmetric 1,3-dipolar cycloaddition reaction between nitrile oxide **38** and acrylamide **39**.

approaches to the synthesis of chiral isoxazolines have been severely limited, in part, due to the problems caused by the reactivity of the nitrile oxide. Thus, study of this particular type of cycloaddition reaction and the possibility of antibody catalysis became very appealing.

Our group reported the first example of an antibody-catalyzed 1,3-dipolar cycloaddition generating 5-acyl-2-isoxazoline **40** from benzonitrile *N*-oxide (**38**) and *N,N*-dimethylacrylamide (**39**) (Scheme 9).[39] The most accepted mechanism for the 1,3-dipolar cycloaddition is asynchronous concerted with a planar and pericyclic nature in the transition state (Figure 2).[40,41,42] The aromatic character of the postulated transition state was mimicked in hapten **42** by a phenyl ring and the relative arrangement of the *N,N*-dimethylamido and the acetamido groups about the planar phenyl core of the hapten could potentially favor the generation of either the 5-substituted product **40** or 4-substituted product **41**. The most efficient isolated antibody, 29G12, catalyzes the regiospecific and enantioselective formation of the 5-acyl-2-isoxazoline **40** in greater than 98% enantiomeric excess. No evidence of product inhibition after multiple turnovers (>50) was observed and the effective molarity of the process was determined to be 26 M. Comparison of the activation parameters for the uncatalyzed and antibody-catalyzed processes reveals that the catalysis is mainly achieved by reduction of the activation enthalpy rather than of the entropy of activation. This observation led to the interpretation that the antibody may bind to a lower-polarity transition state. The crystal structure as well as the substrate specificity of this new catalytic antibody are currently under investigation in our laboratory in order to explain its mechanism and scope of reactivity.

6 Concluding Remarks

Over the past decade, the ability of the immune system to produce high-affinity antibodies to a small chemical hapten has been exploited to generate catalysts for pericyclic cycloaddition reactions. It has been demonstrated that catalytic antibodies provide both restriction of rotational and translational entropy in the substrates as well as hydrogen-bonding interactions that modulate electron densities on the substituents in the transition state. Catalysis of Diels–Alder reaction through activation of the dienophile by a Lewis acid is a well-established synthetic practice. Upon careful analysis of the X-ray crystal structures of several discovered catalytic antibodies, it was found that the evolution of the catalytic efficiency of the immune system showed the same origins for its mechanism. Studies on antibody-catalyzed cycloaddition reactions have also provided useful insights into

the structures of the transition states for such reactions. For instance, the bicyclo[2.2.2]octene motif has been successfully exploited in several cases as a stable transition state surrogate for [4+2] cycloadditions (*vide supra*). The results obtained are in agreement with the postulate that the transition state for the Diels–Alder reaction resembles the boat conformation of a cyclohexene ring.

Despite these newly uncovered biological activities, improvements are needed to broaden the use of antibodies as catalysts for pericyclic cycloadditions in organic synthesis. The principle challenge at present is the production of more efficient biocatalysts. Although good rate accelerations have been observed, improvements are necessary before these biocatalysts will find use in a general synthetic setting. A larger understanding of the structure-function of these catalytic antibodies, and hence the manner in which they achieve catalysis (i.e., via reduction of the enthalpy or entropy of activation) should help in the design of better catalysts for cycloaddition reactions. Improvements in regiochemical and stereochemical control are also critical as it is these two factors that help to determine the utility of an asymmetric chemical catalyst.

With the prevalence of cyclic architecture in natural products that possess medicinal relevance, there still exists a drive to discover new catalytic methods in which these arrays can be synthesized in a regioselective and stereoselective manner. It is in this realm that the future holds promise for the use of catalytic antibodies in synthetic applications, especially as naturally occurring "Diels–Alderase" enzymes are rare. Also, with an increasing emphasis on "green chemistry" in the laboratory, antibodies, as environmentally benign molecules, could find a larger role in synthetic applications. But, the true strength of cycloaddition catalytic antibodies lies in their ability to catalyze the formation of products via disfavored pathways, and it is in this setting that they will find greatest use in the future.

Acknowledgements

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