Electronic Supplementary Information

The crystal structure of the versatile cytochrome P450 enzyme CYP109B1 from *Bacillus subtilis*

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Table S1 Top ranking structures homologous to CYP109B1 identified by Dali search.

PDB code	chain	RMSD Cα [Å]	Z-score	% Identity	Description (donor organism)	Ref
3A4G	A	1.8	47.2	36	Vitamin D_3 hydroxylase (Vdh), *P. autotrophica*	1
2XKR	A	2.1	45.8	34	CYP142, *M. tuberculosis*	2
3EJE	D	1.9	45.7	34	BioI-ACP (C18:1) complex, *B. subtilis*	3
2WIO	A	1.9	45.5	38	EryK, *S. erythraea*	4
2WM5	A	2.0	45.2	34	CYP124, *M. tuberculosis*	5
4UAX	A	2.4	45.1	31	CYP142A2, *M. smegmatis*	6
2BVJ	B	2.1	45.1	38	PikC (CYP107L1), *S. venezuelae*	7
4APY	A	2.1	44.6	31	CYP125A3, *M. smegmatis*	8
2X5L	A	2.1	44.2	31	CYP125A1, *M. tuberculosis*	9
2UUQ	A	1.8	43.9	31	CYP130, *M. tuberculosis*	10

Table S2 Geometry around the heme of native CYP109B1 compared to CYP101A1, CYP101C1, CYP101D1 and CYP101D2

	CYP109B1 PDB:4RM4	CYP101A1 PDB: 3L61	CYP101C1 PDB:3OFT	CYP101D1 PDB:3LXH	CYP101D2 PDB:3NV5
Fe-OH_2 (Å)	2.4	2.2	2.4	2.5	2.2
Fe-S (Å)	2.3	2.3	2.3	2.3	2.3
Fe-NA (Å)	2.1	2.0	2.0	2.0	2.0
Fe-NB (Å)	2.0	2.0	2.0	2.0	2.1
Fe-NC (Å)	2.1	2.1	2.1	1.9	2.1
Fe-ND (Å)	2.1	2.0	2.1	2.1	2.0
∠NA-Fe-S (deg)	100	96	96	100	99
∠NB-Fe-S (deg)	91	89	89	90	88
∠NC-Fe-S (deg)	87	88	88	89	90
∠ND-Fe-S (deg)	97	95	95	100	101

Table S3 A comparison of residues which are found in the active site of CYP109B1 versus those in CYP101A1 (P450cam), CYP101D2 and CYP106A2.

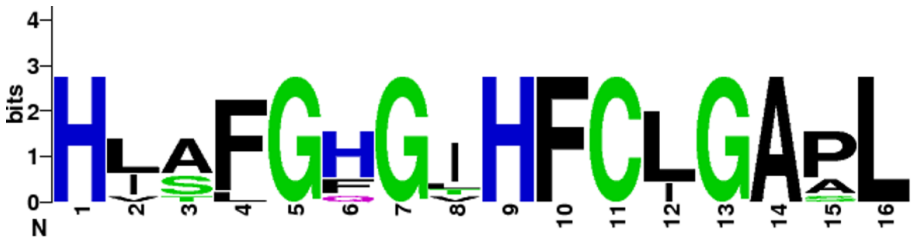
CYP109B1	CYP101A1	CYP101D2	CYP106A2
Asn79	Phe98	Met98	Ile86
Ile82	Thr101	Thr101	Thr89
Asn83	Ser102	Lys102	Glu90
Val166	Thr185	Thr185	Phe173
Leu235	***Leu244***	***Leu250***	***Leu239***
Val238	Val247	Leu253	Gly242
Ala239	*Gly248*	*Gly254*	*Ala243*
Glu242	*Asp251*	*Asp257*	*Glu246*
Pro285	Ser293	Pro299	Asn290
Ala286	Leu294	Val300	Leu291
Pro287	Val295	Val301	Ile292
Val288	Ala296	Ser302	Lys293
Leu289	Asp297	Glu303	Leu294
Arg290	Gly298	Ala304	Asp295
Arg291	***Arg299***	***Arg305***	***Arg296***
Val385	Ile396	Val396	Thr396
Ile386	Ile401	Val402	Gly397

Table S4 A comparison of residues which are found on the surface of PP1957 versus those of a structurally characterised PFOR from *Pseudomonas cepacia* (PDB: 2PIA).

PP1957	PFOR 2PIA
Ser54	Ser58
His100	Glu104
Asp105	Lys109
Ala106	Arg110
Glu217	Glu223
Ser238	Arg244
Glu261	Arg267
Ser291	Arg297
Glu292	Asp298
Ser293	Asp299
Glu294	Glu300
Asp316	Asp320

Figure S1 Sequence logos of conserved regions of (a) the heme binding domain and (b) the I-helix of CYP109B1, CYP109C1, CYP109D1, CYP109D2, CYP109A1 and CYP109 enzymes from *Bacillus amyloliquefaciens* FZB42, *B. cereus* and *B. mojavensis* (created at http://weblogo.berkeley.edu/logo.cgi). The residues span His339 to Leu354 of the heme binding domain and Leu236 to Thr245 of the I-helix in CYP109B1.

(a) Heme binding domain



(b) I helix

Figure S2 The entrance to the substrate access channel on the distal surface of CYP109B1. The heme can be seen from the surface. Positively charge residues on the distal surface are shown in blue and negatively charged residues in red.

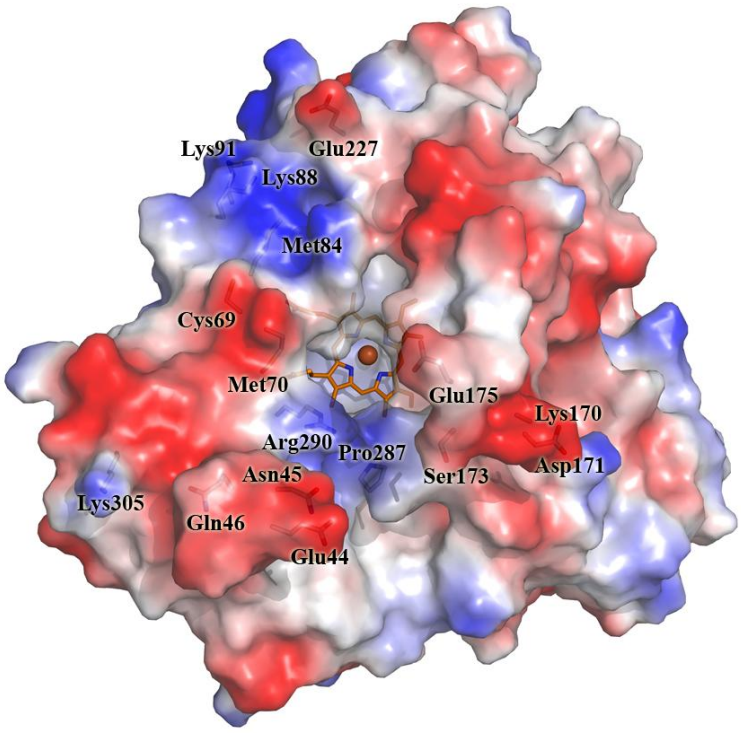
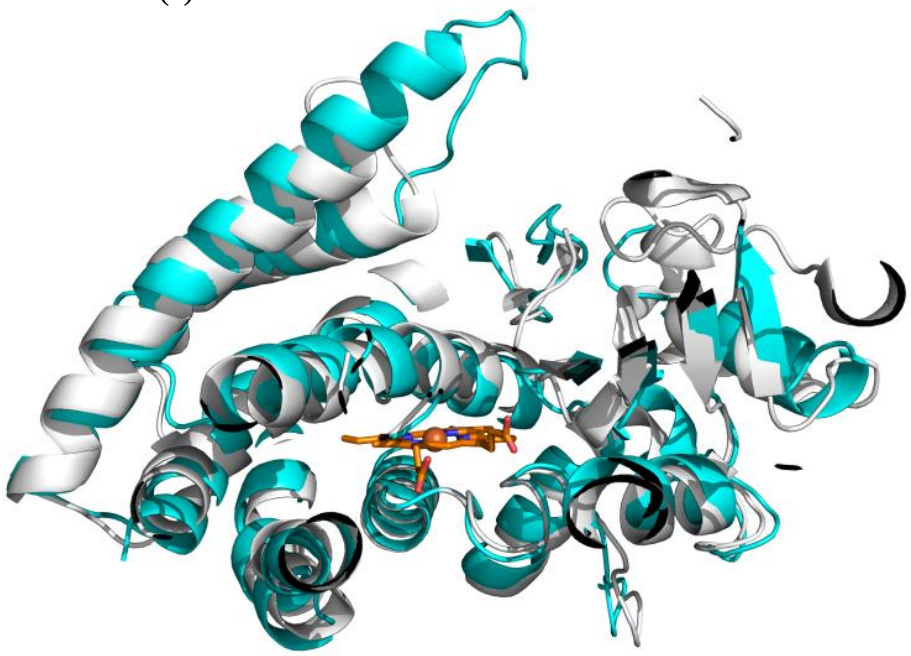


Figure S3 Overlay of the structure of CYP109B1 (cyan) with those of (a) the open CYP101A1 structure (PDB code: 3L62, grey) and (b) the CYP101D2 structure (PDB code: 3NV5, yellow).

(a)



(b)

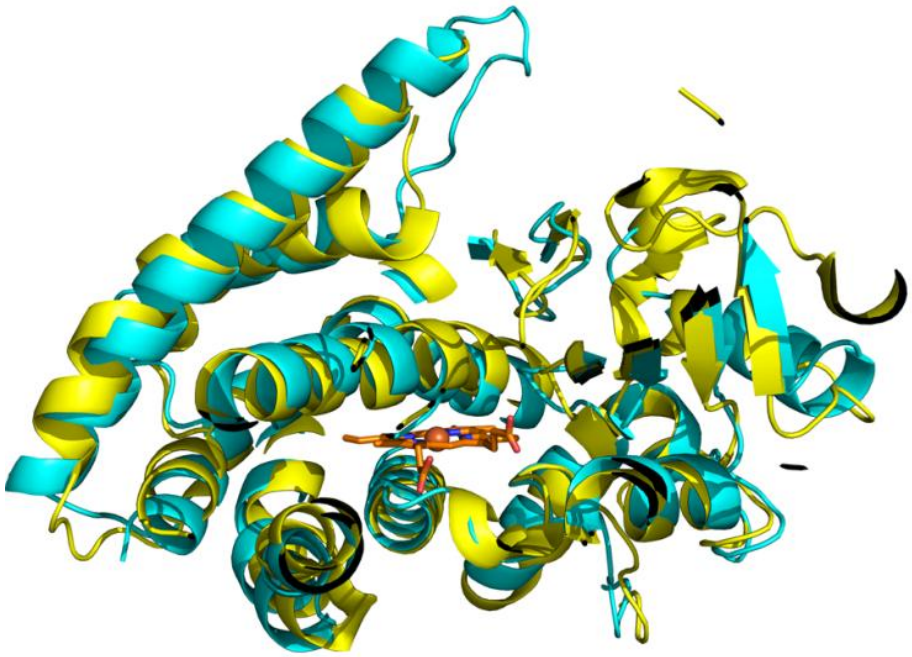


Figure S4 The interactions of the heme group with the surrounding residues of CYP109B1. Water molecules are highlighted in red. Salt bridge and hydrogen bonding interactions are shown as dashed yellow lines.

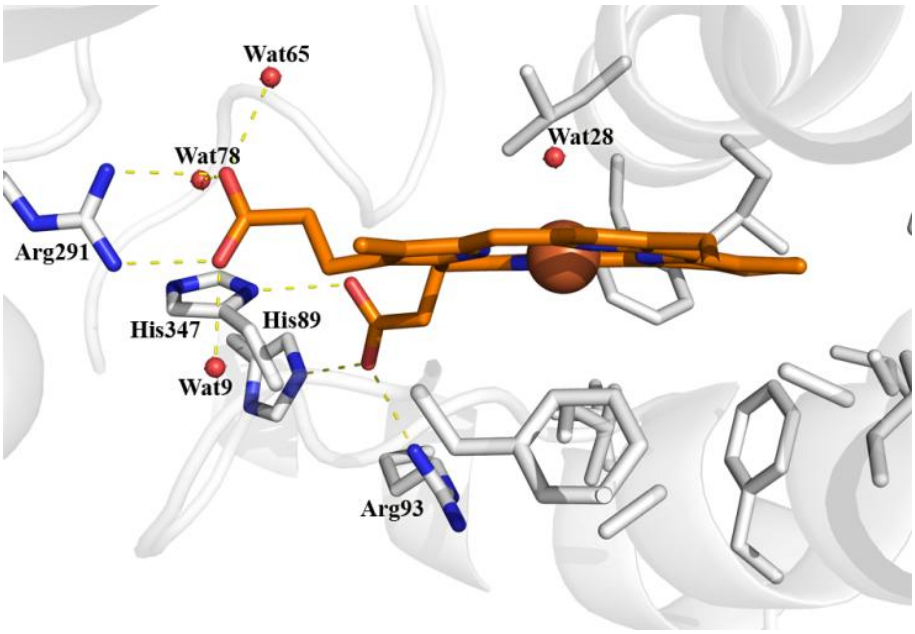
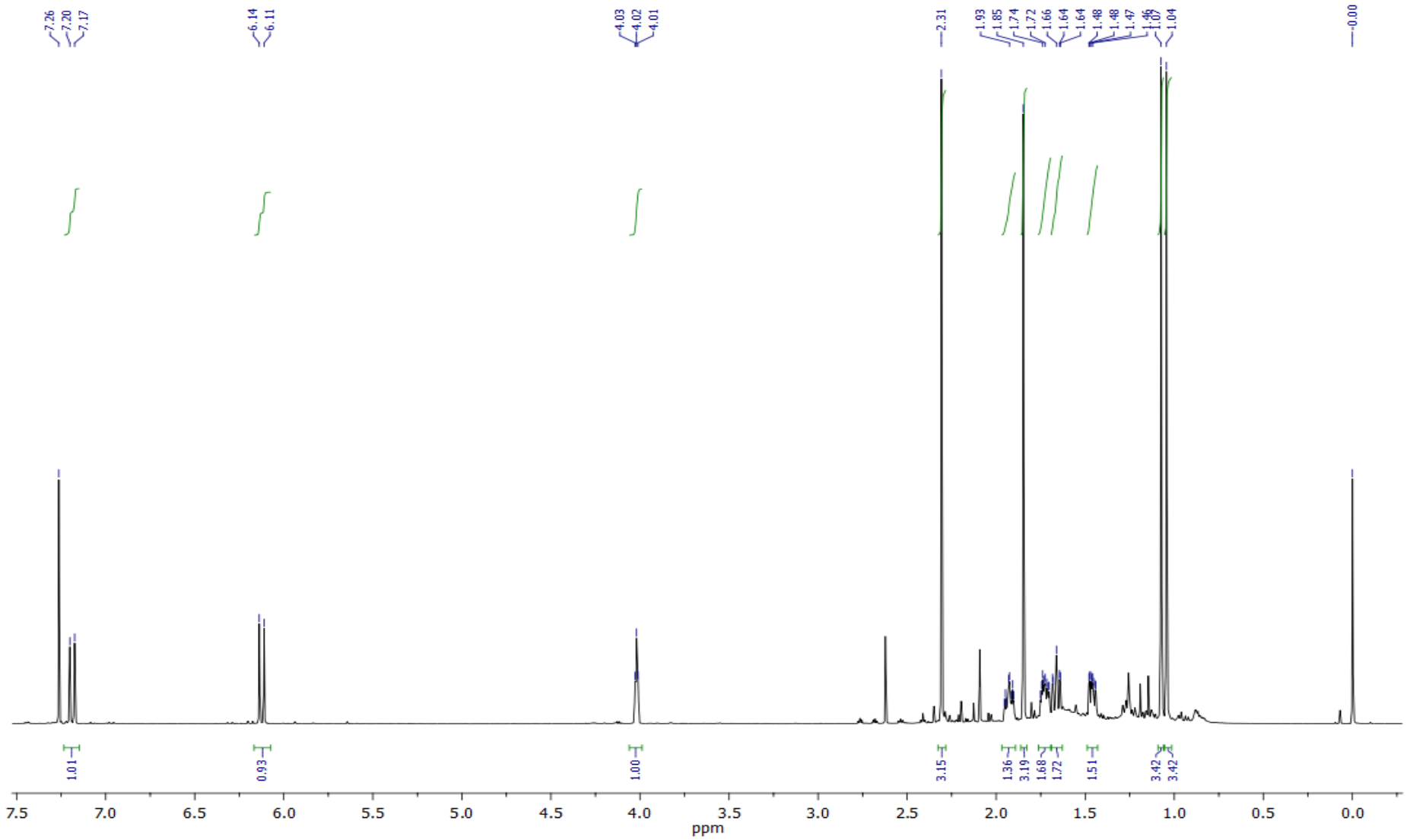
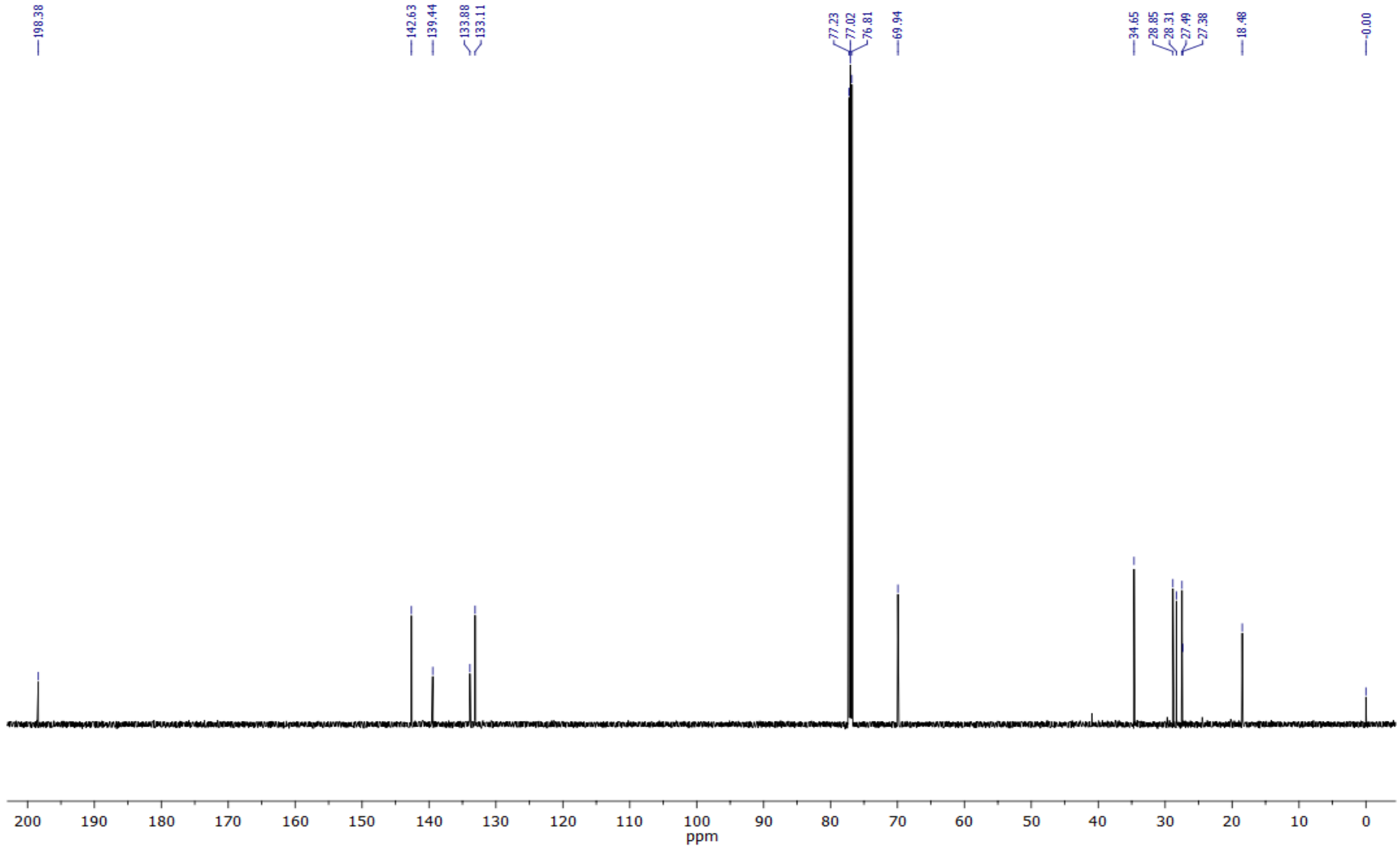


Figure S5 NMR data for characterization of products of β- and α-ionone.

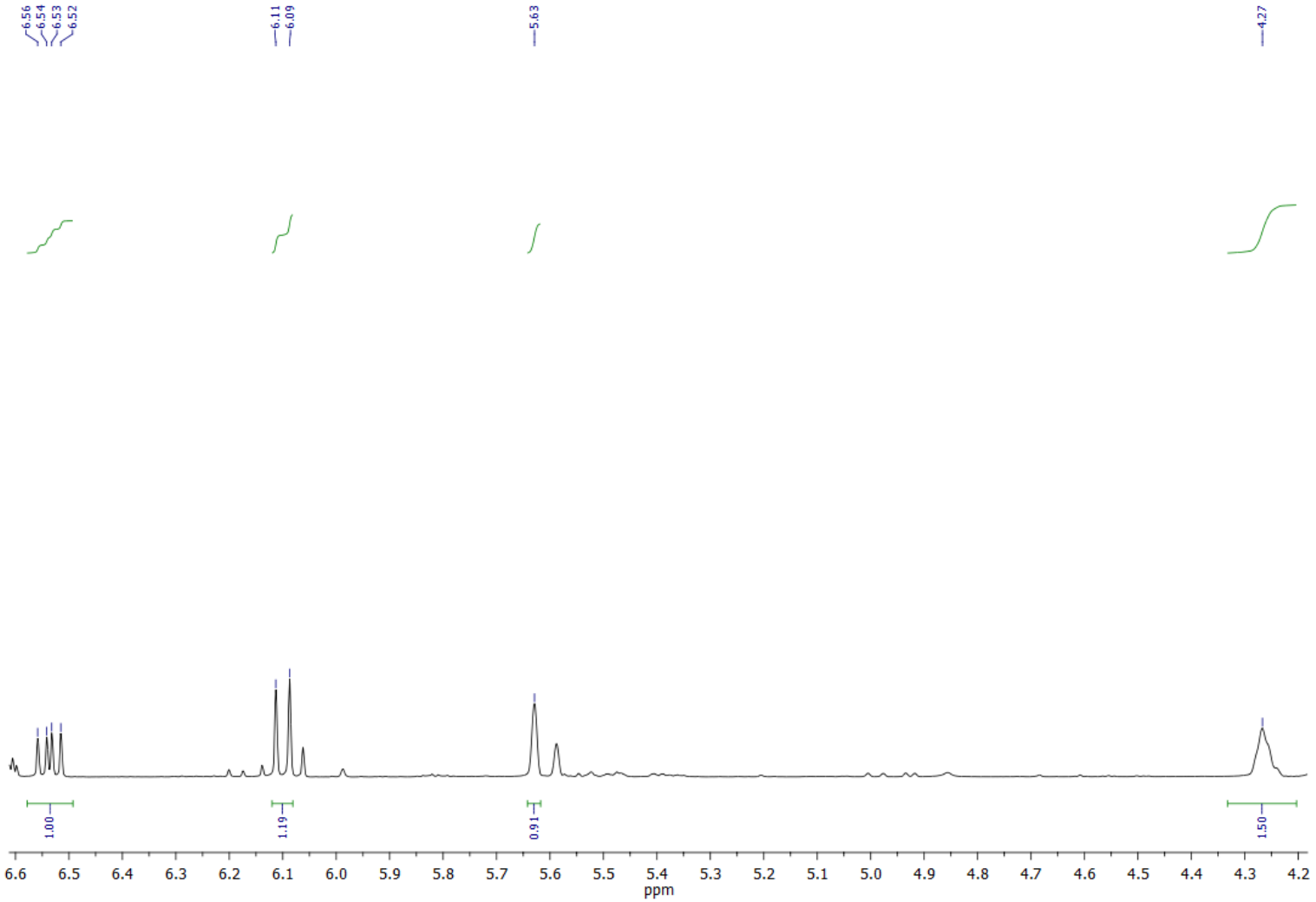
(a) ^{1}H NMR spectrum for 4-hydroxy-β-ionone



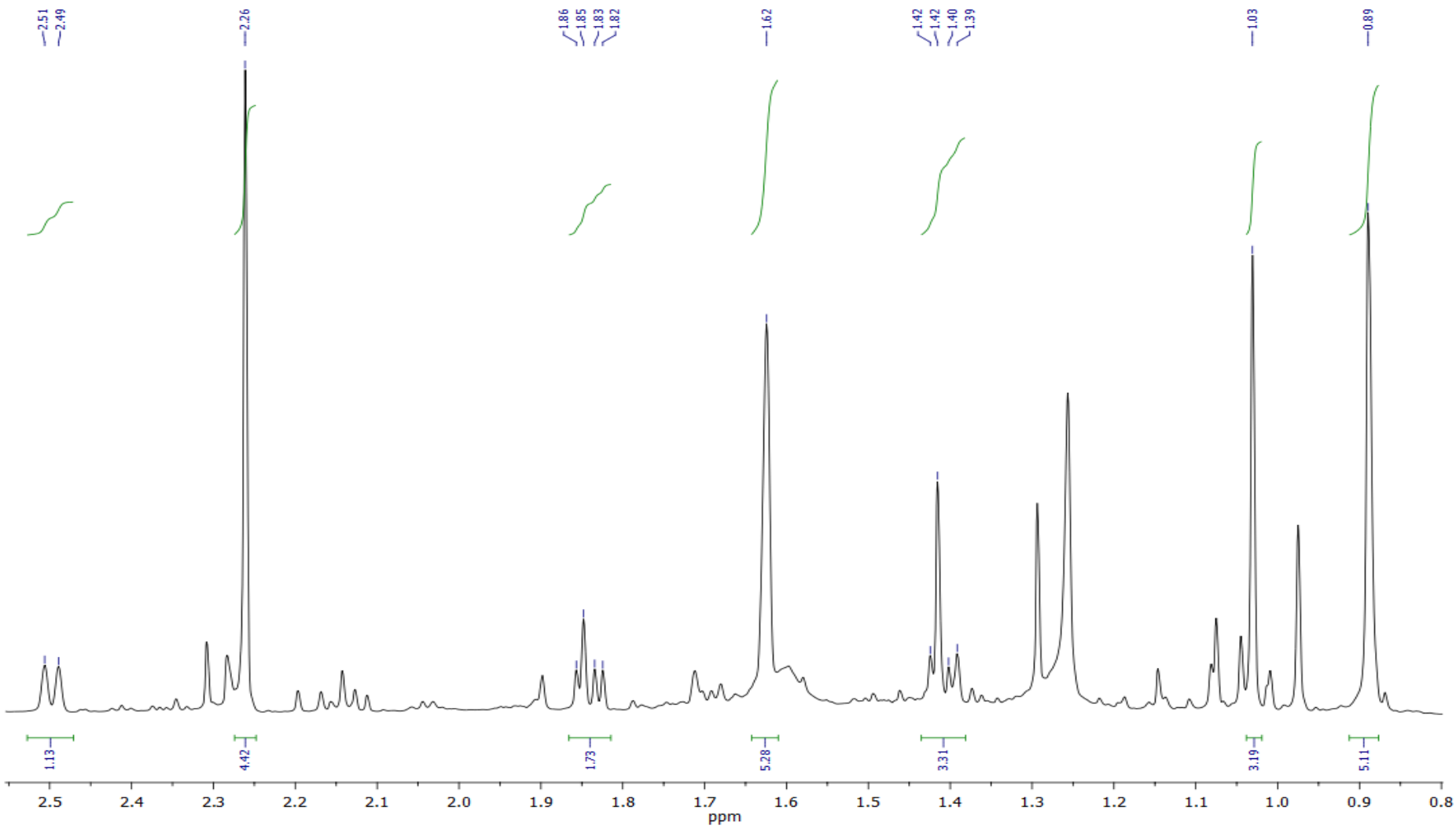
(b) ^{13}C NMR spectrum for 4-hydroxy-β-ionone.



(c) ^{1}H NMR spectrum for the region containing C^{2}, C^{3}, C^{9} and C^{10} *trans*-3-hydroxy-α-ionone



(d) ^{1}H NMR spectrum for the remaining hydrogens of *trans*-3-hydroxy-α-ionone



(e) ^{13}C NMR spectrum for *trans*-3-hydroxy-α-ionone

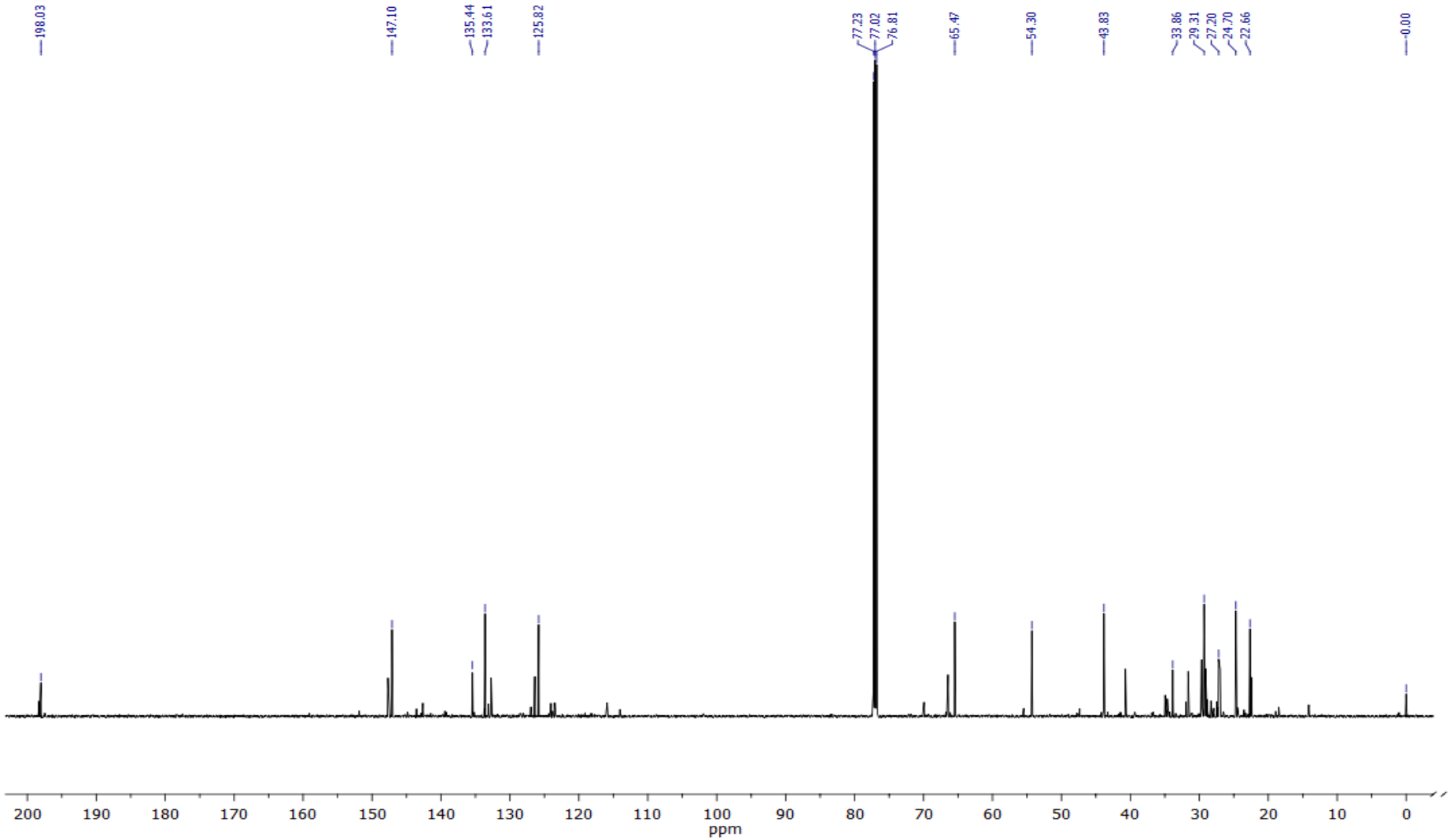
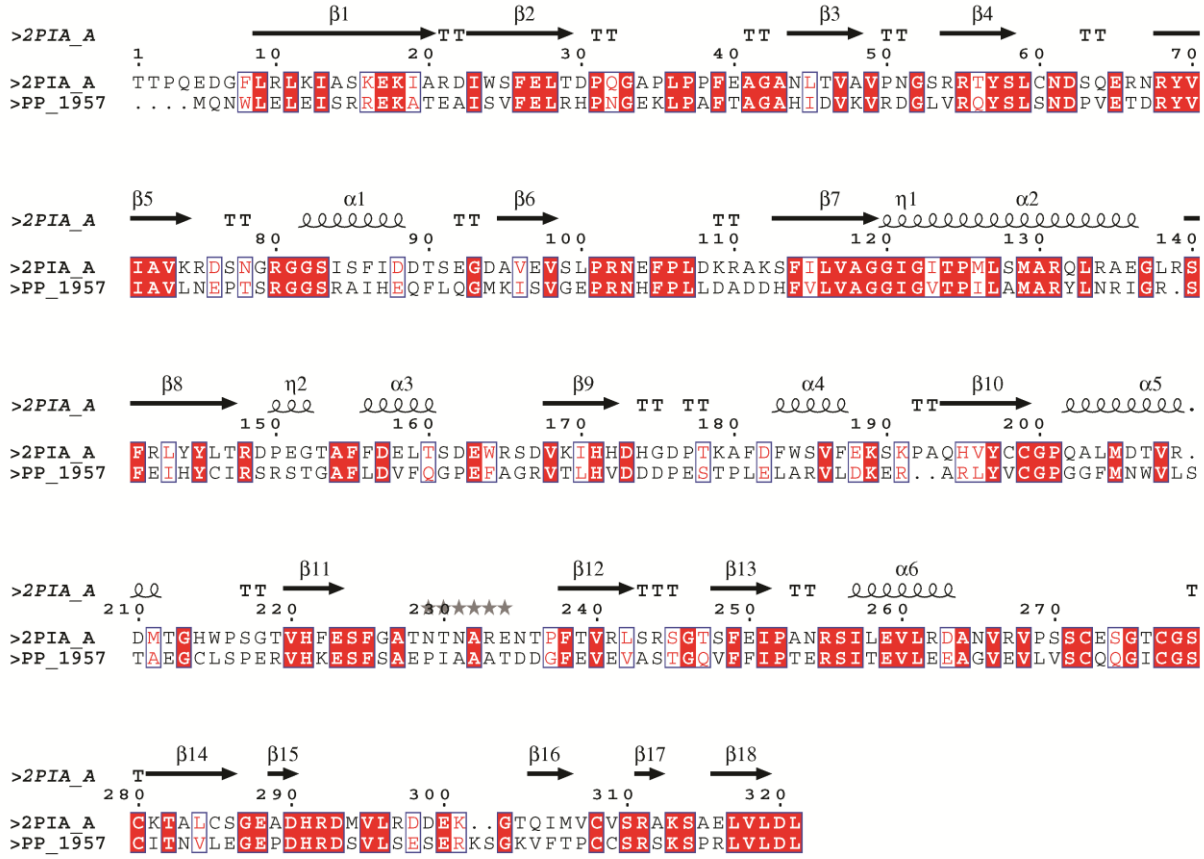


Figure S6 (a) Sequence alignment of the phthalate family oxygenase reductase enzymes from *Pseudomonas putida* KT24440 (PP1957) *Pseudomonas cepacia* (PDB: 2PIA). Conserved residues are highlighted in red boxes with similar residues in red. (b) Structure of amino acids around the [2Fe-2S] cluster of PDB: 2PIA. The cysteine residues and Ser271 are conserved but Ser270, Glu271, Ser274 and Thr276 are Val, Gln, Gln and Ile residues, respectively in PP1957.

(a)



(b)

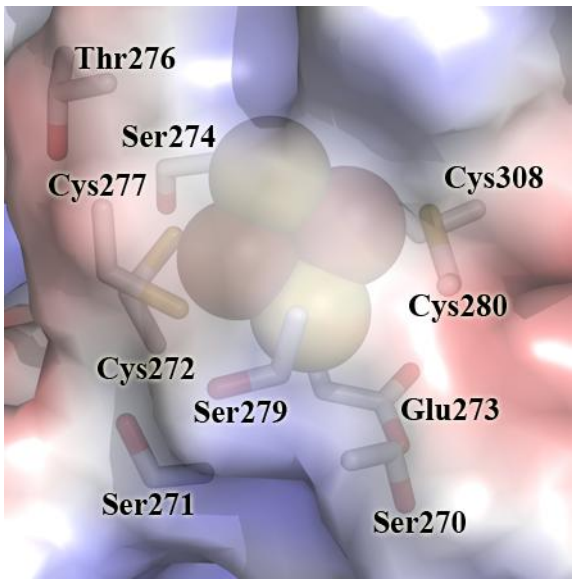
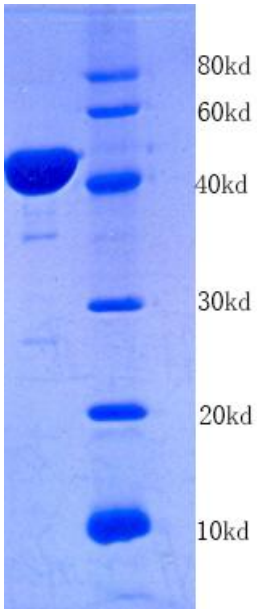


Figure S7 SDS-PAGE analysis of purified CYP109B1



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