

CATALYSIS OF “DIRECT” ASYMMETRIC ALDOL REACTIONS

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INTRODUCTION

The aldol reaction is an important C-C bond forming reaction in organic chemistry and biology. This transformation can create up to two adjacent stereocenters upon joining of a nucleophilic carbonyl donor and an electrophilic carbonyl acceptor. Intensive effort has been invested to develop asymmetric aldol reactions. Three approaches have been taken to address diastereo- and enantioselection issues. Non-catalytic asymmetric aldol reactions usually involve the use of stoichiometric amounts of chiral auxiliaries,¹ while the catalytic enantioselective versions of this reaction include chiral Lewis acid-catalyzed and chiral Lewis base-catalyzed aldol reactions.² However, the former approach suffers from the necessity of additional steps to install and remove the chiral auxiliary, while the latter two methods typically require pre-activation of the donor to a more reactive species, such as silyl enol ether, ketene silyl acetal, or alkyl enol ether.

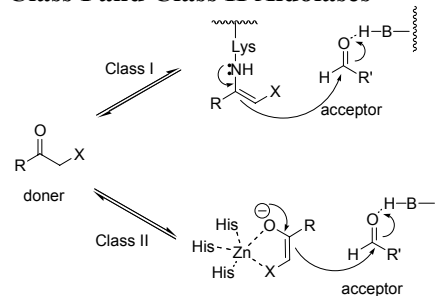
In contrast, nature has developed two classes of aldolases for direct aldol reactions, in which an unmodified ketone donor is added to an aldehyde acceptor.³ Class I aldolases activate the ketone donor via the formation of a Schiff base intermediate with a lysine residue in the active site (Scheme 1). Class II aldolases contain an active site Zn^{2+} cofactor that facilitates the enolate formation by coordinating to the carbonyl oxygen of the ketone donor. Although these enzymes have remarkable catalytic activity, their applications in organic synthesis are still limited by the narrow substrate scope.

Several biological and chemical approaches towards “direct” asymmetric aldol reactions have been developed recently. This report focuses on recent advances in direct asymmetric aldol reactions catalyzed by antibody aldolases, proline, and chiral dinuclear zinc complex.

ANTIBODY ALDOLASES

In class I aldolase catalysis, a lysine residue in the active site plays a central role by covalently linking to the substrate to produce a Schiff base and consequently activating the α -position of the ketone by enamine formation. Recently, several antibodies, which catalyze direct asymmetric aldol reactions through a mechanism similar to that of the class I aldolases, have been generated with broader substrate scope.⁴

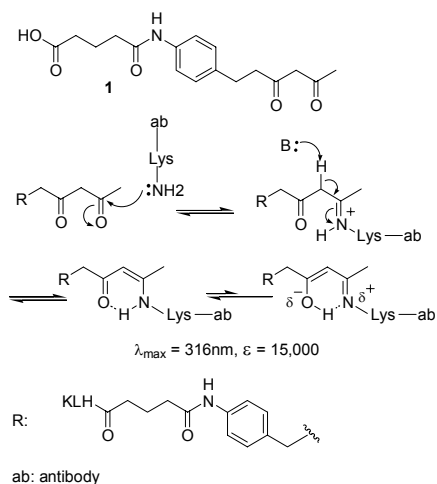
Scheme 1. General Mechanisms for Class I and Class II Aldolases



Antibodies are specific proteins produced by the immune system against foreign substances called antigens. The traditional immunization process has been limited to the use of inert antigens which generally bind non-covalently with antibodies. However, if chemically reactive antigens are used, a covalent bonding can occur between the antibody and the antigen during immunization. Thus, the selection criteria of the immune system are switched from binding affinity to chemical reactivity. Antibodies elicited in response to a reactive antigen normally contain appropriate functional groups capable of forming a covalent bond with the antigen. These antibodies would behave like catalysts through the same covalent bond formation when they interact with a substrate that shares the same chemical reactivity with the antigen used to induce them. Since the chemical reaction occurs early in the selection process, the binding pocket of catalytic antibodies may not be refined to high specificity and these catalytic antibodies can thus accept a broader range of substrates.

The process described above is called reactive immunization.⁵ Reactive antigens used in this process can be prepared by coupling a carrier protein with a reactive hapten. A hapten is a small molecule that can be recognized by antibodies but by itself is incapable of eliciting antibodies unless conjugated with a carrier protein. It was expected that an antibody induced against reactive antigens, which can trap the essential lysine residue in the binding pocket, should also be able to catalyze the aldol reaction. Diketone **1** was chosen as the potentially reactive hapten because it could covalently bind to the lysine residue by forming a stable vinylogous amide (Scheme 2).⁴ In addition, hapten **1** could also act as an entropic trap to induce the binding pocket for both substrates because it has both an aldol acceptor moiety (3-phenylpropionaldehyde) and an aldol donor portion (acetone). Twenty antibodies were produced after immunization of 129 G^{1x+} mice with hapten **1** coupled to the carrier protein—keyhole limpet hemocyanin (KLH). Two out of twenty were found to form the proposed stable adducts by monitoring the characteristic UV absorption of the vinylogous amide around 316 nm. Only those two antibodies, 38C2 (MW~150,000g/mol, Aldrich no. 47,995-0) and 33F12, have shown the catalytic activities in the aldol reaction. An X-ray crystal structure of the antigen-binding fragment of antibody 33F12 has shown that the reactive lysine residue is buried in the hydrophobic microenvironment.⁶

Scheme 2. Mechanism of Trapping the Lysine Residue using Hapten 1



Catalytic antibodies 38C2 and 33F12 generated using reactive immunization have shown great catalytic abilities with a broader substrate scope than natural enzymes. The reaction follows typical Michaelis-Menton kinetics. The characteristic values for k_{cat} of most reactions ranges from 10^{-3} to 5 min,

with rate accelerations of $k_{\text{cat}}/k_{\text{uncat}}$ 10^5 - 10^7 . Except for ketones that are branched at the non-bond forming α -position, small aliphatic ketones are well tolerated. However, the hydrophobic character of the antibody binding pocket does not accept polyhydroxylated aldehydes.⁷

Hapten **2** has been designed to increase the repertoire of antibodies that can catalyze the aldol reaction. Because it has both a reactive diketone and a tetrahedral sulfone moiety that mimics the tetrahedron geometry of the carbon-carbon bond forming transition state (Scheme 3).⁸ It was proposed that antibodies, which can not only form a stable Schiff base but also stabilize the transition state of the C-C bond forming step, would be elicited against hapten **2**. Seventeen antibodies were produced using the same immunization procedure as described above, nine of which have shown catalytic function. This indicates great improvement in comparison to the results of previous studies using hapten **1**, in which, only 2 out of 20, were catalytically active.

Among the 17 antibodies, 93F3 and 84G3 (Aldrich no. 52785-8) have been studied in detail. Antibodies prepared by the two different approaches share similar substrate scope. However, higher catalytic proficiencies of 93F3 and 84G3 over those of 38C2 have been observed (Table 1).⁸

This indicates that the inclusion of transition state analogy into the hapten design indeed contributes to the catalytic efficiency of antibodies.

Another notable difference is the enantioselectivity (Scheme 4).⁸ With antibodies 93F3 and 84G3, the acetone donor attacks on *re*-face of the aldehyde, while with 38C2 aldolases the attack occurs on *si*-face of the aldehyde, providing the opposite enantiomer. More detailed mechanistic studies on the antibody active site environment are needed to explain this reversal of stereochemistry.

Scheme 3. Mechanism of Generation of Antibody Aldolases against Hapten 2

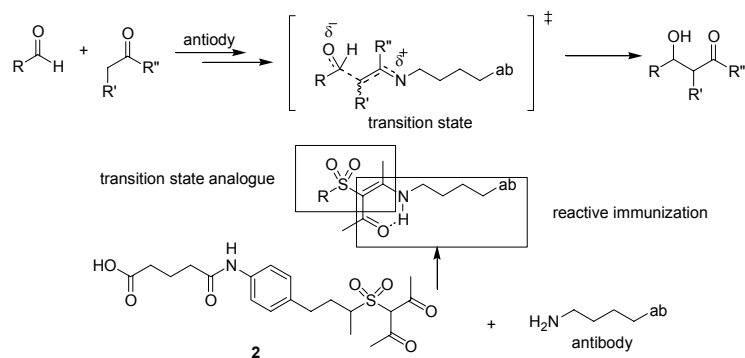
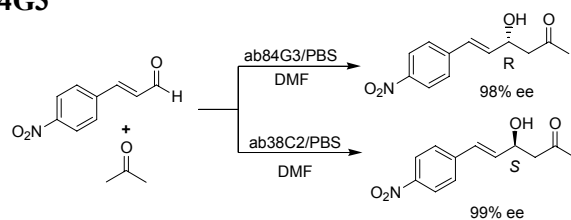


Table 1. Kinetic Parameters for Antibody-Catalyzed Aldol and Retro-Aldol Reactions

entry	Substrate	Antibody	k_{cat} [min ⁻¹]	K_m [μM]	$k_{\text{cat}}/k_{\text{uncat}}$	$(k_{\text{cat}}/K_m)/k_{\text{uncat}}$
1		38C2	0.053	29.5	5.8×10^4	2.0×10^9
		84G3	46.8	10.3	5.2×10^7	5.0×10^{12}
		93F3	43.3	6.5	4.9×10^7	7.4×10^{12}
2		38C2	---	---	---	---
		84G3	81.4	4.2	2.3×10^8	5.4×10^{13}
		93F3	69.6	2.6	1.9×10^8	7.4×10^{13}
3		38C2	6.7×10^{-3}	17	2.9×10^4	1.7×10^9
		84G3	27×10^{-3}	29	1.2×10^5	4.1×10^9
		93F3	33×10^{-3}	34	1.5×10^5	4.4×10^9

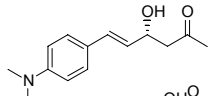
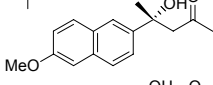
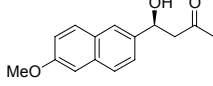
Conditions: For entry 1 and 2: phosphate buffer, pH 7.7
For entry 3: phosphate buffered saline, pH 7.4

Scheme 4. Aldol reaction with Antibody 38C2 and 84G3

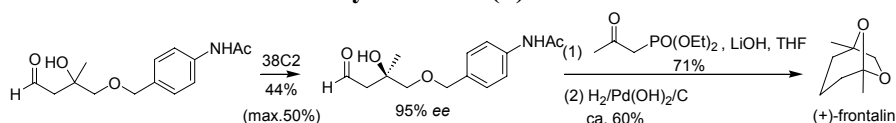


These antibodies catalyze both aldol and retroaldol reactions enantioselectively. They are efficient catalysts for kinetic resolutions of various racemic secondary and tertiary aldols (Table 2).⁹ The challenging targets, enantiomerically enriched tertiary aldols, have been obtained by antibody-catalyzed kinetic resolution. At around 50% conversion, the unconverted β -hydroxy ketones were recovered in high enantioselectivity with up to 99% *ee*. This approach has been used in the synthesis of (+)-frontalin (Scheme 5).⁹

Table 2. Antibody-catalyzed Kinetic Resolution of β -hydroxy Ketones.

entry	product	antibody	time (h)	recovery
1		38C2 (255 mg; 0.025 mol%)	88	1.55 g (49%, >99% <i>ee</i>)
2		84G3 (18 mg; 0.12 mol%)	193	22 mg (44%, 99% <i>ee</i>)
3		84G3 (500 mg; 0.0086 mol%)	65	10 g (50%, >99% <i>ee</i>)

Scheme 5. Enantioselective Synthesis of (+)-Frontalin via Kinetic Resolution



Similar to other proteins, the catalytic antibodies function ideally in aqueous media while synthetically useful organic molecules are often water insoluble. Thus, the application of antibody aldolases in organic synthesis had been limited to the substrates that have both high turnover and high solubility in buffered aqueous solution. To circumvent this problem, List and co-workers recently developed a heterogeneous aqueous/organic solvent system.¹⁰ This allows antibody aldolases to be used for preparative scale. Toluene or chlorobenzene is used as a co-solvent along with the phosphate-buffered saline to increase the substrate solubility. The mixture is shaken during the reaction. After the desired *ee* or recovery is reached, the reaction mixture is cooled to -20 °C, and the organic layer is separated from the frozen aqueous buffer solution. The antibody recovered from the buffer solution can be reused with some loss of activity but still retains enough activity to reach up to 97% *ee*. Large scale reactions (~ 20 g) have been performed following the above procedure (entry 3, Table 2). Even though the heterogeneous aqueous/organic solvent system increases the substrate solubility, a catalytic turnover rate (k_{cat}) of at least 1-10 min⁻¹ is required to be useful for preparative scale organic reactions.

In summary, reactive immunization opens a way to generate catalytic antibodies that can catalyze various reactions with high stereoselectivity. These aldolase antibodies catalyze direct asymmetric aldol or retro-aldol reactions on gram scale under nearly neutral and mild conditions at room temperature. They are commercially available and tolerate a wider substrate scope compared to natural enzymes.

PROLINE CATALYZED ALDOL REACTIONS

Although antibody aldolases are efficient in catalyzing aldol reactions, their applications in organic synthesis are still restricted owing to the long reaction times for large scale reactions and the relatively high price of the antibody catalysts (ab84G3, Aldrich, 10mg, \$125.8). Searches for more convenient and efficient methods using more accessible, small organic molecule as catalysts are being actively carried out.

The class I aldolases activate the ketone donor via enamine formation using active site lysine residue and concomitantly activate the aldehyde acceptor with an active site acidic residue. It would be promising if the catalyst simultaneously activates both the ketone donor and aldehyde acceptor. Amino acids could be potential catalysts since they can form enamines with the amino group and activate aldehydes with the carboxylate group. The studies on structural requirements for efficient amino acid catalysts for aldol reactions revealed that a cyclic secondary amine and appropriate spatial proximity of the carboxylic acid group are necessary.¹¹ Proline has been found to be a powerful amino acid catalyst for various types of aldol reactions.¹²

It is well known that (*S*)-proline can catalyze the intramolecular aldol step of the Robinson annulation to afford the Wieland-Miescher ketone (eq. 1).¹³ Later, Agami found that proline can also catalyze the intramolecular aldol cyclodehydration of achiral diketones (eq. 2).¹⁴ Compared with the catalytic antibodies, proline catalysts provided lower yields and stereoselectivities, but they can be used in large scale production.

Similar to class I aldolases, the proposed mechanism of these intramolecular enantioselective differentiating aldol reactions involve enamine formation.¹⁵ In the first case (eq. 1), owing to the greater reactivity of the less substituted tertiary enamine, activation of the side chain carbonyl group as an enamine would occur followed by nucleophilic attack of the resulting enamine on the carbonyl group in the pro-*S* ring to form the enantiomerically-enriched cyclic product. The enantioselectivity can be explained by a proline-mediated chair-like transition state model **A** (Figure 1).¹⁶ Since an excellent linear correlation between the *ee* of proline and the condensation product was observed, it is unlikely that two proline molecules are involved in the transition state (model **B**) as Agami et al proposed previously.¹⁵ The second case (eq. 2) is more complicated since both of the carbonyl groups

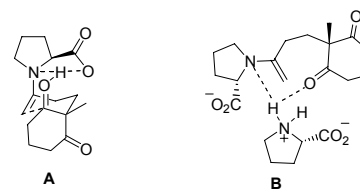
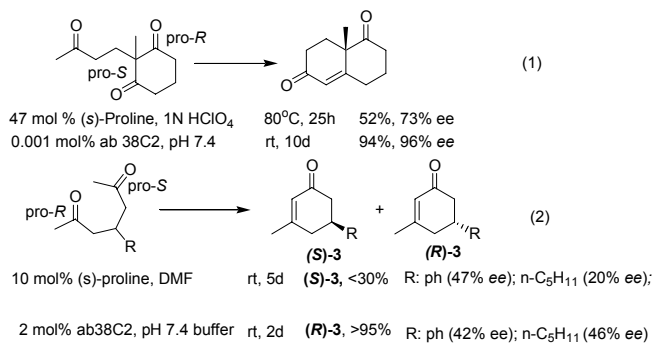


Figure 1. Proposed Transition States for Intramolecular Aldol Reaction

can behave either as an aldol acceptor or as an aldol donor. Either enantiomer can be produced depending on which carbonyl group actually forms the enamine. Not surprisingly, only a slight preference for one enantiomer was observed. The results indicate that the pro-*S* carbonyl group preferentially forms the enamine and attacks the pro-*R* carbonyl group. This observed selectivity needs further explanation.

Direct intermolecular asymmetric aldol reactions catalyzed by proline were first reported by List et al (Table 3).¹⁷ Large excesses of acetone donors were used to suppress undesired self-condensation of aldehydes. In the presence of 30-40 mol % of proline catalysts, the cross aldol reactions proceeded smoothly at room temperature giving moderate to good yields and enantioselectivities. The reaction scope was later extended to the asymmetric aldol reactions of acetone with α -mono- and α -disubstituted aliphatic aldehydes (entry 3 and 4).¹² In contrast to the enantioselectivity of antibody catalysis, these aliphatic aldehydes show even better enantioselectivities than the aromatic aldehydes. However, reactions with α -unsubstituted aldehydes result in modest ee's along with product contamination by self-aldol condensation product (entry 5 and 6).¹⁸ This is presumably due to the difficulty in differentiating the α -protons of the aldehyde and ketone.

The mechanism of proline-catalyzed intermolecular aldol reaction has been extensively studied. Again, the key enamine intermediate is formed by the secondary amine and the ketone donor. This enamine then attacks the aldehyde acceptor activated by a carboxylic acid. The high enantiofacial selectivities of the above reactions can be explained by the highly organized tricyclic hydrogen bonded framework in the proposed Zimmerman-Traxler chair-like transition state A (Figure 2).^{11, 19}

Proline can also catalyze the direct aldol reaction between hydroxyacetone and various aldehydes with good regio- and stereoselectivities (Table 4).²⁰ Anti-1,2 diols were formed with proline catalysis whereas antibody catalysts gave syn-1,2 diols. This

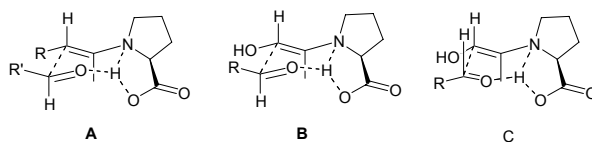
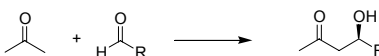


Figure 2. Proposed Transition States for the Intermolecular Aldol reactions

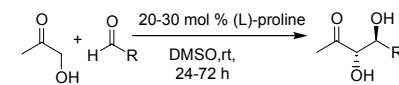
Table 3. Proline-Catalyzed Aldol Reactions with Acetone



entry 1-4: 30-40 mol % (L)-proline, DMSO, rt
entry 5-6: 10-20 mol % (L)-proline, rt, 3-7d

entry	product	yield %	ee (%)
1		62	60
2		68	76
3		97	96
4		85	>99
5		31	67
6		34	73

Table 4. Proline-Catalyzed Aldol Reactions with Hydroxyacetone



entry	product	yield (%)	dr	ee (%)
1		60	>20:1	>99
2		62	>20:1	99
3		95	1.5:1	67
4		38	1.7:1	>97
5		40	2:1	>97

stereoselectivity is consistent with the chair-like transition state **B** (Figure 2) in that the *E*-hydroxyl enamine attacks *re*-face of the aldehyde to form anti-product. Boat-like transition state **C** will lead to syn-product. The poor anti to syn ratios in some cases are presumably attributable to the competing boat-like transition states which may become more favored with less sterically hindered or α -oxygenated aldehydes.

Compared with the results of antibody catalysts, the stereoselectivity of proline catalyst is generally lower. However, proline is readily available in both enantiomeric forms and applicable in large scale production. The main drawbacks of the proline catalyst are the use of excess amount of ketone donors and the formation of dehydration side products in some cases. Nevertheless, proline is a highly promising catalyst for direct asymmetric aldol reactions, and further improvements in stereoselectivities and scope are anticipated.

DINUCLEAR ZINC COMPLEX CATALYZED ALDOL REACTIONS

Several metal-based catalysts containing both Lewis acidic and Brønsted basic functionalities have been reported as class II aldolase mimics.²¹ Recently, Trost et al. reported the development of dinuclear zinc complex (Figure 3).²² It has been postulated that one zinc ion forms the metal enolate while the other zinc acts as a Lewis acid and subsequently coordinates with the aldehyde to form chelation-controlled active complex (Figure 3). The proper orientation of the enolate in this kind of chiral intermediate permits selective enantiofacial attack on the activated aldehyde.

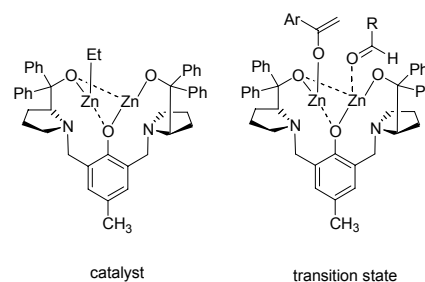


Figure 3. Proposed Structures of Catalyst and Transition State

This catalyst has shown catalytic abilities comparable to the catalytic antibodies and proline catalyst. In addition, it can catalyze the aldol reaction between α -hydroxy ketones and aldehydes using nearly stoichiometric amounts of two substrates.²³ Thus, this newly developed catalyst is approaching the ideal atom-economical version of the asymmetric aldol reaction that both catalytic antibodies and proline catalyst failed to provide. It should be noted that the most challenging α -unsubstituted aldehydes can also be used with some success (Table 5).²⁴

CONCLUSION

Antibodies, proline, and a dinuclear zinc complex, have proven versatile and efficient catalysts for asymmetric aldol reactions in regard to substrate scope and stereochemistry. Although the direct catalytic asymmetric aldol reaction currently is still not widely used to prepare enantiomerically

Table 5. Dinuclear Zinc -Complex-Catalyzed Aldol Reactions

R	Ar	yield (%)	dr	ee (%)
	Ph	89	13:1	92
	Ph	78	9:1	91
	Ph	91	5:1	87
		90	6:1	96

enriched aldol products, this method has achieved noticeable advances in the last five years. More convenient approaches with higher enantioselectivity, better yield, and broader substrate scope are anticipated.

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