Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.

Supplementary material

Reaction system

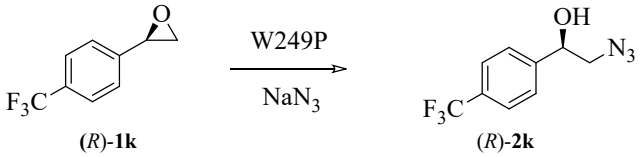
S-1. Reactions in the selected system for kinetic characterization and model development:

A. main reaction: biocatalytic synthesis of (*R*)-2-azido-1-[4-(trifluoromethyl)phenyl]ethanol (**2k**);

B. side reaction: spontaneous chemical synthesis of *rac*-2-azido-2-[4-(trifluoromethyl)phenyl]ethanol (**3k**);

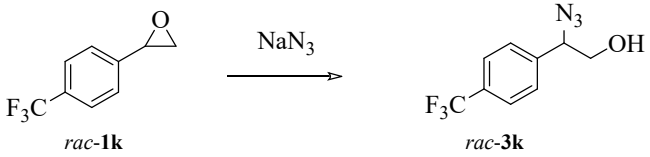
C. side reaction: spontaneous hydrolysis of *rac*-**1k** to *rac*-2-[4-(trifluoromethyl)phenyl]-1,2-ethanediol (**4k**).

A.



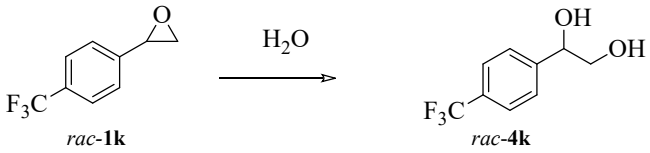
(*R*)-**1k** (*R*)-**2k**

B.



rac-**1k** *rac*-**3k**

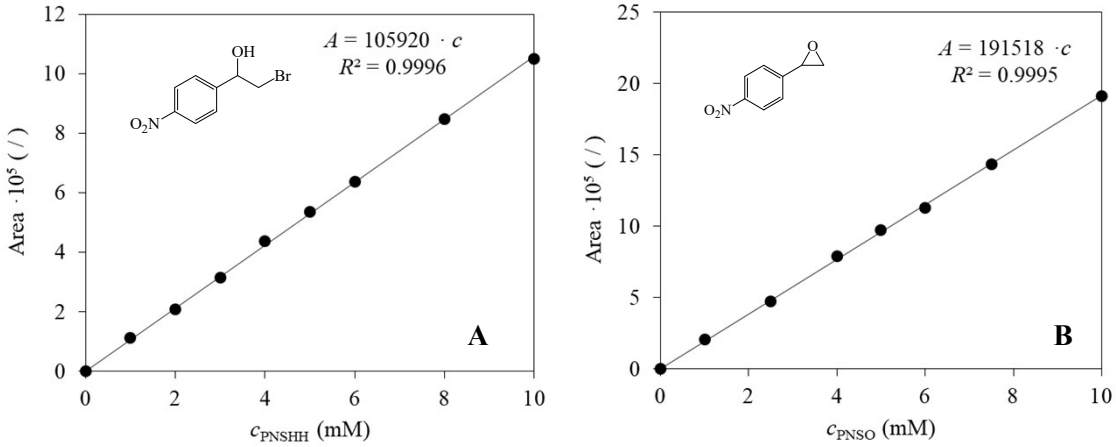
C.



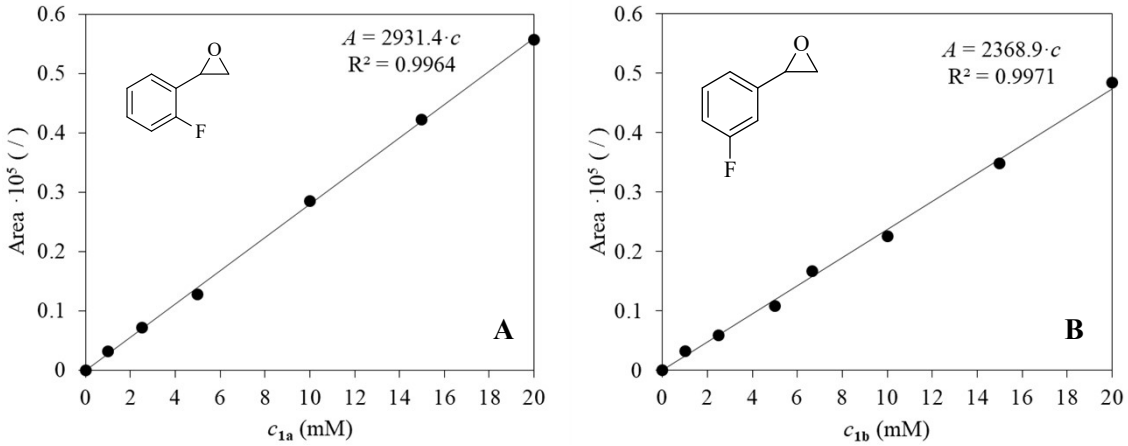
rac-**1k** *rac*-**4k**

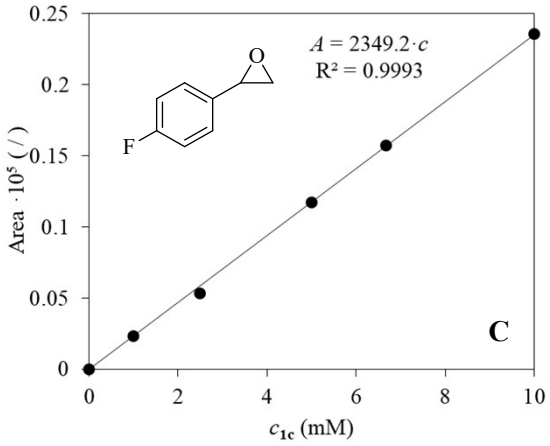
Calibration curves

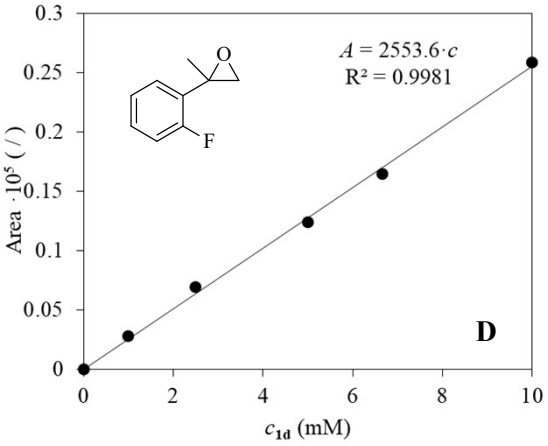
S-2. Calibration curves for **A.** *para*-nitro-2-bromo-1-phenylethanol (PNSHH) and **B.** *para*-nitro styrene oxide (PNSO).

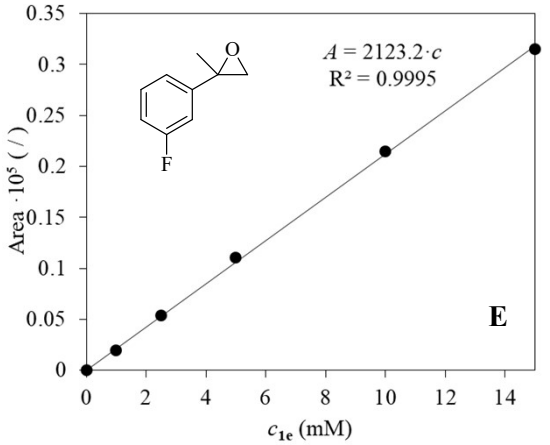


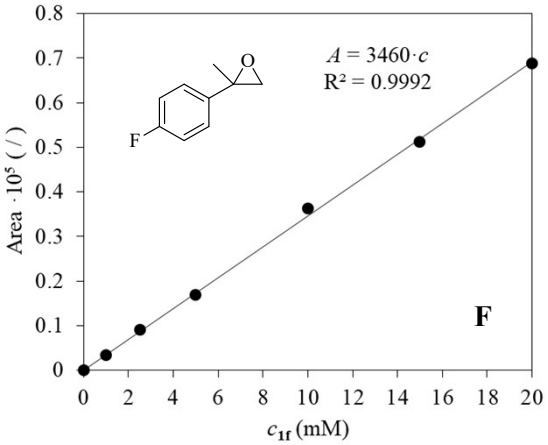
S-3. Calibration curves for the substrates (**1a**-**1k**): **A.** 2-(2-fluorophenyl)oxirane (**1a**); **B.** 2-(3-fluorophenyl)oxirane (**1b**); **C.** 2-(4-fluorophenyl)oxirane (**1c**); **D.** 2-(2-fluorophenyl)-2-methyloxirane (**1d**); **E.** 2-(3-fluorophenyl)-2-methyloxirane (**1e**); **F.** 2-(4-fluorophenyl)-2-methyloxirane (**1f**); **G.**– 2-(2,6-difluorophenyl)oxirane (**1g**); **H.** 2-(3,4-difluorophenyl)oxirane (**1h**); **I.** 2-(2,4-difluorophenyl)oxirane (**1i)**; **J.** 2-(2,4,5-trifluorophenyl)oxirane (**1j**); **K.** 2-[4-(trifluoromethyl)phenyl]oxirane (**1k**).

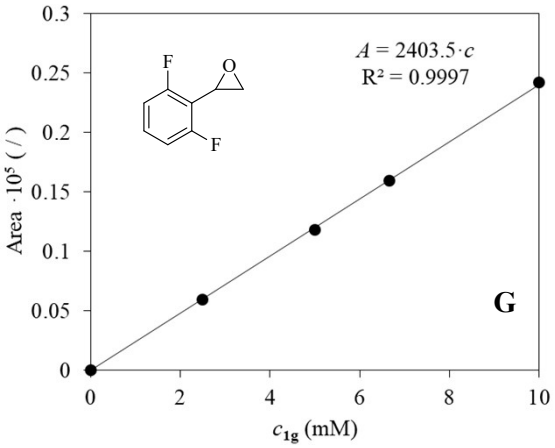


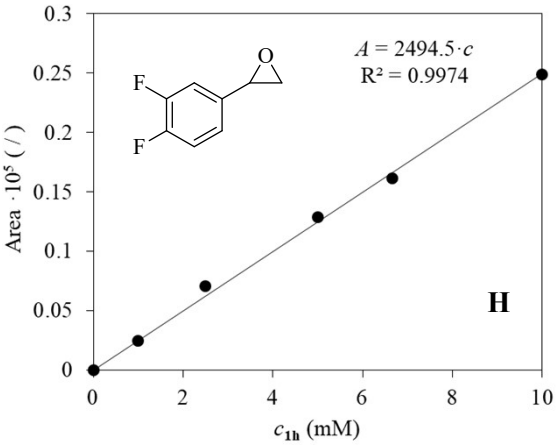

A = 2349.2·c
R² = 0.9993
Area ·10⁵ (/)
c1c (mM)
C

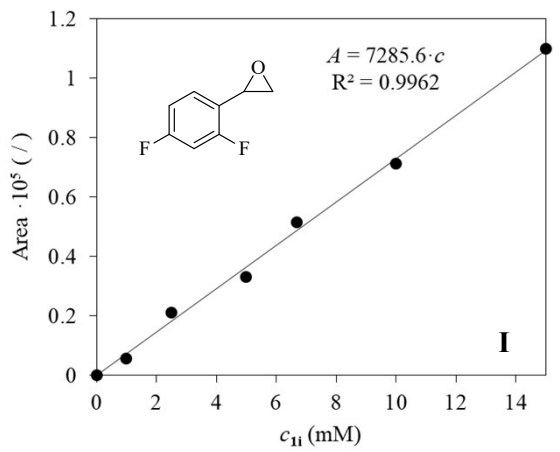

A = 2553.6·c
R² = 0.9981
Area ·10⁵ (/)
c1d (mM)
D

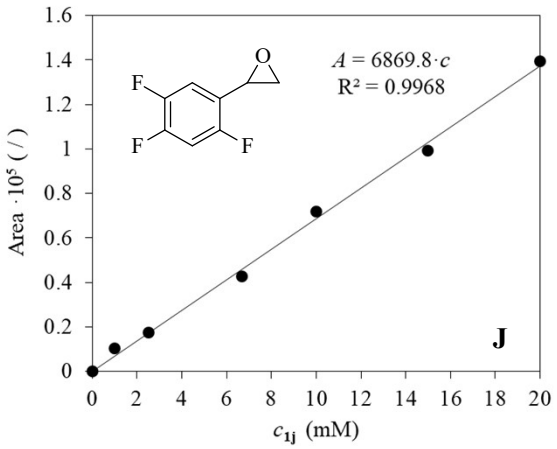

A = 2123.2·c
R² = 0.9995
Area ·10⁵ (/)
c1e (mM)
E

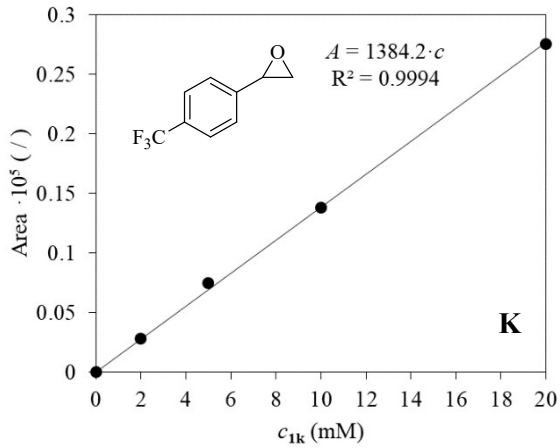

A = 3460·c
R² = 0.9992
Area ·10⁵ (/)
c1f (mM)
F


A = 2403.5·c
R² = 0.9997
Area ·10⁵ (/)
c1g (mM)
G

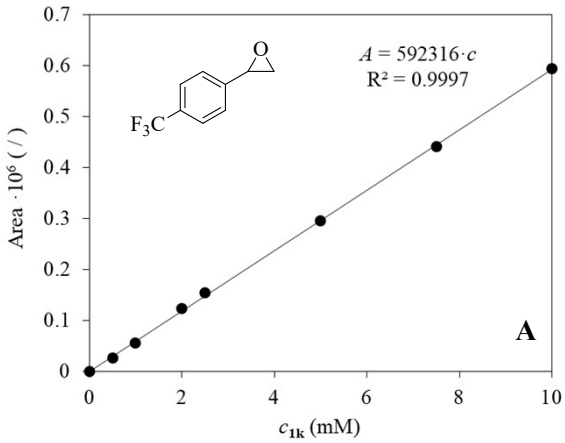

A = 2494.5·c
R² = 0.9974
Area ·10⁵ (/)
c1h (mM)
H

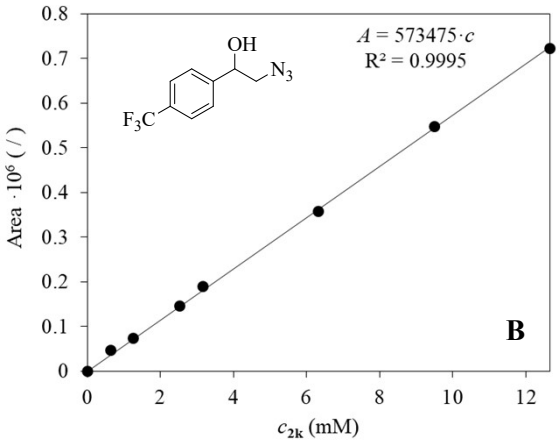




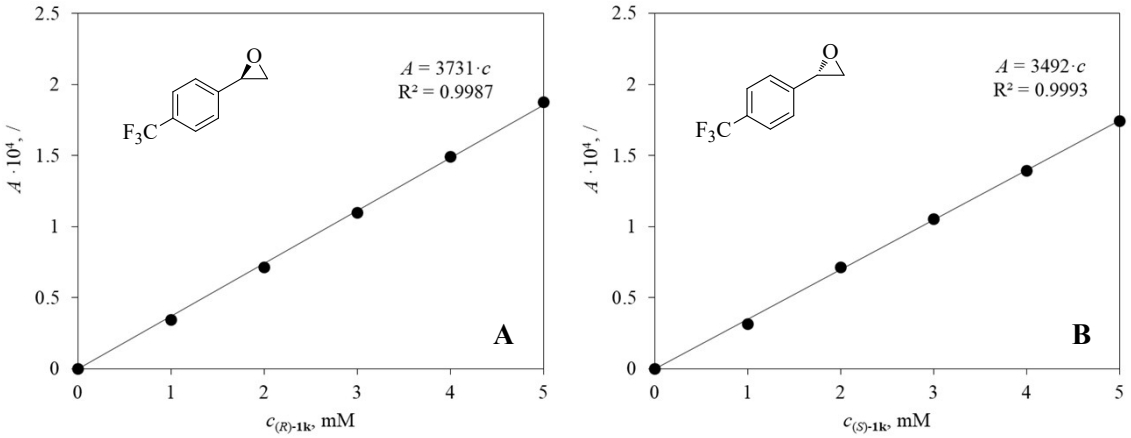


S-4. Calibration curves for the selected system: **A.** substrate (**1k**) and **B.** product (**2k**).

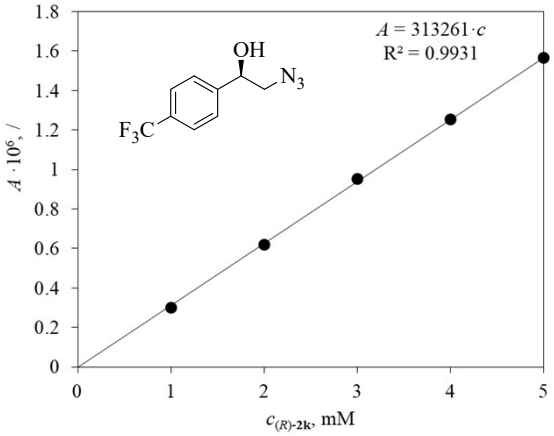




S-5. Calibration curves for individual enantiomers of the substrate: **A.** (*R*)-**1k** and **B.** (*S*)-**1k**

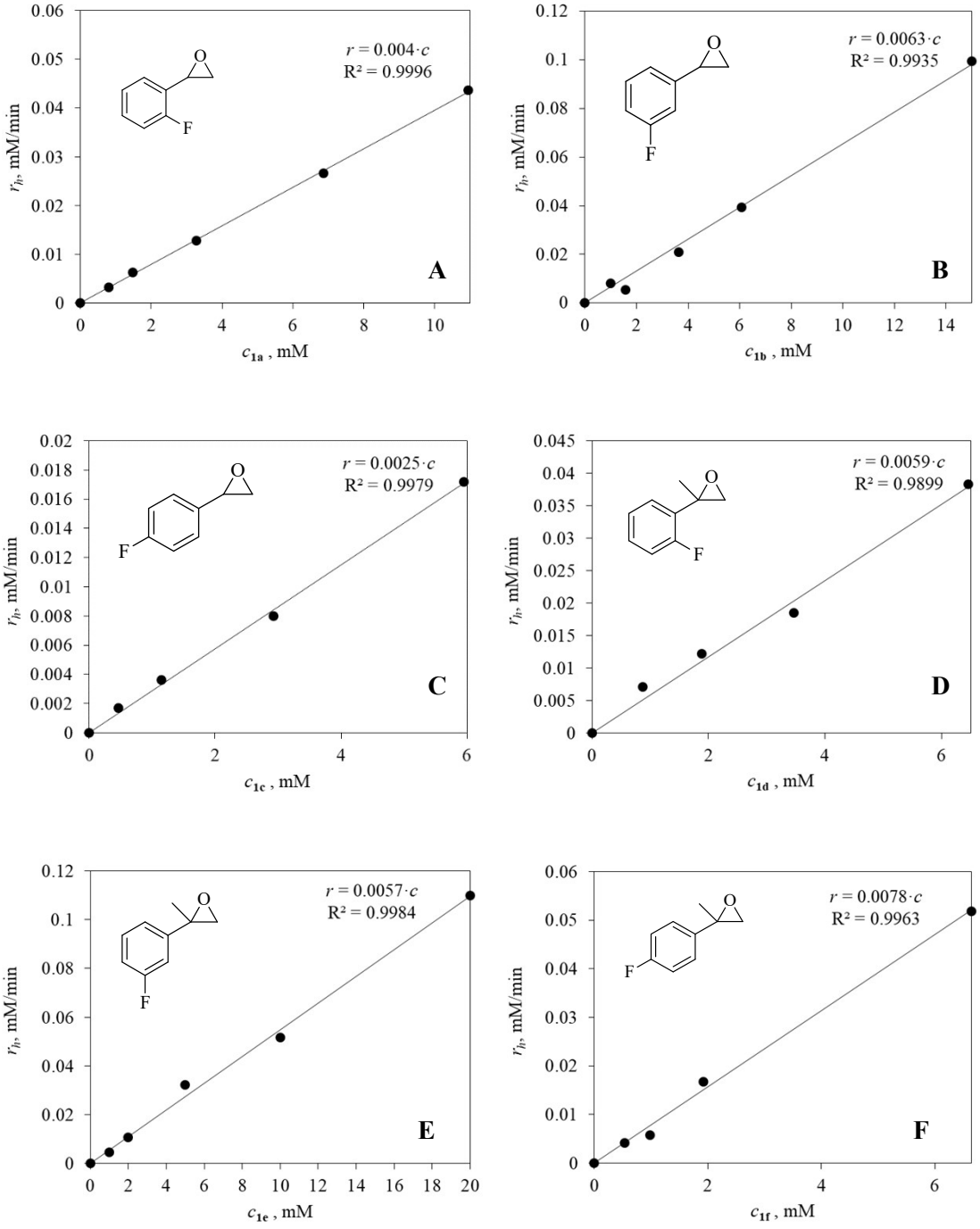


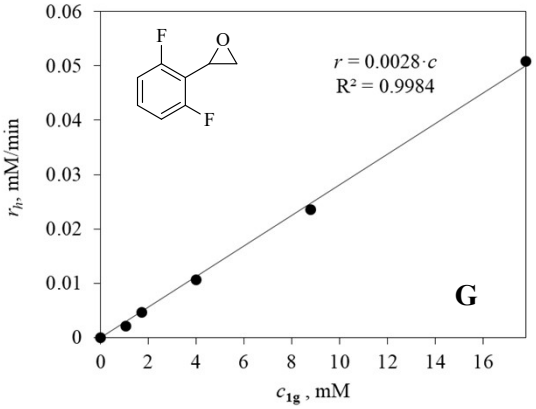
S-6. Product (*R*)-**2k** calibration curve on chiral column

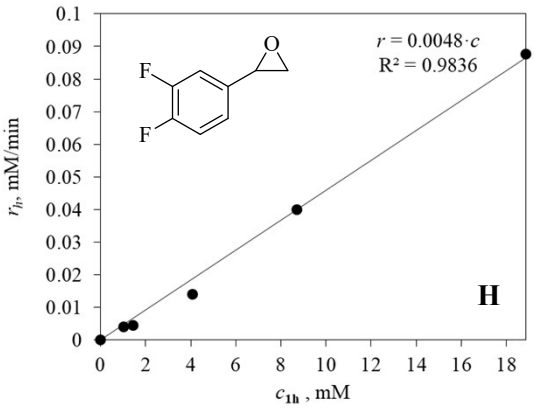


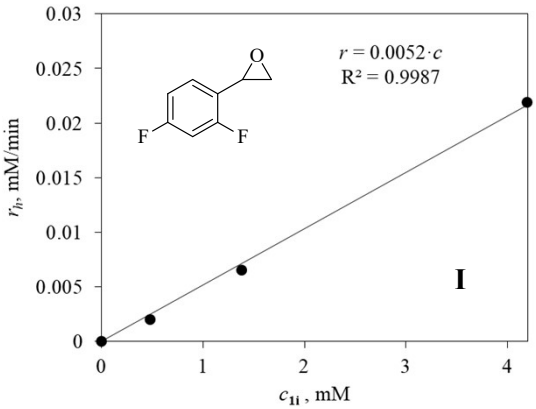
Hydrolysis kinetics

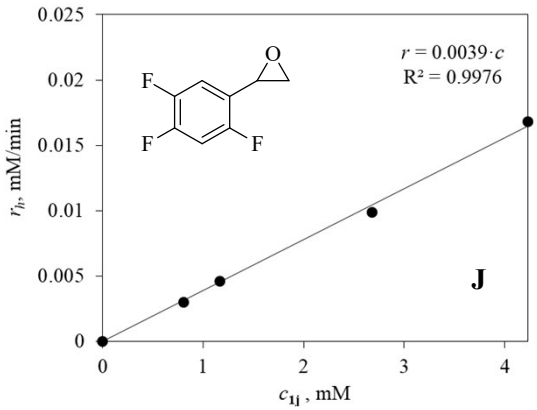
S-7. Determination of hydrolytic constants for substrates **1a**-**1k**. **A. 1a**; **B. 1b**; **C. 1c**; **D. 1d**; **E. 1e**; **F. 1f**; **G. 1g**; **H. 1h**; **I. 1i**; **J. 1j**; **K. 1k**. The graph for **1k** is given in the main paper (*See* 3.5)




0.06
0.05
0.04
0.03
0.02
0.01
0
r_i, mM/min
F
O
F
r = 0.0028·c
R² = 0.9984
G
0 2 4 6 8 10 12 14 16
c_{1g}, mM

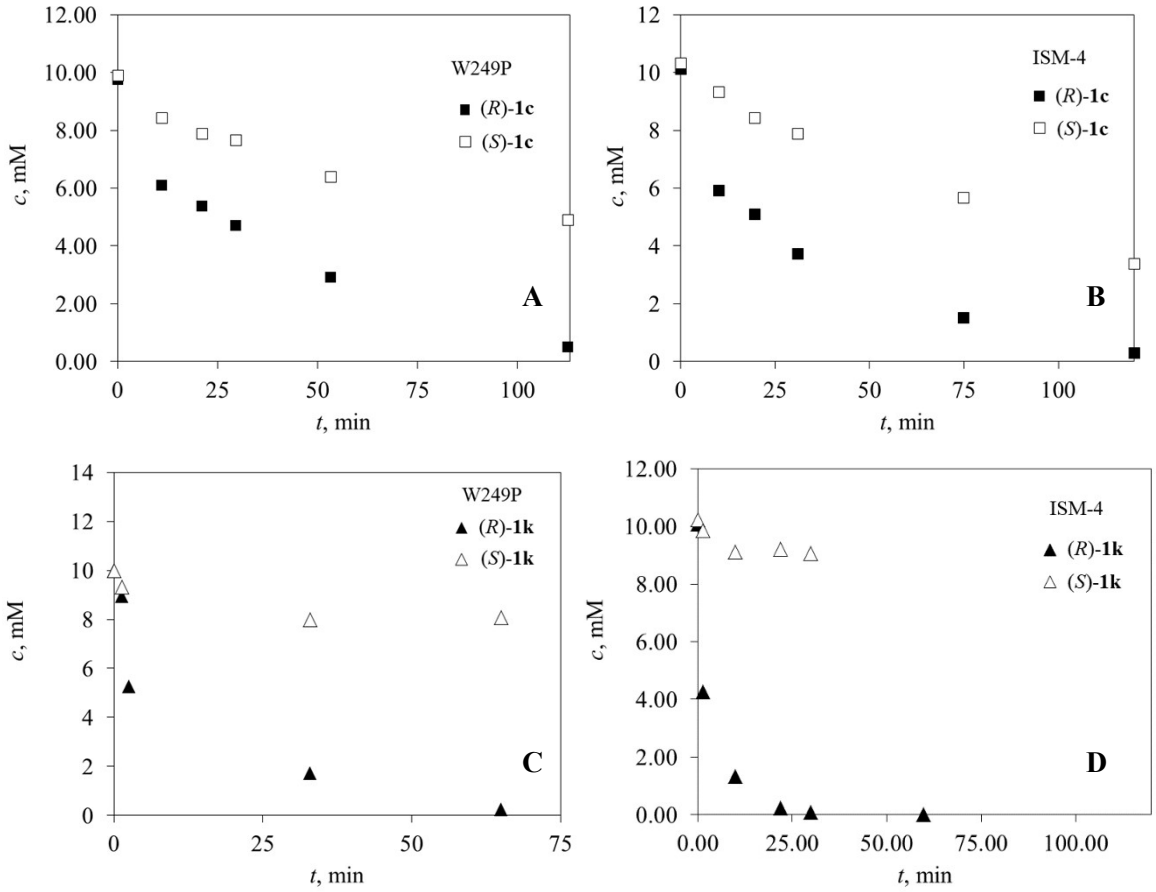

0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
r_i, mM/min
O
F
F
r = 0.0048·c
R² = 0.9836
H
0 2 4 6 8 10 12 14 16 18
c_{1h}, mM


0.03
0.025
0.02
0.015
0.01
0.005
0
r_i, mM/min
O
F
F
r = 0.0052·c
R² = 0.9987
I
0 1 2 3 4
c_{1i}, mM

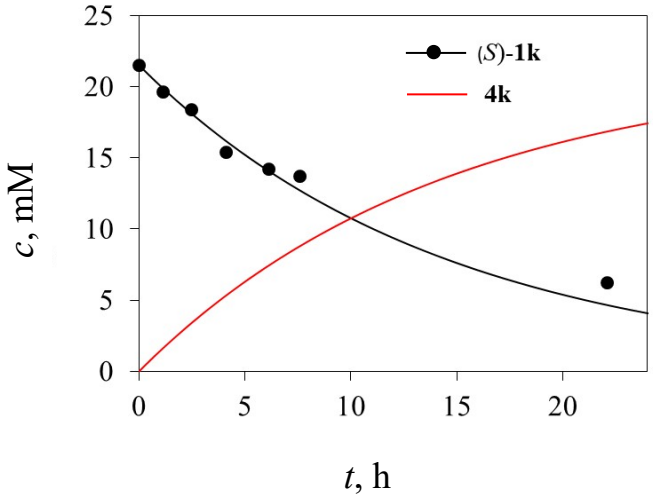

0.025
0.02
0.015
0.01
0.005
0
r_i, mM/min
O
F
F
F
r = 0.0039·c
R² = 0.9976
J
0 1 2 3 4
c_{1j}, mM

Reaction enantioselectivity

S-8. Azidolysis curves of **A. 1c** with W249P, **B. 1c** with ISM-4, **C. 1k** with W249P, **D. 1k** with ISM-4. Legend: black squares (*R*)-**1c**; white squares (*S*)-**1c**; black triangles (*R*)-**1k**; white triangles (*S*)-**1k**. Reaction conditions: 20 mM *rac*-epoxide, 20 mM NaN_3, 10% *v/v* DMSO, V_r = 3 mL, 1000 rpm, 25 °C, γ_{W249P} =1 mg/mL (left panel), γ_{ISM-4} = 2 mg/mL (right panel).



S-9. Proof of complete reaction enantioselectivity, i.e. absence of (*S*)-**1k** azidolysis with W249P enzyme. In order to check whether the enzymatic reaction is completely enantioselective towards (*R*)-**1k**, a reaction in the batch reactor was performed starting with (*S*)-**1k** and high enzyme concentration (3 mg/mL). Even after 24 h, the formation of the biocatalytic product (*S*)-**2k** was not recorded at all. Also, the consumption of the (*S*)-enantiomer over time follows the model-predicted consumption in the hydrolytic reaction, which is another proof that (*S*)-**1k** is not consumed, except in hydrolysis, in any other reaction (including the biocatalytic reaction). Conditions: $c_{(S)\text{-}\mathbf{1k}}$ = 20 mM, $c_{\text{sodium azide}}$ = 20 mM, γ_{W249P} =3 mg/mL. Due to the weak response, **4k** was not monitored experimentally but was confirmed by the model.



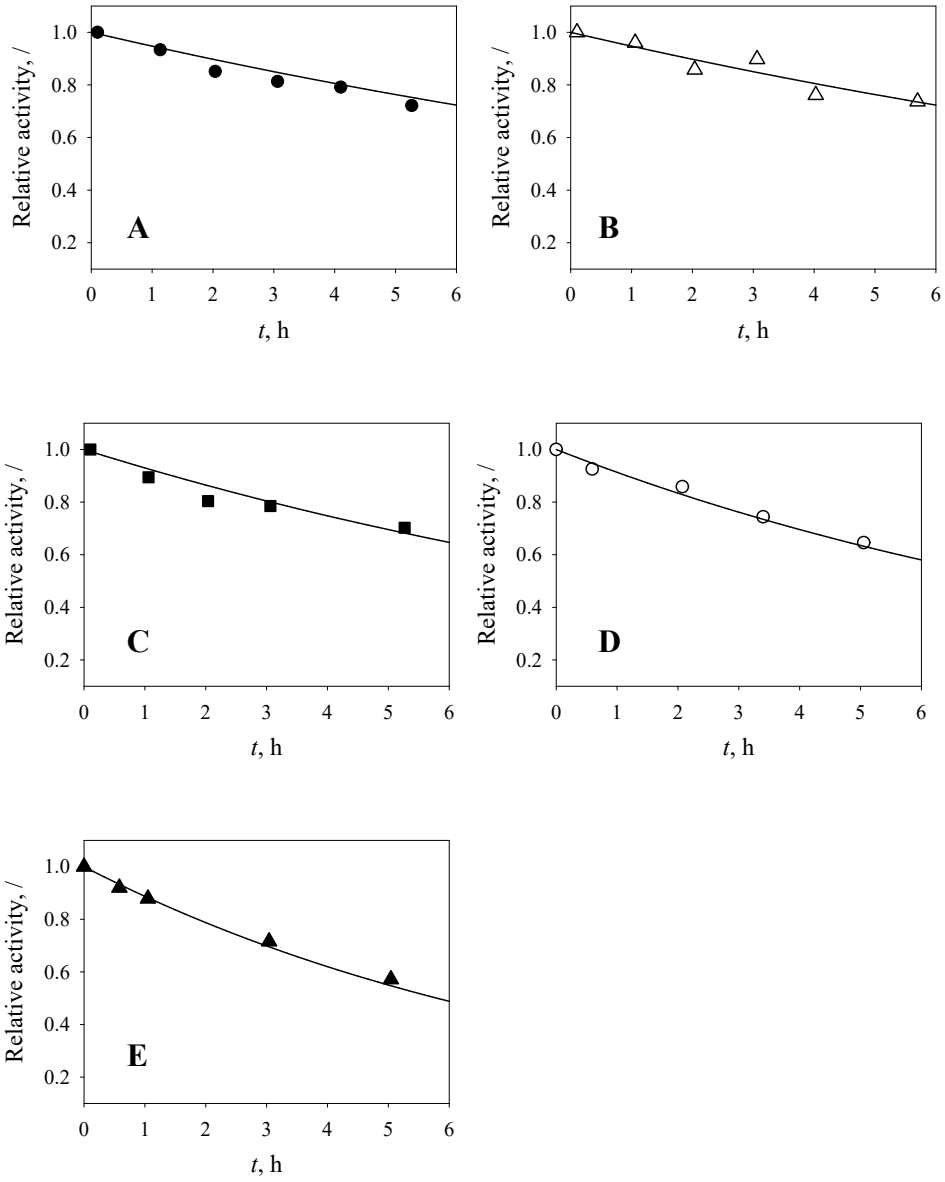
Model validation

S-10. Model validation statistics for experiments conducted in batch reactor (**Fig 3.** in main text) that provides information about fitting of experimental data and models. Validation of mathematical model in batch reactor experiments (V_r = 500 µL; 500 mM Tris-SO_4; pH 7.5; 25 °C; DMSO 10% *v/v*; 1000 rpm) conducted with different initial conditions: **A.** $c_{\text{sodium sodium azide}}$ = 5 mM, $c_{(R)\text{-}\mathbf{1k}}$ = 6.26 mM, γ_{W249P} = 0.30 mg mL^{-1}; **B.** $c_{\text{sodium sodium azide}}$ = 25 mM, $c_{rac\text{-}\mathbf{1k}}$ = 16.5 mM, γ_{W249P} = 0.33 mg mL^{-1}; **C.** $c_{\text{sodium sodium azide}}$ = 10 mM, $c_{rac\text{-}\mathbf{1k}}$ = 20.1 mM, γ_{W249P} = 2.0 mg mL^{-1}; **D.** $c_{\text{sodium azide}}$ = 20 mM, $c_{rac\text{-}\mathbf{1k}}$ = 40.3 mM, γ_{W249P} = 2.0 mg mL^{-1}.

Batch experiment	Standard deviation (σ)	Coefficient of determination (R^2)	Correlation coefficient (ρ)	Model selection criterion (MSC)
Fig 3-A	0.802	0.924	0.953	1.490
Fig 3-B	1.148	0.987	0.977	1.929
Fig 3-C	1.737	0.981	0.907	1.560
Fig 3-D	1.043	0.913	0.955	1.494

S-11. Experimentally determined and model-confirmed enzyme operational stability decay during batch experiments (V_r = 500 µL; 500 mM Tris-SO_4; pH 7.5; 25 °C; DMSO 10% *v/v*; 1000

rpm). Initial conditions: **A.** $c_{\text{sodium sodium azide}}$ = 5 mM, $c_{(R)\text{-}\mathbf{1k}}$ = 6.26 mM, γ_{W249P} = 0.3 mg mL^{-1}; **B.** $c_{\text{sodium azide}}$ = 5 mM, $c_{rac\text{-}\mathbf{1k}}$ = 5.3 mM, γ_{W249P} = 0.2 mg mL^{-1}; **C.** $c_{\text{sodium sodium azide}}$ = 10 mM, $c_{rac\text{-}\mathbf{1k}}$ = 9.2 mM, γ_{W249P} = 0.2 mg mL^{-1}; **D.** $c_{\text{sodium sodium azide}}$ = 25 mM, $c_{rac\text{-}\mathbf{1k}}$ = 16.5 mM, γ_{W249P} = 0.33 mg mL^{-1}; **E.** $c_{\text{sodium sodium azide}}$ = 50 mM, $c_{rac\text{-}\mathbf{1k}}$ = 74.1 mM, γ_{W249P} = 0.4 mg mL^{-1}.



S-12. Table of enzyme operational stability decay rate constants of the first order. Initial conditions: **A.** $c_{\text{sodium azide}}$ = 5 mM, $c_{(R)\text{-}\mathbf{1k}}$ = 6.26 mM, γ_{W249P} = 0.3 mg mL^{-1}; **B.** $c_{\text{sodium azide}}$ = 5 mM, $c_{rac\text{-}\mathbf{1k}}$ = 5.3 mM, γ_{W249P} = 0.2 mg mL^{-1}; **C.** $c_{\text{sodium azide}}$ = 10 mM, $c_{rac\text{-}\mathbf{1k}}$ = 9.2 mM, γ_{W249P} = 0.2 mg mL^{-1}; **D.** $c_{\text{sodium azide}}$ = 25 mM, $c_{rac\text{-}\mathbf{1k}}$ = 16.5 mM, γ_{W249P} = 0.33 mg mL^{-1}; **E.** $c_{\text{sodium azide}}$ = 50 mM, $c_{rac\text{-}\mathbf{1k}}$ = 74.1 mM, γ_{W249P} = 0.4 mg mL^{-1}.

Experiment	$c_{0,\mathbf{1k}}$, mM	k_d, min^{-1}
A	6.26	$0.00106 \pm 5.7 \cdot 10^{-5}$
B	5.26	$0.00095 \pm 1.4 \cdot 10^{-4}$
C	9.22	$0.00121 \pm 1.5 \cdot 10^{-4}$
D	16.5	$0.001512 \pm 7.7 \cdot 10^{-5}$
E	74.1	$0.001992 \pm 6.8 \cdot 10^{-5}$