An Efficient Benchtop System for Multigram-Scale Kinetic Resolutions Using Aldolase Antibodies

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Abstract: The preparative scale kinetic resolution of racemic aldols **1**–**4** using aldolase antibodies 38C2 (Aldrich no. 47995-0) and 84G3 (Aldrich no. 52785-8) is described. These reactions use a biphasic aqueous/organic solvent system that allows the catalyst to be reused. Reaction scales range from miligrams to grams, with 0.0086 to 0.12 mol% of antibody binding sites. Because antibodies 38C2 and 84G3 have opposite enantioselectivities, both aldol product enantiomers are accessible by kinetic resolution.

Keywords: asymmetric synthesis · biphasic catalysis · catalytic antibodies · enantiomeric resolution · retro-aldol reactions

Introduction

When using catalytic antibodies for chemical synthesis, a dilemma often encountered is that many of the interesting and synthetically useful organic molecules have low water solubility, while catalytic antibodies, like all proteins, function ideally in aqueous environments. The potential benefits of using catalytic antibodies for organic synthesis have motivated a variety of researchers to develop optimized systems for circumventing this problem.[1] Proposed solutions include addition of co-solvents such as acetonitrile or methanol to increase substrate solubility,[1a–c] enclosing catalytic antibodies in reverse micelles,[1d] or using a biphasic aqueous/organic solvent system.[1e–f] Broad-scope, enantioselective, aldolase antibodies 38C2 and 84G3 are the most proficient catalytic antibodies ($(k_{cat}/K_M)/k_{uncat}$ up to $10^{13}\,M^{-1}$) currently available.[1b–c, 2–5] These antibodies catalyze both the aldol and the *retro*-aldol reaction enantioselectively. In addition to high turnover numbers (k_{cat}'s up to $1.4\,s^{-1}$),[5b] these antibodies show antipodal enantioselectivity.[5] These attractive features would make them ideal catalysts for organic synthesis of enantiomerically pure aldols on a preparative scale. Here we describe the development of a biphasic aqueous/organic solvent system for the gram-scale *retro*-aldol kinetic resolution of racemic aldols using aldolase antibodies 38C2 and 84G3.

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Initial attempts at using catalytic antibodies for preparative scale synthesis have met with encouraging success.[1a, e, f] The hydrolysis of a cyclic enol ether with antibody 14D9 to produce 1.50 g of an enantiomerically enriched ketone has been reported.[1a] However, the yield was only moderate (62%), and an *ee* of 86% was obtained only after recrystallization. In addition, this gram-scale synthesis was achieved by running a milligram-scale reaction five times, with two dialysis purifications between each reaction.

The more recent gram-scale enantioselective synthesis of an epothilone precursor highlights the synthetic potential of our broad-scope aldolase antibodies.[5a] However, the substrates described were unique in having both high turnover rates and high solubility in buffered aqueous solution ($\approx$50 mM). In parallel with these results, we were interested in developing a simple, practical, and, most importantly, general technique that would allow aldolase antibodies to be used for preparative scale organic synthesis, and would serve as a guide for use of other catalytic antibodies in synthesis.

Results and Discussion

Because of its high turnover rate with 38C2, we chose aldol *rac*-**1** (Cynol)[3, 4] as a target for a gram-scale kinetic resolution via *retro*-aldol reaction to yield (*R*)-**1**, 4-(dimethylamino)cinnamaldehyde, and acetone (Scheme 1). Initially, we treated *rac*-**1** with 38C2 in aqueous solutions containing up to 20% acetonitrile as co-solvent. Although this allows substrate concentrations higher than 10 mM, both enantioselectivity and rate acceleration decreased under these conditions. However, the use of a biphasic toluene/aqueous buffer solvent system yielded aldol (*R*)-**1** in >97% *ee* with 49% recovery (theoret-

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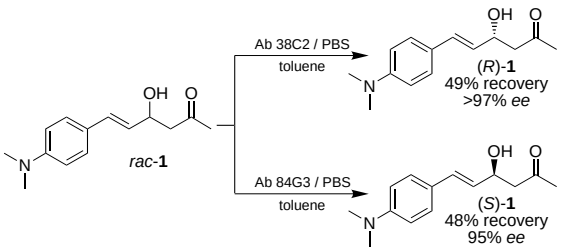
ical maximum for 100% *ee* is 50% recovery). Furthermore, we found that this biphasic system could also be used for 84G3-catalyzed reactions. Kinetic resolution of *rac*-**1** with antibody 84G3 provided (*S*)-**1** in 95% *ee* with 48% recovery (Scheme 1).

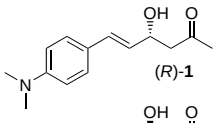
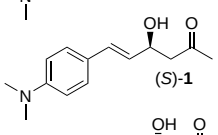
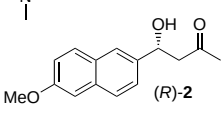
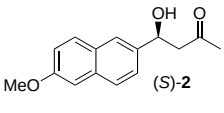
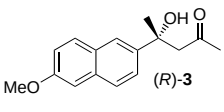
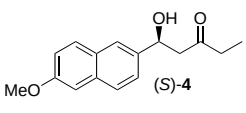


Scheme 1. *retro*-Aldol kinetic resolution of aldol *rac*-**1** with aldolase antibodies 38C2 and 84G3.

Given these encouraging results, we synthesized other enantiomerically pure aldols using this biphasic system. In a typical reaction, a solution of the antibody in phosphate buffered saline (PBS) is added to a solution of the racemic substrate (ca. 50–100 mM) in either toluene or chlorobenzene. The mixture is shaken, while the substrate *ee* is monitored by chiral-phase HPLC. When the desired *ee* is reached, the reaction mixture is cooled (−20 °C), allowing easy separation of the organic layer from the frozen aqueous antibody solution. The aldol product is purified by column chromatography, and the antibody solution is thawed for reuse.

The results for our kinetic resolutions are shown in Table 1. The biphasic procedure was used to synthesize enantiomerically pure aldols by kinetic resolution on a scale of milligrams to grams, with amounts of antibody binding sites ranging from 0.0086 to 0.12 mol%. Aldol *rac*-**2** (Methodol)[4] was resolved with 38C2 (0.10 mol%) to yield (*R*)-**2** (50% recovery, 97% *ee*). To illustrate the potential for catalyst recycling, we chose to synthesize (*S*)-**2** via three sequential resolutions of aldol *rac*-**2** with the same 84G3 catalyst. At the conclusion of each reaction, the apparent relative antibody activity (defined as the reciprocal of the time required to reach 97% *ee*) decreased (Figure 1).

Table 1. Enantiomerically pure aldols prepared by antibody catalyzed kinetic resolution.

Product	Antibody	Time	Recovery[a]
 (*R*)-**1**	38C2 (255 mg; 0.025 mol%)	88 h	1.55 g (49%) >97% *ee*
 (*S*)-**1**	84G3 (16 mg; 0.015 mol%)	340 h	154 mg (48%) 95% *ee*
 (*R*)-**2**	38C2 (15.4 mg; 0.10 mol%)	144 h	25 mg (50%) 97% *ee*
 (*S*)-**2**	84G3 (210 mg; 0.065–0.068 mol%)	91 h 172 h 259 h	469 mg (47%) 441 mg (42%) 458 mg (43%) 97% *ee*
 (*R*)-**3**	38C2 (18 mg; 0.12 mol%)	193 h	22 mg (44%) 99% *ee*
 (*S*)-**4**	84G3 (500 mg; 0.0086 mol%)	65 h	10 g (50%) >99% *ee*

[a] Theoretical maximum for 100% *ee* is 50% recovery.

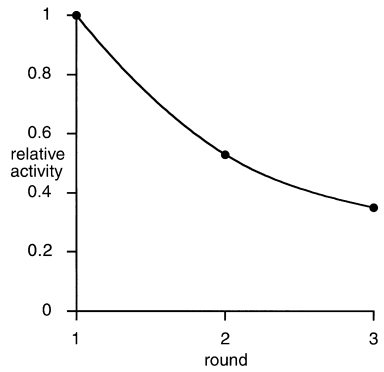


Figure 1. Apparent relative antibody activity (defined as the reciprocal of the time required to reach 97% *ee*) versus time for the *retro*-aldol kinetic resolution of aldol *rac*-**2** with 84G3.

Despite this loss in relative catalyst activity after each reaction, it was still possible to reach 97% *ee* in each iteration. Resolution of tertiary aldol *rac*-**3** (*tert*-Methodol)[1c] with 38C2 (0.12 mol%) provided (*R*)-**3** in 44% recovery and 99% *ee*. A plot of *ee* versus time (Figure 2) shows the data points falling only slightly below an idealized theoretical curve.

Racemic aldols **1**–**3** have k_{cat}'s of 1–5 min^{-1},[3,4] and were used for kinetic resolutions on scales ranging from mg to a few g, using low amounts of catalyst (<0.15 mol%). We believe that these rates represent the minimum substrate turnover required to be useful for preparative scale syntheses, and substrates with higher turnovers can be conveniently synthesized in even larger quantities. Indeed, aldol *rac*-**4** has a k_{cat} of 46.8 min^{-1} for the 84G3-catalyzed *retro*-aldol reaction[5b] and was resolved on a 20 g scale in a reaction volume of 700 mL (Figure 3). A 50% recovery and >99% *ee* was achieved using 500 mg (0.0086 mol%) of 84G3 (from our initial antibody supply of 100 g) as catalyst.[6] This is the largest reaction scale ever performed with an antibody catalyst.

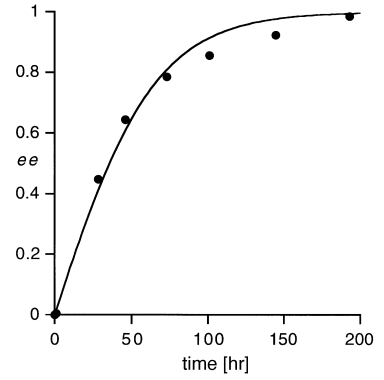


Figure 2. Enantiomeric excess (*ee*) versus time for the *retro*-aldol kinetic resolution of aldol *rac*-**3** with 38C2.

Figure 3. A 20 g scale kinetic resolution of aldol *rac*-**4** using catalytic antibody 84G3.

In summary, we have shown that biphasic organic/aqueous reaction conditions are compatible with preparative scale chemical synthesis with aldolase antibodies 38C2 and 84G3. The limiting factor on the reaction scale is the catalytic turnover rate (k_{cat}), with rates of at least $1-10\ min^{-1}$ required. The biphasic system described here offers many benefits over other previously described methods. Because of increased solubility, typical volumes for biphasic reactions are at least 10 times lower than analogous aqueous systems. Furthermore, these reactions allow facile catalyst recovery and recycling. As with all typical antibody-catalyzed reactions, they proceed at room temperature, and do not require inert atmospheric conditions.

Experimental Section

Racemic aldols **1**–**4** have been described previously.[3, 4, 5b]

Antibody-catalyzed resolutions: The racemic aldols were added to a Teflon tube and dissolved in either toluene or chlorobenzene. To this solution was added the antibody solution in PBS. This mixture was allowed to shake at 250 r.p.m, while the *ee* was monitored by chiral-phase HPLC. When the desired *ee* was reached, the reaction was cooled to $-20\,^{\circ}C$ for several hours to allow the aqueous and organic layers to separate. The organic layer was decanted, and the frozen aqueous layer was allowed to thaw, extracted with either toluene or chlorobenzene (3×3 vol. eq), and EtOAc/(toluene or chlorobenzene) 1:1 (3×3 vol. eq). The combined organic phases were dried (Na_2SO_4), concentrated in vacuo, and purified by column chromatography over silica gel to provide the enantiomerically pure aldols.

HPLC analysis: A 5 μL sample of the organic layer was withdrawn by syringe and dissolved in 500 μL of 10% isopropyl alcohol (IPA)/hexanes. A 10 μL aliquot was injected onto a chiral analytical HPLC column ($1\ mL\,min^{-1}$). Compound *rac*-**1**: Diacel Chiralcel AS, 29% IPA/hexanes, $\lambda = 287$ nm, $t_R = 8.7$ min, $t_S = 12.8$ min). Compound *rac*-**2**: Diacel Chiralcel AS, 20% IPA/hexanes, $\lambda = 230$ nm, $t_S = 43.4$ min, $t_R = 15.6$ min. Compound *rac*-**3**: Diacel Chiralcel AS, 10% IPA/hexanes, $\lambda = 230$ nm, $t_R = 28.0$ min, $t_S = 37.3$ min. Compound *rac*-**4**: Diacel Chiralcel AD, 18% IPA/hexanes, $\lambda = 264$ nm, $t_S = 10.2$ min, $t_R = 9.0$ min.

(*R*)-6-(4-Dimethylamino-phenyl)-4-hydroxy-hex-5-en-2-one ((*R*)-1): 38C2 (255 mg, 3.40 μmol binding site, 0.025 mol%) in PBS (17.0 mL) was added to a solution of *rac*-**1** (3.17 g, 13.6 mmol) in toluene (170 mL). The reaction was stopped at >97% *ee* after 88 h. Column chromatography (hexanes/EtOAc 2:1) furnished (*R*)-**1** (1.55 g, 49%). $[\alpha]_D^{22} = +22.0$ ($c = 1.0$ in $CHCl_3$).

(*S*)-6-(4-Dimethylamino-phenyl)-4-hydroxy-hex-5-en-2-one ((*S*)-1): 84G3 (16 mg, 213 nmol binding site, 0.015 mol%) in PBS (1 mL) was added to a solution of *rac*-**1** (321 mg, 1.38 mmol) in toluene (17 mL). The reaction was stopped at 95% *ee* after 340 h. Column chromatography (hexanes/EtOAc 2:1) furnished (*S*)-**1** (154 mg, 48%). $[\alpha]_D^{22} = -21.8$ ($c = 1.0$ in $CHCl_3$).

(*R*)-4-Hydroxy-4-(6-methoxy-naphthalen-2-yl)-butan-2-one ((*R*)-2): 38C2 (15.4 mg, 205 nmol binding site, 0.10 mol%) dissolved in PBS (5.00 mL) was added to a solution of *rac*-**2** (50 mg, 205 μmol) in toluene (5 mL). The reaction was stopped at 97% *ee* after 144 h. Column chromatography (hexanes/EtOAc 3:1) furnished (*R*)-**2** (25 mg, 50%). $[\alpha]_D^{22} = +52.5$ ($c = 1.0$ in $CHCl_3$).

(*S*)-4-Hydroxy-4-(6-methoxy-naphthalen-2-yl)-butan-2-one ((*S*)-2): 84G3 (210 mg, 2.80 μmol binding site, 0.068 mol%) dissolved in PBS (13.2 mL) was added to a solution of *rac*-**2** (1.00 g, 4.09 mmol) in chlorobenzene (50 mL). The reaction was stopped at 97% *ee* after 91 h. Column chromatography (hexanes/EtOAc 3:1) furnished (*S*)-**2** (469 mg, 47%).

PBS (5 mL) was added to the remaining antibody solution, followed by a solution of *rac*-**2** (1.06 g, 4.34 mmol) in chlorobenzene (50 mL). The reaction reached 97% *ee* after 263 h. Column chromatography (hexanes/EtOAc 3:1) yielded (*S*)-**2** (441 mg, 42%). This was repeated with another solution of *rac*-**2** (1.06 g, 4.34 mmol) in chlorobenzene (50 mL). After 522 h, the reaction reached 97% *ee*. (*S*)-**2** (458 mg, 43%) was isolated by column chromatography. $[\alpha]_D^{22} = -52.9$ ($c = 1.0$ in $CHCl_3$).

(*R*)-4-Hydroxy-4-(6-methoxy-naphthalen-2-yl)-pentan-2-one ((*R*)-3): 38C2 (18.0 mg, 240 nmol binding site, 0.12 mol%) dissolved in PBS (908 μL) was added to a solution of *rac*-**3** (50 mg, 193 μmol) in toluene (2 mL). The reaction was stopped at 99% *ee* after 193 h. Column chromatography (hexanes/EtOAc 3:1) furnished (*R*)-**3** (22 mg, 44%). $[\alpha]_D^{22} = +3.8$ (c = 1.0 in $CHCl_3$).

(*S*)-1-Hydroxy-1-(6-methoxy-naphthalen-2-yl)-pentan-3-one ((S)-4): 84G3 (500 mg, 6.67 μmol binding site, 0.0086 mol%) dissolved in PBS (87.5 mL) was added to a solution of *rac*-**4** (20 g, 77.4 mmol) in toluene (600 mL). The reaction was stopped at >99% *ee* after 65 h. Column chromatography (hexanes/EtOAc 3:1) yielded (*S*)-**4** (10 g, 50%). $[\alpha]_D^{22} = -49.2$ ($c = 1.0$ in $CHCl_3$).

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[6] This antibody supply was produced entirely from cell culture.

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