Supplementary Material (ESI) for Chemical Communications

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Superparamagnetic nanoparticle-supported enzymatic resolution of racemic carboxylates

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Materials and Instrumentation. Chemicals and the lipase were purchased from Acros Organics (Somerville, NJ), and Sigma (St. Louis, MO) and used as received without further purification. Water was obtained from a Milli-Q reagent water system purchased from Millipore Corporation (Milford, MA). ^{1}H NMR and ^{13}C data were obtained on a Varain VXR-300 system with an Oxford wide-bore magnet and the chemical shifts were reported in parts per million (ppm) downfield relative to tetramethylsilane using the residual proton resonance of solvents as the references (^{1}H NMR): $CDCl_3$ δ 7.27; CD_2Cl_2 δ 5.32 and (^{13}C NMR): $CDCl_3$ δ 77.2; CD_2Cl_2 δ 54.0. TEM measurements were accomplished with a Hitachi H7100FA operating at an accelerating voltage of 75 or 100kV. Iron oxide nanoparticle specimens were deposited onto Formvar- and silicon-coated, 200 mesh copper grids (Structure Probe, Inc., West Chester, PA) and the edge of the grid touched to a filter paper to wick away most of the solvent. Images were recorded either on conventional photographic films or captured using a Gatan 789 digital camera. Magnification was calibrated using a MAG*I*CAL high resolution magnification standard accurated to 1 x 10E6 X. The permanent magnet (LifeSep^{TM} 50sx magnetic separator) was purchased from Dexter Magnetic Technologies (Elk Grove Village, IL). Gas chromatography (GC) analyses were performed on a Varian 3900 GC system installed with a thermo-conductive detector and a Zebron ZB-5 capillary GC column of a 0.25 mm ID. Chiral GC analyses using a RT-BetaDEXsm chiral column from Restek Coporation (Bellefonte, PA) were also utilized for determining the e.e. values of the products. A typical GC analysis program used a temperature gradient with an initial column temperature of 60 ^{o}C, which was gradually increased to a temperature of 180 ^{o}C in 30 min followed by a 15 ^{o}C/min increase to the final temperature of 230 ^{o}C. Optical rotation measurements were carried out by using an automatic polarimeter from Rudolph Research (Flaners, NJ). This program was successfully tested for resolving racemic 2-bromopropionic acid. The absolute configurations and e.e. values of the products were determined by comparing our measurements with those reported in the literature.[1]

Immobilization of Lipase to Maghemite Nanocrystals.

Maghemite nanocrystals of 11 nm with narrow size distributions were prepared by following the literature procedures.[2,3] To 200 mg of these nanoparticles in 10 mL of $CHCl_3$ was

added excessive 3-aminopropyltriethoxysilane (2 mL). After 12 h at refluxing temperature, the solution was cooled down to ambient temperature. Magnetic nanoparticles were magnetically concentrated via using an external permanent magnet (LifeSep^{TM} 50sx magnetic separator). The concentrated nanoparticles were washed with 5 x 20 mL $CHCl_3$ and air-dried.

The aforementioned amino-functionalized maghemite nanoparticles were added into a 3 mL phosphate buffer solution (pH 7.4) containing 1 mL of 50 wt% glutaraldehyde. After 12 h at room temperature, magnetic nanoparticles were magnetically concentrated and washed with phosphate buffer 4 x 10 mL. Such nanocomposites were transferred into a phosphate buffer solution (5 mL) with 100 mg of lipase (from *candida rugosa*). After 24 h, nanoparticles were magnetically concentrated and washed with phosphate buffer 3 x 20 mL. The amount of the enzyme immobilized onto nanoparticles were determined by measuring the protein concentrations before and after incubation. A standard BCA assay was adopted for determining solution protein concentrations.[4]

Immobilization of Lipase onto Solid-Phase Resins.

A mixture of chloromethylated polystyrene resins (200 mg, 200-400 mesh) and 50 wt% glutaraldehyde (1 mL) in 3 mL of phosphate buffer (pH 7.4) was stirred at room temperature. After 12 h, resins were separated from solution via filtration. The solid beads were washed with phosphate buffer 5 x 20 mL and transferred into a phosphate solution (pH 7.4) containing 100 mg of lipase (from *candida rugosa*). After 24 h, the resins were isolated via filtration and washed with the buffer 5 x 20 mL. The amount of the lipase immobilized onto the resins was determined to be 1.2 μg per mg of resins.

Nanoparticle-Supported Enzymatic Resolution of Racemic Mixtures.

In a typical experiment, a racemic carboxylate (0.65 mmol) was mixed with excessive *n*-butanol (240 mg, 3.2 mmol) and nanoparticle-supported lipase (30 mg) in hexanes (10 ml). The reaction was maintained at 30 ^{o}C in an incubation shaker. For enzyme stability studies, the catalytic reactions were stopped after seven days by magnetically removing nanoparticles out of reaction solvents. The remaining mixtures in solution were analyzed by a Varian 3900 GC system using thermal conductivity detector (the column temperature was maintained at 100 ^{o}C for 10 min and then was gradually increased to 320 ^{o}C in 11 min). The carboxylate could then be separated from the ester by washing the organic solvent with 0.5 M $NaHCO_3$ aqueous solution. Removal of hexanes *in vacuo* led to the *n*-hexyl ester. The carboxylate could then by recovered by adjusting the pH of the aqueous medium to 1 followed by extracting with CH_2Cl_2 (3 x 30 mL). The combined CH_2Cl_2 solutions were dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo* to yield the carboxylate residue.

Resin-Supported Enzymatic Resolution of Racemic Mixtures

The resin-supported resolution of racemic carboxylates was carried out similarly by following the aforementioned procedures. Usually 225 mg of resins were employed. Isolation of resins was achieved by filtration instead. The isolated esters and carboxylates were subjected to ^{1}H NMR and mass spectrometry analyses. The reaction progress was monitored by GC analyses.

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