

Supporting Information

Selective oxidation of aliphatic C-H bonds in alkylphenols by a chemomimetic biocatalytic system

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SI Methods

Strains and culture conditions. The bacterial strains and plasmids used in this study are listed in Table S3. *Escherichia coli* strains were grown at 37 °C on LB agar (2% w/v) plates or LB liquid broth. *E. coli* DH5 α strain was used for vector construction and plasmid isolation. For protein expression and purification, *E. coli* BL21 (DE3) strain was cultured in Terrific Broth (TB) medium (1) with a rotary shaker (220 rpm). When required, kanamycin was added to a final concentration of 50 μ g/ml.

DNA isolation and manipulation. Plasmids were isolated using the E.Z.N.A.TM Plasmid Miniprep Kit (Omega Biotek, Norcross, GA). DNA fragments were purified using the Wizard SV Gel and PCR Clean-up System (Promega, Madison, USA). Vector transformation, agarose gel electrophoresis and other standard techniques of molecular cloning were performed as previously described (2).

General protein expression, purification and concentration determination. For protein expression, *E. coli* BL21 (DE3) carrying a specific expression vector was grown in LB broth containing 50 μ g/ml kanamycin at 37 °C overnight. Terrific Broth (1 l) supplemented with 4% glycerol was inoculated with a 1:100 dilution of the overnight culture. The culture was then incubated on a rotary shaker (220 rpm) at 37 °C until the OD₆₀₀ reached ~0.6. Next, IPTG (final concentration of 0.2 mM) was added into the culture to induce protein expression. Cultivation continued for additional 20 h at 18 °C.

Protein purification was carried out as described elsewhere (3) with minor modifications. All procedures were performed at 4 °C. The cells were harvested by centrifugation (5000 \times g for 5 min), washed with deionized water for 2 times, and re-suspended in 50 ml of lysis buffer (50 mM NaH₂PO₄, 300 mM NaCl, 10% glycerol and 10 mM imidazole, pH 8.0). After sonication on ice, the crude cell lysate was centrifuged at 12,000 \times g for 30 min to collect the supernatant fraction. Subsequently, 1 ml of Ni-NTA resin (Qiagen, Germany) was added into the supernatant with subsequent incubation at 4 °C on a gentle rotator for 30 min. This slurry was loaded onto an empty column, and washed with ~100 ml wash buffer (50 mM NaH₂PO₄, 300 mM NaCl, 10% glycerol and 20 mM imidazole, pH 8.0) until no proteins were detectable in flow-through by Coomassie Brilliant Blue G-250 assays. Approximately 10 ml of elution buffer (50 mM NaH₂PO₄, 300 mM NaCl, 10% glycerol and 250 mM imidazole, pH 8.0) was

used to elute target proteins from the Ni-NTA resin. The eluent was concentrated with an Amicon Ultra centrifugal filter (Merck KGaA, Darmstadt, Germany) ($7500 \times g$ for 30 min) and buffer exchanged on a PD-10 column (GE Healthcare, Buckinghamshire, UK) with 5 ml desalting buffer (50 mM NaH_2PO_4 , 10% glycerol, pH 7.5). The desalted enzyme solution was again concentrated to about 1 ml using Amicon Ultra centrifugal filter. The protein aliquots were flash frozen by liquid nitrogen and stored at $-80\text{ }^\circ\text{C}$.

Concentrations of non-P450 enzymes (CreH, CreI, CreE, CreF, CreD, CreG and CreC) were determined by the Bradford method using BSA as the standard (4). The concentration of P450 enzymes (wt and mutant CreJ enzymes) was determined according to the method described by Omura (5). Specifically, the CO-bound reduced difference spectrum was recorded by NanoDrop 2000 Spectrophotometer (Thermo Scientific). The concentration of functional P450 was calculated using the extinction coefficient ($\epsilon_{450\text{ nm}-490\text{ nm}}$) of $91000\text{ M}^{-1}\cdot\text{cm}^{-1}$.

Expression and purification of CreJ for crystallization procedures. To facilitate heterologous expression of CreJ, the *creJ* gene with the first 84 nucleotides truncated was cloned into pET28a between *Bam*HI and *Hind*III restriction sites. The shortened protein containing an *N*-terminal His₆-tag was expressed in *Escherichia coli* BL21 (DE3) and purified using a Ni²⁺ Sepharose HP column (GE healthcare). The protein was further purified by anion exchange chromatography with a Q-sepharose column (GE healthcare) and gel filtration using a Superdex 75 column (GE healthcare). The gel filtration result indicates that the recombinant CreJ is monomeric in solution.

CreJ mutagenesis and relative activity determination. Mutation sites were introduced via PCR (Table S4). Specifically, complimentary primer pairs containing the mutated codon of interest were employed in PCR amplifications (35 cycles of $95\text{ }^\circ\text{C}$ for 10 s, $55\text{ }^\circ\text{C}$ for 15 s, and $72\text{ }^\circ\text{C}$ for 2 min; FailSafe system from Epicentre) to yield a linear mutant plasmid from the original pET28a-*creJ* template DNA. Following gel purification, each linear mutant plasmid was treated with homologous recombinase (Hieff Clone TM One Step Cloning Kit, Yeasen, Shanghai, China) following vendor's protocols, followed by transformation into *E. coli* BL21(DE3). The incorporation of the mutated codon was confirmed via Sanger DNA sequencing (GENEWIZ, Suzhou, China).

Wild-type and mutant enzymatic activities were comparatively determined in reactions using **1'** as substrate, at 30 °C for 20 min. A standard reaction mixture contained 1 μM CreJ (or CreJ mutant), 1 μM CreE and 1 μM CreF, 1 mM substrate, and 4 mM NADPH in 100 μL of 50 mM Tris·HCl (pH 8.0). The substrate consumption ratio of each reaction was determined by high-performance liquid chromatography (HPLC). Relative enzymatic activity of each mutant was calculated by comparing substrate consumption ratios to that of CreJ, which was assigned 100% activity. All reactions were carried out in triplicate and the data was reported as the means ± SD.

Substrate binding assays. Spectroscopic substrate binding assays were carried out at room temperature by employing a UV visible spectrophotometer (Infinite M200 pro, TECAN) (6). Protein dissolved in 1 mL of 50 mM sodium phosphate buffer containing 10% glycerol (pH 7.4) at concentration of 1 μM was titrated with 100 mM stock solution of 4-cresol. The final concentration of substrate ranged from 0.2 to 0.8 mM. Data consisting of absorbance differences ΔA ($A_{390\text{ nm}} - A_{420\text{ nm}}$) and corresponding substrate concentration from duplicated experiments were plotted and fitted to the hyperbolic function $\Delta A = A_{\text{max}} \cdot S / (K_D + S)$, where S is the total substrate concentration, A_{max} is the maximal absorption shift at saturation, and K_D is the apparent dissociation constant for the enzyme-substrate complex.

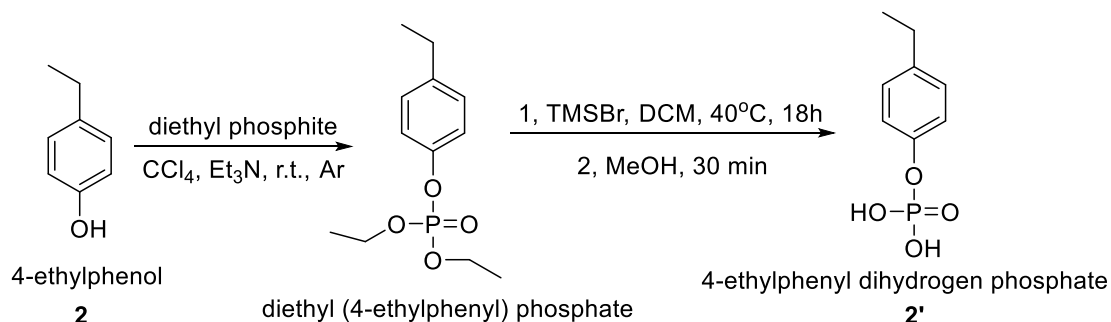
Chiral HPLC analysis of 2a and 7a. The reaction sample was extracted with an equal volume of ethyl acetate. The organic phase was dried by nitrogen stream and re-constituted in an equal volume of methanol. The chiral column (250 × 4.6 mm) was manually packed with the chiral stationary phase, which was prepared by coating chitosan bis(3-chlorophenylcarbamate)-(cyclohexyl urea) on the surface of 3-aminopropyl silica gel according to a previously published method (7).

Chiral analysis was carried out on an Agilent 1260 infinity HPLC system (Agilent Technologies, USA) equipped with a DAD detector. The two enantiomers of **2a** and **7a** were respectively separated on the chiral column described above using an isocratic mobile phase of hexane containing 10% isopropanol over 60 min. The flow rate was set to 1 ml/min and injection volume was 10 μL. The detection wavelength was set to 275 nm. Optical configuration was determined via retention time comparison with the enantiomerically pure authentic standard.

Sources of substrates and authentic standard compounds. Except the phosphorylated compounds that were chemically synthesized (*see below*), all substrates and authentic standard compounds were purchased from different chemical suppliers. In details, **2** was bought from Tokyo Chemical Industry (TCI, Shanghai, China); **3**, **4b**, **6a**, **7b**, **11** and **12** from J&K Scientific Ltd. (Beijing, China); **4** from Acros Organics (Thermo Fisher Scientific, USA); **5**, **6** and **9** from AIKE REAGENT (Chengdu, China); **7** from Sigma-Aldrich (USA), **8** from Toronto Research Chemicals (TRC, Canada); **10** from Fluorochem (Derbyshire, UK), **5b** and racemic **2a** from Bide Pharmatech Ltd. (Shanghai, China); **5a** and the enantiomerically pure *S*-**2a** and *S*-**7a** from Enamine (Ukraine); **2b**, **3b**, **6b** and **6c** from Aladdin (Shanghai, China); racemic **7a** from Accela ChemBio (San Diego, CA, USA). **1'** was synthesized by following the chemical method with reference to the previous report (8).

Chemical synthesis of 4-ethylphenyl phosphate (2'). Compound **2'** was synthesized through chemical methods with reference to previous reports by McKenna and Kenner (9, 10).

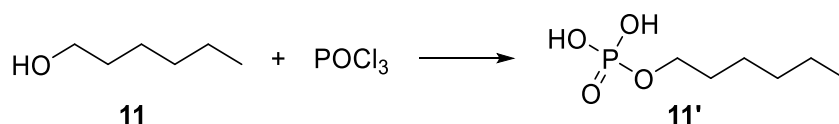
The reaction scheme is as follow:



In details, to a solution of 4-ethylphenol (1.22 g, 10 mmol) dissolved in carbon tetrachloride (10 mL) was added diethyl phosphite (1.55 mL, 12 mmol) at 0°C under argon atmosphere. Triethylamine (1.65 mL, 12 mmol) was carefully added drop-wise to the mixture by using a dropping funnel. The mixture was stirred overnight at room temperature. Water (30 mL) was added and the organic layer was separated. The organic layer was washed twice with dilute hydrochloric acid (2×15 mL), four times with dilute sodium hydroxide solution (4×15 mL), and twice with brine (2×15 mL) before being dried over anhydrous MgSO_4 . Removal of solvent on rotary evaporator gave a crude, which was further purified by silica gel column chromatography using a petroleum ether/ethyl acetate (4/1: v/v) mixture to afford diethyl (4-ethylphenyl) phosphate (2.51 g). Yield: 97 %.

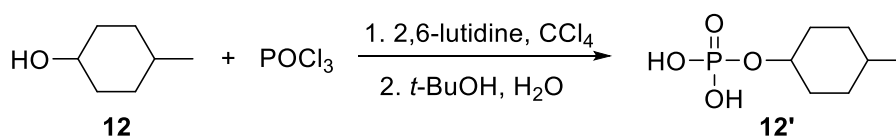
Diethyl (4-ethylphenyl) phosphate (2.51 g, 9.7 mmol) was dissolved in dry CH₂Cl₂ (50 mL). Then bromotrimethylsilane (12.8 mL, 10 equiv. to phosphate ester) was added to the solution and the mixture was heated to reflux for 18 h under argon atmosphere. The solvent was evaporated and the residue was dissolved in anhydrous methanol (50 mL). The solution was stirred for 30 min at 40 °C. Then the resulting solution was concentrated to give **2'** (1.8 g). Yield: 92%. Structure of synthetic **2'** was confirmed by HRMS (Fig. S42) and NMR (Fig. S43 and S44).

Chemical synthesis of 1-hexanol phosphate (11') and 4-methyl cyclohexanol phosphate (12'). Compound **11'** was chemically synthesized using previously reported methods (11). The reaction scheme is as follow:



In details, **11'** was prepared by mixing **11** (5 mmol, 0.51 g) with the reagent phosphorus oxychloride (5 mmol, 0.77 g), by vigorous stirring for 1 h under aspirator vacuum to remove HCl (g), and then heated at 50 °C for 5 h. The reaction was quenched via a dropwise addition of the reaction mixture into excess cold water, and the resultant mixture was then stirred for 5 h at 30 °C. The product was extracted with Et₂O multiple times. The combined extracts were dried with anhydrous MgSO₄, and Et₂O was removed under reduced pressure to afford a colorless oil 0.75 g (yield: 91 %).

Compound **12'** was accessed by following previously described methods (12). The reaction scheme is as follow:



Specifically, **12** (5 mmol, 0.57 g) and 2,6-lutidine (5.5 mmol, 0.59 g) in carbon tetrachloride (2 ml) were added to phosphorous oxychloride (5mmol, 0.77 g) in carbon tetrachloride (10 ml) at 0-10 °C. After an hour, the product was filtered from base hydrochloride and evaporated, yielding the dichloridate as a colorless oil. 4-Methyl cyclohexyl phosphorodichloridate in carbon tetrachloride (5 ml) was treated with *tert*-butyl

alcohol (0.85 g) and water (0.1 ml) for 30 min at 50 °C. Removal of the solvent left an oil which was dissolved in 30 ml water, and extracted with Et₂O several times. The combined extracts were dried with anhydrous MgSO₄, and Et₂O was removed *en vacuo* to give 0.3 g of a white solid (yield: 31 %). The chemical structures of synthetic **11'** and **12'** were confirmed by HRMS (Fig. S29 and S30) and NMR (Fig. S45-S48).

SI Tables

Table S1. Data collection and refinement statistics for CreJ structures.

Parameter	CreJ-1'	CreJ-2'
Data collection ^a		
Space group	C 1 2 1	P 43 21 2
<i>a, b, c</i> (Å)	125.54, 121.14, 125.61	86.07, 86.07, 125.04
α, β, γ	90.0, 90.96, 90.0	90.0, 90.0, 90.0
Wavelength	0.979	0.979
Resolution (Å)	34.56-2.0 (2.03-2.0)	35.45-1.70 (1.73-1.70)
Total reflections	454625 (17470)	687298 (36247)
Unique reflections	125653 (5928)	52282 (2731)
Completeness (%)	99.3 (94.4)	99.8 (100.0)
Multiplicity	3.6 (2.9)	13.1 (13.3)
<i>Mean I/sigma (I)</i>	8.5 (2.1)	20.1 (5.6)
R_{merge}^b	0.106 (0.516)	0.091 (0.505)
Refinement		
$R_{\text{work}}/R_{\text{free}}$ (%)	16.08/19.79	18.15/20.88
No. atoms		
Protein	12765	3252
Ligands	255	56
water	1825	552
B-factors		
Average B-factor	34.00	24.20
Proteins	33.70	22.40
Ligands	23.80	11.20
Solvent	38.10	35.80
r.m.s.d.		
Bond length (Å)	0.01	0.006
Bond angles	1.22	1.15
Ramachandran statistics		
Favored (%)	97	97
Outliers (%)	0.06	0

^a Numbers in parentheses refer to data in the highest-resolution shell.

^b $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I(hkl)_i - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i \langle I(hkl) \rangle}$, where I is the observed intensity, $\langle I(hkl) \rangle$ represents the average intensity, and $I(hkl)_i$ represents the observed intensity of each unique reflection.

Table S2. Reactivity of CreHI, CreJEF and CreD towards different alkylphenols (1-10).

Alkylphenol	CreHI ^a	CreHI + CreJEF ^b	CreHI + CreJEF + CreD ^c
<i>p</i> -cresol (1)	+	+	+
<i>p</i> -ethylphenol (2)	+	+	+
<i>p</i> -vinylphenol (3)	+	+	+
<i>p</i> -propylphenol (4)	+	+	+
<i>p</i> -isopropylphenol (5)	+	+	+
<i>m</i> -cresol (6)	+	+	+
<i>m</i> -ethylphenol (7)	+	+	+
<i>m</i> -vinylphenol (8)	+	-	+
<i>o</i> -cresol (9)	+	-	+
<i>o</i> -ethylphenol (10)	-	-	-

^aEach alkylphenol (1 mM) was phosphorylated by CreHI (10 μ M for each enzyme), in the presence of 20 mM MgCl₂, 2 mM MnCl₂, and 2 mM ATP, at 30 °C for 2 h;

^bFollowing the first stage of phosphorylation by CreHI^a, CreJEF (10 μ M for each protein) and 2 mM NADPH were added at 30 °C for 2 h;

^cFollowing the reactions catalyzed by CreHI^a and CreJEF^b, CreD (10 μ M) was added at 30 °C for 2 h;

“+” represents that substrate consumption or product formation was detected;

“-” represents neither substrate consumption nor product formation was detected.

Table S3. Strains and plasmids used in this study.

Strain, plasmid	Genotype/Phenotype	Source
<i>E. coli</i> BL21(DE3)		Novagen
<i>E. coli</i> DH5 α		Invitrogen
pET28b	Expression vector	Novagen
pET28b- <i>creC</i>	Expression vector of CreC	Du L, <i>et al.</i> , 2016
pET28a- <i>creD</i>	Expression vector of CreD	Li T, <i>et al.</i> , 2014
pET28a- <i>creE</i>	Expression vector of CreE	Li T, <i>et al.</i> , 2014
pET28a- <i>creF</i>	Expression vector of CreF	Du L, <i>et al.</i> , 2016
pET28a- <i>creG</i>	Expression vector of CreG	Li T, <i>et al.</i> , 2014
pET28b- <i>creH</i>	Expression vector of CreH	Du L, <i>et al.</i> , 2016
pET28b- <i>creI</i>	Expression vector of CreI	Du L, <i>et al.</i> , 2016
pET28a- <i>creJ</i>	Expression vector of CreJ	Li T, <i>et al.</i> , 2014
pET28a- <i>creJ</i> -Q83A	Expression vector of CreJ-mutant (Q83A)	Present study
pET28a- <i>creJ</i> -S106A	Expression vector of CreJ-mutant (S106A)	Present study
pET28a- <i>creJ</i> -S109A	Expression vector of CreJ-mutant (S109A)	Present study
pET28a- <i>creJ</i> -R194A	Expression vector of CreJ-mutant (R194A)	Present study
pET28a- <i>creJ</i> -R194K	Expression vector of CreJ-mutant (R194K)	Present study
pET28a- <i>creJ</i> -R194E	Expression vector of CreJ-mutant (R194E)	Present study
pET28a- <i>creJ</i> -S261A	Expression vector of CreJ-mutant (S261A)	Present study
pET28a- <i>creJ</i> -W199A	Expression vector of CreJ-mutant (W199A)	Present study
pET28a- <i>creJ</i> -F264A	Expression vector of CreJ-mutant (F264A)	Present study
pET28a- <i>creJ</i> -I312A	Expression vector of CreJ-mutant (I312A)	Present study
pET28a- <i>creJ</i> -W315A	Expression vector of CreJ-mutant (W315A)	Present study
pET28a- <i>creJ</i> -F416A	Expression vector of CreJ-mutant (F416A)	Present study

Table S4. Primers used for mutagenesis of CreJ.

Mutant	Primer	Sequence
Q83A	Q83A-F	AAATGCAG C AGCCCCAGTCCGCAAG
	Q83A-R	CTGGGGCT G CTGCATTTTCAGAGGA
S106A	S106A-F	TGCATAC G CAGGATTATCAGCTCGT
	S106A-R	ATAATCCT G CGTATGCAGTGAAGCC
S109A	S109A-F	CGGATTAG C AGCTCGTATTCCACCA
	S109A-R	TACGAGCT G CTAATCCGGAGTATGC
R194A	R194A-F	AGATTCC G CAGCGGCCATGACCTGG
	R194A-R	TGGCCGCT G CGGAATCTGACCACCG
R194K	R194K-F	AGATTCC A AAGCGGCCATGACCTGG
	R194K-R	TGGCCGCT T TGGAATCTGACCACCG
R194E	R194E-F	AGATTCC G AAGCGGCCATGACCTGG
	R194E-R	TGGCCGCT T CGGAATCTGACCACCG
S261A	S261A-F	GCTGTAC G CACTGCTTTTTGCGGGG
	S261A-R	AAAGCAGT G CGTACAGCAAAGAAGC
W199A	W199A-F	CATGACC G CAGGCGATCTTAGTGAT
	W199A-R	GATCGCCT G CGGTCATGGCCGCACG
F264A	F264A-F	CCTGCTT G CAGCGGGGCACGAAACA
	F264A-R	GCCCCGCT G CAAGCAGGGAGTACAG
I312A	I312A-F	CGGCTCG G CAGTGGGGTGGCGTCGA
	I312A-R	ACCCCACT G CCGAGCCGGAGTACCG
W315A	W315A-F	CGTGGGG G CACGTTCGAAAAGCATTA
	W315A-R	TTCGACGT G CCCCCACGATCGAGCC
F416A	F416A-F	CCTCTCC G CACGCGTCCCCACTTCT
	F416A-R	GGACGCGT G CGGAGAGGTTCTCCCG

Nucleotides in bold represent the mutated codons.

SI Figures

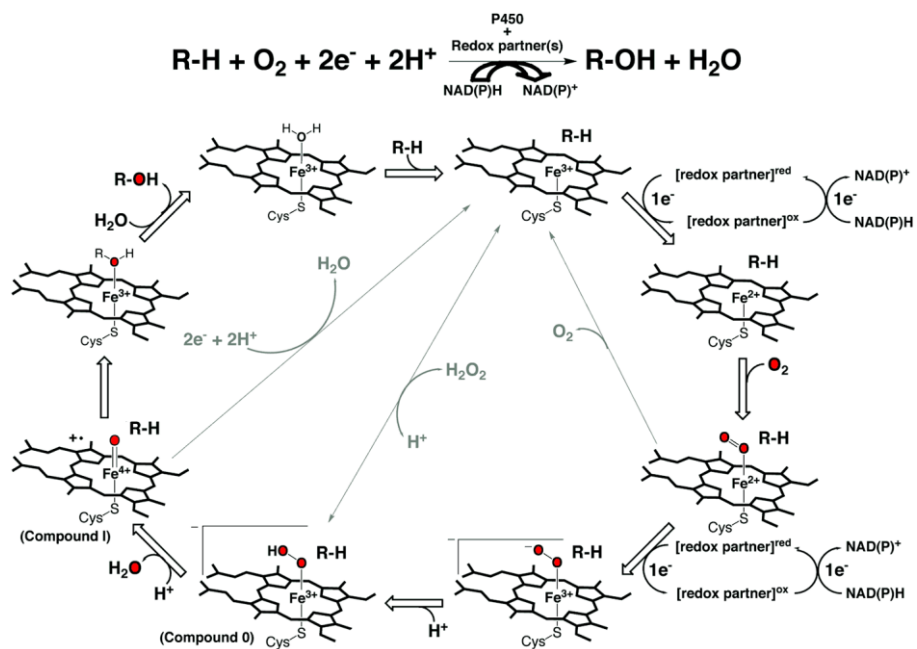


Fig. S1. The P450 catalytic cycle. (Adapted from the previous publication (13))

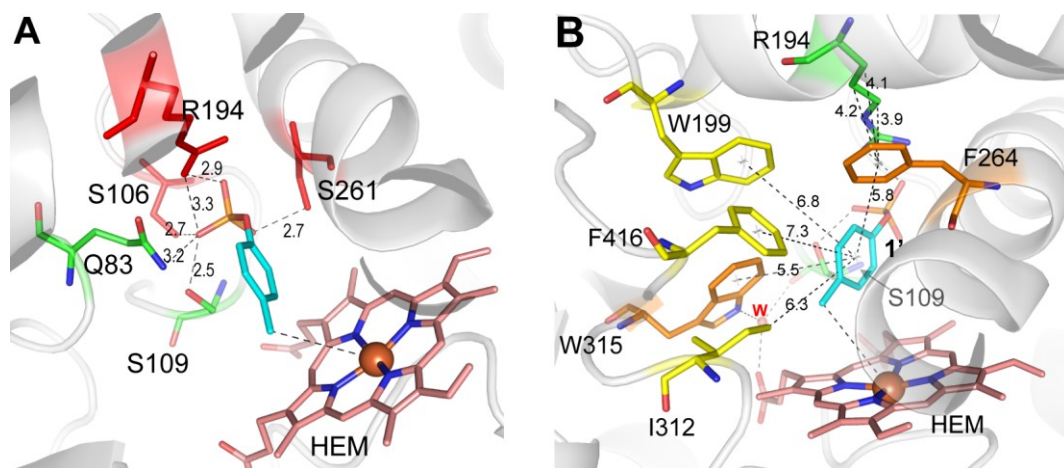


Fig. S2. Steric positions of the residues interacting with the phosphate group (A) and benzyl moiety (B) of 4-cresol.

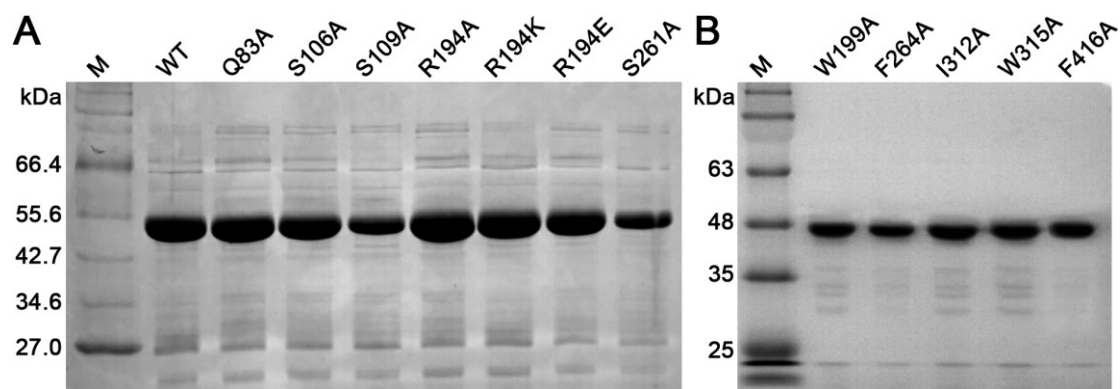


Fig. S3. SDS-PAGE analysis of purified wild-type and mutant CreJ enzymes. (A) Mutants of the residues interacting with the phosphate group of substrate. (B) Mutants of the residues surrounding the benzyl moiety of substrate. M, protein marker.

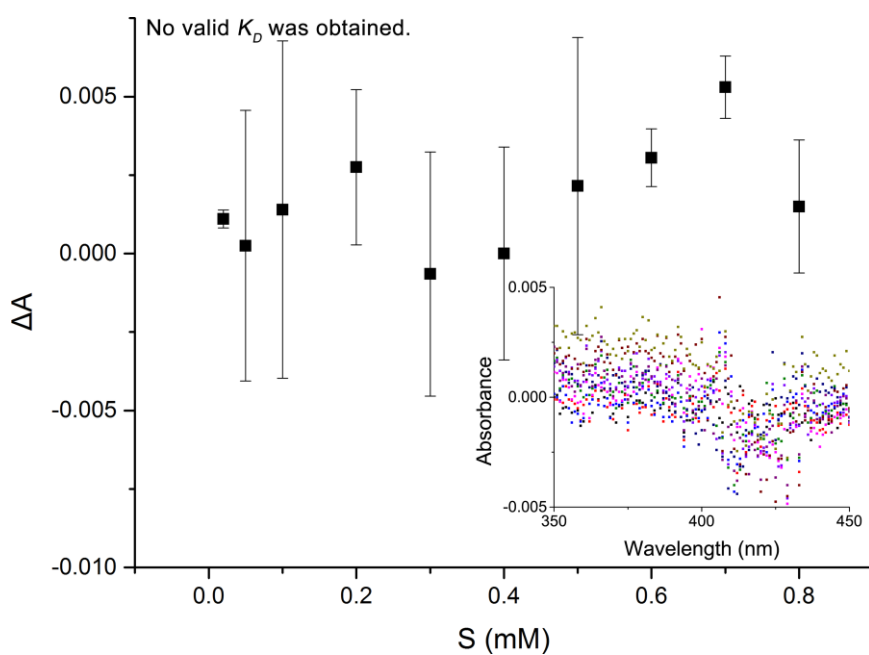


Fig. S4. The binding curve of 4-cresol to the R194A mutant.

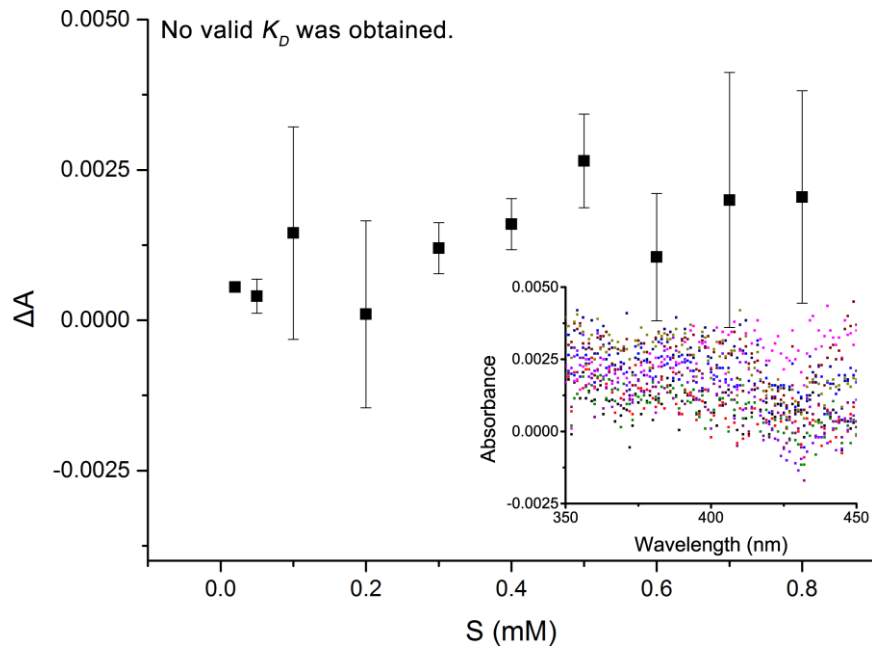


Fig. S5. The binding curve of 4-cresol to the R194K mutant.

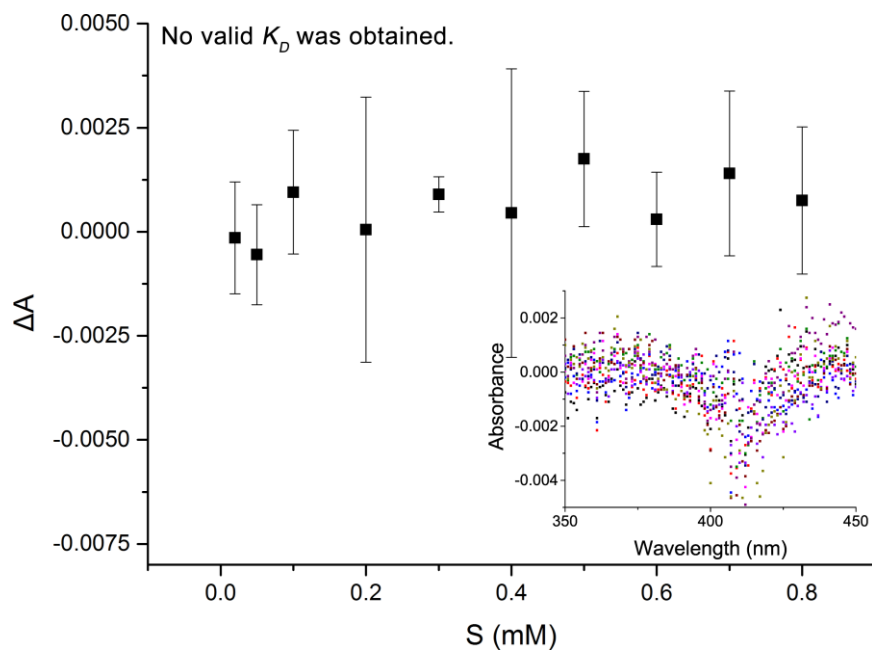


Fig. S6. The binding curve of 4-cresol to the R194E mutant.

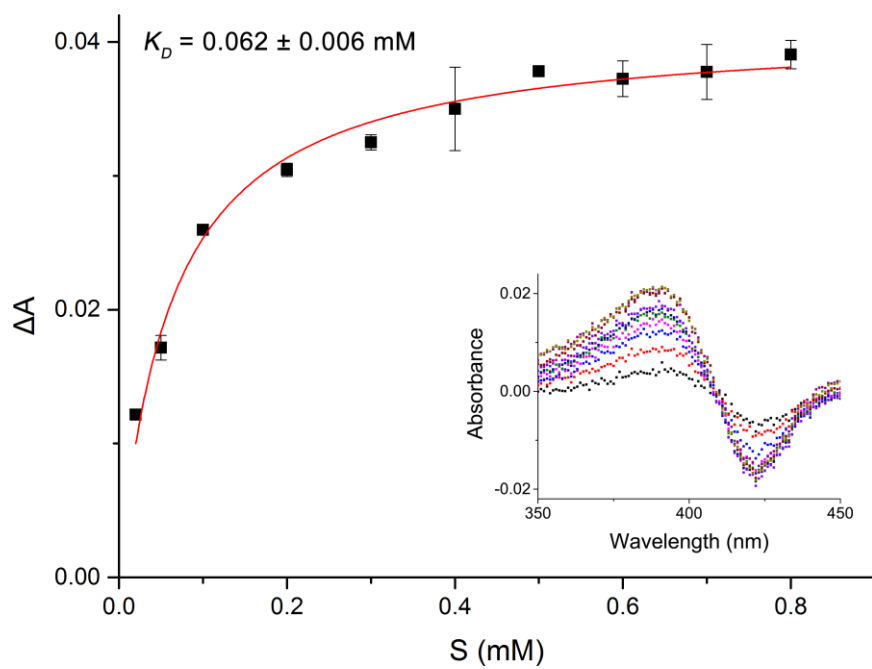


Fig. S7. The binding curve of 4-cresol to wild-type CreJ.

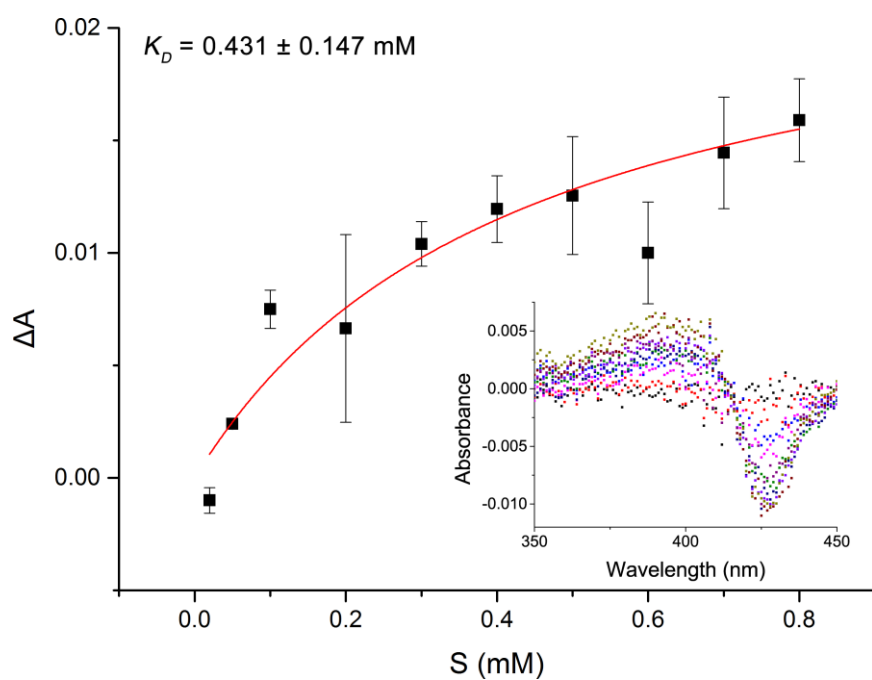


Fig. S8. The binding curve of 4-cresol to the S106A mutant.

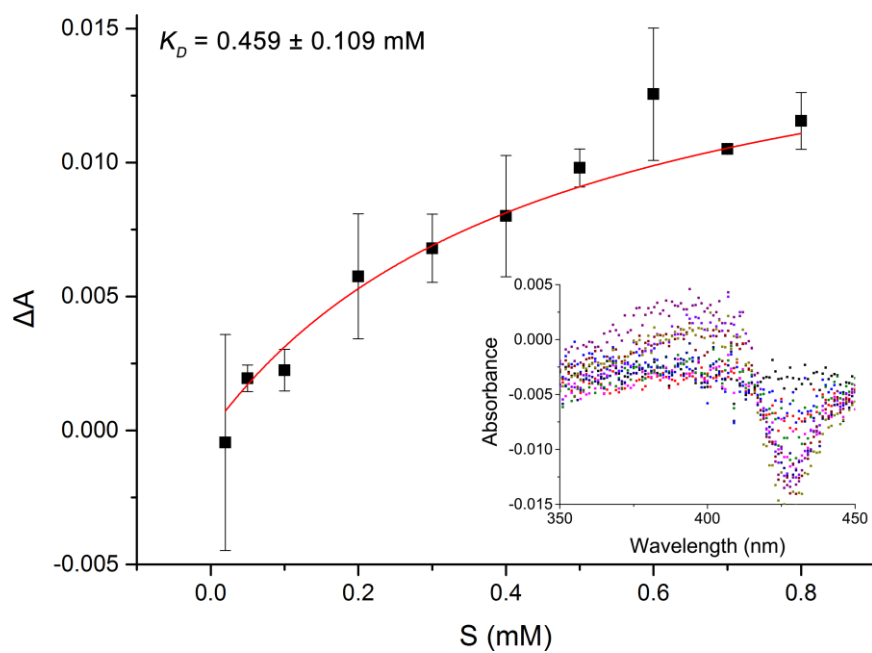


Fig. S9. The binding curve of 4-cresol to the S261A mutant.

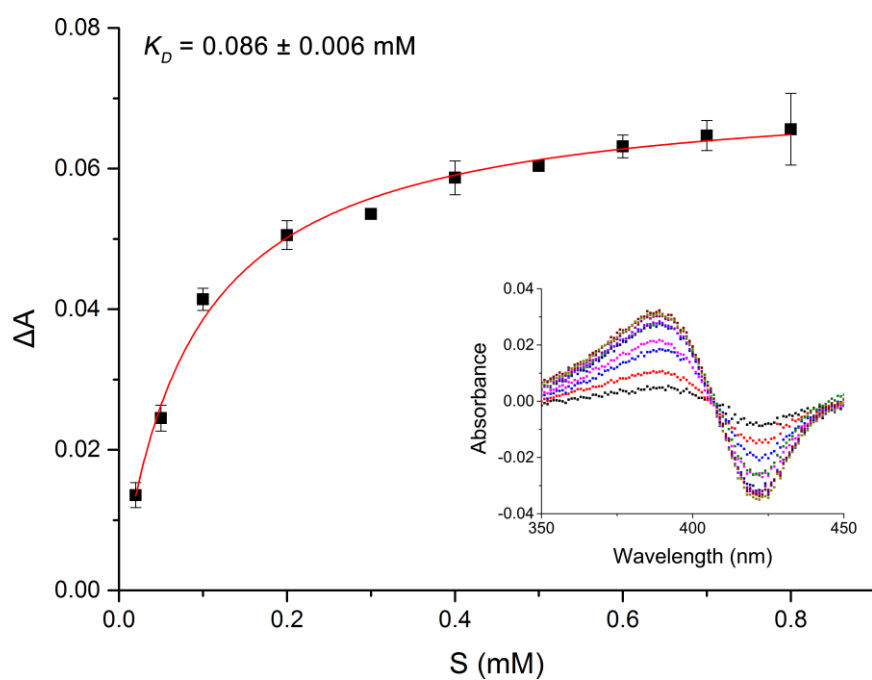


Fig. S10. The binding curve of 4-cresol to the Q83A mutant.

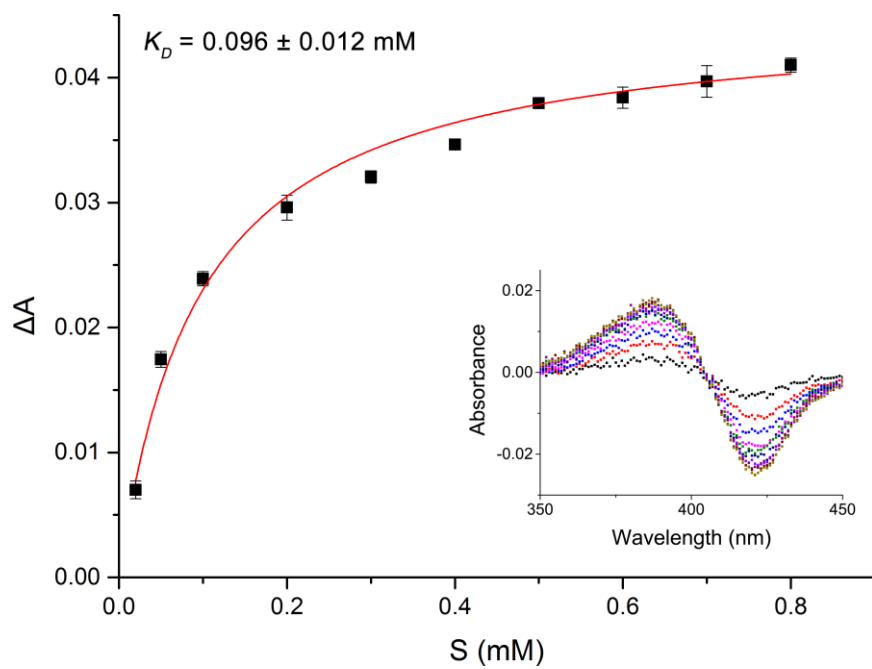


Fig. S11. The binding curve of 4-cresol to the S109A mutant.

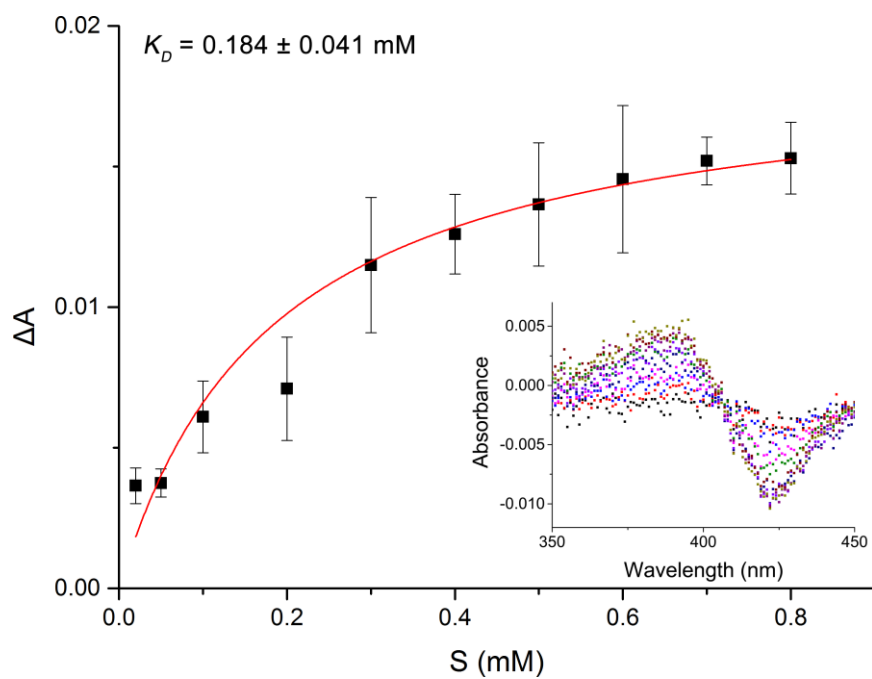


Fig. S12. The binding curve of 4-cresol to the W199A mutant.

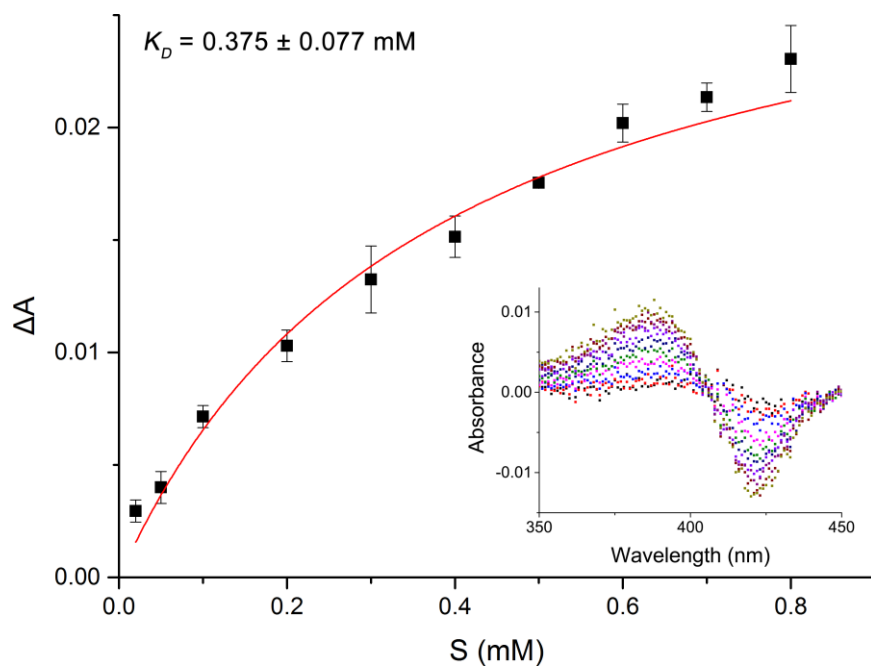


Fig. S13. The binding curve of 4-cresol to the I312A mutant.

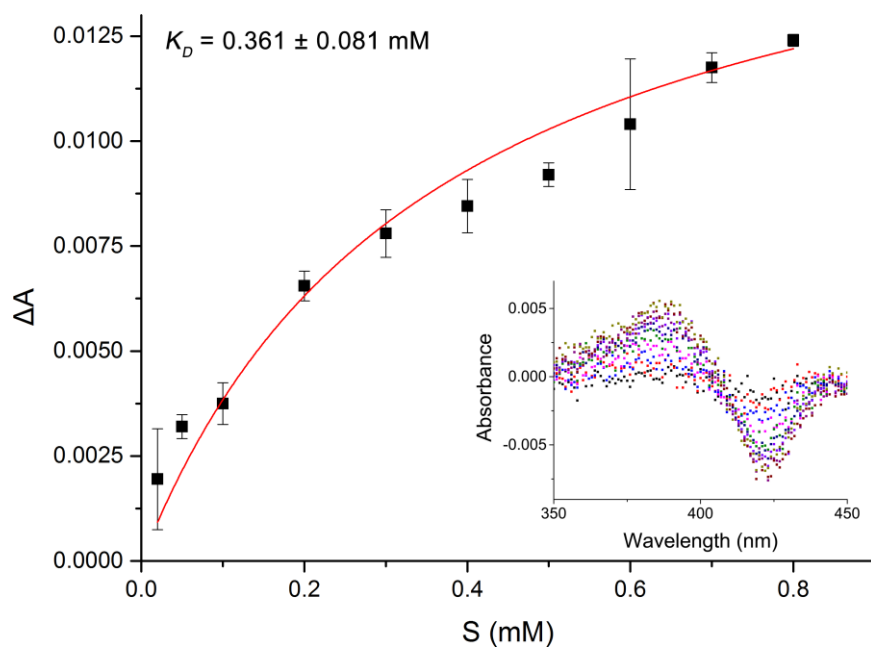


Fig. S14. The binding curve of 4-cresol to the F416A mutant.

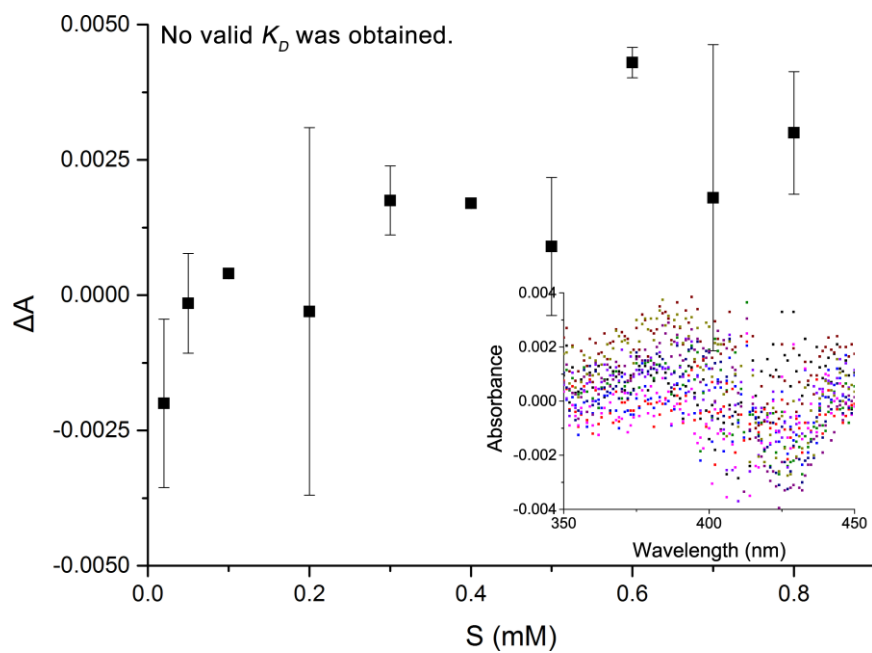


Fig. S15. The binding curve of 4-cresol to the F264A mutant.

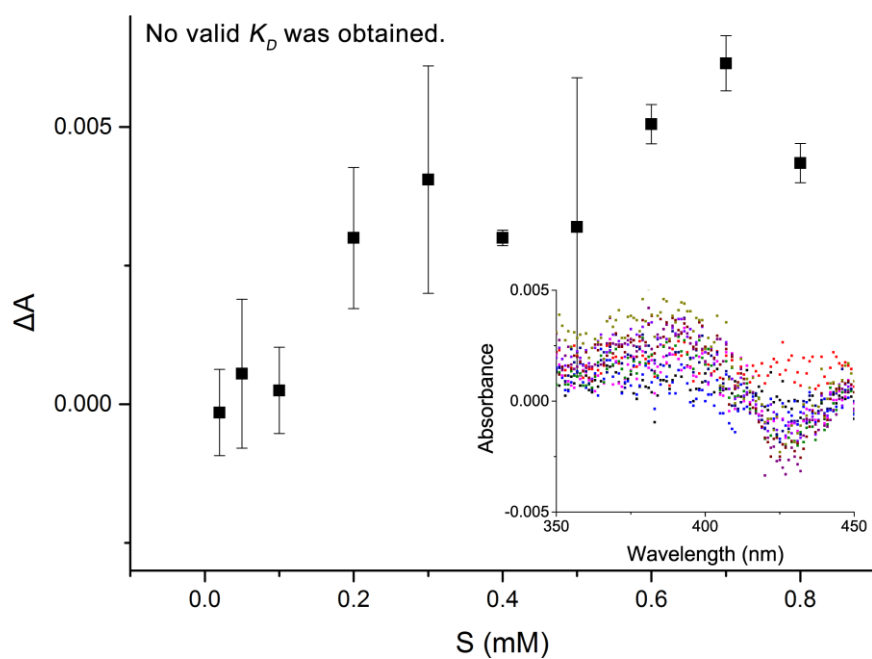


Fig. S16. The binding curve of 4-cresol to the W315A mutant.

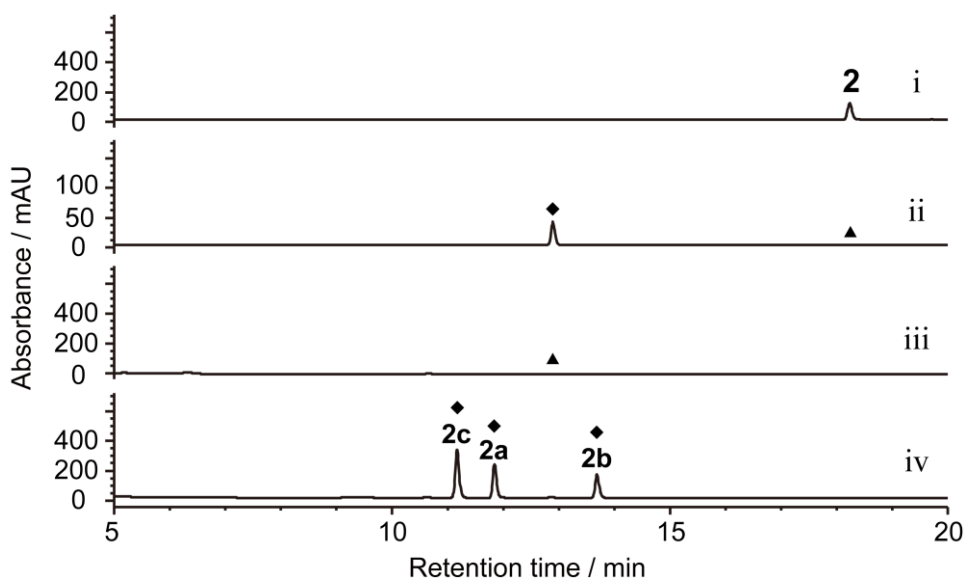


Fig. S17. Preliminary reactivity determination of CreHI, CreJEF and CreD towards 2 and its derivatives. Trace i, Negative control; trace ii, 2 + CreHI; trace iii, the post-CreHI reaction mixture + CreJEF; trace iv, the post-CreHI/CreJEF reaction mixture + CreD. ◆ indicates product generation compared to the upper trace. ▲ indicates significant substrate consumption compared to the upper trace.

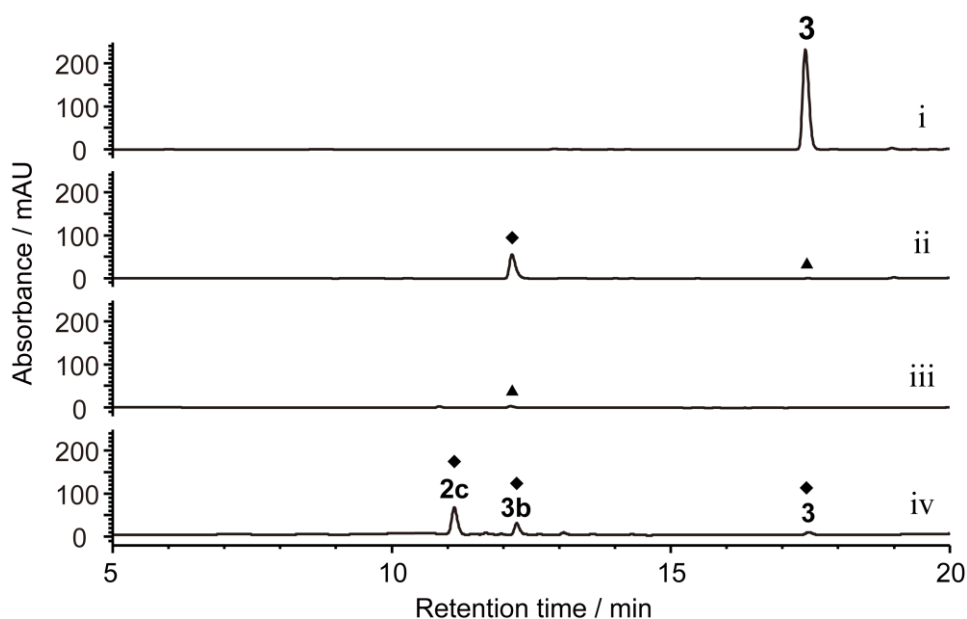


Fig. S18. Preliminary reactivity determination of CreHI, CreJEF and CreD towards 3 and its derivatives. Trace i, Negative control; trace ii, 3 + CreHI; trace iii, the post-CreHI reaction mixture + CreJEF; trace iv, the post-CreHI/CreJEF reaction mixture + CreD. ◆ indicates product generation compared to the upper trace. ▲ indicates significant substrate consumption compared to the upper trace.

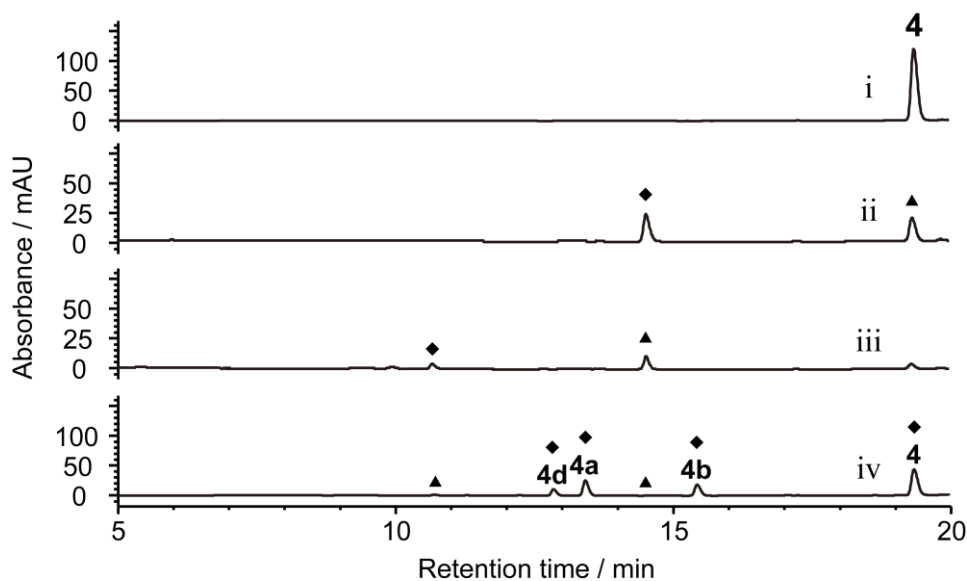


Fig. S19. Preliminary reactivity determination of CreHI, CreJEF and CreD towards 4 and its derivatives. Trace i, Negative control; trace ii, 4 + CreHI; trace iii, the post-CreHI reaction mixture + CreJEF; trace iv, the post-CreHI/CreJEF reaction mixture + CreD. ◆ indicates product generation compared to the upper trace. ▲ indicates significant substrate consumption compared to the upper trace.

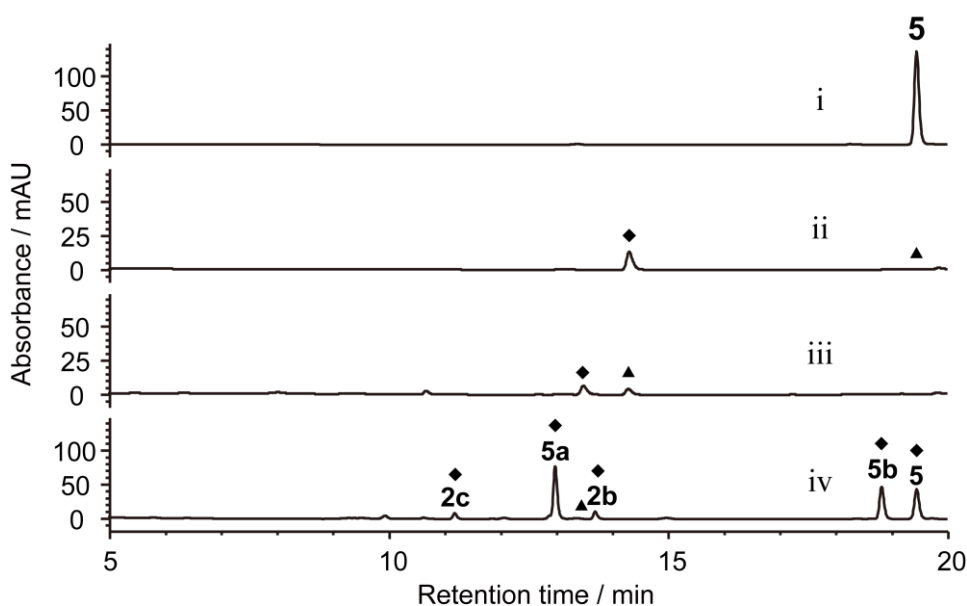


Fig. S20. Preliminary reactivity determination of CreHI, CreJEF and CreD towards 5 and its derivatives. Trace i, Negative control; trace ii, 5 + CreHI; trace iii, the post-CreHI reaction mixture + CreJEF; trace iv, the post-CreHI/CreJEF reaction mixture + CreD. ◆ indicates product generation compared to the upper trace. ▲ indicates significant substrate consumption compared to the upper trace.

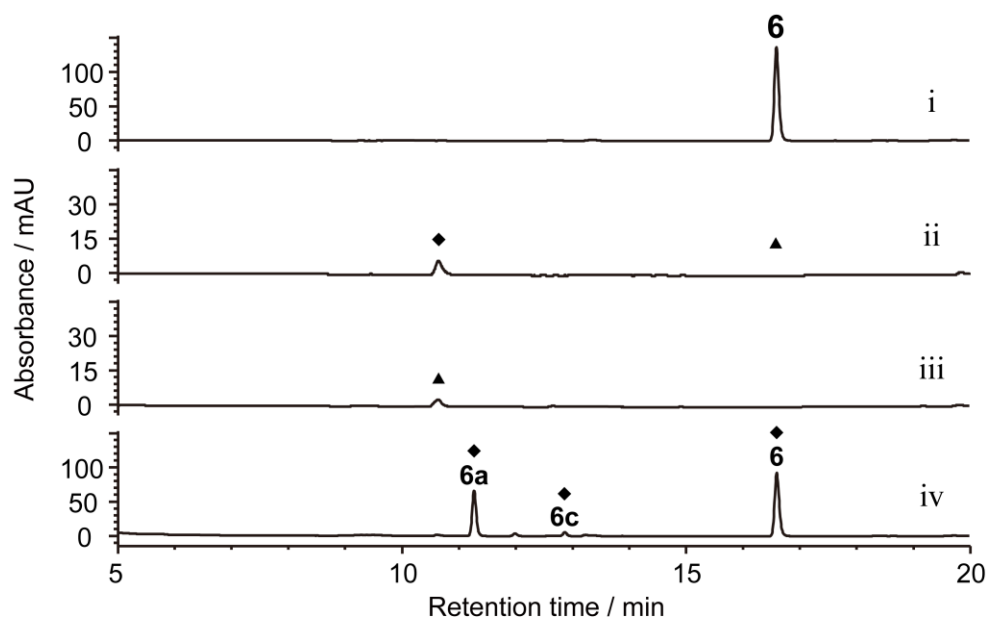


Fig. S21. Preliminary reactivity determination of CreHI, CreJEF and CreD towards 6 and its derivatives. Trace i, Negative control; trace ii, 6 + CreHI; trace iii, the post-CreHI reaction mixture + CreJEF; trace iv, the post-CreHI/CreJEF reaction mixture + CreD. ◆ indicates product generation compared to the upper trace. ▲ indicates significant substrate consumption compared to the upper trace.

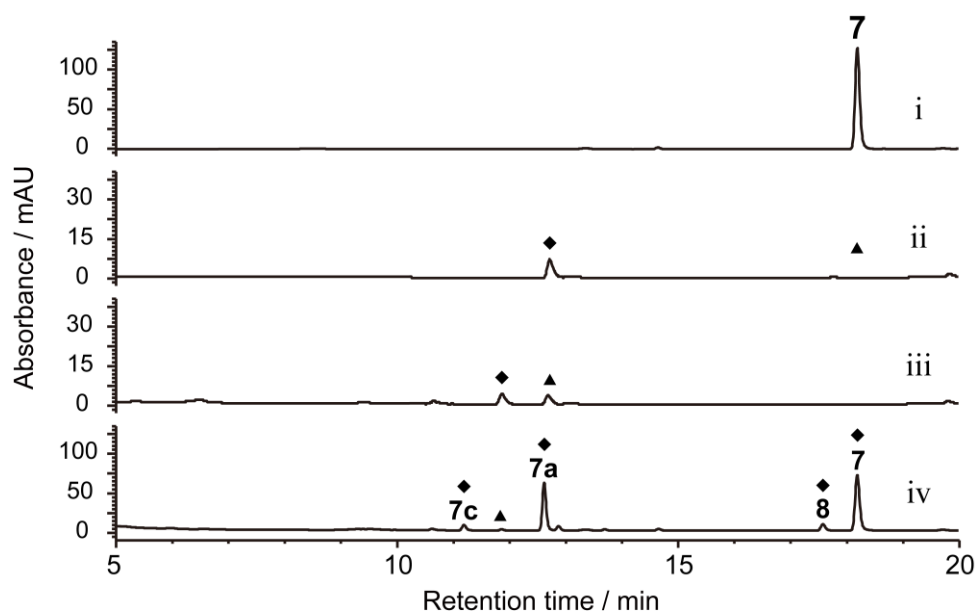


Fig. S22. Preliminary reactivity determination of CreHI, CreJEF and CreD towards 7 and its derivatives. Trace i, Negative control; trace ii, 7 + CreHI; trace iii, the post-CreHI reaction mixture + CreJEF; trace iv, the post-CreHI/CreJEF reaction mixture + CreD. ◆ indicates product generation compared to the upper trace. ▲ indicates significant substrate consumption compared to the upper trace.

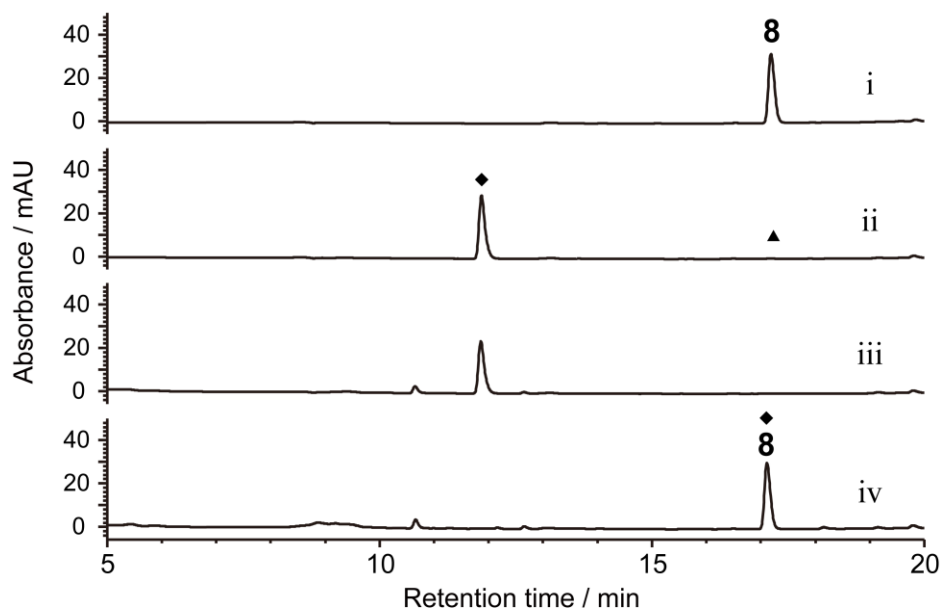


Fig. S23. Preliminary reactivity determination of CreHI, CreJEF and CreD towards **8 and its derivatives.** Trace i, Negative control; trace ii, **8** + CreHI; trace iii, the post-CreHI reaction mixture + CreJEF; trace iv, the post-CreHI/CreJEF reaction mixture + CreD. ◆ indicates product generation compared to the upper trace. ▲ indicates significant substrate consumption compared to the upper trace.

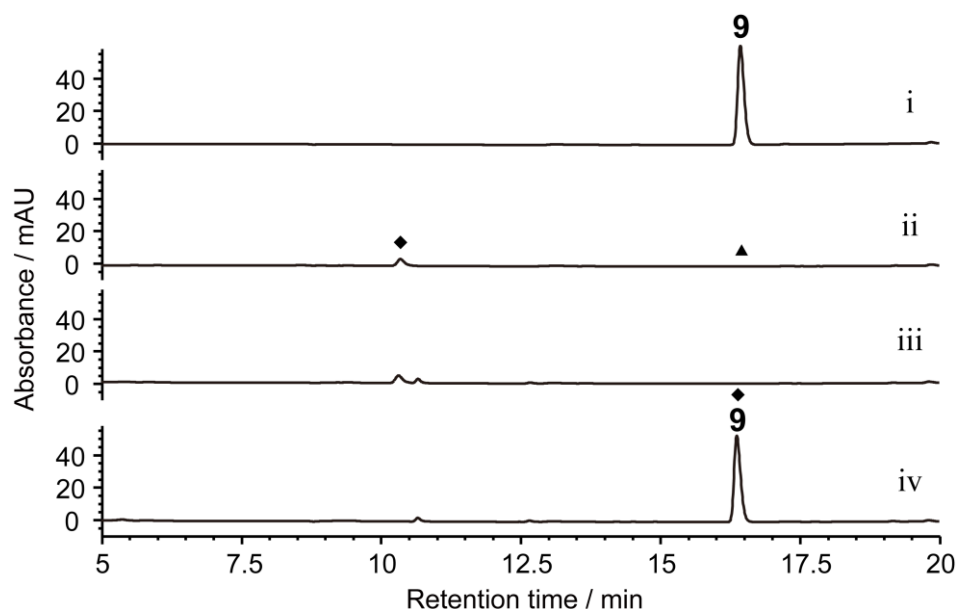


Fig. S24. Preliminary reactivity determination of CreHI, CreJEF and CreD towards **9 and its derivatives.** Trace i, Negative control; trace ii, **9** + CreHI; trace iii, the post-CreHI reaction mixture + CreJEF; trace iv, the post-CreHI/CreJEF reaction mixture + CreD. ◆ indicates product generation compared to the upper trace. ▲ indicates significant substrate consumption compared to the upper trace.

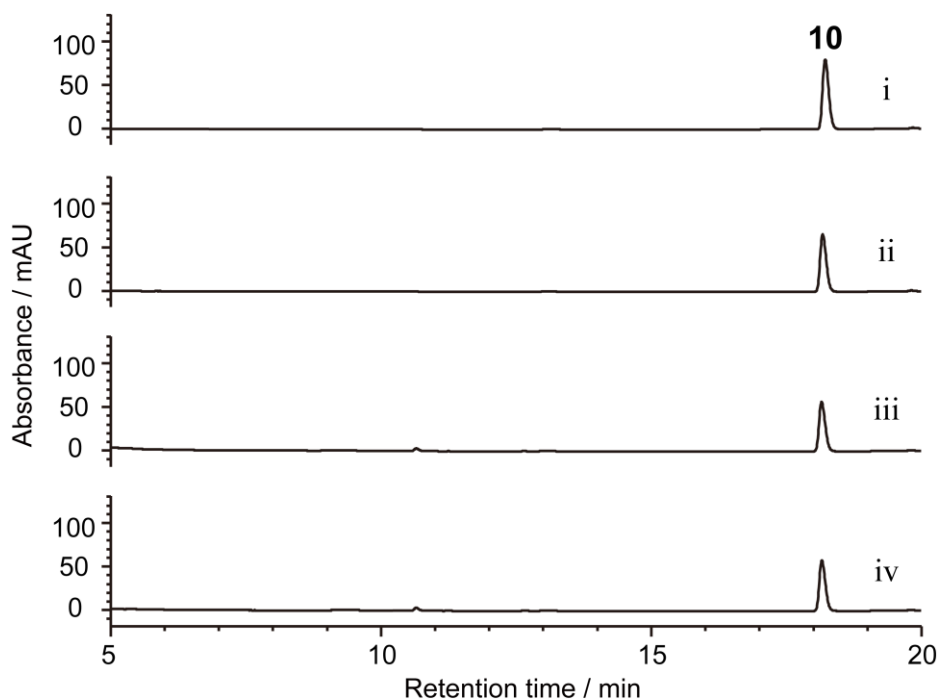


Fig. S25. Preliminary reactivity determination of CreHI, CreJEF and CreD towards 10 and its derivatives. Trace i, Negative control; trace ii, **10** + CreHI; trace iii, the post-CreHI reaction mixture + CreJEF; trace iv, the post-CreHI/CreJEF reaction mixture + CreD.

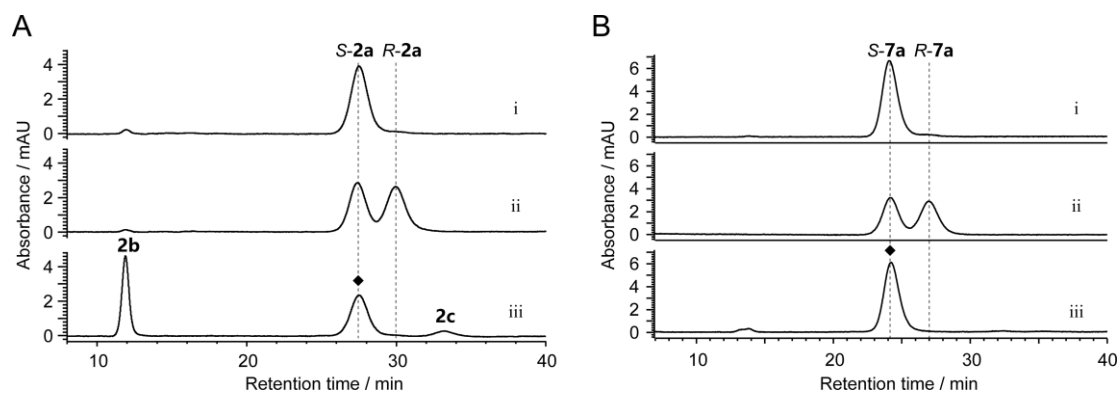


Fig. S26. Determination of the absolute configuration of 2a (A) and 7a (B) by chiral HPLC analysis. (A) Trace i, *S*-**2a** authentic standard; trace ii, racemic **2a** standard; trace iii, the reaction mixture of **2** sequentially reacted with CreHI/CreJEF and CreD. ◆ indicates only *S*-**2a** was produced. (B) Trace i, *S*-**7a** authentic standard; trace ii, racemic **7a** standard; trace iii, the reaction mixture of **7** sequentially reacted with CreHI/CreJEF and CreD. ◆ indicates only *S*-**7a** was produced.

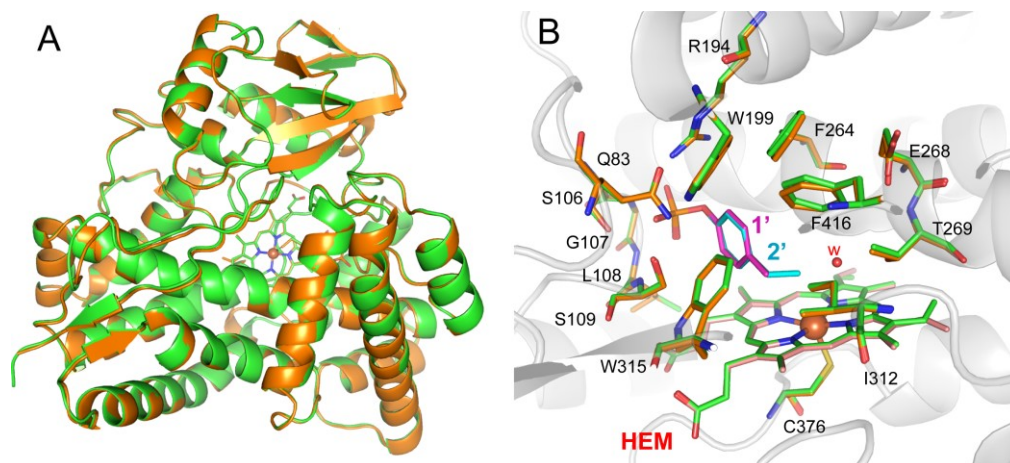


Fig. S27. Structural comparison of CreJ-1' (green, 5GWE) and CreJ-2' (orange, 5XJN). (A) Superimposition of CreJ-1' and CreJ-2'. (B) Conformational alignment of the active site residues. Substrate 1' and 2' are shown in purple and cyan, respectively. “w” represents the water molecule observed only in the CreJ-1' structure.

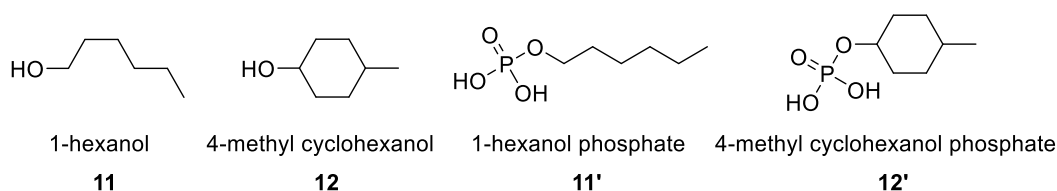


Fig. S28. Structures of non-aromatic 11, 12, 11' and 12'.

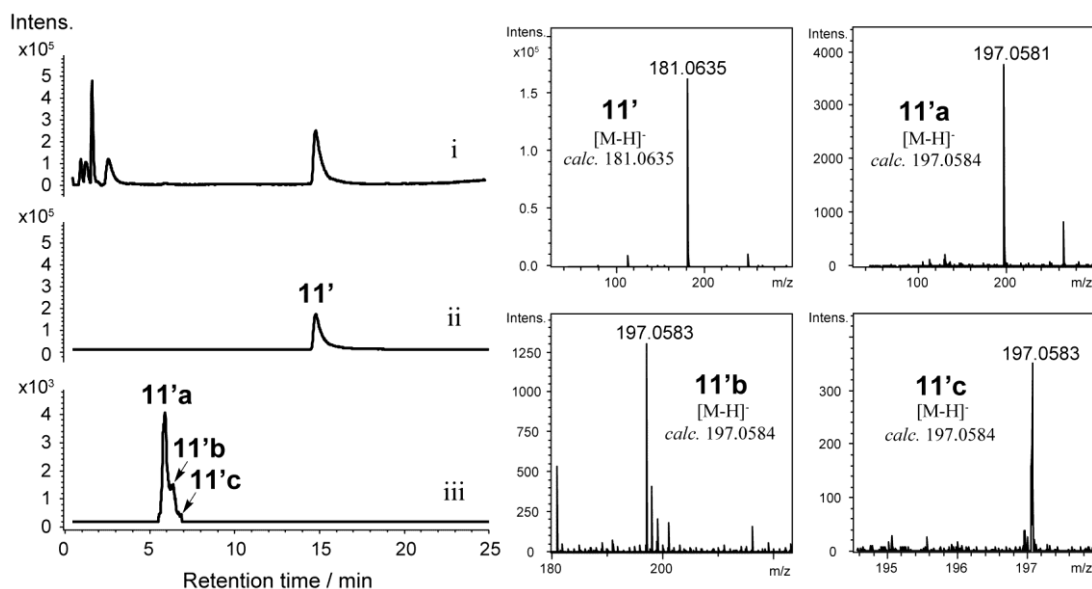


Fig. S29. LC-HRMS analysis of the CreJ reactivity towards 11'. Trace i, the total ion chromatography (TIC) of the CreJEF catalyzed reaction with 11' as substrate; trace ii, the extract ion chromatography at m/z 181; trace iii, the extract ion chromatography at m/z 197. The right panel shows the high resolution mass spectra of 11' and 11'a-c.

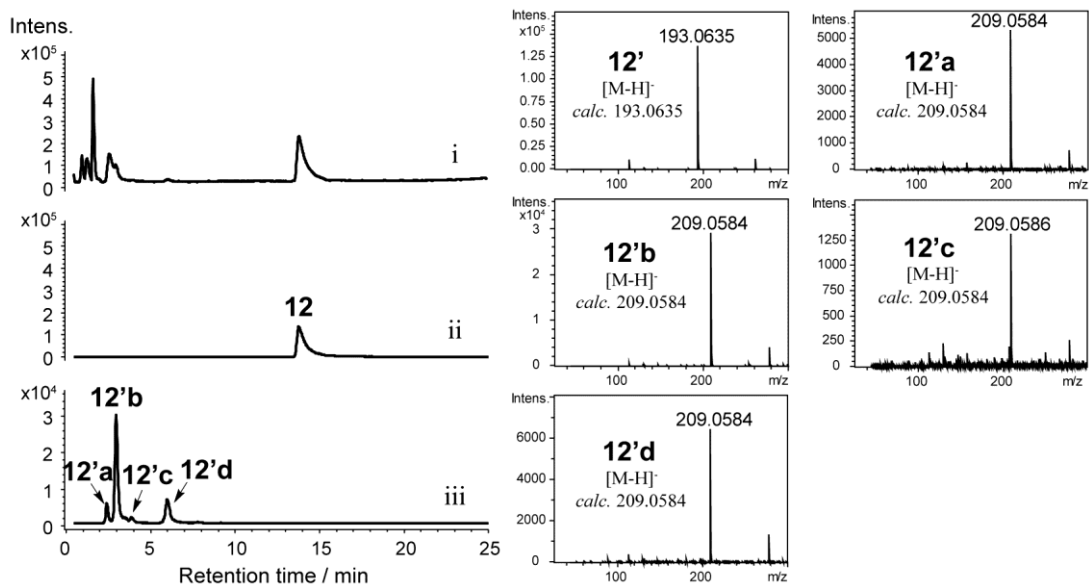


Fig. S30. LC-HRMS analysis of the CreJ reactivity towards 12'. Trace i, the total ion chromatography (TIC) of the CreJEF catalyzed reaction with **12'** as substrate; trace ii, the extract ion chromatography at m/z 193; trace iii, the extract ion chromatography at m/z 209. The right panel shows the high resolution mass spectra of **12'** and **12'a-d**.

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 140 150 160 170 180 190 200 210

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 W P _ 0 6 1 2 5 2 8 3 7 . 1 S I R Q H V T I L L E R M L A R F K . . . A T G D F V R D L A Y D F E T I T I L T L I G A D T G . . K V D T F K R W S D S R A A M T W G D L S D E E Q V P H A H
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 W P _ 0 2 0 3 8 9 6 2 5 . 1 A I R A N V V A Q L E G L L A N P G . . . R R G D L V K D L A Y Q V E T I T I L T L I G A D T S . . Q V D T Y K R W S D S R A A M T W G D L S D E E Q I F P H A H
 W P _ 0 5 3 4 4 7 2 8 2 . 1 E I R E N V R L L D M L A R P E . . . A K G D I V R D L A R D V E T I T I L T L I G E D V D . . Q V D F K R W S D S R A A M T W G D L S D E E Q I F P H A H
 W P _ 0 2 4 3 6 8 4 8 1 . 1 F I R Q N V H I L V T M L A R P E . . . R R G D I V R D L A Y D F E T I T I L T L I G E D V A . . M I P S Y K R W S D S R A A M T W G L S D E E Q I F P H A H
 W P _ 0 2 2 8 7 0 1 6 6 . 1 M I R D N V K A S I Q M L A K P T . . . P H A D I V S V A Y S F E T I T I L T L I G E S T P E T I D Q Y K R W S D S R A A M T W G L S D E E Q I F P H A H
 O D T 1 1 9 7 0 . 1 T I R E N T R T A L Q R M L E S P D . . . R G G D V H D L A F D F E T I T I L T L I G V D P A . . M V P T Y K R W S D S R A A M T W G D L S D E E Q V P H A H
 W P _ 0 4 3 0 5 5 8 0 3 . 1 M I R D N V R T A S I E R M L A K P T . . . P H A D I I A D V A Y S F E T I T I L T L I G E D T P E T V D Q Y K R W S D S R A A M T W S L S D E E Q I F P H A H
 W P _ 0 5 6 7 2 8 8 2 8 . 1 A I R E N T R T A L R A M L E S P D . . . R T G D F L M D V A Y E F E T I T I L T L I G V D P A . . M V P T Y K R W S D S R A A M T W G D L S D E E Q I F P H A H
 B A U 9 9 4 9 8 . 1 A I R E N V D I M I D K M L A A G E . . . P G D I I S D I A N D F E T I T I L T L I G V P I S . . R I P E I K W S G S R A L M T W A E L T D E E Q I F P H A H
 W P _ 0 3 5 8 7 7 8 7 8 . 1 T I R E N T A R L L Q Q M L A H P D . . . A R G D F I T D V A Y D F E T I T I L G L L G V G P E . . M V P T Y K R W S D S R A A M T W G L S D E E Q I F P H A H
 S B T 3 8 7 4 3 . 1 Q I R A N V V R L L K R M L L R A E . . . R T G D L V S D L A I D V E T I T I L T L I G A D P A . . M L S T F K R W S A S R A A M T W G D L S A E E Q V P H A H
 K G A 0 7 7 0 0 . 1 F I R E N A D A L I D A M V A K G G . . . N S G D I I T D V A Y D F E T I T I F A L I G V P N E . . M T D L V K K W A S R A A M T W G D L T D E E Q V P H A H
 G A P 5 6 7 9 4 . 1 F I R Q N V V E L L E K M L A R P E . . . H R G D M V K D L A Y D F E T I T I L T L I G A D V S . . Q V D T F K R W S D S R A A M T W G D L S D E E Q I F P H A H
 W P _ 0 1 9 9 8 6 7 6 8 . 1 R I R E L A I N M I G G F Q A . D G . . . H A E M V R D L A Y D F A F V I F M L L G V P N E . . D V Q Q V K S W A E S R A A M T W G D L T D E E Q I V H A E
 W P _ 0 1 5 0 3 0 0 6 8 . 1 K I R E L A I N M I E D F A A . K G . . . K T N I I K D L A Y D F A Y V I F M L L G V P N E . . E V Q Q V K S W A E S R L L L T W G D L S E D D Q I V H A Q
 W P _ 0 0 9 1 8 4 7 7 9 . 1 E I R N I A I R M I E E L R A G N K . . . Q A E M I S E L A Y D F A L V I F K L L G V P D S . . D V A Q V K S W A E S R L L T W G D L D E D A Q M M H A E
 W P _ 0 1 5 6 6 4 2 2 1 . 1 E I K A I V N Q A I D G F A D R G H . . . A D F F R E F A Y D F A L V L F K L V G V P N M . . D V P R V K S W A V S R A L L T W G D L S D E E Q I V H A H
 W P _ 0 0 9 0 3 1 0 0 6 . 1 E I K A I V N Q A I D A F A A R G H . . . A D F F R E F A Y D F A L V L F K L V G I P N M . . D V P R V K S W A V S R A L L T W G D L S D E E Q V V H A R
 W P _ 0 0 6 6 1 4 2 5 7 . 1 E I K A I V N Q A I D A F A A R G H . . . A D F F R E F A Y D F A L V L F K L V G I P N M . . D V P R V K S W A V S R A L L T W G D L S D E E Q I V H A R
 W P _ 0 1 2 0 4 1 8 5 5 . 1 E I K A I V N R A I D T F A E R G H . . . A D F F R E F A Y D F A L V L F K L V G I P N I . . D V P R V K S W A V S R A L L T W G D L S D E E Q I V H A R
 W P _ 0 6 7 5 4 7 9 3 6 . 1 Q I E A I V D R H L N K I A V A G E . . . C D F F R D V A Y D F A L V L F A L M G I P D E . . D V P K V K W A A S R A L L T W G D L S D E E Q I F P L A E
 W P _ 0 1 3 9 2 6 2 1 9 . 1 D I R A L A I E M I E K F Q D . K K . . . H A E L V K E L Y Y D F A F V I F M L L G V P K E . . E V T Q V K W A I S R M M L T F S D T S E E E Q I F H A R
 W P _ 0 0 7 2 0 4 7 0 0 . 1 Q I E A I I D R H L T K I A A A G E . . . C D F F R D V A Y D F A L V L F A L M G I P D A . . D V P K V K W A A S R A L L T W G L S D E D O P L A H
 W P _ 0 0 9 8 0 9 9 7 5 . 1 K I E A I I D R T L N E A A E L G Q . . . D F F R Q V A Y P F A L V L F T M M G I P D E . . D V P K V K W A V S R A L V T W G L S D E E Q V V H A Q
 W P _ 0 6 4 7 5 0 5 6 6 . 1 Q I R E L I T T M I D R F A S R G H . . . A D L V E A L T H E F A L V I F R L L G I P D A . . D V P R V K W A A S R V F L N F G D L P V S E O V A H A E
 W P _ 0 3 7 6 6 4 3 8 8 . 1 K V W A K A T E L V D A I K . P G Q . . . V D L V S A L T Y P F A Y M I F T F I G F P D E . . D M D M L K S W C G N R I A F S W G R P S G D E O R E I A T
 S C G 0 2 6 4 2 . 1 K V W A K A T E L V D A I K . P G Q . . . V D L V S A L T Y P F A Y M I F T F I G F P D E . . D M D M L K S W C G N R I A F S W G R P S G D E O R E I A T
 W P _ 0 3 0 4 9 7 7 9 6 . 1 K V W A K A T E L V D A I E . P G R . . . V D L V N A L T Y P F A Y M I F T F I G F P D E . . D M D M L K S W C G N R I A F S W G R P S A D E O R E I A T
 W P _ 0 1 4 1 5 8 2 7 9 . 1 K V W A K A T E L V D A V E . P G Q . . . V D L V A A L T Y P F A Y M I F T F I G F P D E . . D M D M L K S W C G N R I A F S W G R P S A D E O R E I A T
 W P _ 0 3 7 8 3 1 1 5 4 . 1 K V W A K A T E L V D A V E . P G Q . . . V D L V A A L T Y P F A Y M I F T F I G F P D E . . D M D M L K S W C G N R I A F S W G R P S A D E O R E I A T
 W P _ 0 3 0 8 8 2 0 3 5 . 1 K V W A K A T E L V D A V E . P G Q . . . V D L V A A L T Y P F A Y M I F T F I G F P D E . . D M D M L K S W C G N R I A F S W G R P S A G E O R E I A T
 W P _ 0 0 9 9 5 1 1 2 7 . 1 T I R A Y V T E M I D R L H Q D R . . . F D I V Q E V T F P F E A T I V F N L I G F P E S . . D M E M L K G L A I N R M A P T W G R S T E A E Q V R I A K

CYP288A2	α9			α10			α11						
	220	*	230	240	250	260	270	280					
CYP288A2	NLVE	YMQE	QR	MVA	DAHAH	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_011013726.1	NLVE	YMQE	QR	MVA	DAHAH	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_060564075.1	NLVE	YMQE	QR	MVA	DAHAH	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_065366652.1	NLVE	YMQE	QR	MVA	DAHAH	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_003860615.1	NLVE	YMQE	QR	MVA	DAHAH	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_063967321.1	NLVE	YMQE	QR	MVA	DAHAH	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_038582779.1	NLVE	YMQE	QR	MVA	DAHAH	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_006287245.1	NLVE	YMQE	QR	MVA	EAHEN	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_044026924.1	NLVE	YMQE	QR	MVA	EAHEN	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_040966998.1	NLVE	YMQE	QR	MVA	DAHAH	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
BAF53637.1	NLVE	YMQE	QR	MVA	EAHEN	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_003854399.1	NLVE	YMQE	QR	MVA	EAHEN	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
BAU94954.1	NLVE	YMQE	QR	MVA	DAHAH	GGDNI	TADL	VRAQ	QE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_066564562.1	NLVE	YMQE	QR	MVA	HAHEH	GGDNI	TADL	VRAQ	ES	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_035109760.1	NLVE	YMQE	QR	MVA	DAHAN	GGDNI	TADL	VRAQ	ES	Q	EITDHEIASLLYSLLF	AGHETT	TTL
EEW50177.1	NLVE	YMQE	QR	MVA	DAHAN	GGDNI	TADL	VRAQ	ES	Q	EITDHEIASLLYSLLF	AGHETT	TTL
BAC17372.1	NLVE	YMQE	QR	MVA	DAHAN	GGDNI	TADL	VRAQ	ES	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_015399966.1	SLVE	YMQE	QR	MVA	EAHAN	GGDSI	TADL	ARAQ	EE	Q	EITDHEIASLLYSLLF	AGHETT	TTL
WP_010144948.1	NLVE	YMQE	QR	LVA	EAHAH	GGDNI	TADL	VRTQ	QE	GG	EISDHEIASLLYSMLF	AGHETT	TTL
WP_026196155.1	NLVE	YMAE	QR	LVA	EAHNS	PTDDI	TSD	LVRF	SE	GG	EISDHEIASLLYSLLF	AGHETT	TTL
WP_062861371.1	NLVE	YMQE	CL	SIVA	DAHEN	GGDSM	TADL	VKAC	SE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_039207235.1	NLVE	YMQE	CL	SIVA	DAHEN	GGDSM	TADL	VKAC	SE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_062241641.1	NLVE	YMQE	CL	SIVA	DAHEN	GGDSM	TADL	VTAQ	SE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_011691841.1	NLVE	YMQE	CL	RLV	KVAHEQ	GGDNI	TADL	VKSC	QE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_024367961.1	NLVE	YMQE	CL	RLV	KVAHEE	GGDNI	TADL	VKSC	QE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_005273409.1	NLVE	YMQE	CL	RLV	VAAHEE	GGDNI	TADL	VKAC	QQ	GA	EITDHEIASVLYSMLF	AGHETT	TTL
WP_043481247.1	NLVE	YMQE	CL	RLV	VAAHEE	GGDNI	TADL	VKSC	QE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_055806675.1	NLVE	YMQE	CL	RLV	VAAHEE	GGDNI	TADL	VKSC	QE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_062289581.1	NLVE	YMQE	CL	RLV	VAAHEE	GGDNI	TADL	VAAQ	AG	GA	EITDHEIASVLYSLLF	AGHETT	TTL
WP_028269402.1	NLVE	YMQE	CL	RLV	AAHEK	GGDNI	TADL	VKAC	QQ	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_028276640.1	NLVE	YMQE	CL	RLV	VAAHEE	GGDNI	TADL	VKAC	QQ	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_026539569.1	NLVE	YMQE	CL	RLV	VAAHEE	GGDNI	TADL	VKSC	QE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_018775007.1	NLVE	YMQE	CL	RLV	VAAHEE	GGDNI	TADL	VKSC	QE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_013600843.1	NLVE	YMQE	CL	RLV	VAAHEE	GGDNI	TADL	VKAC	QQ	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_028265717.1	NLVE	YMQE	CL	RLV	VAAHDD	GGDNI	TADL	VKSC	QQ	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_068734283.1	NLVE	YMAE	CL	RLV	AAHEH	GGDNI	TADL	VKAC	SD	GA	EITDHEIASLLYSLLF	AGHETT	TTL
WP_069950213.1	NLVE	YMQE	CL	RLV	KVAHEE	GGDNI	TADL	VKSC	QE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_007269721.1	NLVE	YMAE	CL	RLV	AAHKKH	GGDNI	TADL	VKAC	TE	GA	EITDHEIASLLYSLLF	AGHETT	TTL
ATS67786.2	NLVE	YMAE	CL	RLV	AAHKKH	GGDNI	TADL	VKAC	TE	GA	EITDHEIASLLYSLLF	AGHETT	TTL
WP_066143328.1	NLVE	YMAE	CL	RLV	AAHAT	EGDSI	TADL	VKAC	NE	GA	EITDHEIASLLYSLLF	AGHETT	TTL
WP_038992926.1	NLVE	YMAE	CL	RLV	AAHAT	EGDSI	TADL	VKAC	NE	GA	EITDHEIASLLYSLLF	AGHETT	TTL
WP_047120326.1	NLVE	YMAE	CL	RLV	AAHAA	GGDSI	TADL	VQAC	KD	GA	EITDHEIASLLYSLLF	AGHETT	TTL
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WP_040795180.1	NLVE	YMAE	CL	RLV	AAHET	RPDSI	LVG	DMIRMG	AD	GP	EITDHEIASLCYSLF	AGHETT	TTL
WP_064080112.1	NLVE	YMAE	CL	RLV	AAHEK	QPDSI	LIG	DLVRAQ	AA	GP	EISDHEIASVGYSLF	AGHETT	TTL
WP_045191440.1	NLVE	YMAE	CL	RLV	AAHEK	QPDSI	LVG	DLVRAQ	QA	GP	EISDHEIASLCYSLF	AGHETT	TTL
WP_066501301.1	NLVE	YMAE	CL	RLV	AAHAE	GGDNI	TADL	VTAQ	KD	GA	EISDHEIASVAYSLF	AGHETT	TTL
WP_052024002.1	NLVE	YMAE	CL	RLV	AAHEK	QPDSI	LIG	DLVRAQ	AA	GP	EISDHEIASVGYSLF	AGHETT	TTL
WP_026453050.1	NLVE	YMAE	CL	RLV	AAHGN	ERDSI	LVG	DLVRAQ	RD	GP	EITDHEIASVCYSLF	AGHETT	TTL
WP_067137052.1	NLVE	YMAE	CL	RLV	AAHEH	ERGSI	LVG	DLVRAQ	AA	GP	EITDHEIASVYSLF	AGHETT	TTL
ELB90053.1	NLVE	YMAE	CL	RLV	AAHEK	QPDSI	LIG	DLVRAQ	AA	GP	EISDHEIASVGYSLF	AGHETT	TTL
WP_029339819.1	NLVE	YMAE	CL	RLV	AAHAK	PRDSI	LVG	DLIRAQ	RA	GP	EITDHEIASVCYSLF	AGHETT	TTL
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WP_066941537.1	NLVE	YMAE	CL	RLV	AAHEH	ERGSI	LVG	DLVRAQ	AA	GP	EITDHEIASVYSLF	AGHETT	TTL
WP_040168028.1	NLVE	YMAE	CL	RLV	AAHES	GGDNI	TADL	VVEAQ	KA	GA	EITDHEIASLCYSLF	AGHETT	TTL
WP_053293979.1	NLVE	YMAE	CL	RLV	AAHET	ERDSI	LVG	DMVRMG	AA	GP	EISDHEIASVCYSLF	AGHETT	TTL
WP_061252837.1	NLVE	YMAE	CL	RLV	AAHEH	ERGSI	LVG	DLVRAQ	AA	GP	EITDHEIASVYSLF	AGHETT	TTL
WP_006335139.1	NLVE	YMAE	CL	RLV	AAHEN	PRDSI	LVG	DLVRAQ	HE	GP	EISDHEIASVCYSLF	AGHETT	TTL
WP_020389625.1	NLVE	YMAE	CL	RLV	AAHET	PDPSI	LVG	DLVRAQ	RD	GP	EITDHEIASVCYSLF	AGHETT	TTL
WP_053447282.1	NLVE	YMQE	CL	RLV	AAHRE	GGDNI	TADL	VVAC	QQ	GA	EITDHEIASVLYSLLF	AGHETT	TTL
WP_024368481.1	NLVE	YMQE	CL	RLV	VAAHEE	AGDSI	TADL	VKAC	SE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_022870166.1	NLVE	YMAE	CL	RLV	VAAHEE	GGDNI	TADL	MVRDQ	EA	GA	EITDHEIASVLYSLLF	AGHETT	TTL
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WP_056728828.1	NLVE	YMAE	CL	RLV	VAAHER	GGDNI	TADL	VVEAQ	DA	GA	EISDHEIASLCYSLF	AGHETT	TTL
BAU99498.1	NLVE	YMAE	CL	RLV	VAAHAK	GGDNI	TADL	VVAC	AE	GA	EISDHEIASVLYSMLF	AGHETT	TTL
WP_035877878.1	NLVE	YMAE	CL	RLV	VAAHEALAAHEALAAQKDAADDEAV	TDNI	LVADL	VRAQ	LA	GA	EISDHEIASVLYSLLF	AGHETT	TTL
SBT38743.1	RLVE	YMAE	CL	RLV	VAAEQS	ERDSI	LVG	DLVRAQ	RG	GP	EISDHEIASVCYSLF	AGHETT	TAL
KGA07700.1	NMVA	YMSF	QK	LVA	DAKVT	PGDNI	VT	DLVNLQ	AA	GE	KISDHEIASVLYSLLF	AGHETT	TTL
GAP56794.1	NLVE	YMQE	CL	RLV	KVAHAE	GGDNI	TADL	VKSC	QE	GA	EISDHEIASVLYSLLF	AGHETT	TTL
WP_019986768.1	NMIX	YWNQ	QNL	VK	LRHEN	PTDDI	FG	DLVRLQ	AE	GE	EISDREIAAMCYSLF	AGHETT	TSL
WP_015030068.1	NMIX	YWNQ	QGL	V	KRKEN	PTDDI	FG	DLVRYQ	AE	GE	EISDREIAAMCYSLF	AGHETT	TSL
WP_009184779.1	NMIX	YWNQ	QK	LVS	DRKEK	MQDDI	FG	DLVVLC	AE	GE	EITDREIAATCYSLF	AGHETT	TSL
WP_015664221.1	NMVE	YWNQ	QRL	V	QRHDD	PTDDI	FG	DLVRLQ	KD	GA	EISDREIAGVLYSALF	AGHETT	TTL
WP_009031006.1	NMVD	YWNQ	QRL	V	QRHDD	PTDDI	FG	DLVRLQ	KE	GA	EISDREIAGVLYSALF	AGHETT	TTL
WP_006614257.1	NMVE	YWNQ	QR	L	QRHDD	PTDDI	FG	DLVRLQ	KD	GA	EISDREIAGVLYSALF	AGHETT	TTL
WP_012041855.1	NMVE	YWNQ	CR	L	QRHDD	PTDDI	FG	DLVRLQ	KD	GA	EISDREIAGVLYSALF	AGHETT	TTL
WP_067547936.1	KMVD	YWDY	CR	G	VAARKEV	PGDDF	FP	DMVQAC	AN	GA	EITDREIAGLMYSVLF	AGHETT	TTL
WP_013926219.1	QVVK	YWDY	CR	E	MVAARRKQV	LGDDF	FP	DLVRLQ	QE	GE	EISDREIAAMCYNLQF	AGHETT	TSL
WP_007204700.1	KMVA	YWSY	CR	L	VAAARKEV	PGDDF	FP	DMVRTC	AE	GG	EITDREIAGLMYSVLF	AGHETT	TTL
WP_009809975.1	GVD	YWAY	CR	L	VDAARREN	PGDDF	FP	DLKAC	AE	GA	EITDREIAGLMYSTLF	AGHETT	TTL
WP_064750566.1	NLVR	YWRQ	C	E	LTEARRKE	PRDDI	FS	ALVALD	DAS	..	ISLDEIAGLVYGGQLT	AGHETT	TSAL
WP_037664388.1	NMTH	YWEY	CEN	F	VARRAA	FVDDFT	S	DLIRIRE	ENF	E	ALSLEDITNVAYGLSF	AGHETT	TSF
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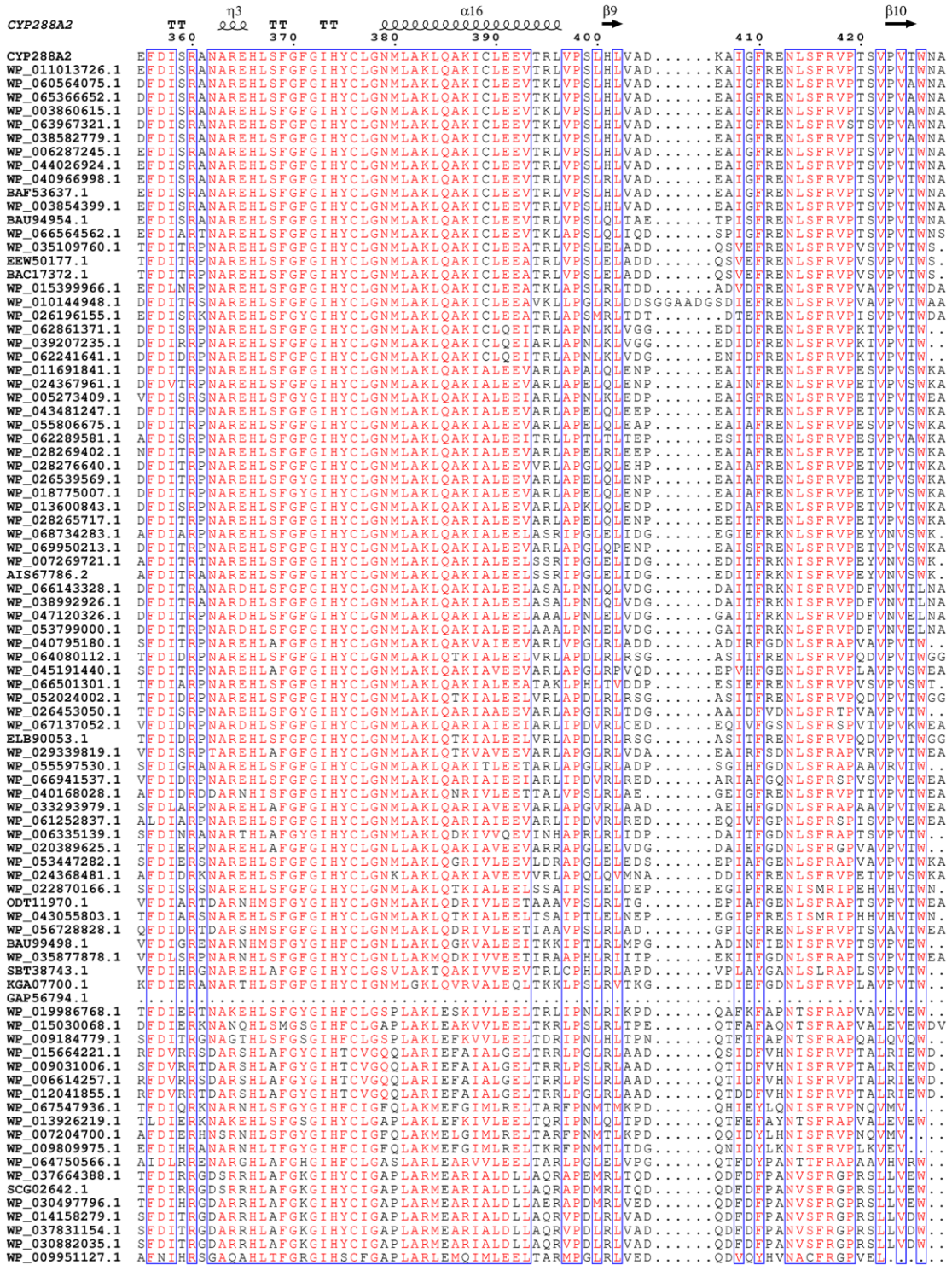


Fig. S31. Protein sequence alignment of CreJ homologues from diverse genera of microorganisms. Conserved residues are highlighted in white display with red background.

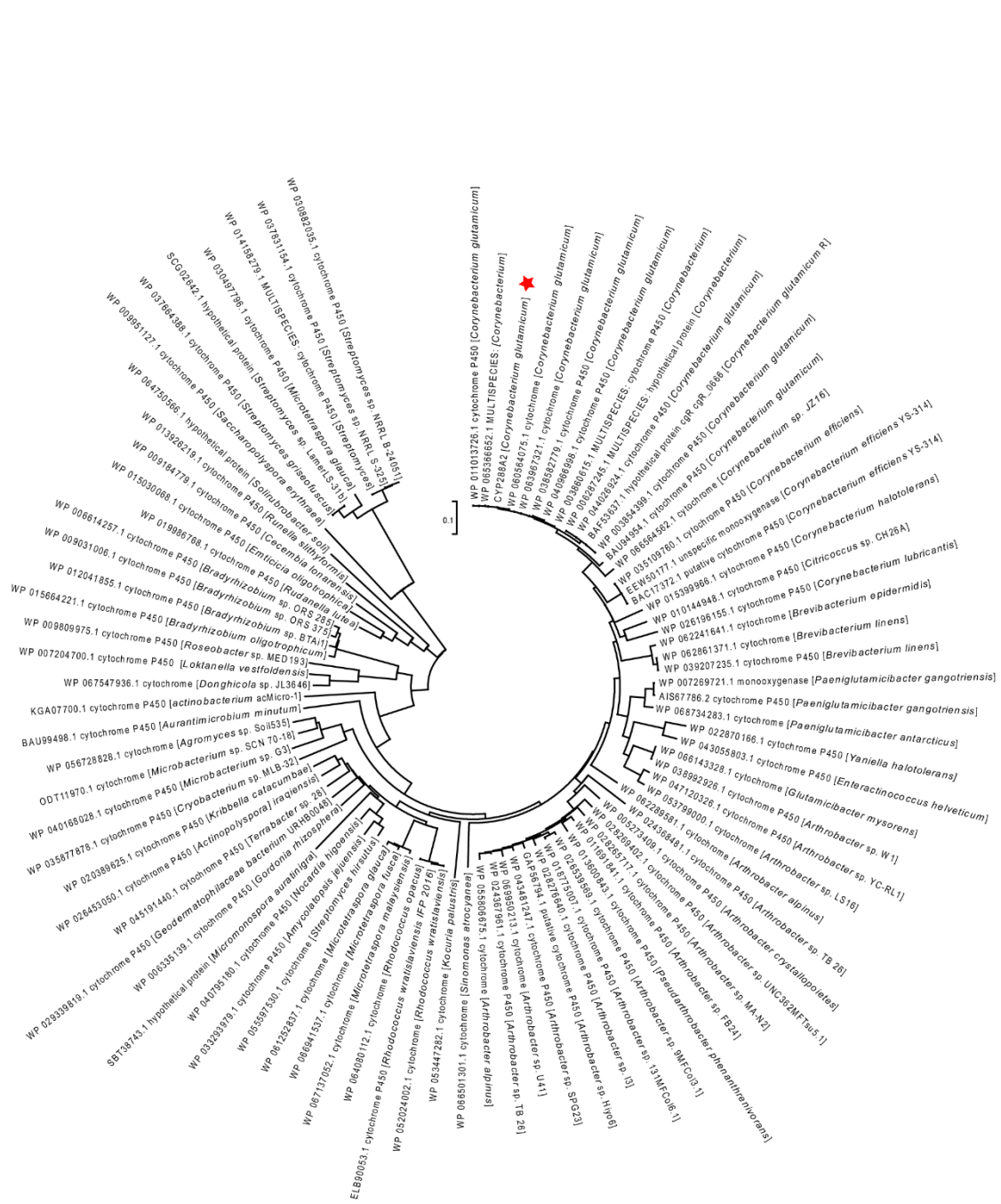


Fig. S32. Phylogenetic tree of CreJ homologues with > 40% protein sequence identity.
 CreJ (*i.e.* CYP288A2) is highlighted with the symbol of star.

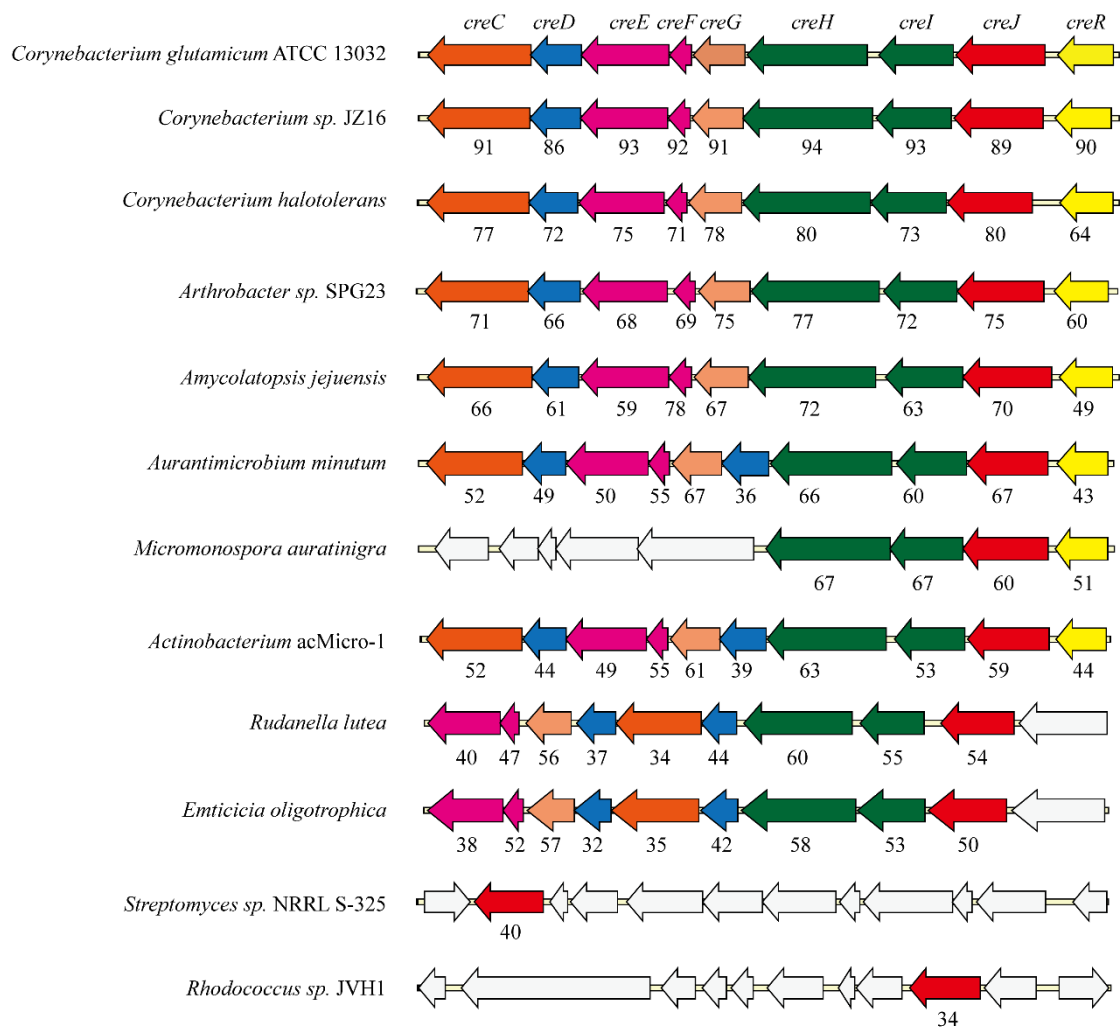


Fig. S33. Representations of cre-like gene clusters from diverse microorganisms. Arrows in a same color represent the homologous genes. Genes without colors have no homology to any cre genes. The number below the arrow indicates the percentage of protein sequence identity between the specific gene with its homologous gene in the cre cluster of *C. glutamicum* ATCC13032.

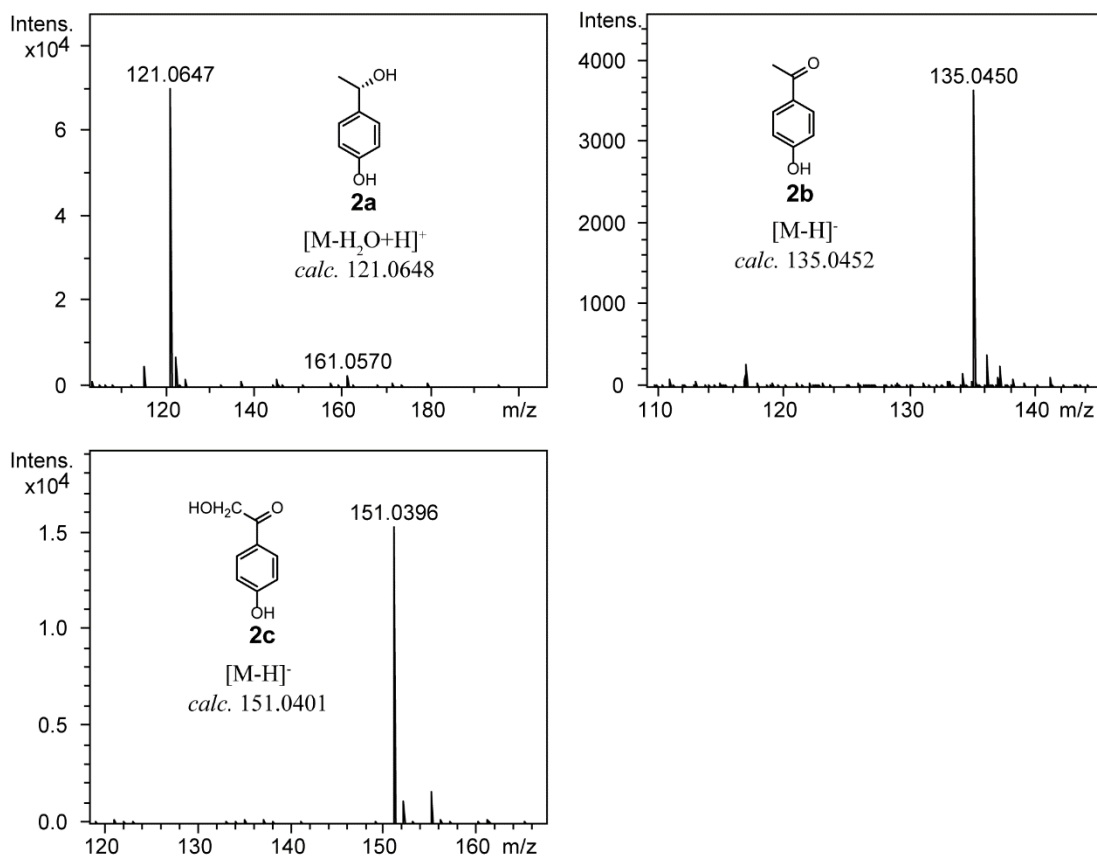


Fig. S34. HRMS spectra of oxidative products from 2.

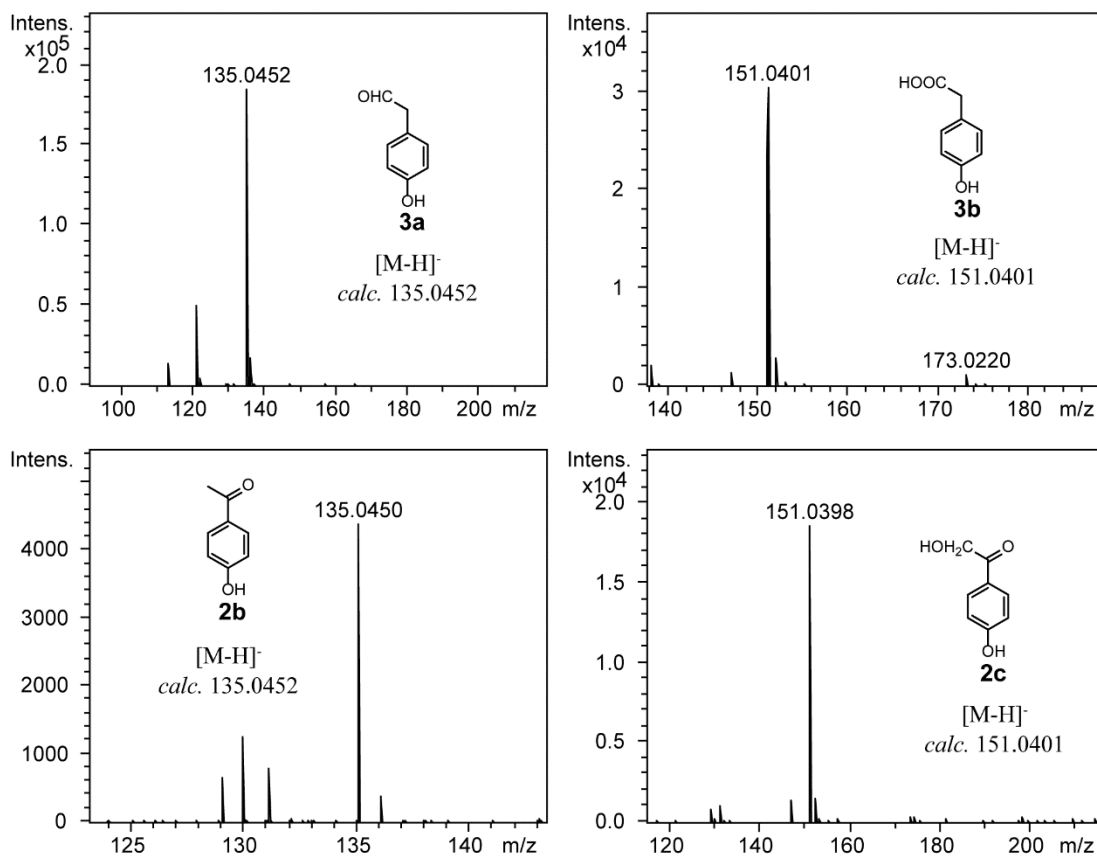


Fig. S35. HRMS spectra of oxidative products from 3.

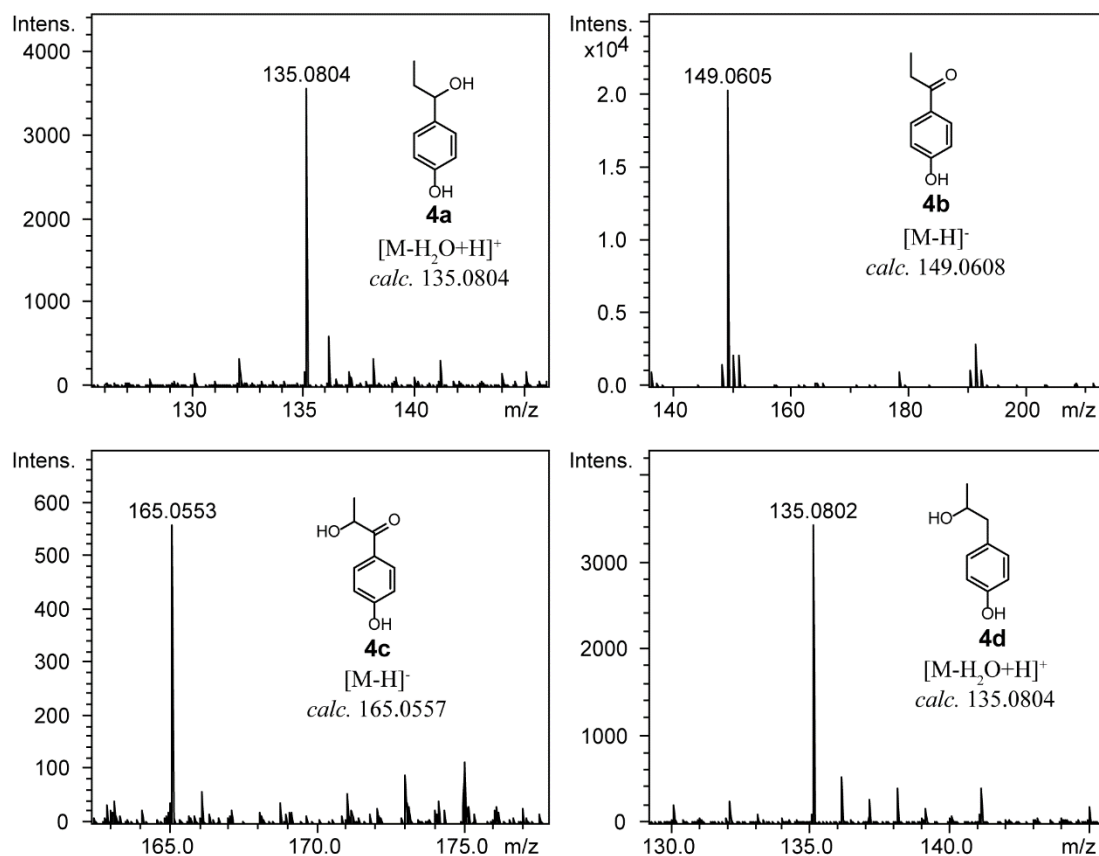


Fig. S36. HRMS spectra of oxidative products from 4.

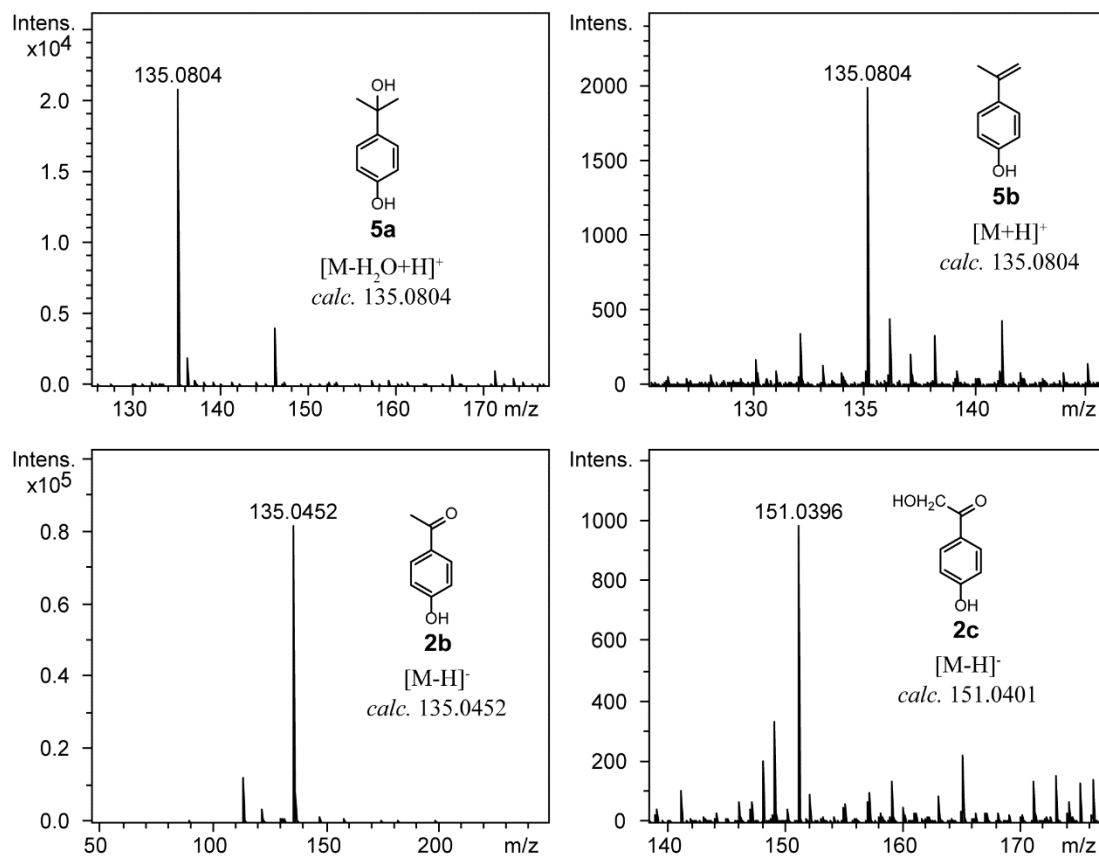


Fig. S37. HRMS spectra of oxidative products from 5.

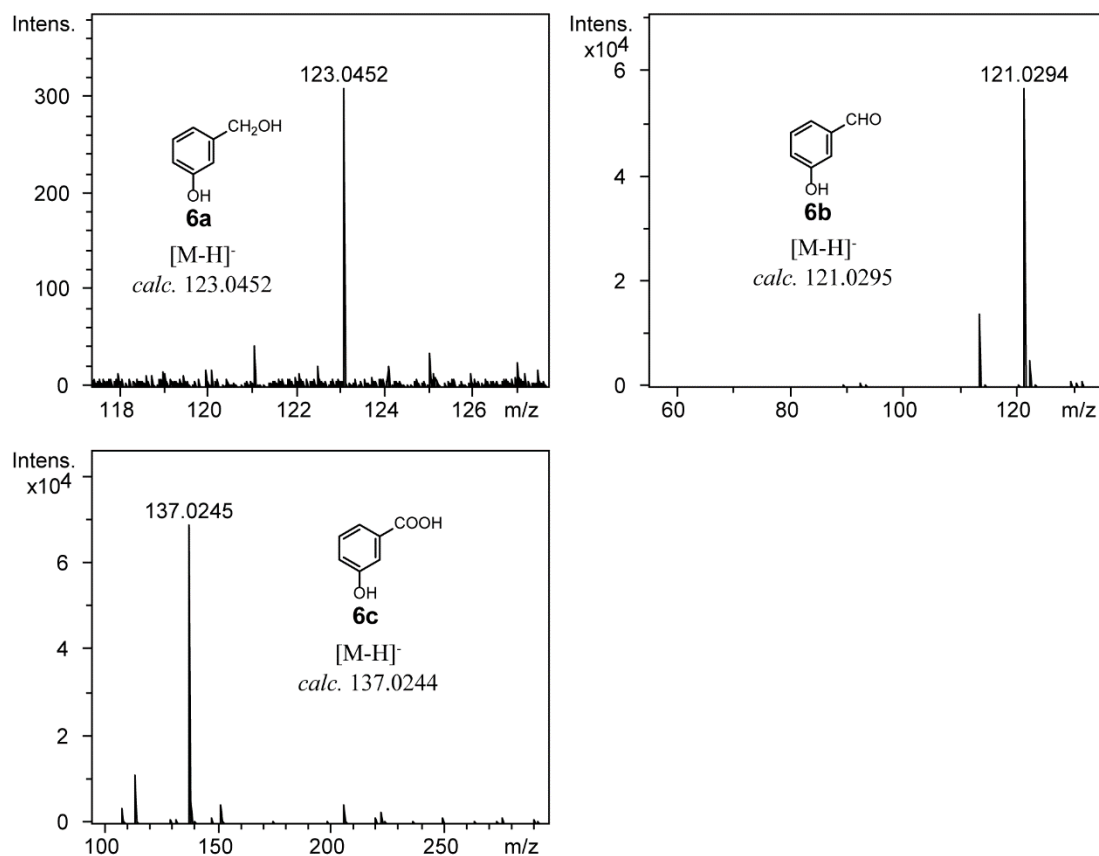


Fig. S38. HRMS spectra of oxidative products from 6.

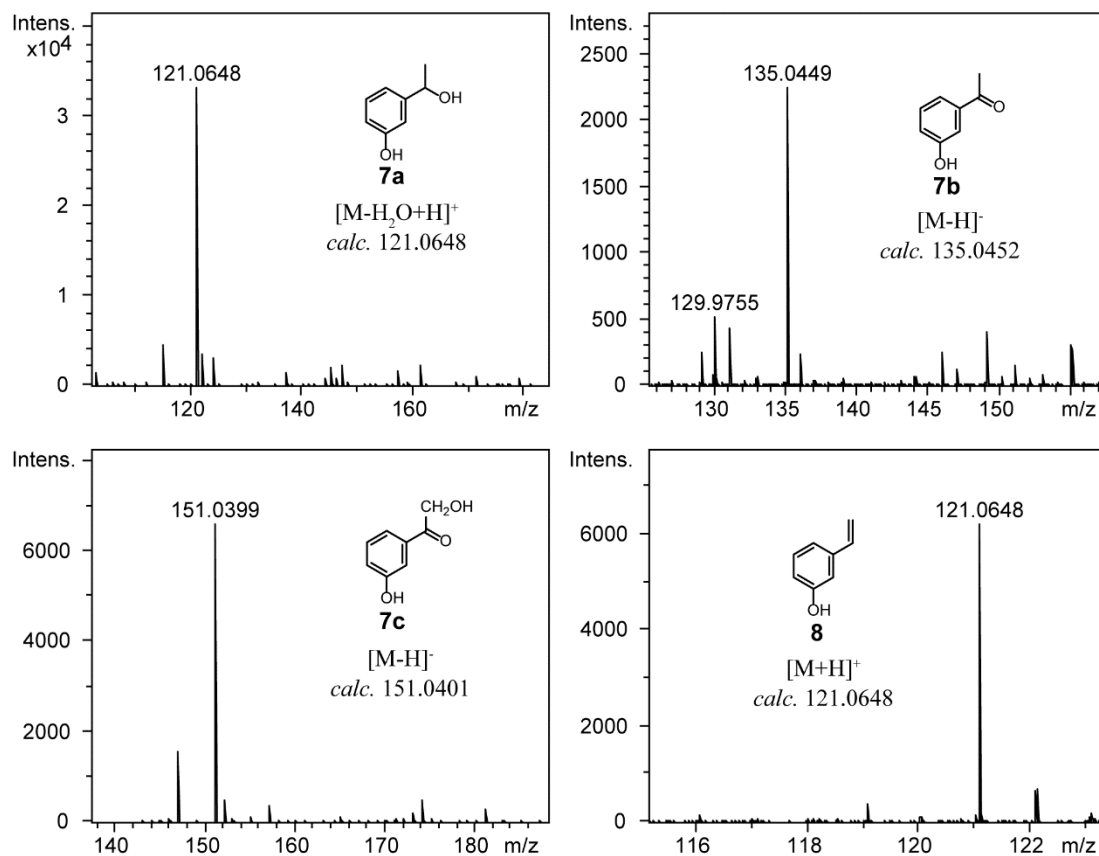


Fig. S39. HRMS spectra of oxidative products from 7.

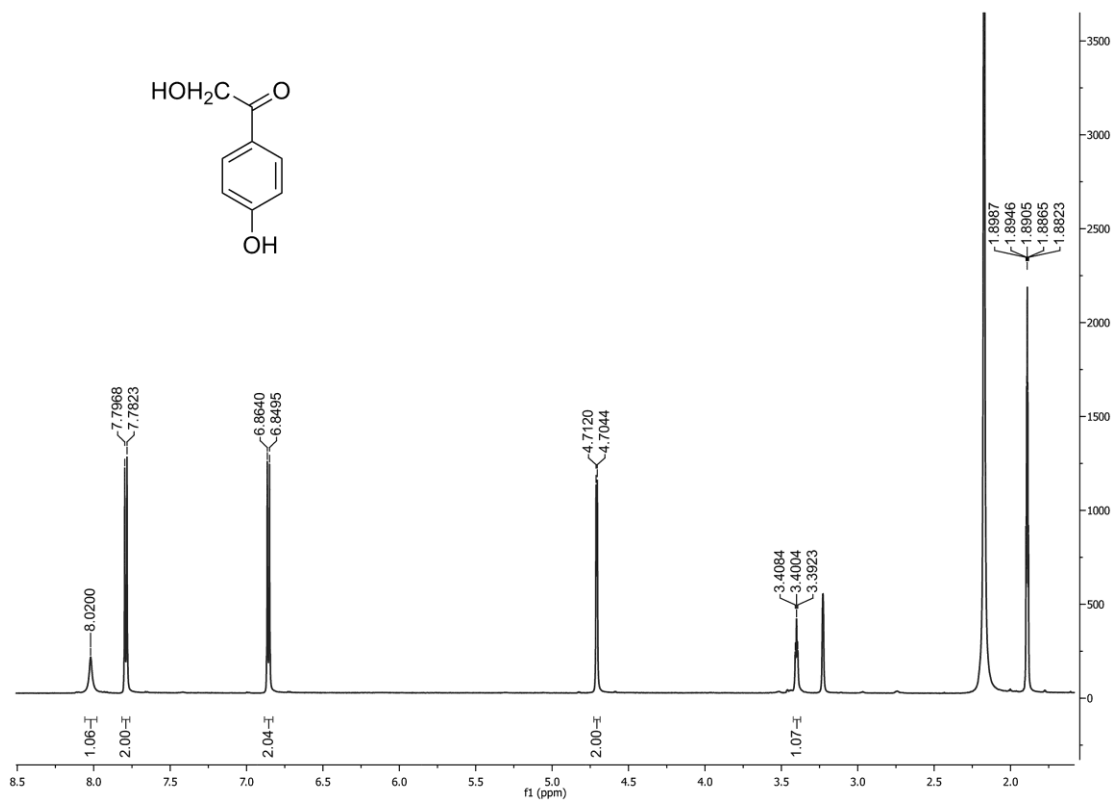


Fig. S40. ^1H NMR spectrum of 2c in CD_3CN .

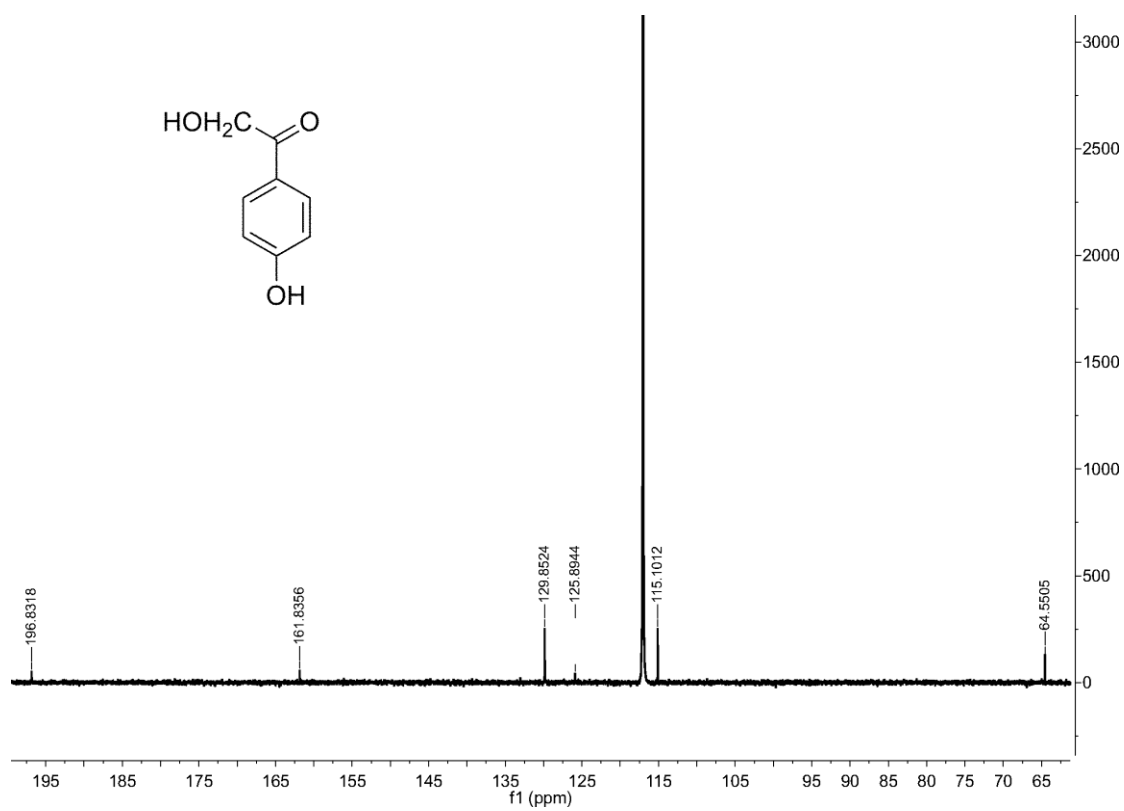


Fig. S41. ^{13}C NMR spectrum of 2c in CD_3CN .

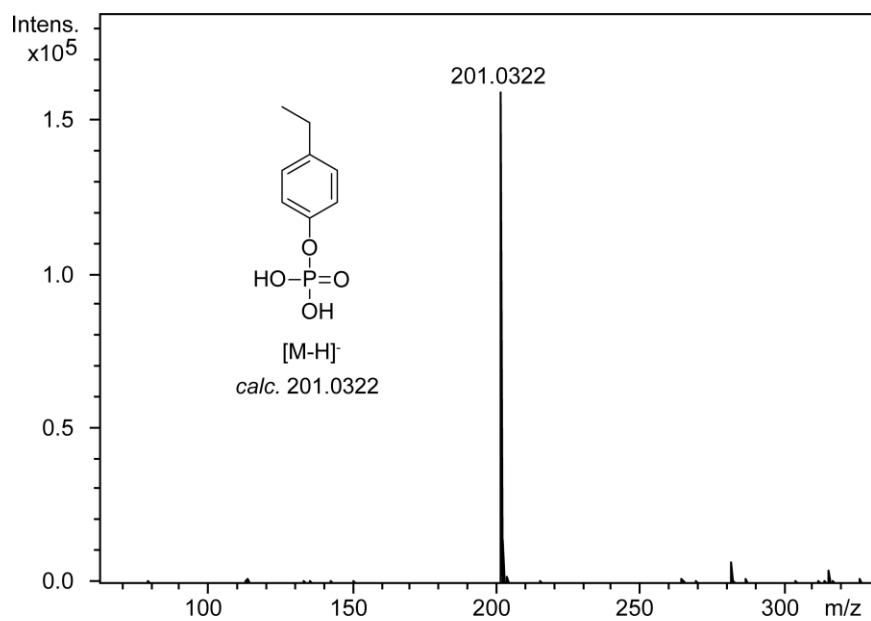


Fig. S42. HRMS of 2'.

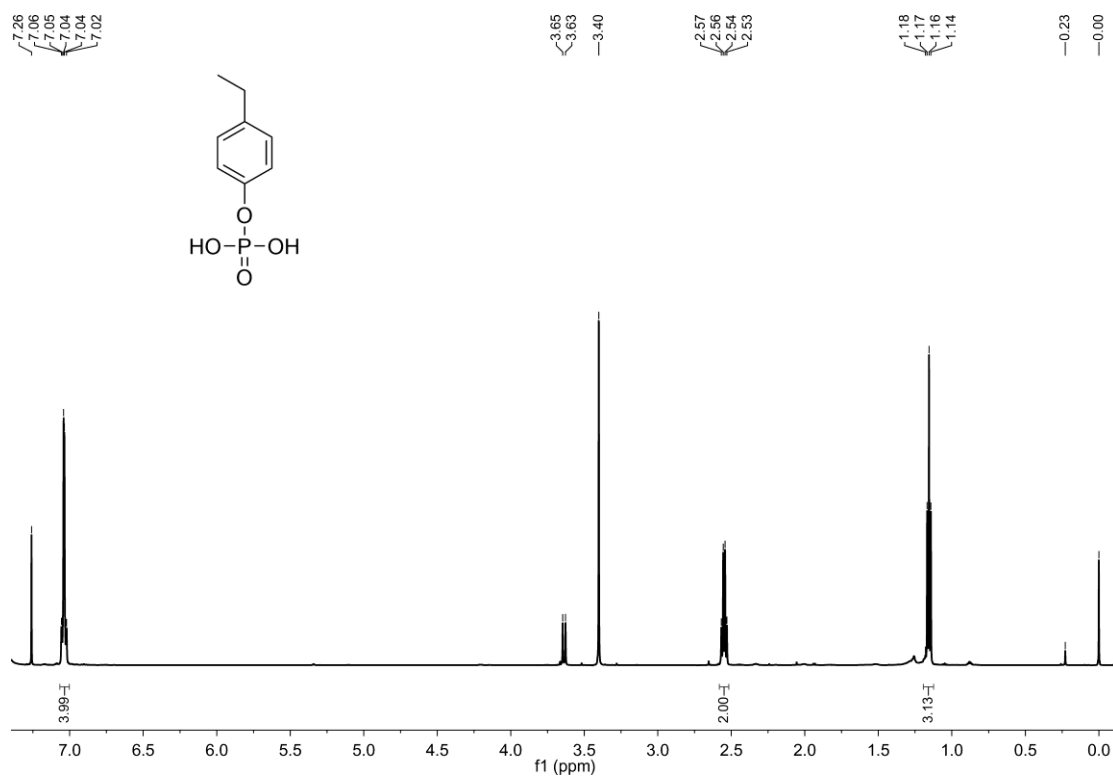


Fig. S43. ¹H NMR spectrum of 2' in CD₃CN.

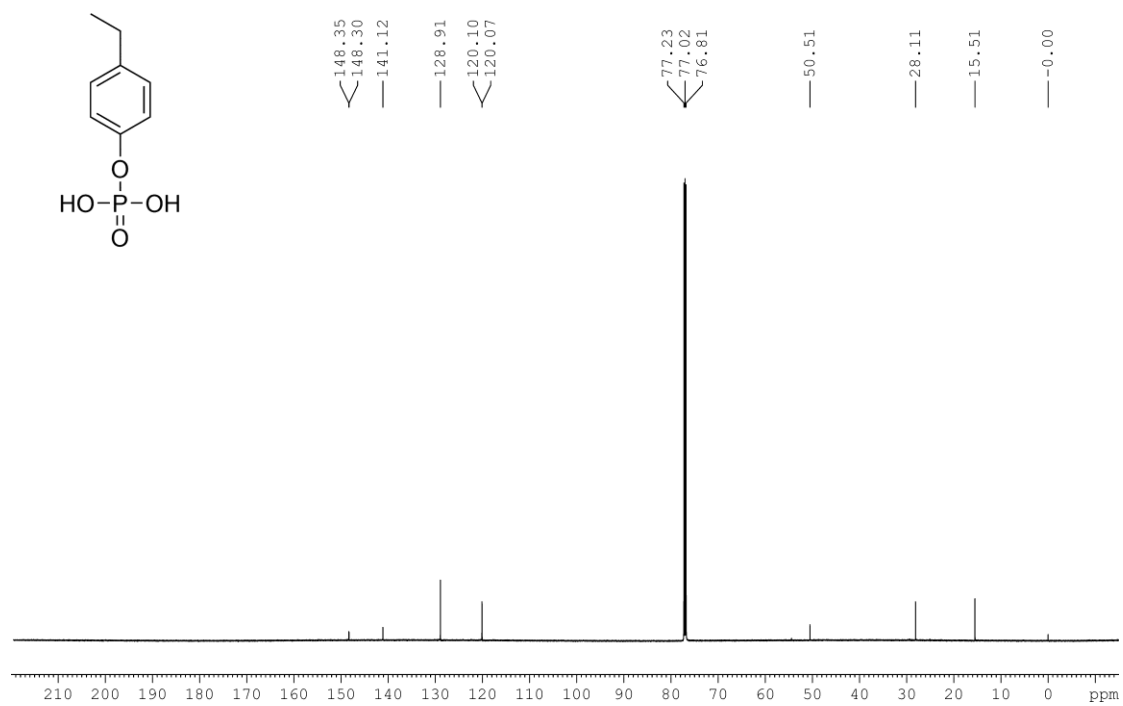


Fig. S44. ^{13}C NMR spectrum of 2' in CD_3CN .

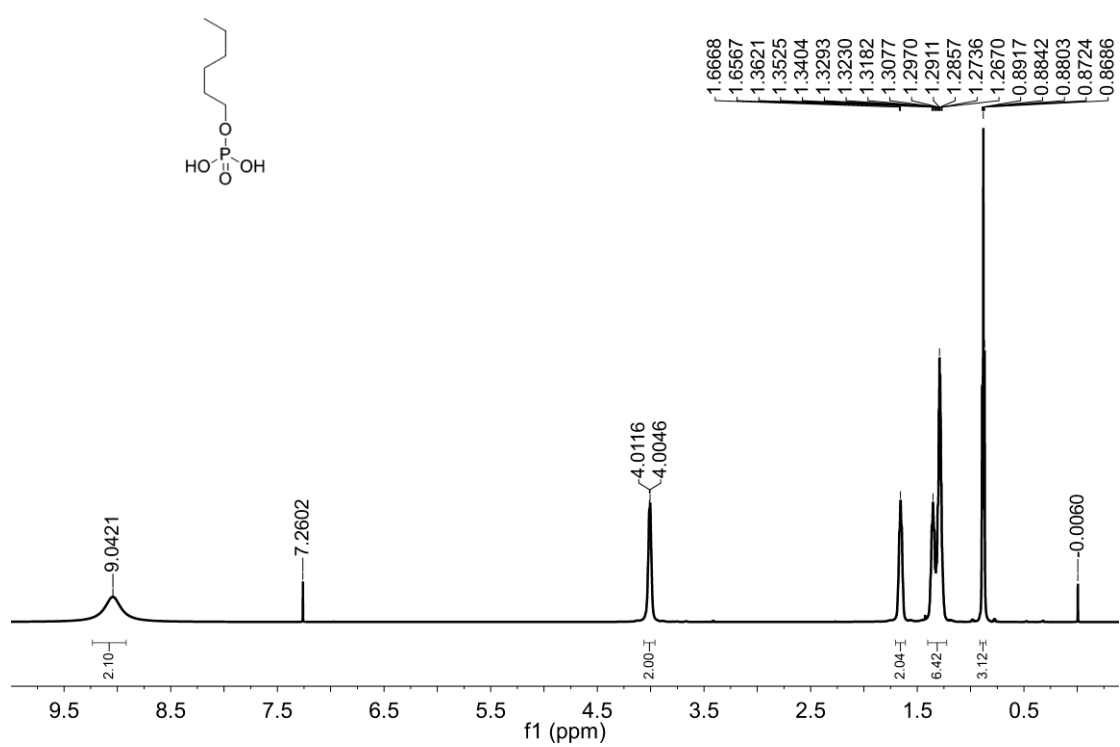


Fig. S45. ^1H NMR spectrum of 11' in CDCl_3 .

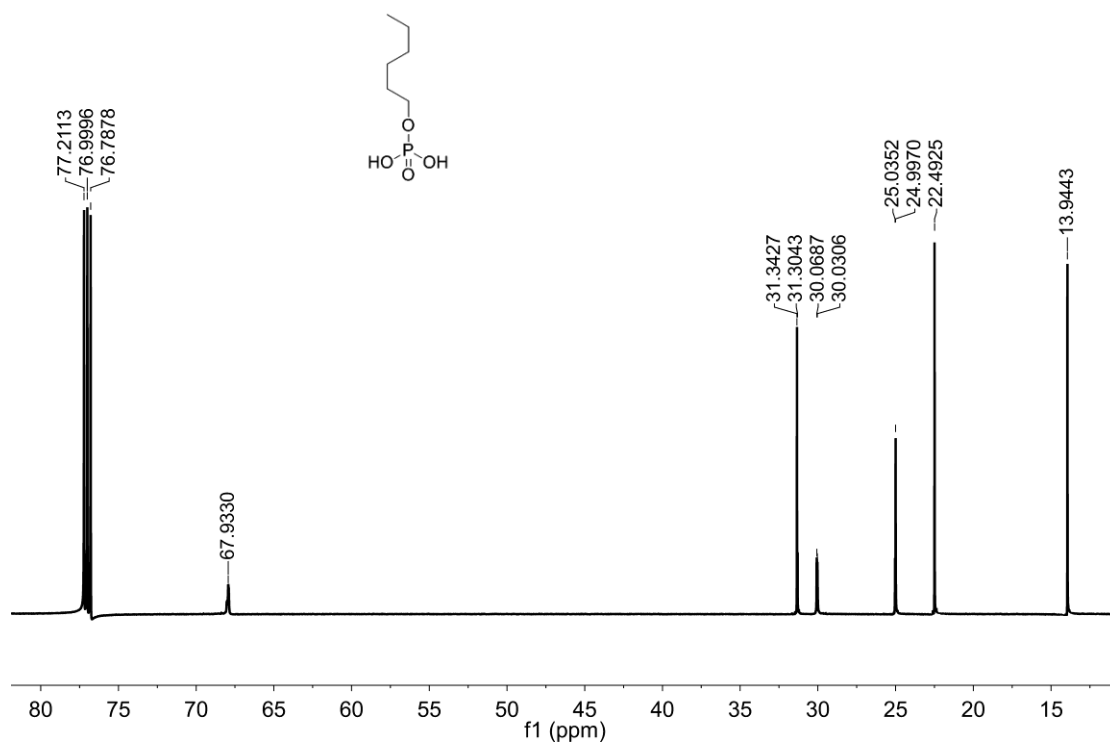


Fig. S46. ¹³C NMR spectrum of 11' in CDCl₃.

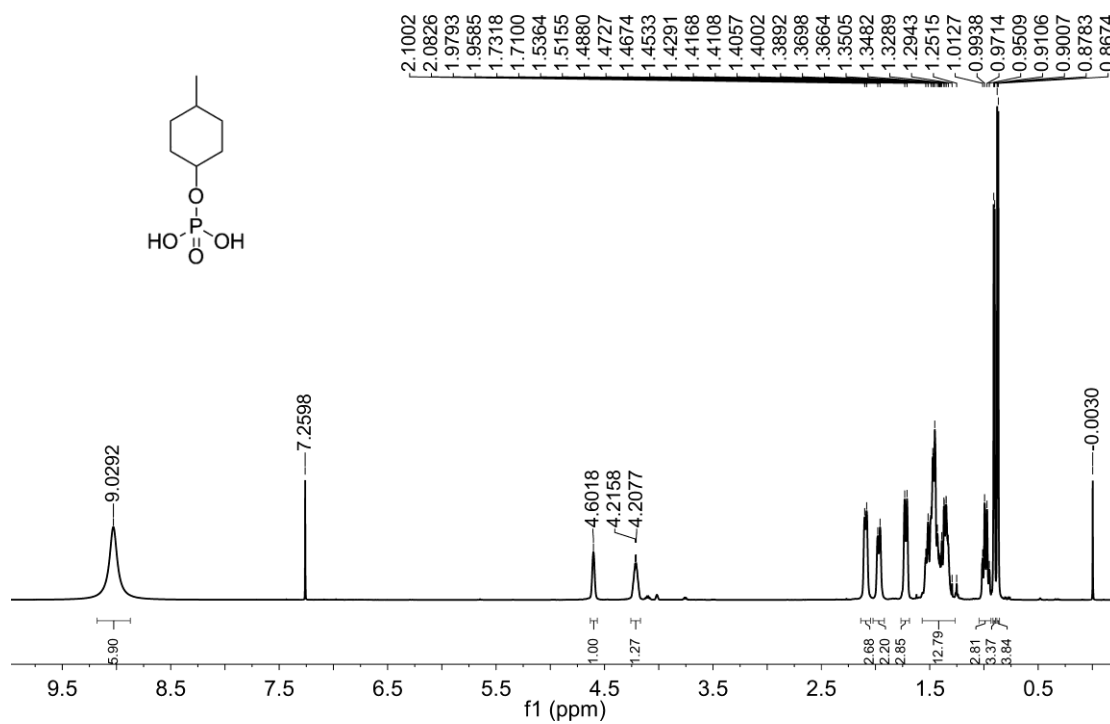


Fig. S47. ¹H NMR spectrum of 12' in CDCl₃.

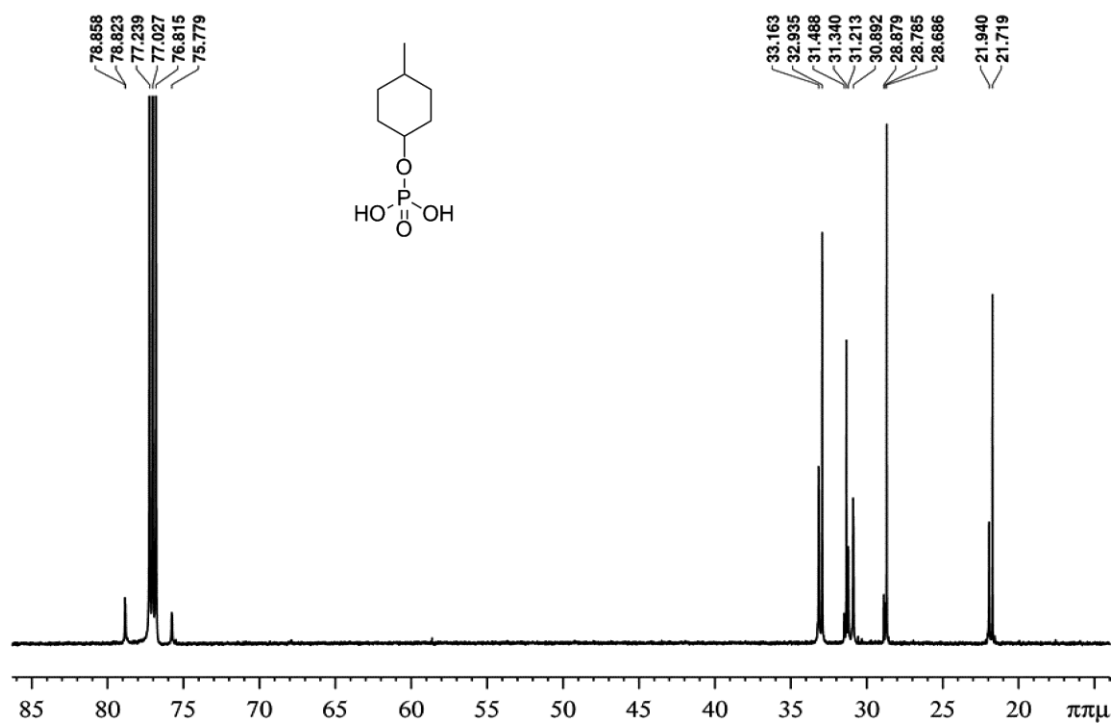


Fig. S48. ^{13}C NMR spectrum of 12' in CDCl_3 .

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