Supplementary Information

For

A ratiometric fluorescence sensor based on enzymatically activatable micellization of TPE derivatives for quantitative detection of alkaline phosphatase activity in serum

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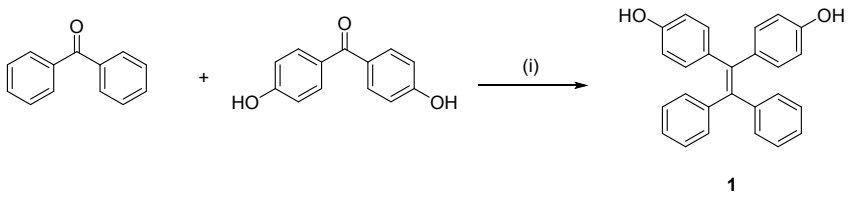
Synthesis of Compound 1



Scheme S1. Synthesis of **1**

Synthesis of compound 1. Benzophenone (3.1 g, 16.8 mmol) and bis(4-hydroxyphenyl)methanone (3.0 g, 14.0 mmol) were dissolved in 150 mL of THF. The resulting solution was deoxygenated with argon for 15 minutes. Then $TiCl_4$ (13.3 g, 70.0 mmol) and zinc powder (9.2 g, 140.0 mmol) were added to the stirred solution under the protection of argon. The reaction mixture was then heated up to 60 ~ 65 ^oC and stirred for 24 hours. The excess zinc residue was removed by flash chromatography using THF as solvent and the filtrate was concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel using ethyl acetate:hexane (1:2) as the eluent. Yield: 64%; ^1H NMR (500 MHz, $CDCl_3$): δ 7.07-6.98 (m, 10H), 6.79 (d, 4H, J = 8.65 Hz), 6.46 (d, 4H, J = 8.7 Hz); ^{13}C NMR (125 MHz, Tetrahydrofuran-d_8): 157.35, 145.80, 142.07, 139.01, 135.96, 133.48, 132.28, 128.36, 126.59, 115.21 ; ESI(+)-MS (*m/z*): [M^+] Calcd. for $C_{26}H_{20}O_2$, 364.44; found, 364.44

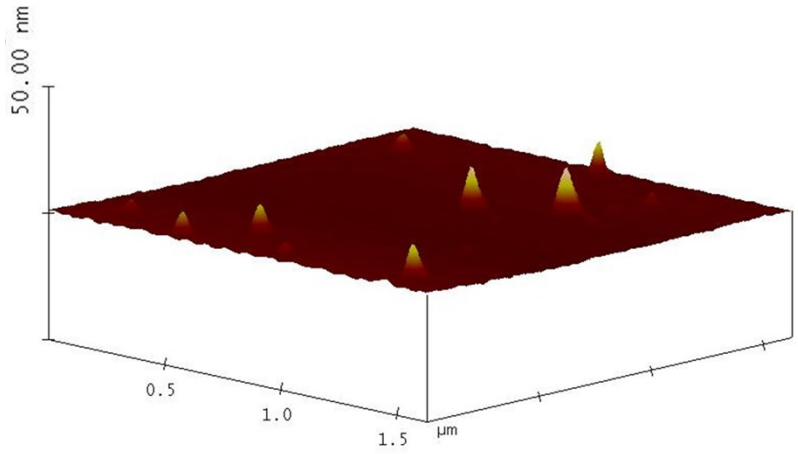


Fig. S1 AFM image of **P-TPE-TG** (1.0×10^{-5} M) on a silicon wafer incubated with ALP (100 mU/mL) for 60 minutes in aqueous solution.

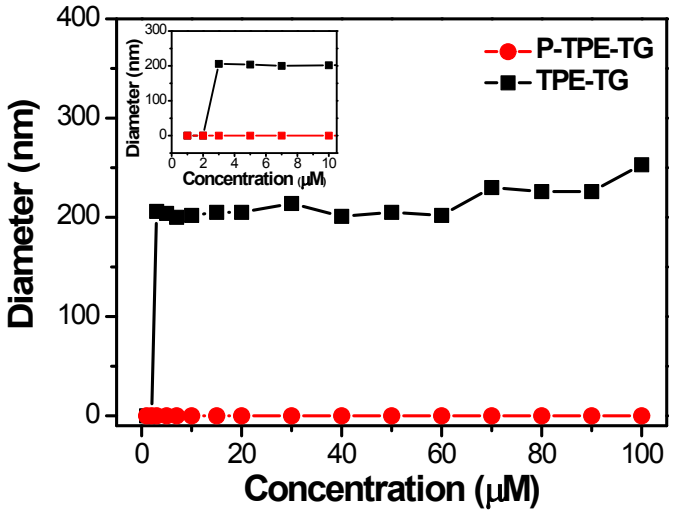


Fig. S2 hydrodynamic radii obtained from DLS of **P-TPE-TG** and **TPE-TG** with increasing concentration (1-100 μM) in 10 mM Tris-HCl buffer solution, pH 7.4.

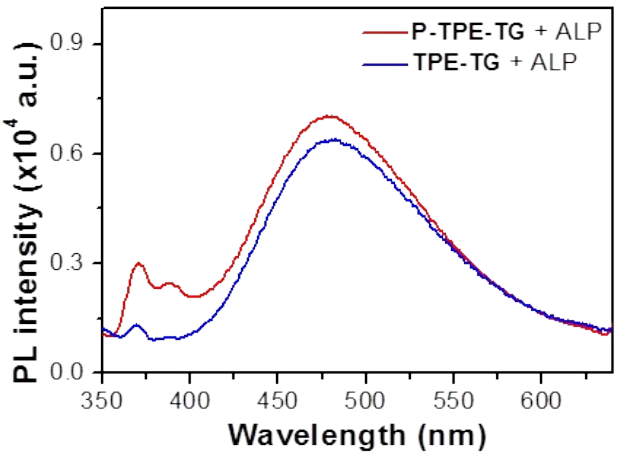


Fig. S3 Fluorescence emission spectra of **P-TPE-TG** (1.0×10^{-5} M) and **TPE-TG** (1.0×10^{-5} M) incubated with ALP (100 mU/mL) for 20 minutes in 10 mM Tris-HCl buffer solution, at 37 ^{o}C, pH 7.4. Excitation at 330 nm.

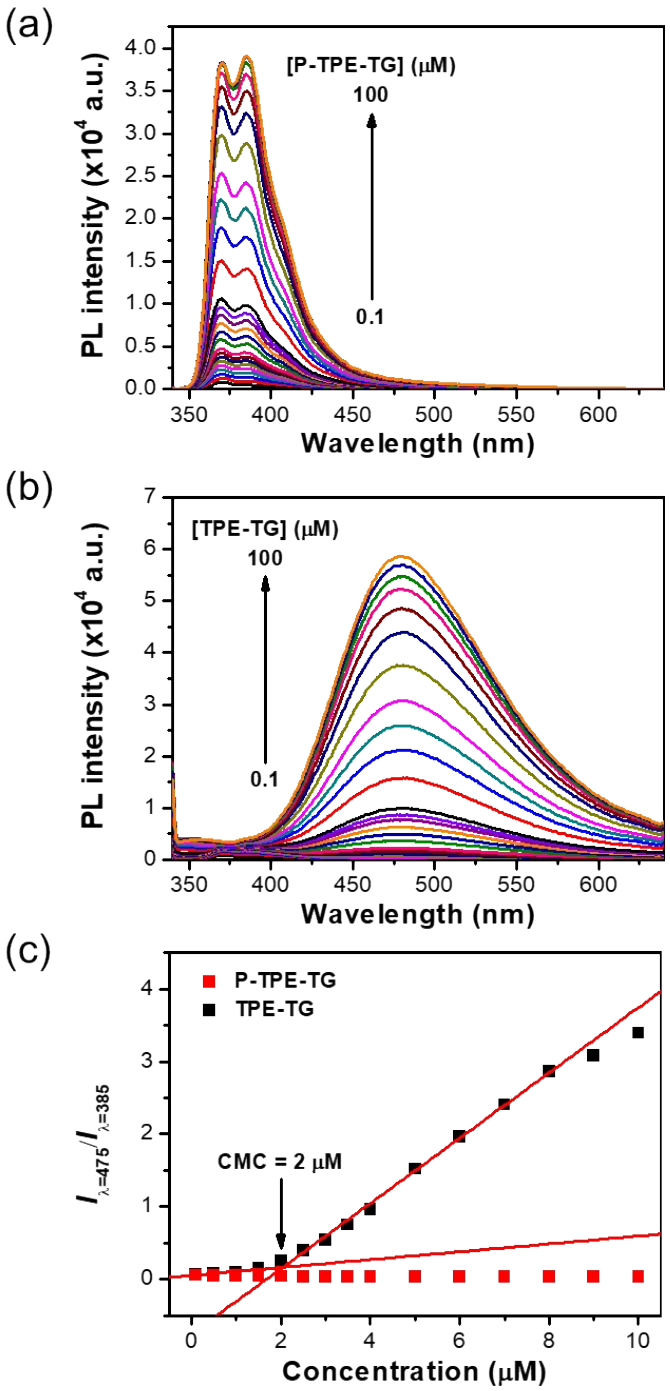


Fig. S4 Fluorescence intensity changes of (a) **P-TPE-TG** and (b) **TPE-TG** with increasing concentration (0.1-100 μM) in 10 mM Tris-HCl buffer solution, pH 7.4; (c) critical micelle concentration (CMC) determined by the change in fluorescence intensity as a function of the concentration.

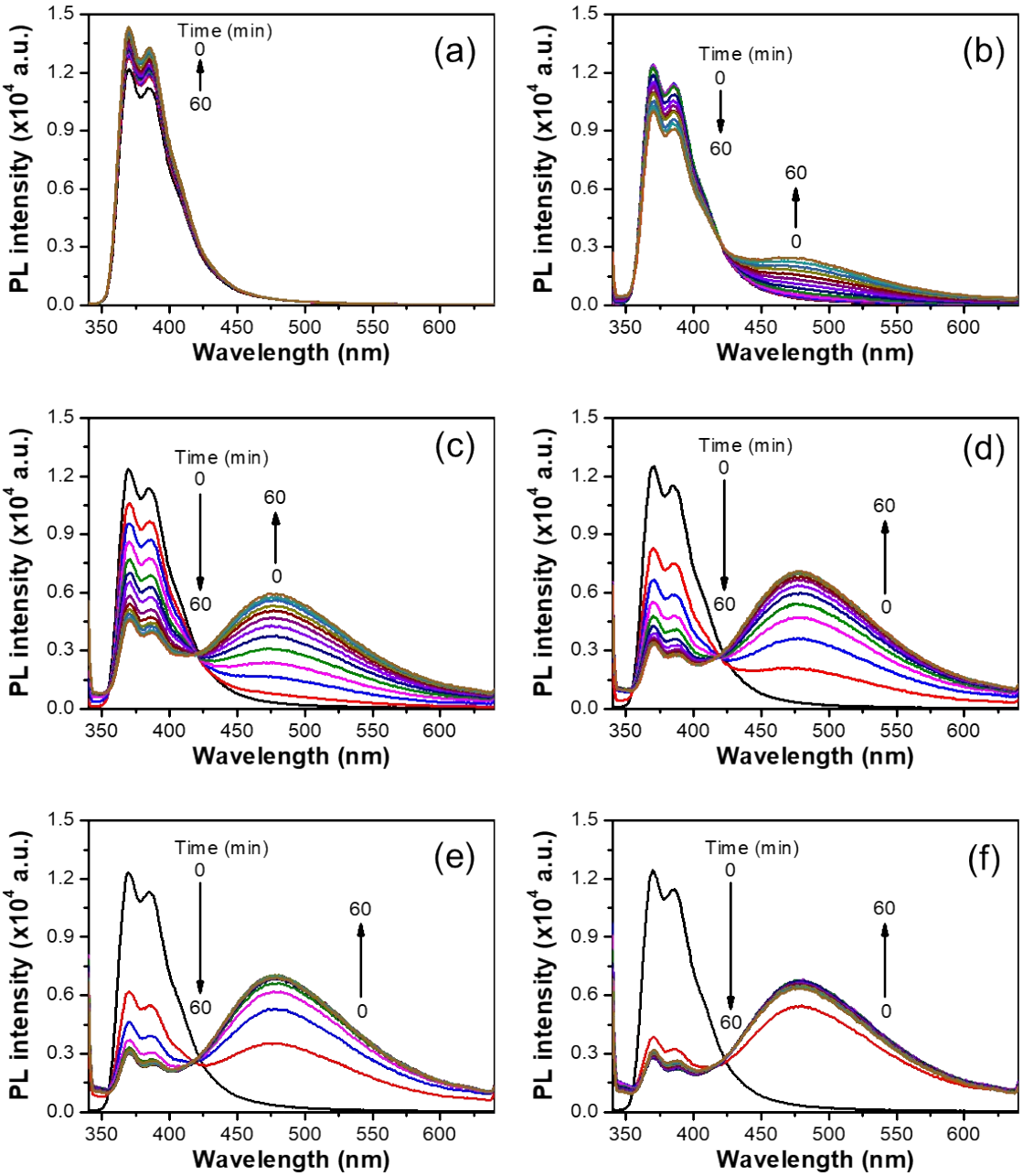


Fig. S5. Time-resolved fluorescence changes of **P-TPE-TG** (10×10^{-6} M) incubated with various concentrations of ALP (a: 0, b: 10, c: 25, d: 50, e: 100 and f: 200 mU/mL) at 37 ^{o}C in 10 mM Tris-HCl buffer solution, pH 7.4 for 60 min. Excitation at 330 nm.

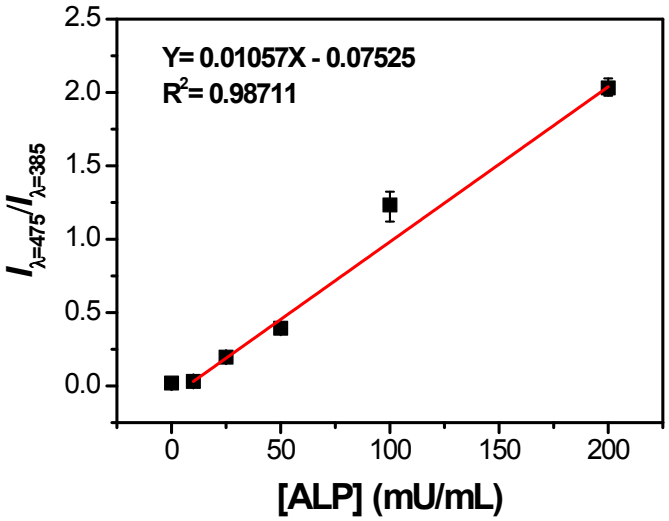


Fig. S6. Titration profile of **P-TPE-TG** (1.0×10^{-5} M) incubated with various concentrations of ALP for 10 min in 10 mM Tris-HCl buffer solution at 37 °C, pH 7.4. Excitation at 330 nm.

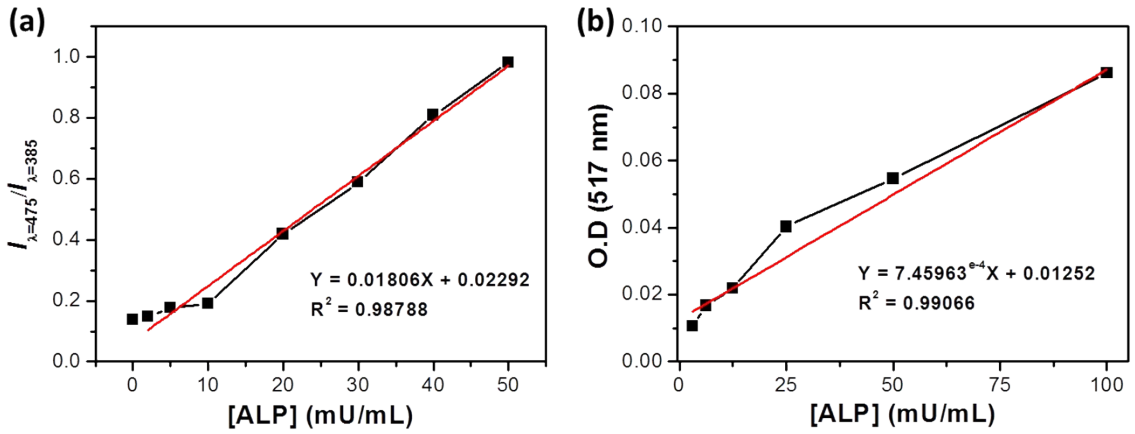
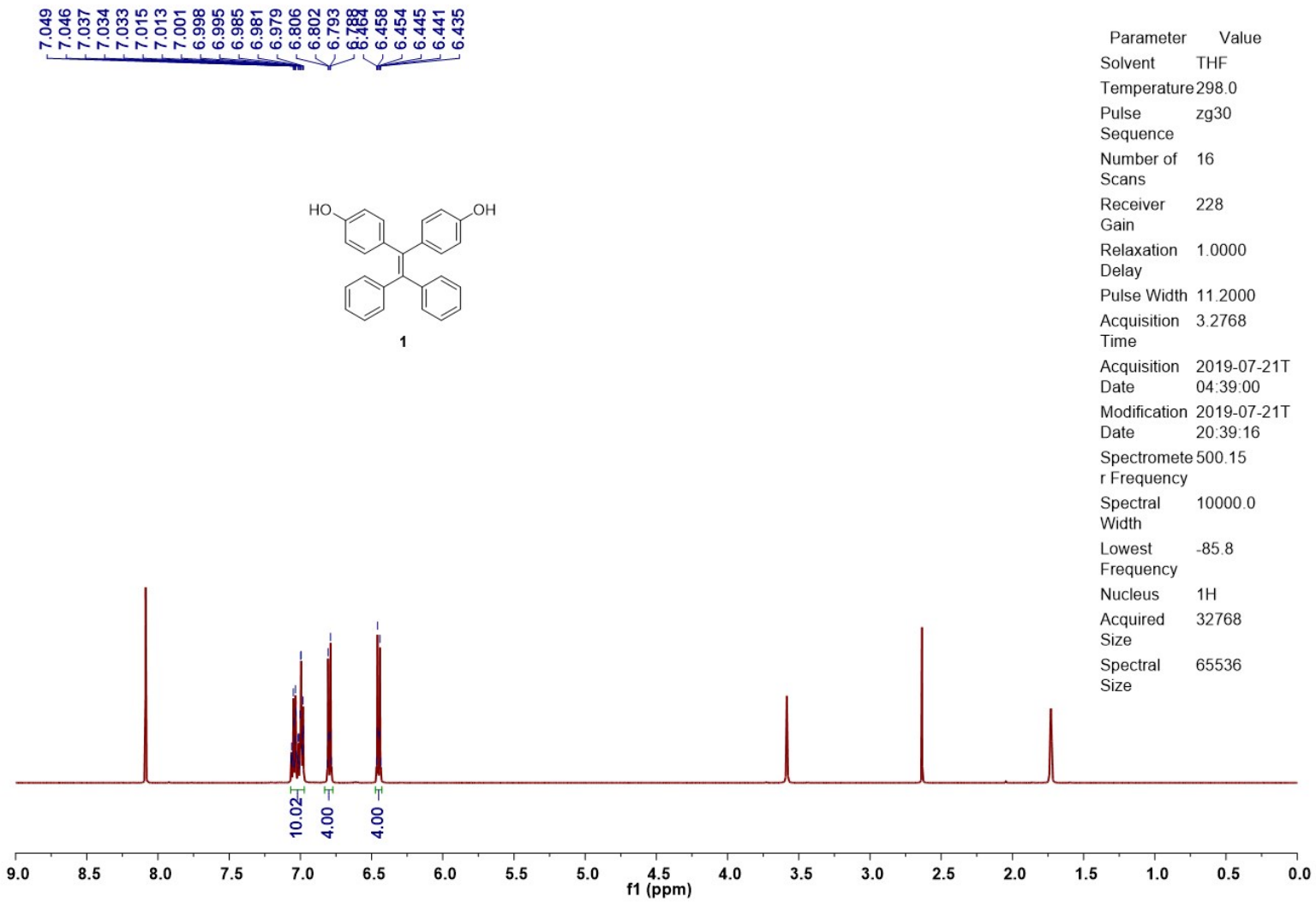


Fig. S7 Titration profiles and calibration plots of (a) **P-TPE-TG** (1.0×10^{-5} M) incubated with various concentrations of ALP for 60 min in diluted serum (10%) at 37 ^{o}C, pH 7.4 and (b) DPP incubated with various concentrations of ALP for 15 min in diluted serum (1.25%).

Table S1. Specification for normal human serum that was purchased from Sigma-Aldrich chemical company. Data was collected from Sigma-Aldrich chemical company sample information.

TEST	Specification
Appearance (Color)	Colorless to Brown-Yellow to Brown
Appearance (Form)	Liquid
pH	7.0 – 9.0
Iron (UG%)	40 – 100
Source: Male donors Within the United States	Conforms
Processing Country of Origin United States	Conforms
Osmolality Expressed in MOSM/KG H_2O	260 - 340
Sterility by USP Guidelines	Pass
Hemoglobin	≤ 25 mg/dl
Mycoplasma Test	None Detected
Endotoxin Level	≤ 10 EU/ml
Cholesterol	80 – 200 mg/dl
Triglyceride	30 – 175 mg/dl
Glucose	50 – 180 mg/dl
Sodium (Na)	100 – 160 MEQ/L
Protein Content	4.0 – 9.0 %
Tested For Infectious Agents	Tested

All donor units are collected in donor centers located in the United States, which are licensed by the FDA.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7.037
7.034
7.033
7.015
7.013
7.001
6.998
6.995
6.985
6.981
6.979
6.806
6.802
6.793
6.458
6.454
6.445
6.441
6.435
HO
OH
1
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Solvent THF
Temperature 298.0
Pulse Sequence zg30
Number of Scans 16
Receiver Gain 228
Relaxation Delay 1.0000
Pulse Width 11.2000
Acquisition Time 3.2768
Acquisition Date 2019-07-21T 04:39:00
Modification Date 2019-07-21T 20:39:16
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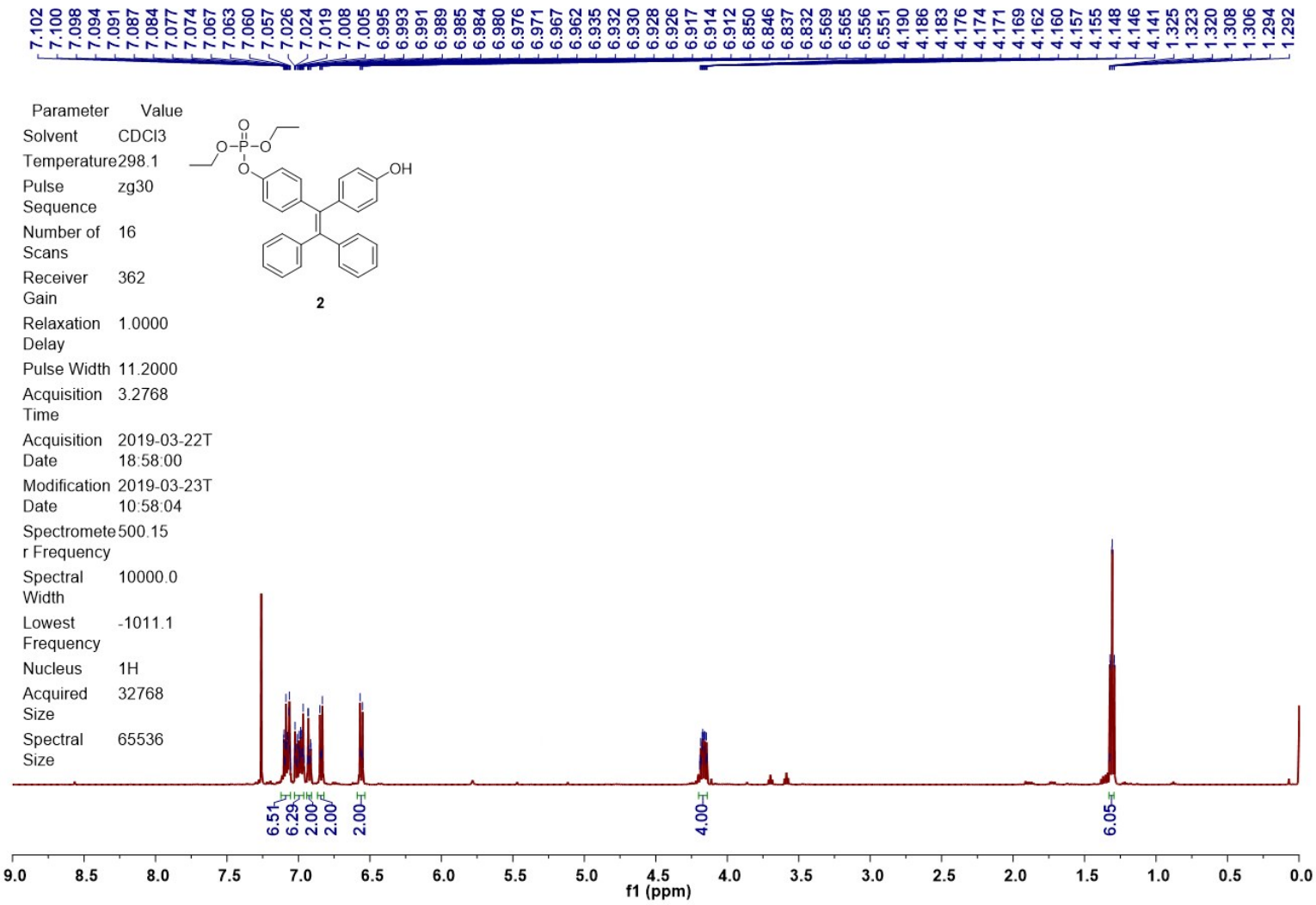
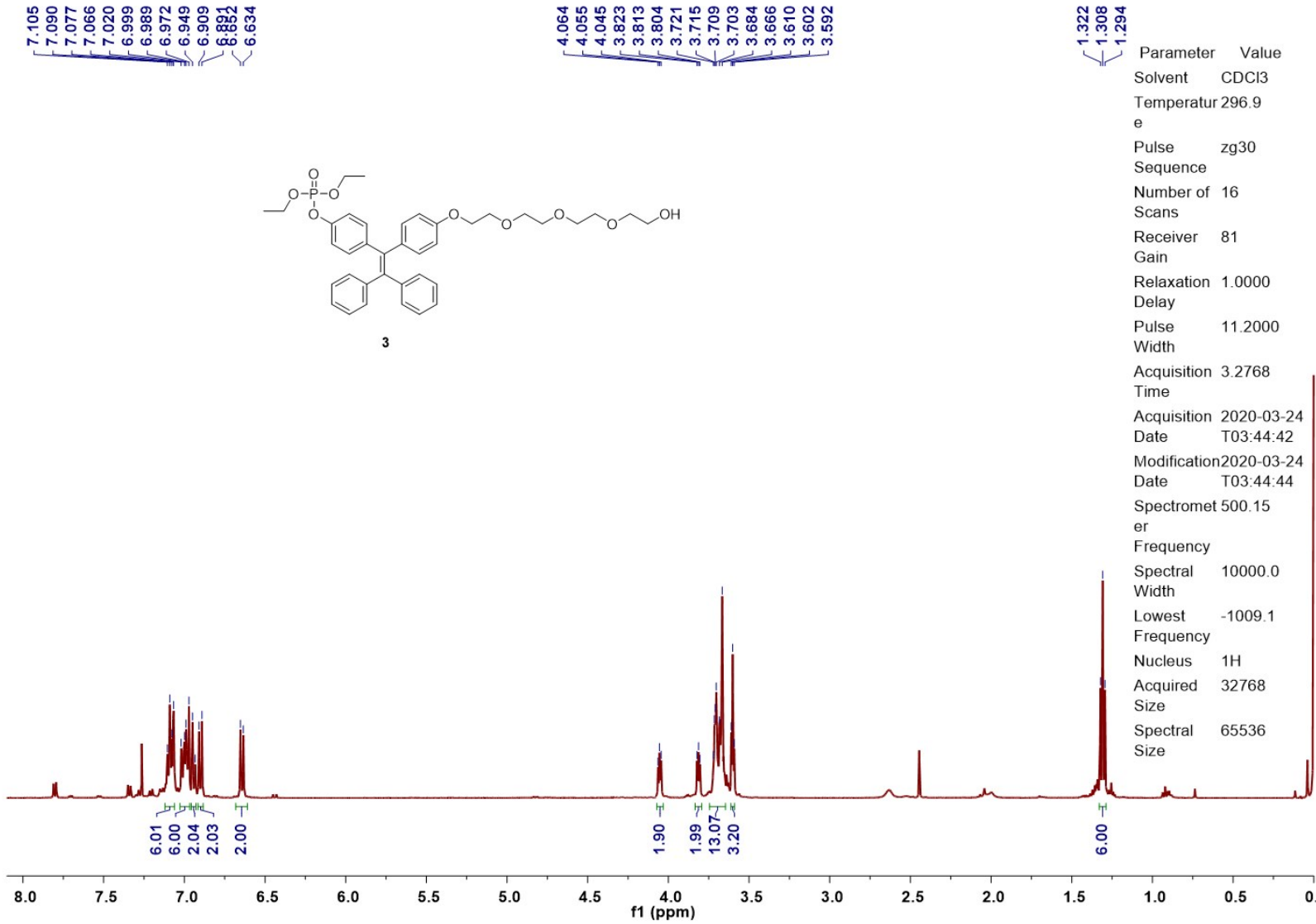
Spectral Width 10000.0
Lowest Frequency -85.8
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
10.02
4.00
4.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


7.102 7.100 7.098 7.094 7.091 7.087 7.084 7.077 7.074 7.067 7.063 7.060 7.057 7.026 7.024 7.019 7.008 7.005 6.995 6.993 6.991 6.989 6.985 6.984 6.980 6.976 6.971 6.967 6.962 6.935 6.932 6.930 6.928 6.926 6.917 6.914 6.912 6.850 6.846 6.837 6.832 6.569 6.565 6.556 6.551 4.190 4.186 4.183 4.176 4.174 4.171 4.169 4.162 4.160 4.157 4.155 4.148 4.146 4.141 1.325 1.323 1.320 1.308 1.306 1.294 1.292
Parameter Value
Solvent CDCl3
Temperature 298.1
Pulse Sequence zg30
Number of Scans 16
Receiver Gain 362
Relaxation Delay 1.0000
Pulse Width 11.2000
Acquisition Time 3.2768
Acquisition Date 2019-03-22T 18:58:00
Modification Date 2019-03-23T 10:58:04
Spectrometer Frequency 500.15
Spectral Width 10000.0
Lowest Frequency -1011.1
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
OH
2
6.51 6.29 2.00 2.00 2.00 4.00 6.05
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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7.090
7.077
7.066
7.020
6.999
6.989
6.972
6.949
6.909
6.634
4.064
4.055
4.045
3.823
3.813
3.804
3.721
3.715
3.709
3.703
3.684
3.666
3.610
3.602
3.592
1.322
1.308
1.294
3
Parameter Value
Solvent CDCl3
Temperature 296.9
Pulse Sequence zg30
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Relaxation Delay 1.0000
Pulse Width 11.2000
Acquisition Time 3.2768
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Modification Date 2020-03-24 T03:44:44
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Spectral Width 10000.0
Lowest Frequency -1009.1
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
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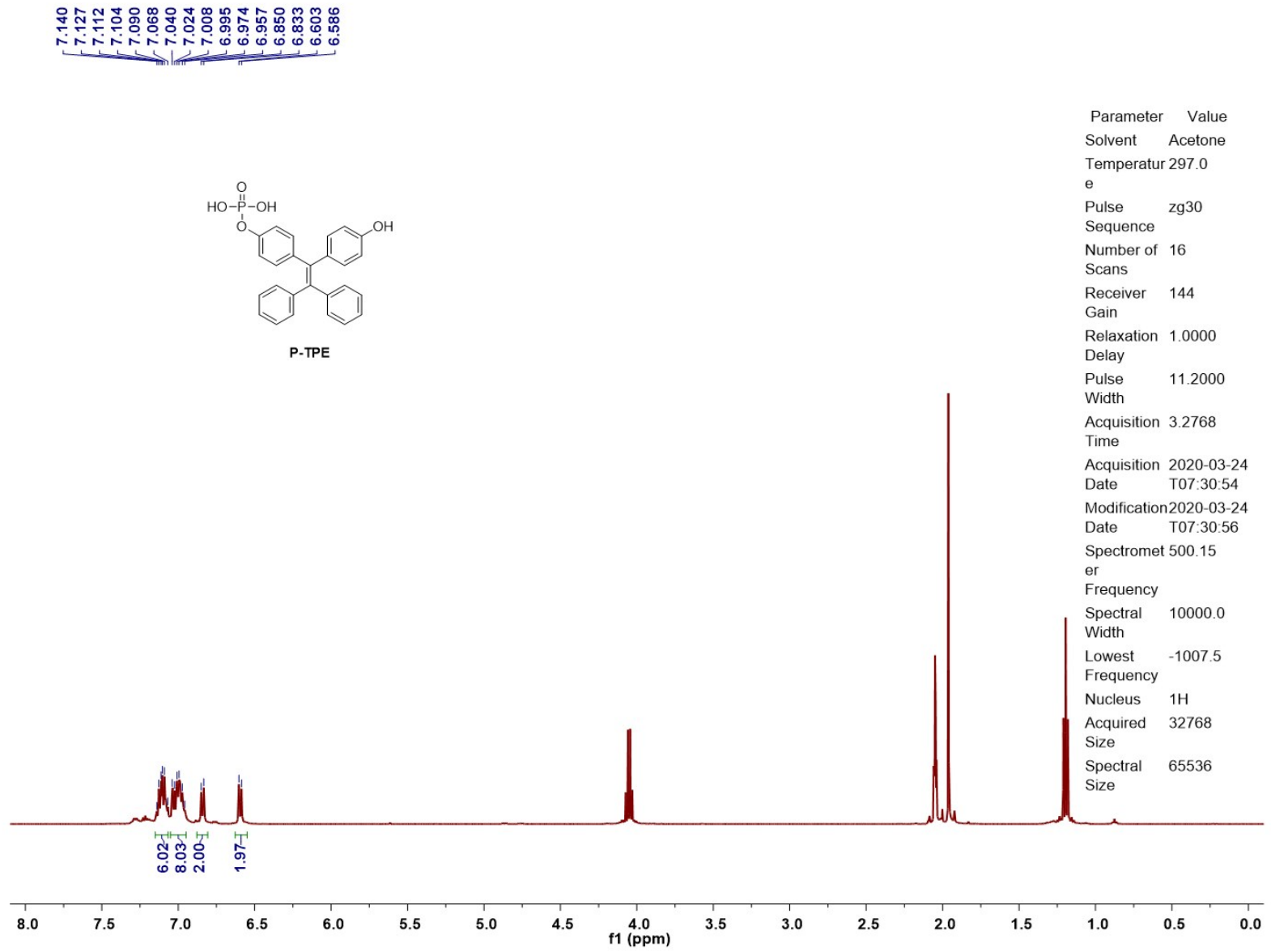
6.00
2.04
2.03
2.00
1.90
1.99
13.07
3.20
6.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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7.112
7.104
7.090
7.068
7.040
7.024
7.008
6.995
6.974
6.957
6.850
6.833
6.603
6.586
HO–P–OH
O
O
OH
P-TPE
Parameter Value
Solvent Acetone
Temperature 297.0
Pulse Sequence zg30
Number of Scans 16
Receiver Gain 144
Relaxation Delay 1.0000
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Modification Date 2020-03-24 T07:30:56
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Spectral Width 10000.0
Lowest Frequency -1007.5
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
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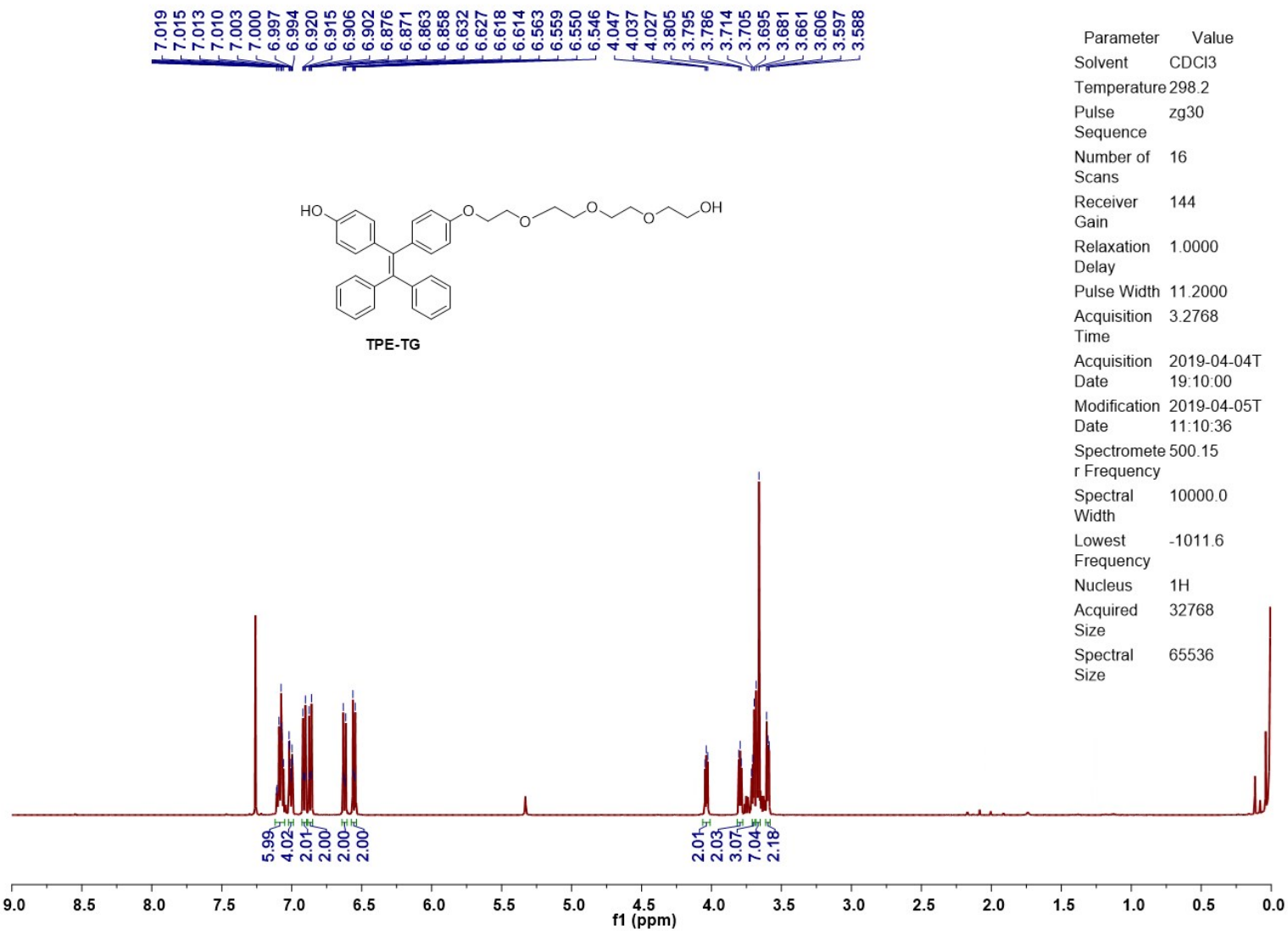
8.03
2.00
1.97
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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7.010
7.003
7.000
6.997
6.994
6.920
6.915
6.906
6.902
6.876
6.871
6.863
6.858
6.632
6.627
6.618
6.614
6.563
6.559
6.550
6.546
4.047
4.037
4.027
3.805
3.795
3.786
3.714
3.705
3.695
3.681
3.661
3.606
3.597
3.588
HO
O
O
O
O
OH
TPE-TG
Parameter Value
Solvent CDCl3
Temperature 298.2
Pulse Sequence zg30
Number of Scans 16
Receiver Gain 144
Relaxation Delay 1.0000
Pulse Width 11.2000
Acquisition Time 3.2768
Acquisition Date 2019-04-04T 19:10:00
Modification Date 2019-04-05T 11:10:36
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Spectral Width 10000.0
Lowest Frequency -1011.6
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
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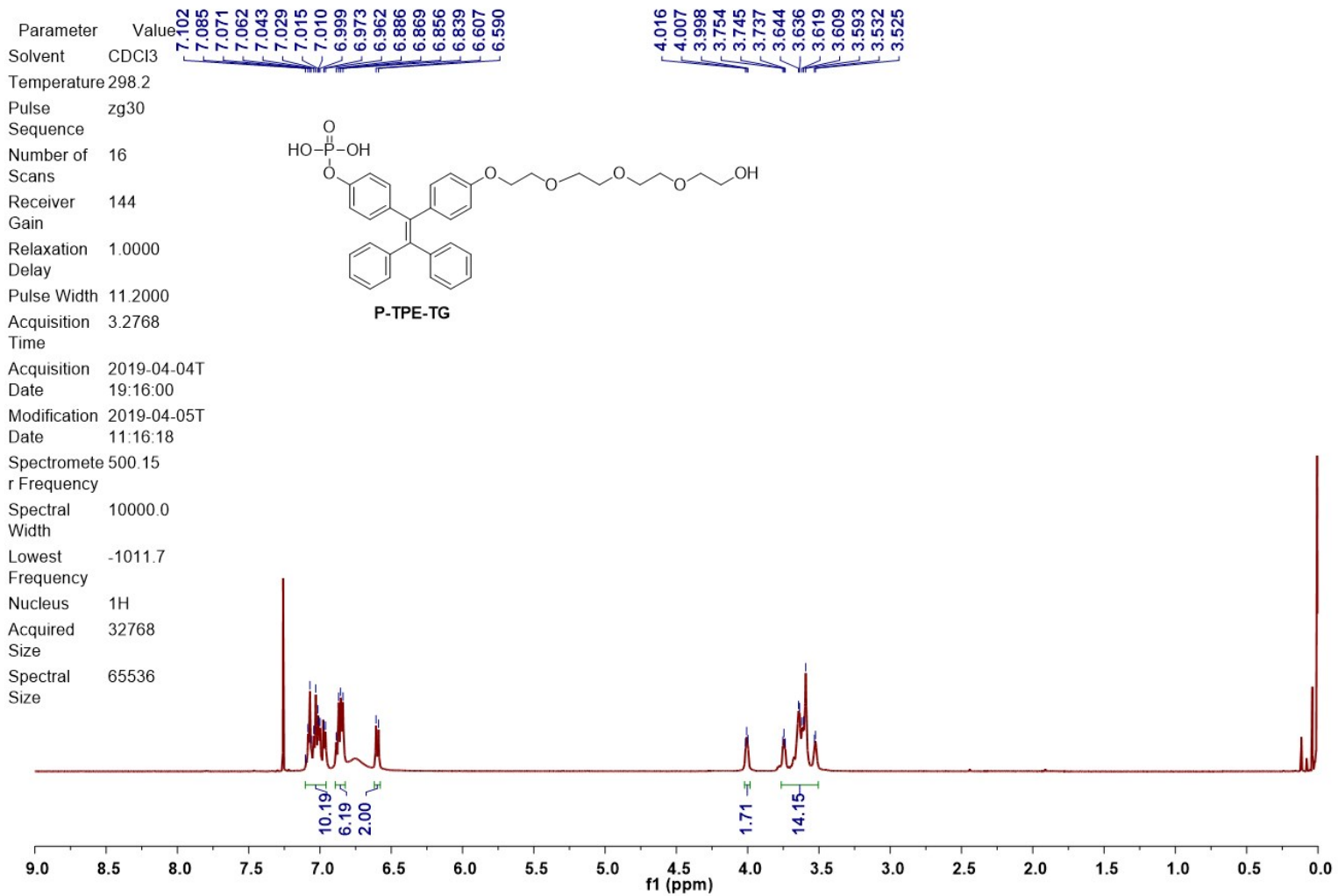
4.02
2.01
2.00
2.00
2.00
2.01
2.03
3.07
7.04
2.18
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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3.754
3.745
3.737
3.644
3.636
3.619
3.609
3.593
3.532
3.525
Parameter Value
Solvent CDCl3
Temperature 298.2
Pulse Sequence zg30
Number of Scans 16
Receiver Gain 144
Relaxation Delay 1.0000
Pulse Width 11.2000
Acquisition Time 3.2768
Acquisition Date 2019-04-04T 19:16:00
Modification Date 2019-04-05T 11:16:18
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Lowest Frequency -1011.7
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
HO–P–OH
O
O
OH
P-TPE-TG
10.19
6.19
2.00
1.71
14.15
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Parameter	Value
Solvent	THF
Temperature	298.8
Pulse Sequence	zgpg30
Number of Scans	2048
Receiver Gain	2050
Relaxation Delay	2.0000
Pulse Width	8.5000
Acquisition Time	1.1010
Acquisition Date	2019-07-21T 06:29:00
Modification Date	2019-07-21T 22:29:54
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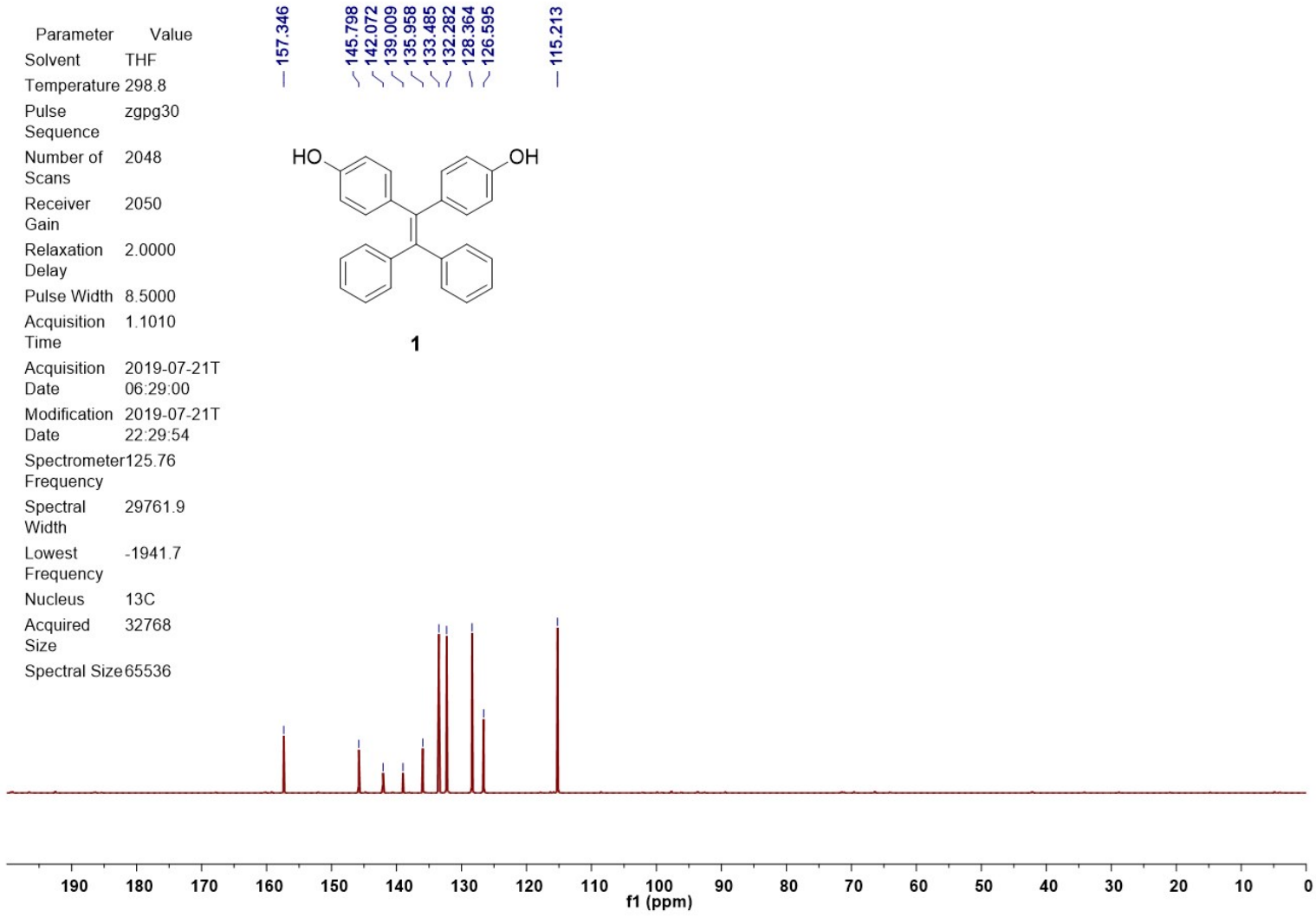
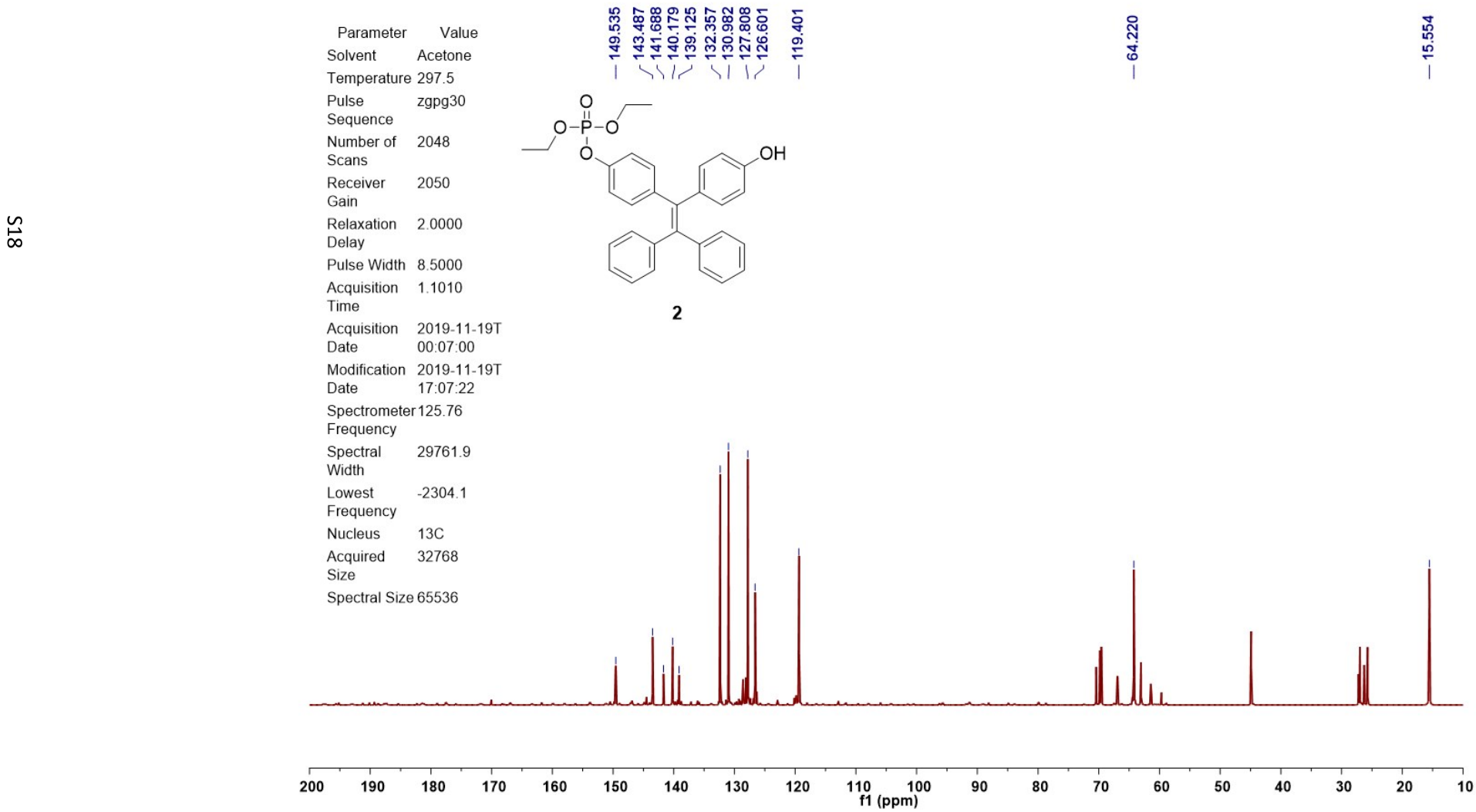
Spectral Width	29761.9
Lowest Frequency	-1941.7
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

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Solvent Acetone
Temperature 297.5
Pulse Sequence zgpg30
Number of Scans 2048
Receiver Gain 2050
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Acquisition Date 2019-11-19T 00:07:00
Modification Date 2019-11-19T 17:07:22
Spectrometer Frequency 125.76
Spectral Width 29761.9
Lowest Frequency -2304.1
Nucleus 13C
Acquired Size 32768
Spectral Size 65536
149.535
143.487
141.688
140.179
139.125
132.357
130.982
127.808
126.601
119.401
64.220
15.554
O
O
P
O
O
O
OH
2
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

Parameter	Value
Solvent	CDCl3
Temperature	299.6
Pulse Sequence	zgpg30
Number of Scans	6144
Receiver Gain	2050
Relaxation Delay	2.0000
Pulse Width	8.5000
Acquisition Time	1.1010
Acquisition Date	2019-07-27T 02:39:00
Modification Date	2019-07-27T 18:39:40
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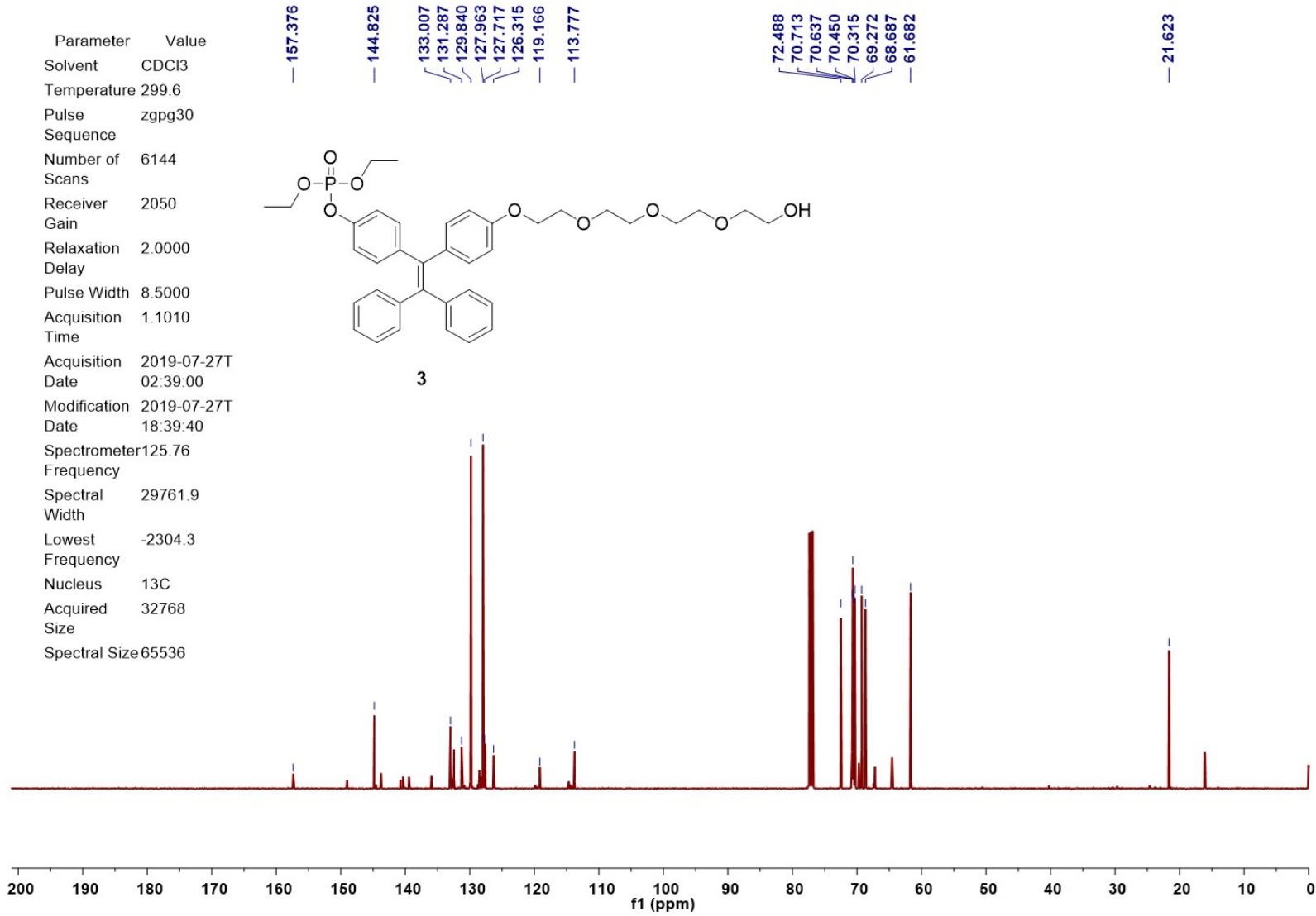
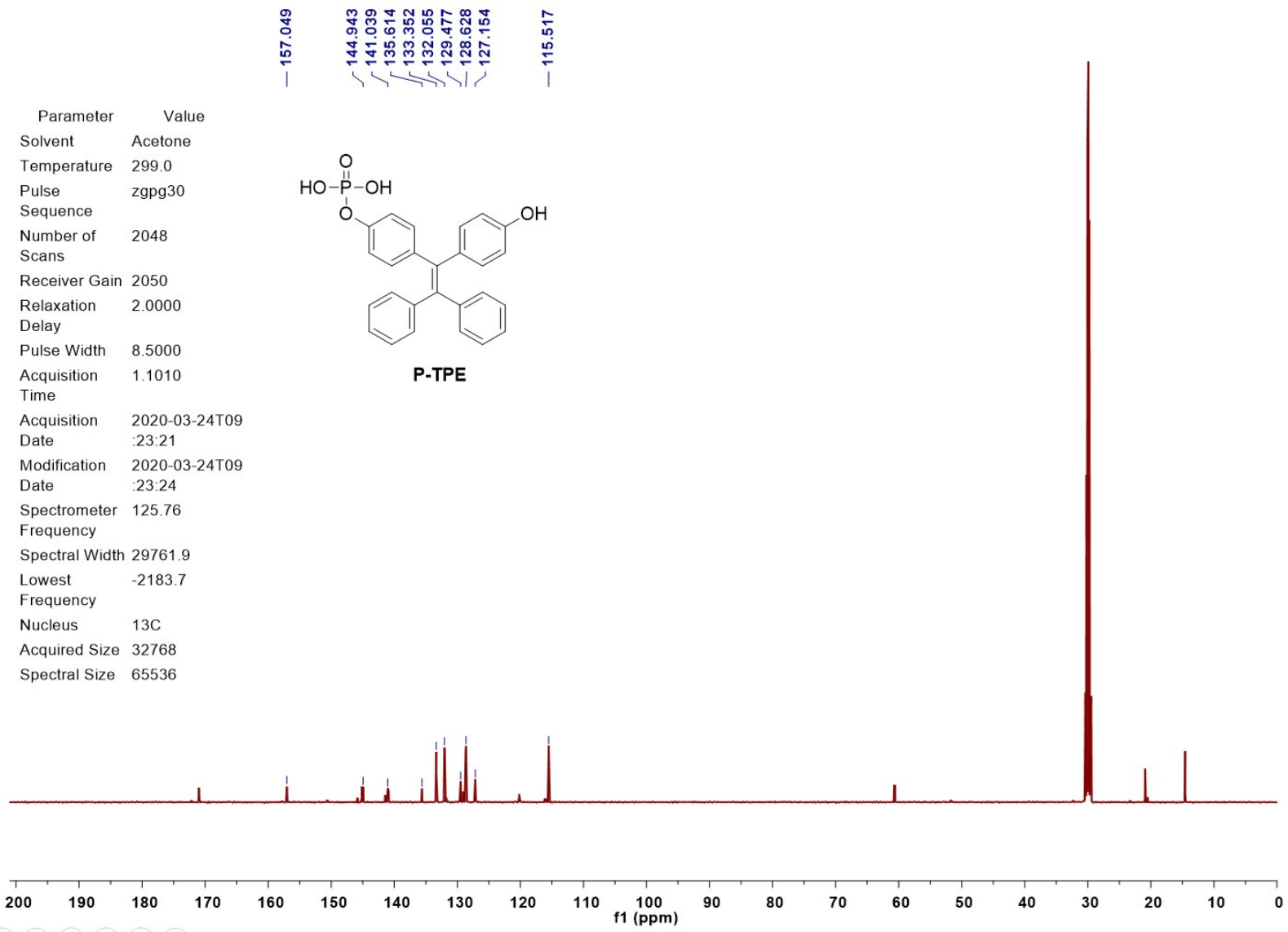
Spectral Width	29761.9
Lowest Frequency	-2304.3
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

3





Parameter	Value
Solvent	Acetone
Temperature	299.0
Pulse Sequence	zgpg30
Number of Scans	2048
Receiver Gain	2050
Relaxation Delay	2.0000
Pulse Width	8.5000
Acquisition Time	1.1010
Acquisition Date	2020-03-24T09:23:21
Modification Date	2020-03-24T09:23:24
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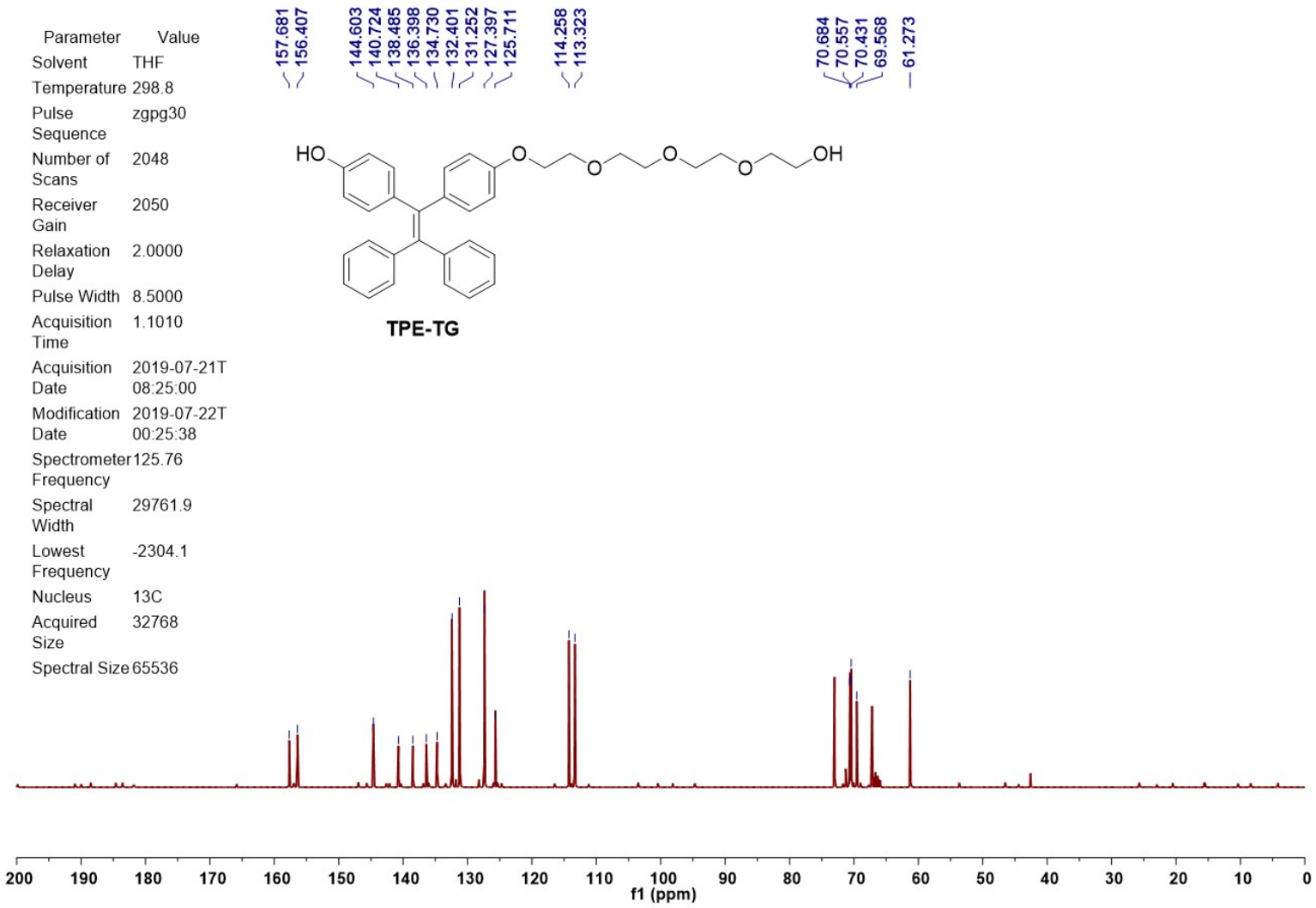
Spectral Width	29761.9
Lowest Frequency	-2183.7
Nucleus	13C
Acquired Size	32768
Spectral Size	65536


157.681
156.407
144.603
140.724
138.485
136.398
134.730
132.401
131.252
127.397
125.711
114.258
113.323
70.684
70.557
70.431
69.568
61.273
Parameter Value
Solvent THF
Temperature 298.8
Pulse Sequence zgpg30
Number of Scans 2048
Receiver Gain 2050
Relaxation Delay 2.0000
Pulse Width 8.5000
Acquisition Time 1.1010
Acquisition Date 2019-07-21T 08:25:00
Modification Date 2019-07-22T 00:25:38
Spectrometer Frequency 125.76
Spectral Width 29761.9
Lowest Frequency -2304.1
Nucleus 13C
Acquired Size 32768
Spectral Size 65536
HO
O
O
O
O
OH
TPE-TG
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

Parameter	Value
Solvent	CDCl3
Temperature	299.6
Pulse Sequence	zgpg30
Number of Scans	6144
Receiver Gain	2050
Relaxation Delay	2.0000
Pulse Width	8.5000
Acquisition Time	1.1010
Acquisition Date	2019-07-27T 02:39:00
Modification Date	2019-07-27T 18:39:40
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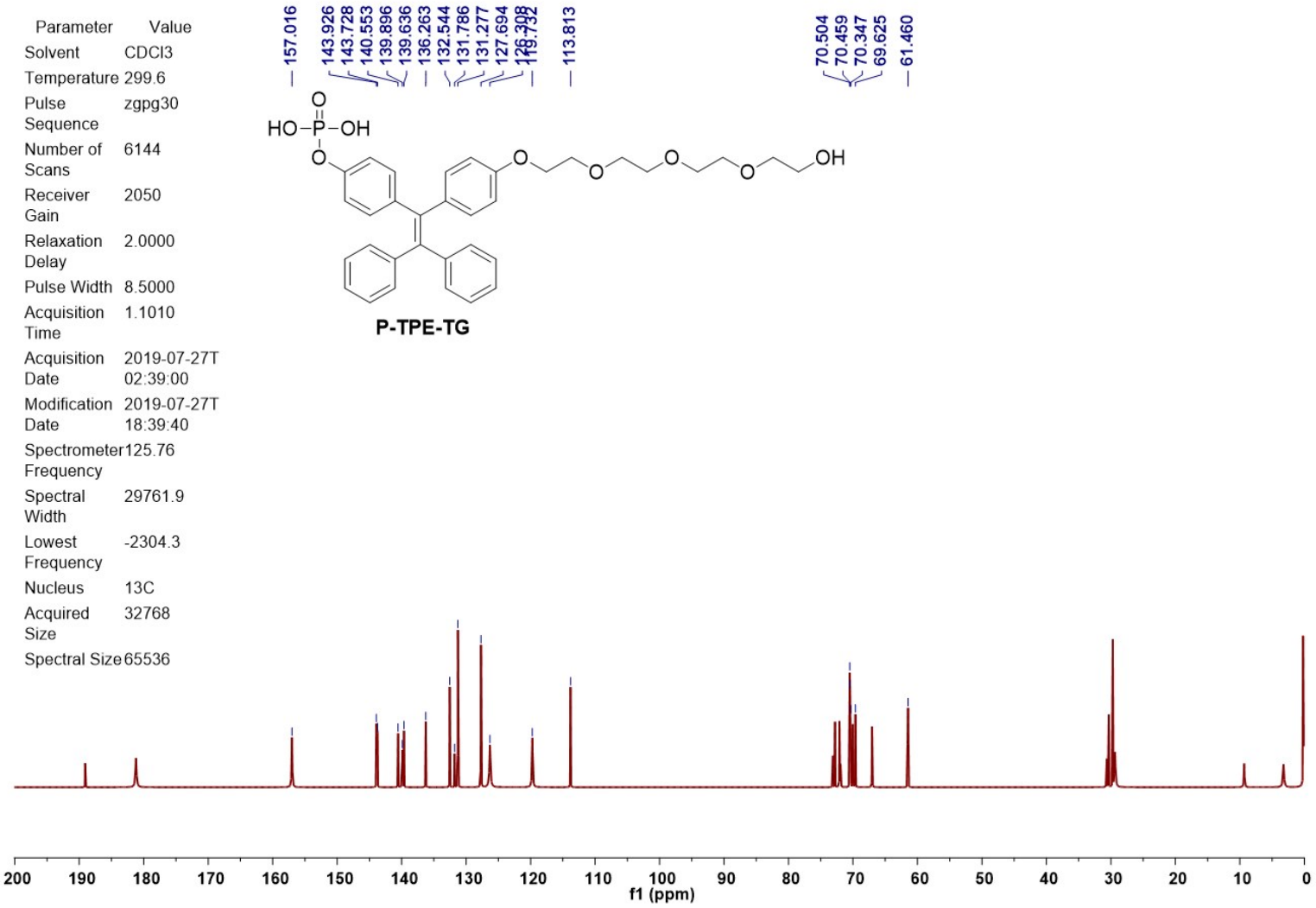
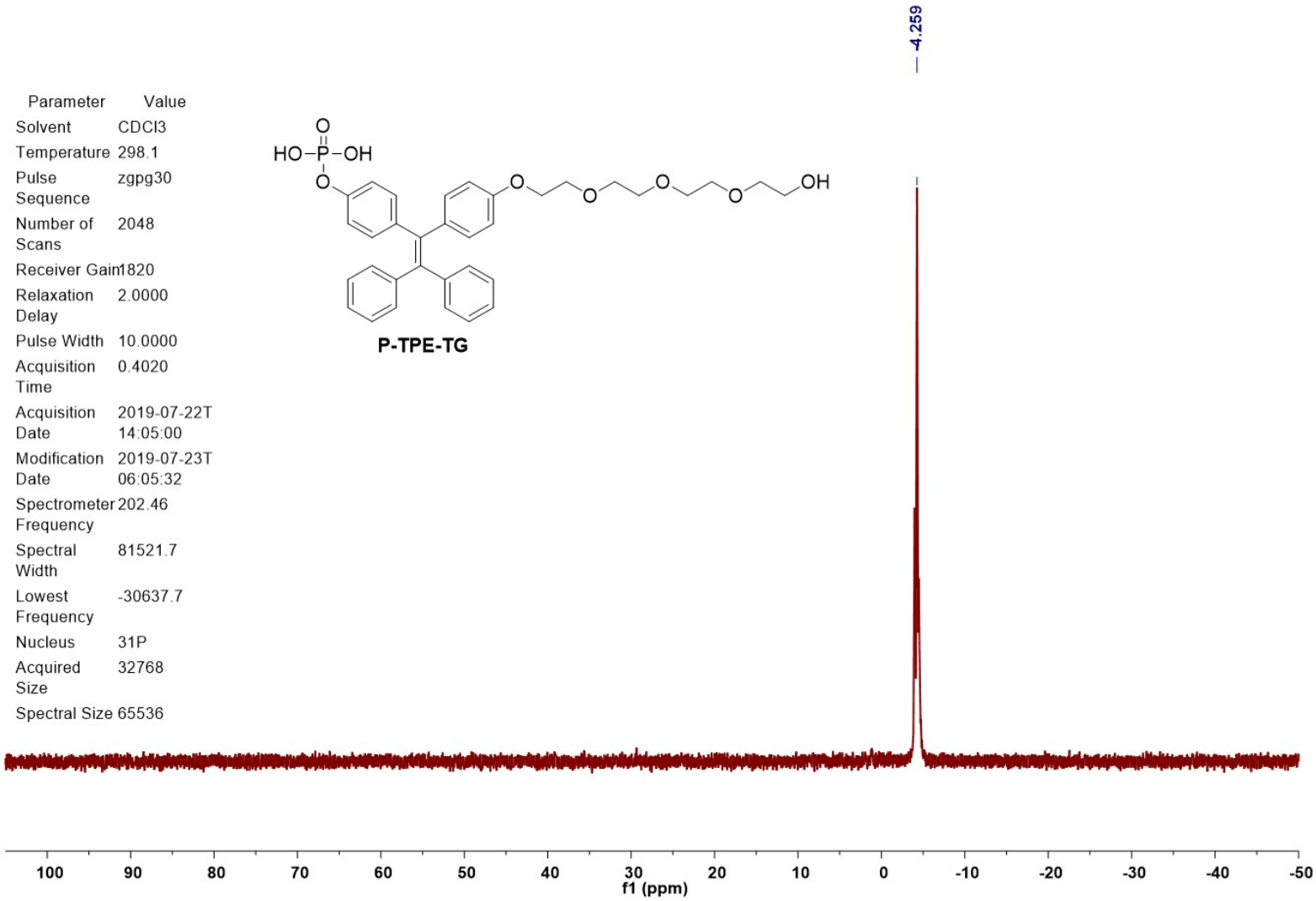
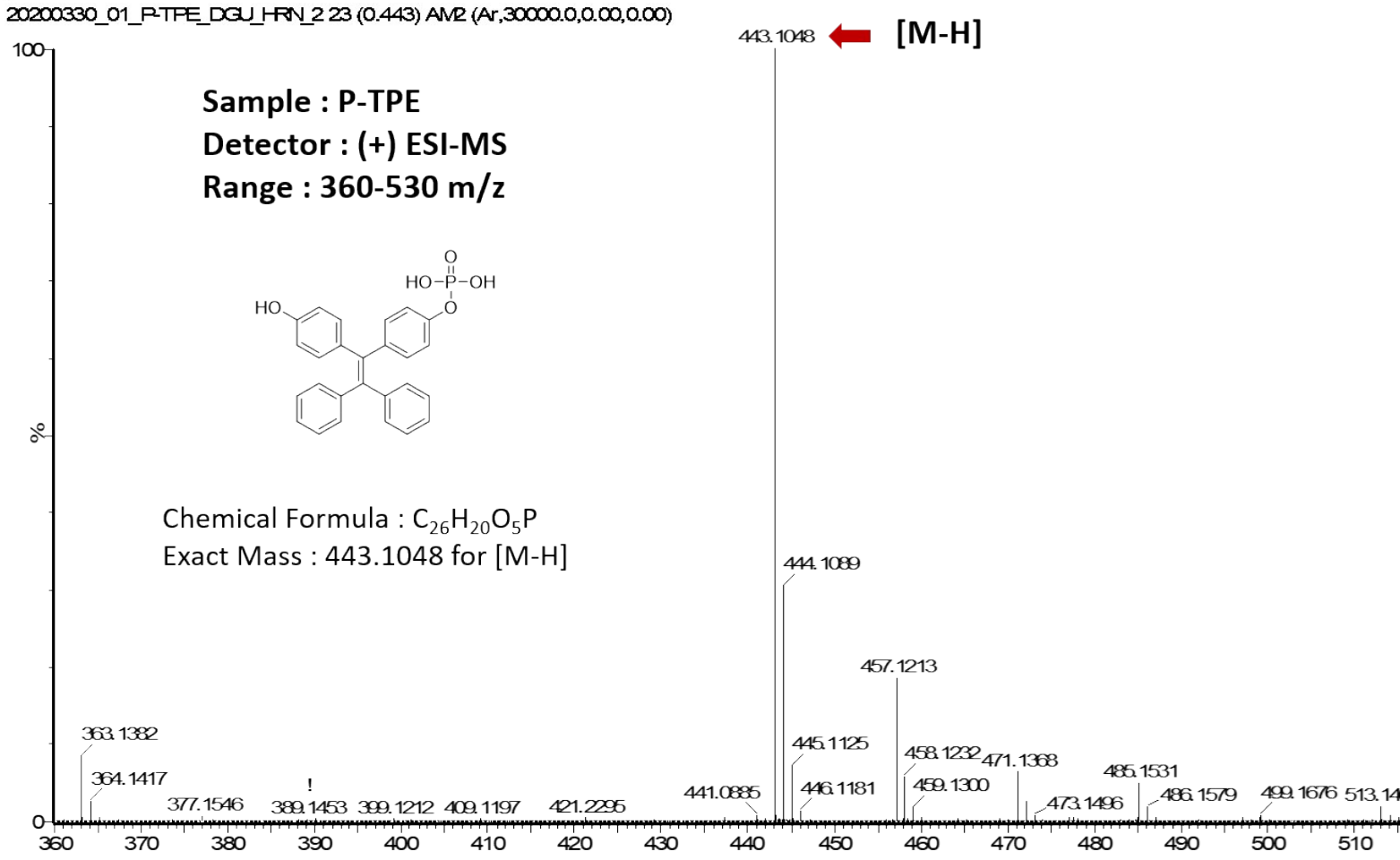
Spectral Width	29761.9
Lowest Frequency	-2304.3
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

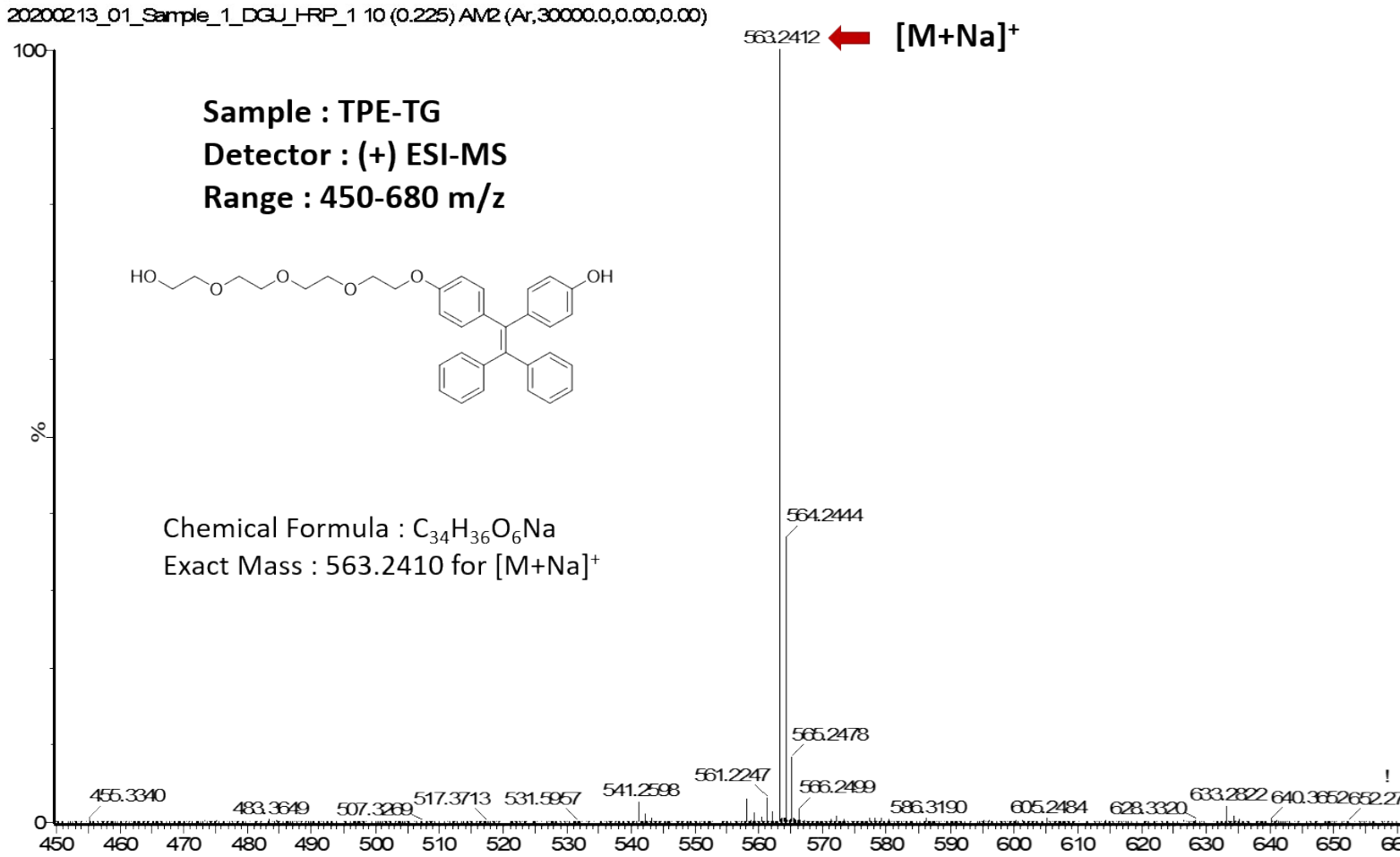


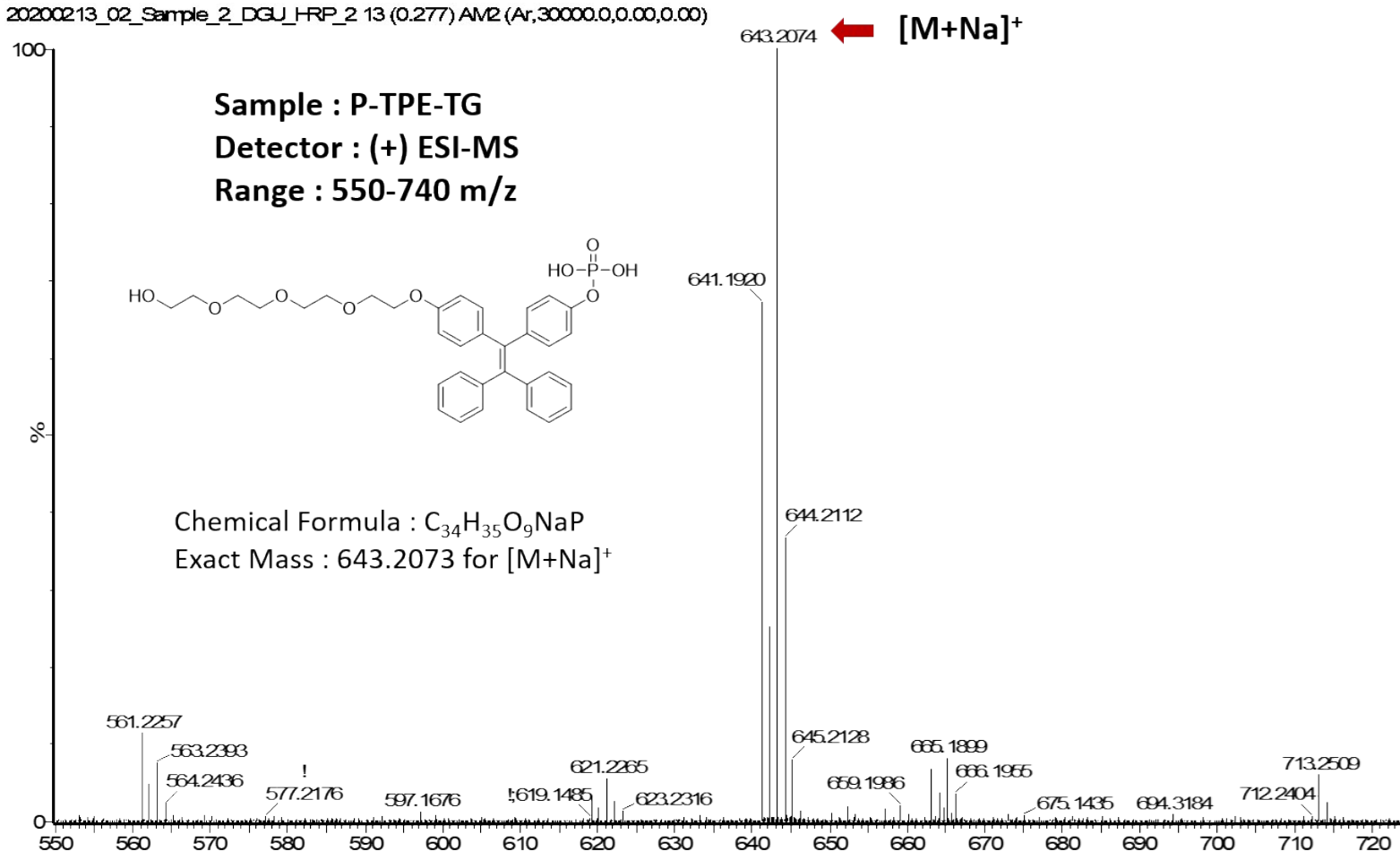
Parameter	Value
Solvent	CDCl3
Temperature	298.1
Pulse Sequence	zgpg30
Number of Scans	2048
Receiver Gain	1820
Relaxation Delay	2.0000
Pulse Width	10.0000
Acquisition Time	0.4020
Acquisition Date	2019-07-22T 14:05:00
Modification Date	2019-07-23T 06:05:32
Spectrometer Frequency	202.46
Spectral Width	81521.7
Lowest Frequency	-30637.7
Nucleus	31P
Acquired Size	32768
Spectral Size	65536

P-TPE-TG




20200330_01_P-TPE_DGU_HRN_2 23 (0.443) AM2 (Ar,30000.0,0.00,0.00)
443.1048
[M-H]
Sample : P-TPE
Detector : (+) ESI-MS
Range : 360-530 m/z
HO
HO–P–OH
O
Chemical Formula : C26H20O5P
Exact Mass : 443.1048 for [M-H]
444.1089
457.1213
363.1382
364.1417
445.1125
458.1232
471.1368
485.1531
459.1300
446.1181
441.0885
377.1546
389.1453
399.1212
409.1197
421.2295
473.1496
486.1579
499.1676
513.14
100
%
0
360
370
380
390
400
410
420
430
440
450
460
470
480
490
500
510


20200213_01_Sample_1_DGU_HRP_1 10 (0.225) AM2 (Ar,30000.0,0.00,0.00)
563.2412
[M+Na]+
100
%
0
Sample : TPE-TG
Detector : (+) ESI-MS
Range : 450-680 m/z
HO
O
O
O
O
OH
Chemical Formula : C34H36O6Na
Exact Mass : 563.2410 for [M+Na]+
564.2444
565.2478
561.2247
566.2499
455.3340
483.3649
507.3269
517.3713
531.5957
541.2598
586.3190
605.2484
628.3320
633.2822
640.3652
652.27
450 460 470 480 490 500 510 520 530 540 550 560 570 580 590 600 610 620 630 640 650


20200213_02_Sample_2_DGU_HRP_2 13 (0.277) AM2 (Ar,30000.0,0.00,0.00)
643.2074
[M+Na]+
Sample : P-TPE-TG
Detector : (+) ESI-MS
Range : 550-740 m/z
641.1920
644.2112
Chemical Formula : C34H35O9NaP
Exact Mass : 643.2073 for [M+Na]+
561.2257
563.2393
564.2436
577.2176
597.1676
619.1485
621.2265
623.2316
645.2128
659.1986
665.1899
666.1955
675.1435
694.3184
712.2404
713.2509
%
100
0
550
560
570
580
590
600
610
620
630
640
650
660
670
680
690
700
710
720