

Supporting Information

Structural and mechanistic insights into enantioselectivity toward near-symmetric esters of a novel carboxylesterase *RoCE*

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Experimental

1. Activity assay

The activity of *RoCE* toward *p*-nitrophenol esters was spectrophotometrically assayed according to optical absorption changes of *p*-nitrophenol at 405 nm. A 200 μ L reaction mixture, including 10 μ L *p*-nitrophenyl acetate (pNPA) (0.5 mM), 10 μ L enzyme solution and 180 μ L PBS buffer (pH 7.0, 100 mM), was incubated at 30°C. The OD₄₀₅ of the reaction mixture was monitored for 3 min. One unit of activity (U) was defined as the amount of *RoCE* required to catalyze the formation of 1 μ mol of *p*-nitrophenol per minute.

2. Enzyme characterization

Influence of pH on activity of *RoCE* was evaluated using above mentioned method except for at different pH values ranging from 4.0 to 10.0, using sodium citrate buffer (pH 4.0–6.0, 100 mM), sodium phosphate buffer (pH 6.0–7.0, 100 mM), Tris-HCl buffer (pH 7.0–9.0, 100 mM) and glycine-NaOH buffer (pH 9.0–10.0, 100 mM).

Effect of temperature on activity of *RoCE* was measured by determining the relative activity toward pNPA at temperature ranges of 20–60°C in PBS buffer (pH 7.0, 100 mM).

The effects of metal ions (Cu²⁺, Al³⁺, Ag⁺, Mn²⁺, Ca²⁺, Ni²⁺, Co²⁺, Zn²⁺, Fe²⁺, and Mg²⁺) and EDTA on the activity of *RoCE* were explored in Tris-HCl buffer (pH 7.0, 100 mM) at 30°C. Appropriate amount of purified *RoCE* was incubated with various metal ions (1 mM) for indicated periods of time. Control experiment with addition of equal volume of Tris-HCl buffer at same incubation time was regarded as 100%. All activity assays were carried out in triplicate.

pNP esters containing acyl chains with different length including acetate, butyrate, caproate, caprylate, laurate, myristate and palmitate were used to determine the substrate specificity. All the activity assays were conducted using the above-mentioned method for at least three times.

3. Theozyme calculation

Reaction complex of enzyme and (S)-THPCE

C	-3.59437400	149.16571100	107.61549200
C	-3.44635300	150.68697200	107.80262300
C	-4.07583200	151.11995600	109.12965400
C	-3.33093800	150.44383400	110.29402800

C	-3.49850200	148.46380000	108.99673500
C	-2.90397900	147.07232800	108.89288100
O	-3.55263300	146.35012400	107.99111300
O	-1.98400200	146.64901200	109.57955700
C	-3.01817500	145.03942800	107.67515200
H	-4.56580800	148.92064800	107.17232900
H	-2.82208400	148.77848400	106.94716800
H	-2.38582000	150.95872700	107.80577700
H	-4.03009300	152.20679200	109.25736300
H	-2.49680100	151.05789900	110.63719800
H	-4.01077500	150.25008300	111.13817600
H	-4.51318100	148.32695100	109.41038800
H	-3.28720800	144.35816600	108.48844100
H	-1.93291200	145.11794200	107.61675800
C	-0.82325700	143.12479800	109.83284900
C	0.50418100	143.84004100	109.99209700
O	1.58515400	143.24570500	109.91375200
N	0.41460000	145.17825700	110.19626200
C	1.60863600	145.99030600	110.31342400
C	1.44519100	147.38058100	110.91574600
O	2.48688100	147.95915800	111.27318600
N	0.22558500	147.93774100	111.01041300
C	-0.05672500	149.21184000	111.69260300
C	0.06324300	149.30863900	107.66927200
C	0.24448200	147.86939900	107.22206400
O	-0.94157600	147.39317200	106.60126600
C	0.07731100	140.39186200	107.50607700
C	0.45909000	141.05186900	106.16992900
C	0.43764700	142.54657400	106.21494000
C	-0.28547100	143.49653200	105.52845500
N	1.25737000	143.27575500	107.06007600
C	0.98602000	144.59348600	106.87021900
N	0.06028700	144.77081700	105.94453300
H	0.98474300	149.68875700	108.12229200
H	-0.19117100	149.94397200	106.81433300
H	-0.73464400	149.38953400	108.41179900
H	1.09665900	147.79635200	106.52814000
H	0.48753300	147.25203700	108.10154300
H	-0.95615200	140.63272300	107.77655300
H	0.16745900	139.30277300	107.43871300
H	0.72242800	140.73470000	108.32287200
H	1.45797500	140.70749400	105.86863900
H	-0.22854300	140.72444400	105.38259800
H	-1.13838500	149.31009900	111.64396900

H	-0.58379700	147.52129500	110.55247400
H	-0.48699100	145.61349200	110.02963900
H	2.33144500	145.46023900	110.93745000
H	2.10396400	146.13275900	109.34182200
H	-1.66379300	143.70654300	110.21866200
H	-0.99253300	142.92411600	108.76979900
H	-0.76944300	142.16415600	110.34990100
H	-1.02066100	143.33869300	104.75192600
H	1.48599200	145.37393900	107.42047300
H	-0.71537900	146.53666100	106.17289700
H	1.78325800	142.92224200	107.85545100
O	-2.71110400	149.20307400	109.90375900
H	-3.90940200	151.21237000	106.96066700
H	-5.13900700	150.84437100	109.13638000
C	0.39021000	149.20235800	113.16173300
H	-0.09194000	148.37330700	113.68894400
H	1.47210000	149.09140700	113.25196700
H	0.08963100	150.14128200	113.63698500
C	0.49646000	150.45247100	110.96225400
O	1.79523700	150.44032500	110.65062100
H	2.20836200	149.57813200	110.93558800
O	-0.20676900	151.41322100	110.73047400
C	-3.61820900	144.61698800	106.35183500
H	-3.31992700	145.31422700	105.56356100
H	-3.25025300	143.62032200	106.08927000
H	-4.71108000	144.58663300	106.40195000

TSI of enzyme and (S)-THPCE

C	-3.58446800	148.95914900	107.31811100
C	-3.62500600	150.49402400	107.41963700
C	-3.93364300	150.91235500	108.85981000
C	-2.82198000	150.39682700	109.79512700
C	-2.94761800	148.35282100	108.58748600
C	-2.15194500	147.05832100	108.35912800
O	-2.99353600	146.19777600	107.61178000
O	-1.52485000	146.55560200	109.35134400
C	-3.36272700	144.97556900	108.23836000
H	-4.59836300	148.55874200	107.20777200
H	-3.01479300	148.64919000	106.44044800
H	-2.65683700	150.91261200	107.12260300
H	-4.01592100	152.00099900	108.95401200
H	-2.05306900	151.15200900	109.96827600
H	-3.24608900	150.10072300	110.76962500
H	-3.74125400	148.04710900	109.29243700

H	-3.67630800	145.15910300	109.27410900
H	-2.51236300	144.28283300	108.27630200
C	-1.45103600	143.01125600	110.84785800
C	-0.06648900	143.59983400	110.66499000
O	0.87507200	142.93007900	110.21824800
N	0.07874800	144.89538500	111.03324300
C	1.33979900	145.58666900	110.80905200
C	1.35713100	147.04748600	111.24366100
O	2.43069700	147.51189600	111.66760400
N	0.22892400	147.76500300	111.10289800
C	0.08683300	149.16309800	111.53526800
C	0.16585400	149.52947100	107.03300800
C	0.13299900	148.07377900	107.46416600
O	-1.09652000	147.42061600	107.10458500
C	0.69604300	140.71770300	105.94039600
C	1.42891500	142.01288100	105.55297800
C	0.78403400	143.24067100	106.11015100
C	0.21739100	144.33806100	105.50707700
N	0.63315100	143.45614600	107.47326700
C	0.00659200	144.62968800	107.66237500
N	-0.25916800	145.18435100	106.48846800
H	1.14488700	149.96148500	107.26949000
H	-0.00879800	149.61932600	105.95514700
H	-0.59040700	150.09756600	107.57459500
H	0.94306200	147.51273200	106.97679500
H	0.27968700	147.98100300	108.54259300
H	-0.33926600	140.73496800	105.58497700
H	1.19680100	139.84786500	105.50384800
H	0.67660300	140.58030800	107.02737300
H	2.47287200	141.95855000	105.88998400
H	1.46136300	142.11306900	104.46302200
H	-0.95656100	149.40310000	111.35110200
H	-0.52142700	147.41769700	110.47395700
H	-0.76098600	145.45923800	111.08547800
H	2.13792000	145.08317200	111.35646000
H	1.61830400	145.55022500	109.74540700
H	-2.21278700	143.76561700	111.05909200
H	-1.72589100	142.46544400	109.94129100
H	-1.42348300	142.29047100	111.67183600
H	0.12561800	144.56927400	104.45711200
H	-0.29570400	145.08104400	108.59835000
H	-0.76845000	146.28255500	106.60038400
H	0.92710900	142.87781900	108.26262200
O	-2.10401300	149.28978800	109.23923500

H	-4.37196100	150.90040200	106.72855500
H	-4.90613000	150.49494400	109.15304500
C	0.39554900	149.35638900	113.02672500
H	-0.24520000	148.69984900	113.62390500
H	1.43755100	149.12787700	113.25613800
H	0.18970500	150.39434300	113.30605500
C	0.88643000	150.16497100	110.67654400
O	2.18399500	149.89358300	110.49056300
H	2.43502100	149.06504300	110.98185300
O	0.37584300	151.16842200	110.22812500
C	-4.49183000	144.37583600	107.41824200
H	-4.16369600	144.19929400	106.38876800
H	-4.81359900	143.42121000	107.84987200
H	-5.35027900	145.05426500	107.39007400

Intermediate and (S)-THPCE

C	-3.64159500	149.01305700	107.41001600
C	-3.69296100	150.53958400	107.58512200
C	-3.99014100	150.88133100	109.04762400
C	-2.86338500	150.32629700	109.94300300
C	-2.93258700	148.34989100	108.61122800
C	-2.03718200	147.15356200	108.22110400
O	-2.86581000	146.22552500	107.46386900
O	-1.40943800	146.59134200	109.22974600
C	-3.74487200	145.42530300	108.23700300
H	-4.65885100	148.61021000	107.33026100
H	-3.11545700	148.75130900	106.48976500
H	-2.72992000	150.97801900	107.29986900
H	-4.08149500	151.96295100	109.19825300
H	-2.11564500	151.08887800	110.17136800
H	-3.27822300	149.94878900	110.89337200
H	-3.67680000	147.95050700	109.32025500
H	-4.72731300	145.91547400	108.32706900
H	-3.33515500	145.30029400	109.24561800
C	-1.55233500	143.03720800	110.64429900
C	-0.15128500	143.59743600	110.51272000
O	0.78682500	142.89946200	110.08766100
N	0.00425400	144.89197800	110.86049100
C	1.26968500	145.57497700	110.62813300
C	1.32563600	147.00416200	111.16049100
O	2.41281300	147.41937900	111.60275600
N	0.20802900	147.74340400	111.07403100
C	0.08669500	149.11053300	111.59516500
C	0.14989000	149.63020900	107.11770700

C	0.11224200	148.14424400	107.43810000
O	-1.14415900	147.54102400	107.10439600
C	0.78323200	140.66030100	106.35101900
C	1.41878400	141.90685900	105.71307800
C	0.72044900	143.17154600	106.09557500
C	0.14195800	144.16657500	105.35075100
N	0.52820200	143.53867000	107.42199700
C	-0.13698300	144.69768600	107.48532600
N	-0.37561900	145.09157600	106.23739600
H	1.15468500	150.02510600	107.30757300
H	-0.10390600	149.80669400	106.06631500
H	-0.55169900	150.16796200	107.75608600
H	0.87528400	147.61147100	106.85151700
H	0.33135600	147.96606300	108.49199700
H	-0.26264100	140.55534900	106.04507800
H	1.32393800	139.75910700	106.04677000
H	0.81191100	140.71300300	107.44509900
H	2.47541500	141.97047000	106.00535300
H	1.40326700	141.82443300	104.62143500
H	-0.95651700	149.37369800	111.44489400
H	-0.53777400	147.44100200	110.40694900
H	-0.83160700	145.46947800	110.83735900
H	2.07465700	145.01919400	111.10935000
H	1.50261600	145.61145800	109.55352100
H	-2.24450300	143.72348000	111.13766800
H	-1.93779600	142.80727700	109.64516200
H	-1.50606800	142.09907600	111.20421600
H	0.06253900	144.28187200	104.28189200
H	-0.49339100	145.26870300	108.35669400
H	-0.87615200	145.98889100	106.11079400
H	0.81518000	143.04428500	108.27906900
O	-2.11720800	149.28641500	109.30709900
H	-4.45033200	150.97406300	106.92274300
H	-4.95579100	150.43968200	109.32800400
C	0.41987000	149.20842900	113.09087300
H	-0.21752400	148.52041900	113.65537000
H	1.46296500	148.95760300	113.28998800
H	0.22711500	150.22834600	113.43806600
C	0.88503600	150.15184900	110.78571100
O	2.18091200	149.88173500	110.58073000
H	2.42558300	149.02236100	111.01962800
O	0.37779300	151.17879000	110.38956400
C	-3.89853900	144.07618400	107.54930900
H	-2.93550600	143.55418500	107.50479300

H	-4.61353600	143.44316800	108.08759900
H	-4.25718700	144.20576700	106.52272800

Reaction complex of enzyme and (R)-THPCE

C	-4.50856900	147.37017700	106.96205500
C	-3.83268600	148.72457000	107.15822800
C	-3.80538800	149.08143100	108.64985500
C	-3.89565100	146.59549600	109.10076900
C	-3.21896400	145.47151800	109.86827000
O	-2.61998300	144.56889300	109.10088600
O	-3.21723400	145.45391000	111.08947100
C	-1.85077900	143.53662900	109.78676000
H	-4.47601800	147.04305000	105.91880800
H	-2.80883300	148.66828800	106.76968000
H	-3.22065000	149.98978600	108.82825500
H	-4.83046600	149.28483500	108.99147300
H	-4.94544400	146.62200000	109.44008200
H	-2.50883100	143.04391200	110.50753900
H	-1.03465900	144.02993700	110.31724100
C	-0.54851300	144.66930300	113.10223400
C	-0.10021900	146.11321300	112.96436200
O	1.01142300	146.48802700	113.33026400
N	-1.02572500	146.96270900	112.42810300
C	-0.80491400	148.38955200	112.36478400
C	-0.33756100	148.97532700	111.03122600
O	-0.45631100	150.18068300	110.81397200
N	0.24873700	148.12017900	110.15883800
C	0.94772300	148.63703200	108.99450400
C	3.27344900	145.92773700	110.15067700
C	2.19576600	144.94698200	110.59944000
O	0.88876600	145.32283700	110.15994400
C	-1.28767300	146.13485100	103.17468800
C	-1.41490300	146.83445100	104.53770900
C	-0.43746200	146.34769600	105.55697400
C	-0.60876800	145.74940600	106.78138100
N	0.93754900	146.44653400	105.40166400
C	1.52810600	145.91825200	106.50997200
N	0.61977300	145.48666500	107.36412200
H	2.42835500	143.92990200	110.24860200
H	2.15139700	144.93317500	111.69065900
H	-1.42426200	145.05388600	103.27984000
H	-2.03983700	146.51195100	102.47413300
H	-0.30255500	146.30476900	102.72392300
H	-1.31122500	147.92101400	104.40574300

H	-2.41633400	146.66831800	104.94584900
H	1.51536600	147.78945200	108.59603400
H	0.41408600	147.14238200	110.41018500
H	-1.90232100	146.57846900	112.09387800
H	-1.71123300	148.93051600	112.65174600
H	-0.02121900	148.62911300	113.09116500
H	-1.56894900	144.50150300	112.75254900
H	0.13415000	144.03221000	112.53381000
H	-0.46921100	144.38392800	114.15589600
H	-1.53876100	145.51004600	107.27502100
H	2.59977100	145.87540900	106.64389000
H	0.84908300	145.26590900	109.17159700
H	1.41674700	146.85244600	104.61080400
H	-5.56754800	147.42504300	107.26693200
H	-4.36357800	149.49055900	106.57968600
C	-3.21508300	147.92668600	109.46449200
O	-3.85293100	146.34380100	107.70650400
H	-2.14440800	147.83244600	109.26244100
H	-3.33225700	148.10452200	110.53755600
C	1.99461800	149.69153000	109.36119200
O	2.35587600	150.58664000	108.63010700
O	2.56297600	149.46060000	110.56719000
H	3.21765100	150.17206900	110.69334100
C	0.02223500	149.16489200	107.89621200
H	3.32593000	145.98817500	109.05587500
H	3.07120800	146.92691900	110.54734600
H	4.25726900	145.60729300	110.51389700
H	-0.58536400	149.98296900	108.28679100
H	0.61421700	149.53929000	107.05748500
H	-0.62235000	148.35657700	107.54655900
C	-1.33178400	142.58678200	108.72771400
H	-0.65126300	143.10284000	108.04464800
H	-0.77996100	141.77381500	109.21214000
H	-2.15251400	142.15080100	108.14928500

TSl of enzyme and (R)-THPCE

C	-3.45041200	148.21436800	106.27935300
C	-3.19709800	149.70821200	106.46921100
C	-3.25683500	150.05694400	107.96144400
C	-2.59639200	147.66917800	108.42912600
C	-1.63644800	146.71518600	109.16653300
O	-1.77418300	145.36520000	108.69143000
O	-1.59529800	146.86943400	110.42509500
C	-2.84769000	144.63882000	109.28155500

H	-3.32650800	147.91264600	105.23402500
H	-2.20347600	149.94996000	106.07003300
H	-2.99366900	151.10805600	108.12654600
H	-4.28800900	149.92824400	108.32141900
H	-3.60901600	147.41060700	108.78338500
H	-3.81325600	145.06263000	108.96386100
H	-2.79325200	144.71529100	110.37311300
C	0.30412000	143.79303000	111.37515700
C	0.63278600	144.71861500	112.54089200
O	1.51268800	144.44519100	113.35161400
N	-0.15339300	145.83104500	112.61386500
C	0.02459700	146.81527100	113.66121300
C	0.37620800	148.23850000	113.21590700
O	0.96533400	148.99882000	113.98285200
N	-0.02265900	148.62262400	111.97672200
C	0.18313100	149.99189300	111.53806000
C	2.12891700	147.45468900	108.76462400
C	0.93260200	146.60520600	109.15917200
O	-0.22702100	147.05892500	108.44204600
C	-0.99296400	141.94702200	104.03679400
C	-1.30141200	143.42352200	103.73384200
C	-0.63686300	144.36815600	104.68191900
C	-1.12870800	145.15656800	105.68783800
N	0.74146400	144.56116000	104.71475900
C	1.03968300	145.43336900	105.70456000
N	-0.07901300	145.80684100	106.29963900
H	1.09991200	145.54227600	108.93816700
H	0.73159700	146.70008500	110.22526500
H	-1.31212100	141.68614200	105.05058400
H	-1.51314800	141.29271000	103.33029900
H	0.07993800	141.73586100	103.95939600
H	-1.01077200	143.66104600	102.70146800
H	-2.38059900	143.59617500	103.79581000
H	0.03461600	149.98189800	110.45024800
H	-0.60675500	148.02298700	111.38714800
H	-0.81345200	146.01438400	111.85613200
H	-0.88041000	146.88908200	114.28173900
H	0.83525600	146.46791000	114.30275200
H	-0.25202300	142.92848300	111.75609000
H	-0.28638400	144.27545400	110.59258300
H	1.24100400	143.41856300	110.95360700
H	-2.13337100	145.32189400	106.03742500
H	2.03579900	145.76203800	105.95764900
H	-0.19547600	146.48512600	107.31578700

H	1.41267900	144.12146400	104.10094400
H	-4.48200900	147.96787100	106.58651900
H	-3.93357300	150.28421600	105.89485100
C	-2.31965400	149.13751100	108.75320400
O	-2.53079700	147.42369100	107.02326900
H	-1.27649700	149.34933500	108.49294600
H	-2.43418000	149.28555600	109.83056900
C	1.62602600	150.44946100	111.74534300
O	1.96016000	151.57169200	112.04918100
O	2.52342700	149.48394000	111.42574400
H	3.39777700	149.87776700	111.60118600
C	-0.79534700	150.98136600	112.17450500
H	2.32245300	147.41201600	107.68470100
H	1.96921400	148.49812700	109.04471700
H	3.02380100	147.10277800	109.28888500
H	-0.65919600	150.98339900	113.25799600
H	-0.61910700	151.99283300	111.80001200
H	-1.82233100	150.68315200	111.94085300
C	-2.72969800	143.19404900	108.82596700
H	-1.78002000	142.76377100	109.15963500
H	-3.54772000	142.59326700	109.23850100
H	-2.76829600	143.12778700	107.73275300

Intermediate and (R)-THPCE

C	-3.48386300	148.22934400	106.30915700
C	-3.31592300	149.72903100	106.54259700
C	-3.38383500	150.02859800	108.04500500
C	-2.58646200	147.66780500	108.44223200
C	-1.56319500	146.75237500	109.15351200
O	-1.67513900	145.39074200	108.63323700
O	-1.60332600	146.84824400	110.43324700
C	-2.75280400	144.63733300	109.16778800
H	-3.35363100	147.96854100	105.25294900
H	-2.34093800	150.03991600	106.14500700
H	-3.18008400	151.08790000	108.23969500
H	-4.40314300	149.83002800	108.40698800
H	-3.58147200	147.34446400	108.78778300
H	-3.71569400	145.03066600	108.80253900
H	-2.75661600	144.71742900	110.26049500
C	0.32324400	143.78096600	111.37105000
C	0.61690500	144.70018900	112.55164500
O	1.47164600	144.41945700	113.38758800
N	-0.16830900	145.81242200	112.60244900
C	-0.01594300	146.79801700	113.65270000

C	0.34008200	148.22009500	113.20661500
O	0.93395800	148.98018900	113.97141600
N	-0.06058500	148.60094000	111.96814600
C	0.16016600	149.96389100	111.52020700
C	2.13200500	147.44124000	108.73311400
C	0.92291700	146.63838900	109.18267800
O	-0.23893000	147.15519800	108.52063600
C	-1.10300300	141.93207500	104.22297700
C	-1.33161000	143.39235300	103.79500200
C	-0.67696800	144.37520900	104.71004800
C	-1.18622200	145.22188200	105.65629000
N	0.70586900	144.53447100	104.78429200
C	1.00344700	145.44085900	105.73623100
N	-0.13278300	145.86768500	106.26274100
H	1.03661000	145.57109800	108.94392300
H	0.79041900	146.72622200	110.26081400
H	-1.48608500	141.76159200	105.23379400
H	-1.61408300	141.24853200	103.53824100
H	-0.03781200	141.67368100	104.22034800
H	-0.97569800	143.54328400	102.76718300
H	-2.40364700	143.61244600	103.78455800
H	0.01377700	149.94655900	110.43221100
H	-0.64176600	147.99532700	111.37407400
H	-0.80533300	146.00250400	111.82295100
H	-0.93531700	146.87048100	114.25221800
H	0.78164700	146.45588400	114.31304100
H	-0.23171700	142.90716600	111.73203000
H	-0.25508800	144.26607400	110.58121100
H	1.27318300	143.42003300	110.96660100
H	-2.19124800	145.43219100	105.97861500
H	1.99454100	145.75664000	106.02041500
H	-0.23265900	146.50848700	107.13691800
H	1.38471700	144.04658700	104.21650500
H	-4.49698200	147.91497600	106.61560500
H	-4.08878200	150.27770500	105.98946300
C	-2.38947400	149.14019600	108.80104300
O	-2.51450200	147.46974200	107.02241300
H	-1.36261700	149.41782600	108.53717500
H	-2.49921500	149.24982500	109.88330200
C	1.60666500	150.40909900	111.72694800
O	1.95325600	151.52935000	112.02484600
O	2.49627500	149.43339900	111.41284400
H	3.37327800	149.82016900	111.59049800
C	-0.80906100	150.96844900	112.14739300

H	2.25796900	147.41899800	107.64154000
H	2.03799300	148.48399100	109.04343300
H	3.04079200	147.03692400	109.19177900
H	-0.67446000	150.97672800	113.23107900
H	-0.62244700	151.97552100	111.76583100
H	-1.83842900	150.67838100	111.91436800
C	-2.57786700	143.19318800	108.72400000
H	-1.63402200	142.78744700	109.10209100
H	-3.39834100	142.57162800	109.09969900
H	-2.56466200	143.12134200	107.62965200

Table S1 Data collection and refinement statistics of *RoCE* crystal.

Item	<i>RoCE</i>
Data collection	
Space group	P 21 21 21
Cell dimensions	48.68 Å, 90.47 Å, 131.36 Å,
a, b, c, α , β , γ	90°, 90°, 90°,
Resolution range (Å)	37.25 - 1.78
Unique reflections	55437
Redundancy	1.0
Completeness (%)	98.5 (37.25-1.78)
Average $I/\sigma(I)$ ^a	3.85 (at 1.78Å)
Wilson B-factor	19.4
CC 1/2	0.994
Refinement	
No. of reflections	52737
Rwork (95% data)	0.170
Rfree (5% data) ^b	0.197
Protein (aa)	273
Average B factors (Å ²)	22.0
RMSD	
Rmsd bonds (Å)	0.013
Rmsd angles (°)	1.789
Ramachandran plot favored (%)	98
Ramachandran plot outliers (%)	0
PDB ID code	7YII

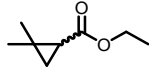
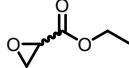
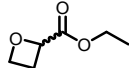
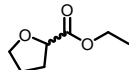
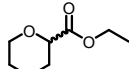
^a Parentheses indicate highest-resolution shells.

^b R_{free} calculated using 5% of reflections that were randomly selected during refinement.

Table S2 List of primers for site-directed mutagenesis.






Primer	Sequence (5'-3')
<i>RoCE-M144S-F</i>	GGTAAAGTTGGTGCAGCAAGCCGTGCC
<i>RoCE-M144S-R</i>	GCCCGGAATTGCGGCACGGCTTGCTGC
<i>RoCE-M144T-F</i>	GGTAAAGTTGGTGCAGCAACCCGTGCC
<i>RoCE-M144T-R</i>	GCCCGGAATTGCGGCACGGGTTGCTGC
<i>RoCE-M144Y-F</i>	GGTAAAGTTGGTGCAGCATATCGTGCC
<i>RoCE-M144Y-R</i>	GCCCGGAATTGCGGCACGATATGCTGC
<i>RoCE-M144C-F</i>	GGTAAAGTTGGTGCAGCATGTCGTGCC
<i>RoCE-M144C-R</i>	GCCCGGAATTGCGGCACGACATGCTGC
<i>RoCE-M144N-F</i>	GGTAAAGTTGGTGCAGCAAATCGTGCC
<i>RoCE-M144N-R</i>	GCCCGGAATTGCGGCACGATTTGCTGC
<i>RoCE-M144Q-F</i>	GGTAAAGTTGGTGCAGCACAGCGTGCC
<i>RoCE-M144Q-R</i>	GCCCGGAATTGCGGCACGCTGTGCTGC
<i>RoCE-M144G-F</i>	GGTAAAGTTGGTGCAGCAGGTCGTGCC
<i>RoCE-M144G-R</i>	GCCCGGAATTGCGGCACGACCTGCTGC
<i>RoCE-V227S-F</i>	CATGGCACCGAGGATAGCAGCGTGGAT
<i>RoCE-V227S-R</i>	ACCGGCGCTCACATCCACGCTGCTATC
<i>RoCE-V227T-F</i>	CATGGCACCGAGGATAGCACCGTGGAT
<i>RoCE-V227T-R</i>	ACCGGCGCTCACATCCACGGTGCTATC
<i>RoCE-V227Y-F</i>	CATGGCACCGAGGATAGCTATGTGGAT
<i>RoCE-V227Y-R</i>	ACCGGCGCTCACATCCACATAGCTATC
<i>RoCE-V227C-F</i>	CATGGCACCGAGGATAGCTGTGTGGAT
<i>RoCE-V227C-R</i>	ACCGGCGCTCACATCCACACAGCTATC
<i>RoCE-V227N-F</i>	CATGGCACCGAGGATAGCAATGTGGAT
<i>RoCE-V227N-R</i>	ACCGGCGCTCACATCCACATTGCTATC
<i>RoCE-V227Q-F</i>	CATGGCACCGAGGATAGCCAGGTGGAT
<i>RoCE-V227Q-R</i>	ACCGGCGCTCACATCCACCTGGCTATC
<i>RoCE-V227G-F</i>	CATGGCACCGAGGATAGCGGTGTGGAT
<i>RoCE-V227G-R</i>	ACCGGCGCTCACATCCACACCGCTATC

Table S3 Chiral GC analysis methods used in this study.

Entry	Substrate	Conditions	(<i>R</i>)-ester	(<i>S</i>)-ester
1		Injector: 280°C, Detector: 280°C Column: 60°C for 3 min, 5°C/min to 120°C and kept for 1 min, 10°C/min to 160°C	9.24 min	8.97 min
2		Injector: 280°C, Detector: 280°C Column: 80°C for 3.5 min, 5°C/min to 98°C	5.91 min	5.50 min
3		Injector: 280°C, Detector: 280°C Column: 100°C for 5 min, 10°C/min to 120°C	5.69 min	5.38 min
4		Injector: 280°C, Detector: 280°C Column: 100°C for 5 min, 10°C/min to 132°C	7.18 min	6.92 min
5		Injector: 280°C, Detector: 280°C Column: 35°C for 5 min, 4°C/min to 60°C, 5°C/min to 100°C, 8°C/min to 120°C, 10°C/min to 160°C, 20°C/min to 165°C	24.5 min	24.7 min

Note: Chiral GC was performed using GC2014 (SHIMADZU Co. Ltd) equipped with CP-Chirasil-Dex CB.

Table S4 Enantioselective resolution of various esters with near-symmetric structure employing M144T.

Substrate	Specific activity /U·mg ⁻¹	Conv. /%	<i>ee</i> _s /%	<i>E</i> value
DMCPE 	3.43±0.05	51.1	>99.9	>200
ORCE 	36.7±0.3	89.2	96.5	3.6
OTCE 	32.4±0.5	81.1	96.6	5.1
THFCE 	80.6±0.8	69.8	96.8	8.9
THPCE 	37.2±0.5	62.6	97.3	15.1

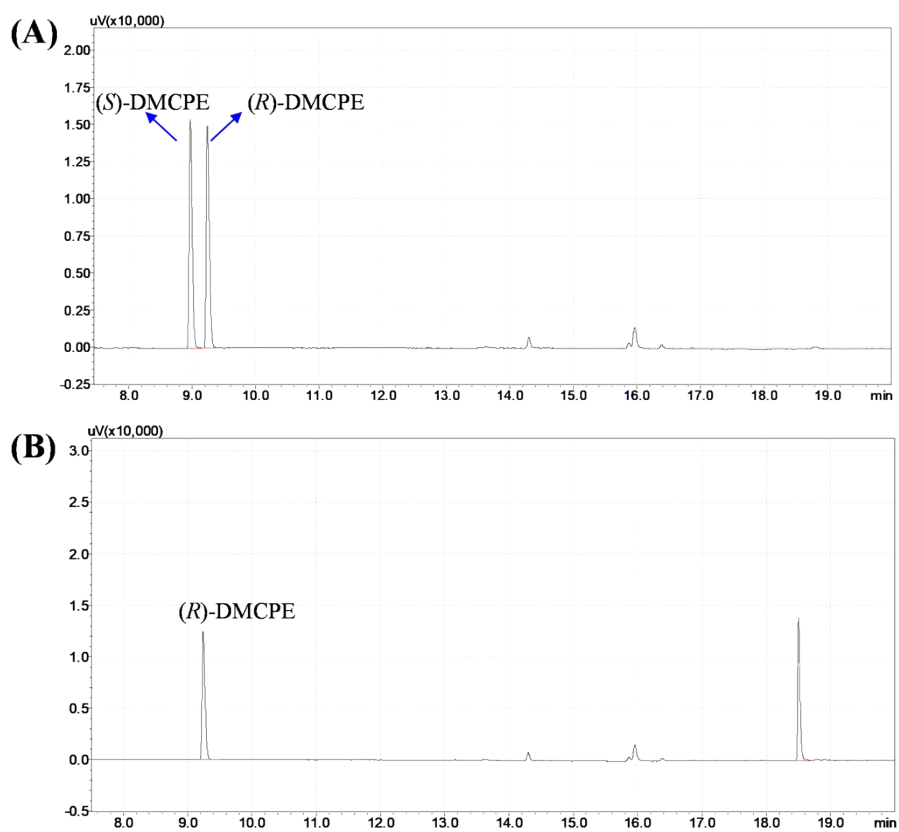


Fig. S1 GC spectra of racemic DMCPE (A) and the (R)-DMCPE produced by recombinant RoCE (B).

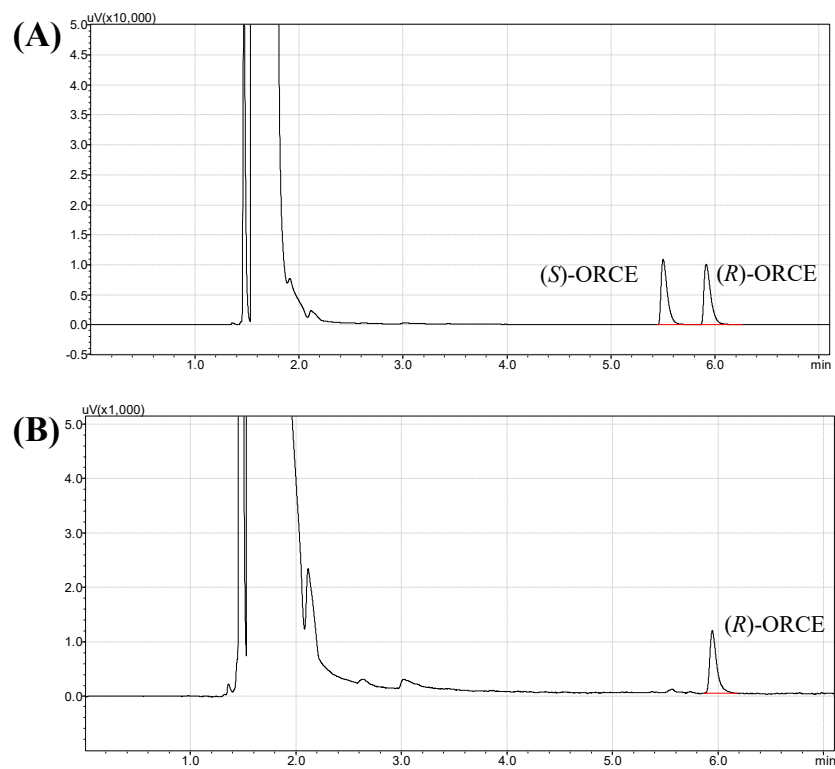


Fig. S2 GC spectra of racemic ORCE (A) and the (R)-ORCE synthesized by recombinant *RoCE* (B).

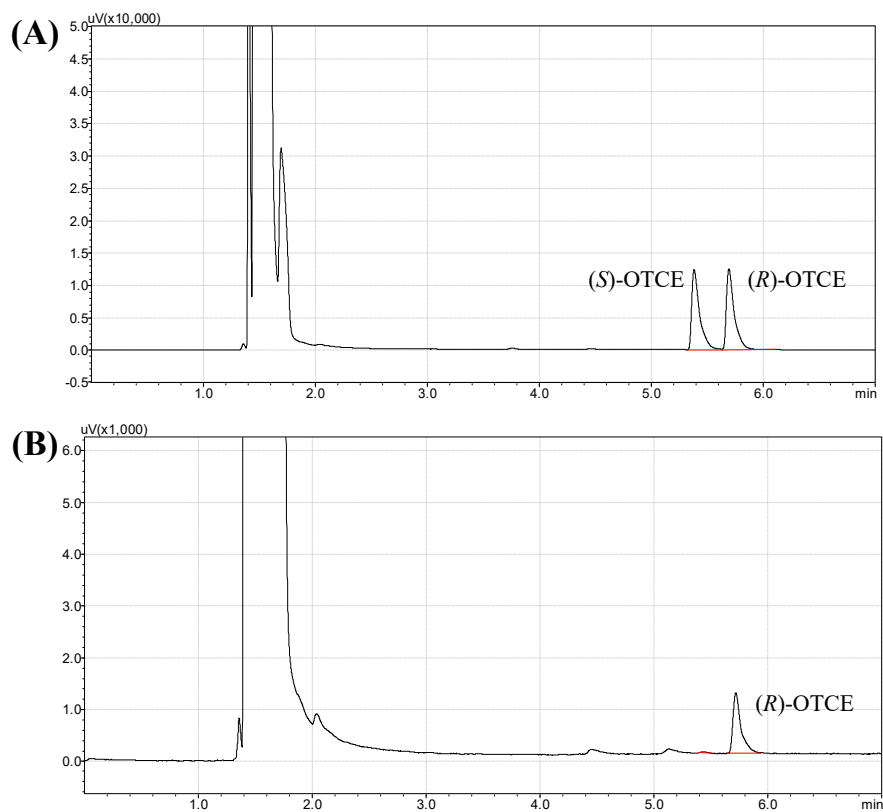


Fig. S3 GC spectra of racemic OTCE (A) and the (R)-OTCE synthesized by recombinant RoCE (B).

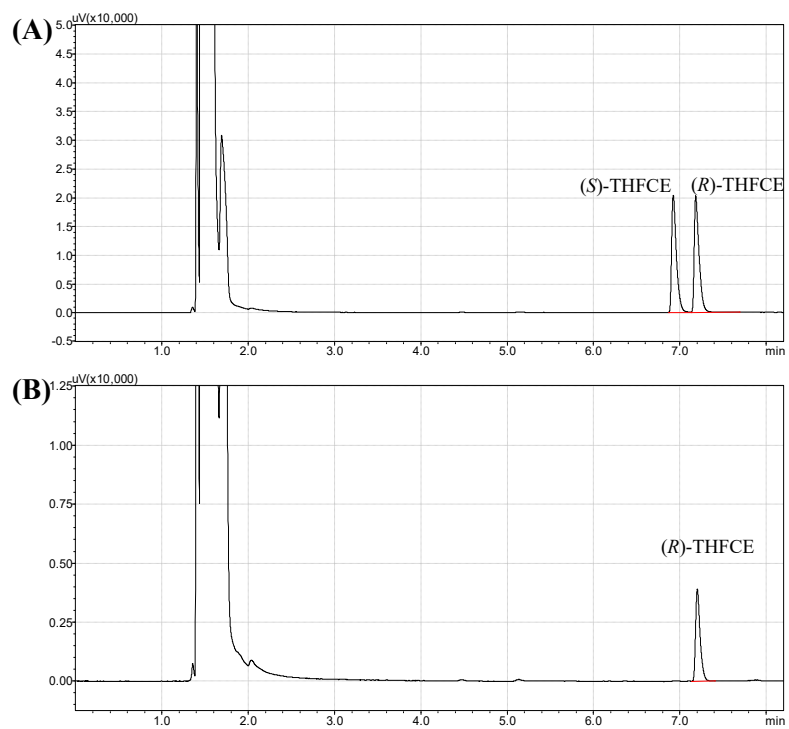


Fig. S4 GC spectra of racemic THFCE (A) and the (*R*)-THFCE synthesized by recombinant *RoCE* (B).

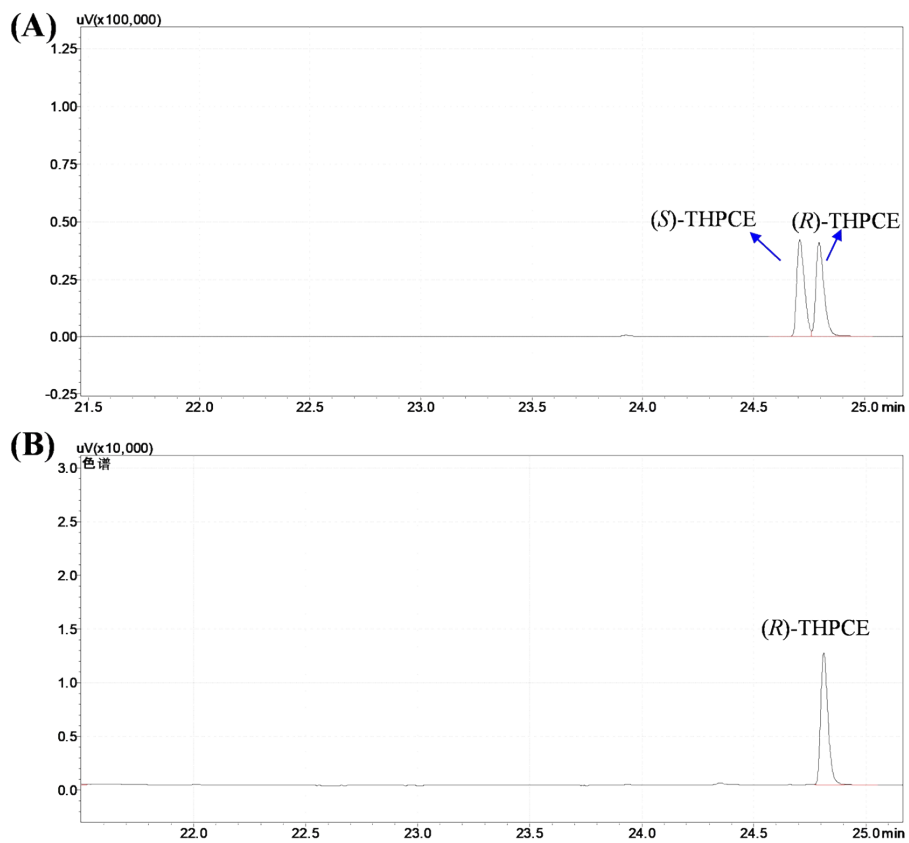


Fig. S5 GC spectra of racemic THPCE (A) and the (R) -THPCE synthesized by recombinant $RoCE$ (B).

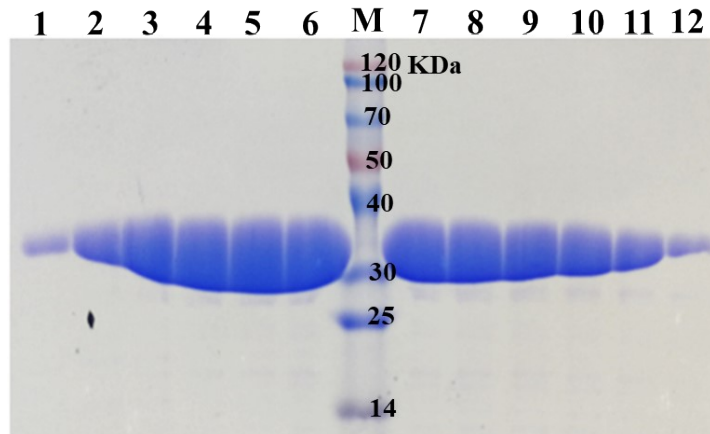


Fig. S6 SDS-PAGE of *RoCE* purified by gel filtration chromatography. Lane M: protein marker, Lanes 1-12: The serial number of the eluents from gel filtration chromatography.

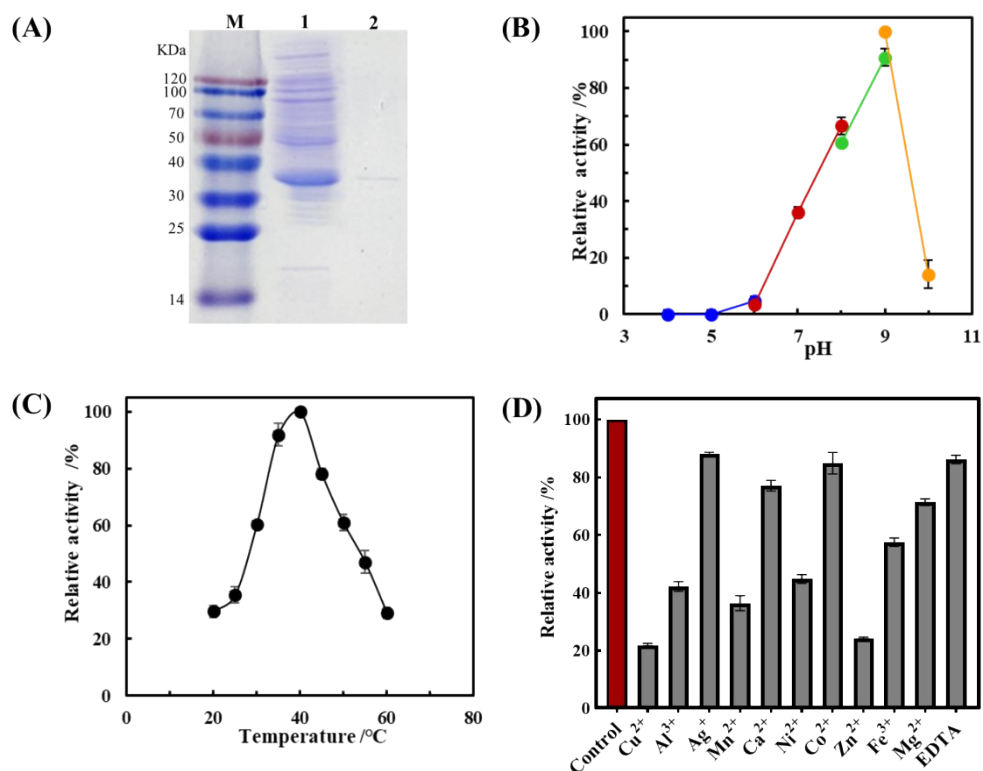


Fig. S7 Enzyme characterization of purified *RoCE*.

(A) SDS-PAGE analysis of *RoCE*. Lane M, protein molecular marker; Lanes 1 & 2, supernatant and precipitant of *E. coli* BL21(DE3)/pET28-*RoCE*; (B) pH-activity profile of purified *RoCE* toward p-nitrophenyl acetate (pNPA), (●): sodium citrate buffer (pH 4.0–6.0), (●): sodium phosphate buffer (pH 6.0–7.0), (●): Tris-HCl buffer (pH 7.0–9.0), (●): Glycine-NaOH buffer (pH 9.0–10.0); (C) Temperature-activity profile of purified *RoCE*; (D) Effect of metal ions on the activity of *RoCE*.

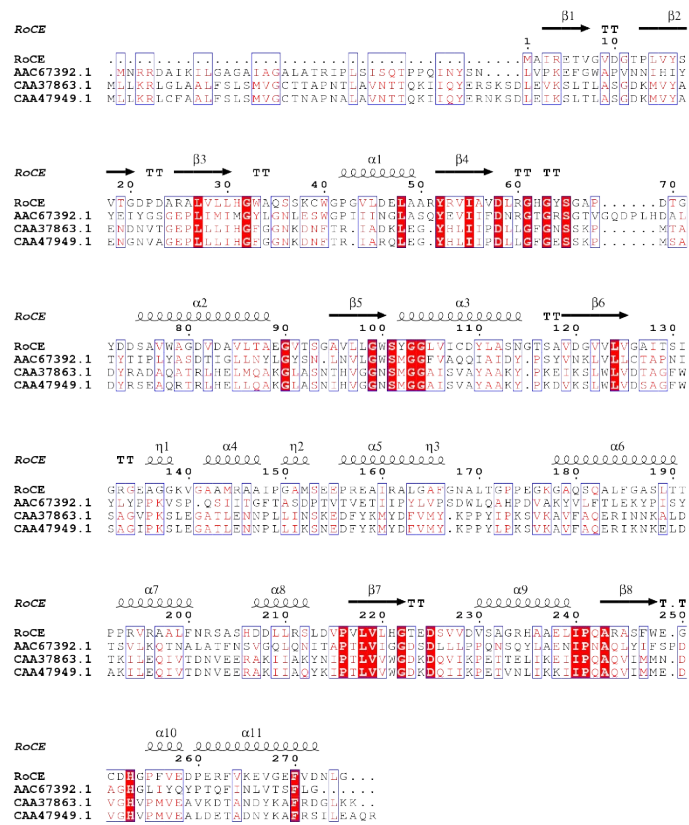


Fig. S8 Multiple sequence alignment of RoCE and homologous carboxylesterases from Family V.

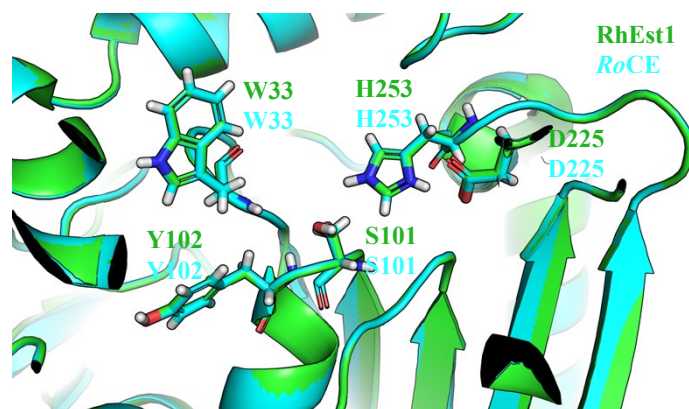


Fig. S9 Structural alignment and enlarged view of *RhEst1* and *RoCE*. Oxyanion hole residues and catalytic triad were illustrated in stick. Green: *RhEst1*, Blue: *RoCE*.

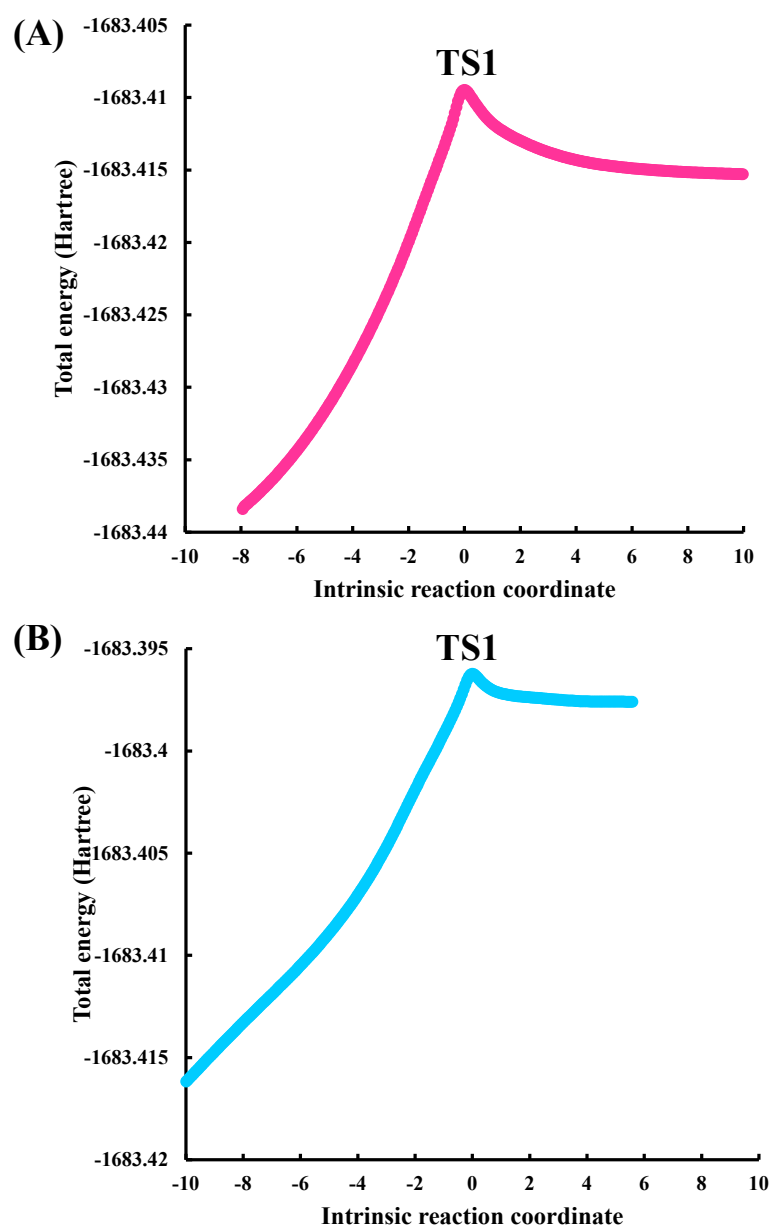


Fig. S10 IRC analysis of (*S*)-THPCE (A) and (*R*)-THPCE (B).

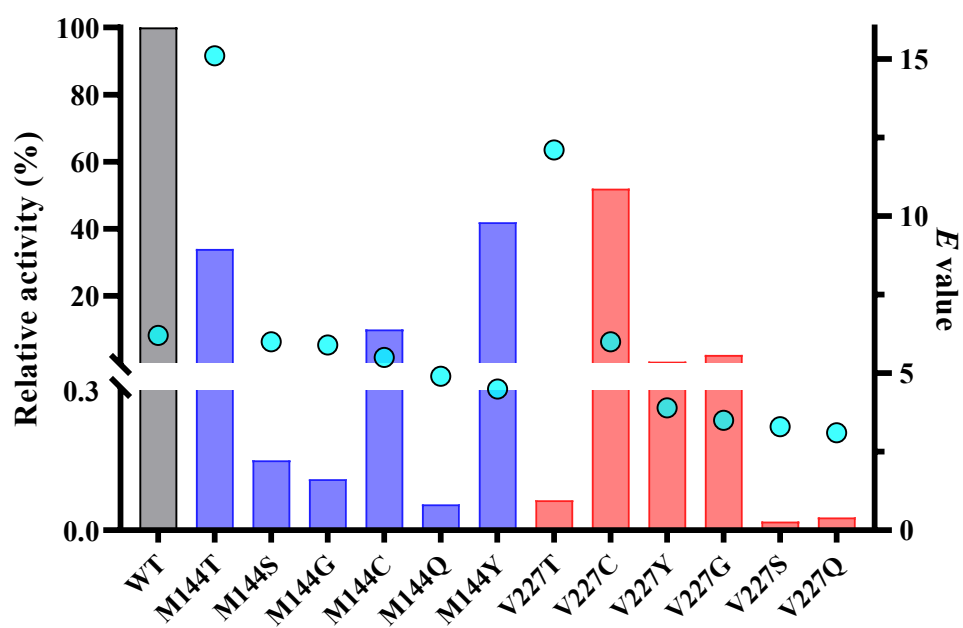


Fig. S11 Relative activity and *E* values of *RoCE* mutants with site-directed mutagenesis at M144 and V227.
 Column: relative activity of WT (grey), M144 mutants (blue) and V227 mutants (red). Filled cycle: *E* values.

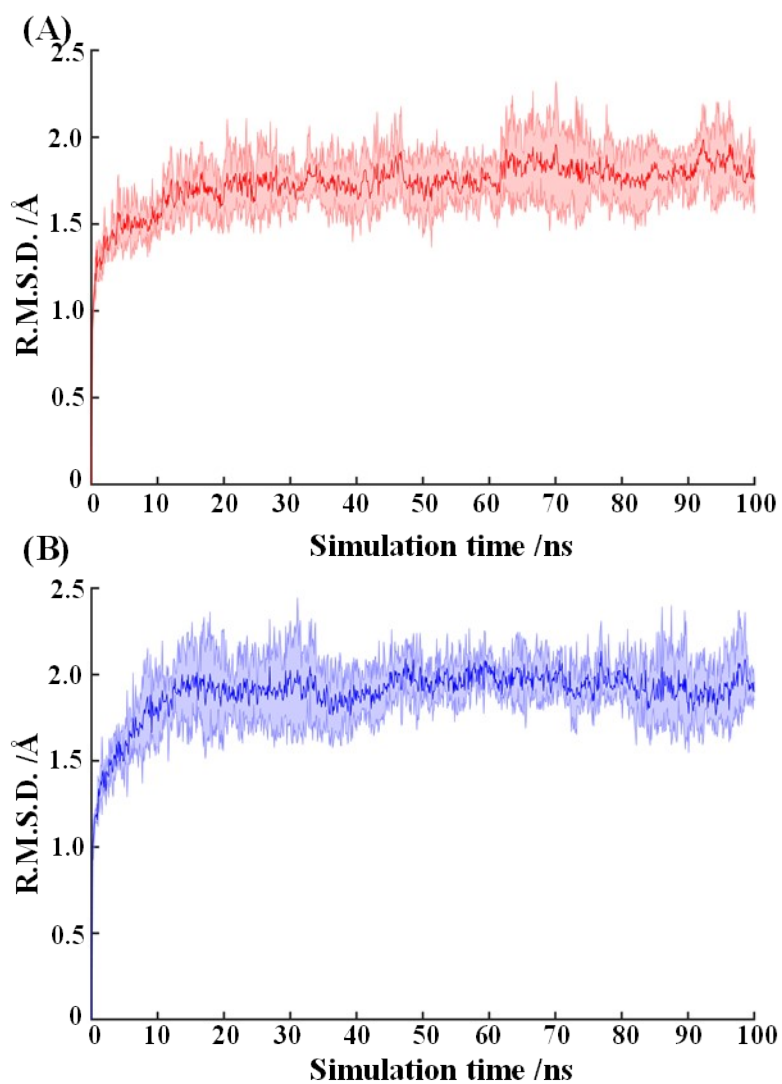


Fig. S12 Root-mean-square deviation of WT with DMCPE during the MD simulation. (A) WT in complex with (*S*)-DMCPE, (B) WT in complex with (*R*)-DMCPE.

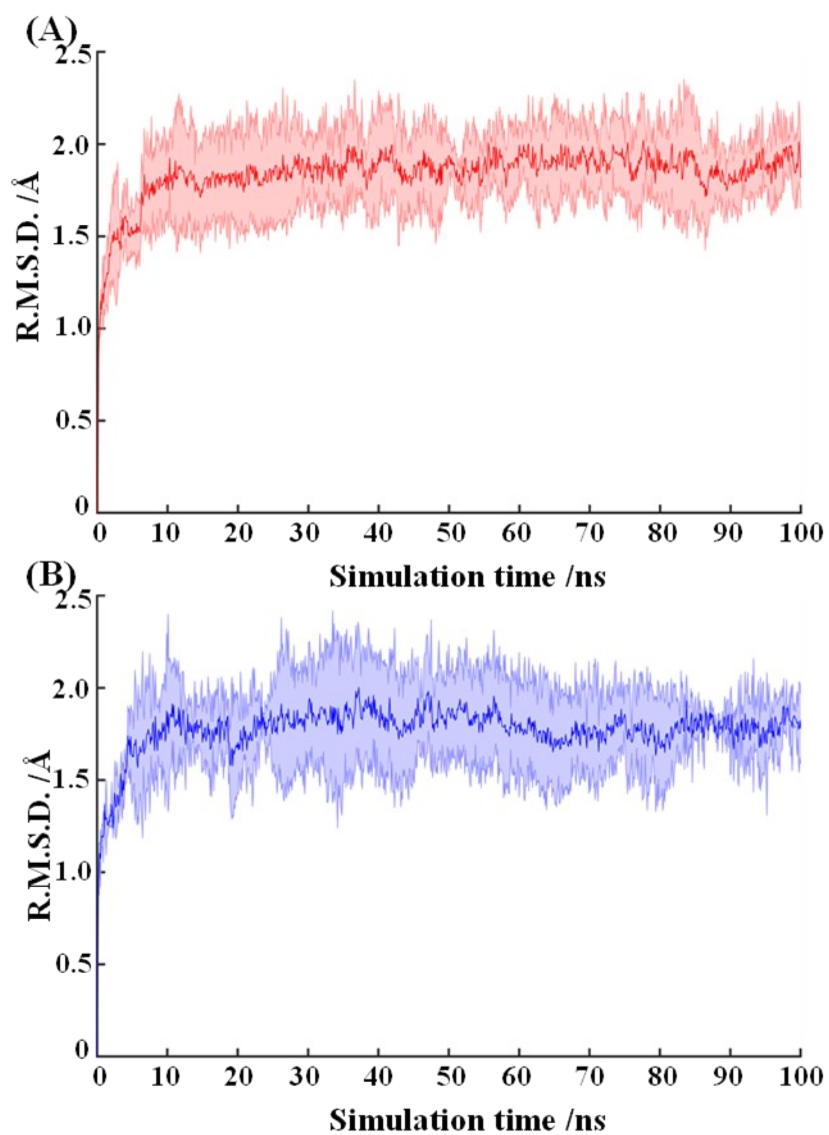


Fig. S13 Root-mean-square deviation of WT with THPCE during the MD simulation.
(A) WT in complex with (*S*)-THPCE, (B) WT in complex with (*R*)-THPCE.

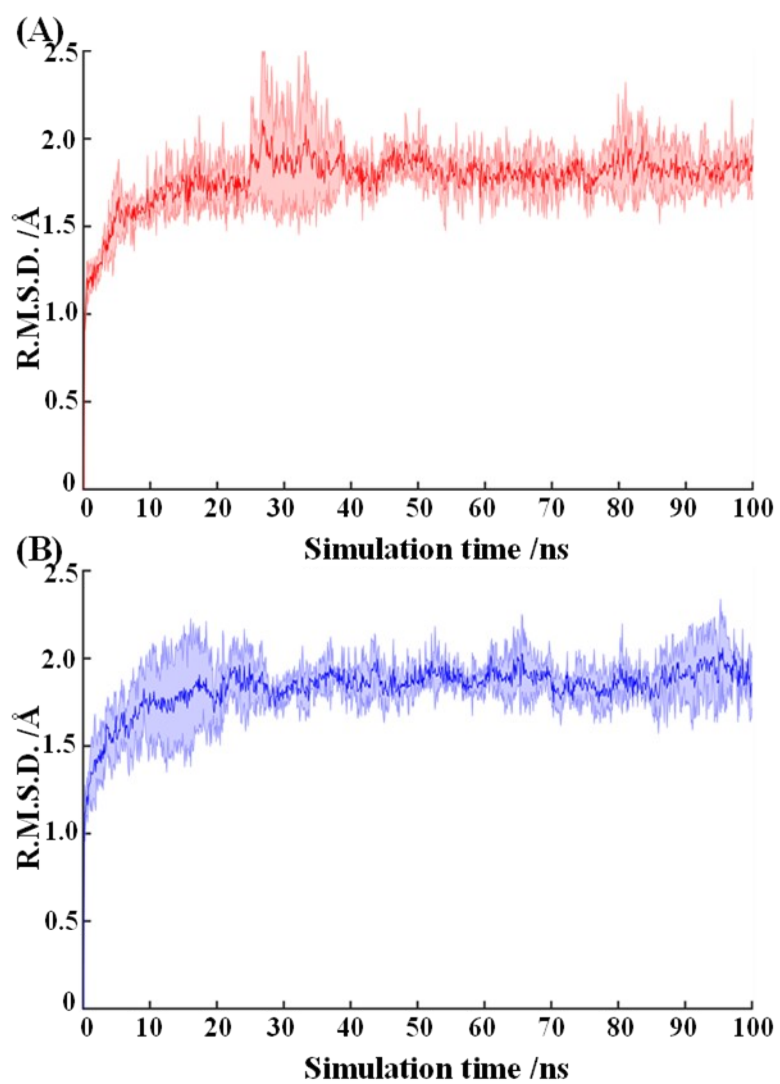


Fig. S14 Root-mean-square deviation of M144T with THPCE during the MD simulation.
(A) M144T in complex with (*S*)-THPCE, (B) M144T in complex with (*R*)-THPCE.

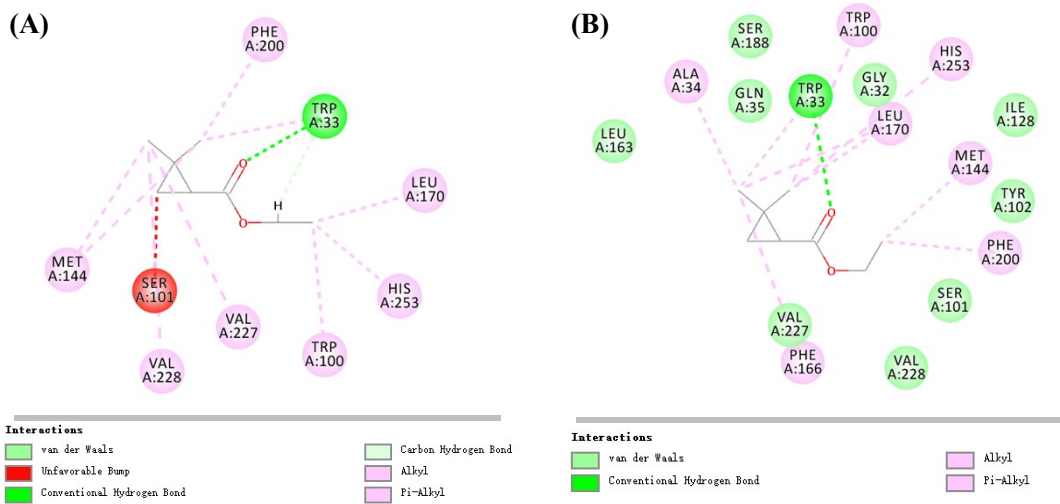


Fig. S15 Interaction analysis of WT with (*R*)-DMCPE (A) and (*S*)-DMCPE (B).

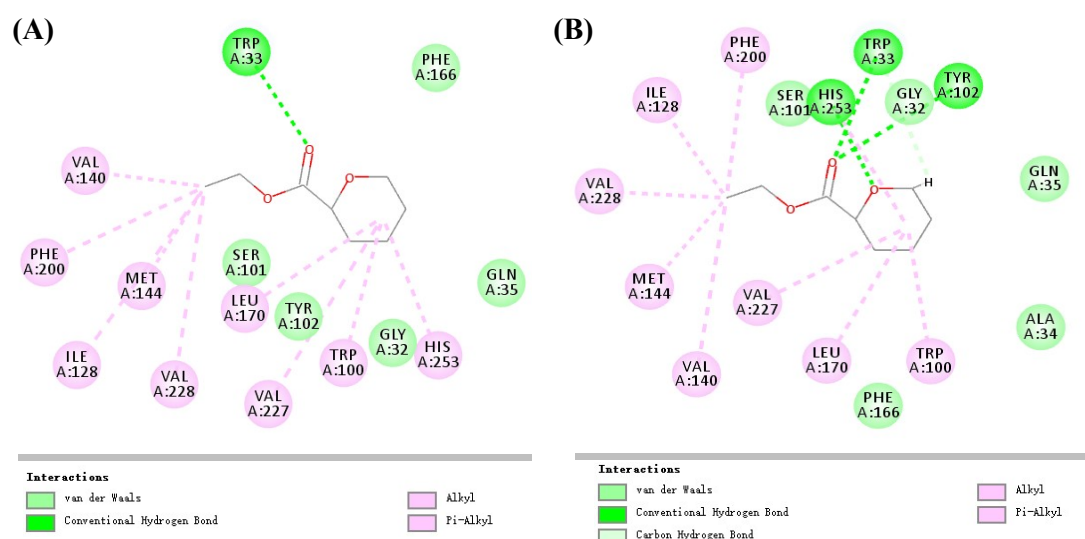


Fig. S16 Interaction analysis of WT with (*R*)-THPCE (A) and (*S*)-THPCE (B).

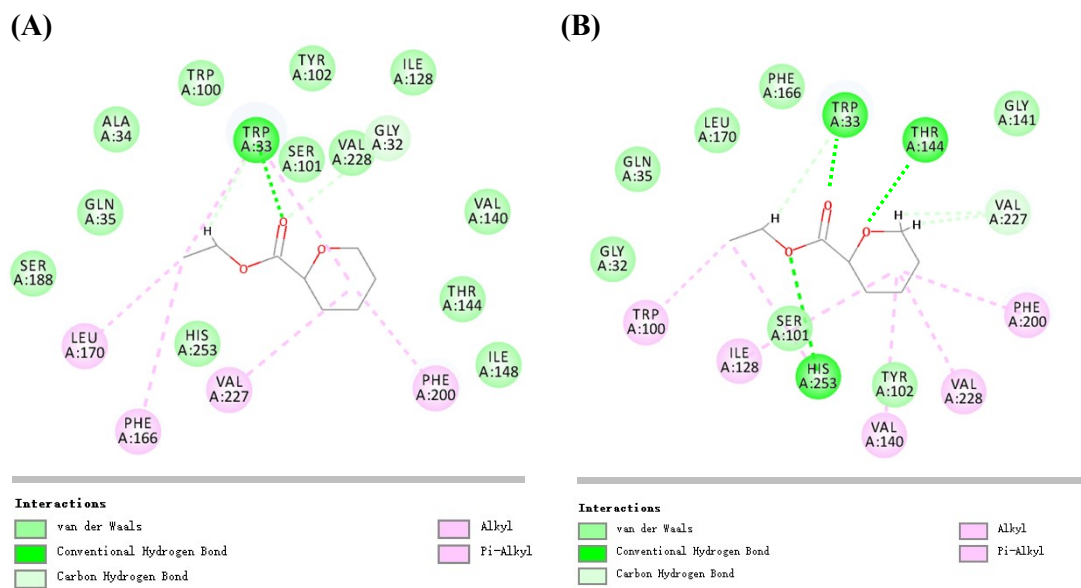


Fig. S17 Interaction analysis of M144T with (*R*)-THPCE (A) and (*S*)-THPCE (B).