Electronic Supplementary Information (ESI)

Novel Immobilization Method of Enzymes Using a Hydrophilic Polymer Support

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Experimental Section

Preparation of copolymer 1: 4-Vinyl benzyl tetraethyleneglycol ether (2.33 g, 7.5 mmol), 4-vinyl benzyl glycidyl ether (1.43 g, 7.5 mmol), V-70 [2,2'-azobis(2,4-dimethyl-4-methoxyvarelonitrile)] (69.5 mg, 0.225 mmol) were mixed in chloroform (4.0 mL). The mixture was stirred for 48 h at 45 ˚C and then cooled to room temperature. The resulting polymer solution was poured slowly into diethyl ether. The supernatant was removed and the precipitated polymer was washed with diethyl ether several times. The polymer was dissolved in dichloromethane and again poured into diethyl ether. This procedure was conducted three times and the material obtained was dried under reduced pressure to afford the desired copolymer (**1**, 2.91 g, 77% yield). The molar ratio of the components was determined by ^{1}H NMR analysis (x/y = 54/46). *Mw*: 29,168, *Mn*: 14,023, *Mw*/*Mn* = 2.08 (gel permeation chromatography)

Preparation of polymer-immobilized lipase (Scheme 1): Polymer **1** (720 mg) was dissolved in dichloromethane (30 mL) at room temperature, and lipase *candida antarctica* (Lipozyme CALB L (liquid type), Novozymes Japan Co. Ltd.) was then added with stirring vigorously to make it dispersed. After dispersion (1 hour), hexane was slowly added and a precipitate was formed. The solvents were removed and the residue was washed with hexane several times. The residue was treated with amine **2** in hexane (100 mL) and heated at 60 ˚C for 12 hours. The solvent was removed by decantation and the residue was washed with dichloromethane and diethyl ether (or ethyl acetate) and dried *in vacuo*, giving polymer-immobilized lipase. The polymer-immobilized lipase thus prepared was used for two batches of the following kinetic resolution (242 mg: for 1 reaction).

Determination of the loading: The protein amount in liquid lipase (Lipozome CALB L) was estimated by Lowry's method (18.8 mg protein/100 mg). The supernatant organic washing solutions used during immobilization process were combined and extracted with water by using a centrifugal separator. The amount of protein in the water solution was estimated by Lowry's method. The loading of protein in polymer-immobilized lipase was estimated by subtracting this amount from the amount of protein contained in liquid lipase originally used (72.5 mg protein/g).

Kinetic resolution using polymer-immobilized lipase (Tables 1, 2): A typical experiment procedure is described for kinetic resolution of phenylethyl alcohol. Polymer-immobilized lipase (242 mg), racemic 1-phenylethyl alcohol (61.1 mg, 0.5 mmol) and vinyl acetate (215 mg, 2.5 mmol) were combined in diethyl ether (3 mL), and the mixture was stirred at 25 ˚C for 24 hours. After completion of the reaction, the organic liquid phase was collected by decantation washing with dichloromethane and diethyl ether several times. The combined organic layers were concentrated and the residue was purified by preparative thin layer chromatography on silica gel to give the acetylated alcohol and the recovered alcohol as well. Enantimeric excess was determined by chiral HPLC analysis. The polymer-immobilized lipase that remained in the flask was dried *in vacuo* and reused for the next trial.

(1*R*)-1-Phenylethyl acetate:[1] ^{1}H NMR ($CDCl_3$) δ = 1.50 (d, 3H, *J* = 5.9 Hz), 2.07 (s, 3H), 5.88 (q, 1H, *J* = 6.4 Hz), 7.25-7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ = 21.3, 22.2, 72.3, 126.1, 127.8, 128.5, 141.6, 170.3. $[\alpha]_D^{20}$ +96.7 (*c* 1.74, $CHCl_3$). HPLC (CHIRALCEL OB, ^{i}PrOH/hexane = 1/400). t_R = 13.4 (*R*) and 15.6 (*S*) min.

(1*S*)-1-Phenylethyl alcohol:[1] ^{1}H NMR ($CDCl_3$) δ = 1.54 (d, 3H, *J* = 7.3 Hz), 1.87 (s, 1H), 4.89 (q, 1H, *J* = 6.4 Hz), 7.25-7.39 (m, 5H); ^{13}C NMR ($CDCl_3$) δ = 25.1, 70.4, 125.3, 127.4, 128.5, 145.8. $[\alpha]_D^{20}$ -52.9 (*c* 1.30, $CHCl_3$). HPLC (CHIRALCEL OB, ^{i}PrOH/hexane = 1/9). t_R = 6.1 (*S*) and 8.1 (*R*) min.

1-(1-Naphthyl)ethyl acetate:[1] ^{1}H NMR ($CDCl_3$) δ = 1.70 (d, 3H, *J* = 6.4 Hz), 2.12 (s, 3H), 6.65 (q, 1H, *J* = 6.4 Hz), 7.45-7.61 (m, 4H), 7.80 (d, 1H, *J* = 8.2 Hz), 7.87 (d, 1H, *J* = 8.2 Hz), 8.08 (d, 1H, *J* = 8.2 Hz); ^{13}C NMR ($CDCl_3$) δ = 21.4, 21.7, 69.4, 123.1, 123.2, 125.3, 125.6, 126.3, 128.4, 128.9, 130.2, 133.8, 137.4, 170.3. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/9). t_R = 5.1 and 6.1 min.

α-Methyl-1-naphthalenemethanol:[2] ^{1}H NMR ($CDCl_3$) δ = 1.67 (d, 3H, *J* = 6.4 Hz), 1.89 (s, 1H), 5.68 (q, 1H, *J* = 6.4 Hz), 7.46-7.55 (m, 3H), 7.68 (d, 1H, *J* = 7.3 Hz), 7.78 (d, 1H, *J* = 8.7 Hz), 7.88 (d, 1H, *J* = 7.8 Hz), 8.12 (d, 1H, *J* = 8.2 Hz); ^{13}C NMR ($CDCl_3$) δ = 24.3, 67.1, 122.0, 123.1, 125.51, 125.53, 126.0, 127.9, 128.9, 130.3, 133.8, 141.3. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/9). t_R = 9.5 and 15.5 min.

1-(2-Naphthyl)ethyl acetate:[3] ^{1}H NMR ($CDCl_3$) δ = 1.62 (d, 3H, *J* = 6.9 Hz), 2.10 (s, 3H), 6.05 (q, 1H, *J* = 6.4 Hz), 7.45-7.50 (m, 3H), 7.80-7.85 (m, 4H); ^{13}C NMR ($CDCl_3$) δ = 21.4, 22.2, 72.4, 124.1, 125.0, 126.0, 126.2, 127.6, 128.0, 128.3, 133.0, 133.1, 139.0, 170.3. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/30). t_R = 6.2 and 7.3 min.

α-Methyl-2-naphthalenemethanol:[2] ^{1}H NMR ($CDCl_3$) δ = 1.58 (d, 3H, *J* = 6.4 Hz), 1.86 (s, 1H), 5.07 (q, 1H, *J* = 6.4 Hz), 7.44-7.52 (m, 3H), 7.81-7.85 (m, 4H); ^{13}C NMR ($CDCl_3$) δ = 25.1, 70.5, 92.5, 123.8, 125.8, 126.1, 127.7, 128.3, 129.8, 132.9, 133.3, 143.2.

α-Methyl-4-biphenylmethanol acetate:[4] δ = 1.58 (d, 3H, *J* = 6.4 Hz), 2.10 (s, 3H), 5.93 (q, 1H, *J* = 6.4 Hz), 7.33-7.46 (m, 5H), 7.58 (d, 4H, *J* = 7.8 Hz); ^{13}C NMR ($CDCl_3$) δ = 21.4, 22.1, 72.1, 126.6, 127.1, 127.3, 127.3, 128.8, 140.7, 140.8, 140.9, 170.4. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/400). t_R = 20.0 and 28.6 min.

α-Methyl-4-biphenylmethanol:[2] ^{1}H NMR ($CDCl_3$) δ = 1.54 (d, 3H, *J* = 6.4 Hz), 1.83 (s, 1H), 4.96 (q, 1H, *J* = 6.4 Hz), 7.33-7.46 (m, 5H), 7.59 (d, 4H, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$) δ = 25.1, 70.2, 125.8, 127.1, 127.2, 128.7, 140.5, 140.8, 144.8. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/30). t_R = 20.2 and 22.0 min.

1,2,3,4-Tetrahydro-1-naphthalenol acetate:[5] ^{1}H NMR ($CDCl_3$) δ = 1.78-2.01 (m, 4H), 2.02 (s, 3H), 2.71-2.90 (m, 2H), 6.00 (t, 1H, *J* = 8.7 Hz), 7.12-7.28 (m, 4H); ^{13}C NMR ($CDCl_3$) δ = 18.8, 21.5, 29.0, 29.1, 70.0, 126.1, 128.1, 129.1, 129.4, 134.5, 137.9, 170.8. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/100). t_R = 5.8 and 6.2 min.

1,2,3,4-Tetrahydro-1-naphthalenol: ^{1}H NMR ($CDCl_3$) δ = 1.66 (s, 1H), 1.74-2.01 (m, 4H) 2.69-2.86 (m, 2H), 4.78 (t, 1H, *J* = 9.2 Hz), 7.09-7.44 (m, 4H); ^{13}C NMR ($CDCl_3$) δ = 18.8, 29.2, 32.3, 68.2, 126.2, 127.6, 128.6, 129.0, 137.1, 138.8. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/150). t_R = 24.4 and 26.4 min.

Indanyl acetate:[6] ^{1}H NMR ($CDCl_3$) δ = 2.06 (s, 3H), 2.04-2.13 (m, 1H), 2.45-2.54 (m, 1H), 2.84-2.91 (m, 1H), 3.07-3.15 (m, 1H), 6.19 (m, 1H), 7.20-7.42 (m, 4H); ^{13}C NMR ($CDCl_3$) δ = 21.3, 30.2, 32.3, 78.3, 124.8, 125.6, 126.7, 128.9, 141.0, 144.4, 171.1. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/400). t_R = 9.2 and 10.2 min.

Indanol:[6] ^{1}H NMR ($CDCl_3$) δ = 1.84-1.92 (m, 1H), 2.38-2.47 (m, 1H), 2.71-2.79 (m, 1H), 2.96-3.03 (m, 1H), 5.18 (t, 1H, *J* = 6.0 Hz), 7.16-7.36 (m, 4H); ^{13}C NMR ($CDCl_3$) δ = 29.8, 36.0, 76.5, 124.2, 124.9, 126.7, 128.3, 143.3, 145.0.

1-(4-Methoxyphenyl)ethyl acetate:[6] ^{1}H NMR ($CDCl_3$) δ = 1.51 (d, 3H, *J* = 6.4 Hz), 2.04 (s, 3H), 3.80 (s, 3H), 5.84 (q, 1H, *J* = 6.4 Hz), 6.87 (d, 2H, *J* = 8.7 Hz), 7.29 (d, 2H, *J* = 8.7 Hz); ^{13}C NMR ($CDCl_3$) δ = 21.4, 21.9, 55.3, 72.0, 113.8, 127.6, 133.7, 159.2, 170.4. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/400). t_R = 15.7 and 16.8 min.

1-(4-Methoxyphenyl)ethanol:[6] ^{1}H NMR ($CDCl_3$) δ = 1.48 (d, 3H, *J* = 6.4 Hz), 3.80 (s, 3H), 4.85 (q, 1H, *J* = 6.4 Hz), 6.88 (d, 2H, *J* = 8.3 Hz), 7.30 (d, 2H, *J* = 8.7 Hz); ^{13}C NMR ($CDCl_3$) δ = 25.0, 55.3, 70.0, 113.8, 126.6, 138.0, 159.0.

1-(4-Bromophenyl)ethyl acetate:[7] ^{1}H NMR ($CDCl_3$) δ = 1.51 (d, 3H, *J* = 6.4 Hz), 2.07 (s, 3H), 5.82 (q, 1H, *J* = 6.8 Hz), 7.22 (d, 2H, *J* = 8.3 Hz), 7.47 (d, 2H, *J* = 8.2 Hz); ^{13}C NMR ($CDCl_3$) δ = 21.3, 22.1, 71. 6, 76.6, 121.7, 127.8, 131.6, 140.7, 170.2. HPLC (CHIRALCEL AD, ^{i}PrOH/hexane = 1/19). t_R = 8.8 and 10.0 min.

1-(4-Bromophenyl)ethanol:[8] ^{1}H NMR ($CDCl_3$) δ = 1.46 (d, 3H, *J* = 6.4 Hz), 4.86 (q, 1H, *J* = 6.4 Hz), 7.24 (d, 2H, *J* = 8.2 Hz), 7.46 (d, 2H, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$) δ = 25.2, 69.8, 121.1, 127.1, 131.5, 144.7.

1-Phenyl-1-propyl acetate:[6] ^{1}H NMR ($CDCl_3$) δ = 0.88 (t, 3H, *J* = 7.8 Hz), 1.73-1.97 (m, 2H), 2.07 (s, 3H), 5.66 (t, 1H, *J* = 6.9 Hz), 7.20-7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ = 9.9, 21.3, 29.3, 126.6, 127.8, 128.4, 140.5, 170.4. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/400). t_R = 8.5 and 10.0 min.

1-Phenyl-1-propanol:[6] ^{1}H NMR ($CDCl_3$) δ = 0.92 (t, 3H, *J* = 7.3 Hz), 1.70-1.88 (m, 2H), 4.59 (t, 1H, *J* = 6.9 Hz), 7.25-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ = 10.1, 31.9, 76.0, 126.0, 127.5, 128.4, 144.6.

2-Acetoxy-4-phenyl-but-3-ene:[9] ^{1}H NMR ($CDCl_3$) δ = 1.39 (d, 3H, *J* = 6.4 Hz), 2.06 (s, 3H), 5.51 (m, 1H), 6.17 (dd, 1H, *J* = 16.0, 6.9 Hz), 6.17 (d, 1H, *J* = 15.6 Hz), 7.23-7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ = 20.4, 21.4, 71.0, 126.6, 127.9, 128.6, 128.8, 131.5, 136.3, 170.4. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/400). t_R = 14.7 and 16.0 min.

***trans*-4-Phenyl-3-buten-2-ol:**[9] ^{1}H NMR ($CDCl_3$) δ = 1.37 (d, 3H, *J* = 6.4 Hz), 4.48 (q, 1H, *J* = 6.4 Hz), 6.26 (dd, 1H, *J* = 15.6, 6.0 Hz), 6.57 (d, 1H, *J* = 15.6 Hz), 7.22-7.39 (m, 5H); ^{13}C NMR ($CDCl_3$) δ = 23.4, 68.9, 126.4, 127.6, 128.6, 129.4, 133.5, 136.7. HPLC (CHIRALCEL OD-H, ^{i}PrOH/hexane = 1/19). t_R = 13.8 and 23.0 min.

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