A Single Antibody Catalyzes Multiple Chemical Transformations upon Replacement of the Functionalized Small Nonprotein Components

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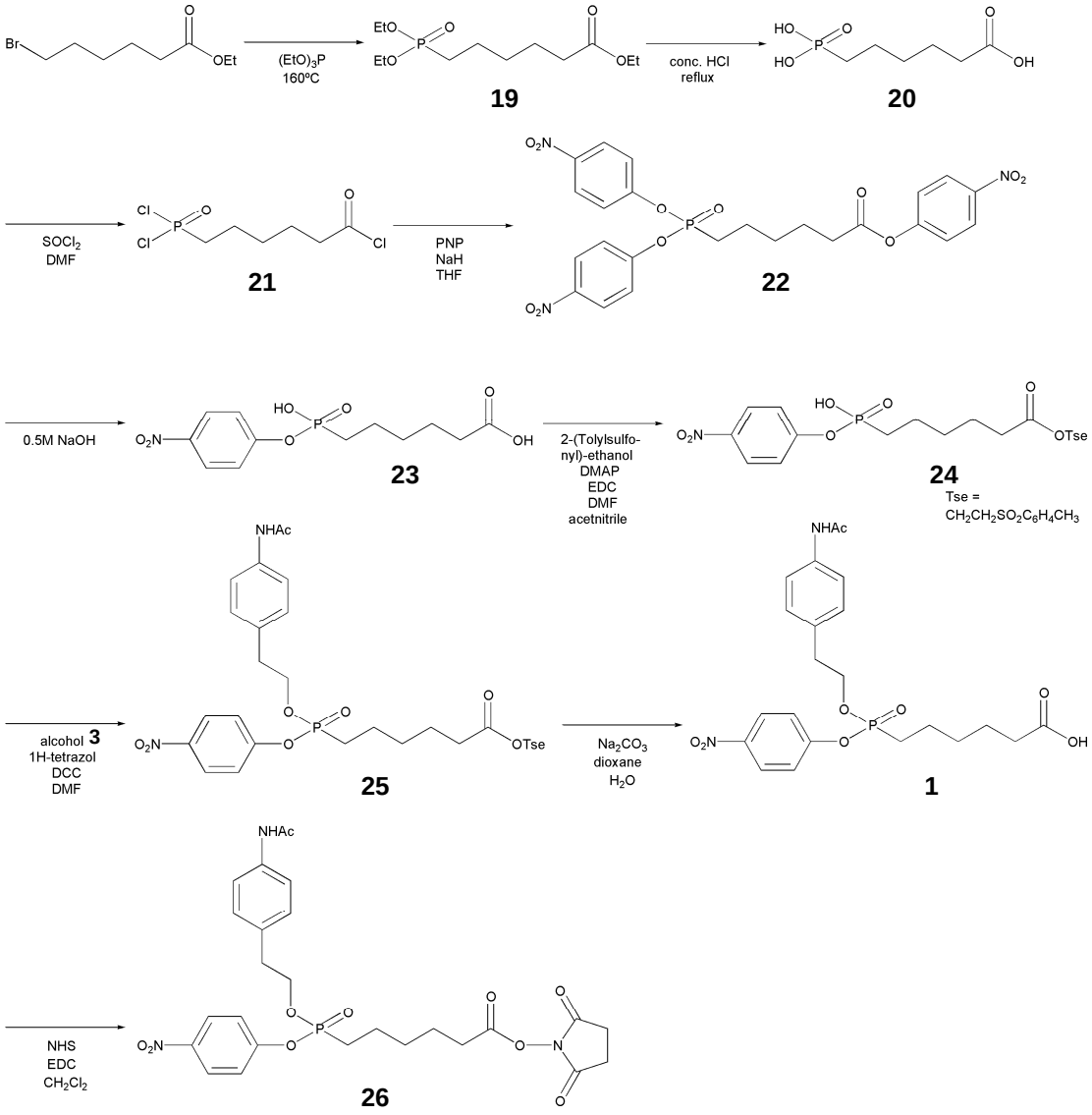
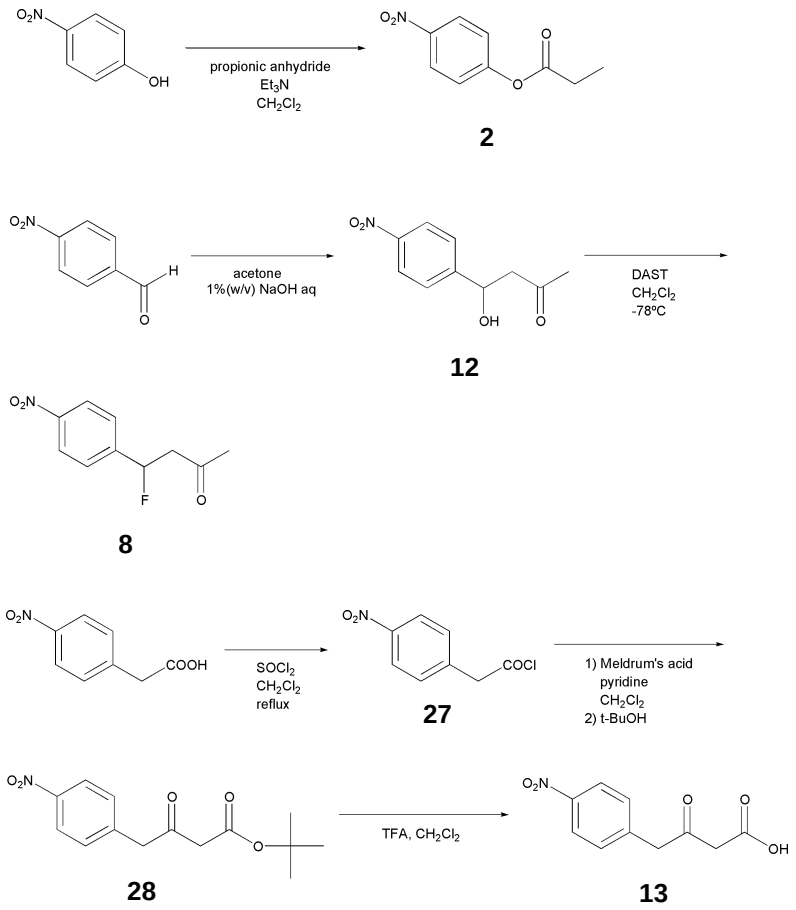
General Methods (synthesis)

All oxygen- or moisture-sensitive reactions were carried out under N_2. Analytical and preparative thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ plates (Merck). Flash chromatography was performed on silica gel 60 (230-400 mesh) (Merck). High-performance liquid chromatography (HPLC) was performed on a Hitachi L2130 equipped with an L2400 UV detector. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-AL400 NMR spectrometer. The spectra were reported in δ downfield from tetramethylsilane. Mass spectra were obtained on a JEOL MS700 mass spectrometer.

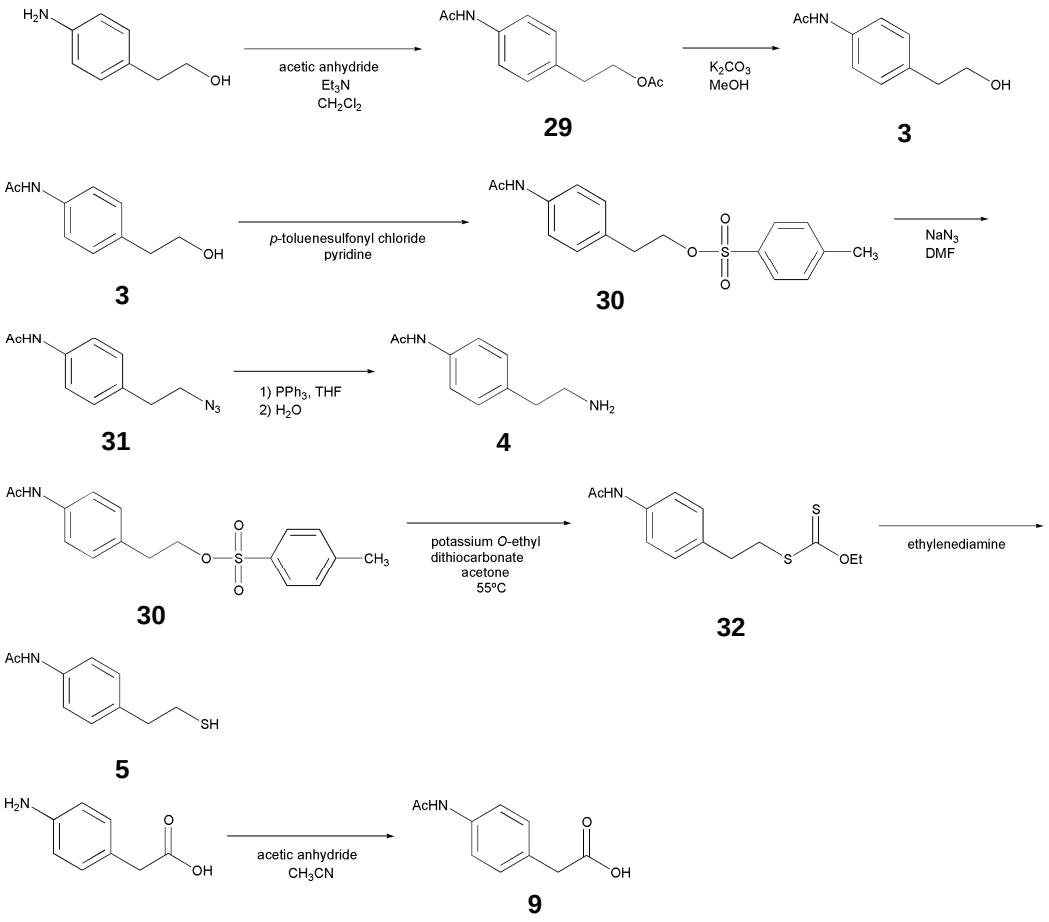
Schematic Summary of Synthesis Operations



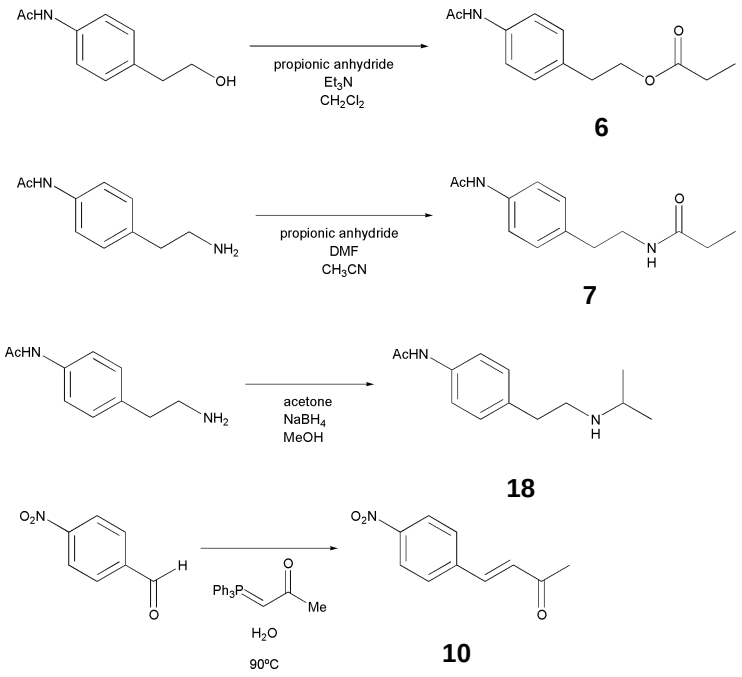
Scheme S1. Synthesis of hapten **1**



Scheme S2. Synthesis of substrates

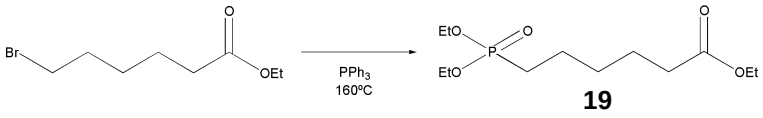


Scheme S3. Synthesis of cofactors



Scheme S4. Synthesis of products

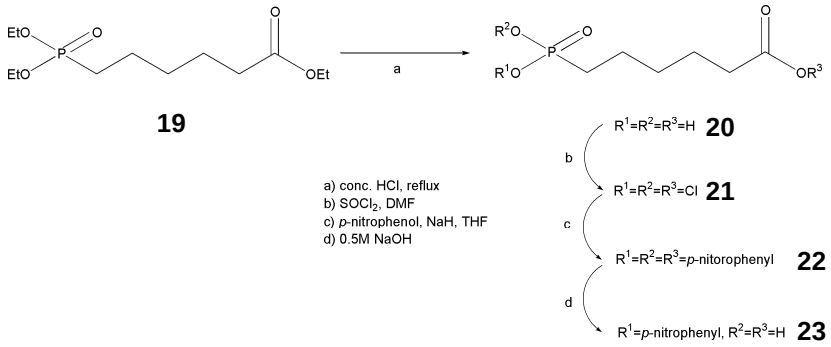
Synthesis of Hapten



19

6-(Diethoxyphosphoryl) hexanoic acid ethyl ester (19)

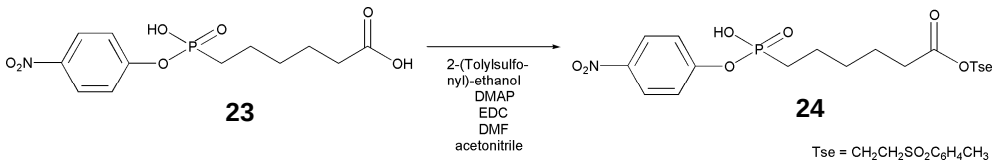
A mixture of ethyl 6-bromohexanoate (9.1 mL, 51.2 mmol) and triethyl phosphate (26.4 mL, 153.6 mmol) was stirred at 160℃ for 14 h. The resulting product was distilled under reduced pressure to afford the desired product **19** (110~135℃/3.1 mmHg, 16.1 g, quant.) as a colorless liquid. ^{1}H-NMR (400 MHz, CD_3OD) : δ 4.11~4.06 (m, 6H), 2.31 (d, *J*=7.3 Hz, 2H), 1.81~1.74 (m, 2H), 1.64~1.55 (m, 4H), 1.45~1.41 (m, 2H), 1.33~1.22 (m, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 171.4, 59.9 (J_{cp}=6.6 Hz), 58.6, 32.6, 28.7 (J_{cp}=16.5 Hz), 24.2 (J_{cp}=139 Hz), 23.2 20.9 (J_{cp}=5.0 Hz), 15.2 (J_{cp}=5.8 Hz), 13.0; HRMS (FAB^+) : $[M+H]^+$ calcd for $C_{12}H_{26}O_5P$, 281.1518; found, 281.1479.



19

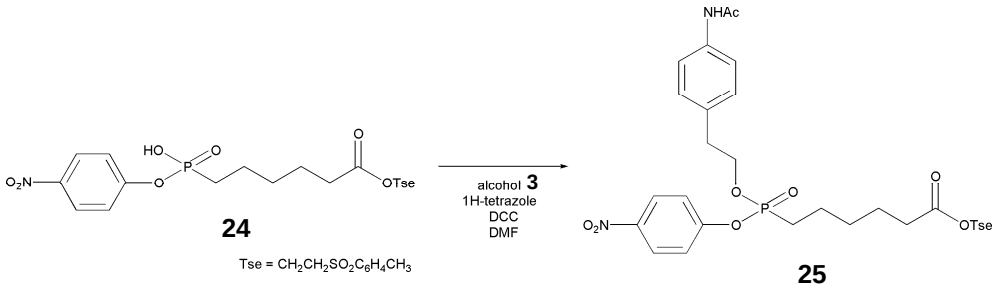
6-[Hydroxy (4-nitro-phenoxy) phosphoryl] hexanoic acid (23)

A mixture of compound **19** (6.00 g, 21.4 mmol) and conc. HCl (120 mL) was stirred under reflux for 15 h. After removal of the solvent, the residue was washed with dioxane (20 mL) and evaporated to dryness. This process was repeated twice. Finally, the solid was washed with toluene (20 mL) and concentrated in vacuo. This process was repeated twice. The crude compound **20** was obtained as a yellow solid (4.20 g). The crude diacid **20** (2.00 g, 10.2 mmol) was suspended in $SOCl_2$ (3 mL, 40.8 mmol) with stirring and DMF (200 μL) was added as catalyst, and the mixture was stirred at 55℃ for 13 h. After cooling, excess $SOCl_2$ was removed in vacuo. The crude compound **21** was obtained as a green oil (2.57 g). To *p*-nitrophenol (5.67 g, 40.8 mmol) in THF (40 mL) was added NaH (60%, dispersion in paraffin liquid 1.67 g (0.98 g), 40.8 mmol) with stirring at room temperature. Stirring was continued until evolution of gas (H_2) ceased. To the mixture was added a solution of the crude trichloride **21** (2.57 g, 10.2 mmol) in THF (40 mL) gradually with vigorous stirring at room temperature and the mixture was stirred for 12 h. The reaction mixture was poured into an ice-aqoeous solution of HCl and then was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated to dryness. Compound **22** was obtained as a brown powder. The triester **22** (5.71 g, 10.2 mmol) was stirred vigorously in 0.5 M aqueous NaOH solution (200 mL) at room temperature for 48 h. After removal of unreacted impurities by filtration, the filtrate was acidified with 0.5 M aqueous HCl solution and evaporated to dryness. The residue was purified by HPLC (YMC-pack ODS-AM AM323:C-18 reverse-phase column, ϕ 10 mm×250 mm, CH_3CN:0.1% aqueous TFA = 20:80, 3.0 mL/min, 254 nm, retention time 15.5 min). The CH_3CN and TFA were removed in vacuo, and the water was removed by lyophilization to give **23** as a white solid (345.5 mg, 11% from **19**). ^{1}H NMR (400MHz, CD_3OD) : δ 8.27 (d, *J*=9.0Hz, 2H), 7.41 (d, *J*=8.1Hz, 2H), 2.30 (t, *J*=7.2Hz, 2H), 2.00~1.47 (m, 8H); ^{13}C NMR (100MHz, CD_3OD) : δ 177.3, 158.1 (J_{CP}=8.3Hz), 145.1, 126.4, 122.1 (J_{CP}=4.1Hz), 34.7, 31.1 (J_{CP}=17Hz), 27.8 (J_{CP}=139Hz), 25.6, 23.7 (J_{CP}=5.0Hz); HRMS (FAB^+) : $[M+H]^+$ calcd for $C_{12}H_{17}O_7NP$, 318.0743; found, 318.0744.



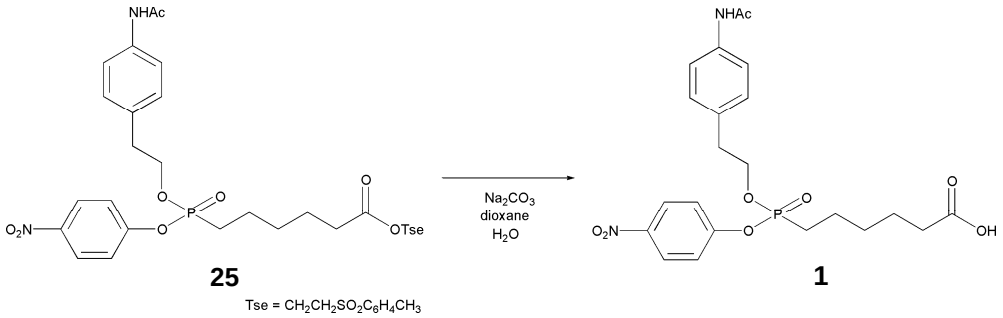
6-[Hydroxy (4-nitrophenoxy) phosphoryl] hexanoic acid 2-(toluene-4-sulfonyl) ethyl ester (24)

2-(*p*-tolylsulfonyl)ethanol (89.3 mg, 0.47 mmol), 4-dimethylaminopyridine (32.8 mg, 0.27 mmol), and EDC (51.3mg, 0.27 mmol) was added to a solution of compound **23** (70.8 mg, 0.22 mmol) in DMF (1.2 mL) and CH_3CN (3.6 mL). The solution was stirred at room temperature for 15 h. After removal of the solvent, the residue was purified by HPLC (YMC-pack ODS-AM AM323:C-18 reverse-phase column, ϕ 10 mm×250 mm, CH_3CN:0.1% aqueous TFA = 40:60, 3.0 mL/min, 254 nm, retention time 13.4 min). The CH_3CN and TFA were removed in vacuo, and the water was removed by lyophilization to give **24** as a colorless oil (102.8 mg, 92 %). ^{1}H NMR (400 MHz, CD_3OD) : δ 8.26 (d, *J*=9.0 Hz, 2H), 7.79 (d, *J*=8.3 Hz, 2H), 7.45 (d, *J*=7.8 Hz, 2H), 7.41 (d, *J*=8.1 Hz, 2H), 4.36 (t, *J*=5.7 Hz, 2H), 3.56 (t, *J*=5.6 Hz, 2H), 2.45 (s, 3H), 2.07 (t, *J*=7.3 Hz, 2H), 1.97~1.35 (m, 8H) ; ^{13}C NMR (100 MHz, $CDCl_3$) : δ 172.6, 154.8 (J_{CP}=9.1 Hz), 145.0, 144.5, 136.2, 129.9, 128.0, 125.5, 121.2 (J_{CP}=4.1 Hz), 57.5, 55.0, 33.4, 29.6 (J_{CP}=17 Hz), 25.7 (J_{CP}=143 Hz), 24.0, 21.7 (J_{CP}=10 Hz), 21.7; HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{21}H_{27}O_9NPS$, 500.1144; found, 500.1155.



6-[[2-(4-Acetylaminophenyl) ethoxy] (4-nitrophenoxy) phosphoryl] hexanoic acid 2-(toluene-4-sulfonyl) ester (25)

Compound **3** (144.6 mg, 0.81 mmol), 1H-tetrazole (11.4 mg, 0.16 mmol), 4-dimethylaminopyridine (11.9 mg, 0.097 mmol), and DCC (166.5 mg, 0.81 mmol) was added to a solution of compound **24** (40.3 mg, 0.081 mmol) in DMF (2.8 mL) and the solution was stirred at room temperature for 6 days. After removal of the solvent, the residue was purified by HPLC (YMC-pack ODS-AM AM323:C-18 reverse-phase column, ϕ 10 mm×250 mm, CH_3CN:0.1% aqueous TFA = 55:45, 3.0 mL/min, 254 nm, retention time 13.6 min). The CH_3CN and TFA were removed in vacuo, and the water was removed by lyophilization to give **25** as a colorless oil (32.2 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 8.16 (d, *J*=9.0 Hz, 2H), 7.80 (d, *J*=8.3 Hz, 2H), 7.44~ 7.37 (m, 4H), 7.27 (d, *J*=12.0 Hz, 2H), 7.12 (d, *J*=8.5 Hz, 2H), 4.44~4.36 (m, 3H), 4.27~4.23 (m, 1H), 3.43 (t, *J*=6.0 Hz, 2H), 2.91 (t, *J*=6.2 Hz, 2H), 2.46 (s, 3H), 2.15 (t, *J*=7.6 Hz, 5H), 1.84~1.76 (m, 2H), 1.55~1.47 (m, 4H), 1.35~1.27 (m, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) : δ 172.7, 168.6, 155.2 (J_{CP}=9.1Hz), 145.1, 144.3, 136.8, 136.1, 132.6, 129.9, 129.3, 128.0, 125.5, 120.8 (J_{CP}= 4.1 Hz), 112.0, 67.3 (J_{CP}=6.6 Hz), 57.4, 55.0, 36.1 (J_{CP}=6.6 Hz), 33.4, 29.7 (J_{CP}=17 Hz), 25.6 (J_{CP}=140 Hz), 24.4, 24.0, 21.8 (J_{CP}=5.0 Hz), 21.7; HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{31}H_{38}O_{10}N_2PS$, 661.1985; found, 661.1983.

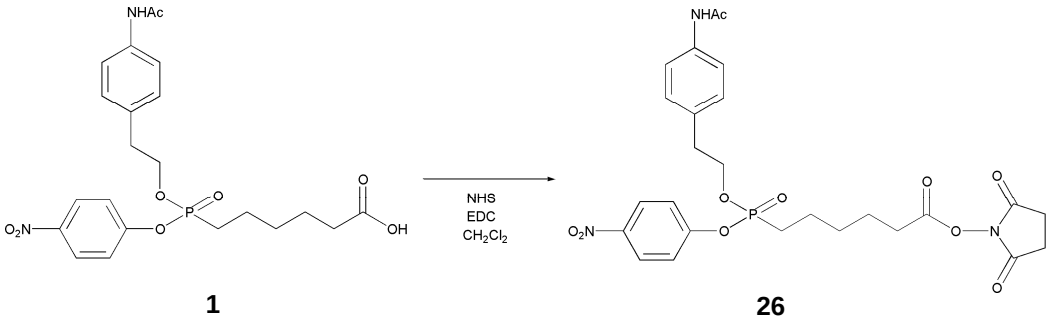


25 **1**

Tse = $CH_2CH_2SO_2C_6H_4CH_3$

6-[[2-(4-Acetylamıno-pnenyı) ethoxy] (4-nitro-phenoxy) phosphoryl] hexanoic acid (1)

A solution of compound **25** (17.9 mg, 0.027 mmol) and Na_2CO_3 (28.6 mg, 0.270 mmol) in dioxane (2.6 mL) and H_2O (260 μL) was stirred at room temperature for 3 days. After removal of the solvent, the residue was purified by HPLC (YMC-pack ODS-AM AM323:C-18 reverse-phase column, ϕ 10 mm × 250 mm, CH_3CN:0.1% aqueous TFA = 55:45, 3.0 mL/min, 254 nm, retention time 6.0 min). The CH_3CN and TFA were removed in vacuo, and the water was removed by lyophilization to give **1** as a colorless oil (11.9 mg, 92%). ^{1}H NMR (400 MHz, CD_3OD) : δ 8.07 (d, *J*=9.0 Hz, 2H), 7.34 (d, *J*=8.5 Hz, 2H), 7.11 (d, *J*=8.5 Hz, 2H), 7.05 (d, *J*=8.5 Hz, 2H), 4.32~4.19 (m, 2H), 2.83 (t, *J*=6.1 Hz, 2H), 2.18 (t, *J*=7.2 Hz, 2H), 2.02 (s, 3H), 1.89~1.80 (m, 2H), 1.52~1.42 (m,4H), 1.35~1.28 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) : δ 177.2, 171.5, 156.3 (J_{CP}=9.1 Hz), 145.8, 138.6, 134.4, 130.4, 126.5, 122.1 (J_{CP}= 4.1 Hz), 121.0, 69.0 (J_{CP}=7.5 Hz), 37.0 (J_{CP}= 6.6 Hz), 34.6, 30.8 (J_{CP}=17 Hz), 25.9 (J_{CP}=139 Hz), 25.4, 23.8, 22.9 (J_{CP}= 5.8 Hz); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{22}H_{28}O_8N_2P$, 479.1583; found, 479.1591.



1 **26**

6-[[2-(4-Acetylaminylphenyl) ethoxy]-(4-nitrophenoxy) phosphoryl] hexanoic acid 2,5-dioxo pyrrolidin-1-yl ester (26)

N-hydroxysuccinimide (7.40 mg, 0.064 mmol) and EDC (4.71 mg, 0.0384 mmol) was added to a solution of compound **1** (15.1 mg, 0.032 mmol) in CH_2Cl_2 (2 mL) and the solution was stirred at room temperature for 10 h. After removal of the solvent, the residue was purified by HPLC (YMC-pack ODS-AM AM323:C-18 reverse-phase column, ϕ 10 mm × 250 mm, CH_3CN:0.1% aqueous TFA = 45:55, 3.0 mL/min, 254 nm, retention time 13.8 min). The CH_3CN and TFA were removed in vacuo, and the water was removed by lyophilization to give **26** as a white solid (12.8 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 8.17 (d, *J*=9.0 Hz, 2H), 7.66 (br. s, 1H), 7.44 (d, *J*= 8.3 Hz, 2H), 7.26 (d, *J*=9.0 Hz, 2H), 7.12 (d, *J*=8.3 Hz, 2H), 4.43~4.35 (m, 1H), 4.29~ 4.22 (m, 1H), 2.93~2.87 (m, 6H), 2.54 (t, *J*=7.3 Hz, 2H), 2.15 (s, 3H), 1.83~1.75 (m, 2H), 1.71~1.63 (m, 2H), 1.60~1.40 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) : δ 169.2, 168.4, 168.3, 155.3 (J_{CP}=8.3 Hz), 144.4, 136.9, 132.7, 129.4, 125.6, 120.8 (J_{CP}= 5.0 Hz), 119.8, 67.2 (J_{CP}=5.8 Hz), 36.1 (J_{CP}= 6.6 Hz), 30.6, 29.2 (J_{CP}=17Hz), 25.7, 25.5 (J_{CP}=140Hz), 24.5, 24.0, 21.7 (J_{CP}=5.8Hz); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{26}H_{31}O_{10}N_3P$, 576.1748; found, 576.1748.

Preparation of Antigens

KLH-hapten

A KLH solution in PBS (10 mg/mL, 1 mL) was added to a stirred solution of **26** (2.9 mg, 0.0050 mmol) in DMF (300 μL) and PBS. After 4 h of stirring at room temperature, the mixture was dialyzed against PBS at 4℃. KLH-hapten conjugate was used for immunization. The antigen was stored at -30℃.

BSA-hapten

A BSA solution in PBS (10 mg/mL, 1 mL) was added to a stirred solution of **26** (3.5 mg, 0.0061 mmol) in DMF (300 μL) and PBS. After 4 h of stirring at room temperature, the mixture was dialyzed against PBS pH 7.4 at 4℃. The concentration of hapten was determined by UV absorbance at 280 nm to give an epitope density of 40 hapten molecules per BSA monomer. BSA-hapten conjugate was used for enzyme-linked immunosorbent assay (ELISA). The antigen was stored at -30℃.

Immunization

Antibody Production and Purification

Five Balb/c mice each received an intraperitoneal injection of KLH-hapten conjugate (100 μg) emulsified in RIBI adjuvant (MPL and TDM emulsion) on days 1, 7 and 14. On day 21, serum was taken from the mice, and the titer was determined by enzyme-linked immunosorbent assay (ELISA). On day 49, the all mice with the high titer received a final intraperitoneal boost with a mixture of KLH-hapten conjugate and RIBI adjuvant. Three days after last boost, the spleen was taken from the mouse, and the cells were fused with 2.4×10^7, 5.0×10^7, 4.9×10^7, 4.5×10^7 P3X63-Ag8.63 myeloma cells by a Simadzu Somatic Hybridizer SSH10 (electrode distance: 1.0 mm; frequency: 1 MHz; primary AC voltage: 80 V; initial time: 10s; pulse width: 40 ms; DC voltage: 920 V; electric field strength: 2.30 $kVcm^{-1}$; secondary AC voltage: 80 V; pulse repeat interval: 1 s; number of pulses: 1; VDC change: +0 V; final time: 10 s; AC voltage decrease rate: 0%; adhesion intensifier: off). Hybridoma cells were plated into eleven 96-well plates; each well contained HAT-RPMI 1640 (100 μL) with fetal bovine serum (20%) and Briclone (5%, ARCHPORT LTD.). After 8-14 days, the plates were analyzed by ELISA for binding to the BSA-hapten conjugate. All positive colonies were subcloned twice according to standard protocols. All cell lines that retained active binding after subcloning were individually grown to 200 mL, and the supernatants were purified by anti-mouse IgG+IgM affinity chromatography (NGK, loaded on in PBS and eluted with 0.2 M Gly-HCl, pH 2.5) to yield purified antibodies. The subclass of each antibody was determined by using a monoclonal isotyping kit purchased from Amersham (RPN 29).

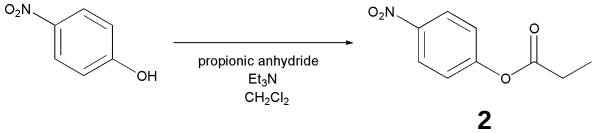
Antibody Affinity Measurement

A competition ELISA protocol was followed to determine dissociation constants of inhibitor for each antibodies (25E2, 27C1). Prior to carrying out the competition ELISA, the optimum antibody concentration and the minimum antigen concentration on the ELISA plate (precoated with dilution series 10-0.078125 μg/mL of the protein-hapten conjugate and then blocked with SKIM MILK) were determined to establish a reproducible titration curve. The optinum antibody concentration is defined as the concentration at which ca. 70% of the antibody (reflecting on the absorbance) binds to the protein-hapten conjugate. The minimum antigen concentration is defined as the minimum saturating concentration binding to the conjugate (reflecting on the absorbance). In our experiments, the concentrations were determined for each antibodies (the optimum antibody concentrations; 25E2, 0.15625 μg/mL, 27C1, 0.15625 μg/mL, the minimum antigen concentrations ; 25E2, 0.625 μg/mL, 27C1, 0.625 μg/mL). Competition ELISA experiments were performed as follows. A 60 μL solution of inhibitor was serially diluted 2-fold across a non-binding plate, containing 30 μL (in PBS, pH 7.4, 10% DMSO) of buffer per well. To each well was then added 30 μL of an antibody solution (the final concentration was preadjusted), and the mixture was equilibrated for 2 hours at 25℃. The resulting antibody-inhibitor solution (50 μL) from each well was transferred to the corresponding well of the ELISA plate binding to the minimum antigen concentration with a multipipeter and incubated for 15min. After the plate washed, standard ELISA protocol was followed. The results were analyzed by a Klotz plot (a double reciprocal plot of the inhibitor-concentration *vs* the ratio of inhibitor-bound antibody to the total added antibody), the slope of the line yielding the K_d for inhibitor.

Table S1. Dissociation constants

Antibody	K_d (**1**)
25E2	27nM
27C1	8.7nM

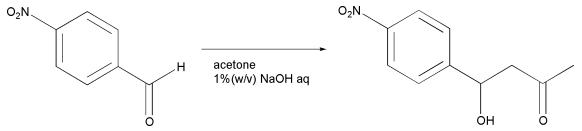
Synthesis of Substrates



2

Propionic acid 4-nitrophenyl ester (2)

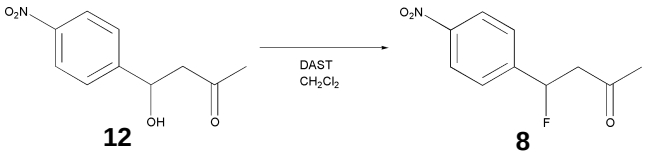
A solution of *p*-nitrophenol (300 mg, 2.16 mmol), propionic anhydride (0.55 mL, 4.31 mmol), and trietylamine (0.36 mL, 2.59 mmol) in CH_2Cl_2 (6 mL) was stirred at room temperature. After 12 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ and was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was diluted with hexane and the precipitate was collected by filtration. Compound **2** was obtained as a white powder (294.7 mg, 70%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.27 (d, *J*=9.0 Hz, 2H), 7.28 (d, *J*=9.3 Hz, 2H), 2.64 (q, *J*=7.6 Hz, 2H), 1.29 (t, *J*=7.6 Hz, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_9H_{10}O_4N$, 196.0609; found, 196.0679.



12

4-Hydroxy-4-(4-nitrophenyl) butan-2-one (12)

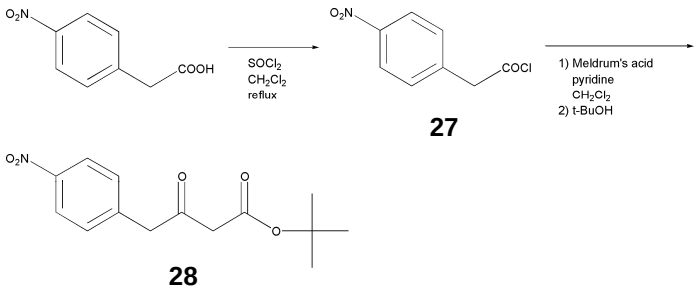
To a solution of *p*-nitrobenzaldehyde (2 g, 13.2 mmol) in 24 mL acetone was added 2.4 mL of a 1% (w/v) aqueous NaOH solution at 0℃. Stirring was continued at 0℃ for 15 min. The solution was then neutralized by addition of 0.5 N aqueous HCl solution and concentrated in vacuo. The residue was dissolved in 50 mL of H_2O and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc:Hexane=1:1) to give **12** as yellow powder (2.3 g, 85%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.21 (d, *J*=8.8 Hz, 2H), 7.54 (d, *J*=8.3 Hz, 2H), 5.29-5.25 (m, 1H), 3.61 (d, *J*=3.4 Hz, 1H), 2.85 (d, *J*=4.4 Hz, 2H), 2.23 (s, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{10}H_{12}O_4N$, 210.0766; found, 210.0818.



12 **8**

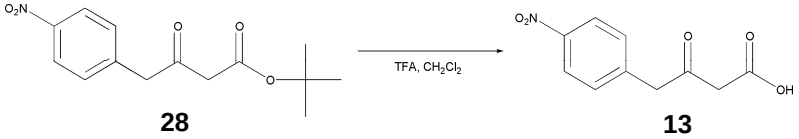
4-Fluoro-4-(4-nitrophenyl) butan-2-one (8)

To a solution of **12** (821 mg, 3.92 mmol) in 5 mL of CH_2Cl_2 at -78℃ was added 1 mL (7.47 mmol) of diethylaminosulfur trifluoride. The solution was allowed to stir for 10 min at -78℃ and then poured into 20 mL of H_2O. Two phases were separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc:Hexane=1:2) to give **8** as a yellow powder (373 mg, 45%). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 8.25 (d, *J*=8. 3Hz, 2H), 7.54 (d, *J*=8.8 Hz, 2H), 6.08 (ddd, *J*=46.5, 8.0, 4.5 Hz, 1H), 3.22 (ddd, *J*=17.1, 15.9, 8.0 Hz, 1H), 2.87 (ddd, *J*=29.0, 17.2, 4.4 Hz, 1H), 2.24 (s, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{10}H_{11}O_3NF$, 212.0723; found, 212.0769.



4-(4-Nitrophenyl)-3-oxobutyric acid *tert*-butyl ester (28)

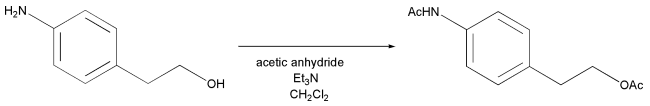
Thionyl chloride (20 mL) was added to a solution of *p*-nitrophenylacetic acid (2.20 g, 12.1 mmol) in CH_2Cl_2 (10 mL) and the suspension was refluxed for 2h. The resulting solution was evaporated and the residue was dissolved in CH_2Cl_2 (15 mL). This solution was added to a stirred solution of Meldrum's acid (1.75 g, 12.1 mmol) and pyridine (1.96 mL, 24.2 mmol) in CH_2Cl_2 (15 mL) at 0℃. After the addition was completed (approximately 1 h), the resulting dark orange solution was stirred for 1 h at 0℃, and then at room temperature for 2 h. The solution was diluted with CH_2Cl_2 (20 mL) and the organic phase was washed with 0.1 N aqueous HCl solution and brine, dried over Na_2SO_4 and evaporated to dryness. The resulting orange solid was suspended in t-butyl alcohol (30mL) and refluxed for 12h. The solution was evaporated and the residue was purified by silica gel column chromatography (EtOAc:Hexane=1:2) to give **28** as a white powder (1.6 g, 47%). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 8.21 (d, *J*=8.8 Hz, 2H), 7.38 (d, *J*=8.8 Hz, 2H), 3.98 (s, 2H), 3.44 (s, 2H), 1.48 (s, 9H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{14}H_{18}O_5N$, 280.1185; found, 280.1217.



4-(4-Nitrophenyl)-3-oxobutyric acid (13)

To a solution of **28** (131 mg, 0.47 mmol) in 2 mL of CH_2Cl_2 at room temperature was added 2 mL of TFA. After 2 h, The solution was evaporated. Compound **13** was obtained as a yellow solid (112 mg, quant.). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 8.22 (d, *J*=8.5 Hz, 2H), 7.39 (d, *J*=8.8 Hz, 2H), 4.01 (s, 2H), 3.60 (s, 2H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{10}H_{10}O_5N$, 224.0559; found, 224.0625.

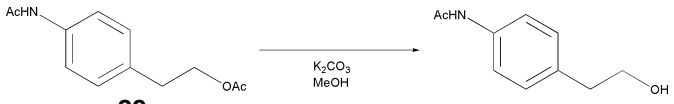
Synthesis of Cofactors



29

Acetic acid 2-(4-acetylaminophenyl) ethyl ester (29)

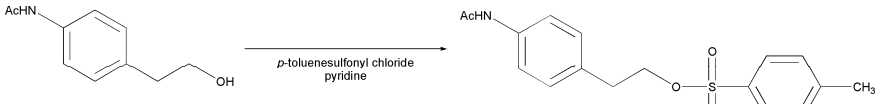
A solution of 4-aminophenethyl alcohol (2.50 g, 18.2 mmol), acetic anhydride (6.05 mL, 63.8 mmol), and triethylamine (5.56 mL, 40.1 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature. After 14h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ and was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was diluted with hexane and the precipitate was collected by filtration. Compound **29** was obtained as a white powder (4.13 g, quant.). ^{1}H-NMR (400MHz, $CDCl_3$) : δ 7.43 (d, *J*=8.3 Hz, 2H), 7.16 (d, *J*=7.1 Hz, 2H), 4.24 (t, *J*=7.0 Hz, 2H), 2.89 (t, *J*=7.0 Hz, 2H), 2.16 (s, 3H), 2.03 (s, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{12}H_{16}O_3N$, 222.1130; found, 222.1160.



29 **3**

***N*-[4-(2-Hydroxyethyl) phenyl] acetamide (3)**

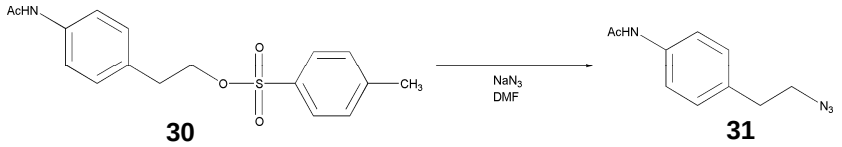
K_2CO_3 (one scoop of small spatula) was added to a solution of **29** (1.50 g, 6.79 mmol) in MeOH (30 mL) and the solution was stirred at room temperature for 3 h. After removal of the solvent, the residue was purified by silica gel column chromatography (MeOH:$CHCl_3$=1:19) to give **3** as a white powder (1.19 g, 98%). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 7.41 (d, *J*=8.3 Hz, 2H), 7.16 (d, *J*=8.3 Hz, 2H), 3.82 (t, *J*=6.5 Hz, 2H), 2.82 (t, *J*=6.5 Hz, 2H), 2.15 (s, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{10}H_{14}O_2N$, 180.1025; found, 180.1037.



3 **30**

Toluene-4-sulfonic acid 2-(4-acethylaminophenyl) ethyl ester (30)

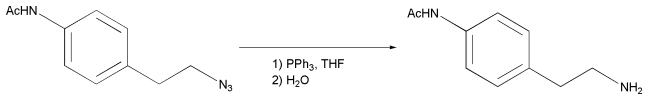
p-Toluenesulfonyl chloride(1.14 g, 6.0 mmol) was added to a solution of **3** (430 mg, 2.4 mmol) in Pyridine (5 mL) and the solution was stirred at room temperature for 2 h. The reaction mixture was poured into aqueous HCl solution and was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by silica gel column chromatography (EtOAc:Hexane=1:1) to give **30** as a white solid (641 mg, 80%). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 7.69 (d, *J*=8.3 Hz, 2H), 7.38 (d, *J*=8.5 Hz, 2H), 7.29 (d, *J*=8.0 Hz, 2H), 7.06 (d, *J*=8.5 Hz, 2H), 4.17 (t, *J*=7.0 Hz, 2H), 2.91 (t, *J*=7.1 Hz, 2H), 2.44 (s, 3H), 2.17 (s, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{17}H_{21}O_4NS$, 334.1113; found, 334.1111.



30 **31**

***N*-[4-(2-Azidoethyl)phenyl] acetamide (31)**

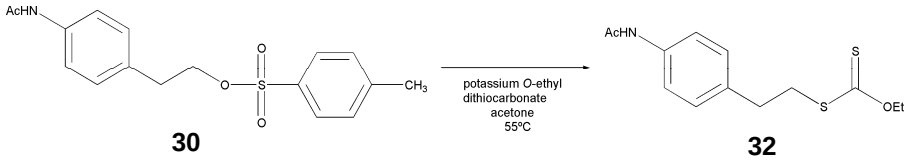
A solution of compound **30** (444 mg, 2.18 mmol) and sodium azide (861 mg, 13.3 mmol) in DMF (25 mL) was stirred at room temperature. After 12 h, the reaction mixture was diluted with EtOAc. The organic layer was extracted with water, washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was diluted with hexane and the precipitate was collected by filtration. Compound **31** was obtained as a yellow powder (265 mg, 98%). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 7.44 (d, *J*=8.3 Hz, 2H), 7.17 (d, *J*=8.3 Hz, 2H), 3.48 (t, *J*=7.1 Hz, 2H), 2.86 (t, *J*=7.2 Hz, 2H), 2.18 (s, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{10}H_{12}N_4$, 205.1089; found, 205.1059.



31 **4**

***N*-[4-(2-Aminoethyl)phenyl] acetamide (4)**

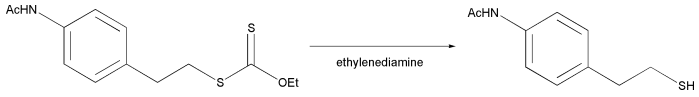
A solution of compound **31** (265 mg, 1.30 mmol) and triphenylphosphine (748 mg, 2.86 mmol) in THF (12 mL) was stirred at room temperature. After 15 h, H_2O (80 μL) was added to the reaction mixture. The solution was stirred at room temperature for 2 h and evaporated to dryness. The residue was purified by silica gel column chromatography (MeOH:$CDCl_3$=1:1) to give **4** as a white powder (214 mg, 80%). ^{1}H-NMR (400 MHz, CD_3OD) : δ 7.41 (d, *J*=8.5 Hz, 2H), 7.15 (d, *J*=8.5 Hz, 2H), 2.94 (t, *J*=6.8 Hz, 2H), 2.71 (t, *J*=7.0 Hz, 2H), 2.17 (s, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{10}H_{15}ON_2$, 179.1184; found, 179.1229.



30 **32**

Dithiocarbonic acid *S*-[2-(4-acetylaminophenyl) ethyl] ester *O*-ethyl ester (32)

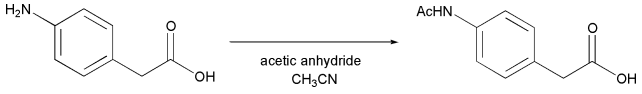
A solution of compound **30** (246 mg, 0.74 mmol), potassium *O*-ethyl dithiocarbonate (271 mg, 1.69 mmol), and acetone (5 mL) was stirred at 55℃ for 1h. The reaction mixture was evaporated to dryness. The residue was washed with water several times to remove unreacted potassium *O*-ethyl dithiocarbonate and the precipitate was collected by filtration. Compound **32** was obtained as a white powder (178 mg, 96%). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 7.43 (d, *J*=8.5 Hz, 2H), 7.20 (d, *J*=8.3 Hz, 2H), 4.66 (q, *J*=7.2 Hz, 2H), 3.33 (t, *J*=7.8 Hz, 2H), 2.95 (t, *J*=7.8 Hz, 2H), 2.18 (s, 3H), 1.43 (t, *J*=7.1 Hz, 2H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{13}H_{18}O_2NS_2$, 284.0779; found, 284.0805.



32 **5**

***N*-[4-(2-Mercaptoethyl)phenyl] acetamide (5)**

A solution of compound **32** (100 mg, 0.35 mmol) and ethylenediamine, anhydrous (3 mL) was stirred at room temperature for 15 min. The reaction mixture was poured into an ice-aqueous solution of H_2SO_4 and then was extracted with CH_2Cl_2. The combined organic layers were washed with 10% (v/v) aqueous solution of H_2SO_4, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by silica gel column chromatography (MeOH:$CHCl_3$=1:19) to give **5** as a white powder (86.1 mg, 63%). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 7.43 (d, *J*=8.5 Hz, 2H), 7.15 (d, *J*=8.5 Hz, 2H), 2.89 (t, *J*=7.3 Hz, 2H), 2.77 (t, *J*=7.7 Hz, 2H), 2.17 (s, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{10}H_{14}ONS$, 196.0796; found, 196.0773.

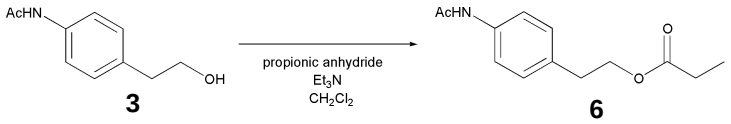


9

(4-Acetylaminopheny) acetic acid (9)

A solution of *p*-aminophenylacetic acid (300 mg, 1.98 mmol) and acetic anhydride (0.41 mL, 4.36 mmol), in CH_3CN (5 mL) was stirred at room temperature for 12 h. After removal of the solvent, the solid was washed with 1 N HCl, extracted with EtOAc, washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by silica gel column chromatography (MeOH:$CHCl_3$=1:4) to give **9** as a pale brown powder (276.8 mg, 72%). ^{1}H-NMR (400 MHz, CD_3OD) : δ 7.48 (d, J=8.8 Hz, 2H), 7.21 (d, J=8.8 Hz, 2H), 3.55 (s, 2H), 2.10 (s, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{10}H_{12}O_3N$, 194.0817; found, 194.0770.

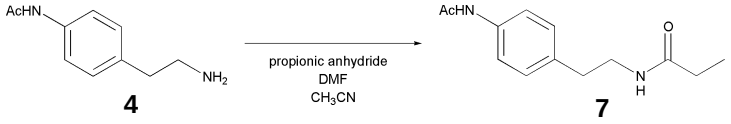
Synthesis of Products



3 6

Propionic acid 2-(4-acetylaminophenyl) ethyl ester (6)

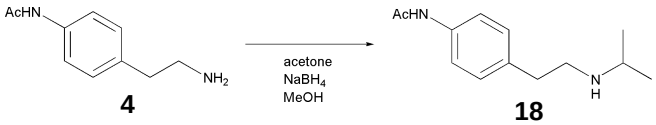
A solution of compound **3** (200 mg, 1.12 mmol), propionic anhydride (0.43 mL, 3.36 mmol), and triethylamine (0.34 mL, 2.46 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature. After 5h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ and was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by silica gel column chromatography (EtOAc:Hexane=1:1) to give **6** as a white powder (231 mg, 88%). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 7.43 (d, *J*=8.3 Hz, 2H), 7.17 (d, *J*=8.3 Hz, 2H), 4.26 (t, *J*=7.0 Hz, 2H), 2.90 (t, *J*=7.0 Hz, 2H), 2.31 (q, *J*=7.6 Hz, 2H), 2.17 (s, 3H), 1.12 (t, *J*=7.6 Hz, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{13}H_{18}O_3N$, 236.1287; found, 236.1286.



4 7

***N*-[2-(4-Acetylaminophenyl) ethyl] propionamide (7)**

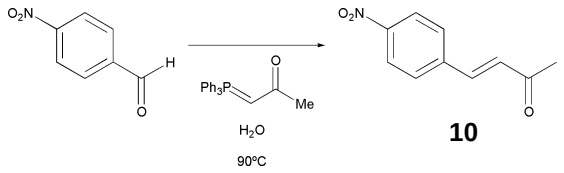
A solution of compound **4** (30 mg, 0.17 mmol), propionic anhydride (0.11 mL, 0.86 mmol), and 4-dimethylaminopyridine (44.2 mg, 0.36 mmol) in DMF (1.5 mL) and CH_3CN (1.5 mL) was stirred at room temperature. After 2 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ and was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated to dryness. The residue was purified by silica gel column chromatography (MeOH:$CHCl_3$=1:4) to give **7** as a white powder (18 mg, 49%). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 7.45 (d, *J*=8.5 Hz, 2H), 7.15 (d, *J*=8.5 Hz, 2H), 3.36 (t, *J*=7.7 Hz, 2H), 2.74 (t, *J*=7.3 Hz, 2H), 2.15 (q, *J*=7.6 Hz, 2H), 2.10 (s, 3H), 1.09 (t, *J*=7.6 Hz, 3H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{13}H_{18}O_2N_2$, 235.1446; found, 235.1476.



4 18

***N*-[4-(2-Isopropylaminoethyl) phenyl] acetamide (18)**

A mixture of compound **4** (30 mg, 0.17 mmol), $NaBH_4$ (20 mg, 0.52 mmol), and acetone (50 μL) in MeOH was stirred at 0℃ for 1h, and then warmed to room temperature and stirred for 20 h. The reaction mixture was poured into H_2O and was extracted with $CHCl_3$. The combined organic layers were dried over $MgSO_4$, and evaporated to dryness. The residue was purified by silica gel column chromatography (MeOH:$CHCl_3$=1:1) to give **18** as a white powder (51.1 mg, quant.). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 7.41 (d, *J*=8.3 Hz, 2H), 7.17 (d, *J*=8.0 Hz, 2H), 2.86-2.75 (m, 5H), 1.04 (d, *J*=6.3 Hz, 6H); HRMS (FAB$^+$) : $[M+H]^+$ calcd for $C_{13}H_{21}ON_2$, 221.1654; found, 221.1653.



4-(4-Nitrophenyl)-but-3-en-2-one (10)

A solution of *p*-nitrobenzaldehyde (500 mg, 3.31 mmol) and 1-(triphenylphosphoranyli-dene)-2-propane (1.59 g, 5 mmol) in H_2O was stirred 90℃ for 3 h. The reaction mixture was cooled to room temperature, extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc:Hexane=1:3) to give **10** as a yellow powder (360.2 mg, 57%). ^{1}H-NMR (400 MHz, $CDCl_3$) : δ 8.27 (d, *J*=9.0 Hz, 2H), 7.70 (d, *J*=8.8 Hz, 2H), 7.54 (d, *J*=16.1 Hz, 1H), 6.82 (d, *J*=16.3 Hz, 1H), 2.43 (s, 3H); HRMS (FAB^+) : $[M+H]^+$ calcd for $C_{10}H_{10}O_3N$, 192.0661; found, 192.0635.

Kinetics Measurement (1)

Screening Assay of Acyl-transfer Reaction (ester 2, alcohol 3) for Catalytically Active Clones

The screening assays were performed in a EYELA DIGITAL UNI ACE UA-100 water bath (±0.1) at 25℃ in a 50 mM Tris-HCl, pH 8.0 with 10% v/v DMSO as a co-solvent. The reaction was followed by monitoring formation of the ester product **6** by the reversed phase HPLC. The analytical HPLC was performed on a Hitachi L-2130 unit equipped with a Hitachi L-2400 UV detector, using a YMC ODS AM303 column eluted with acetonitrile/aqueous TFA (0.1%, 35:65) at a flow rate of 1.0 mL/min with detection at 264 nm. The retention time of **6** was 10.8 min. A solution of alcohol **3** in DMSO (20 mM final concentration) was added to an Eppendorf tube, containing the buffer solution (45 μL) with or without antibodies (10 μM). The reaction was then initiated by addition of a DMSO solution of **2** (10 mM final concentration) into the mixture. After certain time periods, a portion of the reaction mixture (10 μL) was analyzed by the HPLC system *vide supra*. Product concentrations were determined by comparison to product standard curve.

Kinetic Parameters Determination of Acyl-transfer Reaction (25E2, 27C1)

The buffer system, assay procedure, product standard curve, and HPLC assay (**6** R_t = 10.8 min) were the same as described in the previous section.

Antibody 25E2

The 25E2-catalyzed process was investigated under saturating conditions. The antibody-catalyzed rates and the K_m (**2**) were measured at a fixed concentration of **3** (150 mM) and varying concentrations of **2** (2-12 mM). In contrast, the K_m (**3**) was measured at fixed concentration of **2** (10 mM) and varying alcohol **3** concentrations (10-150 mM). Final antibody concentration (10 μM) and temperature (25℃) were maintained throughout the assay. Kinetic parameters [V_{max}, k_{cat}, K_m (**2**), K_m (**3**)] were determined by nonlinear least-squares fitting of the initial rate against **2** or **3** concentrations to a hyperbolic curve described by the Michaelis-Menten equation, respectively.

Antibody 27C1

The 27C1-catalyzed process was investigated under saturating conditions. The antibody-catalyzed rates and the K_m (**2**) were measured at a fixed concentration of **3** (150 mM) and varying concentrations of **2** (1-4 mM). In contrast, the K_m (**3**) was measured at a fixed concentration of **2** (10 mM) and varying alcohol **3** concentrations (10-160 mM). Final antibody concentration (10 μM) and temperature (25℃) were maintained throughout the assay. Kinetic parameters [V_{max}, k_{cat}, K_m (**2**), K_m (**3**)] were determined by nonlinear least-squares fitting of the initial rates against concentrations of **2** or **3** to a hyperbolic curve described by the Michaelis-Menten equation, respectively.

Inhibition Studies of 25E2 and 27C1 with Hapten 1

The buffer system, assay procedure, product standard curve, and HPLC assay were the same as described in the previous section with the exception that the DMSO solution of inhibitor was added [to a final concentration of 160 μM (25E2), 368 μM (27C1)] to a mixture. The assay of 25E2 (10 μM) was performed with fixed concentrations of 10 mM of **2** and 50 mM of **3**. The assay of 27C1 (10 μM) was performed with fixed concentrations of 5 mM of **2** and 25 mM of **3**. DMSO concentration was maintained at 10% v/v.

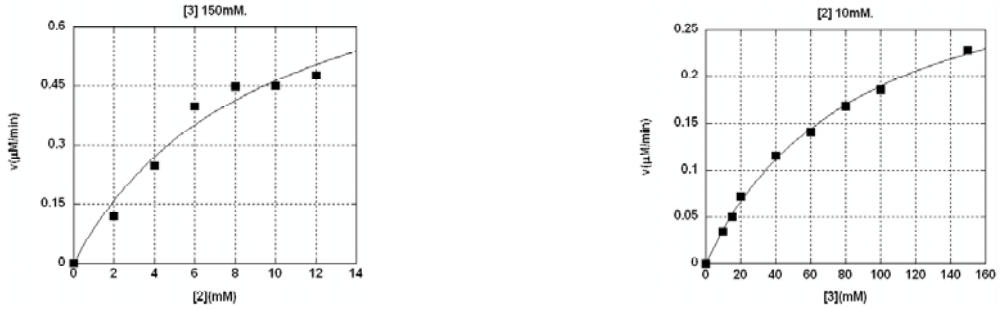


Figure S1. Michaelis-Menten plots of 25E2. Assays were performed at 25℃ in 50 mM Tris-HCl, pH 8.0.

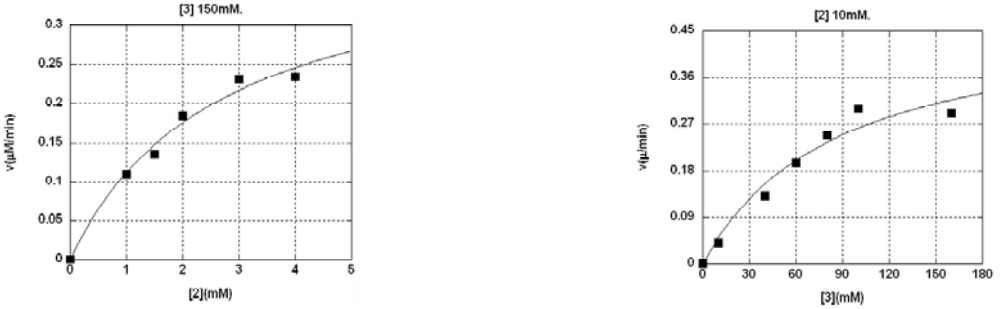


Figure S2. Michaelis-Menten plots of 27C1. Assays were performed at 25℃ in 50 mM Tris-HCl, pH 8.0.

Kinetics Measurements (2)

Screening Assay of Acyl-transfer Reaction (ester 2, amine 4) for Antibodies 25E2 and 27C1

The screening assays were performed in a EYELA DIGITAL UNI ACE UA-100 water bath (±0.1) at 25℃ in a 50 mM Tris-HCl, pH 8.0, buffer system with 10% v/v DMSO as co-solvent. The reaction was followed by monitoring formation of the amide product **7** by the reversed phase HPLC. The analytical HPLC was performed on a Hitachi L-2130 unit equipped with a Hitachi L-2400 UV detector, using a YMC ODS AM303 column eluted with acetonitrile/aqueous TFA (0.1%, 25:75) at a flow rate of 1.0 mL/min with detection at 264 nm. The retention time of **7** was 5.4 min. A solution of **4** in DMSO (250 μM final concentration) was added to an Eppendorf tube, containing the buffer solution (45 μL) with or without antibodies (25E2, 27C1) (10 μM). The reaction was then initiated by addition of a DMSO solution of **2** (250 μM final concentration), into the mixture. After certain time periods, a portion of the reaction mixture (10 μL) was analyzed by the HPLC system as shown previously. Product concentrations were determined by comparison to product standard curve.

Inhibition Studies of 27C1 with Hapten 1

The buffer system, assay procedure, product standard curve, and HPLC assay were the same as described in the previous section with the exception that the DMSO solution of inhibitor **1** was added (to a final concentration of 40 μM). The assay was performed with 10 μM of 27C1, and 250 μM of **2** and **4**. DMSO concentration was maintained at 10% v/v.

Inhibition Studies of 25E2 with Hapten 1

The buffer system and assay procedure were the same as described in the previous section with the exception that the DMSO solution of inhibitor **1** was added (to a final concentration of 40 μM). The analytical HPLC was performed on a Hitachi L-2130 unit equipped with a Hitachi L-2400 UV detector, using a YMC ODS AM303 column eluted with acetonitrile/aqueous TFA (0.1%, 15:85) at a flow rate of 1.0 mL/min with detection at 264 nm. The retention time of **7** was 12.5 min. Product concentrations were determined by comparison to product standard curve. The assay was performed with 10μM of 25E2, and 200 μM of **2** and **4**. DMSO concentration was maintained at 10% v/v.

Kinetic Parameter Determinations of Acyl-transfer Reactions (2) (25E2, 27C1)

The buffer system, assay procedure, product standard curve, and HPLC assay (**7** R_t = 12.5min) were the same as described in the previous section.

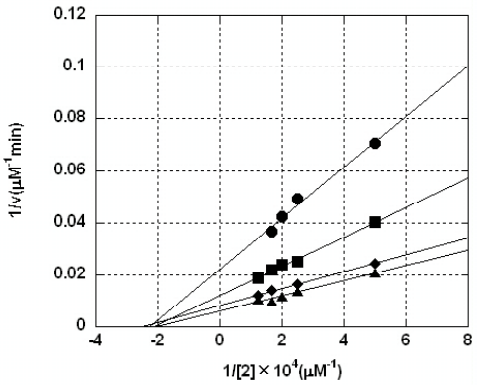
Antibody 25E2

The 25E2-catalyzed process was investigated as a random, rapid equilibrium system (Segal, I. H. *Enzyme Kinetics*; John Wiley & Sons, Inc.: New York, 1975). The antibody-catalyzed rates were measured at fixed concentrations of **4** (1 mM ≤ [**4**] ≤ 4 mM) and varying concentrations of **2** (2 mM ≤ [**2**] ≤ 8 mM). Final antibody concentration (5 μM) and temperature (25℃) were maintained throughout the assay. Kinetic parameters were determined by a two-step analysis. First, Lineweaver-Burk (1/*V vs.* 1/*S*) plots of the raw data were constructed. These *y*-intercepts and slopes were then replotted to yield the actual V_{max} and K_m (**4**) values for the 25E2-catalyzed process. The k_{cat} values were determined from the actual V_{max} values. Similarly, the antibody-catalyzed rates were measured at fixed concentrations of **2** (1 mM ≤ [**2**] ≤ 4 mM) and varying concentrations of **4** (1 mM ≤ [**4**] ≤ 5 mM). Analogous plots were constructed to give kinetic constants for **2**.

Antibody 27C1

The 27C1-catalyzed process was investigated as a random, rapid equilibrium system (Segal, I. H. *Enzyme Kinetics*; John Wiley & Sons, Inc.: New York, 1975). The antibody-catalyzed rates were measured at fixed concentrations of **4** (0.25 mM ≤ [**4**] ≤ 2 mM) and varying concentrations of **2** (0.5 mM ≤ [**2**] ≤ 2 mM). Final antibody concentration (3 μM) and temperature (25℃) were maintained throughout the assay. Kinetic parameters were determined by a two-step analysis. First, Lineweaver-Burk (1/*V vs.* 1/*S*) plots of the raw data were constructed. These *y*-intercepts and slopes were then replotted to yield the actual V_{max} and K_m (**4**) values for the 27C1-catalyzed process. The k_{cat} values were determined from the actual V_{max} values. Similarly, the antibody-catalyzed rates were measured at fixed concentrations of **2** (0.25 mM ≤ [**2**] ≤ 1.5 mM) and varying concentrations of **4** (0.25 mM ≤ [**4**] ≤ 2 mM). Analogous plots were constructed to give kinetic constants for **2**.

A



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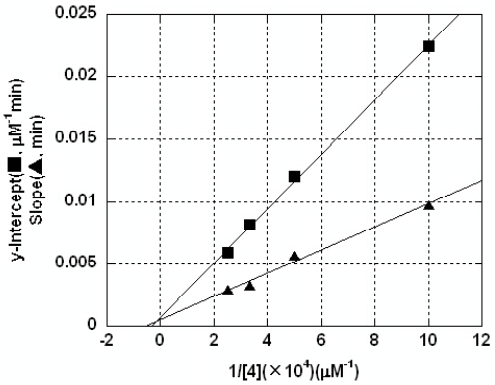

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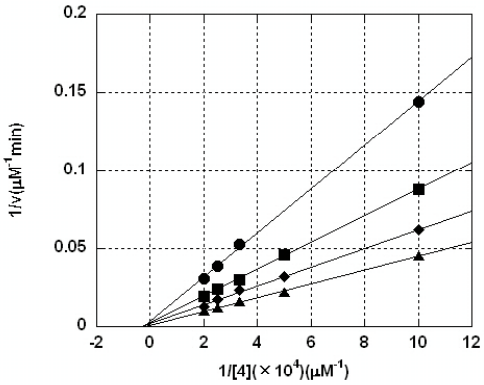

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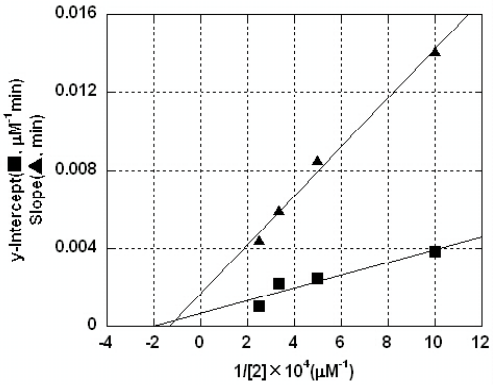


Figure S3. (**A**) Lineweaver-Burk plot with **4** held at four fixed concentrations while **2** was varied over concentrations ranging from 2 to 8mM (●[**4**]=1 mM; ■[**4**]=2 mM; ◆[**4**]=3 mM; ▲[**4**]=4 mM); *v*,velocity. (**B**) Replot of *y* intercepts and slopes of Lineweaver-Burk plots as a function of $[\mathbf{4}]^{-1}$. (**C**) Analogous plot was constructed with **2** held at four fixed concentrations while **4** was varied over concentrations ranging from 1 to 5 mM (●[**2**]=1 mM; ■[**2**]=2 mM; ◆[**2**]=3 mM; ▲[**2**]=4 mM). (**D**) Analogous plots were constructed to give kinetic constants for **2**. Reaction mixtures contained 5 μM of antibody 25E2, 10%(v/v) DMSO, and 50 mM Tris-HCl, pH8.0, and were incubated at 25℃.

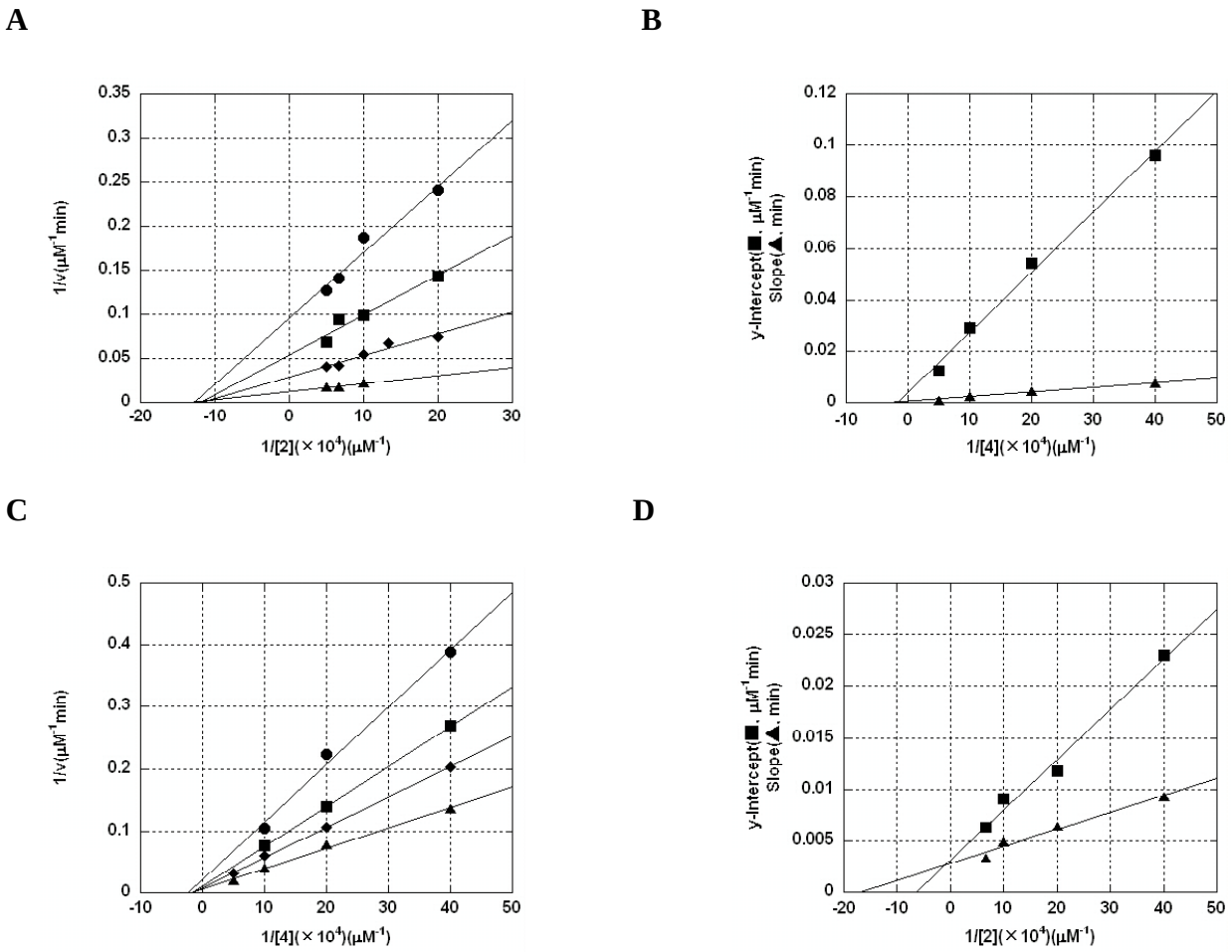


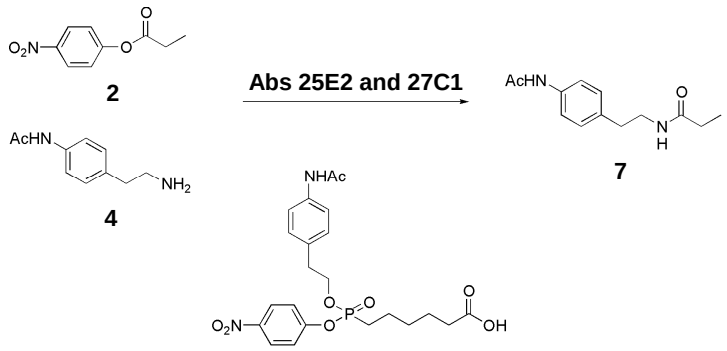
Figure S4. (**A**) Lineweaver-Burk plot with **4** held at four fixed concentrations while **2** was varied over concentrations ranging from 0.5 to 2 mM (●[**4**]=0.25 mM; ■[**4**]=0.5 mM; ◆[**4**]=1 mM; ▲[**4**]=2 mM); *v*, velocity. (**B**) Replot of *y* intercepts and slopes of Lineweaver-Burk plots as a function of $[\mathbf{4}]^{-1}$. (**C**) Analogous plot was constructed with **2** held at four fixed concentrations while **4** was varied over concentrations ranging from 1 to 4 mM (●[**2**]=0.25 mM; ■[**2**]=0.5 mM; ◆[**2**]=1 mM; ▲[**2**]=1.5 mM). (**D**) Analogous plots were constructed to give kinetic constants for **2**. Reaction mixtures contained 3 μM of antibody 27C1, 10%(v/v) DMSO, and 50 mM Tris-HCl, pH8.0, and were incubated at 25℃.

Table S2. Kinetic parameters for the antibody-catalyzed acyl transfer reaction of ester **2** with amine **4**.

	K_m				
	2 (mM)	**4 (mM)**	V_{max} **(μMmin^{-1})**	k_{cat} **(min^{-1} per binding sites)**	$k_{cat}/K_m(2)/k_{uncat}$ $k_{cat}/K_m(4)/k_{uncat}$
25E2	7.4	16.9	1.54×10^3	153.8	1.4×10^4 5.9×10^3
27C1	0.598	4.3	3.04×10^2	50.7	5.5×10^4 7.7×10^3

In the reaction with amine **4**, kinetics were measured at 25°C in 50 mM Tris-HCl, pH 8.0 and 10% DMSO. The V_{max}, k_{cat} and K_m were calculated from initial rates using a random, rapid equilibrium mechanism. The bimolecular non-catalyzed rate constant (k_{uncat}) for the reaction of **2** and **4** was determined by the method of initial rates under the identical conditions, affording $k_{uncat} = 1.53\times10^{-3}$ mM^{-1}min^{-1}.

Measurement of the K_i of hapten 1



Inhibition Studies of 25E2 with Hapten 1

The buffer system and assay procedure were the same as described in the previous section with the exception that the DMSO solution of hapten **1** was added (to a final concentration of 0-40 μM). The analytical HPLC was performed on a Hitachi L-2130 unit equipped with a Hitachi L-2400 UV detector, using a YMC ODS AM303 column eluted with acetonitrile/aqueous TFA (0.1%, 15:85) at a flow rate of 1.0 mL/min with detection at 264 nm. The retention time of **7** was 12.5 min. Product concentrations were determined by comparison to product standard curve. The assay was performed with 10μM of 25E2 and 200 μM of **2** and **4**. DMSO concentration was maintained at 10% v/v.

Inhibition Studies of 27C1 with Hapten 1

The buffer system and assay procedure were the same as described in the previous section with the exception that the DMSO solution of inhibitor **1** was added (to a final concentration of 0-40 μM). The analytical HPLC was performed on a Hitachi L-2130 unit equipped with a Hitachi L-2400 UV detector, using a YMC ODS AM303 column eluted with acetonitrile/aqueous TFA (0.1%, 15:85) at a flow rate of 1.0 mL/min with detection at 264 nm. The retention time of **7** was 12.5 min. Product concentrations were determined by comparison to product standard curve. The assay was performed with 10μM of 27C1 and 500 μM of **2** and **4**. DMSO concentration was maintained at 10% v/v.

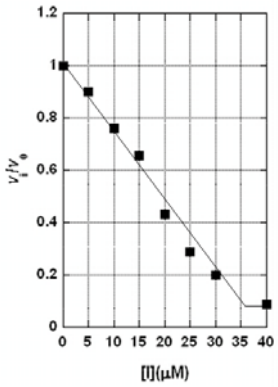


Figure S5. Tight-binding inhibition of antibody 25E2 by **1**. Initial rates were measured at increasing concentrations of **1** in the presence of 25E2 (10μM), **2** (200 μM) and **4** (200 μM). K_i = 110 nM if inhibition is due to one enantiomer only.

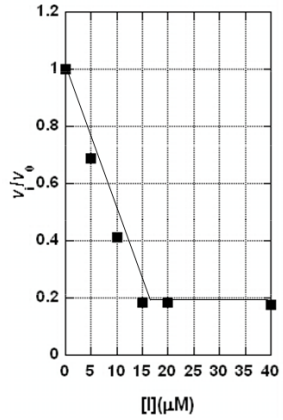


Figure S6. Tight-binding inhibition of antibody 27C1 by **1**. Initial rates were measured at increasing concentrations of **1** in the presence of 27C1 (10μM), **2** (500 μM) and **4** (500 μM).

Antibodies 25E2 and 27C1 demonstrated competitive tight-binding inhibition by hapten **1** (Figure S5 and S6). K_i for **1** was determined at fixed substrate **2** and amine **4** concentrations by fitting the initial rate v_0 to the equation for tight-binding inhibition [Eq. (1)], where v is the initial rate in the presence of hapten **1**, v_0 is the initial rate in the absence of **1**, E is the concentration of functional catalyst, I is the concentration of **1**, K_i' is the apparent inhibition constant defined by Equation (2), and S is the substrate **2** concentration.

$$v = \{(v_0/2E)[E - I - K_i' + (K_i' + I - E)^2 + 4EK_i']^{1/2} \quad (1)$$

$$K_i' = K_i\,(1 + S/K_m) \quad (2)$$

Kinetics Measurements (3)

Screening Assay of Acyl-transfer Reaction (ester 2, thiol 5) for Antibodies 25E2 and 27C1

The screening assays were performed in a EYELA DIGITAL UNI ACE UA-100 water bath (±0.1) at 25℃ in a 50 mM Tris-HCl, pH8.0, buffer system with 10% v/v acetonitrile as a co-solvents. The reaction was followed by monitoring formation of *p*-nitrophenol by the reversed phase HPLC. The analytical HPLC was performed on a Hitachi L-2130 unit equipped with a Hitachi L-2400 UV detector, using a YMC ODS AM303 column eluted with acetonitrile/aqueous TFA (0.1%, 35:65) at a flow rate of 1.0 mL/min with detection at 322 nm. The retention time of *p*-nitrophenol was 9.6 min. A solution of **5** in acetnitrile (250 μM final concentration) was added to an Eppendorf tube, containing the buffer solution (45 μL) with or without antibodies (25E2, 27C1) (10 μM). The reaction was then initiated by addition of an acetonitrile solution of **2** (250 μM final concentration) into the mixture. After certain time periods, a portion of the reaction mixture (10 μL) was analyzed by the HPLC system as shown previously. Product concentrations were determined by comparison to product standard curve.

Inhibition Studies of 25E2 and 27C1 with Hapten 1

The buffer system, assay procedure, product standard curve, and HPLC assay were the same as described previously with the exception that an acetonitrile solution of inhibitor **1**, was added (to a final concentration of 240 μM). The assay was performed with 10 μM of antibodies (25E2, 27C1) and 250 μM of **2** and **5**. Acetonitrile concentrations were maintained at 10% v/v.

The Participation of Thiol 5 in Antibody Catalysis

The buffer system, assay procedure, product standard curve, and HPLC assay were the same as described previously with the exception that **5** was absent. The assay was performed with 10μM of antibodies (25E2, 27C1) and 250μM of **2**. Acetonitrile concentrations were maintained at 10% v/v. Neither 25E2 nor 27C1 showed any rate acceleration of the cleavage of **2**.

Kinetic Parameter Determinations of Acyl-transfer Reaction (3) (25E2, 27C1)

The kinetic assays were performed in a BECKMAN DIODE ARRAY SPECTROPHOTOMETER DU 7500 at 25℃ in a 50 mM Tris-HCl, pH 8.0, buffer system with 10% v/v acetonitrile as a co-solvent. Initial rates were determined spectrophotometically by measuring the absorbance increase by *p*-nitrophenolate at 405nm as a function of time (ε = 14,200 $M^{-1}cm^{-1}$ **2** λ_{max} = 271 nm, **5** λ_{max} = 246 nm).

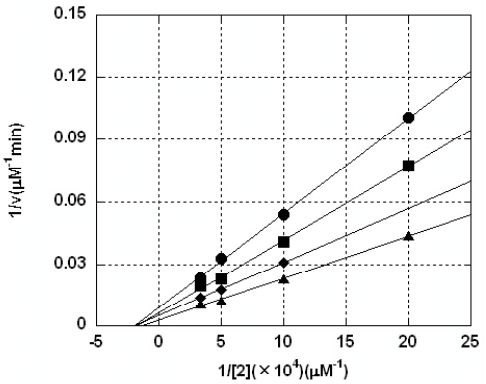
Antibody 25E2

The 25E2-catalyzed process was investigated as a random, rapid equilibrium system (Segal, I. H. *Enzyme Kinetics*; John Wiley & Sons, Inc.: New York, 1975). The antibody-catalyzed rates were measured at fixed concentrations of **5** (0.5 mM ≤ [**5**] ≤ 3 mM) and varying concentrations of **2** (0.5 mM ≤ [**2**] ≤ 3 mM). Final antibody concentration (5 μM) and temperature (25℃) were maintained throughout the assay. Kinetic parameters were determined by a two-step analysis. First, Lineweaver-Burk (1/*V vs.* 1/*S*) plots of the raw data were constructed. These *y*-intercepts and slopes were then replotted to yield the actual V_{max} and K_m (**5**) values for the 25E2-catalyzed process. The k_{cat} values were determined from the actual V_{max} values. Similarly, the antibody-catalyzed rates were measured at fixed concentrations of **2** (0.5 mM ≤ [**2**] ≤ 3 mM) and varying concentrations of **5** (0.5 mM ≤ [**5**] ≤ 3 mM). Analogous plots were constructed to give kinetic constants for **2**.

Antibody 27C1

The 27C1-catalyzed process was investigated as a random, rapid equilibrium system (Segal, I. H. *Enzyme Kinetics*; John Wiley & Sons, Inc.: New York, 1975). The antibody-catalyzed rates were measured at fixed concentrations of **5** (1 mM ≤ [**5**] ≤ 4 mM) and varying concentrations of **2** (0.5 mM ≤ [**2**] ≤ 3 mM). Final antibody concentration (5 μM) and temperature (25℃) were maintained throughout the assay. Kinetic parameters were determined by a two-step analysis. First, Lineweaver-Burk (1/*V vs.* 1/*S*) plots of the raw data were constructed. These *y*-intercepts and slopes were then replotted to yield the actual V_{max} and K_m (**5**) values for the 27C1-catalyzed process. The k_{cat} values were determined from the actual V_{max} values. Similarly, the antibody-catalyzed rates were measured at fixed concentrations of **2** (0.5 mM ≤ [**2**] ≤ 3 mM) and varying concentrations of **5** (1 mM ≤ [**5**] ≤ 6 mM). Analogous plots were constructed to give kinetic constants for **2**.

A



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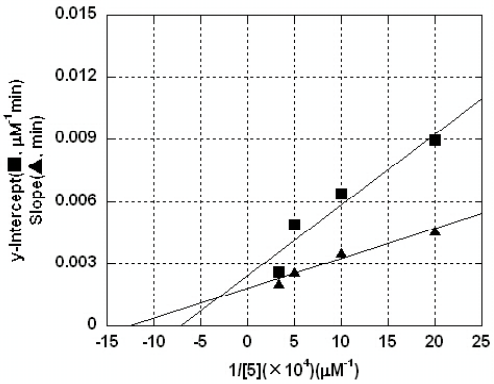

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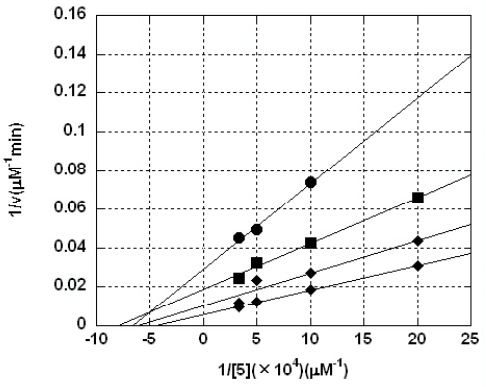

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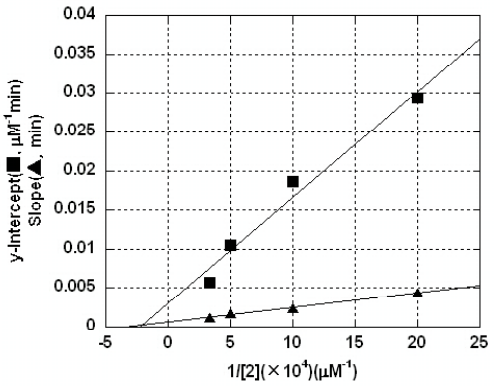


Figure S7. (**A**) Lineweaver-Burk plot with **5** held at four fixed concentrations while **2** was varied over concentrations ranging from 0.5 to 3 mM (●[**5**]=0.5 mM; ■[**5**]=1 mM; ◆[**5**]=2 mM; ▲[**5**]=3 mM); *v*, velocity. (**B**) Replot of *y* intercepts and slopes of Lineweaver-Burk plots as a function of $[\mathbf{5}]^{-1}$. (**C**) Analogous plot was constructed with **2** held at four fixed concentrations while **5** was varied over concentrations ranging from 0.5 to 3 mM (●[**2**]=0.5 mM; ■[**2**]=1 mM; ◆[**2**]=2 mM; ▲[**2**]=3 mM). (**D**) Analogous plots were constructed to give kinetic constants for **2**. Reaction mixture contained 5 μM antibody 25E2, 10%(v/v) acetonitrile, and 50 mM Tris-HCl, pH 8.0, and were incubated at 25℃.

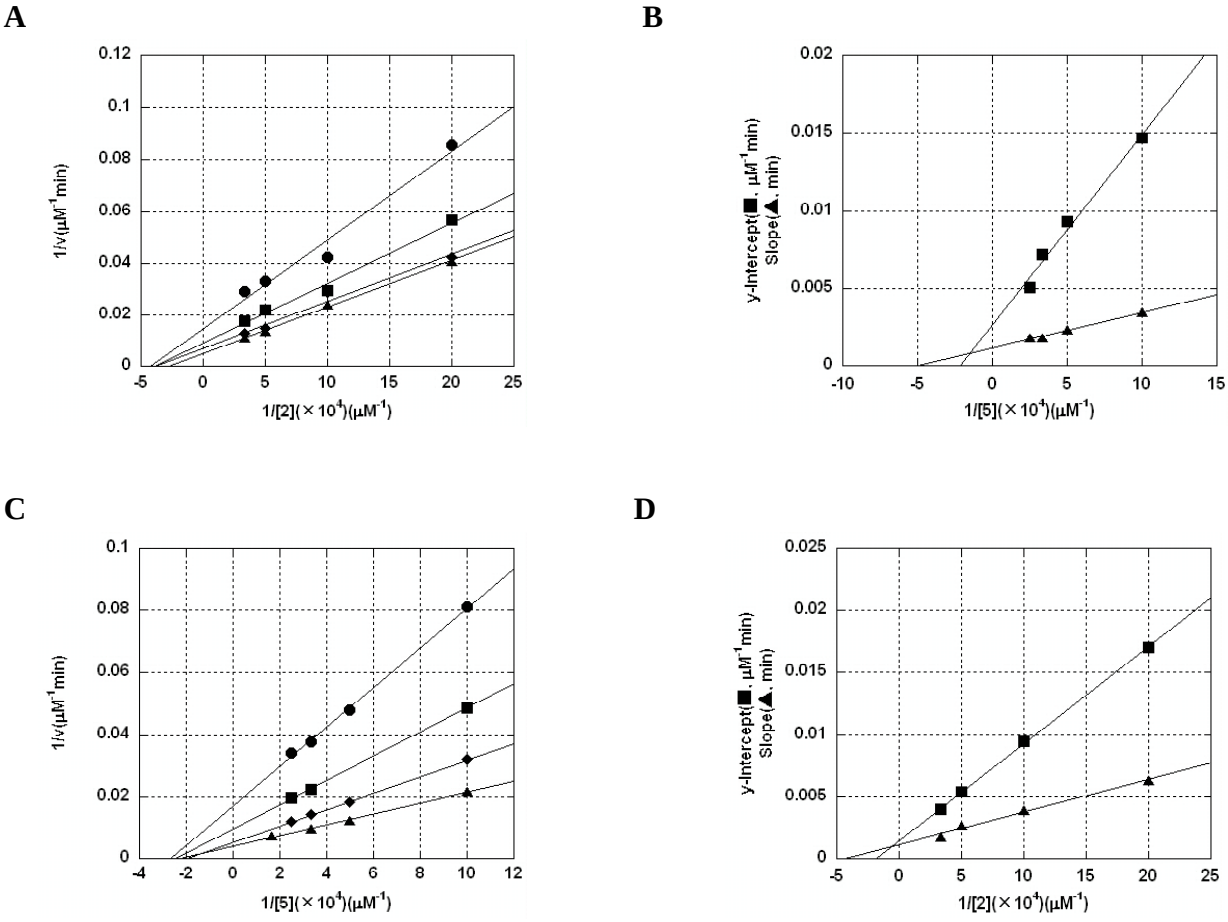


Figure S8. (**A**) Lineweaver-Burk plot with **5** held at four fixed concentrations while **2** was varied over concentrations ranging from 0.5 to 3 mM (●[**5**]=1 mM; ■[**5**]=2 mM; ◆[**5**]=3 mM; ▲[**5**]=4 mM); *v*, velocity. (**B**) Replot of *y* intercepts and slopes of Lineweaver-Burk plots as a function of $[\mathbf{5}]^{-1}$. (**C**) Analogous plot was constructed with **2** held at four fixed concentrations while **5** was varied over concentrations ranging from 1 to 6 mM (●[**2**]=0.5 mM; ■[**2**]=1 mM; ◆[**2**]=2 mM; ▲[**2**]=3 mM). (**D**) Analogous plots were constructed to give kinetic constants for **2**. Reaction mixtures contains 5 μM antibody 27C1, 10%(v/v) acetonitrile, and 50 mM Tris-HCl, pH 8.0, and were incubated at 25℃.

Table S3. Kinetic parameters for the antibody-catalyzed acyl transfer reaction of ester **2** with thiol **5**.

	K_m				
	2 (mM)	**5** (mM)	V_{max} (μMmin^{-1})	k_{cat} (min^{-1} per binding sites)	$k_{cat}/K_m(2)/k_{uncat}$ $k_{cat}/K_m(5)/k_{uncat}$
25E2	2.9	0.802	4.12×10^3	41.2	1.2×10^3 4.2×10^3
27C1	2.4	1.9	6.73×10^3	67.3	2.4×10^3 3.0×10^3

In the reaction with thiol **5**, kinetics were measured at 25°C in 50 mM Tris-HCl, pH 8.0 and 10% CH_3CN. The V_{max}, k_{cat} and K_m were calculated from initial rates using a random, rapid equilibrium mechanism. The bimolecular non-catalyzed rate constants (k_{uncat}) for the reaction of **2** and **5** were determined by the method of initial rates under the identical conditions, affording $k_{uncat} = 1.21\times10^{-2}$ mM^{-1}min^{-1}.

Kinetics Measurments (4)

Screening Assay of β-Elimination Reaction for Antibodies 25E2 and 27C1

The screening assays were performed in a NanoDrop ND-1000 Full-spectrum UV/Vis Spectrophotometer (NanoDrop Technologies, Inc.) at 25℃ in a 10 mM bis-Tris, 100 mM NaCl, pH 6.0, buffer system with 5% v/v acetonitrile and 5% v/v MeOH as co-solvents. Initial rates were determined spectrophotometically by measuring the absorbance increase at 330 nm as a function of time (**8** λ_{max} = 272 nm, **9** λ_{max} = 248 nm, **10** λ_{max} = 312 nm, $\Delta\varepsilon$ (**10**-**8**) (330 nm) = 14,430 $M^{-1}cm^{-1}$). A solution of phenyl acetic acid derivative **9** in MeOH (250 μM final concentration) was added to an Eppendorf tube, containing the buffer solution with or without antibodies (25E2, 27C1) (10 μM). The reaction was then initiated by addition of an acetonitrile solution of β-haloketone **8** (250 μM final concentration) into the mixture.

Inhibition Studies of 25E2 with Hapten 1

The buffer system, assay procedure were the same as described previously with the exception that the inhibitor, as a stock in MeOH, was added (to a final concentration of 250 μM). The assay was performed with 10 μM of antibody (25E2) and 250 μM of **8** and **9**. Acetonitrile and MeOH concentrations were maintained at 5%v/v, respectively.

The Participation of Phenyl Acetic Acid Derivative 9 in Antibody-catalyzed β-Elimination Reaction

The buffer system, assay procedure were the same as described previously in the absence of **9**. The assay was performed with 10 μM of antibody (25E2) and 250 μM of **8**. Acetonitrile and MeOH concentrations were maintained at 5% v/v, respectively. The antibody 25E2 didn't show any rate acceleration of the conversion of **8** to **10**.

Kinetic Parameters Determination of β-Elimination Reaction (25E2)

The kinetic assays were performed under the same conditions as described previously.

Antibody 25E2

The 25E2-catalyzed process was investigated as a random, rapid equilibrium system (Segal, I. H. *Enzyme Kinetics*; John Wiley & Sons, Inc.: New York, 1975). The antibody-catalyzed rates were measured at fixed concentrations of **9** (50 μM ≤ [**9**] ≤ 300 μM) and varying concentrations of **8** (0.3 mM ≤ [**8**] ≤ 1.5 mM). Final antibody concentration (10 μM) and temperature (25℃) were maintained throughout the assay. Kinetic parameters were determined by a two-step analysis. First, Lineweaver-Burk ($1/V$ *vs.* $1/S$) plots of the raw data were constructed. These *y*-intercepts and slopes were then replotted to yield the actual V_{max} and K_m (**9**) values for the 25E2-catalyzed process. The k_{cat} values were determined from the actual V_{max} values. Similarly, the antibody-catalyzed rates were measured at fixed concentrations of **8** (0.3 mM ≤ [**8**] ≤ 1.5 mM) and varying concentrations of **9** (50 μM ≤ [**9**] ≤ 300 μM). Analogous plots were constructed to give kinetic constants for **8**.

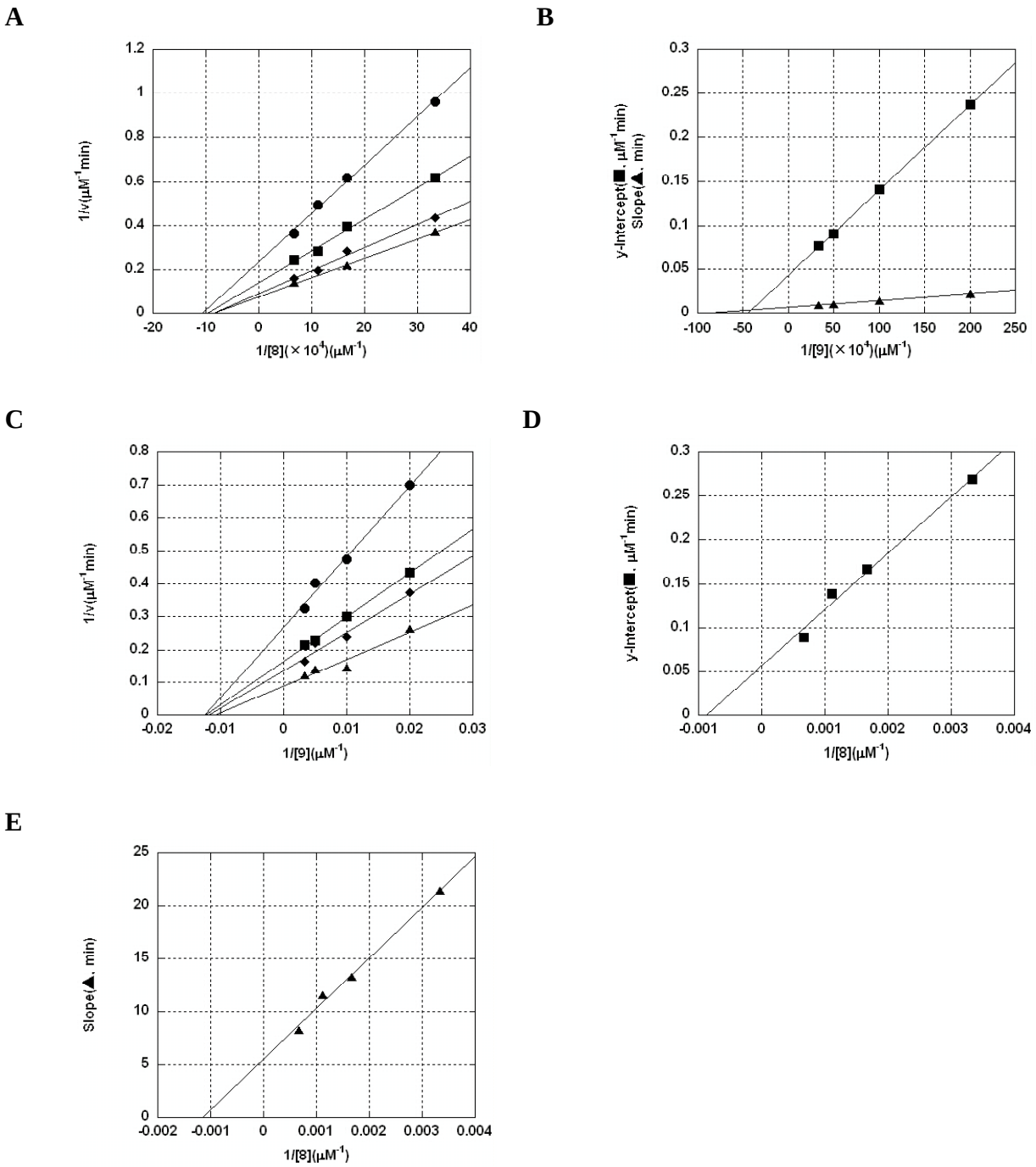


Figure S9. (**A**) Lineweaver-Burk plot with **9** held at four fixed concentrations while **8** was varied over concentrations ranging from 0.3 to 1.5 mM (●[**9**]=50 μM; ■[**9**]=100 μM; ◆[**9**]=200 μM; ▲[**9**]=300 μM); *v*, velocity. (**B**) Replot of *y* intercepts and slopes of Lineweaver-Burk plots as a function of $[\mathbf{9}]^{-1}$. (**C**) Analogous plot was constructed with **8** held at four fixed concentrations while **9** was varied over concentrations ranging from 50 to 300 μM (●[**8**]=0.3 mM; ■[**8**]=0.6 mM; ◆[**8**]=0.9 mM; ▲[**8**]=1.5 mM). (**D**) (**E**) Analogous plots were constructed to give kinetic constants for **8**. Reaction mixtures contained 10 μM antibody 25E2, 5%(v/v) acetonitrile, 5%(v/v) MeOH, and 10 mM bis-Tris 100 mM NaCl, pH6.0, and were incubated at 25°C.

Kinetics Measurments (5)

Screening Assay of Aldol Reaction for Antibodies 25E2 and 27C1

The screening assays were performed in a EYELA DIGITAL UNI ACE UA-100 water bath (±0.1) at 25℃ in a 50 mM Tris-HCl, pH 8.0, buffer system with 5% v/v DMSO and 5% v/v acetone as co-solvents. The reaction was followed by monitoring formation of *β*-hydroxy ketone **12** by the reversed phase HPLC. The analytical HPLC was performed on a Hitachi L-2130 unit equipped with a Hitachi L-2400 UV detector, using a YMC ODS AM303 column eluted with acetonitrile/aqueous TFA (0.1%, 40:60) at a flow rate of 1.0 mL/min with detection at 283 nm. The retention time of **12** was 6.3 min. A solution of amine **4** in DMSO (500 μM final concentration) and *p*-nitrobenzaldehyde **11** in DMSO (500 μM final concentration) was added to an Eppendorf tube, containing the buffer solution (45 μL) with or without antibodies (25E2, 27C1) (10 μM). The reaction was then initiated by addition of acetone (2.5 μL) into the mixture. After certain time periods, a portion of the reaction mixture (10 μL) was analyzed by the HPLC system shown previously. Product concentrations were determined by comparison to product standard curve.

Inhibition Studies of 25E2- and 27C1-catalyzed Aldol Reactions

The buffer system, assay procedure, product standard curve, and HPLC assay were the same as described previously with the exception that a DMSO solution of inhibitor **1** was added (to a final concentration of 160 μM). The assay was performed with antibodies 25E2 (5 μM), 27C1 (10 μM) and 500 μM of *p*-nitrobenzaldehyde **11** and **4**. DMSO and acetone concentrations were maintained at 5%v/v, respectively.

The Participation of Amine 4 in Antibody-catalyzed Aldol Reactions

The buffer system, assay procedure, product standard curve, and HPLC assay were the same as described previously with the exception that amine **4** was absenct. The assay was performed with 10 μM of antibodies (25E2, 27C1) and 500 μM of *p*-nitrobenzaldehyde **11**. DMSO and acetone concentrations were maintained at 5% v/v, respectively. Neither 25E2 nor 27C1 showed any rate acceleration of aldol reaction.

Kinetic Parameters Determination of Aldol Reaction (25E2, 27C1)

The buffer system (50 mM Tris-HCl, pH 8.0 with 5% v/v DMSO and 5% v/v acetone as co-solvents) and HPLC assay (**12** R_t = 6.3 min) were the same as described previously. Samples (14.8 μL) were quenched a certain time periods by being mixed with 0.2μL TFA, immediately analyzed by HPLC. Product concentrations were determined by comparison to product standard curve.

Antibody 25E2

The 25E2-catalyzed process was investigated as a random, rapid equilibrium system (Segal, I. H. *Enzyme Kinetics*; John Wiley & Sons, Inc.: New York, 1975). The antibody-catalyzed rates were measured at fixed concentrations of **4** (5 mM ≤ [**4**] ≤ 20 mM) and varying concentrations of *p*-nitrobenzaldehyde **11** (0.25 mM ≤ [**11**] ≤ 1.5 mM). Final antibody concentration (2.5 μM) and temperature (25℃) were maintained throughout the assay. Kinetic parameters were determined by a two-step analysis. First, Lineweaver-Burk (1/*V vs.* 1/*S*) plots of the raw data were constructed. These *y*-intercepts and slopes were then replotted to yield the actual V_{max} and K_m (**4**) values for the 25E2-catalyzed process. The k_{cat} values were determined from the actual V_{max} values. Similarly, the antibody-catalyzed rates were measured at fixed concentrations of *p*-nitrobenzaldehyde **11** (0.25 mM ≤ [**11**] ≤ 1.5 mM) and varying concentrations of **4** (5 mM ≤ [**4**] ≤ 20 mM). Analogous plots were constructed to give kinetic constants for *p*-nitrobenzaldehyde **11**.

Antibody 27C1

The 27C1-catalyzed process was investigated as a random, rapid equilibrium system (Segal, I. H. *Enzyme Kinetics*; John Wiley & Sons, Inc.: New York, 1975). The antibody-catalyzed rates were measured at fixed concentrations of **4** (5 mM ≤ [**4**] ≤ 20 mM) and varying concentrations of *p*-nitrobenzaldehyde **11** (0.25 mM ≤ [**11**] ≤ 1.5 mM). Final antibody concentration (5 μM) and temperature (25℃) were maintained throughout the assay. Kinetic parameters were determined by a two-step analysis. First, Lineweaver-Burk (1/*V vs.* 1/*S*) plots of the raw data were constructed. These *y*-intercepts and slopes were then replotted to yield the actual V_{max} and K_m (**4**) values for the 27C1-catalyzed process. The k_{cat} values were determined from the actual V_{max} values. Similarly, the antibody-catalyzed rates were measured at fixed concentrations of *p*-nitrobenzaldehyde **11** (0.25 mM ≤ [**11**] ≤ 1.5 mM) and varying concentrations of **4** (5 mM ≤ [**4**] ≤ 20 mM). Analogous plots were constructed to give kinetic constants for *p*-nitrobenzaldehyde **11**.

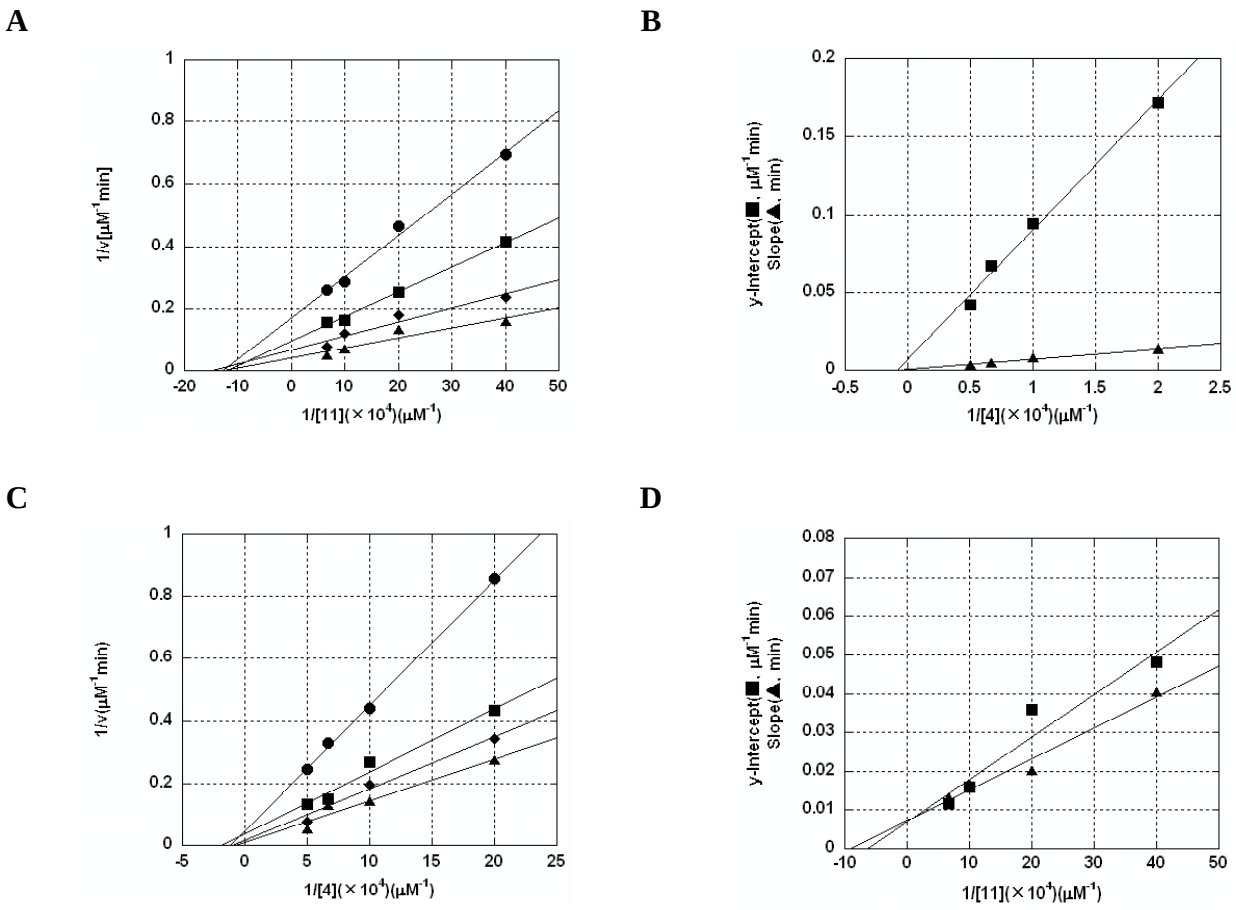


Figure S10. (**A**) Lineweaver-Burk plot with **4** held at four fixed concentrations while **11** was varied over concentrations ranging from 0.25 to 1.5 mM (●[**4**]=5 mM; ■[**4**]=10 mM; ◆[**4**]=15 mM; ▲[**4**]=20 mM); *v*, velocity. (**B**) Replot of *y* intercepts and slopes of Lineweaver-Burk plots as a function of $[\mathbf{4}]^{-1}$. (**C**) Analog plot was constructed with **11** held at four fixed concentrations while **4** was varied over concentrations ranging from 5 to 20 mM (●[**11**]=0.25 mM; ■[**11**]=0.5 mM; ◆[**11**]=1 mM; ▲[**11**]=1.5 mM). (**D**) Analogous plots were constructed to give kinetic constants for **11**. Reaction mixtures contained 2.5μM of antibody 25E2 and 5%(v/v) DMSO, 5% acetone, and 50mM Tris-HCl, pH8.0, and were incubated at 25℃.

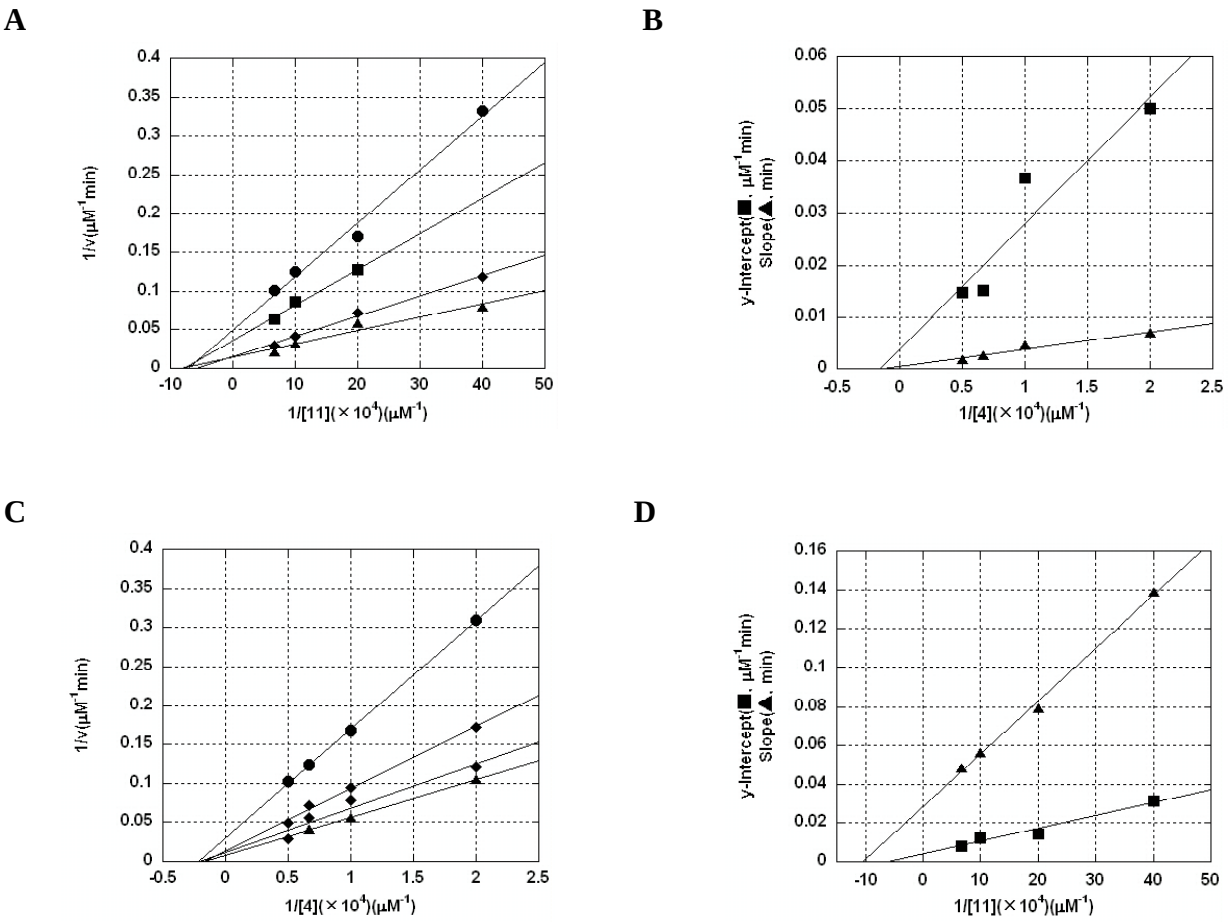


Figure S11. (**A**) Lineweaver-Burk plot with **4** held at four fixed concentrations while **11** was varied over concentrations ranging from 0.25 to 1.5 mM (●[**4**]=5 mM; ■[**4**]=10 mM; ◆[**4**]=15 mM; ▲[**4**]=20 mM); *v*, velocity. (**B**) Replot of *y* intercepts and slopes of Lineweaver-Burk plots as a function of $[\mathbf{4}]^{-1}$. (**C**) Analogous plot was constructed with **8** held at four fixed concentrations while **4** was varied over concentrations ranging from 5 to 20 mM (●[**11**]=0.25 mM; ■[**11**]=0.5 mM; ◆[**11**]=1 mM; ▲[**11**]=1.5 mM). (**D**) Analogous plots were constructed to give kinetic constants for **11**. Reaction mixtures contained 5 μM antibody 27C1, 5%(v/v) DMSO, 5% acetone, and 50 mM Tris-HCl, pH8.0, and were incubated at 25℃.

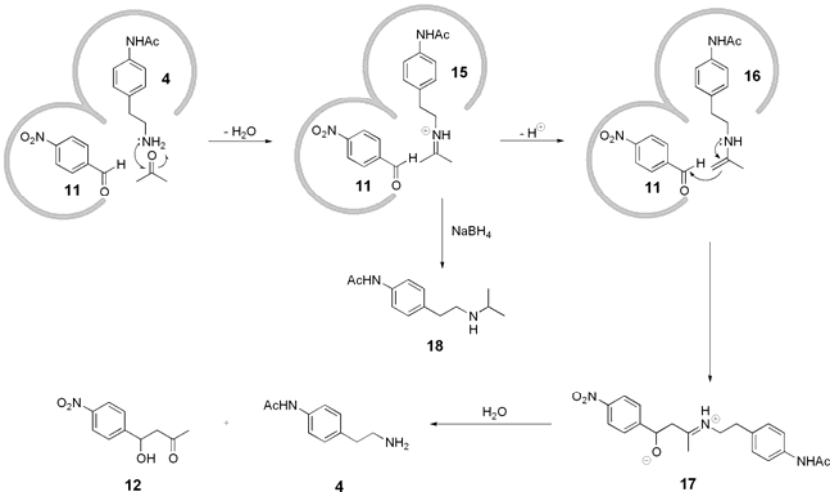
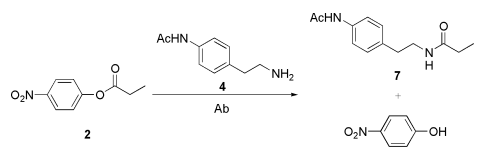
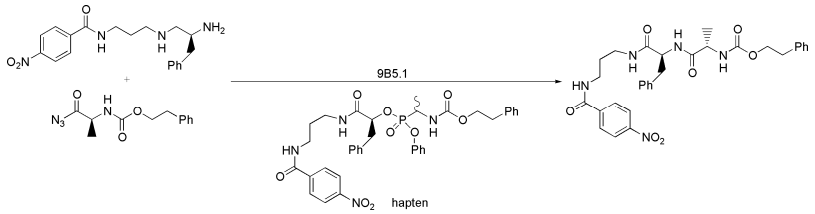
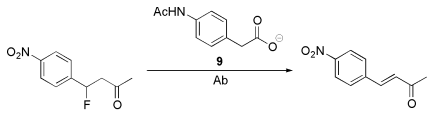
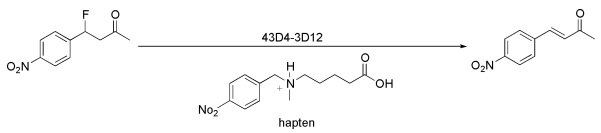
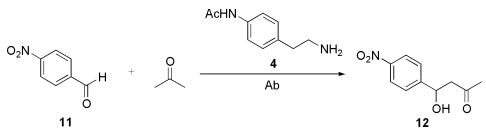
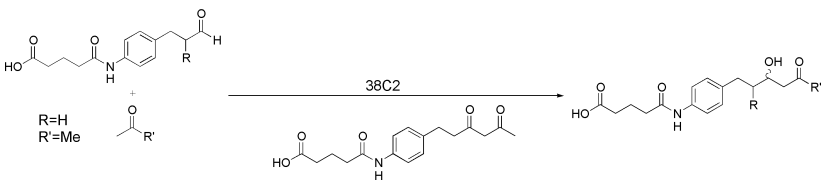


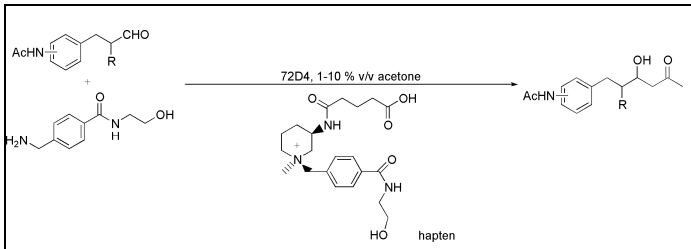
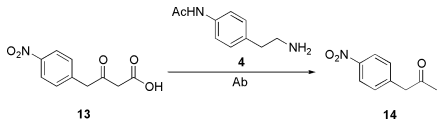
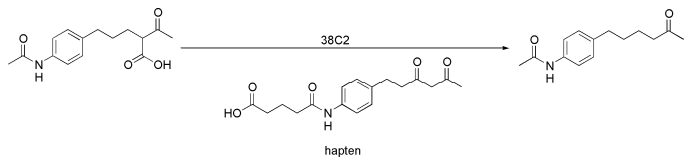
Figure S12. Antibody-catalyzed aldol reaction.

Formation of Isopropylamine 18

The assays were performed in a EYELA DIGITAL UNI ACE UA-100 water bath (±0.1) at 25℃ in a 50 mM Tris-HCl, pH 8.0, buffer system with 5% v/v DMSO, 4% v/v acetone and 10 mM $NaBH_4$. The reaction was followed by monitoring formation of **18** by the reversed phase HPLC. The analytical HPLC was performed on a Hitachi L-2130 unit equipped with a Hitachi L-2400 UV detector, using a YMC ODS AM303 column eluted with acetonitrile/aqueous TFA (0.1%, 15:85) at a flow rate of 1.0 mL/min with detection at 263 nm. The retention time of **18** was 6.4 min. A solution of **4** in DMSO (1 mM final concentration) and $NaBH_4$ in 0.1% (w/v) aqueous NaOH solution (10 mM final concentration) was added to an Eppendorf tube, containing the buffer solution (45 μL) with or without antibodies (25E2, 27C1) (For 25E2, 10 μM; For 27C1, 20 μM), respectively. The reaction was then initiated by addition of acetone (2.0 μL) into the mixture. Pairs of identical antibody solutions, with and without hapten **1** as an inhibitor, were used for measuring the background and catalyzed reaction rates, respectively. Samples (10 μL) were quenched a certain time periods by being mixed with 5 μL (DMSO 4.8 μL, TFA 0.2 μL), immediately frozen, and later analyzed by HPLC.

Table S4. Comparison of the catalytic activities for the antibody-catalyzed reactions between our antibodies and the other "specialized" catalytic antibodies.

Acyl-transfer reaction	catalytic activity
	25E2 $k_{cat}/K_m/k_{uncat} = 1.4\times10^4$ 27C1 $k_{cat}/K_m/k_{uncat} = 5.5\times10^4$
 Jacobsen, J. R.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, 91, 5888.	$k_{cat}/K_m/k_{uncat} = 1.0\times10^4$
β-Elimination reaction	catalytic activity
	25E2 $k_{cat}/K_m/k_{uncat} = 2.4\times10^5$ 27C1 no reaction
 Shokat, K. M.; Leumann, C. J.; Sugasawara, R.; Schultz, P. G. *Nature*, **1989**, 338, 269.	$k_{cat}/k_{uncat} = 8.8\times10^4$
Aldol reaction	catalytic activity
	25E2 $k_{cat}/K_m/k_{uncat} = 4.4\times10^4$ 27C1 $k_{cat}/K_m/k_{uncat} = 4.4\times10^4$
 Wanger, J.; Lerner, R.A.; Barbas III, C.F. *Science*, **1995**, 270, 1797.	$k_{cat}/k_{uncat} = 2.9\times10^4$

 Reymond, J.-L.; Chen, Y. *J. Org. Chem.* **1995**, 60, 6970.	k_{cat}/k_{uncat} = 0.13-1.7$\times 10^2$ (app.)
Decarboxylation reaction	catalytic activity
	27C1 $k_{cat}/K_m/k_{uncat} = 1.3\times 10^5$
 Björnestedt, R.; Zhong, G.; Lerner, R. A.; Barbas III, C. F. *J.Am. Chem. Soc.* **1996**, 118, 11720.	$k_{cat}/k_{uncat} = 1.5\times 10^4$