Supporting Information

Seaweed Natural Products Modify the Host Inflammatory Response via Nrf2 Signaling and Alter Colon Microbiota Composition and Gene Expression

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Supporting Figures

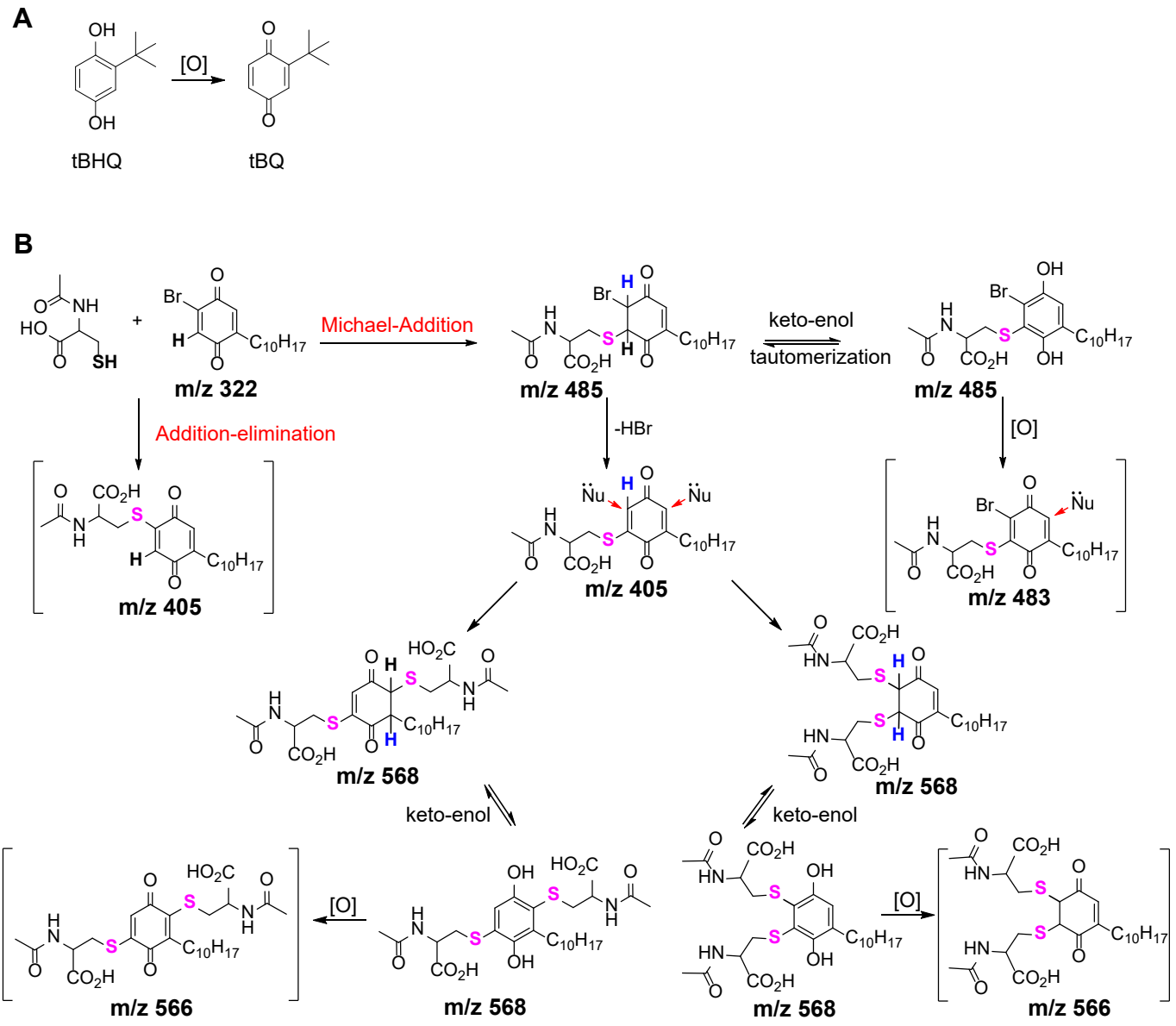


Fig. S1: Structural comparison of CymQ with tBHQ/tBQ and CymQ chemical reactivity towards NAC. *(A)* Structures of tBHQ and its oxidation product tBQ. (*B*) Reactions of CymQ (**5**) with *N*-acetylcysteine (NAC) and Keap1 showing mechanisms for formation of different CymQ-adducts.

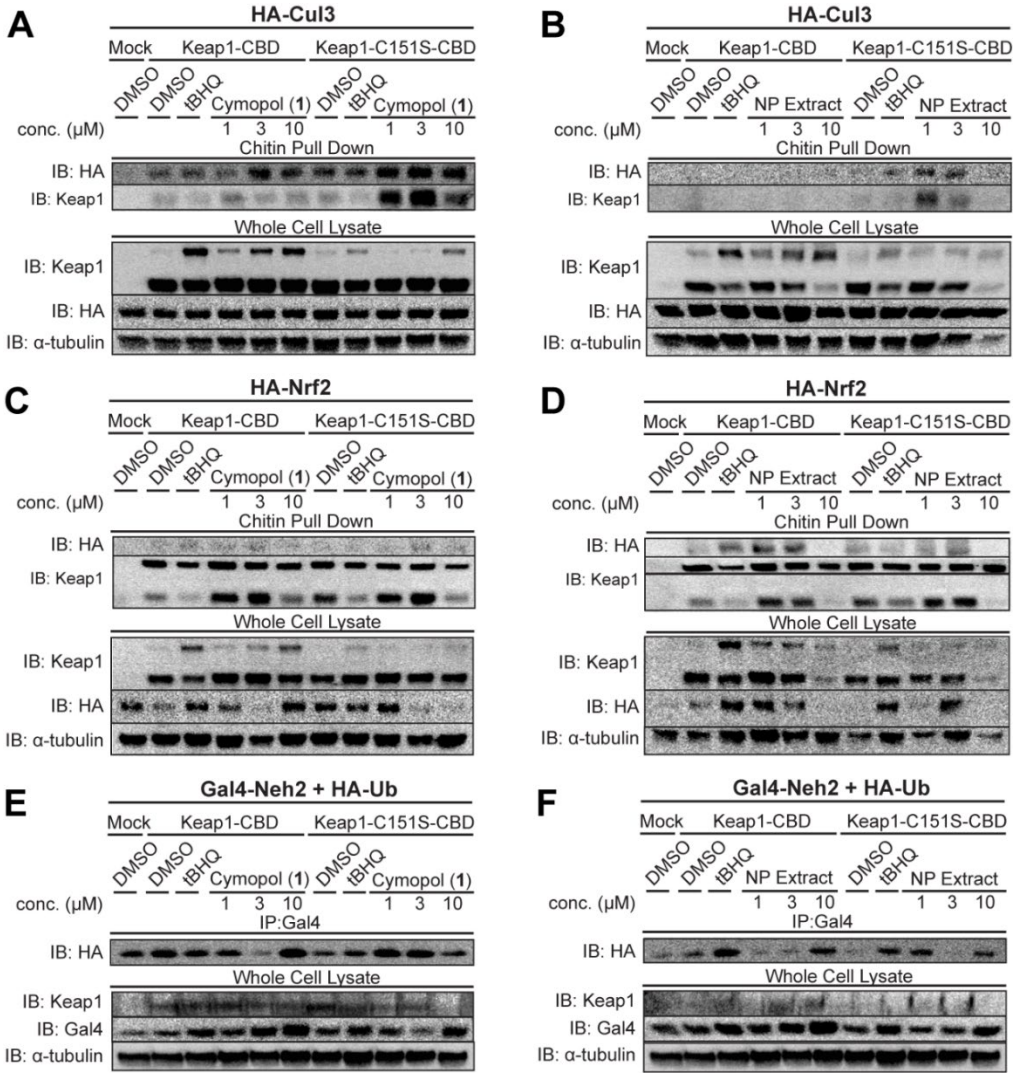


Fig. S2. Keap1 mutant analysis. Cymopol (**1**) and NP extract demonstrate some reactivity towards Keap1 Cys-151. Cells transfected with either mock cDNA-RFP or Keap1-CBD, or Keap1-C151S-CBD and HA-Cul3 or HA-Nrf2 or Gal4-Neh2 and HA-Ub and treated with DMSO control, tBHQ, or increasing concentrations of **1** or the NP extract. Whole cell lysates or protein eluted from pull-down assays (chitin pull down or Gal-4 pull down) were evaluated for changes in binding patterns of the Nrf2/Keap1 pathway. (*A*) Cymopol (**1**) and (*B*) NP extract increase binding of Keap1-Cul3 interaction in a dose response manner. This effect is affected by Cys-151 mutation, indicating the role of this residue in Keap1-Cul3 interaction. In the whole cell lysate, it is seen that **1** and NP extract increase dimerization, and that the effect is decreased in Keap1-C151S, suggesting that cymopols promote dimerization of Keap1 via Cys-151. (*C,D*) Cymopol (**1**) and NP increase dimerization of Keap1 in a dose-response manner. The whole cell lysates indicate that this is dependent on Cys-151, as the mutants do not have an increased dimerization. (*E/F*) To determine whether cymopols reduce ubiquitination of Nr2, cells were transfected with HA-Ub and Gal4-Neh2 and subjected to 24 h treatment with cymopols. Results on ubiquitination trend of Nrf2 remain inconclusive. Since Keap1 was not knocked down before transfections, the effects of the endogenous wild-type Keap1 cannot be ruled out.

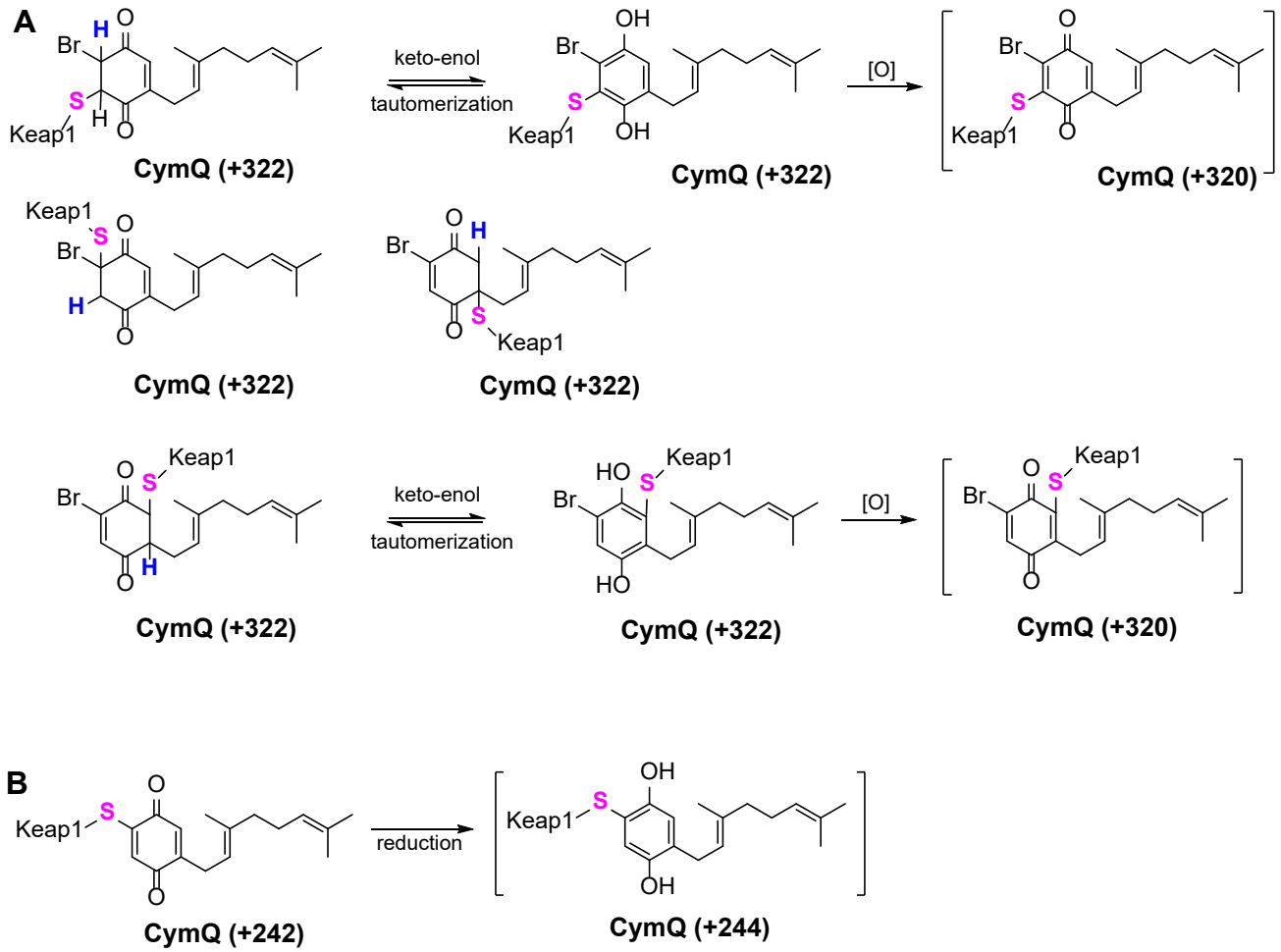
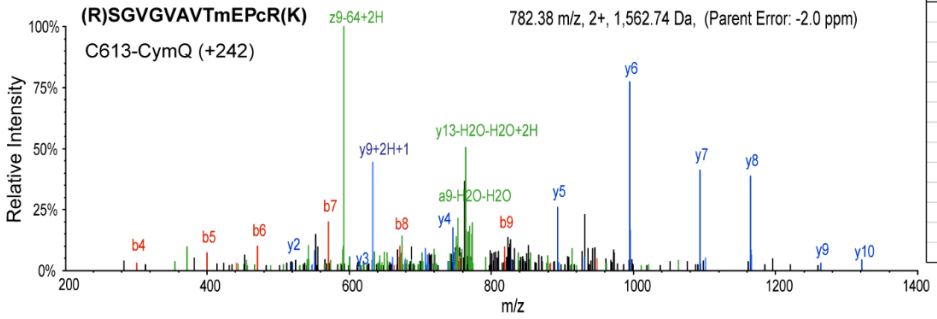


Fig. S3. Potential Keap1 mass tags identified by proteomics and rationale for adduct formation. Scaffold (version Scaffold 4.2.1, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 95.0% probability. Two major mass tags $[M+H]^+$ corresponding to CymQ(+242) and CymQ(+322) were recognized for the most dominant modifications resulting from conjugate (Michael) addition (*A*) and addition-elimination (*B*) reactions.



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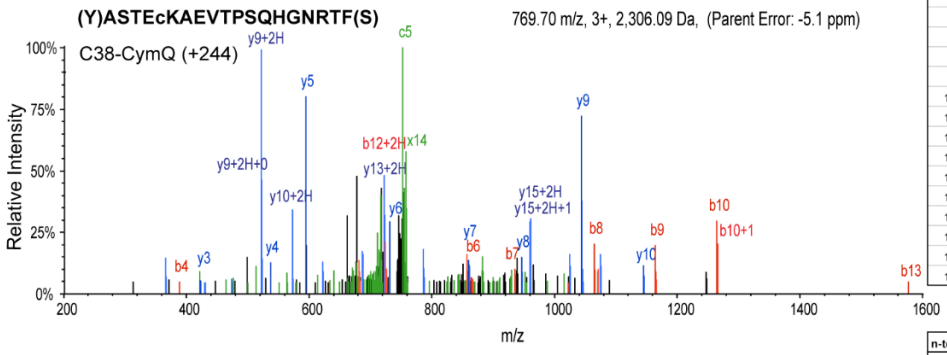
1	88.0		105.1		S	1563.8	782.4	1547.7	774.4	13
2	145.1		162.1		G	1476.7	738.9	1460.7	730.9	12
3	244.1		261.2		V	1419.7	710.4	1403.7	702.3	11
4	301.2		318.2		G	1320.6	660.8	1304.6	652.8	10
5	400.2		417.3		V	1263.6	632.3	1247.6	624.3	9
6	471.3	236.1	488.3	244.1	A	1164.5	582.8	1148.5	574.8	8
7	570.3	285.7	587.4	293.7	V	1093.5	547.3	1077.5	539.3	7
8	671.4	336.2	688.4	344.2	T	994.4	497.7	978.4	489.7	6
9	818.4	409.7	835.4	417.7	M+16	893.4		877.4		5
10	947.5	474.2	964.5	482.2	E	746.4		730.3		4
11	1044.5	522.8	1061.5	530.8	P	617.3		601.3		3
12	1389.6	695.3	1406.7	703.3	C+242	520.3		504.2		2
13	1563.8	782.4	1580.8	790.4	R	175.1		159.1		1



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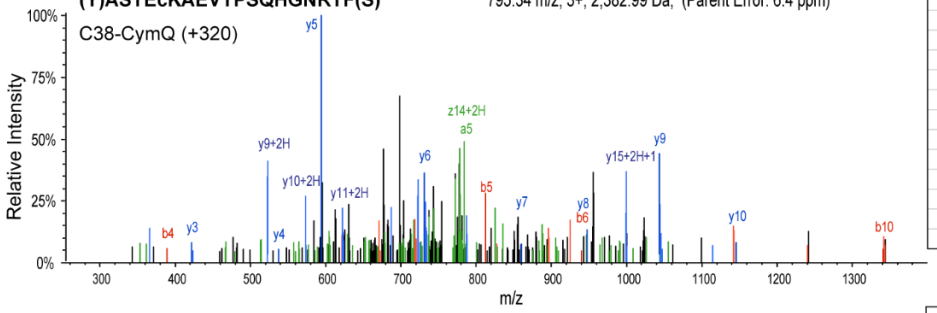
1	72.0	36.5	89.1	44.5	A	2307.1	1154.1	2291.1	1146.1	19
2	159.1	80.0	176.1	88.1	S	2236.1	1118.5	2220.1	1110.5	18
3	260.1	130.6	277.2	138.6	T	2149.0	1075.0	2133.0	1067.0	17
4	389.2	195.1	406.2	203.1	E	2048.0	1024.5	2032.0	1016.5	16
5	736.3	368.7	753.4	376.7	C+244	1919.0	960.0	1902.9	952.0	15
6	864.4	432.7	881.4	440.7	K	1571.8	786.4	1555.8	778.4	14
7	935.5	468.2	952.5	476.2	A	1443.7	722.4	1427.7	714.3	13
8	1064.5	532.8	1081.5	540.8	E	1372.7	686.8	1356.6	678.8	12
9	1163.6	582.3	1180.6	590.3	V	1243.6	622.3	1227.6	614.3	11
10	1264.6	632.8	1281.6	640.8	T	1144.6	572.8	1128.5	564.8	10
11	1361.7	681.3	1378.7	689.4	P	1043.5	522.3	1027.5	514.2	9
12	1448.7	724.9	1465.7	732.9	S	946.5	473.7	930.4	465.7	8
13	1576.8	788.9	1593.8	796.9	Q	859.4	430.2	843.4	422.2	7
14	1713.8	857.4	1730.8	865.4	H	731.4	366.2	715.3	358.2	6
15	1770.8	885.9	1787.9	893.9	G	594.3	297.7	578.3	289.6	5
16	1884.9	942.9	1901.9	951.0	N	537.3	269.1	521.3	261.1	4
17	2041.0	1021.0	2058.0	1029.0	R	423.2	212.1	407.2	204.1	3
18	2142.0	1071.5	2159.1	1079.5	T	267.1	134.1	251.1	126.1	2
19	2307.1	1154.1	2324.1	1162.1	F	166.1	83.6	150.1	75.5	1



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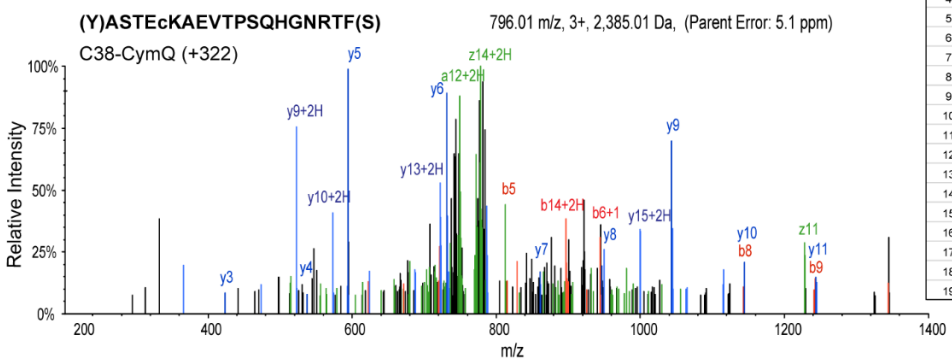
1	72.0	36.5	89.1	44.5	A	2384.0	1192.5	2368.0	1184.5	19
2	159.1	80.0	176.1	88.1	S	2313.0	1157.0	2296.9	1149.0	18
3	260.1	130.6	277.2	138.6	T	2225.9	1113.5	2209.9	1105.5	17
4	389.2	195.1	406.2	203.1	E	2124.9	1062.9	2108.9	1054.9	16
5	812.2	406.6	829.2	414.6	C+320	1995.8	998.4	1979.8	990.4	15
6	940.3	470.7	957.3	478.7	K	1572.8	786.9	1556.8	778.9	14
7	1011.4	506.2	1028.4	514.2	A	1444.7	722.8	1428.7	714.8	13
8	1140.4	570.7	1157.4	578.7	E	1373.6	687.3	1357.6	679.3	12
9	1239.5	620.2	1256.5	628.2	V	1244.6	622.8	1228.6	614.8	11
10	1340.5	670.8	1357.5	678.8	T	1145.5	573.3	1129.5	565.3	10
11	1437.6	719.3	1454.6	727.3	P	1044.5	522.8	1028.5	514.7	9
12	1524.6	762.8	1541.6	770.8	S	947.4	474.2	931.4	466.2	8
13	1653.6	827.3	1670.7	835.3	Q+1	860.4	430.7	844.4	422.7	7
14	1790.7	895.9	1807.7	903.9	H	731.4	366.2	715.3	358.2	6
15	1847.7	924.4	1864.7	932.4	G	594.3	297.7	578.3	289.6	5
16	1961.8	981.4	1978.8	989.4	N	537.3	269.1	521.3	261.1	4
17	2117.9	1059.4	2134.9	1067.4	R	423.2	212.1	407.2	204.1	3
18	2218.9	1110.0	2235.9	1118.0	T	267.1	134.1	251.1	126.1	2
19	2384.0	1192.5	2401.0	1200.5	F	166.1	83.6	150.1	75.5	1



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1	72.0	36.5	89.1	44.5	A	2386.0	1193.5	2370.0	1185.5	19
2	159.1	80.0	176.1	88.1	S	2315.0	1158.0	2299.0	1150.0	18
3	260.1	130.6	277.2	138.6	T	2227.9	1114.5	2211.9	1106.5	17
4	389.2	195.1	406.2	203.1	E	2126.9	1064.0	2110.9	1055.9	16
5	814.2	407.6	831.3	415.6	C+322	1997.8	999.4	1981.8	991.4	15
6	942.3	471.7	959.4	479.7	K	1572.8	786.9	1556.8	778.9	14
7	1013.4	507.2	1030.4	515.2	A	1444.7	722.8	1428.7	714.8	13
8	1142.4	571.7	1159.4	579.7	E	1373.6	687.3	1357.6	679.3	12
9	1241.5	621.2	1258.5	629.3	V	1244.6	622.8	1228.6	614.8	11
10	1342.5	671.8	1359.6	679.8	T	1145.5	573.3	1129.5	565.3	10
11	1439.6	720.3	1456.6	728.3	P	1044.5	522.8	1028.5	514.7	9
12	1526.6	763.8	1543.6	771.8	S	947.4	474.2	931.4	466.2	8
13	1655.7	828.3	1672.7	836.3	Q+1	860.4	430.7	844.4	422.7	7
14	1792.7	896.9	1809.7	904.9	H	731.4	366.2	715.3	358.2	6
15	1849.7	925.4	1866.8	933.4	G	594.3	297.7	578.3	289.6	5
16	1963.8	982.4	1980.8	990.4	N	537.3	269.1	521.3	261.1	4
17	2119.9	1060.4	2136.9	1068.5	R	423.2	212.1	407.2	204.1	3
18	2220.9	1111.0	2238.0	1119.0	T	267.1	134.1	251.1	126.1	2
19	2386.0	1193.5	2403.0	1201.5	F	166.1	83.6	150.1	75.5	1

Fig. S4. Representative mass spectra of the four major CymQ-modifications observed for Cys613 (CymQ+242) and Cys38 (CymQ+244, +320, +322)

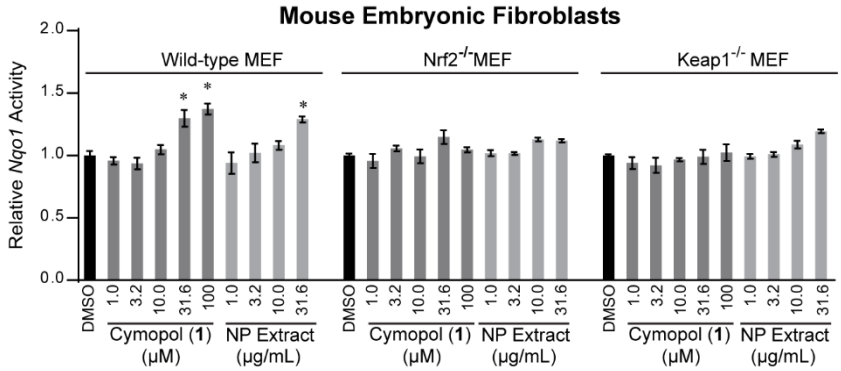


Fig. S5. *Nqo1* activity in MEFs. Cymopol (**1**) and the NP extract increase Nqo1 activity in wild-type MEF cells to a greater extent than in $Nrf2^{-/-}$ and $Keap1^{-/-}$ MEFs. Asterisks indicate $p<0.05$ which correlate to the indicated color. Significant values indicated with the black asterisk indicate concentrations which are increased over DMSO control in the same cell line. MEF cells were chemically induced by pre-treating with IFN-γ and TNF-α. Of note, this assay may be prone to artefacts since it utilizes an enzymatically (Nqo1)-generated hydroquinone (from menadione) that is structurally similar to cymopol to function as a reducing agent of MTT, thus forming formazan, the purple dye from which we detect enzyme activity. Cymopol may act as a substrate.

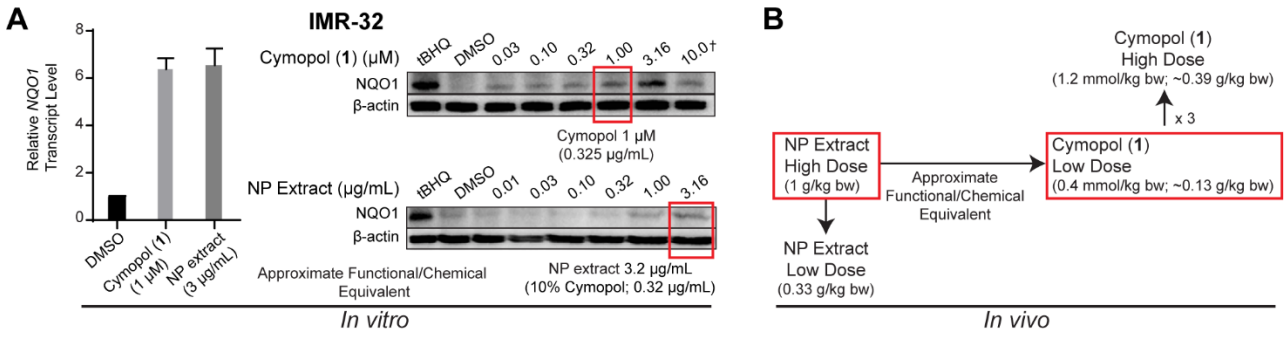


Fig. S6. Estimation of functional equivalents for cymopol and the NP extract and rationale for doses used in the tissue distribution studies. Based on percent yield, the non-polar extract contains ~10% cymopols by weight (0.1 g/kg bw of cymopols in high dose NP extract and 0.03g/kg bw of cymopols in low dose NP extract).

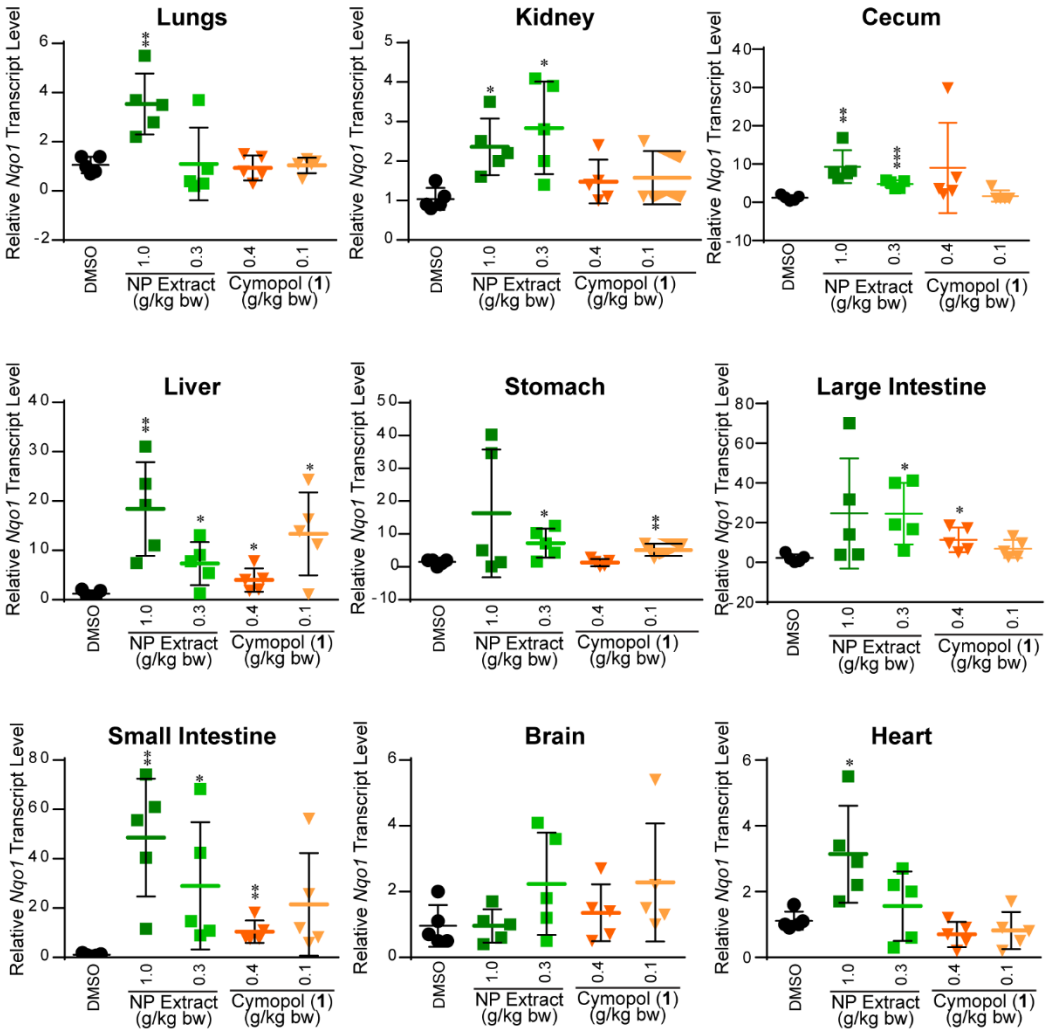


Fig. S7. *Nqo1* transcript levels in various tissues. Mice were gavaged daily with cymopol or NP extract (two different doses each) for 3 days and tissues harvested 12 h after the last treatment. mRNA levels were analyzed by RT-qPCR by TaqMan (endogenous control beta actin) (* indicates p-value < 0.05; ** indicates p-value < 0.005; *** indicates *p*-values < 0.0005).

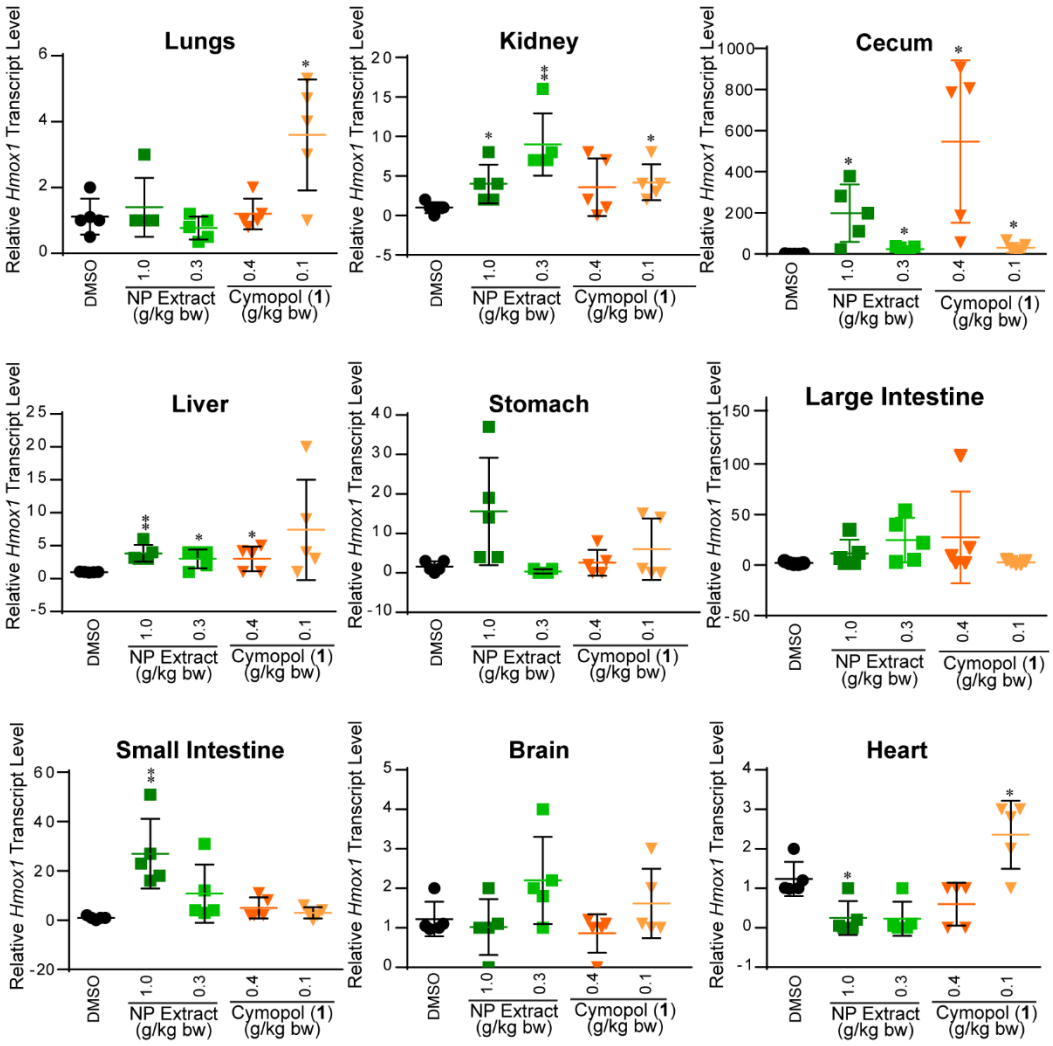


Fig. S8. *Hmox1* transcript levels in various tissues. Mice were gavaged daily with cymopol or NP extract (two different doses each) for 3 days and tissues harvested 12 h after the last treatment. mRNA levels were analyzed by RT-qPCR by TaqMan (endogenous control beta actin) (* indicates p-value < 0.05; ** indicates p-value < 0.005; *** indicates *p*-values < 0.0005).

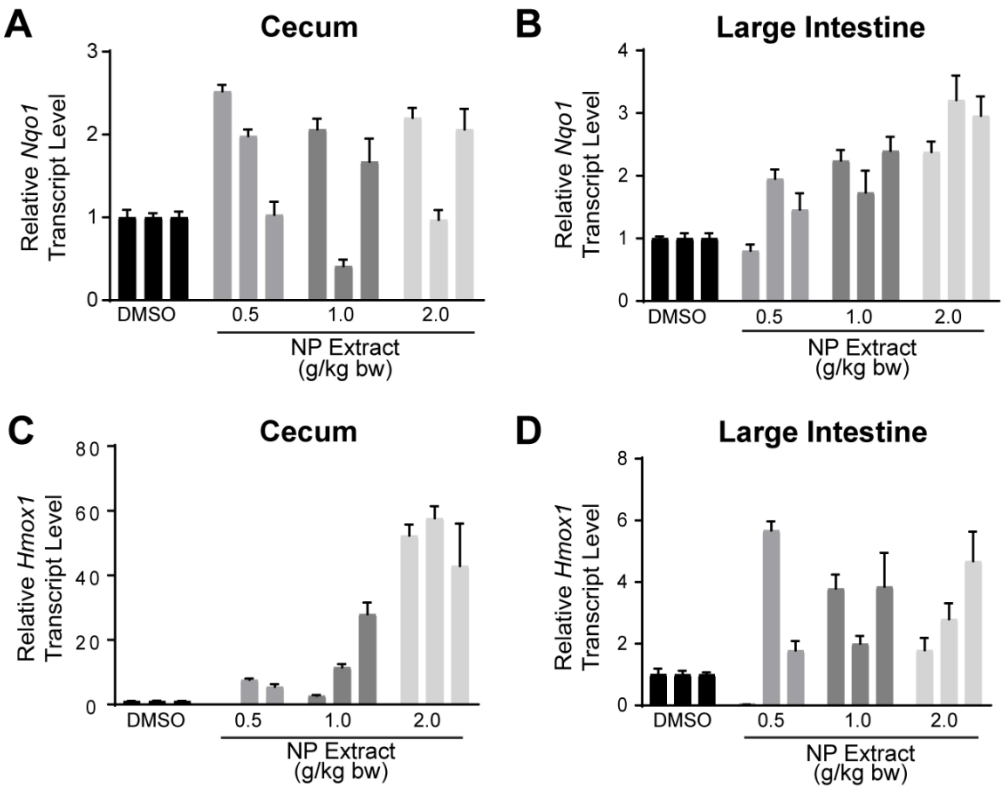


Fig. S9. Dose optimization of NP extract concentration for DSS model. Age-matched WT C57Bl/6 mice (6-8 weeks old) received daily doses of 1.0, 1.5 or 2.0 mg/kg NP extract for 3 days, and cecum and large intestines were harvested 12 h after the last dose. *Nqo1* and *Hmox1* mRNA levels were analyzed by RT-qPCR by TaqMan (endogenous control β-actin).

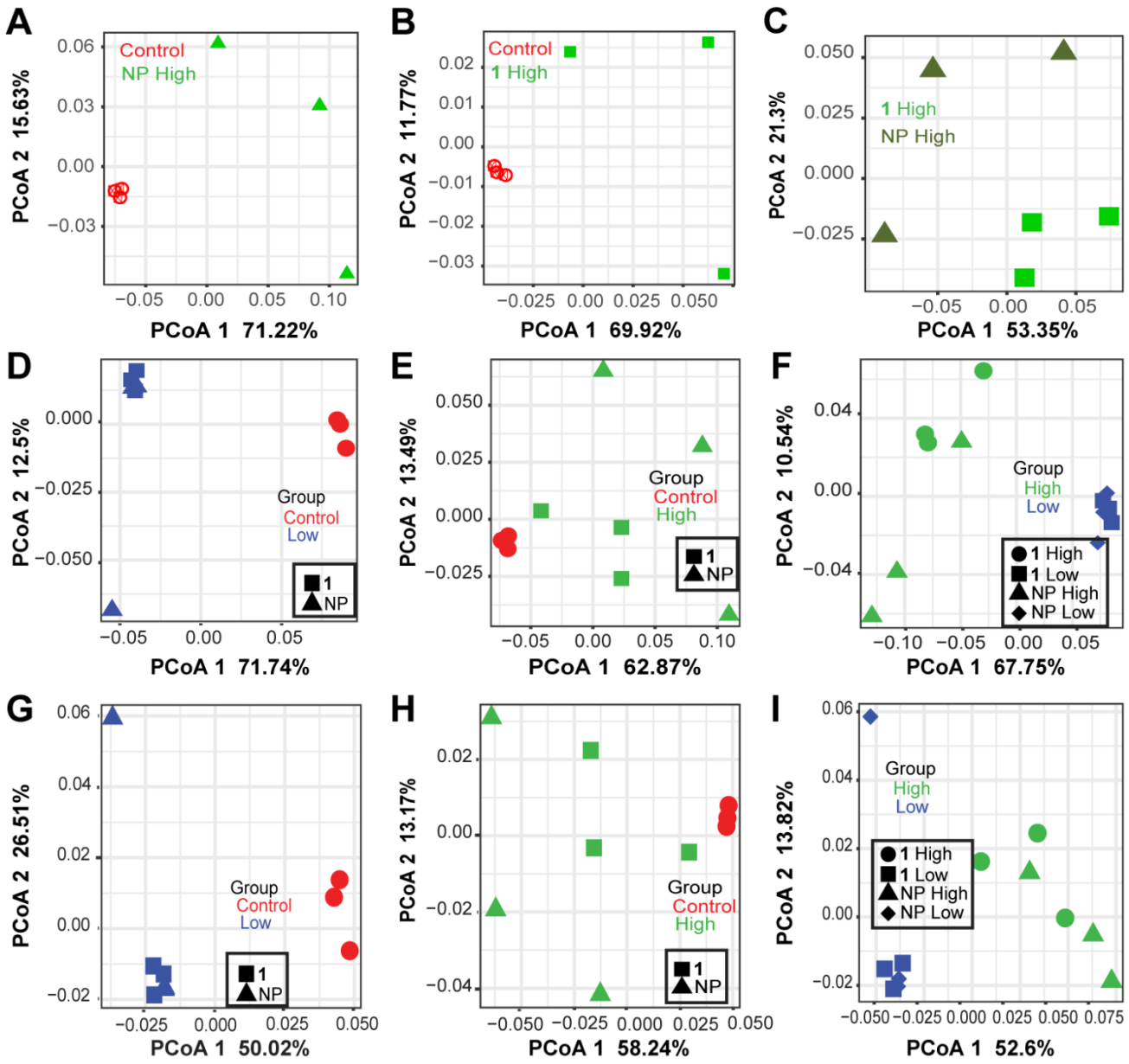


Fig. S10. Analysis of the microbiome in the large intestines using 16S sequences extracted from RNA-seq data. The extract and cymopol (**1**) administration changed the composition of mouse gut microbiota. PCoA analysis using QIIME close-reference OTUs generated from the forward reads shows significant shift in microbial composition between (*A*) control and NP high PCoA1 FDR $p = 0.02$ but not between (*B*) control and **1** high PCoA1 FDR $p = 0.05$ or (*C*) NP high and **1** high PCoA1 FDR $p = 0.18$. PCoA analysis on QIIME close-reference OTUs generated from the reverse reads shows significant shift in bacterial composition between (*D*) control and low groups PCoA1 FDR p = 1.01e-08, (*E*) control and high groups, PCoA1 FDR $p = 0.04$ and (*F*) high and low groups, PCoA1 FDR p = 2.84e-06. The same shift in microbial composition is also detected using centrifuge classified reads (*G*) control and low groups PCoA1 FDR p = 3.62e-06, (*H*) control and high groups, PCoA1 FDR $p = 0.03$ and (*I*) high and low groups, PCoA1 FDR p = 3.1e-5.

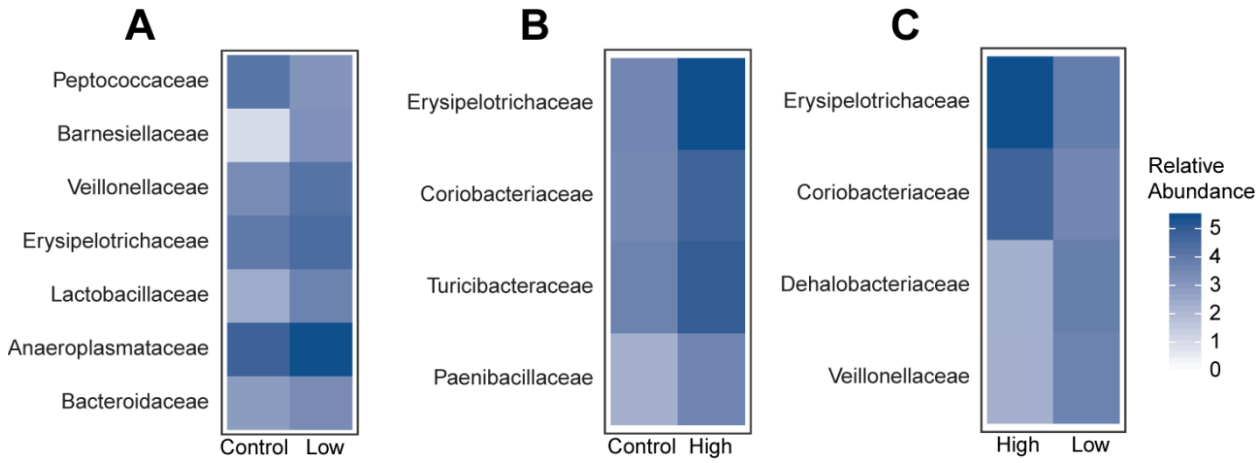


Fig. S11. Heatmap representing the mean $\log_{10}$ normalized relative abundances of bacterial families that were significantly different (FDR $p < 0.05$) between (*A*) control and low groups, (*B*) control and high groups and (*C*) high and low groups.

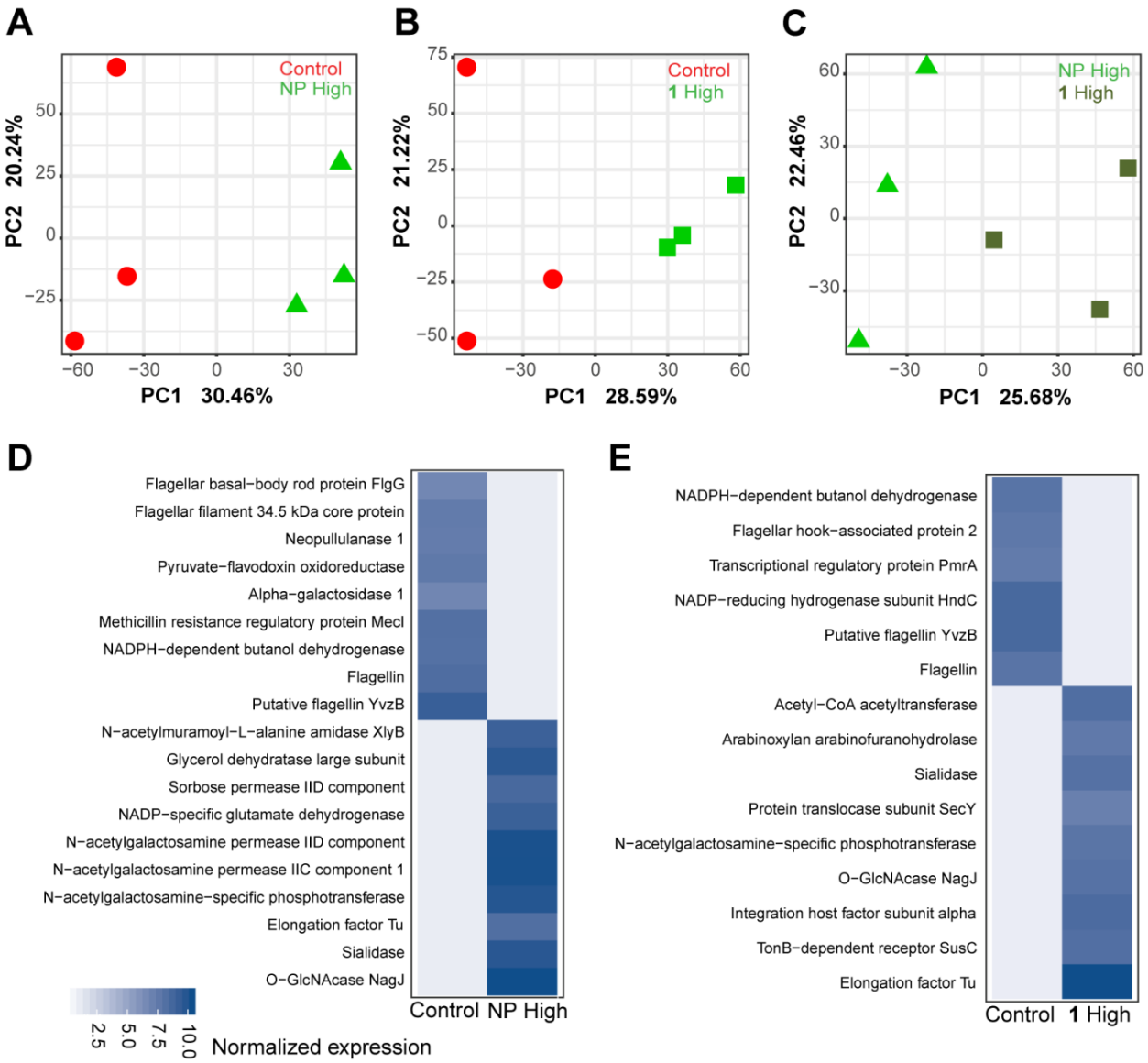


Fig. S12. PCA analysis of microbial gene expression shows significant changes in microbial gene expression between (*A*) control and NP high, PC1 FDR $p = 0.001$, (*B*) control and **1** high, PC1 FDR $p = 0.009$ and (*C*) NP high and **1** high, PC1 FDR $p = 0.03$. Heatmaps showing the mean log2 edgeR normalized gene expression of representative significantly differentially (FDR $p < 0.05$) expressed genes between (*D*) control and NP high, (*E*) control and **1** high. No significantly differentially expressed genes between NP high and **1** high were detected.

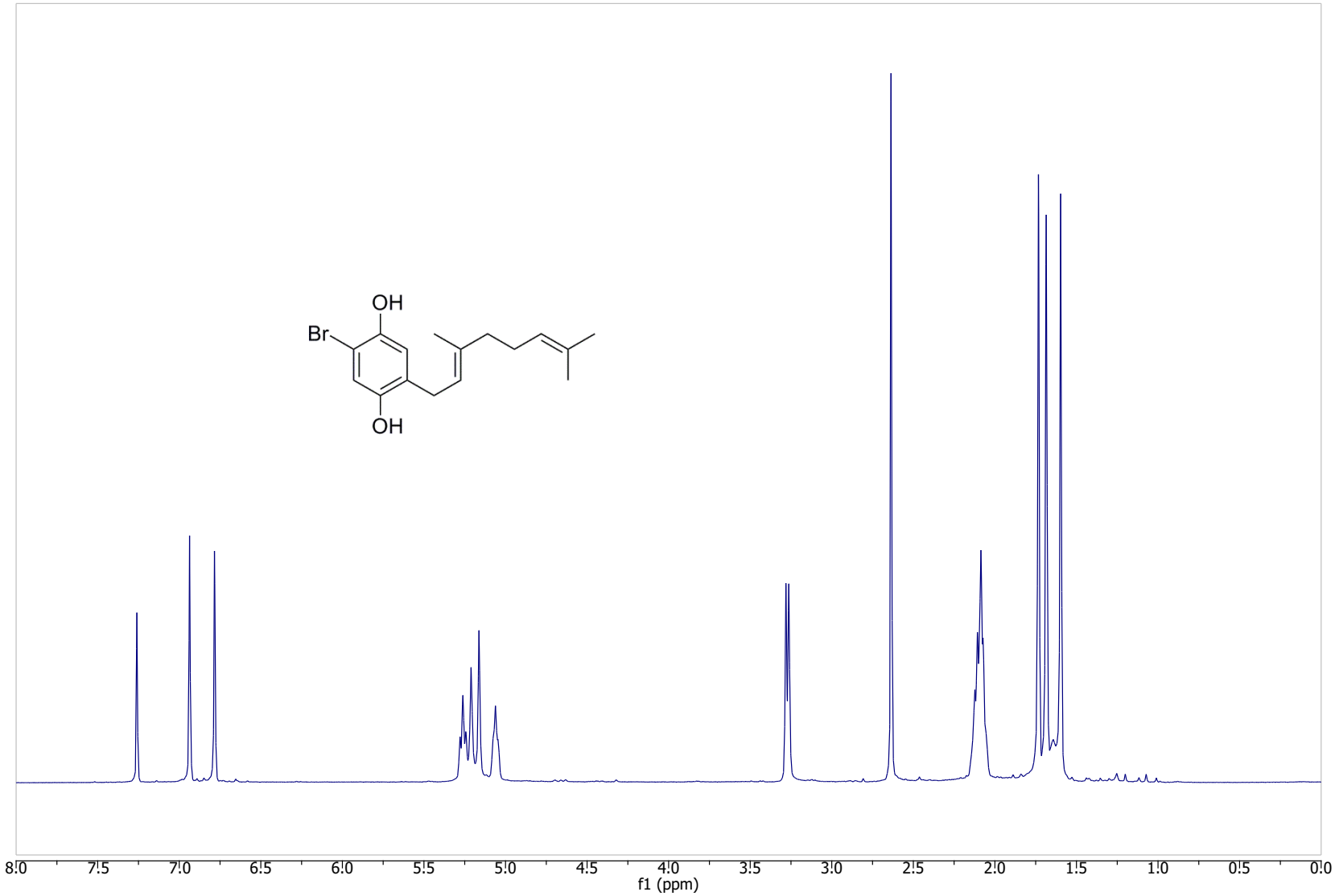
Table S1. Genus-level comparisons between the control, low-dose (**1**, NP) and high-dose (**1**, NP) groups

Genus	group pval	group FDR	change Control/Low	Genus	group pval	group FDR	change Control/High	Genus	group pval	group FDR	change High/Low
Ethanoligenens	9.16E-06	3.39E-04	8.08E-01	*Clostridium*	1.51E-03	2.79E-02	7.84E-01	*Sharpea*	1.78E-05	6.57E-04	7.19E-01
Peptococcus	1.80E-04	3.09E-03	1.34E+00	*Coprobacillus*	8.54E-04	2.79E-02	5.87E-01	*Marvinbryantia*	1.25E-04	2.30E-03	7.75E-01
Roseburia	2.51E-04	3.09E-03	1.22E+00	*Turicibacter*	2.76E-03	3.41E-02	7.63E-01	*Lachnospira*	5.54E-04	5.13E-03	1.84E+00
Anaerotruncus	1.71E-03	1.26E-02	1.34E+00	*Pseudobutyrivibrio*	1.09E-02	1.00E-01	1.48E+00	*Pseudobutyrivibrio*	5.35E-04	5.13E-03	6.68E-01
Sharpea	1.40E-03	1.26E-02	7.57E-01	*Adlercreutzia*	1.39E-02	1.03E-01	8.93E-01	*Clostridium*	1.18E-03	7.28E-03	1.16E+00
Clostridium	2.64E-03	1.40E-02	9.07E-01	*Dehalobacterium*	1.81E-02	1.12E-01	1.72E+00	*Coprobacillus*	9.85E-04	7.28E-03	1.39E+00
Gracilibacter	2.29E-03	1.40E-02	1.05E+00	*Lactobacillus*	2.32E-02	1.15E-01	6.03E-01	*Dehalobacterium*	1.76E-03	9.30E-03	5.91E-01
Lachnospira	5.55E-03	2.57E-02	1.57E+00	*Moryella*	2.48E-02	1.15E-01	8.44E-01	*Ethanoligenens*	2.43E-03	1.12E-02	6.64E-01
Lactobacillus	6.26E-03	2.57E-02	6.55E-01	*Leucobacter*	3.38E-02	1.39E-01	1.37E+00	*Anaerotruncus*	4.11E-03	1.69E-02	1.32E+00
Moryella	9.04E-03	3.34E-02	6.93E-01	*Citrobacter*	4.06E-02	1.50E-01	1.16E+00	*Leucobacter*	1.02E-02	3.77E-02	7.54E-01
Finegoldia	1.51E-02	5.07E-02	1.08E+00	*Butyrivibrio*	6.30E-02	1.60E-01	1.09E+00	*Oscillospira*	1.35E-02	4.53E-02	9.22E-01
Marvinbryantia	1.72E-02	5.31E-02	8.43E-01	*Oscillospira*	6.62E-02	1.60E-01	1.09E+00	*Anaeroplasma*	2.03E-02	6.26E-02	7.71E-01
Anaeroplasma	2.37E-02	6.02E-02	8.73E-01	*Roseburia*	5.73E-02	1.60E-01	1.14E+00	*Moryella*	2.43E-02	6.91E-02	8.29E-01
Bifidobacterium	2.44E-02	6.02E-02	2.46E-01	*Serratia*	6.74E-02	1.60E-01	1.16E+00	*Adlercreutzia*	2.89E-02	7.64E-02	1.09E+00
Blautia	2.28E-02	6.02E-02	1.04E+00	*Trabulsiella*	6.72E-02	1.60E-01	1.14E+00	*Coprococcus*	3.11E-02	7.66E-02	9.70E-01
Coprobacillus	3.43E-02	7.94E-02	8.08E-01	*WH18*	6.90E-02	1.60E-01	1.07E+00	*Butyrivibrio*	5.38E-02	1.24E-01	9.37E-01
Oribacterium	3.88E-02	8.21E-02	1.13E+00	*Gracilibacter*	1.08E-01	2.22E-01	1.12E+00	*Shuttleworthia*	7.48E-02	1.63E-01	1.09E+00
Shuttleworthia	3.99E-02	8.21E-02	1.10E+00	*Oribacterium*	1.04E-01	2.22E-01	1.49E+00	*Oribacterium*	1.11E-01	2.20E-01	7.55E-01
Ruminococcus	9.60E-02	1.87E-01	1.02E+00	*Finegoldia*	1.29E-01	2.52E-01	1.15E+00	*Turicibacter*	1.13E-01	2.20E-01	1.12E+00
Anaerostipes	1.05E-01	1.94E-01	8.85E-01	*Blautia*	1.41E-01	2.61E-01	1.04E+00	*Papillibacter*	1.34E-01	2.48E-01	8.91E-01
Turicibacter	1.22E-01	2.14E-01	8.50E-01	*Papillibacter*	1.67E-01	2.95E-01	1.16E+00	*Citrobacter*	1.80E-01	3.15E-01	8.46E-01
Coprococcus	1.66E-01	2.80E-01	9.76E-01	*Bifidobacterium*	2.01E-01	3.38E-01	2.36E-01	*Gracilibacter*	1.87E-01	3.15E-01	9.41E-01
Butyricimonas	2.45E-01	3.95E-01	9.08E-01	*Lachnospira*	2.23E-01	3.58E-01	8.44E-01	*Dorea*	2.36E-01	3.64E-01	9.62E-01
Dehalobacterium	2.61E-01	4.03E-01	1.02E+00	*Marvinbryantia*	2.40E-01	3.70E-01	1.09E+00	*Lachnobacterium*	2.26E-01	3.64E-01	9.05E-01
Butyrivibrio	3.08E-01	4.42E-01	1.02E+00	*Peptococcus*	2.52E-01	3.72E-01	1.19E+00	*Sphingomonas*	2.51E-01	3.71E-01	1.20E+00
Sphingomonas	3.11E-01	4.42E-01	1.22E+00	*Ethanoligenens*	2.73E-01	3.89E-01	1.22E+00	*Finegoldia*	2.77E-01	3.75E-01	9.36E-01
Papillibacter	3.39E-01	4.65E-01	1.03E+00	*Dorea*	3.69E-01	5.06E-01	1.04E+00	*Roseburia*	2.64E-01	3.75E-01	1.06E+00
Leucobacter	5.00E-01	6.60E-01	1.03E+00	*Anaeroplasma*	4.04E-01	5.28E-01	1.13E+00	*Serratia*	2.92E-01	3.75E-01	8.63E-01
Adlercreutzia	5.63E-01	6.94E-01	9.72E-01	*Lachnobacterium*	4.14E-01	5.28E-01	1.10E+00	*Trabulsiella*	2.94E-01	3.75E-01	8.77E-01
WH18	5.54E-01	6.94E-01	1.03E+00	*Sharpea*	4.41E-01	5.44E-01	1.06E+00	*Ruminococcus*	3.24E-01	4.00E-01	1.01E+00
Oscillospira	6.76E-01	8.07E-01	1.00E+00	*Ruminococcus*	6.56E-01	7.83E-01	1.01E+00	*Anaerostipes*	3.51E-01	4.06E-01	8.67E-01
Dorea	8.37E-01	9.60E-01	1.00E+00	*Coprococcus*	7.03E-01	8.13E-01	1.01E+00	*Peptococcus*	3.43E-01	4.06E-01	1.12E+00
Pseudobutyrivibrio	8.56E-01	9.60E-01	9.92E-01	*Shuttleworthia*	8.32E-01	9.32E-01	1.01E+00	*WH18*	3.77E-01	4.23E-01	9.66E-01
Lachnobacterium	8.84E-01	9.62E-01	9.94E-01	*Anaerostipes*	9.19E-01	9.62E-01	1.02E+00	*Lactobacillus*	3.96E-01	4.31E-01	1.10E+00
Citrobacter	9.23E-01	9.76E-01	9.84E-01	*Anaerotruncus*	9.12E-01	9.62E-01	1.01E+00	*Butyricimonas*	5.57E-01	5.89E-01	9.12E-01
Serratia	9.91E-01	9.91E-01	1.00E+00	*Sphingomonas*	9.36E-01	9.62E-01	1.01E+00	*Blautia*	7.41E-01	7.61E-01	9.94E-01
Trabulsiella	9.88E-01	9.91E-01	9.97E-01	*Butyricimonas*	9.89E-01	9.89E-01	9.97E-01	*Bifidobacterium*	8.06E-01	8.06E-01	1.11E+00

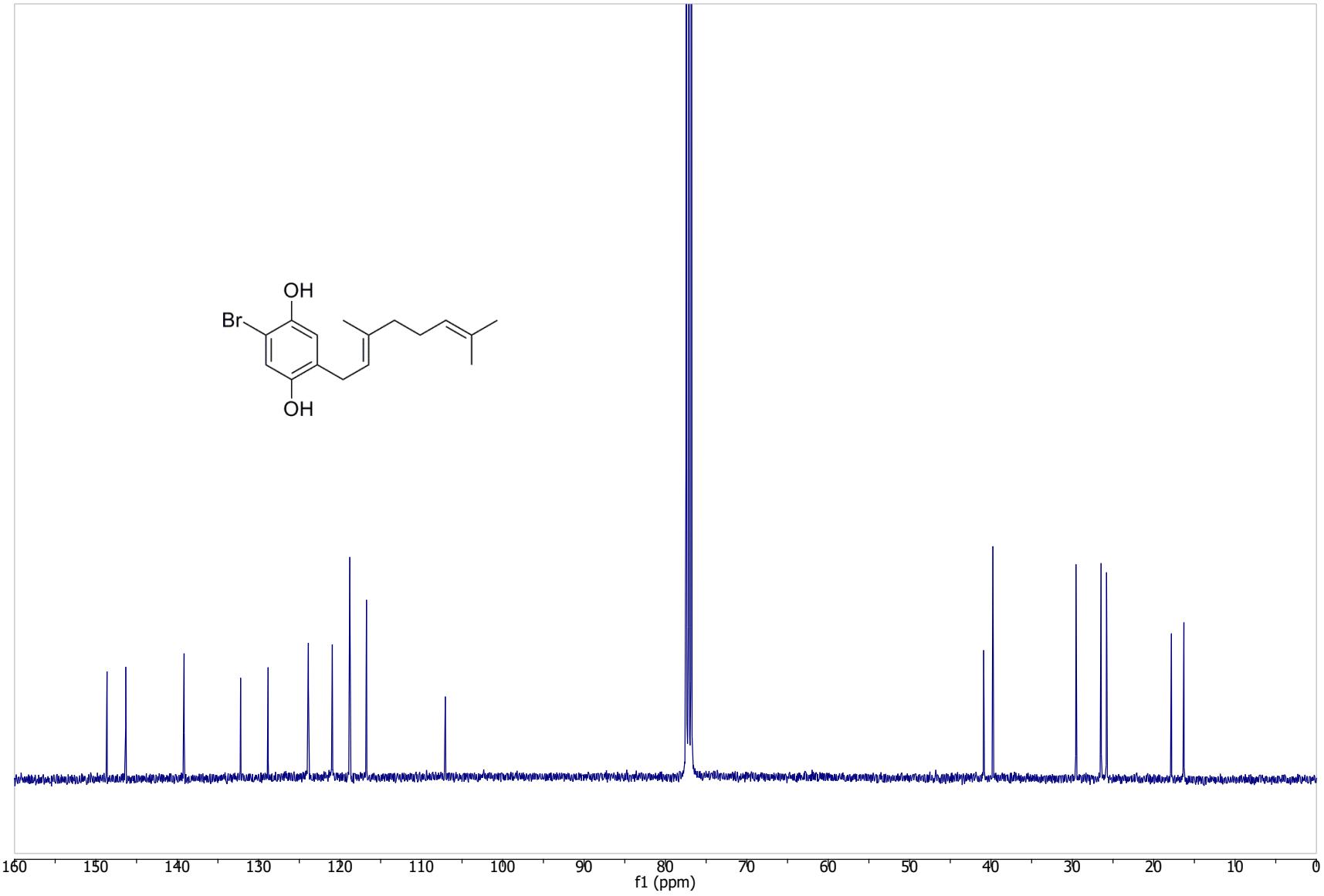
Table S2. Family-level comparisons between the control, low-dose (**1**, NP) and high-dose (**1**, NP) groups

Family	treatment pval	treatment FDR	change Control/Low	Family	treatment pval	treatment FDR	change Control/High	Family	treatment pval	treatment FDR	change High/Low
f__Peptococcaceae	3.10E-05	7.44E-04	1.36E+00	f__Erysipelotrichaceae	2.00E-04	5.00E-03	6.56E-01	f__Erysipelotrichaceae	1.03E-05	2.57E-04	1.39E+00
f__Barnesiellaceae	3.56E-04	4.27E-03	3.41E-01	f__Coriobacteriaceae	2.16E-03	2.02E-02	7.50E-01	f__Coriobacteriaceae	8.06E-05	1.01E-03	1.32E+00
f__Veillonellaceae	5.43E-04	4.34E-03	8.18E-01	f__Turicibacteraceae	2.42E-03	2.02E-02	7.48E-01	f__Dehalobacteriaceae	2.63E-03	1.67E-02	6.10E-01
f__Erysipelotrichaceae	3.79E-03	1.82E-02	9.11E-01	f__Paenibacillaceae	3.49E-03	2.18E-02	6.47E-01	f__Veillonellaceae	2.68E-03	1.67E-02	6.31E-01
f__Lactobacillaceae	3.76E-03	1.82E-02	6.61E-01	f__Lactobacillaceae	1.52E-02	7.60E-02	5.84E-01	f__Barnesiellaceae	1.08E-02	5.39E-02	5.62E-01
f__Anaeroplasmataceae	1.14E-02	4.36E-02	8.79E-01	f__Dehalobacteriaceae	2.04E-02	8.49E-02	1.69E+00	f__Microbacteriaceae	1.75E-02	7.30E-02	7.81E-01
f__Bacteroidaceae	1.27E-02	4.36E-02	8.53E-01	f__Lachnospiraceae	4.39E-02	1.39E-01	1.02E+00	f__Anaeroplasmataceae	3.00E-02	1.07E-01	7.90E-01
f__Aurantimonadaceae	2.54E-02	6.58E-02	1.23E+00	f__Microbacteriaceae	4.45E-02	1.39E-01	1.33E+00	f__Paenibacillaceae	4.65E-02	1.45E-01	1.20E+00
f__Bifidobacteriaceae	2.30E-02	6.58E-02	2.52E-01	f__Enterobacteriaceae	7.45E-02	2.07E-01	1.07E+00	f__Ruminococcaceae	6.48E-02	1.62E-01	9.51E-01
f__Gracilibacteraceae	2.74E-02	6.58E-02	1.06E+00	f__Aurantimonadaceae	8.89E-02	2.22E-01	1.28E+00	f__Turicibacteraceae	6.02E-02	1.62E-01	1.15E+00
f__Tissierellaceae	3.56E-02	7.77E-02	1.09E+00	f__Acidaminobacteraceae	1.44E-01	3.12E-01	1.05E+00	f__Sphingomonadaceae	1.57E-01	3.56E-01	1.25E+00
f__Paenibacillaceae	4.55E-02	9.10E-02	7.76E-01	f__Bifidobacteriaceae	1.87E-01	3.12E-01	2.19E-01	f__Peptococcaceae	1.98E-01	4.13E-01	1.16E+00
f__Turicibacteraceae	1.23E-01	2.28E-01	8.56E-01	f__Gracilibacteraceae	1.85E-01	3.12E-01	1.10E+00	f__Lactobacillaceae	2.37E-01	4.49E-01	1.14E+00
f__S247	1.68E-01	2.89E-01	9.52E-01	f__Ruminococcaceae	1.76E-01	3.12E-01	1.05E+00	f__Paraprevotellaceae	2.51E-01	4.49E-01	3.60E+00
f__Dehalobacteriaceae	2.66E-01	3.55E-01	1.03E+00	f__Tissierellaceae	1.57E-01	3.12E-01	1.12E+00	f__Clostridiaceae	2.76E-01	4.61E-01	1.15E+00
f__Lachnospiraceae	2.30E-01	3.55E-01	1.02E+00	f__Veillonellaceae	2.03E-01	3.18E-01	1.29E+00	f__Enterobacteriaceae	3.00E-01	4.68E-01	9.30E-01
f__Odoribacteraceae	2.56E-01	3.55E-01	9.18E-01	f__Barnesiellaceae	2.62E-01	3.86E-01	6.11E-01	f__Bacteroidaceae	4.19E-01	6.17E-01	8.94E-01
f__Sphingomonadaceae	2.48E-01	3.55E-01	1.24E+00	f__Peptococcaceae	3.01E-01	4.18E-01	1.17E+00	f__Gracilibacteraceae	5.15E-01	7.15E-01	9.69E-01
f__Acidaminobacteraceae	2.98E-01	3.58E-01	1.04E+00	f__Paraprevotellaceae	3.22E-01	4.24E-01	9.64E-02	f__Tissierellaceae	5.84E-01	7.69E-01	9.70E-01
f__Microbacteriaceae	2.94E-01	3.58E-01	1.04E+00	f__Anaeroplasmataceae	4.79E-01	5.71E-01	1.11E+00	f__Aurantimonadaceae	6.78E-01	7.82E-01	9.65E-01
f__Clostridiaceae	6.78E-01	7.74E-01	1.05E+00	f__S247	4.80E-01	5.71E-01	9.59E-01	f__Bifidobacteriaceae	7.19E-01	7.82E-01	1.17E+00
f__Coriobacteriaceae	7.15E-01	7.80E-01	9.87E-01	f__Clostridiaceae	5.31E-01	6.03E-01	9.16E-01	f__Lachnospiraceae	7.01E-01	7.82E-01	1.00E+00
f__Ruminococcaceae	8.32E-01	8.68E-01	9.96E-01	f__Bacteroidaceae	8.30E-01	9.03E-01	9.55E-01	f__Odoribacteraceae	7.05E-01	7.82E-01	9.46E-01
f__Enterobacteriaceae	9.30E-01	9.30E-01	9.91E-01	f__Odoribacteraceae	8.88E-01	9.25E-01	9.70E-01	f__Acidaminobacteraceae	8.39E-01	8.74E-01	9.94E-01
				f__Sphingomonadaceae	9.34E-01	9.34E-01	9.86E-01	f__S247	8.80E-01	8.80E-01	9.93E-01

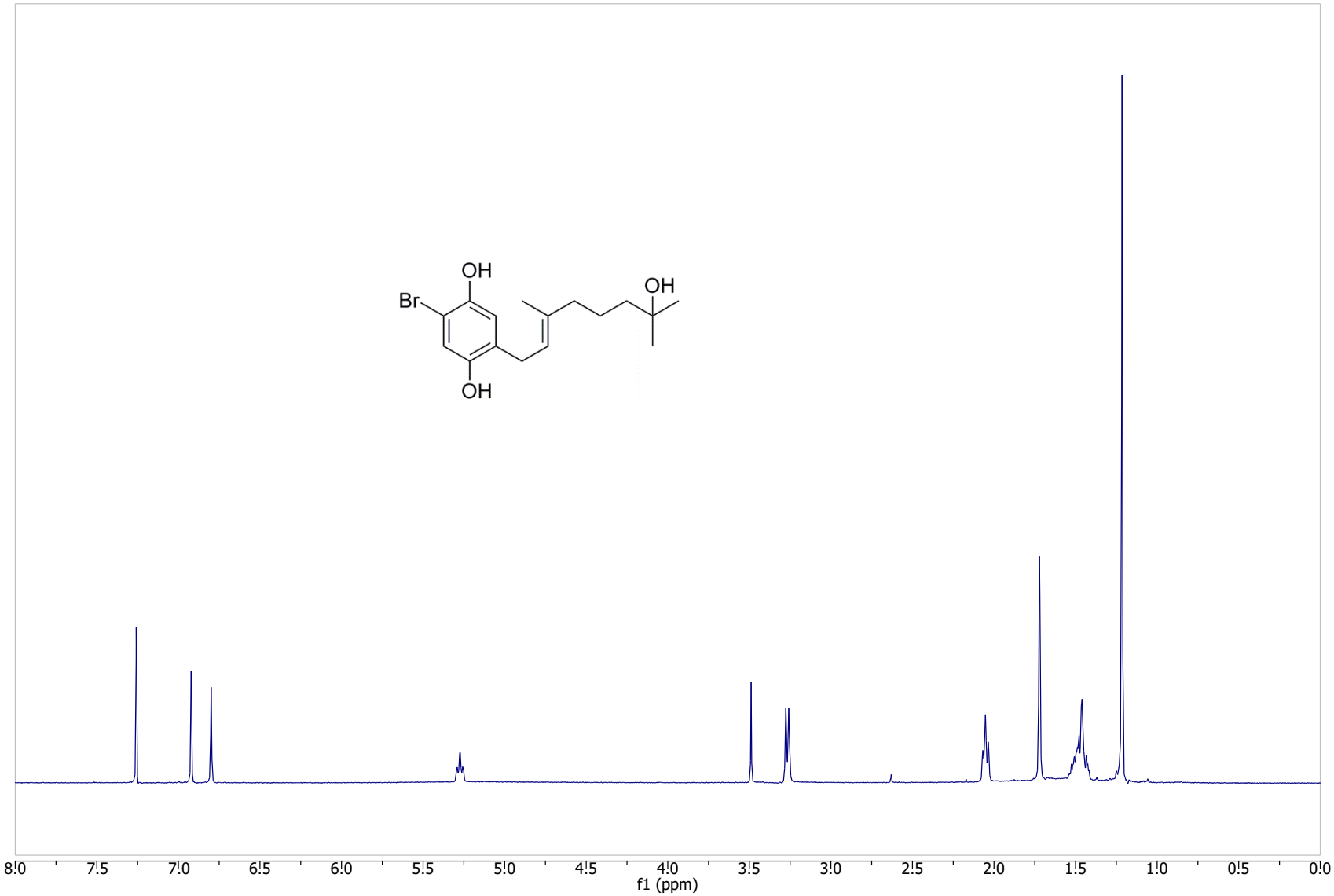
^{1}H NMR Spectrum of Cymopol (**1**) in $CDCl_3$ (400 MHz) at 25 °C



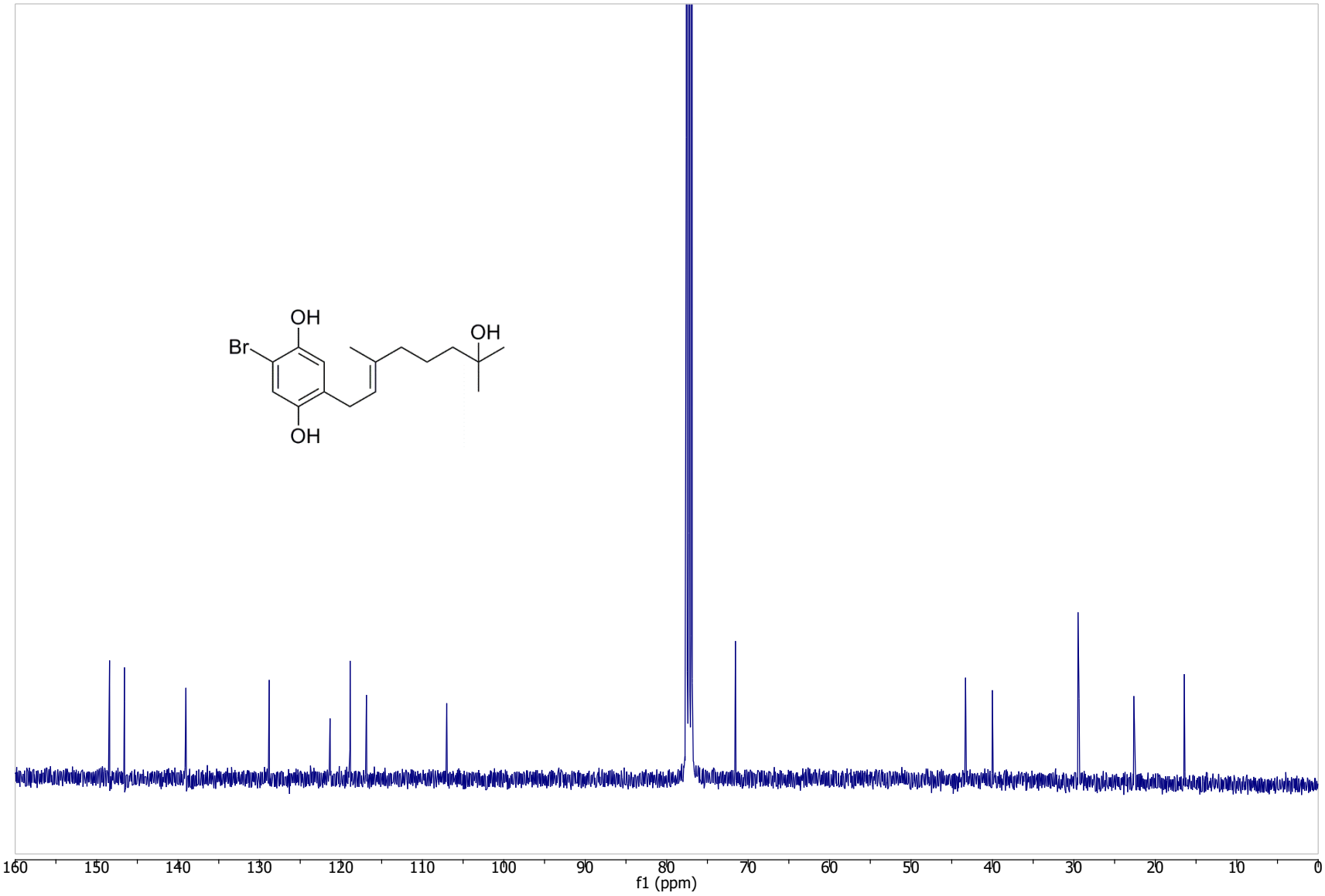
^{13}C NMR Spectrum of Cymopol (**1**) in $CDCl_3$ (100 MHz) at 25 °C



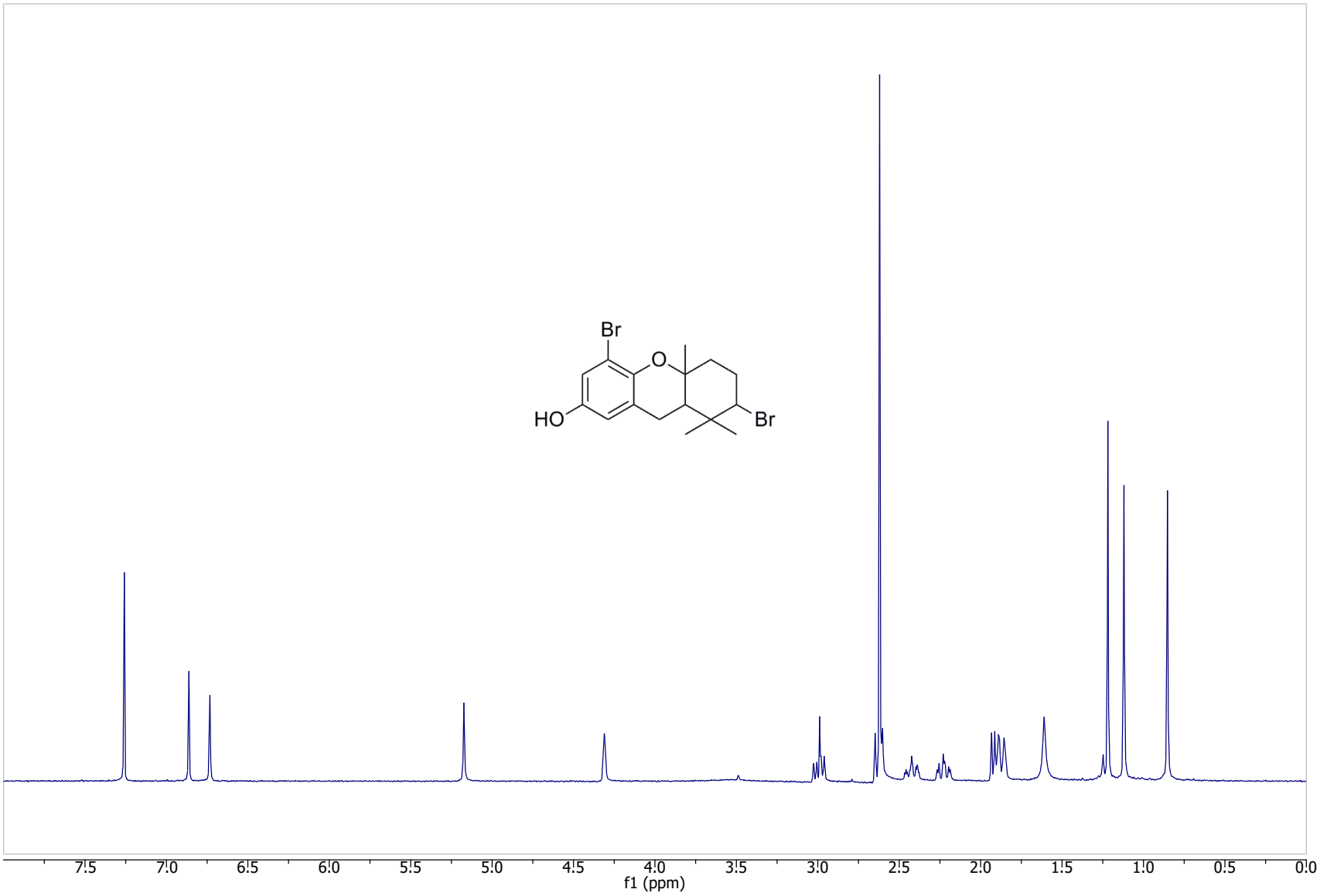
^{1}H NMR Spectrum of 7-Hydroxycymopol (**2**) in $CDCl_3$ (400 MHz) at 25 °C



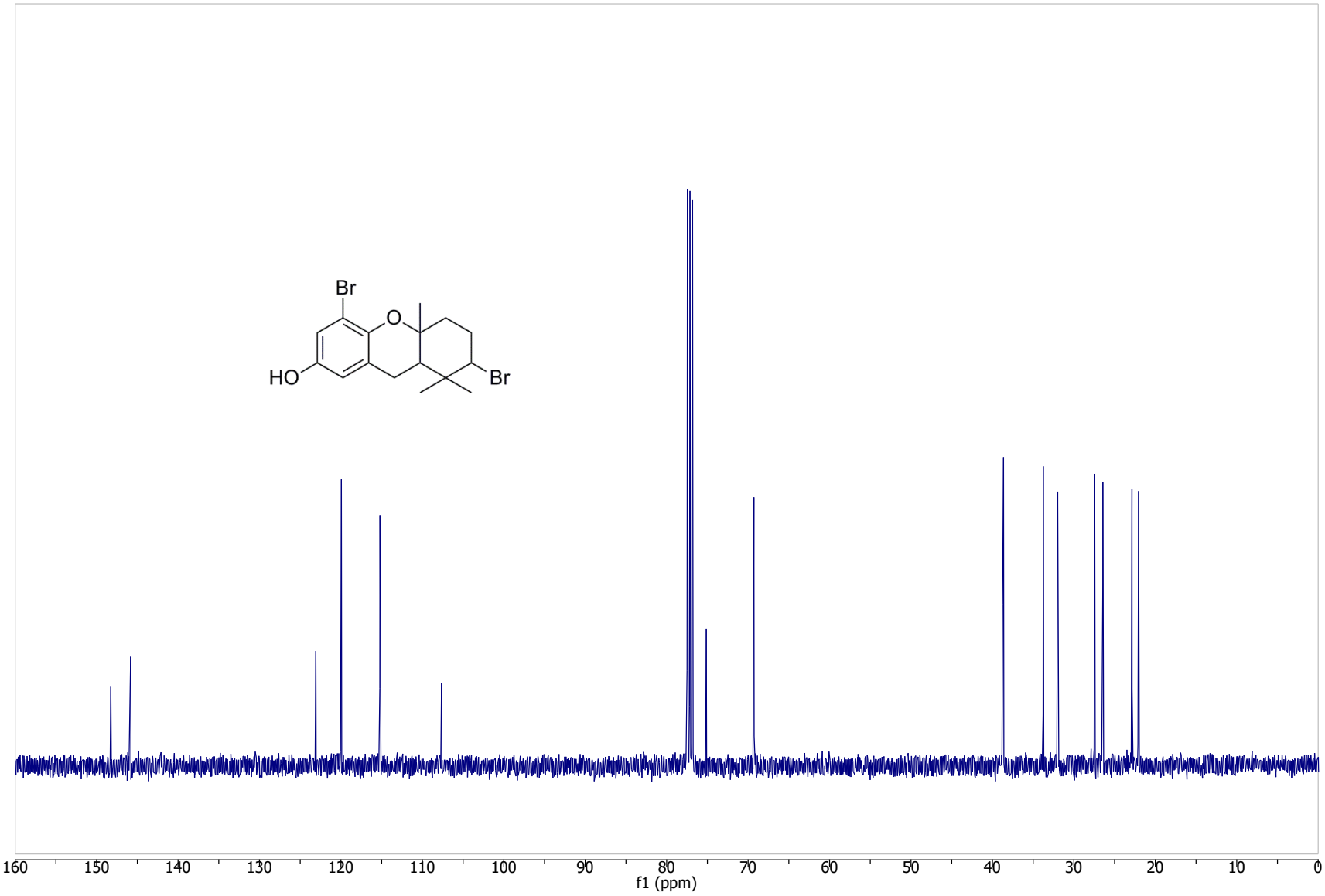
^{13}C NMR Spectrum of 7-Hydroxycymopol (**2**) in $CDCl_3$ (100 MHz) at 25 °C



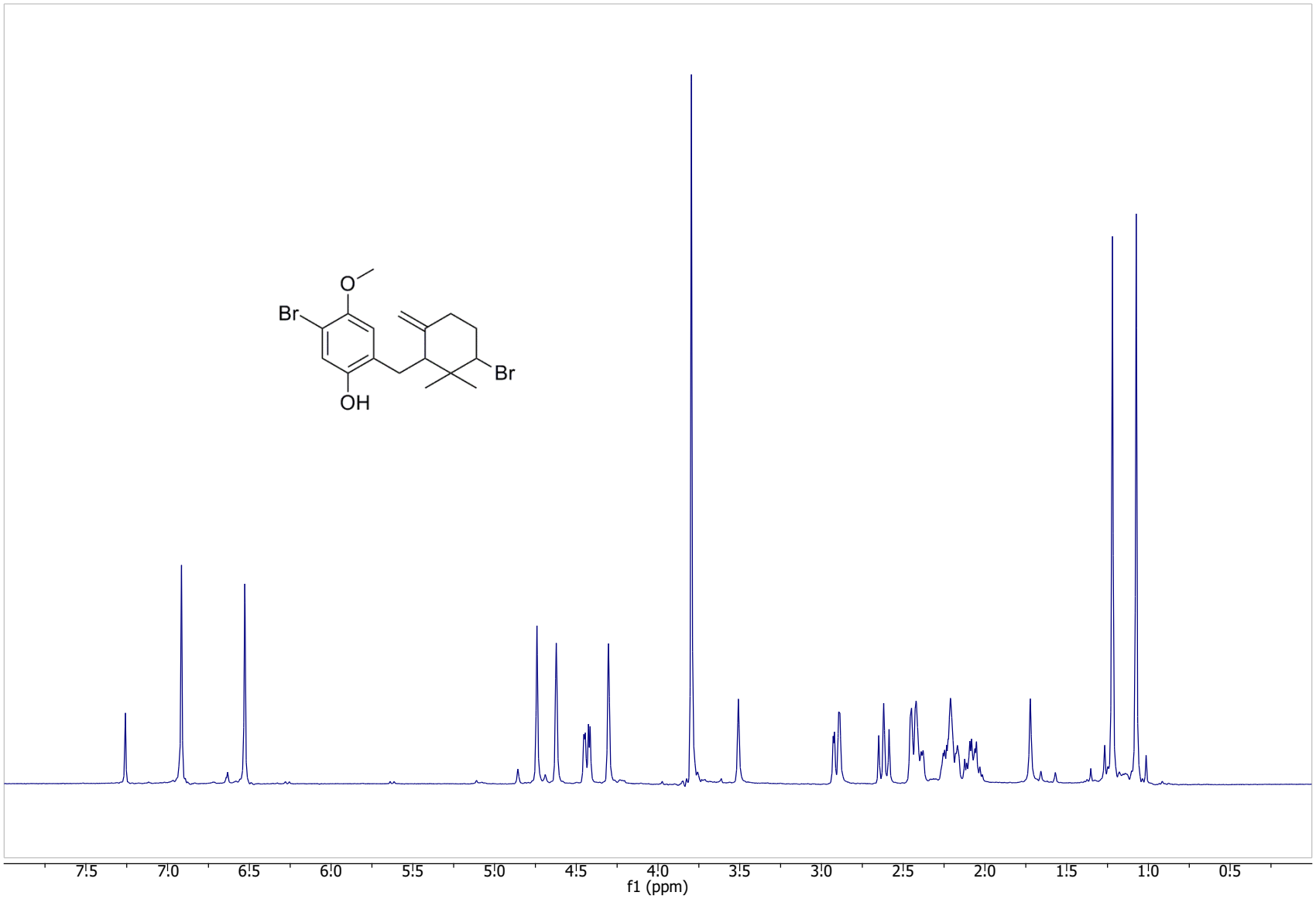
^{1}H NMR Spectrum of Cymobarbatol (**3**) in $CDCl_3$ (400 MHz) at 25 °C



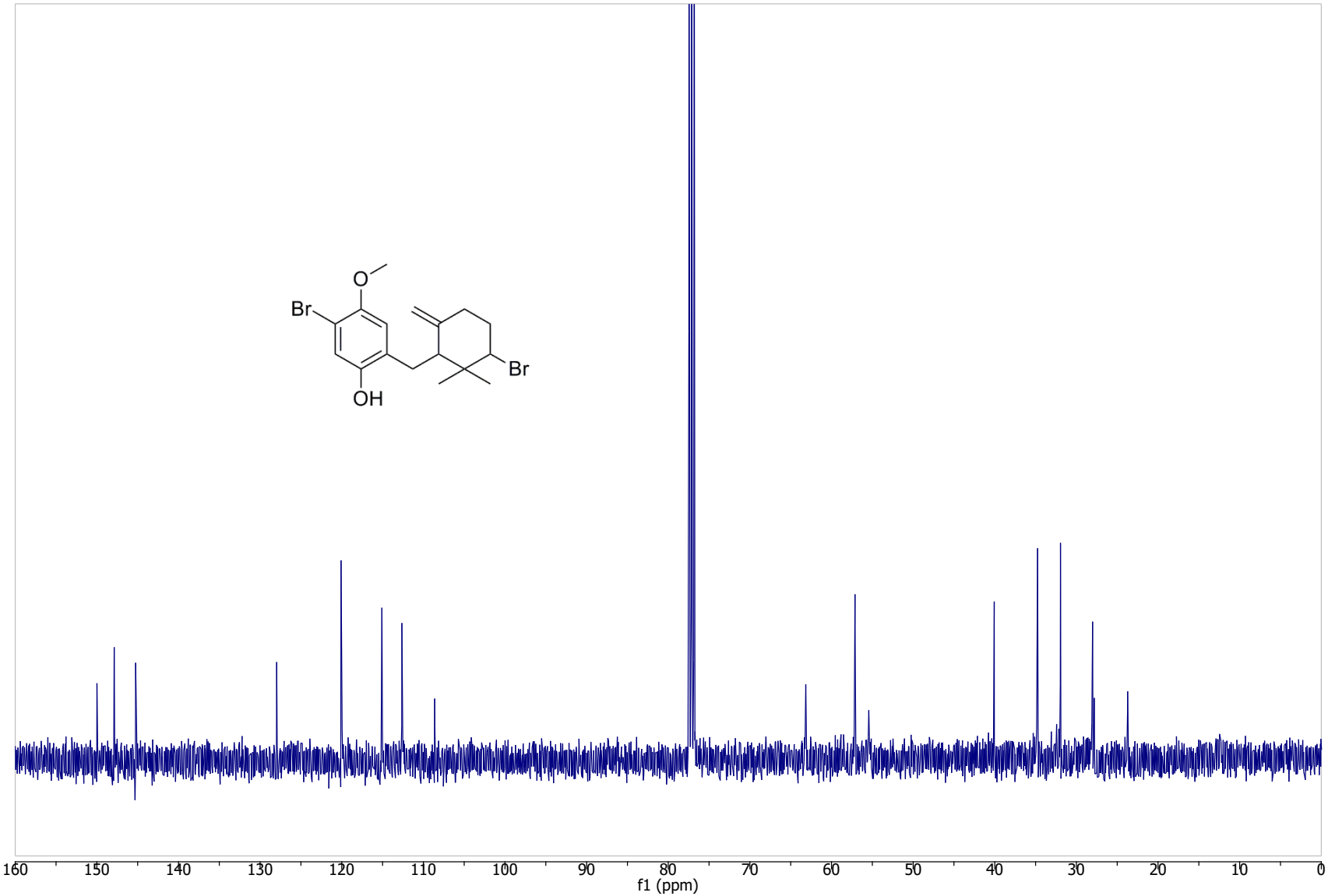
^{13}C NMR Spectrum of Cymobarbatol (**3**) in $CDCl_3$ (100 MHz) at 25 °C



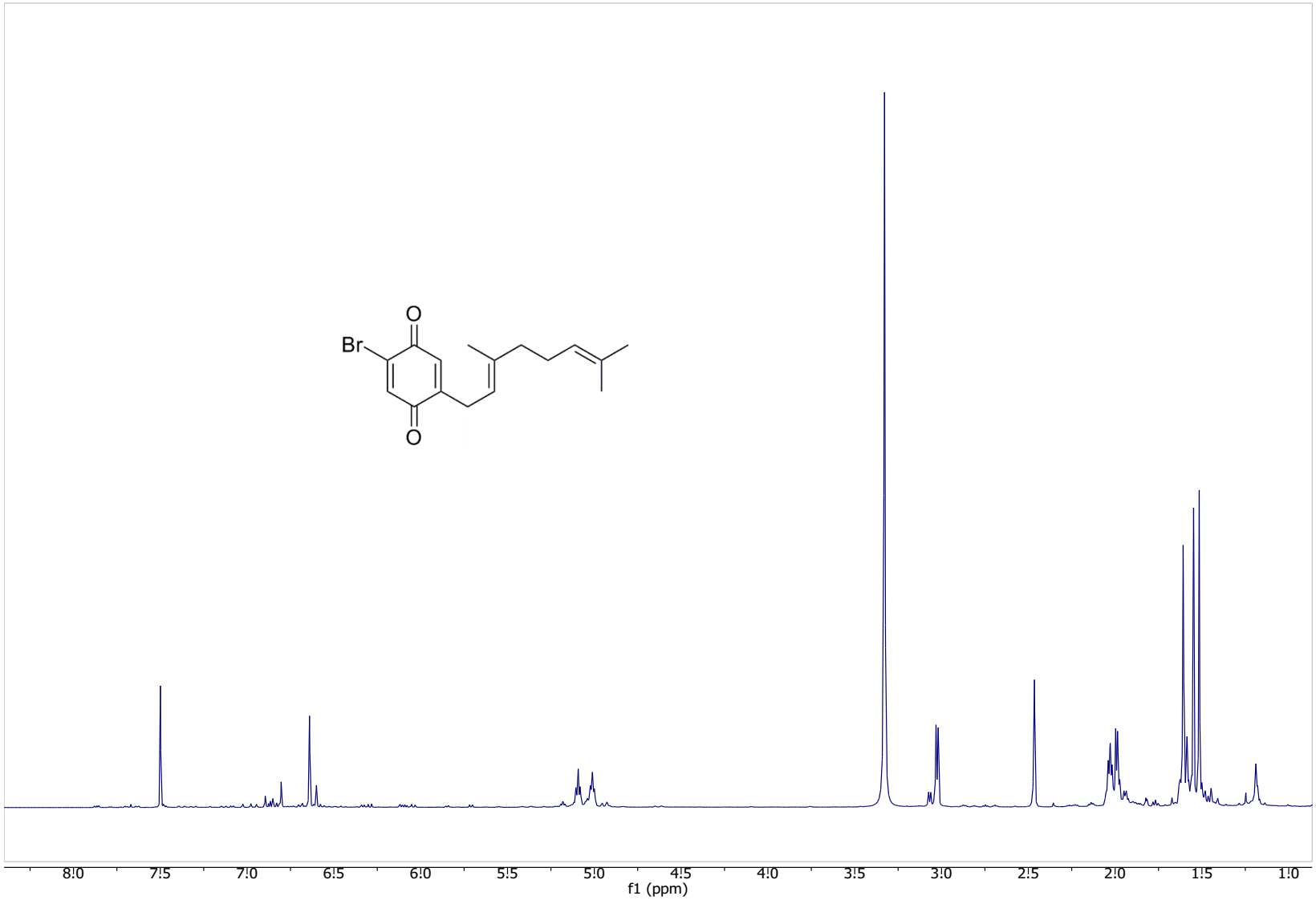
^{1}H NMR Spectrum of Cyclocymopol Monomethyl Ether (**4**) in $CDCl_3$ (400 MHz) at 25 °C



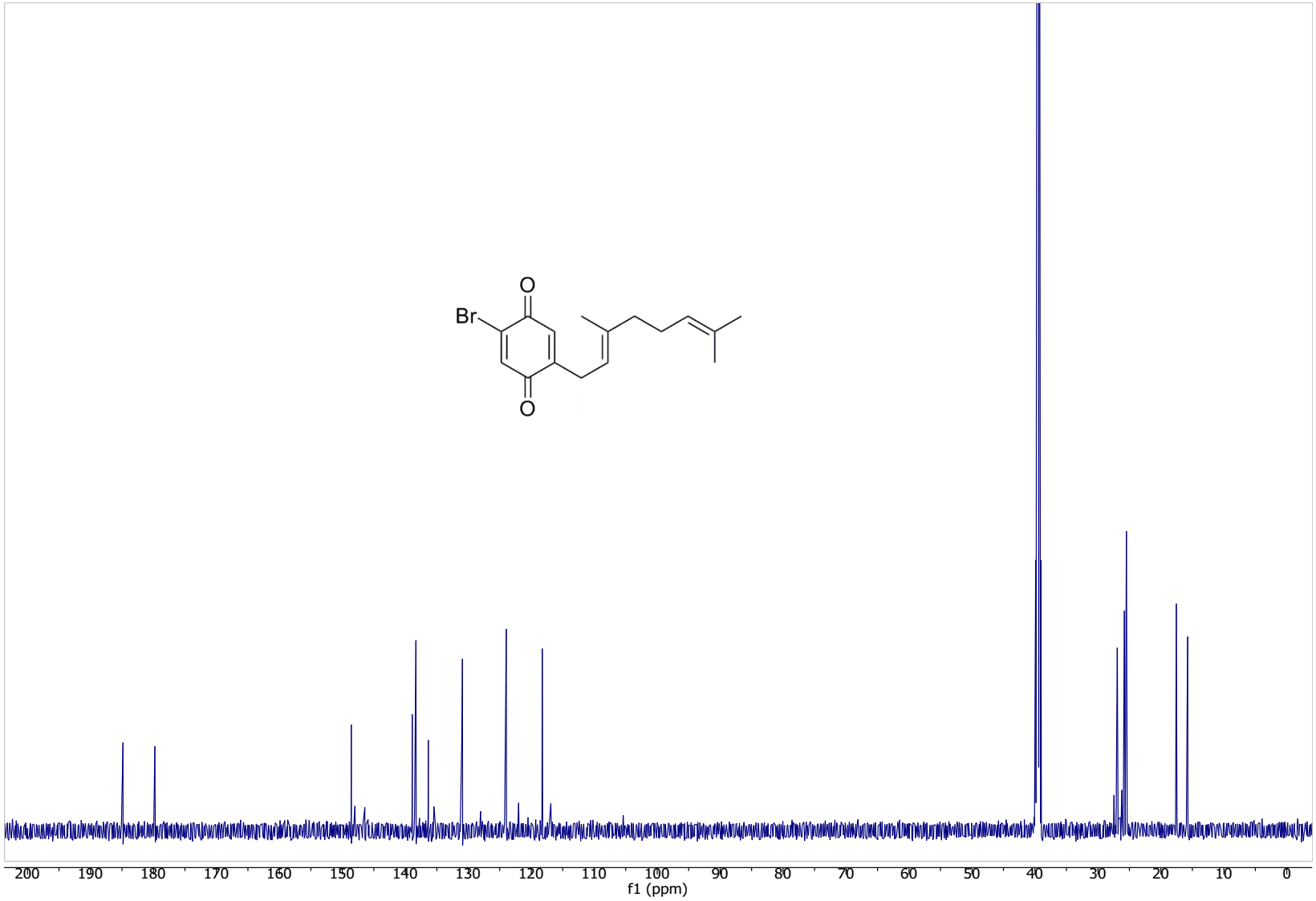
^{13}C NMR Spectrum of Cyclocymopol Monomethyl Ether (**4**) in $CDCl_3$ (400 MHz) at 25 °C



^{1}H NMR Spectrum of Cymopol Quinone (**5**) in d_6-DMSO (600 MHz) at 25 °C



^{13}C NMR Spectrum of Cymopol quinone (**5**) in d_6-DMSO (150 MHz) at 25 °C



SI – Appendix Legends

Movie S1. Neutrophil migration in zebrafish near the injury site (DMSO control)

Movie S2. Neutrophil migration in zebrafish near the injury site (treated with NP extract)