Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.

Chemo-enzymatic oxidative cleavage of isosafrole for the synthesis of piperonal

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SUPPORTING INFORMATION

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Table S1. Factorial design for the enzymatic epoxidation of isosafrole.

n° run	Enzyme [mg]	Isosafrole [M]	H_2O_2 [M]	Isosafrole conversion [%][a]
1	10	0.5	0.3	27
2	20	0.2	0.6	87
3	20	0.5	0.6	56
4	20	0.2	0.3	72
5	10	0.2	0.6	77
6	10	0.5	0.6	47
7	20	0.5	0.3	35
8	10	0.2	0.6	74
9	20	0.5	0.6	55
10	20	0.2	0.6	83
11	10	0.5	0.3	29
12	10	0.2	0.3	48
13	20	0.5	0.3	34
14	10	0.5	0.6	44
15	20	0.2	0.3	77
16	10	0.2	0.3	55

[a] Calculated on the basis of GC/MS analysis

The intervals of the three variables submitted to DOE[1] optimization were chosen on the basis of preliminary data found in the literature on similar reactions[2-4] and considering also our first experiments carried out in batch conditions using the simple method of changing one variable at a time.

Table S2. Results of factorial design experiments.

n° run	Epoxide (8) [%][a]	Monoethyl ethers (11a,b) [%][a]	Diol (10) [%][a]	Monoacetates (9a,b) [%][a]	Isosafrole ((*E*)- and (*Z*)-3) [%][a]
1	3	4	16	4	73
2	16	10	16	45	13
3	7	5	11	33	44
4	6	7	38	21	28
5	18	5	33	21	23
6	7	4	18	18	53
7	3	7	22	3	65
8	16	5	25	28	26
9	7	5	14	29	45
10	14	10	16	43	17
11	3	3	18	5	71
12	4	5	31	8	52
13	3	5	19	7	66
14	7	3	15	19	56
15	8	5	38	26	23
16	5	7	31	12	45

[a] Calculated on the basis of GC/MS analysis

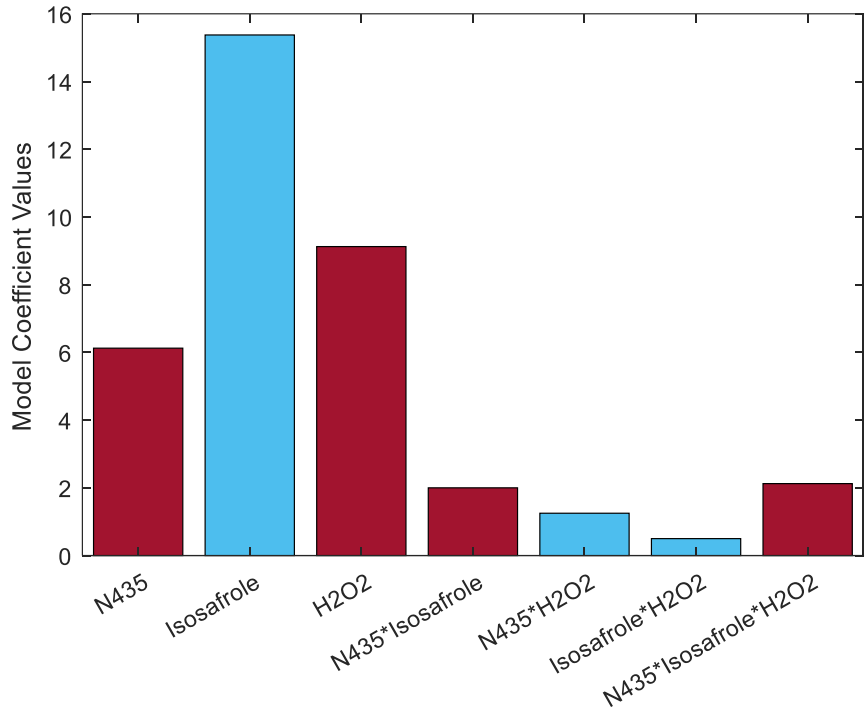


Figure S1. Coefficient plot showing the effects of the three variables (enzyme load, isosafrole concentration and H_2O_2 concentration) and of their interactions on conversion: red=positive value = positive effect; light blue= negative value = negative effect.
(multivariate regression model obtained by DOE analysis: $Y = 56.25 + 6.125X_1 – 15.375X_2 + 9.125X_3 – 2.00X_1X_2 – 1.250X_1X_3 + 0.500X_2X_3 + 2.125X_1X_2X_3$ - enzyme load X_1, isosafrole concentration X_2, H_2O_2 concentration X_3).

From the graph it is possible to observe that isosafrole has the highest influence on the final reaction conversion in a negative way (the increase of isosafrole concentration determines a decrease of conversion, "negative effect"), while hydrogen peroxide and enzyme concentration are less effective, but their increase produces an increase of the final conversion ("positive effect"). Second order interactions between the variables are not effective on final conversion (very low values of 2nd order terms).

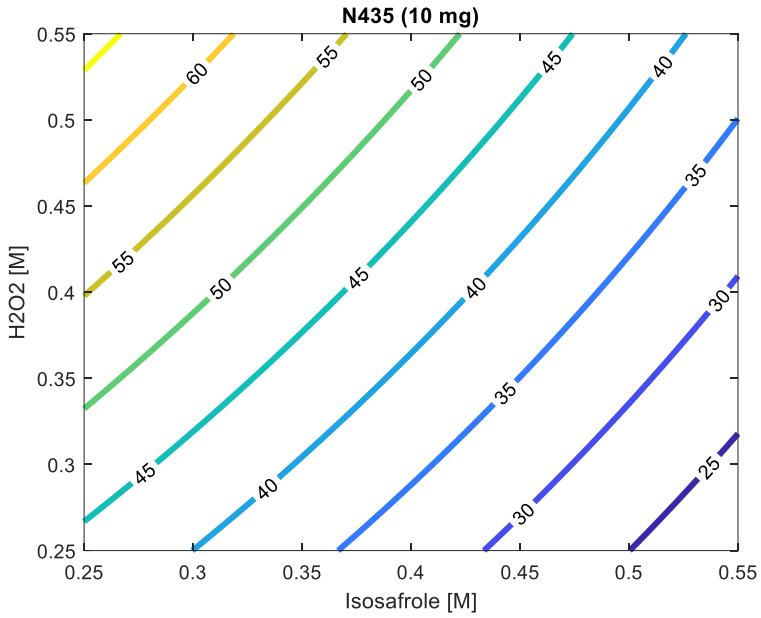


Figure S2. Contour plot of conversion [%] as a function of isosafrole concentration [M] and hydrogen peroxide concentration [M] evaluated at an enzyme load of 10 mg. Lines refer to constant conversion corresponding to the value reported on the labels.

It is possible to observe that the yellow line (corresponding to higher values of conversion) is found in correspondence of low values of isosafrole concentration and high values of hydrogen peroxide concentration.

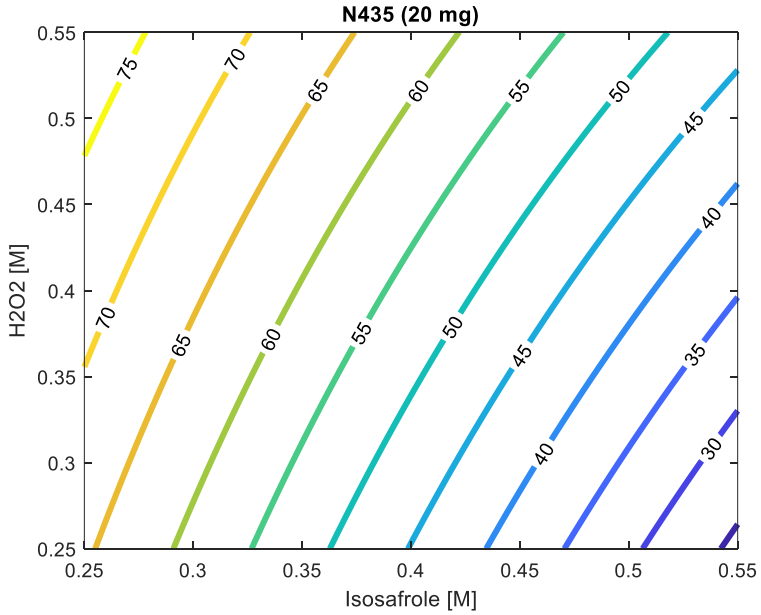
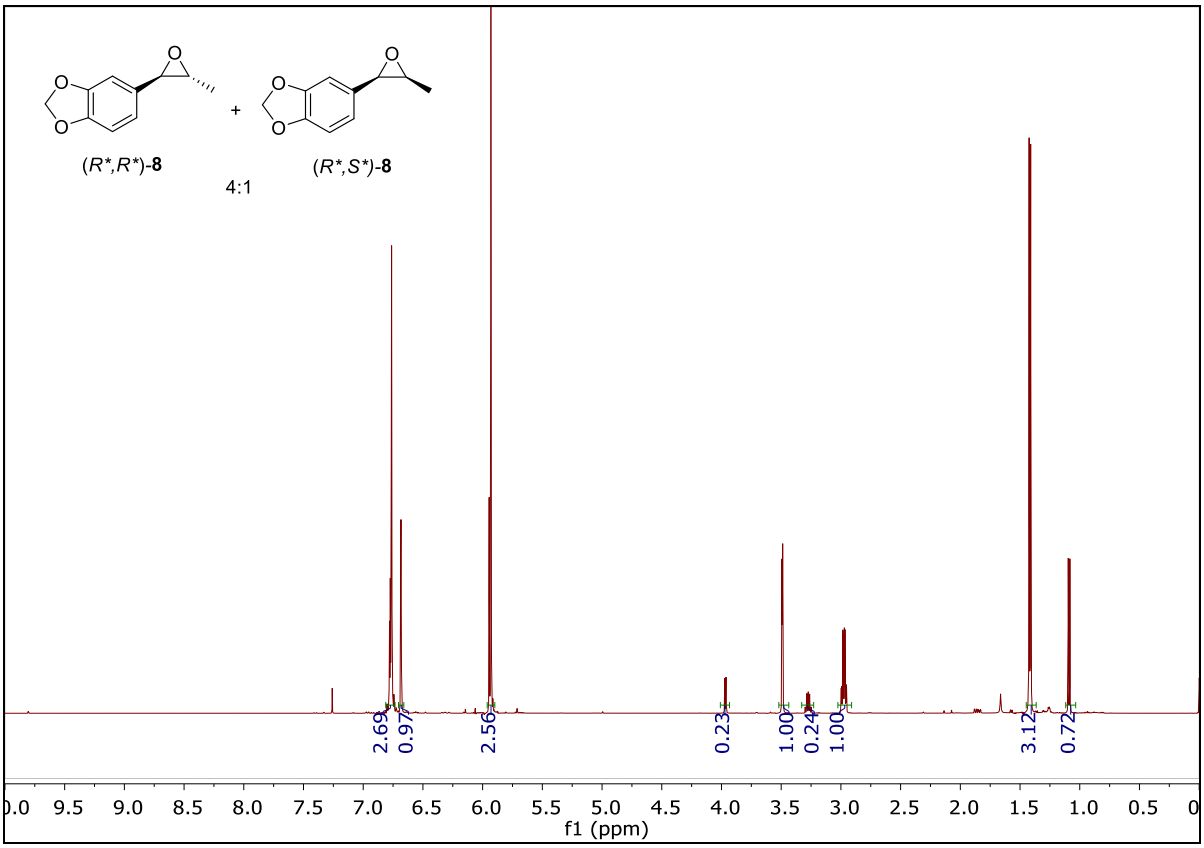
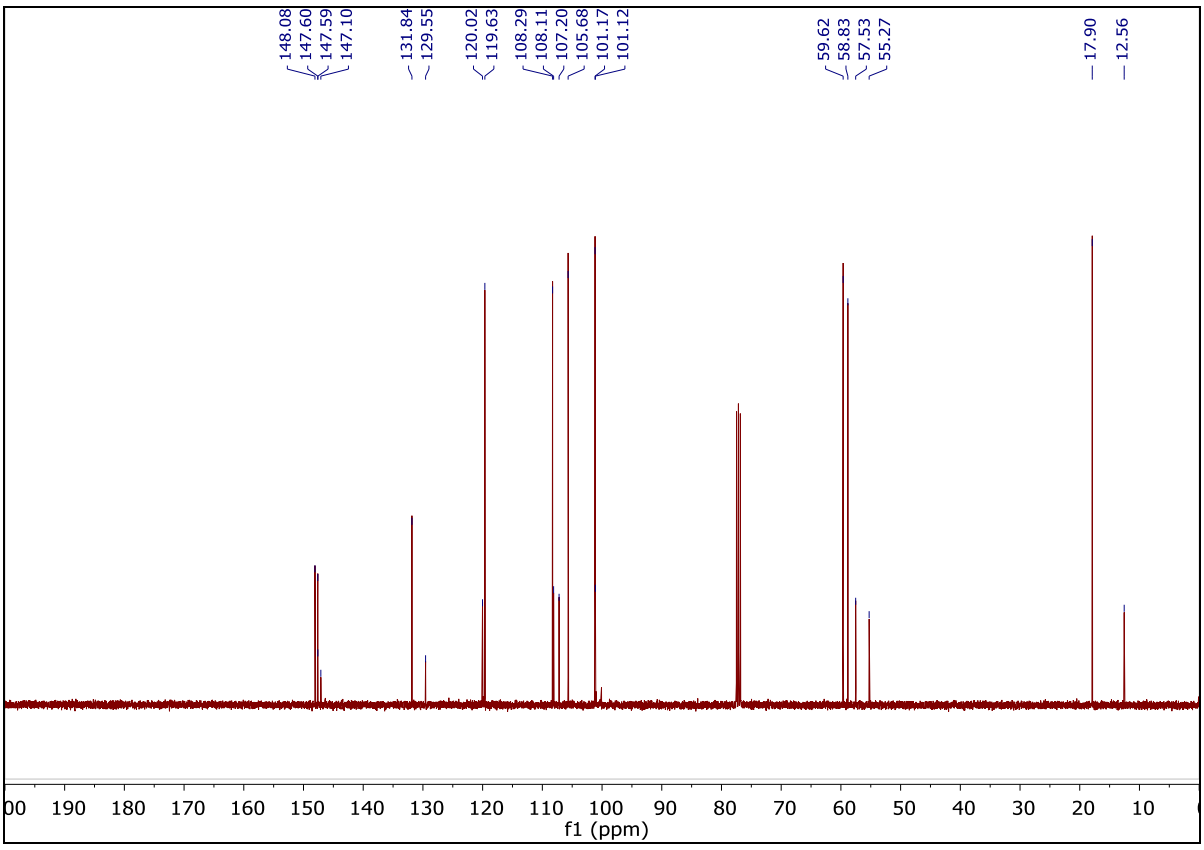


Figure S3. Contour plot of conversion [%] as a function of isosafrole concentration [M] and hydrogen peroxide concentration [M] evaluated at an enzyme load of 20 mg. Lines refer to constant conversion corresponding to the value reported on the labels.

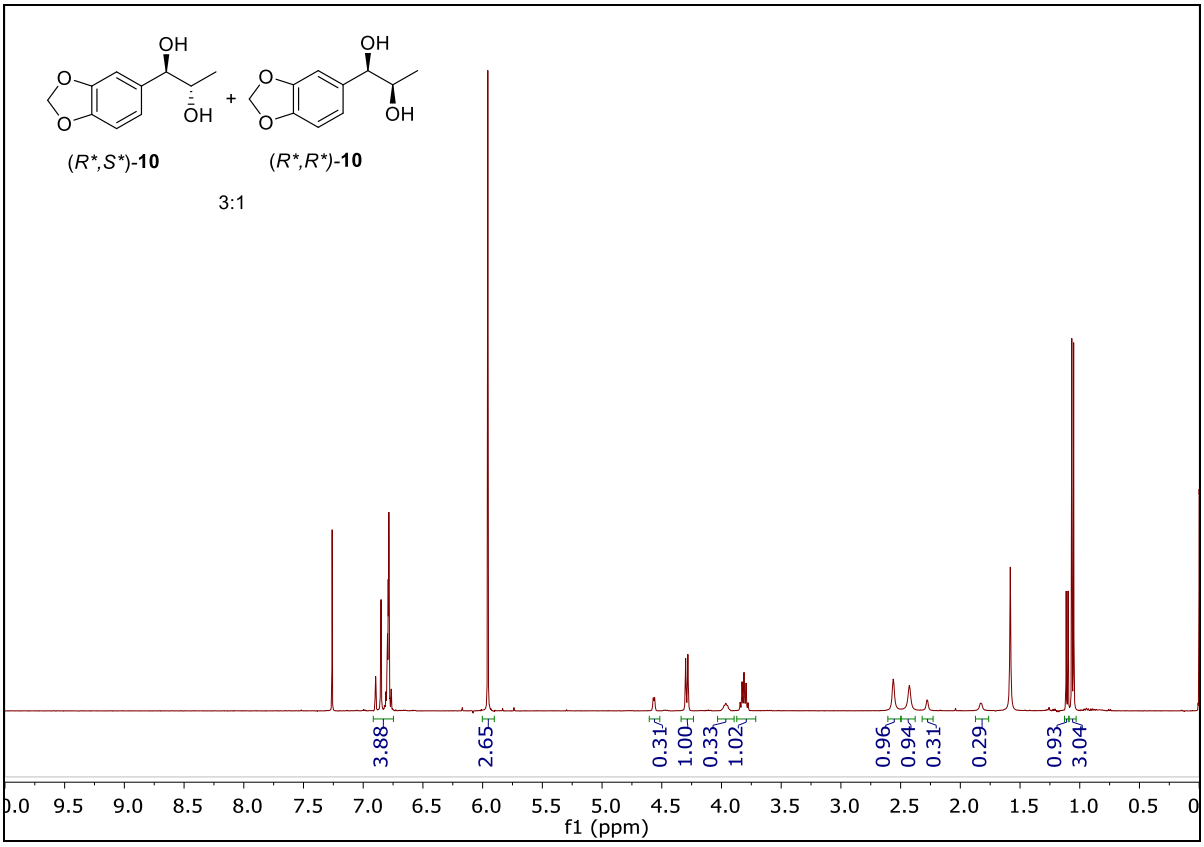
The analysis of the contour plot shows that the best values of conversion (yellow line) are related to low isosafrole concentration and high hydrogen peroxide concentration. With respect to the plot in Figure S2, obtained for a lower lipase amount (10 mg), the conversions in this second plot are higher than the previous ones since the increase of enzyme amount has a positive effect on the final conversion.

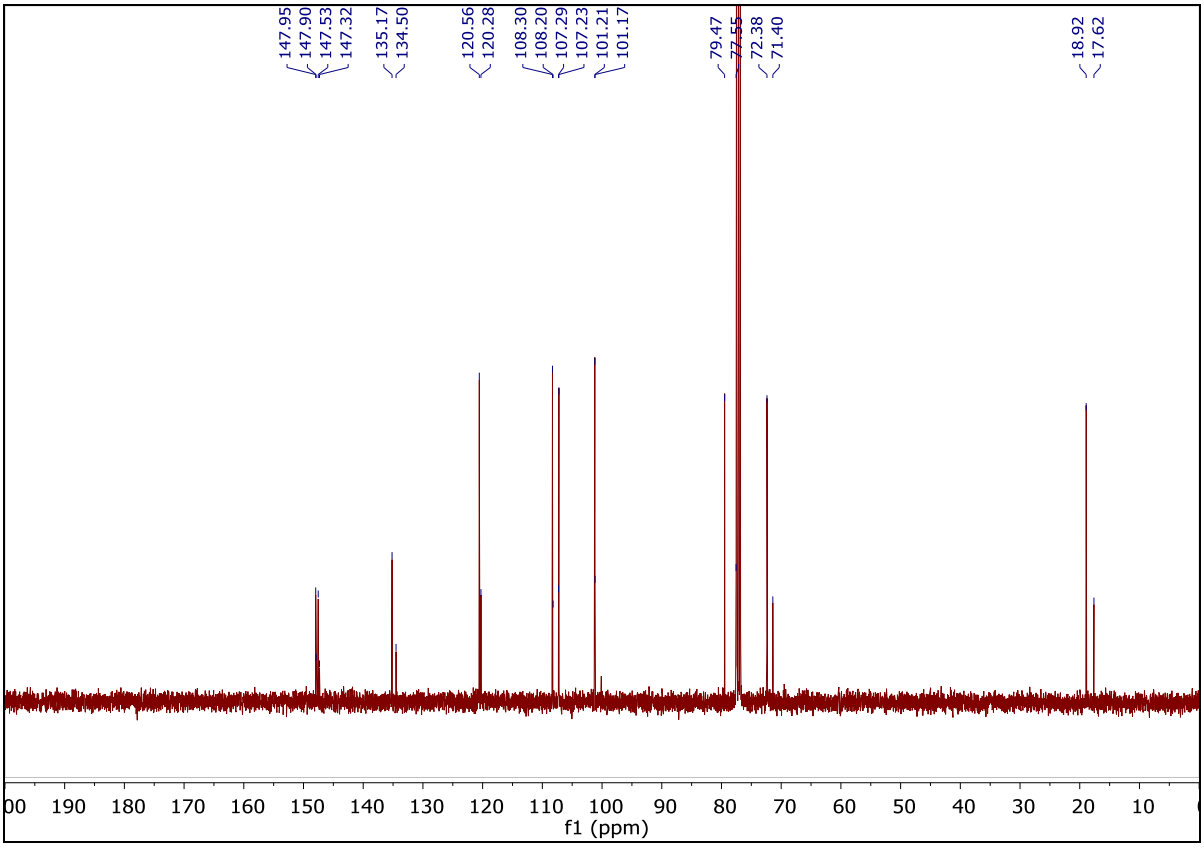
<u>Copies of ^{1}H and ^{13}C NMR spectra</u>: epoxides **8**



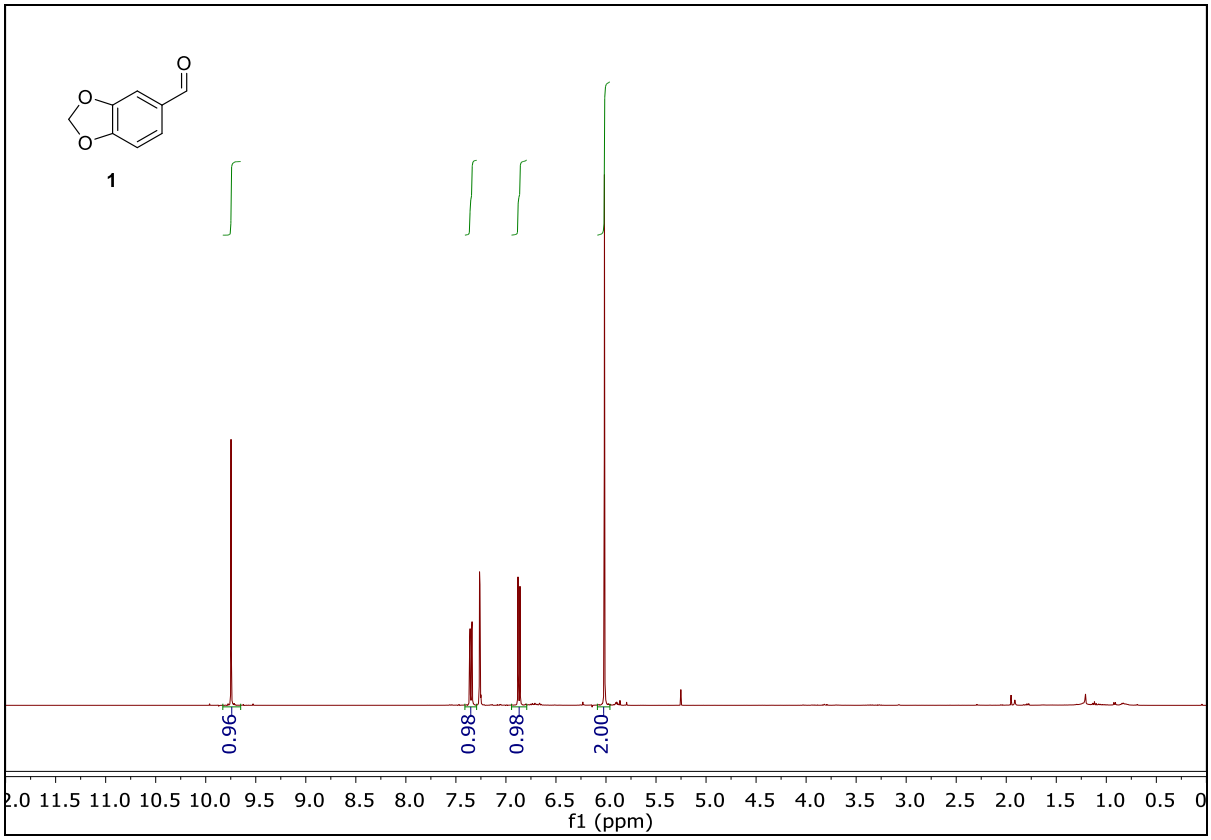


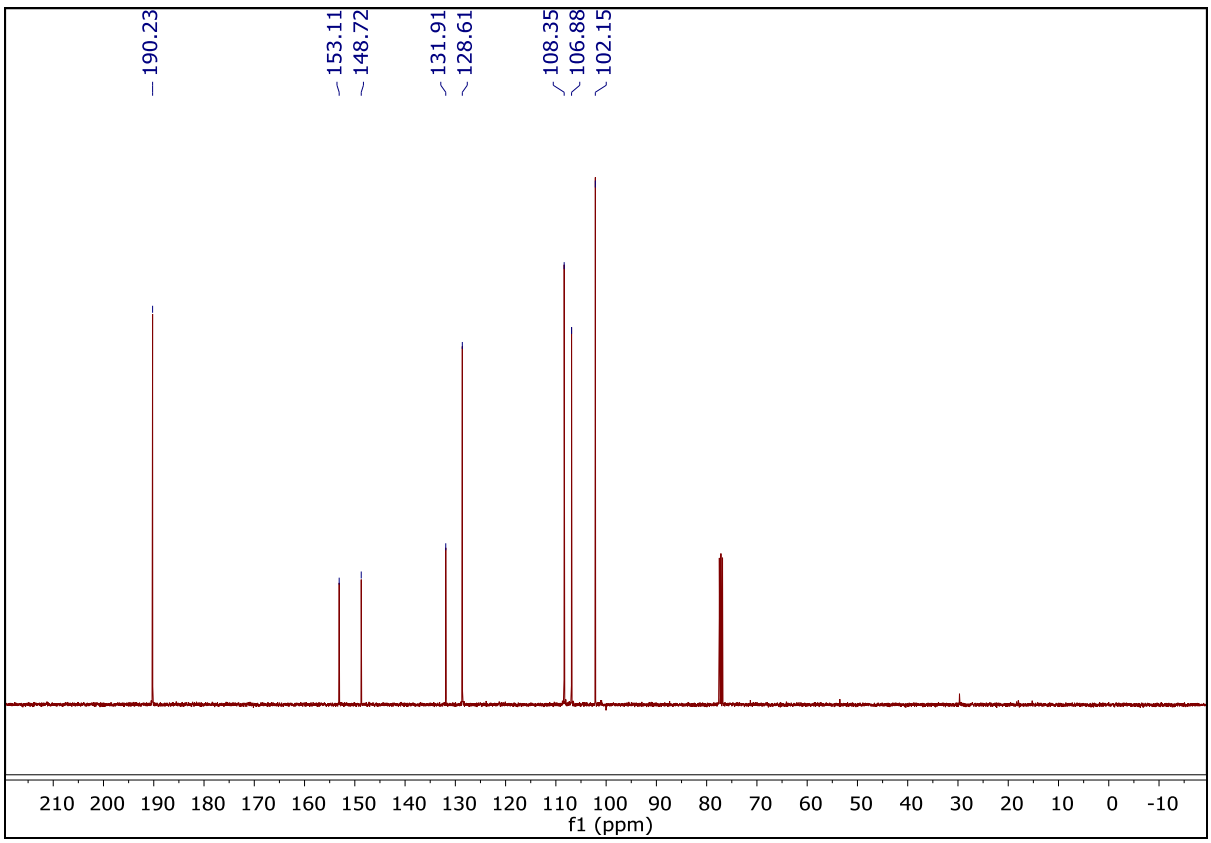
Copies of ^{1}H and ^{13}C NMR spectra: diols **10**





<u>Copies of ^{1}H and ^{13}C NMR spectra</u>: piperonal **1**





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