Aldolase Antibody 38C2: A Biocatalyst Expanding the Scope of Enzymatic Transformations

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Dedicated to Prof. Dr. H. Kunz on the Occasion of his 60th Birthday

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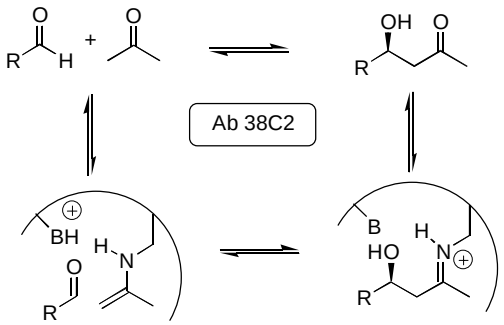
Contents

1. Introduction

Copying Nature's versatility and accuracy in catalyzing chemical reactions has always been an elusive goal for synthetic chemists. In recent years, however, exciting advances at the interface of chemistry and biology have resulted in the successful development of novel artificial enzymes with customized catalytic motifs [1]. In this regard, one of the most promising concepts is the use of catalytic antibodies. Since their discovery in 1986 [2, 3], a number of catalytic antibodies have emerged as valuable tools that offer the opportunity to design traditionally challenging synthetic schemes in organic synthesis [4–7]. By means of a novel technique called reactive immunization [8], Lerner, Barbas and coworkers recently developed two antibodies, Ab 38C2 and Ab 33F12, that catalyze one of the main carbon–carbon bond forming methodologies in organic chemistry – the aldol reaction [9]. These aldolase antibodies use the enamine mechanism utilized by natural occurring class I aldolases, such as fructose-1,6-bisphosphate aldolases (*e.g.* rabbit muscle aldolase). Catalytic antibody 38C2 exhibits a similarly high specificity and efficiency as its natural counterparts, but in contrast, a much wider range of substrates is accepted [10, 11]. In addition to aldol reactions (section 2), a number of other synthetically useful transformations are promoted by antibody 38C2, such as decarboxylation of β-keto acids [12], cyclodehydration (section 3), *retro*-aldol (section 4) and *retro*-Michael reactions (section 5). As a result of its versatility, Ab 38C2 is the first catalytic antibody that is commercially available [13]. Ab 38C2 is very stable and may be used "straight from the jar" in the same manner as a chemical reagent. Therefore, it is now possible for the synthetic versatility of this antibody to be realized by the non-specialist. The biocatalyst operates in buffered aqueous solutions at neutral pH and may be recovered for reuse. This article shall highlight the synthetic utility of this antibody as an artificial enzyme, both *in vitro* and *in vivo.*

2. Aldol Reactions

Catalytic aldol reactions are among the most useful – and challenging – synthetic methods for highly stereocontrolled asymmetric synthesis. Antibody 38C2 mimics the (reversible) catalytic cycle of naturally occurring aldolases. Essential to its catalytic mechanism is a highly reactive lysine residue (LysH93) in the binding pocket of the antibody. It has been suggested that the donor carbonyl initially forms a Schiff base with the Lys ε-amino group, which is subsequently tautomerized to an enamine. The enamine intermediate attacks the acceptor carbonyl, and upon hydrolysis the aldol is released. Generally, the donor attacks the *Si*-face of the acceptor carbonyl, yielding (*R*)-β-hydroxycarbonyls with high enantiomeric excesses (> 95% *ee*) (Scheme 1).



Scheme 1 Proposed mechanism of Ab 38C2 promoted aldol reaction

In an extensive study by Barbas, Lerner and coworkers, the synthetic scope of Ab 38C2 has been investigated [10]. 23 donors, a large variety of aliphatic and aromatic ketones and aldehydes, and 16 carbonyl acceptors, including **1**, **3** and **5**, have already been identified as substrates for Ab 38C2, and at least 200 aldol reactions have been found to be viable using the aldolase antibody. A few examples of the generally highly stereoselective process (up to 99% *ee*) using acetone as donor are shown in table 1. Compound **6** has been used in a total synthesis of epothilones (see section 4).

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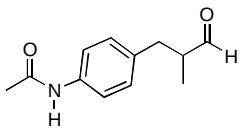
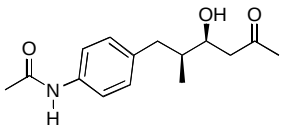
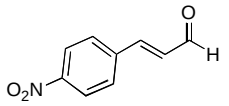
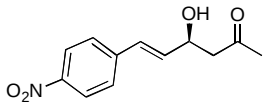
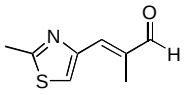
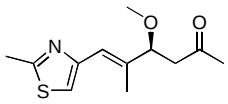
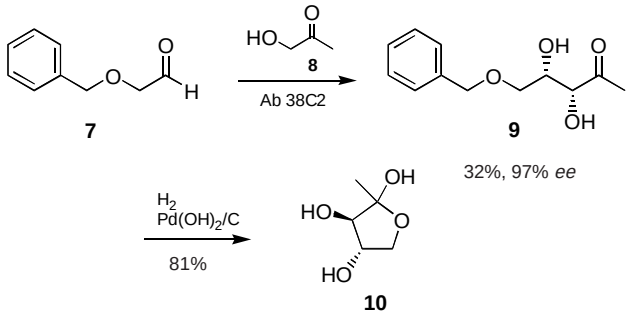
Table 1 Examples of Ab 38C2 catalyzed aldol reactions using acetone as donor [9, 10, 18]

Acceptor	Product
 **1**	 **2** (95% de)
 **3**	 **4** (95% *ee*)
 **5**	 **6** (75% *ee*)

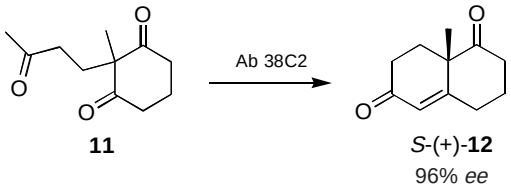
Since the rather lipophilic reactive site of Ab 38C2 does not accept carbohydrates as substrates, the antibody complements the synthetic scope of some naturally occurring aldolases. Surprisingly though, while no other catalyst is capable of using hydroxyacetone (**8**) as donor substrate for aldol reactions, **8** is one of the best aldol donors for antibody 38C2 [10, 14]. Being an attractive substrate for the stereoselective synthesis of polyhydroxy compounds, hydroxyacetone has been subjected to numerous reactions with a set of aldehydes. Interestingly, the stereochemistry of the aldol reaction is reversed (*re*-facial attack), giving *a*-(2*R*,3*S*)-dihydroxy ketones such as **9**. This methodology has been applied to an elegant two step synthesis of 1-deoxy-*L*-xylulose (**10**), which is a key intermediate in the non-mevalonate biosynthesis of terpenes (Scheme 2) [14].



Scheme 2 Efficient two step synthesis of 1-deoxy-*L*-xylulose (**10**)

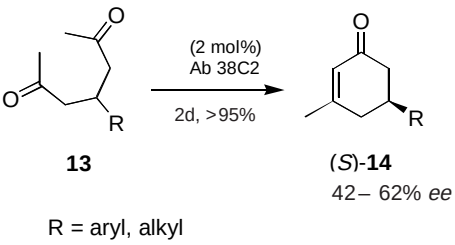
3. Robinson Annulations and Cyclodehydrations

In many aldol reactions catalyzed by Ab 38C2, it has been observed that the antibody catalyzes both the C–C-bond formation as well as a subsequent dehydratation step yielding the aldol condensation products. When conducted in an intramolecular fashion, the antibody catalyzed sequence even resulted in a Robinson annulation, which is known as a valuable method for the preparation of natural compounds, such as terpenes and steroids [15]. A renowned bicyclic enone building block that has been initially prepared by a proline mediated Robinson annulation of **11** is the Wieland-Miescher ketone **12**. As an improvement on the classical "*meso*-trick" reaction, which provides **12** in ca. 70% *ee*, antibody 38C2 successfully generates **12** in essentially pure form (96% *ee*) (Scheme 3) [15].



Scheme 3 Antibody 38C2 catalyzed Robinson annulation. Synthesis of the Wieland-Miescher ketone **12**

Ab 38C2 not only exhibits a great control over prochiral discrimination of *meso*-1,3-diones, but is also capable of an enantiogroup-differentiation of 4-substituted-2,6-heptanediones **13**. The concomitant aldol cyclodehydration of **13** to the corresponding (*S*)-enones **14** is viable, albeit with only with moderate enatiomeric excesses (Scheme 4) [16]. The corresponding 2,5- and 2,7-dione systems are not processed by Ab 38C2 [10].

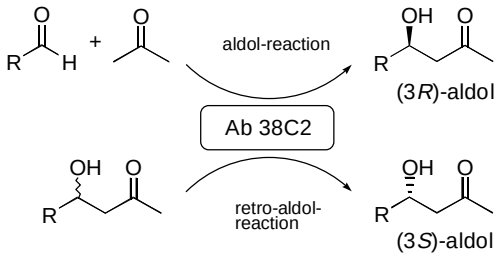


Scheme 4 Ab 38C2 catalyzed cyclodehydrations

4. *Retro*-Aldol Reactions – Kinetic Resolution of Secondary and Tertiary Aldols

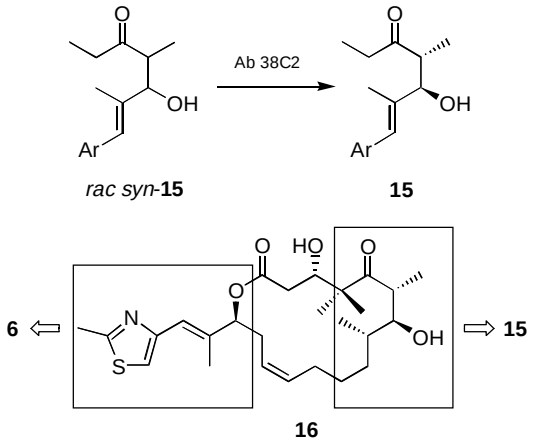
As depicted in scheme 1, all steps of the catalytic cycle leading to aldols are reversible. Due to the great efficiency of Ab 38C2 in promoting aldol reactions, it was envisioned that the antibody may also be a useful catalyst for the retrograde reaction. This reaction would ultimately allow for the kinetic resolution of racemic aldols, a reaction that – surprisingly – is unprecedenced in organic synthesis. Lerner, Barbas and coworkers found that Ab 38C2 in fact very efficiently catalyzes the *retro*-aldol reaction of (3*R*)-hydroxyketones. Therefore, a single antibody catalyst can act on different substrates in the preparation of both aldol enantiomers (Scheme 5) [17].

It is remarkable that the optical purity of the aldols obtained by the *retro*-aldol reaction is sometimes higher than that obtained during the aldol reaction [18]. A careful study of the kinetics of this reaction demonstrated that the reaction



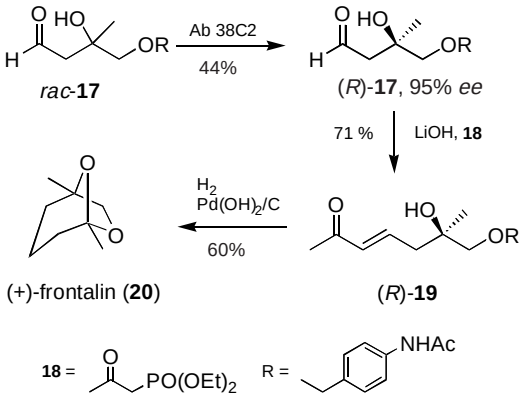
Scheme 5 Enantioselective aldol reaction *vs*. kinetic resolution through retrograde aldol reaction

mechanism is substrate dependent [19]. Nevertheless, the kinetic resolution of racemic aldols has proven to be a valuable alternative strategy for the generation of β-hydroxyketones in general and has been applied to several natural products syntheses. As an example, homochiral aldol **15**, an intermediate in the total synthesis of epothilones A and C, was obtained in essentially optically pure form from *rac syn*-**15** as the unconverted aldol at ca. 50% conversion (ca. 4 h) [18, 20].



Scheme 6 Kinetic resolution of intermediate for the total C (synthesis of epothilone **16**)

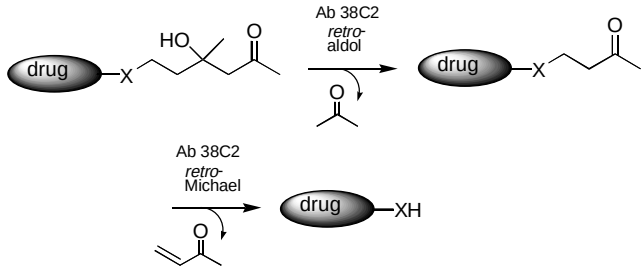
The antibody catalyzed *retro*-aldol reaction also provides a useful alternative route towards enantiomerically pure α,β-dihydroxyketones, which serve as valuable intermediates in the synthesis of several members of the brevicomin group of beetle pheromones [21]. Homochiral hydroxy-substituted quaternary carbon centers represent abundant structural elements in natural products, however, to date no general method for their preparation is available. The kinetic resolution of tertiary aldols using Ab 38C2 may provide a significant contribution to this field [22]. Employing this catalytic enantioselective route, both isomers of mevalonolactone and the side chain of saframycin H have been prepared. (+)-Frontalin (**20**), an important beetle sex pheromone, has also been prepared by means of this approach. Kinetic resolution of *rac*-**17** provided (*R*)-**17** in 44%, (50% max.) with 95% *ee* (Scheme 7) [22]. Subsequent Horner-Emmons-Reaction with **18** and deprotection of enone (*R*)-**19** yielded the pheromone **20** in virtually enantiopure form (Scheme 7) [22].



Scheme 7 Synthesis of (+)-frontalin (**20**)

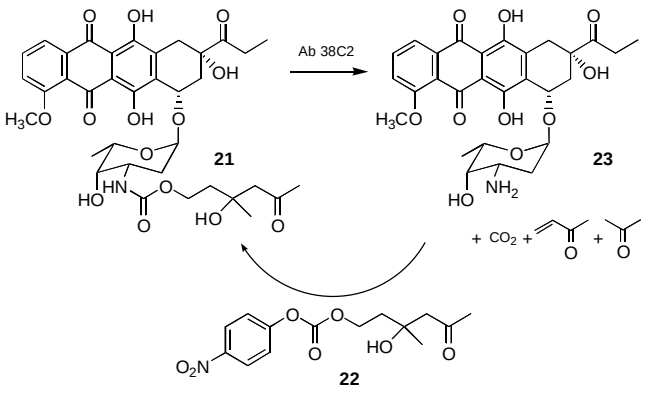
5. In *Vivo* Prodrug Trigger by *Retro*-Aldol-*Retro*-Michael-Reaction

Many potent antitumor leads suffer from misdirected, nonspecific toxicity and thus have severe side effects. As a solution, the cytotoxic drug could be masked ideally as non-toxic prodrug, prior to activation at the tumor site. Recently, it has been found that aldolase antibody 38C2 is uniquely capable of catalyzing a *retro*-Michael reaction of β-heterosubstituted ketones and aldehydes to generate free amine, hydroxyl, or thiol groups. Coupling this to a *retro*-aldol reaction results in a tandem *retro*-aldol-*retro*-Michael reaction. Such a reaction sequence could provide a highly specific prodrug trigger, as this sequence is not promoted by any known naturally occurring biocatalyst (Scheme 8) [23].



Scheme 8 Prodrug trigger by a *retro*-aldol-*retro*-Michael reaction; X = N, O, S

In a single synthetic operation, topoisomerase I and II inhibitor doxorubicin **23** is successfully masked with **22**, and indeed a high ratio between antiproliferative effects of prodoxorubicin and doxorubicin is observed. In the presence of Ab 38C2, carbamate **21** is degraded to but-3-en-2-one, acetone and CO_2 (Scheme 9), as both *in vitro* and *in vivo* studies (mouse model) demonstrate. Ultimately, a bifunctional antibody, integrating a targeting and a catalytic motif, would be a truly powerful tool in cancer therapy [24].



Scheme 9 *In vivo* doxorubicin (**23**) prodrug activation

6. Conclusion

The above examples of Ab 38C2 catalyzed transformations as described above illustrate the large synthetic potential of this highly stereospecific catalytic antibody. Ab 38C2 not only promotes a plethora of aldol and *retro*-aldol reactions, but also catalyzes Robinson annulations and *retro*-Michael reactions. Antibody 38C2 was found to be suitable for addressing problems that are classically difficult to emulate using enzymes or more traditional synthetic methods. The availability and stability of this biocatalyst may pave the way for further explorations on the scope and versatility of this reagent.

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