Antibody-Catalyzed Enantioselective Robinson Annulation

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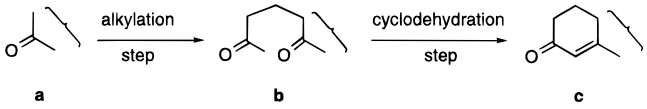
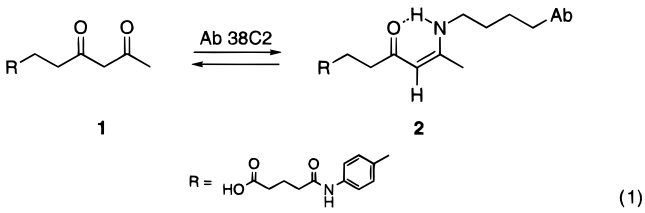
We report an antibody that is remarkable in that it catalyzes both steps of an important synthetic transformation, the Robinson annulation. The Robinson annulation which accomplishes, in net terms, the conversion of **a** → **c** occupies a key role in organic synthesis.[1] In most instances, the overall annulation is comprised of an alkylation (or Michael addition) step leading to **b** followed by a cyclodehydration step to give a cycloalkenone **b** → **c**.



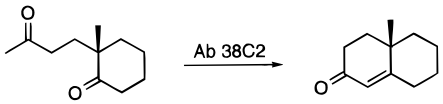
A particularly well-known synthetic intermediate, which is assembled through a Robinson annulation sequence, is the Wieland−Miescher (WM) ketone **6**.[2] The Robinson annulation reaction, in general, and the WM ketone, in particular, have been employed on countless occasions in the synthesis of natural products, notably steroids and terpenoids.[1a−c] For instance, a recent total synthesis of taxol started with the (*S*)-antipode of the WM ketone.[3] For this, as well as other applications,[4,5] the availability of the enantiopure version of the WM ketone is enormously helpful. In practice, however, the enantioselection in the cyclization step of prochiral **5** *en route* to **6** is on the order of 70% enantiomeric excess (ee).[2,6] Fractional crystallization, with attendant losses, is necessary to attain acceptable homogeneity of the desired (*R*)- or (*S*)-antipode of **6** or derivatives thereof. The goals of the research described herein were to explore the possibility of utilizing catalytic antibodies to achieve the entire Robinson annulation sequence (**a** → **b** → **c**) and the cyclodehydration step (**b** → **c**). For the case of the synthesis of the WM ketone, we explore whether enantioselection in the last step (*cf.* **5** → **6**) could be achieved under governance by the catalytic antibody.

Previously, two antibodies 33F12 and 38C2, capable of catalyzing a broad range of intermolecular aldol reactions between ketone donors and aldehyde acceptor substrates, were produced using the strategy of reactive immunization[7,8] with the β-diketone hapten **1**. A carbonyl group of **1** reacts with the ϵ-amino group of the lysine residue within the active site of the antibody to form a tetrahedral hemiaminal which subsequently dehydrates and then tautomerizes to the stable vinylogous amide **2** (eq 1).

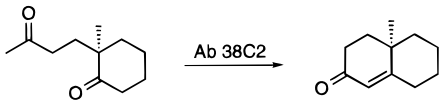


(1)

We first describe an experiment which probed the feasibility of antibody catalysis of the cyclodehydration step while exploring the possibility of kinetic resolution at this step. For these purposes, we prepared the (*S*)- and (*R*)-versions of compound **3** by known chemistry.[9] As shown in Table 1, both antipodes (*S*)- and (*R*)-**3** were efficient substrates for the annulation reaction with Ab38C2, leading to (*S*)- and (*R*)-**4**, respectively (eqs 2 and 3). We took this powerful catalysis of



(2)



(3)

the cyclodehydration step to be a positive omen for the feasibility of at least the cyclodehydration phase of the Robinson annulation under mediation by Ab38C2. Surprisingly, the values of k_{cat} for the cyclization of each enantiomer are quite comparable. Hence, the catalytic antibody is not kinetically responsive to the enantiosense of the remote C2-stereogenic center of the cyclohexanone moiety.

From this perspective, we addressed the WM ketone problem. Here, three issues were under consideration. First, the gross feasibility of cyclodehydration step had to be determined. Thus, the vulnerability of the WM ketone to retro-Claisen type cleavages[10] is well-known. Second was the critical issue as to whether enamine produced by the chiral catalytic antibody could differentiate the two ring ketones flanking the prochiral quaternary carbon at C2. Finally, the possibility of mediation by Ab38C2 of the Michael phase (*cf.* **a** → **b**) would be determined.

Antibody catalysis of the formation of the annulation product **6** and consumption of the triketone **5** were monitored by reversed-phase high-performance liquid chromatography (RP-HPLC). The intramolecular aldol condensation catalyzed by both antibodies followed Michaelis−Menten kinetics. We chose

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Table 1. Kinetic Parameters for Antibody-Catalyzed Annulation Reactions[a]

substrate	k_{cat} (min^{-1})	K_m (mM)	k_{un} (min^{-1})	k_{cat}/k_{un}
(*S*)-**3**	0.186	12.4	nd	nd
(*R*)-**3**	0.126	2.45	nd	nd
5	0.086	2.34	2.4×10^{-8}	3.6×10^{6}

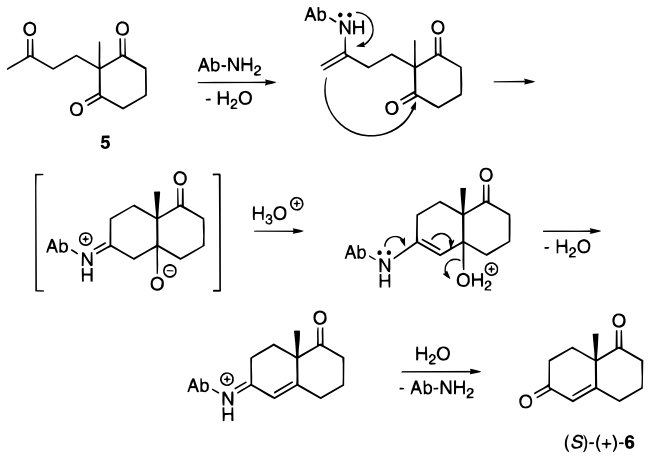
[a] Conditions: pH 6.5 in the presence of 10 μM active sites of 38C2.

to study catalysis by the antibody Ab38C2 in detail. The Michaelis constant K_m and catalytic rate constant k_{cat} values were determined to be 2.34 mM and 8.6×10^{-2} min^{-1}, per active site, respectively. The background rate of this annulation reaction in the absence of antibody was 2.4×10^{-8} min^{-1} providing a rate enhancement of 3.6×10^{6} (k_{cat}/k_{un}). Other antibodies that bound hapten **5** with high affinity albeit in a noncovalent fashion were also studied and were found not to catalyze this reaction. Since bovine serum albumin, BSA, is known to possess a lysine residue within a permissive hydrophobic binding pocket and is also known to catalyze a variety of reactions, we studied catalysis of this reaction by BSA. No catalysis above background was observed when the antibody was substituted by a molar equivalent of BSA. Traditionally, the WM ketone **6** is synthesized using optically active proline in organic solvent.[2,6,12] No significant catalysis was observed when L-proline substituted for antibody in our aqueous reaction, even when provided at a concentration equimolar with substrate. To confirm that catalysis proceeded as designed, we studied the reaction in the presence of the mechanism-based inhibitor 2,4-pentanedione. When 1 equiv of 2,4-pentanedione was incubated with Ab38C2 prior to the aldol condensation assay, the catalytic activity was inhibited 71% even though 5000 equiv of triketone **5** was employed. This result demonstrates that the intramolecular aldol condensation takes place at the active site of the antibody with the essential participation of the ϵ-amino group of the lysine residue. The proposed mechanism for this reaction is shown (Scheme 1). We failed to observe accumulation of an aldol addition intermediate suggesting that the elimination step occurs within the active site of the catalyst, perhaps with assistance of the enamine intermediate shown.

The enantioselectivity of this antibody-catalyzed cyclization (the final step of the Robinson annulation) was studied by chiral normal-phase HPLC analysis, chemical shift reagent (europium tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate]) ^{1}H NMR analysis,[12] and optical measurement of product obtained from a 100 mg scale reaction. Only a single enantiomer was detected in HPLC and NMR studies, suggesting **6** was formed in >95% ee. The product demonstrated a 96% optical purity by polarimetry. The enantioselectivity of the antibody-catalyzed Robinson annulation is much higher than that obtained with optically active proline induced annulation in organic solvent (*ca.* 70% ee).[2,6]

To investigate the possibility of conducting the entire Robinson annulation transformation through catalytic antibody mediation, we studied the reaction of 2-methyl-1,3-cyclohexanedione with methyl vinyl ketone in aqueous medium in

Scheme 1



the presence of Ab38C2. Indeed, the one-flask annulation could be conducted. Catalysis of the Michael addition step was modest, $k_{cat}/k_{un} = 125$, and was not inhibited by the addition of 2,4-pentanedione.

In summary, we have successfully used reactive immunization to generate catalysts to achieve the cyclodehydration step of the Robinson annulation reaction and, indeed, the entire process. The catalyst, which uses the enamine mechanism of natural class I aldolase enzymes, demonstrates an extremely high rate enhancement and control over prochiral discrimination. These results underscore the ability of the process of reactive immunization to generate efficient biocatalysts. It seems likely that catalysts generated using the strategy of reactive immunization will have advantages in terms of mechanistic programming and scope over antibody catalysts generated through the traditional transition-state analog approach alone. Since the selective pressure for antibody induction in the reactive immunization strategy is focused on chemical reactivity, the catalysts may not be highly refined with respect to noncovalent contacts which do not contribute much to the chemical reactivity of the antibody pocket. The end result can be, as is the case for the catalysts here, catalytic activity coupled with a permissive binding pocket—the key components of useful synthetic catalysts. Having demonstrated the ability of this catalyst to accomplish complex transformations in series, we expect it to be used to prepare even less accessible compounds. Toward this end, this catalyst will be commercially available from Aldrich Chemical Company, thus allowing the chemical community to further define its scope.

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Supporting Information Available: Experimental details for the preparation, HPLC purification, and determination of optical purity of **6** and Michaelis−Menten plots for substrates (*R* and *S*)-**3** and -**6** (4 pages). See any current masthead page for ordering and Internet access instructions.

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