Complementary and selective oxidation of hydrocarbon derivatives by two cytochrome P450 enzymes of the same family

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Supporting Information

Table S1 Substrate binding, turnover and coupling efficiency data for CYP101B1 and CYP101C1 with cyclic alkanes and cyclododecanol. The CYP101B1 H85F variant was analysed with certain cyclic alkanes (***B1 H85F***). The *in vitro* turnover activities were measured using a ArR:Arx: CYP101B1/CYP101C1 concentration ratio of 1:10:1 (0.5 mM CYP enzyme, 50 mM, pH 7.4). N is the NADH oxidation rate, PFR the product formation rate, C is the coupling efficiency and TTN, total turnover number. The data are reported as mean ± S.D. (n = 3), and rates are given in nmol.nmol-CYP^{-1}.min^{-1}. - Not measured or not able to be determined accurately. n.p: no product.

Substrate	**CYP101B1/ CYP101C1**	**HS %**	**K_d (µM)**	**N**	**PFR**	**C (%)**
cyclohexane	CYP101B1	15	-	61 ± 9	2 ± 1	3
	CYP101C1	**-**	**-**	**-**	**-**	**-**
cyclooctane	CYP101B1	35	31 ± 4	391 ± 11	209 ± 17[a]	49
	CYP101C1	**-**	**-**	**90 ± 13**	**18 ± 7**	**20**
	B1 H85F	***80***	***1.6 ± 0.2***	***581 ± 19***	***282 ± 23***	***54***
cyclodecane	CYP101B1	40	0.88 ± 0.04	397 ± 15	42 ± 34[b]	11
	CYP101C1	**-**	**-**	**164 ± 5**	**17 ± 7**	**13**
	B1 H85F	***90***	***0.28 ± 0.04***	***518 ± 12***	***43 ± 6***	***14***
cyclododecane	CYP101B1	30	0.16 ± 0.03	174 ± 5	15 ± 3	9
	CYP101C1	**-**	**-**	**84 ± 10**	**2 ± 1**	**2**
	B1 H85F	**-**	**-**	***129 ± 13***	***15 ± 1***	***12***
cyclododecanonol	CYP101B1	90	-	198 ± 7	41 ± 1[c]	21
	CYP101C1	**-**	**-**	**280 ± 5**	**31 ± 20[d]**	**11**

[a] TTN 3400 ± 150; [b] TTN 420 ± 75; [c] TTN 1360 ± 90; [d] TTN 650 ± 40.

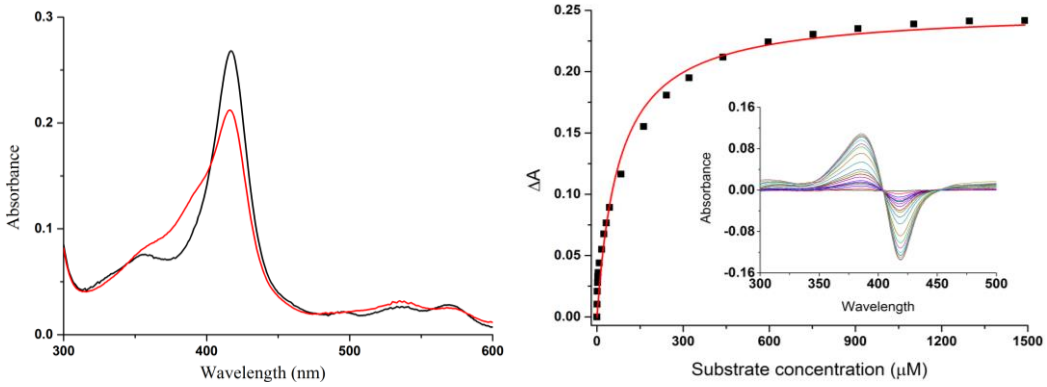
Table S2 ^{1}H and ^{13}C NMR signals of the HC-OH and HC-O groups which have been reported ($CDCl_3$) [1, 2].

Hydroxy ketone	HC-OH (C) (δ)	Hemiacetal	HC-O (C) (δ)
5-hydroxycyclononanone	3.70 (71.3)	1-hydroxy-10-oxabicyclo [4.3.1]decane	4.08 (73.7)
5-hydroxycyclodecanone	3.75 (69.9)	1-oxabicyclo[6.3.1] undecan-1-ol	4.0 (73.6)
6-hydroxycyclodecanone	**3.83 (69.9)**	**1-oxabicyclo[6.3.1] undecan-1-ol**	**4.07 (76.6)**
2-hydroxycyclodecanone	3.97-4.27	-	-

Table S3 Additional turnover and coupling efficiency data for CYP101B1 and CYP101C1 substrates containing the ester directing group. The turnover activities were measured as described in Table S1. The data are reported as mean ± S.D. (n = 3). Rates are given in nmol.nmol-CYP^{-1}.min^{-1}.

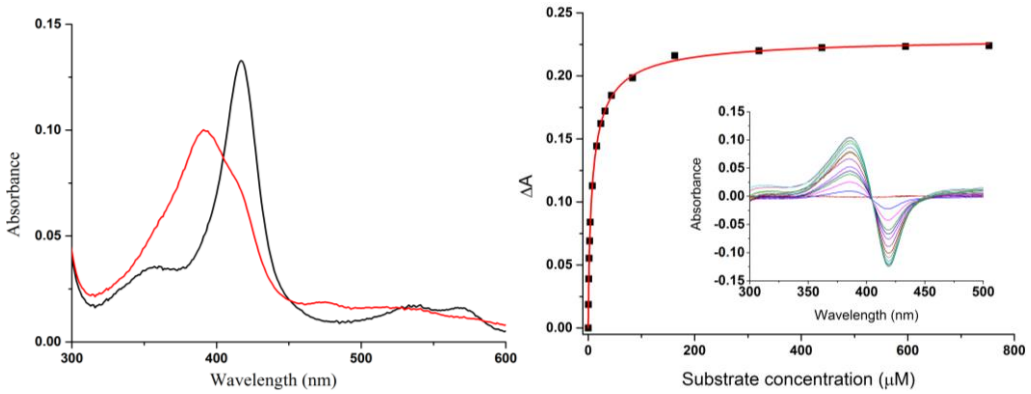
Substrate	CYP101B1/ CYP101C1	NADH	PFR	Coupling (%)	TTN
cyclohexyl isobutyrate	CYP101B1	383 ± 26	220 ± 62	57	3230 ± 65
	CYP101C1	**106 ± 11**	**20 ± 2**	**19**	**144 ± 10**
ethylcyclohexyl acetate	CYP101B1	667 ± 29	274 ± 15	42	7250 ± 1720
	CYP101C1	**284 ± 35**	**56 ± 12**	**21**	**421 ± 60**
cyclooctyl isobutyrate	CYP101B1	732 ± 11	626 ± 30	75	4660 ± 1700
	CYP101C1	**301 ± 2**	**25 ± 5**	**9**	**186 ± 75**

Spin-state shifts and dissociation constants (K_d) with CYP101B1



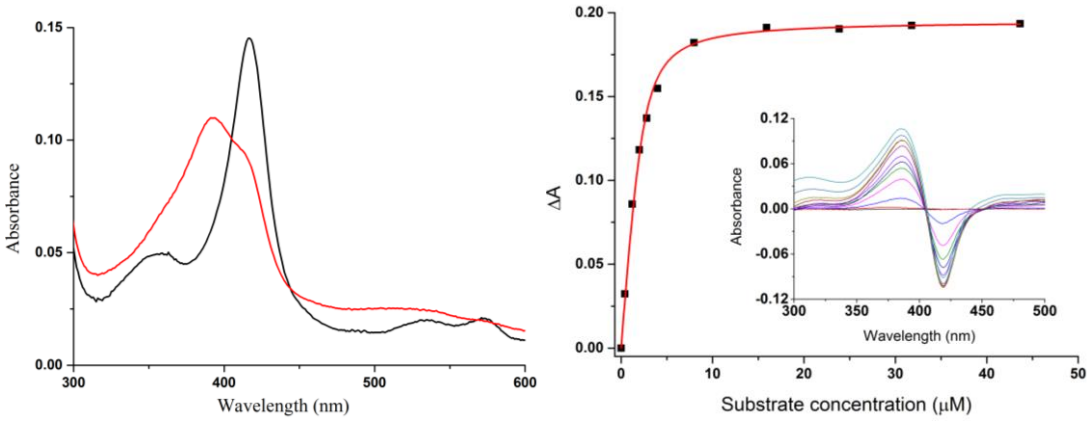
(i) (ii)

Figure S1 a (i) The spin-state shifts (red) of CYP101B1 after addition of cyclononanone. **(ii)** The dissociation constant analysis with cyclononanone (K_d = 46 μM).



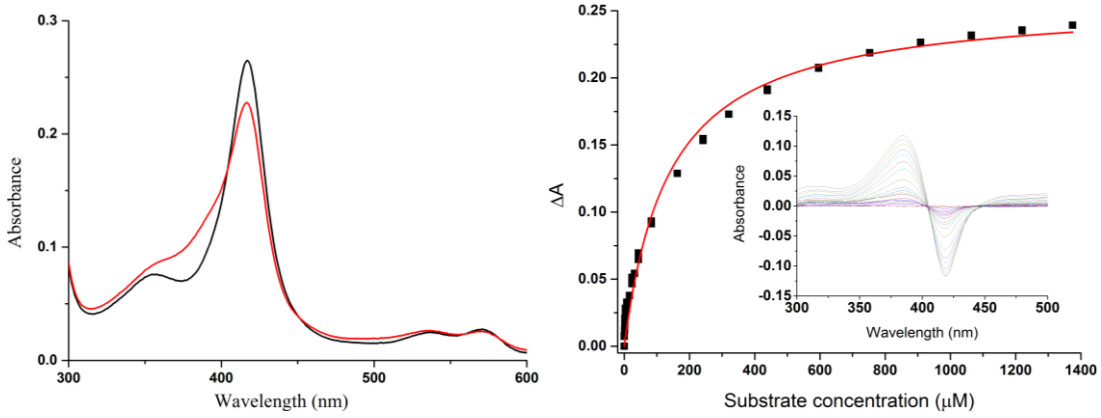
(i) (ii)

Figure S1 b (i) The spin-state shift (red) of CYP101B1 after addition of cycloundecanone. (ii) The dissociation constant analysis with cycloundecanone (K_d = 8.4 μM).



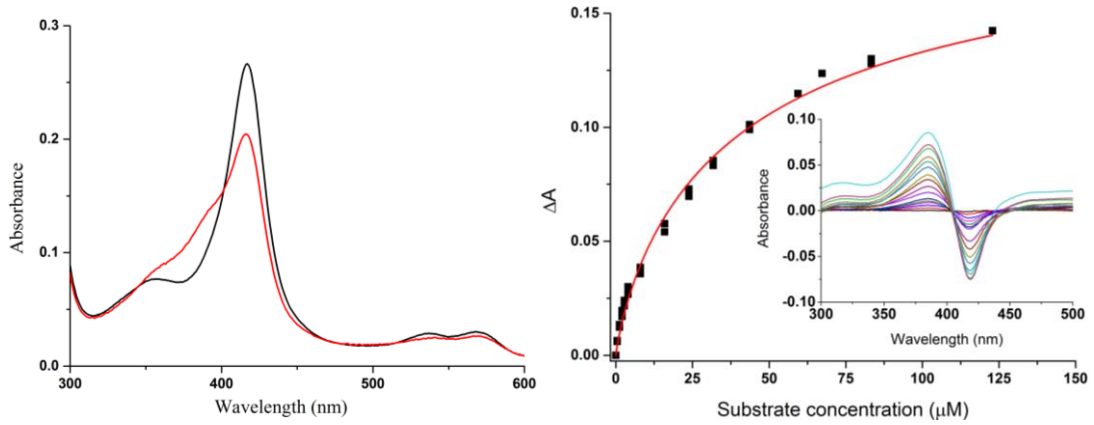
(i) (ii)

Figure S1 c (i) The spin-state shift (red) of CYP101B1 after addition of cyclopentadecanone. **(ii)** The dissociation constant analysis with cyclopentadecanone (2.3 μM CYP101B1/K_d = 0.5 μM). The concentration of enzyme used in the binding study and the dissociation constant (K_d) are provided in brackets.



(i) (ii)

Figure S1 d (i) The spin-state shifts (red) of CYP101B1 after addition of 2-nonanone. **(ii)** The dissociation constants analysis with 2-nonanone (K_d = 140 μM).



(i) (ii)

Figure S1 e (i) The spin-state shifts (red) of CYP101B1 after addition of 2-undecanone. **(ii)** The dissociation constants analysis with 2-undecanone (K_d = 44 μM).

GC and GC-MS analysis of CYP101B1 and CYP101C1 *in vitro* and *in vivo* turnovers

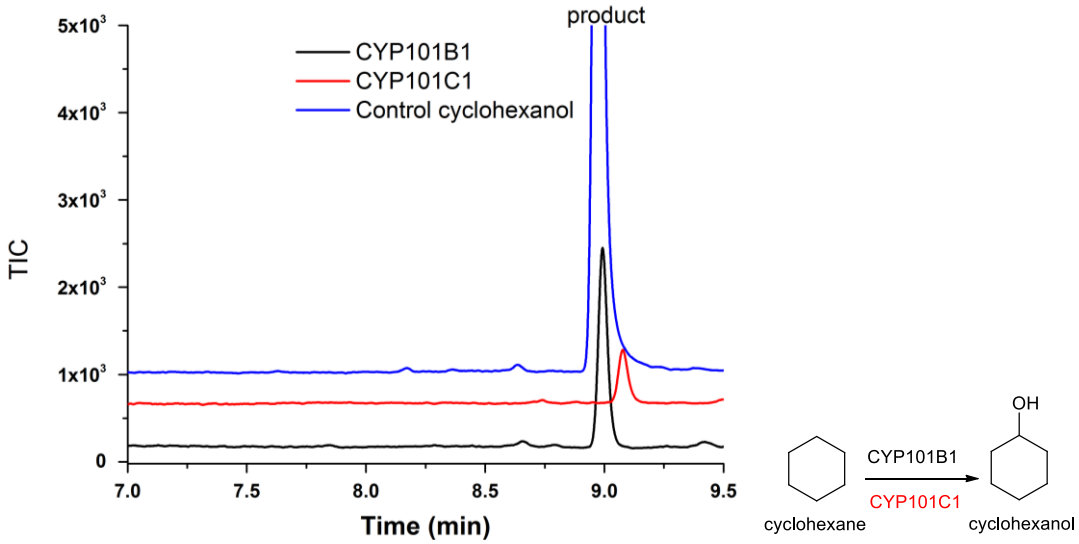


Figure S2 a GC analysis of the turnover of cyclohexane by CYP101B1 (black) and CYP101C1 (red). Cyclohexane (RT 2.05 min, not shown) and the product; cyclohexanol (8.9 min). The chromatogram (CYP101C1) was offset along the x and y-axes for clarity.

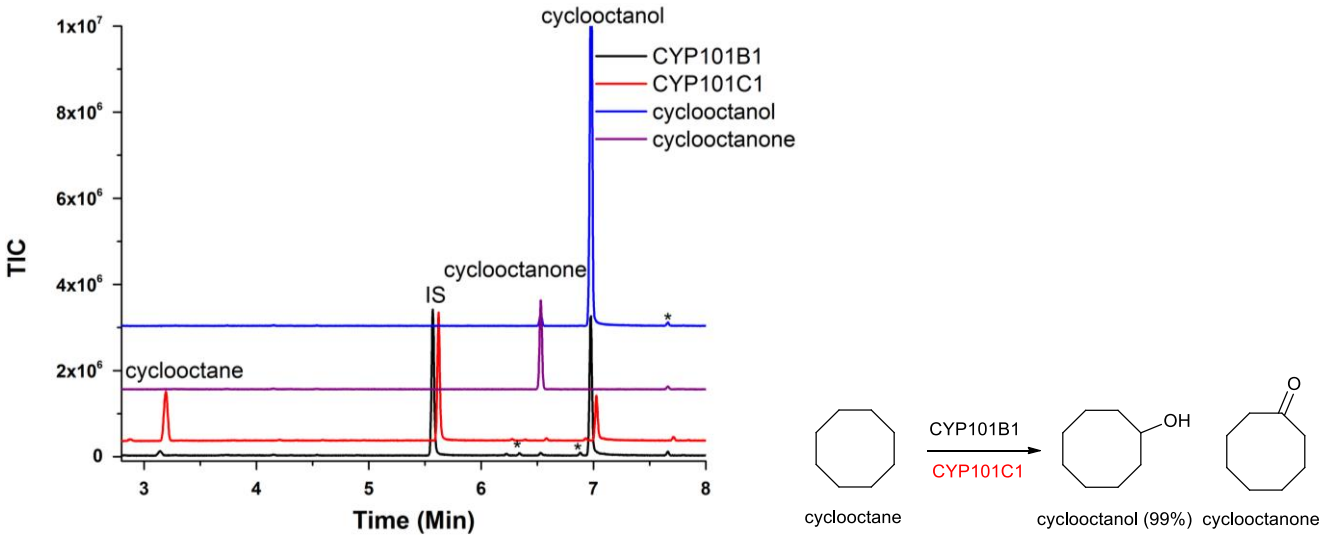
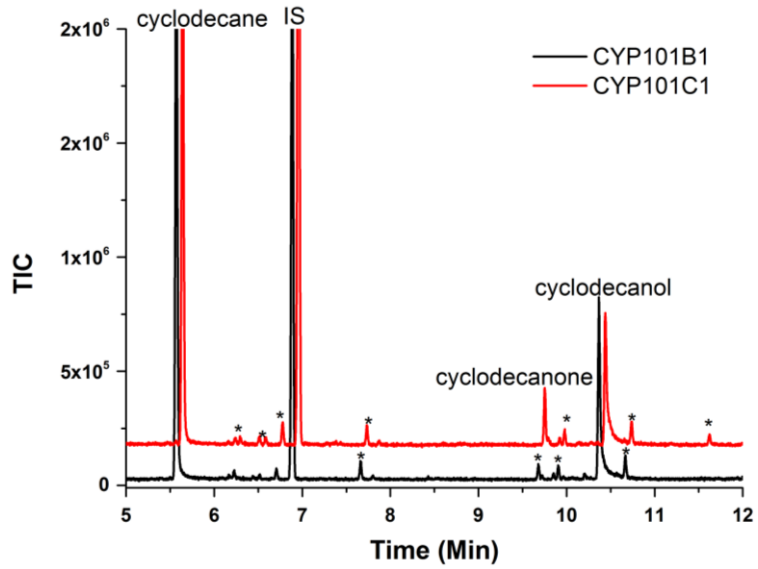
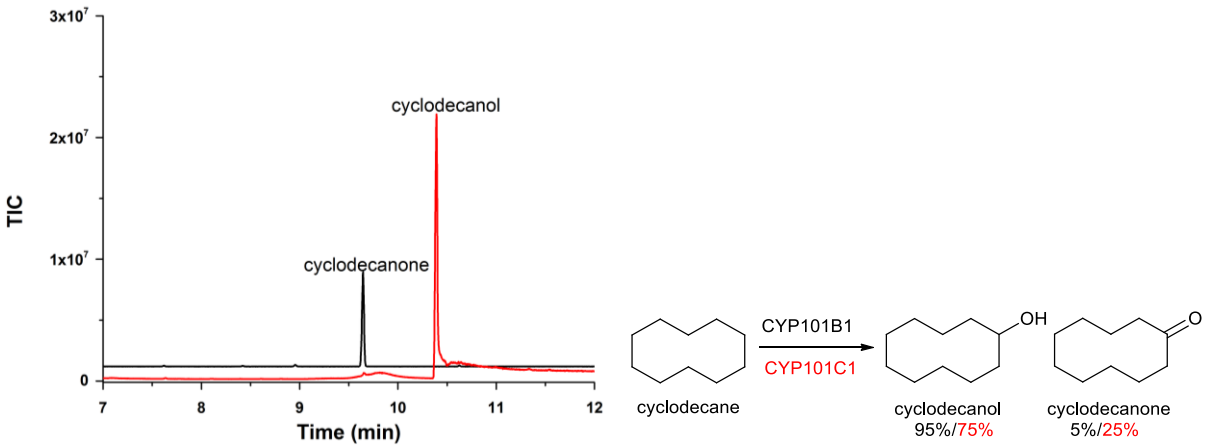


Figure S2 b GC-MS analysis of the turnovers of cyclooctane by CYP101B1 (black) and CYP101C1 (red). Cyclooctane (RT 3.2 min) and the products; cyclooctanone (6.6 min) and cyclooctanol (6.9 min). The chromatogram (CYP101C1) was offset along the x and y-axes for clarity.

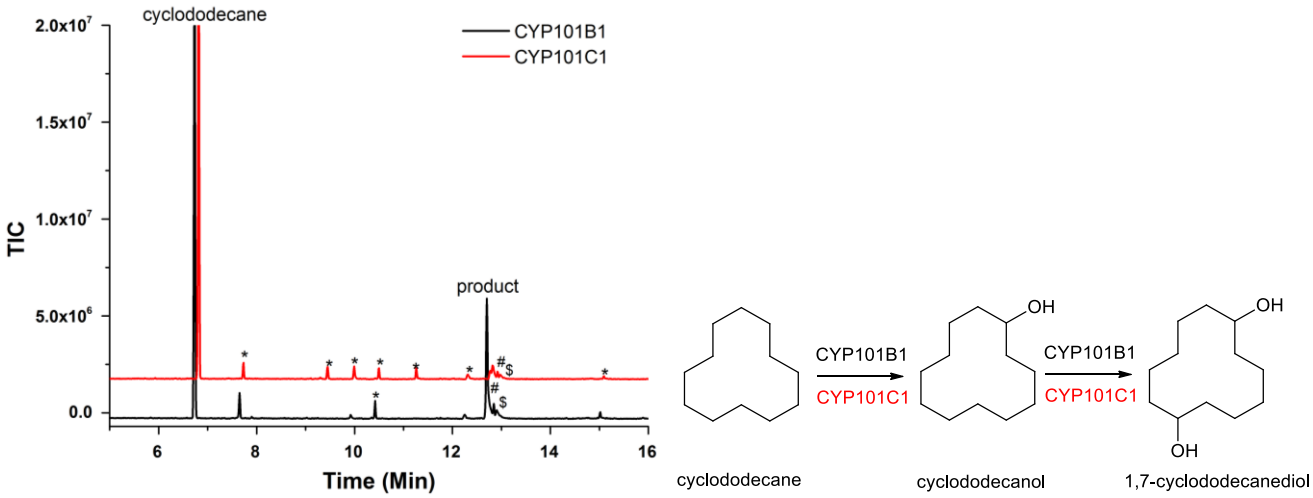


(i)



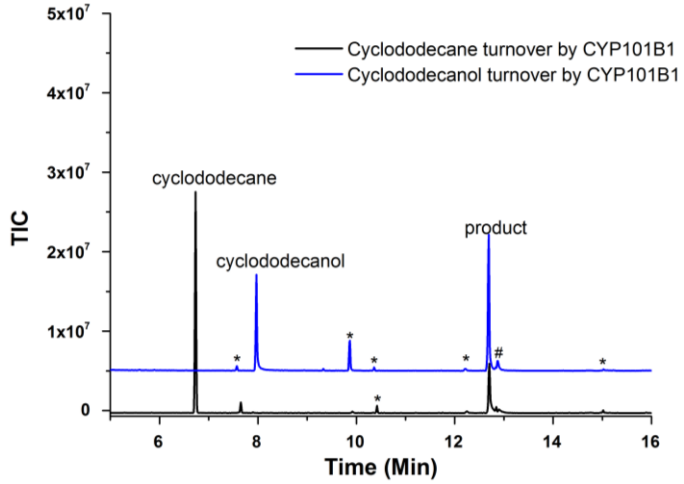
(ii)

Figure S2 c (i) GC-MS analysis of the turnovers of cyclodecane by CYP101B1 (black) and CYP101C1 (red). Cyclodecane (RT 5.1 min) and the products; cyclodecanone (9.7 min) and cyclodecanol (10.45 min). The chromatogram (CYP101C1) was offset along the x and y-axes for clarity. **(ii)** The control of cyclodecanone, and cyclodecanol generated from the reduction of cyclodecanone by $NaBH_4$. Impurities are labelled (*).

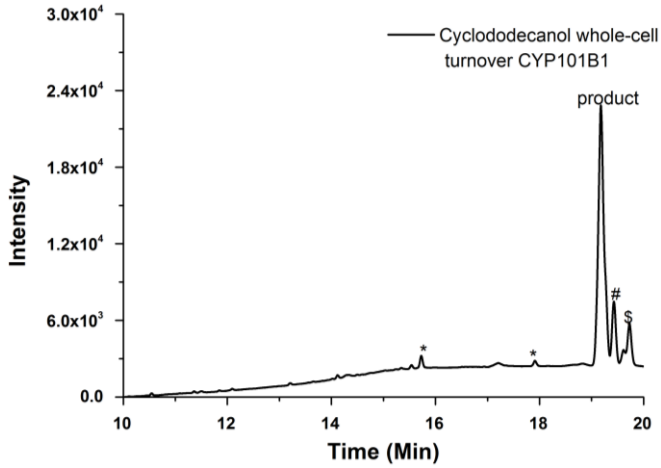


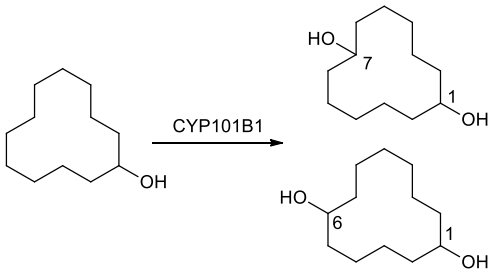
(i)

Figure S2 d (i) GC-MS analysis of cyclododecane *in vitro* turnovers by CYP101B1 (black) and CYP101C1 (red). Cyclododecane (RT 6.8 min) and the products; 1,7-cyclododecanediol (RT 12.9 min), 1,6-cyclododecanediol and unknown metabolite (RT 12.95 min and RT 13.0 min) labelled as # and $. The chromatogram (CYP101C1) was offset along the x and y-axis for clarity (Figure continued overleaf).



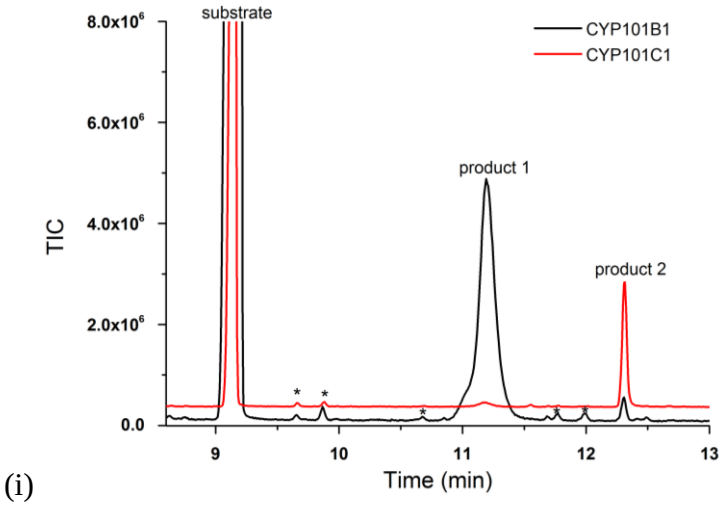
(ii)



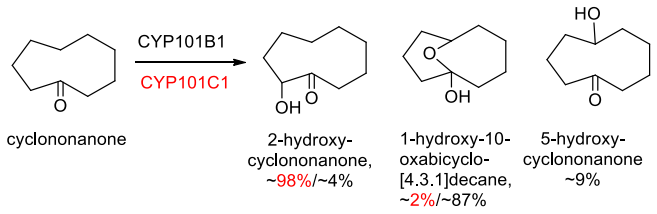


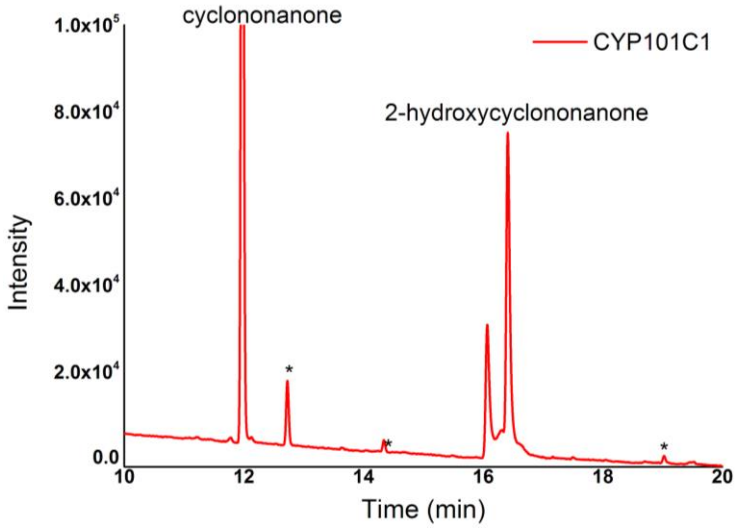
(iii)

Figure S2 d (ii) GC-MS analysis of cyclododecanol turnover with CYP101B1. Cyclododecanol (RT 7.9 min) and the products; 1,7-cyclododecanediol (RT 12.9 min) and 1,6-cyclododecanediol (RT 12.95 min) labelled as #. **(iii)** The GC analysis of the whole-cell turnover of cyclododecanol. Cyclododecanol (RT 10.2 min) and the product; 1,7-cyclododecanediol (RT 19.6 min) and the minor metabolites; 1,6-cyclododecanediol and an unknown (RT 19.65 min$^{\#}$ and RT 19.7 min$^{\$}$). Impurities are marked (*).



(i)





(ii)

Figure S2 e (i) GC-MS analyses of the *in vitro* turnovers of cyclononanone by CYP101B1 and CYP101C1. Cyclononanone (RT 9.1 min) and the products; 1-hydroxy-10-oxabicyclo[4.3.1]decane in equilibrium with 5-hydroxycyclononanone (RT 11.2 min) and 2-hydroxycyclononanone (RT 12.2 min). **(ii)** The chiral GC analysis of the *in vitro* turnover of cyclononanone by CYP101C1. Cyclononanone (RT 12.0 min) and the product 2-hydroxycyclononanone (two enantiomers: RT 16.4 min and RT 16.5 min). Impurities are labelled (*).

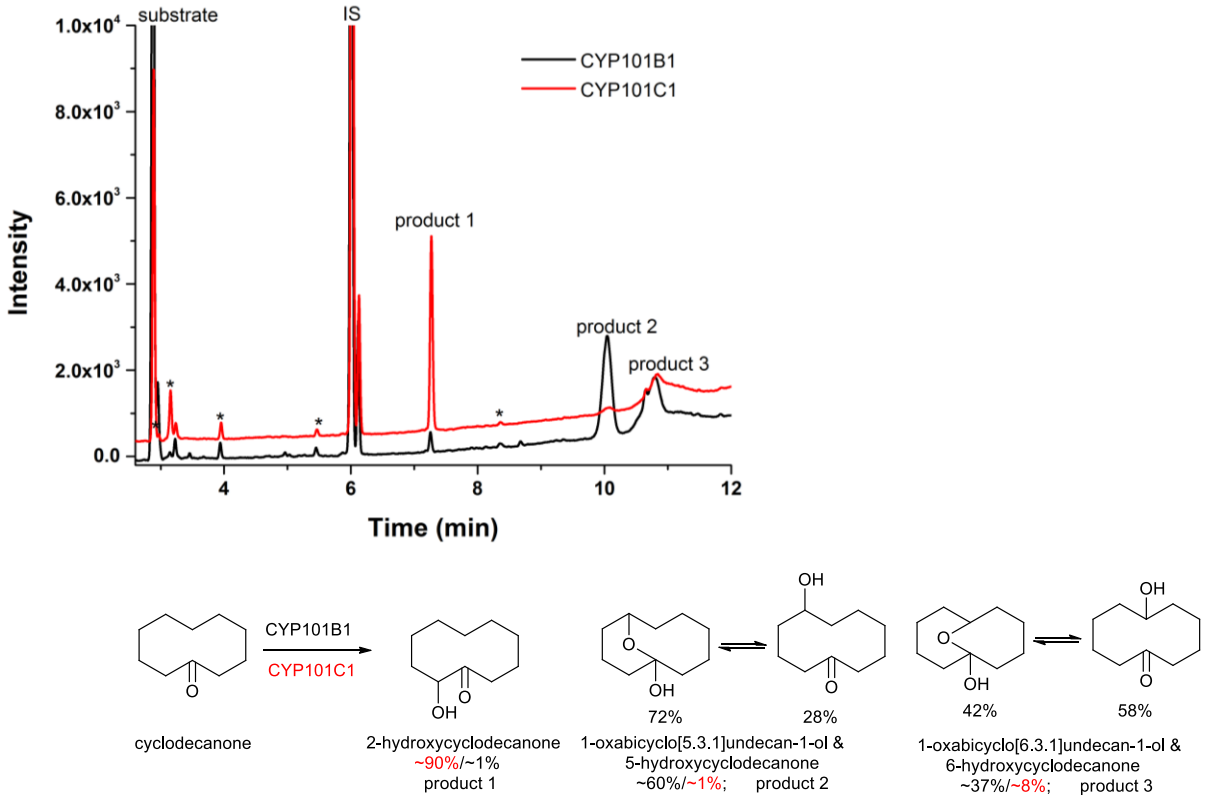
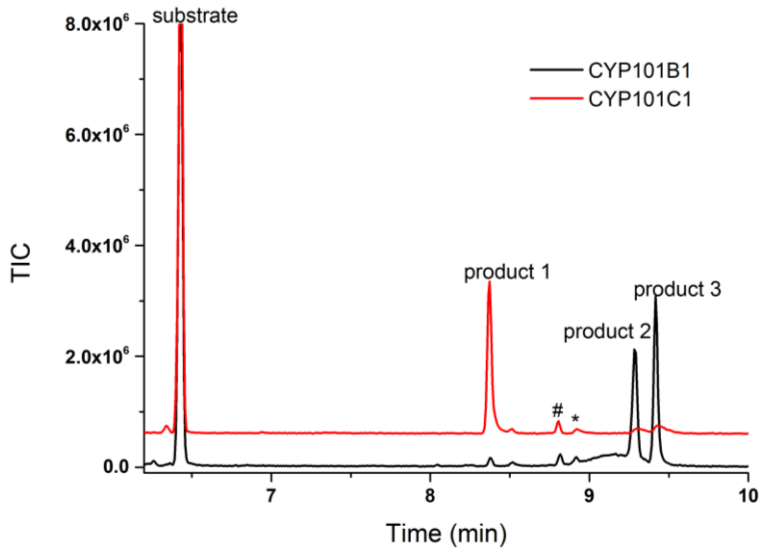
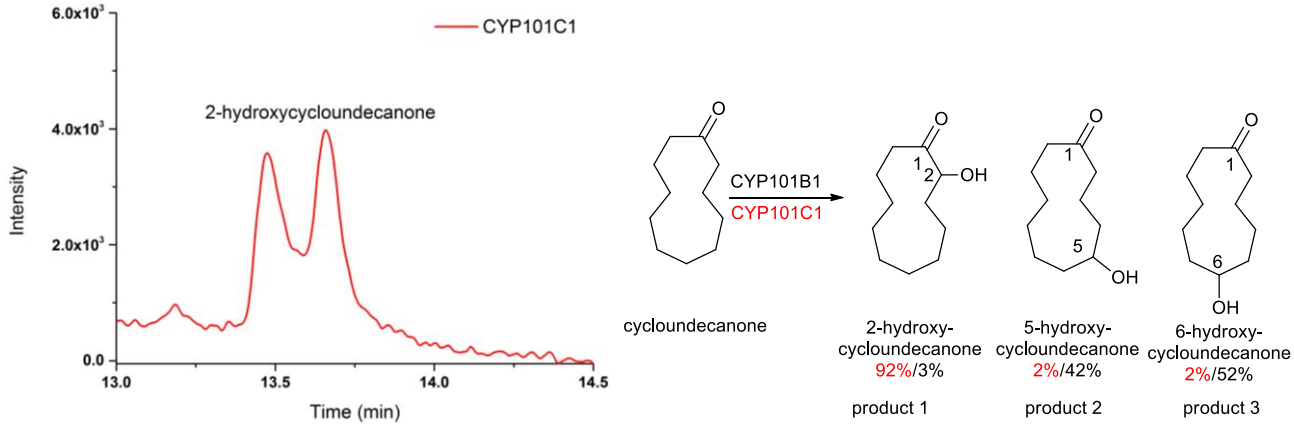


Figure S2 f GC-FID analyses of the *in vitro* turnovers of cyclodecanone by CYP101B1 and CYP101C1. Cyclodecanone (RT 2.9 min) and the products; 2-hydroxycyclodecanone (RT 7.2 min); 1-oxabicyclo[5.3.1]undecan-1-ol and 5-hydroxycyclodecanone (RT 10.0 min); a mixture of 1-oxabicyclo[6.3.1]undecan-1-ol and 6-hydroxycyclodecanone (RT 10.9 min). Impurities are labelled (*).

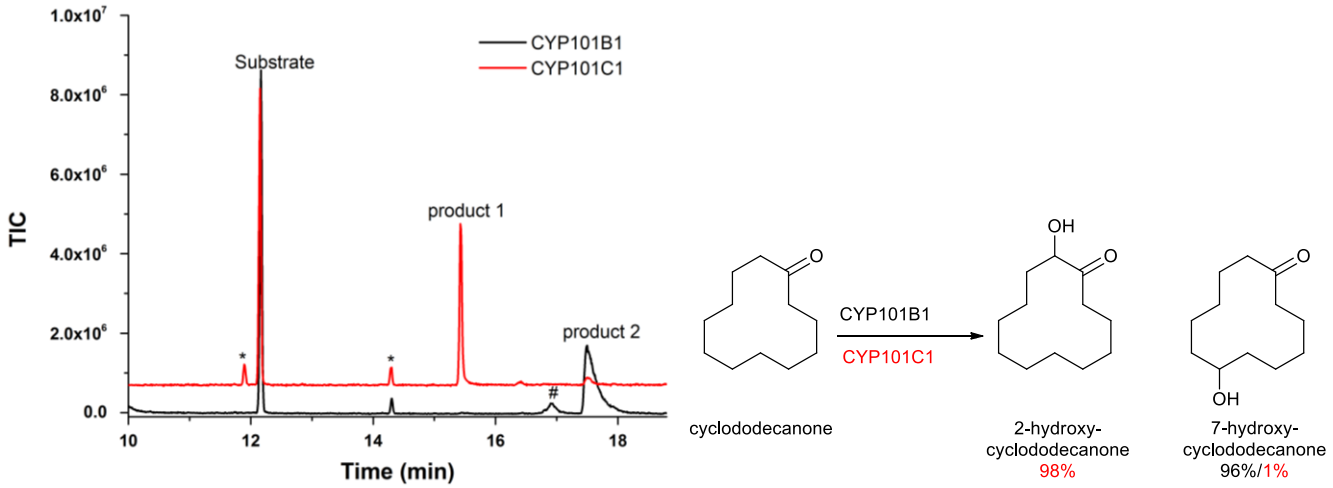


(i)

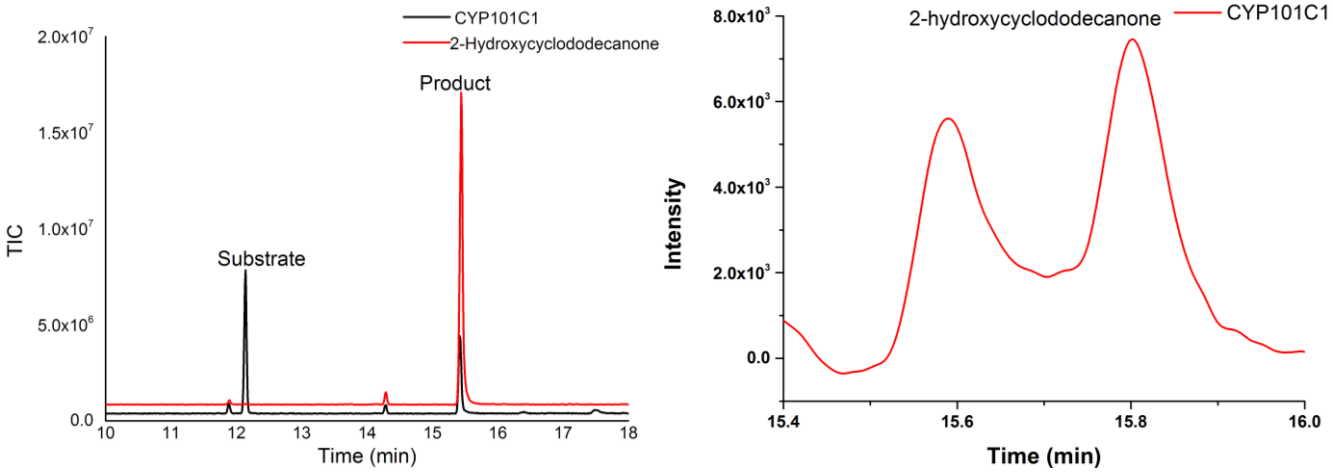


(ii)

Figure S2 g (i) GC-MS analyses of the *in vitro* turnovers of cycloundecanone by CYP101B1 and CYP101C1. Cycloundecanone (RT 6.4 min) and product 1; 2-hydroxycycloundecanone (RT 8.4 min), product 2; 5-hydroxycycloundecanone (RT 9.3 min) and product 3; 6-hydroxycycloundecanone (RT 9.45 min). There is minor (≤4%) unidentified product[#] in both enzymes turnover at RT 8.8 min. **(ii)** The chiral GC analysis of *in vitro* turnover of cycloundecanone by CYP101C1. Cycloundecanone (RT 9.4 min; not shown) and the product; 2-hydroxycycloundecanone (two enantiomers RT 13.5 min and RT 13.6 min). Impurities are labelled (*).



(i)



(ii) (iii)

Figure S2 h (i) GC-MS analyses of the *in vitro* turnovers of cyclododecanone by CYP101B1 (black) and CYP101C1 (red). Cyclododecanone (RT 12.1 min) and the product 1; 2-hydroxycyclododecanone (RT 15.5 min), product 2; 7-hydroxycyclododecanone (RT 17.7 min). There is a minor product[#] in CYP101B1 enzyme turnover at RT 16.9 min. **(ii)** The authentic commercial standard (red) coeluted with CYP101C1 turnover (black) in GC-MS. **(iii)** Chiral GC analysis of the *in vitro* turnover of cyclododecanone by CYP101C1. Cyclododecanone (RT 10.2 min; not shown) and the product; 2-hydroxycyclododecanone (two enantiomers; RT 15.6 min and 15.8 min). Impurities are labelled (*).

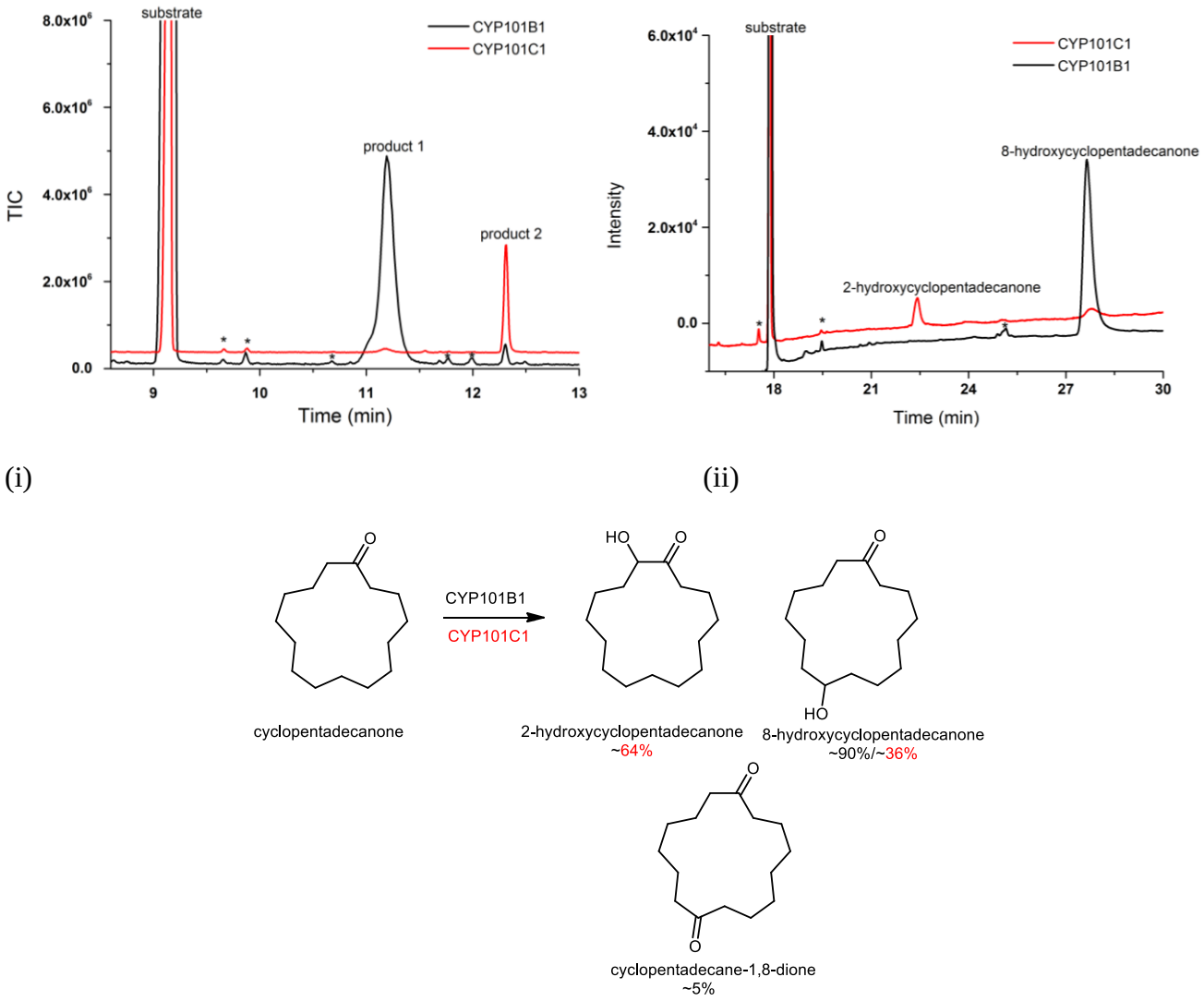


Figure S2 i (i) GC-MS analysis of the *in vitro* turnover of cyclopentadecanone by CYP101B1 and CYP101C1. Cyclopentadecanone (RT 13.1 min) and the product 1; 2-hydroxycyclopentadecanone (RT 14.6 min), product 2; 8-hydroxycyclopentadecanone (RT 15.6 min) [3]. There is a minor product[#] in CYP101B1 enzyme turnover at RT 15.1 min was assigned as cyclopentadecane-1,8-dione (≤5%) [3]. **(ii)** The chiral GC analysis of *in vitro* turnover of cyclopentadecanone by CYP101C1 and CYP101B1. Impurities are labelled (*).

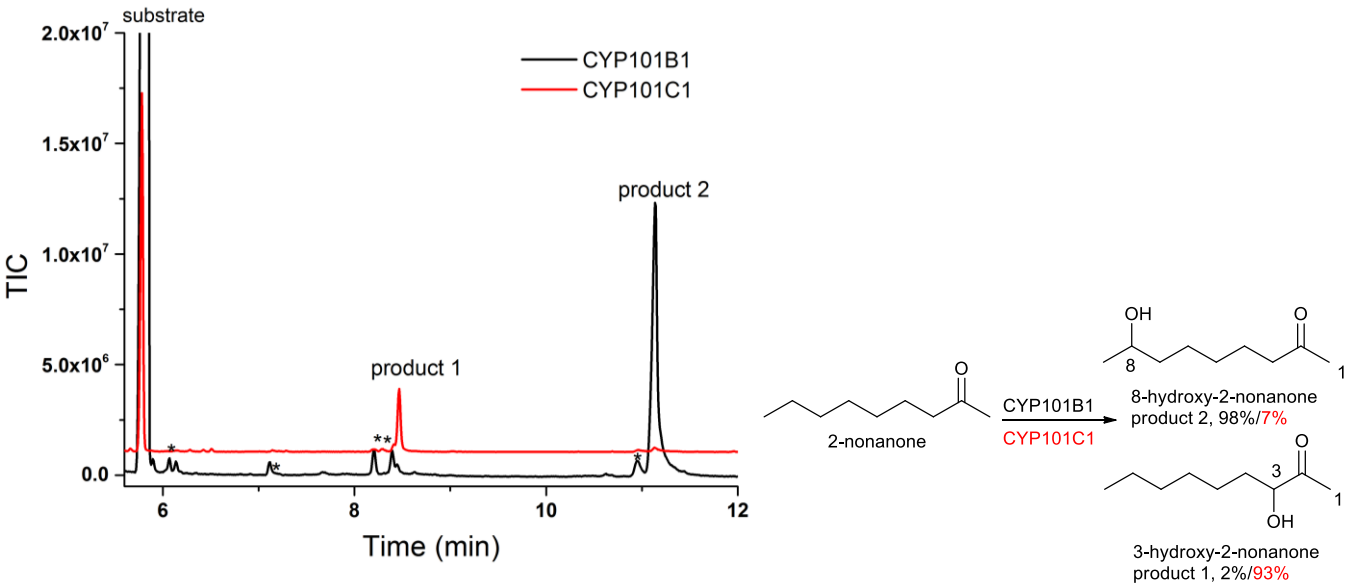


Figure S2 j GC-MS analyses of the *in vitro* turnovers of 2-nonanone by CYP101B1. 2-Nonanone (RT 5.8 min); the major products 8-hydroxy-2-nonanone (RT 11.25 min; m^+/z =158.20) and 3-hydroxy-2-nonanone (RT 8.5 min; m^+/z =158.65). The chromatogram (CYP101C1) was offset along the x and y-axes for clarity.

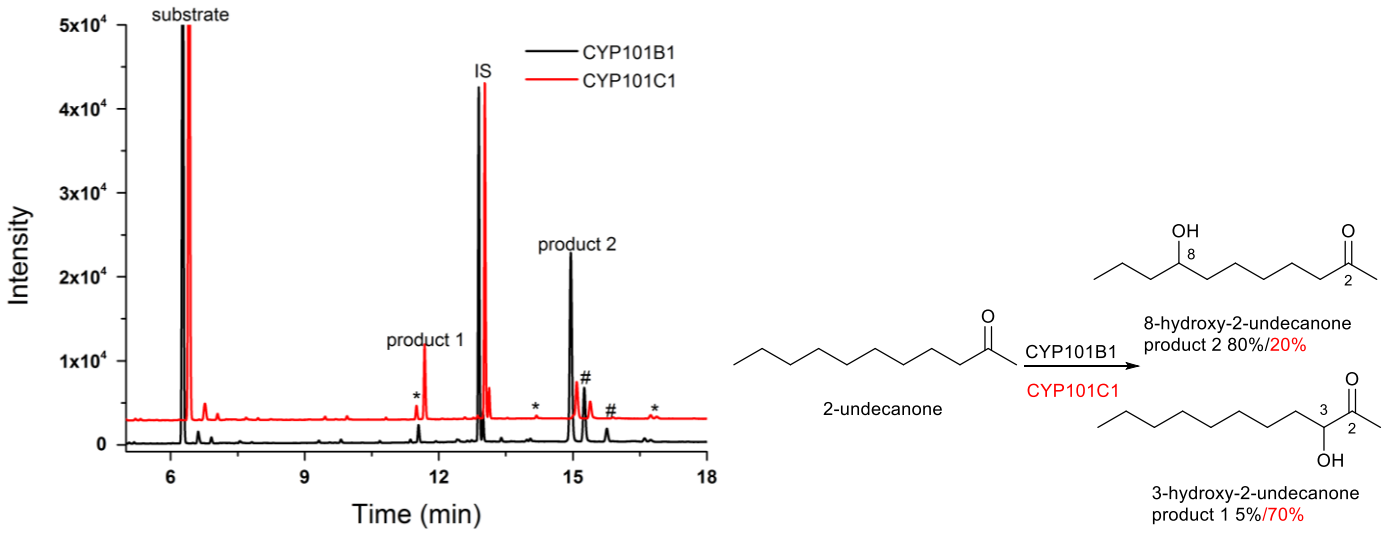


Figure S2 k GC analyses of *in vitro* turnovers of 2-undecanone by CYP101B1 (black) and CYP101C1 (red). 2-Undecanone (RT 6.2 min) and the product 1: 3-hydroxy-2-undecanone (RT 11.5 min), product 2: 8-hydroxy-2-undecanone (RT 14.95 min), and proposed 7-hydroxy-2-undecanone[#] (RT 15.2 min; based on carbon signal (74.92 ppm) intensity in ^{13}C NMR) and 9-hydroxy-2-undecanone[#] (RT 15.9 min, based on C9 (75.92 ppm) carbon signal intensity in the ^{13}C NMR). The chromatogram (CYP101C1) was offset along the x and y-axes for clarity.

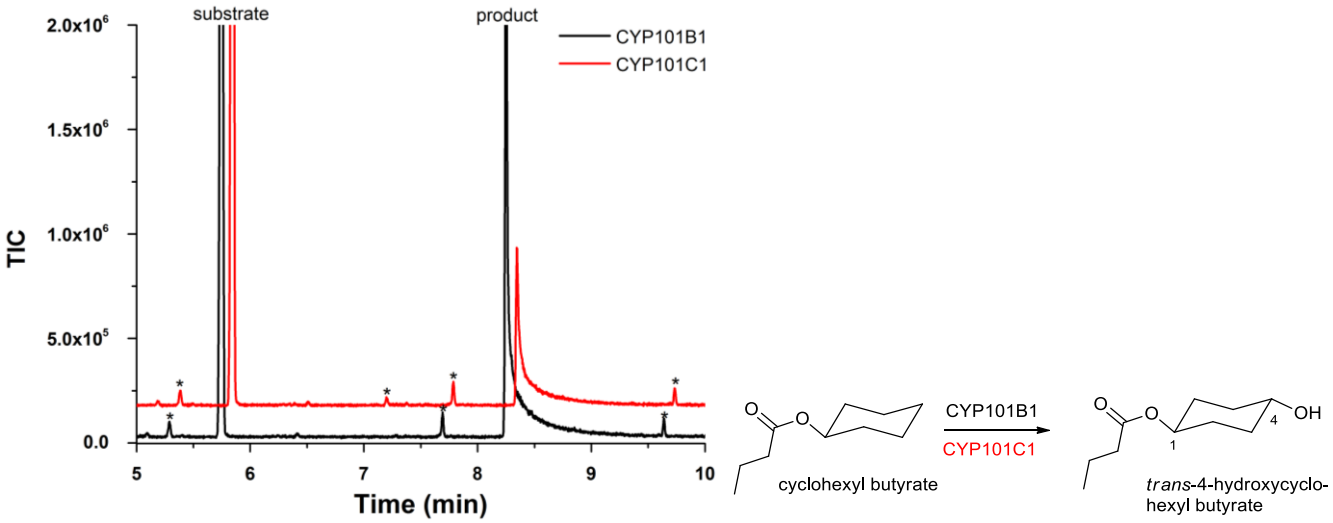


Figure S2 l GC-MS analysis of *in vitro* turnover of cyclohexyl butyrate by CYP101B1 and CYP101C1. Cyclohexyl butyrate (RT 5.7 min) and the product; *trans*-4-hydroxycyclohexyl butyrate (RT 8.5 min). The chromatogram (CYP101C1) was offset along the x and y-axes for clarity.

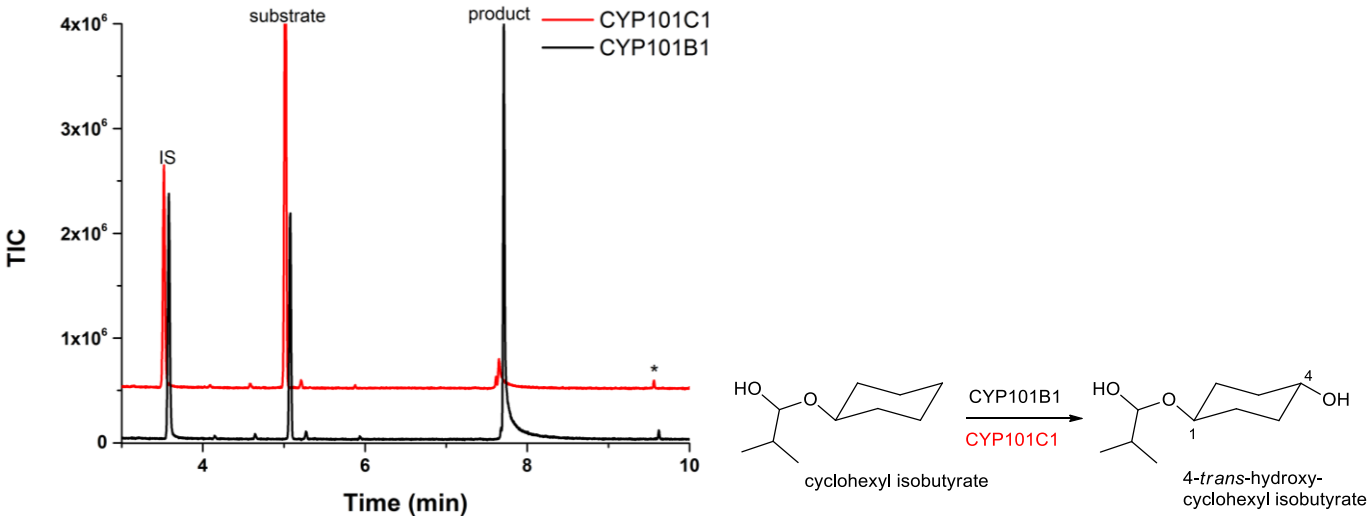


Figure S2 m GC-MS analysis of *in vitro* turnover of cyclohexyl isobutyrate by CYP101B1 and CYP101C1. Cyclohexyl isobutyrate (RT 5.1 min) and the product; *trans*-4-hydroxycyclohexyl isobutyrate (RT 7.9 min). The chromatogram (CYP101C1) was offset along the x and y-axes for clarity.

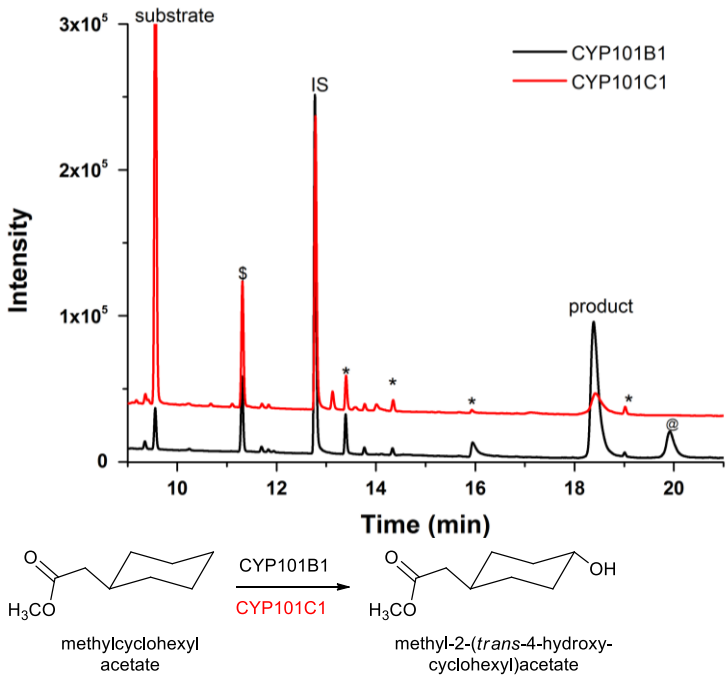


Figure S2 n GC analyses of *in vitro* turnovers of methylcyclohexyl acetate by CYP101B1 and CYP101C1. Methylcyclohexyl acetate (RT 9.8 min) and the product; methyl-2-(*trans*-4-hydroxycyclohexyl)acetate (RT 18.5 min), and a peak for esterification of the substrate to ethylcyclohexyl aceate[$] (RT 11.5 min) and product of ethylcyclohexyl acetate oxidation[@] (RT 19.9 min).

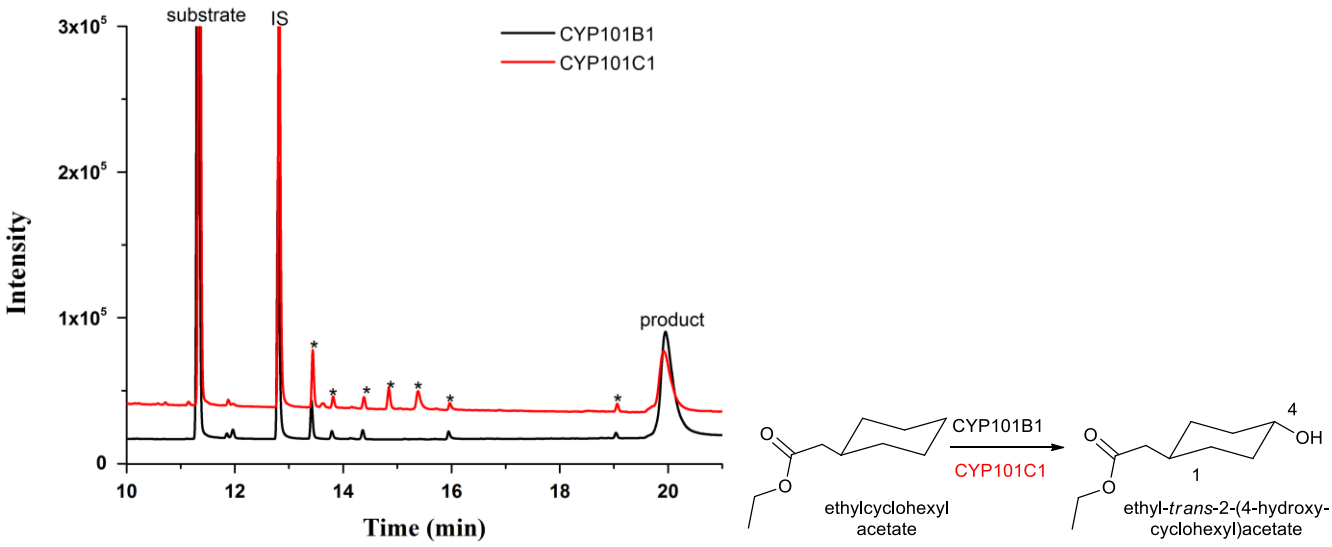


Figure S2 o GC analyses of the *in vitro* turnovers of ethylcyclohexyl acetate by CYP101B1 and CYP101C1. Ethylcyclohexyl acetate (RT 11.5 min) and the product; ethyl-*trans*-2-(4-hydroxycyclohexyl)acetate (RT 20.1 min).

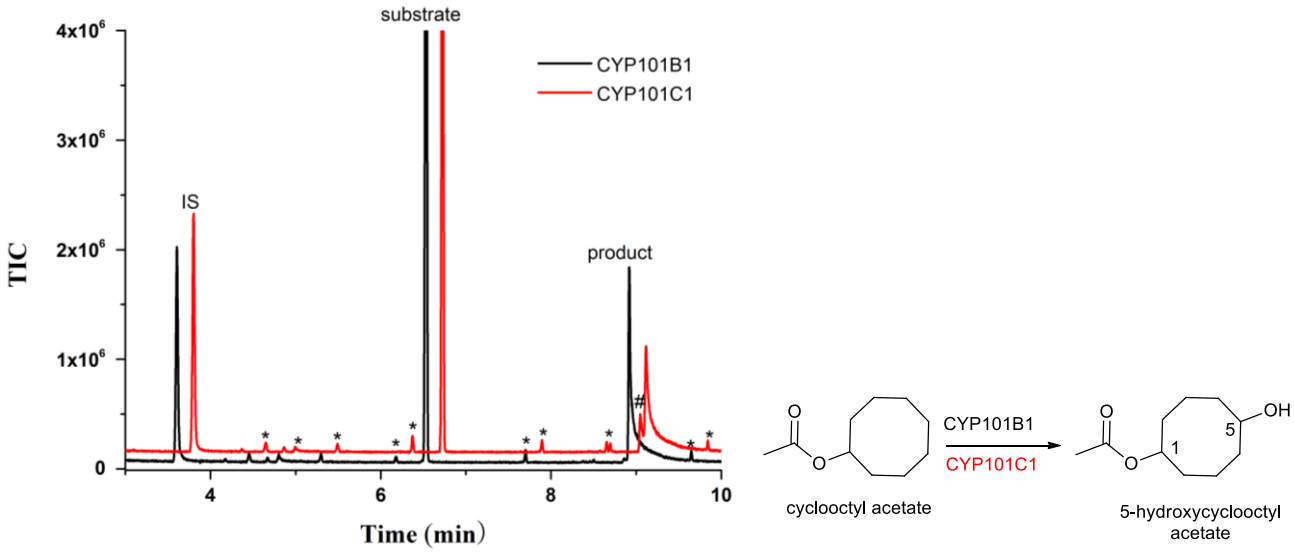


Figure S2 p GC-MS analyses of *in vitro* turnovers of cyclooctyl acetate by CYP101B1 and CYP101C1. Cyclooctyl acetate (RT 6.5 min) and the product; 5-hydroxycyclooctyl acetate (RT 8.95 min), and a minor peak at RT 8.8 min$^{\#}$ with CYP101C1 turnover which was not formed in large enough yield to be identified using NMR but that was assigned as a further oxidation product based on its spectrum (m^+/z = 184.10). The chromatogram (CYP101C1) was offset along the x and y-axes for clarity.

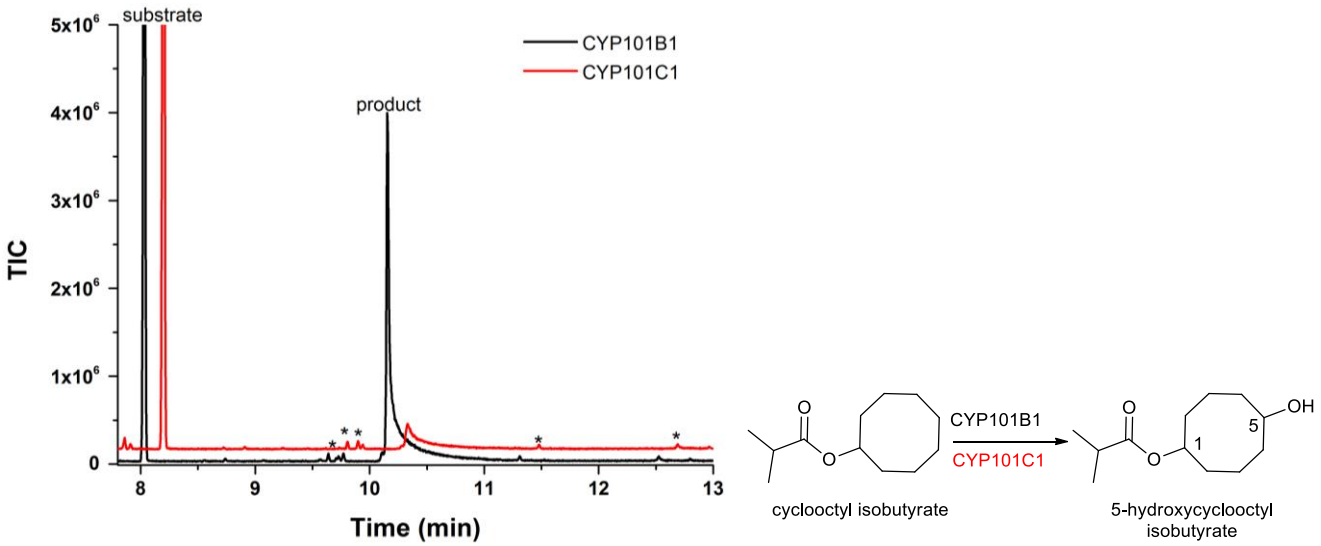


Figure S2 q GC-MS analyses of the *in vitro* turnovers of cyclooctyl isobutyrate by CYP101B1 and CYP101C1. Cyclooctyl isobutyrate (RT 8.0 min) and the product; 5-hydroxycyclooctyl isobutyrate (RT 10.2 min; *trans*). The chromatogram (CYP101C1) was offset along the x and y-axes for clarity.

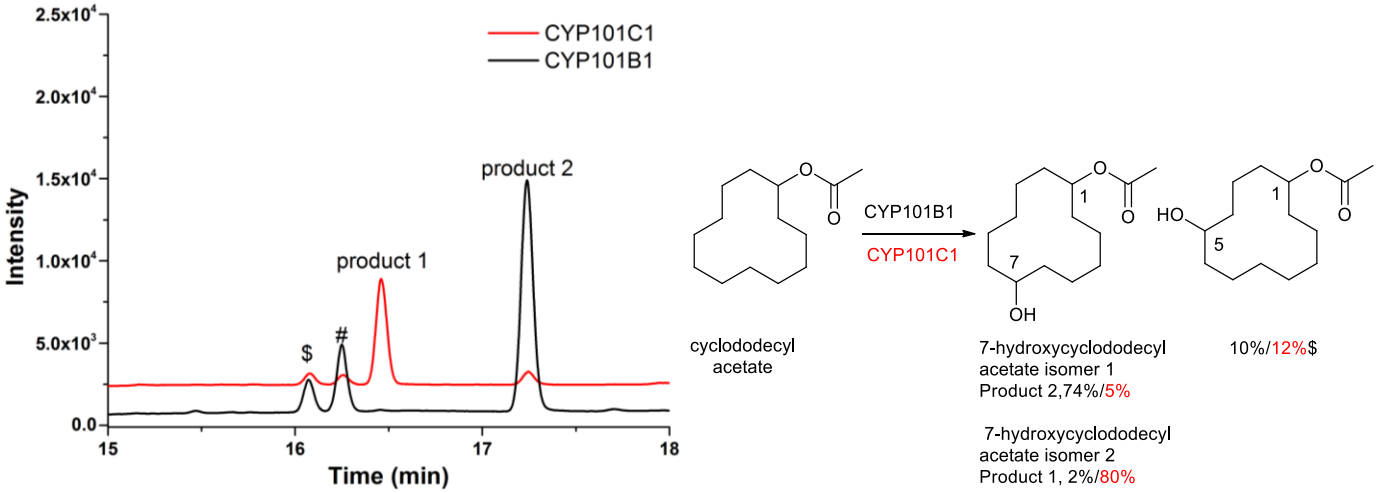


Figure S2 r GC analyses of *in vitro* turnovers of cyclododecyl acetate by CYP101B1 and CYP101C1. Cyclododecyl acetate (RT 5.6 min; not shown) and the products; product 2 has been identified as 7-hydroxycyclododecyl acetate (RT 17.3 min; *trans* isomer) [4]. The other metabolites have been proposed as being 5-hydroxycyclododecyl acetate[$] (RT 16.2 min) and unidentified metabolite[#] (RT 16.4 min); and 7-hydroxycyclododecyl acetate (RT 16.5 min; *cis* isomer) [4].

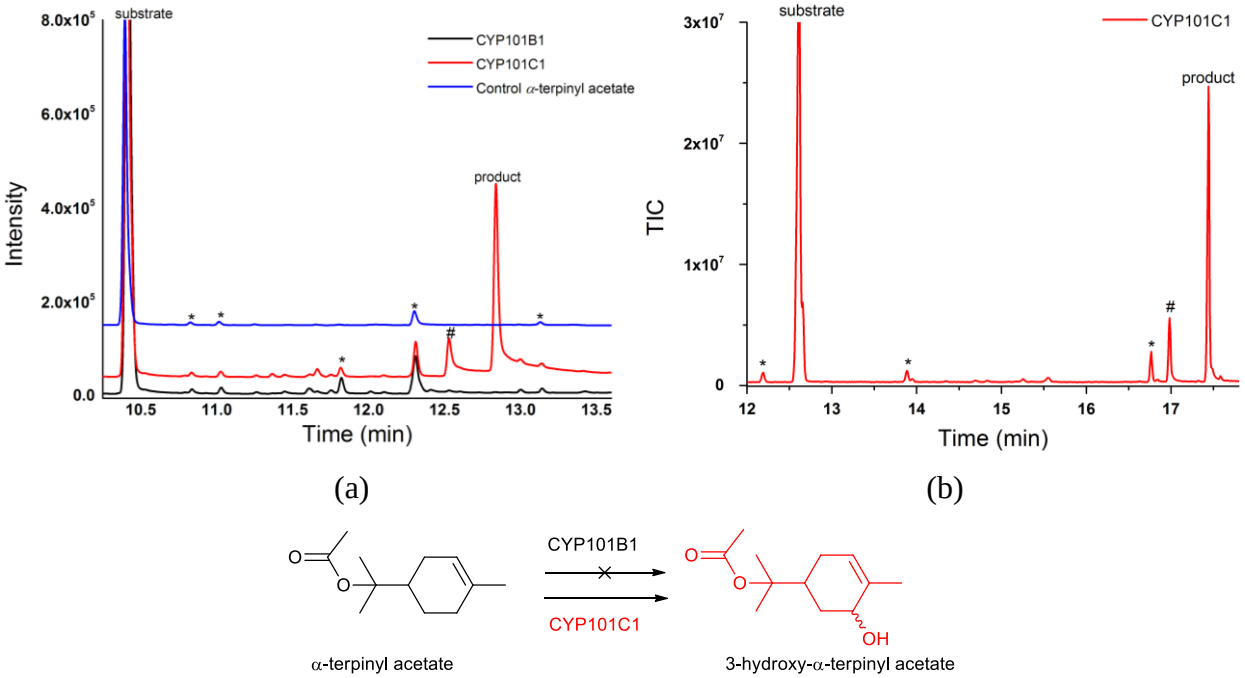


Figure S2 s (i) GC analysis of the *in vitro* turnover of *α*-terpinyl acetate by CYP101B1 and CYP101C1. *α*-Terpinyl acetate (RT 10.4 min) and the products; 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate (5-hydroxy-α-terpinyl acetate; RT 12.8 min; CYP101C1) and its other diastereomer[#] (RT 12.5 min). **(ii)** GC-MS analysis of the *in vitro* turnover of *α*-terpinyl acetate by CYP101C1. *α*-Terpinyl acetate (RT 12.6 min) and the product; 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate (RT 17.4 min; CYP101C1). Based on the NMR the other peaks arising could either be the other diastereomer[#] (RT 17.0 min) of this product or arise from ester hydrolysis. The impurities are marked (*).

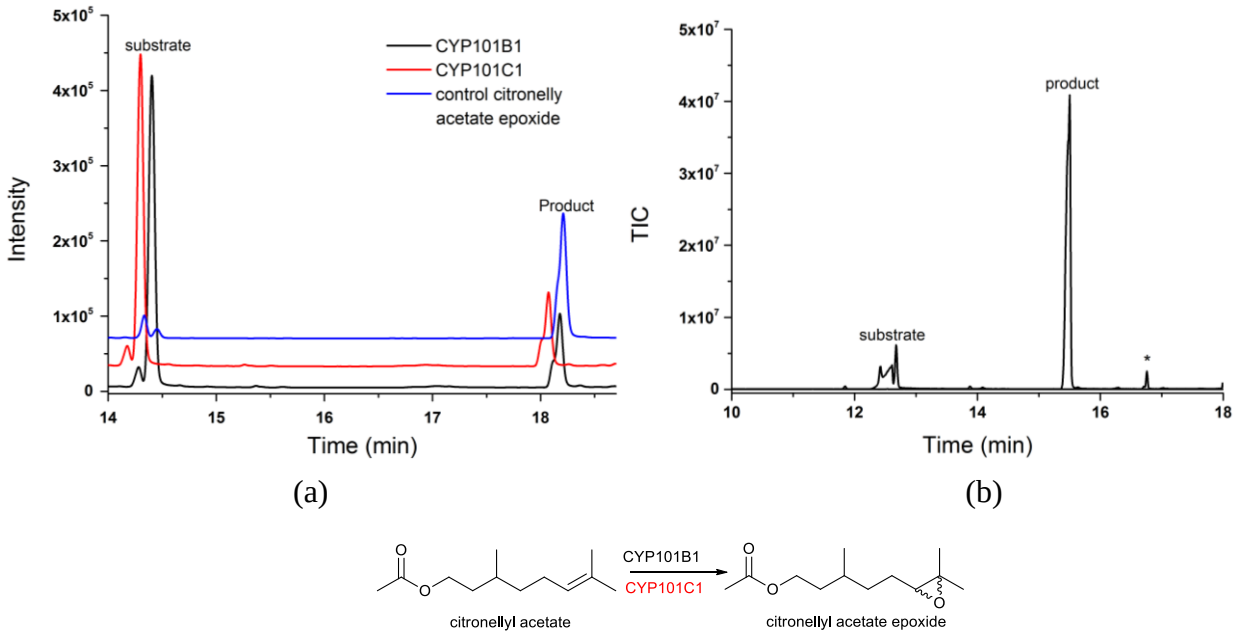
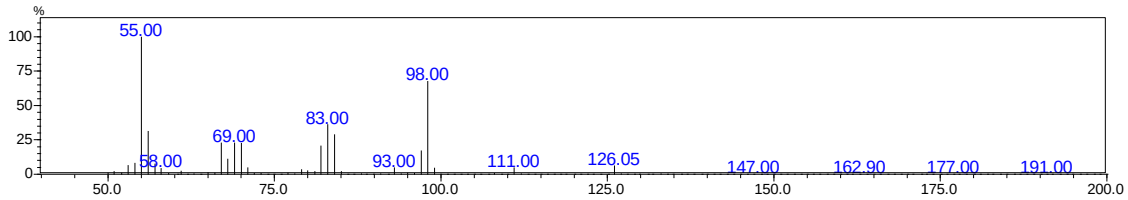


Figure S2 t (i) *In vitro* turnover of citronellyl acetate by CYP101B1 and CYP101C1, coeluted with a synthesised epoxide in GC. Citronellyl acetate (RT 14.4 min) and the product; citronellyl acetate epoxide (RT 18.3 min). The chromatogram (CYP101C1) was offset along the x and y-axes for clarity. **(ii)** GC-MS analysis of epoxide synthesis reaction of citronellyl acetate. Citronellyl acetate (RT 12.7 min) and the product; citronellyl acetate epoxide (RT 15.5 min). The impurities are marked (*).

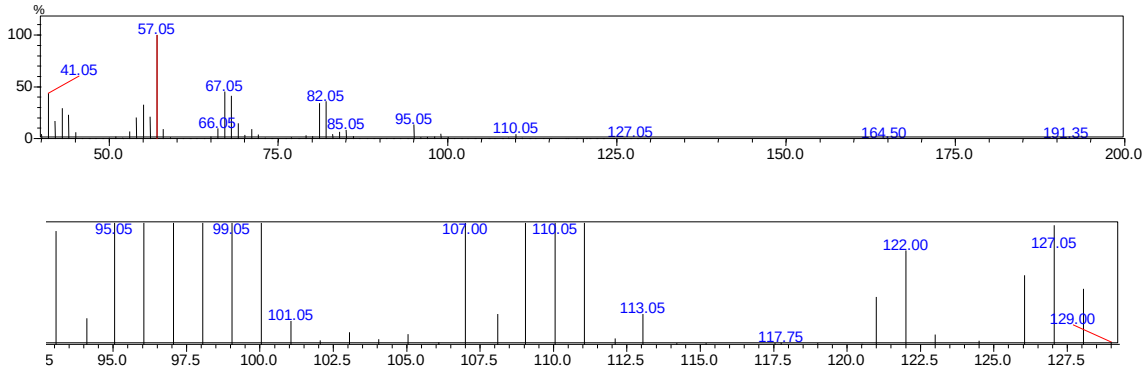
Figure S3 MS analysis of the substrates and products

Figure S3 a MS analysis of cyclooctane products

MS analysis of cyclooctanone RT 6.6 min (m^+/z = 126.05)



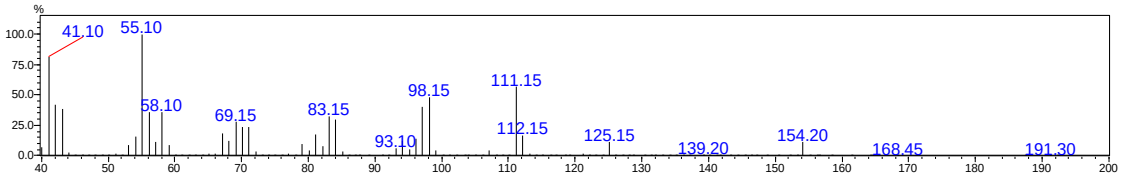
MS analysis of cyclooctanol RT 6.9 min (m^+/z = 128.05)



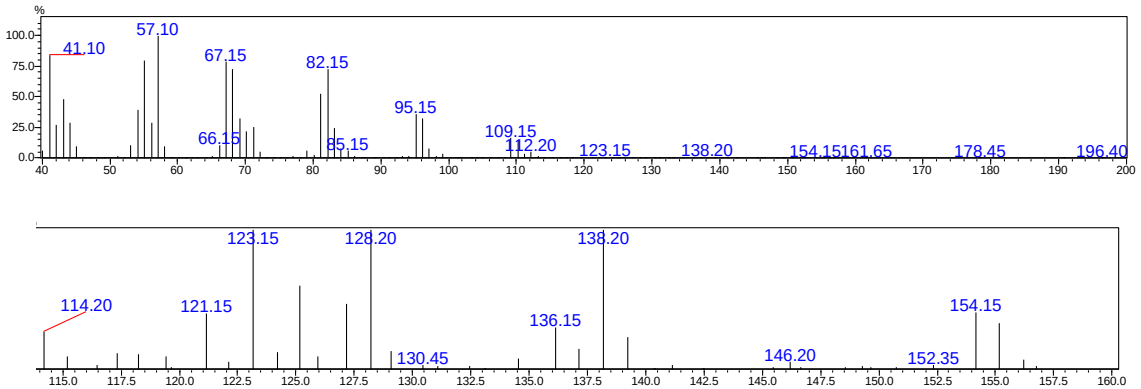
zoomed in version

Figure S3 b MS analysis of cyclodecane and its products

MS analysis of cyclodecanone RT 9.7 min (m^+/z = 154.20)



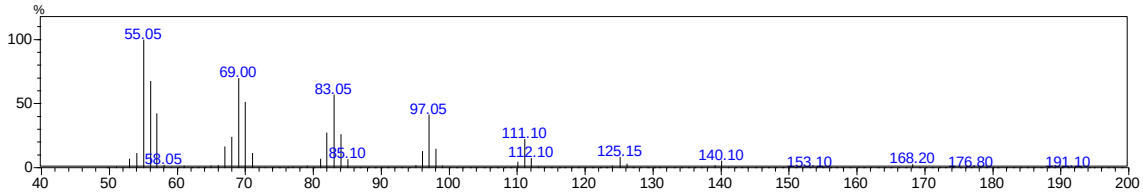
MS analysis of cyclodecanol RT 10.45 min (m^+/z = 156.20)



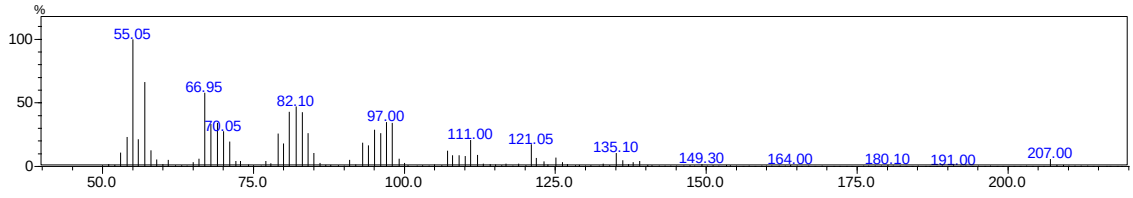
zoomed in version

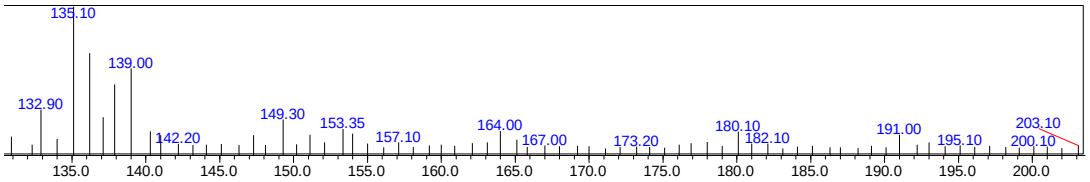
Figure S3 c MS analysis of cyclododecane, cyclododecanol and their products

MS analysis of cyclododecane RT 6.8 min (m^+/z = 168.20)



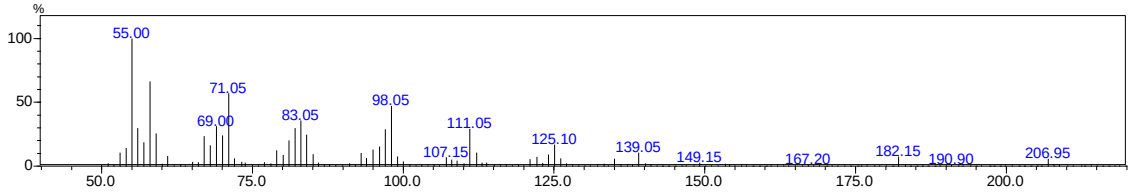
MS analysis of 1,7-cyclododecanediol RT 12.9 min (m^+/z = 200.10)



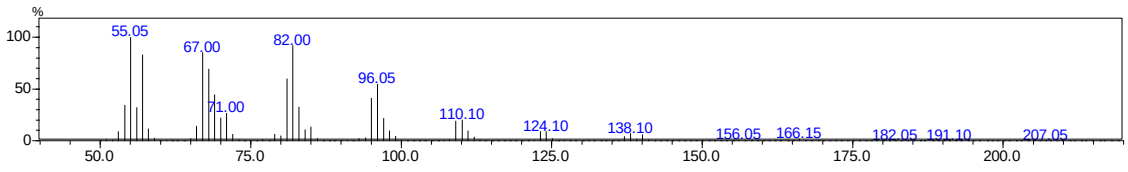


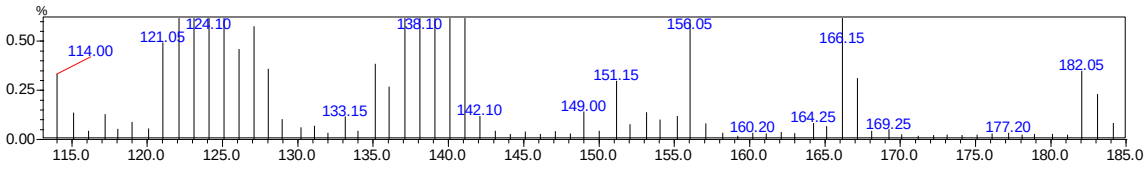
zoomed in version

MS analysis of cyclododecanone RT 7.6 min (m^+/z = 182.15)



MS analysis of cyclododecanol RT 7.9 min (m^+/z = 184.2)

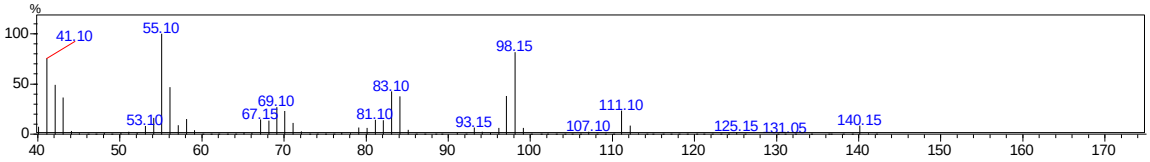




zoomed in version

Figure S3 d MS analysis of cyclononanone and its products

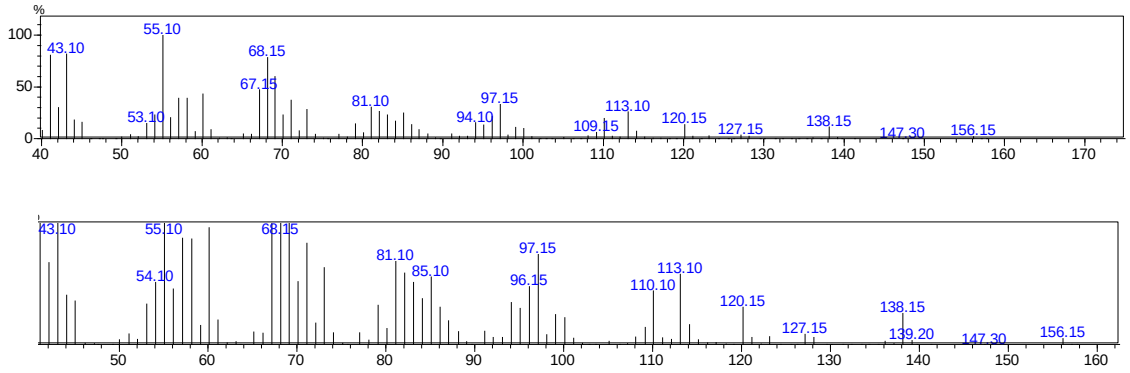
MS analysis of cyclononanone RT 9.1 min (m^+/z = 140.15; CYP101B1)



MS analysis of 1-hydroxy-10-oxabicyclo[4.3.1]decane RT 11.2 min (m^+/z = 156.15) [5].

Experimental m^+/z = 156.15, 138.15, 120.15, 113.10, 97.15, 94.10, 81.10, 71.10, 69.15, 68.15, 67.15, 55.10, 43.10.

Reported [5] m^+/z = 156 (17), 113 (64), 110 (66), 97 (81), 71 (63), 69 (84), 68 (97), 55 (100).

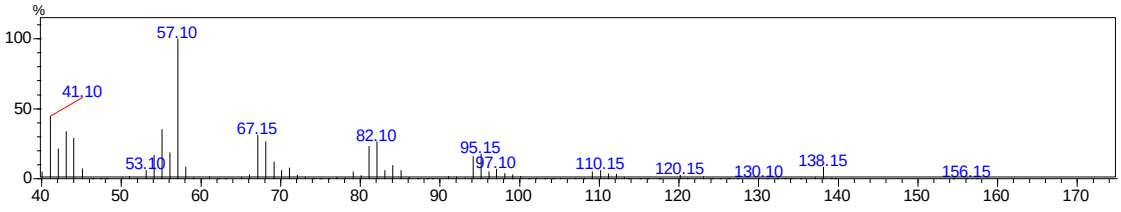


zoomed in version

MS analysis of 2-hydroxycyclononanone RT 12.2 min (m^+/z = 156.15; CYP101C1) [6].

Experimental m^+/z = 156.15, 138.15, 120.15, 110.15, 95.15, 94.10, 82.10, 67.15, 57.10, 41.10.

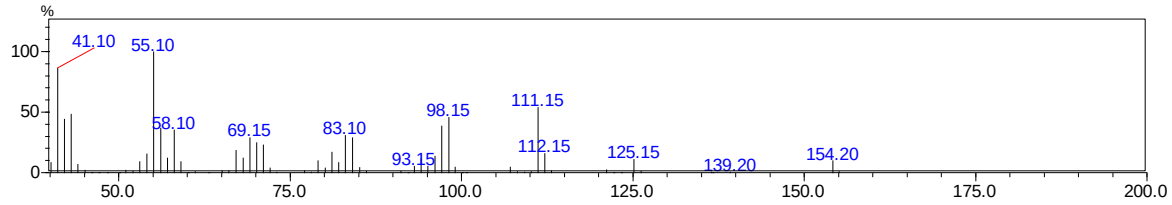
Reported m^+/z = 156, 138, 120, 110, 95, 95, 82, 67, 57, and 41 (WSS: Spectral data were obtained from Wiley Subscription Services, Inc. (US)).



zoomed in version

Figure S3 e MS analysis of cyclodecanone and its products

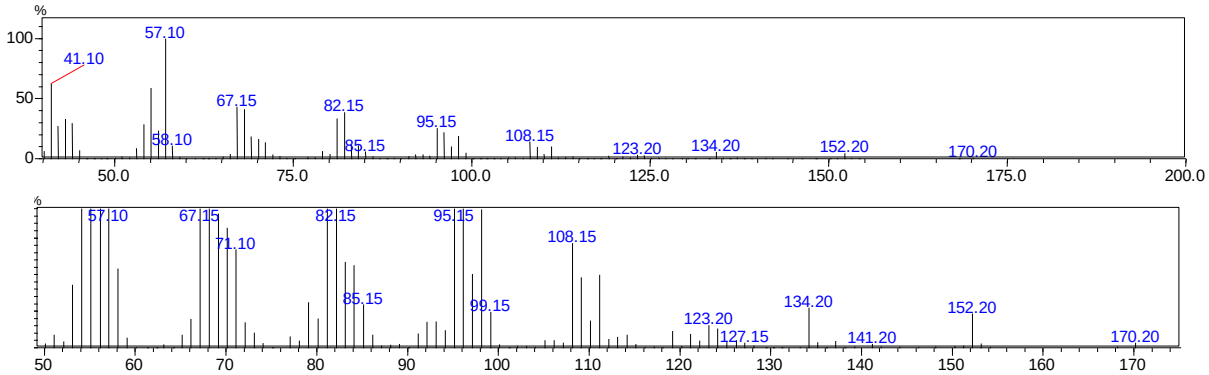
MS analysis of cyclodecanone RT 7.5 min (m^+/z = 154.20; GC-FID RT 2.9 min).



MS analysis of 2-hydroxycyclodecanone RT 10.6 min (m^+/z = 170.2; CYP101C1; GC-FID RT 7.2 min).

Experimental m^+/z = 170.2, 152.20, 134.2, 111.1, 108.15, 98.1, 96.10, 95.15, 82.15, 81.10, 67.15, 57.10 and 41.10.

Reported [7] m^+/z = 170, 152, 134, 111, 98, 81, 57 and 41.



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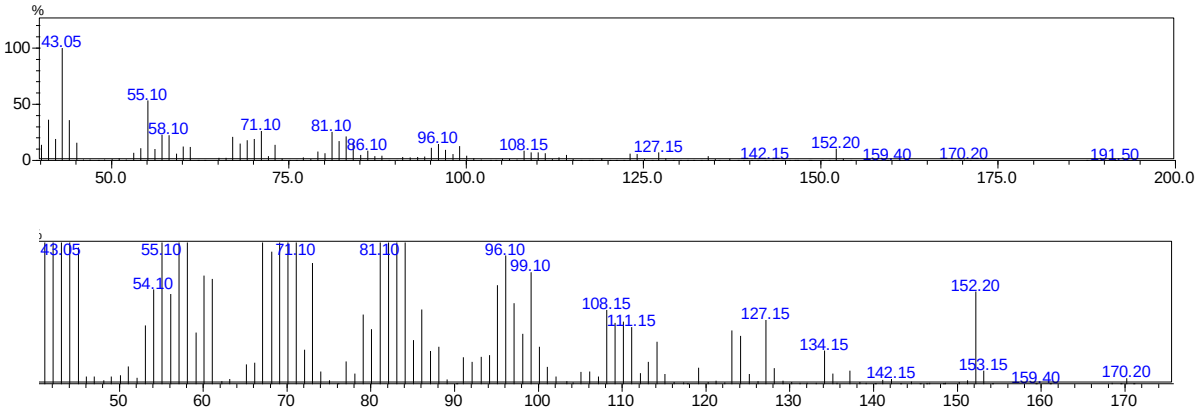
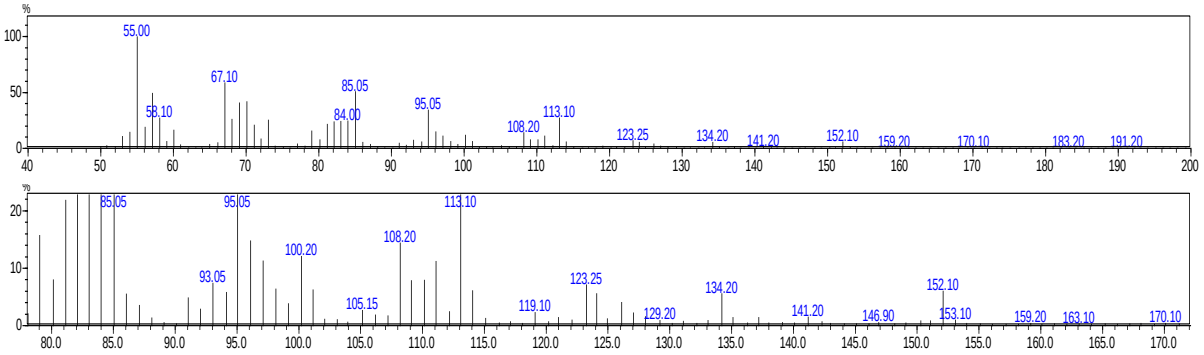
MS analysis of 1-oxabicyclo[5.3.1]undecan-1-ol and 5-hydroxycyclodecanone RT 13.4 min (m^+/z = 170.20; CYP101B1; GC-FID RT 10.0 min) [1].



zoomed in version

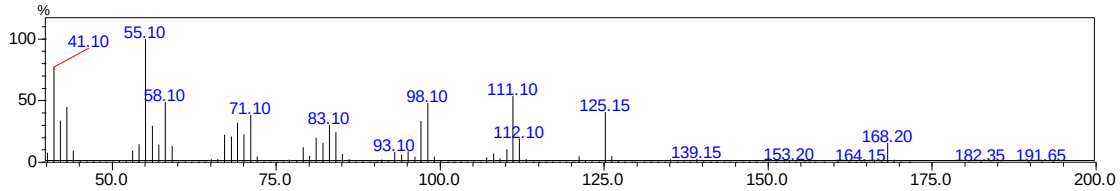
MS analysis of 1-oxabicyclo[6.3.1]undecan-1-ol and 6-hydroxycyclodecanone RT 13.7 min (m^+/z = 170.1; CYP101B1; GC-FID RT 10.9 min).



zoomed in version

Figure S3 f MS analysis of cycloundecanone and its products

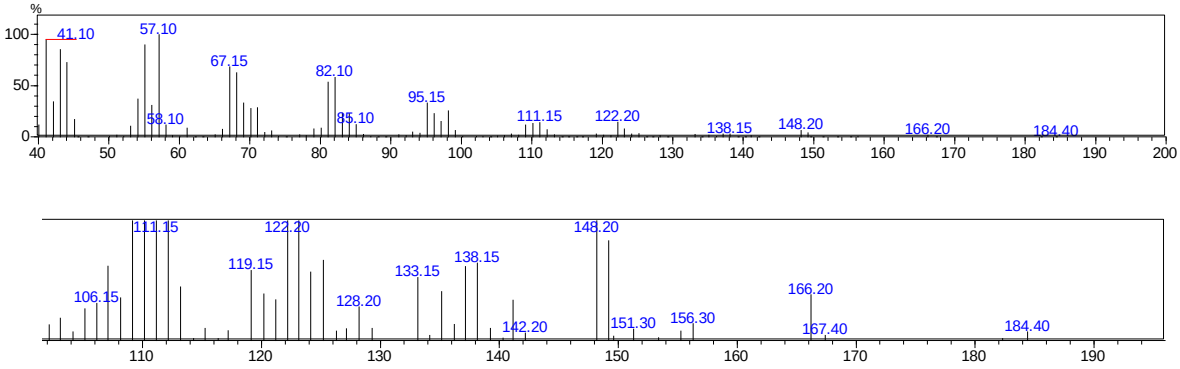
MS analysis of cycloundecanone RT 6.4 min (m^+/z = 168.20).



MS analysis of 2-hydroxycycloundecanone RT 8.4 min (m^+/z = 184.40; CYP101C1).

Experimental m^+/z = 184.40, 166.20, 148.20, 138.15, 133.15, 122.20, 119.15, 111.15, 98.15, 95.15, 82.10, 81.15, 68.10, 67.15, 57.10, 55.15 and 41.10.

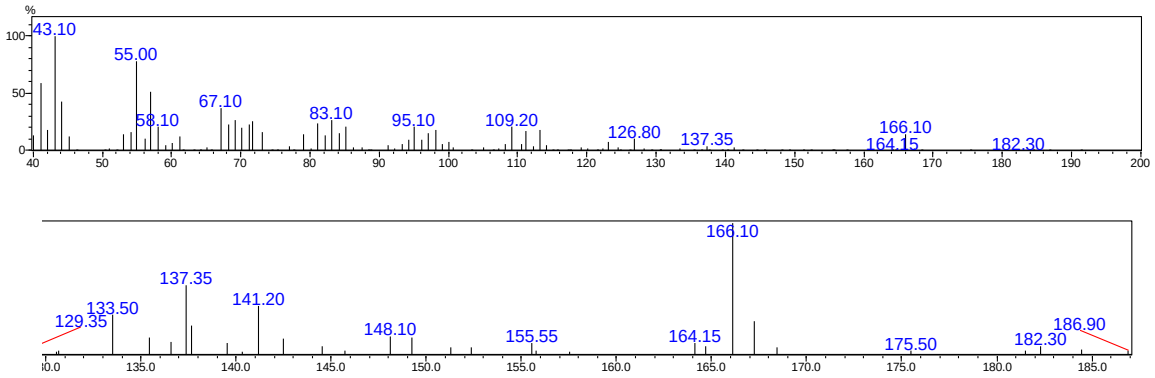
Reported [2] m^+/z = 184, 119, 111, 98, 85, 81, 68 and 55.



zoomed in version

MS analysis of further oxidation product of CYP101B1 turnover at RT 8.8 min (m^+/z = 182.30).

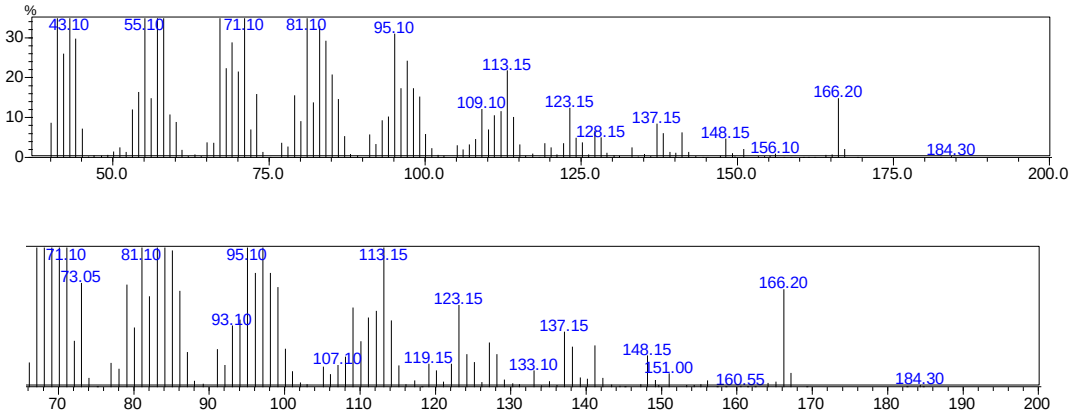
Experimental m^+/z = 182.30, 166.10, 155.55, 148.10, 137.35, 133.50, 109.20, 98.10, 95.10, 83.10, 67.10, 58.10, 55.0 and 43.10.



zoomed in version

MS analysis of product 5-hydroxycycloundecanone RT 9.3 min (m^+/z = 184.3; CYP101B1).

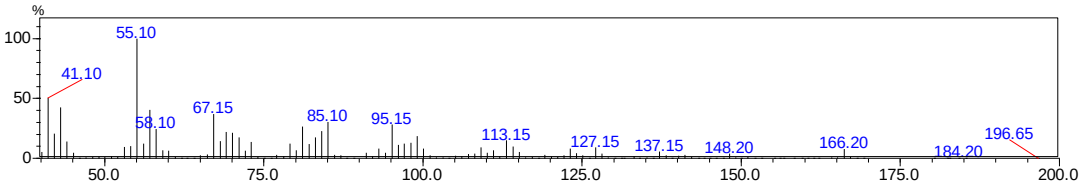
Experimental m^+/z = 184.3, 166.20, 148.15, 137.15, 128.15, 127.15, 123.15, 113.15, 109.10, 81.10, 71.10, 67.15, 55.10 and 41.10.



zoomed in version

MS analysis of product 6-hydroxycycloundecanone RT 9.45 min (m^+/z = 184.2; CYP101B1).

Experimental m^+/z = 184.2, 166.20, 148.20, 137.15, 127.15, 123.15, 113.15, 109.10, 98.15, 95.15, 85.10, 81.15, 67.15, 58.10, 55.10 and 41.10.



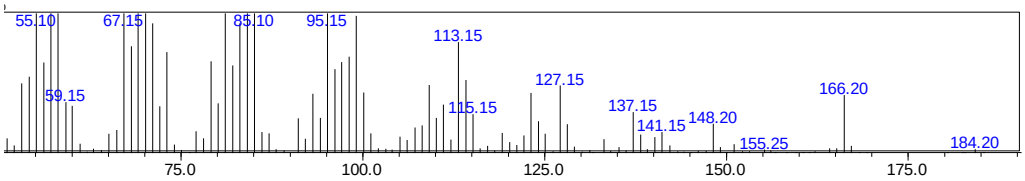


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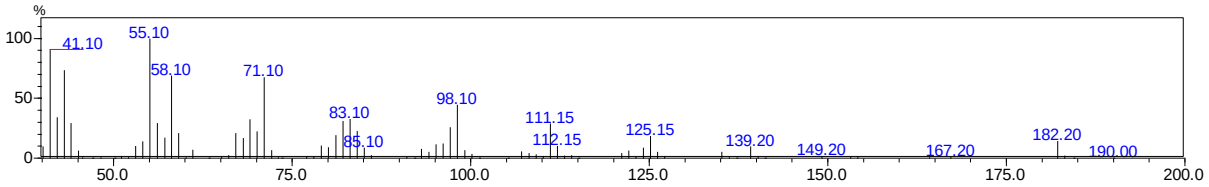
Figure S3 g MS analysis of cyclododecanone and its products

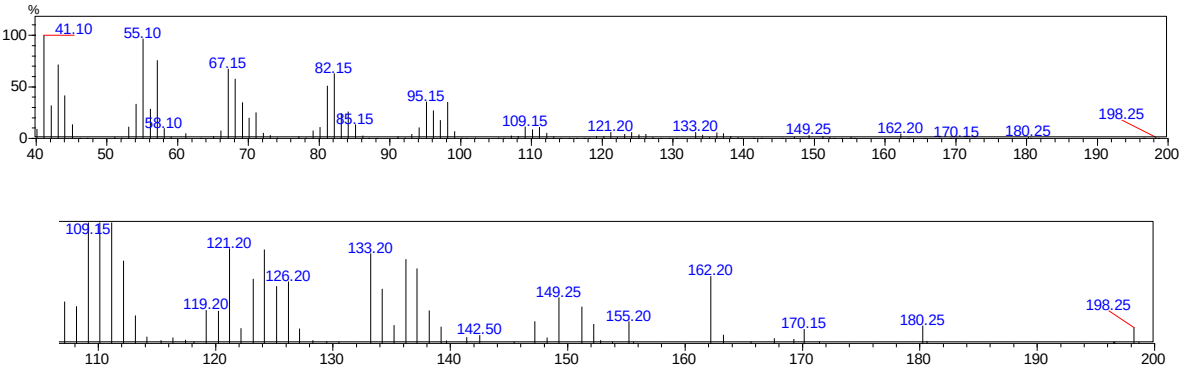
MS analysis of cyclododecanone RT 12.1 min (m^+/z = 182.2).



MS analysis of 2-hydroxycyclododecanone RT 15.5 min (m^+/z = 198.25; CYP101C1) [2, 7].

Experimental m^+/z = 198.25, 180.25, 170.15, 162.20, 149.25, 136.10, 133.20, 124.10, 123.10, 133.20, 121.20, 111.10, 109.15, 98.15, 95.15, 82.15, 81.10, 68.10, 67.15, 57.10, 55.10 and 41.10.

Reported [2] m^+/z = 198, 180, 136, 124, 111, 98, 96, 95, 82, 81, 68, 67 and 57.



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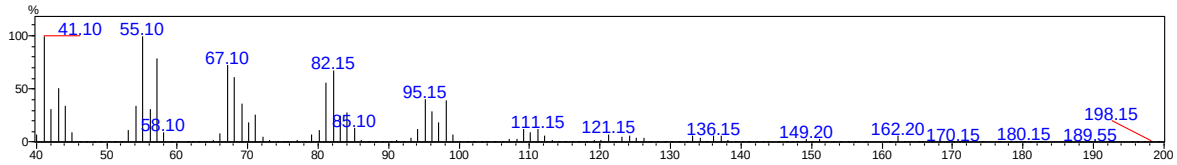
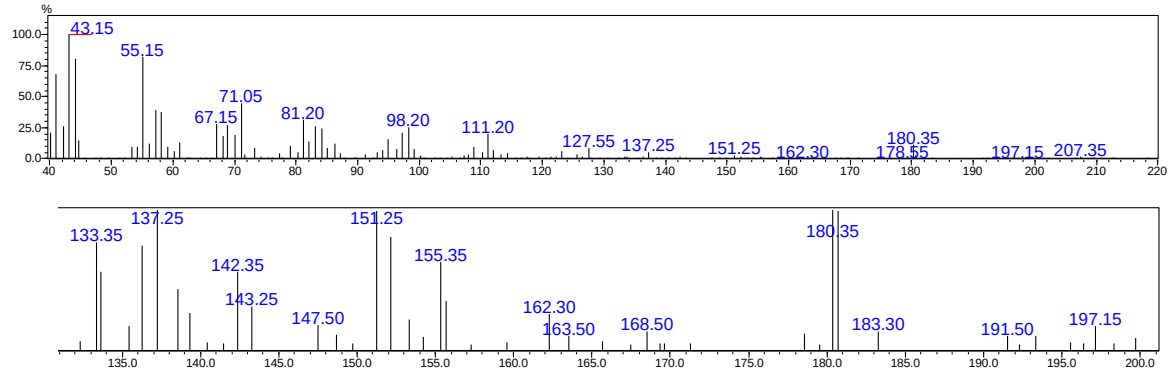
MS analysis of 2-hydroxycyclododecanone standard (m^+/z = 198.15).



MS analysis of minor unidentified product at RT 16.9 min (m^+/z = 198.35; CYP101B1).

Experimental m^+/z = 198.35, 180.35, 162.30, 155.35, 151.25, 137.25, 127.55, 111.20, 98.20, 81.20, 71.05, 67.15. 55.15 and 43.15

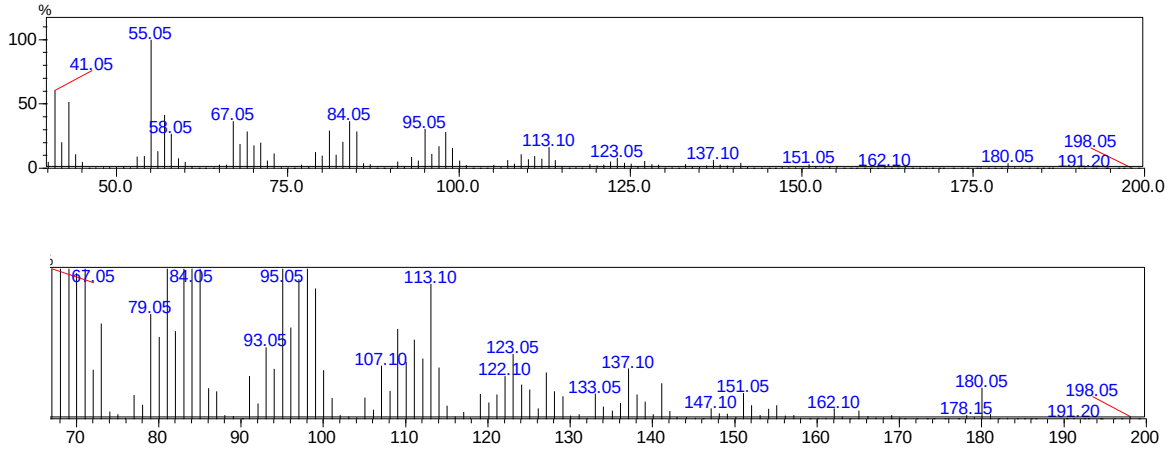


zoomed in version

MS analysis of 7-hydroxycyclododecanone RT 17.7 min (m^+/z = 198.50; CYP101B1) [3].

Experimental m^+/z = 198.05, 180.05, 162.10, 151.05, 137.1, 123.05, 113.1, 95.05, 84.05, 84.05, 81.10, 67.05. 55.00 and 41.10

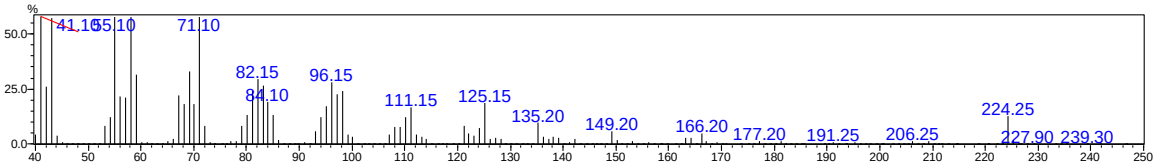
Reported [3] m^+/z = 198, 180, 109, 81 and 55.



zoomed in version

Figure S3 h MS analysis of cyclopentadecanone and its products

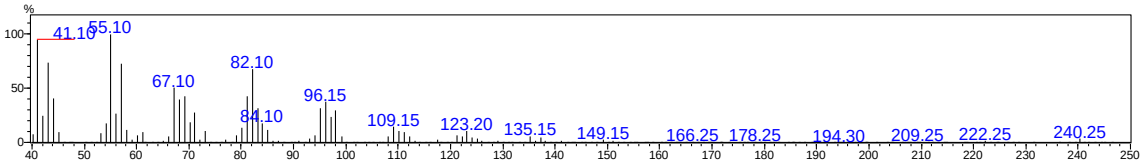
MS analysis of cyclopentadecanone RT 13.1 min (m^+/z = 224.25).

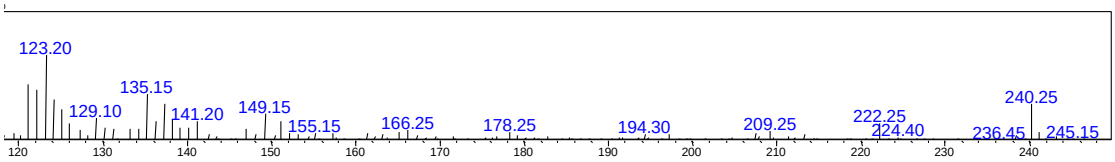


MS analysis of 2-hydroxycyclopentadecanone RT 14.6 min (m^+/z = 240.25; CYP101C1) [7, 8].

Experimental m^+/z = 240.25, 222.25, 166.25, 152.10, 149.15, 135.15, 124.10, 123.20, 110.10, 109.15, 96.15, 82.10, 67.10, 55.10 and 41.10.

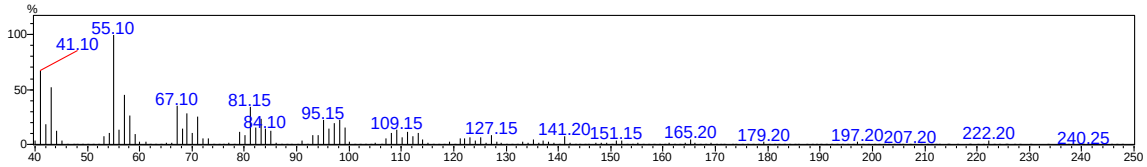
Reported [7, 8] m^+/z = 240, 222, 166, 152, 138, 124, 110, 96, 82 and 55.





zoomed in version

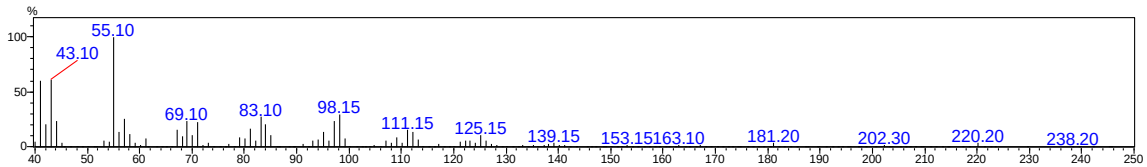
MS analysis of 8-hydroxycyclopentadecanone RT 15.6 min (m^+/z = 240.25; CYP101B1) [3].

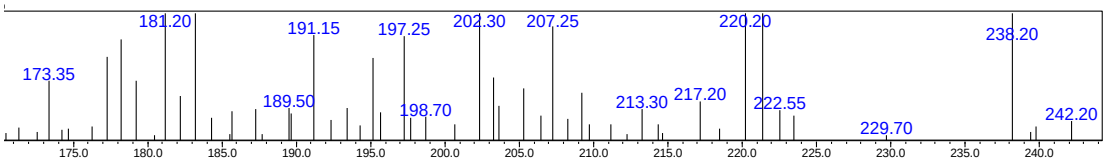


MS analysis of cyclopentadecanone minor product (cyclopentadecane-1,8-dione) CYP101B1 RT 15.1 min (m^+/z = 238.20; CYP101B1) [3].

Experimental m^+/z = 238.20, 220.20, 181.20, 125.15, 112.10, 111.15, 98.15, 84.15, 83.10, 69.10, 55.10 and 43.10.

Reported [3] m^+/z = 238, 181, 126, 112, 111, 98 and 84.





zoomed in version

Figure S3 i MS analysis of cyclohexyl acetate product

MS analysis of *trans*-4-hydroxycyclohexyl acetate RT 10.6 min (m^+/z = 158.00)

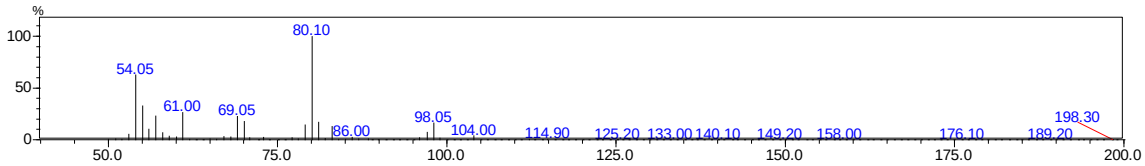
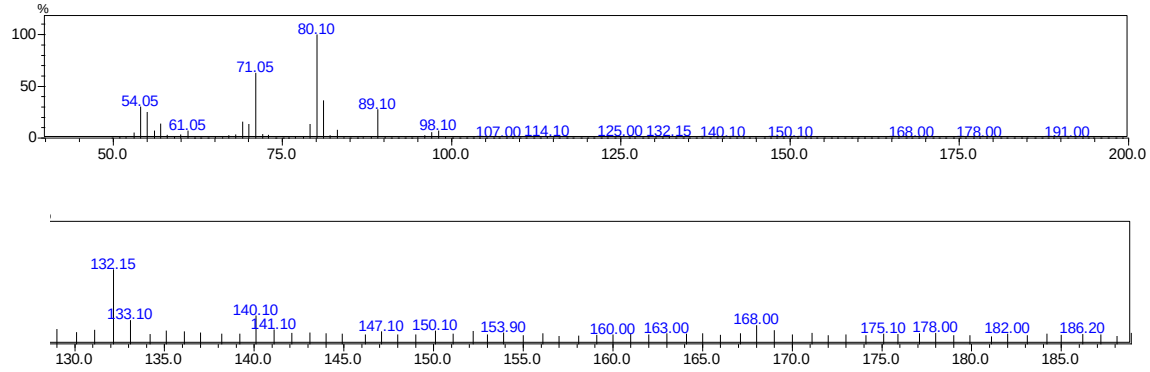


Figure S3 j MS analysis of cyclohexyl butyrate product

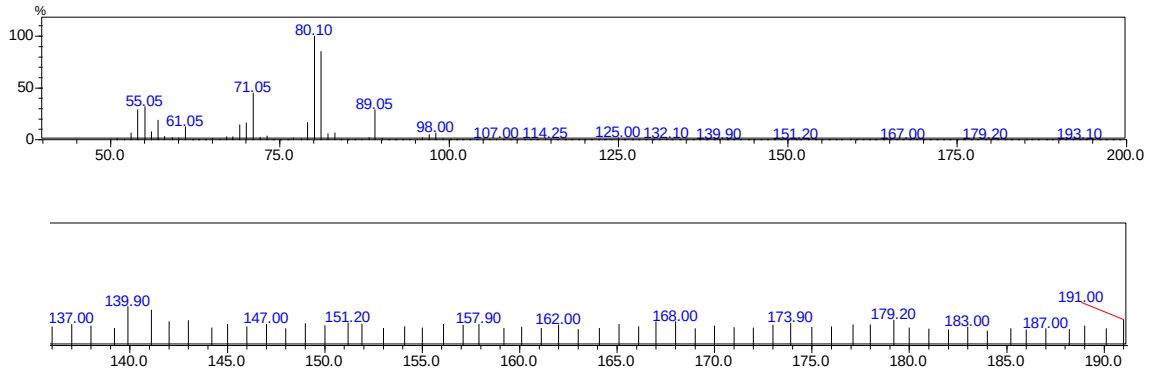
MS analysis of *trans*-4-hydroxy cyclohexyl butyrate RT 8.5 min (m^+/z = 186.20)



zoomed in version

Figure S3 k MS analysis of cyclohexyl isobutyrate product

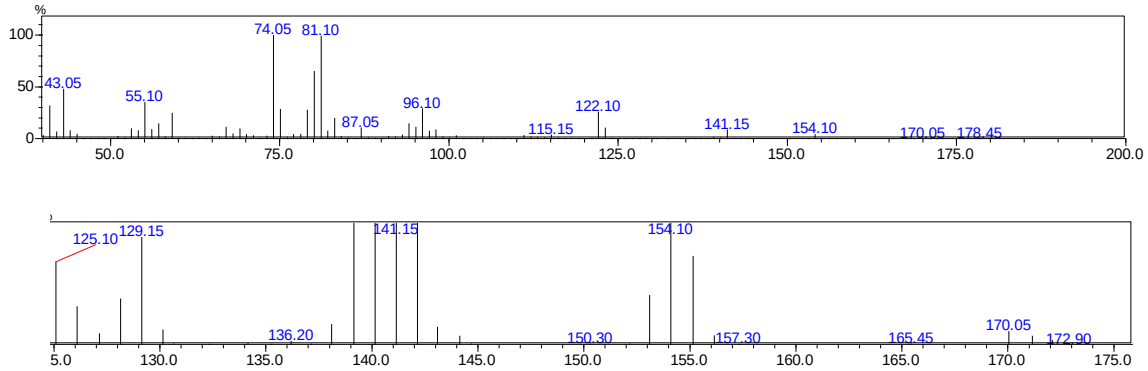
MS analysis of *trans*-4-hydroxycyclohexyl isobutyrate RT 7.9 min (m^+/z =186.0)



zoomed in version

Figure S3 l MS analysis of methyl cyclohexylacetate product

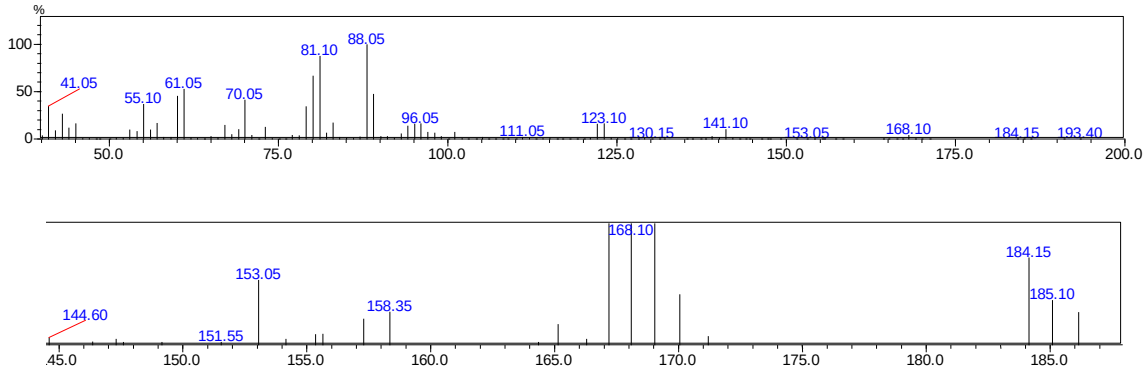
MS analysis of methyl-2-(*trans*-4-hydroxycyclohexyl)acetate RT 9.9 min (m^+/z = 172.0; RT 18.5 min in GC-FID).



zoomed in version

Figure S3 m MS analysis of ethylcyclohexyl acetate products

MS analysis of ethyl-*trans*-2-(4-hydroxycyclohexyl)acetate RT 7.0 min (m^+/z = 186.15; RT 20.1 min in GC-FID).



zoomed in version

MS analysis of further oxidation product RT 6.9 min (m^+/z = 184.25).

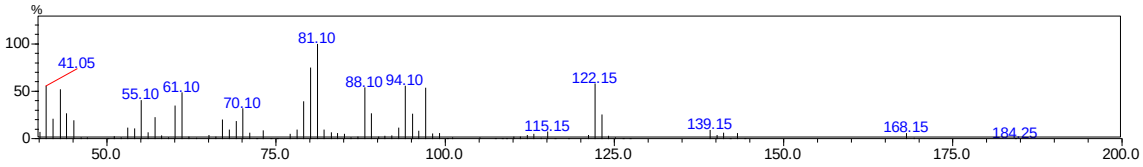
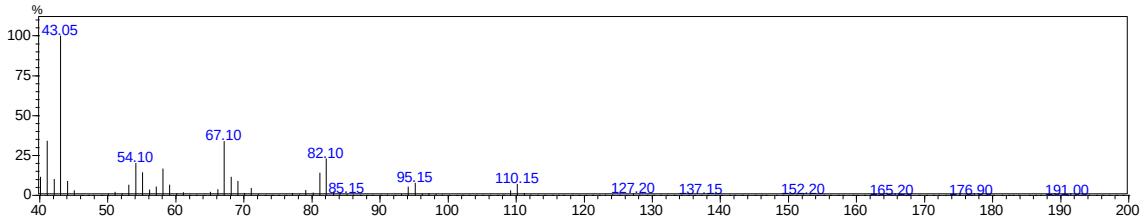
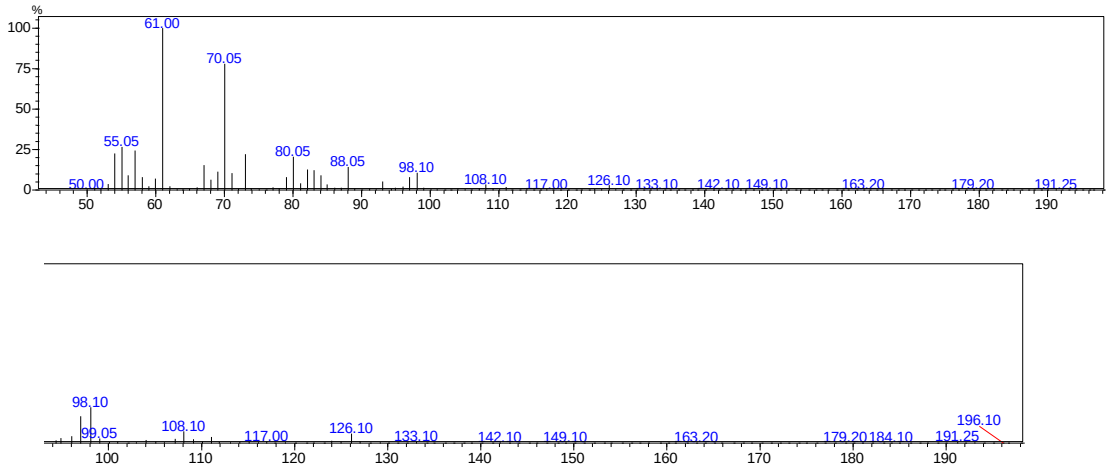


Figure S3 n MS analysis of cyclooctyl acetate and its products

MS analysis of cyclooctyl acetate RT 6.5 min (m^+/z = 170.10).

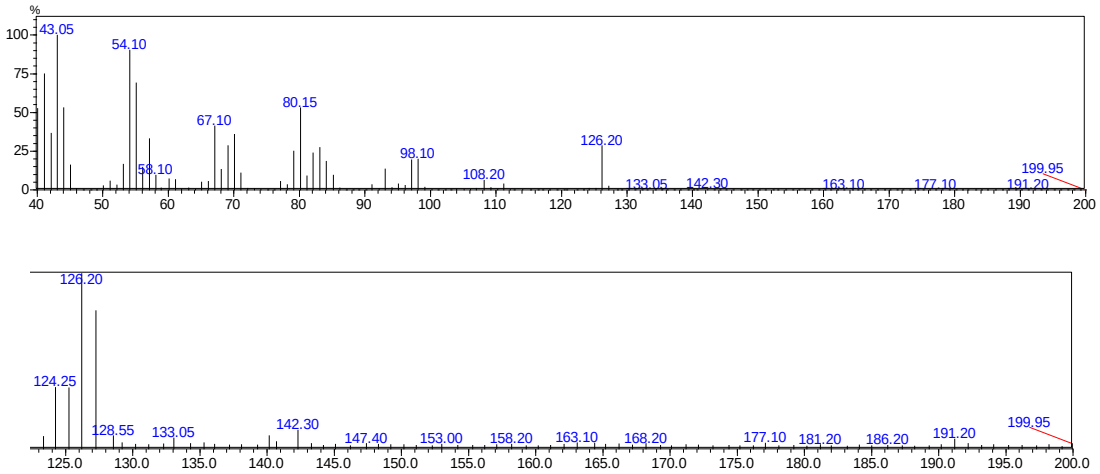


MS analysis of further oxidation product cyclooctyl acetate RT 8.8 min (m^+/z = 184.10; CYP101C1)



zoomed in version

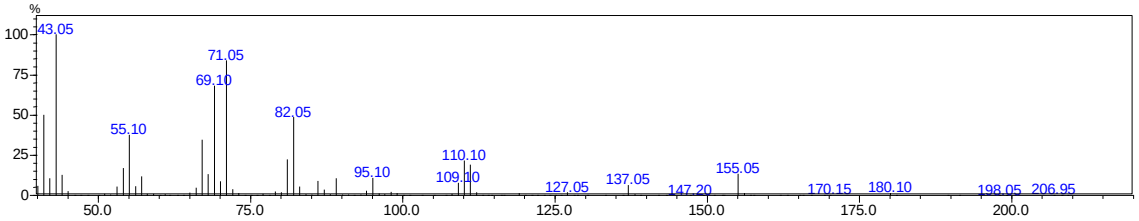
MS analysis of 5-hydroxycyclooctyl acetate RT 8.95 min (m^+/z = 186.20).



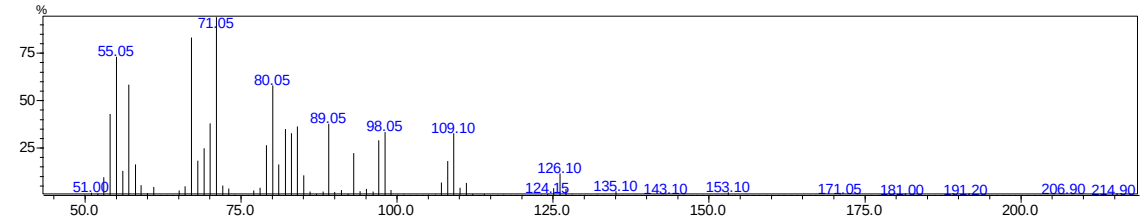
zoomed in version

Figure S3 o MS analysis of cyclooctyl isobutyrate and its products

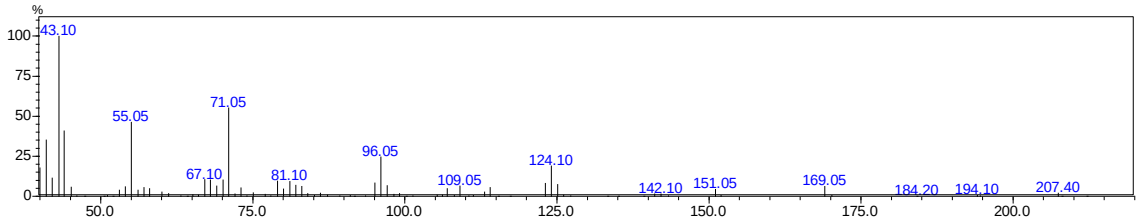
MS analysis of cyclooctyl isobutyrate RT 8.0 min (m^+/z = 198.05).

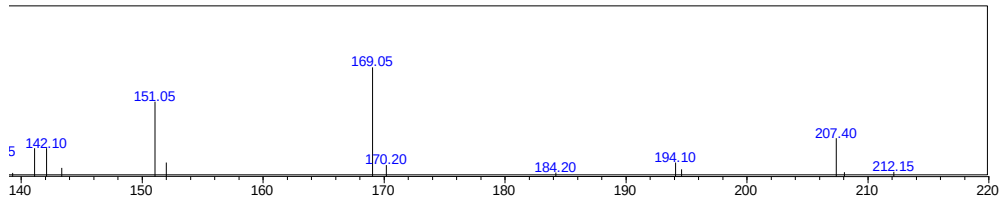


MS analysis of 5-hydroxycyclooctyl isobutyrate RT 10.2 min (m^+/z = 214.9).



MS analysis of further oxidation product of cyclooctyl isobutyrate RT 10.1 min (m^+/z = 212.15).

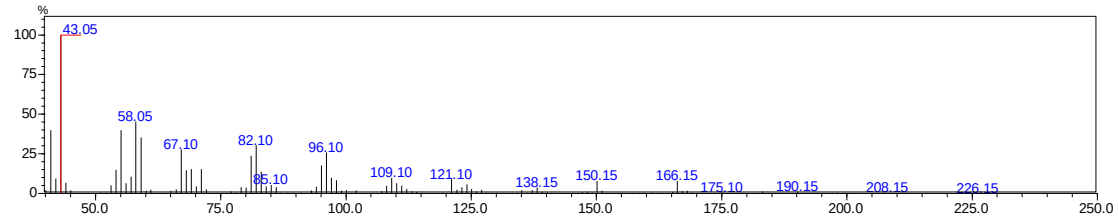




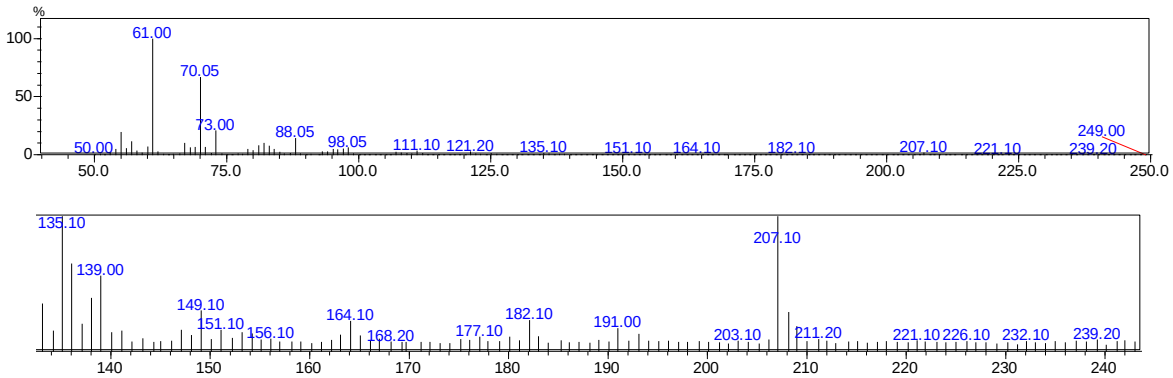
zoomed in version

Figure S3 p MS analysis of cyclododecyl acetate and its products

MS analysis of cyclododecyl acetate RT 9.6 min GC-MS (m^+/z = 226.15; RT 5.6 min in GC).

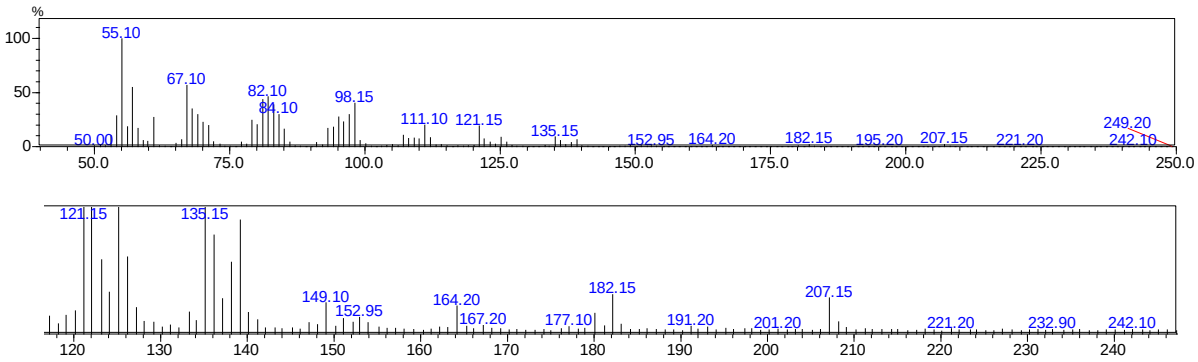


MS analysis of 7-hydroxycyclododecyl acetate (*cis*) RT 12.8 min GC-MS (m^+/z = 242.0; CYP101C1; RT 16.5 min in GC).



zoomed in version

MS analysis of 7-hydroxycyclododecyl acetate (*trans*) RT 13.6 min (m^+/z = 242.10; CYP101B1; RT 17.3 min in GC).



zoomed in version

MS analysis of 5-hydroxycyclododecyl acetate RT 12.4 min (RT 16.2 min GC).

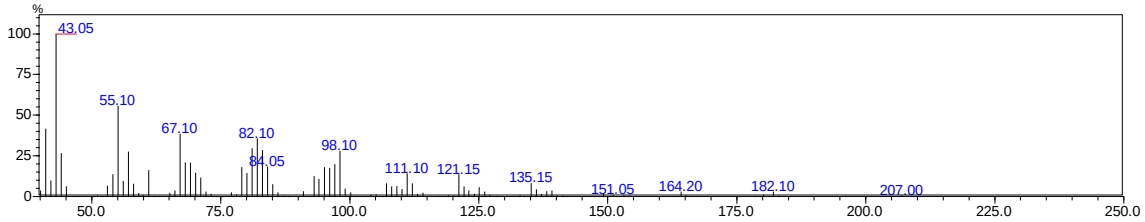
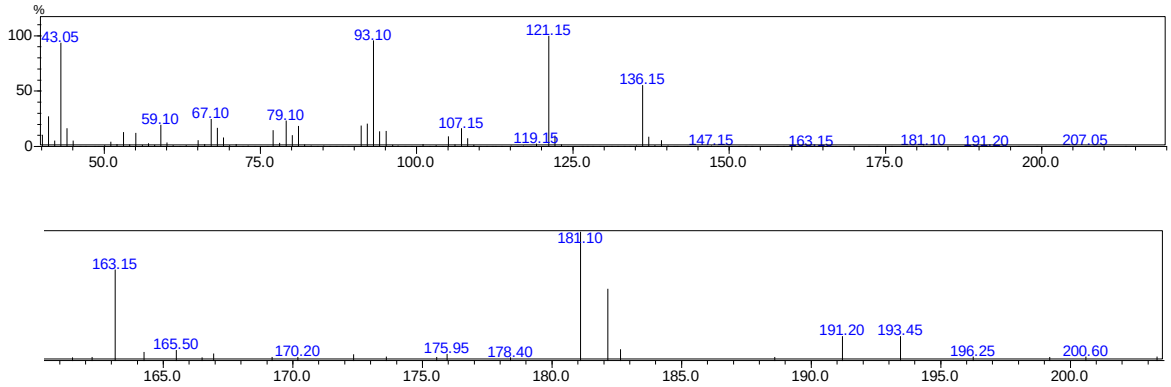


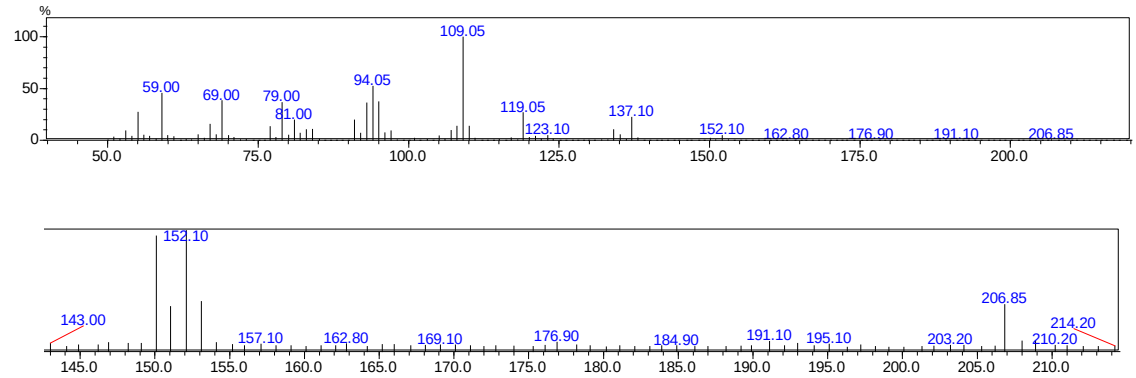
Figure S3 q MS analysis of *α*-terpinyl acetate and its products

MS analysis of *α*-terpinyl acetate RT 12.6 min (m^+/z = 196.25).



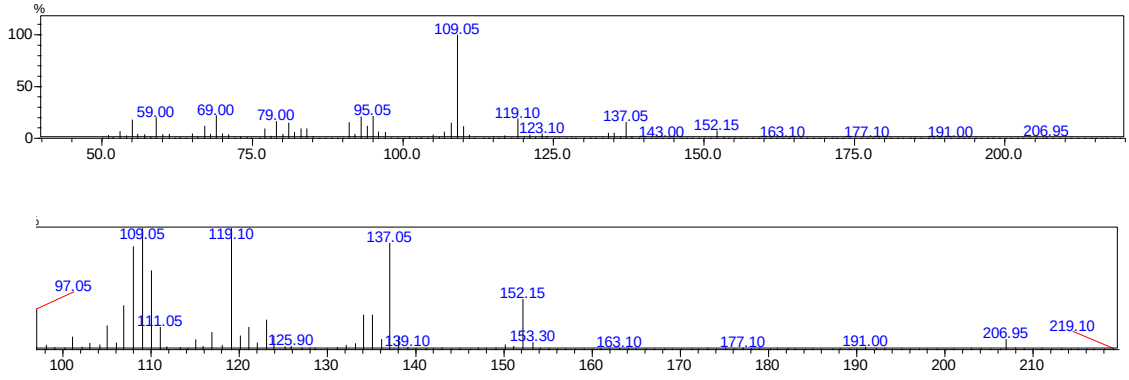
zoomed in version

MS analysis of 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate at RT 17.4 min (m^+/z = 212.10; CYP101C1).



zoomed in version

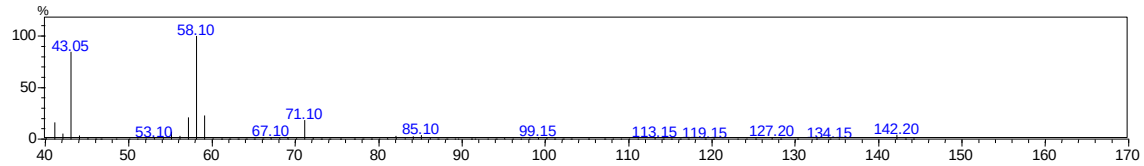
MS analysis of 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate (other diastereomer) at RT 17.0 min (m^+/z = 212.10; CYP101C1).



zoomed in version

Figure S3 r MS analysis of 2-nonanone and its products

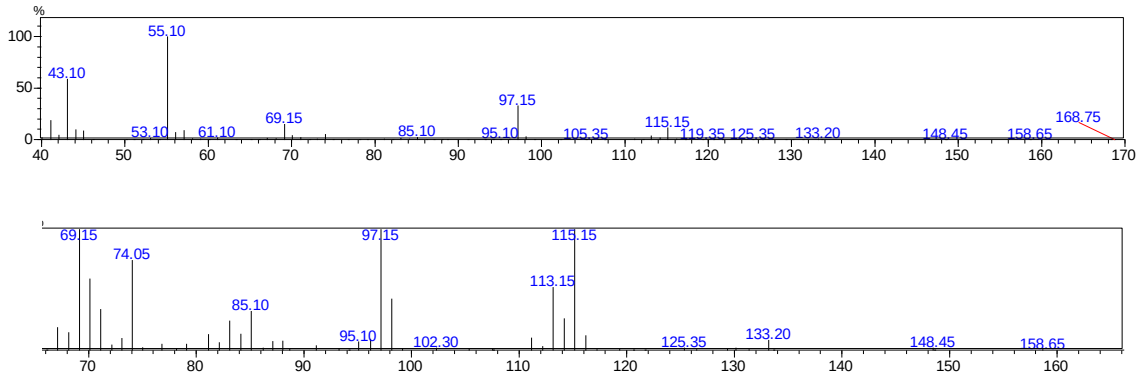
MS analysis of 2-nonanone RT 5.8 min (m^+/z = 142.05).



MS analysis of 3-hydroxy-2-nonanone RT 8.5 min (m^+/z = 158.65; CYP101C1).

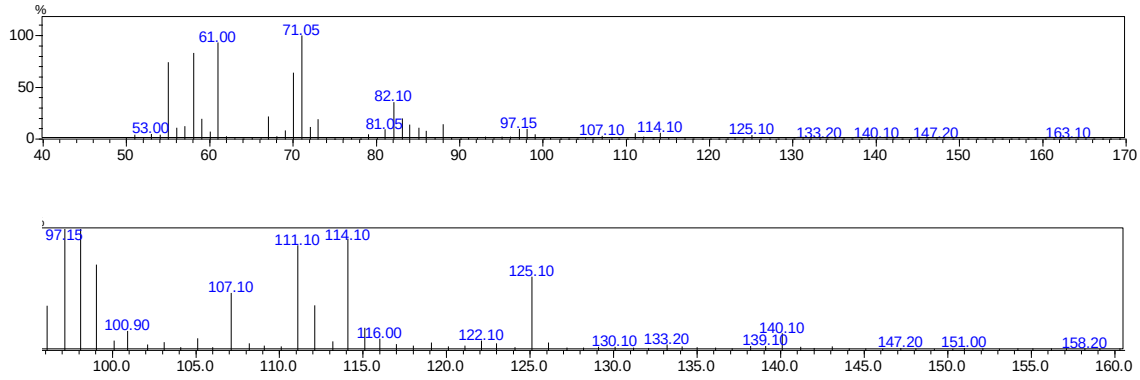
Experimental m^+/z = 158.65, 115.15, 97.15, 85.10, 74.05, 71.15, 69.15, 55.10, 43.10.

MS fragmentation pattern was similar to that of 3-hydroxy-2-undecanone.



zoomed in version

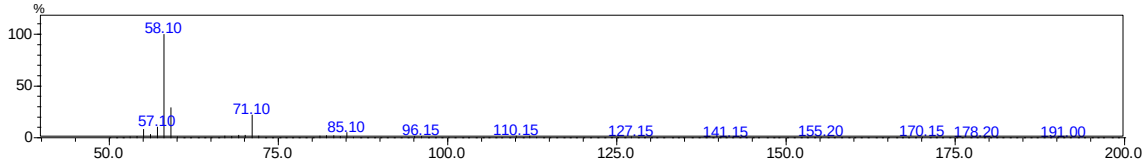
MS analysis of 8-hydroxy-2-nonanone RT 11.25 min (m^+/z = 158.20; CYP101B1).



zoomed in version

Figure S3 s MS analysis of 2-undecanone and its products

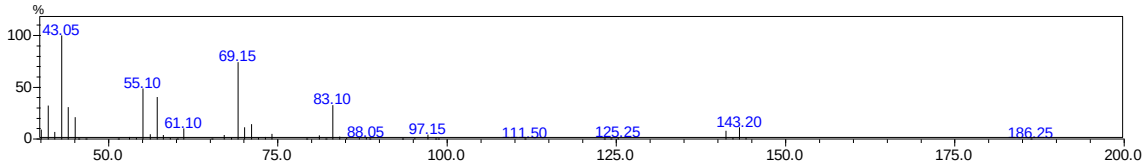
MS analysis of 2-undecanone RT 6.2 min (m^+/z = 170.25).



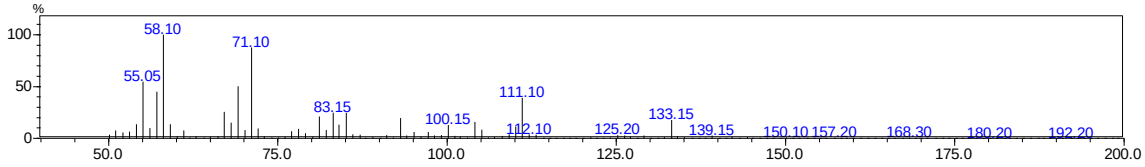
MS analysis of 3-hydroxy-2-undecanone RT 11.5 min (m^+/z = 186.25; CYP101C1) [7].

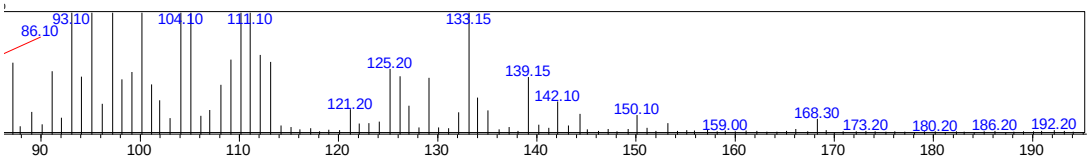
Experimental m^+/z = 186.25, 143.20, 125.25, 111.50, 97.15, 83.10, 69.15, 55.10, 43.05.

Reported [7] m^+/z = 186, 143, 125, 111, 97, 83, 69, 55 and 43.



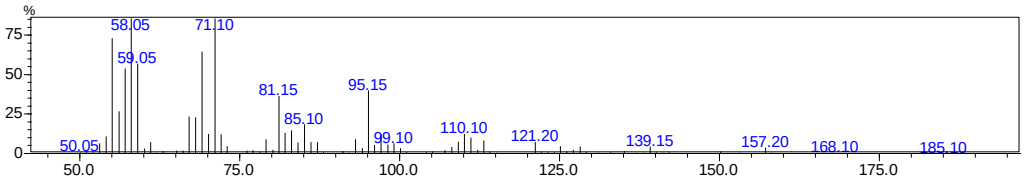
MS analysis of 8-hydroxy-2-undecanone RT 14.95 min (m^+/z = 186.20; CYP101B1).

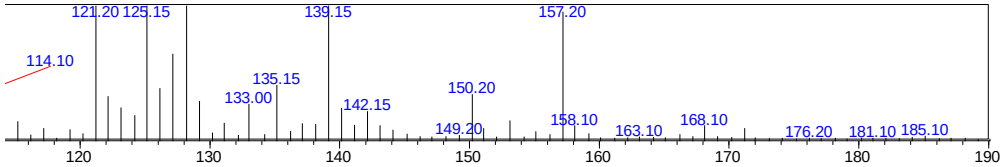




zoomed in version

MS analysis of a mixture of unidentified metabolites of CYP101B1 turnovers at RT 15.2 min and 15.9 min.

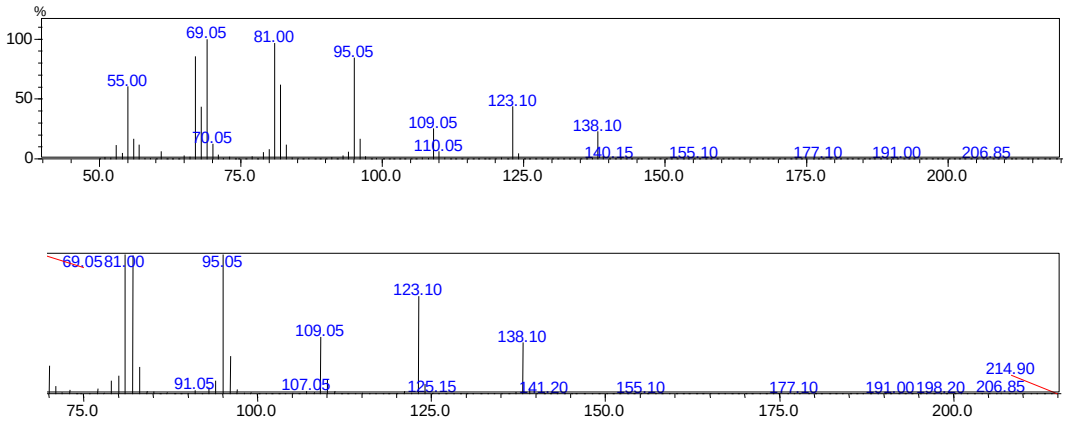




zoomed in version

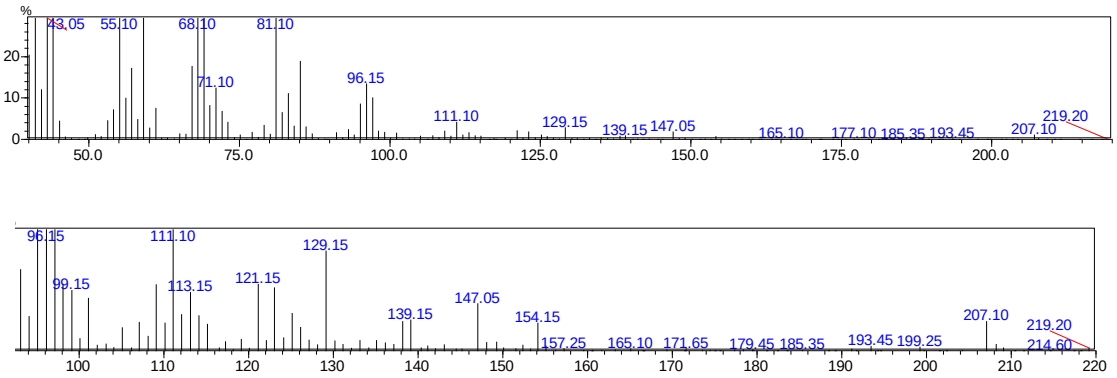
Figure S3 t MS analysis of citronellyl acetate and its product

MS analysis of citronellyl acetate RT 12.7 min (m^+/z = 198.40).



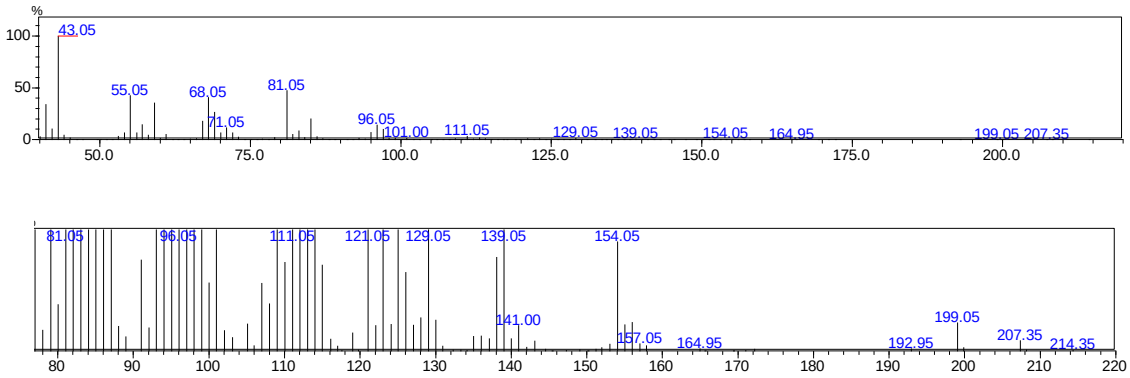
zoomed in version

MS analysis of citronellyl acetate epoxide RT 15.5 min (m^+/z = 214.60).



zoomed in version

MS analysis of citronellyl acetate epoxide synthesised using *m*CPBA RT 15.5 min (m^+/z = 214.35).



zoomed in version

NMR Analysis of CYP101B1 and CYP101C1 Turnover Products

Data for 2-hydroxycyclononanone [6]:

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.28-4.21 (m, 1H, H2), 3.7-3.72 (m, 1H, O**H** (C2)), 2.83-2.73 (m, 1H, H9), 2.37-2.22 (m, 2H, H3 & H9), 2.12-2.01 (m, 1H, H3), 1.89-1.81 (m, 2H, H6 & H8), 1.68-1.57 (m, 3H, H4, H7 & H8), 1.49-1.42 (m, 3H, H4, H6 & H7), 1.39-1.32 (m, 1H, H5), 1.31-1.25 (m, 1H, H5).

^{13}C NMR (126 MHz, $CDCl_3$) δ 219.16 (C1), 79.86 (C2), 39.81 (C9), 31.16 (C3), 27.91 (C4), 27.42 (C6), 27.33 (C8), 26.64 (C5), 23.14 (C7).

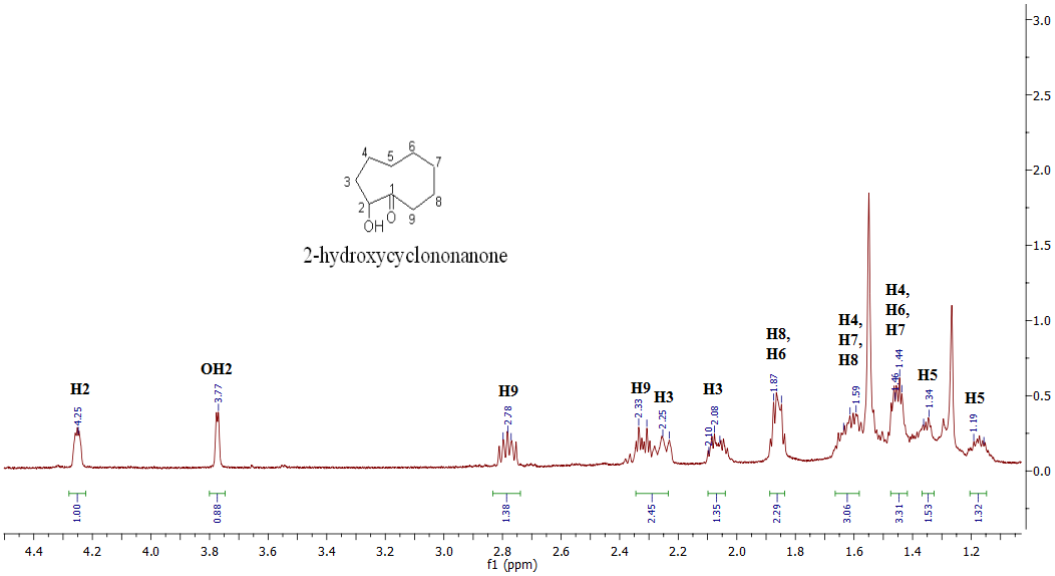
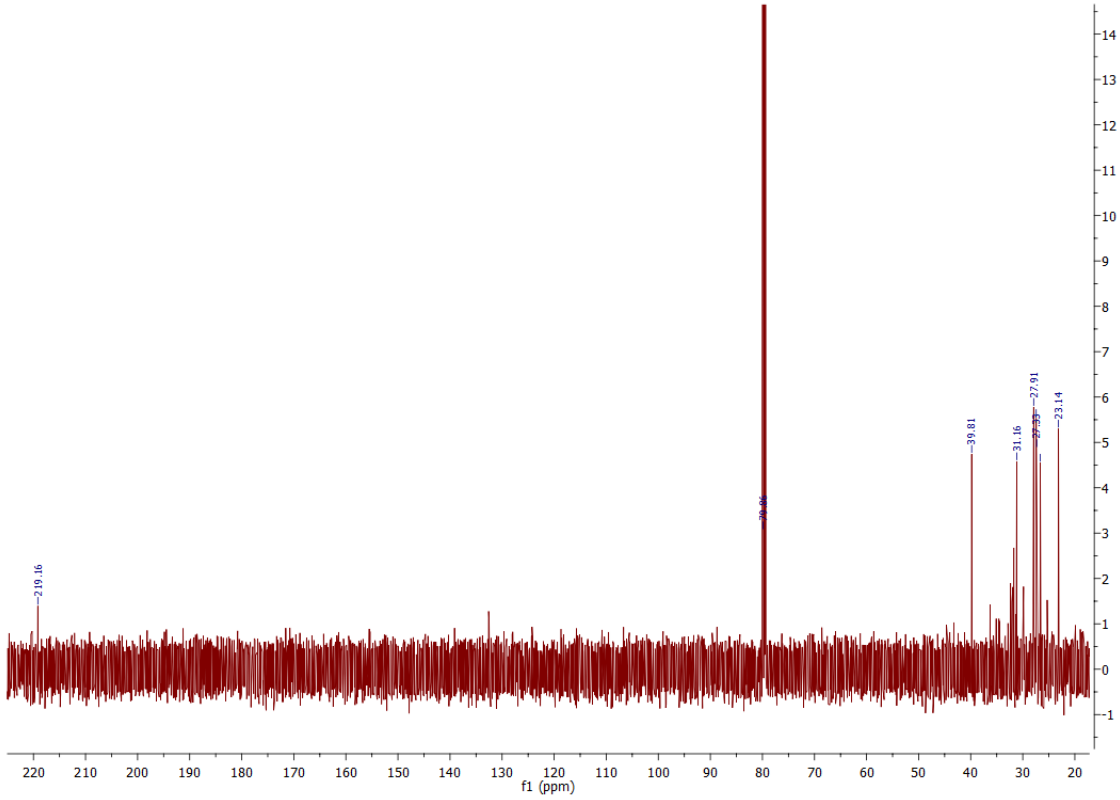
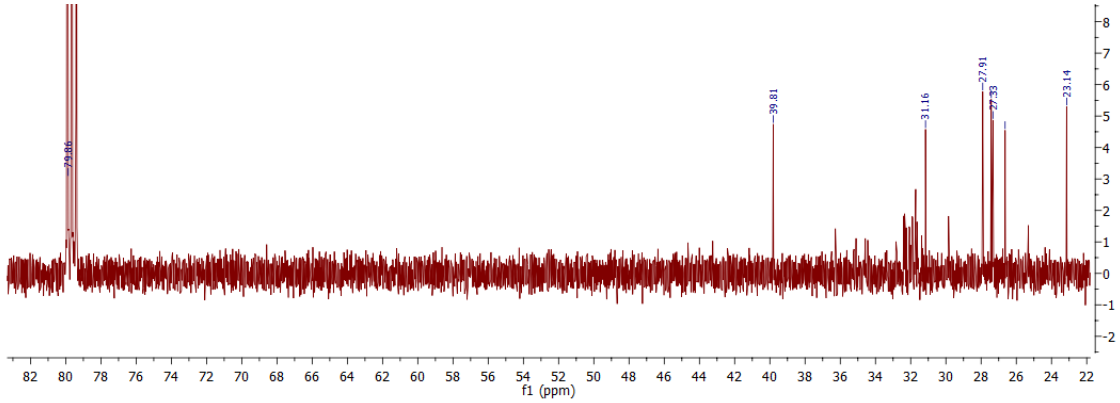


Figure S4 a (i) ^{1}H NMR of 2-hydroxycyclononanone (CYP101C1) [6].



(a)



(b)

Figure S4 a (ii) ^{13}C NMR of 2-hydroxycyclononanone. **(b)** Zoomed in version of the 20 to 80 ppm region to highlight the carbon peaks.

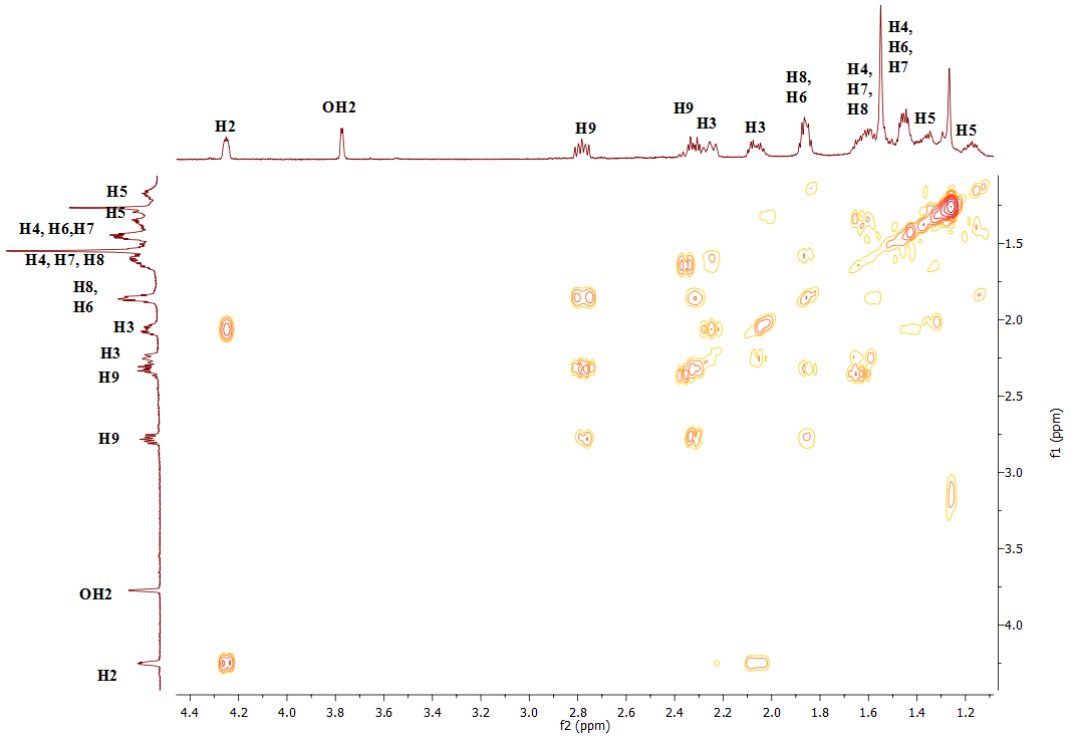


Figure S4 a (iii) gCOSY NMR of 2-hydroxycyclononanone.

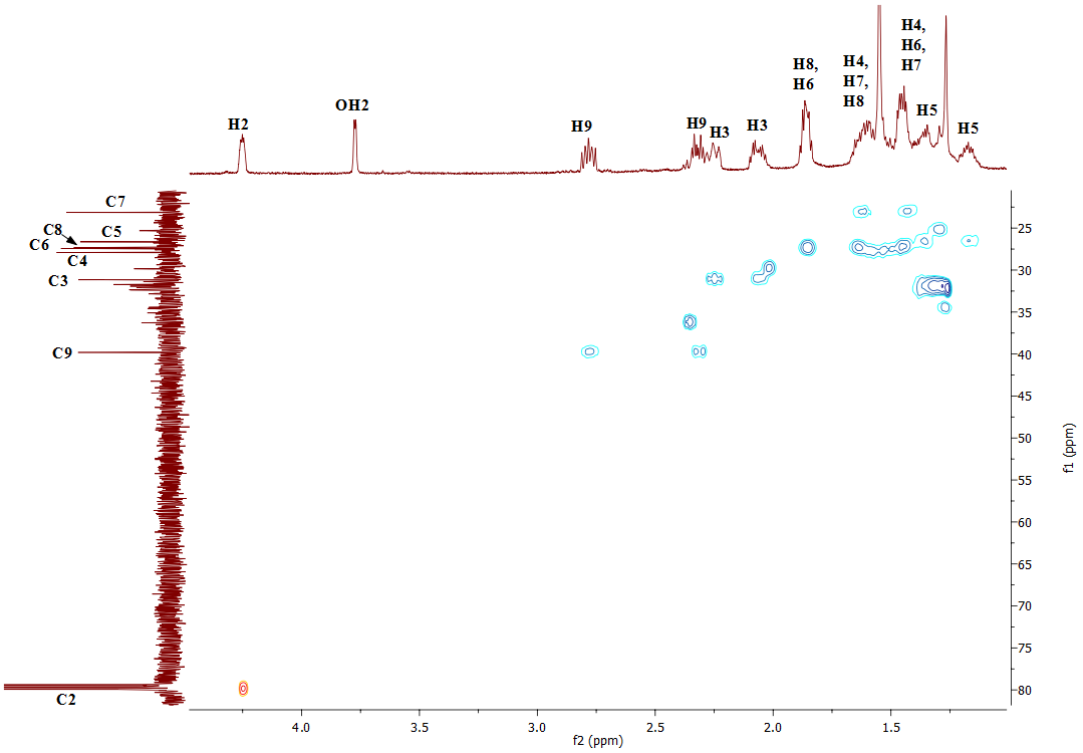


Figure S4 a (iv) HSQC NMR of 2-hydroxycyclononanone.

NMR for 1-hydroxy-10-oxabicyclo[4.3.1]decane [1, 5]:

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.15-4.02 (m, 1H, H5), 2.42 (br s, 1H, O**H**1 (C1)), 2.13-2.03 (m, 1H, H9), 1.95-1.85 (m, 3H, H2, H3 & H8), 1.82-1.70 (m, 4H, H3, H4, H6 & H7), 1.70-1.61 (m, 3H, H6, H7 & H9), 1.55-1.46 (m, 2H, H2 & H8), 1.40-1.34 (m, 1H, H4).

^{13}C NMR (151 MHz, $CDCl_3$) δ 100.36 (C1), 75.11 (C5), 39.22 (C2), 38.48 (C9), 32.85 (C6), 30.26 (C4), 23.92 (C7), 22.60 (C8), 20.21 (C3).

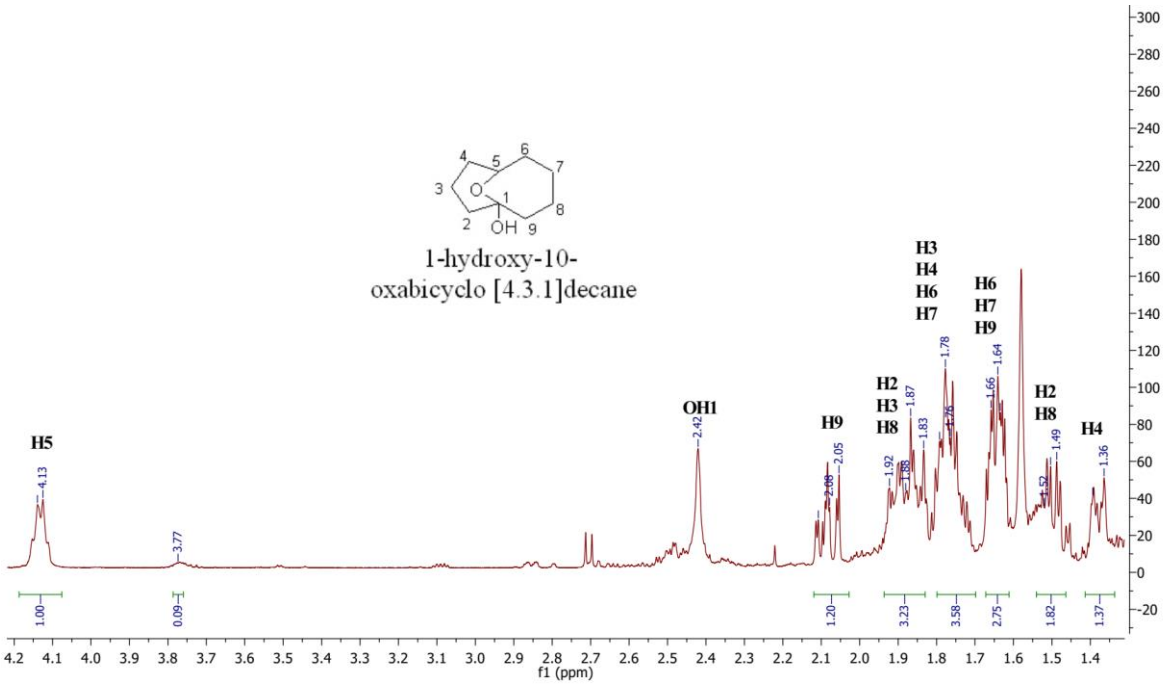


Figure S4 b (i) ^{1}H NMR of 1-hydroxy-10-oxabicyclo[4.3.1]decane (cyclononanone; CYP101B1) [1, 5].

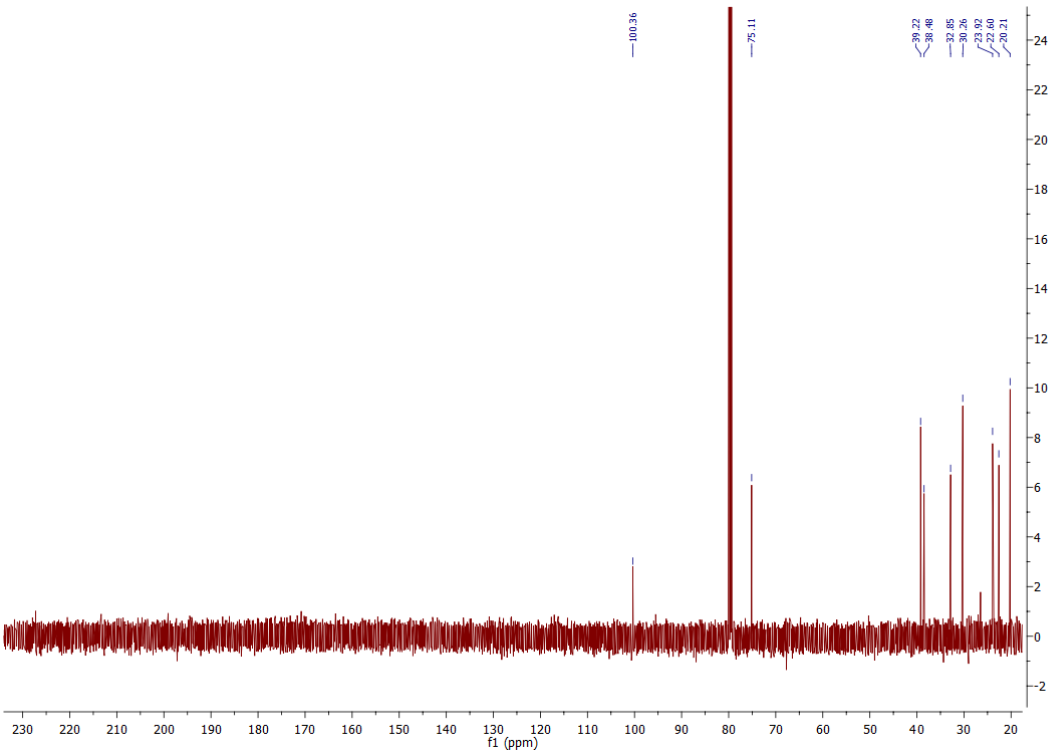


Figure S4 b (ii) ^{13}C NMR of 1-hydroxy-10-oxabicyclo[4.3.1]decane (cyclononanone; CYP101B1) [1, 5].

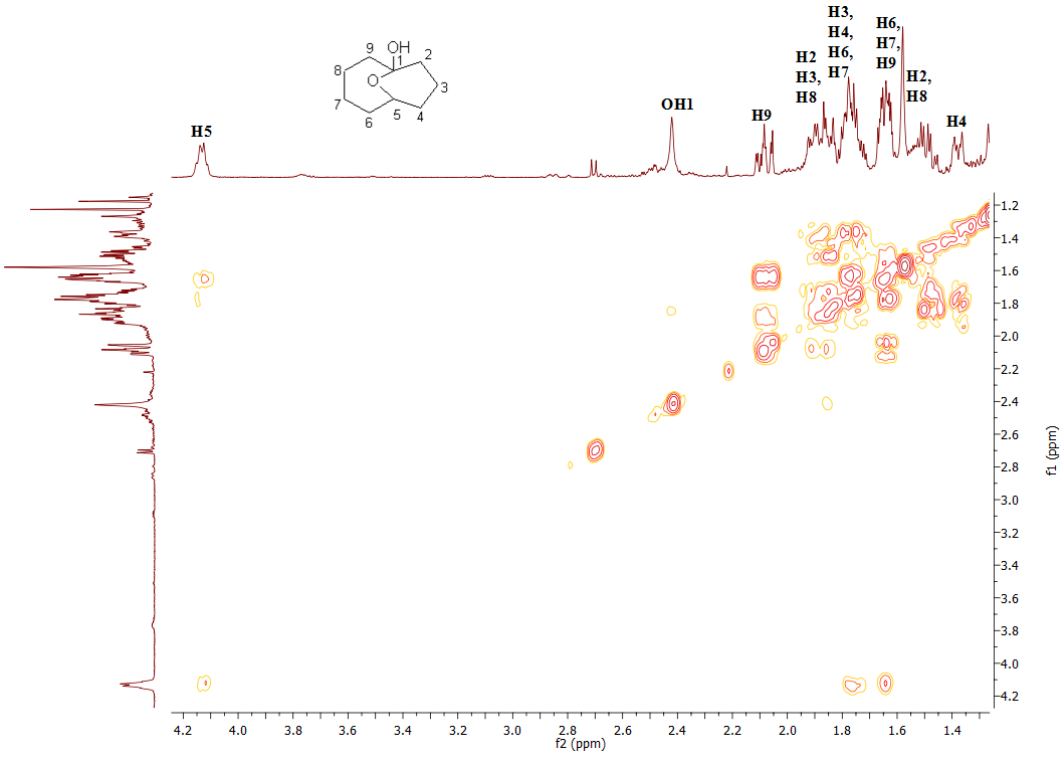


Figure S4 b (iii) gCOSY NMR of 1-hydroxy-10-oxabicyclo[4.3.1]decane (cyclononanone; CYP101B1).

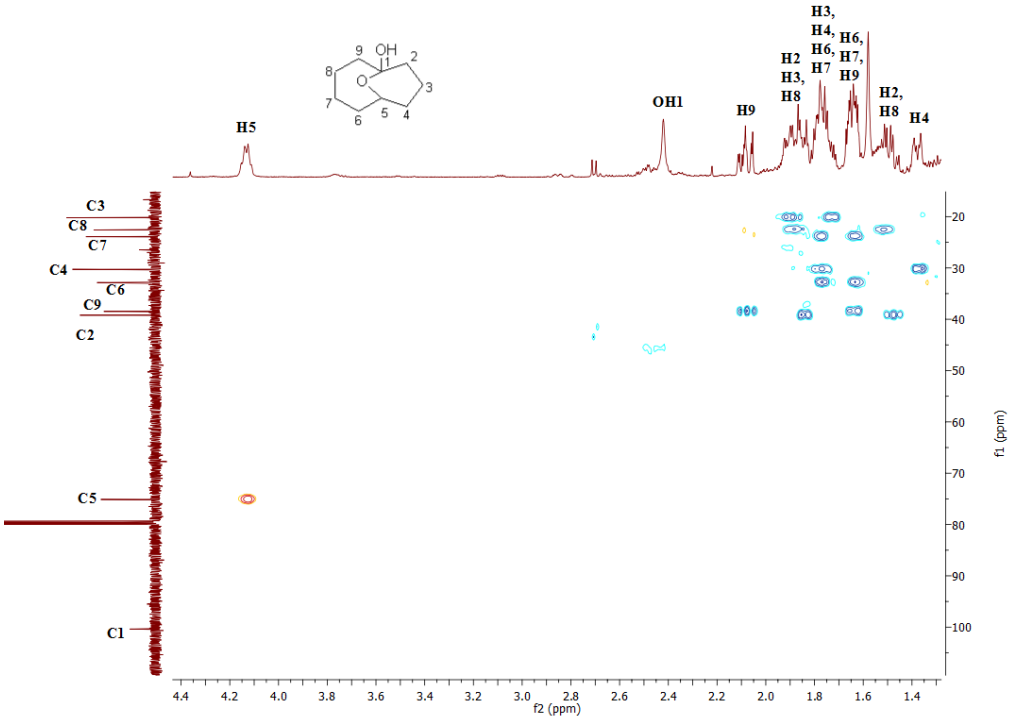


Figure S4 b (iv) HSQC NMR of 1-hydroxy-10-oxabicyclo[4.3.1]decane (cyclononanone; CYP101B1).

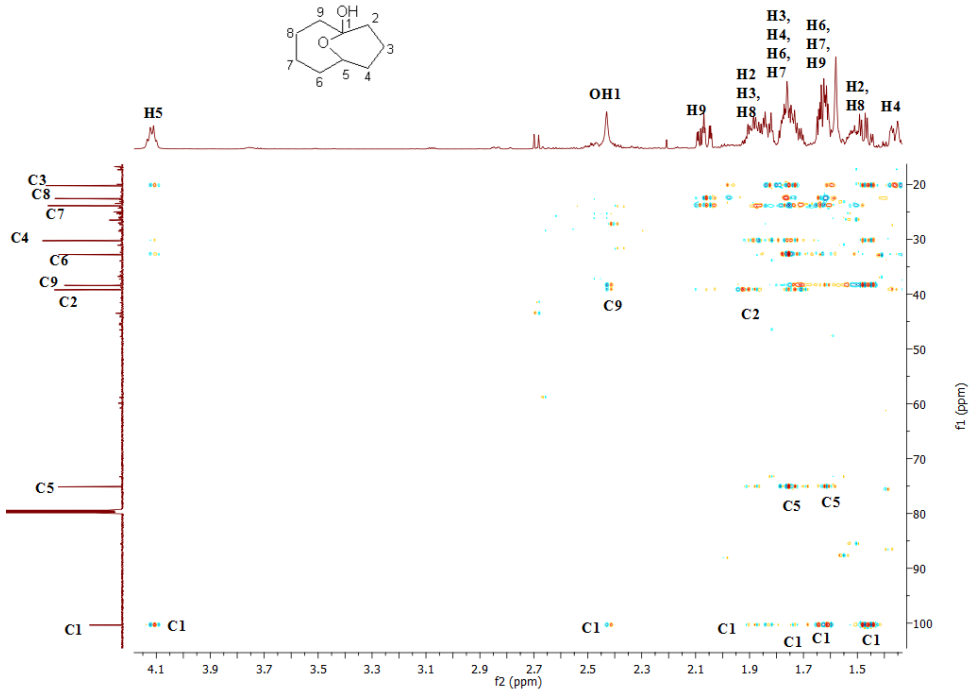


Figure S4 b (v) Zoomed in HMBC NMR of 1-hydroxy-10-oxabicyclo[4.3.1]decane to highlight the C-H interactions in 18 to 108 ppm region (cyclononanone; CYP101B1).

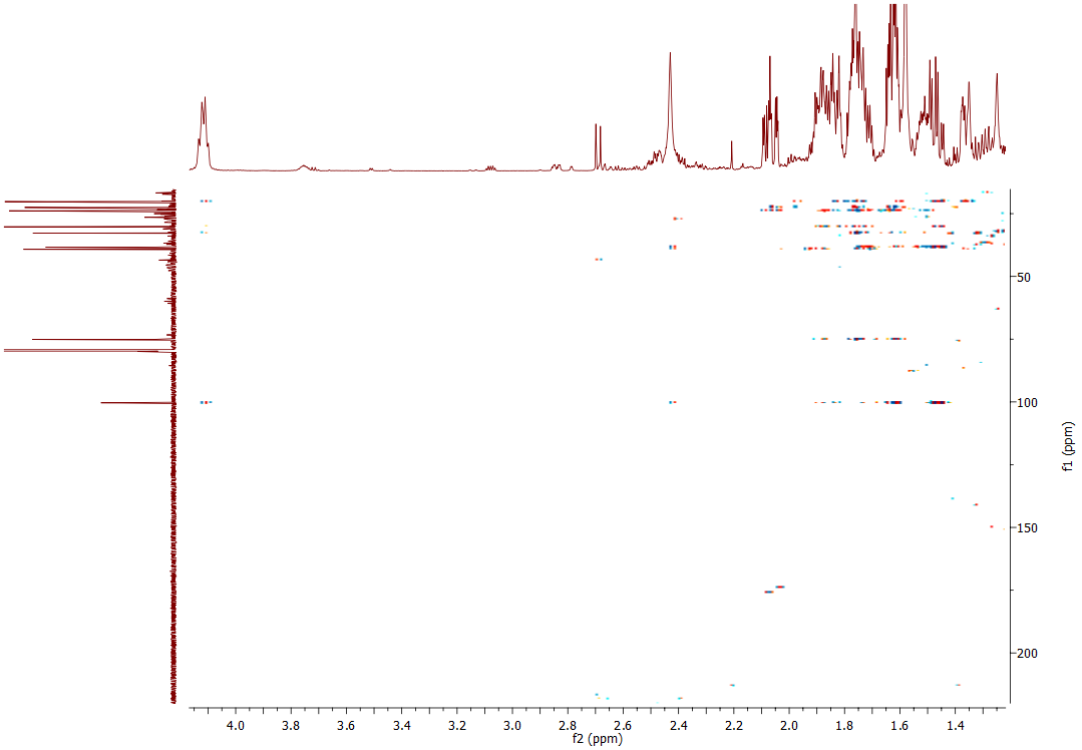


Figure S4 b (vi) HMBC NMR of 1-hydroxy-10-oxabicyclo[4.3.1]decane (cyclononanone; CYP101B1).

Data for 2-hydroxycyclodecanone [7]:

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.29-4.17 (m, 1H, H2), 3.88-3.76 (m, 1H, O**H**2 (C2)), 3.24-3.11 (m, 1H, H10), 2.32-2.06 (m, 4H, 2xH3, H9, H10), 1.74-1.59 (m, 3H, H5, H6 & H9), 1.55-1.47 (m, 1H, H4), 1.46-1.31 (m, 6H, H4, H5, H6, 2xH7 & H8), 1.12-0.99 (m, 1H, H8).

^{13}C NMR (126 MHz, $CDCl_3$) δ 216.62 (C1), 79.11 (C2), 37.67 (C10), 31.73 (C3), 28.84 (C4), 27.55 (C8), 25.73 (C7), 25.45 (C5), 25.37 (C9), 23.12 (C6).

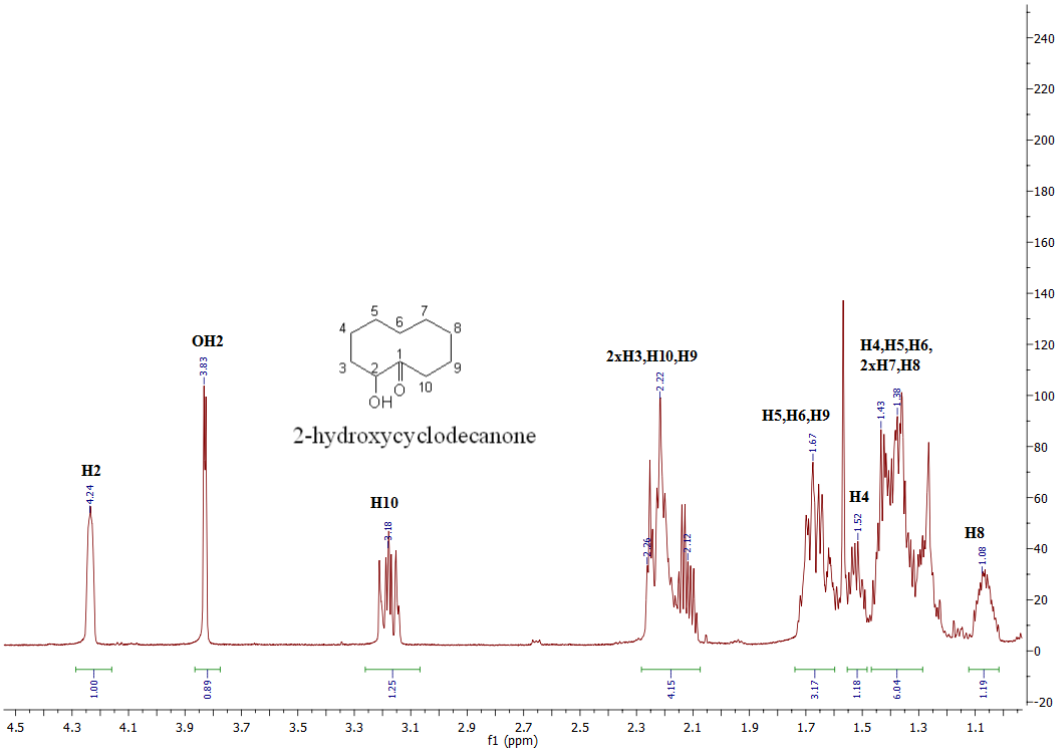


Figure S4 c (i) ^{1}H NMR of 2-hydroxycyclodecanone (CYP101C1).

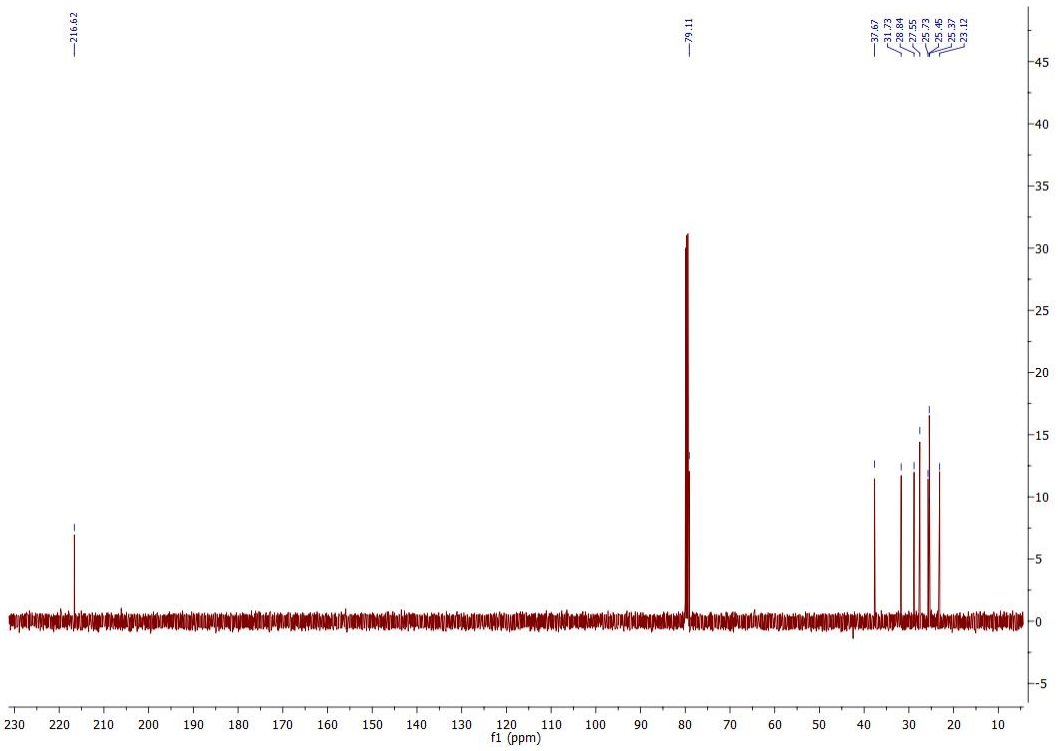


Figure S4 c (ii) ^{13}C NMR of 2-hydroxycyclodecanone (CYP101C1).

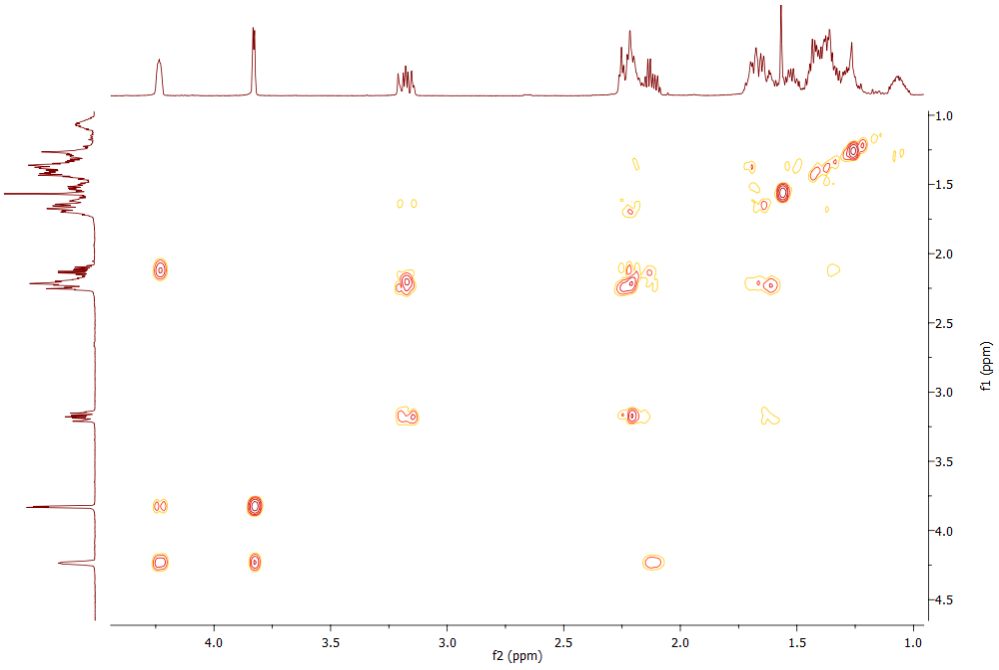


Figure S4 c (iii) gCOSY NMR of 2-hydroxycyclodecanone (CYP101C1).

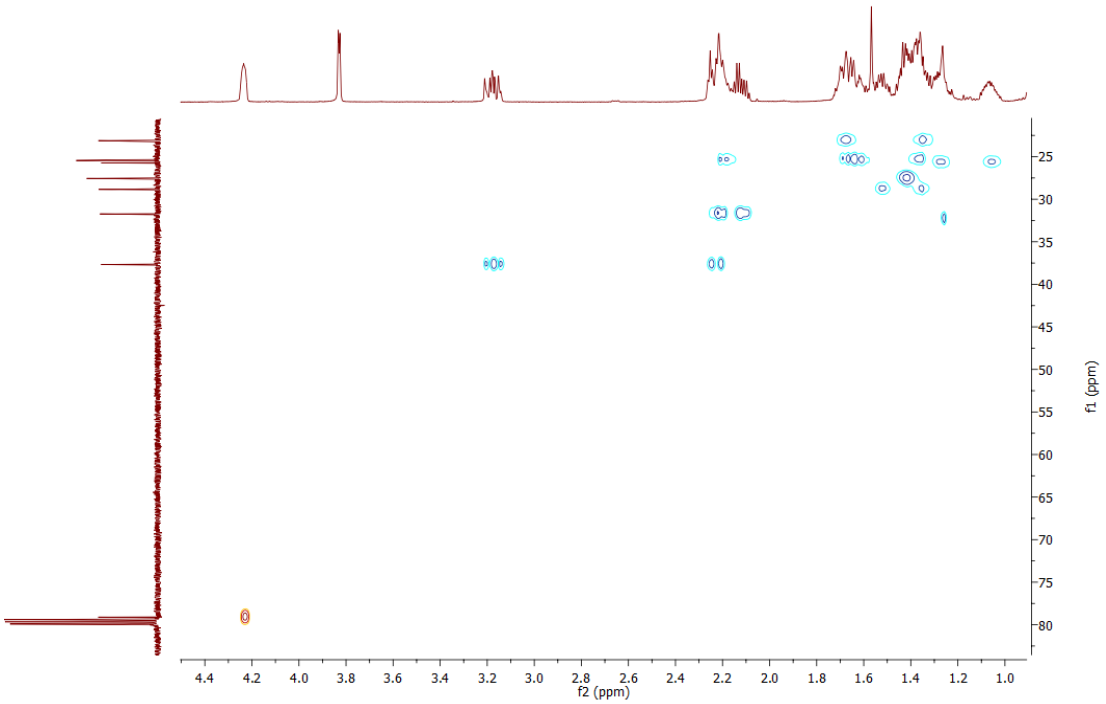


Figure S4 c (iv) HSQC NMR of 2-hydroxycyclodecanone (CYP101C1).

NMR for 2-hydroxycycloundecanone [2, 6, 9]:

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.30-4.24 (m, 1H, H2), 3.6-3.44 (m, 1H, O**H2** (C2)), 2.96-2.75 (m, 1H, H11), 2.37-2.25 (m, 1H, H11), 2.13-1.95 (m, 2H, H3 & H10), 1.79-1.5 (m, 4H, H4, H8, H9 & H10), 1.42-1.10 (m, 10H, H3, H4, 2xH5, 2xH6, 2xH7, H8 & H9).

^{13}C NMR (126 MHz, $CDCl_3$) δ 216.74 (C1), 79.19 (C2), 39.90 (C11), 32.22 (C3), 29.45 (C7), 29.36 (C9), 29.01 (C8), 28.83 (C5), 26.38 (C6), 24.72 (C10), 23.40 (C4).

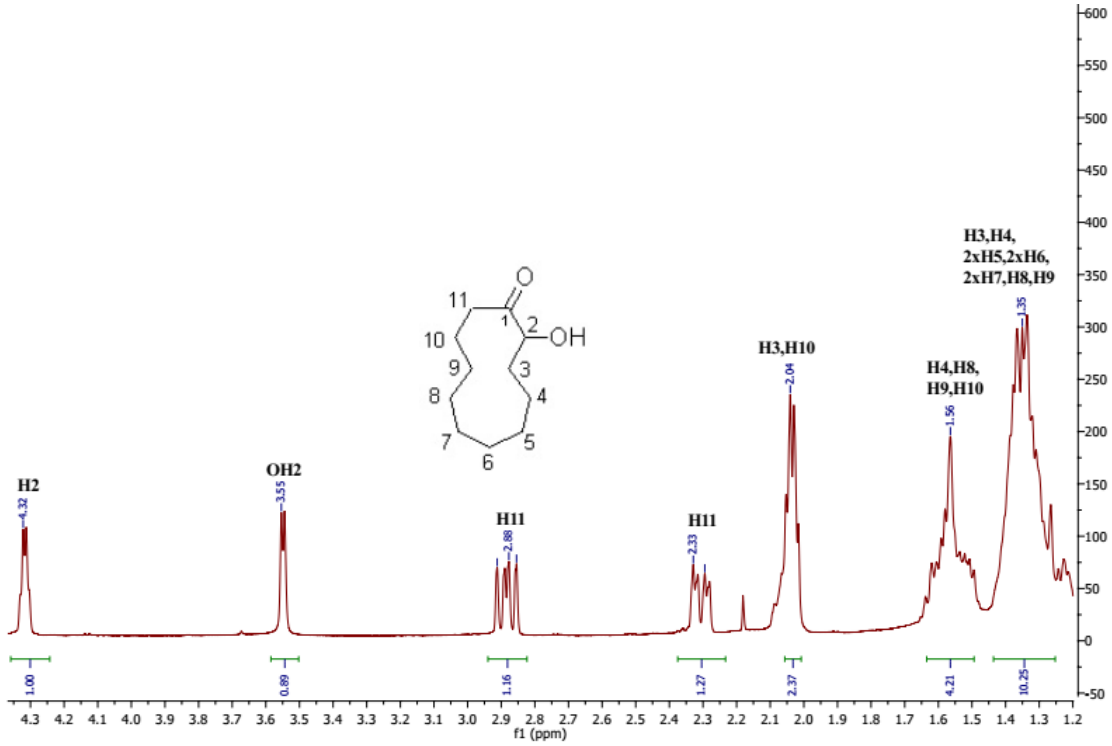


Figure S4 d (i) ^{1}H NMR of 2-hydroxycycloundecanone (CYP101C1).

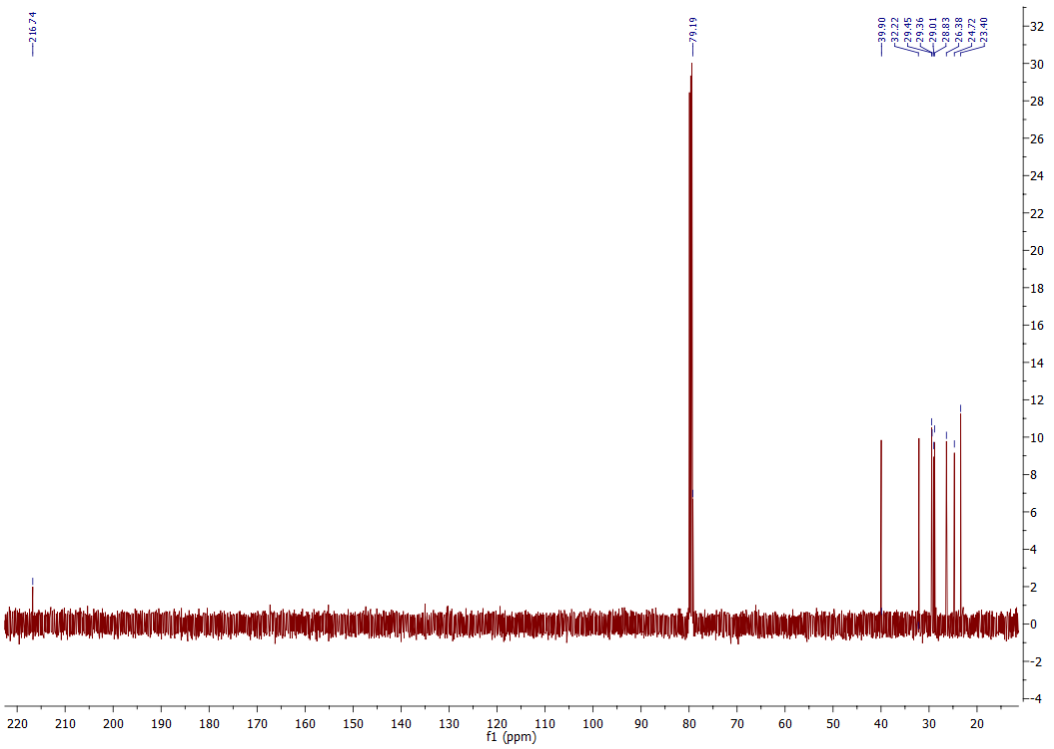


Figure S4 d (ii) ^{13}C NMR of 2-hydroxycycloundecanone (CYP101C1).

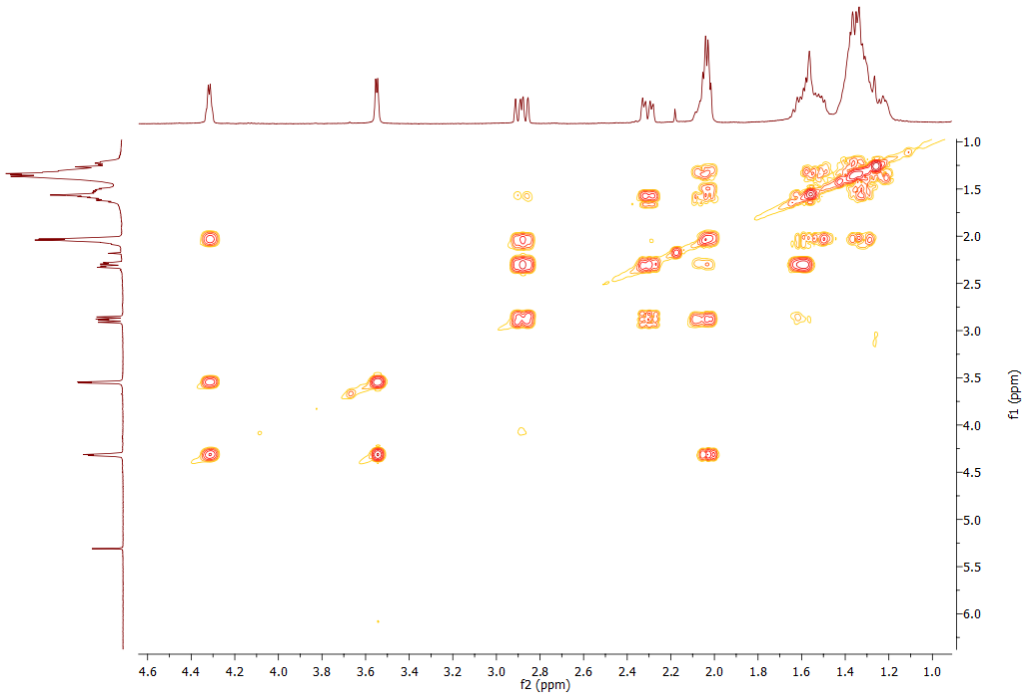


Figure S4 d (iii) gCOSY NMR of 2-hydroxycycloundecanone (CYP101C1).

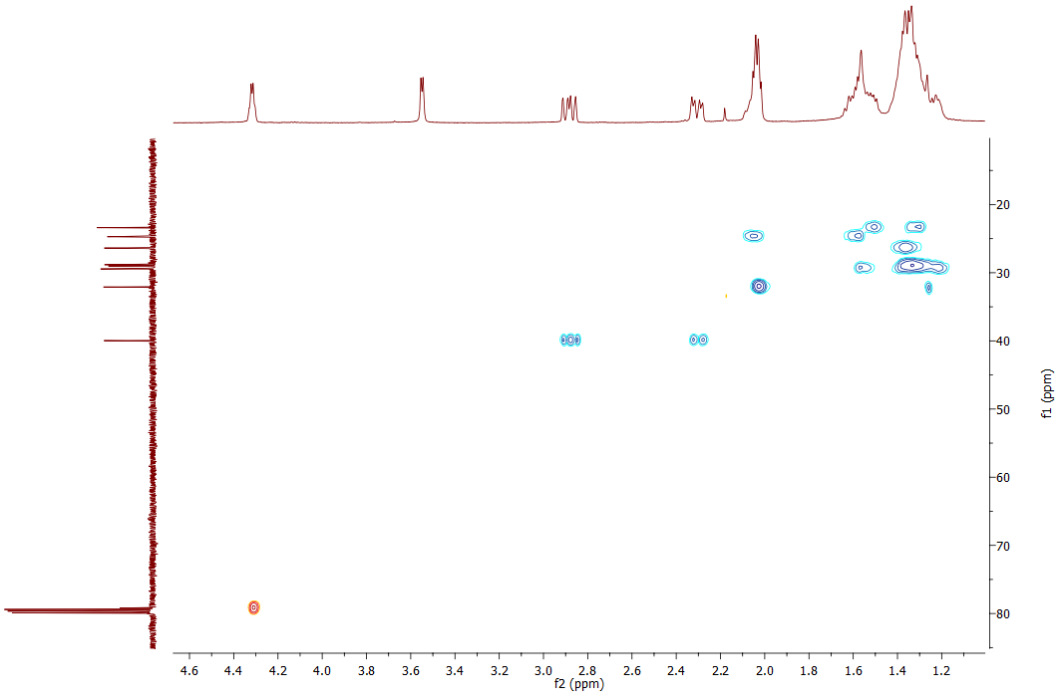


Figure S4 d (iv) HSQC NMR of 2-hydroxycycloundecanone (CYP101C1).

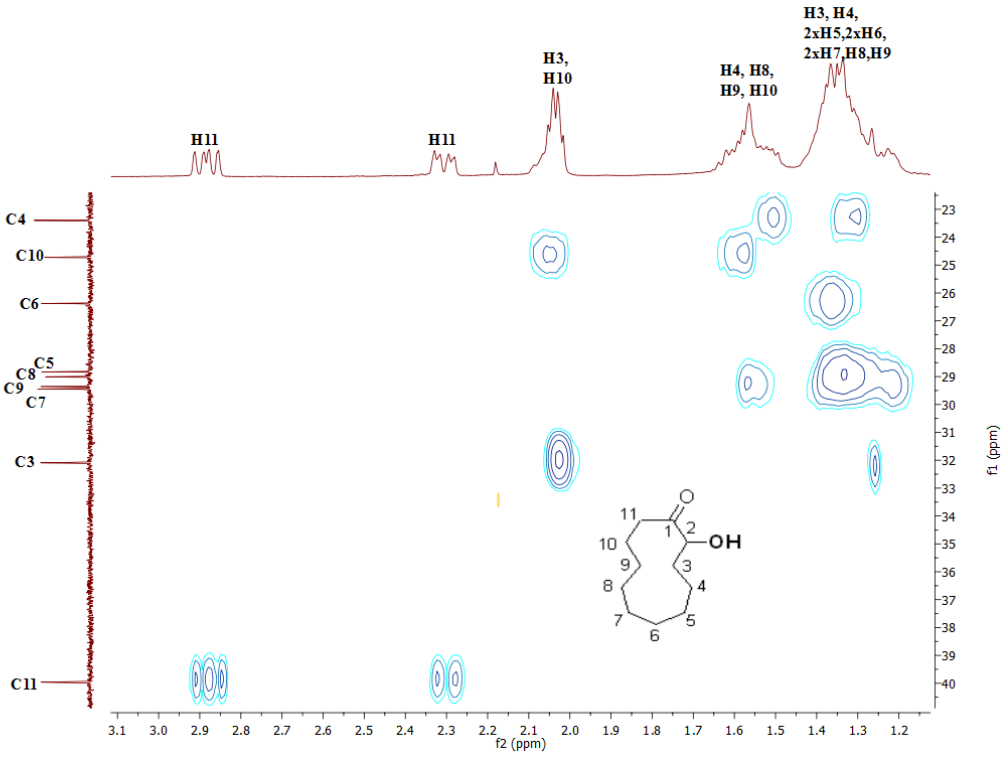
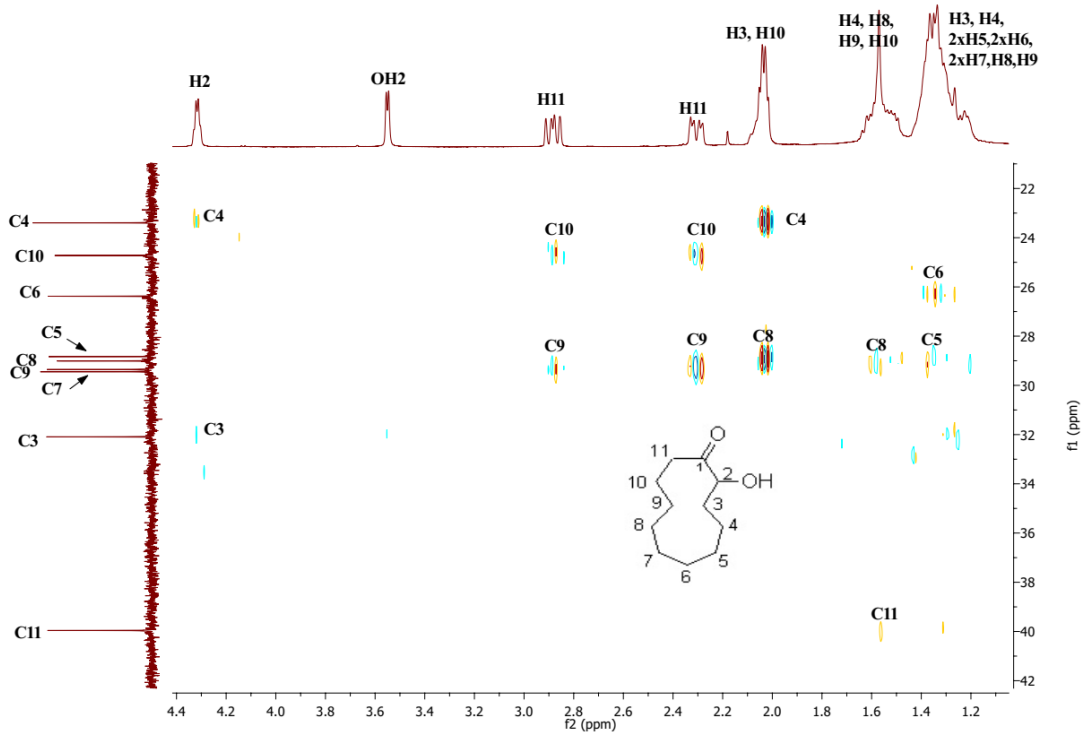
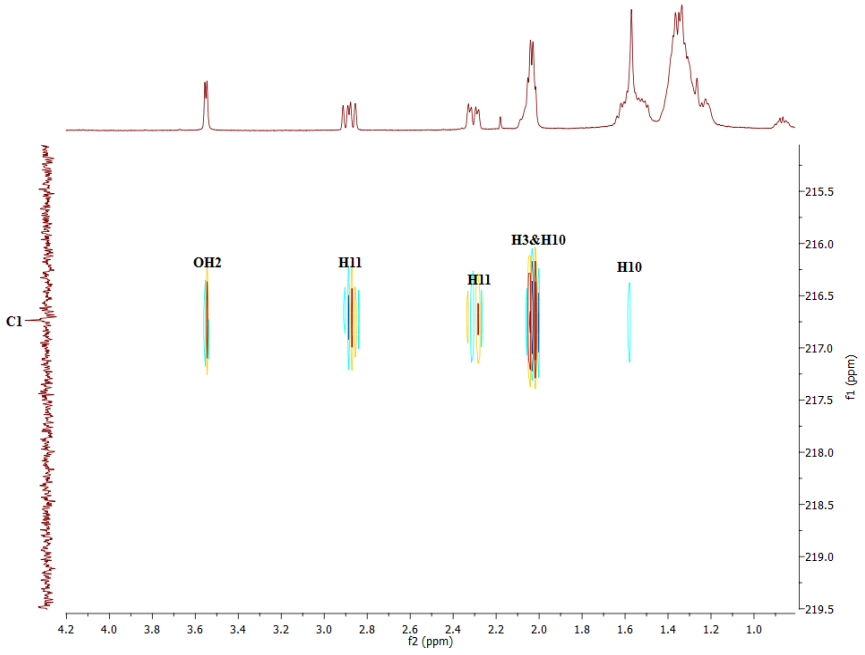


Figure S4 d (v) Zoomed in HSQC NMR to highlight the C-H correlations in 20-40 ppm region.



Zoomed in 20-80 ppm region (HMBC NMR)



Zoomed in C=O region of HMBC NMR. The product was confirmed as 2-hydroxy metabolite as C1 showed strong connections with H2, O**H2**, H3, H10 and H11.

Figure S4 d (vi) Zoomed in HMBC NMR of 2-hydroxycycloundecanone.

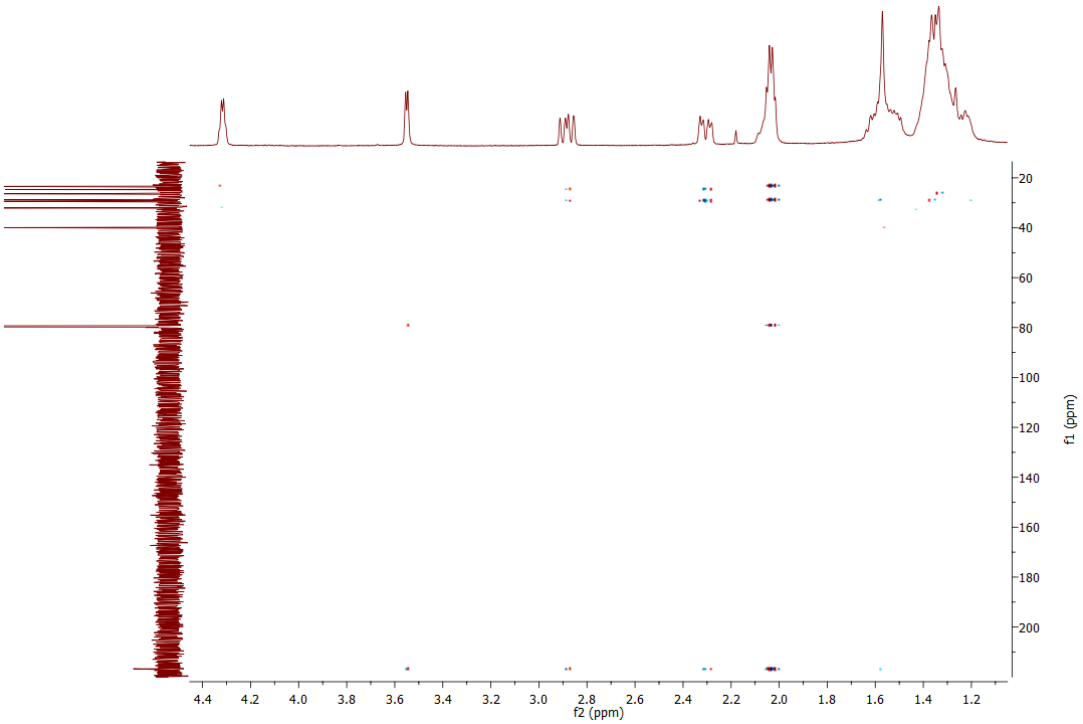


Figure S4 d (vii) HMBC NMR of 2-hydroxycycloundecanone (CYP101C1).

NMR for 5-hydroxycycloundecanone:

^{1}H NMR (600 MHz, $CDCl_3$) δ 3.76-3.70 (m, 1H, H5), 2.55-2.42 (m, 4H, 2xH2 & 2xH11), 1.89-1.82 (m, 2H, 2xH3), 1.81-1.74 (m, 1H, H10), 1.73-1.63 (m, 2H, H4 & H10), 1.61-1.55 (m, 1H, H4), 1.54-1.50 (m, 2H, H6 & H9), 1.48-1.42 (m, 1H, H6), 1.40-1.33 (m, 3H, H7, H8 & H9), 1.31-1.26 (m, 2H, H7 & H8).

^{13}C NMR (151 MHz, $CDCl_3$) δ 216.69 (C1), 72.36 (C5), 45.02 (C11), 44.97 (C2), 37.77 (C4), 36.57 (C6), 28.68 (C9), 27.71 (C8), 25.0 (C10), 24.85 (C7), 22.33 (C3).

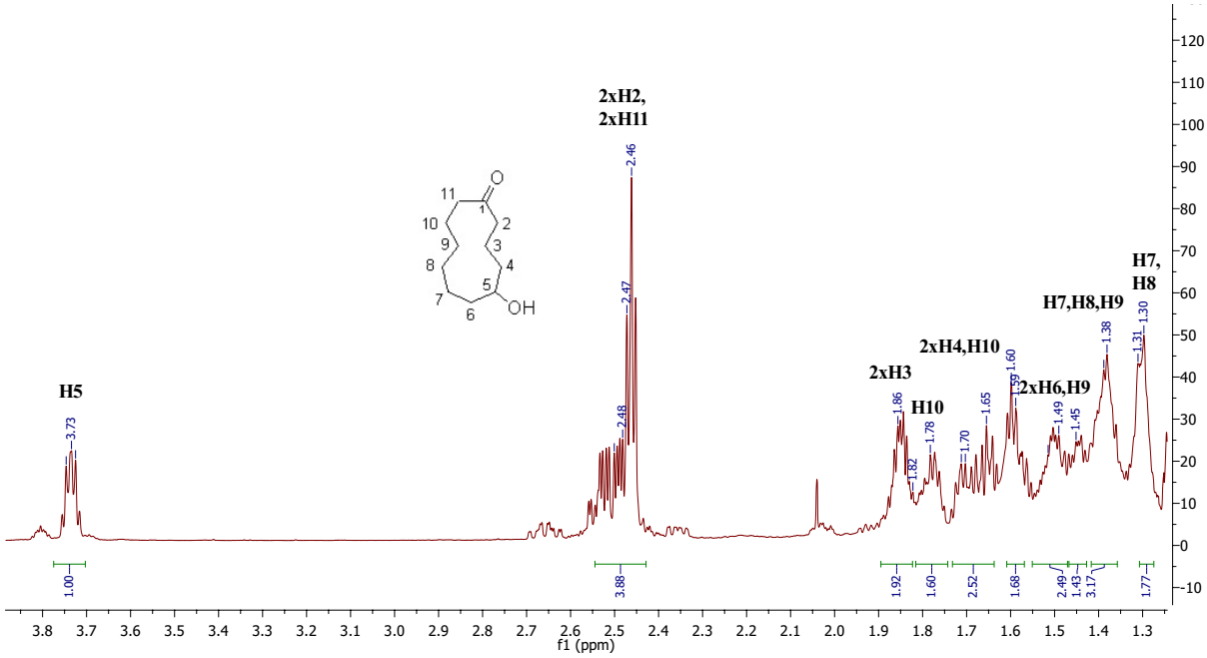


Figure S4 e (i) ^{1}H NMR of 5-hydroxycycloundecanone (CYP101B1; GC-MS RT 9.25 min).

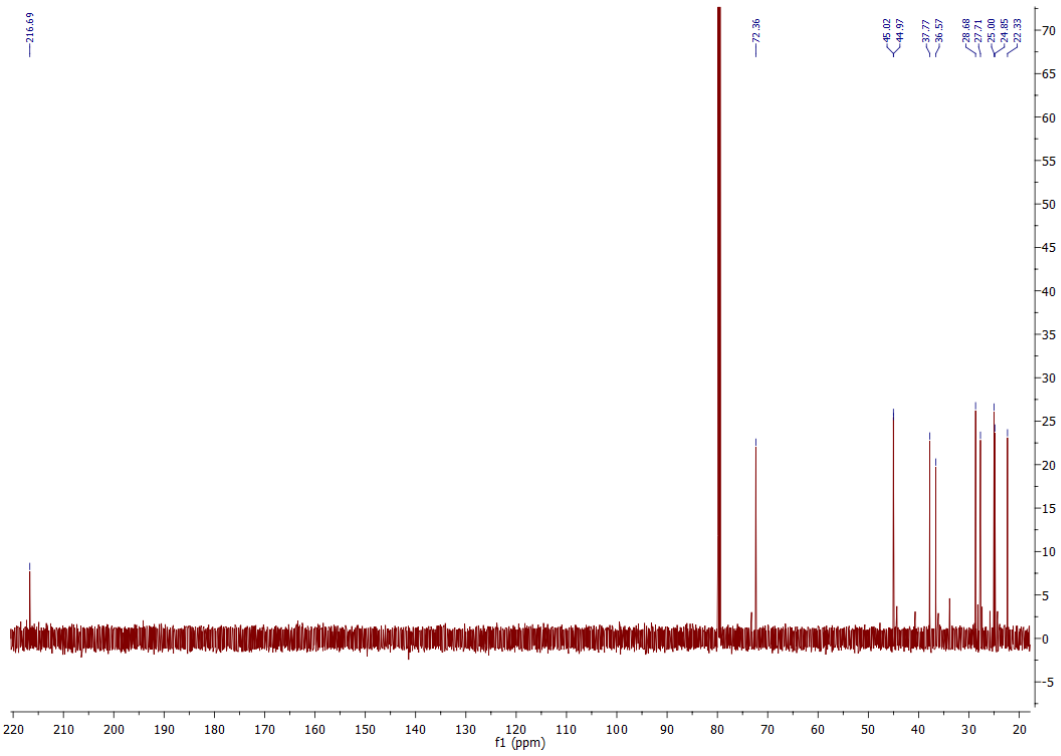


Figure S4 e (ii) ^{13}C NMR of 5-hydroxycycloundecanone.

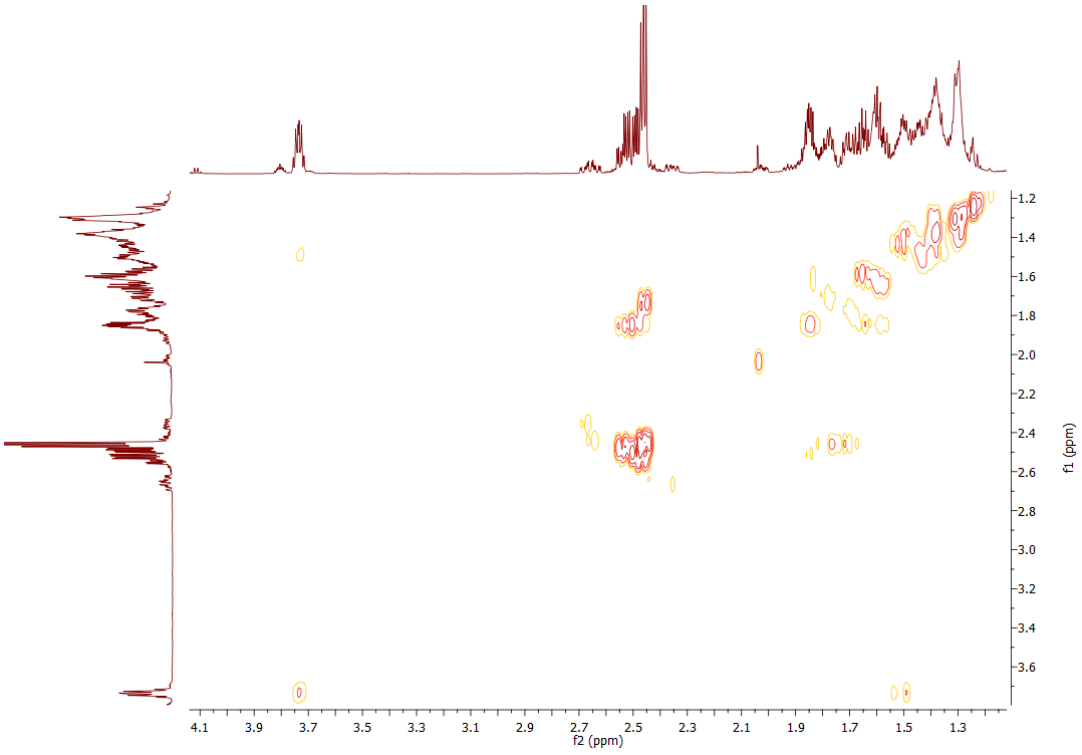


Figure S4 e (iii) gCOSY NMR of 5-hydroxycycloundecanone.

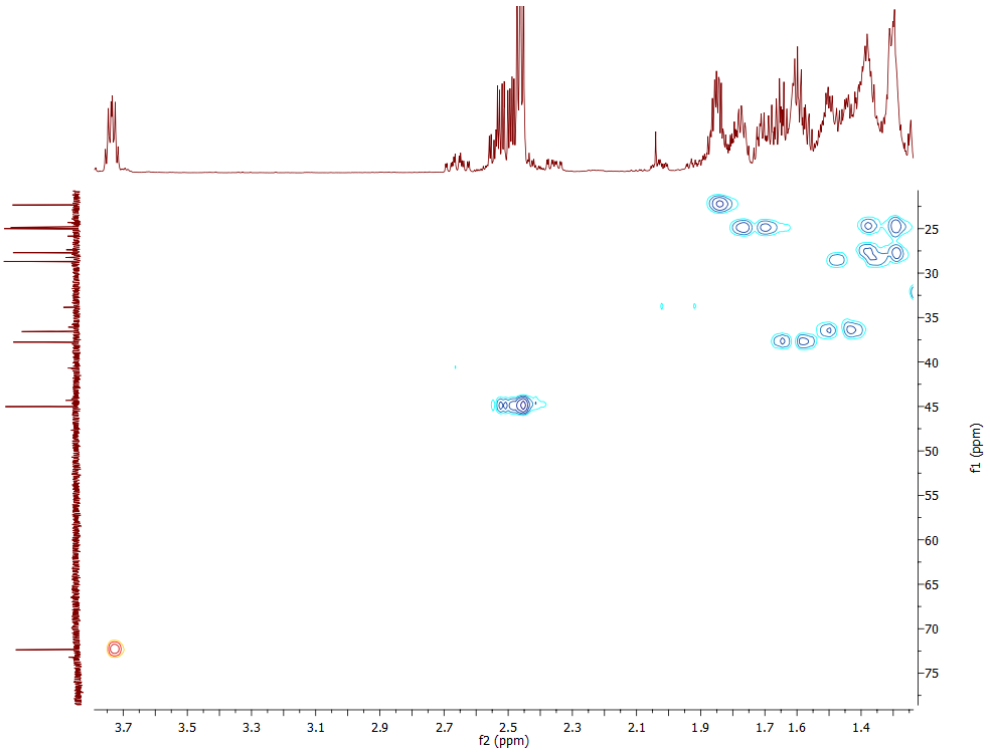
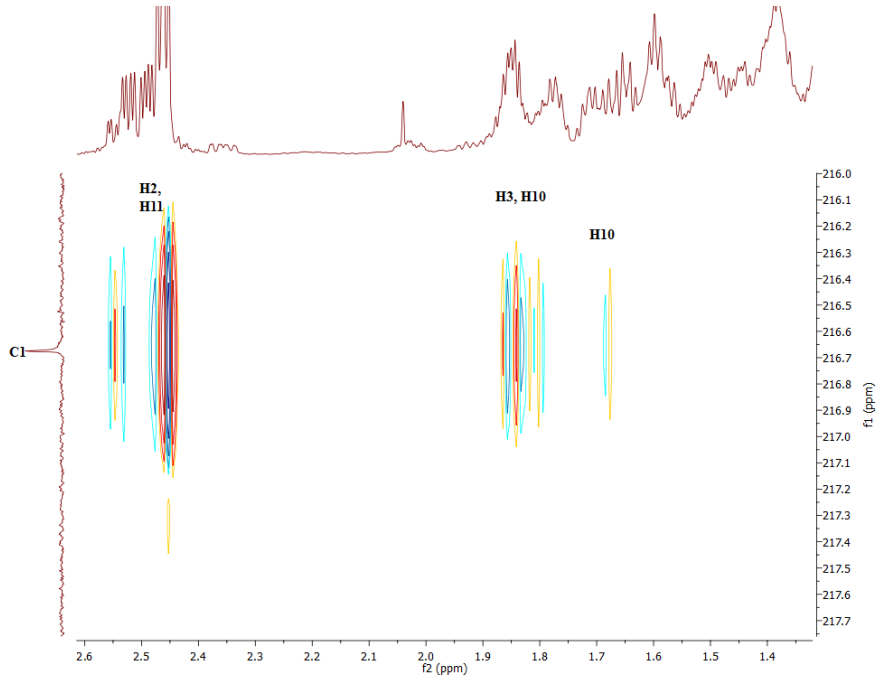
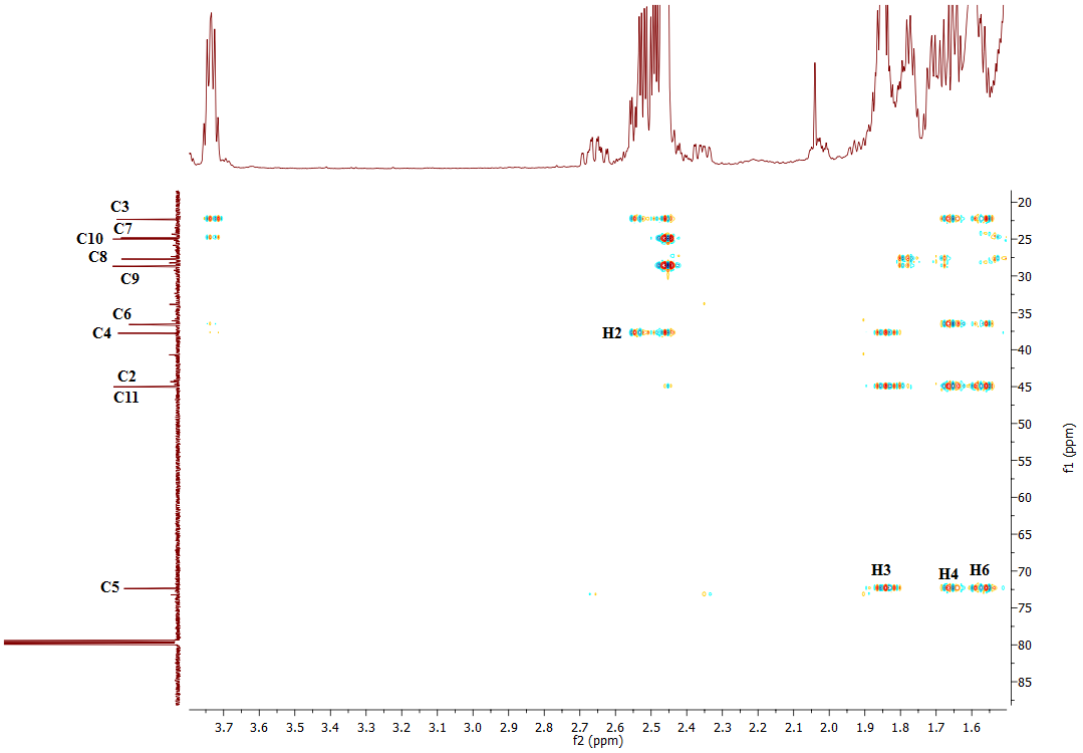


Figure S4 e (iv) HSQC NMR of 5-hydroxycycloundecanone.



Zoomed in C=O region of HMBC NMR to highlight the C1 correlations with the H2, H3, H10 and H11.



Zoomed in HMBC NMR to highlight the C-H interactions in 25 to 90 ppm region. The product was assigned as 5-hydroxycycloundecanone as C5 have strong correlations with H3, H4 and H6. H4 was confirmed by the interaction between C4 with H2 peak in the HMBC NMR.

Figure S4 e (v) HMBC NMR of 5-hydroxycycloundecanone (CYP101B1).

NMR for 6-hydroxycycloundecanone:

^{1}H NMR (600 MHz, $CDCl_3$) δ 3.72-3.66 (m, 1H, H6), 2.60-2.36 (m, 4H, 2xH2 & 2xH11), 1.84-1.72 (m, 4H, 2xH3, 2xH10), 1.63-1.56 (m, 2H, 2xH5), 1.54-1.48 (m, 1H, H9), 1.47-1.42 (m, 3H, H4 & 2xH7), 1.41-1.33 (m, 4H, H4, 2xH8 & H9).

^{13}C NMR (151 MHz, $CDCl_3$) δ 216.91 (C1), 72.73 (C6), 44.71 (C2), 44.17 (C11), 35.81 (C7), 34.79 (C5), 29.23 (C9), 25.18 (C10), 25.0 (C3), 24.42 (C4), 23.89 (C8).

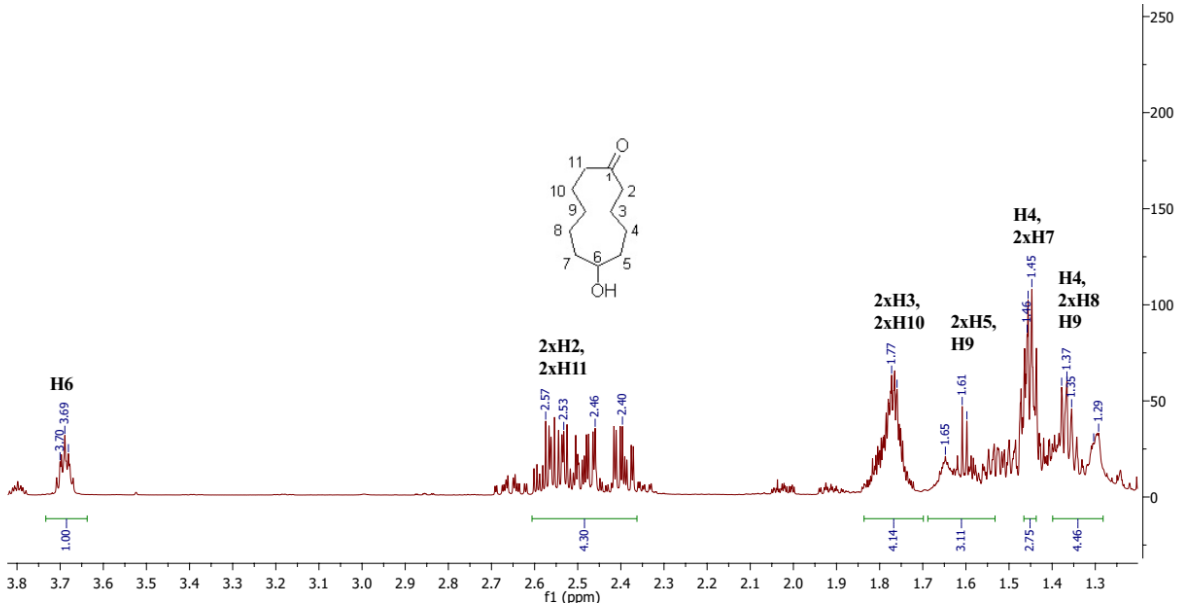


Figure S4 f (i) ^{1}H NMR of 6-hydroxycycloundecanone (CYP101B1).

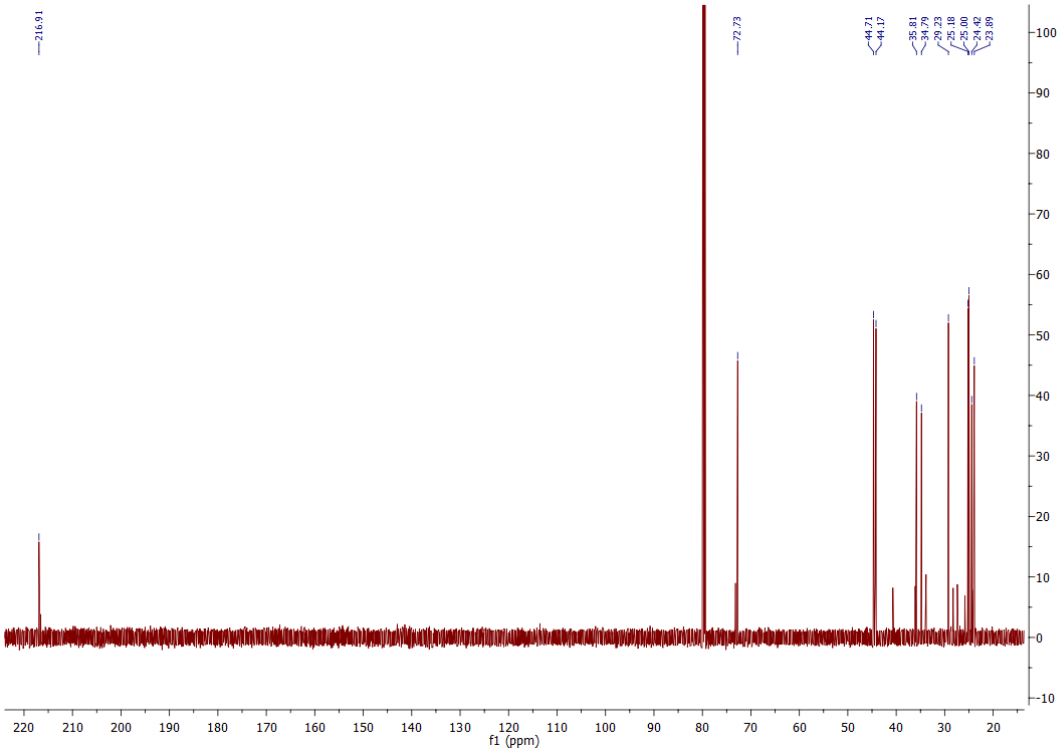


Figure S4 f (ii)^{13}C NMR of 6-hydroxycycloundecanone.

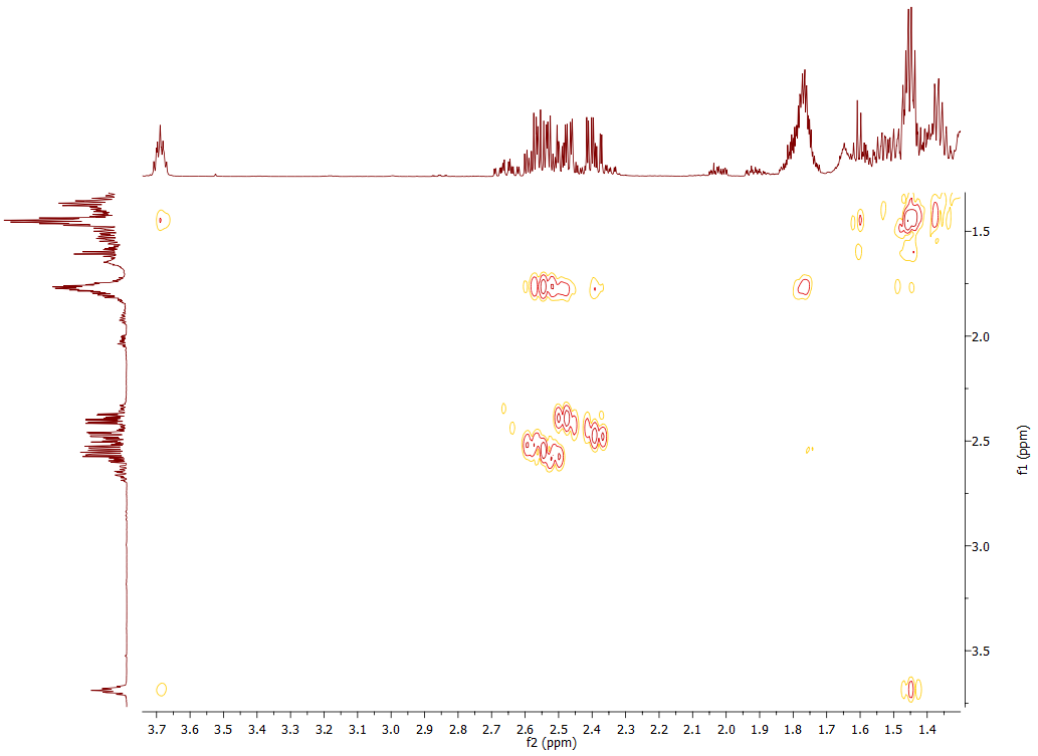


Figure S4 f (iii) gCOSY NMR of 6-hydroxycycloundecanone.

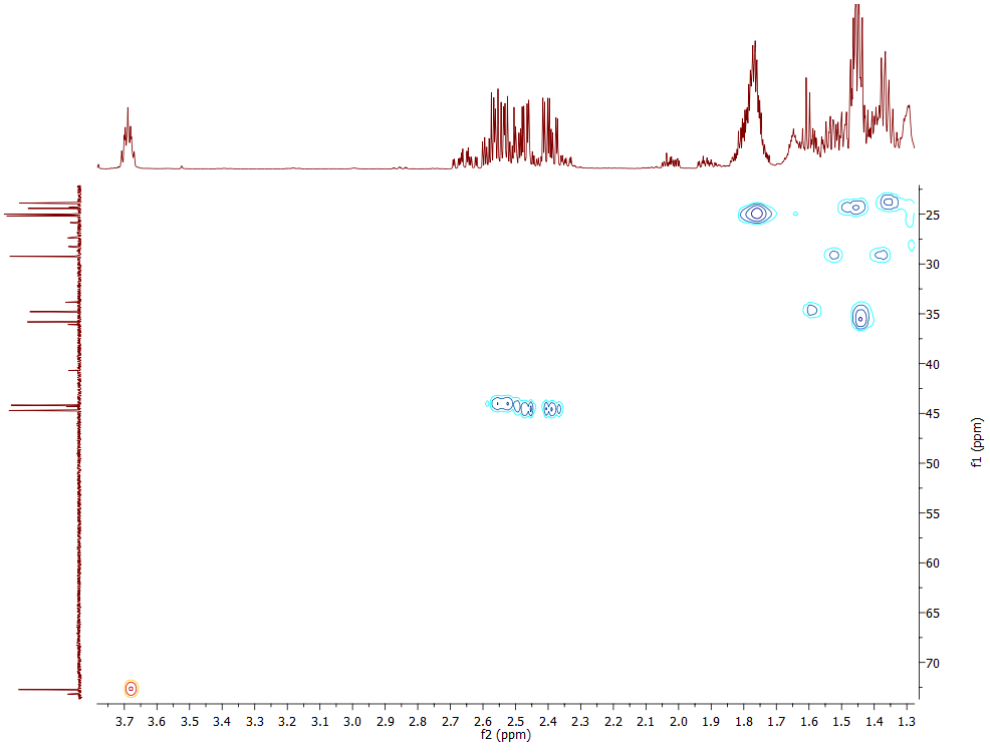
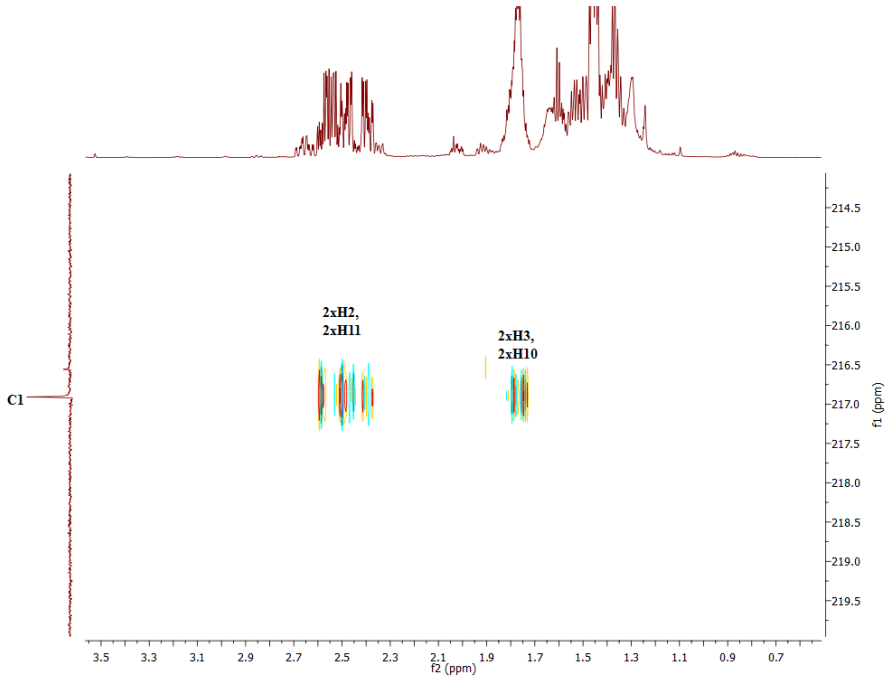
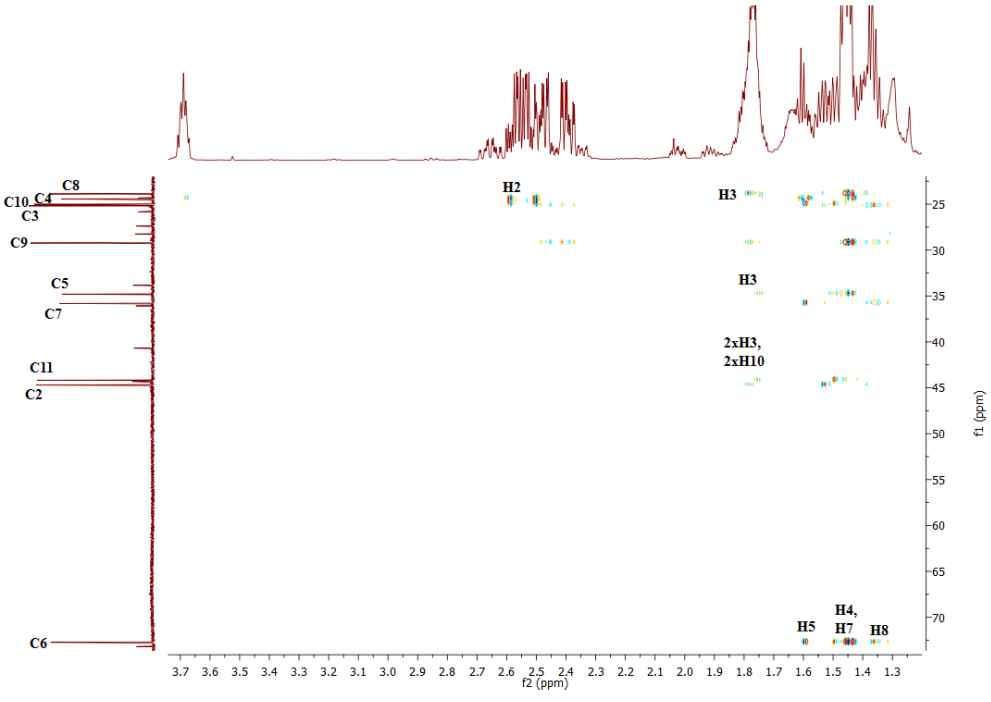


Figure S4 f (iv) HSQC NMR of 6-hydroxycycloundecanone.



Zoomed in C=O region, which highlights the correlations of C1 with the H2, H11, H3 and H10.



Zoomed in HMBC NMR to highlight the C-H interactions in 20 to 80 ppm region. The product was confirmed 6-hydroxycycloundecanone as C6 showed correlations with H4, H5, H7 and H8. C4 and C5 were assigned due to the interactions with H2 and H3, respectively. The H4 and H5 proton peaks in ^{1}H NMR were identified using the HSQC NMR.

Figure S4 f (v) HMBC NMR of 6-hydroxycycloundecanone.

NMR for 2-hydroxycyclododecanone [2, 3, 7, 9]:

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.44-4.34 (m, 1H, H2), 3.58-3.50 (m, 1H, O**H2** (C2)), 3.01-2.96 (m, 1H, H12), 2.26-2.18 (m, 1H, H10), 2.17-2.1 (m, 1H, H12), 1.99-1.87 (m, 2H, 2xH3), 1.60-1.50 (m, 1H, H4), 1.21-1.46 (m, 13H, 2xH5, 2xH6, 2xH7, 2xH8, 2xH9, H10 & 2xH11), 0.77-0.93 (m, 1H, H4).

^{13}C NMR (126 MHz, $CDCl_3$) δ 215.55 (C1), 79.18 (C2), 36.93 (C12), 33.39 (C3), 28.76 (C5), 28.72 (C11), 26.60, 25.34 (C8 & C9), 25.11 (C6), 24.73 (C7), 24.05 (C10), 21.35 (C4).

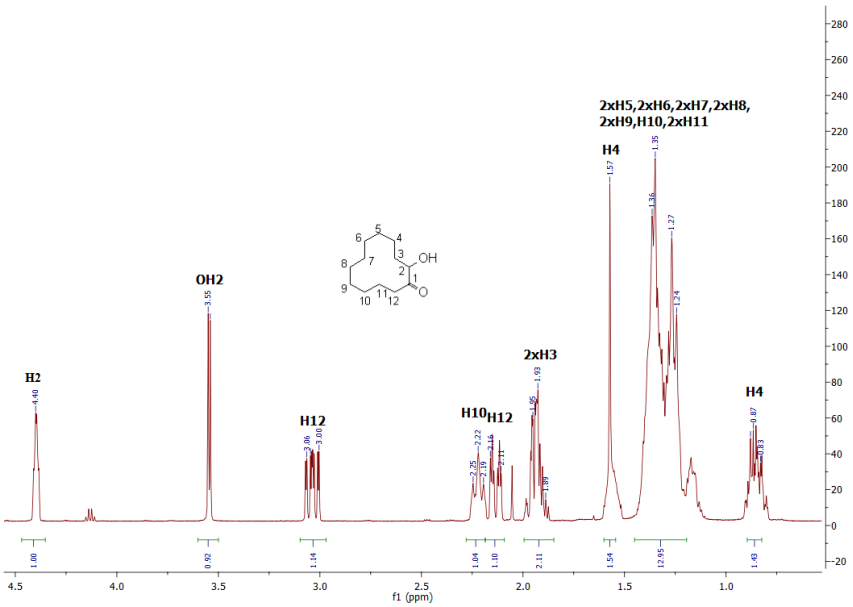


Figure S4 g (v) ^{1}H NMR of 2-hydroxycyclododecanone (CYP101C1).

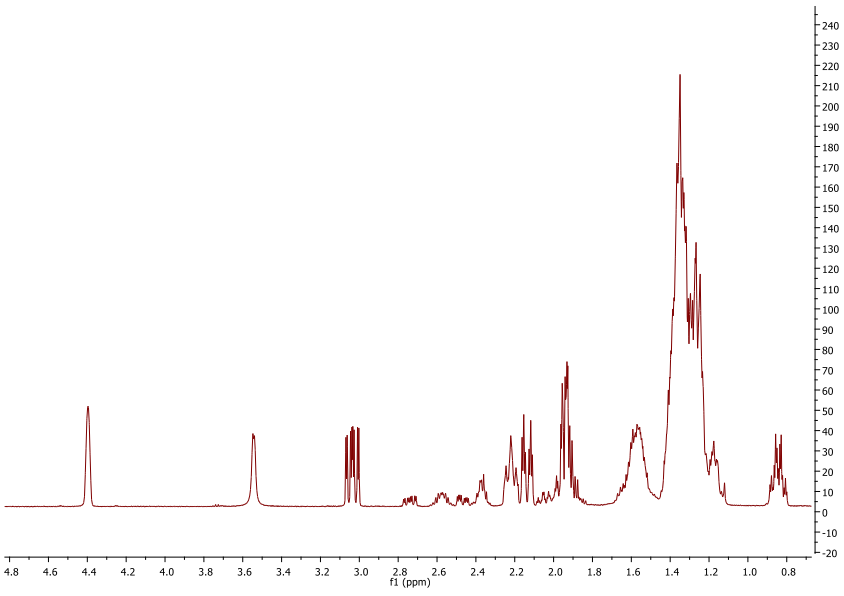


Figure S4 g (ii) ^{1}H NMR of 2-hydroxycyclododecanone (standard).

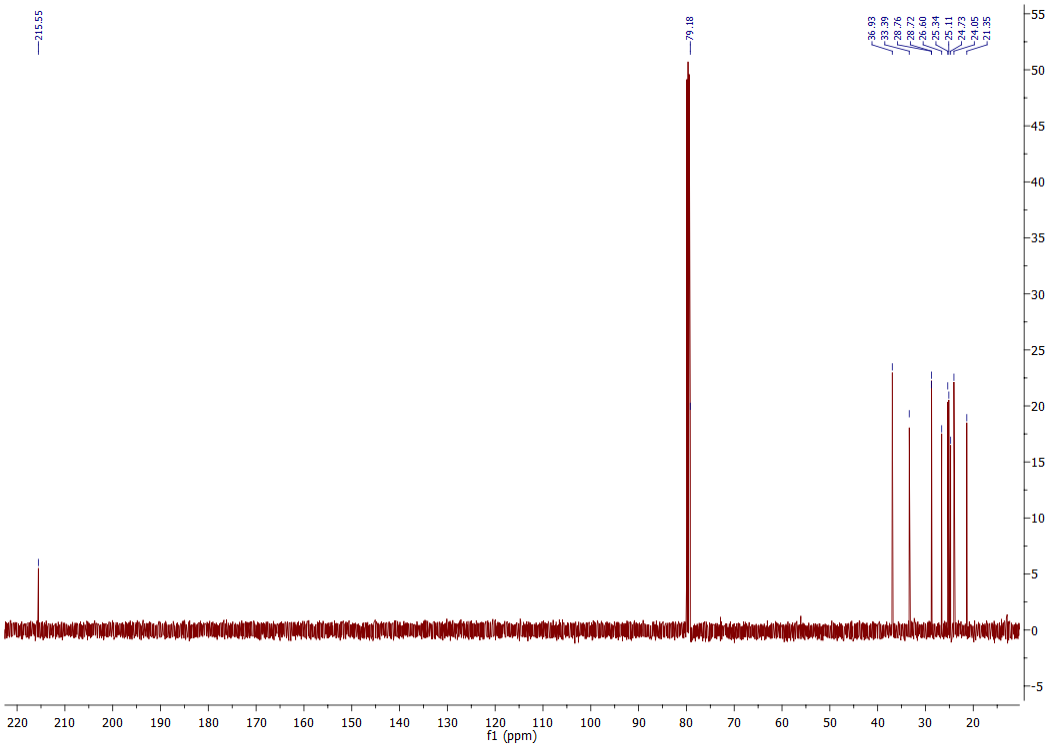


Figure S4 g (iii) ^{13}C NMR of 2-hydroxycyclododecanone (CYP101C1).

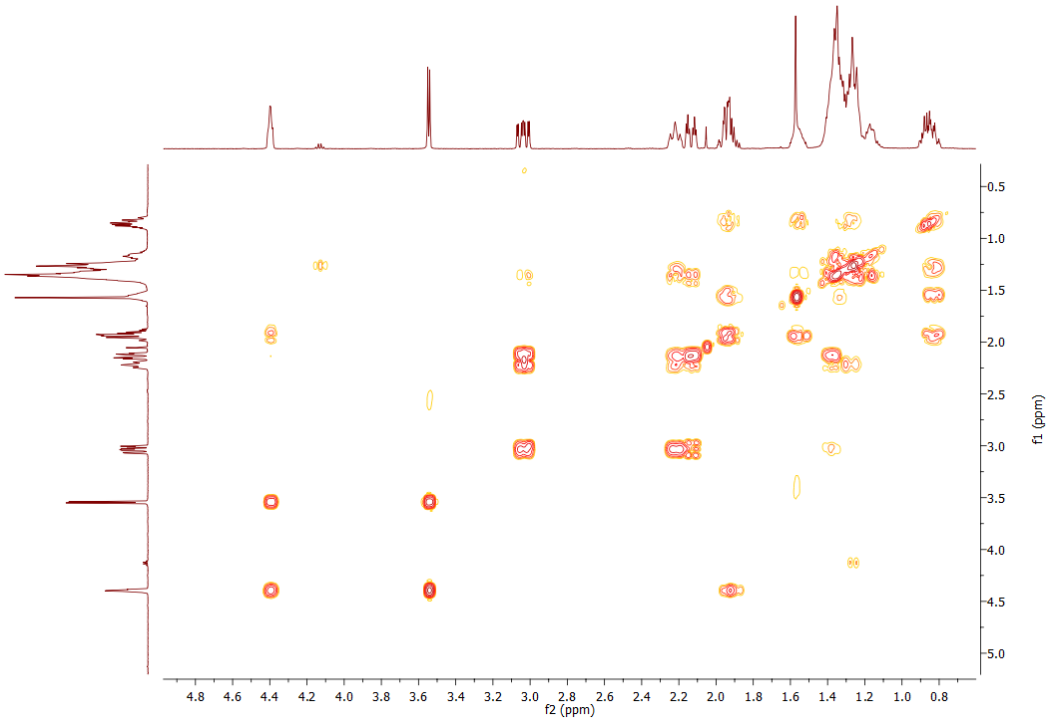


Figure S4 g (iv) gCOSY NMR of 2-hydroxycyclododecanone (CYP101C1).

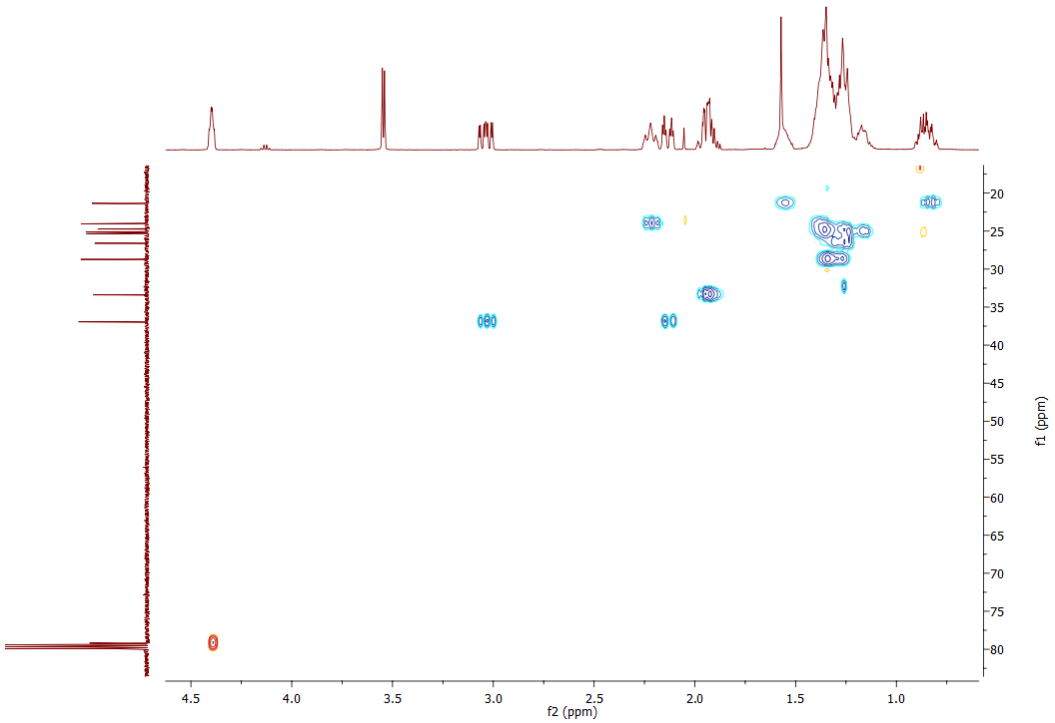
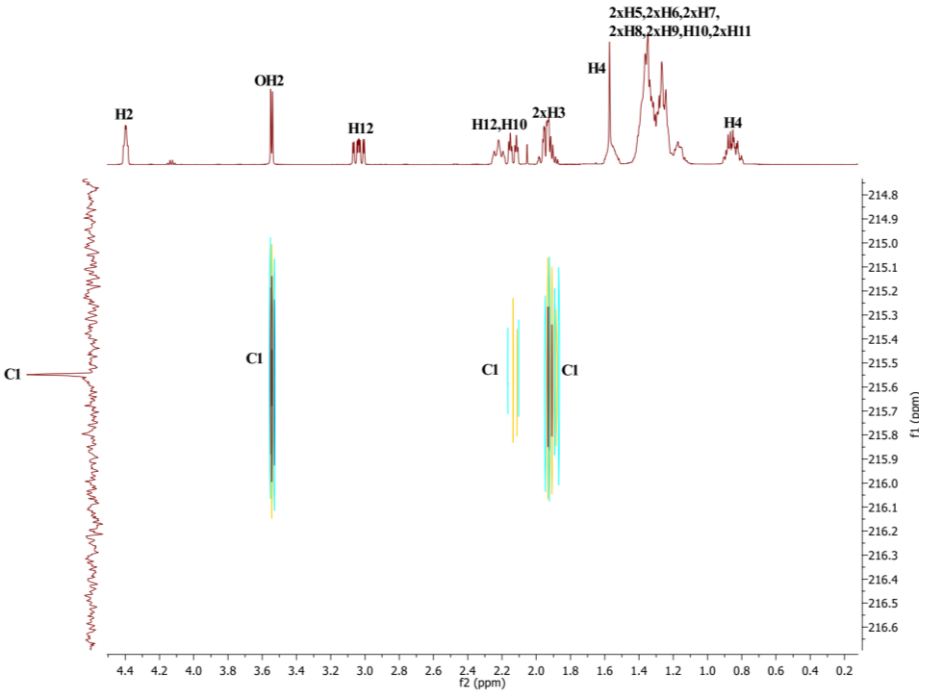
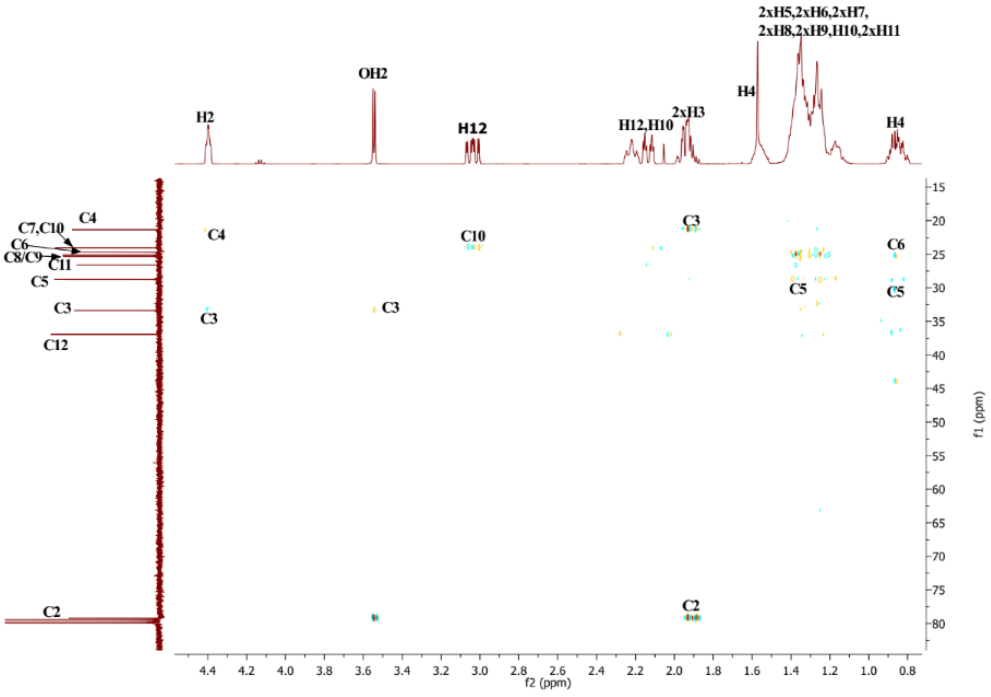


Figure S4 g (v) HSQC NMR of 2-hydroxycyclododecanone (CYP101C1).



Zoomed in HMBC NMR to highlight the interactions of the C=O region. The product was confirmed 2-hydroxy due to the correlations of C1 (215.55 ppm) with O**H2** (3.58-3.50 ppm), H12 (3.01-2.96 ppm), H12 (2.17-2.1 ppm) and H3 (1.99-1.87 ppm).



Zoomed in the region 15-80 ppm to highlight the interactions of C4 and C3 with H2 (4.44-4.34 ppm).

Figure S4 g (vi) HMBC NMR of 2-hydroxycyclododecanone (CYP101C1).

NMR for 8-hydroxycylopentadecanone [3]:

^{1}H NMR (600 MHz, $CDCl_3$) δ 3.71-3.64 (m, 1H, H8), 2.47-2.33 (m, 4H, 2xH2 & 2xH15), 1.74-1.60 (m, 4H, 2xH3 & 2xH14), 1.58-1.43 (m, 4H, 2xH7 & 2xH9), 1.40-1.25 (m, 14H, 2xH4, 2xH5, 2xH6, 2xH10, 2xH11, 2xH12 & 2xH13).

^{13}C NMR (151 MHz, $CDCl_3$) δ 215.30 (C1), 72.70 (C8), 44.86 (C2), 44.56 (C15), 37.35 (C7), 37.30 (C9), 30.18 (C5), 30.16 (C13), 29.64 (C4), 29.63 (C11), 29.51 (C12), 26.26 (C3), 25.90 (C6), 25.70 (C14), 25.45 (C10).

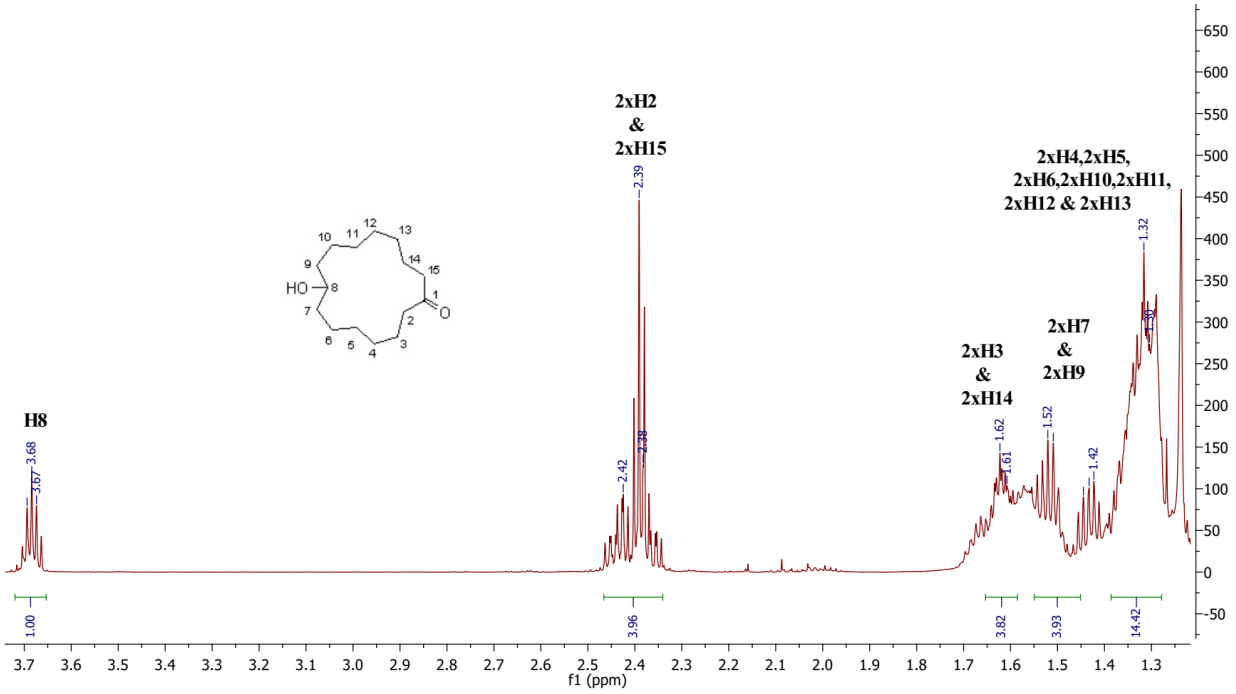
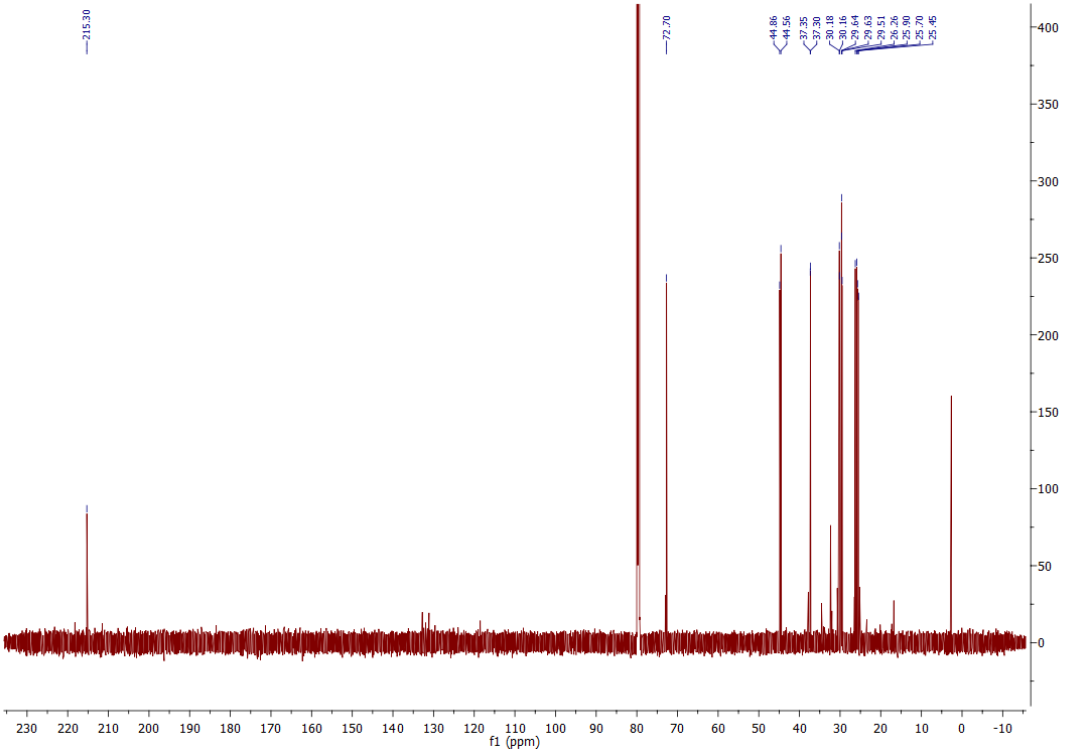
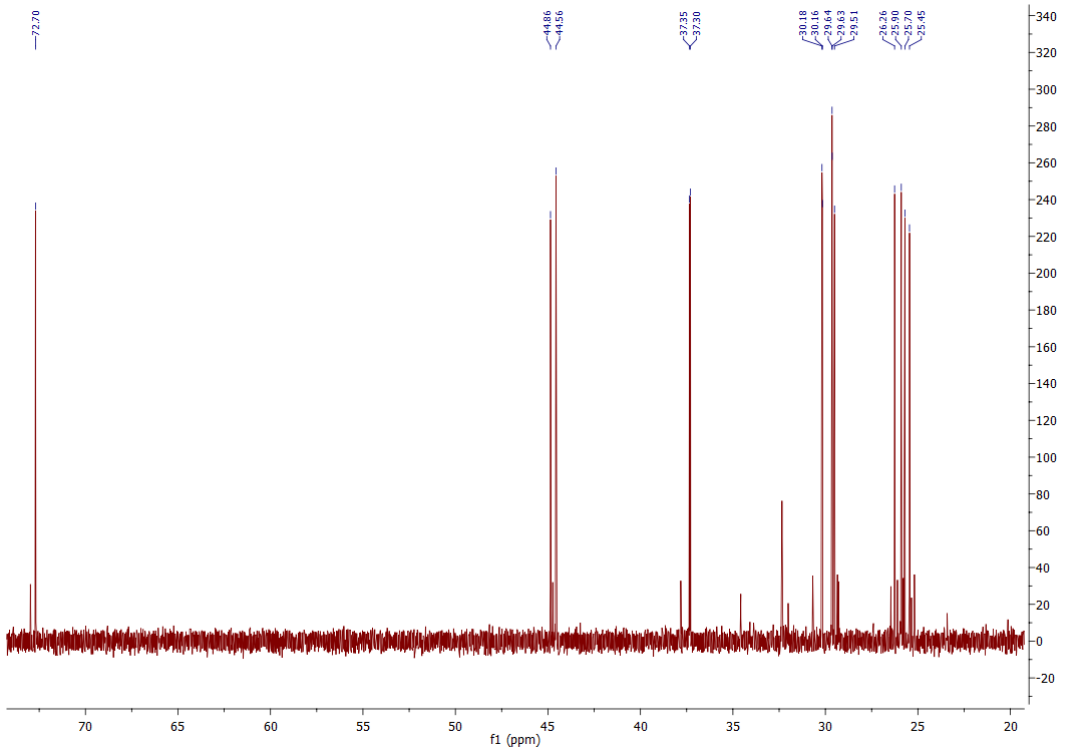


Figure S4 h (i) ^{1}H NMR of 8-hydroxycyclopentadecanone (CYP101B1).



(a)



(b)

Figure S4 h (ii) ^{13}C NMR of 8-hydroxycyclopentadecanone and some minor peaks were also observed indicating one or two minor metabolites (5%) might present. **(b)** Zoomed in ^{13}C NMR of 8-hydroxycyclopentadecanone from the 25 to 73 ppm region.

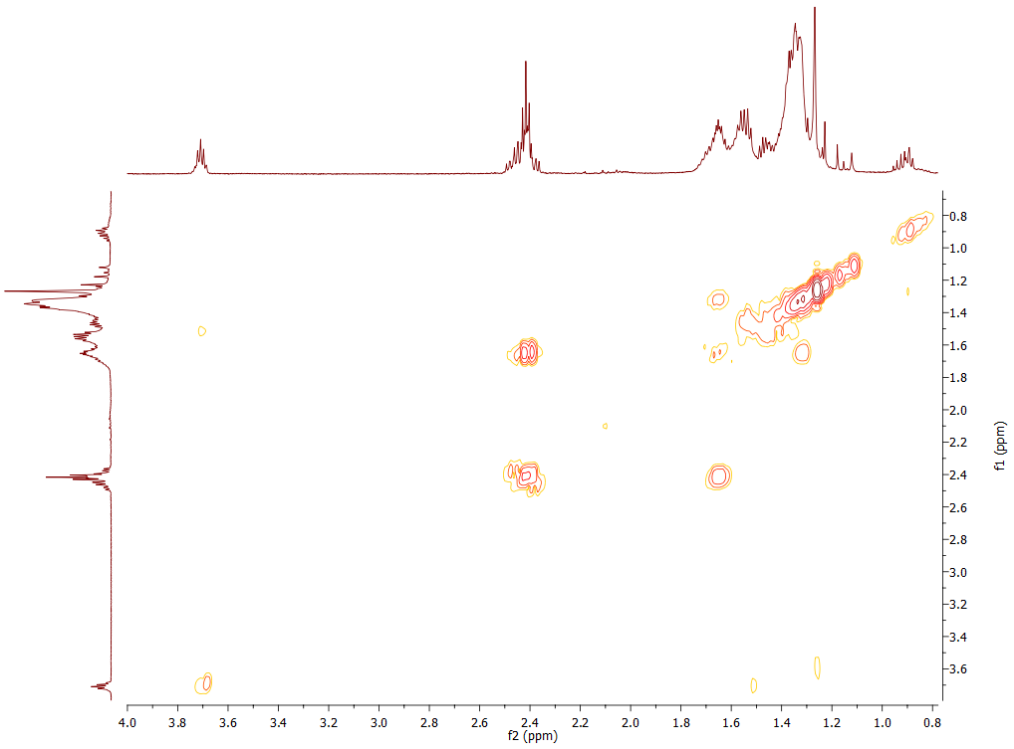


Figure S4 h (iii) gCOSY NMR of 8-hydroxycyclopentadecanone (CYP101B1).

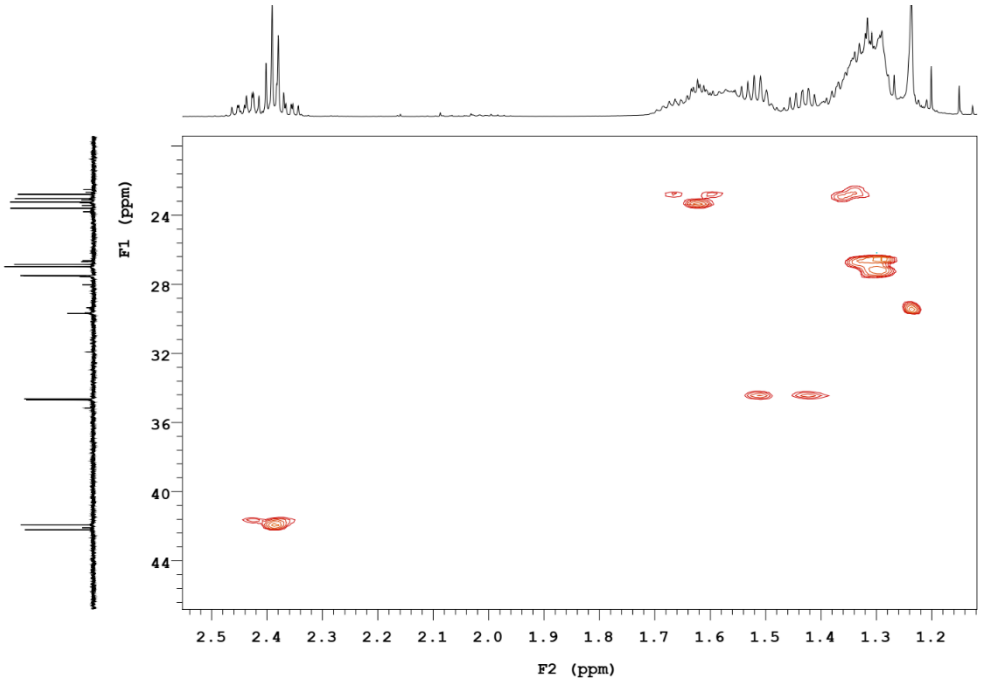


Figure S4 h (iv) Zoomed in (20 to 50 ppm region) HSQC NMR of 8-hydroxycyclopentadecanone (CYP101B1).

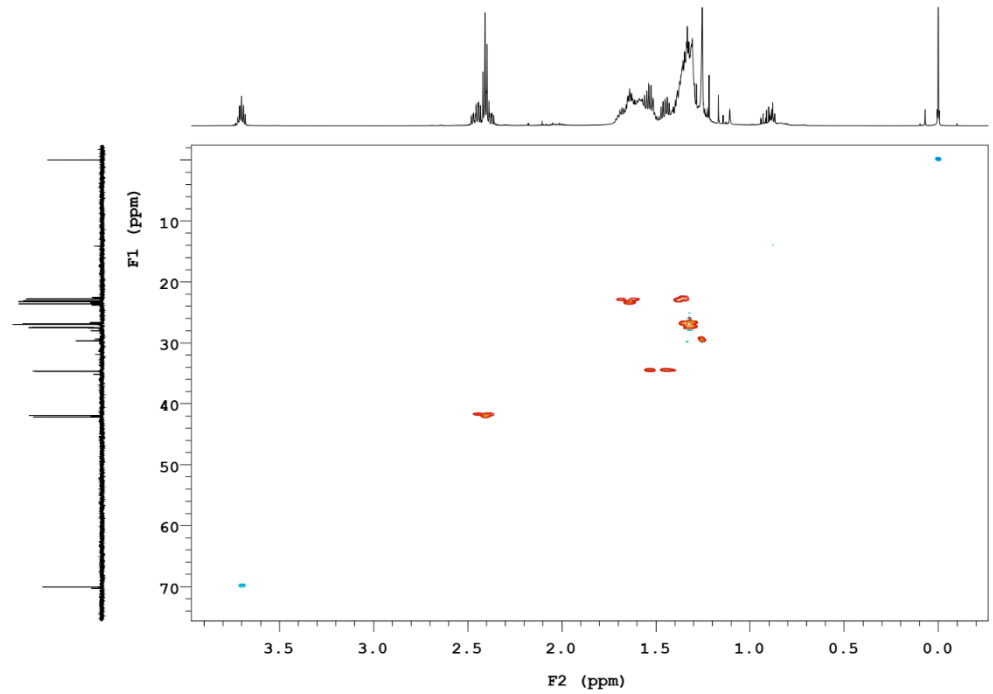


Figure S4 h (v) HSQC NMR of 8-hydroxycyclopentadecanone (CYP101B1).

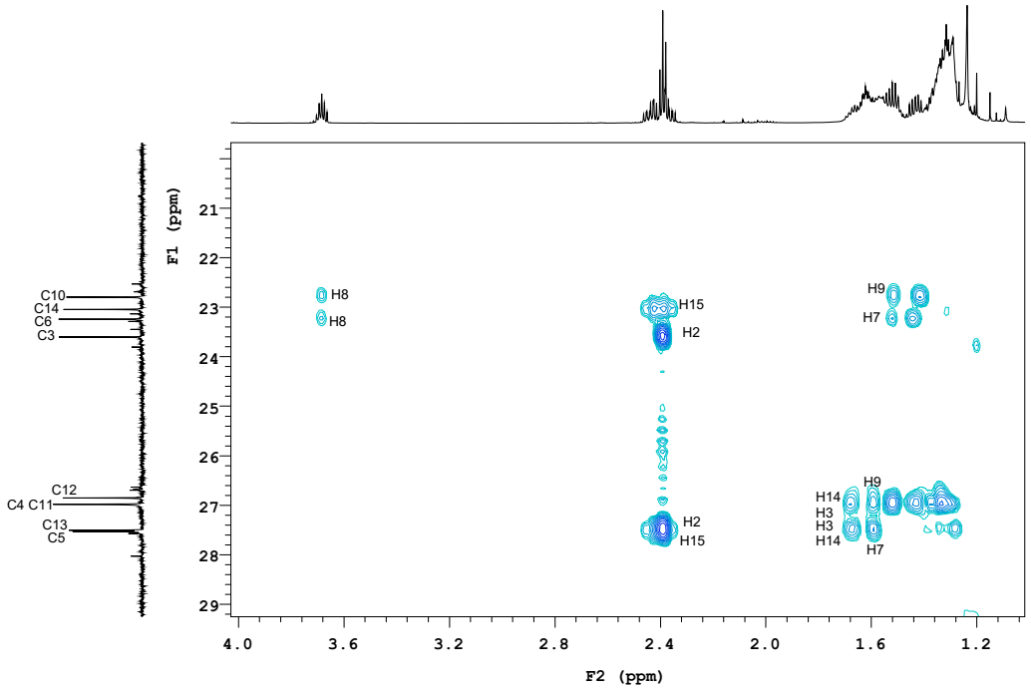


Figure S4 h (vi) Zoomed in (20 to 30 ppm region) HMBC NMR of 8-hydroxycyclopentadecanone which highlighted the correlations of carbons with the protons.

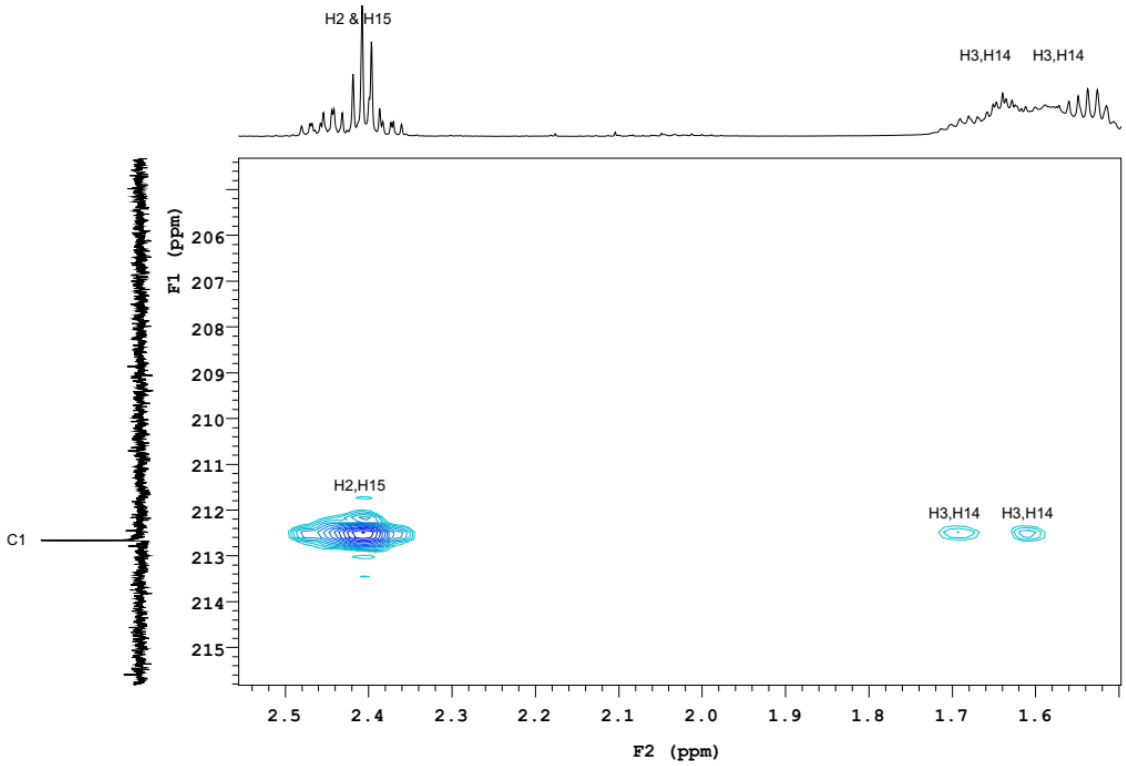


Figure S4 h (vii) Zoomed in C=O (215.30 ppm) region of HMBC NMR of 8-hydroxycyclopentadecanone which highlighted the correlations of C1 (215.30 ppm) with H2, H3, H14 and H15.

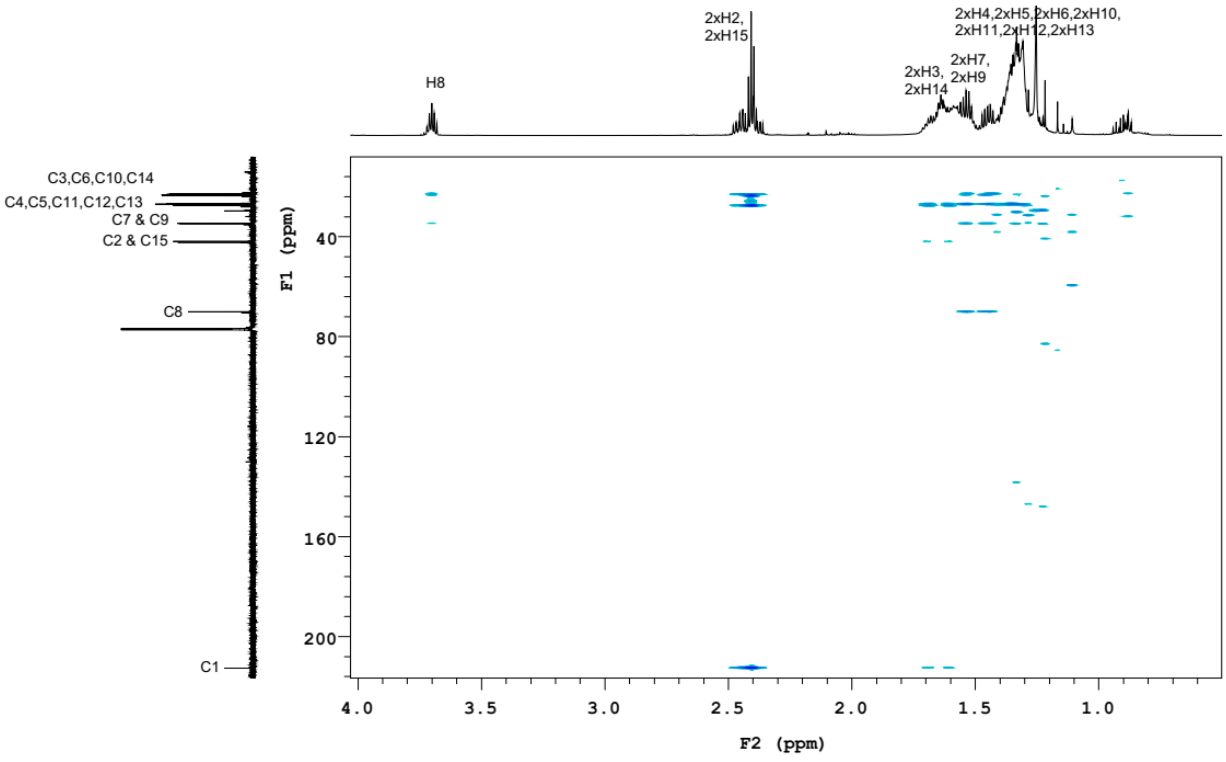


Figure S4 h (viii) HMBC NMR of 8-hydroxycyclopentadecanone

NMR for 8-hydroxy-2-nonanone:

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.86-3.73 (m, 1H, H8), 2.44 (*t*, *J* = 7.4 Hz, 2H, 2xH3), 2.14 (s, 3H, 3xH1), 1.69-1.59 (m, 2H, 2xH4), 1.48-1.39 (m, 3H, H6 & 2xH7), 1.37-1.29 (m, 3H, 2xH5 & H6), 1.19 (d, *J* = 6.2 Hz, 3H, 3xH9).

^{13}C NMR (126 MHz, $CDCl_3$) δ 211.86 (C2), 70.71 (C8), 46.30 (C3), 41.73 (C7), 32.54 (C1), 31.77 (C5), 28.17 (C6), 26.38 (C4), 26.21 (C9).

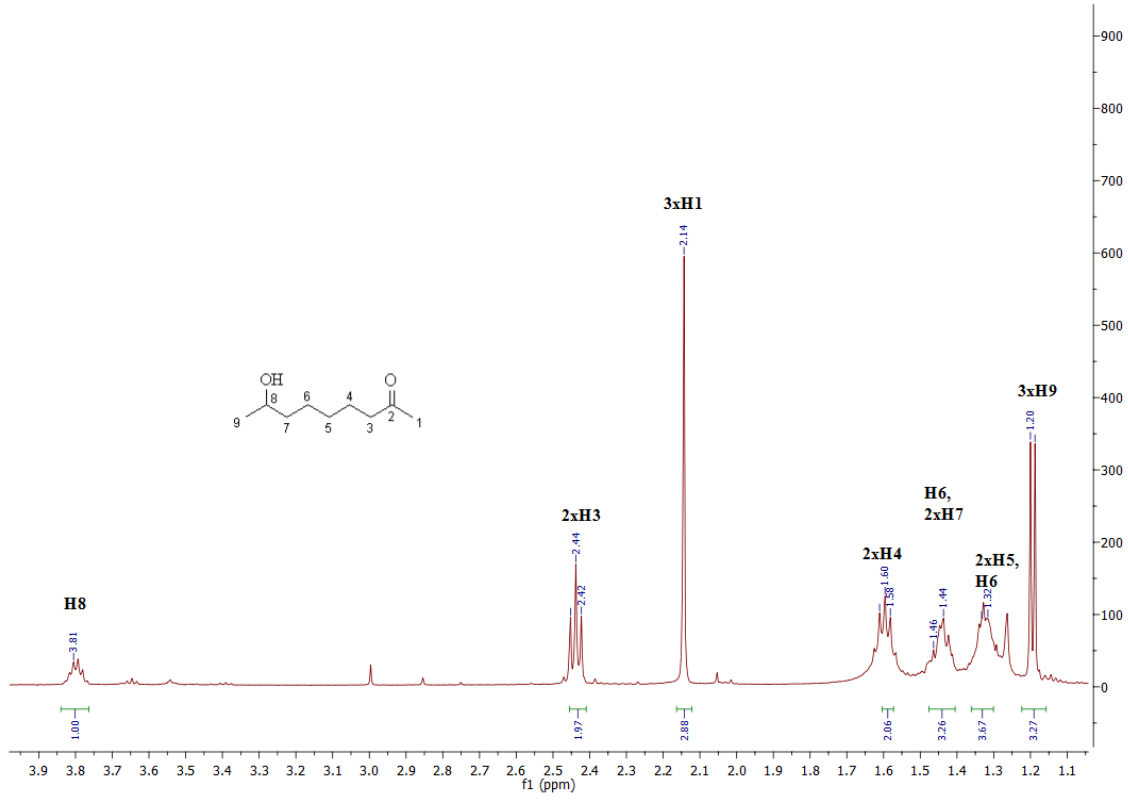


Figure S4 i (i) ^{1}H NMR of 8-hydroxy-2-nonanone (CYP101B1).

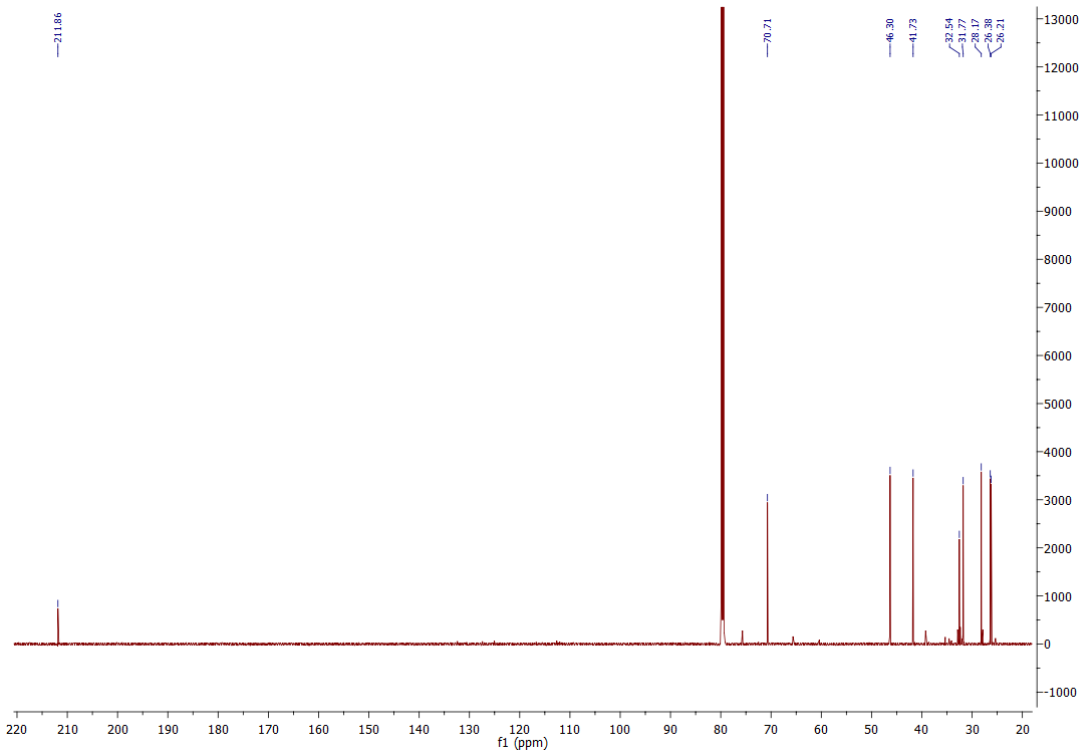


Figure S4 i (ii) ^{13}C NMR of 8-hydroxy-2-nonanone (CYP101B1).

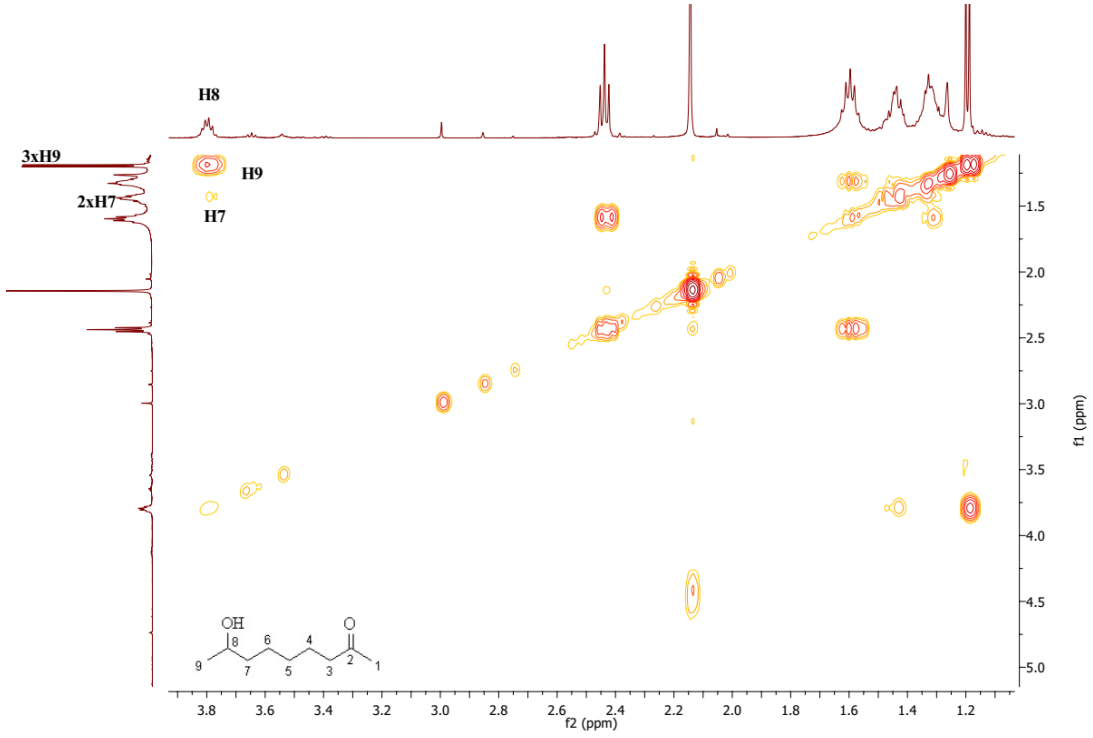


Figure S4 i (iii) gCOSY NMR of 8-hydroxy-2-nonanone (CYP101B1).

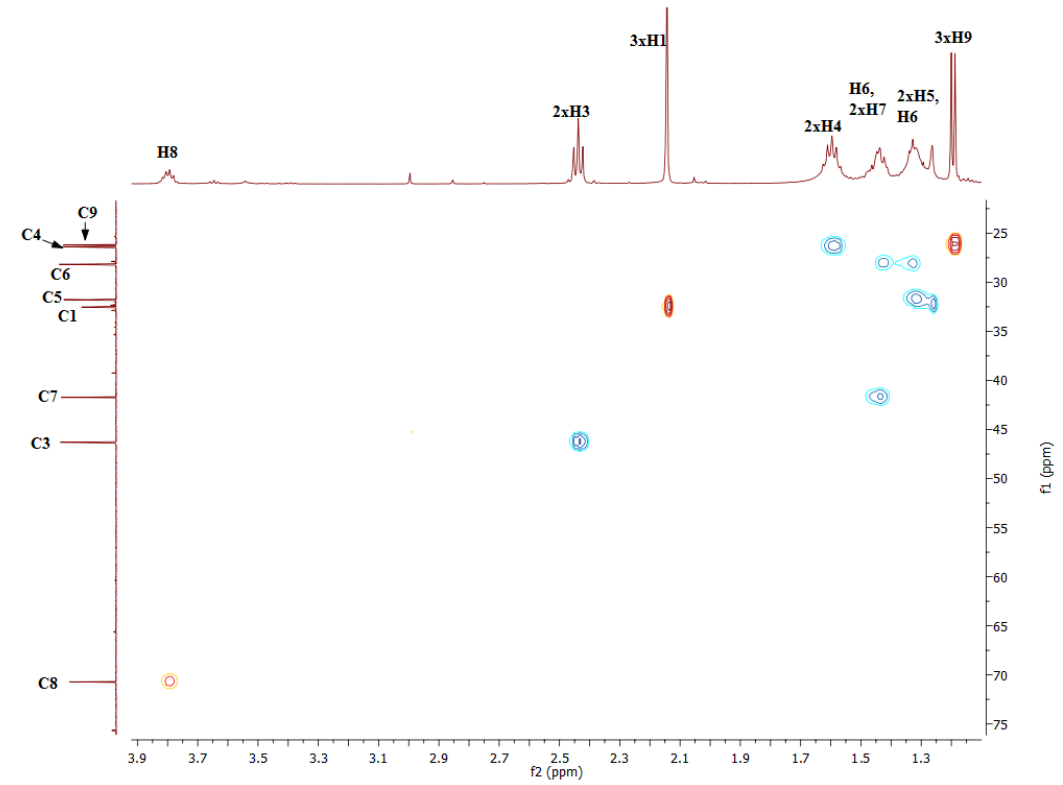


Figure S4 i (iv) HSQC NMR of 8-hydroxy-2-nonanone (CYP101B1).

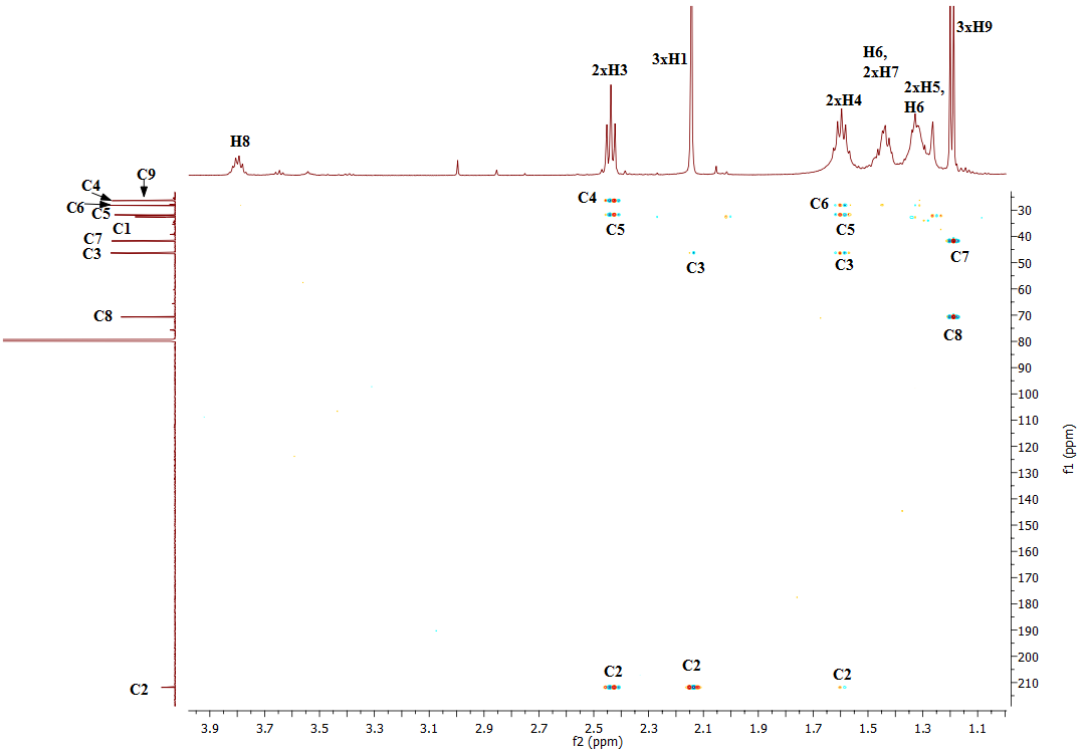


Figure S4 i (v) HMBC NMR of 8-hydroxy-2-nonanone (CYP101B1).

NMR for 8-hydroxy-2-undecanone:

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.63-3.53 (quintet, 1H, H8), 2.44 (dd, J = 15.5, 7.8 Hz, 2H, 2xH3), 2.14 (s, 3H, 3xH1), 1.65-1.53 (m, 2H, 2xH4), 1.52-1.37 (m, 6H, H6, 2xH7, 2xH9 & H10), 1.36-1.28 (m, 4H, 2xH5, H6 & H10), 0.96-0.88 (m, 3H, 3xH11).

^{13}C NMR (126 MHz, $CDCl_3$) δ C2 (211.83), C8 (74.26), C3 (46.32), C9 (42.38), C7 (39.90), C1(32.50), C5 (31.84), C6 (28.06), C4 (26.42), C10 (21.53), C11 (16.78).

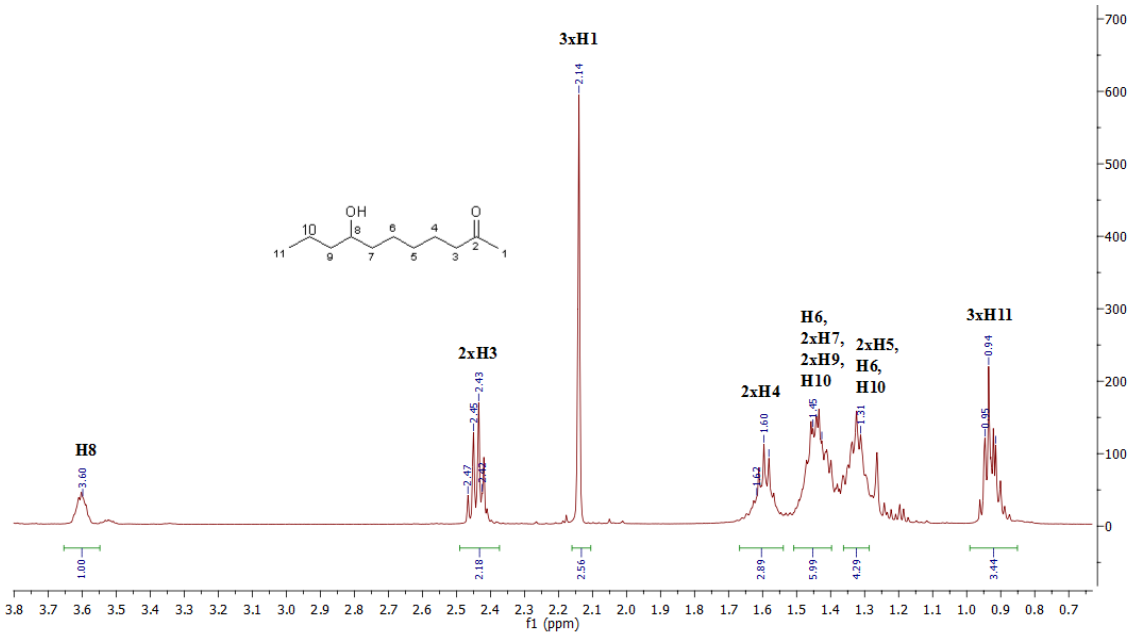
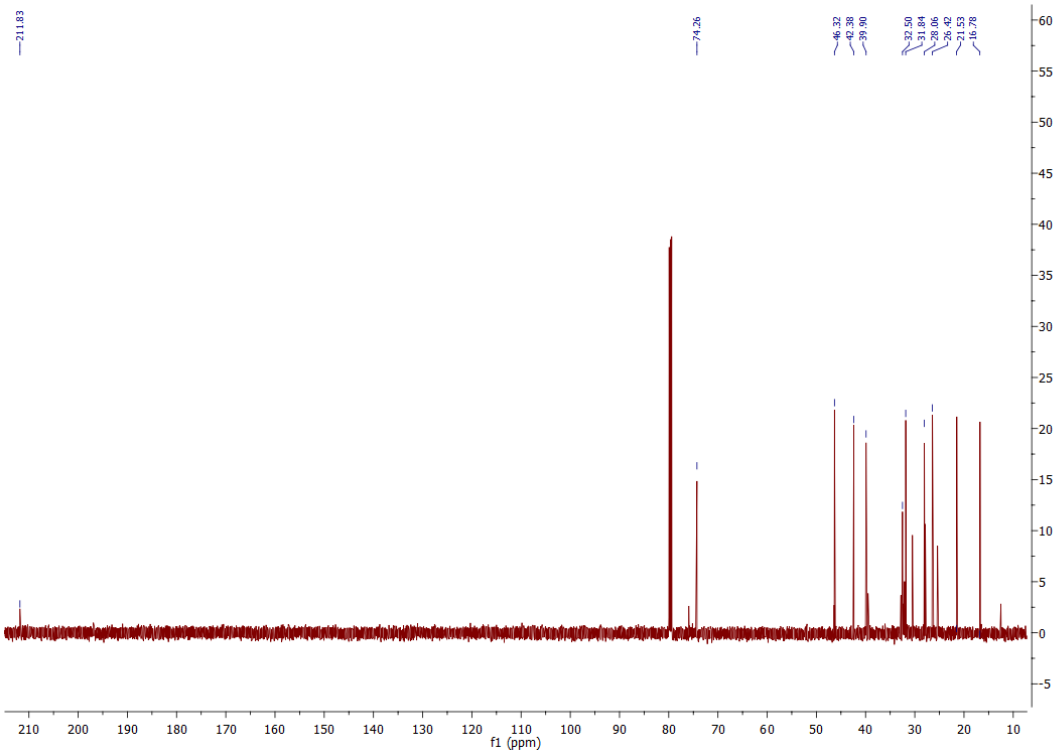
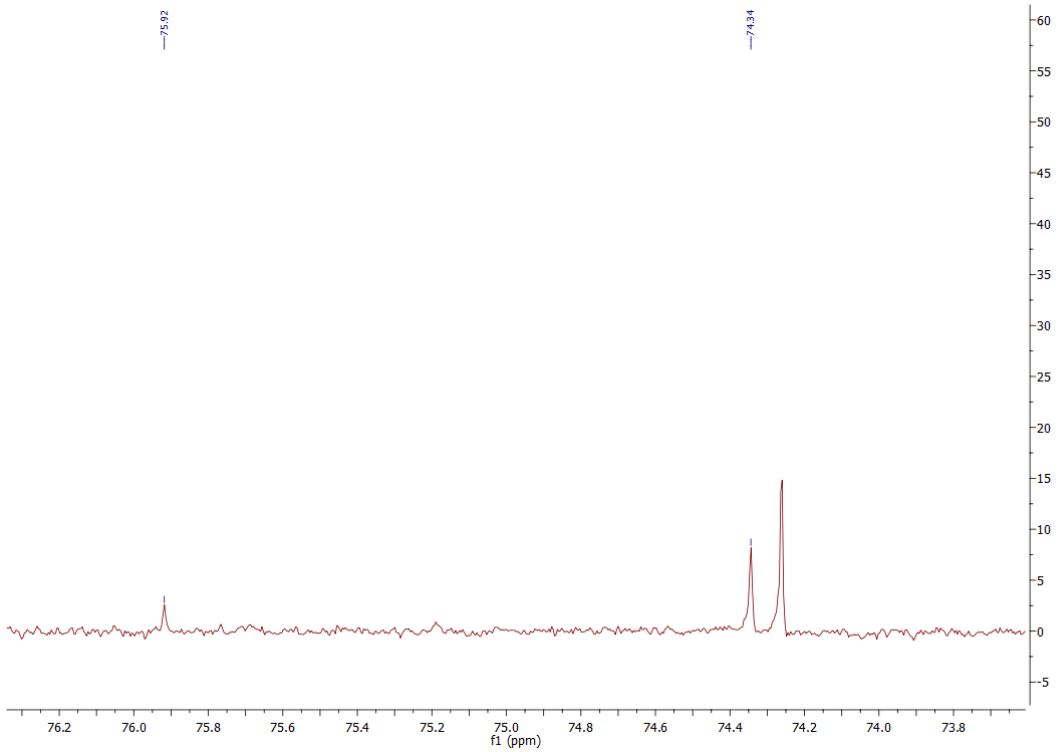


Figure S4 j (i) ^{1}H NMR of 8-hydroxy-2-undecanone (CYP101B1).



(ii)



(iii)

Figure S4 j (ii) and **(iii)** ^{13}C NMR of 8-hydroxy-2-undecanone. The zoomed in ^{13}C NMR of the region 70-76 ppm to highlight the carbon peaks of minor metabolites.

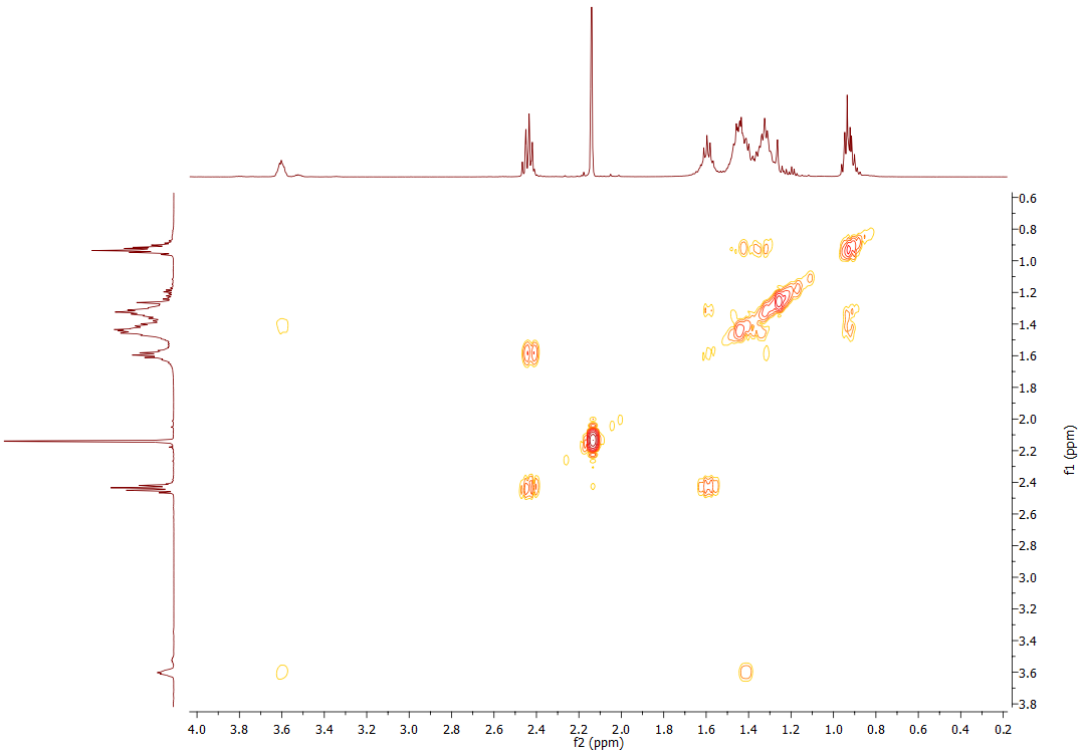


Figure S4 j (iv) gCOSY NMR of 8-hydroxy-2-undecanone (CYP101B1).

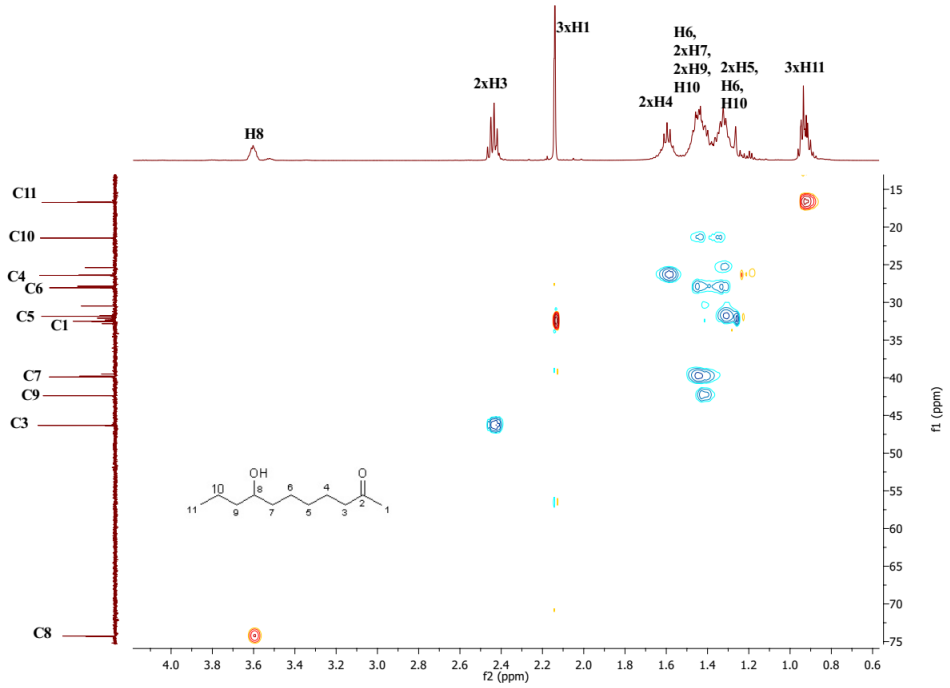
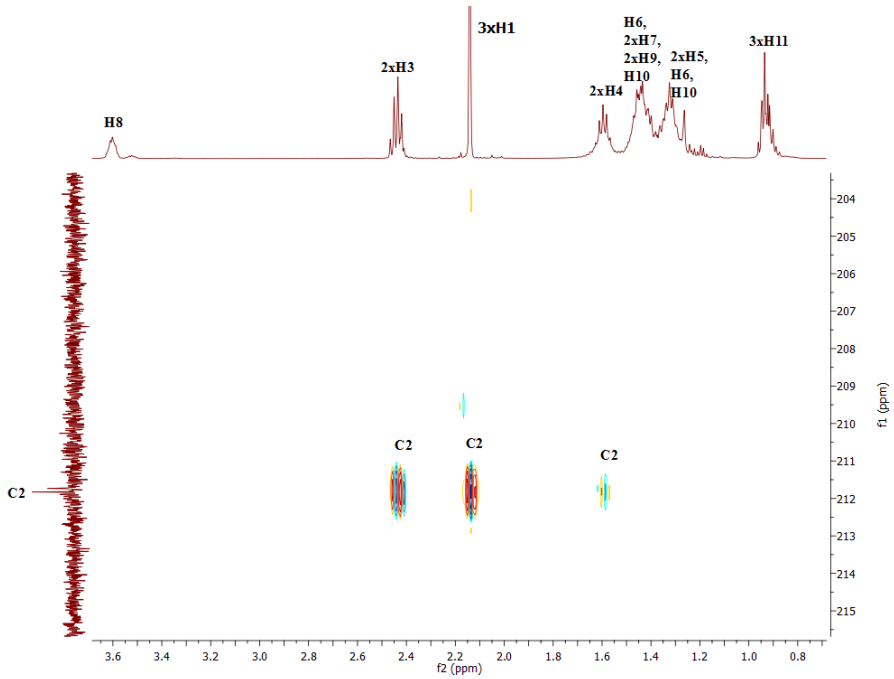
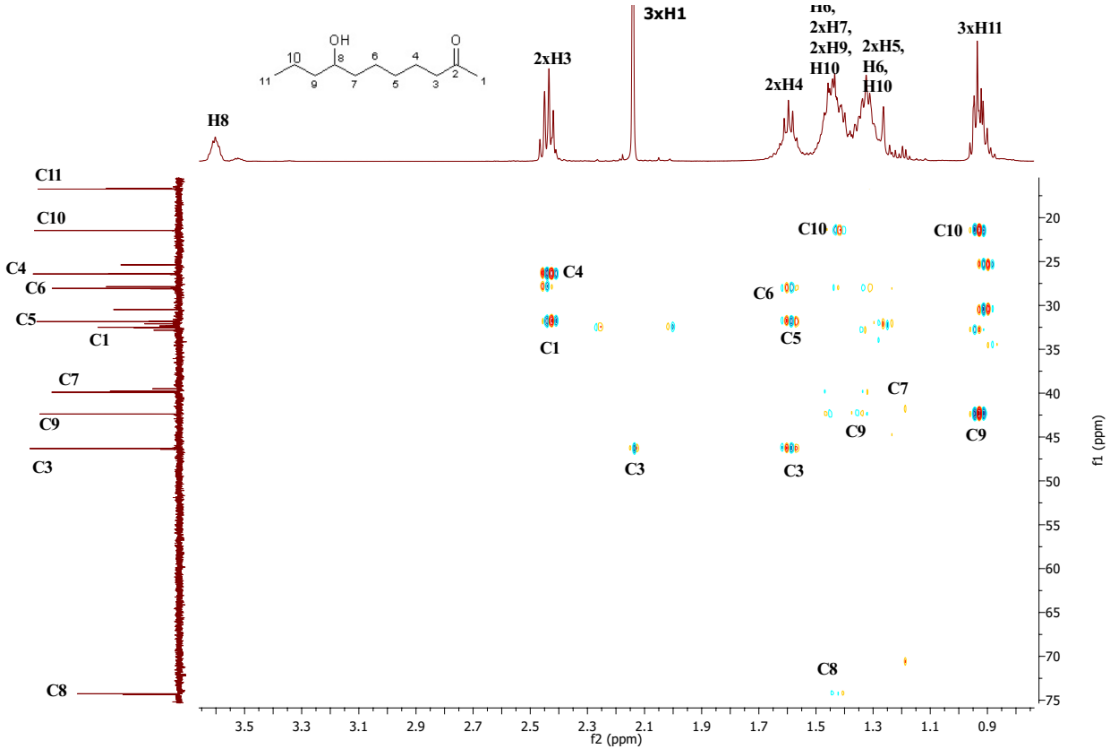


Figure S4 j (v) HSQC NMR of 8-hydroxy-2-undecanone (CYP101B1).



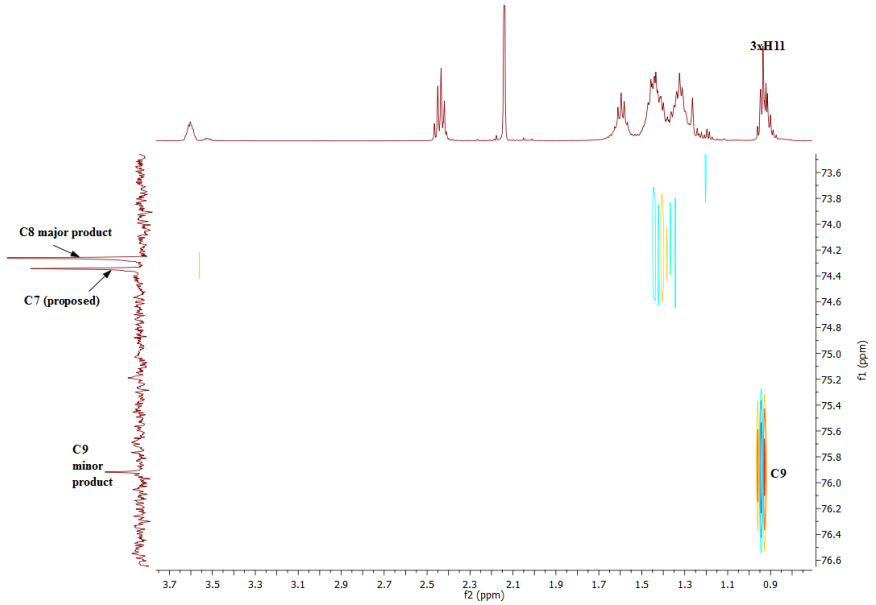
(vi)

Zoomed in version of HMBC NMR to highlight the interactions of C2 with H3, H1 and H4.

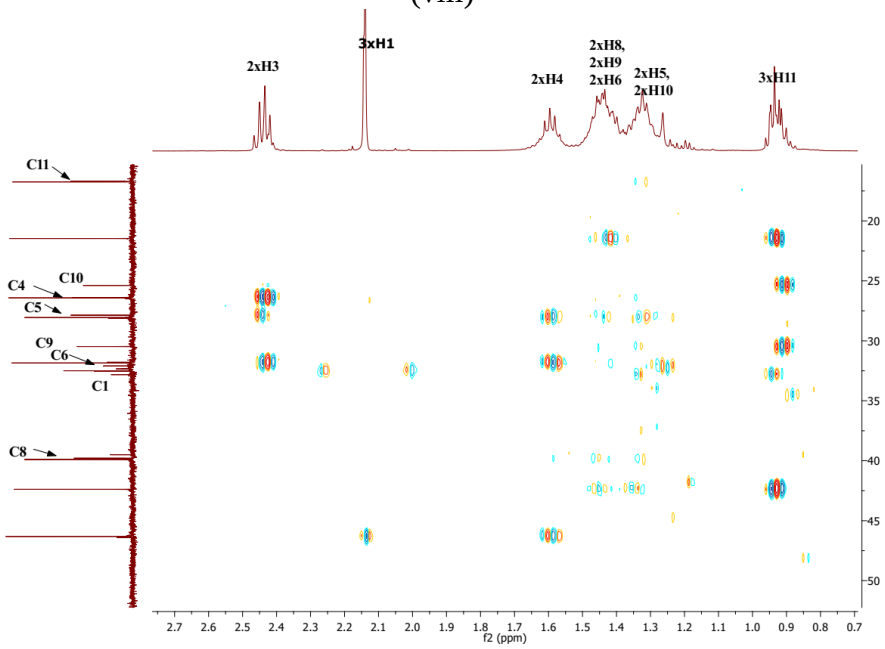


(vii)

Figure S4 j (vi) and **(vii)** HMBC NMR of 8-hydroxy-2-undecanone (CYP101B1). The product was confirmed 8-hydroxy-2-undecanone as carbon signal (74.26 ppm) showed correlations with the protons H6, H7, H9 and H10 (1.52-1.37 ppm).



(viii)



(ix)

Figure S4 j (viii) and **(ix)** Zoomed in the HMBC NMR of 8-hydroxy-2-undecanone to highlight the C-H interactions of minor metabolites. The peak at 75.94 ppm showed a strong correlation with H11 protons and this product was assigned as a 9-hydroxy metabolite. The minor signal at 74.34 ppm displayed the interaction with the protons at 1.52-1.28 ppm. Other carbon signals C1, C4, C5, C6, C8, C9, C10, C11 of this metabolite were identified using the C-H correlations in the HMBC NMR. Hydroxylation did not occur at C3 as 74.34 ppm carbon signal has no correlation with H4 peak. Therefore the product was presumed to be a 7-hydroxy metabolite. However, full characterisation of these metabolites were not possible due to noise and low yield.

NMR for 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate:

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.52-5.41 (m, 1H, H3), 4.20 (brd *s*, 1H, H5), 2.28-2.19 (m, 1H, H1), 2.19-2.13 (m, 1H, H6), 2.02-1.97 (m, 4H, H2 & 3xH12), 1.92-1.85 (m, 1H, H2), 1.76 (s, 3H, 3xH7), 1.48-1.41 (2s, 6H, 3xH9 & 3xH10), 1.35-1.20 (m, 1H, H6).

^{13}C NMR (126 MHz, $CDCl_3$) δ C11 (173.12), C4 (139.06), C3 (126.18), C8 (86.64), C5 (73.78), C1 (44.56), C6 (36.97), C2 (29.36), C9/C10 (25.97), C9/C10 (25.64), C12 (25.08), C7 (21.26).

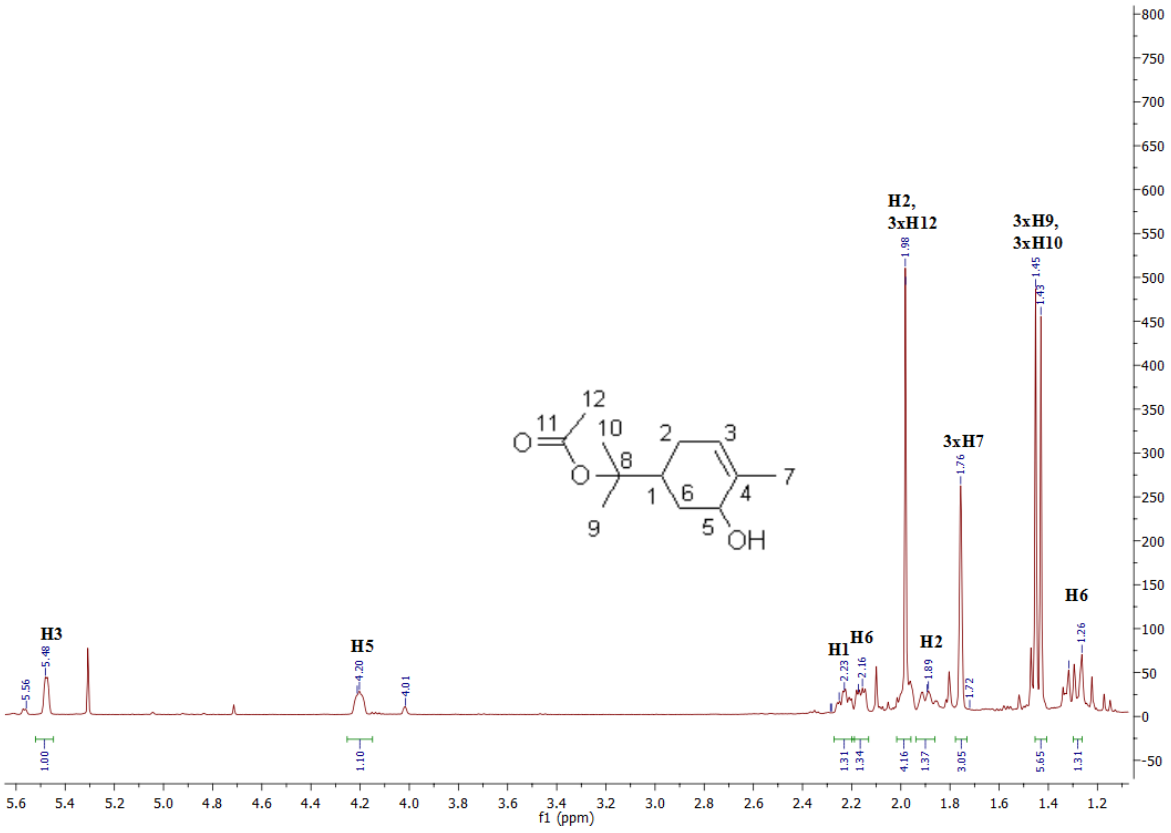


Figure S4 k (i) ^{1}H NMR of 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate (CYP101C1). The minor peaks at 5.56 ppm and 4.01 ppm indicated the presence of other diastereomer of 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate.

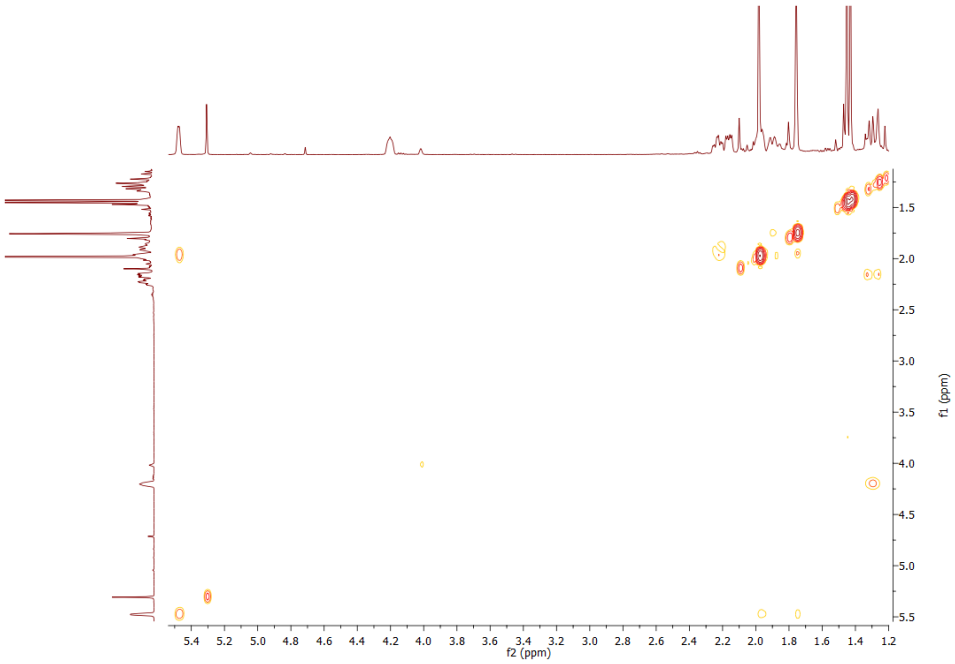


Figure S4 k (ii) gCOSY NMR of 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate (CYP101C1).

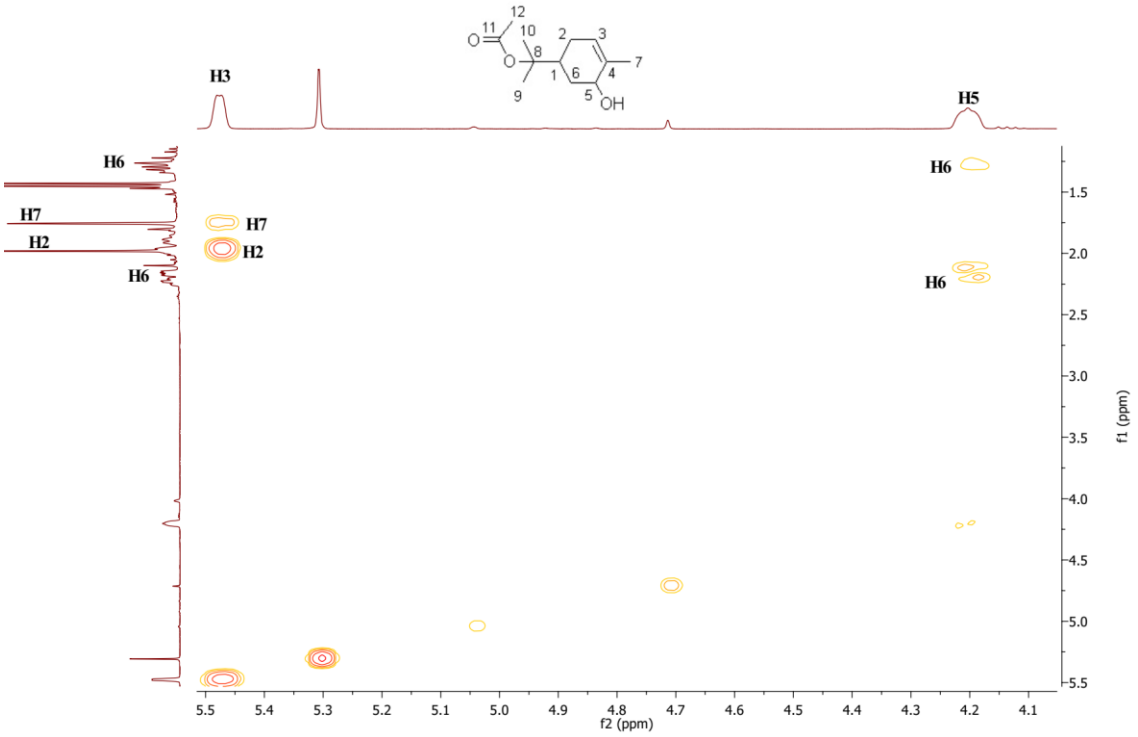
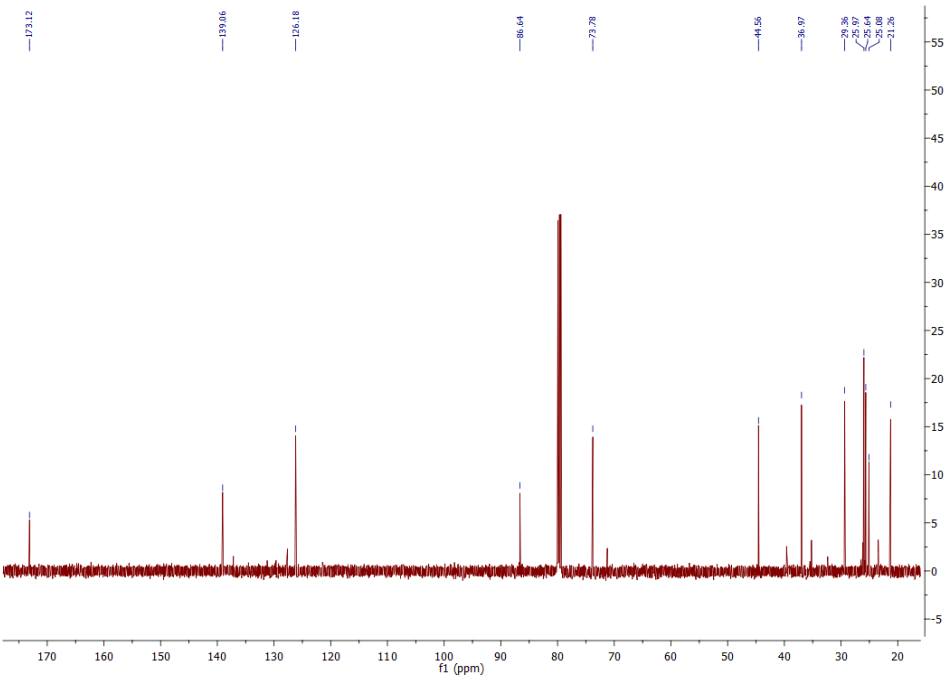
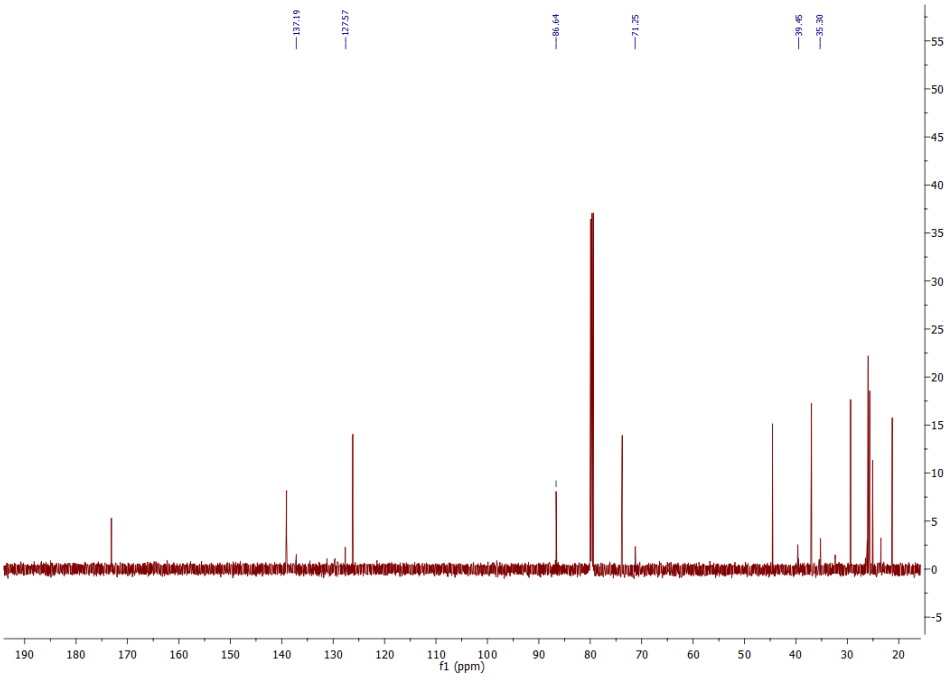


Figure S4 k (iii) Zoomed in gCOSY NMR region of 4.1 ppm to 5.55 ppm to highlight the interactions. The product was confirmed due to strong coupling of H5 (4.21 ppm) with H6 (2.19-2.13 ppm) and H6 (1.35-1.20). H3 showed strong coupling with H2 and a weak coupling with the H7.



(iv)



(v)

Figure S4 j (iv) and **(v)** ^{13}C NMR of 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate (CYP101C1). Highlighted the signals of minor product which was assigned as the other diastereomer of 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-, *α*-acetate.

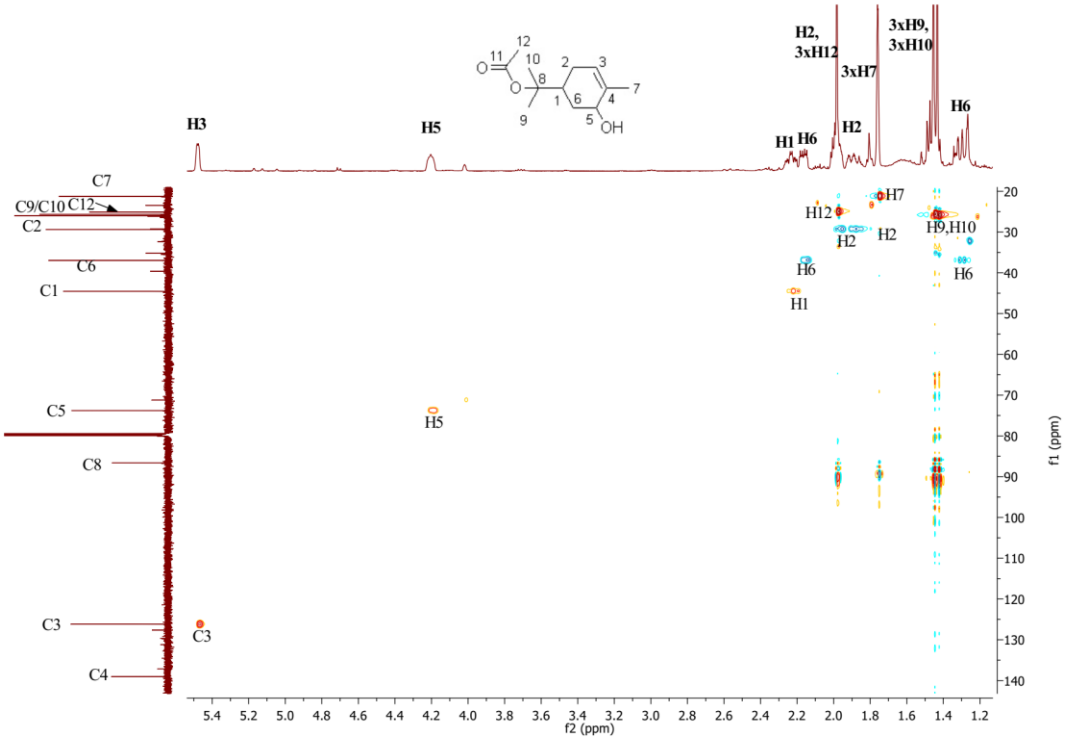


Figure S4 j (vi) HSQC NMR of 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate (CYP101C1), highlighted the C-H interactions.

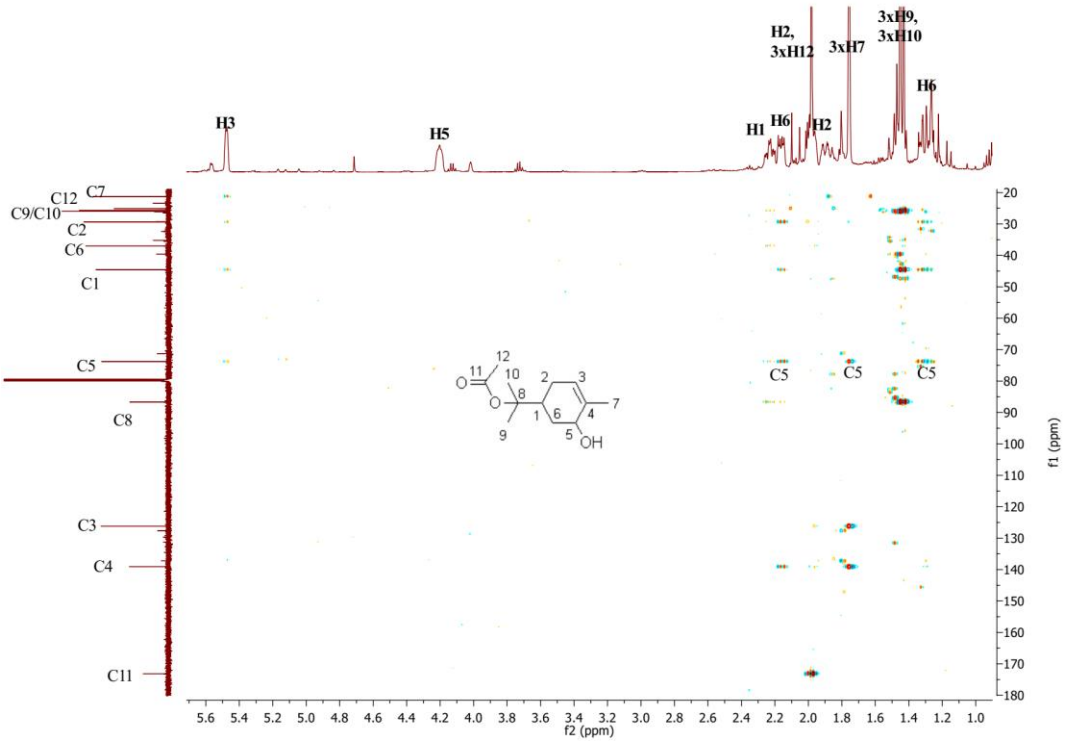


Figure S4 j (vii) HMBC NMR of 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate (CYP101C1).

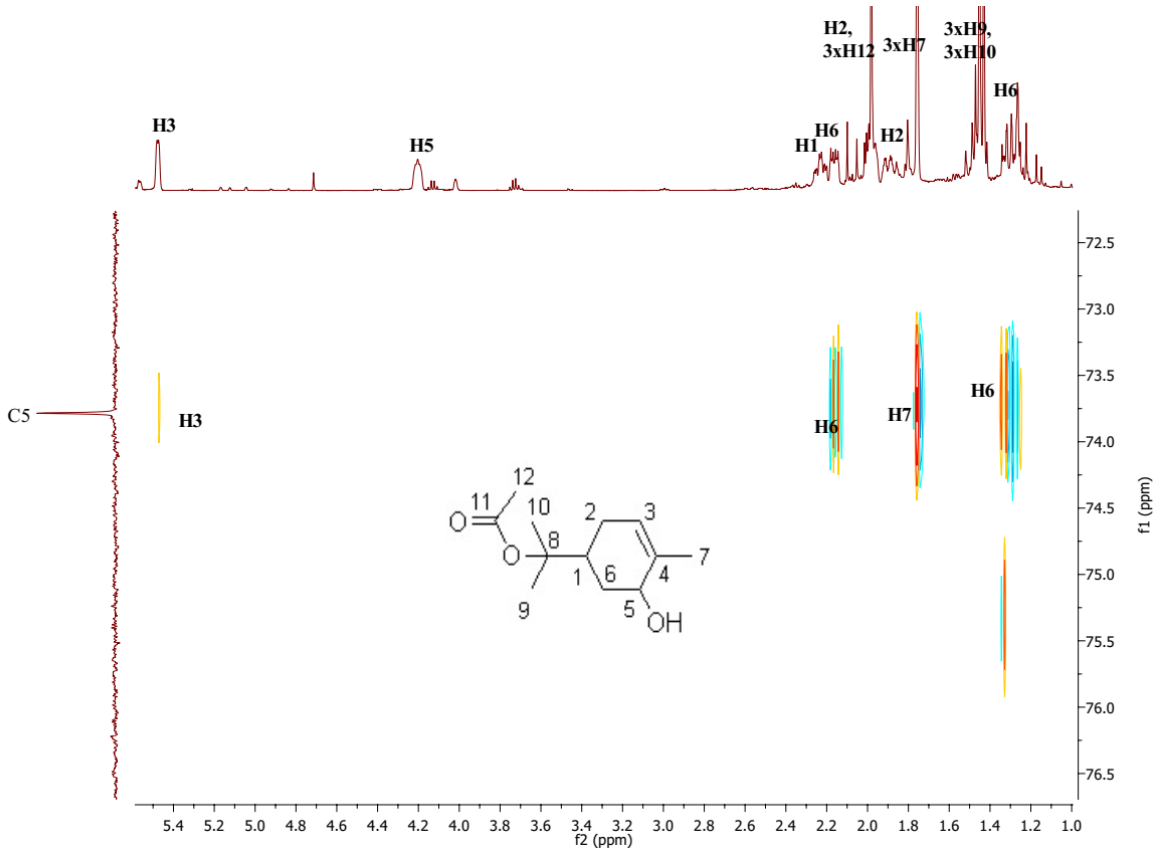


Figure S4 j (viii) HMBC NMR of 3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate (CYP101C1), highlighted the correlations of C5 with H3, H6 and H7.

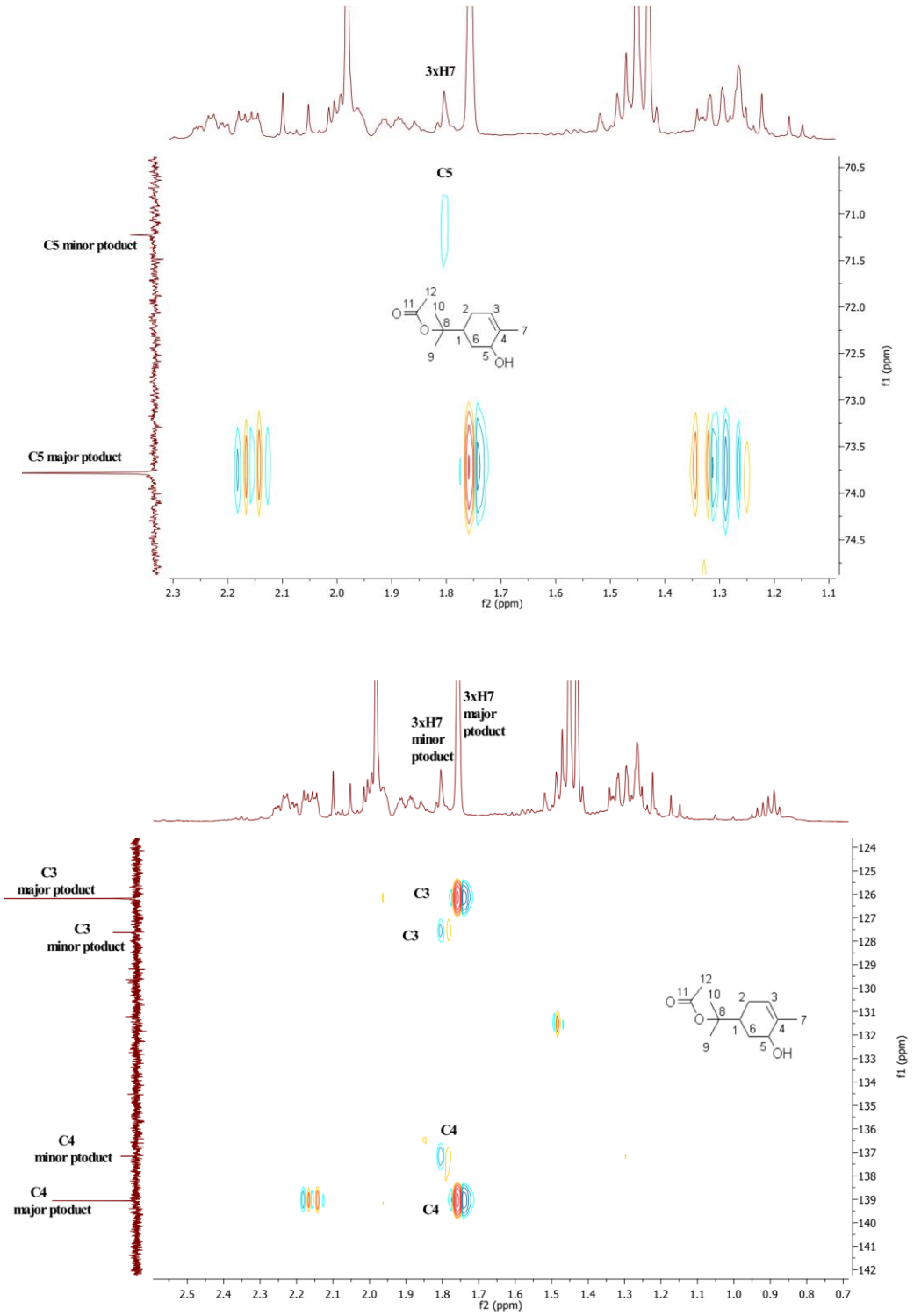


Figure S4 j (ix) Zoomed in HMBC NMR of major product (3-cyclohexene-1-methanol, 5-hydroxy-*α*,*α*,4-trimethyl-,*α*-acetate) to highlight the minor product signals. The minor product was presumed to be either diastereomer of the main product or arises from ester hydrolysis (sobrerol) as the C5 signal showed a correlation with H7 as well as the presence of two alkene carbons peaks at 120 to 140 ppm region in the ^{13}C NMR.

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