Supporting Information

Highly efficient asymmetric reduction of ketopantolactone to D-(-)-pantolactone by *Escherichia coli* cells expressing recombinant conjugated polyketone reductase and glucose dehydrogenase in a fed-batch biphasic reaction system

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Table S1 Plasmids used in this study

Plasmid	Relevant genotype or characteristic	Reference or source
pET28a (+)	P_{T7lac}, Ori (ColE1), Kan^R	Novagen [a]
pET30a (+)	P_{T7lac}, Ori (ColE1), Kan^R	Novagen
pETDuet-1	Two MCS [b], P_{T7lac}, Ori (ColE1), Amp^R	Novagen
pACYCDuet-1	Two MCS [b], P_{T7lac}, Ori (P15A), Cm^R	Novagen
pCDFDuet-1	Two MCS [b], P_{T7lac}, Ori (CloDF13), Sm^R	Novagen
pRSFDuet-1	Two MCS [b], P_{T7lac}, Ori (RSF1030), Kan^R	Novagen
pET28a-CduCPR	Expression vector, pET28a(+) derivative, Kan^R, containing the conjugated polyketone reductase gene from *C. dubliniensis* CD36 (*Cdu*CPR)	Cheng et al., 2019
pEBsuGDH	Expression vector, pET28a(+) derivative, Kan^R, containing the glucose dehydrogenase gene from *Bacillus subtilis* (*Bsu*GDH)	Cheng et al., 2019
pCDFDuet-CduCPR	Expression vector, pCDFDuet derivative, Sm^R, containing the *Cdu*CPR gene in the first MCS	This study
pRSFDuet--CduCPR	Expression vector, pRSFDuet derivative, Kan^R, containing the *Cdu*CPR gene in the first MCS	This study
pACYCDuet-CduCPR	Expression vector, pACYCDuet derivative, Cm^R, containing the *Cdu*CPR gene in the first MCS	This study
pETDuet-CduCPR	Expression vector, pETDuet derivative, Amp^R, containing the *Cdu*CPR gene in the first MCS	This study
pACYCDuet-CduCPR-BsuGDH	Expression vector, pACYCDuet derivative, Cm^R, containing the *Cdu*CPR gene in the first MCS and BsuGDH in the second MCS	This study

[a] Merck KGaA, Darmstadt, Germany. [b] MCS: multiple cloning site.

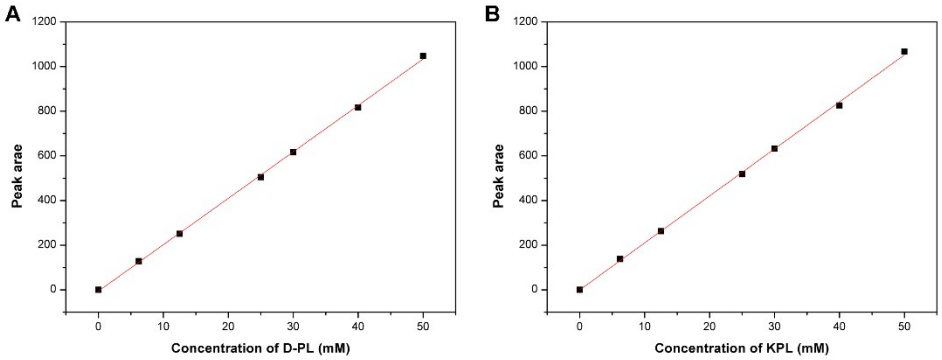

Fig. S1. The standard curves of D-PL (A) and KPL (B) by GC (external standard method). The concentrations of KPL, and D-PL were determined by gas chromatography (Agilent 7890A gas chromatography, Agilent Technologies Inc., USA) equipped with an FID detector and chiral capillary Rt-βDEXm column (30 m × 0.25 mm, Restek, USA).[11] The initial temperature was regulated at 60 °C, and then heated to 150 °C at 10 °C/min and held for 5 min. Nitrogen gas was set at 2.0 ml/min as carrier.

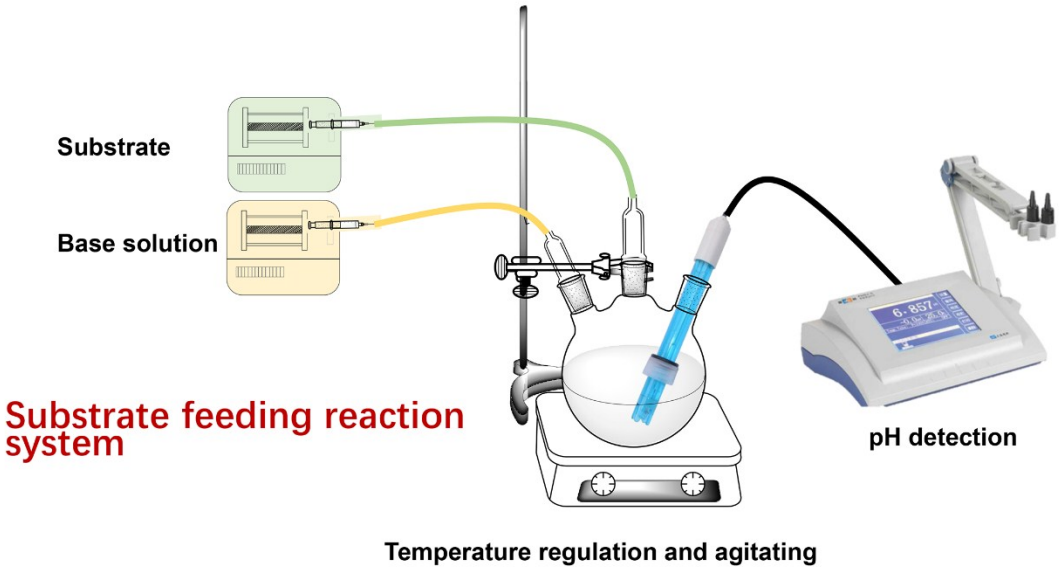

Fig. S2. The gram-scale synthesis of (D)-PL by a substrate feeding reaction strategy

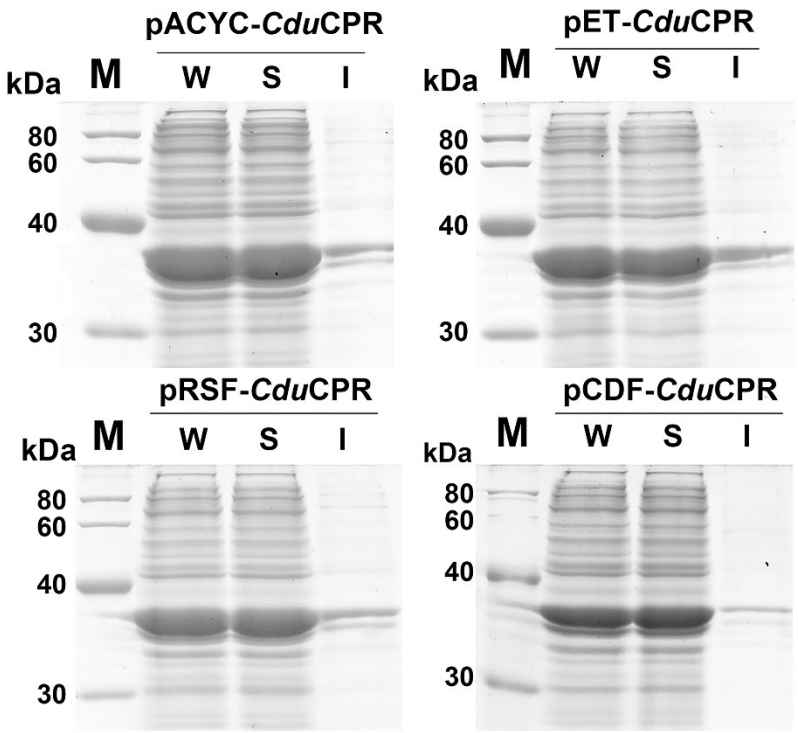

Fig. S3. SDS-PAGE of E. coli containing different recombinant plasmids. Lane M: protein molecular mass marker (ProteinRuler^{TM} I, 12–80 kDa, TransGen Biotech, China), W: whole cell proteins, S: soluble region and I: insoluble region.

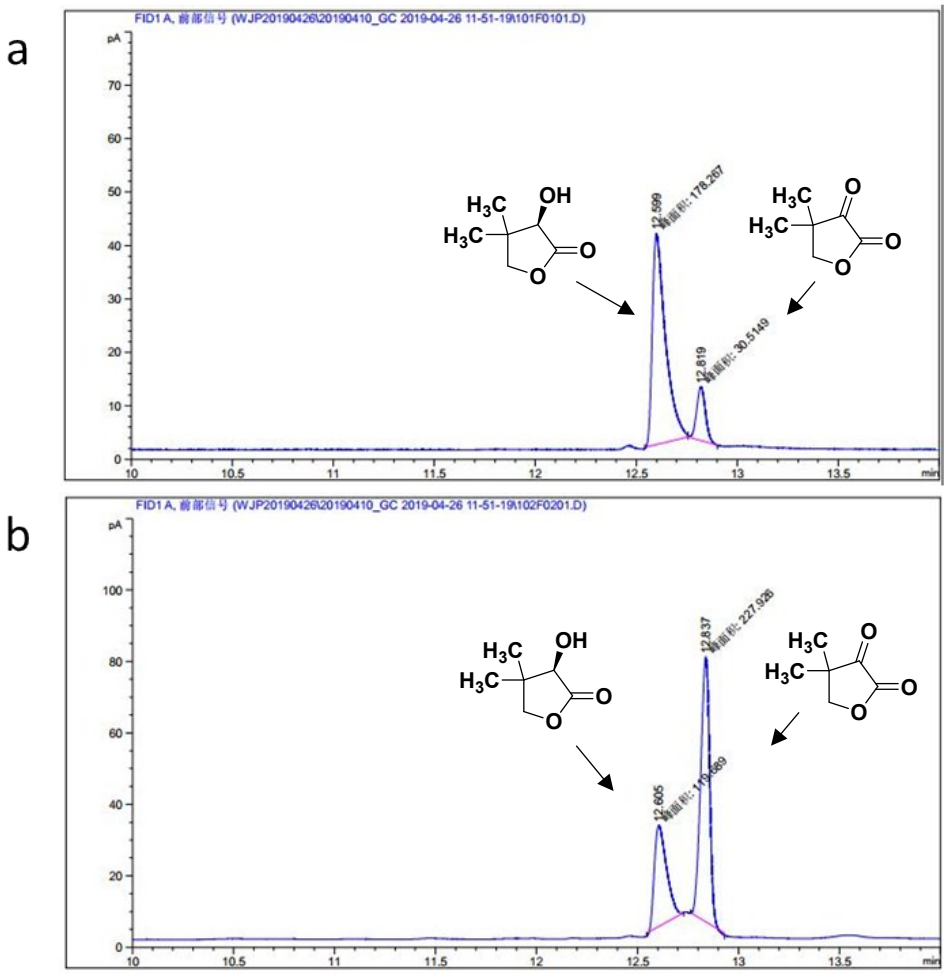

Fig. S4. GC analysis of product catalyzed in pure aqueous buffer. (a) without lactonization, (b) lactonization.

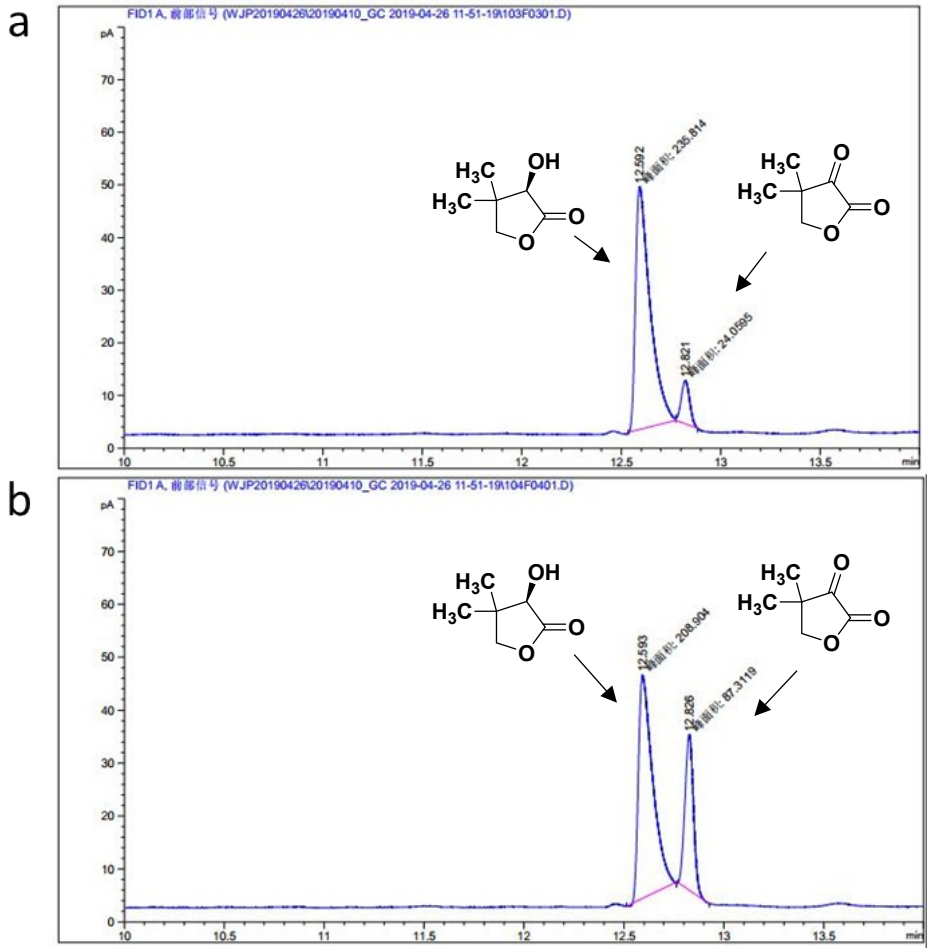

Fig. S5. GC analysis of product catalyzed in a biphasic reaction system containing 10% (*v/v*) tetrahydrofuran. (a) without lactonization, (b) lactonization.

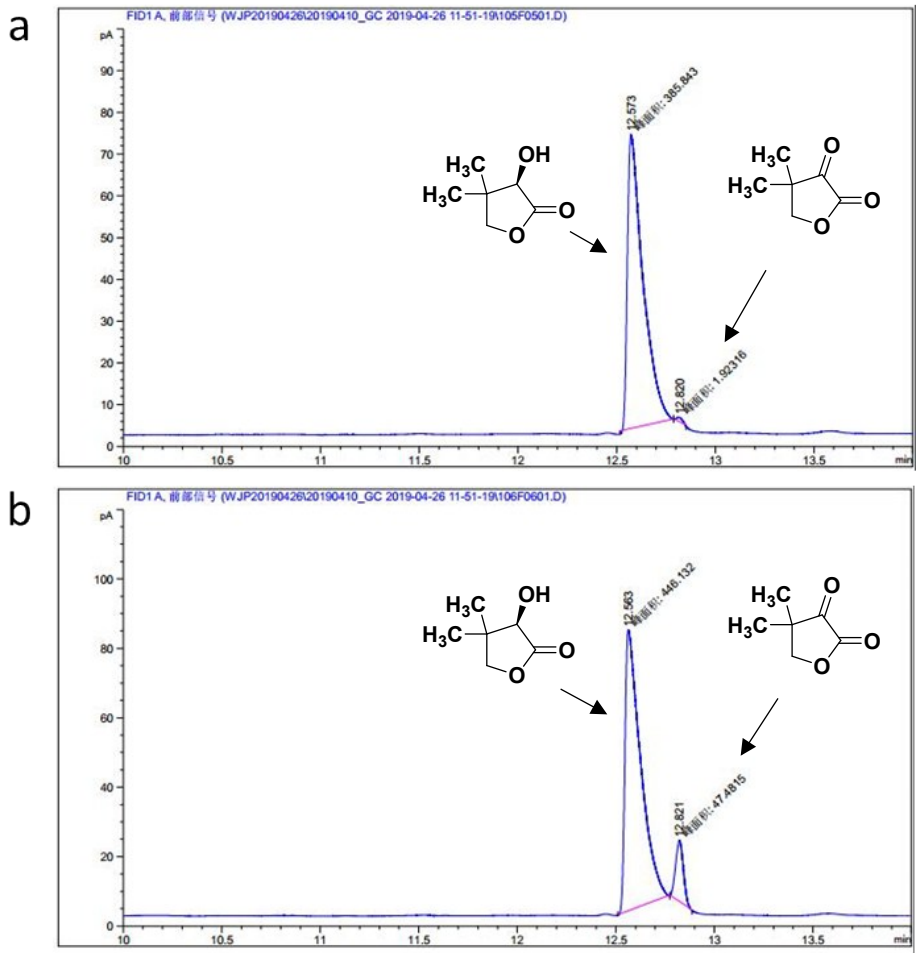

Fig. S6. GC analysis of product catalyzed in a biphasic reaction system containing 10% (*v/v*) dichloromethane. (a) without lactonization, (b) lactonization.

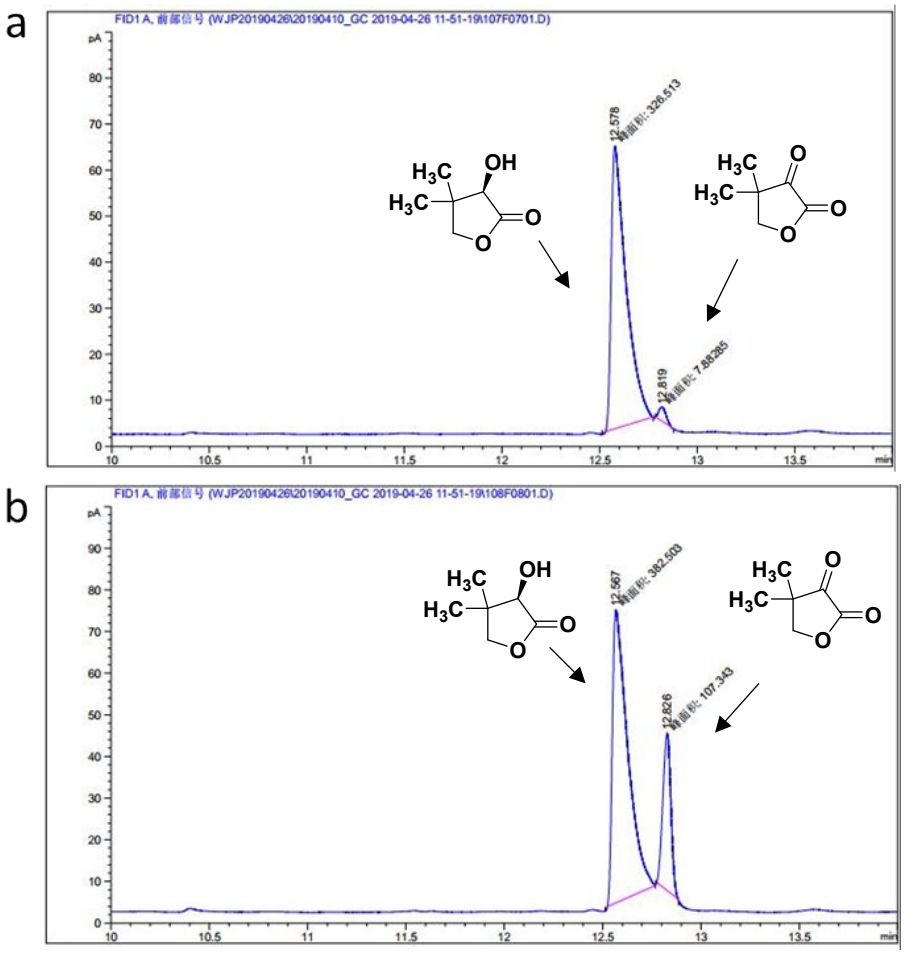

Fig. S7. GC analysis of product catalyzed in a biphasic reaction system containing 10% (*v/v*) toluene. (a) without lactonization, (b) lactonization.

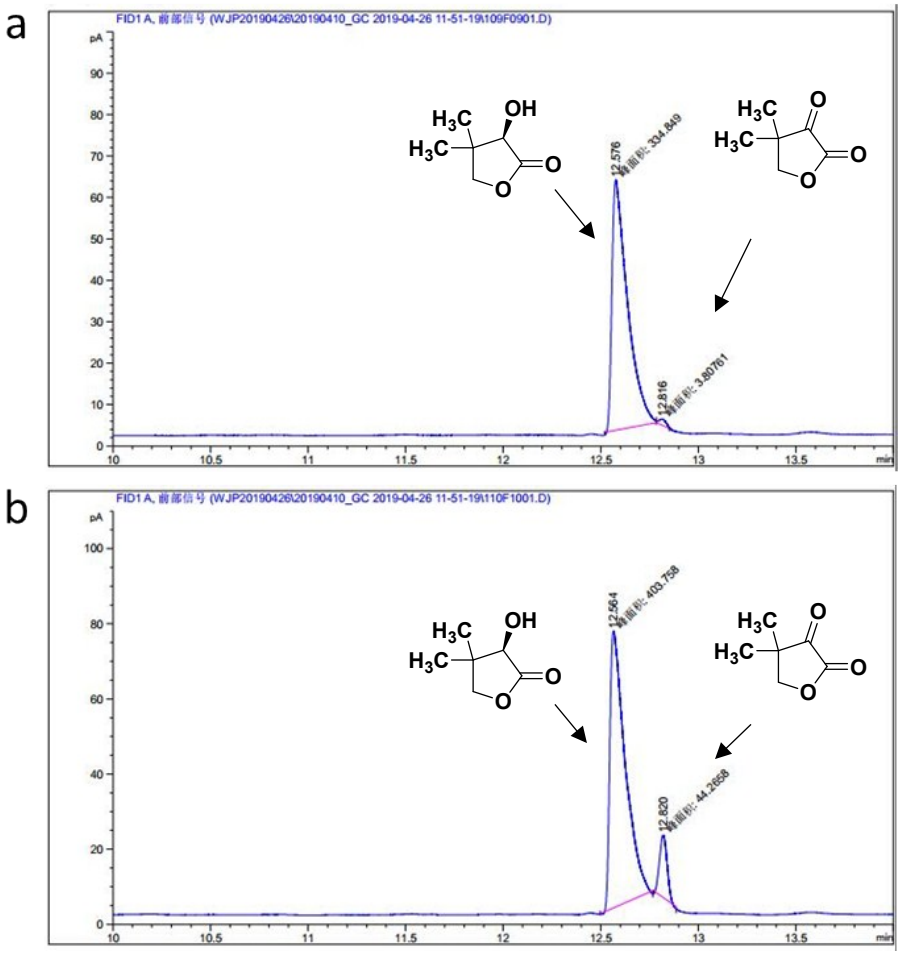

Fig. S8. GC analysis of product catalyzed in a biphasic reaction system containing 10% (*v/v*) carbon tetrachloride. (a) without lactonization, (b) lactonization.

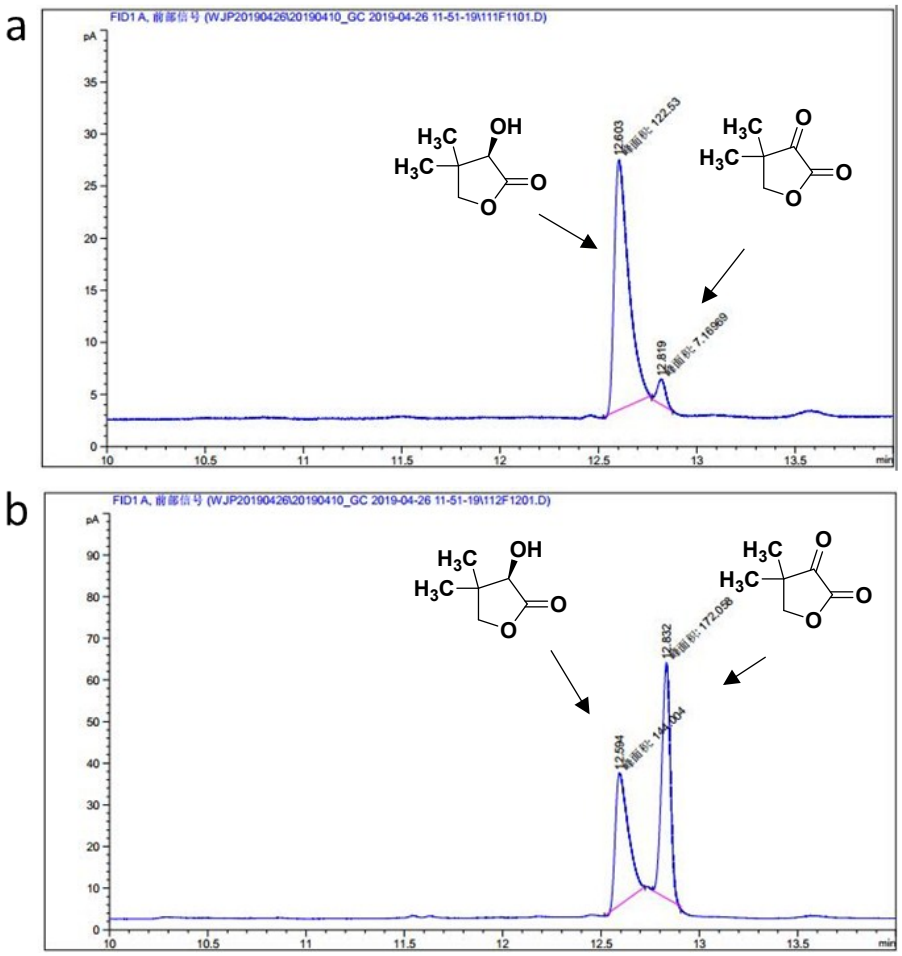

Fig. S9. GC analysis of product catalyzed in a biphasic reaction system containing 10% (*v/v*) hexane. (a) without lactonization, (b) lactonization.

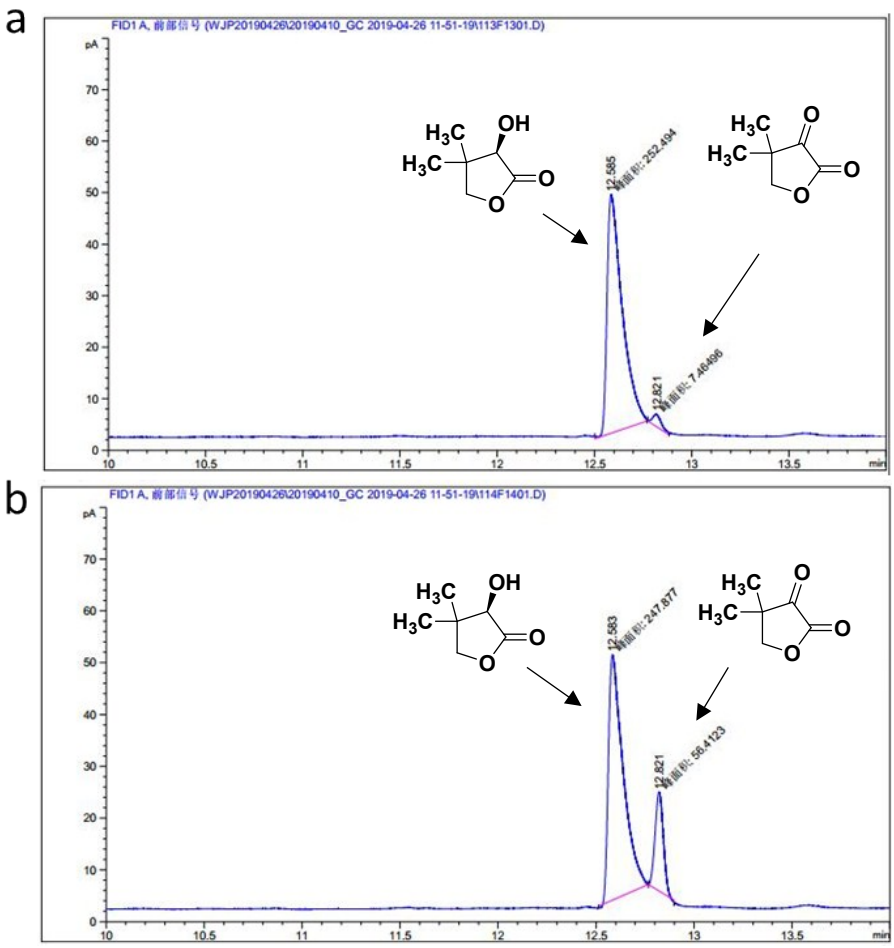

Fig. S10. GC analysis of product catalyzed in a biphasic reaction system containing 10% (*v*/*v*) methyl tert-butyl ether. (a) without lactonization, (b) lactonization.

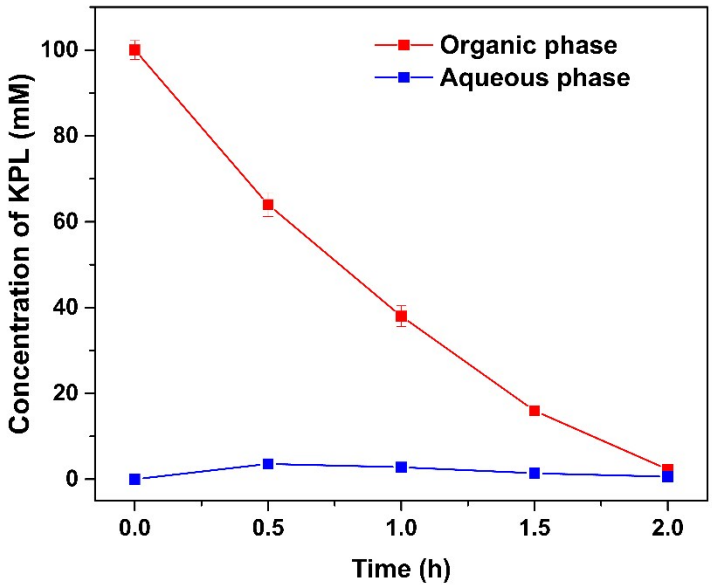

Fig. S11. The KPL concentration curves in organic phase and aqueous phase in the reaction process.

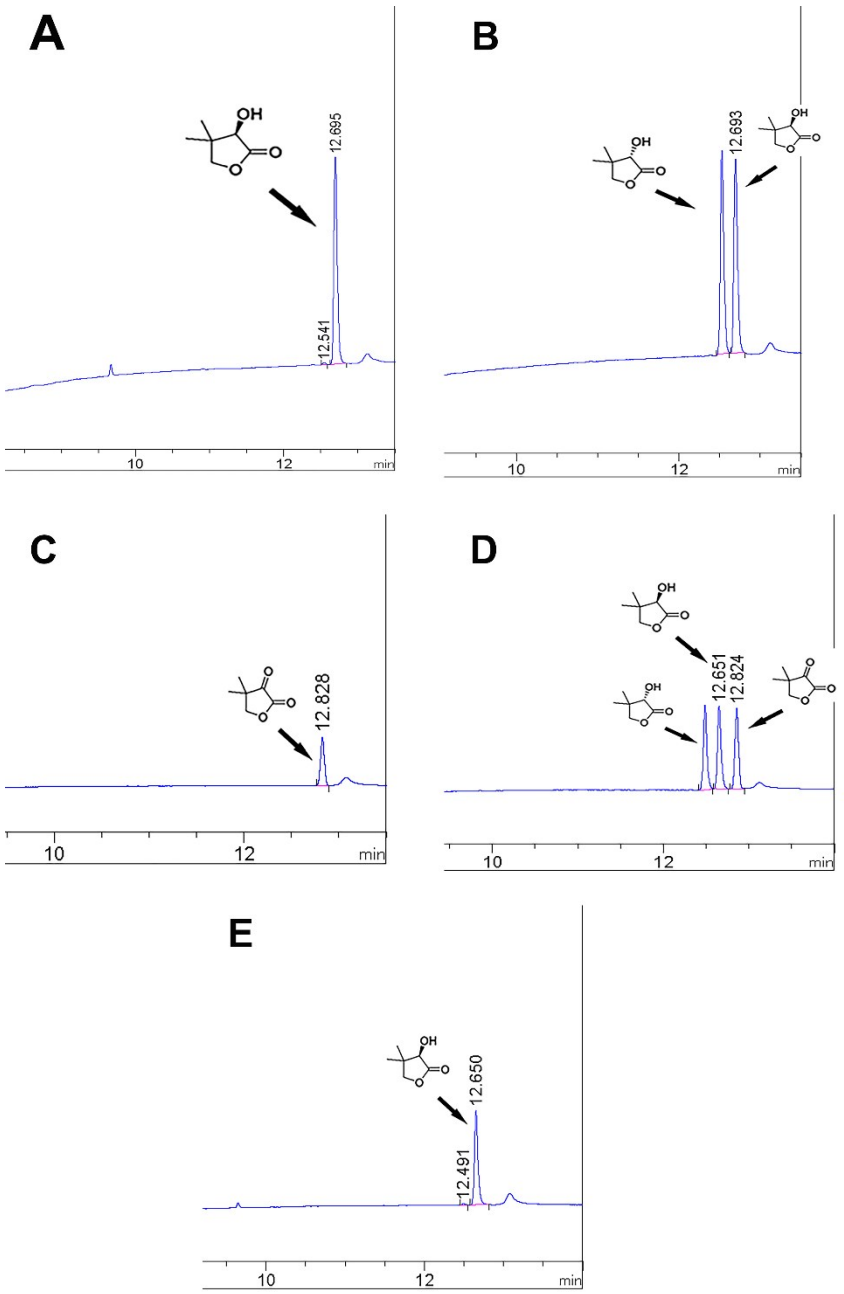

Fig. S12. Gas chromatogram of standard compounds and the synthesized product. (A) D-pantolactone (D-PL), (B) racemic pantolactone (DL-PL), (C) ketopantolactone (KPL), (D) the mixture of DL-PL and KPL and (E) the synthesized D-PL by E. coli expressing recombinant *Cdu*CPR and *Bsu*GDH.

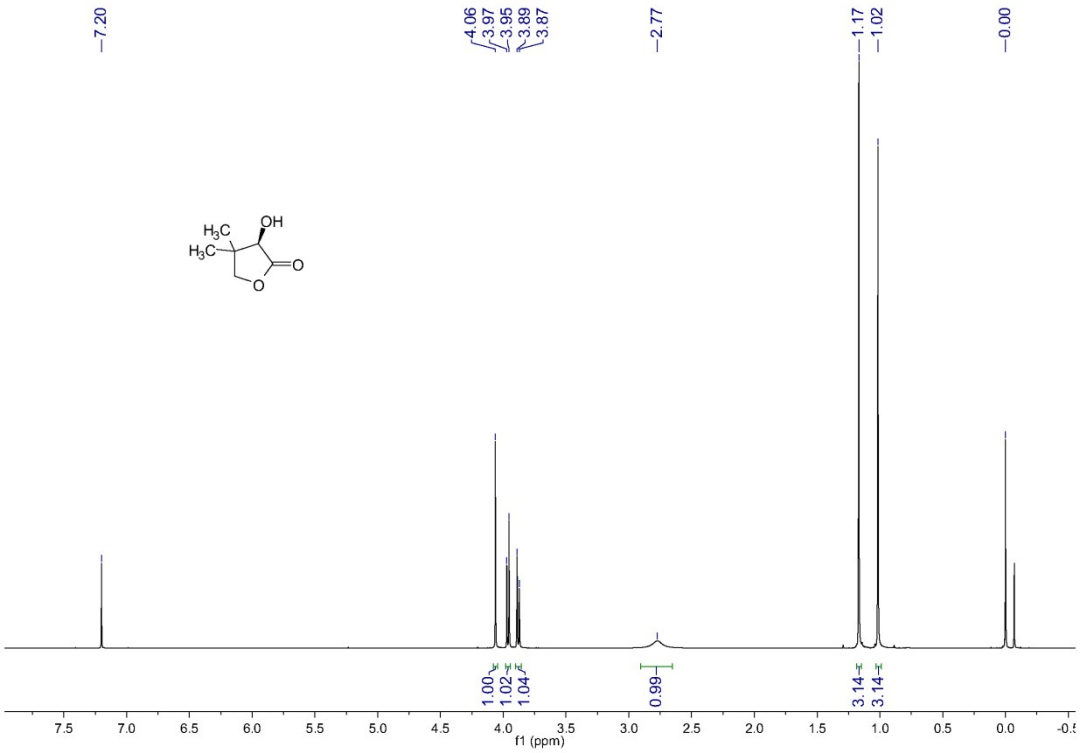

Fig. S13. ^{1}H NMR spectrum of the purified product (*R*)-PL catalyzed using whole recombinant *E. coli* cells harboring pACYCDuet-CduCPR-BsuGDH. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.06 (s, 1H), 3.96 (d, J = 8.9 Hz, 1H), 3.88 (d, J = 8.9 Hz, 1H), 2.77 (s, 1H), 1.17 (s, 3H), 1.02 (s, 3H).

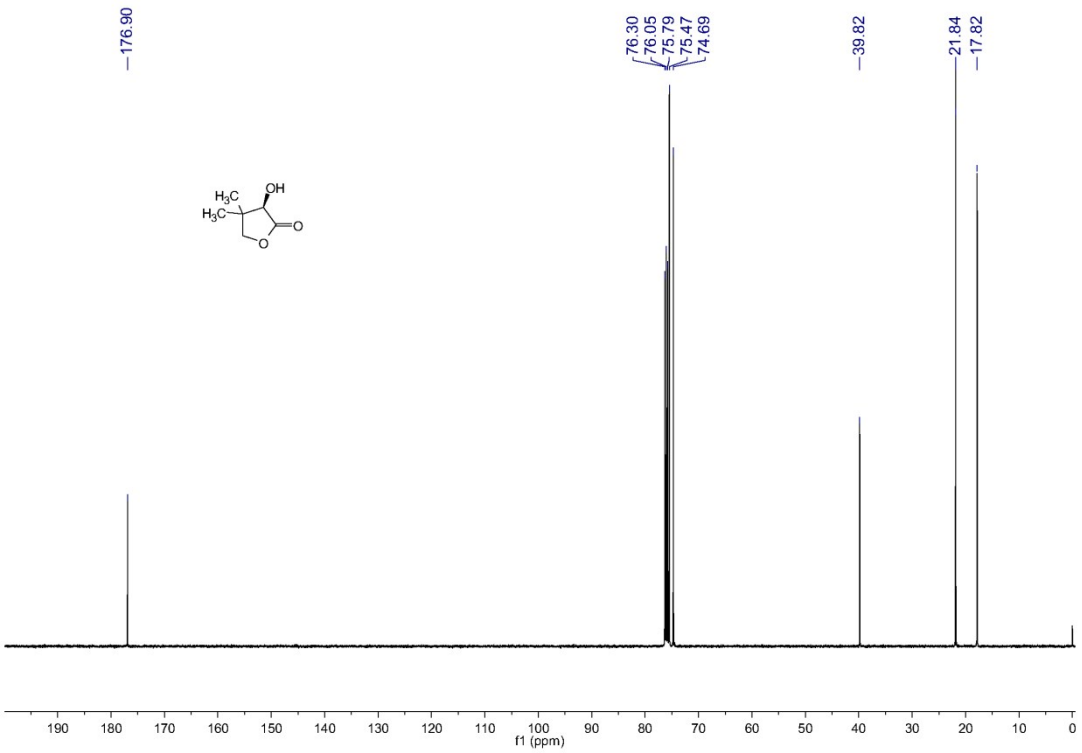

Fig. S14. ^{13}C NMR spectrum of the purified product (*R*)-PL catalyzed using whole recombinant *E. coli* cells harboring pACYCDuet-CduCPR-BsuGDH. ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.90 (s), 75.47 (s), 74.69 (s), 39.82 (s), 21.84 (s), 17.82 (s).

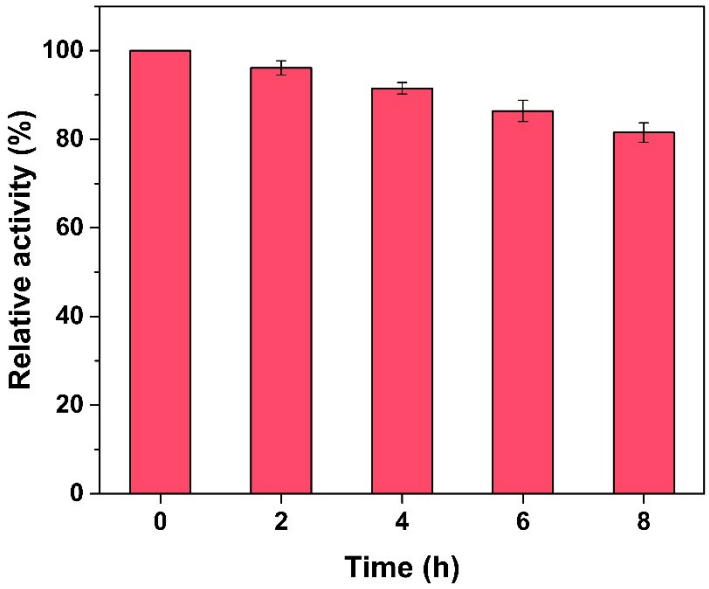

Fig. S15. The change of the biocatalyst activity in the fed-batch biphasic reaction