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ANTIBODY-MEDIATED REGIO- AND ENANTIOSELECTIVE RESOLUTION OF A GLYCEROL DERIVATIVE

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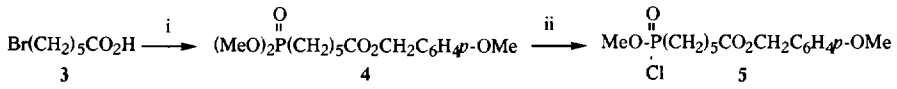
Abstract: The antibody-mediated regio- and enantioselective hydrolysis of a glycerol derivative is reported. (*R*)-2-Acetoxy-1-(3-nitrobenzyloxy)glycerol (80% ee) was obtained from the (*R,S*)-2,3-diacetoxy glycerol derivative in 36% yield at the antibody-catalyzed kinetic resolution step.

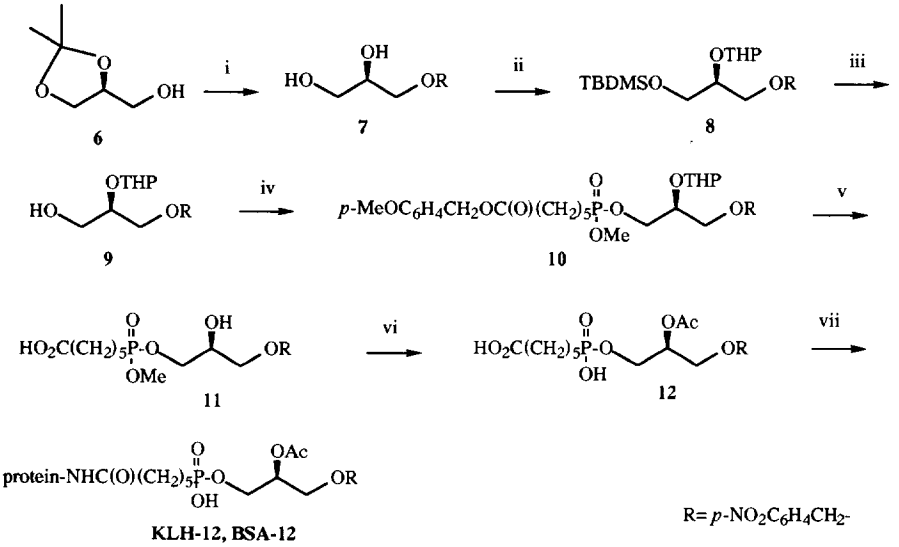
Optically active glycerol derivatives are useful as starting materials for the syntheses of several types of chiral medicines, including (*S*)-propranolol and related compounds, [1] PAF antagonists and their derivatives,[2] 4-amino-3-hydroxybutyric acid (GABOB)[3] as its simple derivatives, lipopeptides[4] and many biologically active compounds.[5] So far many synthetic methods for chiral glycerol derivatives have been established. Although the asymmetric syntheses of glycerol derivatives by enzymatic reactions have been documented,[6] kinetic resolution by an enzyme catalyst is still a useful method for the synthesis of optically active glycerol derivatives.

High levels of regio- and stereoselectivity are one of the greatest advantages of using an antibody catalyst.[7] Chemoselective hydrolysis of glycerol derivatives having several nearly equivalent reaction coordinates is particularly difficult to control. In this paper, we describe the antibody-mediated regio- and enantioselective hydrolysis of the (*R,S*)-2,3-diacetoxy glycerol derivative.

Synthesis of Hapten. We designed haptenic phosphonate transition state analog **12** to generate catalytic antibodies that enantioselectively hydrolyze **(*R,S*)-1** to the alcohol **(*R*)-2**. Enantiomerically pure hapten **12** that possesses a phosphonate group mimicking that the tetrahedral intermediate for ester hydrolysis of a glycerol derivative was prepared in nine steps from (*S*)-2,3-*O*-isopropylidene glycerol **6**, as outlined in Scheme 1. The hydroxyl group of **6** was protected as the *p*-nitrobenzyl group by treatment with *p*-nitrobenzyl bromide, Ag_2O and *t*-butylammonium iodide (TBAI) in 49% yield, and then the isopropylidene group was hydrolyzed by 60% acetic acid to give **7** in 80% yield. The primary hydroxyl group of **7** was selectively protected by *t*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in 81% yield, and then the protection of the remaining hydroxyl group was carried out with dihydropyran (DHP) and PPTS to afford **8** in 92% yield. Treatment of **8** with *t*-butylammonium fluoride (TBAF) gave monoalcohol **9** in 72% yield. Phosphorylation of the hydroxyl group of **9** by **5**, having the five-carbon spacer as the linker in hapten synthesis, was successfully accomplished in the presence of NEt_3 and DMAP to afford phosphonate **10** in 65% yield. Removal of the *p*-methoxybenzyl group and THP groups of **10** were simultaneously carried out with trifluoroacetic acid to give **11** in 79% yield. After acetylation of **11** with Ac_2O and pyridine in 80% yield, treatment with trimethylsilyl bromide gave hapten **12** in 95% yield, FAB-MS (3-NBA matrix) : m/z ($M+H^+$) 448.



Conditions: i) 1) *p*-$MeOC_6H_4CH_2OH$, DCC, DMAP, CH_2Cl_2, 0°C then rt, 15 h (yield 86%), 2) $(MeO)_3P$, *p*-cymene, 170°C, 24 h (yield 74%); ii) $(COCl)_2$, CH_2Cl_2, rt, 18 h (quant.).



Conditions: i) 1) *p*-$NO_2C_6H_4CH_2Br$, Ag_2O, TBAI, MS4A, CH_2Cl_2, rt, 15 h (yield 49%); 2) 60% AcOH, 80-90°C, 2 h (yield 80%); ii) 1) TBDMSCl, imidazole, CH_2Cl_2, rt, 15 h (yield 81%); 2) DHP, PPTS, CH_2Cl_2, rt, 15 h (yield 92%); iii) TBAF, THF, rt, 1 h (yield 72%); iv) **5**, NEt_3, DMAP, 0°C then rt, 15 h CH_2Cl_2 (yield 65%); v) TFA-CH_2Cl_2 (1:10), rt, 2 h (yield 79%); vi) 1) Ac_2O-pyridine (1:2), rt, 24 h (yield 80%), 2) TMSBr, CH_3CN, 40-50°C, 45 h (yield 95%); vii) 1) KLH or BSA,EDC in DMF-H_2O, 2) dialysis, NaCl buffer, pH7.4.

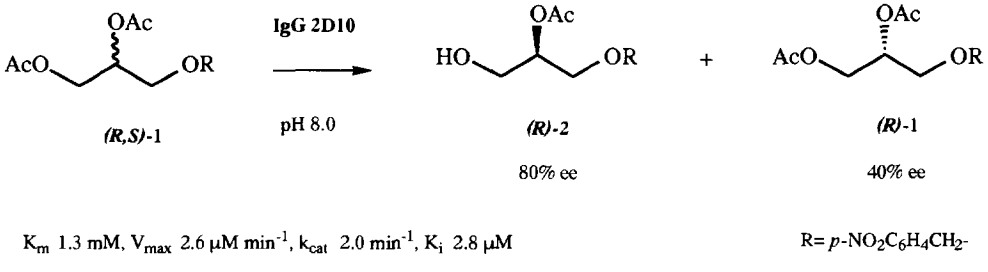
Scheme 1

Antibody Production. Hapten **12** was coupled to the carrier proteins keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA) using a water-soluble carbodiimide (EDC) to provide the corresponding protein conjugates. The precipitates were purified by chromatography on Sephadex G-25. The KLH conjugate was used as an antigen, and the BSA conjugate was used in ELISA experiments for measuring serum titer and hapten affinity. Balb/c mice were immunized with the KLH conjugate of **12**, and hybridomas were prepared from the immunized spleenocytes using standard hybridoma protocols.[8] We obtained five stable hybridoma cell lines that exhibited binding specificity for **BSA-12**. Samples of monoclonal antibodies were prepared by *in vivo* ascites production and purified from ascites fluid to homogenecity by ammonium sulfate precipitation followed by protein G affinity chromatography. The antibodies were dialyzed in PBS at pH 7.4. The homogenecity of each antibody was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie blue staining.

Catalytic Assay and Kinetics. Antibodies that bound to **BSA-12** were screened for the ability to catalyze the hydrolysis of **(*R,S*)-1**. The reaction was performed using 1.3 μM of antibody[9] and 100-750 μM of **(*R,S*)-1** in 10% DMSO/0.2 M Tris (pH 8.0) at 30°C by monitoring production of **(*R*)-2**[10] by reverse-phase high-pressure liquid chromatography (HPLC) and *N*-ethylbenzamide as the internal reference.[11] As a result, three of five antibodies were found to accelerate, over background hydrolysis,[12] the hydrolytic degradation

of **(*R,S*)-1** to the alcohol **(*R*)-2**, and the most effective antibody 2D10 was characterized in further detail. Antibody 2D10 displayed saturation kinetics described by the Michelis-Menten equation in the hydrolysis of **(*R,S*)-1**. The kinetic parameters of 2D10 from the Lineweaver-Burk plot were afforded values of K_m=1.3 mM, V_{max}=2.6 mM min^{-1} and k_{cat}=2.0 min^{-1}, respectively. Furthermore, hydrolysis of **(*R,S*)-1** by 2D10 is competitively inhibited with the addition of chiral phosphonate **12** (K_i= 2.8 μM).

Kinetic Resolution. When the reaction was performed using antibody 2D10 (1.3 μM) and **(*R,S*)-1** (1 mM) in 5% DMSO/0.2 M Tris (pH 8.0) at 4°C, 80% ee of **(*R*)-2** (36% hydrolysis conversion) was obtained. The enantiomeric excess of diester **(*R*)-1** and monoester **(*R*)-2** was measured by HPLC using a chiral column.[13]



(R,S)-1 *(R)*-2 80% ee *(R)*-1 40% ee

K_m 1.3 mM, V_{max} 2.6 μM min^{-1}, k_{cat} 2.0 min^{-1}, K_i 2.8 μM R= *p*-$NO_2C_6H_4CH_2$-

Conclusion. We demonstrated the generation of antibodies that regio- and enantioselectively hydrolyze a glycerol derivative to afford the optically active glycerol derivative. This procedure represents an efficient method for the optical resolution of glycerol derivatives.

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9. Protein concentration was determined by measurement of the absorbance at 280 nm.
10. The enantiomerically pure **(*R*)-2** was prepared from (*R*)-3-*t*-butyldimethylsilyloxy-1-(3-nitrobenzyloxy)glycerol in 2 steps, i) Ac_2O, pyridine, CH_2Cl_2, 93% yield, ii) CF_3CO_2H-H_2O (1:1), 72% yield. **(*R*)-2:** ^1H NMR(270MHz) δ: 2.10 (s, 3H), 3.57-3.66 (m, 2H), 4.08-4.14 (m, 1H), 4.18-4.22 (m, 2H), 4.68 (s, 2H), 7.50, 8.22 (d, *J*=8.6Hz, each 2H). $[\alpha]_D$ + 1.0 °(*c*=0.28, $CHCl_3$).
11. Assay conditions: 100-750 μM **(*R,S*)-1**, 1.3 μM Ab 2D10 in 0.2 M Tris, pH 8.0, 30°C. Product formation was followed by RP-HPLC (YMC ODS A-303, 250x4.6 mm, 254 nm, 0.6 mL/min, H_2O/CH_3CN (40:60), 0.05% CF_3CO_2H, t_R (Internal Reference) = 6.6 min, t_R **((*R,S*)-1)** = 11.9 min, t_R **((*R*)-2)**= 7.4 min. Retention time of (*S*)-3-acetoxy-1-(3-nitrobenzyloxy)glycerol was 5.6 min.
12. The first -order kinetic constant of the background reaction (k_{uncat}) was $8.5x10^{-3}$ min^{-1} (30°C, pH 8.0).
13. The enantioselectivity was measured by HPLC analysis using a column packed with DAICEL CHIRALCEL AD (n-Hexane-IPA=10:1) at 1.0 mL/min. The two enantiomeric products **(*S*)-2** and **(*R*)-2** appeared at 66.9 and 68.6 min, respectively.

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