Electronic Supplementary Information

Discovery of hydrolytic catalysts in a peptidocalixarene library by binding assay with a transition state analogue of the hydrolysis.

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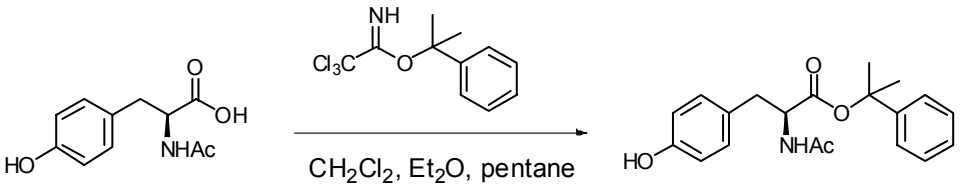
Experimental details and characterization of compounds

Contents

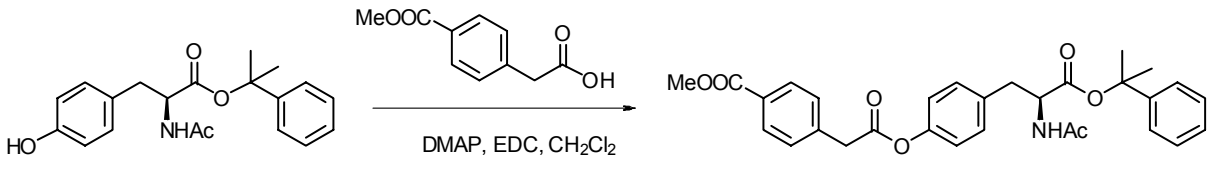
1. Preparation of substrates and authentic samples for quantitative HPLC analyses

All reactions involving air- and moisture-sensitive reagents were carried out using oven dried glassware and standard syringe-septum cap techniques. Routine monitorings of reaction were carried out using glass-supported Merck silica gel 60 F254 TLC plates. Column chromatography was performed on Kanto Chemical Silica Gel 60N (spherical, neutral 40–50 μm) with the solvents indicated. All solvents and reagents were used as supplied with following exceptions. Measurements of optical rotations were performed with a JASCO DIP-370 automatic digital polarimeter. Melting points were taken on a Yanaco MP-3 micro melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were measured with Mercury-300 (300 MHz), GX-400 (400 MHz) spectrometers. Chemical shifts were expressed in ppm using Me_4Si ($\delta = 0$) as an internal standard. The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint) broad (br). Infrared (IR) spectral measurements were carried out with a JASCO FT/IR-4100 spectrometer (ATR method). Low- and High-resolution mass (HRMS) spectra were measured on a JEOL JMS-DX 303/JMA-DA 5000 SYSTEM high resolution mass spectrometer.



1a

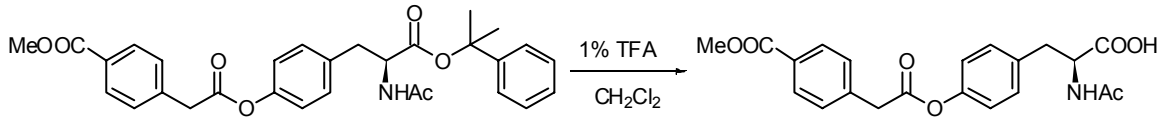
To a stirred solution of *N*-Acetyl-L-tyrosine (1.82 g, 8.06 mmol) in 4:5 CH_2Cl_2/Et_2O mixed solvent (100 mL) was added 2-phenylisopropyltrichloroacetamidate[1] (11 mL of *n*-pentane solution (1.25 mol L^{-1}), 13.8 mmol) at rt. After stirring for 5 h, the mixture was evaporated. The residue was purified by column chromatography (hexane/EtOAc, 1:1) to give **1a** (1.95 g, 5.81 mmol, 70% yield) as a colorless amorphous oil.
$[\alpha]_D^{21}$= +39.5 (*c* 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.21 (5H, m), 6.92 (2H, d, *J* = 8.0 Hz), 6.63 (2H, d, *J* = 8.0 Hz), 5.91 (1H, d, *J* = 6.5 Hz), 4.79 (1H, td, *J* = 6.5, 6.5 Hz), 3.05 (1H, dd, *J* = 14.0, 6.1 Hz), 2.87 (1H, dd, *J* = 14.0, 6.1 Hz), 1.85 (3H, s), 1.71 (3H, s), 1.69 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8 (s), 170.7 (s), 155.6 (s), 144.7 (s), 130.4 (d), 128.3 (d), 127.3 (s), 126.9 (d), 124.3 (d), 115.4 (d), 83.7 (s), 53.7 (d), 37.2 (t), 28.8 (q), 27.8 (q), 22.9 (q). IR (ATR): 3326, 1729, 1651, 1613 cm^{-1}. MS (FAB): *m/z* 342 $[M+H]^+$, 364 $[M+Na]^+$, 380 $[M+K]^+$. HRMS (FAB): calcd for $C_{20}H_{24}O_4N$ $[M+H]^+$, 342.1705, Found 342.1725.



1a **1b**

To a stirred solution of **1a** (949.8 mg, 2.78 mmol), 2-(4-(methoxycarbonyl)phenyl)acetic acid[2] (648.3 g, 3.34 mmol) and 4-(dimethylamino)pyridine (DMAP) (34.2 mg, 0.279 mmol) in CH_2Cl_2 (15 mL) was added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) (1.60 g, 8.47 mmol) at rt. After stirring for 5 h, the reaction was quenched by addition of saturated aqueous NH_4Cl and the mixture was diluted with ethyl acetate. The phases were separated and the aqueous phase was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography

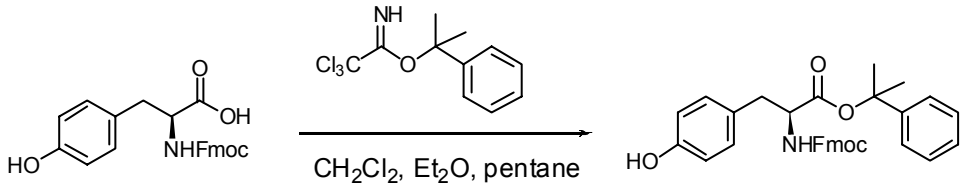
($CHCl_3$/MeOH, 20:1) to give **1b** (971.4 mg, 1.88 mmol, 68% yield, 2 steps) as a colorless oil.
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (2H, d, *J* = 8.5 Hz), 7.45 (2H, d, *J* = 8.5 Hz), 7.35-7.20 (5H, m), 7.13 (2H, d, *J* = 8.5 Hz), 6.97 (2H, d, *J* = 8.5, Hz), 5.88 (1H, d, *J* = 7.7 Hz), 4.83 (1H, dt, *J* = 7.7, 6.2 Hz), 3.92 (3H, s), 3.91 (2H, s), 3.14 (1H, dd, *J* = 14.0, 6.2 Hz), 3.06 (1H, dd, *J* = 14.0, 6.2 Hz), 1.92 (3H, s), 1.75 (3H, s), 1.71 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (s), 169.7 (s), 169.2 (s), 166.7 (s), 149.5 (s), 144.6 (s), 138.4 (s), 133.9 (s), 130.4 (d), 129.9 (d), 129.4 (d), 129.2 (s), 128.2 (d), 127.3 (d), 124.3 (d), 121.2 (d), 83.5 (s), 53.3 (d), 52.1 (q), 41.2 (t), 37.2 (t), 28.8 (q), 27.5 (q), 23.0 (q). IR (ATR) : 3295, 1722, 1658 cm^{-1}. MS (FAB): *m*/*z* 518 $[M+H]^+$, 540 $[M+Na]^+$, 556 $[M+K]^+$. HRMS (FAB): calcd for $C_{30}H_{31}O_7NNa$ $[M+Na]^+$, 540.1998, Found 540.2029.



82%

1b **1**

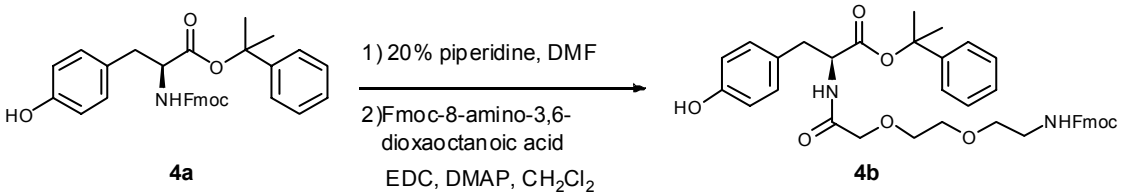
A solution of **1b** (971.4 mg, 1.88 mmol) in 1% TFA-CH_2Cl_2 (20 mL) was stirred for 2 h., The mixture was concentrated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$/MeOH, 20:1 to 10:1) to give **1** (611.7 mg, 1.53 mmol, 82 %) as a colorless solid.
mp 174.0–176.0°C. $[\alpha]_D^{22}$ = +34.0 (*c* 1.00, MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 8.01 (2H, d, *J* = 8.5 Hz), 7.49 (2H, d, *J* = 8.5 Hz), 7.24 (2H, d, *J* = 8.6 Hz), 7.00 (2H, d, *J* = 8.6 Hz), 4.65 (1H, dd, *J* = 8.9, 4.9 Hz), 3.98 (2H, s), 3.90 (3H, s), 3.19 (1H, dd, *J* = 14.0, 4.9 Hz), 2.95 (1H, dd, *J* = 14.0, 8.9 Hz), 1.90 (3H, s). ^{13}C NMR (75 MHz, CD_3OD) δ 174.8 (s), 173.5 (s), 171.6 (s), 168.6 (s), 151.4 (s), 141.1 (s), 136.6 (s), 131.6 (d), 131.11 (d), 131.08 (d), 130.6 (s), 122.8 (d), 55.3 (d), 52.9 (q), 41.9 (t), 38.0 (t), 22.6 (q). IR (ATR): 1743, 1718, 1700, 1650 cm^{-1}. MS (FAB): *m*/*z* 400 $[M+H]^+$, 422 $[M+Na]^+$, 438 $[M+K]^+$. HRMS (FAB): calcd for $C_{21}H_{22}O_7N$ $[M+H]^+$, 400.1397, Found 400.1397.



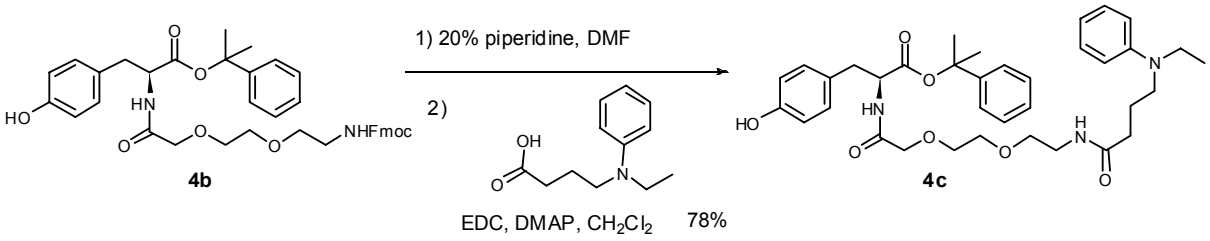
78%

4a

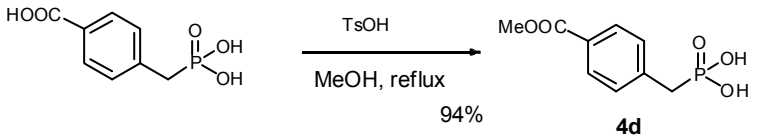
To a stirred solution of Fmoc-L-tyrosine (1.68 g, 4.17 mmol) in 4:5 CH_2Cl_2/Et_2O mixed solvent (90 mL) was added 2-phenylisopropyltrichloroacetamidate[1] (6.7 mL of *n*-pentane solution (1.25 mol L^{-1}), 8.33 mmol) at rt. After stirring for 5 h, the reaction was quenched by addition of H_2O (70 μL). The mixture was evaporated and the residue was purified by column chromatography (hexane/EtOAc, 3:1) to give **4a** (1.72 g, 3.29 mmol, 78% yield) as a colorless solid.
mp 125.0–126.0°C. $[\alpha]_D^{20}$ = +11.6 (*c* 1.00, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.75 (2H, d, *J* = 7.4 Hz), 7.54 (2H, d, *J* = 7.4 Hz), 7.39 (2H, t, *J* = 7.4 Hz), 7.35-7.25 (7H, m), 7.00 (2H, d, *J* = 8.2 Hz), 6.72 (2H, d, *J* = 8.2 Hz), 5.23 (1H, d, *J* = 8.1 Hz), 5.08 (1H, brs), 4.60 (1H, dt, *J* = 8.1, 8.1 Hz), 4.40 (1H, dd, *J* = 7.0, 10.7 Hz), 4.30 (1H, dd, *J* = 7.0, 10.7 Hz), 4.18 (1H, t, *J* = 7.0 Hz), 3.08 (1H, dd, *J* = 8.1, 13.9 Hz), 3.01 (1H, dd, *J* = 8.1, 13.9 Hz), 1.77 (3H, s), 1.75 (3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) d 170.5 (s), 155.8 (s), 155.1 (s), 144.7 (s), 143.7 (s), 143.6(s), 141.2(s), 130.5 (d), 128.3 (d), 127.7 (d), 127.3 (d), 127.0 (d), 125.1 (d), 124.3 (d), 119.9 (d), 115.4 (d), 83.7 (s), 67.1 (t), 55.3 (d), 47.0 (d), 37.4 (t), 28.6 (q), 27.9 (q). IR (ATR): 3333, 1698, 1614 cm^{-1}. MS (FAB): *m*/*z* 544 $[M+Na]^+$, 560 $[M+K]^+$. HRMS (FAB): calcd for $C_{33}H_{31}O_5NNa$ $[M+Na]^+$, 544.2100, Found 544.2105.



A solution of **4a** (1.1462 g, 2.20 mmol) in 20% piperidine-DMF (20 mL) was stirred for 30 min. The mixture was concentrated under reduced pressure. The residue was roughly purified by column chromatography (AcOEt) to give a Fmoc deprotected amine (656.3 mg) as a colorless oil. The amine was dissolved in CH_2Cl_2 (10 mL). To the solution was added Fmoc-8-amino-3,6-dioxaoctanoic acid (849.9 mg, 2.20 mmol), 4-(dimethylamino)pyridine (DMAP) (26.8 mg, 0.219 mmol) and *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) (1.01 g, 5.74 mmol). After stirring for 1 h, the reaction was quenched by addition of saturated aqueous NH_4Cl and the mixture was diluted with CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (hexane/EtOAc, 1:1) to give **4b** (1.3536 g, 2.03 mmol, 92% yield, 2 steps) as a colorless oil.
$[\alpha]_D^{23}$ = +13.8 (*c* 0.91, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (2H, d, *J* = 7.4 Hz), 7.59 (2H, d, *J* = 7.4 Hz), 7.39 (2H, t, *J* = 7.4 Hz), 7.32-7.18 (6H, m), 7.11 (1H, d, *J* = 8.5 Hz), 6.97 (2H, d, *J* = 8.2 Hz), 6.70 (2H, d, *J* = 8.2 Hz), 5.37 (1H, t. *J* = 5.5 Hz), 4.90 (1H, dt, *J* = 8.5, 6.0 Hz), 4.42 (2H, d, *J* = 6.9 Hz), 4.21 (1H, t, *J* = 6.7 Hz), 3.92 (2H, s), 3.53-3.18 (8H, m), 3.13 (1H, dd, *J* = 14.0, 6.3 Hz), 3.00 (1H, dd, *J* = 14.0, 6.3 Hz), 1.77 (3H, s), 1.76 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.94 (s), 169.91 (s), 156.5(s), 155.7 (s), 144.4 (s), 143.5 (s), 140.9 (s), 130.2 (d), 127.9 (d), 127.3 (d), 126.9 (d), 126.7 (d), 126.3 (s), 124.7 (d), 124.0 (d), 119.6 (d), 115.2 (d), 83.3 (s), 77.2 (d), 70.6 (t), 69.7 (t), 69.69 (t), 69.5 (t), 66.3 (t), 52.9 (d), 46.8 (d), 40.4 (t), 37.0 (t), 28.1 (q), 27.6 (q). IR (ATR): 3328, 1700, 1663, 1614 cm^{-1}. MS (FAB): *m*/z 689 $[M+Na]^+$, 705 $[M+K]^+$. HRMS (FAB): calcd for $C_{39}H_{42}O_8N_2Na$ $[M+Na]^+$, 689.2838, Found 689.2817.

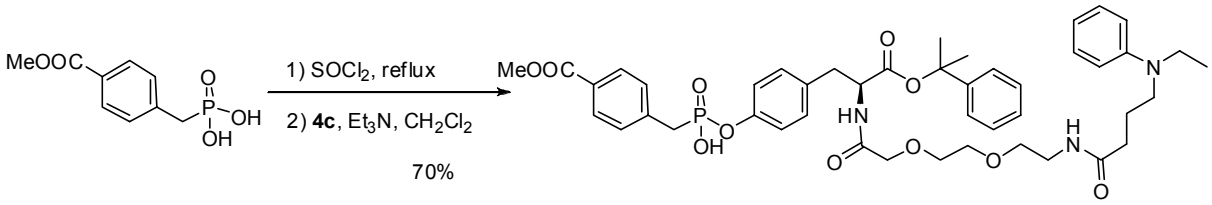


A solution of **4b** (892.1 mg, 1.34 mmol) in 20% piperidine-DMF (15 mL) was stirred for 30 min. The mixture was concentrated under reduced pressure. The residue was roughly purified by column chromatography (AcOEt to AcOEt/MeOH) to give a Fmoc deprotected amine (519.0 mg) as a colorless oil. The amine was dissolved in CH_2Cl_2 (10 mL). To the solution was added 4-(ethyl(phenyl)amino)butanoic acid[3] (237.8 mg, 1.15 mmol), 4-(dimethylamino)pyridine (DMAP) (14.5 mg, 0.115 mmol) and *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) (659.8 g, 3.45 mmol). After stirring for 2 h, the reaction was quenched by addition of saturated aqueous NH_4Cl and the mixture was diluted with CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (hexane/EtOAc, 1:1) to give **4c** (658.7 mg, 1.04 mmol, 78% yield, 2 steps) as a colorless oil.
$[\alpha]_D^{23}$ = +9.33 (*c* 1.05, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (1H, brs), 7.36-7.15 (7H, m), 6.99 (2H, d, *J* = 8.5 Hz), 6.75 (2H, d, *J* = 8.5 Hz), 6.68-6.61 (3H, m), 6.38 (1H, brt, *J* = 5.8 Hz), 4.94 (1H, dt, *J* = 8.5, 6.3 Hz), 3.91 (1H, d, *J* = 15.9 Hz), 3.89 (1H, d, *J* = 15.9 Hz), 3.55-3.14 (13H, m), 2.97 (1H, dd, *J* = 14.0, 6.8 Hz), 2.10 (2H, t, *J* = 7.3 Hz), 1.86 (2H, quint, *J* = 7.3 Hz), 1.76 (3H, s), 1.75 (3H, s), 1.11 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1 (s), 170.3 (s), 169.9 (s), 155.9 (s), 147.5 (s), 144.4 (s), 130.1 (d), 128.9 (d), 128.0 (d), 126.9 (d), 126.0 (s), 123.9 (d), 115.3 (d), 115.2 (d), 111.7 (d), 83.4 (s), 70.6 (t), 69.64 (t), 69.6 (t), 69.5 (t), 52.8 (d), 49.2 (t), 44.4 (t), 38.9 (t), 37.1 (t), 33.0 (t), 28.5 (q), 27.7 (q), 23.0 (t), 11.8 (q); IR (ATR) : 3305, 1733, 1648 cm^{-1}; MS (FAB): *m*/*z* 634 $[M+H]^+$,656 $[M+Na]^+$, 672 $[M+K]^+$; HRMS (FAB): calcd for $C_{36}H_{48}O_7N_3$ $[M+H]^+$, 634.3493, Found 634.3491.



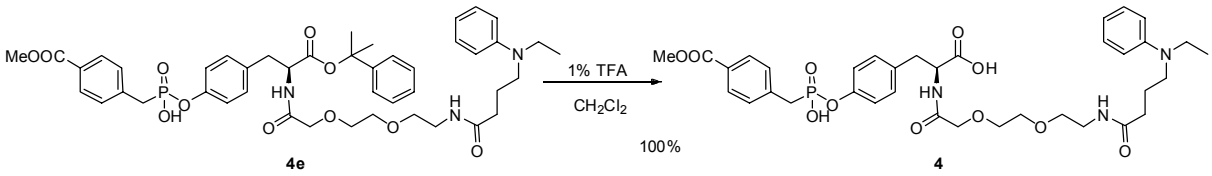
4d

To a stirred solution of 4-(phosphonomethyl)benzoic acid[4] (513.4 mg, 2.37 mmol) in MeOH (5 mL) was added *p*-toluenesulfonic acid monohydrate (10.0 mg, 0.052 mmol). After refluxing for 23 h, the reaction mixture was concentrated. The residue was purified by reversed phase HPLC (H_2O/MeOH, 7:3, column COSMOSIL^{TM}, 5C_{18}-PAQ) to give **4d** (511.5 mg, 2.22 mmol, 94%) as colorless solid.
mp 153.0–155.0°C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.94 (2H, d, *J* = 8.0 Hz), 7.41 (2H, dd, *J* = 8.0, 1.7 Hz), 3.88 (3H, s), 3.20 (2H, d, *J* = 22.2 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 168.7 (s), 140.8 (s), 140.7 (s), 131.5 (d), 131.4 (d), 130.8 (d), 129.8 (s), 52.9 (q), 36.4 (dt, $J_{P\text{-}C}$ = 133.4 Hz). IR (ATR): 2959, 1720, 1280 cm^{-1}. MS (CI): *m*/*z* 231 $[M+H]^+$. HRMS (CI): calcd for $C_9H_{12}O_5P$ $[M+H]^+$, 231.0422, Found 231.0437.



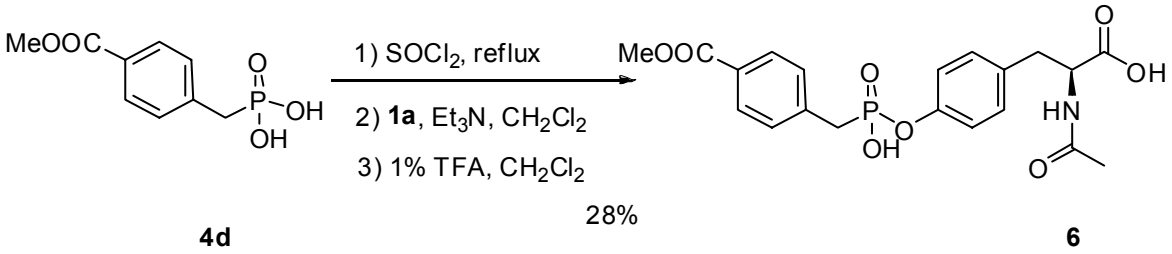
4d **4e**

A solution of **4d** (141.8 mg, 0.66 mmol) in $SOCl_2$ (4 mL) was refluxed for 24 h. The mixture was concentrated under reduced pressure. To the residue was added **4c** (277.2 mg, 0.44 mmol) and Et_3N (0.2 mL, 1.42 mmol) in CH_2Cl_2 (2 mL). After refluxing for 6 h, the reaction mixture was concentrated. The residue was roughly purified by column chromatography ($CHCl_3$/MeOH, 100:0 to 5:1) and then purified by normal phase HPLC ($CHCl_3$/MeOH, 8:2, column COSMOSIL^{TM}, 5SL-II) to give **4e** (258.2 mg, 0.031 mmol, 66%) as colorless oil.
$[\alpha]_D^{22}$ = +37.9 (*c* 0.68, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (2H, d, *J* = 7.9 Hz), 7.45-7.41 (4H, m), 7.39-7.29 (6H, m), 7.25-7.21 (1H, m), 7.17 (2H, d, *J* = 8.2 Hz), 7.15 (1H, t, *J* = 8.0 Hz), 7.03 (2H, d, *J* = 8.4 Hz), 4.87 (1H, dt, *J* = 7.8, 5.3 Hz), 3.99 (1H, d, *J* = 15.8 Hz), 3.89 (1H, d, *J* = 15.8 Hz), 3.89 (3H, s), 3.54-3.41 (4H, m), 3.34-3.00 (12H, m), 2.04 (2H, m), 1.79 (3H, s), 1.785 (3H, s), 1.72-1.59 (2H, m), 0.92 (3H, t, *J* = 7.2 Hz). IR (ATR): 3382, 1717, 1657, 1607, 1281 cm^{-1}. MS (FAB): *m*/*z* 846 $[M+H]^+$, 868 $[M+Na]^+$, 884 $[M+K]^+$. HRMS (FAB): calcd for $C_{45}H_{57}O_{11}N_3P$ $[M+H]^+$, 846.3731, Found 846.3732.

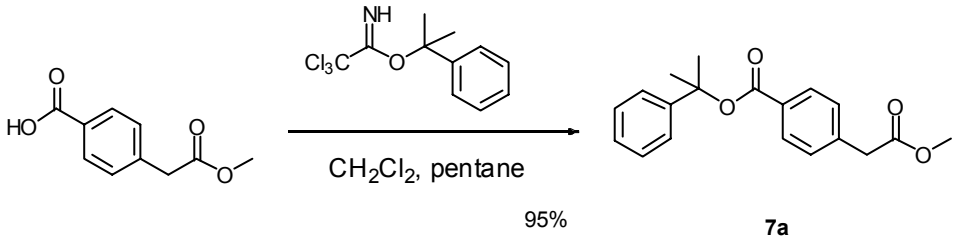


4e **4**

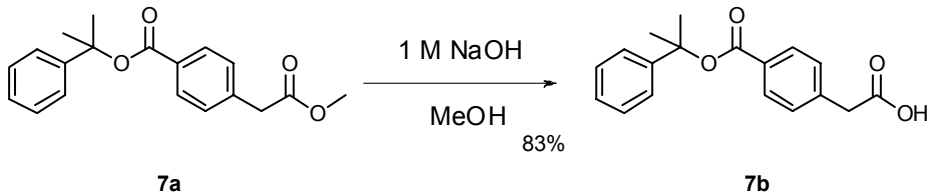
A solution of **4e** (18.6 mg, 0.022 mmol) in 1% trifluoroacetic acid (TFA) - CH_2Cl_2 (5 mL) was stirred for 20 min. The mixture was concentrated under reduced pressure. The residue was purified by reversed phase HPLC (1% TFA-H_2O/MeCN, 7:3, column COSMOSIL^{TM}, C_{18}-PAQ) to give **4e** (16.3 mg, 0.022 mmol, 100%) as colorless oil.
$[\alpha]_D^{18}$ = +20.3 (*c* 0.34, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (2H, d, *J* = 8.3 Hz), 7.61-7.51 (5H, m), 7.46 (2H, d, *J* = 6.8 Hz), 7.17 (2H, d, *J* = 8.6 Hz), 7.07 (2H, d, *J* = 8.4 Hz), 4.73-4.70 (1H, m), 3.96 (2H, d, *J* = 2.2 Hz), 3.65-3.56 (6H, m), 3.53-3.44 (6H, m), 3.37-3.33 (2H, m), 3.23 (1H, dd, *J* = 14.1, 4.8 Hz), 3.04 (1H, dd, *J* = 14.1, 8.0 Hz), 2.26 (2H, td, *J* = 6.6, 1.5 Hz), 1.66 (2H, quint, *J* = 7.2 Hz), 1.03 (3H, t, *J* = 7.2 Hz). IR (ATR) : 1716, 1663, 1650, 1280 cm^{-1}. MS (FAB): *m*/*z* 728 $[M+H]^+$, 750 $[M+Na]^+$, 766 $[M+K]^+$. HRMS (FAB): calcd for $C_{36}H_{46}O_{11}N_3PNa$ $[M+Na]^+$, 750.2767, Found 750.2750.



A solution of **4d** (101.2 mg, 0.44 mmol) in $SOCl_2$ (5 mL) was refluxed for 11 h. The mixture was concentrated under reduced pressure. To the residue was added **1a** (150.1 mg, 0.44 mmol) and Et_3N (0.15 mL, 1.07 mmol) in CH_2Cl_2 (5 mL). After refluxing for 4 h, the reaction mixture was concentrated. The residue was roughly purified by column chromatography ($CHCl_3$/MeOH, 20:1) to give the phosphoric ester as colorless oil. A solution of the phosphoric ester in 1% trifluoroacetic acid (TFA) - CH_2Cl_2 (10 mL) was stirred for 10 min. The mixture was concentrated under reduced pressure. The residue was purified by reversed phase HPLC (1% TFA-H_2O/MeCN, 3:1, column COSMOSIL^{TM}, C_{18}-PAQ) to give **6** (52.8 mg, 0.012 mmol, 28%) as colorless solid.
mp 88.0–90.5°C. $[\alpha]_D^{22}$ = +22.1 (*c* 1.00, MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 7.97 (2H, d, *J* = 7.7 Hz), 7.45 (2H, dd, *J* = 7.7 Hz), 7.20 (2H, d, *J* = 8.5 Hz), 7.06 (2H, d, *J* = 8.5 Hz), 4.62 (1H, dd, *J* = 9.1, 5.2 Hz), 3.89 (3H, s), 3.41 (2H, d, *J* = 22.4 Hz), 3.17 (1H, dd, *J* = 14.0, 5.2 Hz), 2.91 (1H, dd, *J* = 14.0, 9.1 Hz), 1.90 (3H, s). ^{13}C NMR (75 MHz, CD_3OD) δ 174.8 (s), 173.5 (s), 168.6 (s), 151.1 (d, $J_{P\text{-}C}$ = 8.6 Hz), 139.5 (d, $J_{P\text{-}C}$ = 9.4 Hz), 135.5 (s), 131.8 (d), 131.6 (dd, $J_{P\text{-}C}$ = 6.6 Hz), 130.9 (dd, $J_{P\text{-}C}$ = 2.8 Hz), 130.2 (d, $J_{P\text{-}C}$ = 3.7 Hz), 121.8 (dd, $J_{P\text{-}C}$ = 4.3 Hz), 55.4 (d), 52.9 (q), 37.9 (t), 36.1 (dt, $J_{P\text{-}C}$ = 137.7 Hz), 22.6 (q). IR (ATR): 3261, 1717, 1656, 1280 cm^{-1}; MS (FAB): *m/z* 436 $[M+H]^+$, 458 $[M+Na]^+$, 474 $[M+K]^+$. HRMS (FAB): calcd for $C_{20}H_{22}O_8NPNa$ $[M+Na]^+$, 458.0981, Found 458.0998.

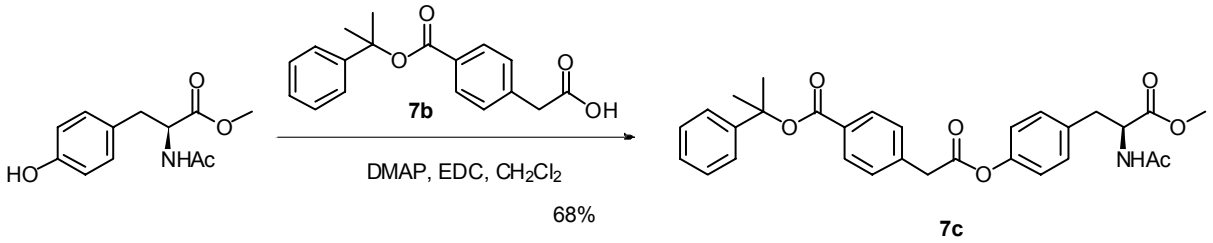


To a stirred solution of 2-(4-(methoxycarbonyl)phenyl)acetic acid[2] (1.02 g, 5.27 mmol) in CH_2Cl_2 (10 mL) was added 2-phenylisopropyltrichloroacetamidate[1] (7.2 mL of *n*-pentane solution (1.25 mol L^{-1}), 9.00 mmol) at rt. After stirring for 10 h, the reaction was quenched by addition of H_2O and the mixture was diluted with CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (hexane/AcOEt, 5:1 to 3:1) to give **7a** (1.56 mg, 5.02 mmol, 95% yield) as a colorless oil.
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (2H, d, *J* = 8.2 Hz), 7.43-7.39 (2H, m), 7.36-7.21 (5H, m), 3.69 (3H, s), 3.68 (2H, s), 1.90 (6H, s).



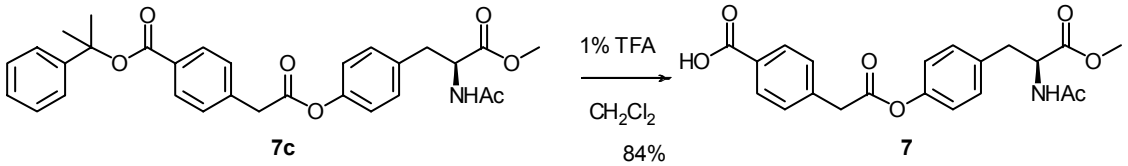
To a stirred solution of **7a** (43.0 mg, 0.138 mmol) in MeOH (3 mL) was added 1 mol L^{-1} NaOH aqueous solution (1.0 mL 1.0 mmol) at rt. After stirring for 3 h, the reaction was quenched by addition of saturated aqueous NH_4Cl and the mixture was diluted with CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (hexane/EtOAc, 2:1 to 1:1) to give **7b** (34.2 mg, 0.115 mmol, 83% yield) as a colorless solid.

mp 116.0–117.0°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (2H, d, *J* = 8.3 Hz), 7.43-7.40 (2H, m), 7.38-7.21 (5H, m), 3.71 (2H, s), 1.91 (6H, s). IR (ATR): 3100, 1712 cm^{-1}. MS (CI): *m/z* 298 $[M]^+$, 299 $[M+H]^+$. HRMS (CI): calcd for $C_{18}H_{19}O_4$ $[M+H]^+$, 299.1284, Found 299.1281.



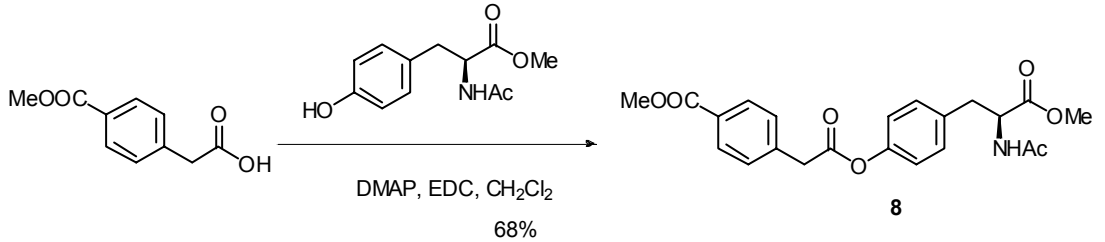
7c

To a stirred solution of *N*-acetyl-L-tyrosine methyl ester (136.9 mg, 0.459 mmol), **7b** (108.9 mg, 0.459 mmol) and 4-(dimethylamino)pyridine (DMAP) (5.6 mg, 0.046 mmol) in CH_2Cl_2 (10 mL) was added *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) (175.9 mg, 0.918 mmol) at rt. After stirring for 3 h, the reaction was quenched by addition of saturated aqueous NH_4Cl and the mixture was diluted with CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography ($CHCl_3$/MeOH, 9:1) to give **7c** (161.6 mg, 0.312 mmol, 68%) as a colorless oil.
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (2H, d, *J* = 8.2 Hz), 7.46-7.42 (4H, m), 7.37-7.32 (2H, m), 7.28-7.23 (1H, m), 7.09 (2H, d, *J* = 8.7 Hz), 6.99 (2H, d, *J* = 8.7 Hz), 5.93 (1H, d, *J* = 7.6 Hz), 4.87 (1H, dt, *J* = 7.6, 5.7 Hz), 3.91 (2H, s), 3.72 (3H, s), 3.15 (1H, dd, *J* = 13.8, 5.7 Hz), 3.08 (1H, dd, *J* = 13.8, 5.7 Hz), 1.99 (3H, s), 1.92 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9 (s), 169.7 (s), 169.3 (s), 164.8 (s), 149.6 (s), 145.6 (s), 138.1 (s), 133.6 (s), 130.6 (s), 130.1 (d), 129.9 (d), 129.3 (d), 128.3 (d), 127.0 (d), 124.2 (d), 121.4 (d), 82.3 (s), 53.0 (d), 52.3 (q), 41.3 (t), 37.1 (t), 28.7 (q), 23.0 (q). IR (ATR) : 3280, 1746, 1717, 1663 cm^{-1}; MS (FAB): *m/z* 518 $[M+H]^+$, 540 $[M+Na]^+$, 556 $[M+K]^+$. HRMS (FAB): calcd for $C_{30}H_{32}O_7N$ $[M+H]^+$, 518.2178, Found 518.2175.



7c 7

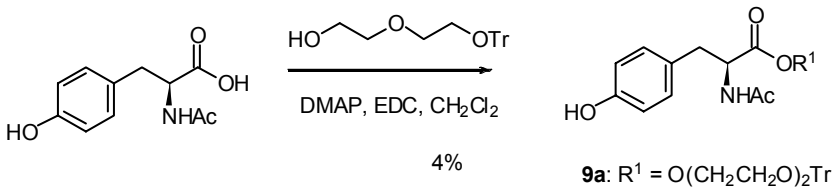
A solution of **7c** (96.4 mg, 0.186 mmol) in 1% trifluoroacetic acid (TFA) - CH_2Cl_2 (10 mL) was stirred for 2 h. The mixture was concentrated under reduced pressure. The residue was purified by normal phase HPLC ($CHCl_3$/MeOH, 9:1, column COSMOSIL^{TM}, 5SL-II) to give **7** (62.7 mg, 0.157 mmol, 84%) as colorless solid.
mp 129.5–131.0°C. $[\alpha]_D^{22}$ = +2.30 (*c* 0.50, MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 8.02 (2H, d, *J* = 8.5 Hz), 7.49 (2H, d, *J* = 8.5 Hz), 7.22 (2H, d, *J* = 8.5, 1.9 Hz), 7.01 (2H, dt, *J* = 8.5 Hz), 4.65 (1H, dd, *J* = 8.8, 5.8 Hz), 3.99 (2H, s), 3.68 (3H, s), 3.14 (1H, dd, *J* = 13.9, 5.8 Hz), 2.96 (1H, dd, *J* = 13.9, 8.8 Hz), 1.90 (3H, s). ^{13}C NMR (75 MHz, CD_3OD) δ 173.7 (s), 173.5 (s), 171.6 (s), 169.8 (s), 151.4 (s), 140.8 (s), 136.3 (s), 131.5 (d), 131.3 (d), 131.2 (s), 131.0 (d), 122.9 (d), 55.4 (d), 53.0 (q), 41.9 (t), 37.9 (t), 22.5 (q). IR (ATR): 3316, 1741, 1710, 1648, 1612 cm^{-1}; MS (CI): *m/z* 400 $[M+H]^+$. HRMS (CI): calcd for $C_{21}H_{22}O_7N$ $[M+H]^+$, 400.1396, Found 400.1404.



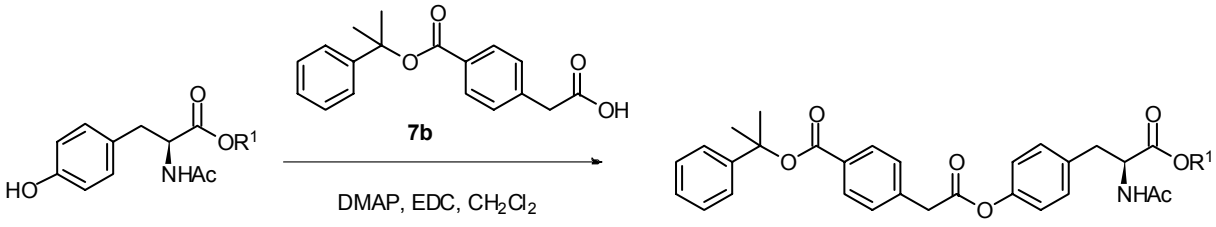
8

To a stirred solution of *N*-acetyl-L-tyrosine methyl ester (280.4 mg, 1.18 mmol), 2-(4-(methoxycarbonyl)phenyl)acetic acid[2] (229.5 mg, 1.18 mmol) and 4-(dimethylamino)pyridine (DMAP) (14.5 mg, 0.118 mmol) in CH_2Cl_2 (10 mL) was added *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) (453.1 mg, 2.36 mmol) at rt. After stirring for 6 h, the reaction was quenched by addition

of saturated aqueous NH_4Cl and the mixture was diluted with CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography ($CHCl_3$/MeOH, 9:1) to give **8** (477.9 mg, 1.16 mmol, 68%) as a colorless oil.
$[\alpha]_D^{21}$ = +3.03 (*c* 0.95, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (2H, d, *J* = 8.2 Hz), 7.45 (2H, d, *J* = 8.2 Hz), 7.08 (2H, d, *J* = 8.5 Hz), 6.98 (2H, d, *J* = 8.5 Hz), 5.92 (1H, d, *J* = 7.7 Hz), 4.86 (1H, dt, *J* = 7.7, 5.7 Hz), 3.92 (3H, s), 3.91 (2H, s), 3.71 (3H, s), 3.14 (1H, dd, *J* = 13.7, 5.7 Hz), 3.08 (1H, dd, *J* = 13.7, 5.7 Hz), 1.98 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7 (s), 169.7 (s), 169.1 (s), 166.5 (s), 149.4 (s), 138.3 (s), 133.7 (s), 130.0 (d), 129.7 (d), 129.2 (d), 129.0 (s), 121.2 (d), 53.0 (d), 52.1 (q), 51.9 (q), 41.0 (t), 36.9 (t), 22.7 (q). IR (ATR) : 1723, 1660 cm^{-1}. MS (CI): *m*/*z* 414 $[M+H]^+$. HRMS (CI): calcd for $C_{22}H_{24}O_7N$ $[M+H]^+$, 414.1553, Found 414.1562.

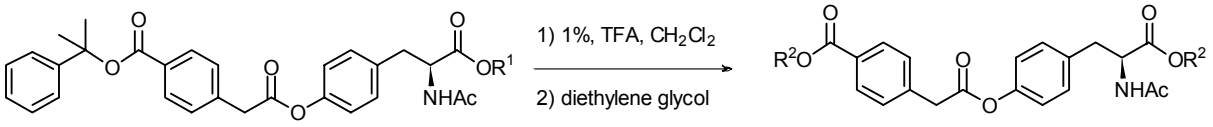


To a stirred solution of *N*-acetyl-L-tyrosine (640.7 mg, 2.87 mmol), diethylene glycol monotrityl ether[5] (1.51 g, 4.31 mmol) and 4-(dimethylamino)pyridine (DMAP) (36.5 mg, 0.299 mmol) in CH_2Cl_2 (10 mL) and DMF (0.5 mL) was added *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) (1.65 g, 8.61 mmol) at rt. After stirring for 3 h, the reaction was quenched by addition of saturated aqueous NH_4Cl and the mixture was diluted with CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography ($CHCl_3$/AcOEt, 3:1) to give **9a** (63.9 mg, 0.115 mmol, 4%) as a colorless oil.
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47-7.43 (6H, m), 7.31-7.16 (9H, m), 6.93 (2H, d, *J* = 8.5 Hz), 6.65 (2H, d, *J* = 8.4 Hz), 6.00 (1H, d, *J* = 7.8 Hz), 4.88 (1H, dt, *J* = 7.8, 5.6 Hz), 4.35 (1H, dt, *J* = 12.3, 5.0 Hz), 4.26 (1H, dt, *J* = 12.3, 5.0 Hz), 3.73 (2H, t, *J* = 5.0 Hz), 3.67 (2H, t, *J* = 5.0 Hz), 3.25 (2H, t, *J* = 5.0 Hz), 3.07 (1H, dd, *J* = 14.0, 5.6 Hz), 2.98 (1H, dd, *J* = 14.0, 5.6 Hz), 1.91 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7 (s), 170.3 (s), 155.6 (s), 143.9 (s), 130.3 (s), 128.6 (d), 127.8 (d), 127.0 (d), 126.7 (s), 115.5 (d), 86.6 (s), 70.6 (t), 68.8 (t), 64.6 (t), 63.2 (t), 53.3 (d), 37.0 (t), 22.9 (q). IR (ATR): 3294, 1738, 1654 cm^{-1}. MS (FAB): *m*/*z* 576 $[M+Na]^+$, 592 $[M+K]^+$. HRMS (FAB): calcd for $C_{34}H_{35}NO_6Na$ $[M+Na]^+$, 576.2362, Found 576.2380.



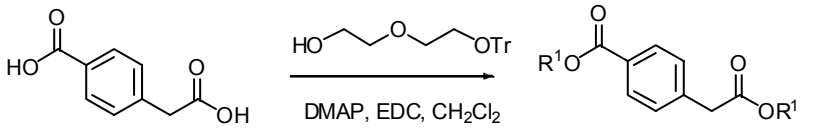
To a stirred solution of **9a** (75.8 mg, 0.137 mmol), **7b** (40.8 mg, 0.137 mmol) and 4-(dimethylamino)pyridine (DMAP) (1.8 mg, 0.015 mmol) in CH_2Cl_2 (10 mL) was added *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) (78.9 mg, 0.412 mmol) at rt. After stirring for 3 h, the reaction was quenched by addition of saturated aqueous NH_4Cl and the mixture was diluted with CH_2Cl_2. The phases were separated and the aqueous phase was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography ($CHCl_3$/AcOEt, 1:0 to 3:1) to give **9b** (93.1 mg, 0.112 mmol, 82%) as a colorless oil.
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (2H, d, *J* = 8.2 Hz), 7.49-7.42 (10H, m), 7.39-7.18 (12H, m), 7.08 (2H, d, *J* = 8.5 Hz), 6.93 (2H, d, *J* = 8.4, 1.9 Hz), 5.93 (1H, d, *J* = 7.7 Hz), 4.88 (1H, dt, *J* = 7.7, 5.6 Hz), 4.34 (1H, dt, *J* = 11.8, 4.7 Hz), 4.25 (1H, dt, *J* = 11.8, 4.7 Hz), 3.89 (2H, s), 3.71 (2H, t, *J* = 4.7 Hz), 3.66 (2H, t, *J* = 5.0 Hz), 3.24 (2H, t, *J* = 5.0 Hz), 3.14 (1H, dd, *J* = 14.1, 5.6 Hz), 3.07 (1H, dd, *J* = 14.1, 5.6 Hz), 1.91 (9H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4 (s), 169.6 (s), 169.2 (s), 164.8 (s), 149.6 (s), 145.7 (s), 143.9 (s), 138.2 (s), 133.7 (s), 130.6 (s), 130.3 (d), 129.9 (d), 129.3 (d), 128.6 (d), 128.3 (d), 127.7 (d), 127.0 (d), 127.0 (d), 124.2 (d), 121.3 (d), 86.5 (s), 82.3 (s), 70.6 (t), 68.8 (t), 64.6 (t), 63.3 (t), 53.0 (d), 41.3 (t), 37.0 (t), 28.7 (q), 23.0 (q). IR (ATR): 1748,

1719, 1674, 1664 cm^{-1}. MS (FAB): *m/z* 856 $[M+Na]^+$, 872 $[M+K]^+$. HRMS (FAB): calcd for $C_{52}H_{51}NO_9Na$ $[M+Na]^+$, 856.3461, Found 856.3444.



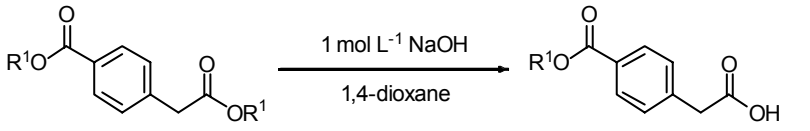
9b: $R^1 = O(CH_2CH_2O)_2Tr$ **9**: $R^2 = O(CH_2CH_2O)_2H$

A solution of **9b** (59.2 mg, 0.071 mmol) in 1% trifluoroacetic acid (TFA) - CH_2Cl_2 (10 mL) was stirred for 30.mim. The mixture was concentrated under reduced pressure. The residue was roughly purified column chromatography ($CHCl_3$/MeOH, 9:1) to give crude carboxylic acid (52.6 mg) as colorless oil. To a stirred the carboxylic acid, diethylene glycol (75.2 mg, 0.709 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.8 mg, 0.007 mmol) in CH_2Cl_2 (3 mL) was added *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) (48.9 mg, 0.255 mmol) at rt. After stirring for 24 h, the reaction was quenched by addition of H_2O (10 μL). The mixture was evaporated and purified by normal phase HPLC ($CHCl_3$/MeOH, 95:5, column COSMOSIL^{TM}, 5SL-II) to give **9** (31.1 mg, 0.055 mmol, 78%) as colorless solid..
$[\alpha]_D^{21}$ = +1.02 (*c* 0.62, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (2H, d, *J* = 8.2 Hz), 7.45 (2H, d, *J* = 8.2 Hz), 7.13 (2H, d, *J* = 8.5 Hz), 6.97 (2H, d, *J* = 8.5 Hz), 6.23 (1H, d, *J* = 8.0 Hz), 4.85 (1H, dt, *J* = 8.0, 6.0 Hz), 4.51-4.48 (2H, m), 4.29 (1H, ddd, *J* = 12.1, 5.5, 4.0 Hz), 4.22 (1H, ddd, *J* = 12.1, 5.2, 4.1 Hz), 3.91 (2H, s), 3.85-3.82 (2H, m), 3.76-3.72 (2H, m), 3.69-3.62 (6H, m), 3.55-3.52 (2H, m), 3.11 (2H, d, *J* = 6.0 Hz), 1.97 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4 (s), 170.0 (s), 169.4 (s), 166.2 (s), 149.6 (s), 138.6 (s), 133.8 (s), 130.3 (d), 130.1 (d), 129.4 (d), 129.1 (s), 121.4 (d), 72.4 (t), 72.4 (t), 69.1 (t), 68.5 (t), 64.3 (t), 64.1 (t), 61.7 (t), 61.5 (t), 53.2 (d), 41.3 (t), 36.9 (t), 22.9 (q). IR (ATR) : 3350, 1738, 1717, 1657 cm^{-1}; MS (FAB): *m/z* 562 $[M+H]^+$, 584 $[M+Na]^+$, 600 $[M+K]^+$; HRMS (FAB): calcd for $C_{28}H_{36}NO_{11}$ $[M+H]^+$, 562.2289, Found 562.2294.



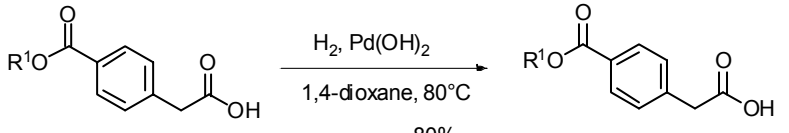
10a: $R^1 = O(CH_2CH_2O)_2Tr$

To a stirred solution of 4-(carboxymethyl)benzoic acid[6] (53.9 mg, 0.299 mmol), diethylene glycol monotrityl ether[5] (195.6 mg, 0.561 mmol) and 4-(dimethylamino)pyridine (DMAP) (5.6 mg, 0.046 mmol) in CH_2Cl_2 (3 mL) was added *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) (555.1 mg, 2.895 mmol) at 30 ˚C. After stirring for 14 h, the reaction was quenched by addition of saturated aqueous NH_4Cl. The phases were separated and the aqueous phase was extracted with AcOEt. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (hexane/AcOEt, 2:1) to give **10a** (178.1 mg, 0.212 mmol, 71%) as a colorless oil.
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (2H, d, *J* = 8.0 Hz), 7.49-7.44 (12H, m), 7.32-7.17 (20H, m), 4.48 (2H, t, *J* = 4.8 Hz), 4.28 (2H, t, *J* = 4.8 Hz), 3.85 (2H, t, *J* = 4.8 Hz), 3.74-3.63 (8H, m), 3.24 (2H, t, *J* = 5.1 Hz), 3.23 (2H, t, *J* = 5.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8 (s), 166.3 (s), 144.0 (s), 139.0 (s), 129.9 (d), 129.3 (d), 128.9 (s), 128.7 (d), 128.2 (d), 127.8 (d), 127.0 (d), 126.9 (d), 86.5 (s), 70.7 (t), 70.6 (t), 69.2 (t), 69.0 (t), 64.2 (t), 63.3 (t), 63.3 (t), 41.1 (t). IR (ATR): 1720 cm^{-1}. MS (FAB): *m/z* 863 $[M+Na]^+$, 879 $[M+K]^+$. HRMS (FAB): calcd for $C_{55}H_{52}O_8Na$ $[M+Na]^+$, 863.3560, Found 863.3563.



10a: $R^1 = O(CH_2CH_2O)_2Tr$ **10b**: $R^1 = O(CH_2CH_2O)_2Tr$

To a stirred solution of **10a** (395.1 mg, 0.470 mmol) in 1,4-dioxane (5 mL) was added 1 mol L^{-1} NaOH (2.35 mL, 2.35 mmol) at rt. After stirring for 1.5 h, the reaction was neutralized with 1 mol L^{-1} HCl (2.35 mL) at 0 ˚C. After addition of saturated aqueous NaH_2PO_4 and AcOEt, the phases were separated and the aqueous phase was extracted with AcOEt. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography ($CHCl_3$/AcOEt, 4:1 to 1:1) to give **10b** (217.1 mg, 0.425 mmol, 90%) as a colorless oil.
^{1}H NMR (300MHz, $CDCl_3$) δ 8.02 (2H, d, *J* = 8.1 Hz), 7.48-7.45 (6H, m), 7.32-7.18 (11H, m), 4.50 (2H, t, *J* = 4.7 Hz), 3.86 (2H, t, *J* = 4.7 Hz), 3.71 (2H, t, *J* = 5.2 Hz), 3.69 (2H, s), 3.25 (2H, t, *J* = 5.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.4 (s), 166.3 (s), 144.0 (s), 138.4 (s), 130.0 (d), 129.5 (d), 129.2 (s), 128.7 (d), 127.8 (d), 126.9 (d), 86.6 (s), 70.7 (t), 69.2 (t), 64.3 (t), 63.3 (t), 40.9 (t). IR (ATR): 1717 cm^{-1}. MS (FAB): *m*/*z* 533 $[M+Na]^+$, 549 $[M+K]^+$. HRMS (FAB): calcd for $C_{32}H_{30}O_6Na$ $[M+Na]^+$, 533.1940, Found 533.1952.



10b: $R^1 = O(CH_2CH_2O)_2Tr$ **10c**: $R^2 = O(CH_2CH_2O)_2H$

To a stirred solution of **10b** (14.2 mg, 0.0278 mmol) in 1,4-dioxane (1 mL) was added $Pd(OH)_2$ / C (20 %, 12.5 mg) at 80 ˚C under hydrogen atmosphere. After stirring for 8 h, the mixture was filtered through Celite and concentrated. The residue was purified by column chromatography ($CHCl_3$/MeOH, 9:1) to give **10c** (6.0 mg, 0.0223 mmol, 80%) as a colorless oil.
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (2H, d, *J* = 8.0 Hz), 7.34 (2H, d, *J* = 8.0 Hz), 4.48 (2H, t, *J* = 4.6 Hz), 3.84 (2H, t, *J* = 4.6 Hz), 3.76 (2H, t, *J* = 4.6 Hz), 3.69 (2H, s), 3.65 (2H, t, *J* = 4.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (s), 166.4 (s), 139.3 (s), 129.9 (d), 129.5 (d), 128.8 (s), 72.4 (t), 69.2 (t), 64.0 (t), 61.6 (t), 41.0 (t). IR (ATR): 1717 cm^{-1}. MS (CI): *m*/*z* 269 $[M+H]^+$. HRMS (CI): calcd for $C_{13}H_{17}O_6$ $[M+H]^+$, 269.1025, Found 269.1024.

2. Preparation of peptidocalix[4]arene library 5

Preparation of solid-supported peptidocalix[4]arene library **5** has been reported reviously.[7]

3. Experimental procedure for the screening of the library 5 for binding to an aniline-labeled transition state analogue 4

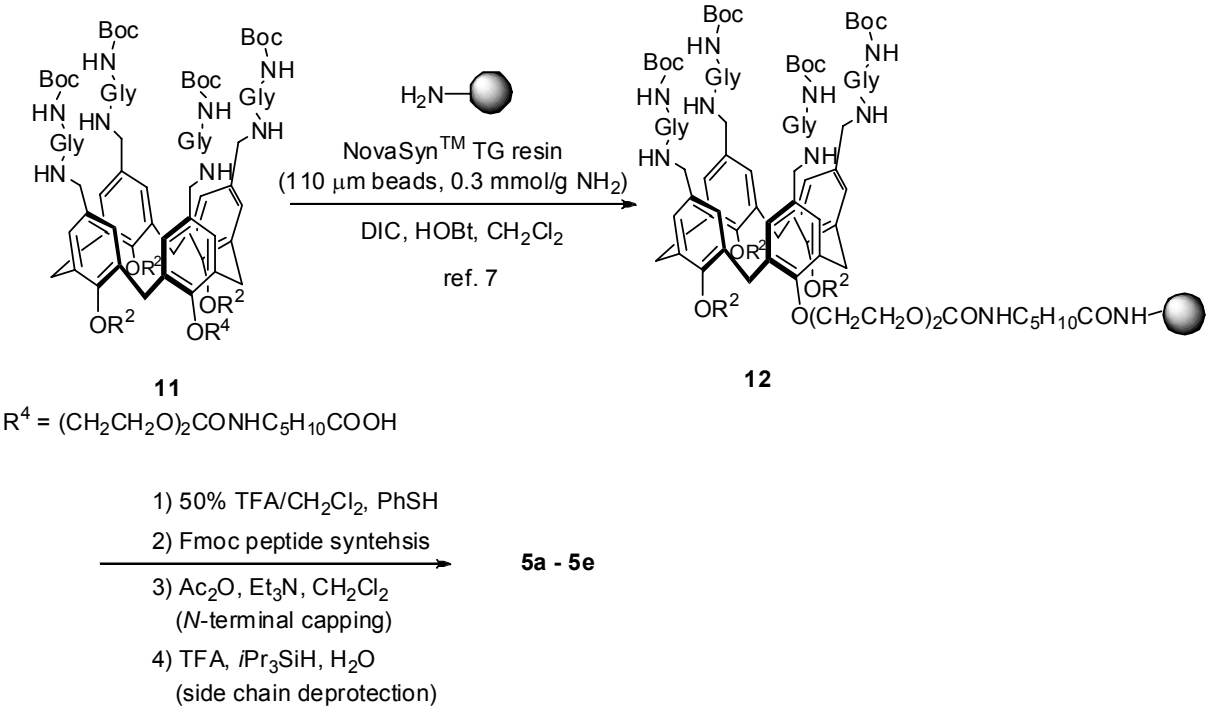
5 mg of the solid supported library **5** (ca. 3 copies for each sequence) was preincubated with 0.6 mL of phosphate buffer (pH 6.86) in an Eppendorf tube for 1 h. The buffer was decanted and 0.6 mL of a transition state analogue **4** (3.5 mmol L^{-1} in the phosphate buffer) was added to the tube. After agitation for 15 h, the mixture was poured into 0.5 mL of Ultrafree^{TM} MC microcentrifuge tube (0.45 μm filter unit).[8] The resin was filtered and washed several times with the phosphate buffer by using centrifuge until the washing buffer was not active for the Trinder reaction. The resin was transferred to an Eppendorf tube. 0.2 mL of 4-aminoantipyrine (1.0 mmol L^{-1} in water) and 0.2 mL of horseradish peroxidase (10 unit mL^{-1} in water) were added to the tube. After shaking several times, 0.2 mL of H_2O_2 (1.0 mmol L^{-1} in water) was added and incubated for 5 min at 38 °C. The beads were placed onto Petri dish. Relatively deep colored 30 beads were isolated manually under low-power microscope. Peptide sequence on colored beads was identified as previously reported manner.[7] Exact sequences of all the 30 peptides are as follows. (Table 1)

Table 1. Peptide sequences of all the 30 library members binding to a transition state analogue **4**.[a]

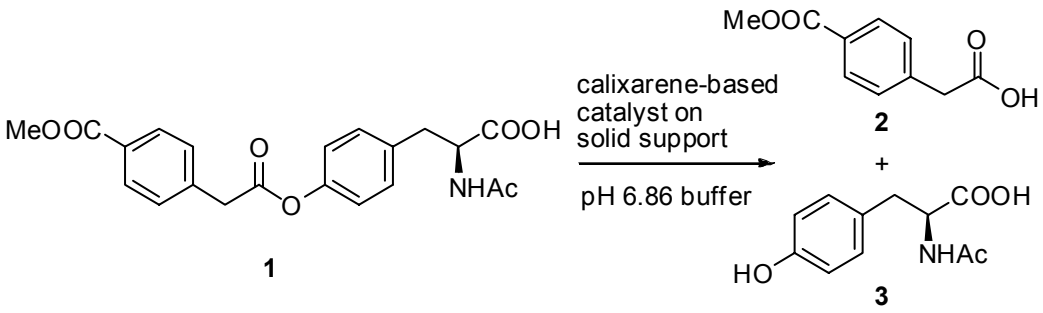
entry	AA_1	AA_2	AA_3
1	His	Arg	His
2	His	Arg	His
3	Pro	Arg	His
4	Leu	Arg	His
5	Ser	Arg	His
6	Tyr	Arg	His
7	Lys	Arg	His
8	Tyr	Lys	His
9	Ser	Lys	His
10	Pro	Lys	His
11	Pro	Lys	His
12	Leu	Lys	His
13	Asn	Lys	His
14	Lys	Pro	His
15	Arg	Pro	His
16	Leu	Ser	His
17	Lys	Ser	His
18	Lys	Tyr	His
19	Lys	Leu	His
20	Lys	Asn	His
21	Tyr	Lys	Arg
22	Trp	Lys	Arg
23	Trp	Trp	Arg
24	Lys	Asn	Trp
25	Lys	Asn	Trp
26	Lys	Asn	Tyr
27	Leu	Lys	Tyr
28	Ser	His	Lys
29	His	Tyr	Lys
30	Lys	Trp	Leu

4. Synthesis of solid-supported peptidocalix[4]arenes 5a – 5e

Peptidocalix[4]arene **5a** – **5e** was synthesized following scheme. **12** was prepared according to our previous work.[7] Synthesis of **5a – 5e** from **12** was typical Fmoc peptide synthesis on a solid support.



5. Concentration-time profile of the hydrolysis of 1 with 5a – 5e



1.00 mg of the solid supported peptidocalix[4]arene **5a** (0.155 μmol) was preincubated with 0.6 mL of phosphate buffer (pH 6.86, 30 μmol of phosphate ion) in an Eppendorf tube for 1 h. The buffer was removed and buffer solution of a substrate **1** (0.6 mL, 1.25 mmol L^{-1} in the phosphate buffer, 0.75 μmol) was added to the tube. The hydrolysis was performed at 30 ± 0.2 °C in a constant temperature bath. After agitation for 3, 6, 24 h, 5 μL of the reaction mixture was injected into HPLC system equipped with a UV detector (254 nm) to quantitatively analyze the product **2**. The analytical conditions and HPLC chart were shown in figure S-1. The peak area (retention time: 4.96 min) was related to concentration of **2** by calibration data of the authentic sample. The data for the hydrolysis with **5a** – **5e** was summarized in Table S-1. Their concentration-time profile was shown in Figure 3 in the main text.

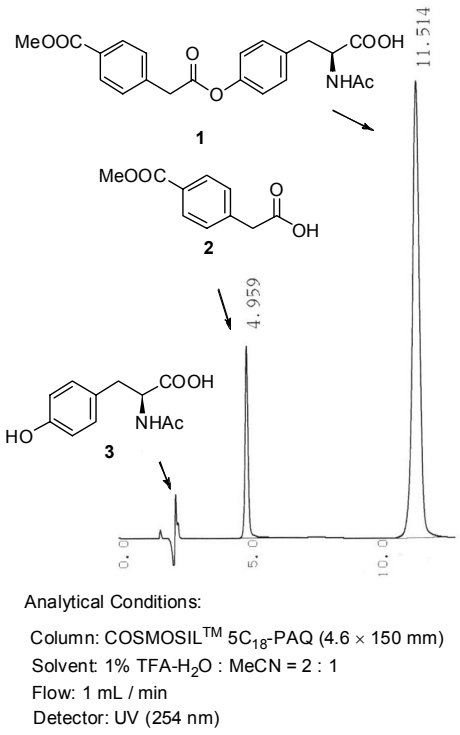


Figure S-1 HPLC chart and analytical conditions

Table S-1 Time course of the hydrolysis of **1** in the presence of the catalyst **5a** – **5e.**

catalyst	concentration of **2** / 10^{-4} mol L^{-1}		
	3 h	6 h	24 h
5a	1.36	2.70	9.67
5b	0.88	1.68	5.99
5c	0.48	1.06	3.70
5d	0.44	0.84	3.08
5e	0.37	0.73	2.53
background	0.44	0.77	2.42

6. Determination of kinetic parameters of the hydrolysis of 1 catalyzed by 5a

1.00 mg of the solid supported peptidocalix[4]arene **5a** (0.155 μmol) was preincubated with 0.6 mL of phosphate buffer (pH 6.86, 30 μmol of phosphate ion) in an Eppendorf tube for 1 h. The buffer was removed and a 2.0 mmol L^{-1} solution of a substrate **1** (0.6 mL, in the phosphate buffer, 1.2 μmol) was added to the tube. The hydrolysis was performed at 30 ± 0.2 °C in a constant temperature bath. After agitation for 0.5 h, 1.0 h, 1.5 h, 2.0 h, and 2.5 h, 5 μL of the reaction mixture was injected into HPLC system equipped with a UV detector (254 nm) to quantitatively analyze the product **2**. The peak area (retention time: 4.96 min) was related to concentration of **2** by calibration data of the authentic sample. Same experimental procedures described above were repeated with different concentrations (0.0, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0 and 8.0 mol L^{-1}). Initial rates for the hydrolysis at individual concentrations were calculated from concentration of **2**. These procedures were repeated three times.

The data was summarized in Table S-2. Michaelis-Menten Plot relating the initial rate (v_0) to the substrate concentration [**1**] was shown in Figure S-3 (blue line). Michaelis constant (K_m) and maximum rate (V_{max}) were calculated as 1.59×10^{-3} mol L^{-1} and 2.43×10^{-8} mol L^{-1} sec^{-1} (r = 0.977) respectively using nonlinear least squares regression (software Kaleidagraph). k_{cat} was derived from V_{max} and concentration of the catalyst **5a** (0.155 μmol / 0.6 mL = 2.58×10^{-4} mol L^{-1}). The value was calculated to be 9.40×10^{-5} sec^{-1} according to the equation: k_{cat} = V_{max} / [**5a**]. k_{uncat} was calculated to be 1.75×10^{-6} sec^{-1} as pseudo-first-order reaction from kinetic experiments in the absence of **5a**. Thus, the rate enhancement (k_{cat}/k_{uncat}) for the hydrolysis was calculated to be 53.7.

Table S-2 Initial rate of the hydrolysis of **1** with various concentrations in the presence of the catalyst **5a.**

concentration of **1**	initial rate (v_0) / 10^{-8} mol L^{-1} s^{-1}			
/ 10^{-3} mol L^{-1}	trial 1	trial 2	trial 3	average
2.0	1.37	1.18	1.26	1.27
2.5	1.45	1.55	1.49	1.50
3.0	1.54	1.66	1.66	1.62
3.5	1.62	1.83	1.72	1.72
4.0	1.78	1.88	1.71	1.79
5.0	1.85	1.92	1.86	1.88
6.0	1.90	1.90	1.89	1.90
7.0	1.98	1.86	1.88	1.91
8.0	2.23	1.98	1.91	2.04

7. Quantitative analysis of inhibitory activity of the transition state analogue 6

Same experiments described above were performed in the presence of transition state analogue **6** (3 mmol L^{-1}). The data was summarized in Table S-3. Michaelis-Menten Plot relating the initial rate (v_0) to the substrate concentration [**1**] was shown in Figure S-3 (red line). Lineweaver-Burk plots in the presence or absence of **6** were shown in Figure 4 in the main text. Two lines intersected on (– +) quadrant, indicating that the mixed-type inhibition. The inhibition constant was determined by following equation (1). Inhibition constant (K_m) was calculated using nonlinear least squares regression (software Kaleidagraph) according to equation 1 ([I] = [**6**] = 3.0×10^{-3} mol L^{-1}, V_{max} = 2.43×10^{-8} mol L^{-1} sec^{-1}). The plot and regression curve are shown in Figure S-3 (red line, r = 0.994). As the result, the inhibition constants was estimated as follows: K_i: 1.8×10^{-3} mol L^{-1}, K_i': 1.2×10^{-2} mol L^{-1}, K_s: 1.2×10^{-3} mol L^{-1}, K_s': 8.0×10^{-3} mol L^{-1}.

$$v_0 = \frac{\dfrac{V_{max}}{\left(1+\dfrac{[I]}{Ki'}\right)}}{1+\dfrac{\left(\dfrac{Ks}{[S]}\right)\left(1+\dfrac{[I]}{Ki}\right)}{\left(1+\dfrac{[I]}{Ki'}\right)}} \quad \text{..... (1)}$$

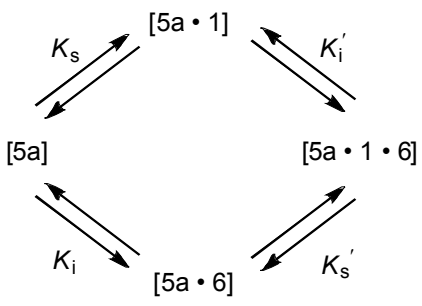


Figure S-2 The mixed type inhibition.

Table S-3 Initial rate of the hydrolysis of **1** with various concentrations in the presence of the catalyst **5a** and transition state analogue **6** (3 mmol L^{-1})**.**

concentration of **1** / 10^{-3} mol L^{-1}	initial rate (v_0) / 10^{-8} mol L^{-1} s^{-1}			
	trial 1	trial 2	trial 3	average
2.0	0.81	0.92	0.79	0.84
2.5	0.88	1.02	0.95	0.95
3.0	0.95	1.11	0.98	1.02
3.5	1.02	1.26	1.13	1.14
4.0	1.09	1.29	1.10	1.16
5.0	1.20	1.35	1.20	1.25
6.0	1.24	1.43	1.25	1.30
7.0	1.32	1.59	1.35	1.42
8.0	1.42	1.67	1.39	1.49

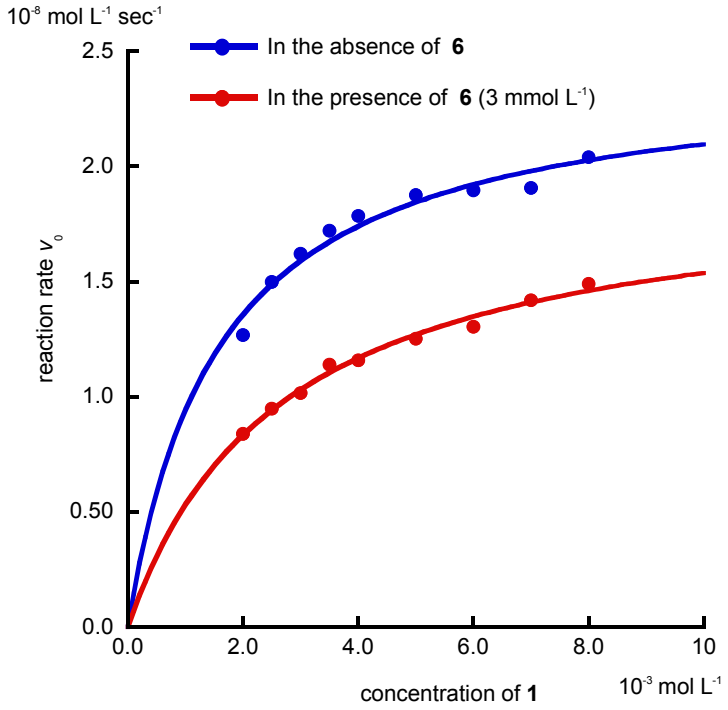


Figure S-3 Michaelis-Menten Plot for the hydrolysis of **1** with **5a** in the absence or presence of transition state analogue **6**.

8. Determination of kinetic parameters of the hydrolysis of 7 catalyzed by 5a

Experimental procedures were the same as those of **1**. The peak area of 4-(carboxymethyl)benzoic acid (retention time: 2.6 min, solvent: 0.5% TFA-H_2O / MeCN = 79 : 21, flow: 1.8 mL / min) was related to its concentration by calibration data of the authentic sample. The data was summarized in Table S-4. Michaelis-Menten Plot relating the initial rate (v_0) to the substrate concentration [**7**] was shown in Figure S-4. Michaelis constant (K_m) and maximum rate (V_{max}) were calculated as 1.86×10^{-3} mol L^{-1} and 3.22×10^{-8} mol L^{-1} sec^{-1} (r = 0.960) respectively, using nonlinear least squares regression (software Kaleidagraph). k_{cat} was derived from V_{max} and concentration of the catalyst **5a** (0.155 μmol / 0.6 mL = 2.58×10^{-4} mol L^{-1}). The value was calculated to be 1.25×10^{-4} sec^{-1} according to the equation: $k_{cat} = V_{max}$ / [**5a**]. k_{uncat} was calculated to be 2.91×10^{-6} sec^{-1} as pseudo-first-order reaction from kinetic experiments in the absence of **5a**. Thus, the rate enhancement (k_{cat}/k_{uncat}) for the hydrolysis was calculated to be 42.9.

Table S-4 Initial rate of the hydrolysis of **7** with various concentrations in the presence of the catalyst **5a.**

concentration of **7**	initial rate (v_0) / 10^{-8} mol L^{-1} s^{-1}			
/ 10^{-3} mol L^{-1}	trial 1	trial 2	trial 3	average
2.0	1.20	1.67	1.68	1.52
2.5	1.52	1.95	1.99	1.82
3.0	1.94	2.04	2.22	2.07
3.5	2.06	2.28	2.26	2.20
4.0	2.19	2.22	2.36	2.26
6.0	2.52	2.41	2.59	2.51
8.0	2.64	2.55	2.29	2.49

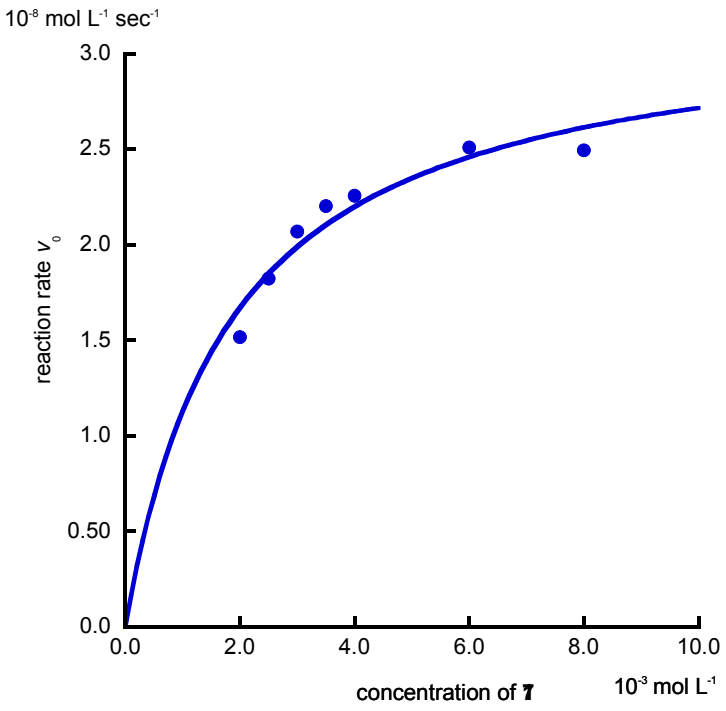


Figure S-4 Michaelis-Menten Plot for the hydrolysis of **7** with **5a**

9. Determination of kinetic parameters of the hydrolysis of 9 catalyzed by 5a

Experimental procedures were the same as those of **1**. The peak area of **10c** (retention time: 3.3 min, solvent: 1% TFA-H_2O / MeCN = 79 : 21, flow: 1.5 mL / min) was related to its concentration by calibration data of the authentic sample. The data was summarized in Table S-5. Michaelis-Menten Plot relating the initial rate (v_0) to the substrate concentration [**9**] was shown in Figure S-5. Michaelis constant (K_m) and maximum rate (V_{max}) were calculated as 4.02×10^{-3} mol L^{-1} and 4.70×10^{-9} mol L^{-1} sec^{-1} (r = 0.980) respectively, using nonlinear least squares regression (software Kaleidagraph). k_{cat} was derived from V_{max} and concentration of the catalyst **5a** (0.155 μmol / 0.6 mL = 2.58×10^{-4} mol L^{-1}). The value was calculated to be 1.82×10^{-5} sec^{-1} according to the equation: $k_{cat} = V_{max}$ / [**5a**]. k_{uncat} was calculated to be 1.23×10^{-6} sec^{-1} as pseudo-first-order reaction from kinetic experiments in the absence of **5a**. Thus, the rate enhancement (k_{cat}/k_{uncat}) for the hydrolysis was calculated to be 14.8.

Table S-5 Initial rate of the hydrolysis of **9** with various concentrations in the presence of the catalyst **5a.**

concentration of **9**	initial rate (v_0) / 10^{-8} mol L^{-1} s^{-1}			
/ 10^{-3} mol L^{-1}	trial 1	trial 2	trial 3	average
2.0	0.13	0.15	0.14	0.14
2.5	0.16	0.18	0.18	0.17
3.0	0.19	0.23	0.21	0.21
3.5	0.21	0.24	0.25	0.23
4.0	0.22	0.25	0.27	0.24
6.0	0.23	0.34	0.29	0.29
8.0	0.33	0.29	0.30	0.30

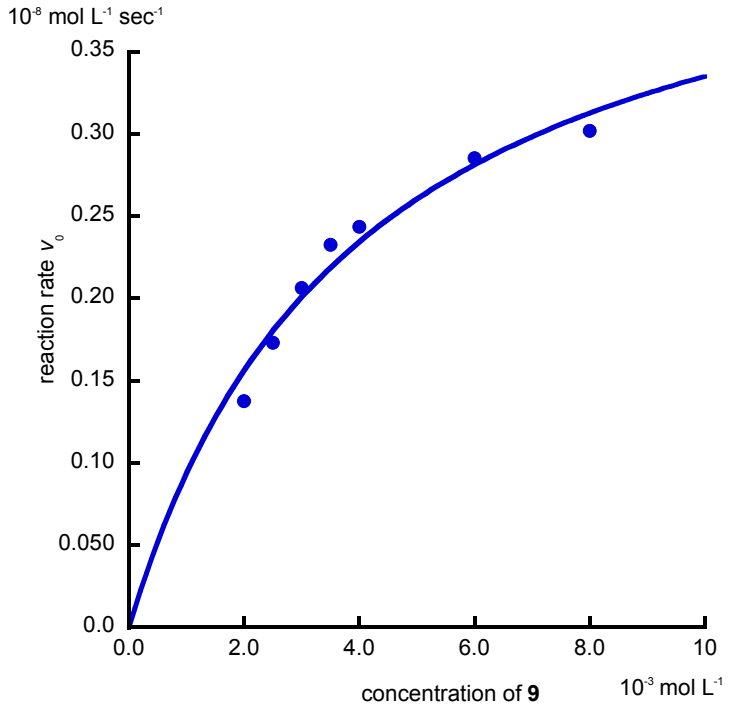


Figure S-5 Michaelis-Menten Plot for the hydrolysis of **9** with **5a**

Notes and references

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8) Ultrafree^{TM} MC microcentrifuge tube was purchased from Millipore Corporation.