Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 2, pp. 297-302, 1994
Elsevier Science Ltd

0960-894X/94 $6.00+0.00

0960-894X(93)E0076-D

AUTOMATION OF ANTIBODY CATALYSIS: A PRACTICAL METHODOLOGY FOR THE USE OF CATALYTIC ANTIBODIES IN ORGANIC SYNTHESIS

Charles G. Shevlin,* Susan Hilton, and Kim D. Janda*

Departments of Molecular Biology and Chemistry
The Scripps Research Institute
10666 North Torrey Pines Road
La Jolla, California 92037

Abstract: The first large scale regio- and stereoselective synthesis catalyzed by an antibody is described which features automation as an attractive means for the general utilization of antibodies in larger reaction scales. A biphasic catalytic antibody system (5% aqueous hexane) is coupled with an automation device which facilitates the multigram synthesis of the desired compound.

Since their inception, catalytic antibodies[1] have been generated which catalyze a large range of reactions[2] varying from the simple hydrolysis of carbonates and esters to more difficult transformations such as the control of regio- and enantioselective reactions.[3] One of the goals of catalytic antibody research is to provide tailor-made catalysts for the synthetic chemist. These antibodies would then catalyze reactions which are difficult or impossible to carry out by other means.[4] In the future, catalytic antibodies may help alleviate the painstaking task of separating unwanted isomers during a synthesis by providing a kinetic preference for the desired isomer. The power of this capability becomes clear when one considers that the efficacy of many drugs depends on the delivery of an enantiomerically pure compound.[5]

Until recently, reactions involving the use of catalytic antibodies have been restricted to aqueous solvent systems. However, we have demonstrated that antibodies are capable of catalyzing reactions at the interface of organic media when in the presence of a small amount of aqueous buffer.[6] The value of such a biphasic system becomes obvious when one considers that

most compounds of interest to the synthetic organic chemist have only limited solubility in water. In addition to possible solubility limitations, one must also contend with the product inhibition associated with many antibody systems. Thus we set out to investigate the feasibility of performing gram scale synthetic reactions using catalytic antibodies which would circumvent these problems i.e. *the development of protocols for antibody-catalyzed reactions in organic solvents (Biphasic Antibody Catalysis Automated, BACA)under conditions that minimize product inhibition.*

After examining several potential candidates, antibody 26D9 was selected as a suitable catalyst, and epoxide **1** its substrate.[8] Our enthusiasm for employing this catalytic antibody was based on its ability to catalyze ring closure reactions in a regio and stereoselective fashion.[4a] Such O-heterocycles are frequent and important targets for synthesis either as final products or as useful synthetic intermediates in natural product total synthesis.[9] Thus, when a solution of hexane containing a racemic mixture of epoxide **1** is placed in the presence of 26D9 and 5% aqueous buffer, tetrahydropyran **2** is formed in 96% ee as determined by HPLC [10] (Figure 1). The initial rate of ring closure catalyzed by 26D9, when measured as a function of **1**, followed Michaelis-Menten kinetics.[11] The Michaelis constant K_m , the catalytic rate constant k_{cat}, and k_{cat}/k_{uncat} were 590 μM, 7.96 min^{-1} and 3.65 X 10^4 respectively.

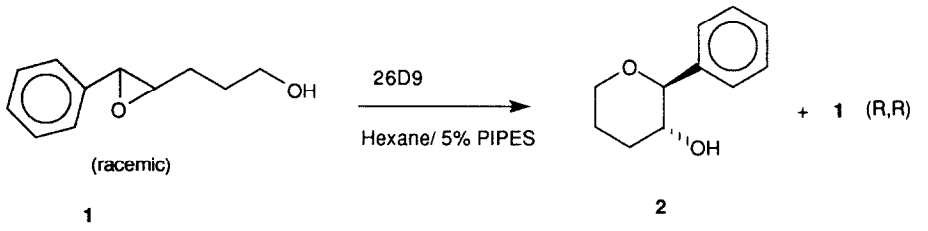

Figure 1. Reaction of **1** with antibody 26D9

The ring closure reaction of **1** has two potential pathways. It can close to form either the 5-membered tetrahydrofuran **3** or the tetrahydropyran **2** (Figure 2).[12] Acid catalysis of the ring closure of **1** results only in the formation of **2**, whereas reaction of **1** in the presence of 26D9

results only in the S,R isomer of **2**[13] and the R,R isomer of **1** in 96% ee.[15] Furthermore **1** and **2** are easily separated using flash column chromatography.[18] Taken together these results demonstrate that the reaction is not only regio and stereoselective, but that 26D9 is also able to easily discriminate between the enantiomers of **1** leading to a kinetic resolution of these epoxides.

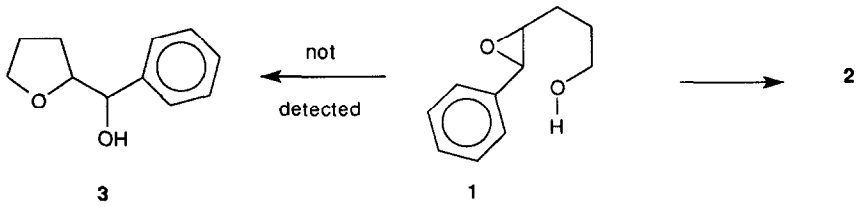

Figure 2. The two possible pathways of **1** in the presence of acid.

The synthesis of **2** from **1** was carried out on a 2.2 gram scale using the apparatus as shown in Figure 3. We constructed this device in order to automate antibody catalysis, and hence demonstrate not only their synthetic practicality, but also the possible industrial feasibility for the use of catalytic antibodies in organic synthesis. The robotics of the events described herein are governed by the microprocessor/timer.[19] In our example, vessel **A** contains a 3.8 mM solution of epoxide **1** in hexane and vessel **B** contains a 135 mM solution of 26D9 (100 ml of PIPES buffer). An 800 ml portion of solution **A** is automatically transferred to vessel **B** under positive argon pressure (any inert gas can be used here) through solenoid valve $\mathbf{V}_1$ and at this time begins to rock, via a solenoid-controlled ($\mathbf{V}_5$) air driven motor, at a rate of 90 swings per minute through an angle of 90° for 2 hr. After this event, a ten minute wait period is provided so that the two phases can separate. The organic layer containing the products is then transferred to vessel **C** under positive pressure through solenoid valve $\mathbf{V}_4$ and the entire process is automatically repeated 4-times with 127-turnovers per period. Throughout this period little degradation of catalyst was

observed (< 5%). Once the reaction is complete, the volume of solvent in vessel **C** is reduced under negative pressure leaving behind the products **1**(R,R) (945 mg) and **2**(R,S) (957 mg).

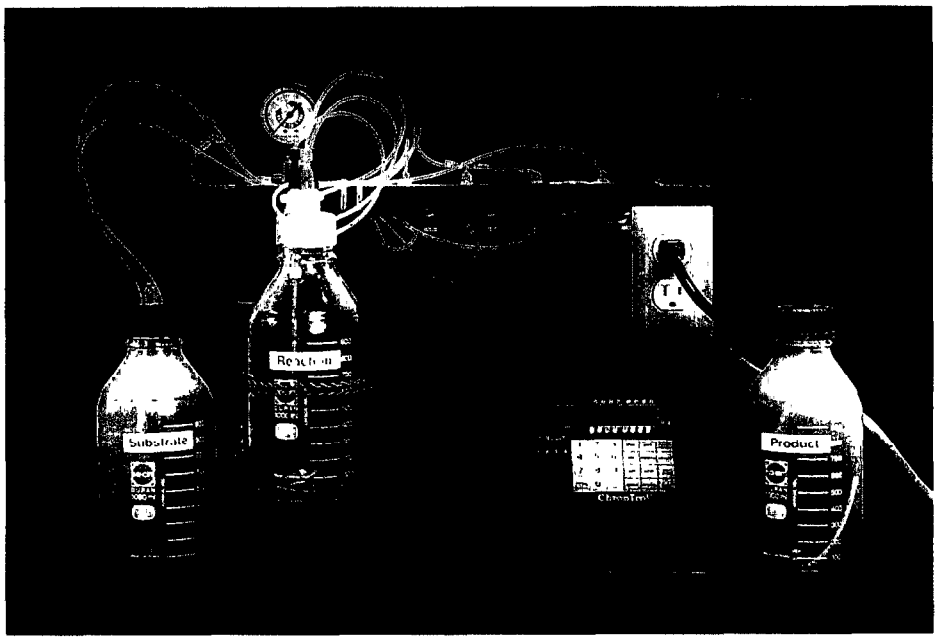

Figure 3. BACA apparatus.

The major advantages of using our biphasic system are its elimination of product inhibition and substrate insolubility. However, in some instances a homogenous aqueous system maybe advantageous. It is also possible to employ our apparatus in a homogeneous-aqueous catalytic antibody system. Using a similar, however non-automated method pioneered by Whitesides for enzymes (Membrane Enclosed Enzymatic Catalysis)[20], we tested how well our device would perform in a membrane enclosed antibody system at a 100 mg scale synthesis. As reported by Whitesides, the reaction rates can be significantly decreased (we saw a relative rate reduction of

approximately three fold) which we and others speculate to be the result of a slow rate of diffusion of the substrate across the membrane. There was also a significant reduction in the enantioselectivity (24% ee), we ascribe this to the now significant uncatalyzed rate of reaction. Futhermore this system requires a rather arduous extraction step. While untenable in our case, the aqueous system might be useful in other catalytic antibody systems where the substrate is insoluble or incompatible with organic solvents.

In summary, we have demonstrated the use of a catalytic antibody for a regio- and enantioselective large scale synthesis. Although an antibody-catalyzed multigram synthesis can be performed using routine laboratory glassware, the automation device described here demonstrates the potential of catalytic antibodies in an industrial setting.

Acknowledgements. This work was supported in part by the National Science Foundation (KDJ) and the Alfred P. Sloan Foundation (KDJ).

CITATIONS:

1. (a) Tramontano, A.; Janda, K.D.; Lerner, R. A. *Proc. Natl. Acad. Sci. USA*, **1986**, *83*, 6736. (b) Tramontano, A.; Janda, K.D.; Lerner, R.A. *Science*, **1986**, *234*, 1566. (c) Pollack, S.J.; Jacobs, J.W.; Schultz, P. G. Science, **1986**, *234*, 1570.
2. Lerner, R. A.; Benkovic, S. J.; Schultz, P. G. *Science* **1991**, *252*, 659.
3. Schultz, P. G.; Lerner, R. A. *Acc. Chem. Res.***1993**, *26*, 391.
4. (a) Janda, K. D.; Shevlin, C. G.; Lerner, R. A. *Science* **1993**, *259*, 490. (b) Hsieh, L. C.; Yonkovich,S.; Kochersperger, L.; Schultz, P. G. *Science* **1993**, *260*, 337.
5. Stinson, S.C. *Chem. Eng. News*, **1992**, 46.
6. Ashley, J. A., Janda, K. D.; *J. Org. Chem.***1992**, *57*, 6691. We have also enjoyed success in the immobilization of antibodies allowing catalysis under non-aqueous conditions.[7]
7. Janda, K. D., Ashley, J.A.; Jones, T.M.; McLeod, D.A.; Schloeder, D.M.; Weinhouse, M.I. *J. Am. Chem. Soc.***1990**, *112*, 8886.
8. A more detailed substrate specificity of antibody 26D9 will be the subject of a forthcoming paper.
9. Nicolaou, K.C.; Prasad, C.V.C.; Somers, P.K.; Hwang, C.K. *J. Am. Chem. Soc.* **1989**, 111, 5330, and references cited within.
10. We used a DAICEL chiral pak AD with a hexane-isopropanol isocratic program of 97/3 at 1 ml/min using a wavelength of 254 nM.

11. The kinetic assays were performed using HPLC (conditions and column described in (5)) using an external standard of 5-phenyl-1-pentanol (2.28×10^{-4} M) to calculate the amount of product formed. The details of this protocol have been previously described (4a).
12. Baldwin, J. E. *J. Chem. Soc. Chem. Commun.***1976**, 734.
13. This is probably due to the incipient benzylic-stabilized carbocation of **1** as proposed by Nicolaou for the analogous allyl epoxides.[14]
14. Nicolaou, K. C.; Prasad, C. V. C.; Somers, P. K.; Hwang, C. K. *J. Am. Chem. Soc.***1989**, *111*, 5330.
15. Determination of the absolute configuration was carried out by preparation of both antipodes of **1**.[16,17] With both optically pure isomers in hand it was possible to identify which isomer was used by 26D9 and which product was formed by coinjection with the racemic mixtures of both **1** and **2**.
16. Sharpless, K. B. *et al. J. Org. Chem.***1992**, *57*, 2768.
17. Kolb, H. C.; Sharpless, K. B. *Tetrahedron***1992**, *48*, 10515.
18. (a) Still, W. C.; Kahn, M.; Mitra, A. J. *J. Org. Chem.***1978**, *48*, 2923. (b) The separation was carried out using an isocratic mobile phase (hexane-ethyl acetate 1:1, R_f **1**= 0.27, R_f **2** = 0.42) on Kieselgel 60 silica.
19. ChronTrol® model XT, San Diego, CA.
20. Bednarski, M. D.: Chenault, H. K.; Simon, E. S.; Whitesides, G. M. *J. Am. Chem. Soc.***1987**, *109*, 1283.

(*Received in USA* 19 *November* 1993)