Supplementary Information

Synthesis of terminal ribose analogues of adenosine 5'-diphosphate ribose (ADPR) as probes for the Transient Receptor Potential (TRP) cation channel TRPM2

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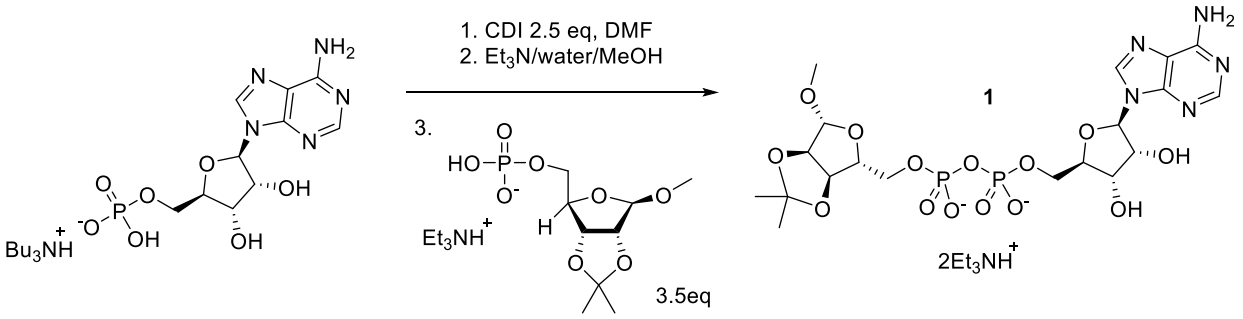
§ = Equal contribution, ¶= Equal contribution

Contents:

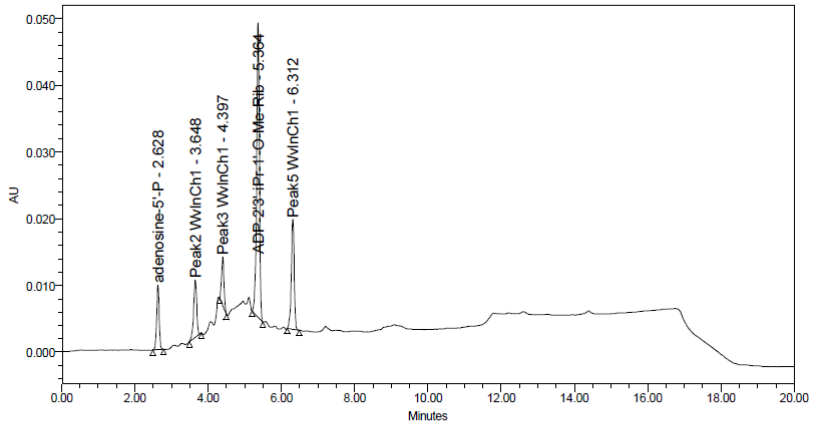
Supplementary Figure 1: Testing of suitable coupling procedure for pyrophosphate bond formation.[1]

a)



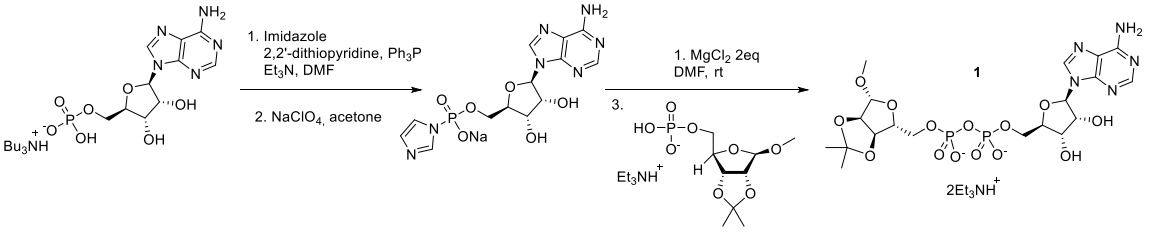
Example of HPLC analysis of the coupling reaction (Peak 4 = product – compound **1**)

SAMPLE INFORMATION			
Sample Name:	obb-57-own	Acquired By:	Joanna
Sample Type:	Unknown	Date Acquired:	09/02/2016 11:46:59 AM
Vial:	29	Acq. Method Set:	RP18 LC
Injection #:	1	Date Processed:	25/02/2016 9:57:54 AM
Injection Volume:	10.00 ul	Processing Method:	test protected ADPR
Run Time:	20.0 Minutes	Channel Name:	WvlnCh1
Sample Set Name:	2016_02_08_obb57own	Proc. Chnl. Descr.:	PDA 259.1 nm



	Peak Name	RT	Area	% Area	Height
1	adenosine-5'-P	2.628	46143	10.11	9658
2	Peak2 WvlnCh1	3.648	50166	10.99	8606
3	Peak3 WvlnCh1	4.397	34789	7.62	7294
4	ADP-2'3'-iPr-1'-O-Me-Rib	5.364	235493	51.59	44239
5	Peak5 WvlnCh1	6.312	89866	19.69	16515

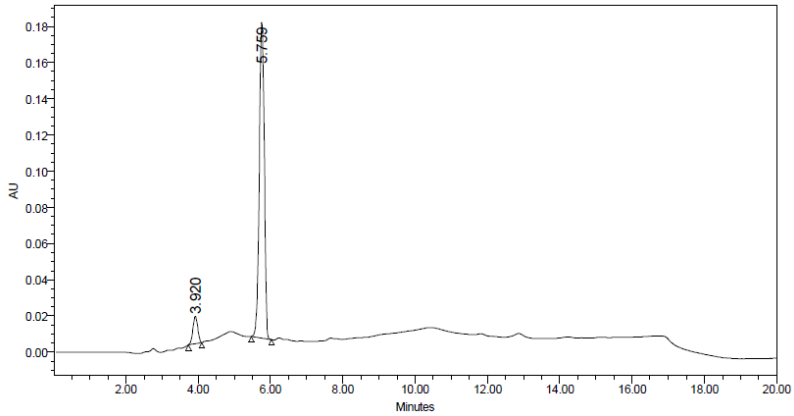
b) Dabrowski-Tumanski procedure[2]



Dabrowski-Tumanski procedure[2]: An example of HPLC analysis of the coupling reaction (Peak 4 = product – compound **1**)

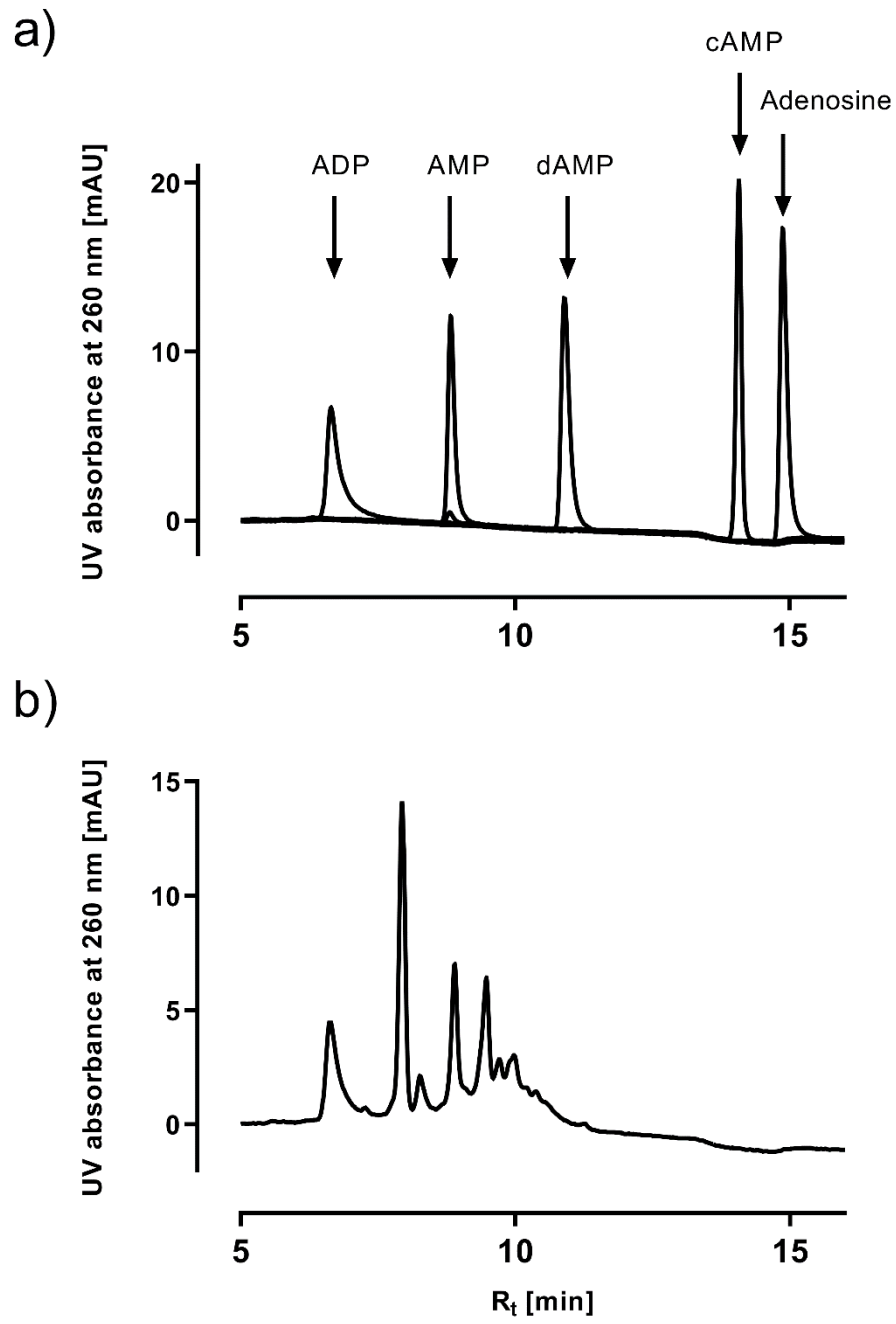
SAMPLE INFORMATION

Sample Name:	2016_02_24_obb59-own2	Acquired By:	Joanna
Sample Type:	Unknown	Date Acquired:	24/02/2016 4:47:39 PM
Vial:	63	Acq. Method Set:	RP18 LC
Injection #:	1	Date Processed:	25/02/2016 10:08:17 AM
Injection Volume:	10.00 ul	Processing Method:	pyr coup from imidazolide
Run Time:	20.0 Minutes	Channel Name:	WvlnCh1
Sample Set Name:	2016_02_24_obb59_test	Proc. Chnl. Descr.:	PDA 259.1 nm



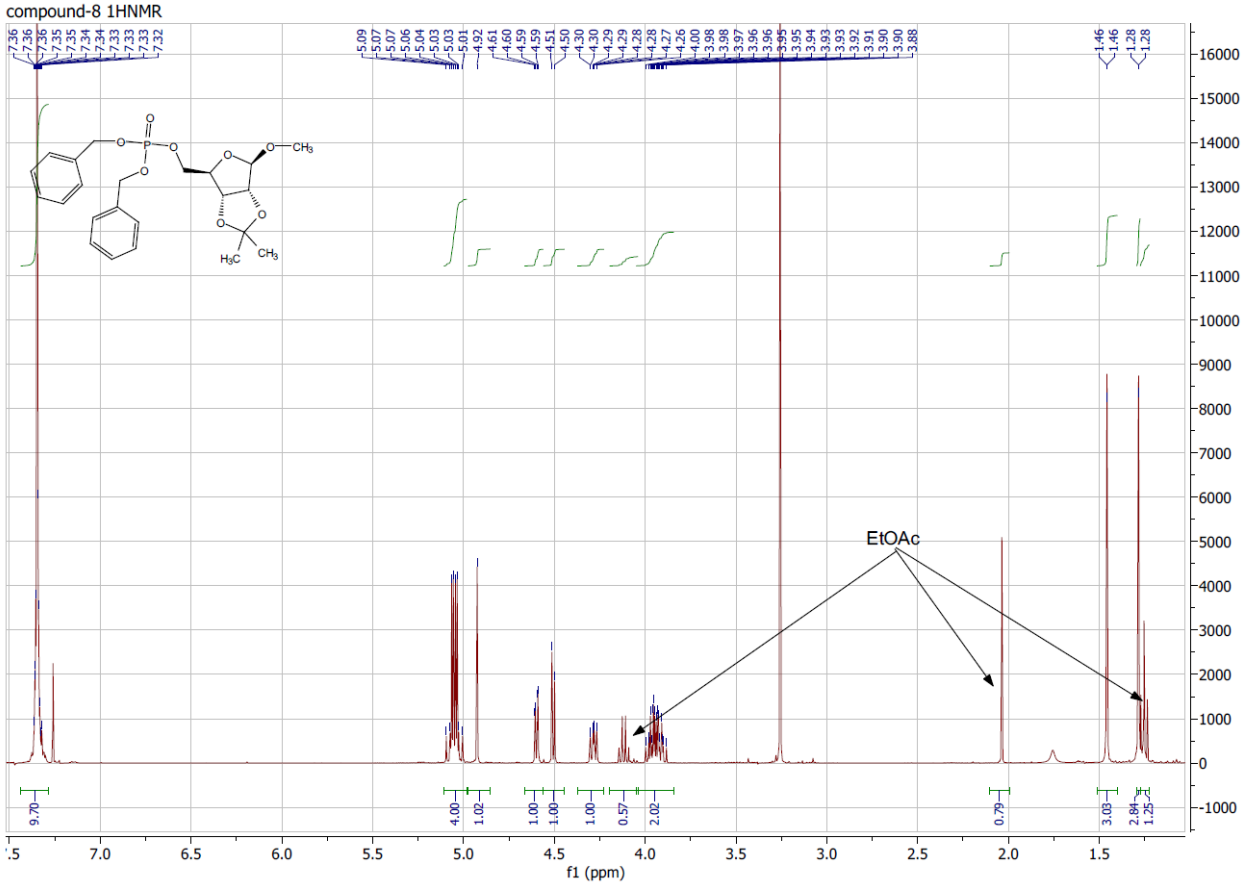
	Peak Name	RT	Area	% Area	Height
1		3.920	139613	7.63	15110
2	adenosine-5'-P-imidazolide	4.226			
3		5.759	1690715	92.37	174335
4	ADP-2'3'-iPr-1'O-Me-Rib	6.401			

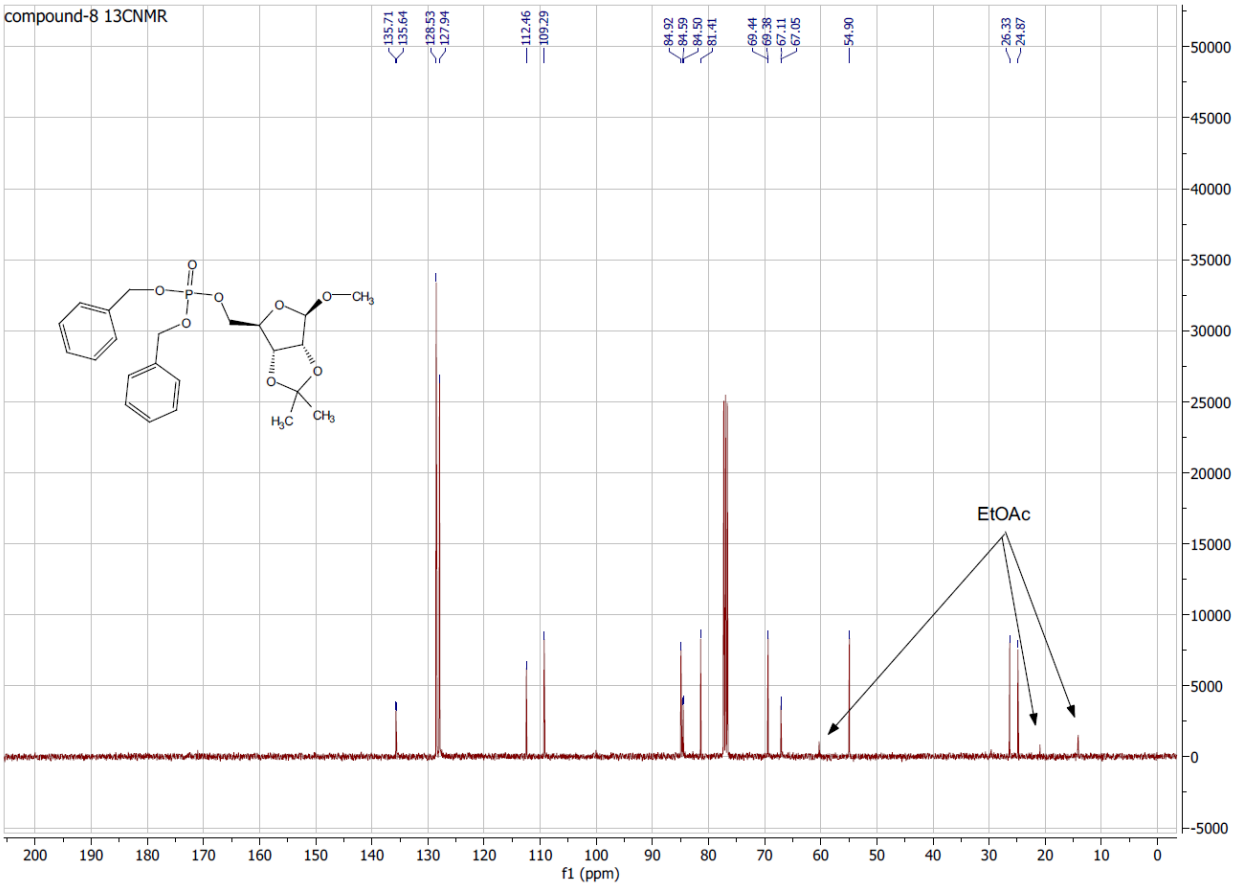
Supplementary Figure 2: HPLC trace showing decomposition of 2″-deoxy-ADPR

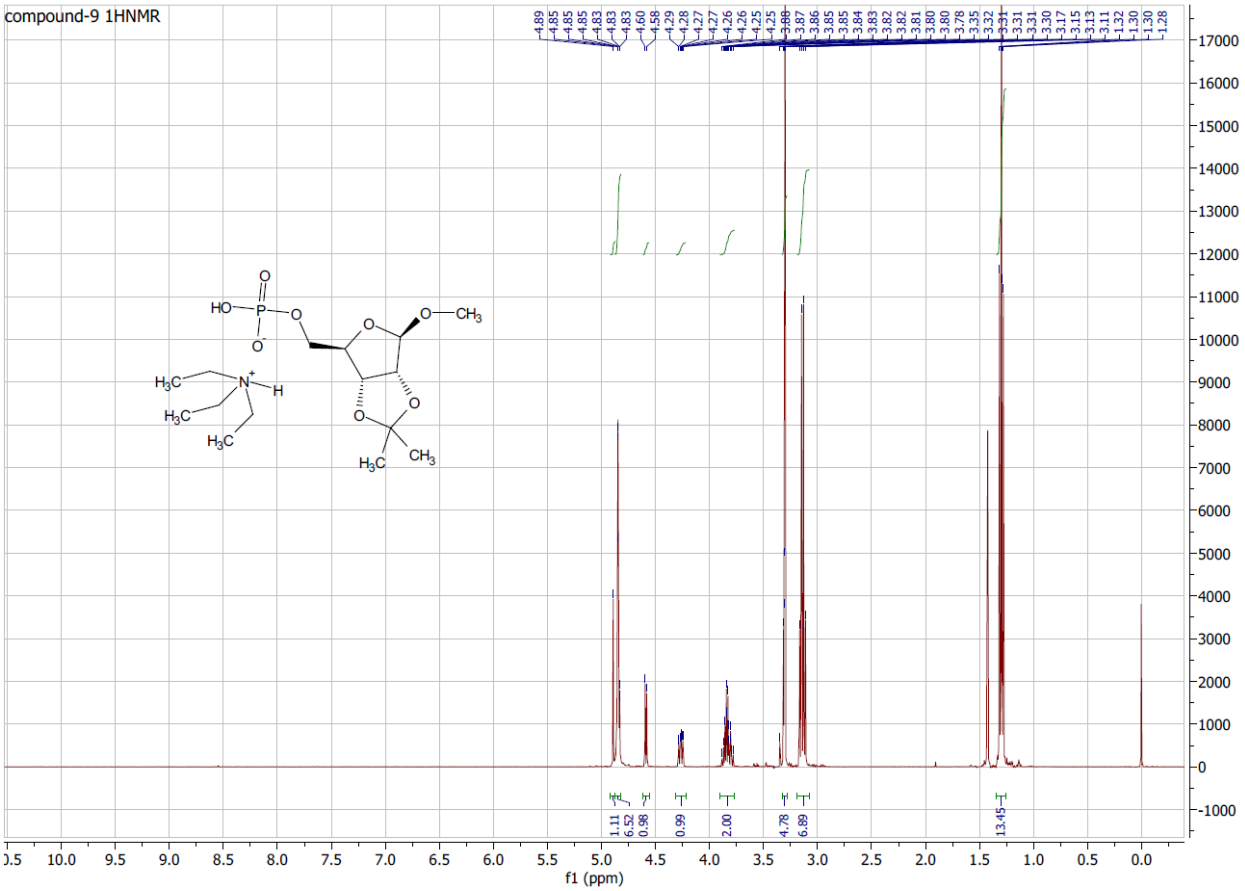


Integrity of 2″-deoxy-ADPR after transport and reconstitution was checked by RP-HPLC analysis on a 1260 Infinity system (Agilent Technologies). Samples and standards (ADP, AMP, dAMP, cAMP, Adenosine 250 pmol each) were run on a 250 mm × 4.6 mm Multohyp C18 5-µm column (Chromatographie Service) with a 4.0 mm × 3.0 mm guard cartridge containing a C18 ODS filter element (Phenomenex) at a flow rate of 0.8 mL/min with buffer (20 mmol/l KH_2PO_4, pH 6) with a linear gradient of methanol from 0 to 50% Methanol over 22.5 min. Adenine nucleotides were detected at 260 nm. Peaks were integrated using the ChemStation Software (Rev. C.01.05; Agilent Technologies). a) Chromatograms of standards (250 pmol each). b) Chromatogram of a preparation of 2″-deoxy-ADPR after freeze drying, transport and reconstitution in 10 mmol/l HEPES pH7.2. The sample should have contained 1 nmol of 2″-deoxy-ADPR, but eluted from the column as multiple peaks, one of the fragments co-elutes with ADP.

^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR data for compounds **8-12**, **14-19, 21-27, and 30**



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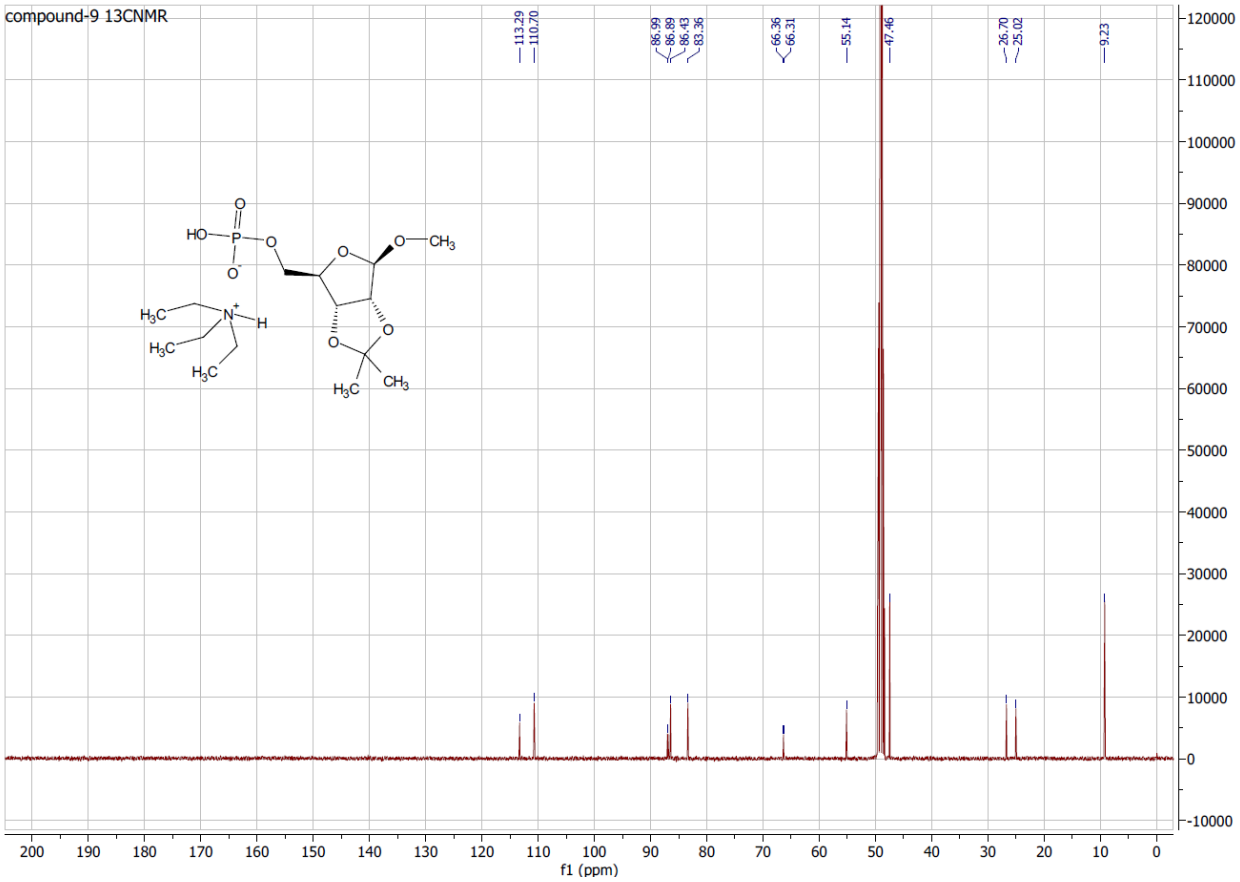
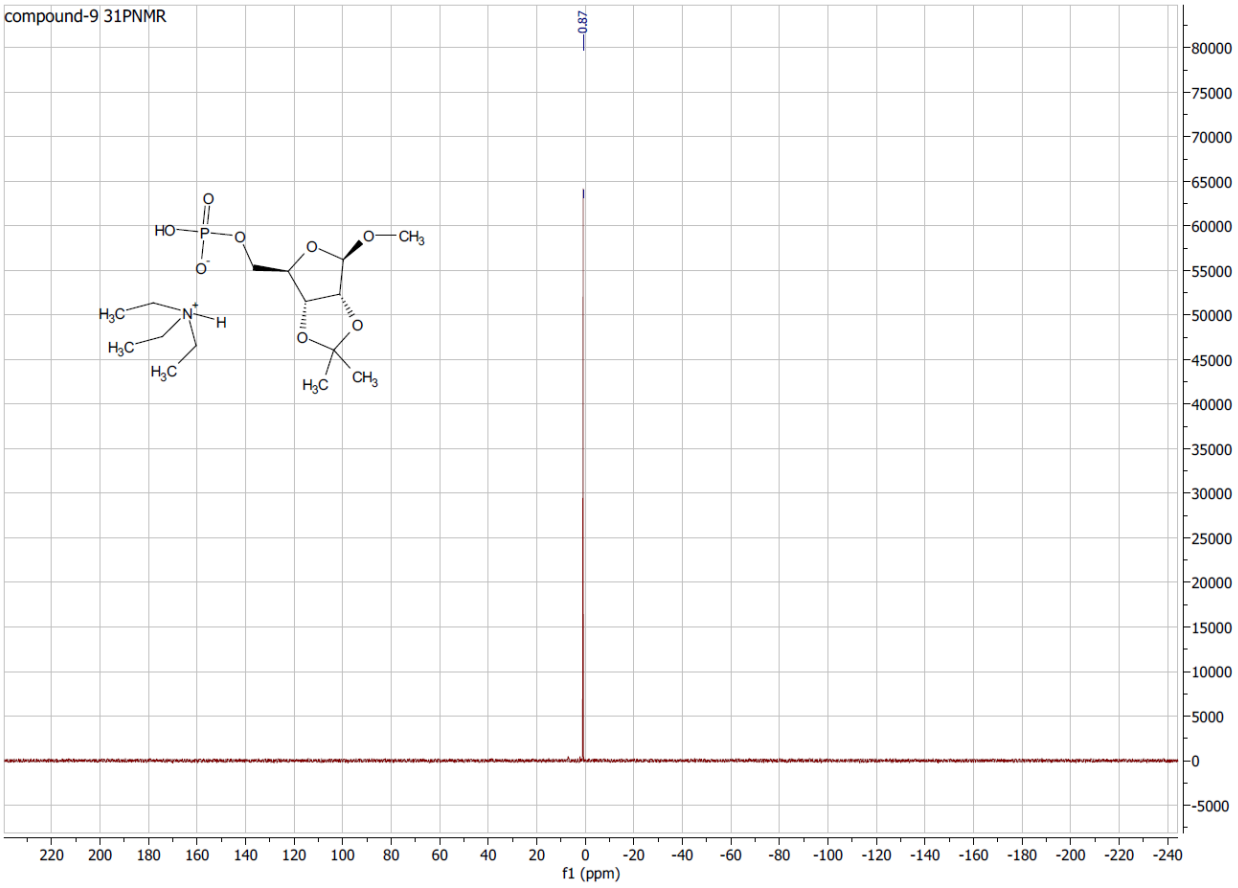
f1 (ppm)


compound-9 13CNMR
f1 (ppm)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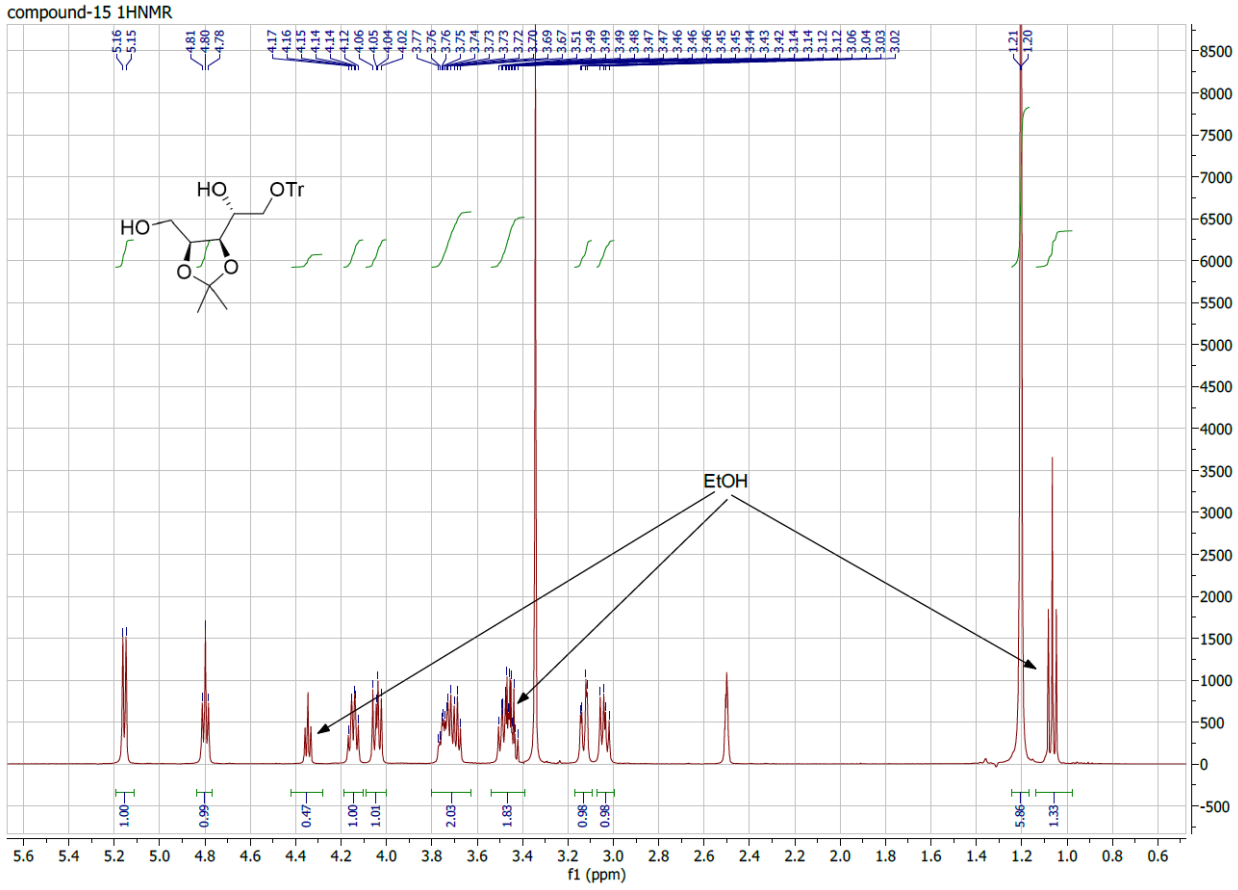
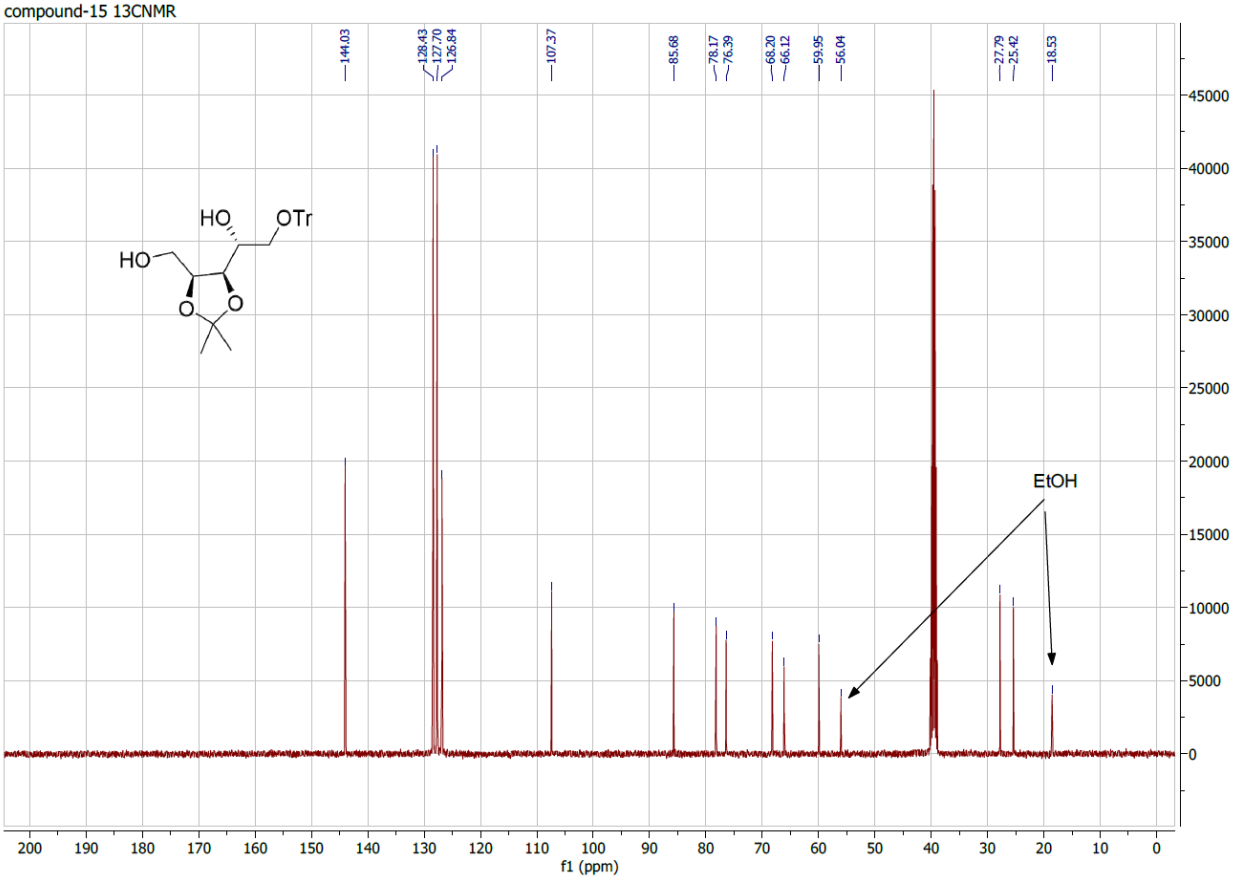
0.87
f1 (ppm)


compound-15 1HNMR
HO
OTr
HO
EtOH
f1 (ppm)

compound-15 13CNMR



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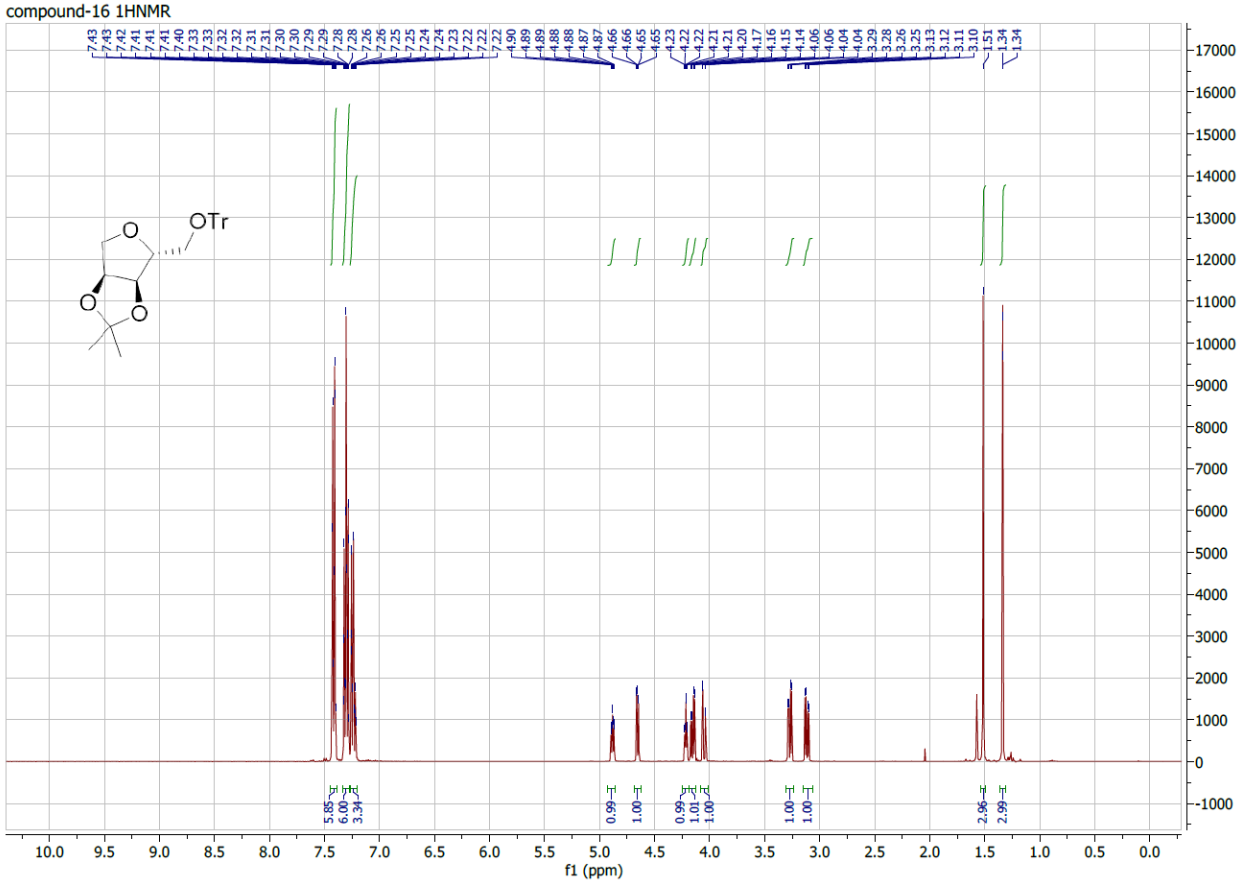
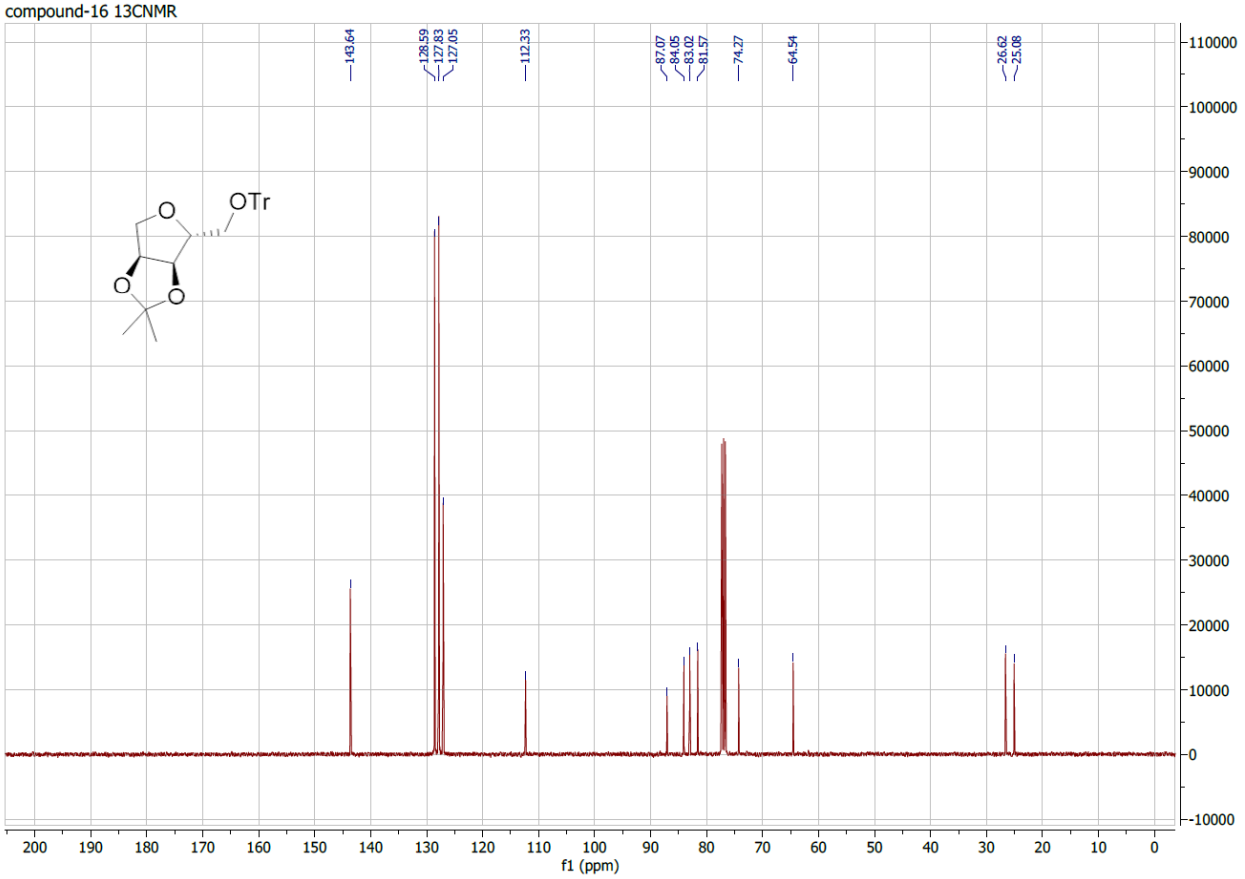
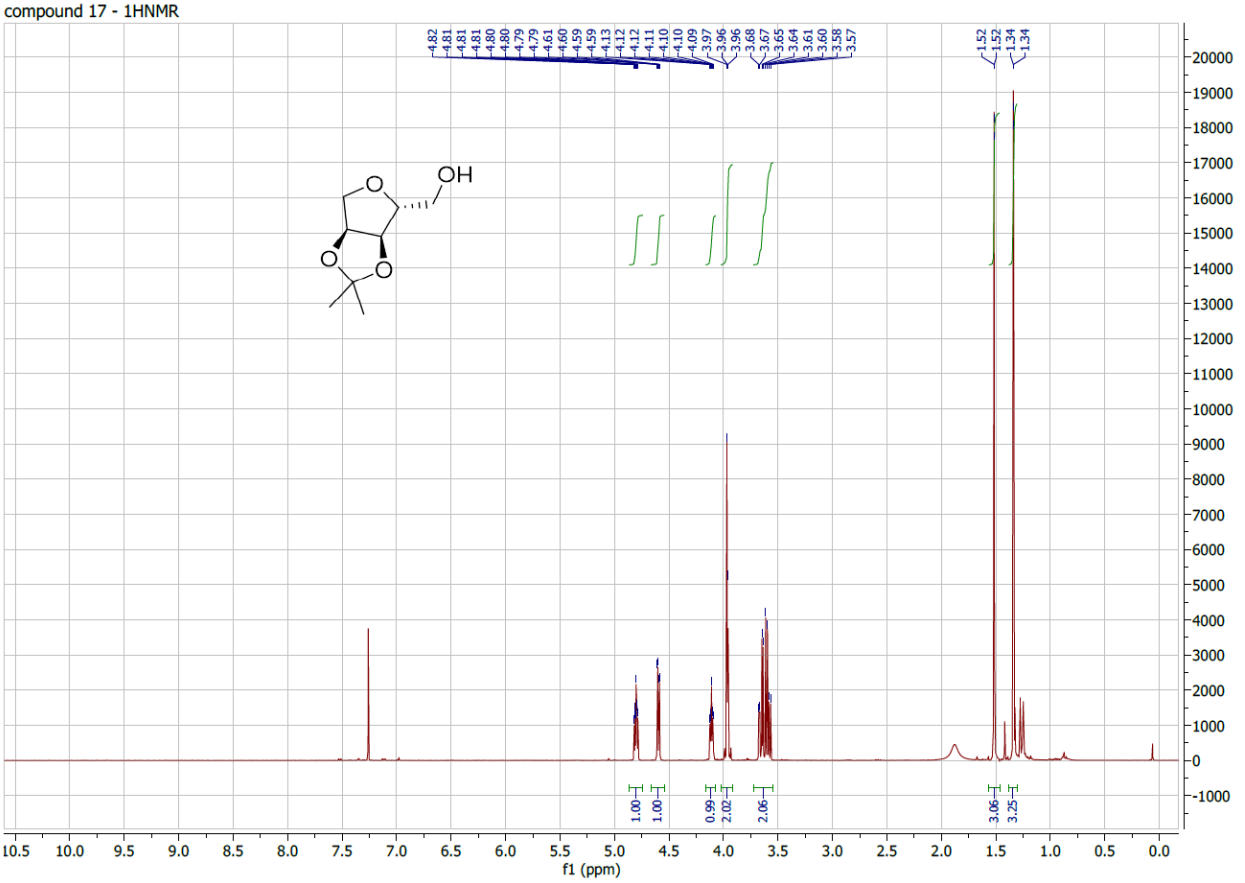


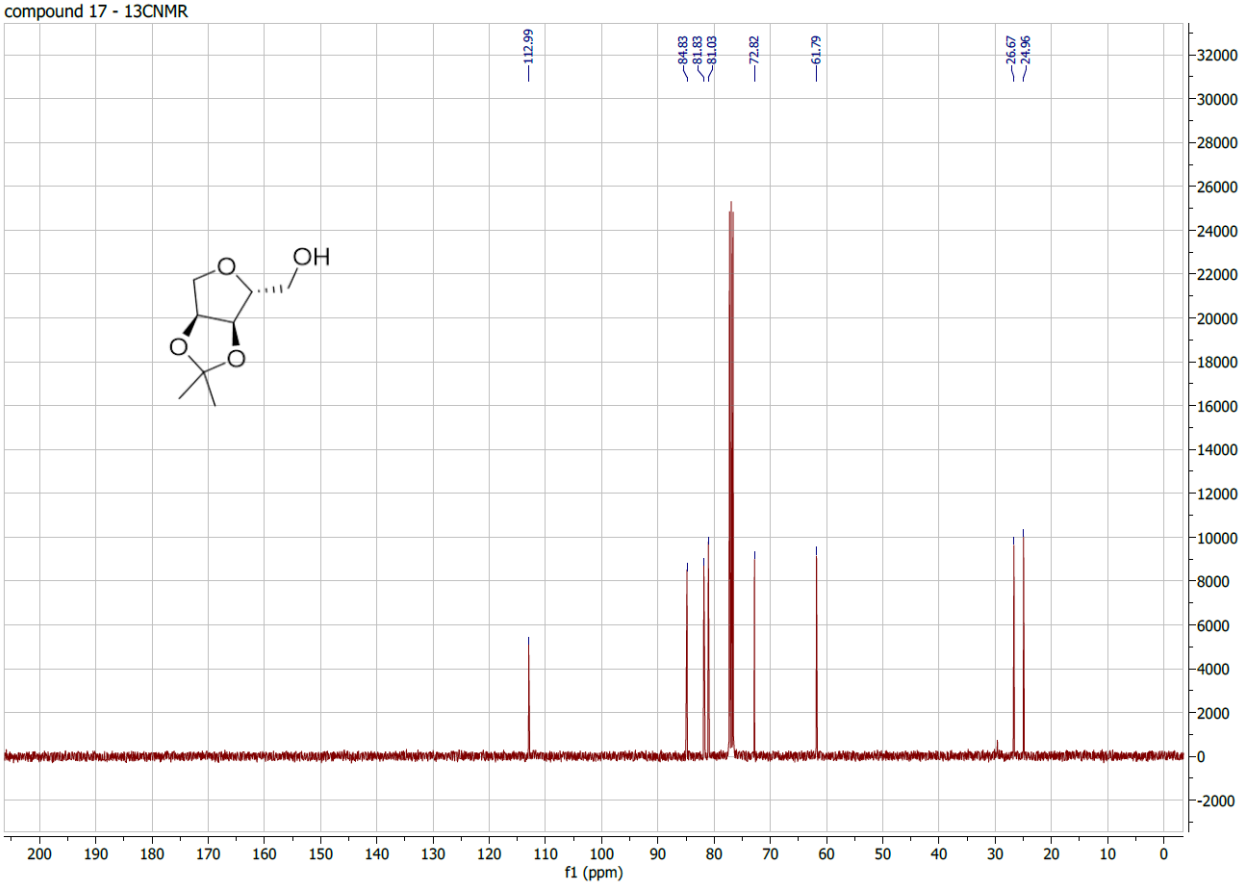
compound-16 13CNMR



compound 17 - 1HNMR



compound 17 - 13CNMR



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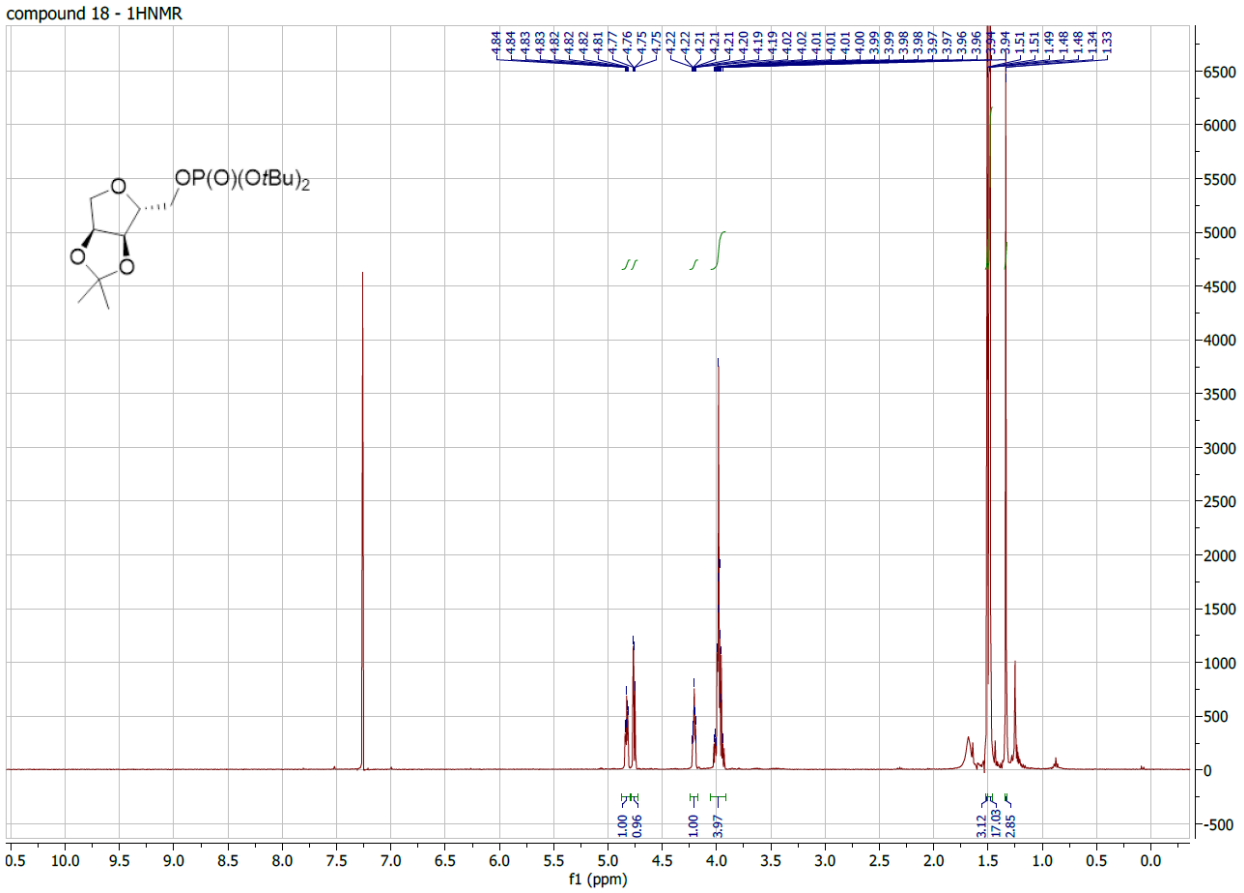
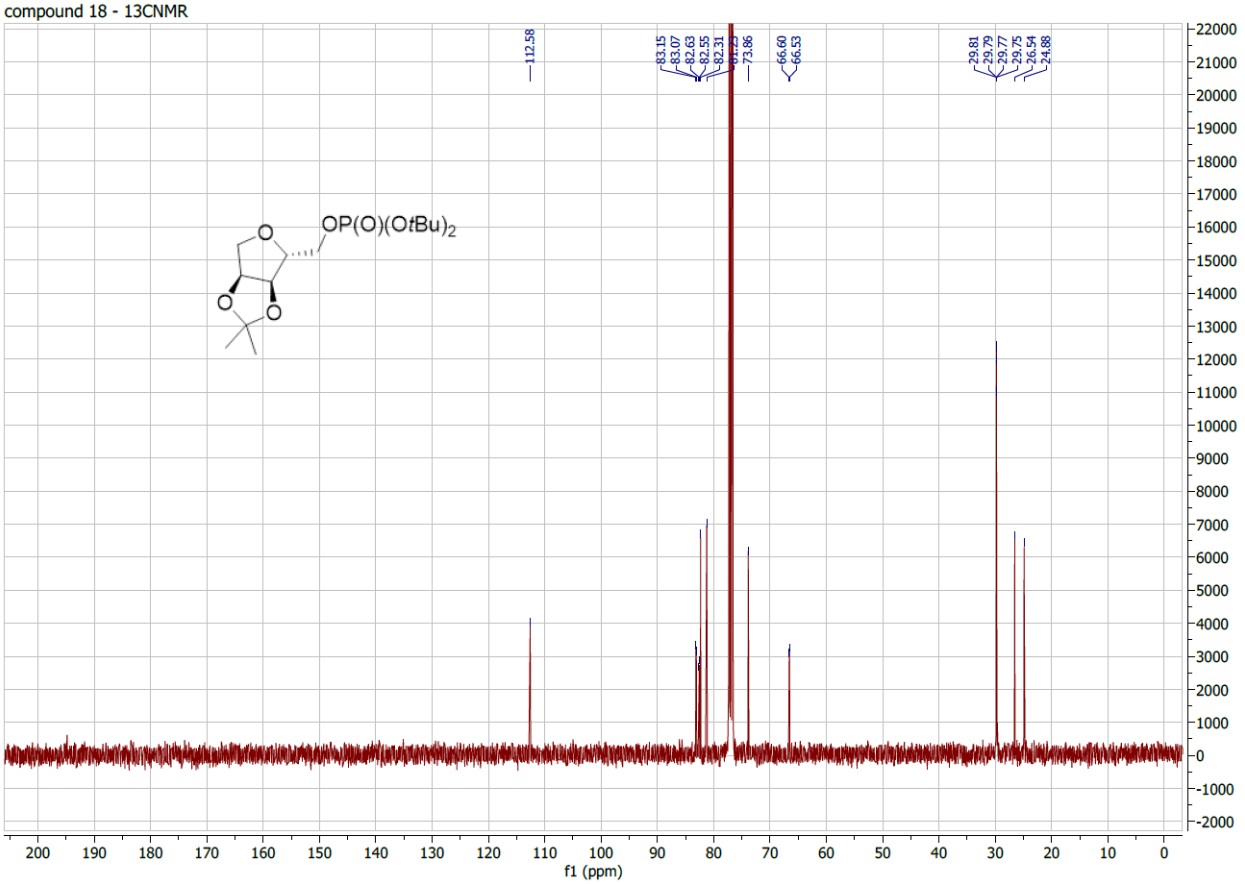
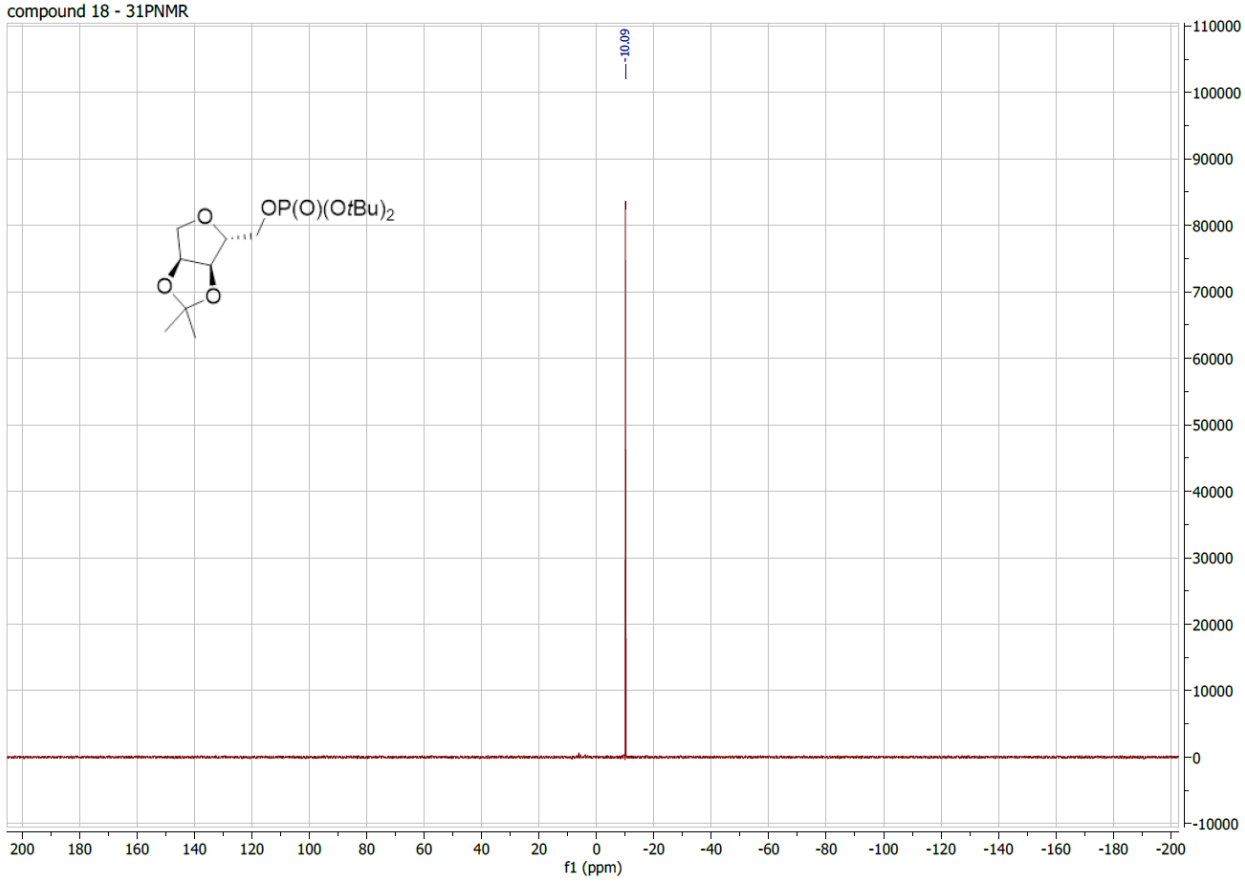


compound 18 - 13CNMR



compound 18 - 31PNMR



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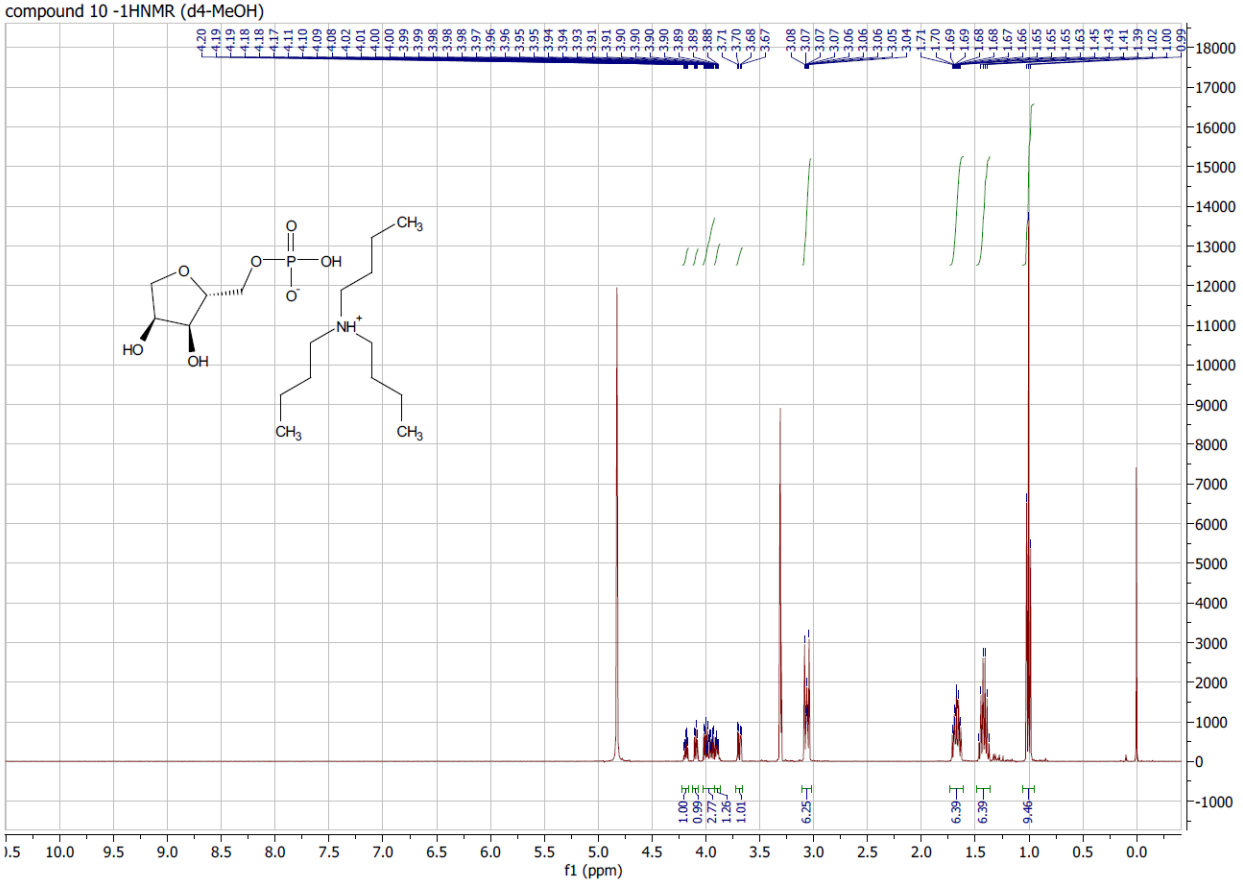

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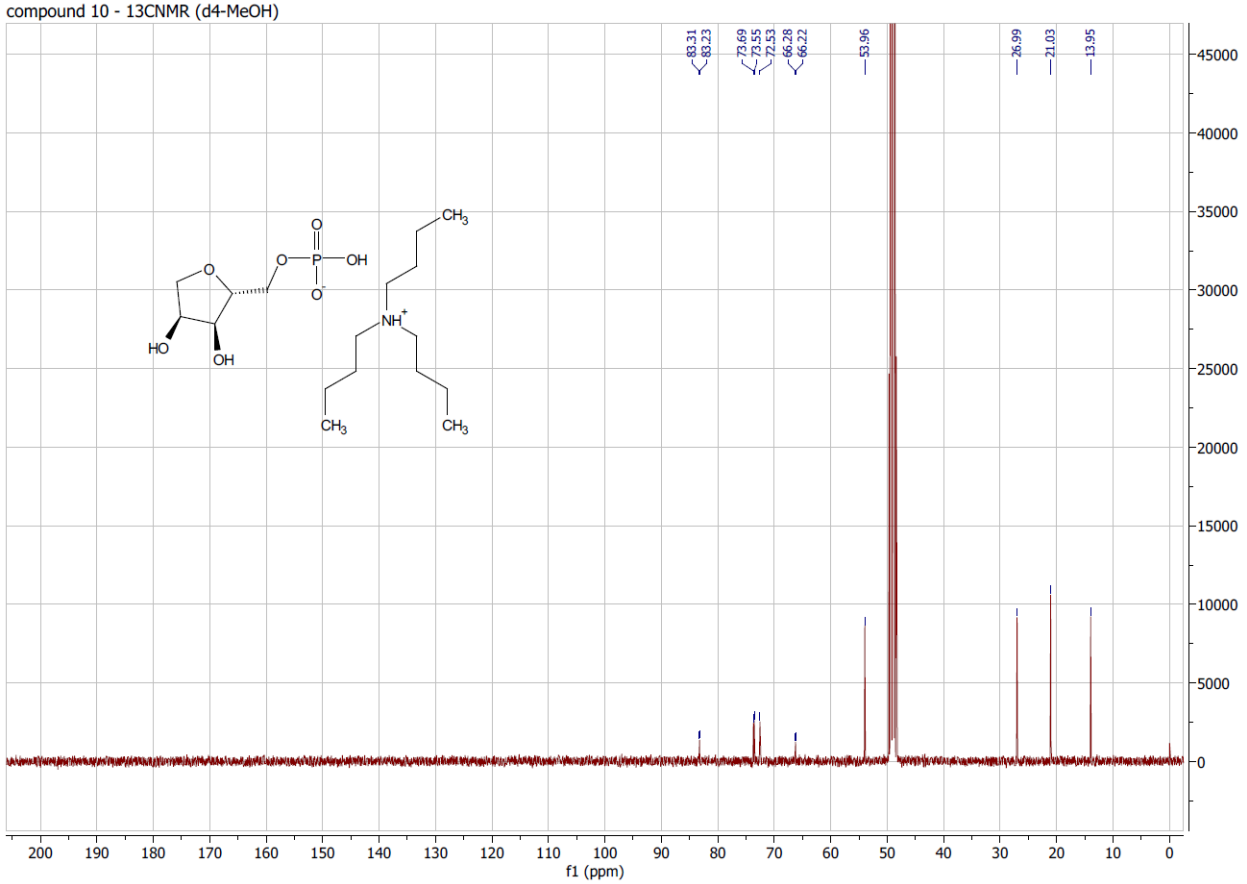
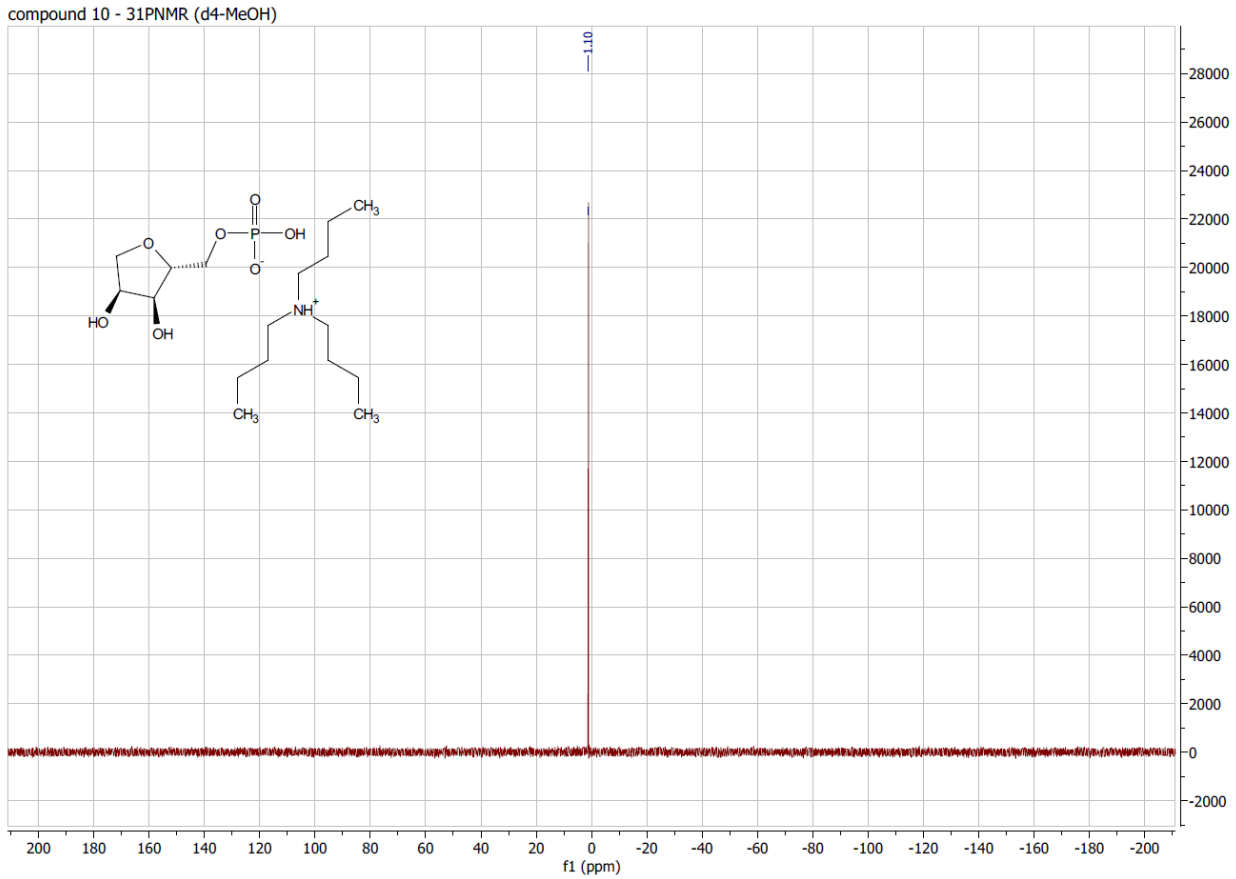
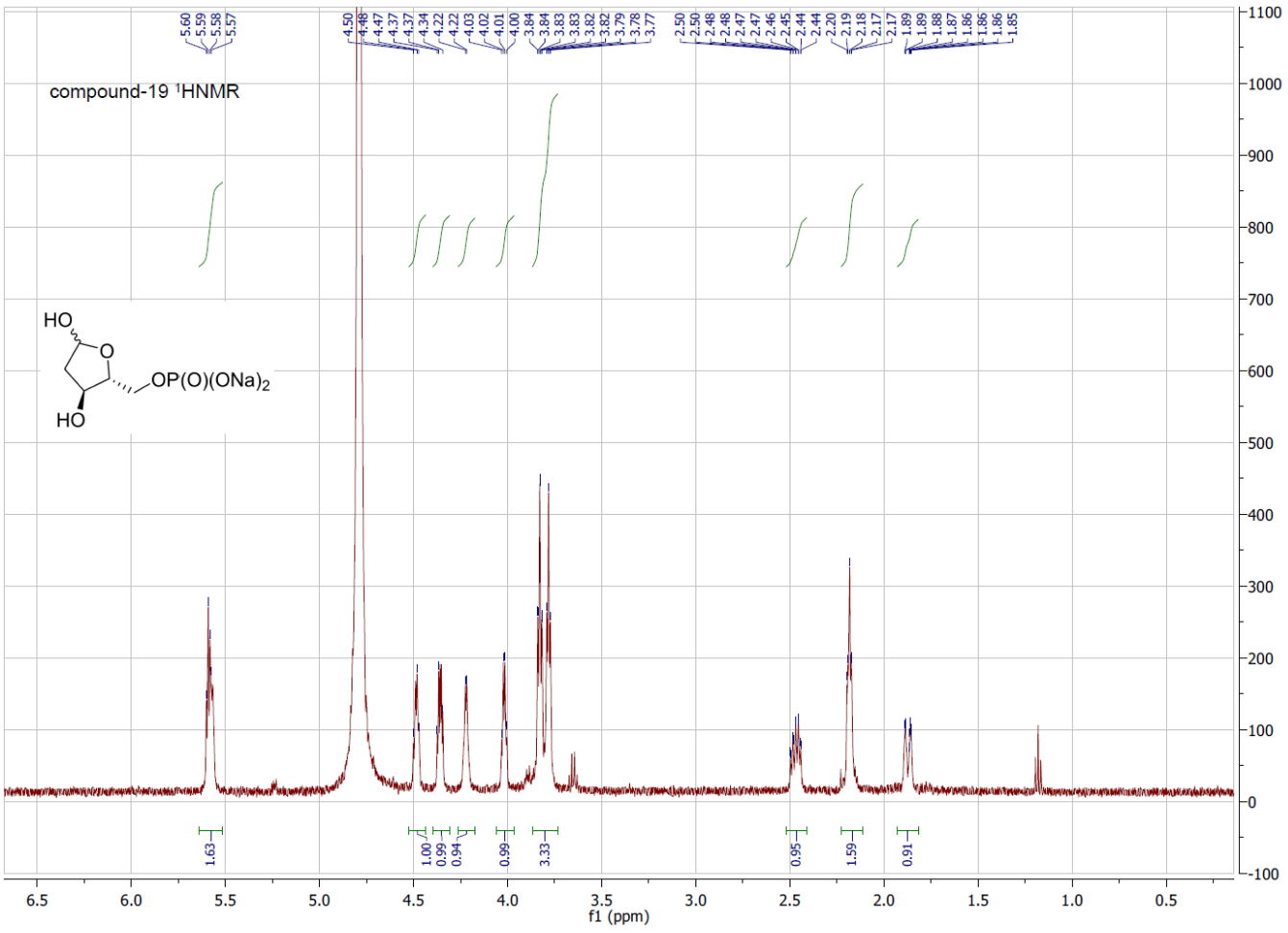
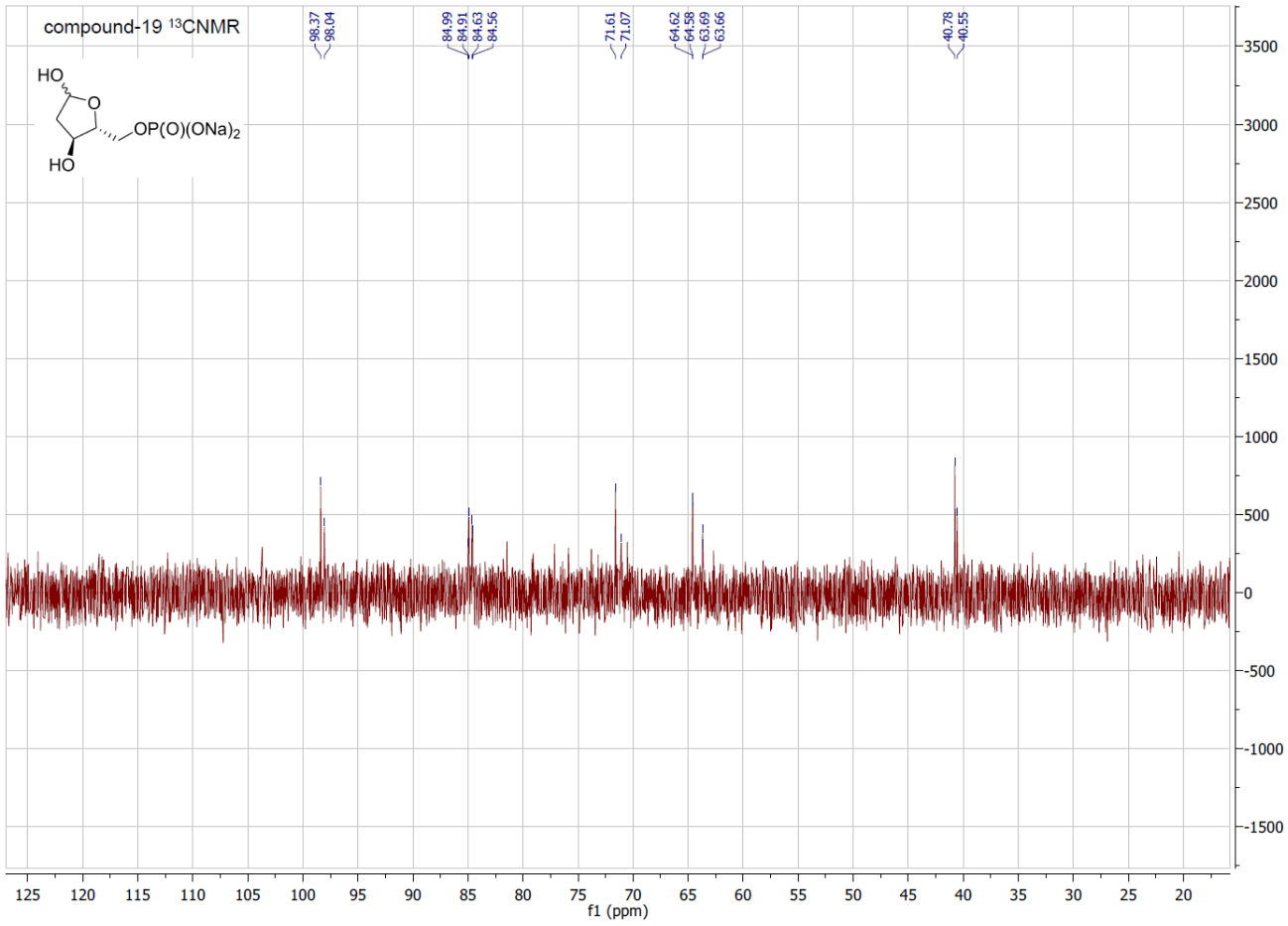
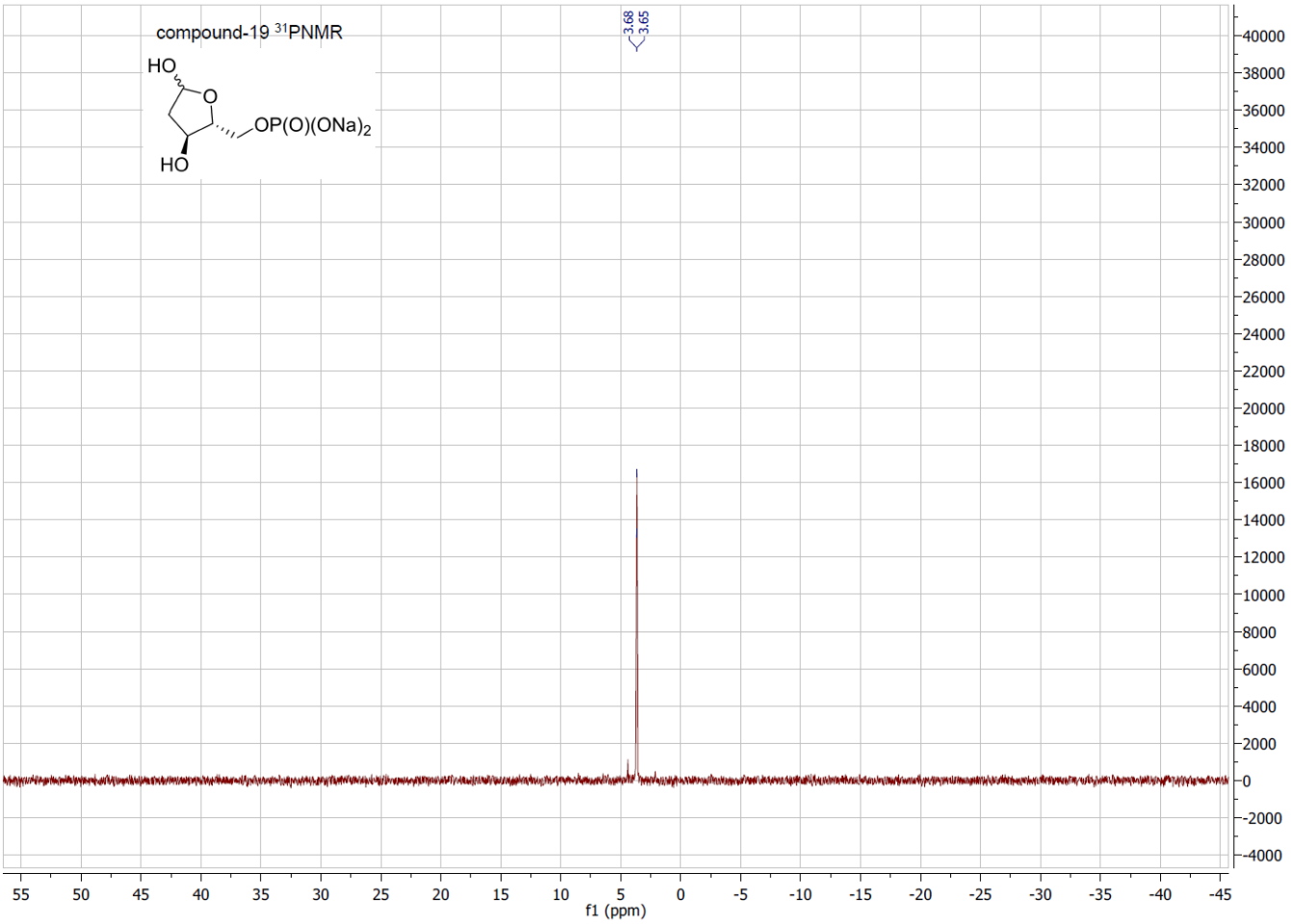


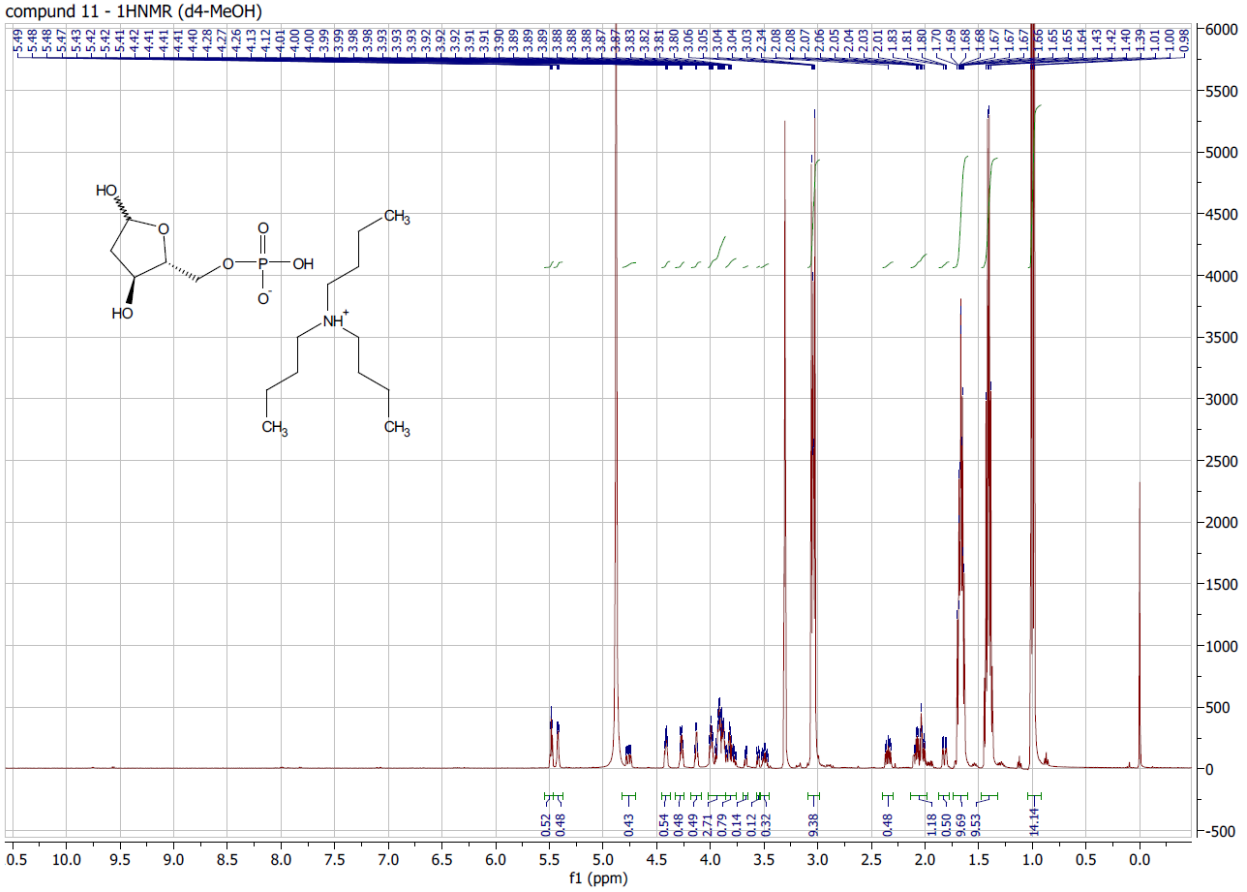

compound 10 - 31PNMR (d4-MeOH)
1.10
f1 (ppm)


compound-19 ¹HNMR
OP(O)(ONa)2
f1 (ppm)

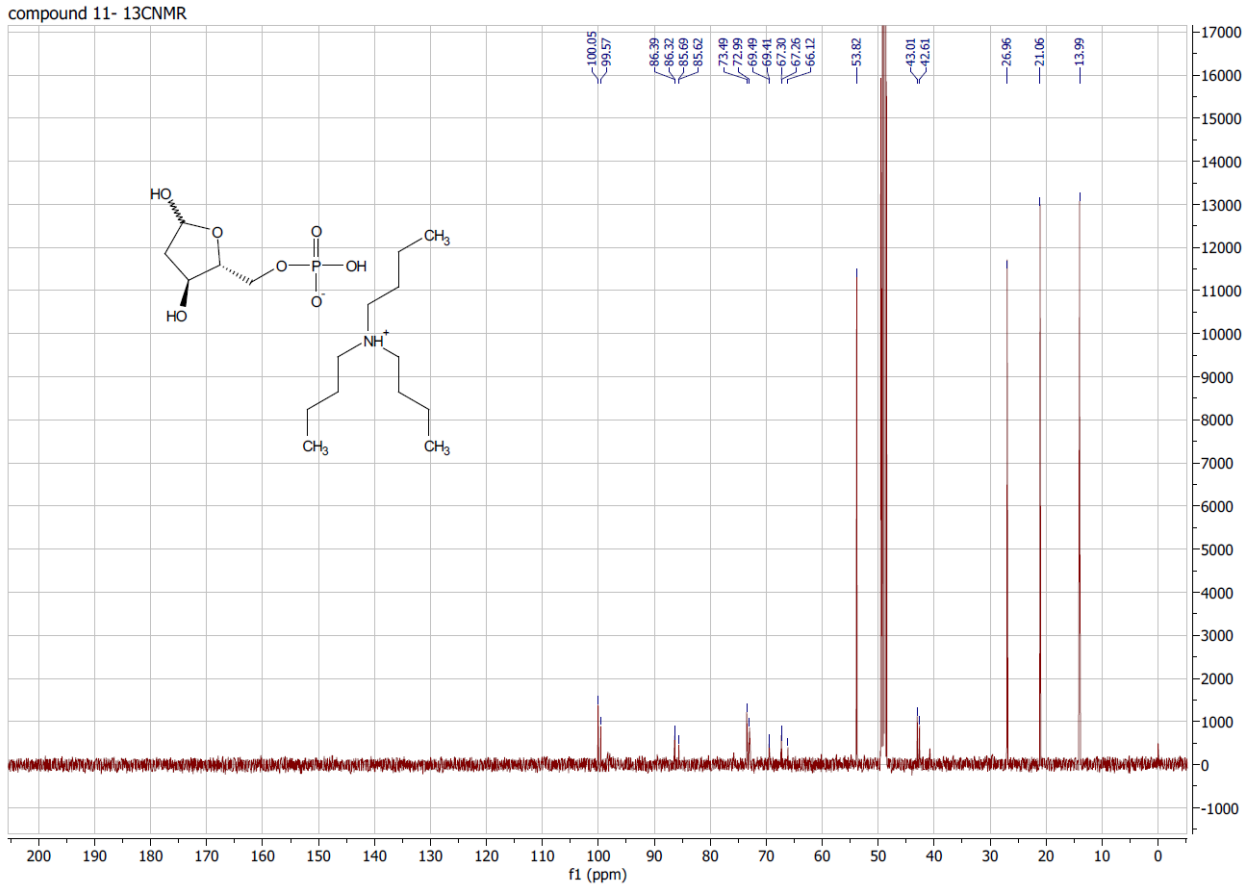

compound-19 13CNMR
HO
O
OP(O)(ONa)2
HO
98.37
98.04
84.99
84.91
84.63
84.56
71.61
71.07
64.62
64.58
63.69
63.66
40.78
40.55
f1 (ppm)


compound-19 31PNMR
HO
O
OP(O)(ONa)2
HO
3.68
3.65
f1 (ppm)

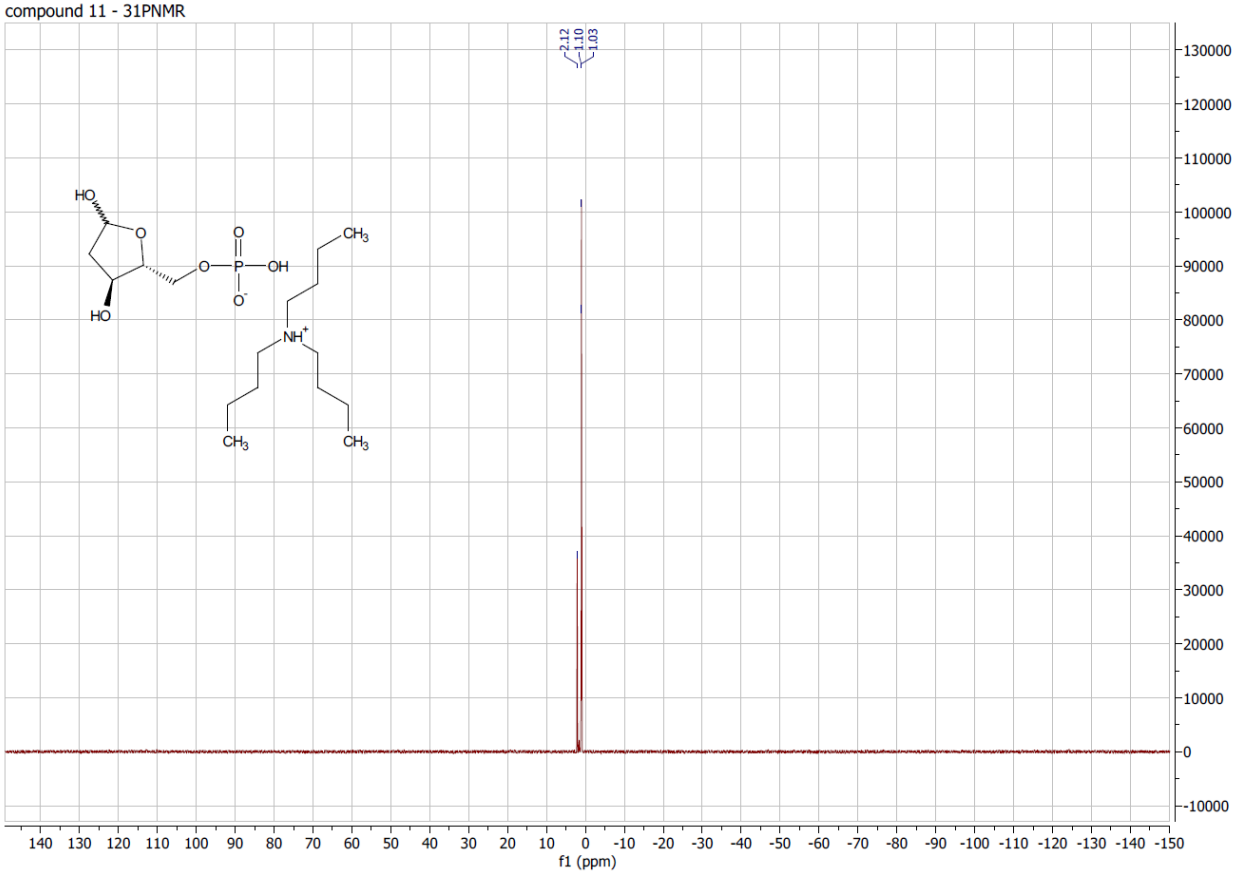
compund 11 - 1HNMR (d4-MeOH)



compound 11- 13CNMR



compound 11 - 31PNMR



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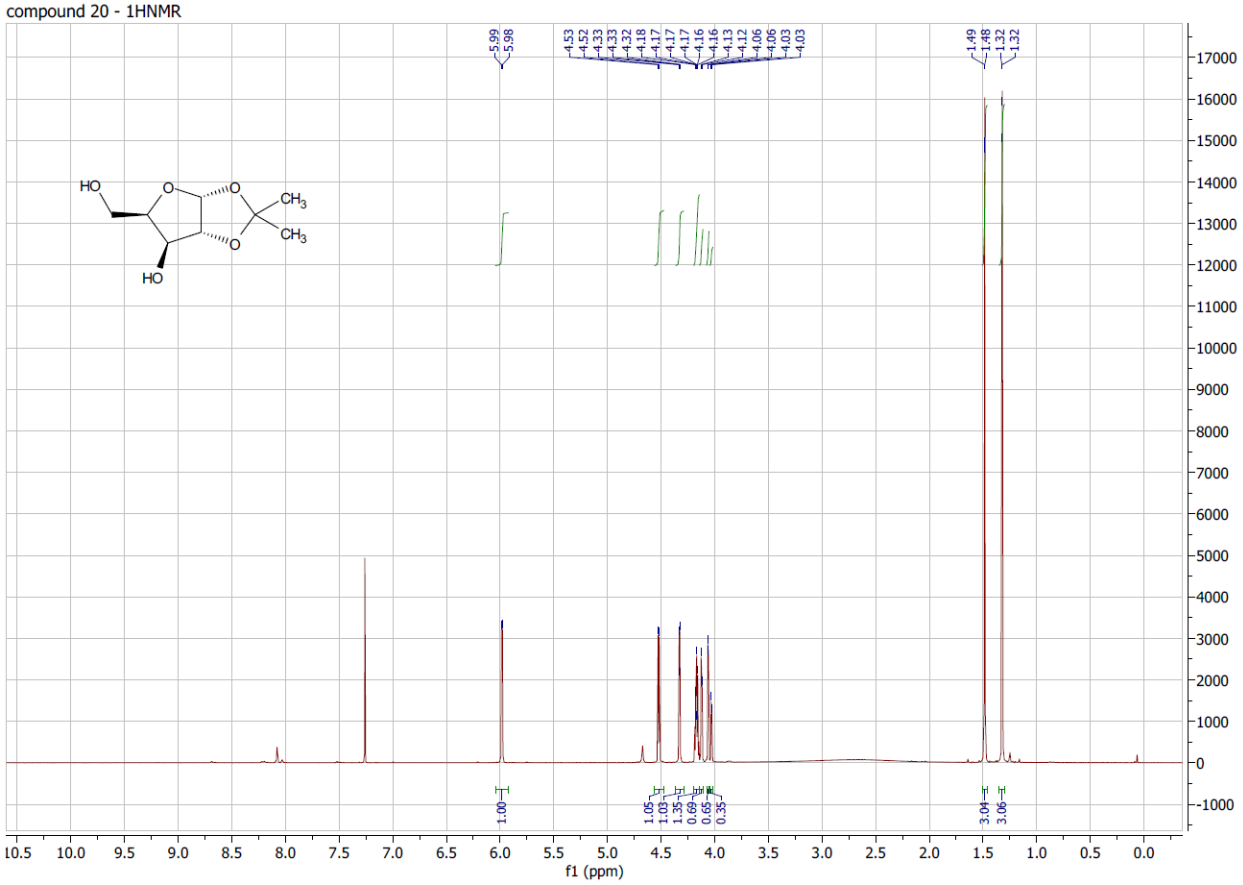

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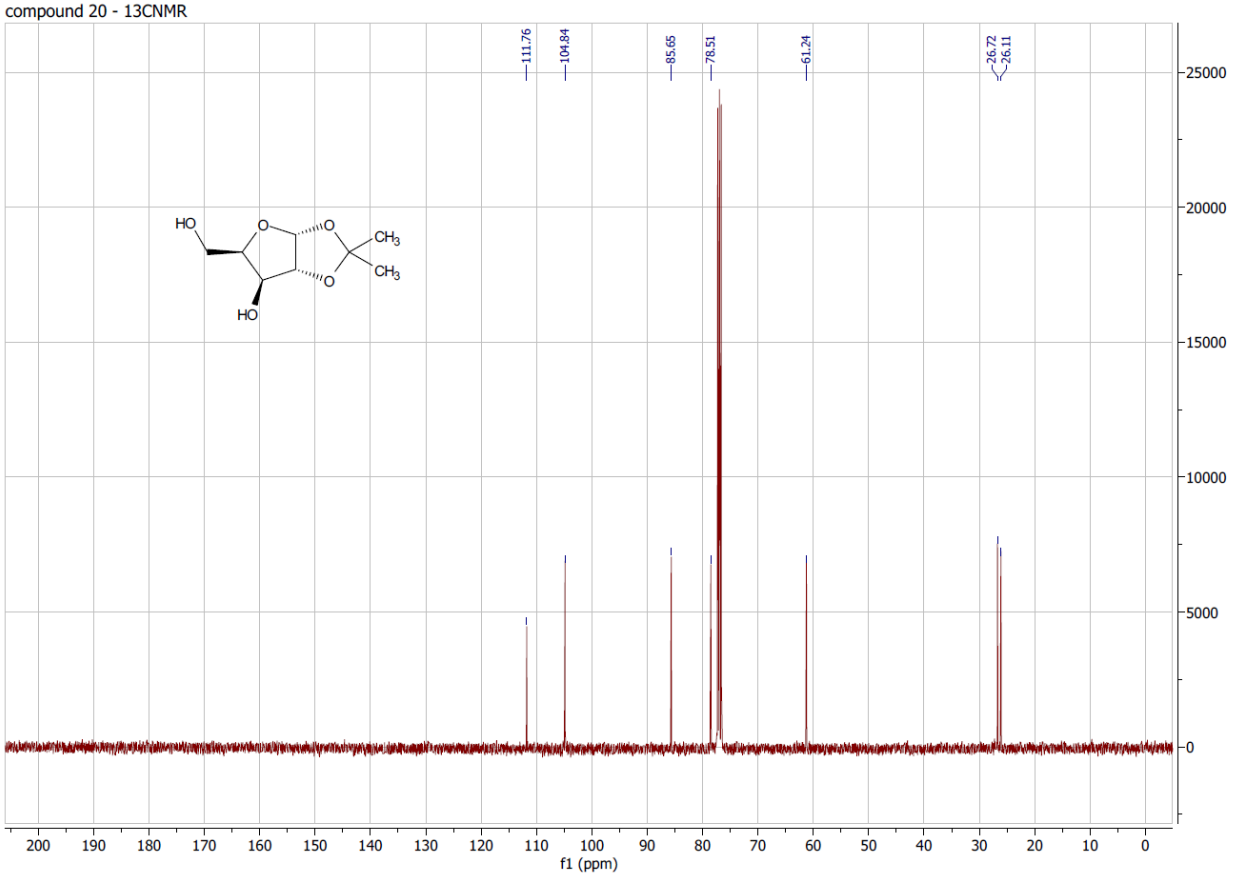

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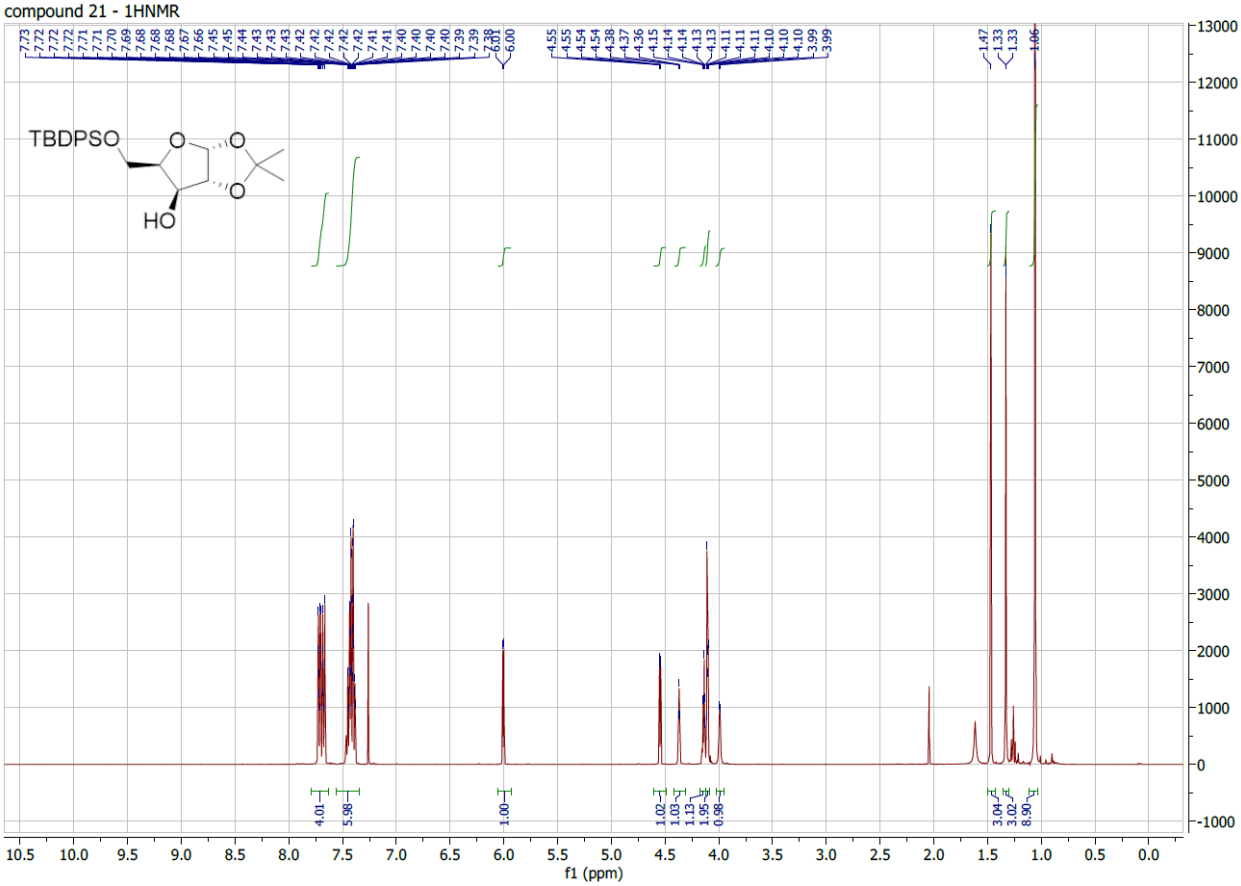

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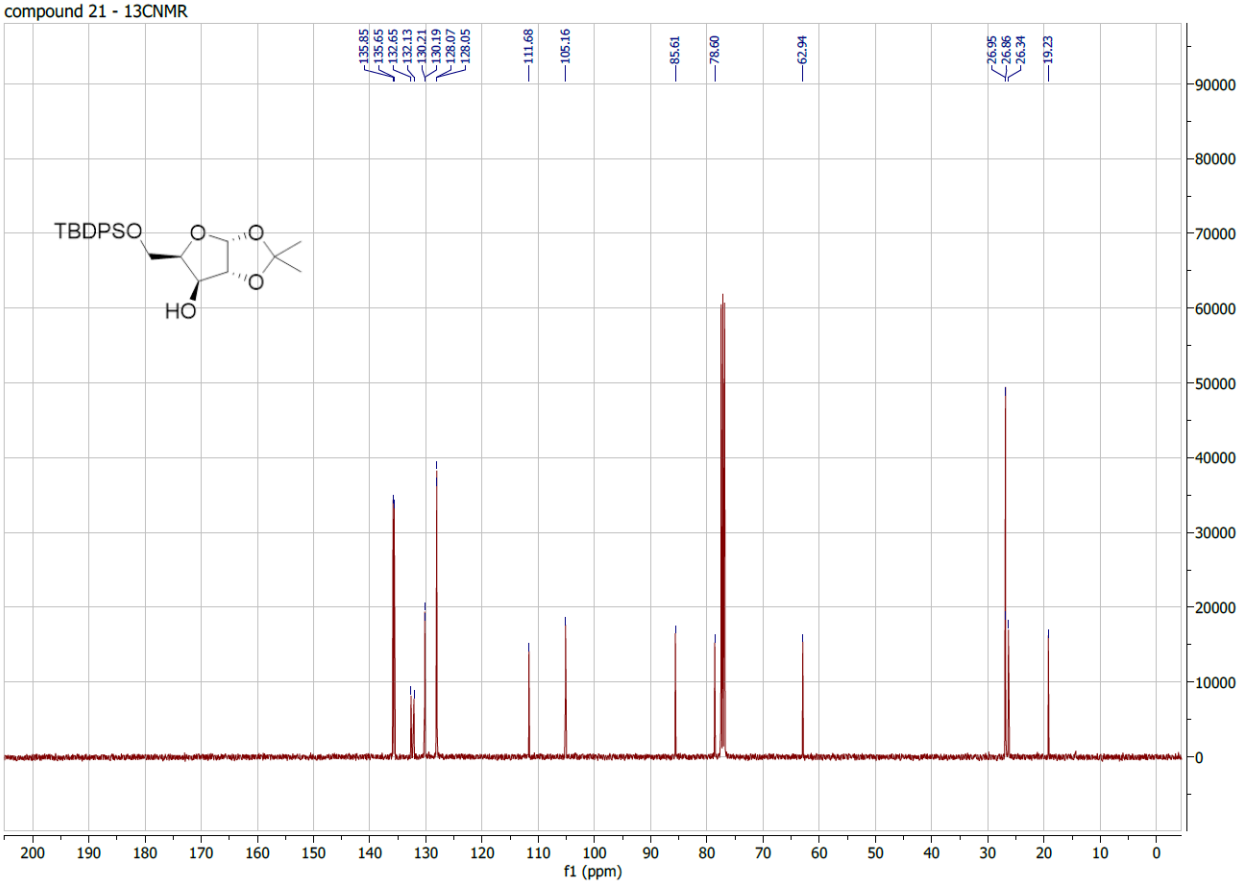

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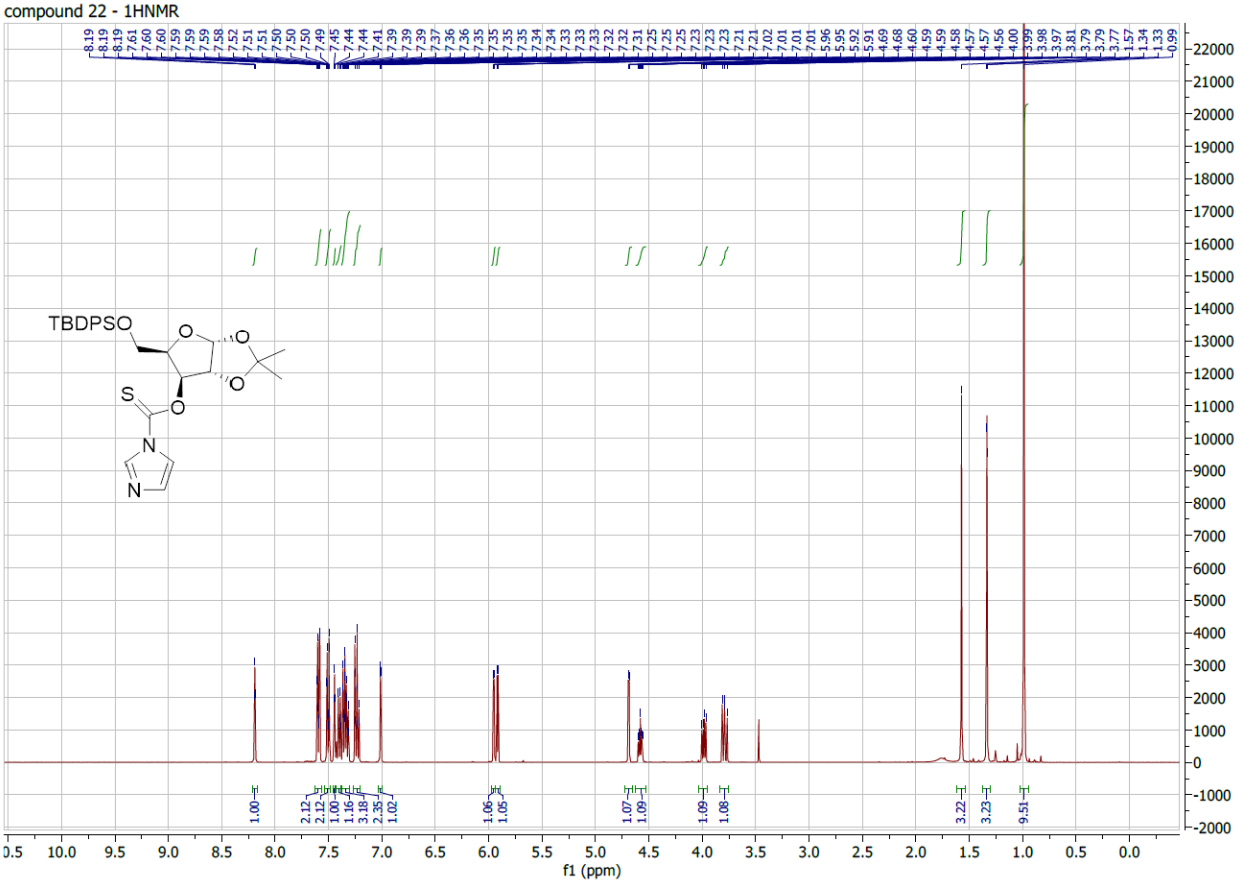

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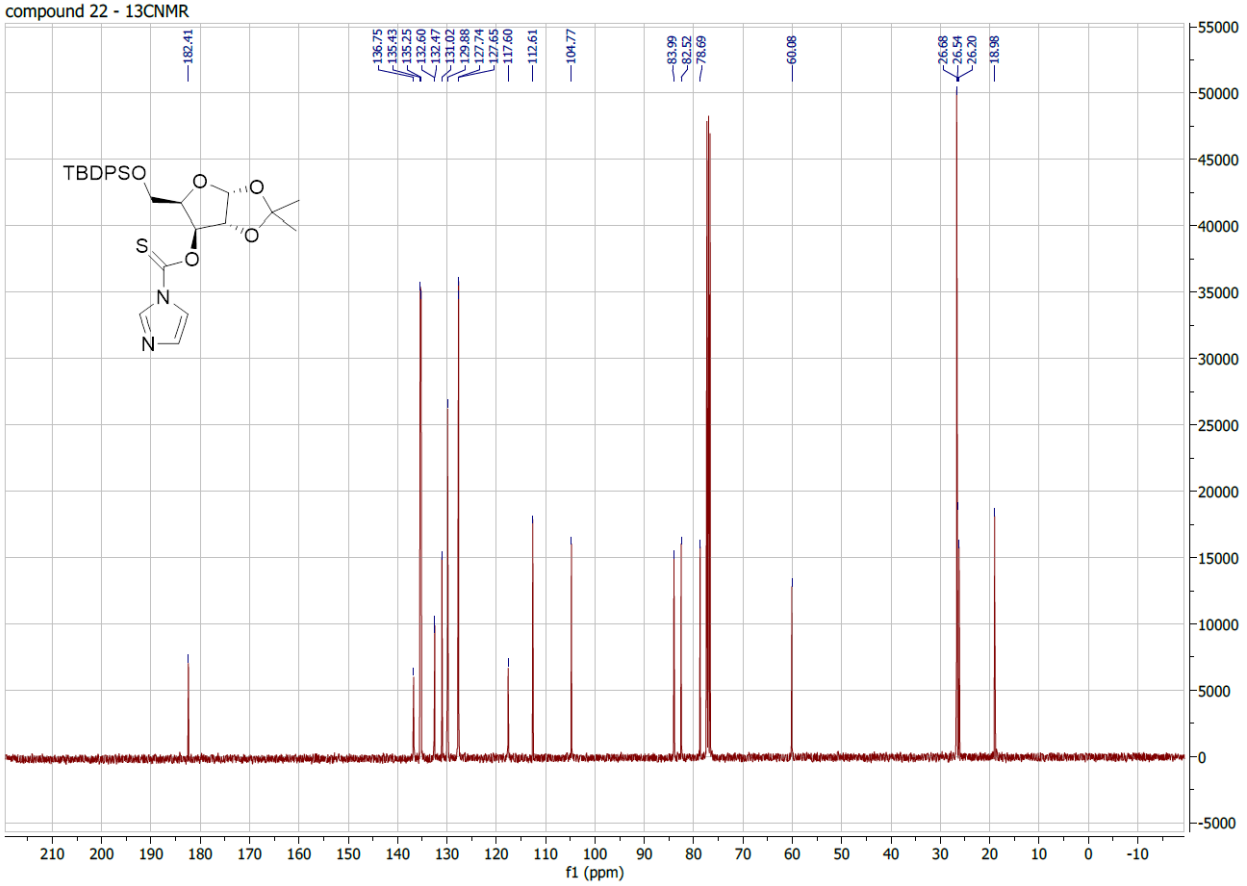

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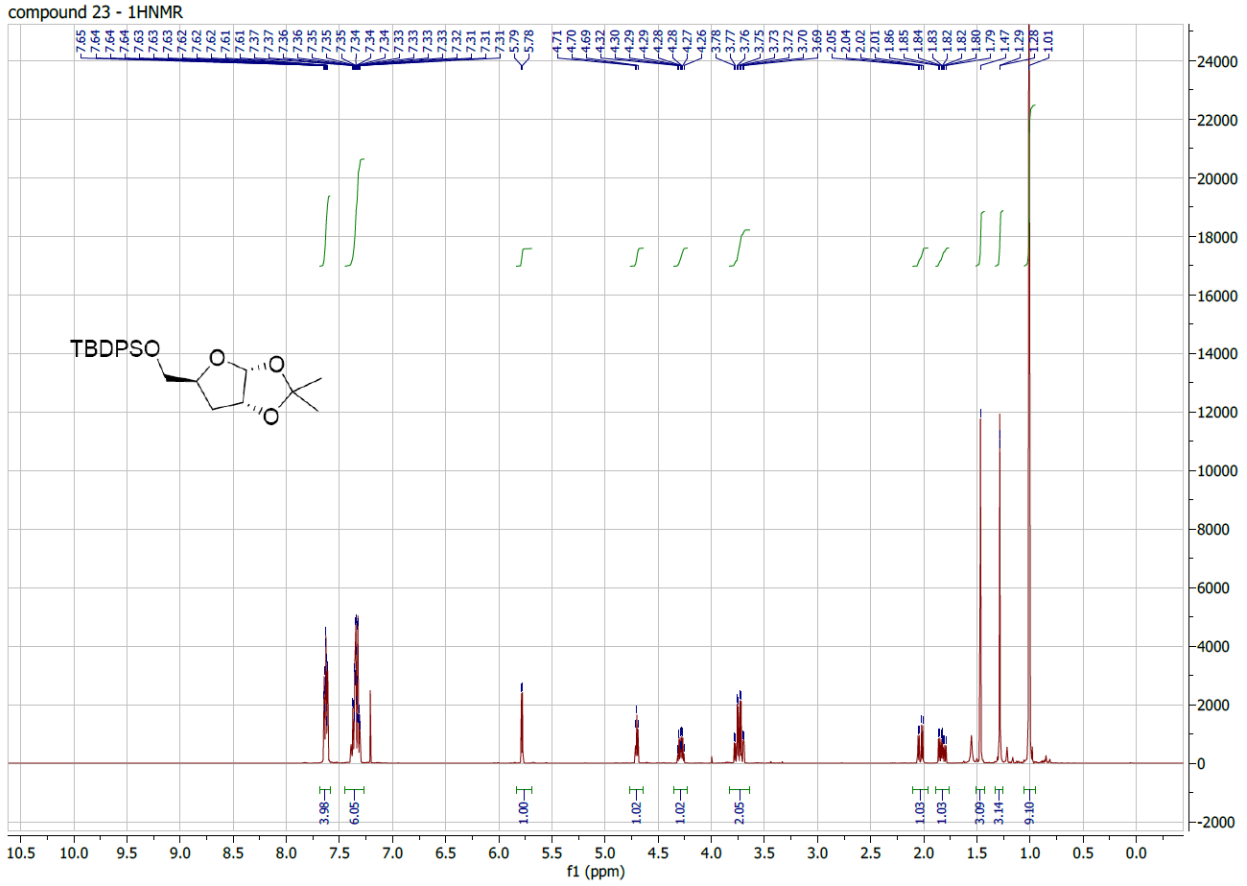

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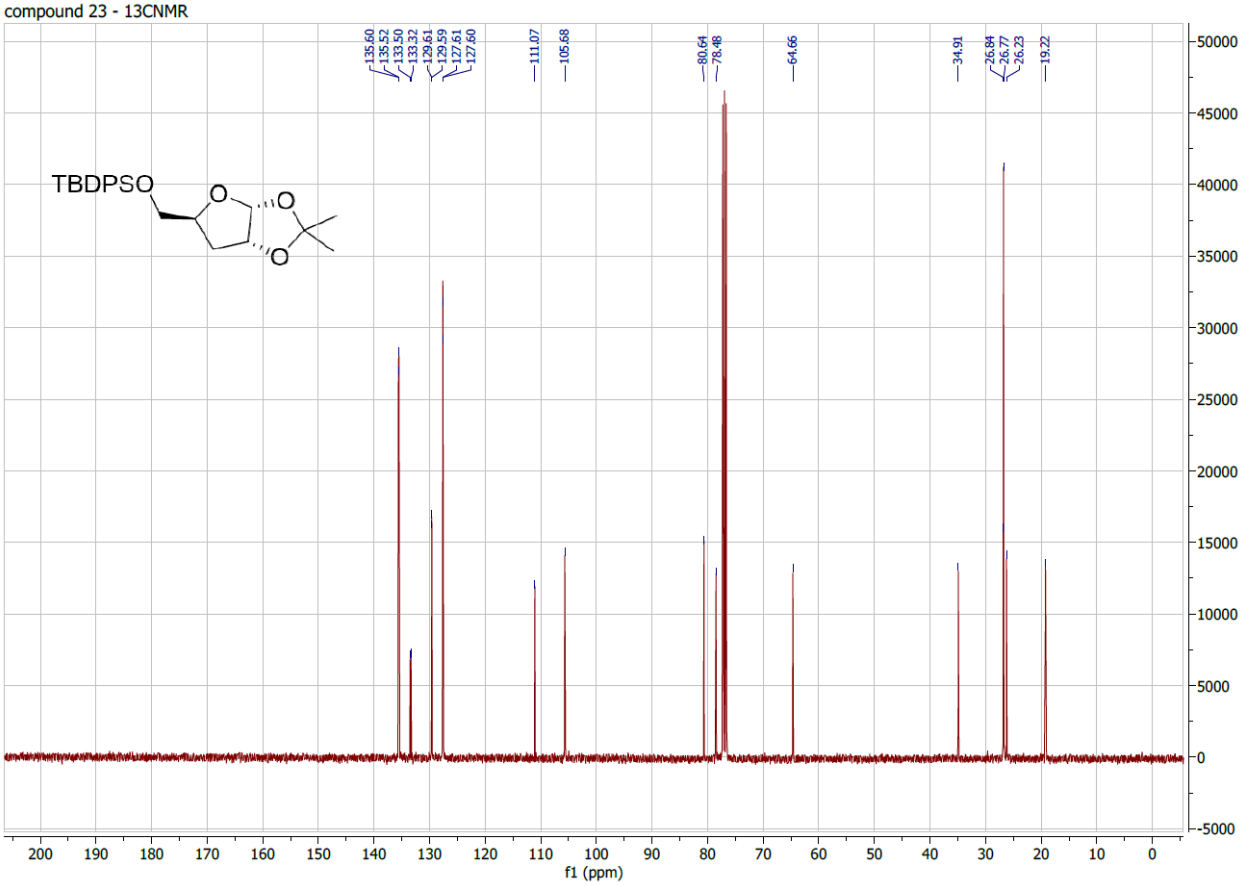
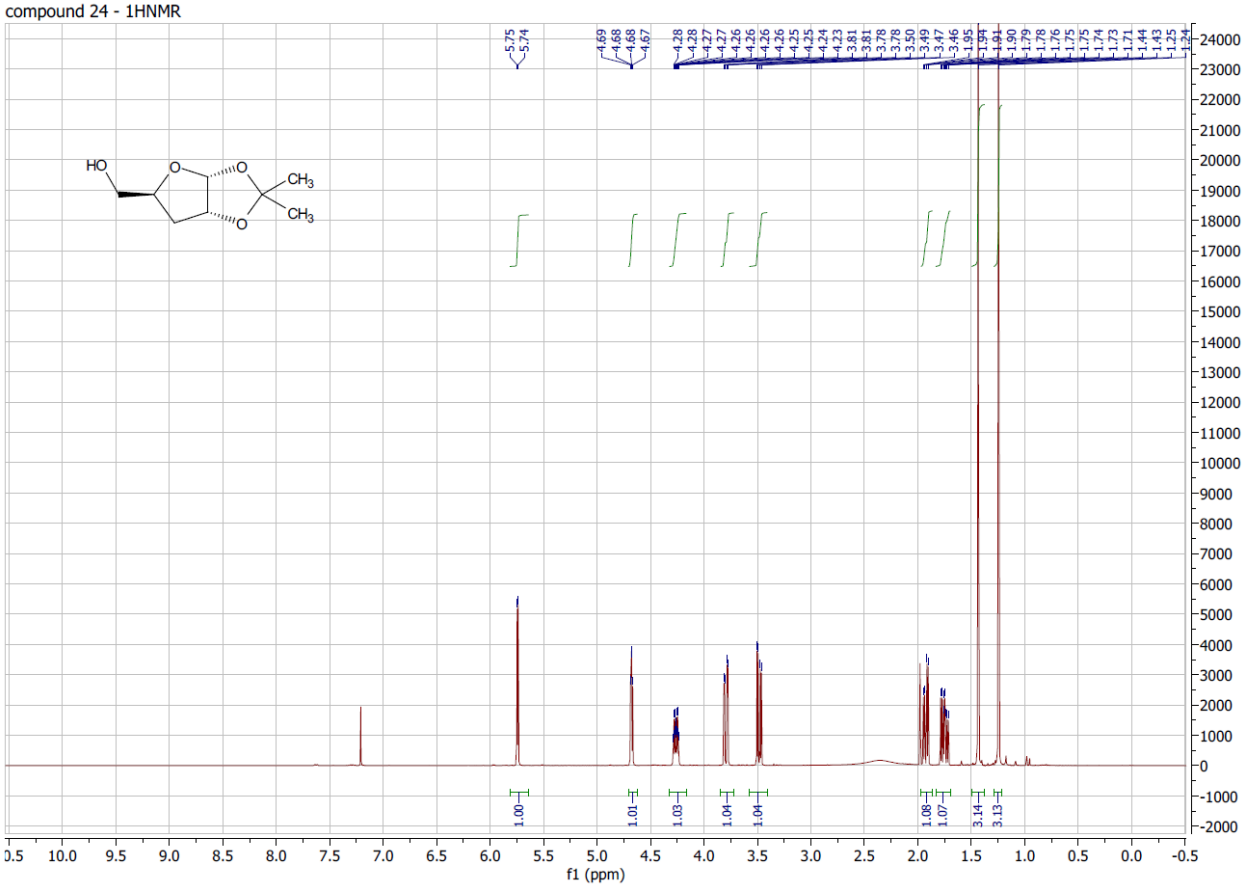


compound 24 - 1HNMR



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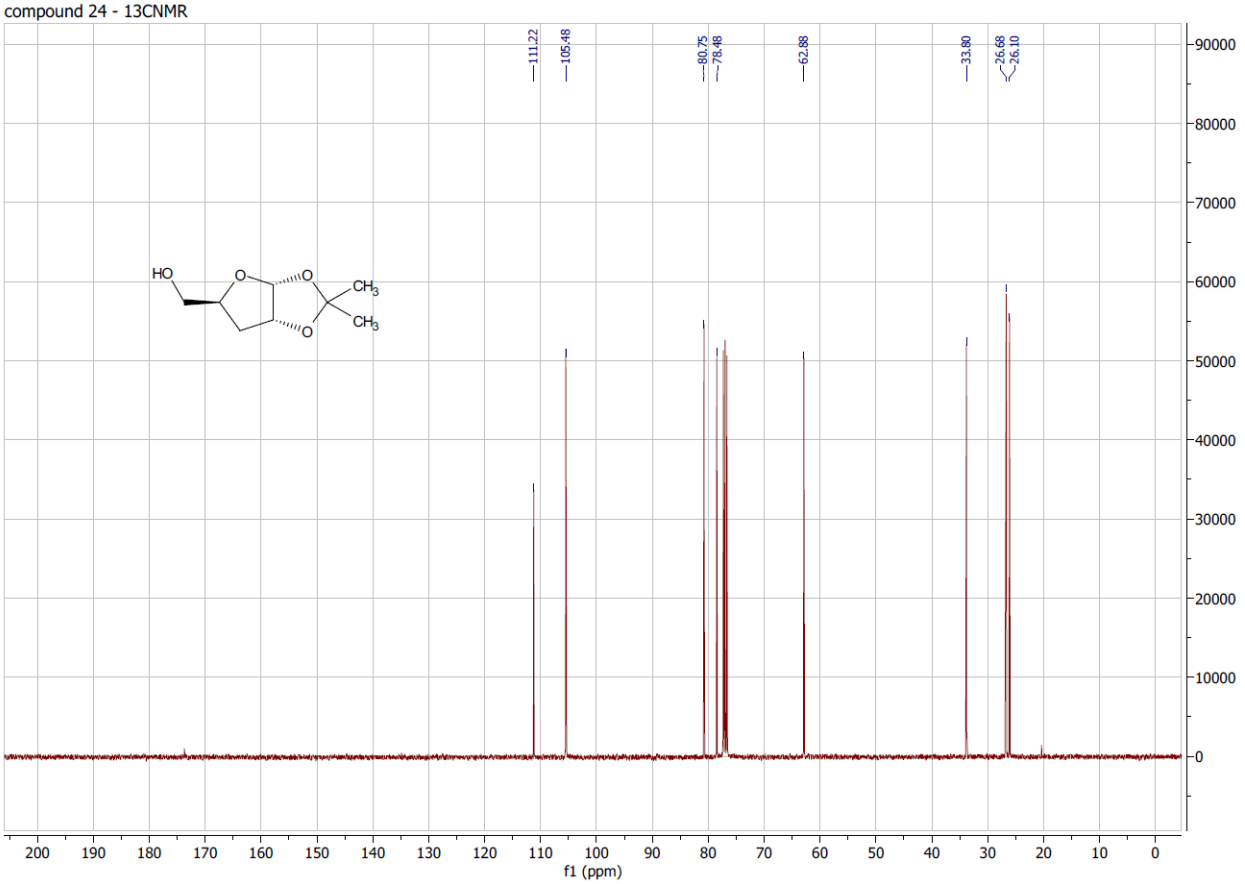
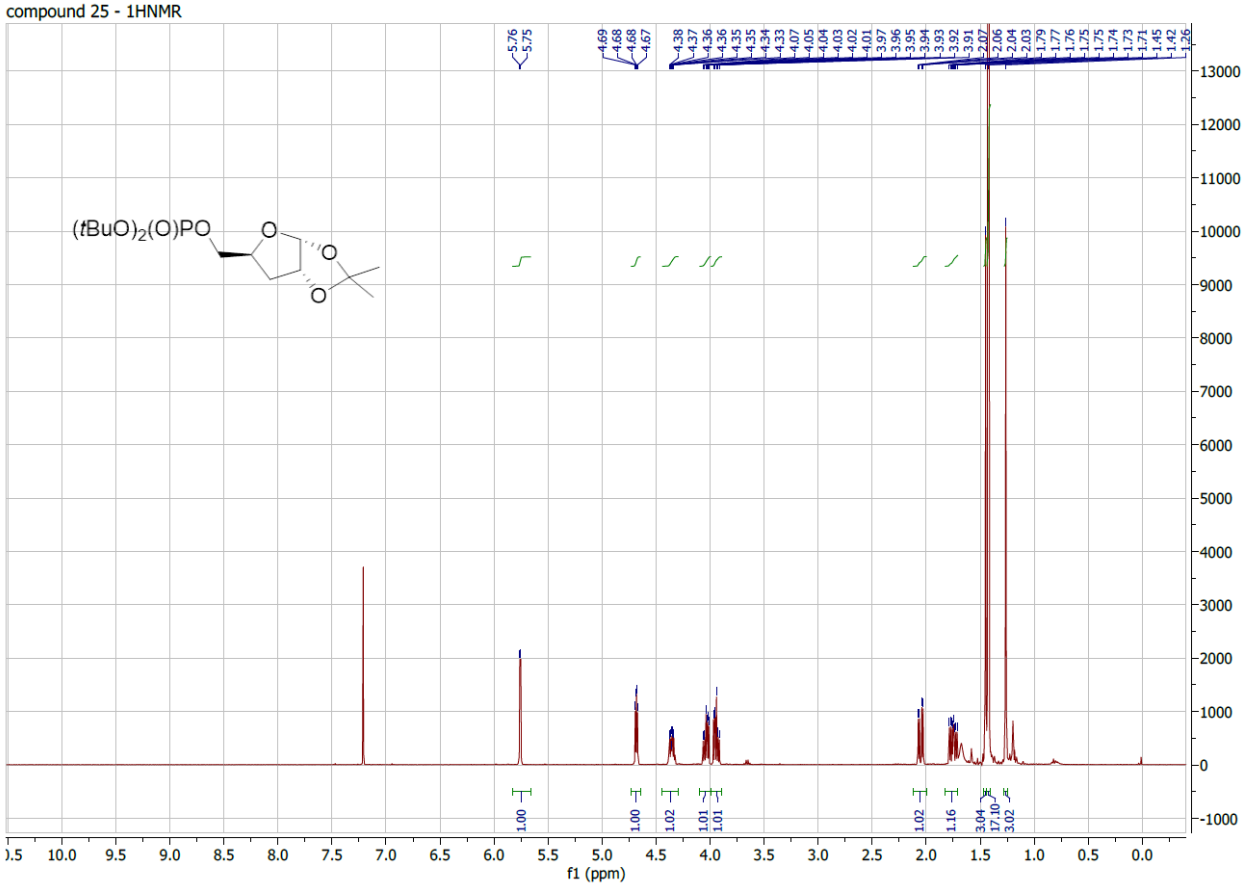
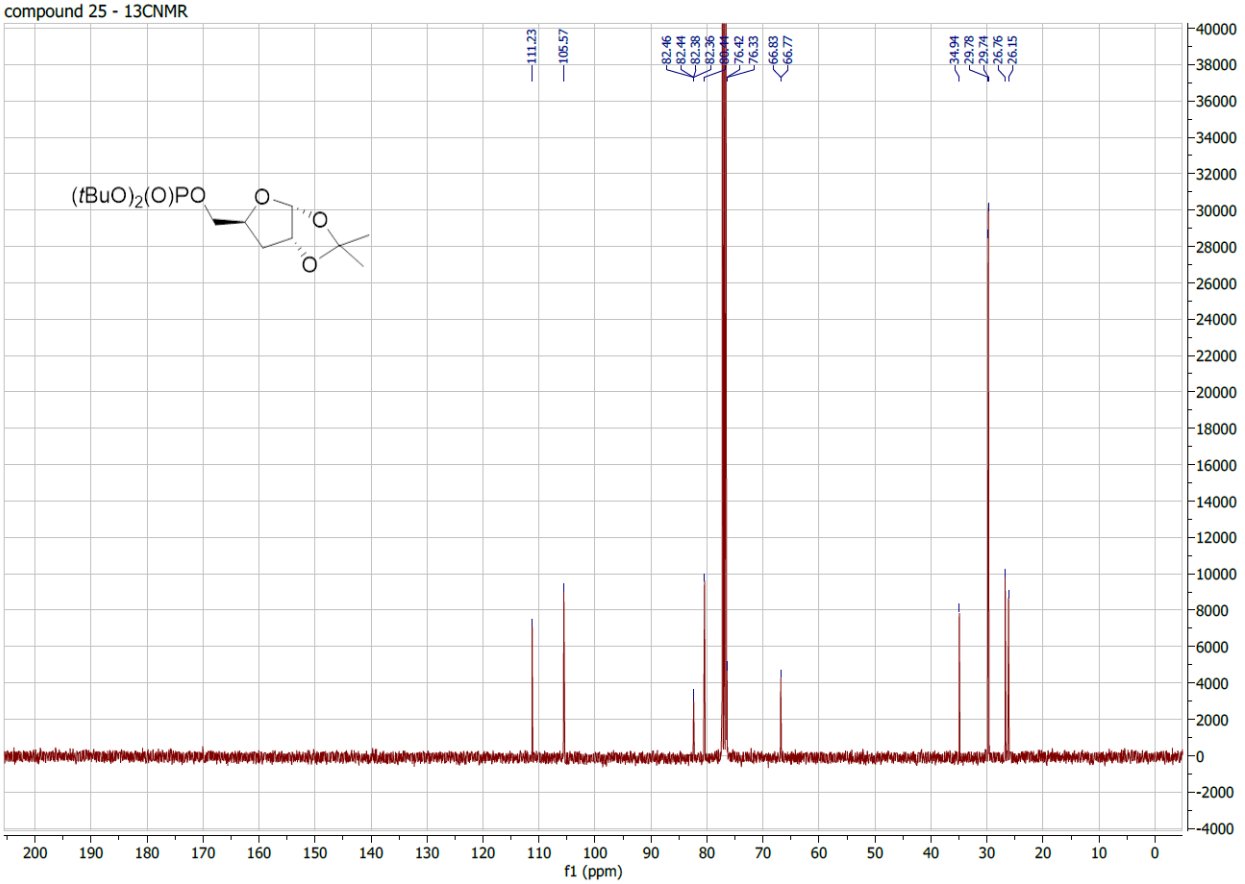


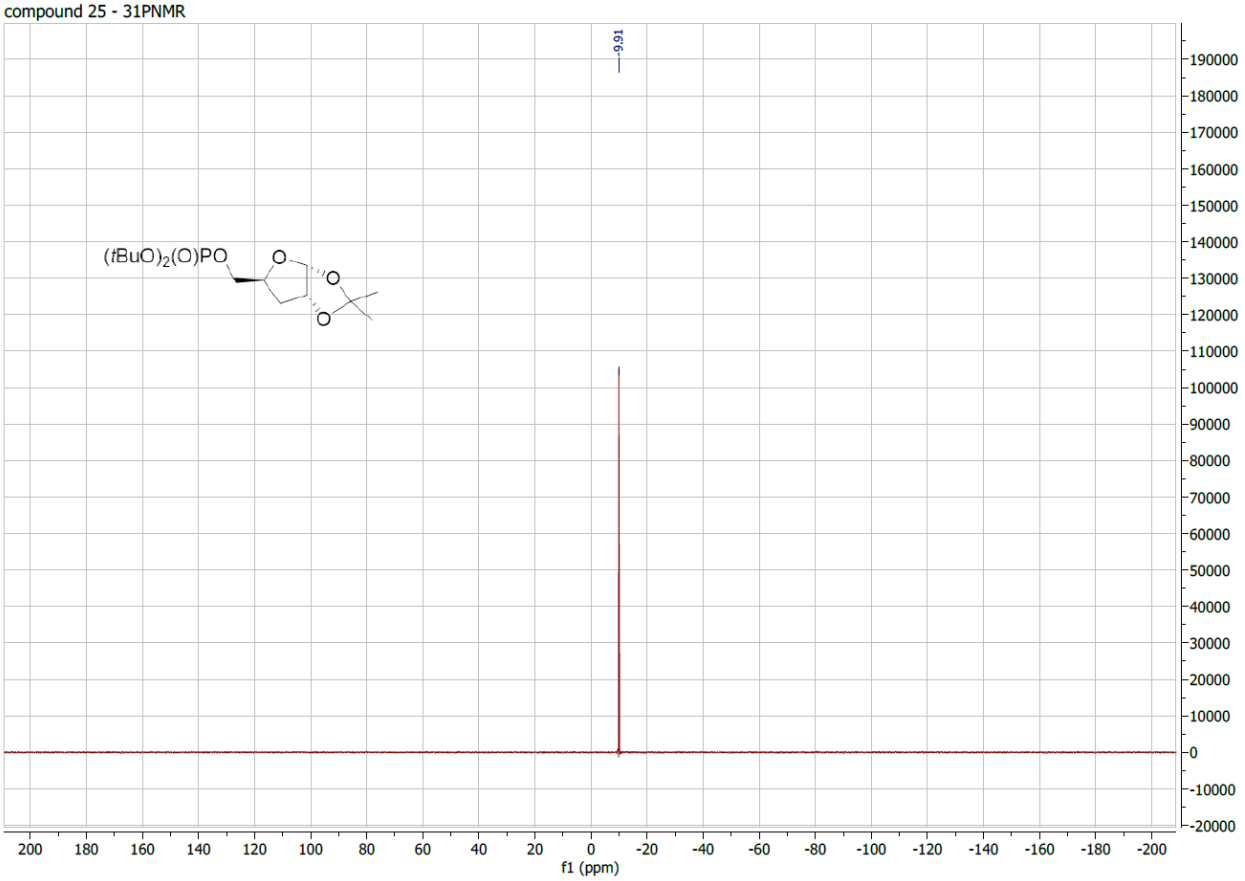
compound 25 - 1HNMR



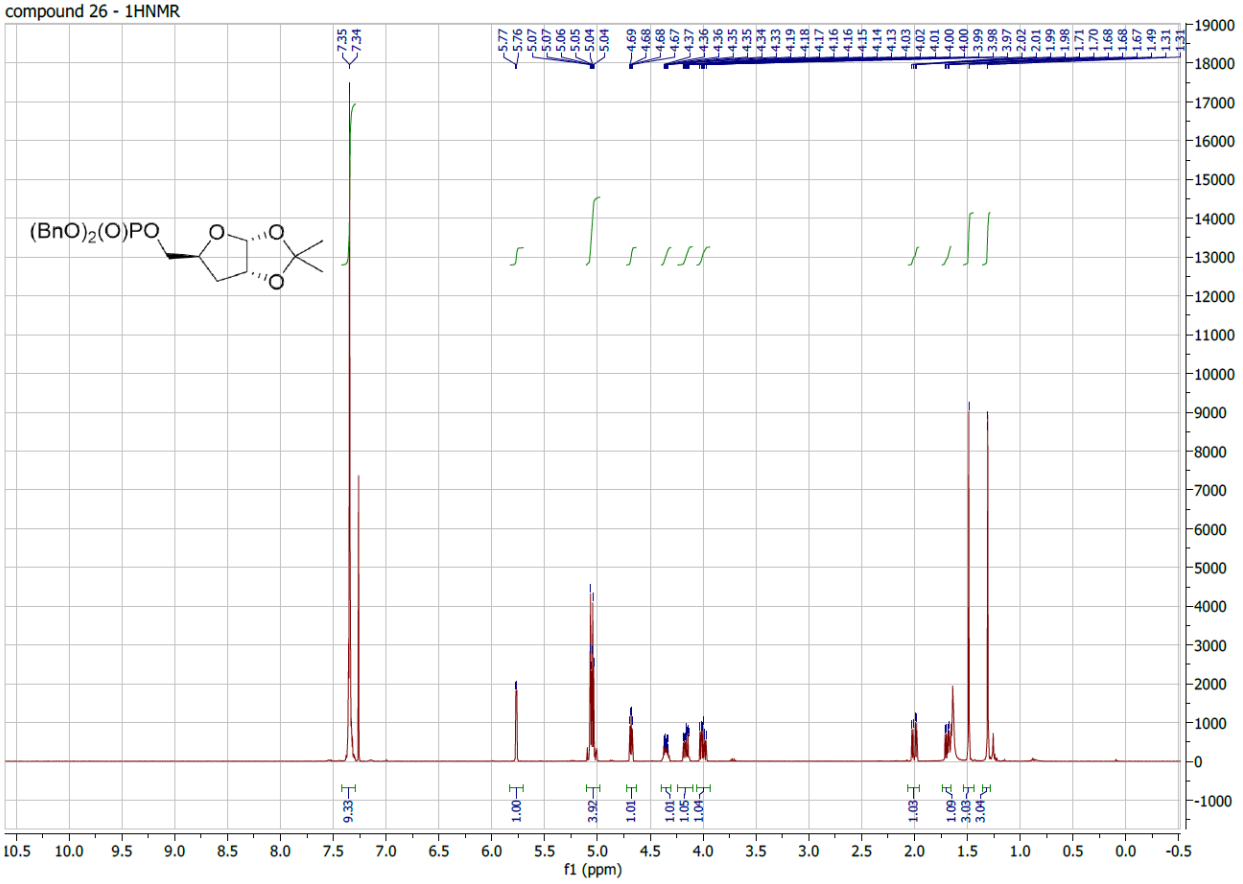
compound 25 - 13CNMR



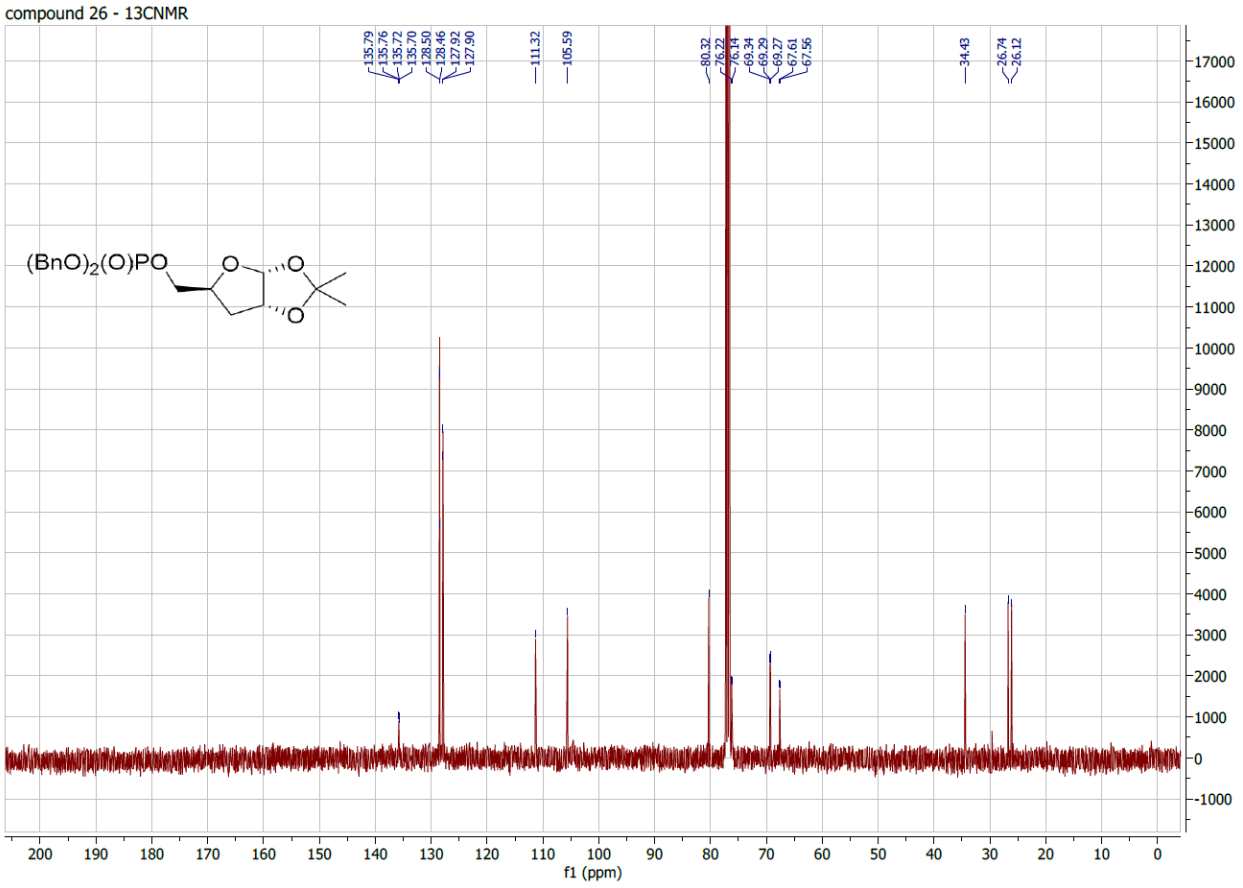
compound 25 - 31PNMR



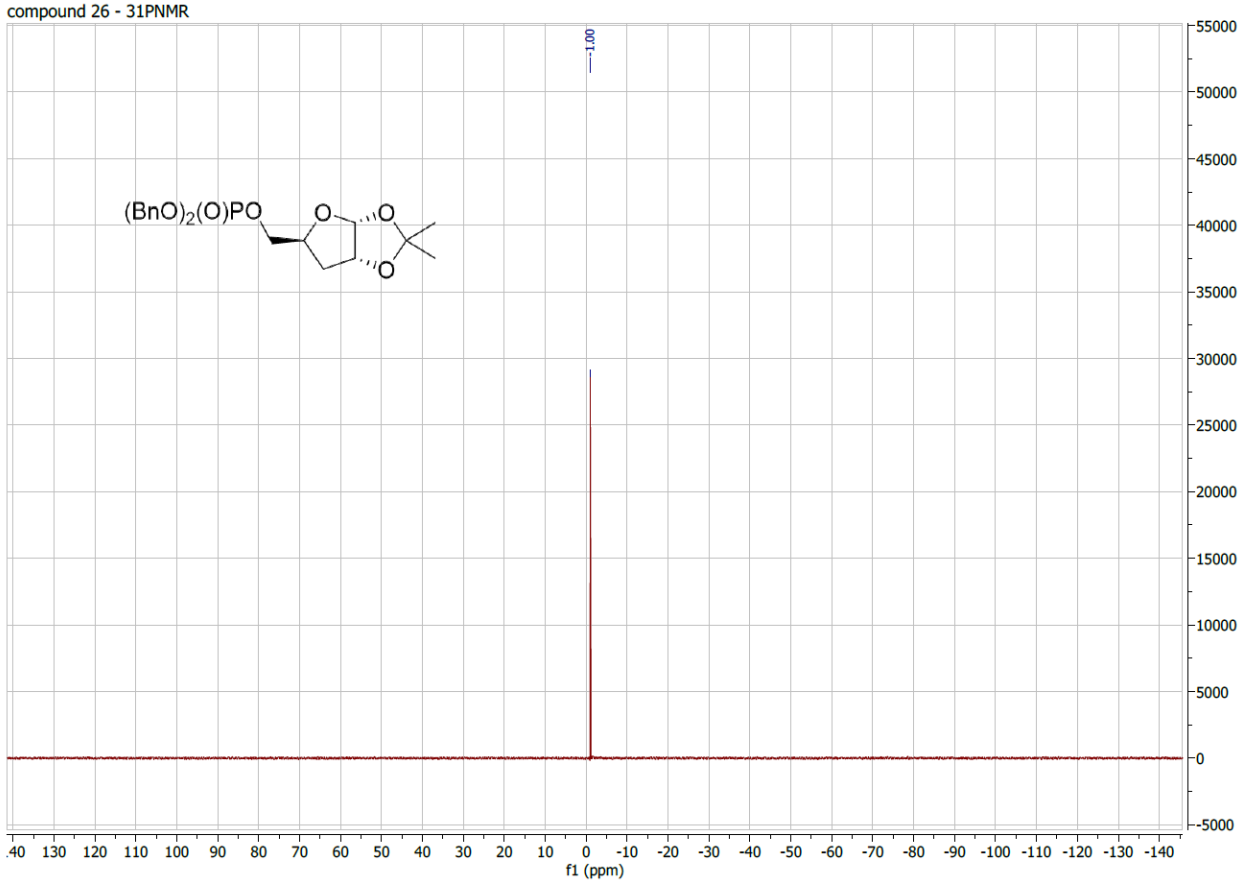
compound 26 - 1HNMR



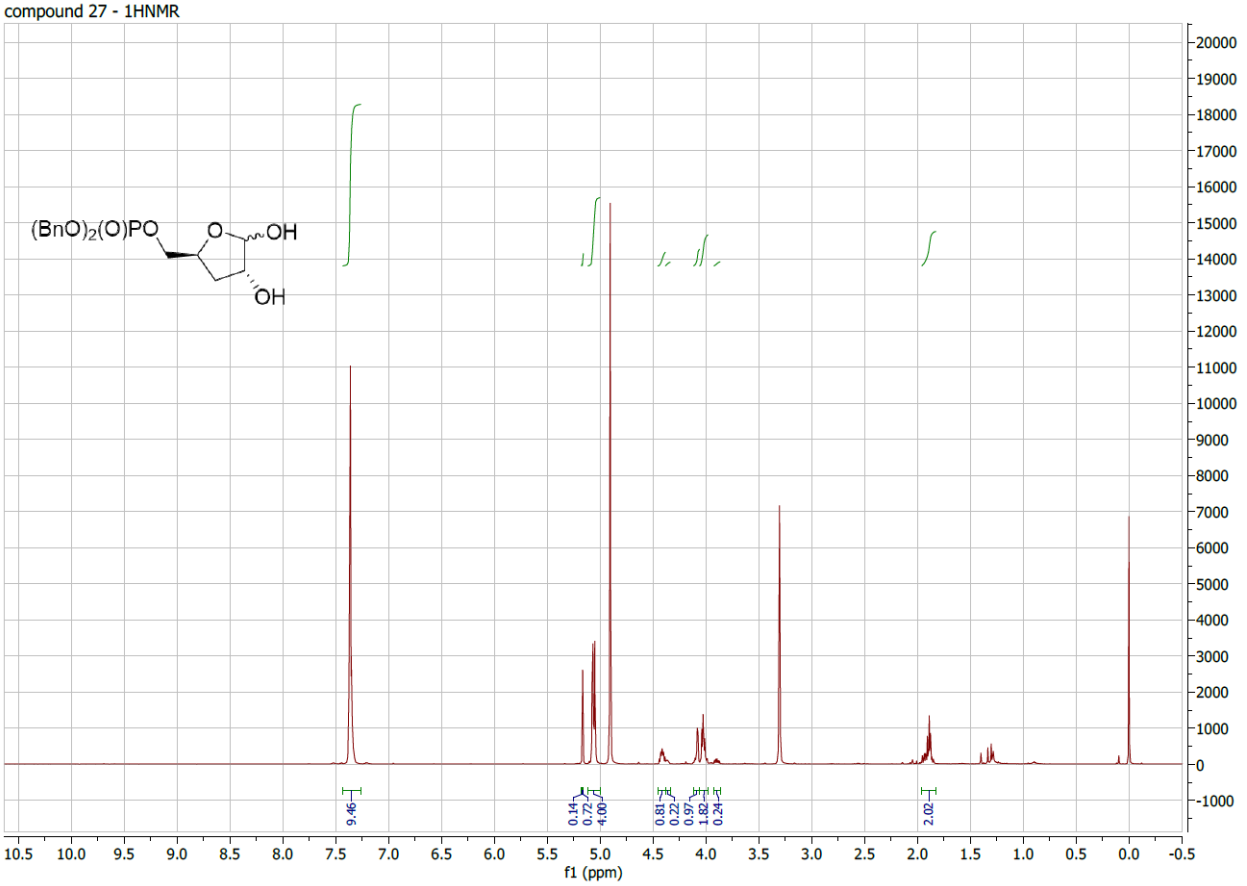
compound 26 - 13CNMR



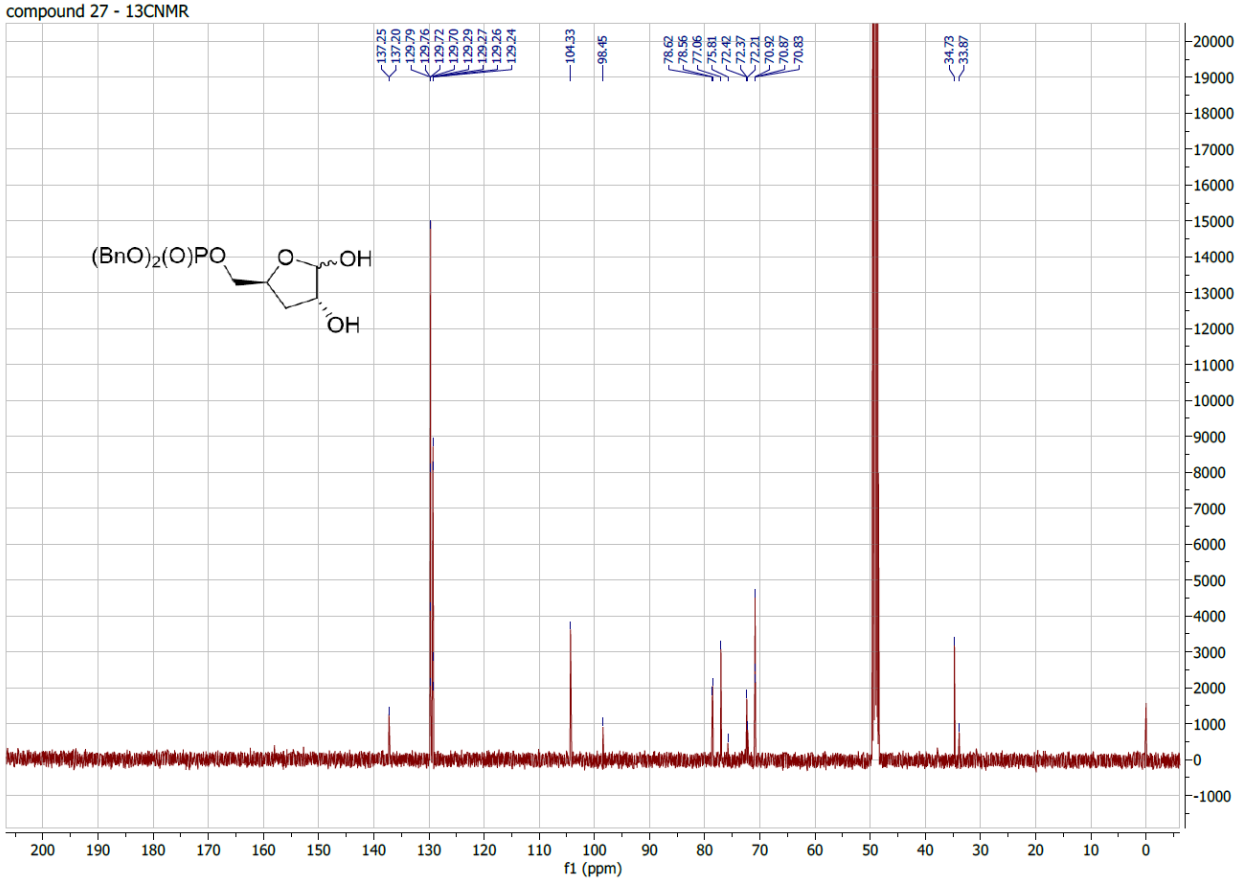
compound 26 - 31PNMR



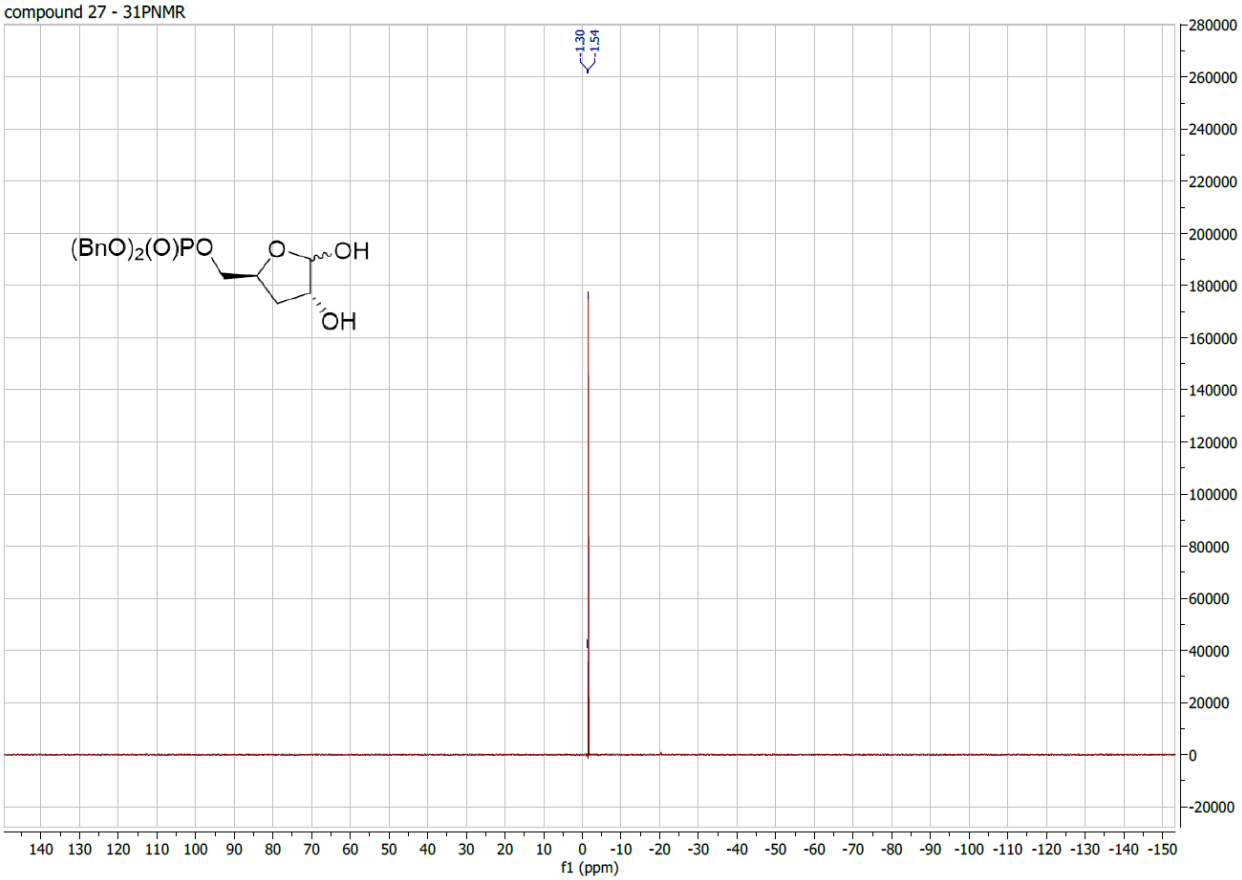
compound 27 - 1HNMR



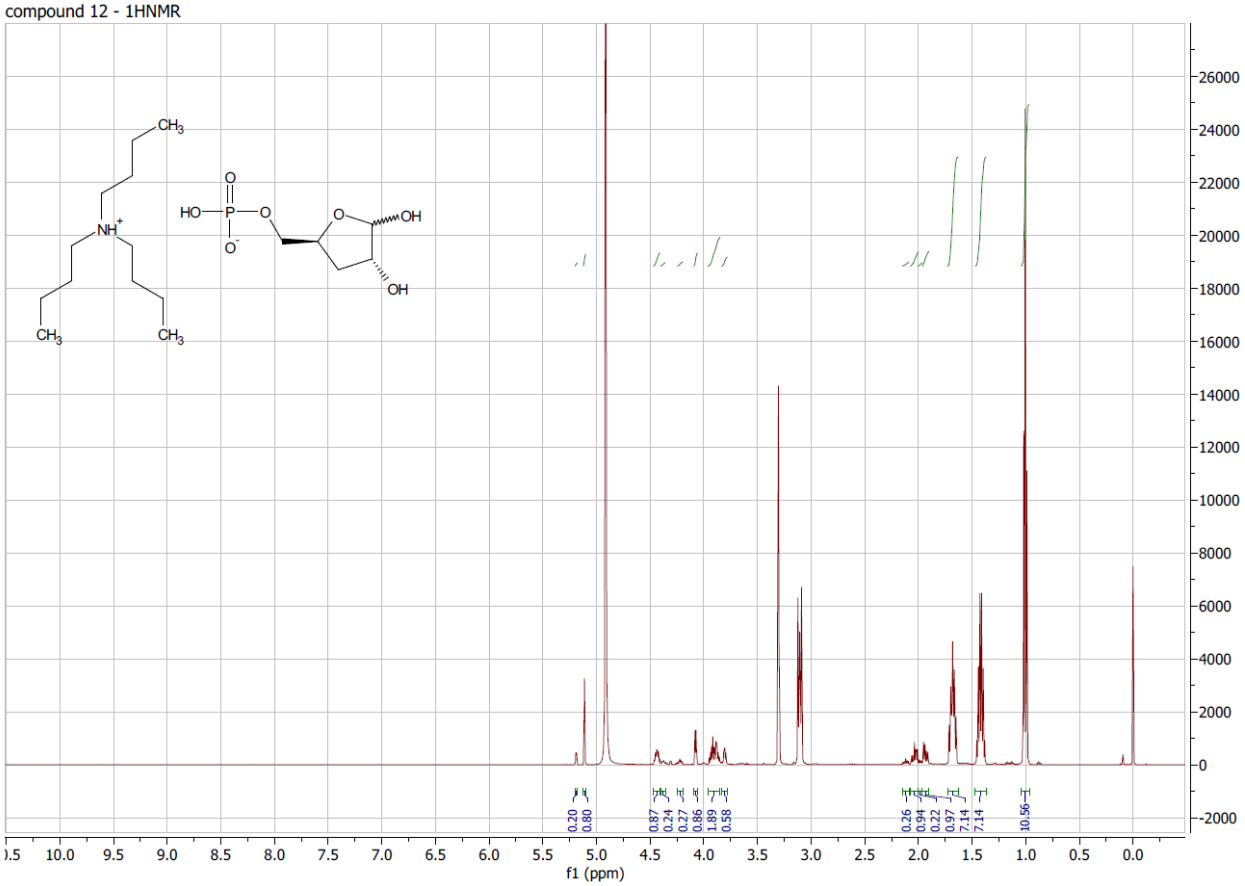
compound 27 - 13CNMR



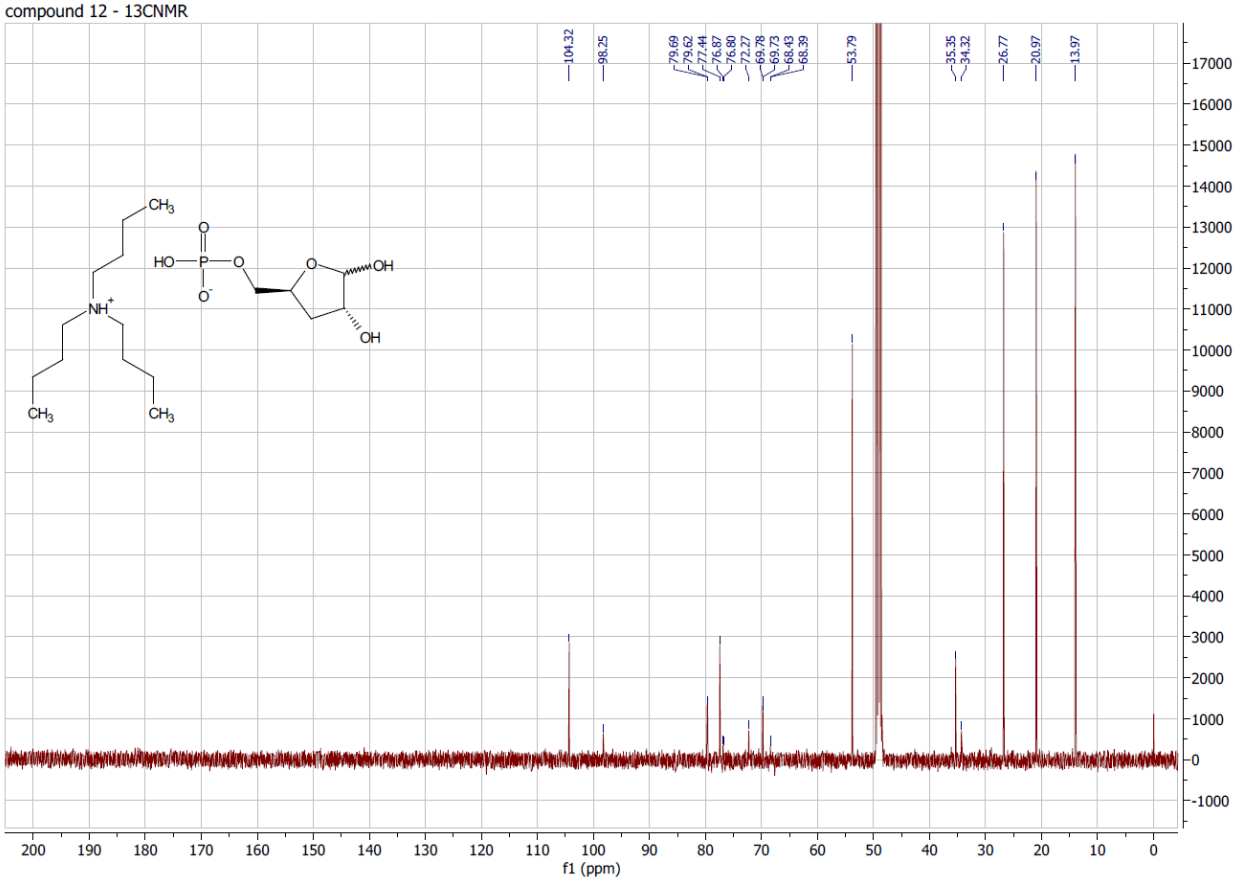
compound 27 - 31PNMR



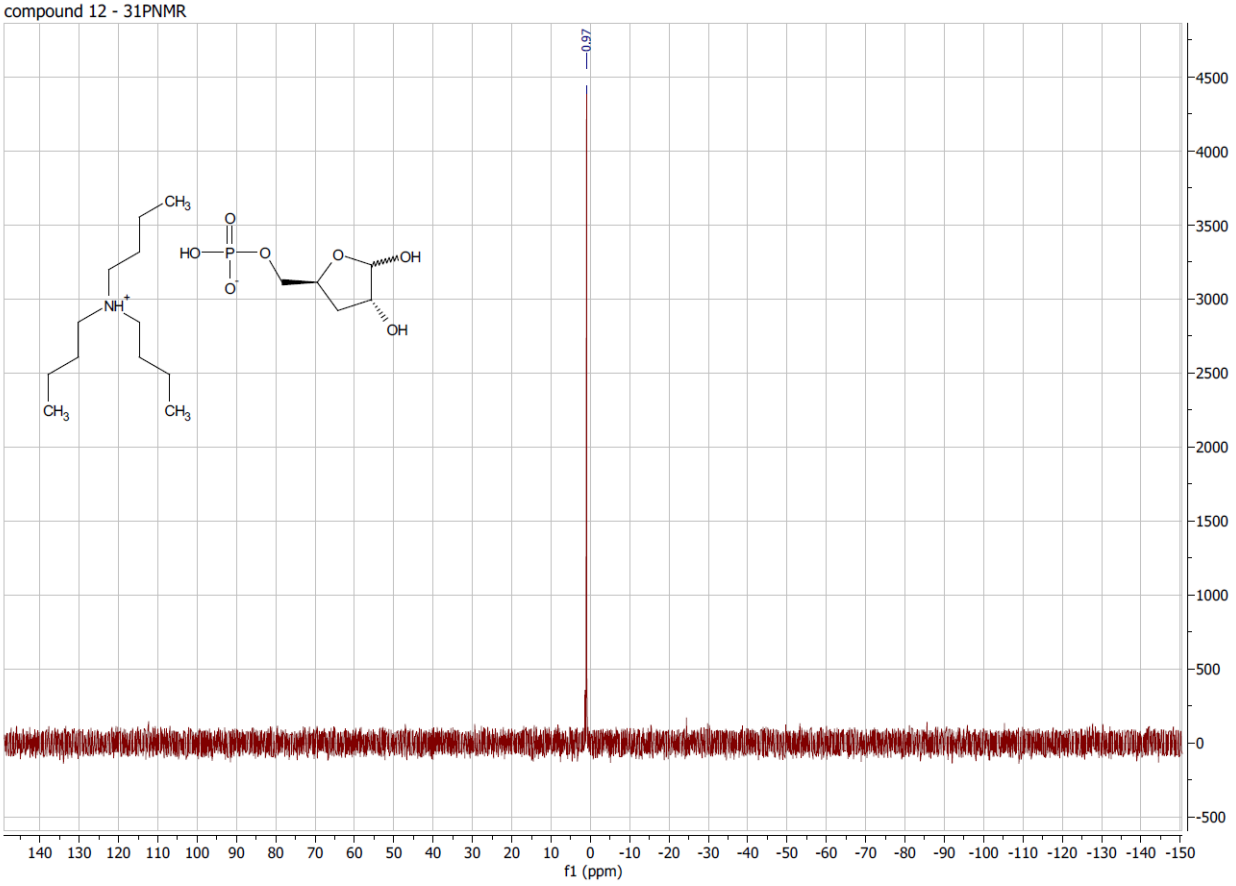
compound 12 - 1HNMR



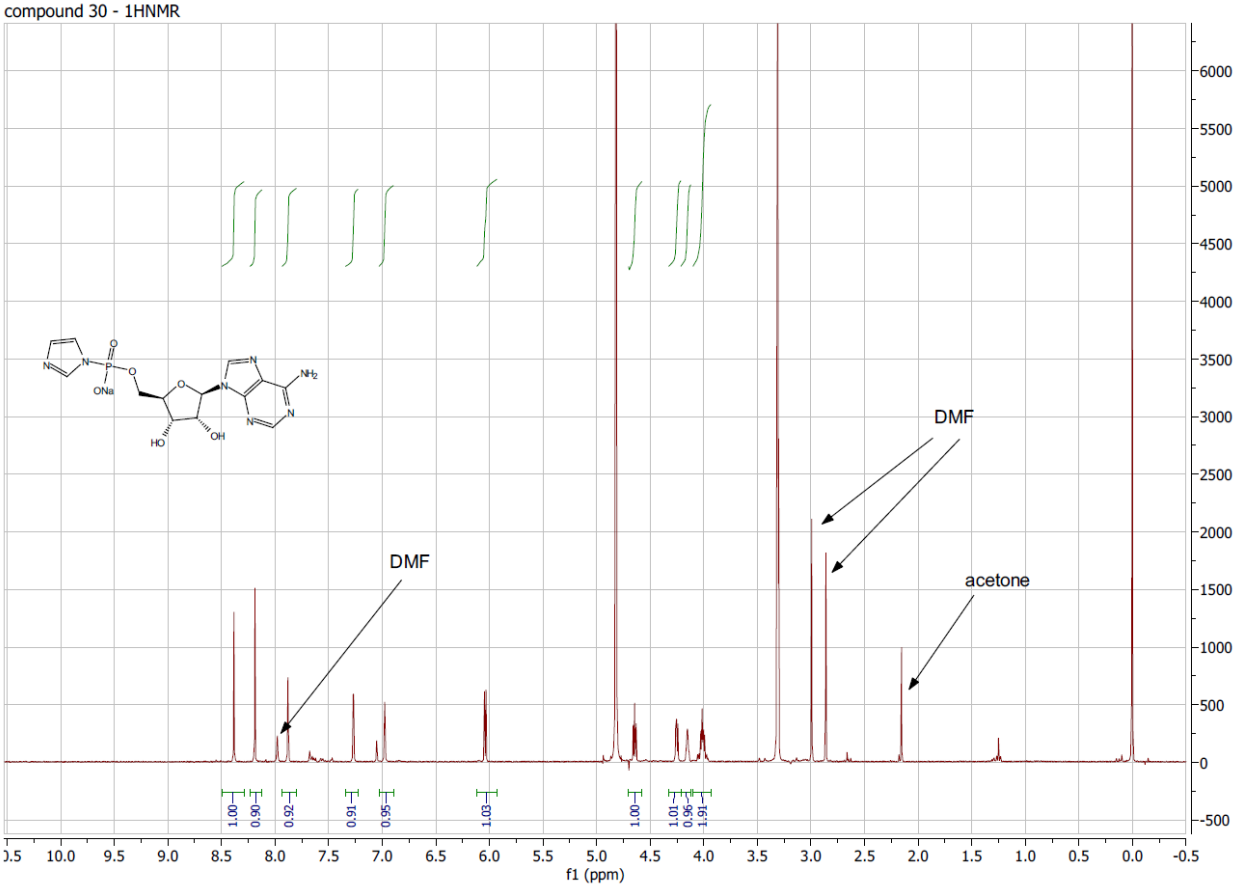
compound 12 - 13CNMR



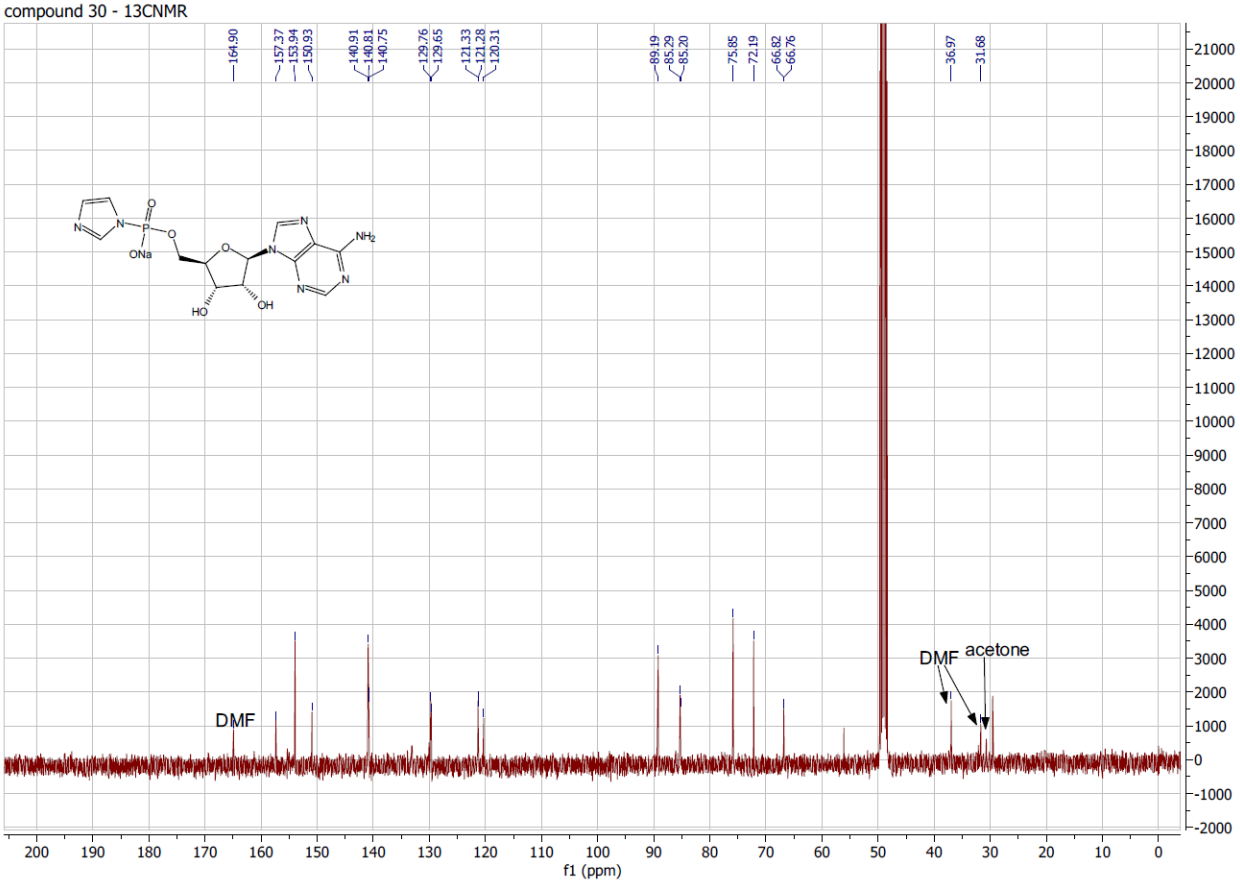
compound 12 - 31PNMR



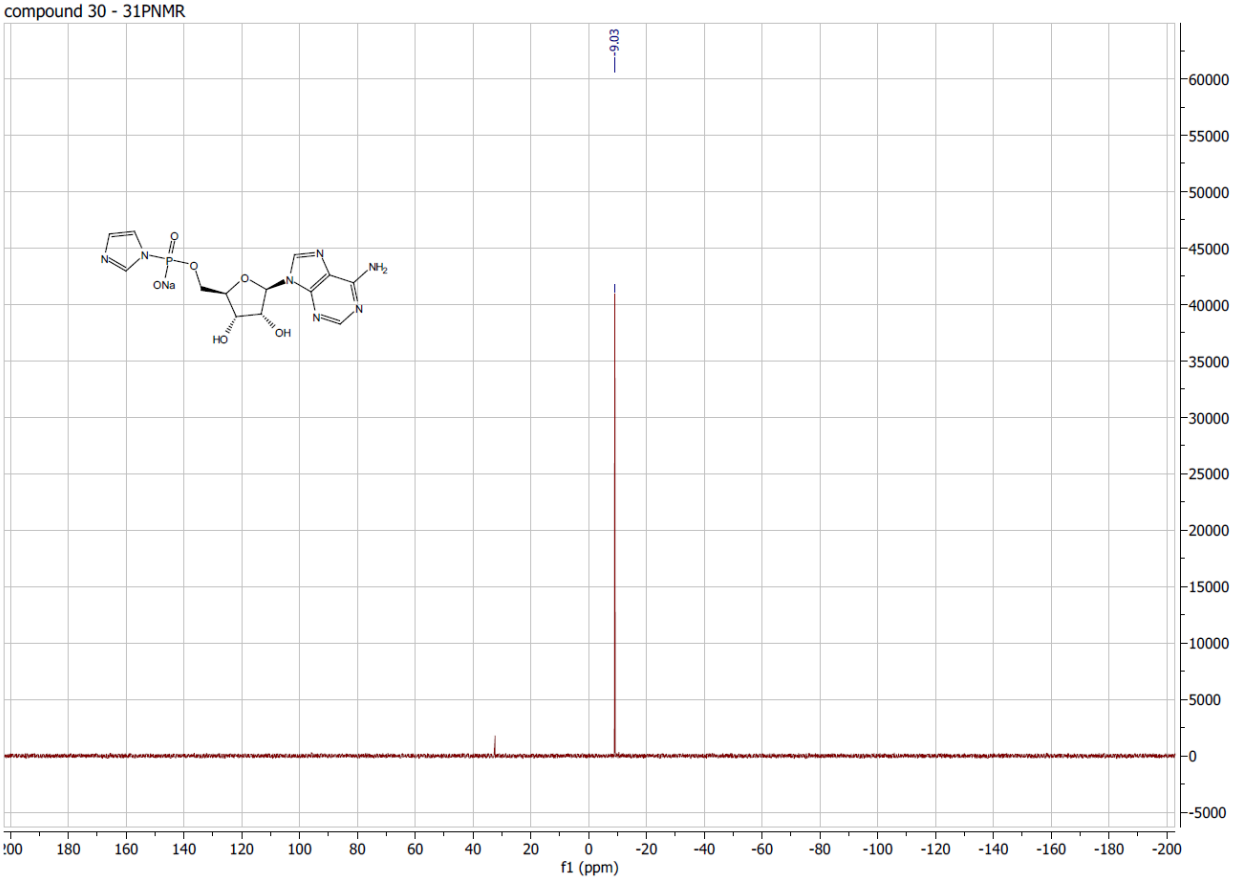
compound 30 - 1HNMR

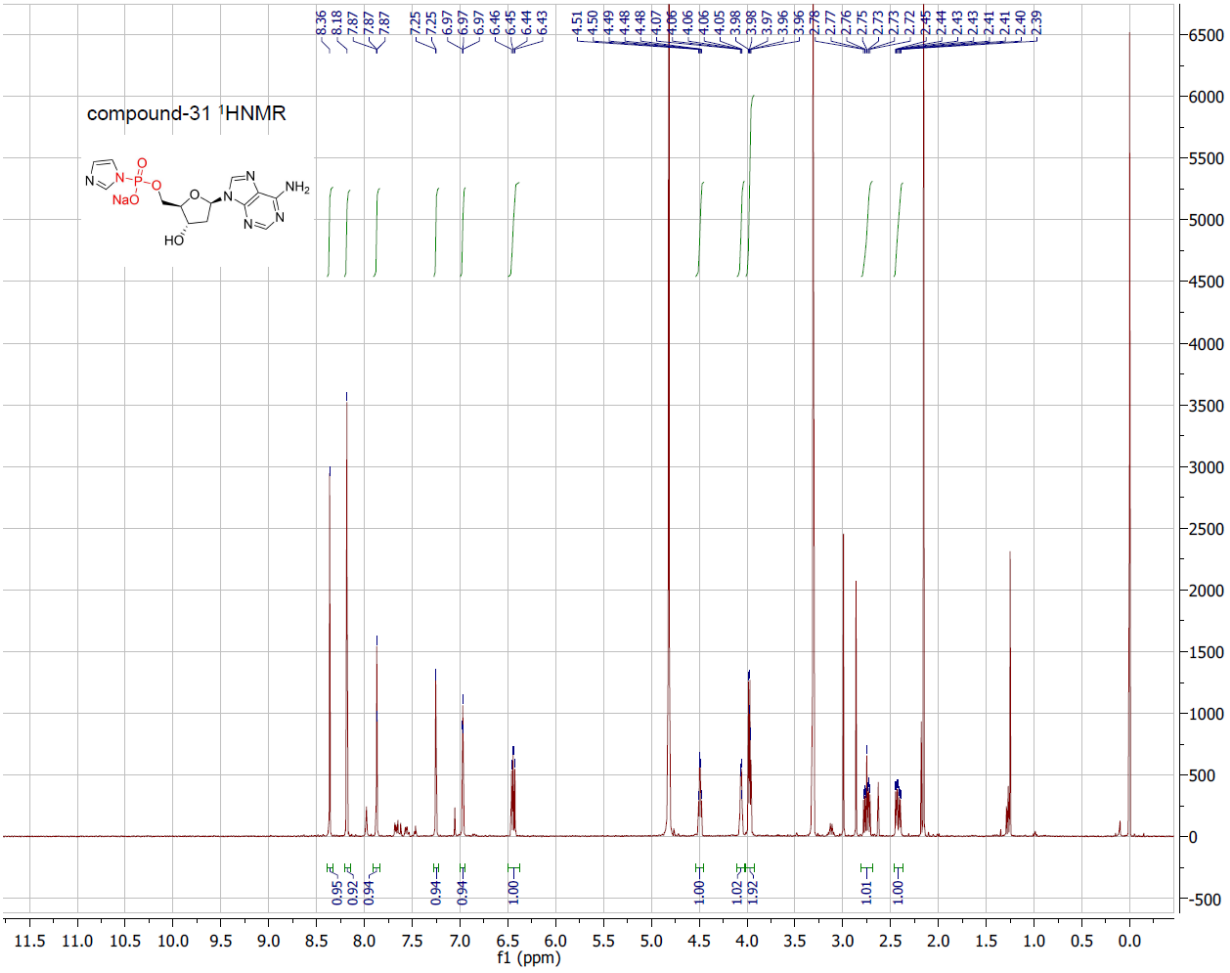


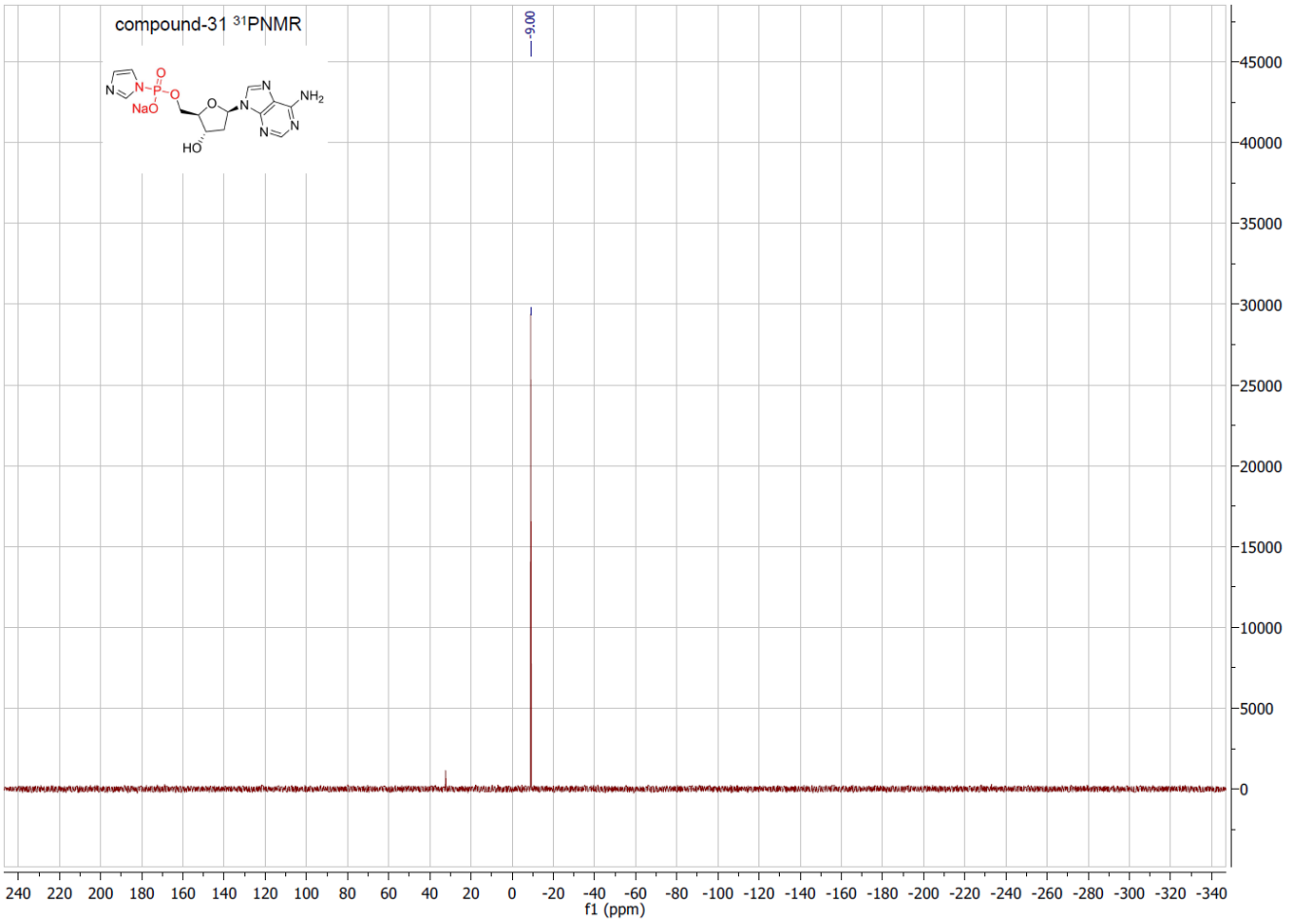
compound 30 - 13CNMR

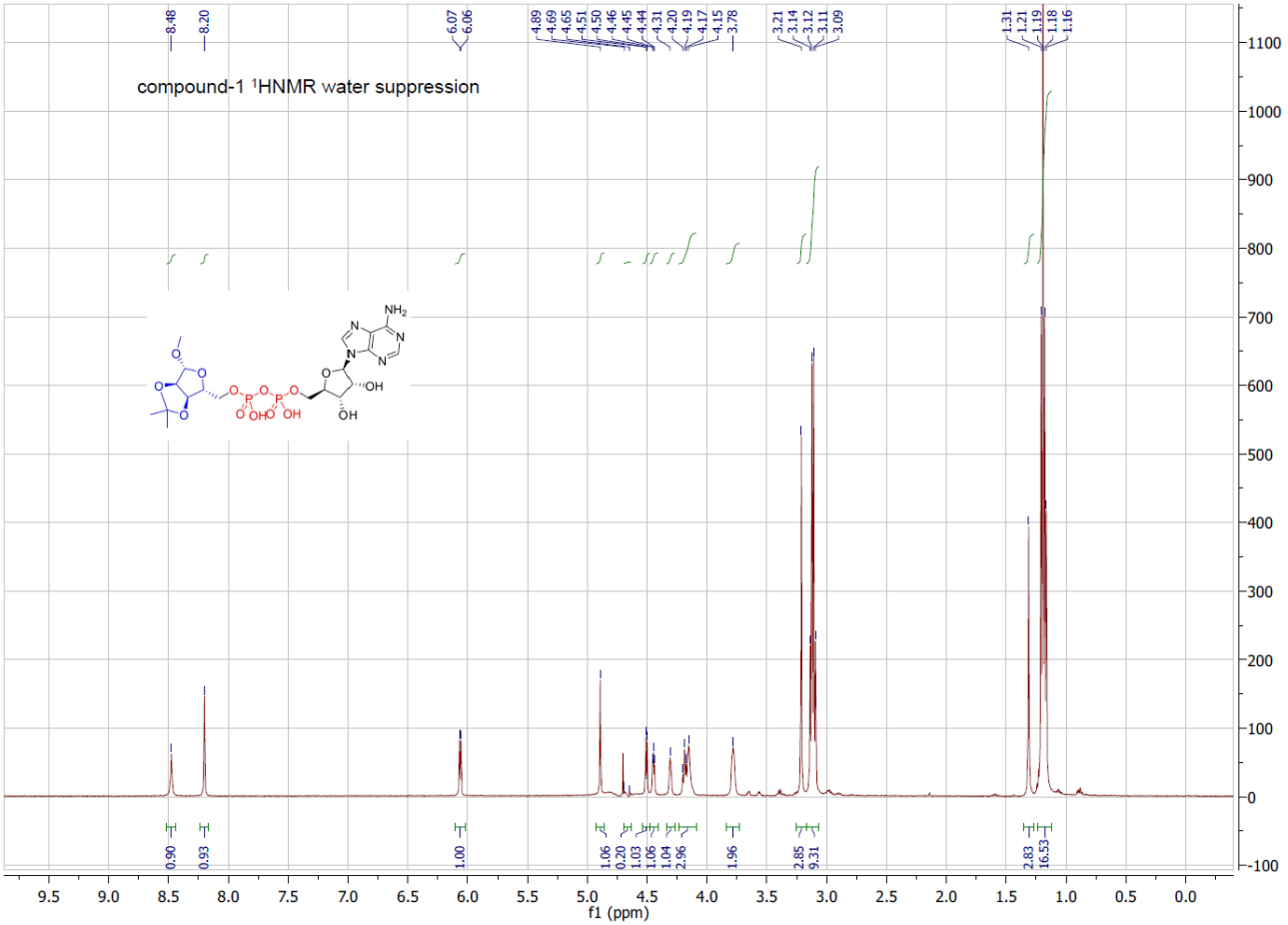


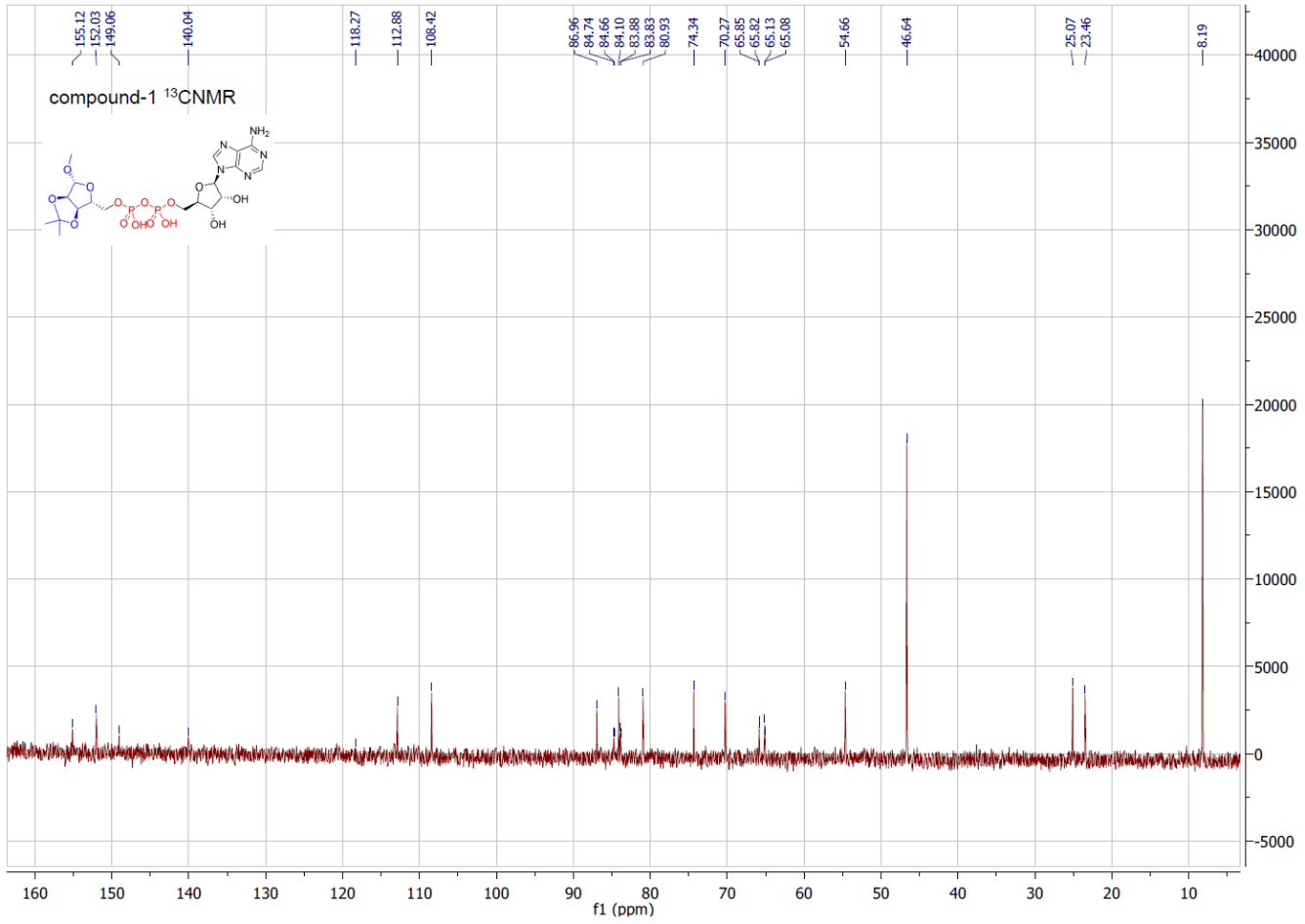
compound 30 - 31PNMR

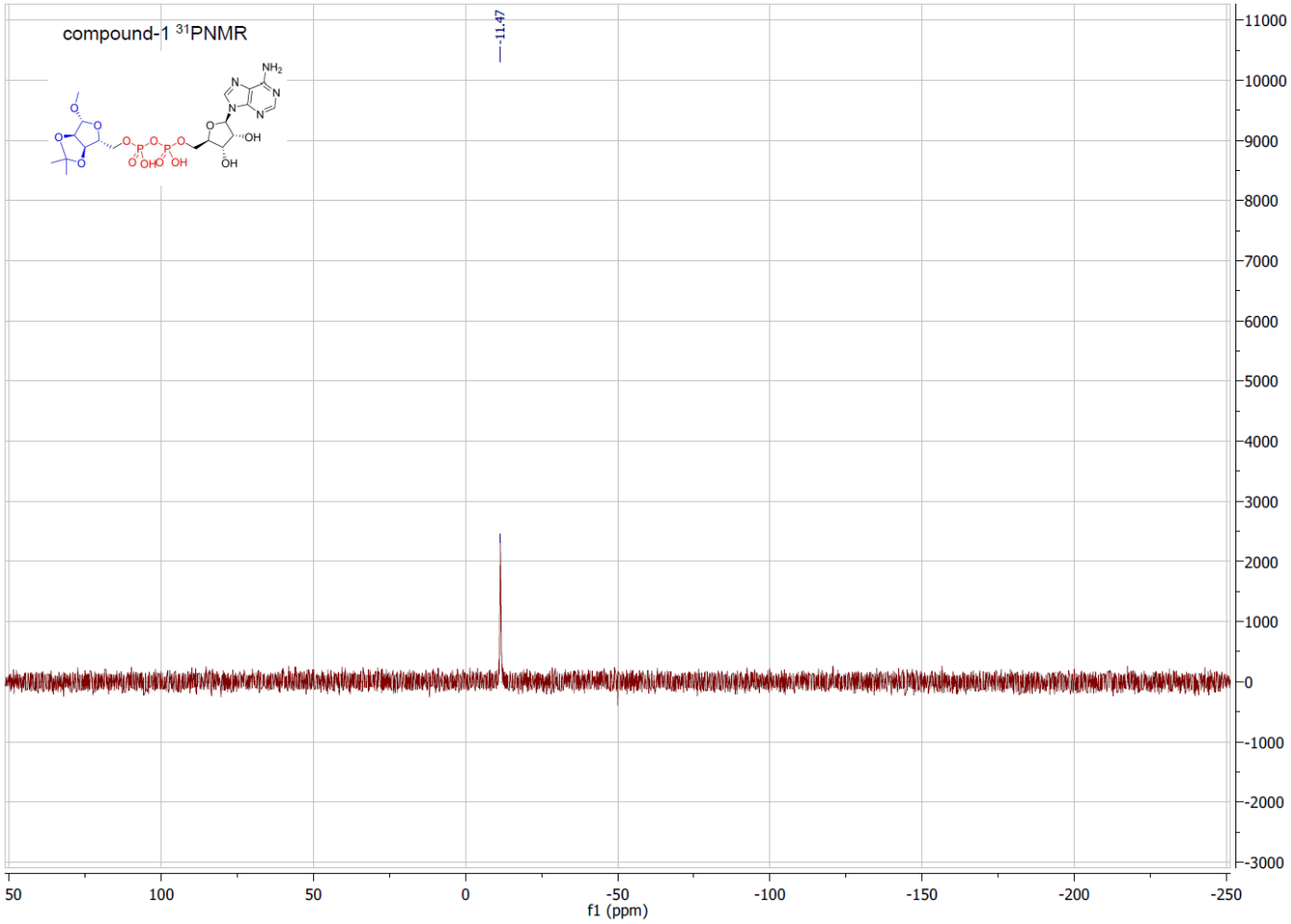


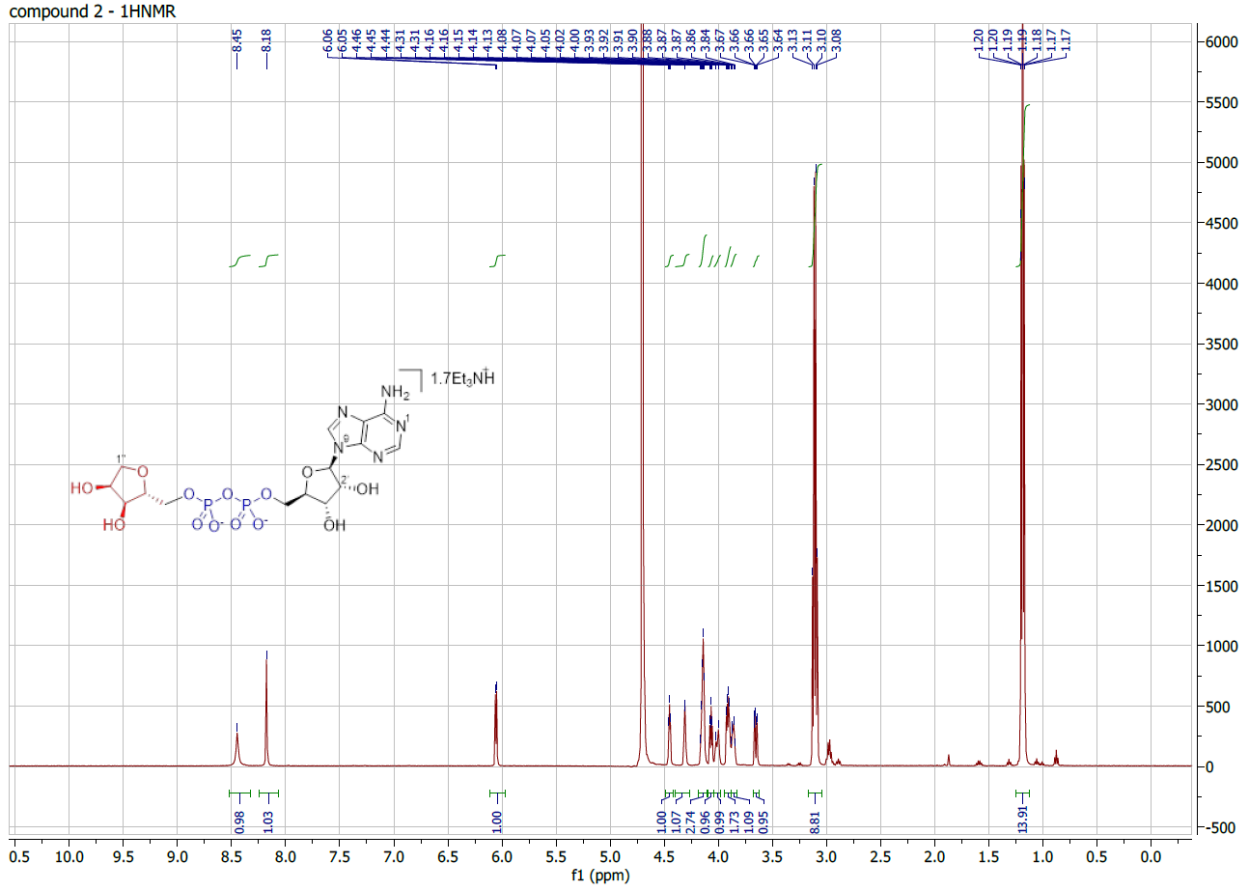

compound-31 1HNMR
f1 (ppm)


compound-31 31PNMR
-9.00
f1 (ppm)

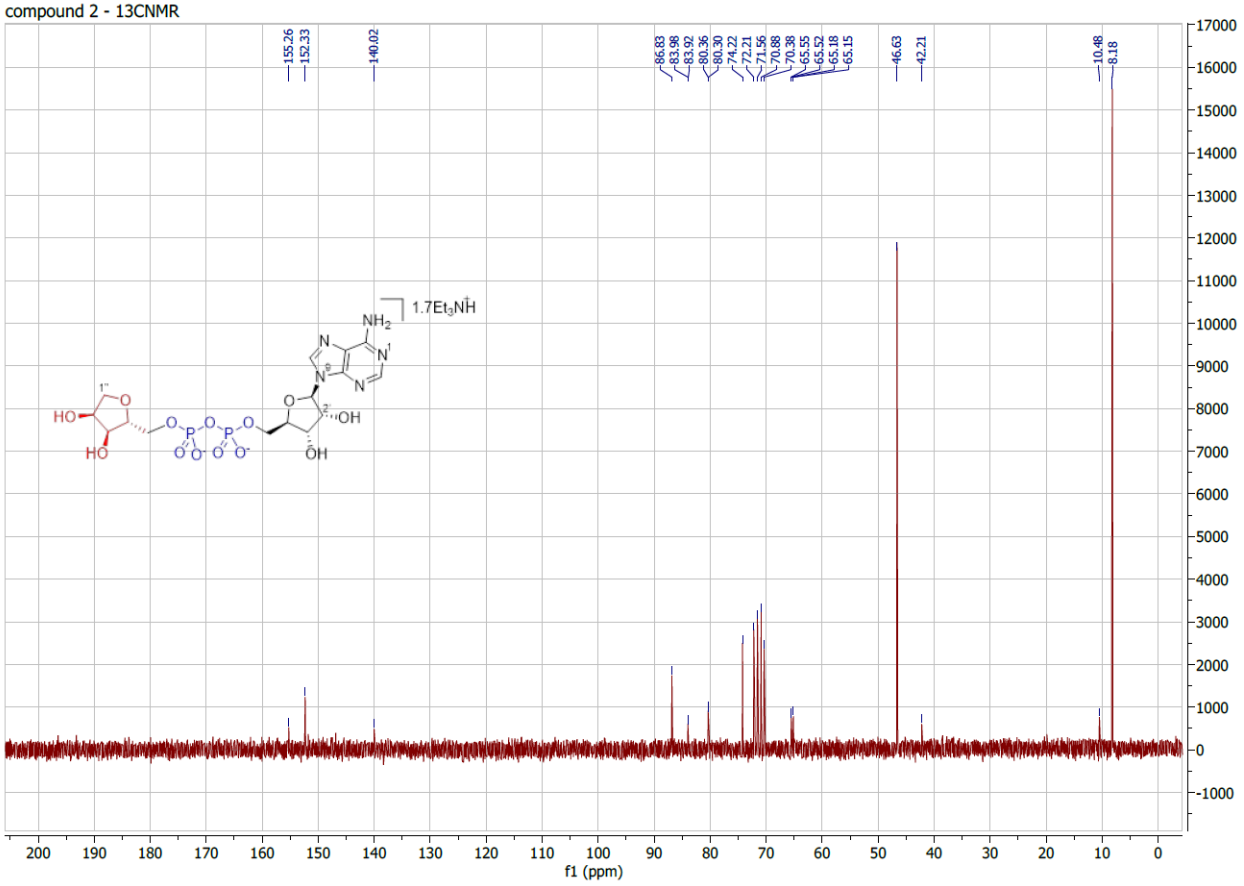

compound-1 ¹HNMR water suppression
8.48
8.20
6.07
6.06
4.89
4.69
4.65
4.51
4.50
4.46
4.45
4.44
4.31
4.20
4.19
4.17
4.15
3.78
3.21
3.14
3.12
3.11
3.09
1.31
1.21
1.19
1.18
1.16
0.90
0.93
1.00
1.06
0.20
1.03
1.06
1.04
2.96
1.96
2.85
9.31
2.83
16.53
f1 (ppm)


compound-1 ¹³CNMR
155.12
152.03
149.06
140.04
118.27
112.88
108.42
86.96
84.74
84.66
84.10
83.88
83.83
80.93
74.34
70.27
65.85
65.82
65.13
65.08
54.66
46.64
25.07
23.46
8.19
f1 (ppm)

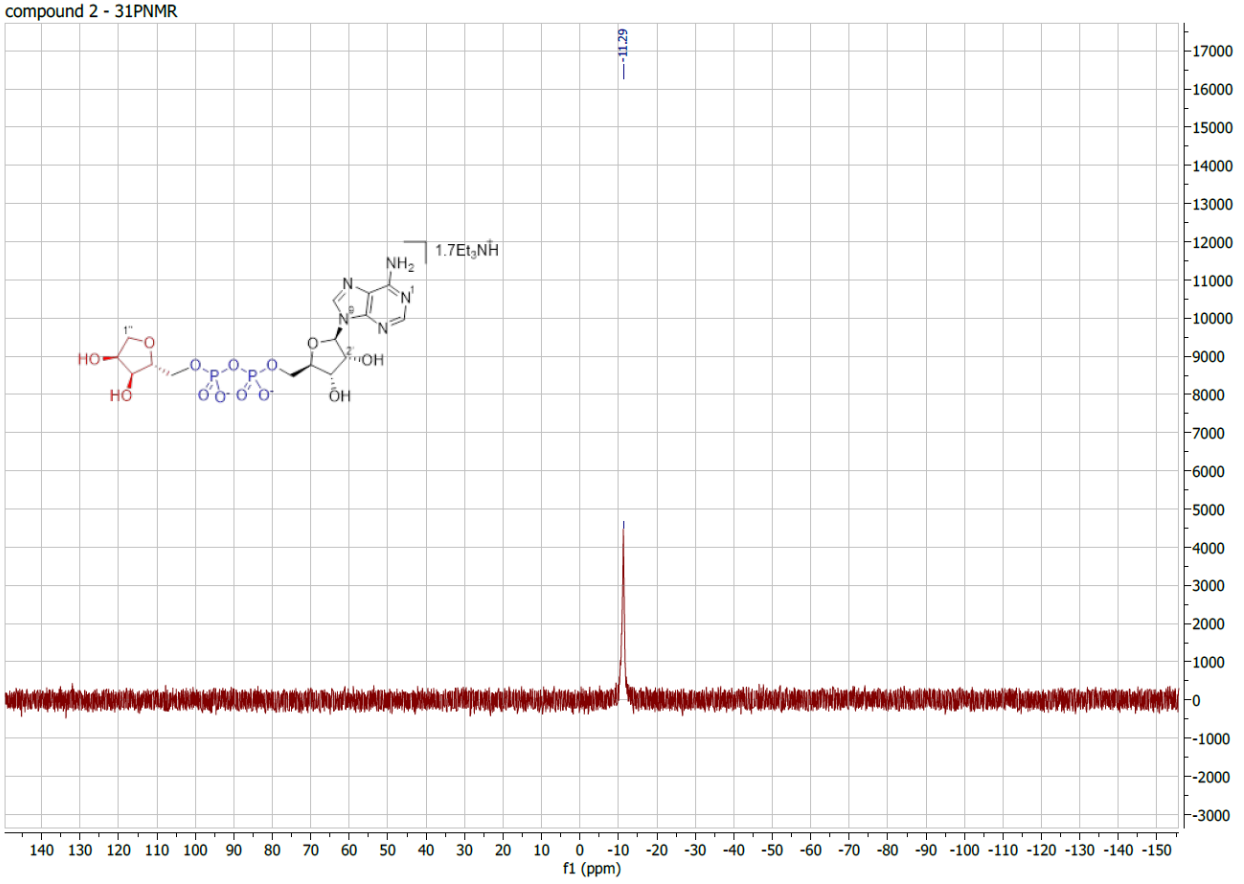

compound-1 31PNMR
-11.47
f1 (ppm)

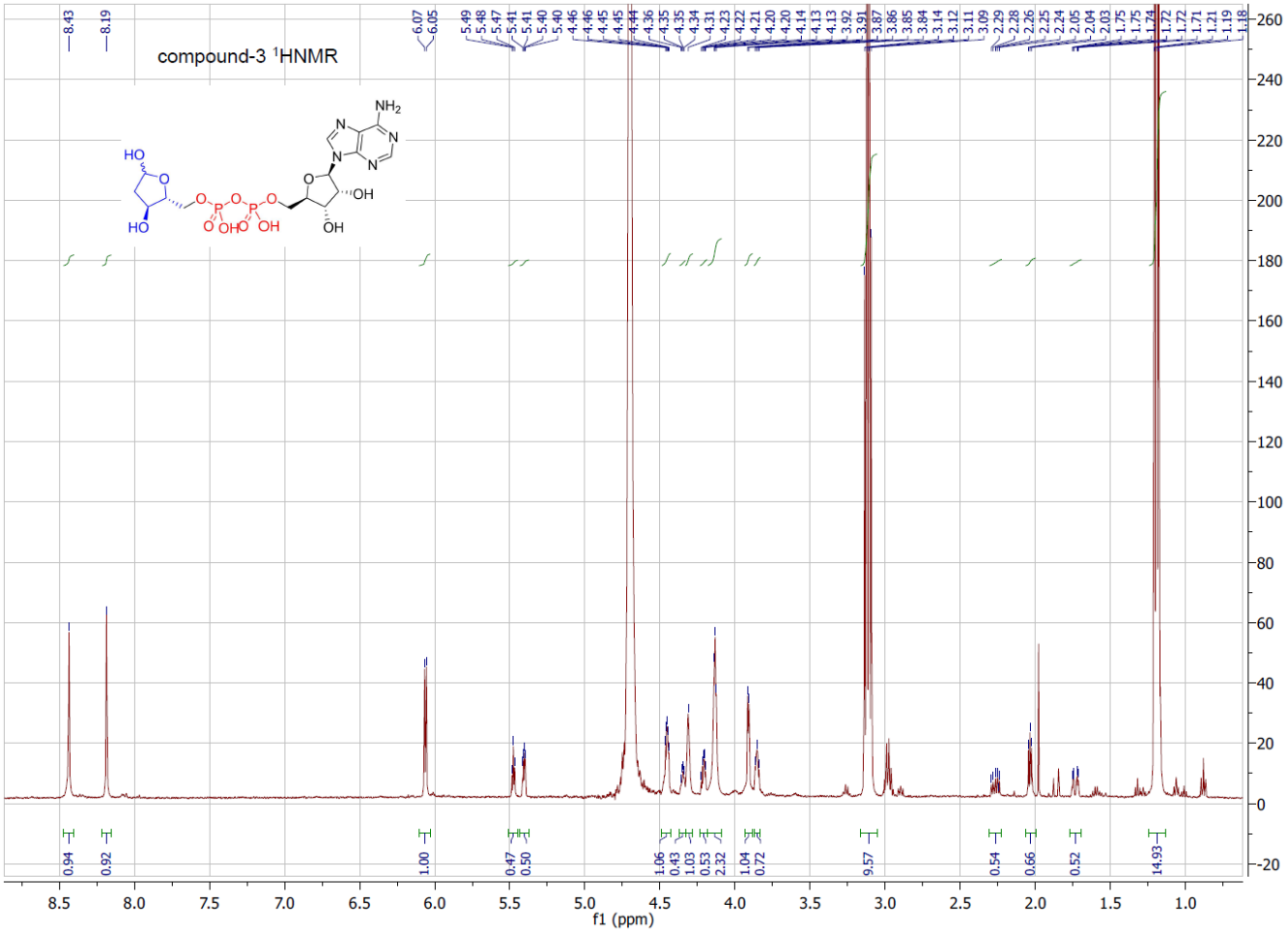

compound 2 - 1HNMR
1.7Et3NH
f1 (ppm)

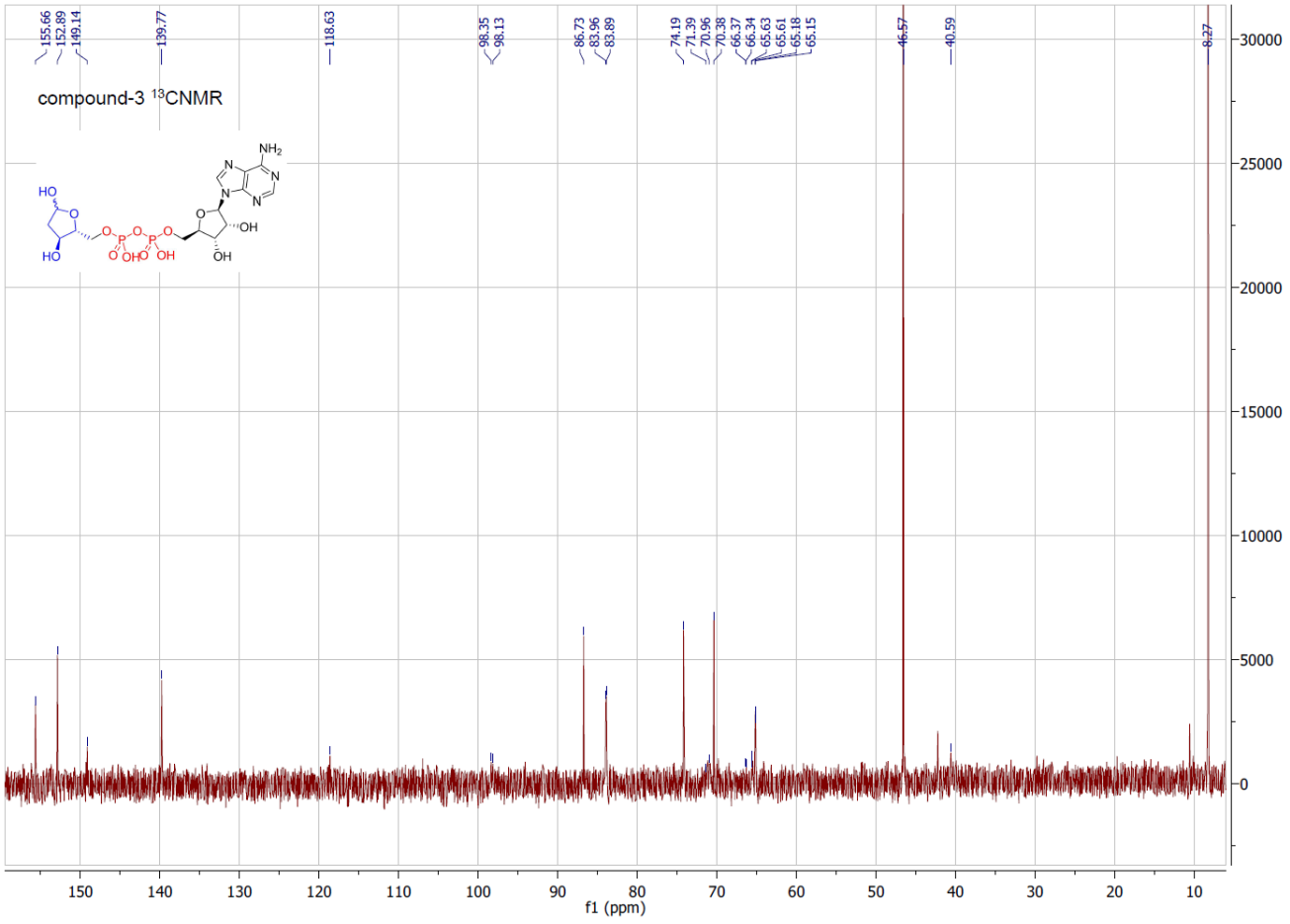
compound 2 - 13CNMR

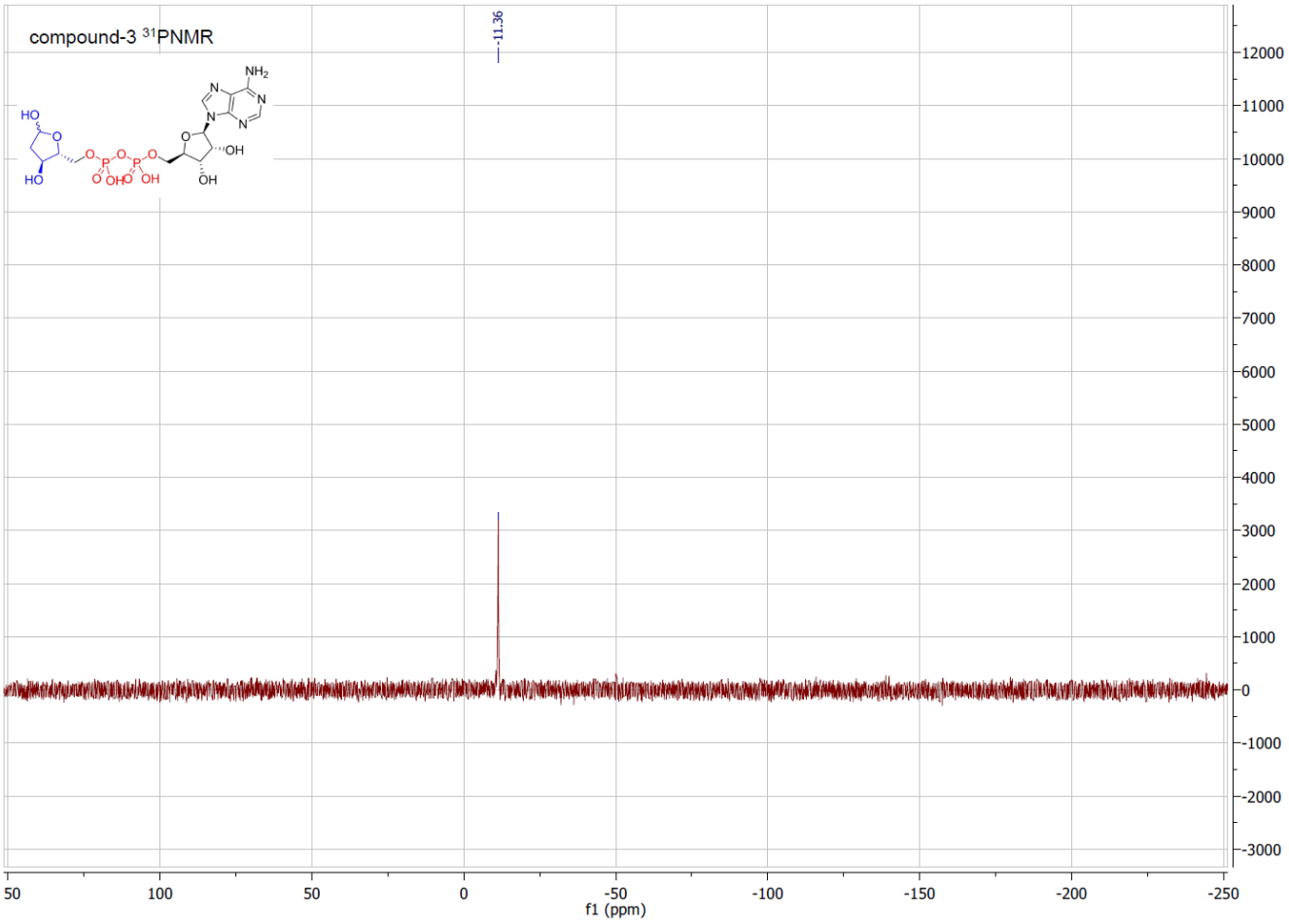


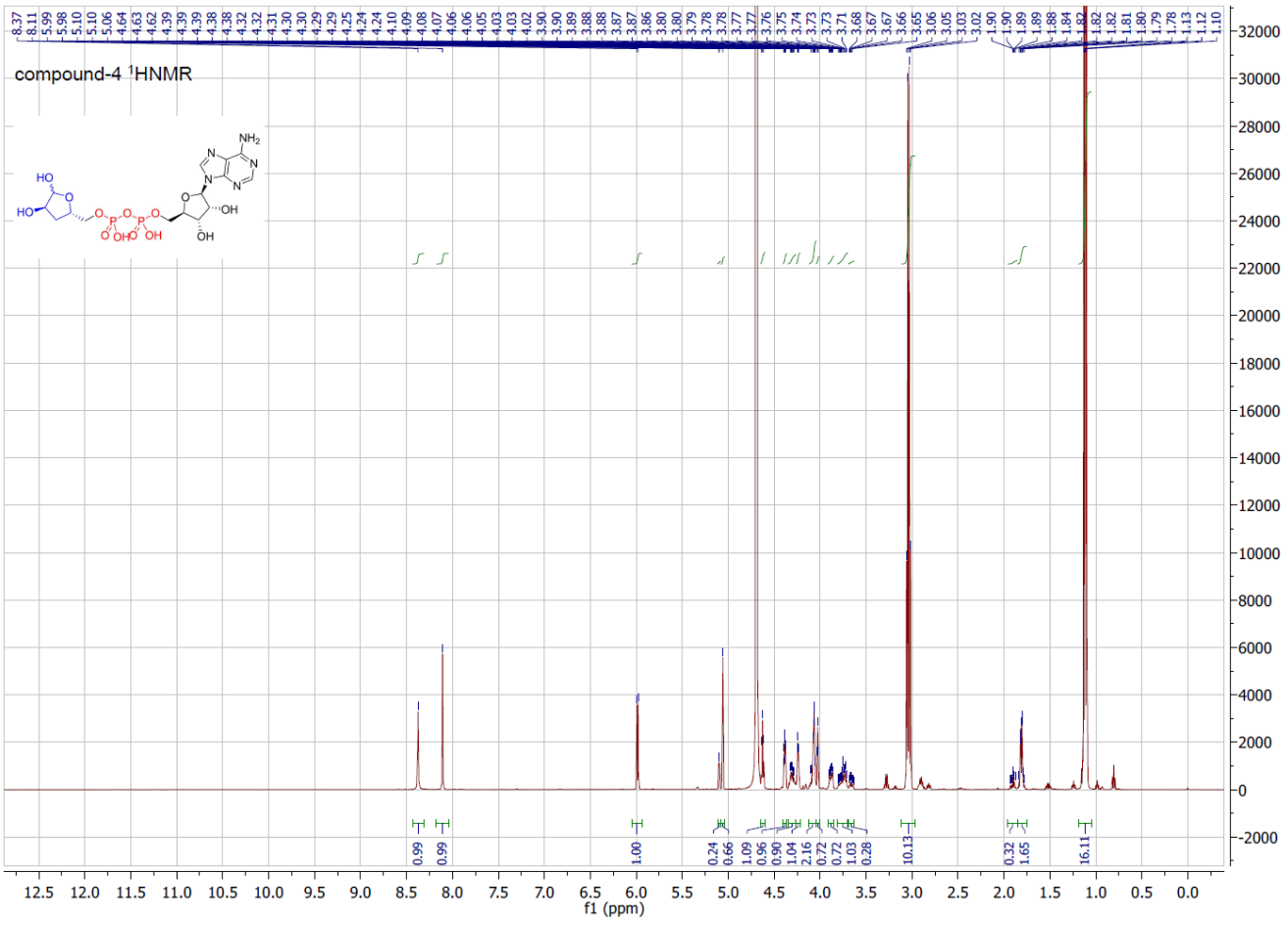
compound 2 - 31PNMR

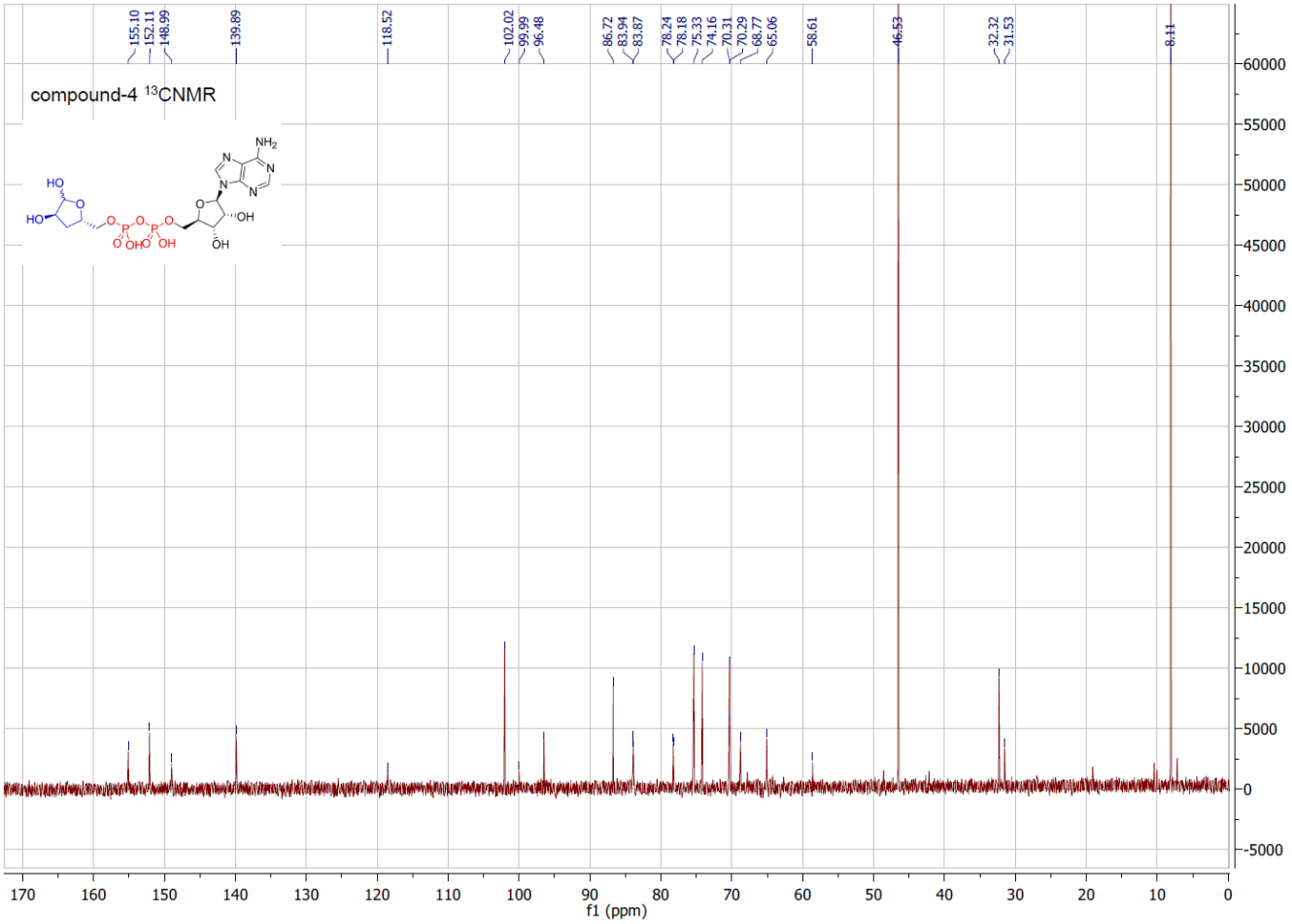


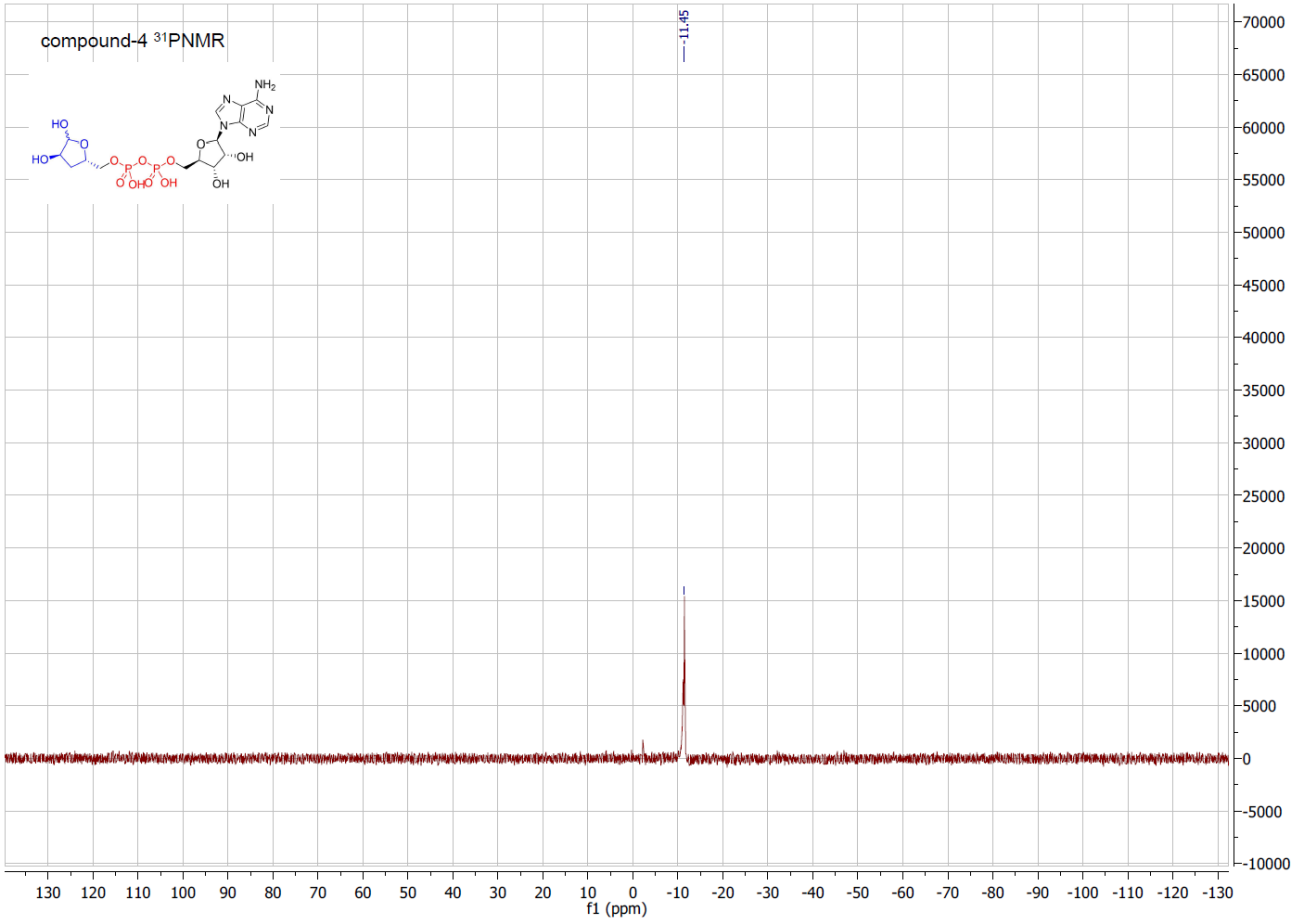

compound-3 ^{1}HNMR
f1 (ppm)

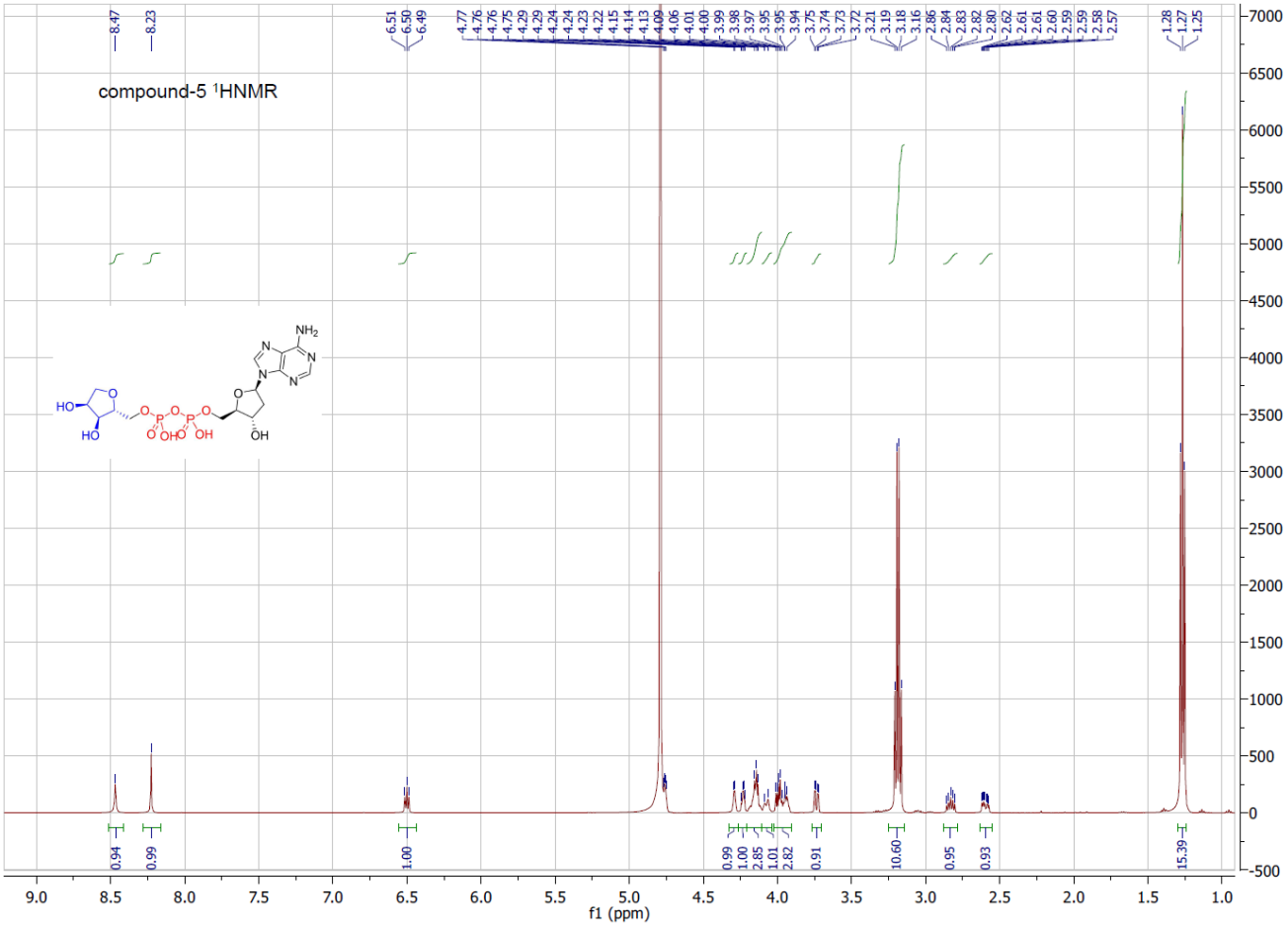

compound-3 ^{13}CNMR
f1 (ppm)

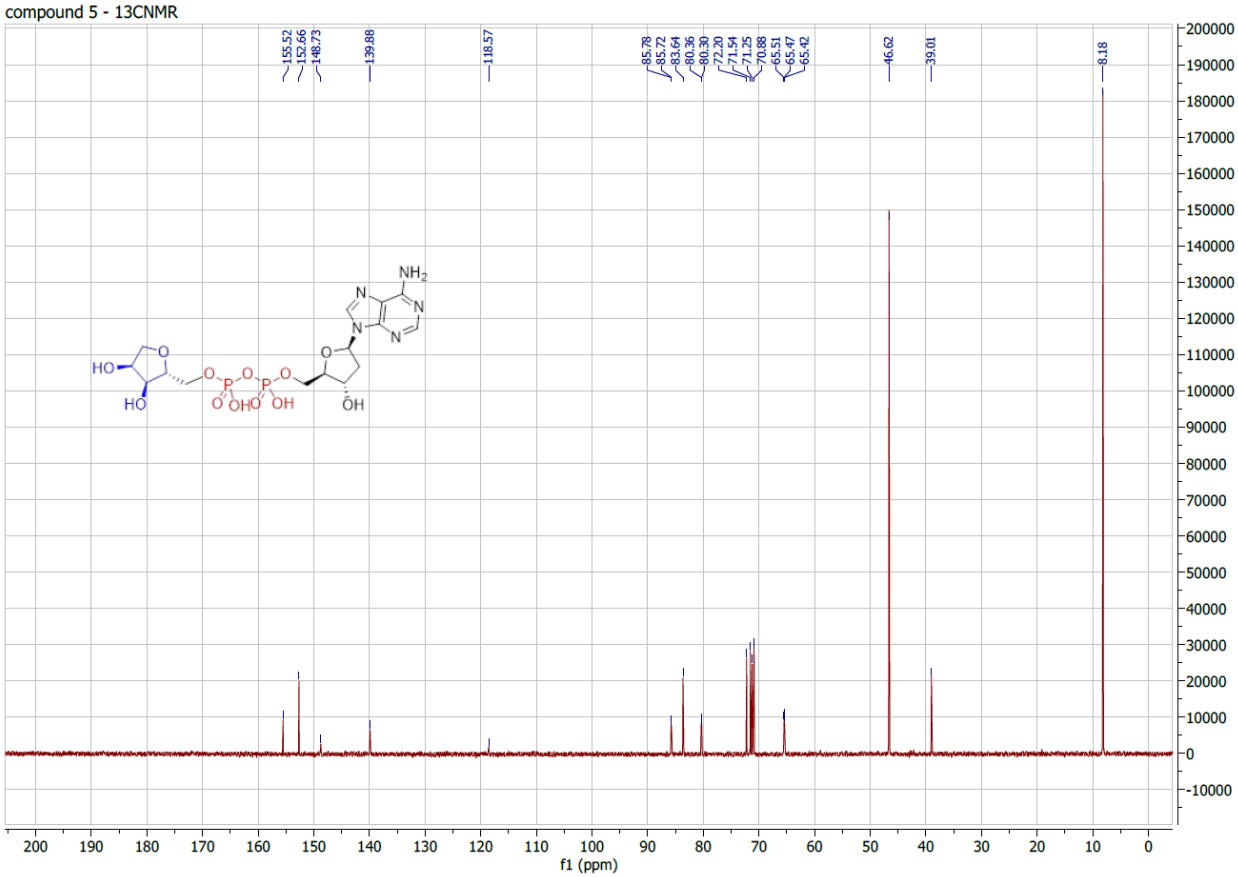

compound-3 31PNMR
-11.36
f1 (ppm)

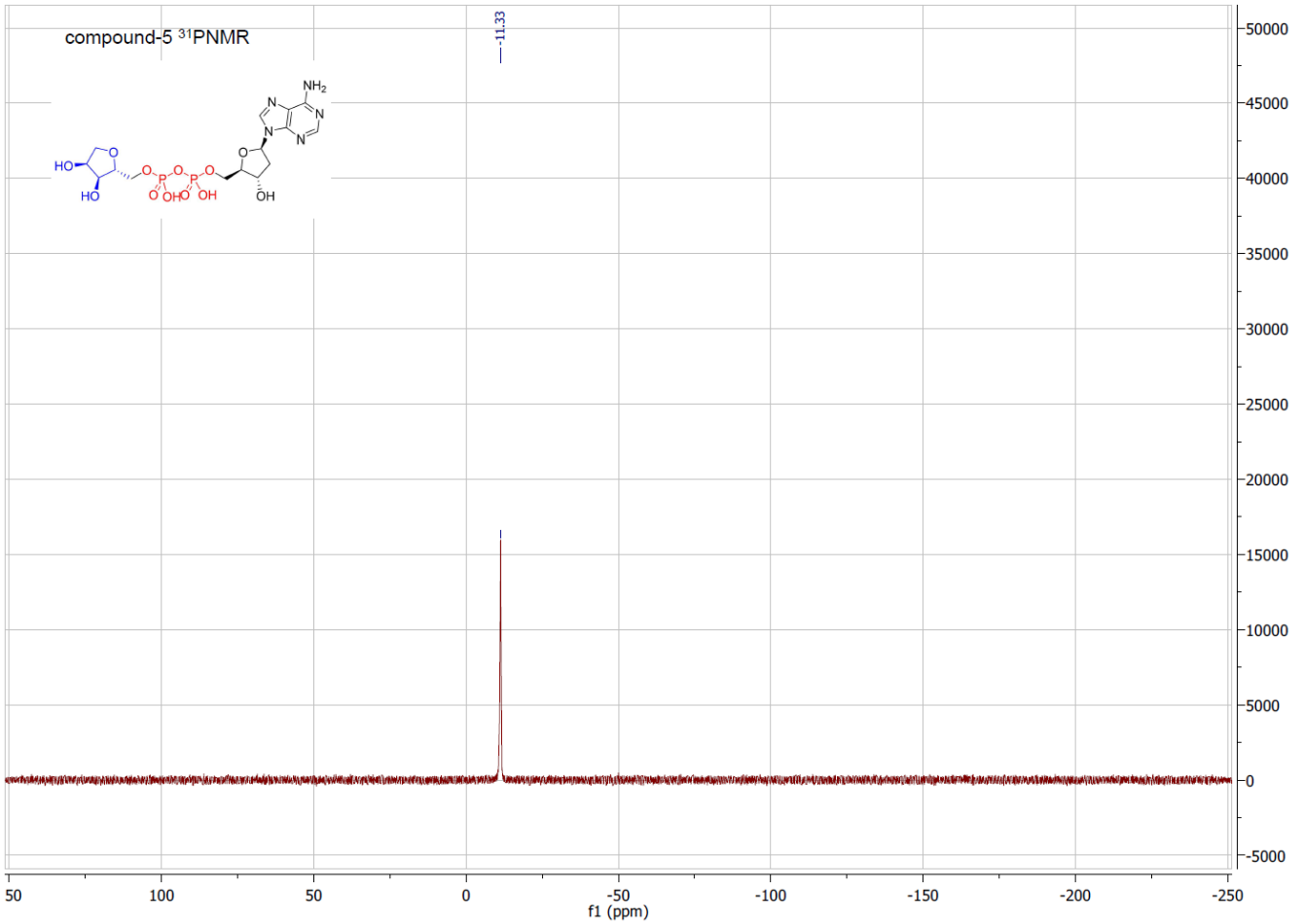

compound-4 1HNMR
f1 (ppm)

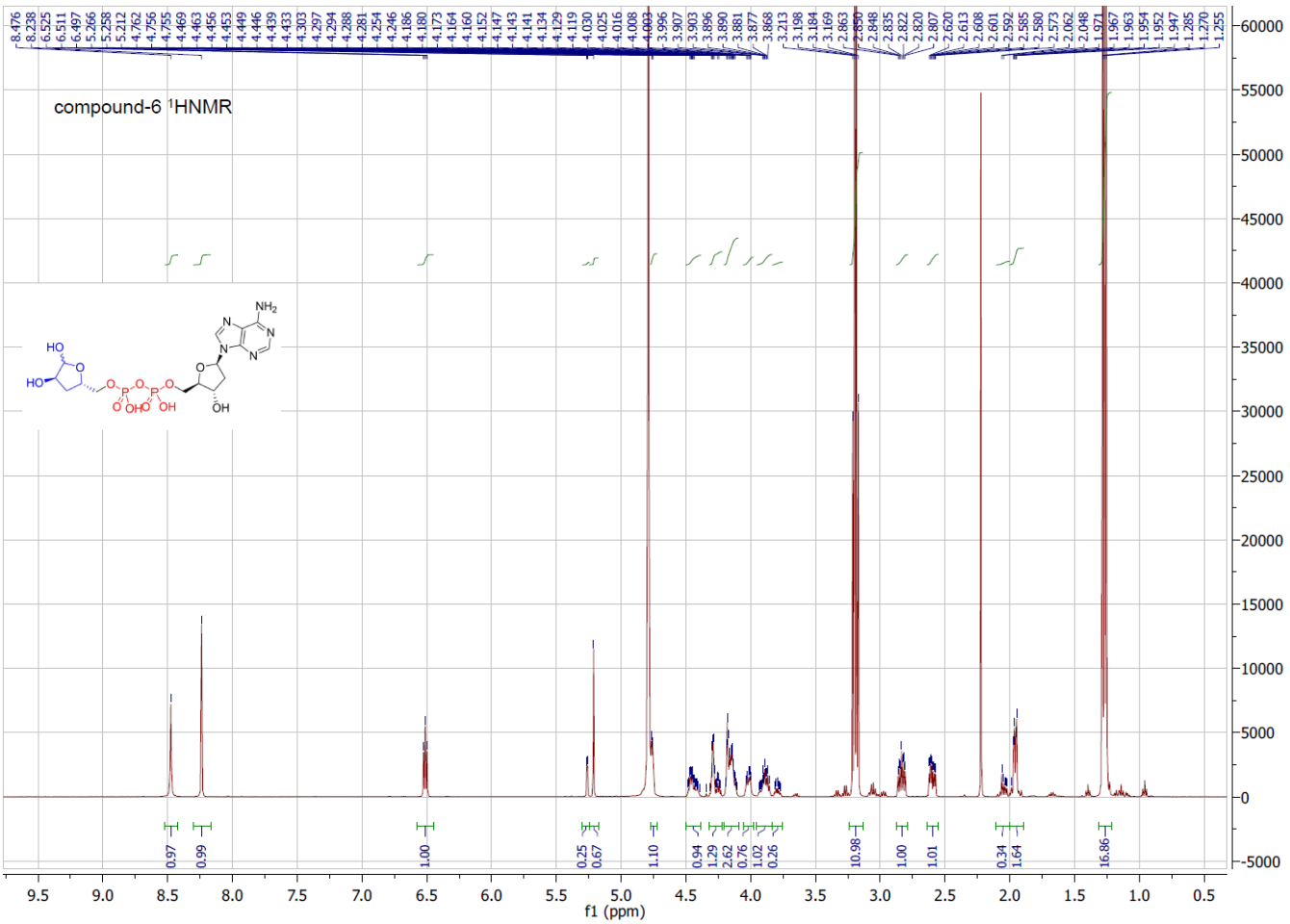

compound-4 ^{13}CNMR
155.10
152.11
148.99
139.89
118.52
102.02
99.99
96.48
86.72
83.94
83.87
78.24
78.18
75.33
74.16
70.31
70.29
68.77
65.06
58.61
46.53
32.32
31.53
8.11
f1 (ppm)

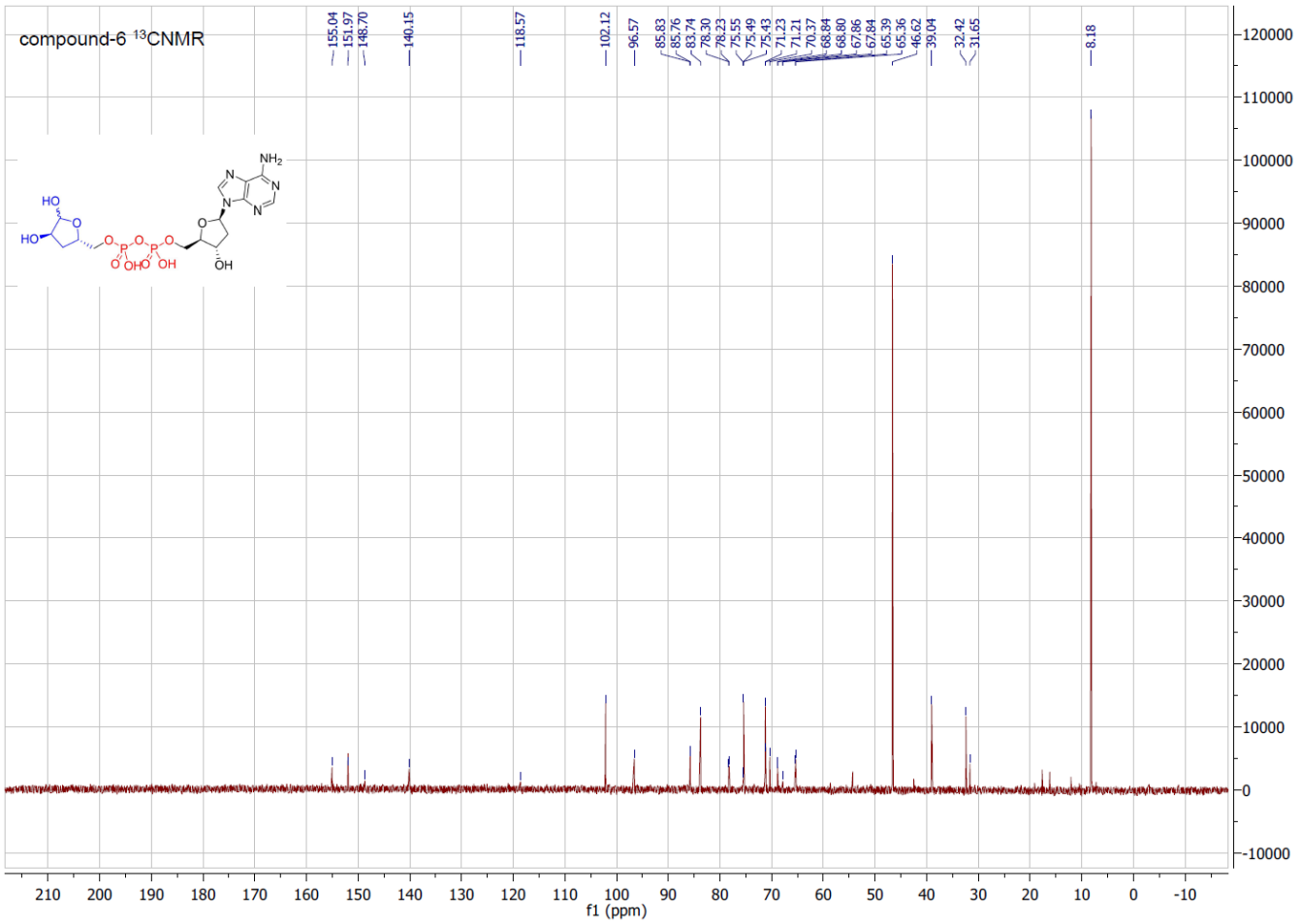

compound-4 ^{31}PNMR
-11.45
f1 (ppm)

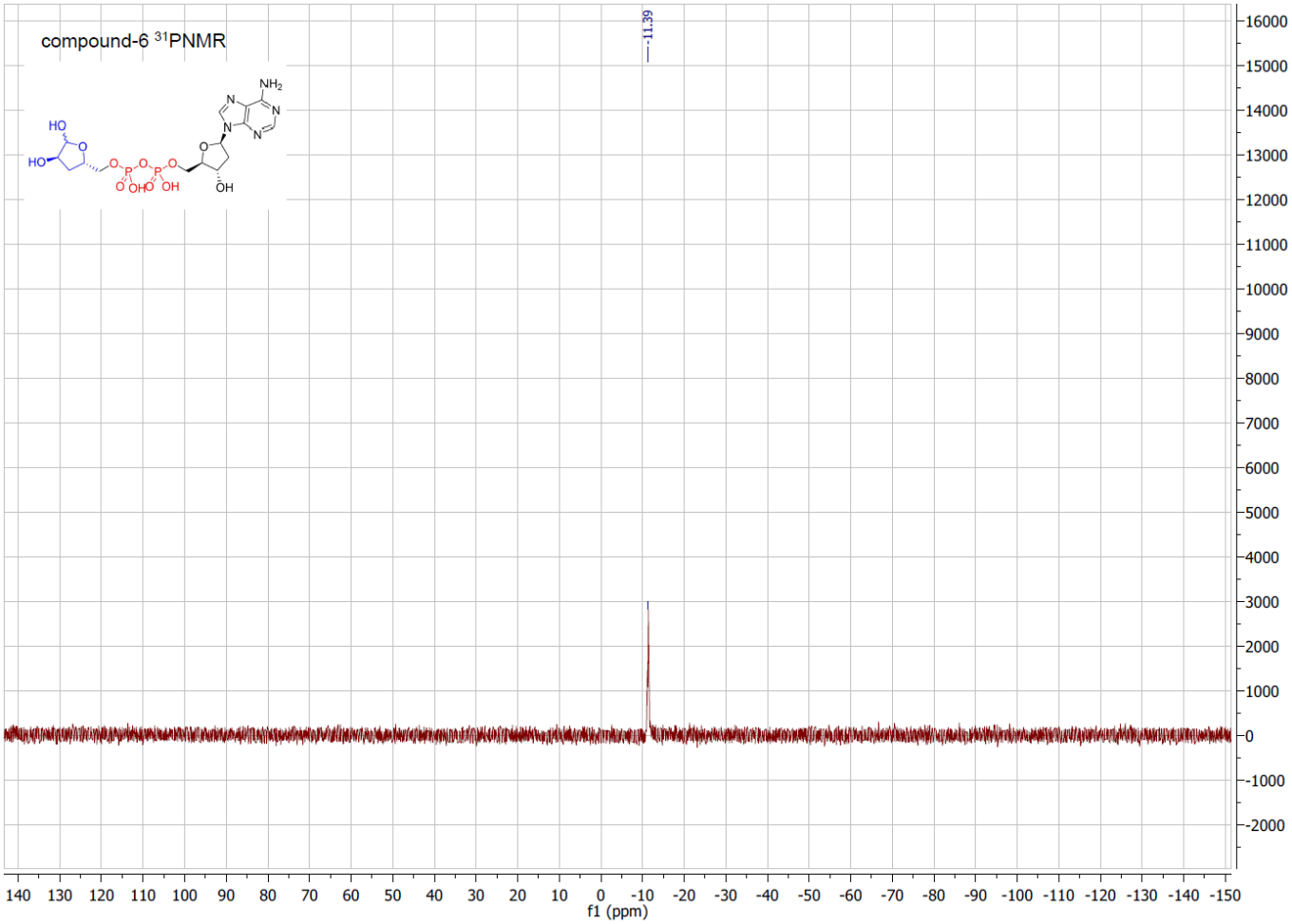

compound-5 ¹HNMR
f1 (ppm)


compound 5 - 13CNMR
f1 (ppm)

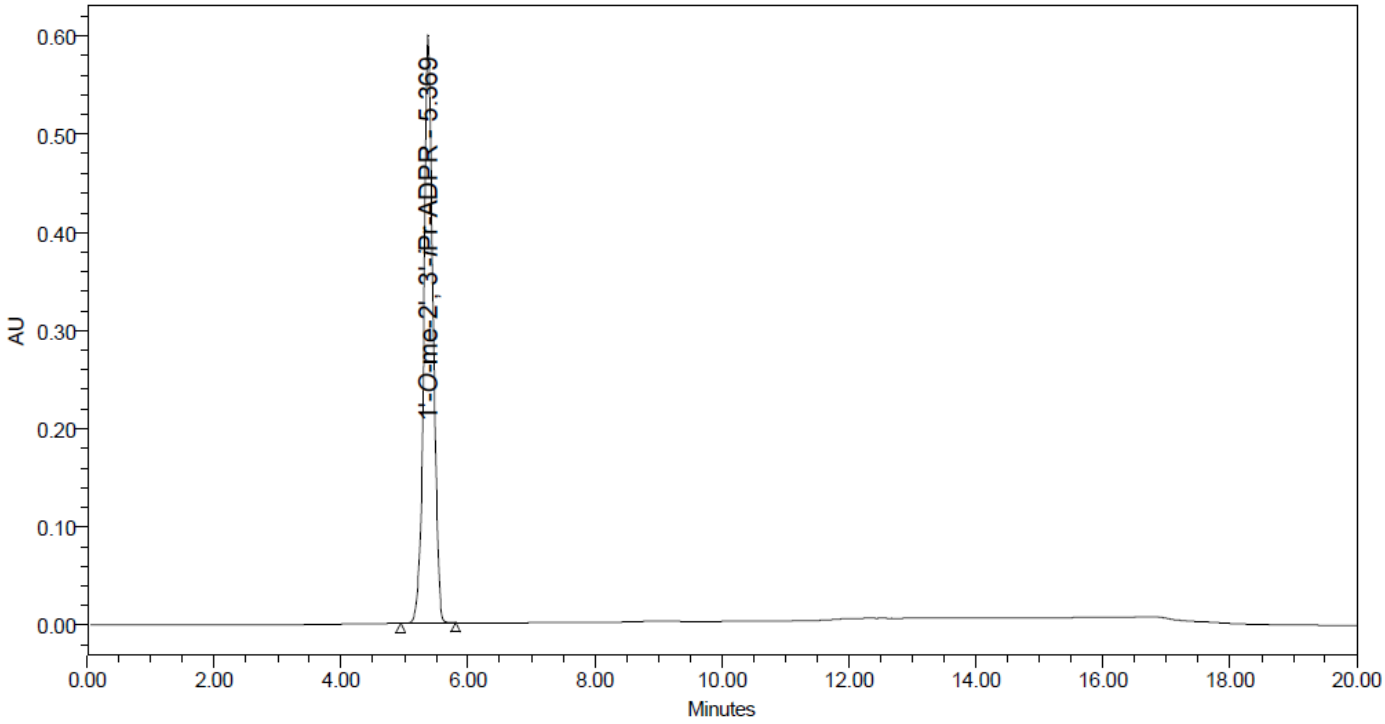

compound-5 31PNMR
-11.33
f1 (ppm)


compound-6 1HNMR
f1 (ppm)


compound-6 13CNMR
155.04
151.97
148.70
140.15
118.57
102.12
96.57
85.83
85.76
83.74
78.30
78.23
75.55
75.49
75.43
71.23
71.21
70.37
68.84
68.80
67.86
67.84
65.39
65.36
46.62
39.04
32.42
31.65
8.18
f1 (ppm)

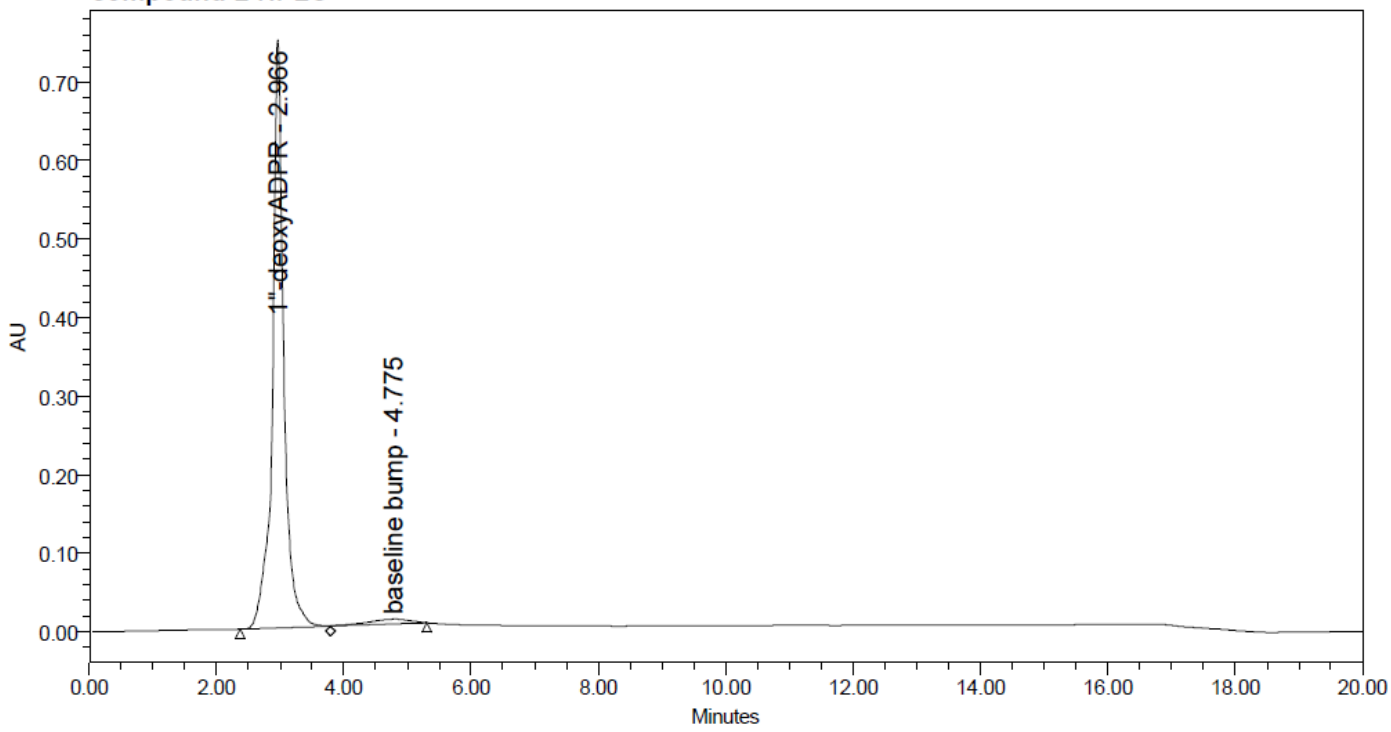

compound-6 31PNMR
-11.39
f1 (ppm)

compound-1 HPLC



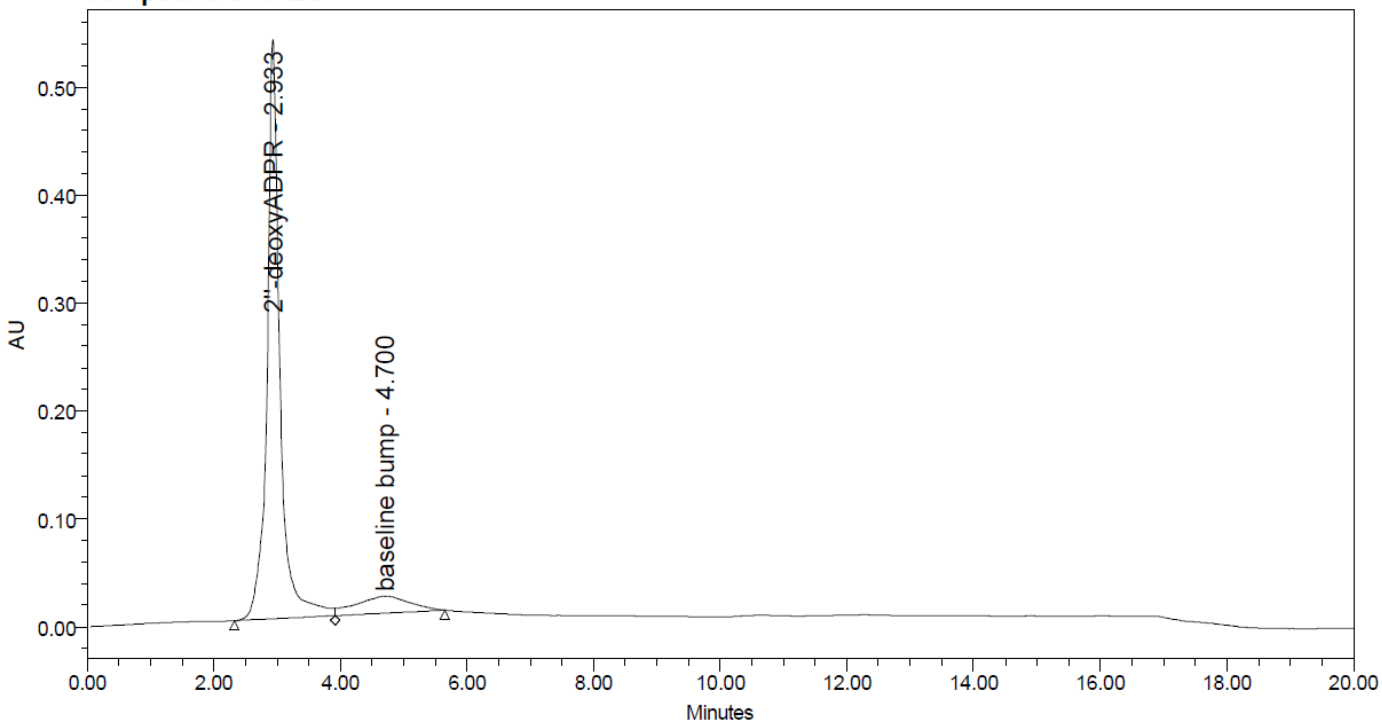
	Peak Name	RT	Area	% Area	Height
1	compound-1	5.369	6016865	100.00	599602

compound-2 HPLC



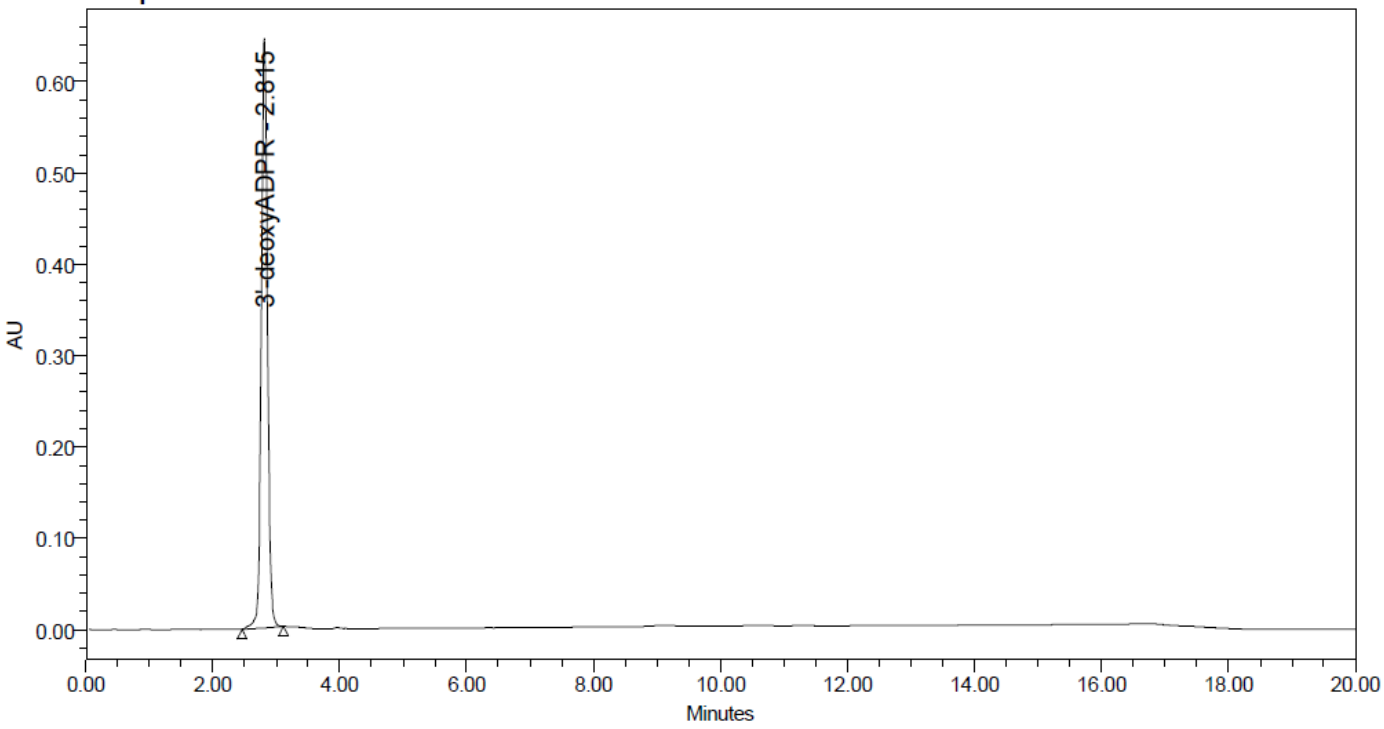
	Peak Name	RT	Area	% Area	Height
1	compound-2	2.966	9281122	97.09	748450
2	baseline bump	4.775	278307	2.91	6239

compound-3 HPLC



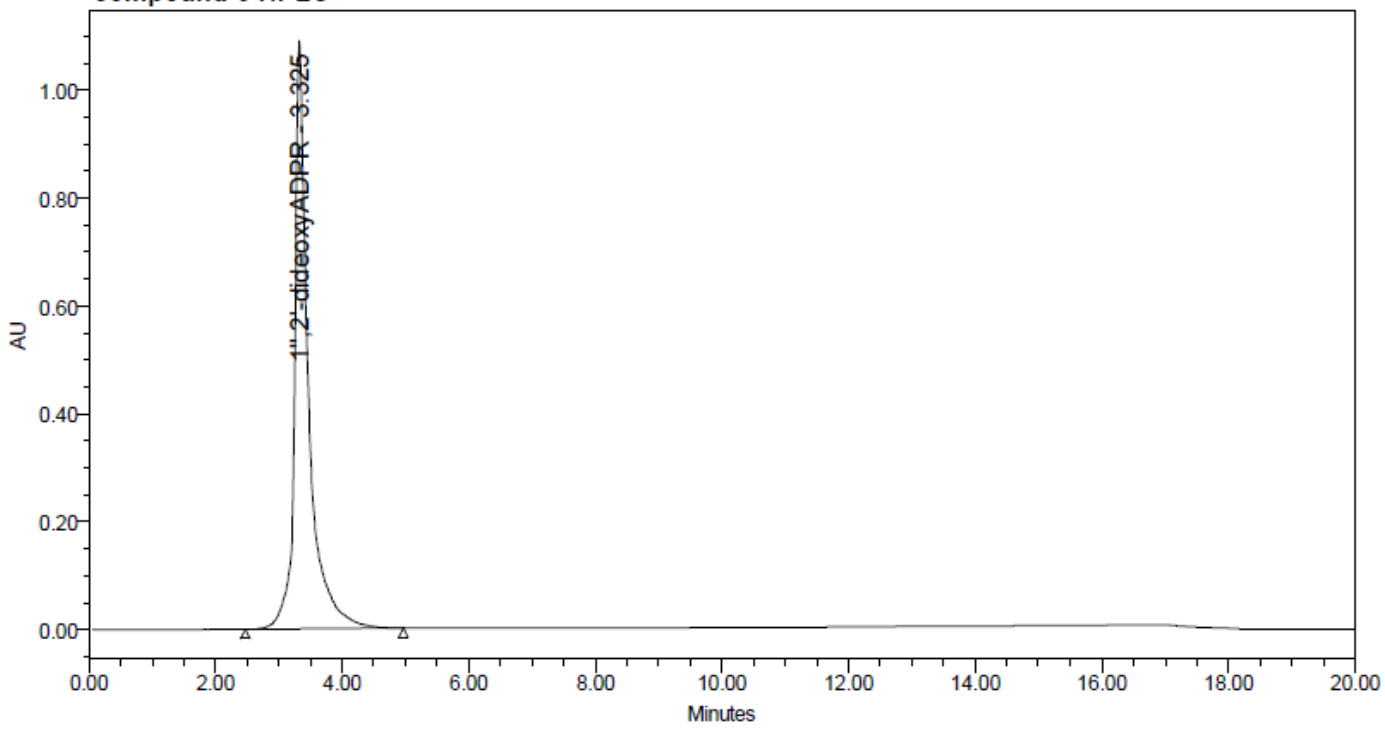
	Peak Name	RT	Area	% Area	Height
1	2''-deoxyADPR	2.933	7595191	88.97	536539
2	baseline bump	4.700	941594	11.03	15903

compound-4 HPLC



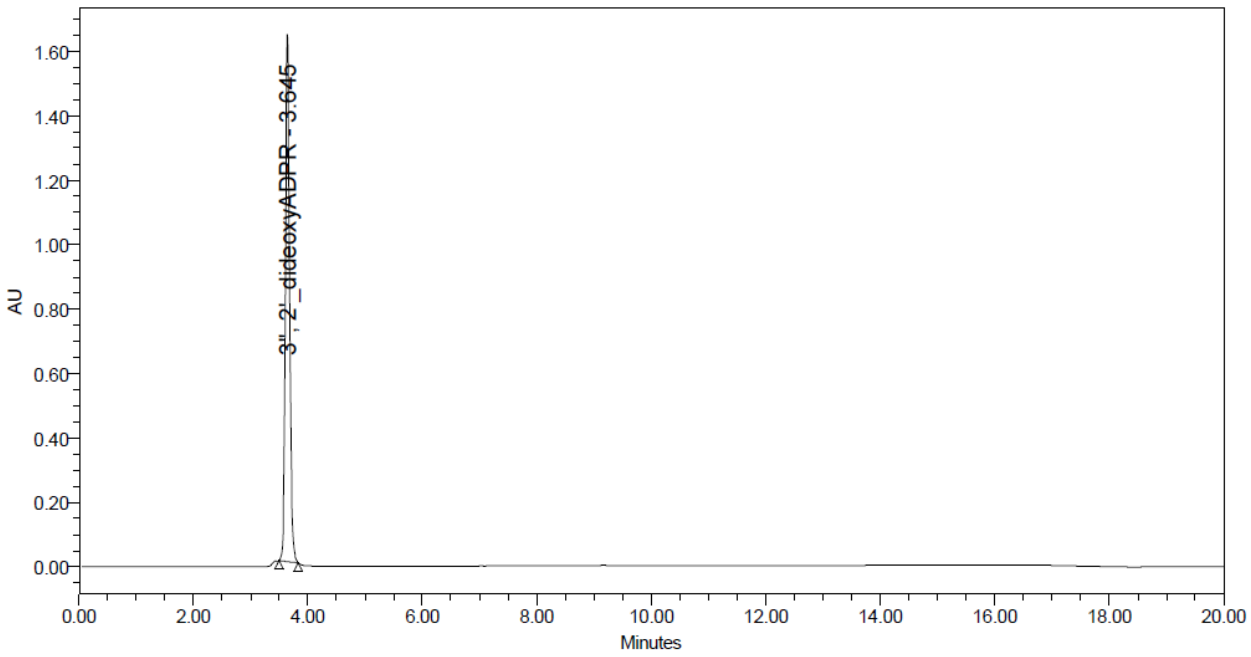
	Peak Name	RT	Area	% Area	Height
1	compound-4	2.815	4332210	100.00	645567

compound-5 HPLC



	Peak Name	RT	Area	% Area	Height
1	compound-5	3.325	16556203	100.00	1090063

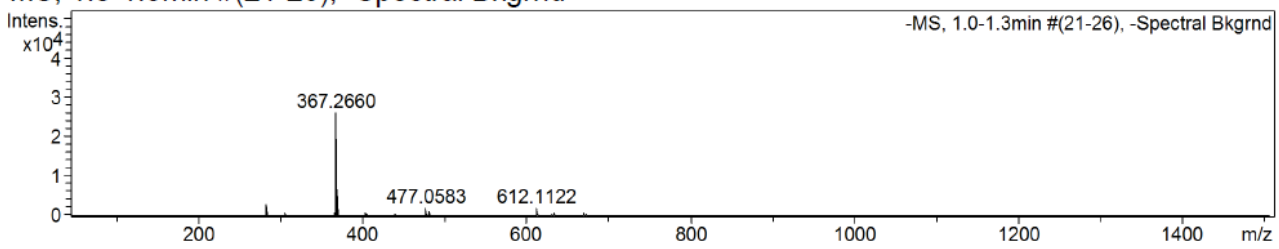
compound-6 HPLC



	Peak Name	RT	Area	% Area	Height
1	Imp_1	2.996			
2	2d_AMP	3.282			
3	compound-6	3.645	8580452	100.00	1642392
4	Bis_2d_AMP	4.044			
5	2d_AMP_imidazolide	4.346			

compound-1 MS

-MS, 1.0-1.3min #(21-26), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	283.0606	2716	10.5	96	4956.2
2	305.5533	663	2.6	26	869.3
3	367.2660	25957	100.0	1585	18013.9
4	368.2699	6576	25.3	414	4528.8
5	369.2739	844	3.3	48	577.2
6	403.2415	745	2.9	46	524.5
7	477.0583	1972	7.6	128	3833.1
8	481.2580	1042	4.0	61	2251.9
9	612.1122	2025	7.8	156	5845.0
10	670.0612	604	2.3	53	1118.4

Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

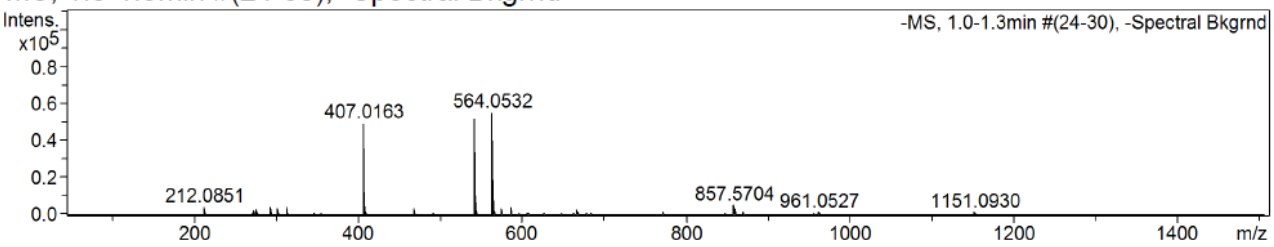
Expected Formula C19 H29 N5 O14 P2 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	612.1122	612.110799	-1.40	0.0278	C 19 H 28 N 5 O 14 P 2

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

compound-2 MS

-MS, 1.0-1.3min #(24-30), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	212.0851	4199	7.6	42	1273.9
2	292.9828	4115	7.5	172	504.8
3	407.0163	49405	89.6	2851	7410.6
4	408.0200	6499	11.8	368	982.6
5	542.0716	51608	93.6	3540	3320.9
6	543.0747	10245	18.6	777	650.1
7	564.0532	55139	100.0	3871	2714.5
8	564.5546	5698	10.3	467	279.0
9	565.0575	10283	18.6	760	500.9
10	857.5704	5209	9.4	559	654.1

Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

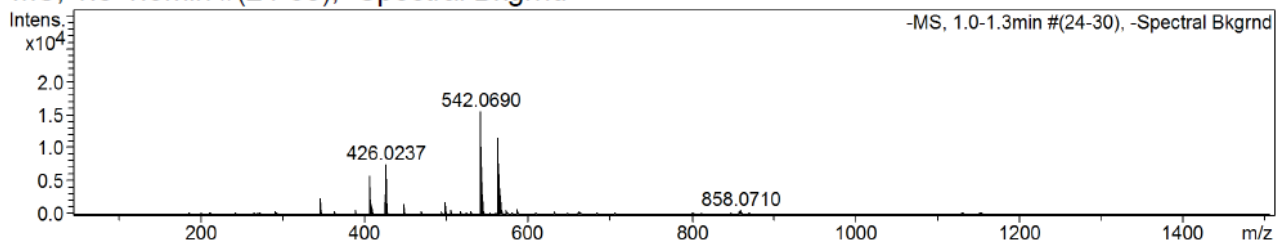
Expected Formula C15 H23 N5 O13 P2 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	542.0716	542.068934	-4.00	0.0066	C 15 H 22 N 5 O 13 P 2

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

compound-3 MS

-MS, 1.0-1.3min #(24-30), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	346.0562	2290	14.9	110	999.9
2	407.0157	5676	36.8	299	1993.8
3	408.0199	2235	14.5	117	787.7
4	426.0237	7460	48.4	364	2800.8
5	542.0690	15407	100.0	1087	1707.3
6	543.0753	7202	46.7	519	787.8
7	544.0799	3131	20.3	249	338.1
8	564.0527	11620	75.4	912	1001.8
9	565.0579	6015	39.0	451	513.4
10	566.0639	2940	19.1	198	248.4

Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

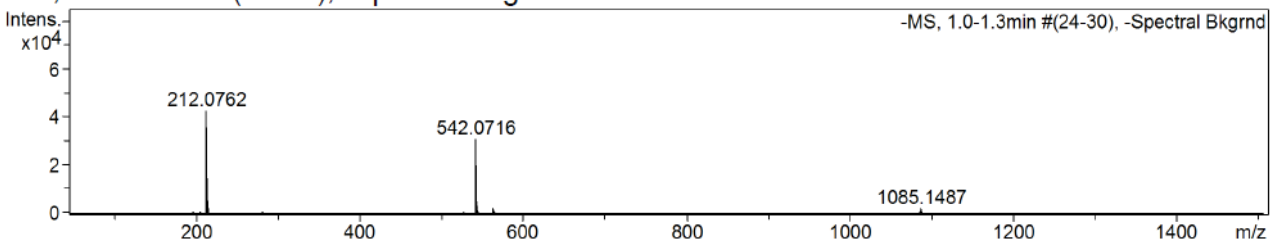
Expected Formula C15 H23 N5 O13 P2 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	566.0639	566.066529	5.60	0.0058	C 15 H 23 N 5 Na 1 O 13 P 2

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

compound-4 MS

-MS, 1.0-1.3min #(24-30), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	211.9125	488	1.2	25	420.9
2	212.0762	42326	100.0	1126	36566.6
3	213.0775	6505	15.4	128	5691.5
4	214.0706	2317	5.5	38	2053.8
5	542.0716	30786	72.7	2127	17385.3
6	543.0728	5061	12.0	379	2813.5
7	544.0775	1224	2.9	72	669.9
8	564.0562	2302	5.4	149	964.1
9	1085.1487	2005	4.7	270	742.2
10	1086.1562	706	1.7	82	261.9

Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

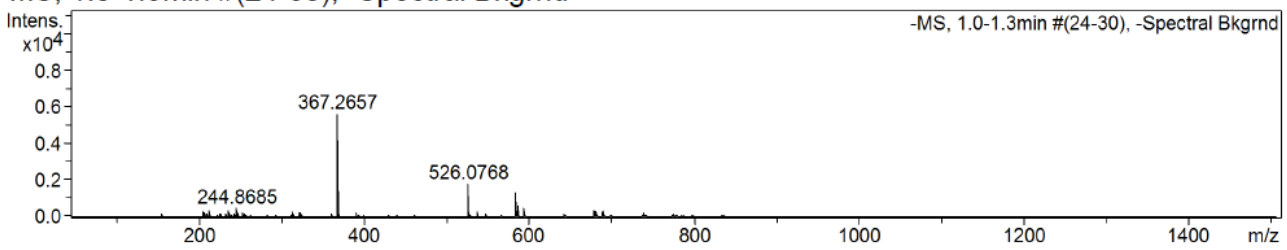
Expected Formula C15 H23 N5 O13 P2 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	542.0716	542.068934	-3.90	0.0108	C 15 H 22 N 5 O 13 P 2

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

compound-5 MS

-MS, 1.0-1.3min #(24-30), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	212.0740	369	6.6	5	1406.7
2	244.8685	471	8.4	16	1214.9
3	367.2657	5596	100.0	305	6685.7
4	368.2686	1470	26.3	78	1749.1
5	526.0768	1777	31.8	107	2795.6
6	584.0244	1324	23.7	84	1305.0
7	585.0318	379	6.8	26	372.3
8	586.0266	592	10.6	38	579.8
9	594.0596	495	8.8	25	471.3
10	679.9457	374	6.7	32	279.6

Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

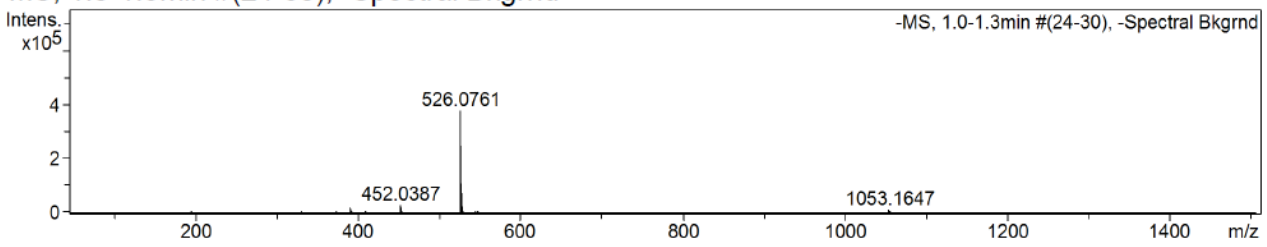
Expected Formula C15 H23 N5 O12 P2 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	526.0768	526.074019	-4.20	0.0397	C 15 H 22 N 5 O 12 P 2

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

compound-6 MS

-MS, 1.0-1.3min #(24-30), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	373.0112	6549	1.7	364	113.9
2	391.0239	17827	4.7	1107	315.0
3	452.0387	28988	7.7	1779	498.8
4	453.0509	5804	1.5	296	99.5
5	525.9448	15147	4.0	1455	439.0
6	526.0761	375790	100.0	17196	10923.0
7	527.0795	109768	29.2	6220	3244.2
8	528.0839	19457	5.2	1450	584.7
9	548.0597	6850	1.8	317	312.3
10	1053.1647	10652	2.8	1505	1686.5

Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C15 H23 N5 O12 P2 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

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