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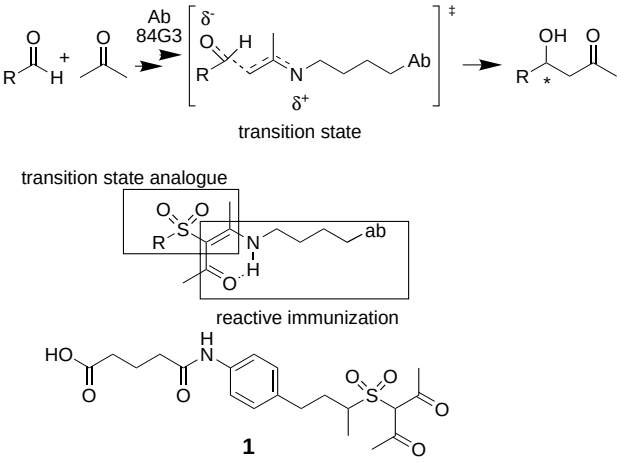
Unprecedented Regiocontrol Using An Aldolase I Antibody**

Virginie Maggiotti, Marina Resmini, and Véronique Gouverneur*

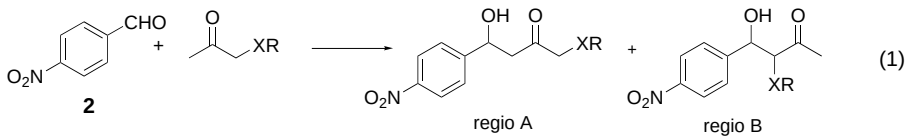
Dedicated to Professor Guy Ourisson

The aldol reaction is one of the most powerful methods for forming carbon – carbon bonds.[1] The value of the reaction relies upon the degree of regio-, diastereo-, and enantioselectivity that one could control. The two approaches towards catalytic asymmetric aldol reactions are biocatalysis and chemical catalysis with Lewis acids, Lewis bases, or small molecules.[2] Each has specific advantages and limitations and they should thus be regarded as complementary. By combining reactive immunization and transition state analogy into a single hapten, the groups of Lerner and Barbas developed a series of aldolase antibodies which use the enamine mechanism of natural occurring class I aldolases.[3] Here we report our findings that antibody 84G3 (ab84G3) raised against the β-diketo sulfone hapten **1**[4] (Scheme 1) is an effective asymmetric catalyst for the regio- and enantiocontrolled formation of a series of disfavored aldol products, therefore controlling reactivity in a unique way.



Scheme 1. Mechanism of the antibody-catalyzed aldol reaction (top) and reactive immunization with **1** for the generation of ab84G3 (bottom).

An inherent challenge to the use of unmodified unsymmetrical ketones in cross aldol reactions is the control of the regioselectivity. To study the regioselectivity of the cross aldol reaction in the presence of ab84G3, *para*-nitrobenzaldehyde **2** was chosen as the standard acceptor aldehyde [Eq. (1)]. As



(1)

3 X = CH_2, R = Me
4 X = CH_2, R = Et
5 X = O , R = Me
6 X = S , R = Me

7 X = CH_2, R = Me
9 X = CH_2, R = Et
11 X = O, R = Me
13 X = S, R = Me

8 X = CH_2, R = Me
10 X = CH_2, R = Et
12 X = O, R = Me
14 X = S, R = Me

can be seen from Table 1, the unsymmetrical aliphatic ketones **3** – **6** undergo the aldol coupling in the presence of ab84G3. Analysis of the reaction mixtures by high-performance liquid chromatography (HPLC) indicated that in each case the antibody-catalyzed reaction occured exclusively at the less substituted carbon atom (products **7**, **9**, **11**, **13**), independently of the presence of heteroatoms in the donor ketones. This reactivity contrasts with the results obtained for the uncatalyzed reactions carried out under the same conditions (PBS, pH 7.4, Table 1). Indeed, the uncatalyzed reactions occurred preferentially at the more substituted carbon atom resulting in a mixture of *syn* and *anti* stereoisomers (products **8**, **10**, **12**, **14**). The other regioisomer is not observed or is formed only as a minor product.

[*] Dr. V. Gouverneur, V. Maggiotti
University of Oxford
The Dyson Perrins Laboratory
South Parks Road, Oxford OX1 3QY (UK)
Fax: (+44) 1865-275-644
E-mail: veronique.gouverneur@chem.ox.ac.uk
Dr. M. Resmini
Queen Mary, University of London
Department of Chemistry
Mile End Road, London E1 4NS (UK)

[**] This work was supported by the University of Oxford (grant B(RE)9862 to V.G. and grant to V.M.) and the Royal Society of Chemistry (RSRG 20750 grant to V.G.). We thank Professor R. A. Lerner for supplying a very generous quantity of antibody 84G3.

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  1433-7851/02/4106-1012 $ 17.50+.50/0

Table 1. Aldol reactions of *para*-nitrobenzaldehyde **2** with unsymmetrical ketones **3**–**6** at pH 7.4 (phosphate-buffered saline, PBS) [Eq. (1)].

Entry	Ketone	Conditions	Conversion [%][a]	Ratio [%][a]
1	**3**	RT, 3 h	0	0/0
2	**3**	9 mol % ab84G3, RT, 3 h	88	100/0[b]
3	**4**	RT, 26 h	1	0/100
4	**4**	9 mol % ab84G3, RT, 26 h	66	99/1[b]
5	**5**	RT, 15 h	54	4/96
6	**5**	0°C, 41 h	0	0/0
7	**5**	25 mol % ab84G3, 0°C, 41 h	76	100/0[b]
8	**6**	RT, 3 h	49	0/100
9	**6**	0°C, 1 h 40 min	9	10/90
10	**6**	25 mol % ab84G3, 0°C, 1 h 40 min	58	98/2[b]

[a] Assigned by HPLC. [b] Regio A/regio B [see Eq. (1)] as a mixture of *syn* and *anti* isomers. [b] Uncorrected ratio.

Previous studies in which other aldolase antibodies were used showed that, in the reaction with unsymmetrical ketones like 2-butanone and 2-pentanone, these antibodies exhibit some control of the regioselectivity of the aldol addition by preferential formation of the most substituted enamine.[5] In addition, it is reported that α-heteroatom-substituted ketones like α-hydroxyacetone show a much higher level of regioselectivity with reactions occurring almost exclusively at the carbon atom bearing the oxygen atom.[6] Aldol reactions with the unsymmetrical, sulfur-containing ketone **6** in the presence of aldolase I antibodies or enzymes have not been reported.[7] Chemically, it is known that aldol couplings of ketone **6** with aldehydes always proceed preferentially at the carbon atom bearing the sulfur atom.[8] Antibody 84G3 is unique in the sense that it is capable of reversing the regioselectivity for all these reactions. The regioselectivity was proven unambiguously by carrying out all the reactions under conditions that suppress any background reaction and assigned by comparison of the HPLC retention times of the products with those of independently chemically synthesized standards.[9]

To gain more detailed information about this unusual reactivity, several aldol and retro-aldol reactions were chosen for further characterization. The kinetic experiments were all performed at room temperature and the results are collected in Table 2. For the aldol condensations (entries 1 and 2), the reactions were performed under pseudo-first order conditions by keeping the concentration of the ketones constant and in large excess. Initial rates (v_i) were determined by monitoring the formation of the products by HPLC. The adherence of the uncorrected initial rate (v_i) versus $[S]_0$ data (S = substrate) to the Michaelis–Menten equation was verified by the linearity of the $[S]_0/v_i$ against $[S]_0$ plot for both aldol and retro-aldol reactions. Determination of k_{cat}/k_{uncat} was not possible for the forward aldol reactions because in the uncatalyzed reactions,

Table 2. Kinetic parameters for selected antibody-catalyzed aldol and retro-aldol reactions.

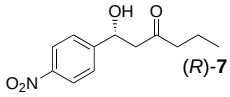
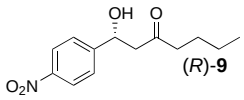
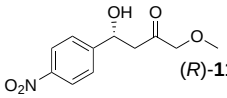
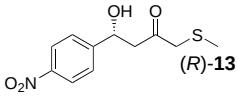
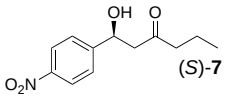
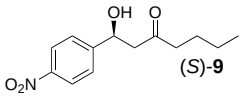
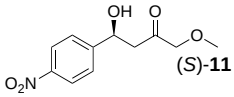
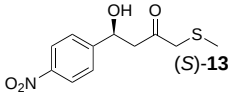
Entry	Substrates	k_{cat} [min^{-1}]	K_m [μM]	k_{cat}/k_{uncat}	$(k_{cat}/K_m)/k_{uncat}$ [M^{-1}]
1	**5** + **2**	0.040 (±0.001)	390 (±23)	–	–
2	**6** + **2**	0.089 (±0.001)	217 (±7)	–	–
3	(±)-**11**	0.099 (±0.004)	170 (±14)	5.5×10^4	3.2×10^8
4	(±)-**13**	0.810 (±0.02)	69 (±3)	4.3×10^5	6.2×10^9

the formation of regioisomer A was negligible under our assay conditions. The failure to form the regioisomer resulting from a reaction on the less substituted carbon atom in the absence of the antibody illustrates the potential of ab84G3 to catalyze an otherwise undetectable reaction.

For the antibody-catalyzed retro-aldolization of the racemic substrates (±)-**11** and (±)-**13** the kinetic results are shown in entries 3 and 4 of Table 2. The catalytic proficiencies $[k_{cat}/K_m]/k_{uncat}$ compare favorably with the efficiency of aldolase antibodies for other retro-aldol reactions.[3] All antibody-catalyzed aldol and retro-aldol reactions were inhibited by the addition of an equimolar amount of 2,4-pentanedione. Further control experiments were carried out using bovin serum albumin (BSA). No catalysis of the aldol coupling was observed for these reactions. These results are consistent with a covalent catalytic mechanism in which a reactive amine is programmed in ab84G3.

HPLC analysis on a chiral stationary phase demonstrated that the typical enantioselectivities of ab84G3-catalyzed aldol or retro-aldol reactions are greater than 94 % (Table 3). For the aldol condensation of *para*-nitrobenzaldehyde with ketones **3**–**6**, the aldol products (*R*)-**7**–(*R*)-**13** are formed and this reflects an attack on the *si* face of the aldehyde. For the retro-aldol reactions, HPLC analysis indicated that at approximately 50 % conversion, the isolated unconverted aldol substrates possessed the *S* configuration ((*S*)-**7**–(*S*)-**13**).

Table 3. *ee* values for antibody-catalyzed aldol and retro-aldol reactions.

Product	Method	Conversion [%]	*ee* [%][a]
 (*R*)-**7**	aldol		94
 (*R*)-**9**	aldol		98[b]
 (*R*)-**11**	aldol		98
 (*R*)-**13**	aldol		97
 (*S*)-**7**	retro-aldol	55	97
 (*S*)-**9**	retro-aldol	50	94[b]
 (*S*)-**11**	retro-aldol	47	97
 (*S*)-**13**	retro-aldol	50	96

[a] Attribution by HPLC (Chiralcel OD or OJ column). [b] No baseline separation of the two enantiomers.

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These results demonstrate that both aldol enantiomers could be accessed through aldol or retro-aldol reactions using the same antibody 84G3. In order to assign the absolute configurations, the enantiopure products were synthesized by independent chemical asymmetric synthesis for compounds **9, 11**, and **13**.[9] The absolute configuration of compound **7** was assigned by analogy with compound **9**.

In conclusion, we described here the first aldolase antibody, ab84G3, capable of rerouting the regioselectivity of a series of cross aldol reactions which led to the formation of the otherwise disfavored products. This new reactivity highlights the scope of the reactive immunization strategy developed by the groups of Lerner and Barbas for catalyst design. This work further increases the repertoire and efficiency of antibody-catalysed aldol reactions. Further studies on the reactivity of ab84G3 are currently in progress.

Received: October 24, 2001 [Z18117]

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Biocatalytic Asymmetric Hydrogen Transfer**

Wolfgang Stampfer, Birgit Kosjek, Christian Moitzi, Wolfgang Kroutil,* and Kurt Faber

Driven by the increased public awareness on hazards derived from chemical production, environmentally benign oxidation methods have gained increasing importance.[1] In this context, the reduction of ketones at the expense of a sacrificial secondary alcohol and the corresponding reverse reaction (known since the 1920s as the Meerwein – Ponndorf – Verley reduction (MPVRed) and Oppenauer-oxidation (OOx), respectively, constitute typical "green" redox reactions.[2] As a consequence, they have been re-investigated recently[3] to replace the previously employed metal alkoxides with catalysts showing improved efficiency,[3c] better recoverability,[3g] avoiding aldol condensation as side reaction,[3f] and water-soluble analogues.[3b] Asymmetric variants have been pursued by using enantioselective hydride-transfer methods based on chiral transition metal complexes[3e] or chiral hydride sources.[3a]

All biocatalytic methods for the asymmetric hydrogen transfer are based on alcohol dehydrogenases requiring nicotinamide cofactors. They have several advantages over the chemical methods, such as 1) their intrinsic asymmetry, 2) absence of side reactions, such as aldol condensation, and 3) they operate under essentially mild reaction conditions. However, their large-scale application has been impeded by the requirement for cofactor-recycling.[4] Since the sacrificial secondary alcohol used as cosubstrate (for MPVRed) or the carbonyl compound (for OOx) has to be employed in excess to drive the reaction from equilibrium towards completion, cosubstrate inhibition is common in such a "coupled-substrate" approach based on the use of a single enzyme.[5] Although this drawback has been surmounted to some extent by using a second dehydrogenase, which is highly specific for the sacrificial cosubstrate,[6] these so-called "coupled-enzyme" methods are rather complex and require the handling of isolated enzymes and cofactor(s). As a consequence, biochemical MPVReds and OOxs on a large scale are limited by the use of fermenting cells[7] and/or low (co)substrate concentration(s).[8]

We have recently isolated a highly enantioselective secondary-alcohol dehydrogenase[9] from *Rhodococcus ruber* DSM 44541, which is exceptionally stable towards organic solvents. The activity of the enzyme remains high at concentrations of up to 20 % (v/v) acetone and 50 % (v/v) 2-propanol. This activity enables the use of the enzyme for MVRed and OOx in the "coupled-substrate" approach. For preparative-

[*] Dipl.-Ing. Dr. W. Kroutil, Dipl.-Ing. W. Stampfer, Mag. B. Kosjek, C. Moitzi, Prof. Dr. K. Faber
Department of Chemistry, Organic and Bioorganic Chemistry
University of Graz
Heinrichstrasse 28, 8010 Graz (Austria)
Fax: (+43) 316-380-9840
E-mail: Wolfgang.Kroutil@uni-graz.at

[**] This study was performed within the Spezialforschungsbereich "Biokatalyse", and financial support by the Fonds zur Förderung der wissenschaftlichen Forschung (Vienna, project No. F115) and the Federal Ministry of Science (Vienna) is gratefully acknowledged.

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