iElectronic Supplementary Information

Continuous flow synthesis of lipophilic cations derived from benzoic acid as new cytotoxic chemical entities in human head and neck carcinoma cell lines

Mabel Catalán[a]**; Vicente Castro-Castillo[b]**; Javier Gajardo-de la Fuente[b] ; Jocelyn Aguilera[c] ; Jorge Ferreira[a] ; Ricardo Ramires-Fernandez[d] ; Ivonne Olmedo[e] ; Alfredo Molina-Berríos[c] ; Charlotte Palominos[a] ; Marcelo Valencia[a] ; Marta Domínguez[f] ; José A. Souto[f]*; José A. Jara[c]*.

[1]Clinical and Molecular Pharmacology Program, Institute of Biomedical Sciences (ICBM), Faculty of Medicine, Universidad de Chile, Santiago, 8380453, Chile.

[2] Department of Organic and Physical Chemistry, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santos Dumont 964, Santiago 8380494, Chile.

[3] Institute for Research in Dental Sciences (ICOD), Faculty of Dentistry, Universidad de Chile, Santiago, 8380492, Chile.

[4] Department of Pathology and Oral Medicine, Faculty of Dentistry, Universidad de Chile, Santiago 8380492, Chile.

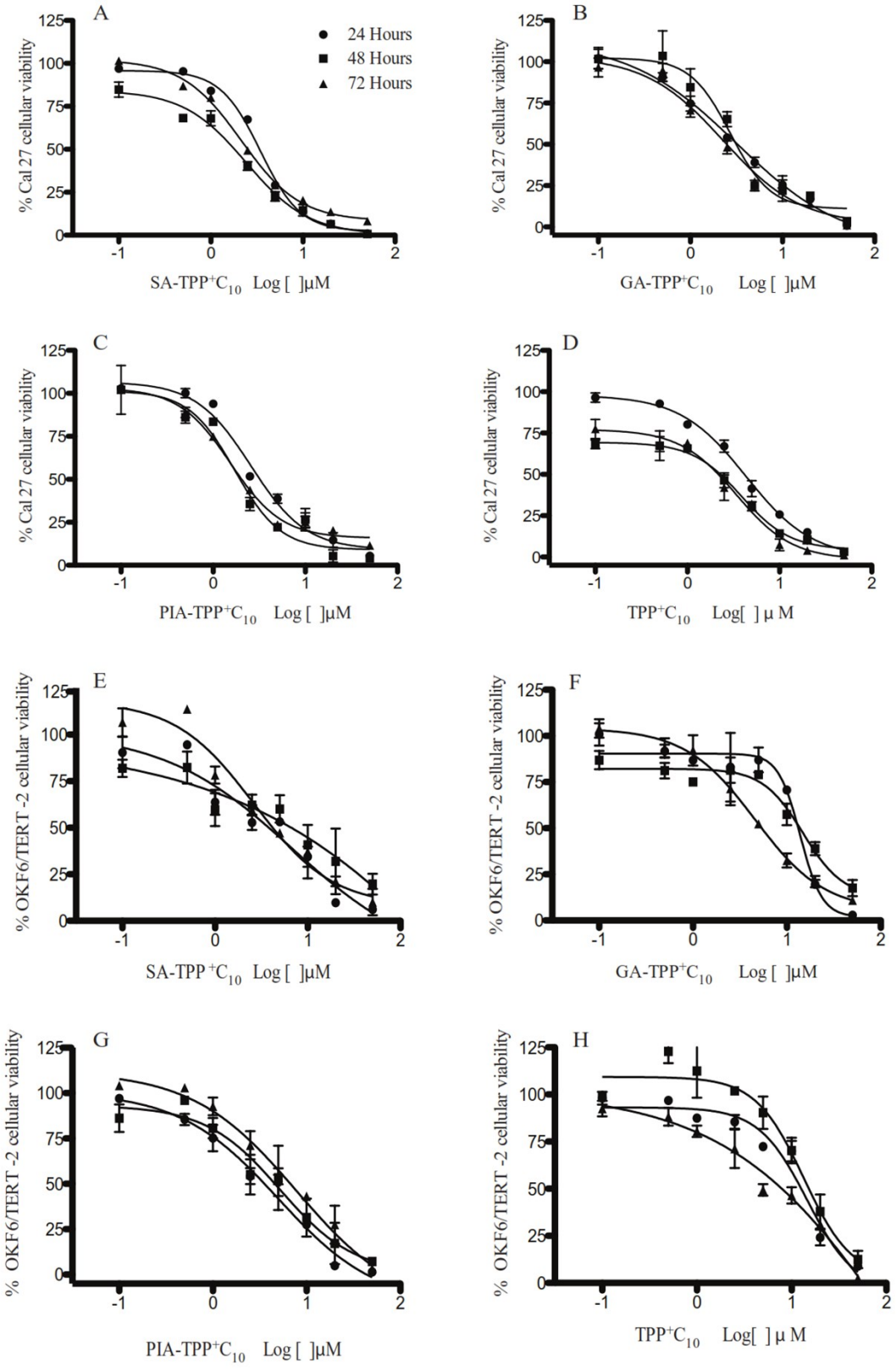
[5] Dentistry School, Universidad Mayor, Santiago 8340585, Chile.

[6] Physiopathology Program, Institute of Biomedical Sciences (ICBM), Faculty of Medicine, Universidad de Chile, Santiago 8380453, Chile.

[7] Departamento de Química Orgánica, Facultad de Química, CINBIO and IIS Galicia Sur, Universidade de Vigo, E-36310, Vigo, Spain.

*Corresponding Authors: Phone: +56 2 29781730. E-mail: jsandovalj@u.uchile.cl
souto@uvigo.es

**These authors contributed equally in this manuscript.


A
24 Hours
48 Hours
72 Hours
% Cal 27 cellular viability
SA-TPP+C10 Log [ ]µM
B
% Cal 27 cellular viability
GA-TPP+C10 Log [ ]µM
C
% Cal 27 cellular viability
PIA-TPP+C10 Log [ ]µM
D
% Cal 27 cellular viability
TPP+C10 Log[ ] µ M
E
% OKF6/TERT -2 cellular viability
SA-TPP +C10 Log [ ]µM
F
% OKF6/TERT -2 cellular viability
GA-TPP+C10 Log [ ]µM
G
% OKF6/TERT -2 cellular viability
PIA-TPP+C10 Log [ ]µM
H
% OKF6/TERT -2 cellular viability
TPP+C10 Log[ ] µ M

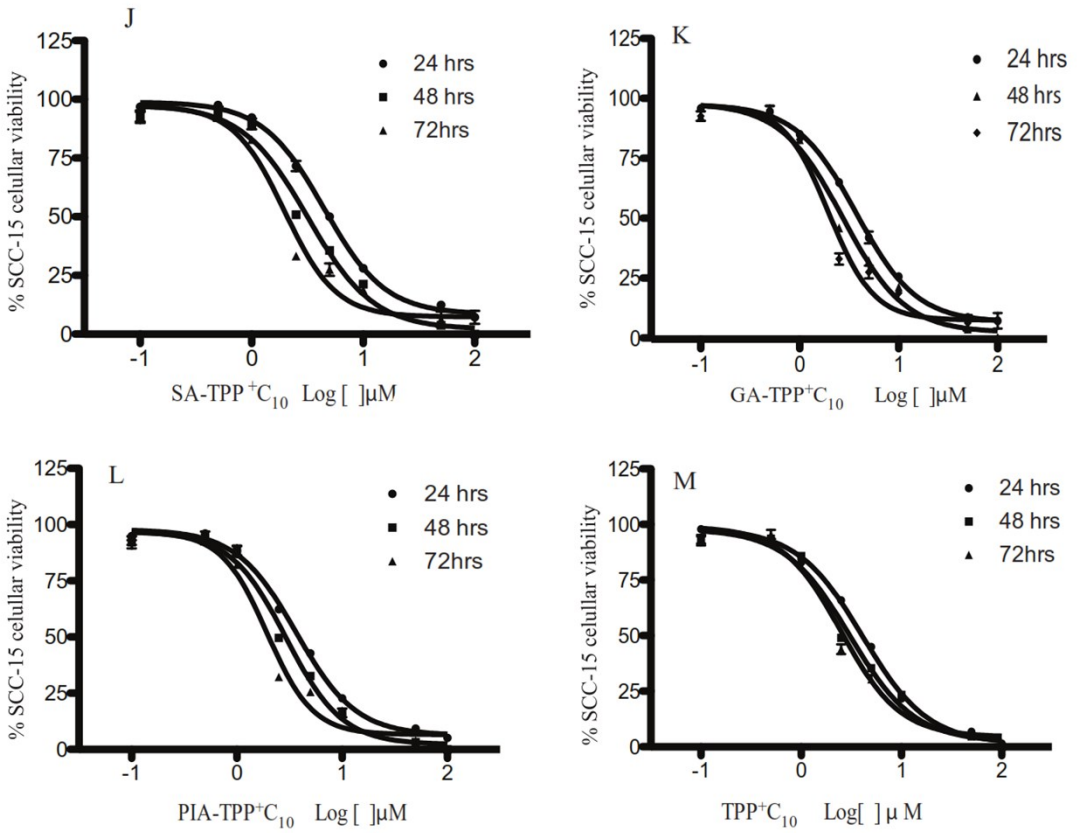
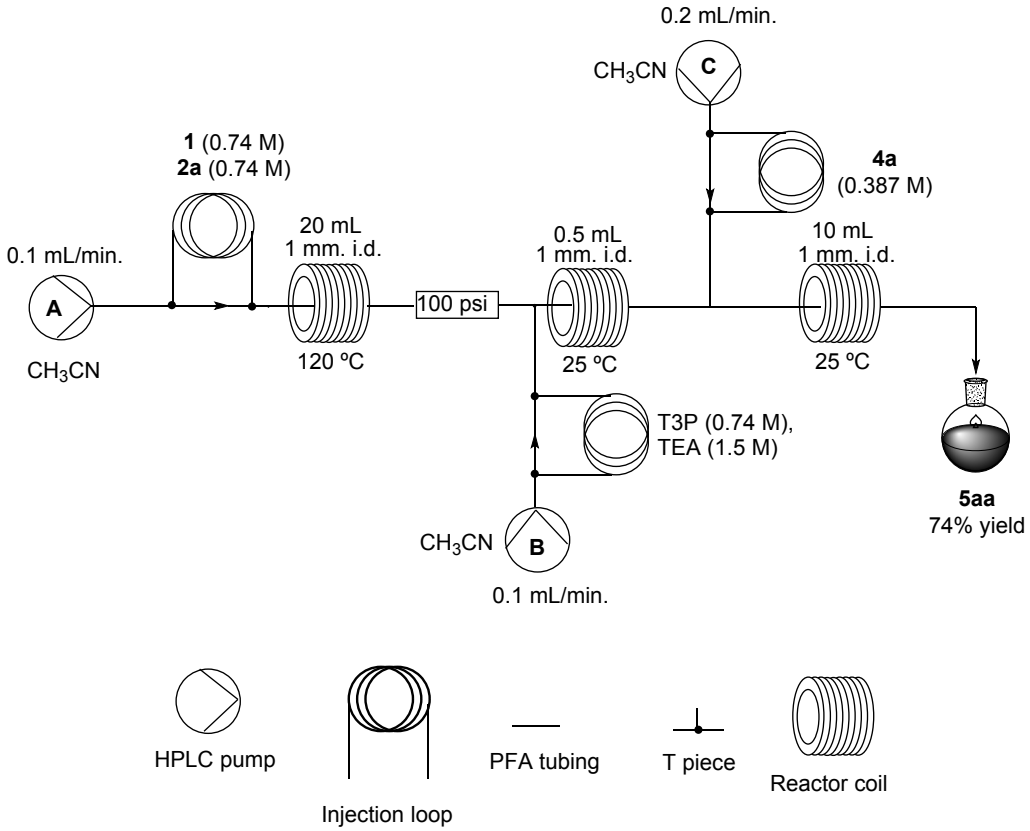


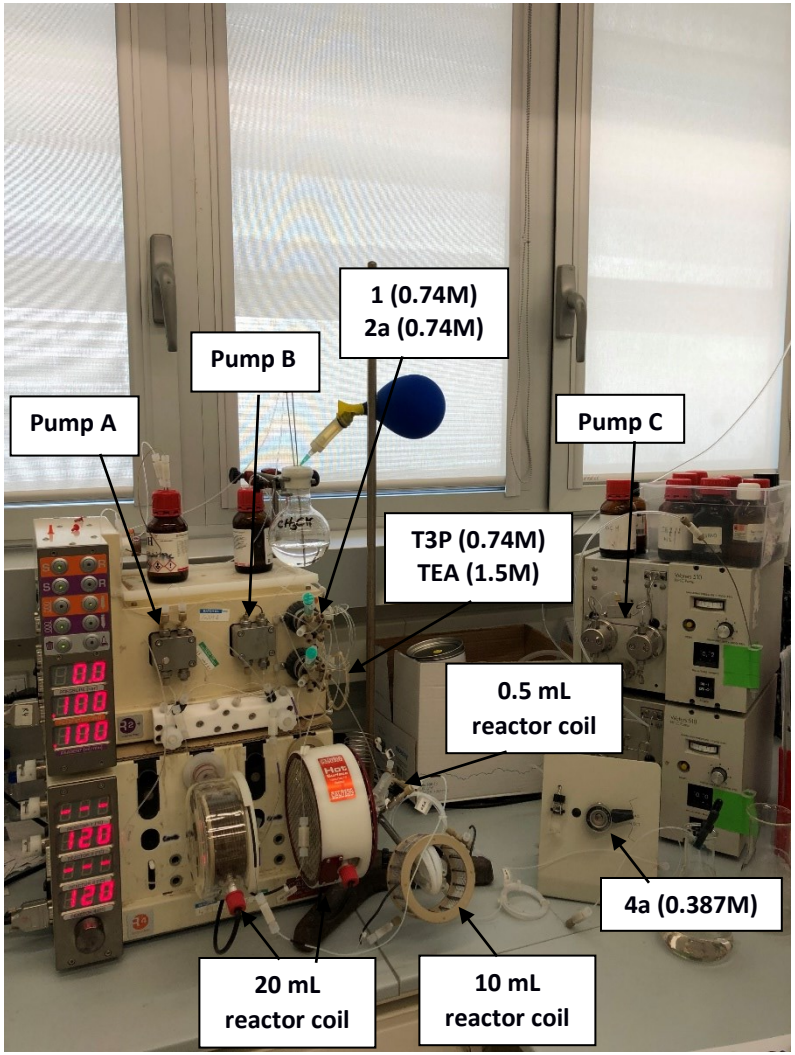
Figure S1: **Cytotoxic activities of compounds in cancer cell lines (Cal 27, SCC-15 and HEp-2)**. (A), (E) and (J) correspond to the activity of **SA-TPP$^+$C$_{10}$** in Cal 27, SCC-15 and HEp-2. (B), (F) and (K) correspond to the activity of **GA-TPP$^+$C$_{10}$** in Cal 27, SCC-15 and HEp-2. (C), (G) and (L) correspond to the activity of **PIA-TPP$^+$C$_{10}$** in Cal 27, SCC-15 and HEp-2. (D), (H) and (M) correspond to the activity of **TPP$^+$C$_{10}$** in Cal 27, SCC-15 and HEp-2. Each compound was incubated with increasing concentrations of the compounds for 24, 48 and 72 h. Viability was assessed by the MTT reduction assay, as described in Material and Methods. The percent viability was calculated from the reduction of MTT (read as absorbance at $\lambda_{570\ nm}$) relative to that observed in cells incubated only with vehicle (DMSO). Values are expressed as mean ± SEM (n=3) and semi-logarithmic dose-response curves were obtained using the GraphPad Prism software (version 5).

	Cell lines											
	Cal 27			Hep-2			SCC-15			OKF-6/TERT		
	24 h.	48 h.	72 h.	24 h.	48 h.	72 h.	24 h.	48 h.	72 h.	24 h.	48 h.	72 h.
Compound	IC_{50}±SD (μM)			IC_{50}±SD (μM)			IC_{50}±SD (μM)			IC_{50}±SD (μM)		
SA-TPP^+C_{10}	3.4±0.01*	2.3±0.06*	2.1±0.02*	2.17±0.31	4.28± 0.12	1.43±0.02	4.5± 0.4	3.2± 0,2	2.0± 0.1	16.9± 0.4[a]	12.7± 0.2[a]	3.2± 0.4[a]
GA-TPP^+C_{10}	2.8±0.02*	2.2±0.02*	2.2±0.04*	4.29 ±0.30	5.66 ± 1.09	2.31 ±0.39	3.3± 0.3***	2.7± 0.1*	1.9± 0.2	13.6± 0.2[a]	13.2± 0.4[a]	12.2± 0.3[a]
PIA- TPP^+C_{10}	2.5±0.02*	1.6±0.01*	1.6±0.04*	7.48 ±0.08	8.28 ± 0.27	3.94 ± 0.71	3.7±0.05	2.9±0.07	1.9±0.04***	6.9± 0.04[a]	7.5± 0.1[a]	11.7± 0.3[a]
TPP^+C_{10}	5.5± 0.04	3.4± 0.1	3.2± 0.1	9.93 ±0.53	12.52± 0.94	4.70 ± 0.67	4.1±0.01	3.0± 0.09	2.5±0.08	15.4± 0.5	13.1± 0.4	12.2± 0.3
BA-C_{10}	≥ 300	243 ± 1.1***	254± 1.5***	n.d	n.d	n.d	≥ 300	≥ 300	≥ 300	≥ 300	≥ 300	≥ 300
PIA-C_{10}	≥ 300	254± 3.3***	267± 3.4***	n.d	n.d	n.d	≥ 300	≥ 300	≥ 300	≥ 300	≥ 300	≥ 300
Cisplatin (CDDP)	55± 0.3	47±0.9	40± 0.6	n.d	5.2±0.7	n.d	15.4±0.3	12.5± 1.2	8.3±0.7	7.3±0.1	3.2±0.2	1.1±0.1

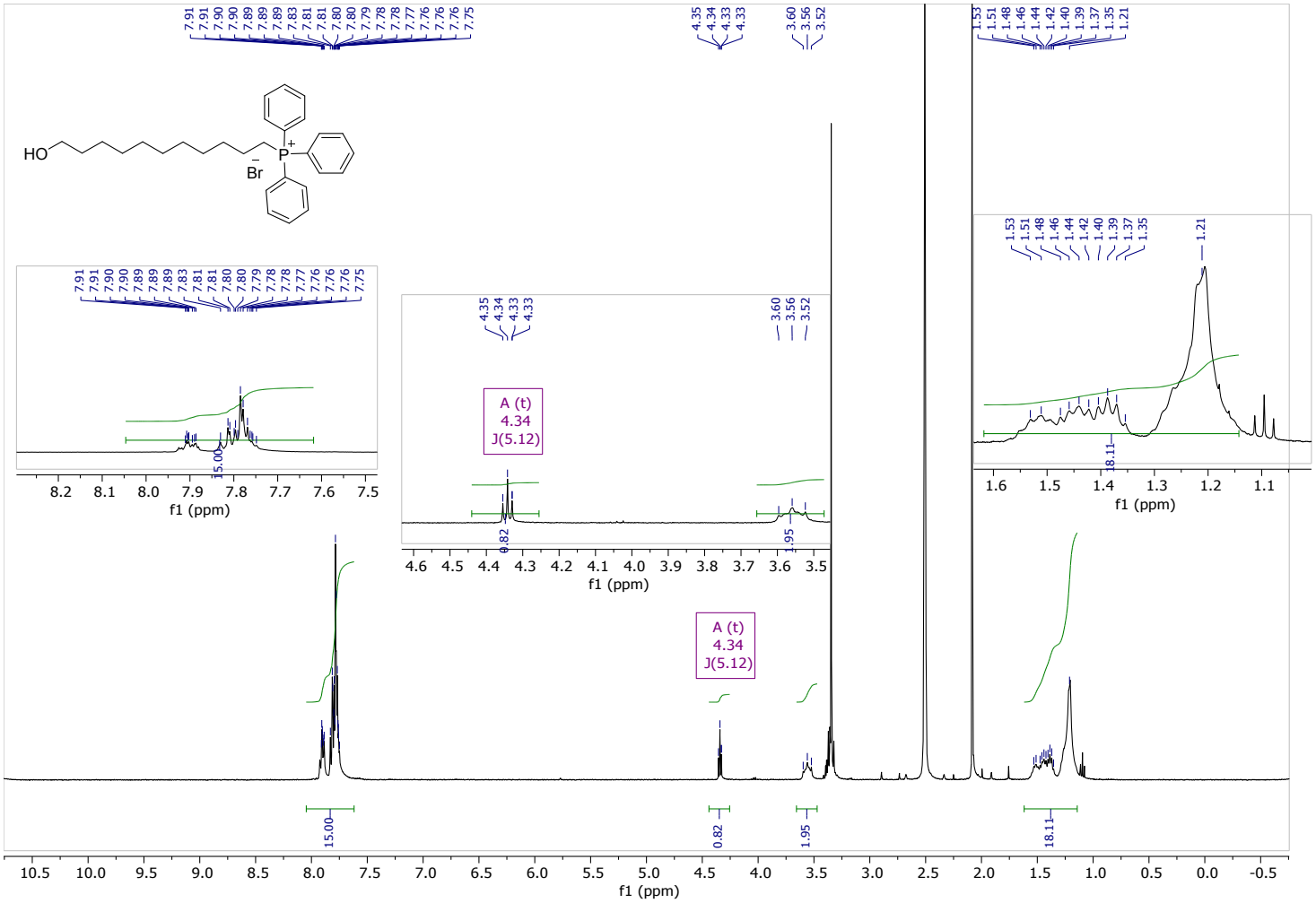
Table 1S. The cytotoxic effect of TPP^+-benzoates on HNSCC cell lines (Cal27, HEp-2, SCC-15) and oral keratinocyte (OKF-6/TERT). IC_{50} = the concentration required to induce cytotoxicity effects in 50% of HNSCC cells and oral keratinocyte cell lines after 24, 48 and 72 h of treatment. Compounds **BA-C_{10}** (benzoic acid decyl ester), and **PIA-C_{10}** (2,3-dihydroxybenzoic acid decyl ester) were used as controls to evaluate the effects of the inclusion of theTPP^+ moiety in the compounds (structures in figure 1). Each assay was performed in triplicate. Key: *$p \leq 0.05$ with respect to the effect of **TPP^+C_{10}** (3,4,5-trihydroxybenzoic acid decyl ester) at the same cell line and time; ***$p \leq 0.001$ with respect to the effect of **TPP^+C_{10}** at the same cell line and time; a= $p \leq 0.05$ with respect to the effect at the same time in the Cal 27 cell line.

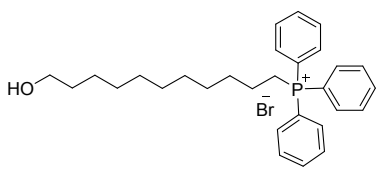
Detailed flow scheme.





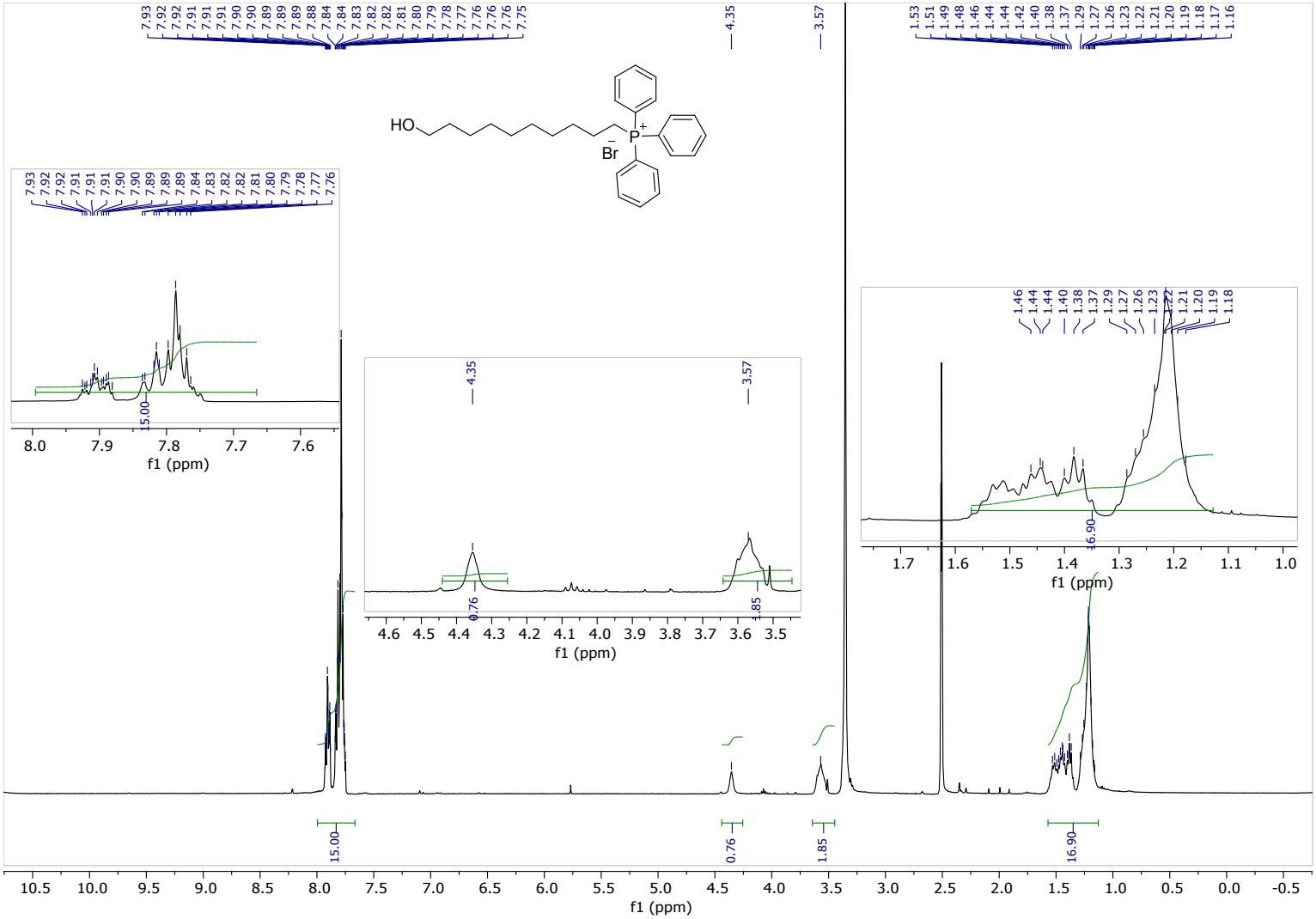
Triphenyl(11)oxy)-undecyl)phosphonium bromide (**3a**)

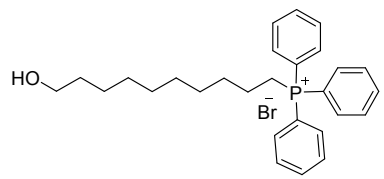



HO
P
Br

24.04
0 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30

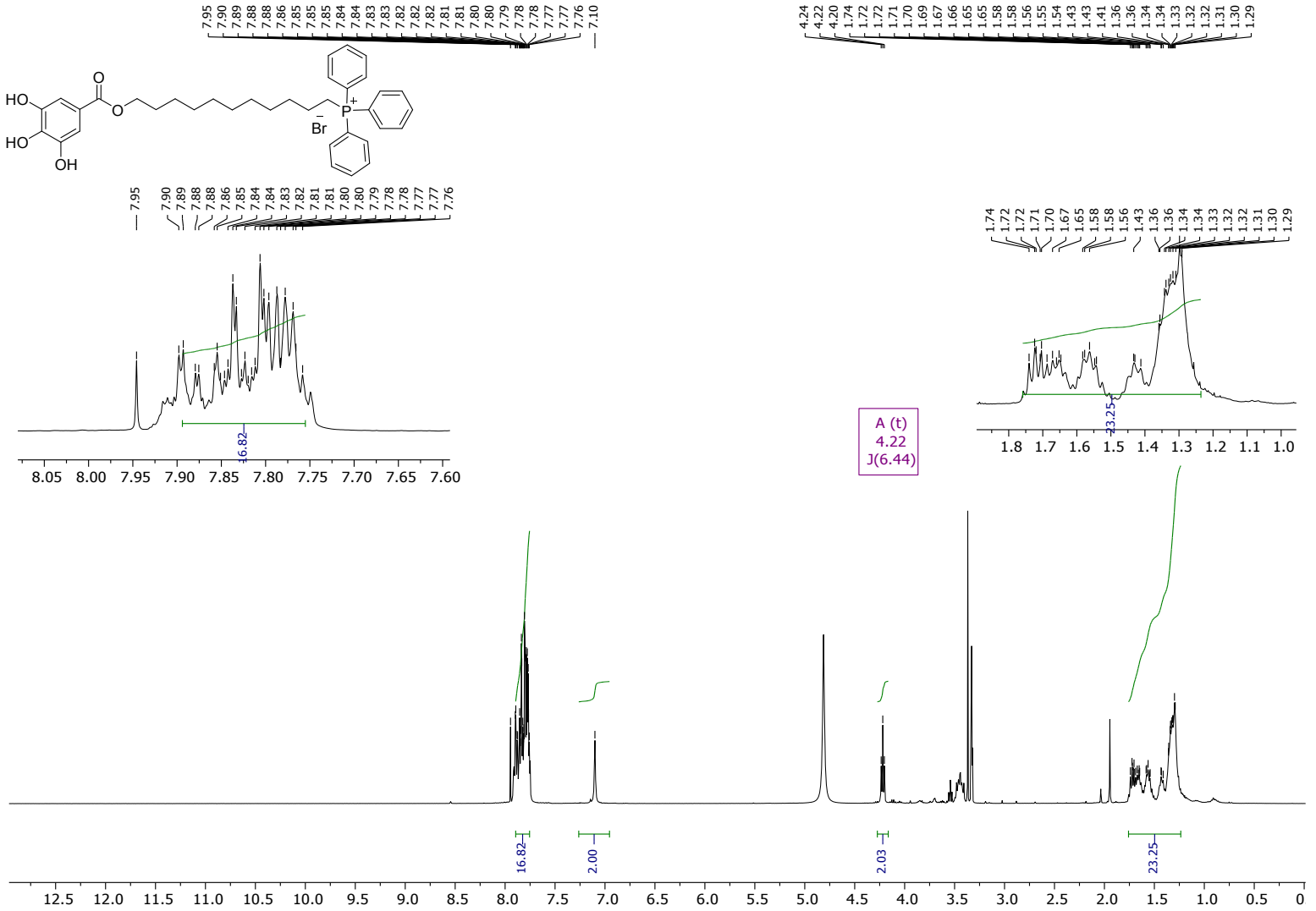
Triphenyl(10)oxy)-decyl)phosphonium bromide (**3b**)

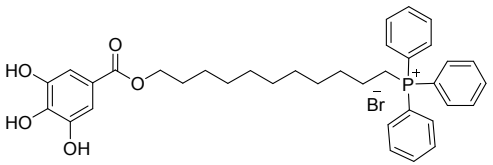



HO
P
+
Br
−

24.03
0
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30

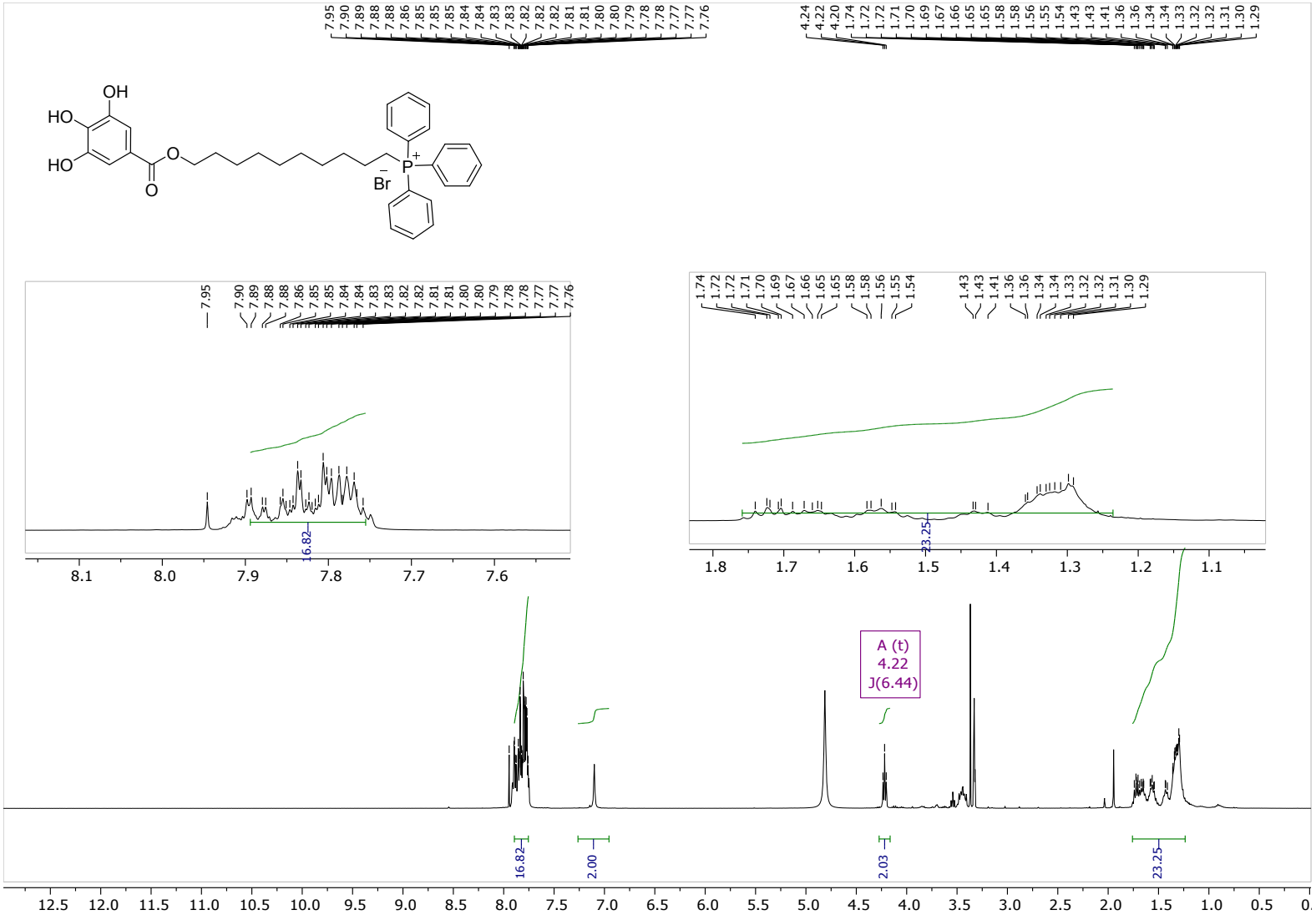
Triphenyl(11-((3,4,5-trihydroxybenzoyl)oxy)-undecyl)phosphonium bromide (**TPP^{+}C$_{11}$, 5aa**)

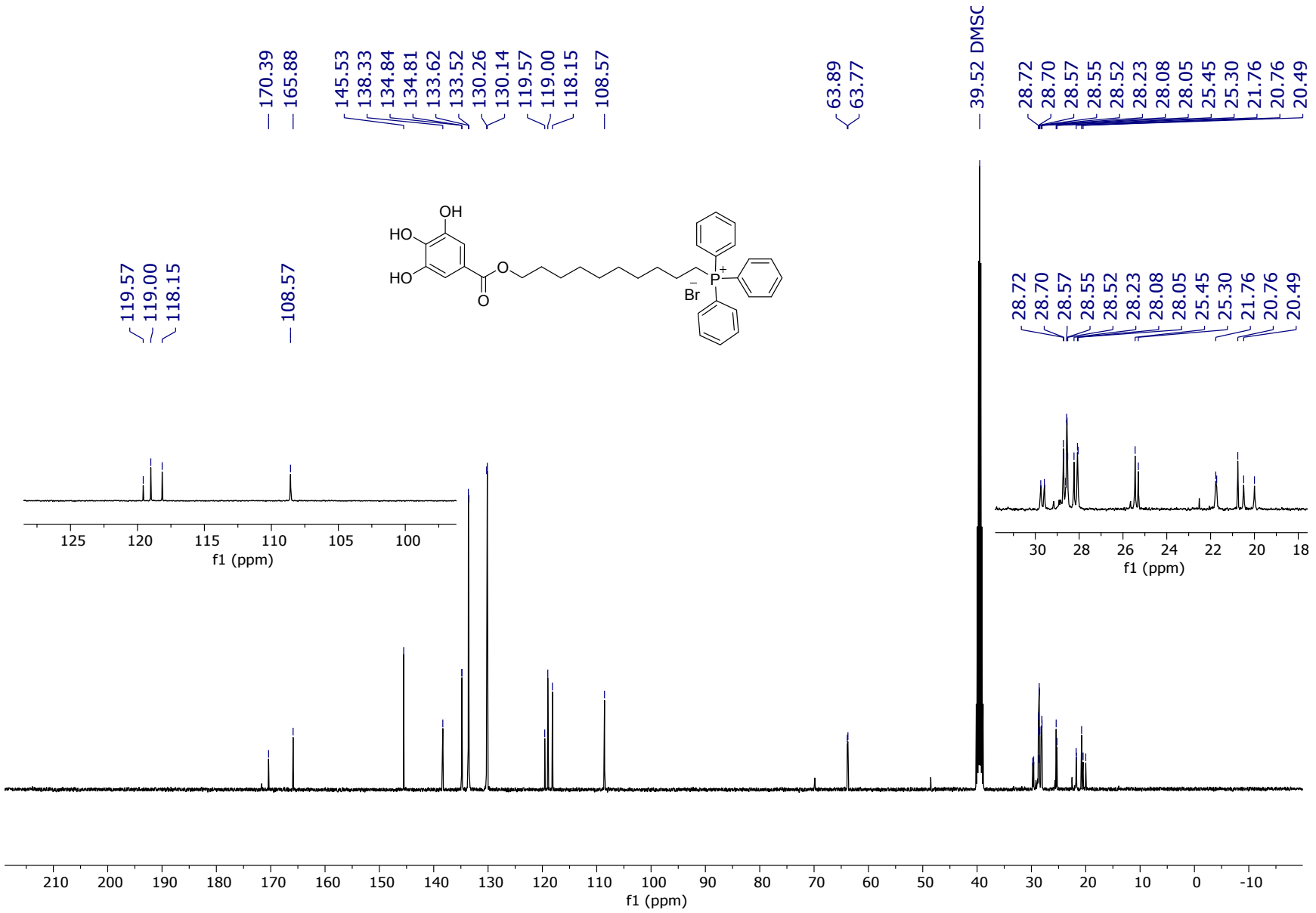



HO
HO
OH
O
O
Br
P

24.04
0
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30

Triphenyl(10-((3,4,5-trihydroxybenzoyl)oxy)-decyl)phosphonium bromide (**TPP^{+}C$_{10}$, 5ba**).

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145.53
138.33
134.84
134.81
133.62
133.52
130.26
130.14
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108.57
63.89
63.77
39.52 DMSO
28.72
28.70
28.57
28.55
28.52
28.23
28.08
28.05
25.45
25.30
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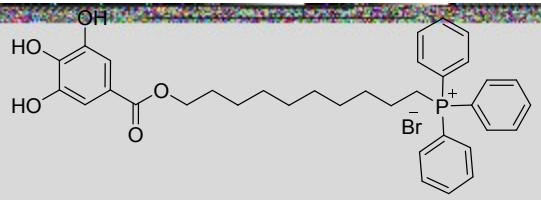
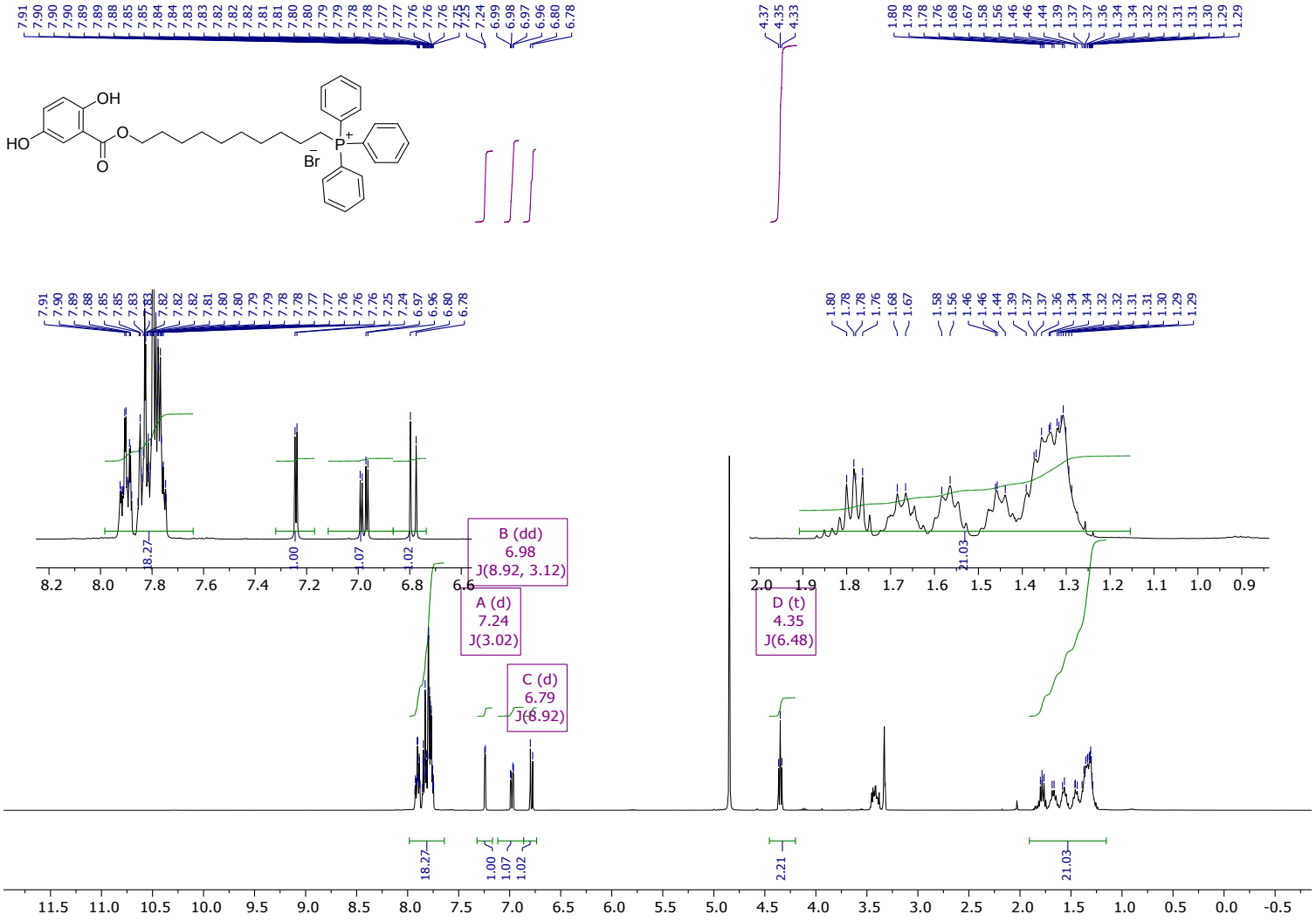
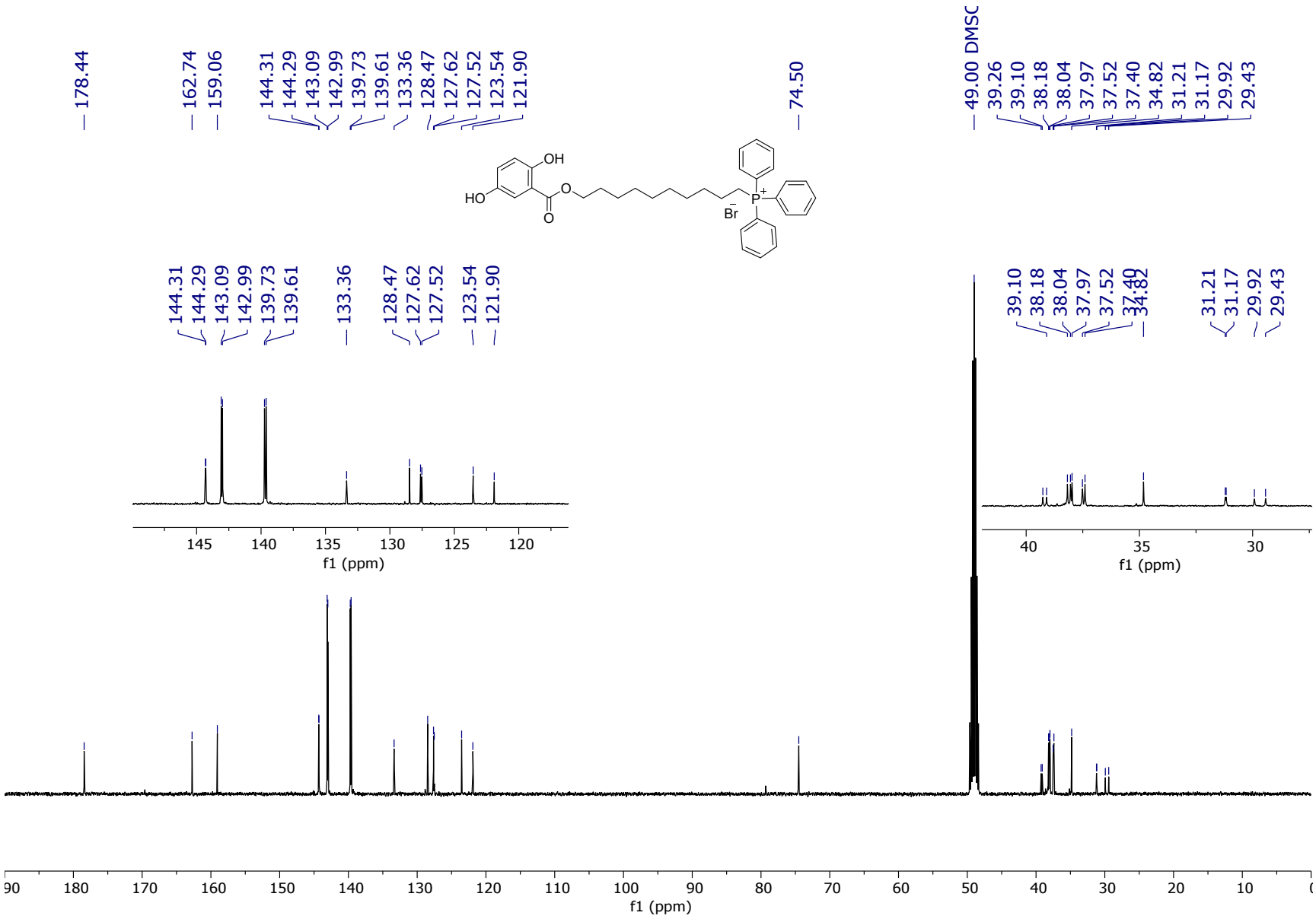
20.76
20.49
OH
HO
HO
O
O
P
Br
f1 (ppm)
f1 (ppm)
f1 (ppm)


OH
HO
HO
O
O
P
+
Br
−

Triphenyl(10-((2,5-dihydroxybenzoyl)oxy)-decyl)phosphonium bromide (**GA-TPP^{+}C$_{10}$, 5bb**).

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159.06
144.31
144.29
143.09
142.99
139.73
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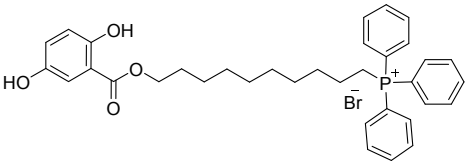
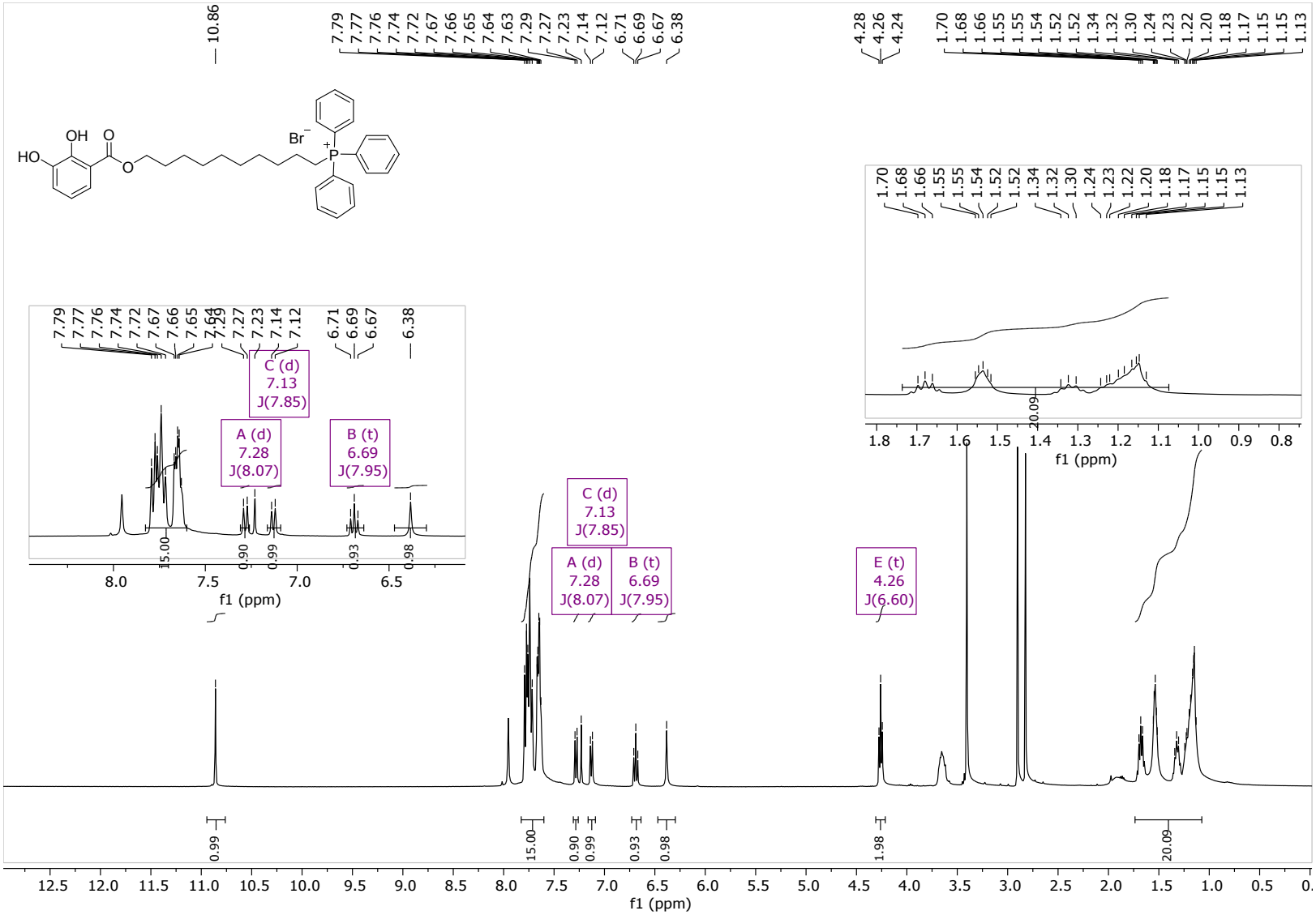
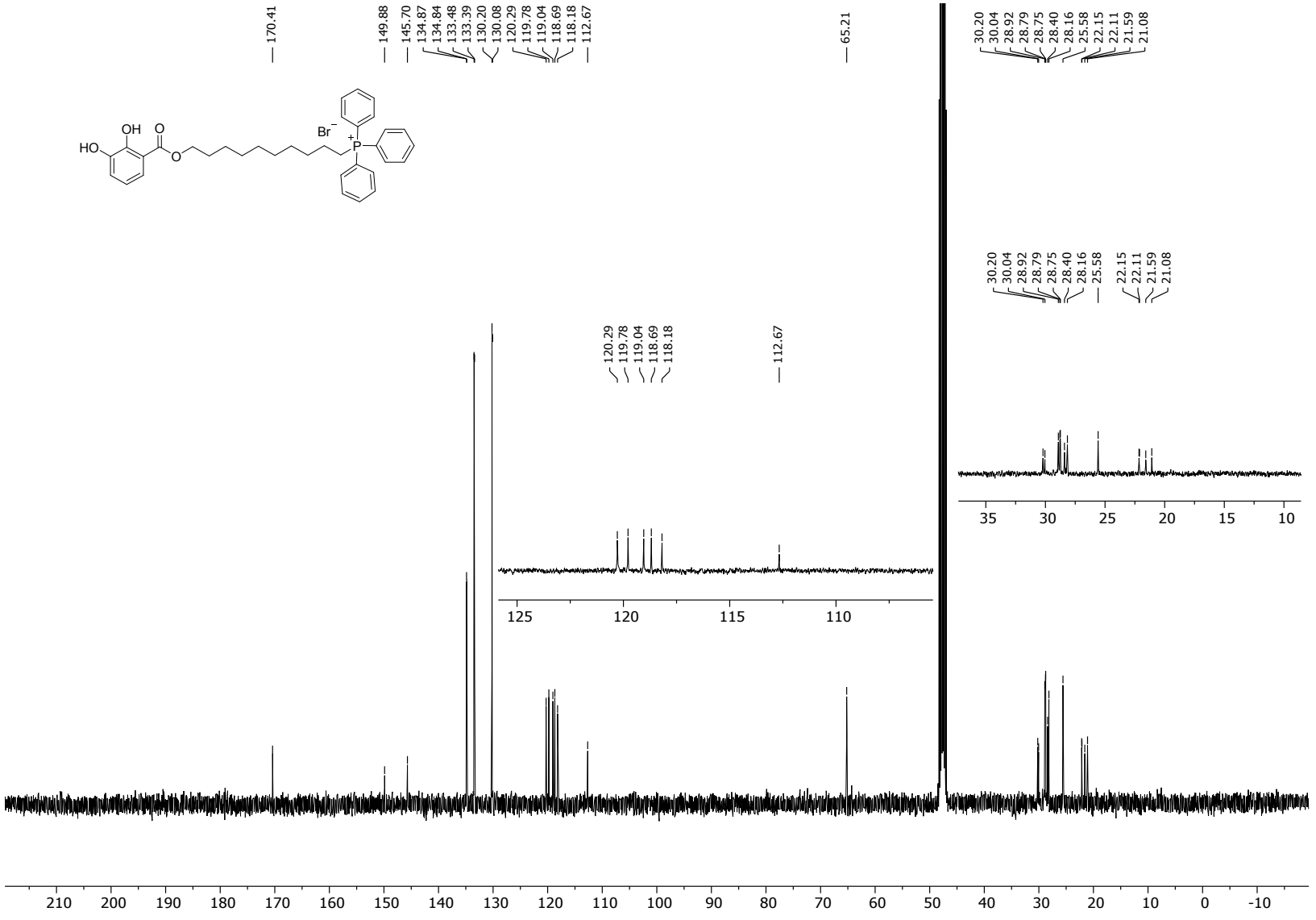
133.36
128.47
127.62
127.52
123.54
121.90
74.50
49.00 DMSO
39.26
39.10
38.18
38.04
37.97
37.52
37.40
34.82
31.21
31.17
29.92
29.43
OH
HO
O
O
P+
Br−
f1 (ppm)
f1 (ppm)
f1 (ppm)


OH
HO
O
O
P+
Br-

24.07
0
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30

Triphenyl(10-((2,3-dihydroxybenzoyl)oxy)-decyl)phosphonium bromide (**PIA-TPP^{+}C$_{10}$, 5bd**)

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145.70
134.87
134.84
133.48
133.39
130.20
130.08
120.29
119.78
119.04
118.69
118.18
112.67
65.21
30.20
30.04
28.92
28.79
28.75
28.40
28.16
25.58
22.15
22.11
21.59
21.08
OH
O
HO
O
Br⁻
P
210
200
190
180
170
160
150
140
130
120
110
100
90
80
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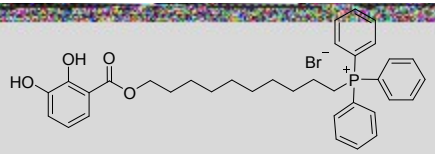
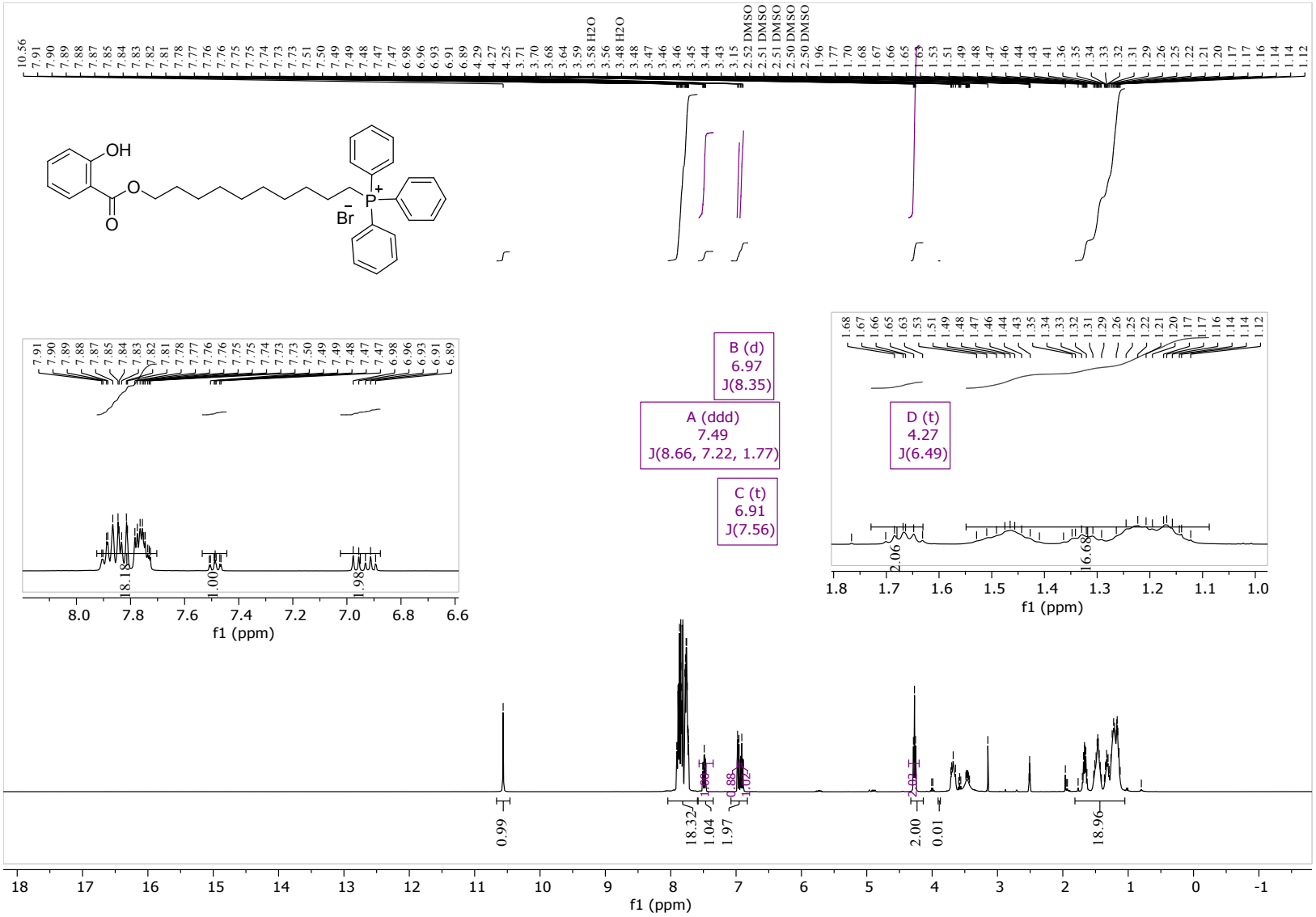
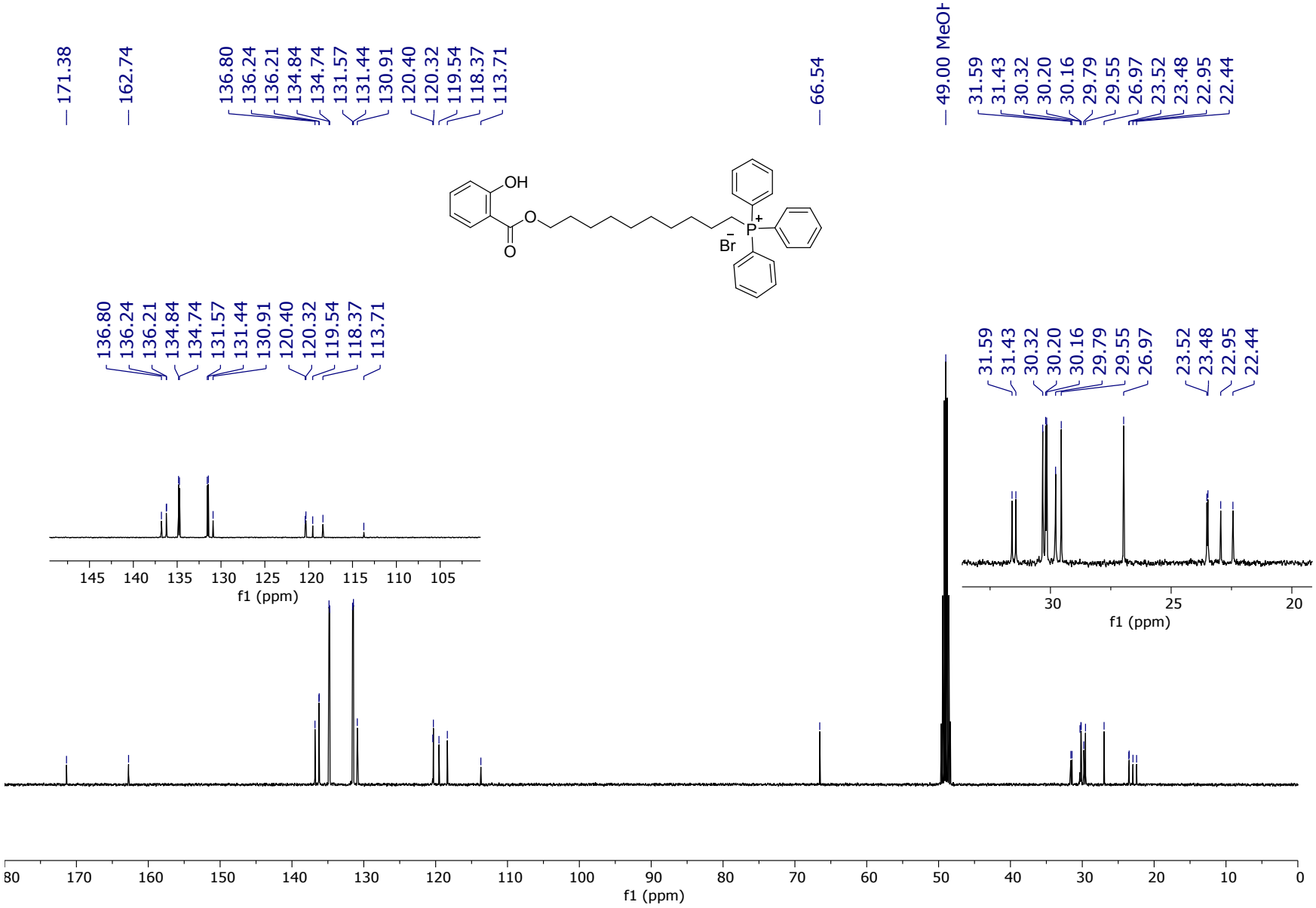
30
25
20
15
10


OH
O
HO
O
Br
−
+
P

Triphenyl(10-((2-hydroxybenzoyl)oxy)-decyl)phosphonium bromide (**SA-TPP^{+}C$_{10}$, 5be**).

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136.24
136.21
134.84
134.74
131.57
131.44
130.91
120.40
120.32
119.54
118.37
113.71
66.54
49.00 MeOH
31.59
31.43
30.32
30.20
30.16
29.79
29.55
26.97
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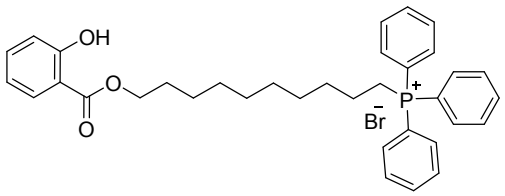
22.44
OH
O
O
P
Br
f1 (ppm)
f1 (ppm)
f1 (ppm)


OH
O
O
P+
Br−

24.03
0
210
200
190
180
170
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100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30