prepared by the methods reported previously. Other starting materials were commercially available and were purified by standard methods before use.

General Procedure for Cross-Carbonylation of 1 and 2. A mixture of 1 (2.5 mmol), 2 (2 mmol), and $PdCl_2(PPh_3)_2$ (14 mg, 0.02 mmol) in benzene (2.5 mL)–triethylamine (2.5 mL) was added into a 50-mL stainless steel autoclave and carbon monoxide (15 atm, 213 psi at room temperature) was charged. Then, the mixture was magnetically stirred at 120 °C for 18 h. After cooling, the mixture was poured into dilute hydrochloric acid, extracted with ether, washed with water, and dried over magnesium sulfate. The product was isolated by column chromatography on silica gel using hexane–ethyl acetate as eluant.

3-Benzoyl-2-butyl-5-phenylfuran (3): oil; ^1H NMR δ 0.94 (t, 3 H, J = 7.3 Hz), 1.40 (qt, 2 H, J = 7.3, 7.3 Hz), 1.75 (tt, 2 H, J = 7.3, 7.3 Hz), 2.97 (t, 2 H, J = 7.3 Hz), 7.24–7.84 (m, 10 H); ^{13}C NMR δ 13.75, 22.36, 27.70, 30.34, 106.60, 121.92, 123.70, 127.70, 128.34, 128.73, 129.00, 130.07, 132.08, 139.20, 151.44, 162.90, 191.24; MS m/z 304 (M^+). Anal. Calcd for $C_{21}H_{20}O_2$: C, 82.15; H, 6.91. Found: C, 82.31; H, 6.61.

3-Benzoyl-2-ethyl-5-phenylfuran (4): oil; ^1H NMR δ 1.34 (t, 3 H, J = 7.3 Hz), 3.00 (q, 2 H, J = 7.3 Hz), 6.79 (s, 1 H), 6.80–7.85 (m, 10 H); ^{13}C NMR δ 12.33, 21.65, 106.54, 121.50, 123.72, 127.70, 128.34, 128.44, 128.72, 128.97, 132.18, 139.21, 151.46, 163.69, 191.15; MS m/z 276 (M^+). Anal. Calcd for $C_{19}H_{16}O_2$: C, 82.57; H, 5.85. Found: C, 82.47; H, 5.77.

3-Benzoyl-2-ethyl-5-(4-methylphenyl)furan (5): oil; ^1H NMR δ 1.34 (t, 3 H, J = 7.3 Hz), 2.37 (s, 3 H), 2.99 (q, 2 H, J = 7.3 Hz), 6.73 (s, 1 H), 7.19 (d, 2 H, J = 7.8 Hz), 7.46–7.57 (m, 5 H), 7.83–7.86 (m, 2 H); ^{13}C NMR δ 12.38, 21.29, 21.68, 105.82, 121.48, 123.74, 127.39, 128.35, 129.00, 129.43, 132.18, 137.66, 139.30, 151.73, 163.42, 191.34; MS m/z 290 (M^+). Anal. Calcd for $C_{20}H_{18}O_2$: C, 82.72; H, 6.26. Found: C, 82.45; H, 6.32.

2-Butyl-3-(4-methylbenzoyl)-5-phenylfuran (6): oil; ^1H NMR δ 0.93 (t, 3 H, J = 7.3 Hz), 1.40 (qt, 2 H, J = 7.3, 7.3 Hz), 1.74 (tt, 2 H, J = 7.3, 7.3 Hz), 2.44 (s, 3 H), 2.98 (t, 2 H, J = 7.3 Hz), 6.79 (s, 1 H), 7.25–7.40 (m, 5 H), 7.64–7.77 (m, 4 H); ^{13}C NMR δ 13.76, 21.61, 22.38, 27.70, 30.28, 106.62, 122.11, 123.70, 127.63, 128.72, 129.03, 129.23, 130.13, 136.53, 142.95, 151.33, 162.57, 190.99; MS m/z 318 (M^+). Anal. Calcd for $C_{22}H_{22}O_2$: C, 82.97; H, 6.98. Found: C, 82.68; H, 7.06.

2-Butyl-3-(4-chlorobenzoyl)-5-phenylfuran (7): oil; ^1H NMR δ 0.94 (t, 3 H, J = 7.3 Hz), 1.41 (qt, 2 H, J = 7.3, 7.3 Hz), 1.75 (tt, 2 H, J = 7.3, 7.3 Hz), 2.99 (t, 2 H, J = 7.3 Hz), 7.25–7.47 (m, 5 H), 7.64–7.80 (m, 4 H); ^{13}C NMR δ 13.74, 22.37, 27.74, 30.21, 106.18, 121.61, 123.74, 127.82, 128.68, 128.76, 129.89, 130.42, 137.48, 138.56, 151.62, 163.14, 189.87; MS m/z 338 (M^+). Anal. Calcd for $C_{21}H_{19}O_2Cl$: C, 74.43; H, 5.66; Cl, 10.46. Found: C, 74.20; H, 5.67; Cl, 10.60.

2-Ethyl-3-(2-thienylcarbonyl)-5-phenylfuran (8): mp 69–71 °C (from hexane); ^1H NMR δ 1.35 (t, 3 H, J = 7.3 Hz), 3.05 (q, 2 H, J = 7.3 Hz), 6.99 (s, 1 H), 7.17 (dd, 1 H, J = 2.9, 4.9 Hz), 7.25–7.43 (m, 3 H), 7.67–7.79 (m, 4 H); ^{13}C NMR δ 12.42, 21.62, 105.75, 121.18, 123.79, 127.82, 127.90, 128.79, 130.05, 132.85, 133.30, 145.05, 151.73, 163.65, 182.10; MS m/z 282 (M^+). Anal. Calcd for $C_{17}H_{14}O_2S$: C, 72.30; H, 5.01; S, 11.36. Found: C, 72.28; H, 4.96; S, 11.11.

Rearrangement of 2a. (a) The alkynone **2a** (200 mg, 1 mmol) in triethylamine (3 mL) was heated under nitrogen in a 50-mL stainless steel autoclave at 120 °C for 18 h. After evaporation of the solvent, the product mixture was chromatographed on silica gel using hexane–ethyl acetate as eluant. The furan **9** (49 mg, 25%) was obtained from the first fraction as an oil: ^1H NMR δ 0.94 (t, 3 H, J = 7.3 Hz), 1.41 (qt, 2 H, J = 7.3 Hz), 1.67 (tt, 2 H, J = 7.3 Hz), 2.68 (t, 2 H, J = 7.3 Hz), 6.06 (d, 1 H, J = 3.2 Hz), 6.54 (d, 1 H, J = 3.2 Hz), 7.18–7.25 (m, 1 H), 7.33–7.37 (m, 2 H), 7.61–7.71 (m, 2 H); ^{13}C NMR δ 13.82, 22.28, 27.87, 30.23, 105.61, 106.80, 123.31, 126.67, 128.55, 131.27, 152.07, 156.45; MS m/z 200 (M^+). The second fraction contained **2a** (88 mg, 45%).

(b) A mixture of **2a** (400 mg, 2 mmol) and $PdCl_2(PPh_3)_2$ (14 mg, 0.02 mmol) in benzene (2.5 mL)–triethylamine (2.5 mL) was heated in a 50-mL stainless steel autoclave under carbon monoxide (15 atm, 213 psi at room temperature) at 120 °C for 18 h. Formation of **9** (27 mg, 7%) and **10** (58 mg, 15%) was confirmed by GC and GC-MS analyses. An authentic sample of **10** was prepared by treatment of **2a** (2 mmol) with $RuCl_2(PPh_3)_3$ (20 mg, 0.02 mmol) in refluxing acetonitrile (5 mL) for 15 h.[20]

1-Phenyl-(2*E*,4*E*)-octa-2,4-dien-1-one (10):[21] oil; ^1H NMR δ 0.93 (t, 3 H, J = 7.3 Hz), 1.48 (qt, 2 H, J = 7.3, 7.3 Hz), 2.21 (t, 2 H, J = 7.3 Hz), 6.21–6.35 (m, 2 H), 6.87 (d, 1 H, J = 15.1 Hz), 7.52–7.56 (m, 4 H), 7.92–7.94 (m, 2 H); ^{13}C NMR δ 13.66, 21.89, 35.20, 123.56, 128.32, 128.47, 129.26, 132.46, 138.29, 145.41, 146.31, 190.91; MS m/z 200 (M^+).

Reaction of the Furan 3 or 4 with Hydrazine. A mixture of **3** or **4** (1 mmol) and hydrazine hydrate (1.0 g) in ethylene glycol (2 mL) was heated at 150 °C for 2 h. After workup, the corresponding pyrazole was isolated by column chromatography on silica gel using hexane–ethyl acetate as eluant. The pyrazoles **16** and **17** may, at least in solution, exist as mixture of annular tautomers, as usual N-unsubstituted pyrazoles:[22] The C_3 and C_5 carbons in their ^{13}C NMR spectra showed more than two peaks (very week), respectively. Probably, there are also aggregates.[22]

4-(Benzoylmethyl)-3(5)-butyl-5(3)-phenylpyrazole (16): mp 144–146 °C (from hexane–benzene); ^1H NMR δ 0.90 (t, 3 H, J = 7.3 Hz), 1.36 (qt, 2 H, J = 7.3, 7.3 Hz), 1.62 (tt, 2 H, J = 7.3, 7.3 Hz), 2.57 (t, 2 H, J = 7.3 Hz), 4.20 (s, 2 H), 7.34–7.45 (m, 8 H), 7.54–7.57 (m, 2 H); ^{13}C NMR 13.78, 22.50, 25.40, 30.81, 34.06, 107.99, 127.93, 128.00, 128.24, 128.57, 128.68, 132.22, 133.09, 136.58, 197.31, (C_3 and C_5 128.30, 128.48, 147.66, 147.73); MS m/z 318 (M^+). Anal. Calcd for $C_{21}H_{22}N_2O$: C, 79.20; H, 6.98; N, 8.80. Found: C, 79.62; H, 7.00; N, 8.56.

4-(Benzoylmethyl)-3(5)-ethyl-5(3)-phenylpyrazole (17): mp 119–120 °C (from hexane–benzene); ^1H NMR δ 1.25 (t, 3 H, J = 7.3 Hz), 2.60 (q, 2 H, J = 7.3 Hz), 4.20 (s, 2 H), 7.34–7.45 (m, 8 H), 7.54–7.56 (m, 1 H), 7.92–7.94 (m, 2 H); ^{13}C NMR δ 13.02, 18.93, 34.03, 107.60, 127.95, 128.00, 128.24, 128.55, 128.64, 132.27, 133.08, 136.53, 197.35, (C_3 and C_5 128.12, 128.15, 128.33, 128.41, 147.69, 147.72, 148.72); MS m/z 290 (M^+). Anal. Calcd for $C_{19}H_{18}N_2O$: C, 78.58; H, 6.26; N, 9.65. Found: C, 78.20; H, 6.19; N, 9.55.

Registry No. **1a**, 591-50-4; **1b**, 624-31-7; **1c**, 637-87-6; **1d**, 1003-09-4; **2a**, 27259-10-5; **2b**, 65236-43-3; **2c**, 108462-79-9; **3**, 142395-76-4; **4**, 142395-77-5; **5**, 142395-78-6; **6**, 142395-79-7; **7**, 142395-80-0; **8**, 142395-81-1; **9**, 80866-26-8; **10**, 96412-99-6; **16**, 142395-82-2; **17**, 142395-83-3; $PdCl_2(PPh_3)_2$, 13965-03-2.

(19) Tohda, Y.; Sonogashira, K.; Hagihara, N. *Synthesis* **1977**, 777.

(20) Inoue, Y.; Imaizumi, S. *J. Mol. Catal.* **1988**, *49*, L19.

(21) Bestmsnn, H. J.; Schmidt, M.; Schobert, R. *Synthesis* **1988**, 49.

(22) Elguero, J. *Pyrazoles and Their Benzo Derivatives, in Comprehensive Heterocyclic Chemistry*; Potts, K. T., Ed.; Pergamon Press: Oxford, 1984; Vol. 4, p 167.

Specificity of Antibody-Catalyzed Transesterifications Using Enol Esters: A Comparison with Lipase Reactions

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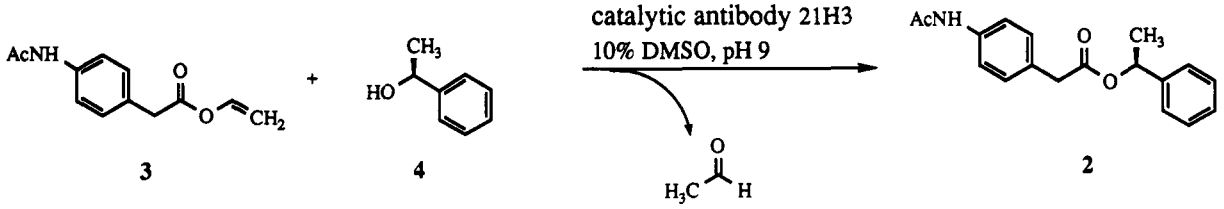
The rapidly growing field of catalytic antibodies has become an effective approach to catalyst design.[1] Since the active site of a catalytic antibody is induced by the designed hapten, the substrate specificity and stereoselectivity of antibody catalysis are therefore expected to be

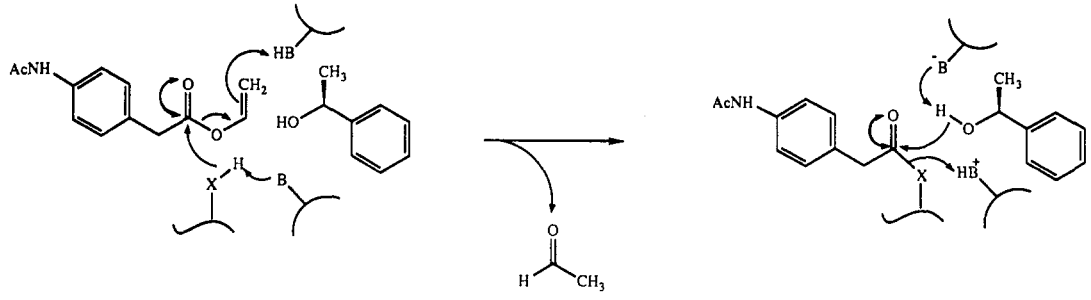
(1) Lerner, R. A.; Benkovic, S. J.; Schultz, P. G. *Science* **1991**, *252*, 659 and references cited therein.

0022-3263/92/1957-4756$03.00/0

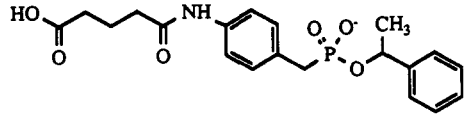
Scheme I



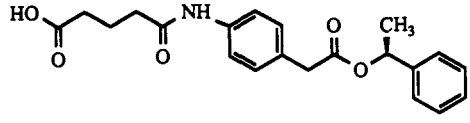
Scheme II. Proposed Induced-Fit Mechanism for 21H3-Catalyzed Transesterification



predictable and programmable. A monoclonal antibody (21H3)[2,3] raised against phosphonate 1, for example, not



1



2

only catalyzes the expected hydrolysis of the ester substrate 2 (k_{cat} = 0.09 min^{-1}) but also the acyl transfer in water from enol ester 3 to (*S*)-4 (k_{cat} = 6.4 min^{-1}) (Scheme I) and some analogous aromatic alcohols.[2,3] The latter reaction occurs via an induced-fit mechanism; i.e., the antibody would not accelerate the hydrolysis of 3 unless a nonalcohol compound structurally similar to (*S*)-4 is present.[3] The reaction was believed to proceed through a covalent intermediate[2,3] as evidenced by the burst kinetic study, the competition analysis, the pH–rate titration study and the ping-pong kinetic behavior. A proposed mechanism for the transesterification is shown in Scheme II where a general base and a general acid along with an unidentified group for the possible formation of an acyl enzyme intermediate are indicated. With the use of free 21H3, no transesterification was observed in anhydrous solvents such as dichloromethane. In contrast, lipase-catalyzed transesterifications only occur in the absence of water.[4] To further probe the specificity of 21H3, some enol esters and alcohols structurally related to (*S*)-4 were synthesized and tested as substrates for transesterification. Since 21H3 was not previously used in preparative synthesis (only HPLC analyses were carried out),[2,3] the preparation of some optically active esters based on 21H3-catalyzed transesterifications were included in this report. The results were compared with lipase reactions with the use of some selected substrates.

Results and Discussion

Racemic (trifluoromethyl)phenylcarbinol (5) reacted with 3 faster than racemic 4 in the presence of 21H3 under the same condition (10% DMSO, pH 9.0). Its enantiomers were subsequently explored. While (*S*)-5 gave no acceleration compared to (*S*)-4, (*R*)-5, which is the geometrical equivalent of (*S*)-4, reacted 1.4-fold faster than (*S*)-4 with 3 (Table I). The increase of rate for (*R*)-5 is not clear. Several other alcohols were also tested as substrates, and the results are shown in Table I. Apparently the antibody accepts 6-membered-ring aromatic alcohols. Furan or cyclohexyl derivatives structurally related to 4 are poorly accepted. Another interesting finding is that nitrogen can replace C in the aromatic ring at the position next to the branch. A ring with two nitrogens or with the N in position 3 or 4 is, however, poorly accepted. In all cases, the stereochemistry of acceptable alcohol substrate is the same as that of 4. The antibody is very selective for the enol ester. Compounds 17 and 18 are not acceptable for 21H3.

The antibody-catalyzed transesterification was then carried out in aqueous dimethyl sulfoxide (DMSO) solution to prepare some esters on mg scales. The esters of (*S*)-4 and (*S*)-6 were prepared from the corresponding racemates in 90% ee. The ester of (*R*)-5 was, however, obtained in 50% ee (Scheme III) after 40% conversion of 1 equiv of *rac*-5. Further studies indicated that a competing 21H3-catalyzed hydrolysis of the ester products occurred during the transesterification reactions. Although the enantioselectivity in the antibody reaction with (*R*)- vs (*S*)-5 is very high (~30), the ester products formed are sensitive to the antibody-catalyzed hydrolysis and the (*R*) vs (*S*) selectivity in the hydrolysis is also high. The product ee, therefore, is lower than expected in the preparative synthesis.

The antibody reaction of 3 with 12 (Scheme IV) was selective for the (*R*) enantiomer, and surprisingly the ester of the primary alcohol was isolated. If the diol 12 is placed

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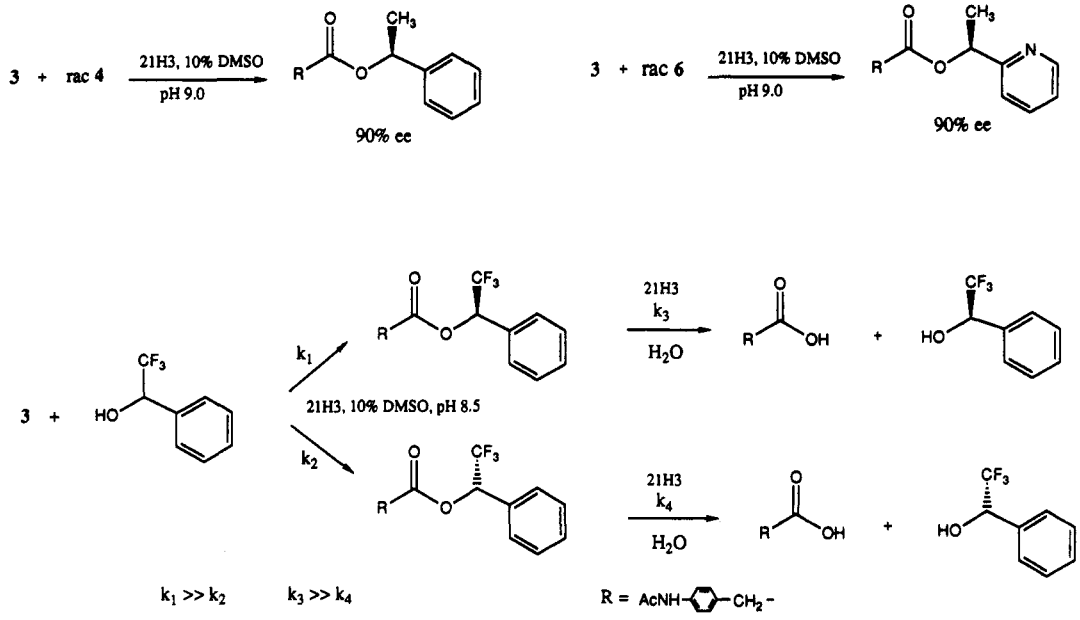
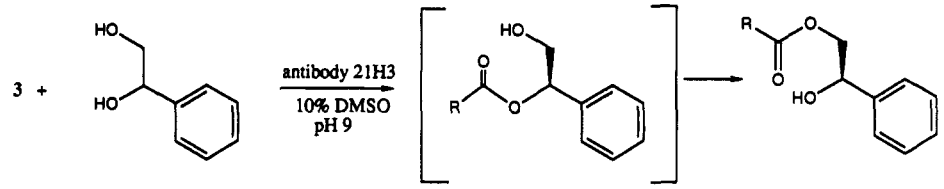
(3) Wirsching, P.; Ashley, J. A.; Benkovic, S. J.; Janda, K. D.; Lerner, R. A. *Science* **1991**, *252*, 680.

(4) Laumen, K.; Schneider, P. *J. Chem. Soc., Chem. Commun.* **1988**, 598.

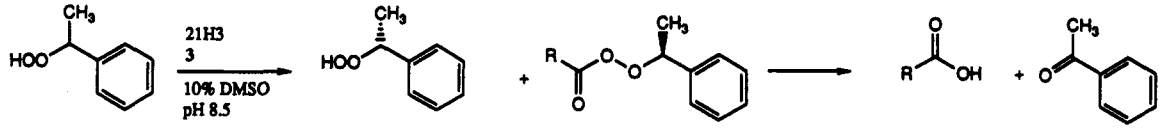
Scheme III



Scheme IV



Scheme V

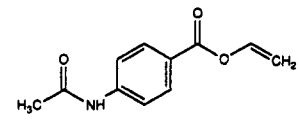


R = AcNH-C6H4-CH2-

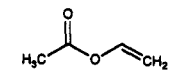
in the active site as is 4, the ester bond should be formed at the secondary alcohol group of (*R*)-12. It is possible that the secondary alcohol ester is formed first followed by an acyl migration to the primary position. Further supports of this argument come from the reactions with (*S*)-12, 13, and 15. Compound 13 is a much better substrate than 15 and (*S*)-12, indicating the orientations of the phenyl and the nucleophilic hydroxyl groups in the active site are the same as that of (*S*)-4. The acyl migration was also observed in a separate NMR study of the synthetic 2-*O*-acyl-(*R*)-12, as evidenced by the upfield shift of the benzylic proton.

The hydroperoxide 14 is also a good substrate for the antibody-catalyzed transesterification (Scheme V), and as predicted, the (*S*)-enantiomer[5] is a preferred substrate. The ester product, however, could not be isolated because it decomposes to acetophenone spontaneously.

We then turned our attention to lipase-catalyzed transesterifications. Enol esters 3 and 17 are not substrates for lipase SAM II in aqueous solution or in CH_2Cl_2. Vinyl acetate (18) was then used as a substrate for transesterifications. All reactions were carried out in anhydrous CH_2Cl_2 because in aqueous solution the lipase only catalyzes the hydrolysis of 18.



17



18

Consistent with previous lipase reactions,[4,5] the (*R*)-enantiomers of compounds 4 and 14 were selectively acetylated, and the acetate of (*R*)-4 and unreacted (*S*)-14 were obtained in >98% ee and 74% ee, respectively, at 50% conversion. Compound 6 was also selectively resolved via the lipase reaction. The fluorocompound (*R*)- or (*S*)-5 was, however, not a substrate for the lipase in CH_2Cl_2,[4] presumably due to the weak nucleophilicity of the OH group in CH_2Cl_2. Comparison of the kinetic data for the lipase-catalyzed reactions and 21H3-catalyzed reactions with 3, it was found that 21H3 in aqueous solution is as effective as lipase SAM II in CH_2Cl_2 (Table II). It is interesting that the antibody reaction was only effective in aqueous solution while the lipase reaction must be carried out in organic solvents.

In summary, this study suggests that an induced antibody for hydrolysis can take a vinyl ester of the acyl

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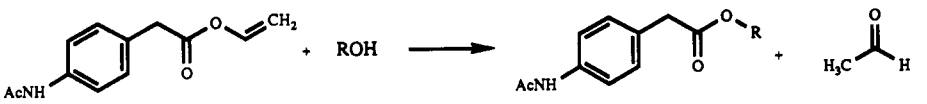
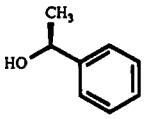
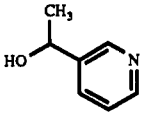
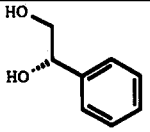
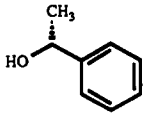
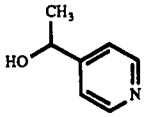
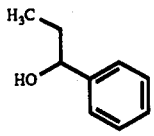
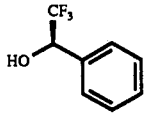
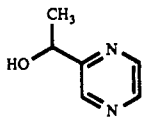
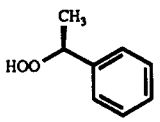
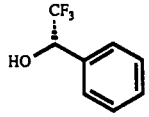
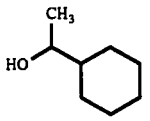
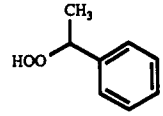
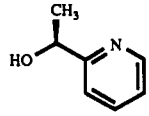
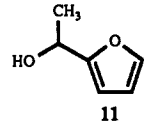
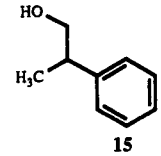
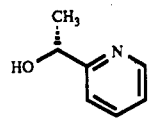
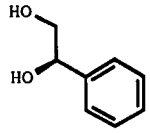
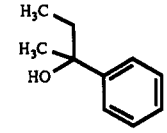
Table I. Substrates Tested for the Transesterification of Enol Ester 3 in Aqueous Solution and Their Relative Velocities Compared to (*S*)-4 Measured at 1 mM Enol Ester and 2 mM Alcohol. The Values in Parentheses Were Determined with 1 mM Enol Ester and 1 mM Alcohol



ROH:	V_{rel}	ROH:	V_{rel}	ROH:	V_{rel}
 (S)-4	100 (100)	 7	< 5 (< 5)	 (S)-12	<5
 (R)-4	2(Lit)	 8	< 5 (< 5)	 13	33
 (R)-5	141 (194)	 9	10 (< 5)	 (S)-14	118
 (S)-5	<5 (<5)	 10	< 5 (< 5)	 14	90
 (S)-6	53 (105)	 11	15 (< 5)	 15	<5
 (R)-6	7 (<5)	 (R)-12	122	 16	<5

component related to the hapten and a broad spectrum of alcohols structurally related to the alcohol portion of the designed substrates for transesterification in aqueous solution with very high stereoselectivity. This study further illustrates the advantages of using enol esters as acyl transfer reagents[6] in enzyme and antibody-catalyzed reactions. The reactions are very effective and can be performed in aqueous or organic solvents. The irreversible nature of the reaction improves the enantioselectivity, whereas in a reversible transesterification, the reverse reaction tends to reduce the enantiomeric excess of the desired products due to the principle of microscopic reversibility. Another advantage is that the released acetaldehyde does not inactivate the catalyst, is easy to remove, and would not cause product inhibition as observed with other esters such as trifluoroethyl acetate (Table II). One disadvantage is that the enol ester is not stable in aqueous solution and must be added in several portions to the reaction mixture.

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Experimental Methods

Preparation of Enol Ester 3. A mixture of 496.5 mg (2.570 mmol) of *N*-acetyl-4-aminophenylacetic acid,[2] 30.0 mg (0.9414 mmol) of $Hg(OAc)_2$, and 4 μL of concd H_2SO_4 in 5 mL vinyl acetate was refluxed for 6 h under an inert atmosphere. After the reaction was quenched with NaOAc the solvent was removed under reduced pressure and the residue was dried under vacuum. Finally, the mixture was purified via silica gel chromatography (CH_2Cl_2/AcOEt = 10/1) to give 424.5 mg (75%) of **3**. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.18 (s, 3 H, CH_3); 3.66 (s, 2 H, CH_2); 4.59 (dd, 1 H, cis-H at CH_2, ^3J = 6.3 Hz, ^2J = 1.6 Hz); 4.90 (dd, 1 H, trans-H at CH_2, ^3J = 13.9 Hz, ^2J = 1.6 Hz); 7.16 (br, s, 1 H, NH); 7.23–7.30 (m, 3 H, CH of vinyl and H-3 and H-5 of aromatic ring); 7.48 (d, 2 H, H-2 and H-6 of aromatic ring). Anal. Calcd for $C_{12}H_{13}O_3N$: C, 66.06; H, 5.96; N, 5.96. Found: C, 66.04; H, 5.94; N, 5.95.

Preparation of the Alcohols. All racemic alcohols except 1-phenyl-1,2-ethanediol (Aldrich) were prepared via reduction

Table II. Comparison of Antibody 21H3 and Lipase SAM II Catalyzed Transesterifications

catalyst	ester	alcohol	solvent[a]	k_{cat}, min^{-1}	K_m ester	K_m alcohol	K_i
antibody[b]	3	(S)-4	10% DMSO in aqueous soln, pH 9	6.4	3.0 mM	7.3 mM	no[c]
lipase	18	(R)-4	CH_2Cl_2	6.2	0.40 M	0.18 M	no[c]
lipase	$CH_3CO_2CH_2CF_3$	(R)-4	CH_2Cl_2	1.2	0.10 M	0.34 M	75 mM[d]

[a] See Experimental Section for details. [b] Data taken from ref 3. [c] The released acetaldehyde is not an inhibitor. In the lipase reaction with 1.33 M 18 and 0.33 M *rac*-4, it proceeded smoothly until 50% of the alcohol was consumed and the isolated ester was >98% ee. [d] A product inhibition caused by the released trifluoroethanol was encountered. Under the same conditions as *c* with the use of this ester instead of 18, the reaction stopped at 25% conversion of the alcohol, and the isolated ester was >98% ee.

of the appropriate keto compound with $NaBH_4$ in methanol at room temperature. The reaction time ranged between 2 and 12 h, and the yields were 75–99%.

(*S*)- and (*R*)-1-Phenylethanol were from Sigma. (*S*)- and (*R*)-1-phenyl-1,2-ethanediol were from Aldrich. The buffer used in the antibody reaction was ATE (0.1 M Aces, 0.052 M Tris, 0.052 M ethanolamine, pH 9.0) or BICINE (100 mM, pH 8.5).

(*R*)-2,2,2-Trifluoro-1-phenylethanol ((*R*)-**5** 93% ee) was prepared from trifluoroacetophenone via reduction with the NADPH-dependent alcohol dehydrogenase from *Thermoanaerobium brockii* (TADH, from Sigma) according to the procedure described previously.[7] The (*S*)-enantiomer was prepared in >98% ee using the NAD-dependent alcohol dehydrogenase from *Pseudomonas* sp. PEP.[8] (*R*)-1-(2-Pyridyl)-ethanol (>97% ee) was prepared similarly from the corresponding ketone using the NADP-dependent alcohol dehydrogenase from *L. kefir*.[9] In all cases, the reduced cofactor was regenerated in the presence of 10% isopropanol.

(*S*)-1-(2-Pyridyl)ethanol ((*S*)-**6**) was prepared from the corresponding racemic alcohol via lipase-catalyzed resolution. To a solution of *rac*-1-(2-pyridyl)ethanol (166 mg, 1.35 mmol) and 1 mL of vinyl acetate in 3 mL of CH_2Cl_2 was added lipase SAM II (30 mg, Amano Pharmaceutical Co.), and the mixture was stirred for 12 h at room temperature. At this point 60% of the starting material was consumed. The enzyme was filtered off and washed with ethyl acetate. The solvent was then removed, and the products were separated via silica gel chromatography (hexane/EtOAc (2:1–1:1)) to give the ester of (*R*)-**6** and unreacted alcohol (*S*)-**6**. Alcohol: The ^{1}H-NMR spectrum was identical to that described above, $[\alpha]^{23}_D = -51.4$ (*c* = 2.63, EtOH), 92% ee compared to the value reported.[10]

Lipase PS-800 was also used for this reaction. After 60% conversion, (*S*)-**6** was obtained in 90% ee and the ester of (*R*)-**6** was obtained in 60% ee.

Experiments with Antibody 21H3-Catalyzed Transesterifications. All substrates were first screened via UV-assay to determine the relative rate (relative to (*S*)-1-phenylethanol). The reactions were typically carried out as follows: In a 1-mL UV cell were mixed together 846 μL of a solution of 2.5 mg of β-NADH and 19.4 mg of alcohol dehydrogenase (ADH, from Bakers yeast, EC 1.1.1.1, 195 units/mg solid, 220 units/mg protein, Sigma) in 100 mM BICINE-buffer, pH 8.5, 20 μL of a 100 mM solution of the appropriate alcohol in DMSO, 70 μL of DMSO and 54 μL of 21H3 solution in PBS, pH 7.5 (concn 11.9 mg/mL), and the mixture was equilibrated for 10 min. The reaction was then started by the addition of 10 μL of a 100 mM solution of enol ester **3** in DMSO, mixed, and put into a Beckman DU-70 spectrophotometer equipped with an automatic cell changer. The reaction was followed over 40 min, and the decrease of absorbance at 340 nm (ϵ_{NADH} = 6.22 mM^{-1} cm^{-1}) was recorded. The initial rates were calculated for the first 4 min. A control reaction was performed simultaneously with 900 μL of the NADH/ADH solution in BICINE, 20 μL of a 100 mM solution of the same alcohol in DMSO, 70 μL of DMSO and 10 μL of a 100 mM solution of enol ester **3** in DMSO.

The formation of the ester was verified by HPLC-analysis on a Gilson-HPLC system interfaced with an IBM-computer with an analytical reversed-phase C-18 column (Scientific Glass Engineering PTY.LTD (SGE)). The flow rate was 0.5 mL/min: solvent A, dd-water with 0.1% TFA; solvent B, acetonitrile (HPLC-grade; Fisher) with 0.1% TFA. For the assays the following solvent program was used: 0–14 min, 50% solvent B to 90% solvent B; 14–16 min, 90% solvent B to 50% B. Typically, a benzophenone solution in CH_3CN–0.1% TFA/H_2O–0.1% TFA (1:1) was added to the samples as an internal standard. The retention time for enol ester **3** was 7.2 min and that for benzophenone was 13.6 min.

Antibody 21H3-Catalyzed Transesterification with *rac*-1-Phenylethanol. To 16.3 mL of ATE buffer in a Centriprep-100 concentrator (Amicon) was added 1.2 mL of antibody 21H3 solution in PBS (11.5 mg/mL), 0.15 mL of a 400 mM solution of *rac*-phenylethanol in DMSO, and 1.65 mL of DMSO. While being gently stirred, a solution of 400 mM enol ester **3** in DMSO was added in intervals: 30 μL at the start, 30 μL after 10 min, 60 μL after 15 min, 60 μL after 25 min, 60 μL after 35 min, and 60 μL after 45 min, totaling 300 μL. The reaction mixture became cloudy because of precipitation. After 5 h the reaction mixture was filtered, and the filtrate was saturated with NaCl and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, and after evaporation of the solvent the crude product was purified by preparative TLC (AcOEt/*n*-hexane = 1/1) to yield 4.8 mg of ester **2** (28% yield). The ee was measured to be 92% via HPLC on a Chiralcel OC column (Daicel Chemical Industries, Ltd.) (70:30 hexane/2-propanol; flow rate, 0.3 mL/min; retention time: 30 min for (*R*)-enantiomer and 40 min for (*S*)-enantiomer, as compared to the synthetic (*S*)-ester and the rac ester). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.50 (d, 3 H, *J* = 6.6 Hz, CH_3 (alcohol)), 2.18 (s, 3 H, Ac); 3.59 (d, 2 H, *J* = 1.6 Hz, methylene protons), 5.87 (g, 1 H, *J* = 6.6 Hz, CH of alcohol); 7.11 (s, 1 H, NH), 7.21 and 7.43 (AA′, BB′, 4 H, *J* = 8.4 Hz, aromatic protons of acid moiety), 7.24–7.31 (m, 5 H, aromatic protons of alcohol moiety). HRFABMS (M + Cs^+) for $C_{18}H_{19}NO_3$: expected, 430.0419; observed, 430.0406. Anal. Calcd: C, 72.97; H, 6.42; N, 4.39. Found: C, 72.90; H, 6.40; N, 4.40.

Antibody-Catalyzed Transesterification with *rac*-1-Phenyl-2,2,2-Trifluoroethanol. To a stirring solution of 6.768 mL of 100 mM BICINE buffer (pH 8.5) was added 432 μL of antibody 21H3 in PBS buffer (11.5 mg/mL), 160 μL of DMSO, and 50 μL of a 400 mM solution of the alcohol in DMSO. Enol ester **3** in DMSO (400 mM, 100 μL) was then added in 20-μL portions every 10 min to the mixture. The reaction mixture was first clear, later opaque. After 7 h, the antibody was spun off (Centriprep-100, Amicon, 500 g) and the supernatant was saturated with NaCl and extracted with AcOEt. After drying over $MgSO_4$ the organic layer was evaporated and the product purified by preparative TLC (EtOAc/*n*-hexane = 1/1) to yield 1.9 mg (30% yield) of the ester of (*R*)-**5** with 50% ee (determined by comparing with synthetic rac ester and enantiomerically enriched (*R*)- and (*S*)-enantiomers) on an analytical Chiralcel OC column (Daicel Chemical Industries, Ltd.) with hexane/2-propanol (6/4) at a flow rate of 0.2 mL/min. Retention times: (*S*)-enantiomer, 36 min; (*R*)-enantiomer, 42 min. The same ee value was found after reaction for 30 min, and with a concentration of 2 mM alcohol and 1 mM enol ester. When the reaction was run for 18 h in BICINE buffer (2 mM alcohol, 1 mM enol ester) the (*S*)-enantiomer was formed as a major product indicating the competing hydrolysis reaction of the ester products. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.18 (s, 3 H, Ac), 3.73 (d, 2 H, *J* = 3.6 Hz, methylene protons of acid moiety), 6.12 (q, 1 H, *J* = 6.9 Hz, CH of alcohol moiety), 7.13 (s, 1 H, NH), 7.22 and 7.47 (AA′, BB′, 4 H, aromatic

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protons acid moiety), 7.26–7.40 (m, 5 H, aromatic protons of alcohol moiety). The NMR spectrum was identical to an authentic sample prepared from (*R*)-**5** and (*S*)-**5**. HRFABMS (M + Cs^+) for $C_{18}H_{16}O_3NF_3$: expected, 484.0137; observed, 484.0137. Anal. Calcd: C, 61.71; H, 4.57; N, 3.71. Found: C, 61.75; H, 4.50; N, 3.70. The ester showed no racemization in ATE or BICINE buffer.

Antibody-Catalyzed Transesterification with 1-(2-Pyridyl)ethanol. To 180 μL of a 100 mM solution of enol ester **3** in DMSO was added a solution containing 7.5 mL of BICINE, 0.5 mL of the antibody solution, and 0.36 mL of the alcohol solution in DMSO (100 mM) and 0.4 mL of DMSO. The reaction progress was monitored by TLC (EtOAc/MeOH = 10/1). After 6 h the reaction mixture was extracted with ethyl acetate. The aqueous layer was saturated with NaCl and again extracted with ethyl acetate. The combined organic layer was dried with $MgSO_4$ and after evaporation of the solvent the product was isolated by preparative TLC (EtOAc/MeOH = 10/1) to yield 1.5 mg (30% yield) of the desired product. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.58 (d, 3 H, J = 6.6 Hz, CH_3 of alcohol moiety); 2.18 (s, 3 H, Ac); 3.66 (s, 2 H, benzylic protons of acid moiety); 5.92 (q, 1 H, C-H alcohol); 7.12 (s, 1 H, NH); 7.17–7.23 (m, 6 H, H-3, H-5 phenyl, H-3, H-4 pyridyl); 7.45 (d, 2 H, J = 9.5 Hz, H-2, H-6 phenyl); 7.63 (dd, 1 H, J = 8.8 Hz, J = 6.3, H-5 pyridyl); 8.57 (d, 1 H, J = 5.7 Hz, H-6 pyridyl). HRFABMS (M + Cs^+) for $C_{17}H_{18}N_2O_3$: expected, 431.0372; observed, 431.0372. Anal. Calcd: C, 68.92; H, 6.08; N, 8.78. Found: C, 68.89; H, 6.04; N, 8.77. The enantiomeric excess was determined to be 92% by HPLC as described above.

Preparation and Test of Enol Esters 17 and 18. Enol ester **17** was prepared in 74% yield, using the same procedure described for **3**. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.23 (s, 3 H, Ac); 4.69 (dd, 1 H, H-2 of vinyl: J(H-2, H-2′) = 1.6 Hz, J(H-2, H-1) = 6.3 Hz); 5.06 (dd, 1 H, H′-2 of vinyl: J(H-2′, H-2) = 1.6 Hz, J(H-2′, H-1) = 14 Hz); 7.27 (s, 1 H, NH); 7.50 (dd, 1 H, H-1 vinyl, J(H-1, H-2) = 6.3 Hz, J(H-1, H′-2) = 13.8 Hz); 7.63 (d, 2 H, J = 8.7 Hz, H-2 and H-6 of aromatic moiety), 8.07 (d, 2 H, H-3 and H-5 of aromatic moiety, J = 8.8 Hz). To test **17** as a substrate for antibody 21H3, 846 μL of ATE buffer, 54 μL of 21H3 solution in PBS (11.9 mg/mL), 20 μL of 100 mM solution of (*S*)-1-phenylethanol in DMSO, and 70 μL of DMSO were mixed and equilibrated over 10 min. The reaction was then started by adding 10 μL of 100 mM enol ester **17** in DMSO and followed by TLC and HPLC. No formation of the ester was observed. The enol ester **17** is about twice as stable as enol ester **3** ($t_{1/2}$ = 20 h at pH 8.5 and 16 h at pH 9, 25 °C). The same conditions were used to test **18** (10 μL of a 100 mM solution of vinyl acetate in DMSO were added). No transesterification or hydrolysis was observed.

Hydrolysis of Enol Ester 3 with Lipase SAM II and with 21H3. A mixture containing 850 μL of ATE, 50 μL of a solution containing 3.2 mg of SAM II in 600 μL of ATE, 90 μL of DMSO, and 10 μL of a 100 mM solution of enol ester **3** in DMSO was stirred gently at room temperature. Samples of 50 μL of the reaction solution were taken and mixed with 50 μL of a 0.15 mM solution of benzophenone in CH_3CN and analyzed by HPLC. No enzymatic hydrolysis was found. The same result was obtained with 21H3.

Attempted Lipase-Catalyzed Transesterification of Racemic 1-Phenylethanol with Enol Ester 3. A mixture containing 1.33 M of enol ester **3**, 0.33 M of 1-phenylethanol, and 3.33 mg/mL of lipase in CH_2Cl_2 was stirred for 3 days, and the reaction was monitored by TLC and/or HPLC. Nine lipases were tested for this reaction in CH_2Cl_2 (lipase SAM II, lipase PS-800, *Candida cylindracea* lipase, lipase MAP 10 from *Mucor* species, lipase N, *porcine pancreatic* lipase, lipase P, lipase AP 12 (*Aspergillus niger*), lipase FAP (*Rhizopus japonics*). None of them showed transesterification.

Attempted Transesterification Using Antibody 21H3 in CH_2Cl_2. A mixture containing 920 μL of CH_2Cl_2, 20 μL of 100 mM (*S*)-1-phenylethanol in CH_2Cl_2, and 10 μL of 100 mM enol ester **3** in CH_2Cl_2 (enol ester and alcohol can be mixed together) was added 54 μL of 21H3 (11.9 mg/mL in PBS). The reaction was followed by TLC and HPLC. No transesterification took place after 5 days.

Kinetics. For the antibody-catalyzed transesterifications, the initial rates were determined by following the formation of acetaldehyde or the ester product according to the reported procedures.[3] For lipase-catalyzed reactions, K_m and V_{max} were determined from Lineweaver–Burke plots and K_i was determined from a Dixon plot.

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Solvolytic Kinetic Studies by ^{19}F NMR

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Over the years we and others have employed the trifluoroacetate leaving group in a number of solvolytic kinetic studies. In terms of reactivity, the trifluoroacetate leaving group lies between the commonly used *p*-nitrobenzoate and tosylate leaving groups.[1] It is comparable to chloride in leaving-group ability. One of the main advantages of this leaving group lies in the ease of preparation of trifluoroacetates by reaction of alcohols with trifluoroacetic anhydride in the presence of amine bases. We have used gas chromatographic methods[2] for measuring rates, as well as spectrophotometric kinetic methods.[3] Conductometric methods have also been used to measure rates,[4,5] as well as titrimetric methods.[1,6] During the course of solvolytic studies, we have encountered systems where rates could not be easily determined by these kinetic methods. We now report a simple method for determination of solvolytic rates of trifluoroacetates ($ROCOCF_3$), as well as triflates ($ROSO_2CF_3$) and triflones (RSO_2CF_3), by ^{19}F NMR spectroscopy.

Since titrimetric methods for determination of rate constants are not useful in the commonly used solvent acetic acid, and gas chromatographic methods are unsuccessful for thermally unstable trifluoroacetates, we have turned our attention to ^{19}F NMR spectroscopy as a simple kinetic method. We have found that solvolytic rates for a variety of substrates (**1**–**8**) can be determined in a variety of solvents using this method. The ionic trifluoroacetate ion usually has a significantly different chemical shift than the covalent trifluoroacetate. Shift differences range from a relatively small value of 0.028 ppm (7.9 Hz) for **5** to 0.713 ppm for **1**. This allows facile determination of rate constants. Another advantage of this method lies in the sensitivity of modern Fourier transform spectrometers which allows rates to be determined using very small quantities (typically 5 mg or less) of substrate. Deuterated solvents are not necessary since spectra can be recorded in the unlocked mode. Figure 1 shows typical data for cumyl trifluoroacetate, **1**, in methanol and the corresponding first-order kinetic plot for this relatively reactive substrate. Such high correlations (r > 0.9997) are routine

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