Supporting Information for:

Enantioselective Polyclonal Antibodies: a Cheap and Efficient Tool for the Fast Determination of the Activity and Enantioselectivity of Catalysts.

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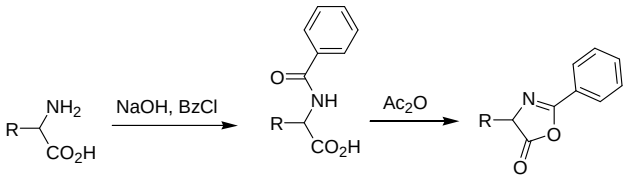
• **General:** Organic solvents (Aldrich) were used without further purification. Purifications of reactions products were carried out by flash chromatography using Merck silica gel (40-63 µm). Infrared spectra (IR) were obtained on a Perkin Elmer system 2000 FTIR spectrophotometer and are reported as wavelength numbers (cm^{-1}). Infrared spectra were collected by preparing a KBr pellet containing the title compound. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) were measured on a Brucker Avance 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ) downfield from residual solvents peaks and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t), …. Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). Electrospray mass spectra were obtained using an ESI/TOF Mariner Mass Spectrometer. Unless otherwise noted, all other commercially available reagents and solvents were used without further purification.

• Synthesis and analytical data of azlactones

Azlactones were prepared in two steps starting from racemic amino acids (scheme S1):

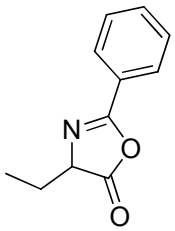


Scheme S1. Preparation of oxazolones

General procedure : 1.7 mmol (1 equiv.) of amino acid were dissolved in 2 mL of a 2 M NaOH aqueous solution. To this mixture were added 240 mg (1.7 mmol, 1 equiv.) of benzoyl chloride. The solution was stirred at room temperature until complete homogenisation, and then acidified to pH 2 by adding 1 M HCl solution. After 2 hours, the product was extracted by EtOAc and the organic phase was dried on $MgSO_4$. Solvent was removed under reduced pressure and the residue was purified on preparative HPLC (column: XBridge™ Prep C18 5 µm, elution : gradient H_2O/CH_3CN 95/05 to 05/95) to afford pure *N*-benzoylated amino acid.

1 mmol of pure *N*-Bz amino acid was then dissolved in 3.3 mL of Ac_2O, the mixture was stirred at 65°C for 2 hours. After evaporation of excess of Ac_2O, azlactone product was obtained quantitatively.

<u>4-ethyl-2-phenyloxazol-5(4H)-one</u>



$C_{11}H_{11}NO_2$.

MW: 189,21 $g \cdot mol^{-1}$.

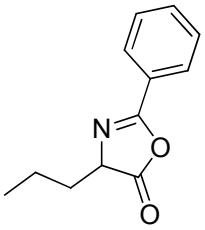
White solid

Global yield : 90%.

Mp: 49-50°C.

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): δ = 1,04 (t, 3H, *J* = 7,2 Hz), 1,96-1,89 (m, 1H), 2,13-2,08 (m, 1H), 4,83 (dd, 1H, *J* = 13,2 Hz, *J* = 6,4 Hz), 6,71 (d, *J* = 7,2 Hz), 7,52-7,46 (m, 2H), 7,82 (d, 2H, *J* = 7,2 Hz), 8,10 (d, 1H, *J* = 7,2 Hz).
^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): δ= 9,4, 24,8, 66,2, 127,8, 128,7, 130,5, 133,7, 167,5, 176,4.
MS (IE, 70 eV): m/z (%)= 51(15), 77(44), 105(100), 130(25), 161(30), 189(12).
IR (NaCl, cm^{-1}): 2972, 2936, 1721, 1644, 1603, 1577, 1535, 1489.
HRMS: *m/z* calcd for $C_{11}H_{12}NO_2$: 190.0868; found: 190.0870

2-phényl-4-propyloxazol-5(4H)-one



$C_{12}H_{13}NO_2$.

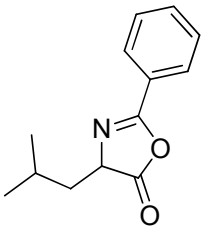
MW: 203,24 g·mol^{-1}.

White solid

Global yield : 91%.

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): δ = 0,99 (t, 3H, *J* = 7,2 Hz), 1,50-1,59 (m, 2H), 1,82-1,88 (m, 1H), 1,96-2,03 (m, 1H), 4,42 (t, 1H, *J* = 6 Hz), 7,49 (t, 2H, *J* = 7,6 Hz), 7,58 (t, 1H, 7,2 Hz, H_{ar}), 8,00 (d, 2H, 8 Hz, H_{ar}).
^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): δ= 13,6, 18,6, 33,5, 65,2, 125,9, 127,8, 128,7, 132,6, 161,5, 178,5.
MS (IE, 70 eV): m/z (%)= 77(53), 105(100), 147(36), 203(19).
IR (KBr, cm^{-1}): 1819, 1654, 1324.
HRMS: *m/z* calcd for $C_{12}H_{14}NO_2$: 205.1103; found: 205.1106

4-isobutyl-2-phenyloxazol-5(4H)-one



$C_{13}H_{15}NO_2$.

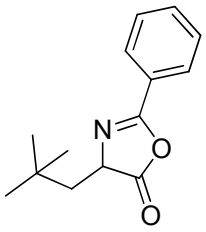
MW: 217,26 g·mol^{-1}.

White solid

Global yield : 98%.

Mp: 54-56°C.
^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): δ = 1,02 (d, 6H, *J* = 6,4 Hz), 1,67-1,72 (m, 1H), 1,82-1,87 (m, 1H), 2,01-2,10 (m, 1H), 4,41 (dd, 1H, *J* = 9,2 Hz, *J* = 6 Hz), 7,49 (t, 2H, *J* = 8 Hz), 7,56 (t, 1H, *J* = 7,6 Hz), 8,00 (d, 2H, *J* = 7,2 Hz).
^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): δ= 31,4, 33,1, 64,4, 126,3, 127,9, 128,4, 128,5, 132,7, 140,1, 161,9, 178,5.
MS (IE, 70 eV): m/z (%)= 77(52), 105(100), 147(34), 161(31), 174(26), 217(17).
IR (NaCl, cm^{-1}): 2959, 2872, 1722, 1641, 1538.
HRMS: *m/z* calcd for $C_{13}H_{16}NO_2$: 218.1181; found: 218.1178

4-neopentyl-2-phenyloxazol-5(4H)-one



$C_{14}H_{17}NO_2$.

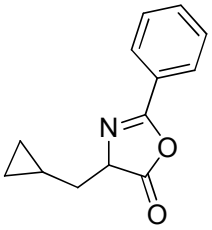
MW: 231,29 g·mol^{-1}.

White solid

Global yield : 98%.

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): δ = 1,10 (s, 9H), 1,60 (dd, 1H, *J* = 14 Hz, *J* = 10 Hz), 1,95 (dd, 1H, *J* = 14 Hz, *J* = 3,2 Hz), 4,41 (dd, 1H, *J* = 10 Hz, *J* = 3,2 Hz), 7,46 (t, 2H, *J* = 8 Hz), 7,57 (t, 1H, *J* = 7,6 Hz), 8,00 (d, 2H, *J* = 7,2 Hz).
^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): δ = 29,6, 30,8, 45,7, 63,4, 126,1, 127,8, 128,7, 132,5, 160,8, 179,9.
MS (IE, 70 eV): m/z (%)= 77(56), 105(100), 147(42), 174(43), 231(27).
IR (NaCl, cm^{-1}) : 2956, 2908, 2870, 1825, 1653, 1322, 1295, 1240.
HRMS: *m/z* calcd for $C_{14}H_{18}NO_2$: 232.1338; found: 232.1342

4-(cyclopropylmethyl)-2-phenyloxazol-5(4H)-one



$C_{13}H_{13}NO_2$.

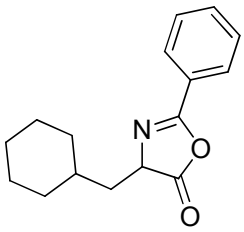
MW: 215,25 g·mol^{-1}.

White solid

Global yield : 90%.

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): δ = 0,19-0,21 (m, 2H), 0,47-0,50 (m, 2H), 0,89-0,93 (m, 1H), 1,73-1,80 (m, 1H), 2,02-2,10 (m, 1H), 4,45 (t, 1H, *J* = 5,6 Hz, CH), 7,50 (t, 2H, *J* = 7,6 Hz), 7,59 (t, 1H, *J* = 7,2 Hz), 8,02 (d, 2H, *J* = 7,6 Hz).
MS (IE, 70 eV): m/z (%)= 51(20), 55(89), 77(66), 105(100), 161(16), 186(20), 187(23), 215(16).
IR (KBr, cm^{-1}): 1819, 1724, 1654, 1616, 1577, 1542, 1330, 1284, 1242, 1218.
HRMS: *m/z* calcd for $C_{13}H_{14}NO_2$: 216.1025; found: 216.1026

4-(cyclohexylmethyl)-2-phenyloxazol-5(4H)-one



$C_{16}H_{19}NO_2$.

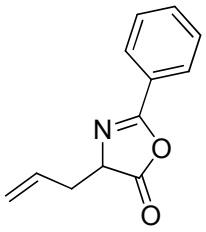
MW: 257,33 g·mol^{-1}.

White solid

Global yield : 98%.

Mp: 57-58°C.
^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): δ = 0,90-1,90 (m, 13H), 4,45 (dd, 1H, *J* = 8,8 Hz, *J* = 5,6 Hz), 7,49 (t, 2H, *J* = 7,6 Hz), 7,58 (t, 1H, *J* = 7,2 Hz), 8,01 (d, 2H, *J* = 7,2 Hz).
^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): δ = 32,6, 33,4, 34,2, 39,5, 63,3, 126,0, 127,8, 128,7, 132,6, 161,4, 179,1.
MS (IE, 70 eV): m/z (%)= 55(17), 77(48), 105(100), 147(54), 148(20), 161(22), 174(29), 257(24).
IR (NaCl, cm^{-1}) : 2923, 2851, 1828, 1721, 1653, 1449, 1322, 1296.
HRMS: *m/z* calcd for $C_{16}H_{20}NO_2$: 258.1494; found: 258.1497

4-allyl-2-phenyloxazol-5(4H)-one



$C_{12}H_{11}NO_2$.

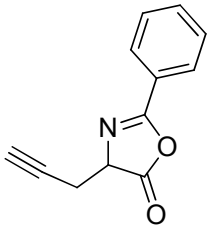
MW: 201,22 g·mol^{-1}.

Yelow solid

Global yield : 91%.

Mp: 40-41°C.
^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): δ = 2,69-2,62 (m, 1H), 2,87-2,79 (m, 1H), 4,51 (t, 1H, *J* = 6 Hz), 5,17 (d, 1H, *J* = 10,4 Hz), 5,25 (dd, 1H, *J* = 15,6 Hz, *J* = 3,6 Hz), 5,85-5,75 (m, 1H), 7,49 (t, 2H, *J* = 8 Hz), 7,59 (t, 1H, *J* = 7,6 Hz), 8,00 (d, 2H, *J* = 7,2 Hz).
^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): δ = 35,3, 65,3, 119,8, 127,9, 128,7, 131,3, 132,8, 132,9, 162,0, 177,5.
MS (IE, 70 eV): m/z (%)= 51(12), 77(38), 105(100), 106(9), 160(14), 173(26), 201(6).
HRMS: *m/z* calcd for $C_{12}H_{12}NO_2$: 202.0868; found: 202.0864

2-phenyl-4-(prop-2-ynyl)oxazol-5(4H)-one



$C_{12}H_9NO_2$.

MW 199,21 g·mol^{-1}.

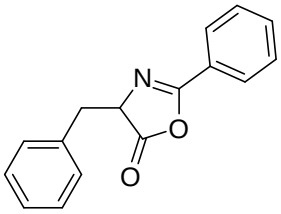
Yelow solid

Global yield : 96%.

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): δ = 2,03 (t, 1H, *J* = 2,4 Hz), 2,89 (ddd, 1H, *J* = 16,8 Hz, *J* = 5,2 Hz, *J* = 2,4 Hz), 2,98 (ddd, 1H, *J* = 16,8 Hz, *J* = 5,2 Hz, *J* = 2,4 Hz), 4,57 (t, 1H, *J* = 5,2 Hz), 7,50 (m, 3H), 8,03 (dd, 2H, *J* = 7,2 Hz, *J* = 1,2 Hz).
^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): δ = 21,5, 64,0, 71,7, 125,4, 128,0, 128,7,133,0, 162,8, 176,5.
MS (IE, 70 eV): m/z (%)= 51(27), 52(6), 77(60), 105(100), 106(12), 128(8), 160(63), 161(8), 171(21), 199(5).
IR (NaCl, cm^{-1}): 3295, 1732, 1645, 1603, 1577, 1529, 1489, 1221.

HRMS: *m/z* calcd for $C_{12}H_{10}NO_2$: 200.0712; found: 200.0716

4-phenethyl-2-phenyloxazol-5(4H)-one



$C_{16}H_{13}NO_2$.

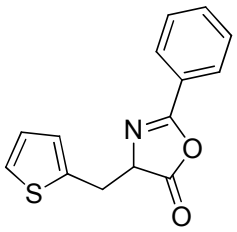
MW: 251.28 g·mol^{-1}.

White solid

Global yield : 90%.

Mp: 68-70°C.
^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): δ = 2,39 (dd, 2H, *J* = 10 Hz, *J* = 6 Hz), 4,41 (dd, 1H, *J* = 7,6 Hz, *J* = 6 Hz), 7,30-7,21 (m, 5H), 7,43 (t, 2H, *J* = 7,2 Hz), 7,51 (t, 2H, *J* = 7,2 Hz), 8,02 (d, 2H, *J* = 7,2 Hz).
^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): δ = 33,1, 64,4, 126,3, 127,9, 128,4, 128,5, 132,7, 140,1, 161,9, 178,2.
IR (KBr, cm^{-1}): 1726, 1640, 1521.
HRMS: *m/z* calcd for $C_{16}H_{14}NO_2$: 252.1025; found: 252.1028

2-phényl-4-(thiophen-2-ylmethyl)oxazol-5(4H)-one



$C_{14}H_{11}NO_2S$.

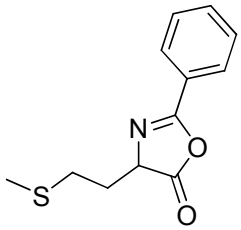
MW: 257,31 g·mol^{-1}.

Yellow solid

Global yield : 90%.

Mp: 45-48°C.
^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): δ = 3,49 (dd, 1H, *J* = 15,2 Hz, *J* = 6,0 Hz), 3,60 (dd, 1H, *J* = 15,2 Hz, *J* = 4,8 Hz), 4,71 (t, 1H, *J* = 5,6 Hz), 6,90 (dd, 1H, *J* = 5,2 Hz, *J* = 3,6 Hz), 6,93 (d, 1H, *J* = 2,8 Hz), 7,13 (dd, 1H, *J* = 5,2 Hz, *J* = 0,8 Hz), 7,48 (t, 2H, *J* = 8,0 Hz), 7,58 (t, 1H, *J* = 7,6 Hz), 7,97 (d, 2H, *J* = 7,6 Hz).
^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): δ = 125,2, 126,8, 127,1, 127,2, 128,0, 128,7, 132,9, 136,2, 166,3, 174,0.
MS (IE, 70 eV): m/z (%)= 51(11), 77(32), 97(100), 98(11), 99(9), 105(19), 257(9).
IR (NaCl, cm^{-1}) : 2927, 1728, 1643, 1529, 1488.
HRMS: *m/z* calcd for $C_{12}H_{14}NO_2S$: 258.0589; found: 258.0592

4-(2-(méthylthio)éthyl)-2-phenyloxazol-5(4H)-one



$C_{12}H_{13}NO_2S$.

MW: 235,30 g·mol^{-1}.

White solid

Global yield : 98%
Mp: 68-70°C.
^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): δ = 2,12 (s, 3H), 2,30-3,00 (m, 4H), 4,61 (t, 1H, *J* = 6,0 Hz), 7,49 (t, 2H, *J* = 8,0 Hz), 7,58 (t, 1H, *J* = 7,6 Hz), 8,00 (d, 2H, *J* = 7,2 Hz).
^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): δ = 15,1, 30,0, 30,3, 63,6, 128,0, 128,4, 128,5, 132,8, 162,1, 178,3.
MS (IE, 70 eV): m/z (%)= 77(52), 105(100), 161(49), 174(77), 235(18).
IR (KBr, cm^{-1}): 1731, 1629, 1577, 1539, 1231, 1219, 1191, 1176.
HRMS: *m/z* calcd for $C_{12}H_{14}NO_2S$: 236.0745; found: 236.0742

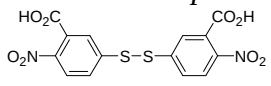
• Reagents for immunoassays

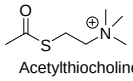
Buffers

Composition of the EIA buffer: phosphate buffer 0.1 M, NaCl 0.15 M, BSA 0.1% (w/w) and sodium azide 0.01% (w/w), pH 7.4
Composition of the washing buffer: phosphate buffer 0.01 M, tween-20 0.05% (w/w), pH 7.4

AcetylCholinesterase

Acetylcholinesterase (AChE, EC 3.1.1.7) was purified as G_4 form from *Electrophorus electricus* as previously described.[1]


DTNB


Acetylthiocholine

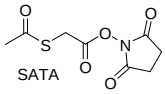
Ellman's reagent

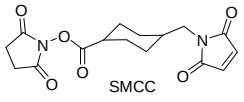
1g of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Sigma), 8.5 g of NaCl and 2.2 g of acetylthiocholine (Sigma) were dissolved in 100 mL of phosphate buffer 0.1 M, pH 7,4.

Microtiter plates

Maxisorb (Nunc, Roskilde, Denmark) microtiter plates were coated by direct adsorption to the solid support with polyclonal mouse anti-rabbit antibody as follow: 200 µL/well of a 10 µg/mL of polyclonal mouse anti-rabbit antibody (Jackson Immuno. Research Laboratories Inc.) in 50 µM phosphate buffer were incubated at room temperature for 12 h. The plates were then washed using washing buffer and 300 µL of a 1mg/mL of BSA solution were added. Plates were storred at 4°C.

Preparation of N-Bz-Lys/AChE conjugate


SATA


SMCC

1 µmole of *N*-Bz-Lysine (racemate) and 12 µmoles of SATA were dissolved in 400 µL of 0,1 M borate buffer, pH 9. After 30 min reaction at room temperature, derivatized *N*-Bz-Lys was purified on Sep-Pack column using MeOH/AcOH 96/04 (v/v) as elution solvent. After evaporation of the solvents, the residue was dissolved in phosphate buffer (0.1 M, pH 6) and 200 µL of a 1 M solution of NH_2OH (pH 7) were added. 2.5 nmoles of this thiolated *N*-Bz-Lys were then reacted with 0.25 nmole of SMCC-AChE conjugate previously prepared by reacting SMCC (10 equiv.) with AChE in

[1] J. Massoulié, A. McMillan, K.N.F. Shaw, *Biochem. Biophys. Acta* **1957**, *25*, 422.

borate buffer 0,1 M, pH 9. The mixture was incubated at 30°C for 3 hours. *N*-Bz-AChE conjugate was then purified on Biogel A 0,5 M and stored at -80°C.

Production of anti-N-Bz-Lys pAbs

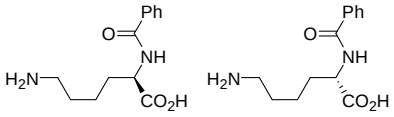
BSA-(*R*) *N*Bz-Lys and BSA-(*S*) *N*-Bz-Lys conjugates were prepared according to the following procedure: 5 mg of BSA and 1 µmol of *N*-Bz-Lys were dissolved in 2 mL of 0.1 M phosphate buffer, pH 7. 8 µL (80 nmols) of a 25% (v/v) aqueous solution of glutaraldehyde were then added and stirred at room temperature overnight. The mixture was then dialyzed against 0.1 M phosphate buffer, pH 7.

Immunizations were carried out under standard protocols:

Polyclonal antibodies against (*R*) and (*S*) *N*-Bz-Lys were produced in rabbits by subcutaneous injection of the above described BSA-conjugates emulsified in complete Freund's adjuvant. Two Rabbits for each *N*-Bz-Lys conjugates were immunized four times, at intervals of two weeks.

The immune responses were followed by analysing serum samples in ELISA assays (see above). The results (table T1) indicate that: *i*) among the four immunized rabbits, only one did not produced pAbs with sufficient enantioselectivity, *ii*) stereoselective pAbs might be obtained in only two weeks following immunization.

Table T1. Binding properties of pAbs for (*R*) and (*S*)-Bz-Lys.



	Rabbit	Serum	Weeks after injection	B/Bo 50% (µM)	B/Bo 50% (µM)	Enantio-selectivity*
anti (*R*) pAbs.	L1729	S1	2	**0.95**	3.48	72.7%
		S2	4	**0.57**	1.32	56.8%
		S3	6	**0.51**	2.73	81.3%
		S4	8	**0.42**	1.52	72.4%
	L1730	S1	2	**0.20**	75.89	99.8%
		S2	4	**0.27**	68.90	99.6%
		S3	6	**0.22**	57.00	99.6%
		S4	8	**0.14**	92.42	99.9%
	Rabbit	Serum	Weeks after injection	B/Bo 50% (µM)	B/Bo 50% (µM)	Enantio-selectivity
anti (*S*) pAbs	L1727	S1	2	56.60	**0.28**	99.5%
		S2	4	22.35	**0.19**	99.2%
		S3	6	9.50	**0.09**	98.9%
		S4	8	3.43	**0.06**	98.3%
	L1728	S1	2	303.66	**0.38**	99.9%
		S2	4	66.44	**0.19**	99.7%
		S3	6	36.00	**0.10**	99.8%
		S4	8	43.52	**0.07**	99.9%

*(1 - [$K_{d,\,app}$ (*R*)/ $K_{d,\,app}$ (*S*)]) x 100 for anti (*R*) pAbs and (1 - [$K_{d,\,app}$ (*S*)/ $K_{d,\,app}$ (*R*)]) x 100 for anti (*S*) pAbs.

Characterization of anti-*N*-Bz-Lys pAbs : $K_{d,\ app}$ determination.

$K_{d,\ app}$ determinations were carried out by competitive immunoassays according to standard protocols previously described.[2] Briefly, in wells of a microtitre plate previously coated (direct adsorption to the solid support) with polyclonal mouse anti-rabbit antibody (Jackson Immuno. Research Laboratories Inc.), 50 µL of *N*-Bz-Lys solutions prepared in EIA buffer at a range of concentration from 10 nM to 10 µM were added to a solution containing 50 µL of the enzymatic tracer *N*-Bz-Lys/AChE and 50 µL of anti (R) or (S)-*N*-Bz-Lys pAbs (sera diluted $1/10^6$) in EIA buffer. After 12 h of incubation at 4°C, the plates were washed and Ellman's reagent was added. The absorbance related to the solid phase bound AChE activity was measured at 414 nm. Results are expressed as B/Bo(%), where B and Bo represent the amount of solid phase-bound tracer in the presence or absence of competitor respectively, as a function of the logarithm of the dose. Calibration curves were fitted using a linear log-logit transformation. All measurements were made in duplicate. $K_{d,\ app}$ values are defined as the concentration of competing antigen that results in half-maximal OD 414 nm (B/Bo = 50%).

Polyclonal antibodies display good affinities and enantioselectivities toward a panel of *N*-Bz amino acids, none of the corresponding azlactones were bound by these pAbs therefore avoiding interference by the substrates during the screening.

• Screening procedure and results

Lipase-catalyzed enantioselective hydrolysis of azlactones: Lipases (sigma) were first dried overnight on P_2O_5 in order to remove traces of water. To 20 µL of anhydrous solvent (THF, CH_2Cl_2, toluene, hexane, CH_3CN or CH_3CN/TEA) containing 9.85 µmols (1 equiv.) of azlactone and 29.55 µmols (3 equiv.) of H_2O were added 2 enzymatic units of lipase. The suspension was stirred vigorously at 37°C for 72 h. Reaction were run on microtiter plates.

Screening procedure: the above described crude catalyzed reaction mixtures were diluted in EIA buffer to get a range of final concentrations from 10 nM to 10 µM. 50 µL of these diluted solutions, 50 µL of the enzymatic tracer *N*-Bz-Lys/AChE and 50 µL of anti (*R*) or (*S*)-*N*-Bz-Lys pAbs (sera diluted $1/10^6$ in EIA buffer) were the added in wells of a microtiter plate previously coated with polyclonal mouse anti-rabbit antibody. After 12 h of incubation at 4°C, the plates were washed and 300 µL of Ellman's reagent were added. The absorbance related to the solid phase bound AChE activity was measured at 414 nm. The calculation of the concentrations of each *N*-Bz-amino acids enantiomer were carried out using standard curves obtained with authentic *N*-Bz-amino acids samples.

Complete screening results:

The 11 azlactones were reacted with 9 lipases in the presence of 3 equivalents of water. Control experiments without lipase were carried out in order to look at possible spontaneous

[2] Taran, F.; Frobert, Y.; Créminon, C.; Grassi, J.; Olichon, D.; Mioskowski, C.; Pradelles, P. *Clin.Chem.* **1997**, *43*, 363-368.

hydrolysis of azlactones. Five solvents and one base (TEA) were assayed leading to a total of 660 combinations run and screened in a parallel manner. Results are presented in a colour code format for clarity reason (figure F1).

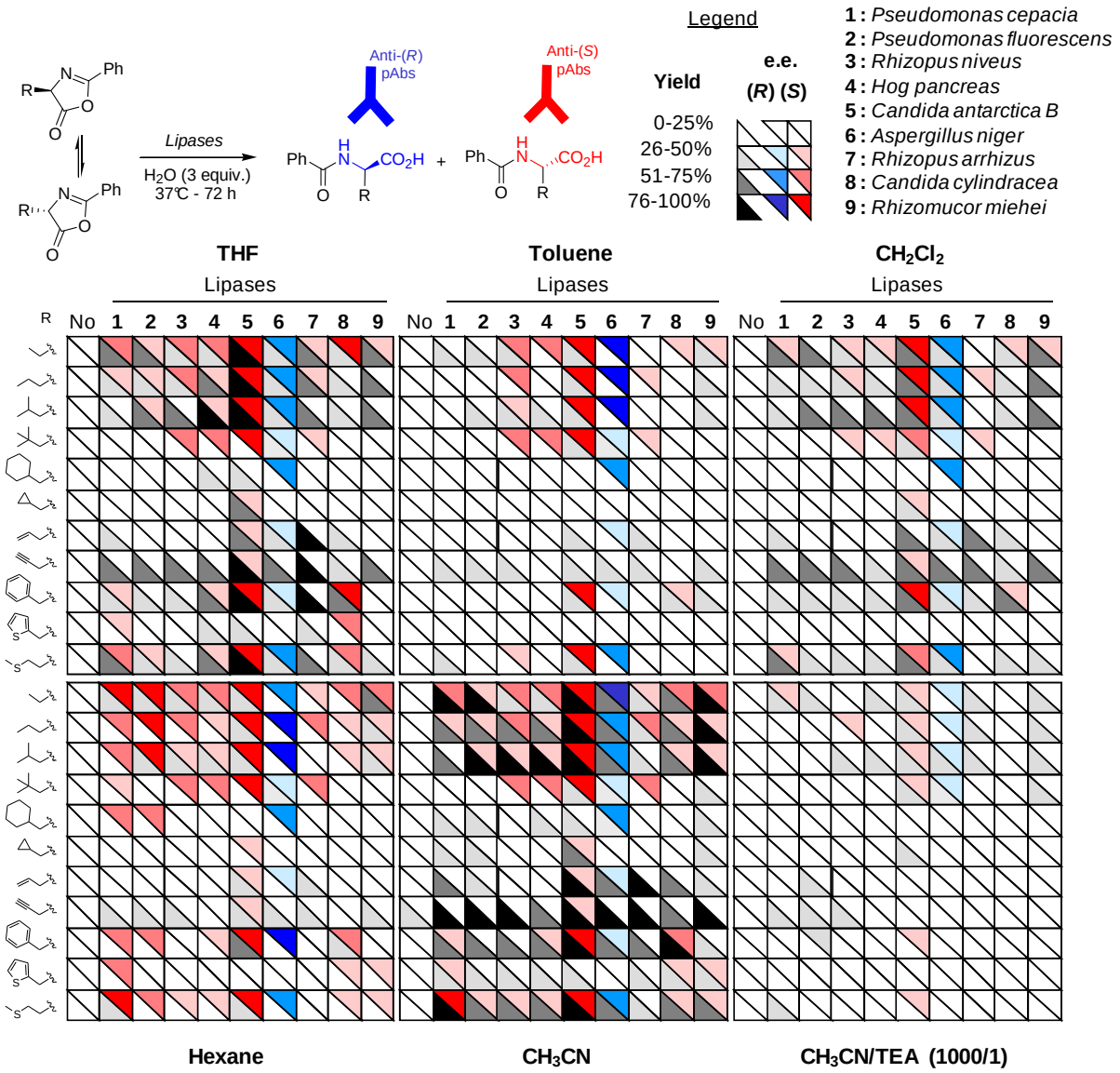


Figure F1. Complete screening results.

• HPLC analysis

Chiral HPLC analysis were carried out on a Chiralpak AD-H column using hexane/EtOH (95/05) + TFA 0,1% as eluant, 1 mL/min., 35°C, detection UV 220 nm.

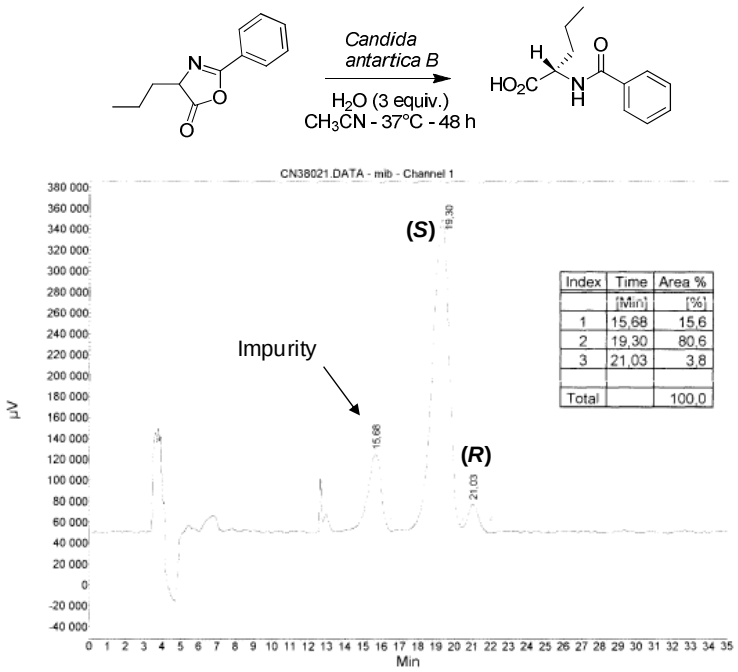


Figure F3. Example of HPLC chiral separation. Analysis of crude *Candida antartica B* – catalysed ring opening reaction.