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Antibody-Catalyzed Asymmetric Intramolecular Michael Addition of Aldehydes and Ketones to Yield the Disfavored Cis-Product

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The world's first commercially available catalytic antibody, 38C2 (Ab38C2), is perhaps the most promiscuous antibody catalyst generated to date. The antibody was found to efficiently catalyze aldol and retro-aldol reactions of a remarkably broad range of substrates[1] with excellent enantioselectivity through an enamine class I aldolase mechanism.[2] The antibody was raised against the β-diketone hapten **1**, which served as a chemical trap to imprint a unique lysine residue (**2**) with an ϵ-amino group with a pK_a of 5.8 (Scheme 1). The X-ray structure[3] of the antibody binding site suggests an interaction between a tyrosine residue and one of the carbonyl groups of the hapten (Scheme 1). Antibody 38C2 is also capable of catalyzing retro-Michael reactions of β-alkoxy ketones,[4−8] a direct Michael addition of acetone to a maleimide derivative,[9a] and mimics the classic organocatalytic Wieland−Miescher ketone synthesis.[9]

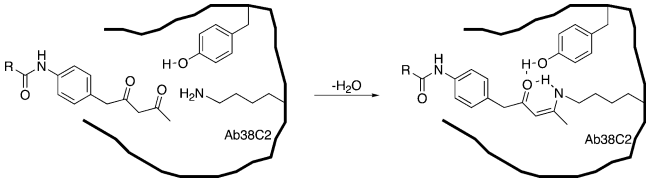
The recent report by List[10] of chiral imidazolidinone-catalyzed intramolecular Michael reactions has promoted us to determine whether antibody 38C2 could catalyze the intramolecular Michael addition of aldehydes and ketones to enones. To our delight, incubation of formyl-enone **I** (R′ = H, R = H) or methyl ketone-enone (R′ = H, R = CH_3) with antibody 38C2 in phosphate-buffered saline (PBS), pH 7.4, indeed generated the expected Michael product **II** (Scheme 2).

The substrates for the antibody-catalyzed reactions were synthesized through a Wittig coupling in a biphasic solvent system using methylene chloride and 2 N sodium hydroxide as shown in Scheme 3. The Wittig salt **III** was dissolved in the aqueous phase and quickly formed the corresponding ylide. The keto-aldehyde (R = CH_3) or the dialdehyde (R = H) were added in methylene chloride. The reaction was mixed for several hours, and after workup the enone product was purified by standard column chromatography techniques. Racemic reference type **II** products were prepared by incubation of the enones with piperidine in DMF.

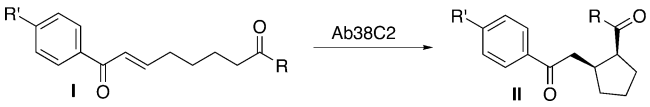
Several aldehyde-enones and methyl ketone-enones were suitable substrates for the antibody-binding site and afforded the intramolecular Michael products with excellent diastereo- and enantioselectivity (Table 1). The cis/trans ratio and the enantioselectivity were determined by standard RP-HPLC and chiral-phase HPLC (AD-RH column), respectively.

Interestingly, the antibody reaction product was, in all examples, predominantly the thermodynamically unfavored cis-diastereoisomer. In contrast, the asymmetric intramolecular Michael reaction catalyzed by imidazolidinones of formyl-enone **3** afforded almost exclusively the trans-diastereoisomer ((*S*)-proline gave a 2:1 trans/cis ratio with only 15% ee of *trans*-**3a**).[10] As presented in Table 1, antibody 38C2 also catalyzed the intramolecular Michael addition of methyl ketones (**6**−**9**) with very high enantio- and diastereoselectivity. Incubation of MacMillan imidazolidinone with ketone-enone **6** in THF for 3 days did not afford any Michael addition product.

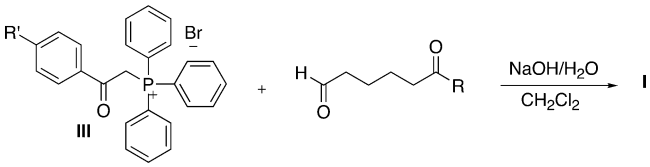
Scheme 1. Mechanism of Trapping the ϵ-Amino-lysine Residue in the Antibody Binding (**2**) Site Using the β-Diketone Derivative Hapten **1** (R = $CH_2CH_2CH_2COOH$)



Scheme 2. Antibody-Catalyzed Intramolecular Michael Addition of Ketones and Aldehydes to Enones



Scheme 3. General Synthesis of the Enone Substrates for the Antibody-Catalyzed Intramolecular Michael Addition Reactions



The k_{cat} and K_m were determined from Lineweaver−Burk plots using Michaelis−Menten analysis (Table 2). Full graphical data are available in the Supporting Information. While the ketones reacted at a relatively moderate rate, aldehyde **3** yielded the intramolecular Michael product **3a** with a k_{cat} of 8.73 min^{-1} (Table 2). To date, this is the highest measured rate for antibody catalysis of a C−C bond-forming reaction. The antibody-catalyzed reaction was up to 5 orders of magnitude faster than the background reaction in buffer alone. This relatively high ratio will be adequate for preparative-scale antibody reactions to generate enantiomerically pure products.[12] In general, the aldehyde-enones were less stable (or more reactive) under these buffer conditions than the methyl ketone-enones, and their reactions had higher k_{cat} values. Ketone-enone **6** exhibited the highest rate enhancement with a k_{cat}/k_{uncat} ratio of 350,000, very high cis/trans product selectivity (90/1), and an excellent enantiomeric excess value of 97%.

Scheme 4 illustrates our proposed mechanism for the antibody catalysis of the intramolecular Michael addition. The ϵ-amino group of the lysine residue (**IV**) presumably reacts with the formyl or the methyl ketone to form a nucleophilic enamine (**V**), that then reacts with the enone to generate the Michael addition product (**VI**). The imine in **VI** is hydrolyzed to release a carbonyl moiety (**VII**) and to regenerate the free ϵ-amino-lysine residue. The X-ray structure

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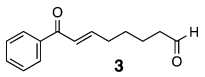
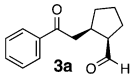
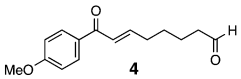
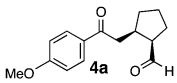
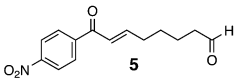
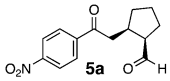
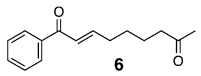
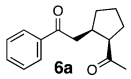
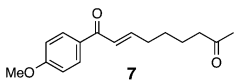
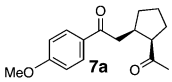
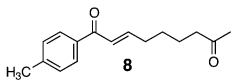
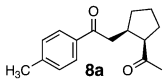
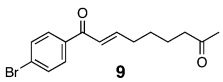
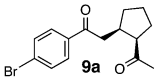
Table 1. Enantioselective Intramolecular Michael Addition Catalyzed by Antibody 38C2

Substrate	Product	*cis/trans*	*ee*
 **3**	 **3a**	12/1[a]	98%
 **4**	 **4a**	3/1[a]	88%
 **5**	 **5a**	2/1[a]	96%
 **6**	 **6a**	90/1	97%
 **7**	 **7a**	90/1	98%
 **8**	 **8a**	30/1	98%
 **9**	 **9a**	90/1	94%

[a] The cis/trans ratio of aldehydes **3a**−**5a** decreased over time since the antibody also catalyzes epimerization at the α carbon.[11] This phenomenon is not observed in ketones **6a**−**9a**.

Table 2. Kinetic Parameters for Intramolecular Michael Addition Catalyzed by Antibody 38C2[a]

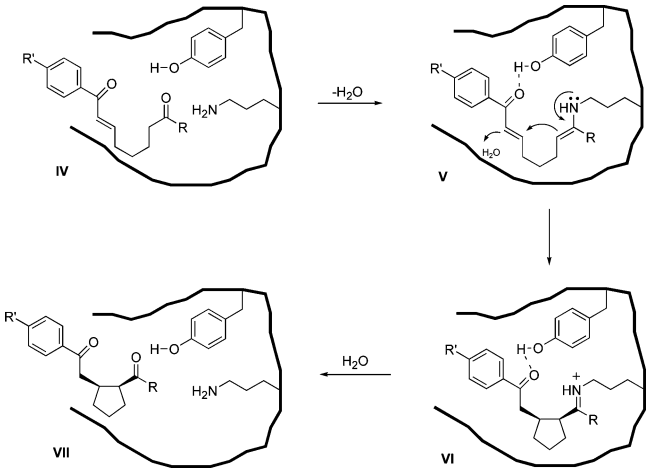
substrate	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/k_{uncat}
3	47	8.73	244,428
4	235	0.18	3,333
5	36	2.92	51,228
6	336	0.14	350,000
7	709	0.09	30,479
8	656	0.09	134,285
9	44	0.025	22,910

[a] Antibody reactions were performed in PBS (50 mM buffer, 100 mM sodium chloride), pH 7.4 with 5% acetonitrile at room temperature.

of antibody 33F12, a very similar antibody to 38C2, shows a tyrosine residue near the active lysine.[3] In our proposed mechanism, the tyrosine hydroxyl residue acts as a Brønsted acid catalyst that activates the enone toward the Michael addition of the nucleophilic enamine. The antibody binding site tolerates a variety of ketone- and aldehyde-enone substrates with different para substituents on the aromatic ring. However, the substrates with the fastest reaction rates among the aldehydes and the ketones were the nonsubstituted enones **3** and **6**, respectively. We have used only aryl-enones as substrates since they are easily monitored using HPLC with a UV detector.

In summary, we have described novel antibody-catalyzed intramolecular Michael addition reactions of aldehydes and ketones to enones. The reactions were enantioselective and diastereoselective

Scheme 4. Suggested Mechanism for Antibody 38C2-Catalyzed Intramolecular Michael Addition of Aldehydes and Ketones to Enones



with high ee values and cis/trans ratios. To the best of our knowledge, no other Michael-addition methods offer such selectivity for the thermodynamically unfavored cis-diastereoisomers.

While organocatalytic Michael addition products are known, antibody 38C2 is the only catalyst known to act on both aldehyde and ketone substrates to produce enantiomeric pure cis-cyclopentane products. This study highlights the dynamic interplay between bioorganic chemistry and organocatalysis.

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Supporting Information Available: Full experimental details, characterization data of all new compounds and antibody assay conditions, full graphical data used to generate Table 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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