*Tetrahedron: Asymmetry* Vol. 4, No. 5, pp. 943-946, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00
Pergamon Press Ltd

# Immobilization of Substrates in Enzyme-Catalyzed Hydrolysis

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(*Received* 11 *February* 1993; *accepted* 16 *March* 1993)

**Abstract:** A new technique - immobilzation of substrates on solid supports - was applied to the synthesis of a new potent optically pure serotonin receptor antagonist.

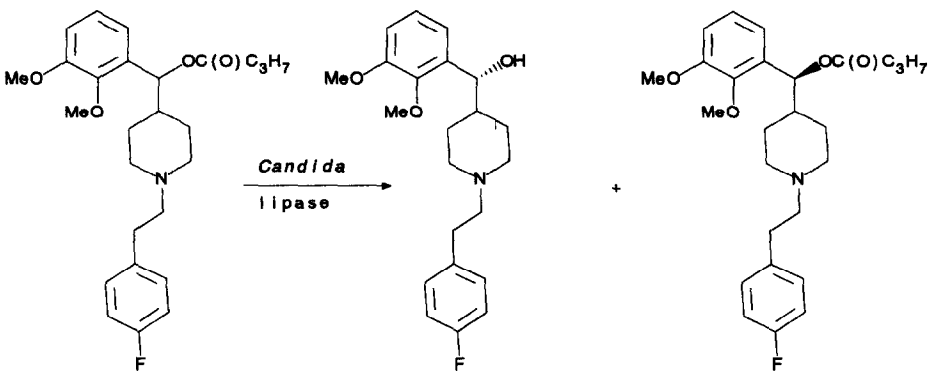
The use of enzymes as catalysts for stereoselective transformations[1] has recently gained in popularity, especially, in the synthesis of optically pure drugs[2]. One of the most developed techniques in the preparation of optically pure building blocks is enzyme-catalyzed hydrolysis of the corresponding racemic esters[1]. This technique has successfully been used for the synthesis of β-adrenergic blocking agents[3], nonsteroidal antiinflammatory drugs[4], serotonin receptor antagonists[5], adenosine receptor agents[6], and several other synthetic optically pure pharmaceuticals[7].

Since many of the esters are highly lipophilic compounds, the substrate solubility in the aqueous reaction mixture presents a serious problem. This problem can be alleviated by using enzymes in aqueous-organic biphasic mixtures[8], or by addition of water-miscible organic solvents. All these methods have their advantages and drawbacks, the main problem being the denaturation of enzymes by an organic co-solvent.
Following the proposal by Nieduziak and Carr[5] we decided to investigate the lipase-catalyzed hydrolysis by adsorbing the ester substrate on a solid support.

In this paper we report the investigation of this technique in the synthesis of a new serotonin receptor antagonist MDL 100907 ((*R*)-**1**)[9]. This (*R*)-enantiomer is much more active both *in vitro* and *in vivo* than its (*S*)-counterpart and the well known achiral serotonin receptor antagonist, ketanserin. Even more importantly, MDL 100907 shows extremely high selectivity towards the 5-$HT_2$ receptor[9].

In general, enantiomerically pure alcohols can be prepared by two major procedures: enzyme-catalyzed hydrolysis of esters in water or acylation of alcohols in organic solvents.[1c]

Attempts to resolve the racemic **1** by lipase-catalyzed stereoselective acylation with vinyl acetate or acetic anhydride in organic solvents were unsuccessful due to extremely low reaction rates. Among several lipases[10] screened for the stereoselecive hydrolysis of **2** in aqueous buffer, only lipase from *Candida rugosa* (formerly known as *Candida cylindracea*) showed somewhat modest activity (Scheme). Even in this case the conversion of **2** was only 13% after 4 days at 45°C (Table). Attempts to increase the reaction rate by using a methanol/aqueous buffer mixture (1:5) or *tert*-butyl methyl ether (*t*-BuOMe) saturated with buffer as the reaction media failed to increase the yield of (*R*)-**1**. The use of enzyme immobilized on Celite in the latter solvent system also showed no improvement.




**2** (*R*)-**1** (*S*)-**2**

As a consequence we decided to try a new approach - immobilization of a substrate on a solid support. In a typical experiment 230 mg of a solid support (Table) was added to 80 mg of **2** dissolved in 2 mL *t*-BuOMe. After evaporation of the ether, the substrate-coated silica was transferred to 5 mL of 0.1 M phosphate buffer (pH 7.0) containing 11 mg of partially purified enzyme.[11] The progress of the lipase-catalyzed reaction, as well as the optical purity of **1** and **2** were determined by HPLC using a Chiralcel OD column (Daicel) with a mobile phase of heptane:octanol:diethylamine (80:20:0.1) at a flow rate 1mL/min and detection at 280 nm. The results are presented in the Table.

Table. Enzyme-Catalyzed Hydrolysis Using Substrate Immobilized on Different Inorganic Supports.

| Support | (*R*)-**1** (%) after 96 h | (*R*)-**1** (liquid/solid) | Ester (**2**) (liquid/solid) |
|---|---|---|---|
| $SiO_2$ | 38 | 2:1 | 1:10 |
| $Al_2O_3$ (neutral) | 26 | 2:3 | 1:37 |
| $Al_2O_3$ (acid) | 31 | 1:1 | 1:22 |
| $Al_2O_3$ (basic) | 34 | 1:2 | 1:31 |
| Zorbax $C_8$ | 30 | 2:1 | 1:21 |
| Vydac $C_{18}$ | 37 | 4:1 | 1:16 |
| Celite | 33 | 5:1 | 1:6 |
| No support | 13 | | |

The adsorption of **2** on different inorganic supports facilitates the lipase-catalyzed resolution. Although an immobilized substrate remains insoluble in water, adsorption on supports seems to prevent its aggregation and apparently makes a substrate more accessible to the enzyme. The type of support has no effect on the degree of conversion or the rate of the reaction. In nearly all the cases the conversion of **2** to (*R*)-**1** was more than 30% after 96 h incubation and the optical purity of (R)-**1** was at least 99% ee. In no case could (*S*)-**1** be found in the reaction mixture by the analytical method used.

In order to demonstrate the feasibility of the proposed substrate immobilization technique on large scale, 90g of **2** adsorbed on 270g silica was hydrolyzed by lipase for 4 days at 45°C. As a result 23g of (*R*)-**1** (36% yield[12]; 96% ee) was obtained.

This example indicates that immobilization of substrates on a solid support has several advantages over the use of water-saturated organic solvents or mixtures of water and water-miscible organic solvents for hydrolytic reactions. First, the operating stability and activity of the enzyme may be higher since no organic solvent or any other additive is added. Secondly, higher enzyme concentrations can be achieved compared to the biphasic systems .

Another important feature which this method, at least in principle, can offer is the ability to regulate the partition of both a substrate and a product between a solid support and a solution simply by changing the support. In an ideal situation a lipophilic ester would remain on the support and a more hydrophilic alcohol would be released into solution. If this goal can be achieved, then the immobilization of substrates on solid supports will provide an excellent method not only for facilitating enzyme-catalyzed hydrolysis but also for facilitating the isolation of products.

The results presented in this paper indicate that indeed the distribution of the remaining substrate (ester) and the product (alcohol) between the reaction medium (liquid) and a support (solid) is influenced by the chosen support (Table). One can see that the major portion of the ester (up to 97%) remains adsorbed on the support whereas the partition of the alcohol is more even[13]. The portion of (*R*)-**1** in buffer varies from 84% (Celite) to 30% (basic $Al_2O_3$).

Although these ratios are not high enough to warrant the use of these supports as a new separation procedure at the present time, the mechanism of the adsorption of the substrates and the products on solid supports is worth special study. Clearly, several parameters such as hydrophobicity, pore size and particle size of a support, as well as the distribution of a substrate inside the support may critically affect the partition of the reactants and products during hydrolysis. If these relations are properly studied and understood this technique may become an important tool for enzyme-catalyzed hydrolysis in general and for resolution of optically pure compounds in particular.

## REFERENCES AND NOTES.

1. (a) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071-1140. (b) Boland, W.; Froβl, C.; Lorenz, M. *Synthesis* **1991**, 1049-1072. (c) Klibanov, A.M. *Acc. Chem. Res.* **1990**, *23*, 114-120. (d) Crout, D.H.G. and Christen, M. *In Modern Synthetic Methods* **1989**; Scheffold,

R. Ed.; Springer-Verlag:Berlin, 1989; pp 1-114. (e) Sih, C.J.; Wu, S.-H. *Top. Sterechem.* **1989**, *19*, 63-125.

2. (a) Margolin, A.L. *CHEMTECH* **1991**, 160-167. (b) Margolin, A.L. *Enz. Microb. Technol.* **1993**, in press.
3. (a) Hamaguchi, S.; Asada, M.; Hasegawa, J.; Watanabe, K. *Agric. Biol. Chem.* **1985**, *49*, 1661-1667. (b) Terao, Y.; Murata, M.; Achiwa, K.; Nishio, T.; Akamtsu, M.; Kamimura, M. *Tetrahedron Lett.* **1988**, *29*, 5173-5176.
4. (a) Mustaers, J.H.G.M.; Kooreman, H.J. *Rec. Trav. Chim. Pays-Bay* **1991**, *110*, 185-188. (b) Battistel, E.; Bianchi, D. Cesti, P.; Pina, C. *Biotechnol. Bioeng.* **1991**, *38*, 659-664. (c) Ahmar, M.; Girard, C.; Bloch, R. *Tetrahedron Lett.* **1989**, *30*, 7053-7056. (d) Gu, Qu-M.; Chen, C.-S.; Sih, C.J. *Tetrahedron Lett.* **1986**, *27*, 1763-1766.
5. (a) Nieduzak, T.R.; Carr A.A. *Tetrahedron:Asymmetry* **1990**, *1*, 535-536. (b) Cregge, R.J., Wagner, E.R., Freedman, J. and Margolin, A.L. *J. Org. Chem.* **1990**, *55*, 4237-4238.
6. Delinck, D.L.; Margolin, A.L. *Tetrahedron Lett.* **1990**, *31*, 6797-6798.
7. (a) Dike, S.; Ner, D.H.; Kumar, A. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 383-386. (b) Clark, J.E.; Fischer, P.; Schumacher, D.P. *Synthesis* **1991**, 891-894. (c) Kalaritis, P.; Regenye, R.W.; Partridge, J.J.; Coffen, D. *J. Org. Chem.* **1990**, *55*, 812-815. (d) Hughes, D.L.; Bergan, J.J.; Amato, J.S.; Reider, P.J.; Grabowski, E.J.J. *J. Org. Chem.* **1989**, *54*, 1287-1788. (e) Sih, C.J.; Gu, Qu-M.; Fulling, G.; Wu, S.-H.; Reddy, D.R. *Dev. Ind. Microb.* **1988**, *29*, 221-229. (f) Chenevert, R.; Thiboufot, S. *Synthesis* **1989**, 444-446.
8. (a) Akita, H.; Enoki, Y.; Yamada, H.; Oishi,T. *Chem. Pharm. Bull.* **1989**, *37*, 2876-2878. (b) Hirose, Y.; Kariya, K.; Sasaki, I.; Kurono, Y.; Ebiike, H.; Achiwa, K. *Tetrahedron Lett.* **1992**, *33*, 7157-7160.
9. Carr, A.A.; Kane, J.M.; Hay, D.H.; Schmidt, C.J. U.S. Patent 5,134,149, July 28, **1992**.
10. Lipases from *Pseudomonas* sp. (AK and P) and *Asperguillus niger* (AP and APF12) from Amano as well as porcine pancreatic lipase (Sigma) were not active in this reaction.
11. Crude commercial enzyme (Sigma) was partially purified by precipitation with ammonium sulfate as described (Wu, S.-H.; Guo, Z.-W.; Sih, C.J. J. *Am. Chem. Soc.***1990**, *112*, 1990-1995) dialyzed (100g of crude enzyme gave 230 mL solution) and lyophilized to 250 mg of a solid.
12. Both the solid and aqueous layers were extracted by EtOAc. The combined organic solutions were concentrated to a residue which was purified by flash chromatography. Recrystallization from cyclohexane gives (*R*)-1 as a white solid: mp 113-114°C; 1H NMR ($CDCl_3$) δ 6.7-7.2 (m, 7H, Aryl), 4.63 (d, 1H, J=8.5 Hz, CHO), 3.87 (s, 6H, $OCH_3$'s), 3.1 (m, 1H), 2.9 (m, 1H), 2.7 (m, 2H), 2.5 (m, 3H), 1.8-2.1 (m, 3H), 1.7 (m, 1H), 1.2-1.6 (m,3H); MS (CI, $CH_4$); m/z (rel. intensity) 374 ($MH^+$, 65%), 356 (68), 364 (27), 342 (6), 322 (8), 264 (100), 236 (7); $[\alpha]_D^{20}$ + 14.0° (c 0.49, $CHCl_3$); Anal Calcd for $C_{22}H_{28}FNO_3$ (373.5): C, 70.75; H, 7.56, N, 3.75. Found: C, 70.53; H, 7.73; N, 3.63.
13. After 4 days of incubation the reaction mixtures were centrifuged (5 min at 4000 rpm). Both supernatants and solid supports were extracted individually with EtOAc (30 mL) and analyzed by HPLC to determine the amounts of **2** and **1**.