

Catalysts

Supporting Information

**Production of 14 α -hydroxyprogesterone using a
steroidal hydroxylase from *Cochliobolus lunatus*
expressed in *Escherichia coli***

Supporting Information

Text: NMR data of 11 β -OH PROG and 14 α -OH PROG.

Table S1: List of primers using in this article.

Figure S1: ProSA Z-Score result of the ΔP -450_{lun} model.

Figure S2: Ramachandran Plot of the structure of ΔP -450_{lun}.

Figure S3: ¹H-NMR spectrum of isolated 11 β -OH PROG (600 MHz, CDCl₃).

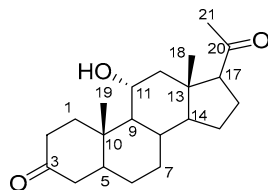
Figure S4: ¹³C-NMR spectrum of isolated 11 β -OH PROG (600 MHz, CDCl₃).

Figure S5: ¹H-NMR spectrum of isolated 14 α -OH PROG (600 MHz, CDCl₃).

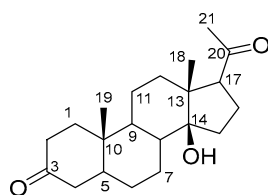
Figure S6: ¹³C-NMR spectrum of isolated 14 α -OH PROG (600 MHz, CDCl₃).

Figure S7: Binding pocket properties of ΔP -450_{lun} and ΔP -450_{lun}-F297W.

Text: NMR data of 11 β -OH PROG and 14 α -OH PROG



11 β -OH-Prog, ^1H NMR (600 M, CDCl_3 , δ ppm): 0.91 (3H, s, H-18), 1.45 (3H, s, H-19), 2.13 (3H, s, H-21), 4.41 (1H, dd, $J = 3.2$ Hz, 6.3 Hz; H-11 α), 5.68 (1H, s, H-4); ^{13}C NMR (150 M, CDCl_3 , δ ppm): 209.0 (C-20), 199.4 (C-3), 171.9 (C-5), 122.4 (C-4), 68.2 (C-11), 63.8 (C-17), 57.5 (C-14), 56.4 (C-9), 48.3 (C-12), 42.9 (C-13), 39.2 (C-10), 35.0 (C-1), 33.8 (C-2), 32.6 (C-6), 32.0 (C-7), 31.3 (C-8), 31.2 (C-21), 24.3 (C-15), 22.7 (C-16), 21.0 (C-19), 15.9 (C-18).



14 α -OH-Prog, ^1H NMR (600 M, CDCl_3 , δ ppm): 0.79 (3H, s, H-18), 1.20 (3H, s, H-19), 2.13 (3H, s, H-21), 3.23 (1H, t, $J = 8.8$ Hz; H-17 α), 5.74 (1H, s, H-4); ^{13}C NMR (150 M, CDCl_3 , δ ppm): 210.3 (C-20), 199.5 (C-3), 170.3 (C-5), 124.0 (C-4), 85.2 (C-14), 59.4 (C-17), 47.9 (C-13), 46.3 (C-9), 38.6 (C-10), 38.3 (C-8), 35.8 (C-1), 34.0 (C-2), 33.4 (C-12), 32.6 (C-6), 31.5 (C-21), 30.9 (C-15), 27.1 (C-7), 21.4 (C-11), 20.1 (C-16), 17.2 (C-18), 17.2 (C-19).

Table S1: List of primers using in this article

Primer	Sequence (5' - 3')
CPR _{lun} -F	AAAAAAC <u>CATATG</u> ATGAGTGCCGATCCGTATGG
CPR _{lun} -R	AAAAAA <u>AAGCTT</u> TAAACTCCAAACGTCTTC
P-450 _{lun} -F	AAAAAAG <u>CTAGC</u> ATGGATACCCAGACTGTCGAG
P-450 _{lun} -R	AAAAAA <u>CTTAAG</u> TAACTCCAAACGTCTTCCTG
CPR _{lunΔ31} -F	AAAAAA <u>CATATG</u> AGTGCCGATCCGTATG
CPR _{lunΔ31} -R	AAAAAA <u>AAGCTT</u> TAAACTCCAAACGTCTTC
P-450 _{lunΔ29} -F	AAAAAAG <u>CTAGC</u> ATGCCCAAACACTACAACCAATG
P-450 _{lunΔ29} -R	AAAAAA <u>CTTAAG</u> TAACTCCAAACGTCTTCCTG
P-450 _{lun} _D2A-F	AAAAAAG <u>CTAGC</u> ATGGCGACCCAGACTGTCGAG
P-450 _{lun} _D2A-R	AAAAAA <u>CTTAAG</u> TAACTCCAAACGTCTTCCTG
P-450 _{lun} _AKKTS-F	AAAAAAG <u>CTAGC</u> ATGGCGAAGAAGACCAGCCCCAAACTACAAC CAATG
P-450 _{lun} _AKKTS-R	AAAAAA <u>CTTAAG</u> TAACTCCAAACGTCTTCCTG
P-450 _{lun} _ALLAVFL-F	AAAAAAG <u>CTAGC</u> ATGGCGCTGCTGCTGGCGGTTTTTCTGCCCAA CTACAACCAATG
P-450 _{lun} _ALLAVFL-R	AAAAAA <u>CTTAAG</u> TAACTCCAAACGTCTTCCTG
P108A-F	AGTTTTGCGGAGGCTCTTACCGAGGAC
P108A-R	AGAGCCTCCGCAAAACTGAGAACATCG
E109A-F	AGTTTCCAGCGGCTCTTACCGAGGAC
E109A-R	AGAGCCGCTGGAAAACACTGAGAACATCG
L122A-F	TACACACATGCGTCGATTGAAAACCCAC
L122A-R	AATCGACGCATGTGTGATTGATTCCAAG
S294A-F	CCAGCTTGCGCTGATCTTCGCCGC
S294A-R	GAAGATCAGCGCAAGCTGGGTTTTGGCG
F297A-F	TCTGATCGCGGCCGCCATTACACG
F297A-R	GCGGCCGCGATCAGAGAAAGCTG
T364A-F	CGGACCTGCGATGACATCCTTTACC
T364A-R	ATGTCATCGCAGGTCCGGTGAATCGC
S367A-F	ATGACAGCGTTTACCCGCCGTGCCCGTAAAG
S367A-R	GGCGGGTAAACGCTGTCATGGTAGGTC
S368A-F	ATGACATCCGCGACCCGCCGTGCCCGTAAAG
S368A-R	GGCGGGTCCGCGATGTCATGGTAGGTC
E109NNK-F	AGTTTTCCANNK KG CTCTTACCGAGGAC
E109NNK-R	AGAGCMNNGGAAAACACTGAGAACATCG
F297NNK-F	TCTGATC NNK GCCGCCATTACACG
F297NNK-R	GCGGCMNNGATCAGAGAAAGCTG
F297W-F	TCTGATCTGGGCCGCCATTACACG
F297W-R	GCGGCCAGATCAGAGAAAGCTG

Underline: restriction endonuclease cleavage site; Bold: mutant site.

Overall model quality

Z-Score: **-8.25**

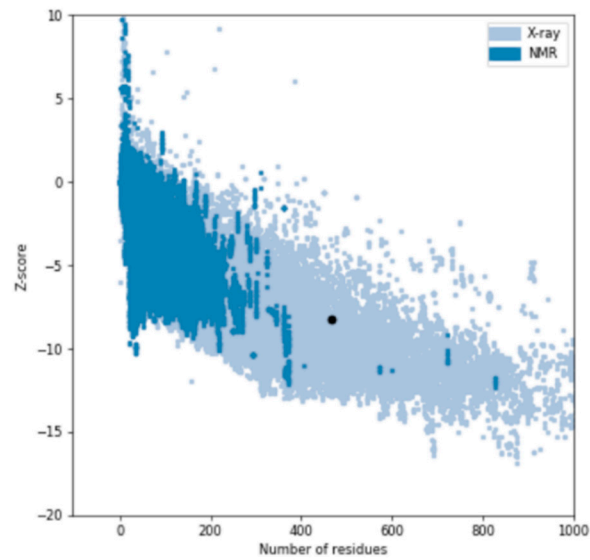
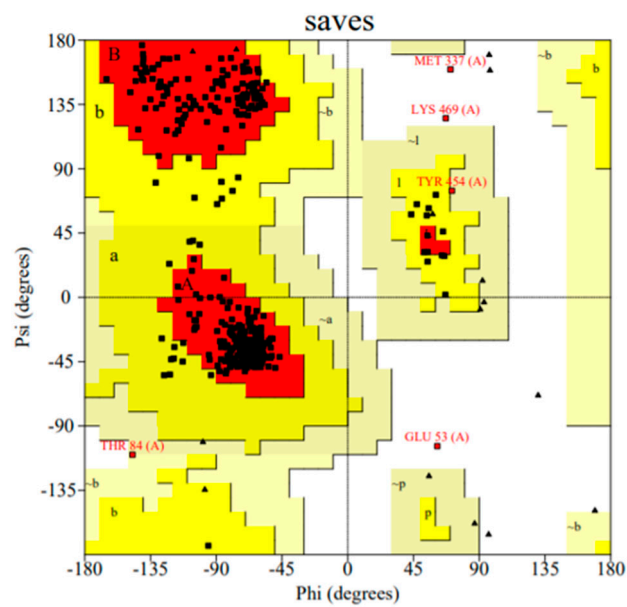


Figure S1: ProSA Z-Score result of the ΔP -450_{lun} model.

PROCHECK

Ramachandran Plot



Plot statistics

Residues in most favoured regions [A,B,L]	376	91.0%
Residues in additional allowed regions [a,b,l,p]	32	7.7%
Residues in generously allowed regions [-a,-b,-l,-p]	2	0.5%
Residues in disallowed regions	3	0.7%
Number of non-glycine and non-proline residues	413	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	20	
Number of proline residues	31	
Total number of residues	466	

Figure S2: Ramachandran Plot of the structure of ΔP -450_{lun}.

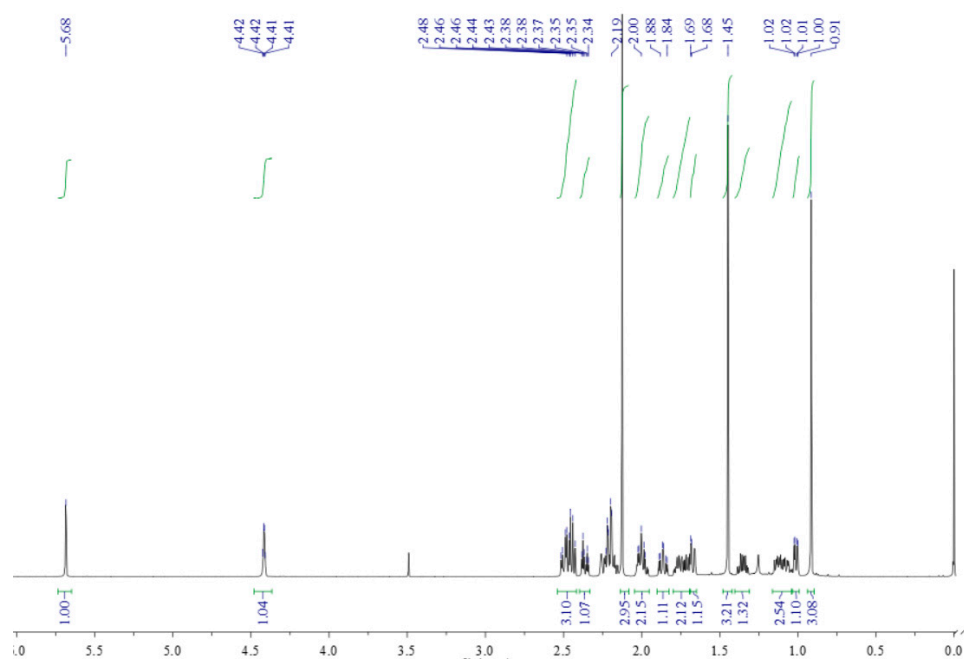


Figure S3: ¹H-NMR spectrum of isolated 11β-OH PROG (600 MHz, CDCl₃).

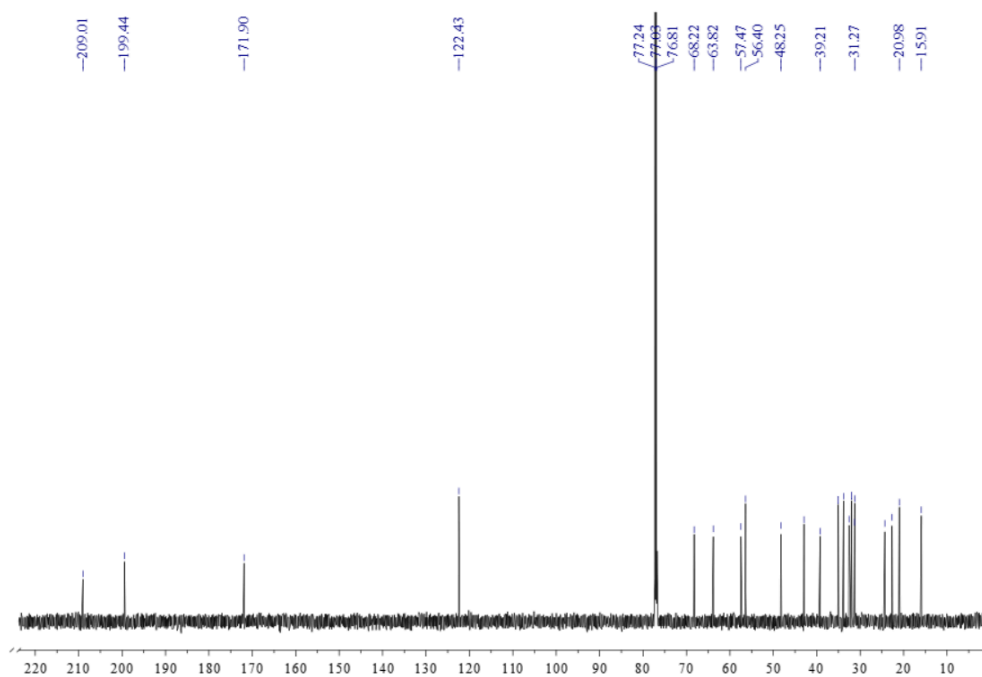


Figure S4: ¹³C-NMR spectrum of isolated 11β-OH PROG (600 MHz, CDCl₃).

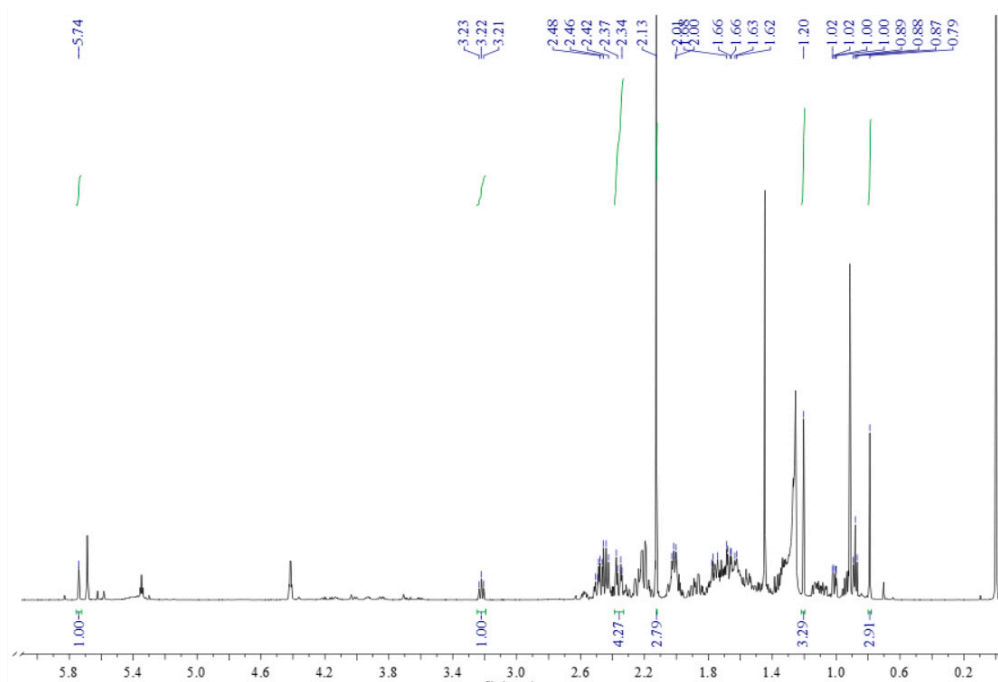


Figure S5: ¹H-NMR spectrum of isolated 14α-OH PROG (600 MHz, CDCl₃).

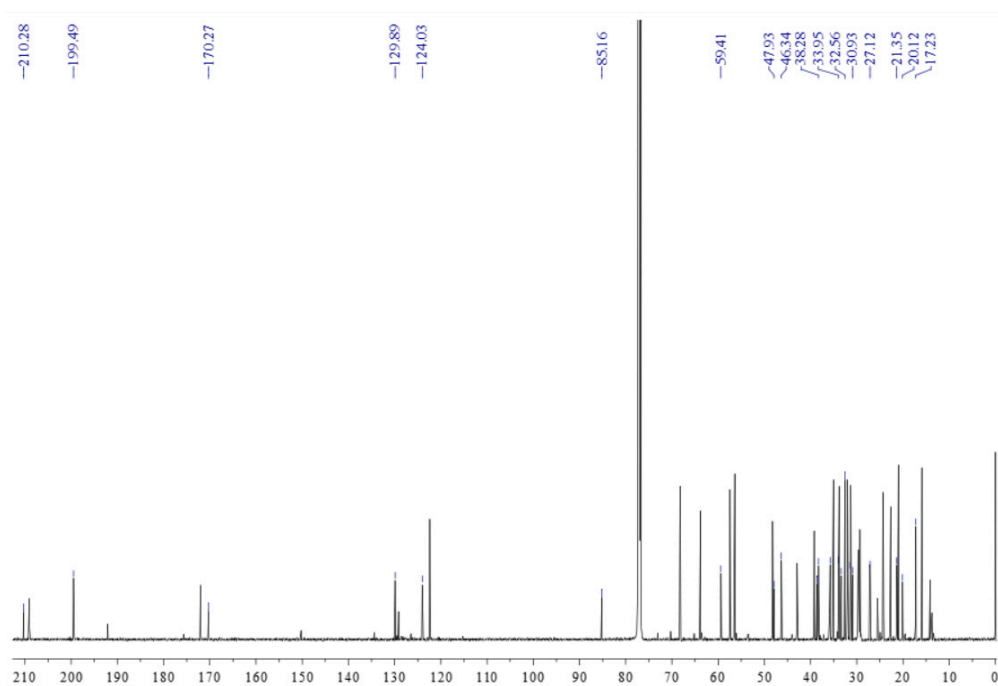
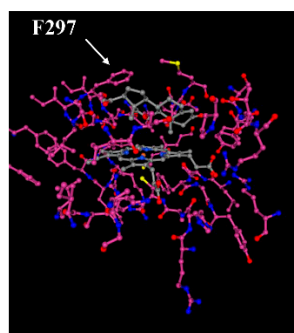
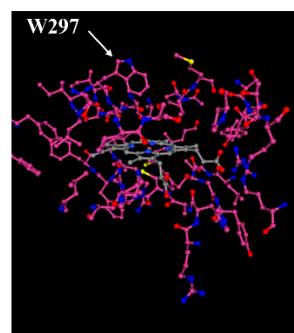


Figure S6: ¹³C-NMR spectrum of isolated 14α-OH PROG (600 MHz, CDCl₃).

A

Acceptors: 39
Depth: 29.88 Å
Donors: 25
Hydrophobicity: 0.72
Metal: 1
Protein Heavy Atoms: 339
Surface: 1519.90 Å²
Surface-Volume-Ratio: 1.00
Volume: 1526.27 Å³

B

Acceptors: 35
Depth: 28.15 Å
Donors: 21
Hydrophobicity: 0.74
Metal: 1
Protein Heavy Atoms: 338
Surface: 1412.57 Å²
Surface-Volume-Ratio: 1.04
Volume: 1357.82 Å³

Figure S7: Binding pocket properties of ΔP -450_{lun} (A) and ΔP -450_{lun}-F297W (B)