

**Towards novel efficient and stable nuclear import signals: synthesis and properties of trimethylguanosine cap analogs
modified within the 5',5'-triphosphate bridge**

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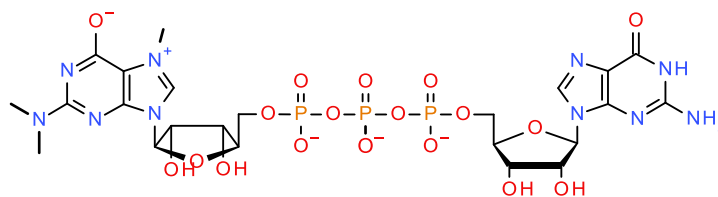
³ *Laboratory of Biological Physics, Institute of Physics, Polish Academy of Sciences, 32/46 Lotnikow Ave., 02-668 Warsaw, Poland*

Supplementary information

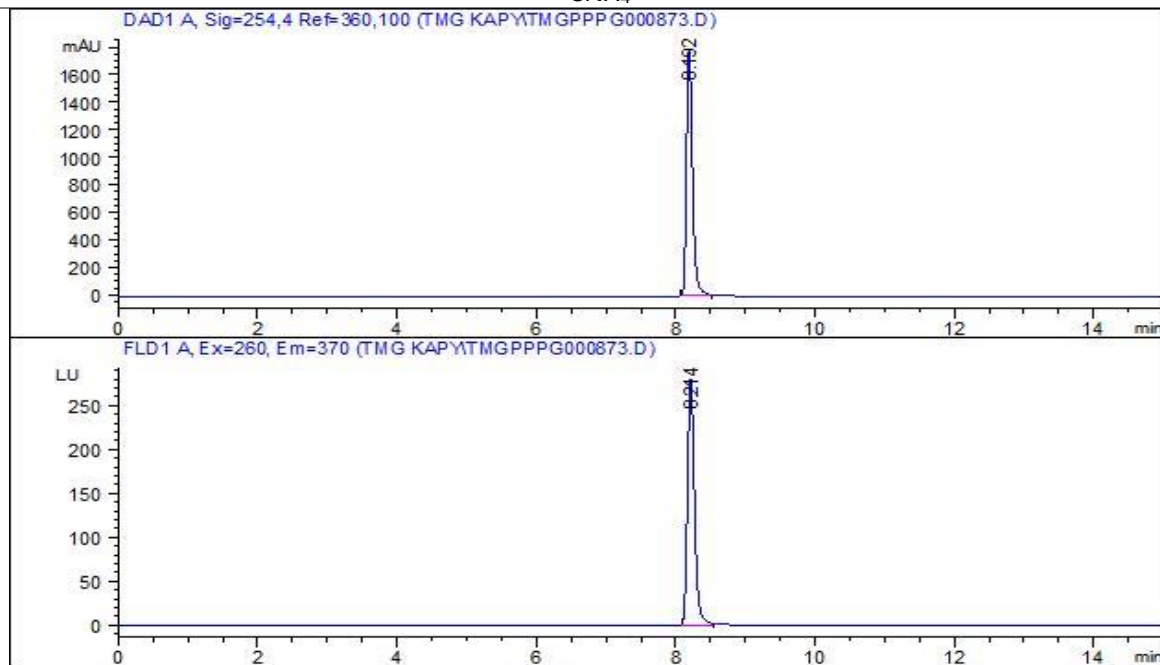
Equipment and experiment conditions	
RP HPLC	Agilent Tech. 1200 Series, Supelcosil LC-18-T 25cm x 4.6mm 5 μ m HPLC column, 0-25% MeOH gradient in 0.05 M CH ₃ COONH ₄ pH 5.9 within 0-15 min, flow 1.3 mL, det. 254 nm
MS ES TOF	Micromass FTMS spectrometer, electrospray ionization
¹ H NMR	Varian UNITY-plus 399.94 MHz, int. TSP, compound conc. ~2 mg/mL, FID signals we analyzed using ACD/1D NMR Processor
³¹ P NMR	Varian UNITY-plus 161.92 MHz, ext 85 % H ₃ PO ₄ , compound conc. ~2 mg/mL, FID signals we analyzed using ACD/1D NMR Processor

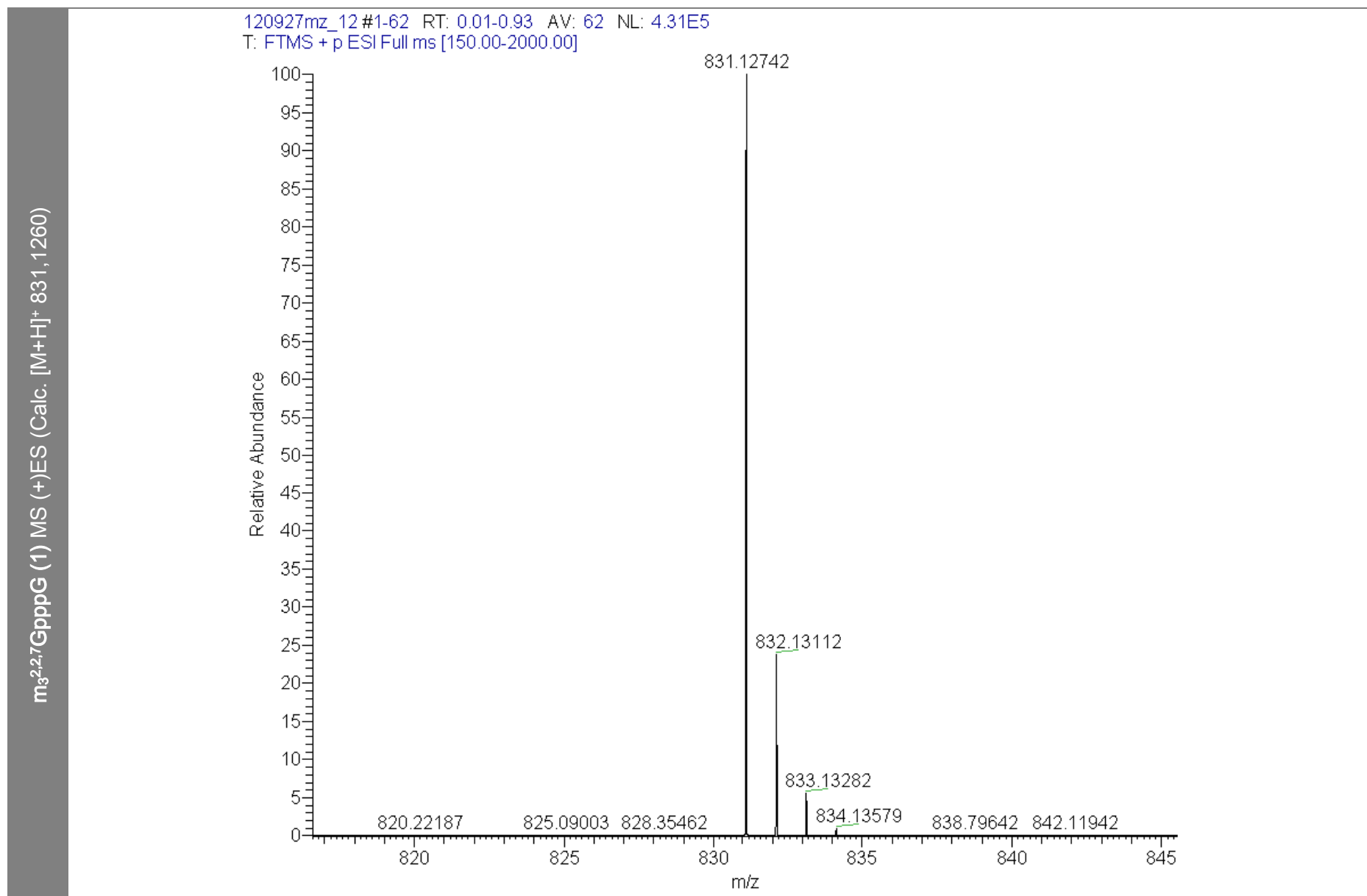
$m_3^{2,2,7}$ GpppG (1) RP HPLC

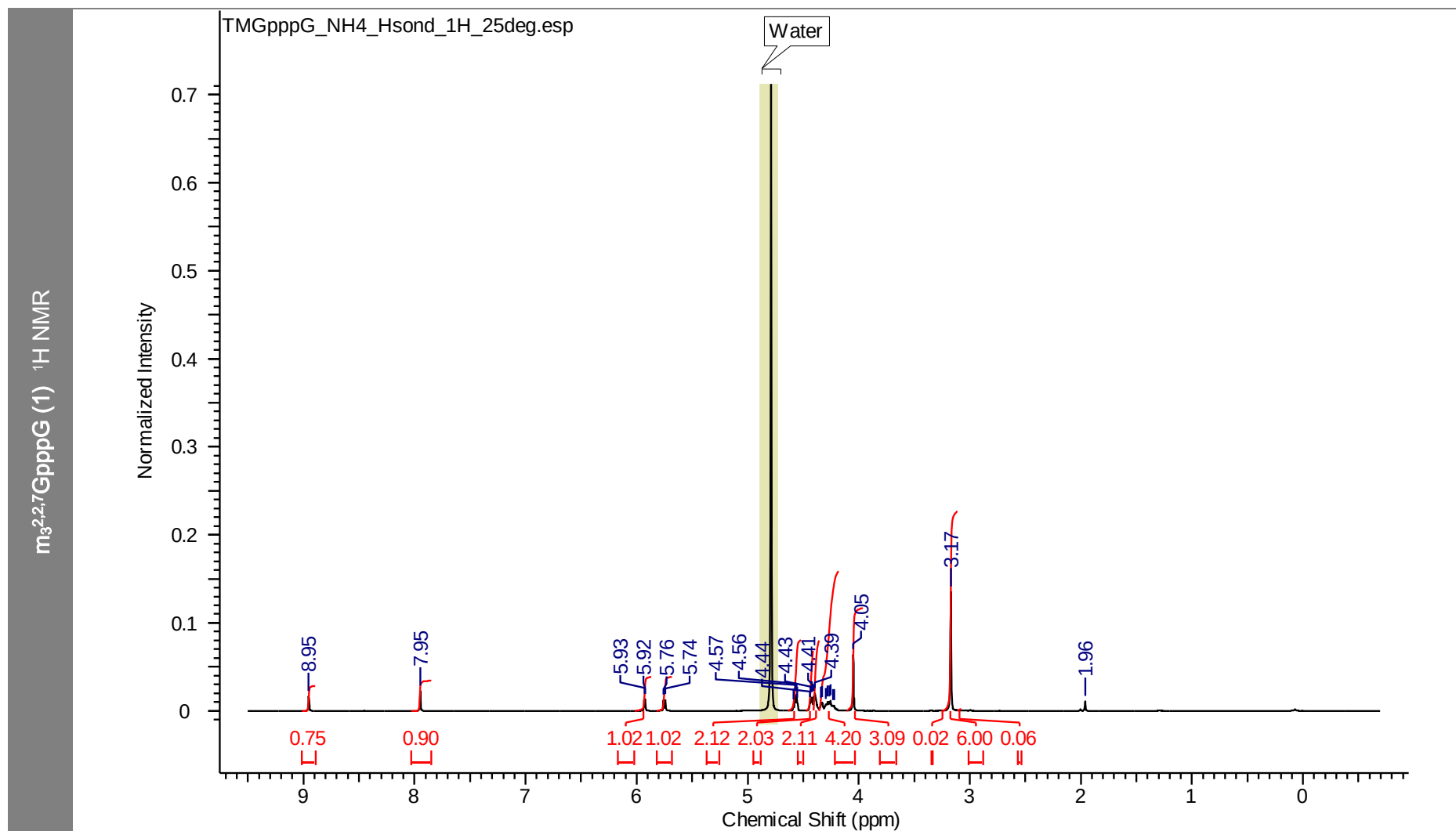
$m_3^{2,2,7}$ GpppG (1)

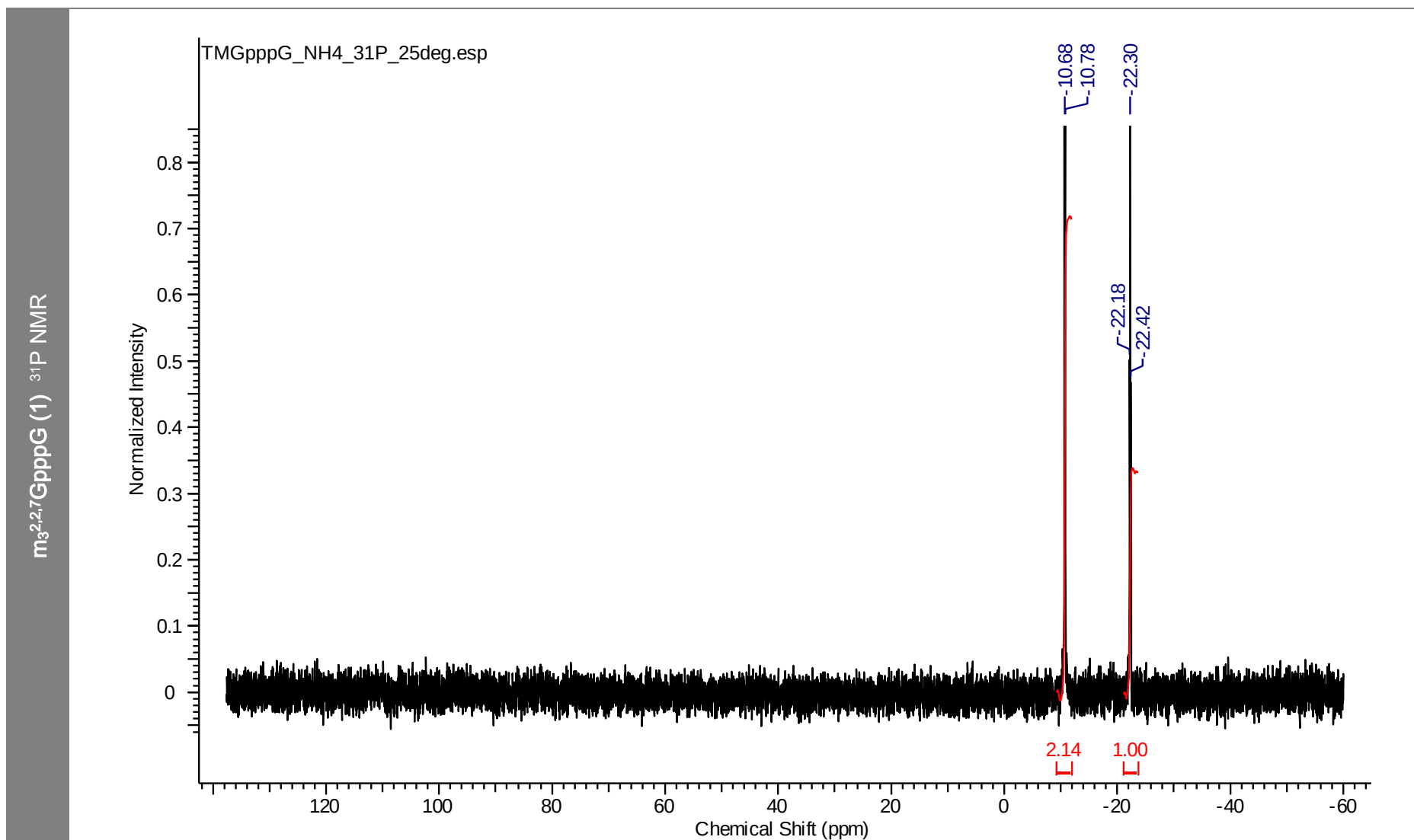


3NH_4^+



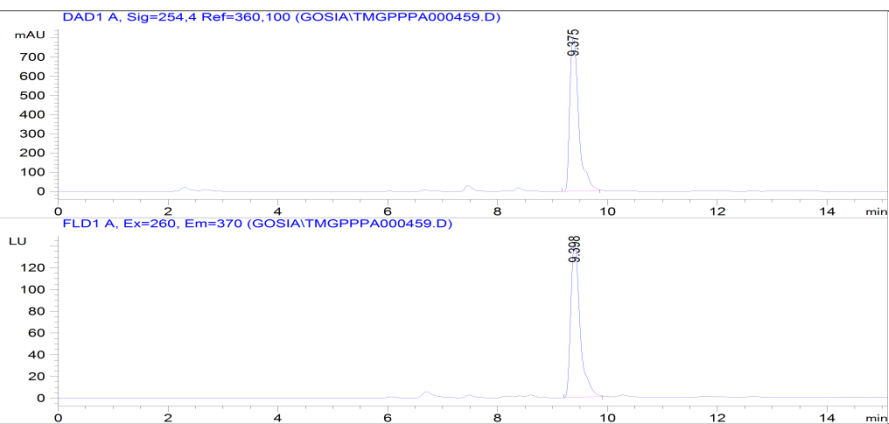
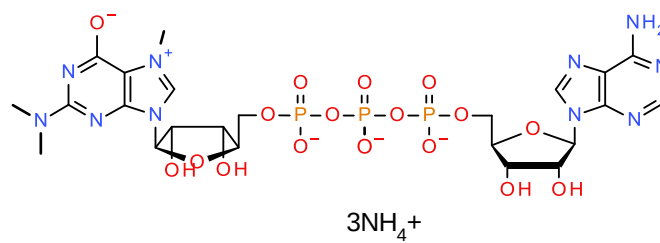


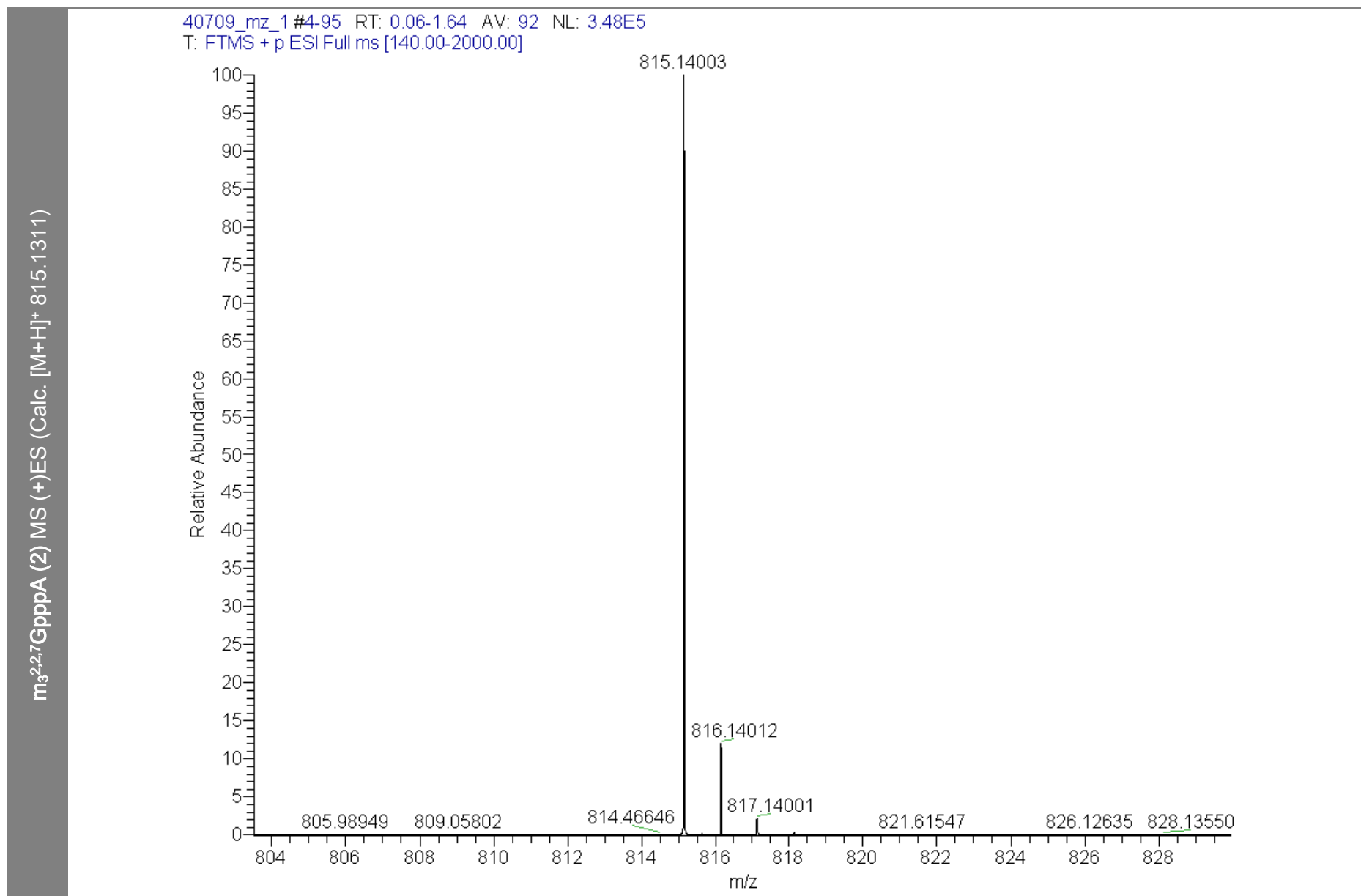




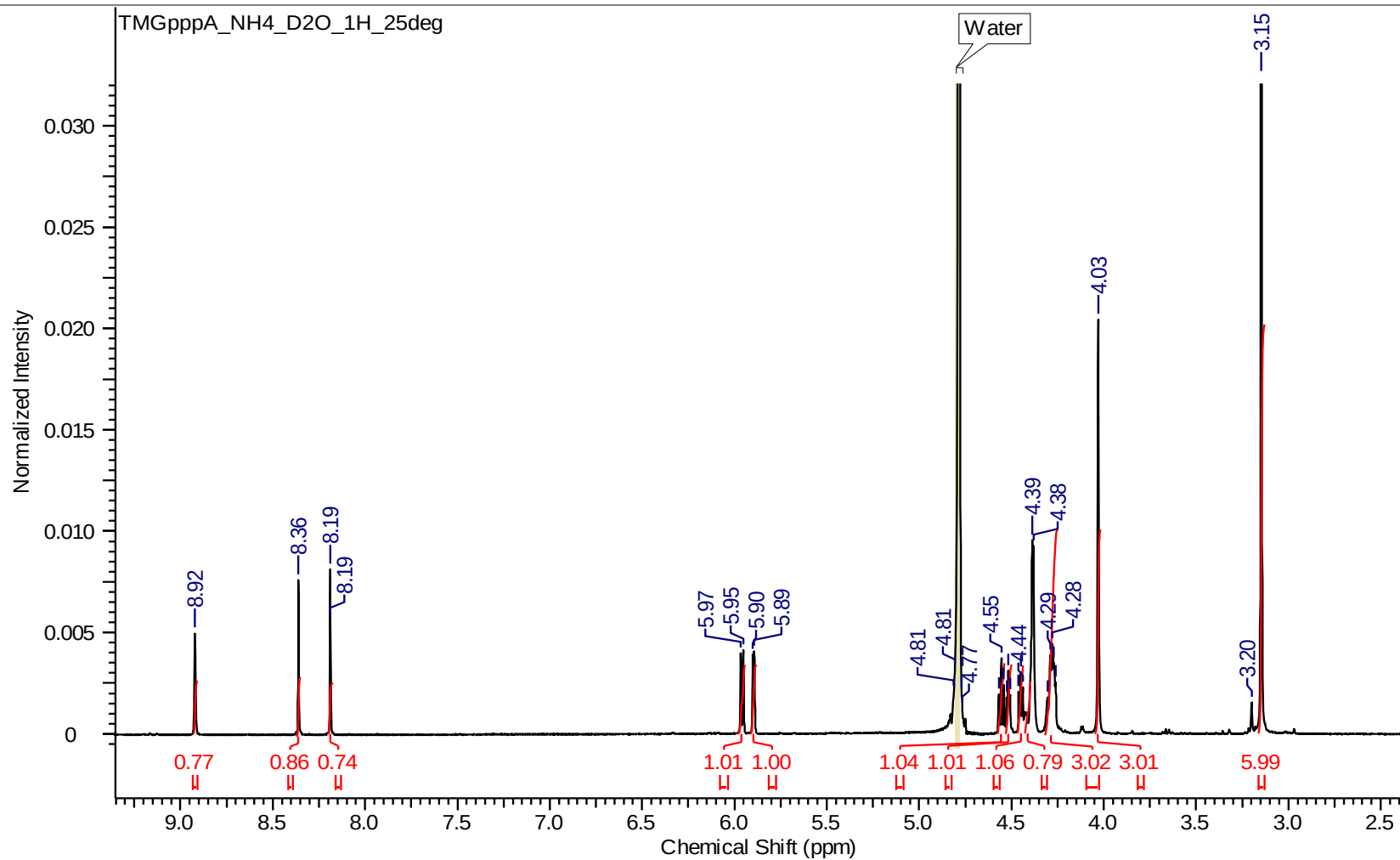
m₃^{2,2,7}GpppA (2) RP HPLC

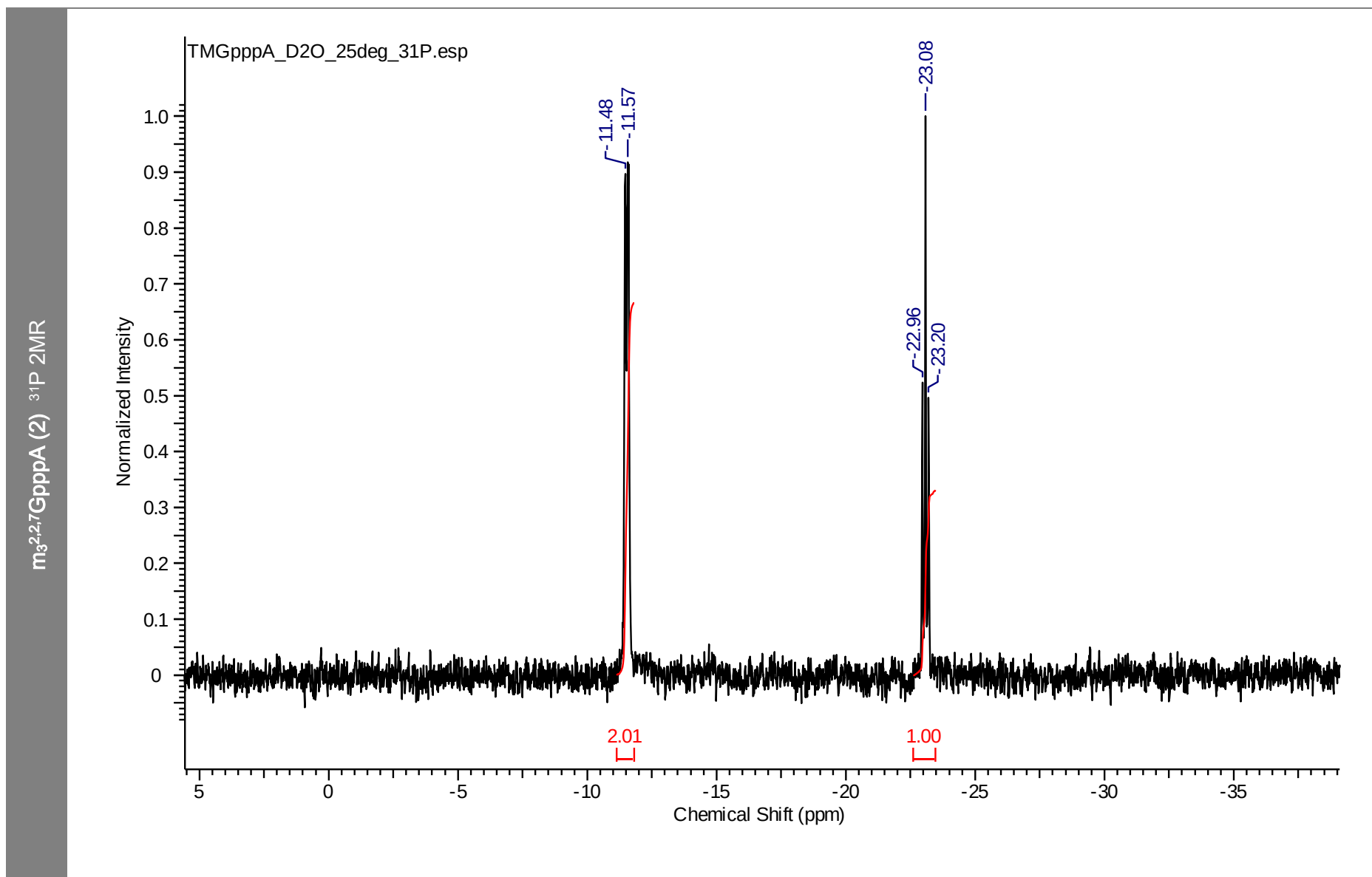
m₃^{2,2,7}GpppA (2)





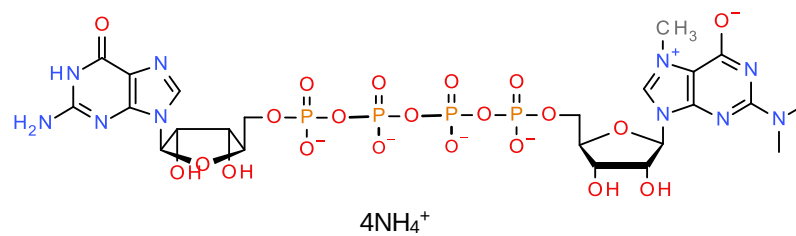
m₃^{2,2,7}GpppA (2) ¹H NMR



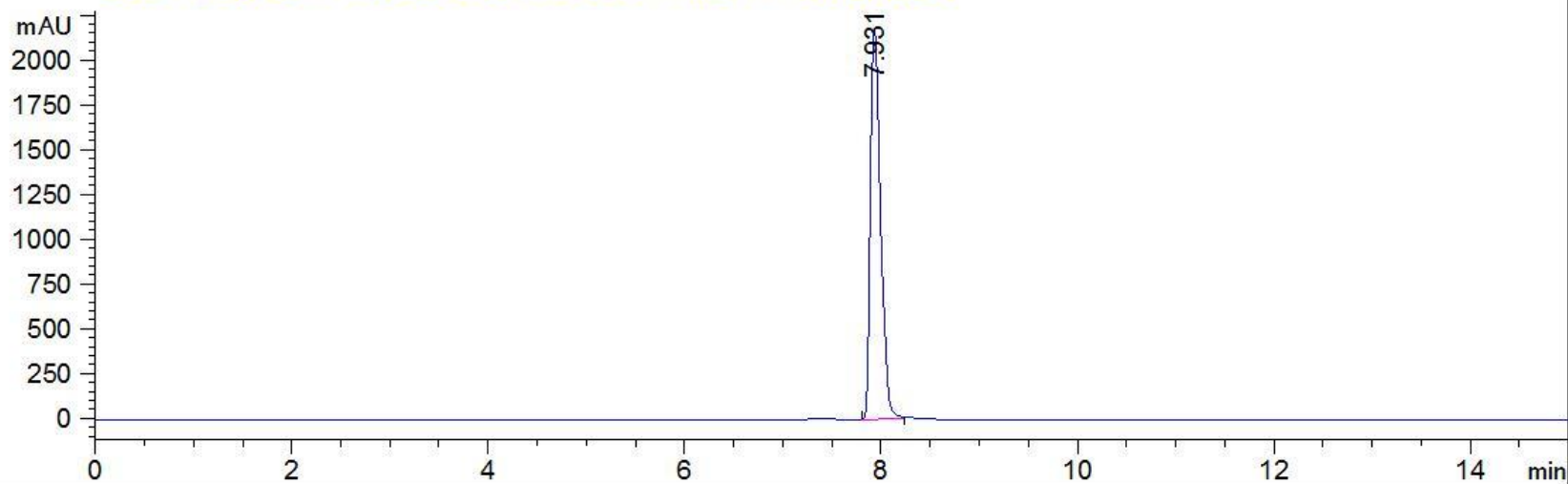


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m₃^{2,2,7}GppppG NH₄⁺ salt (3)



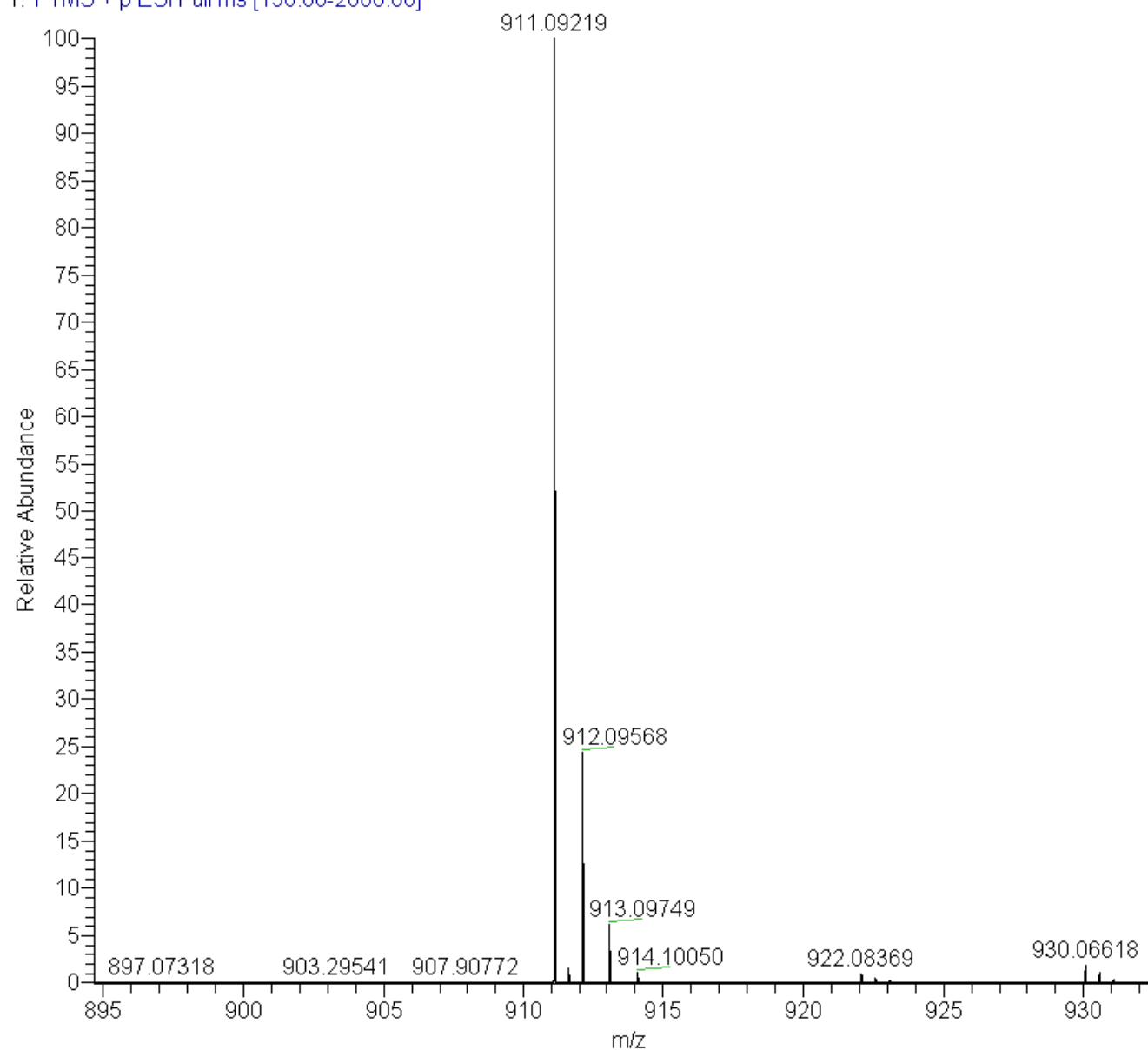
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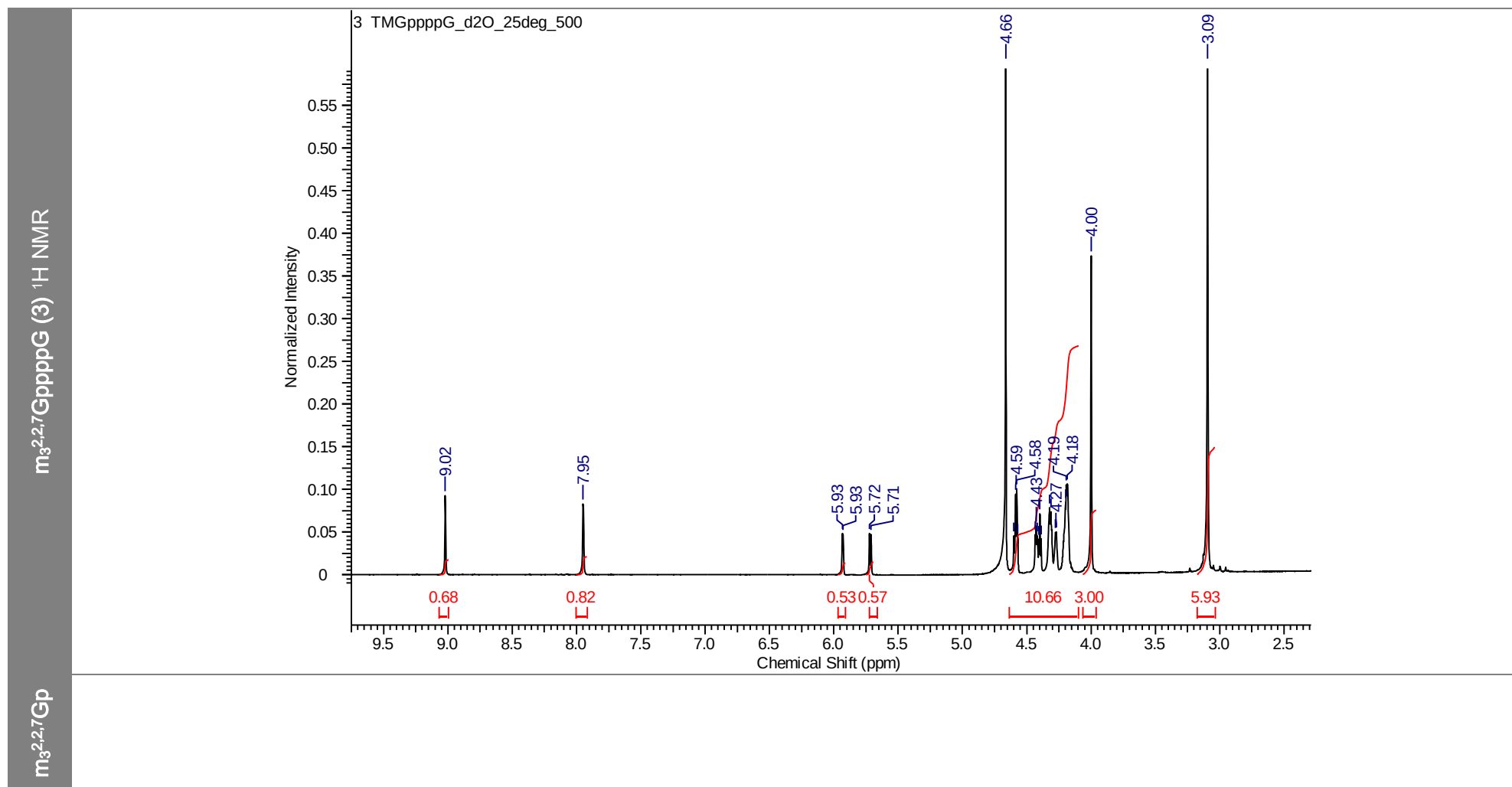


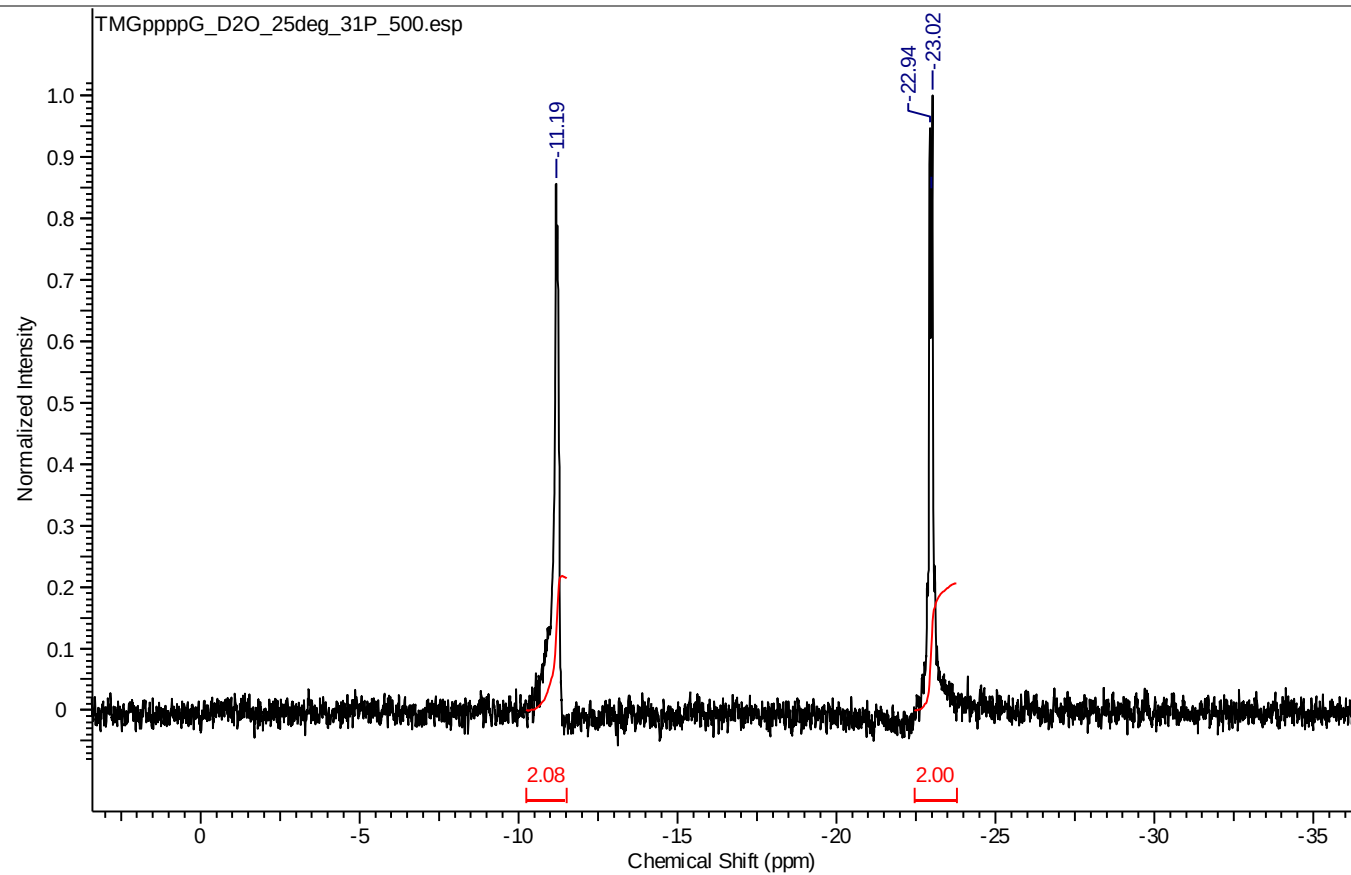
m₃^{2,2,7}GppppG (3) RP HPLC

$m_3^{2,2,7}$ GppppG (3) MS (+)ES (Calc. [M+H]⁺ 911.0923)

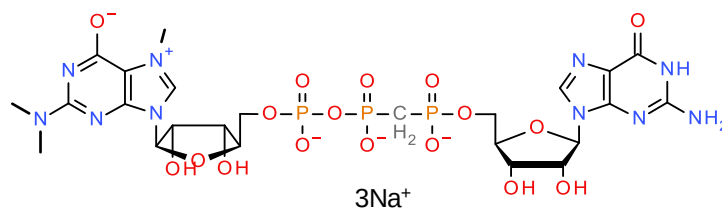
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T: FTMS + p ESI Full ms [150.00-2000.00]



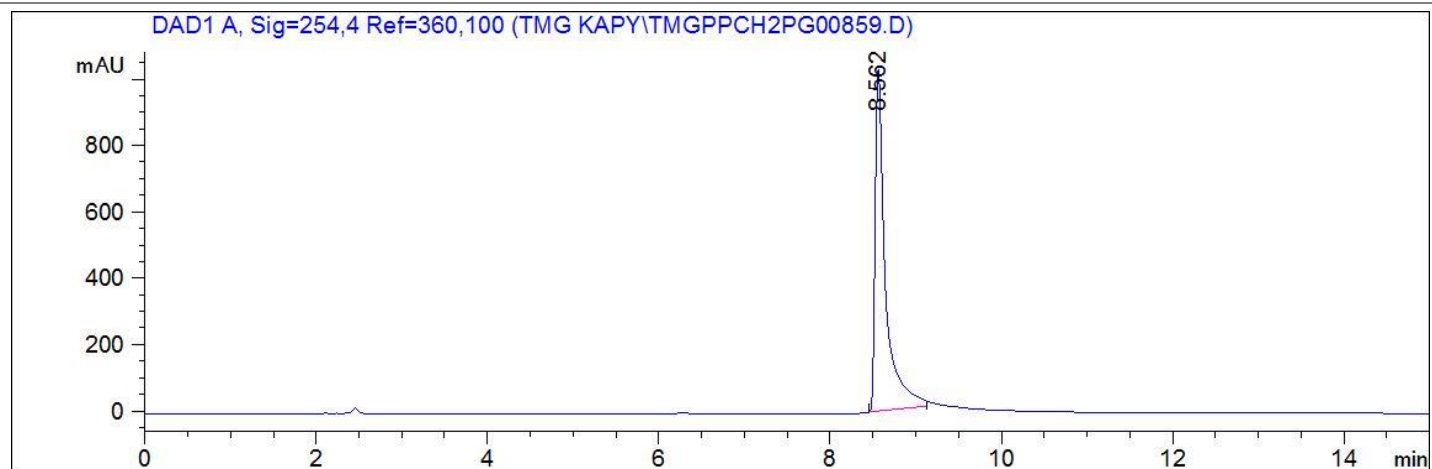




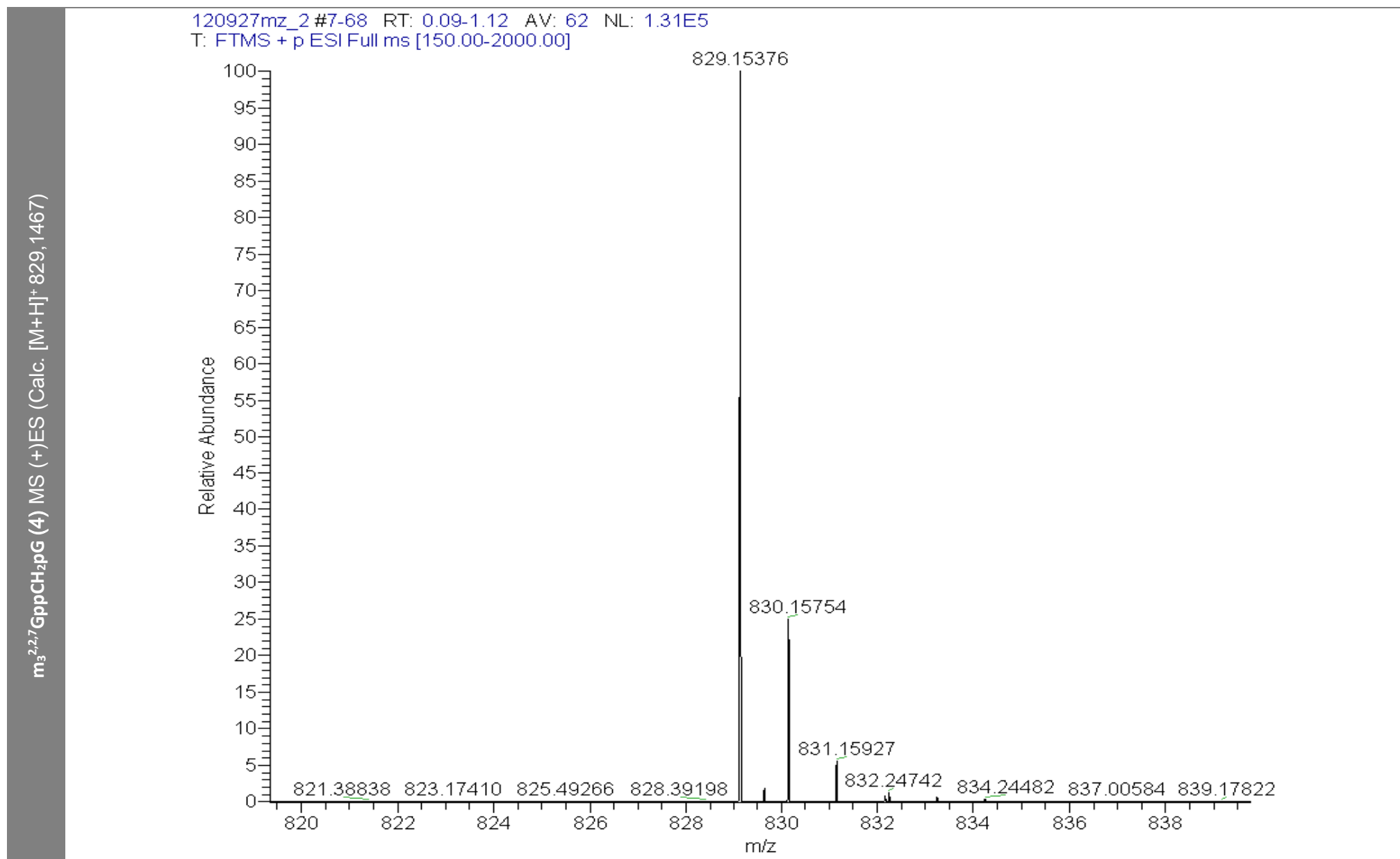
m₃^{2,2,7}GppCH₂pG Na⁺ salt (4)

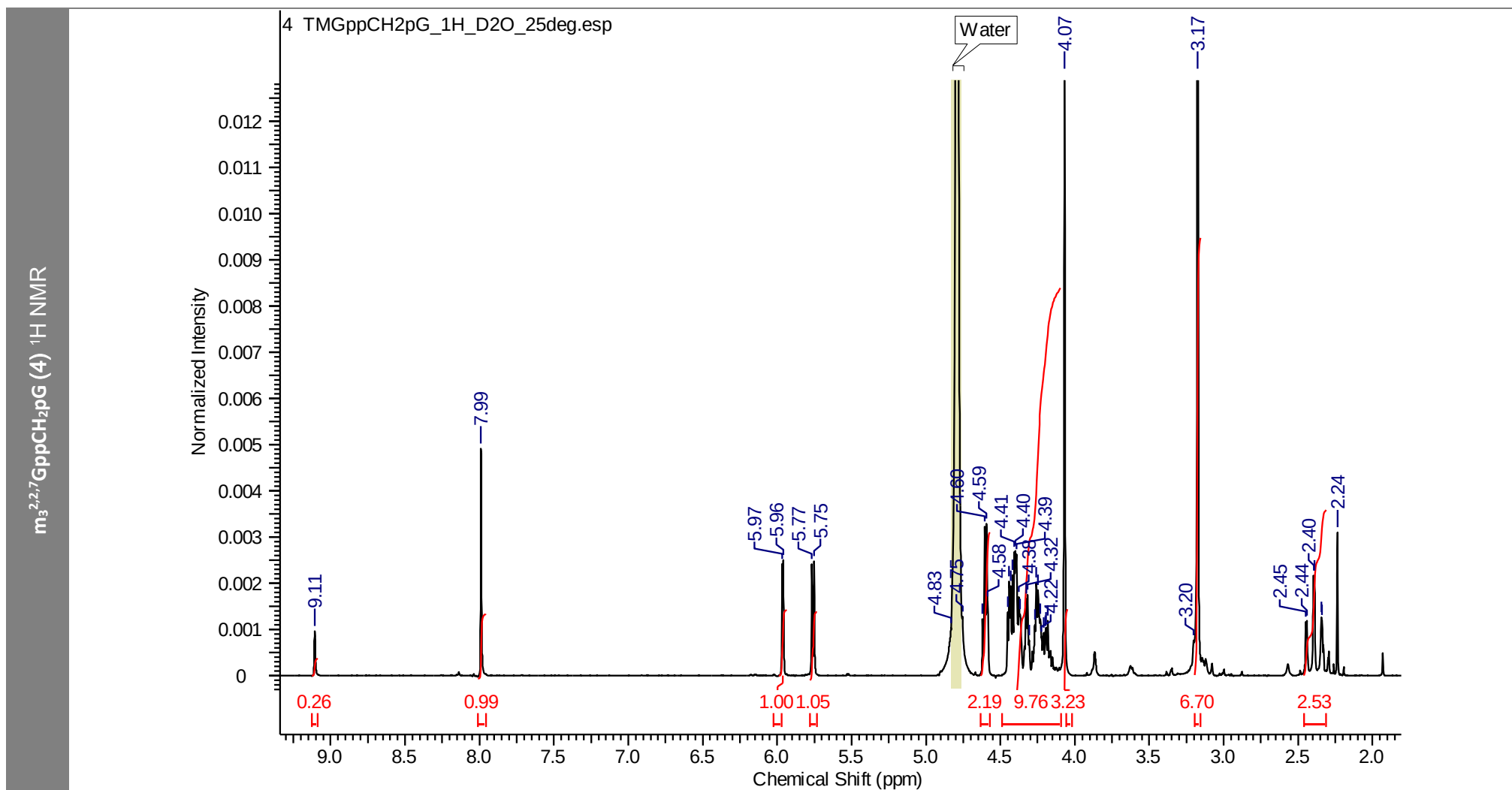


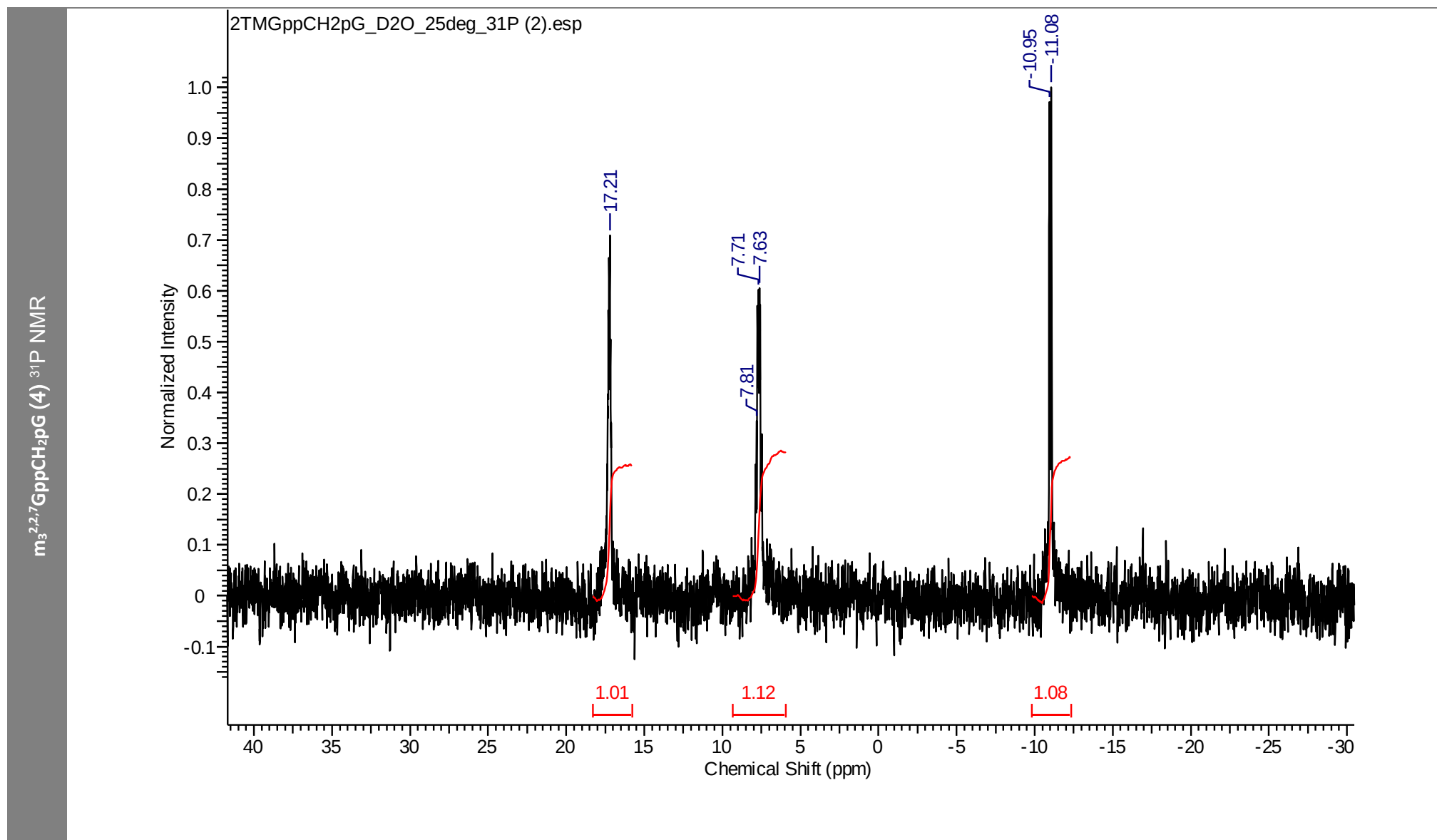
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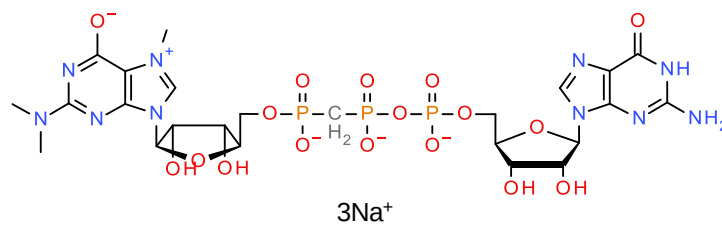
m₃^{2,2,7}GppCH₂pG (4) RP HPLC



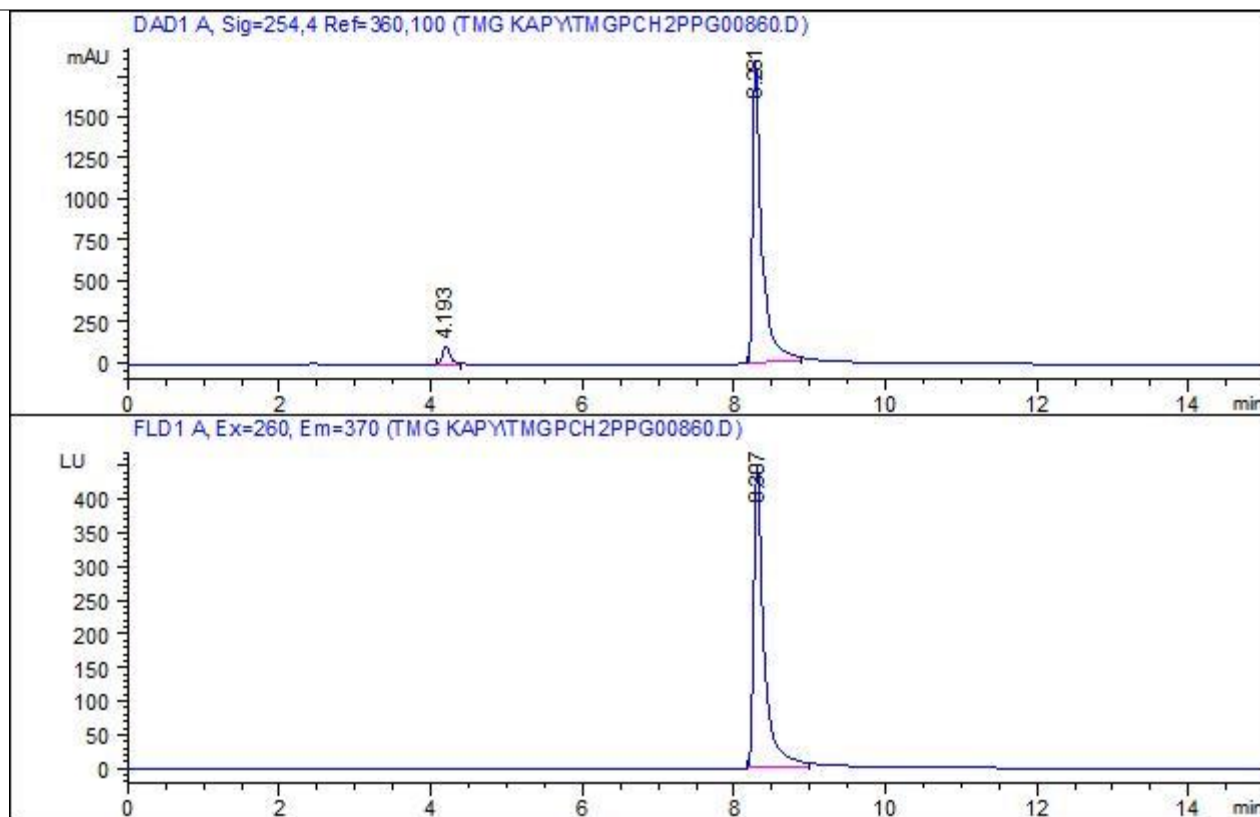




$m_3^{2,2,7}$ GpCH₂ppG Na⁺ salt (5)

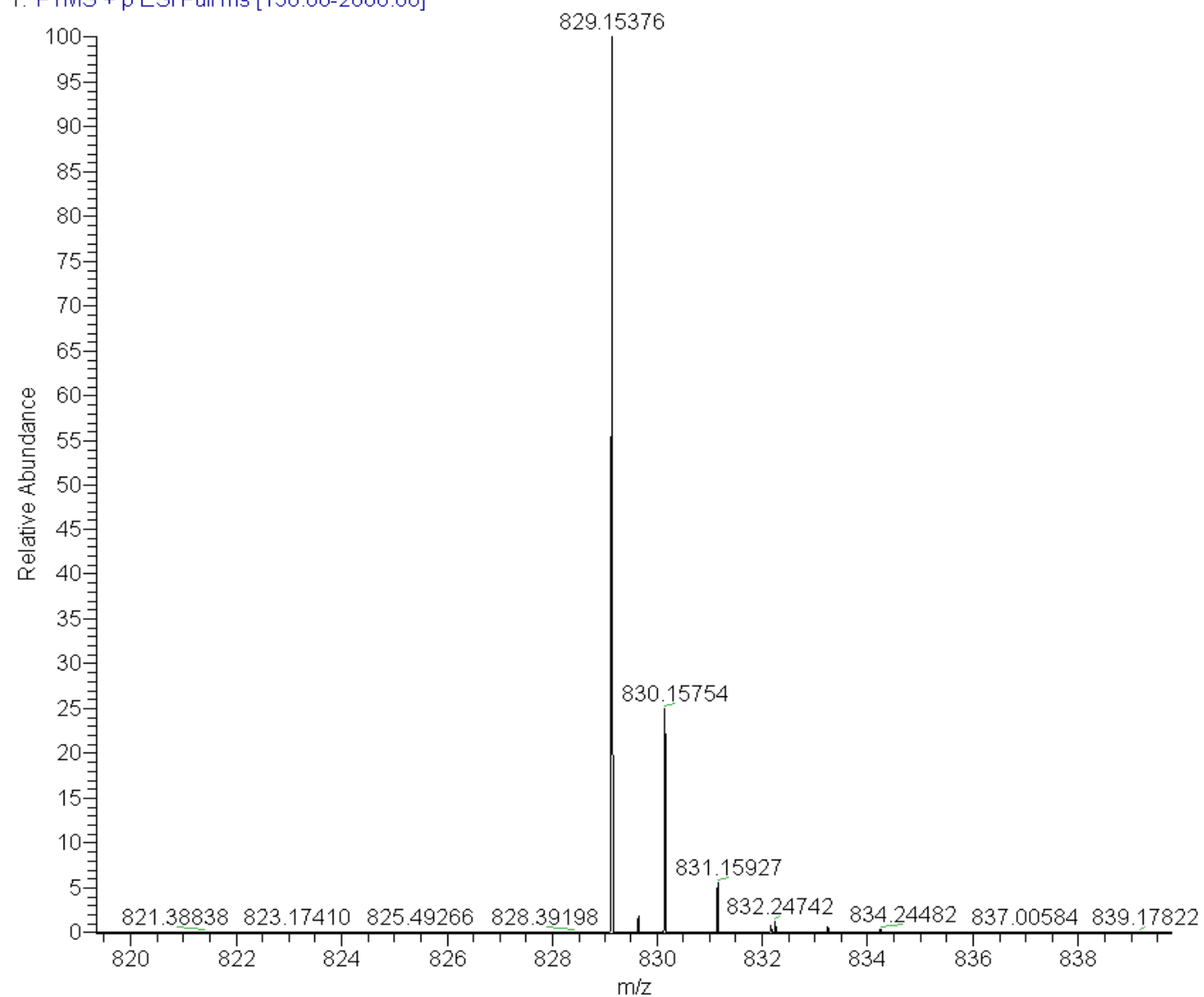


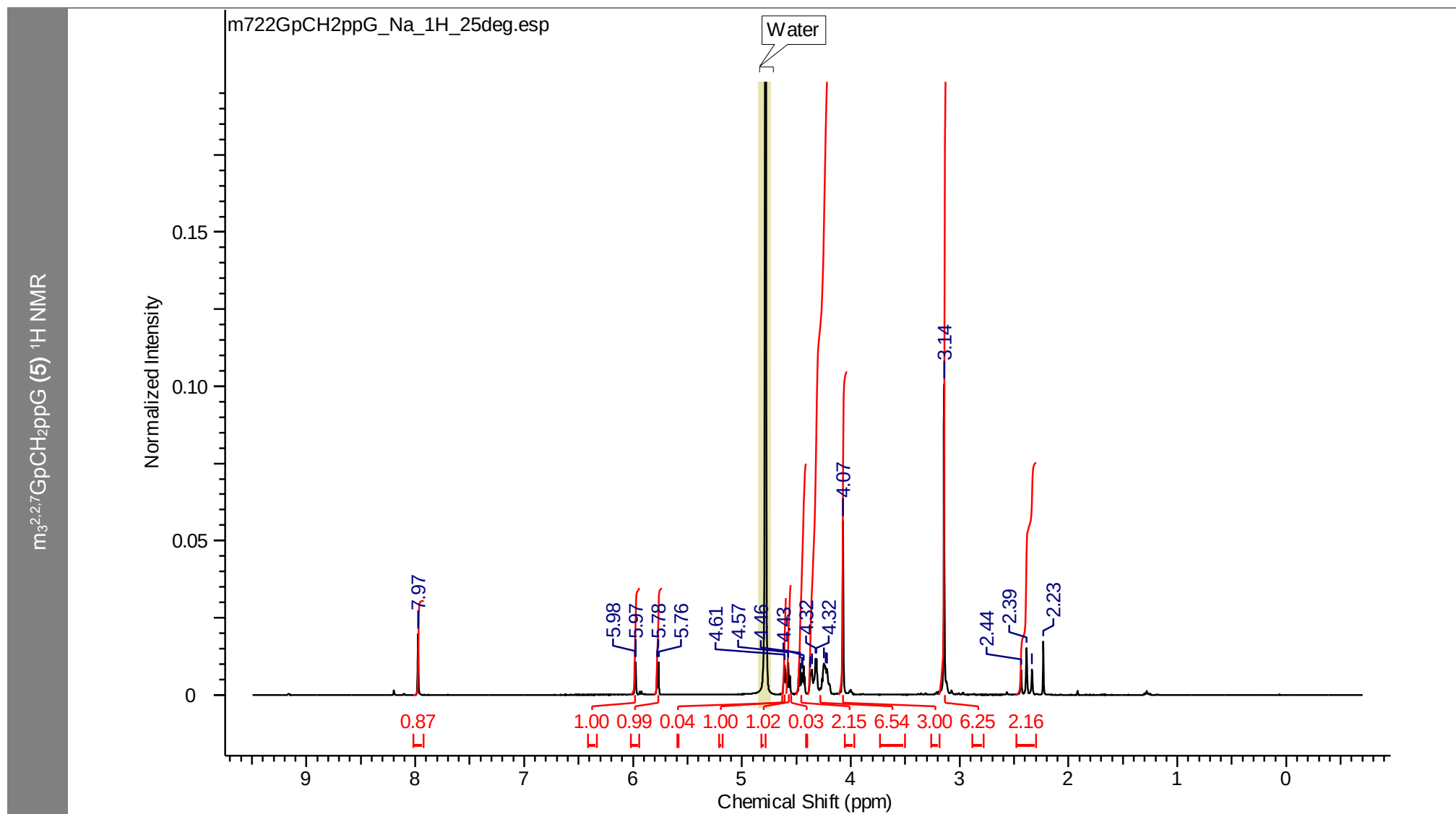
$m_3^{2,2,7}$ GpCH₂ppG (5) RP HPLC

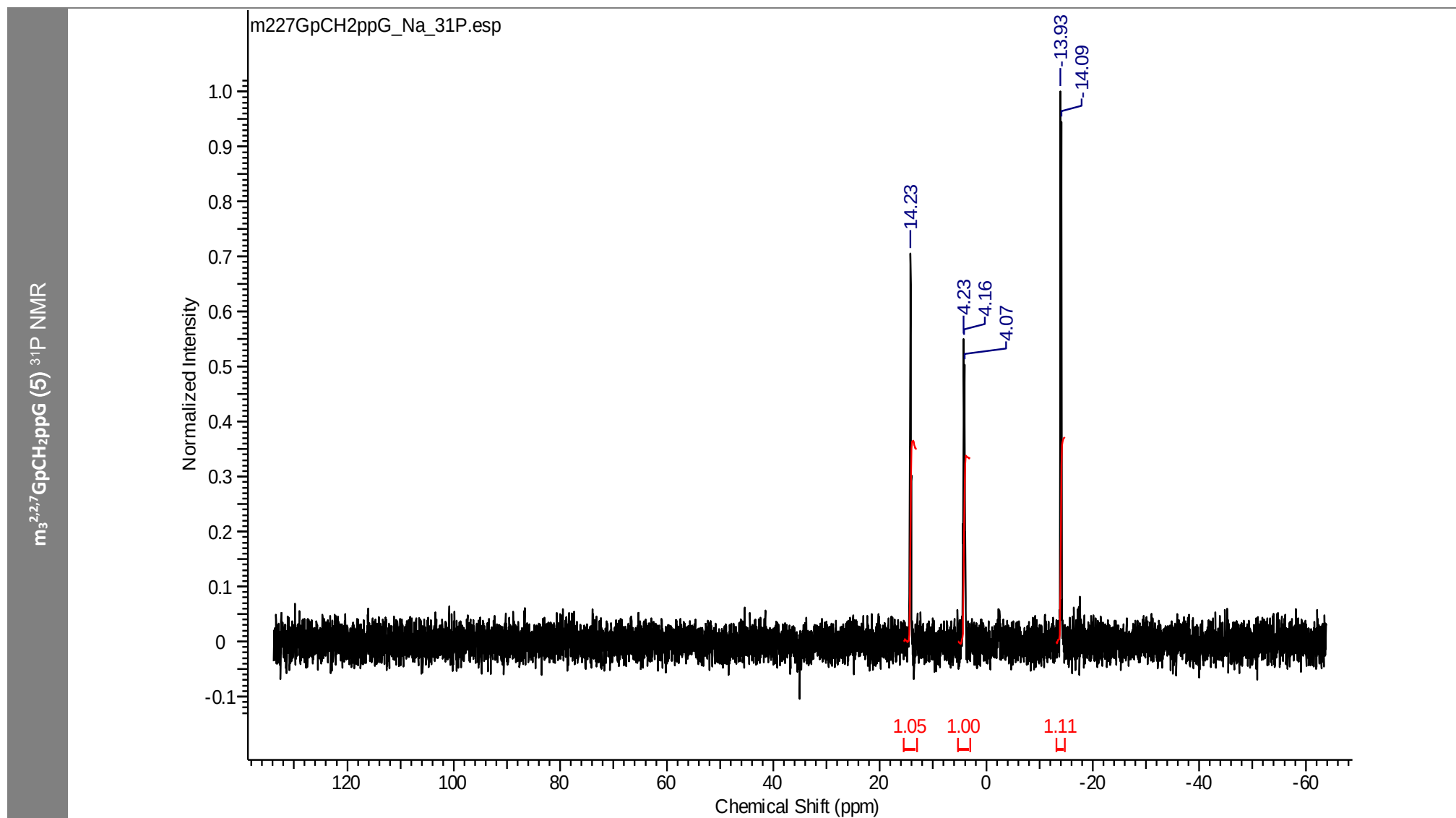


$m_3^{2,2,7}$ GpCH₂ppG (5) MS (+)ES (Calc. [M+H]⁺ 829,1467)

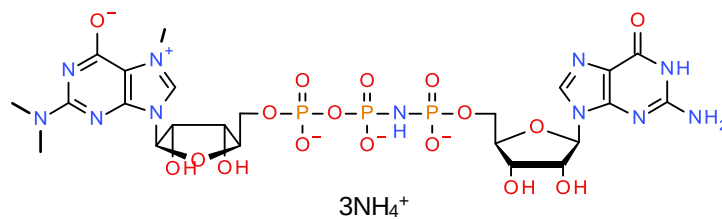
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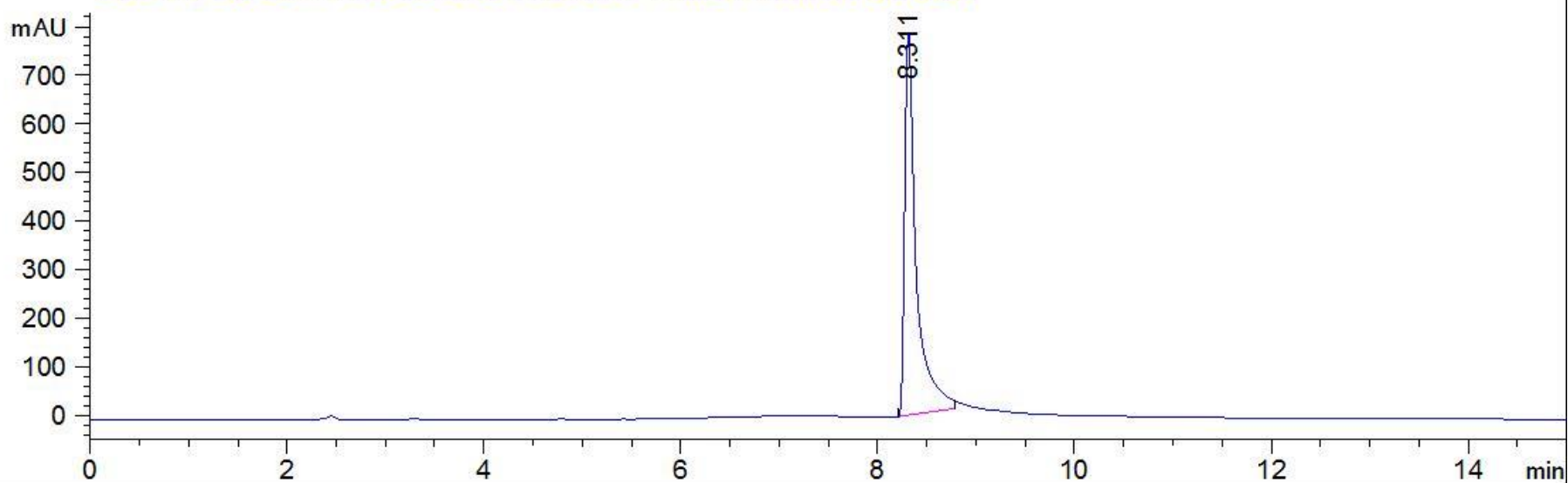




$m_3^{2,2,7}$ GppNHpG NH_4^+ salt (6)

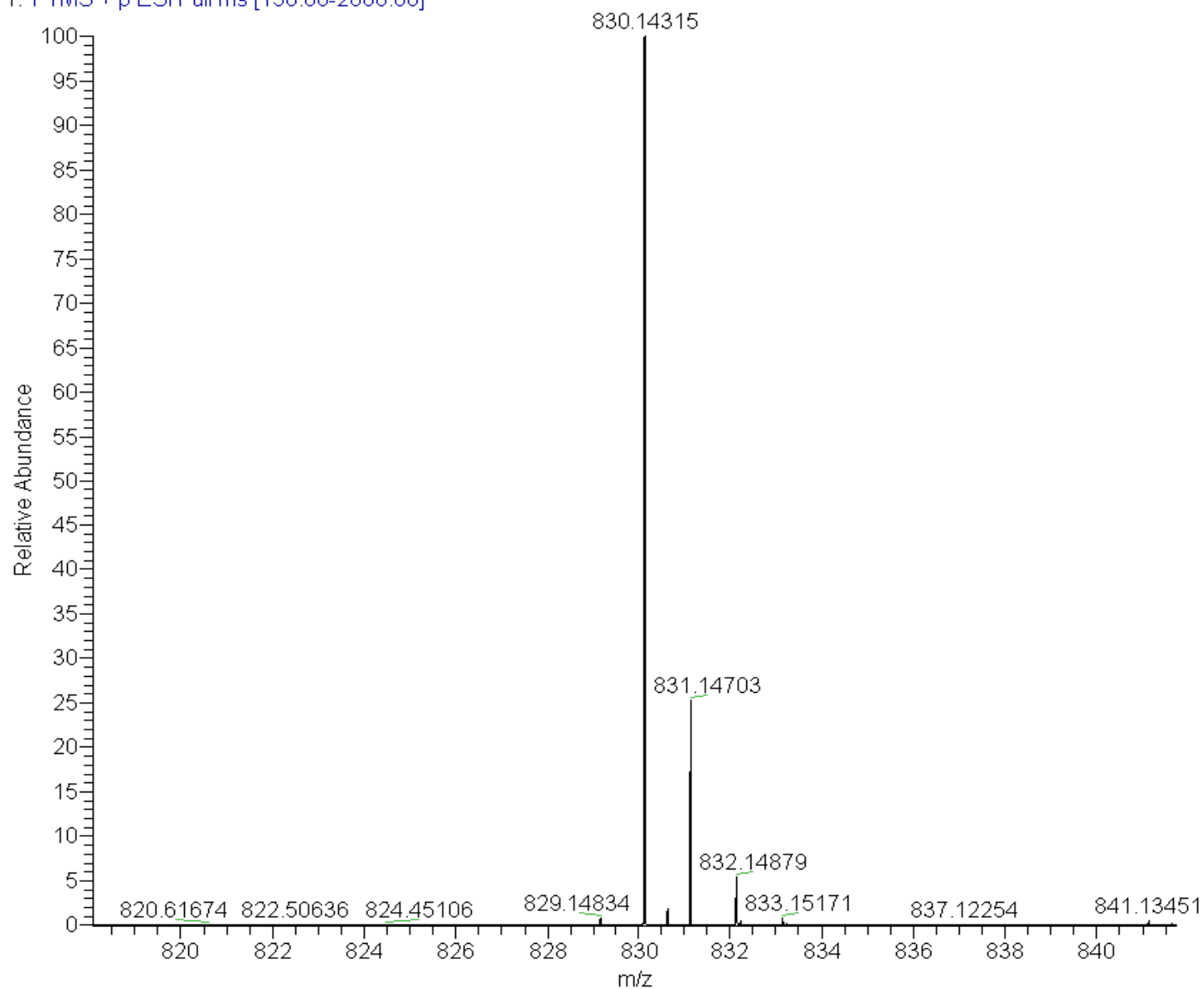


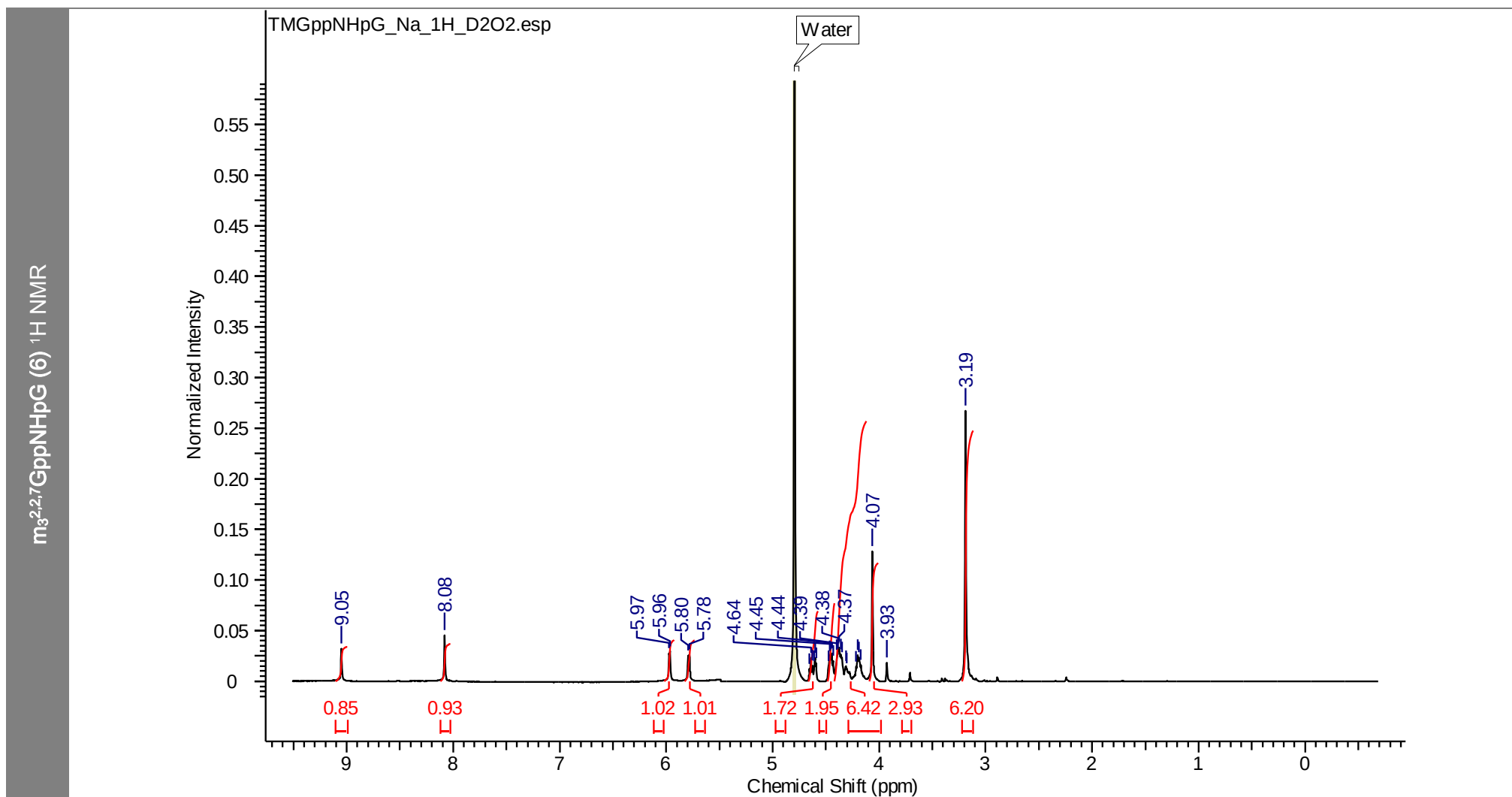
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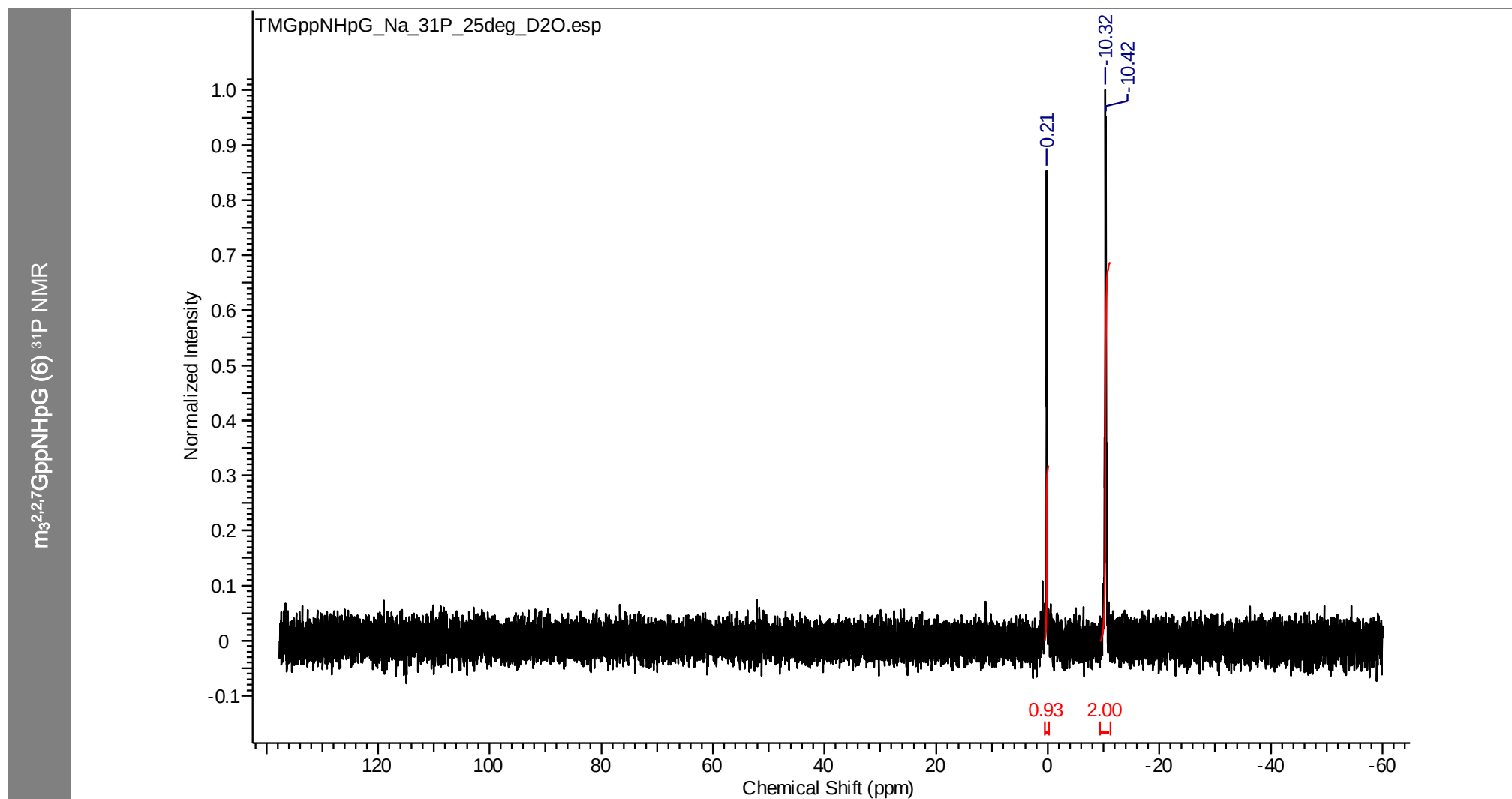


$m_3^{2,2,7}$ GppNHpG (6) MS (+)ES (Calc. [M+H]⁺ 830,1420)

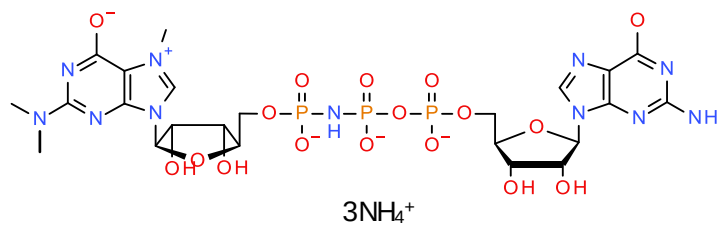
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T: FTMS + p ESI Full ms [150.00-2000.00]



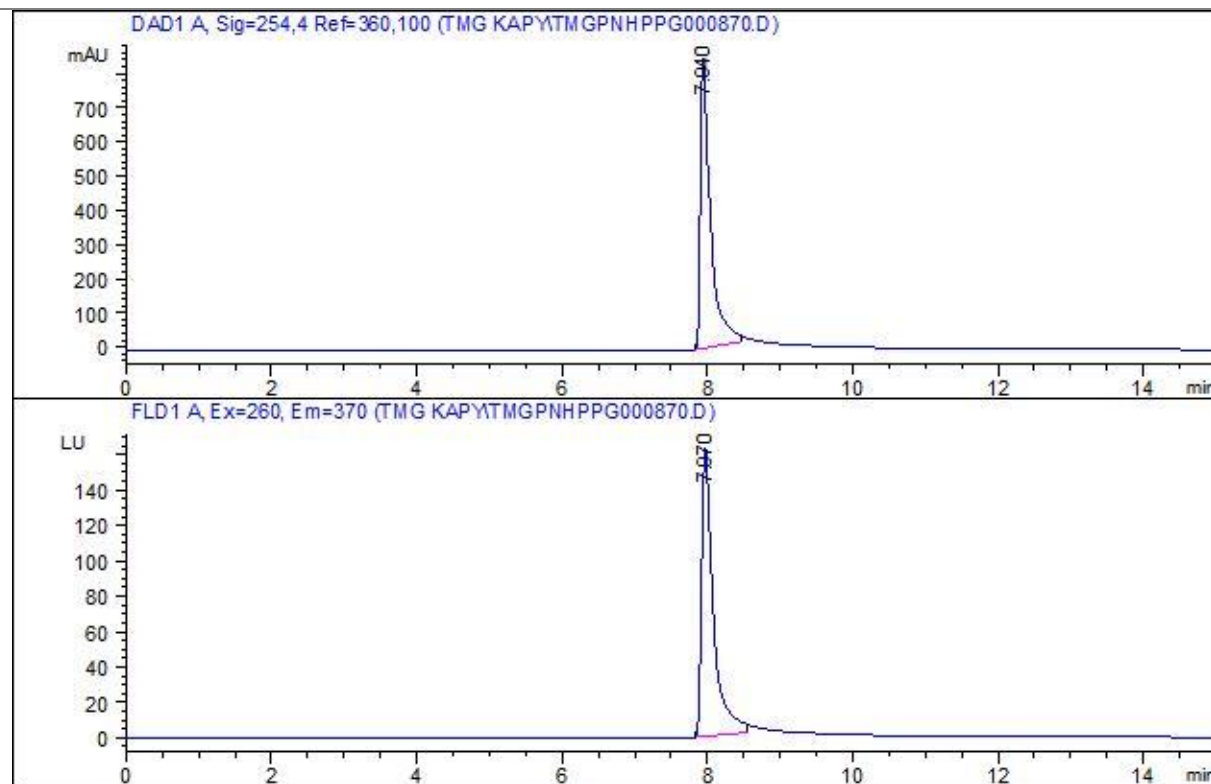




m₃^{2,2,7}GpNHppG (7)

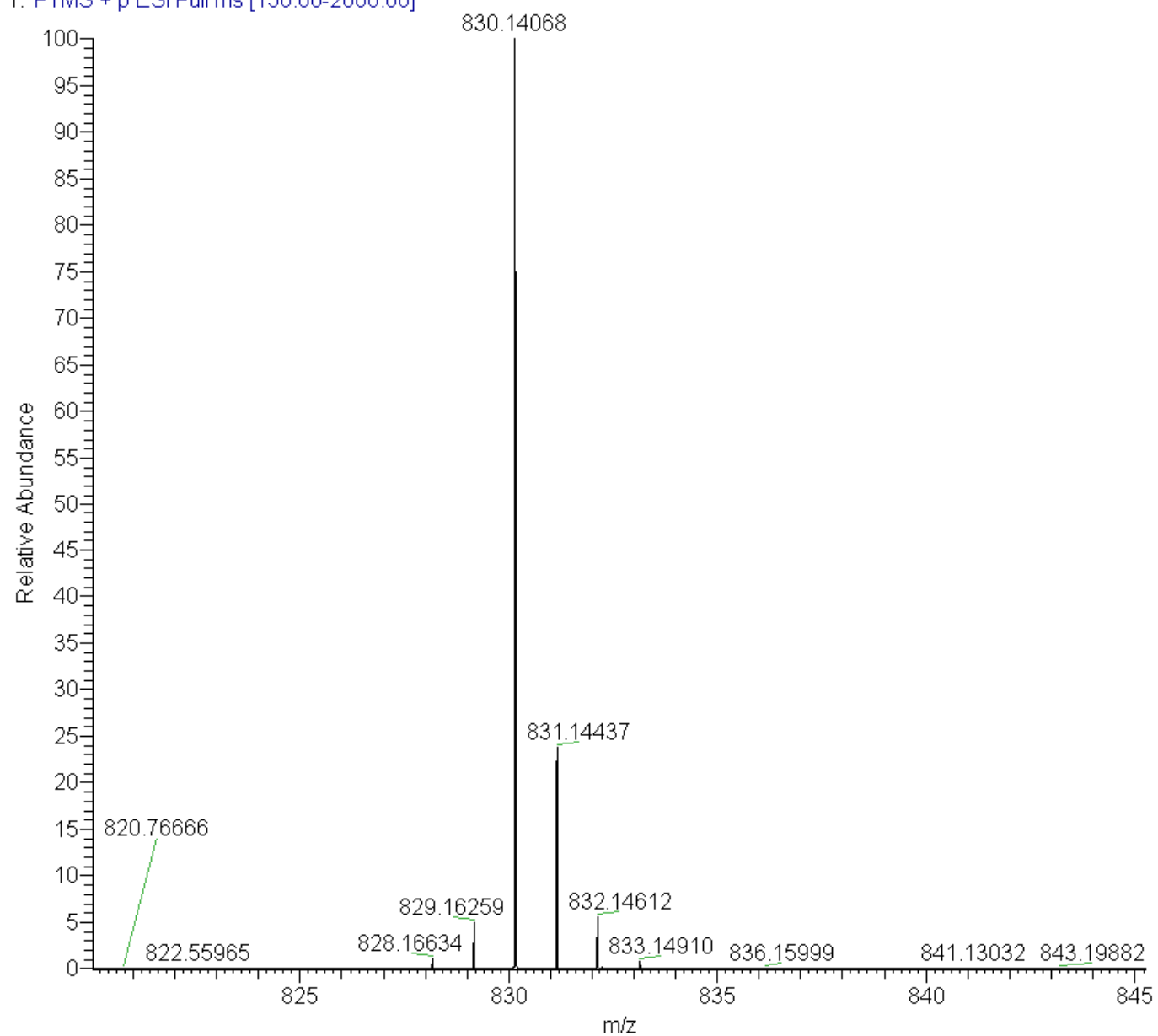


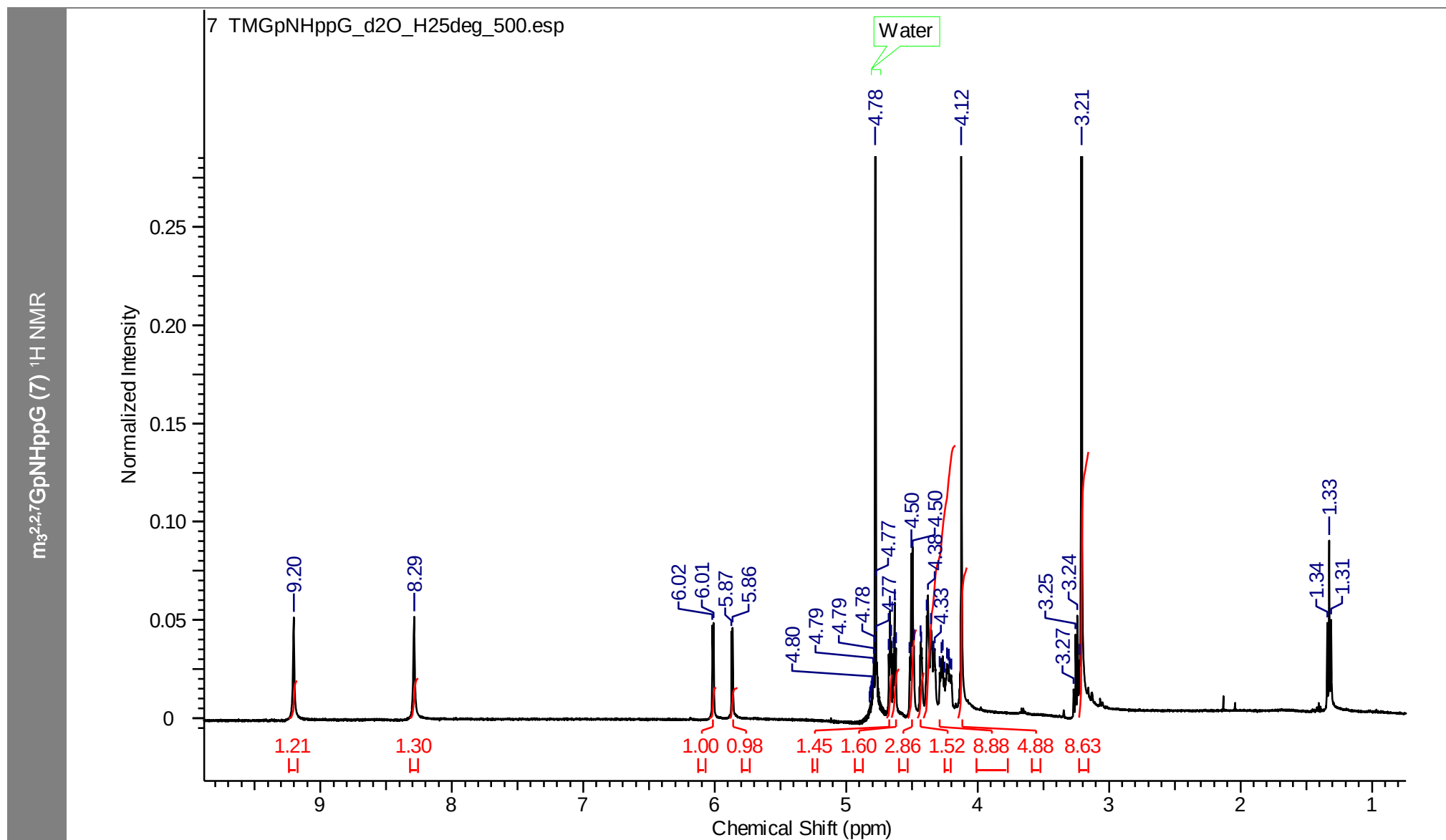
m₃^{2,2,7}GpNHppG (7) RP HPLC

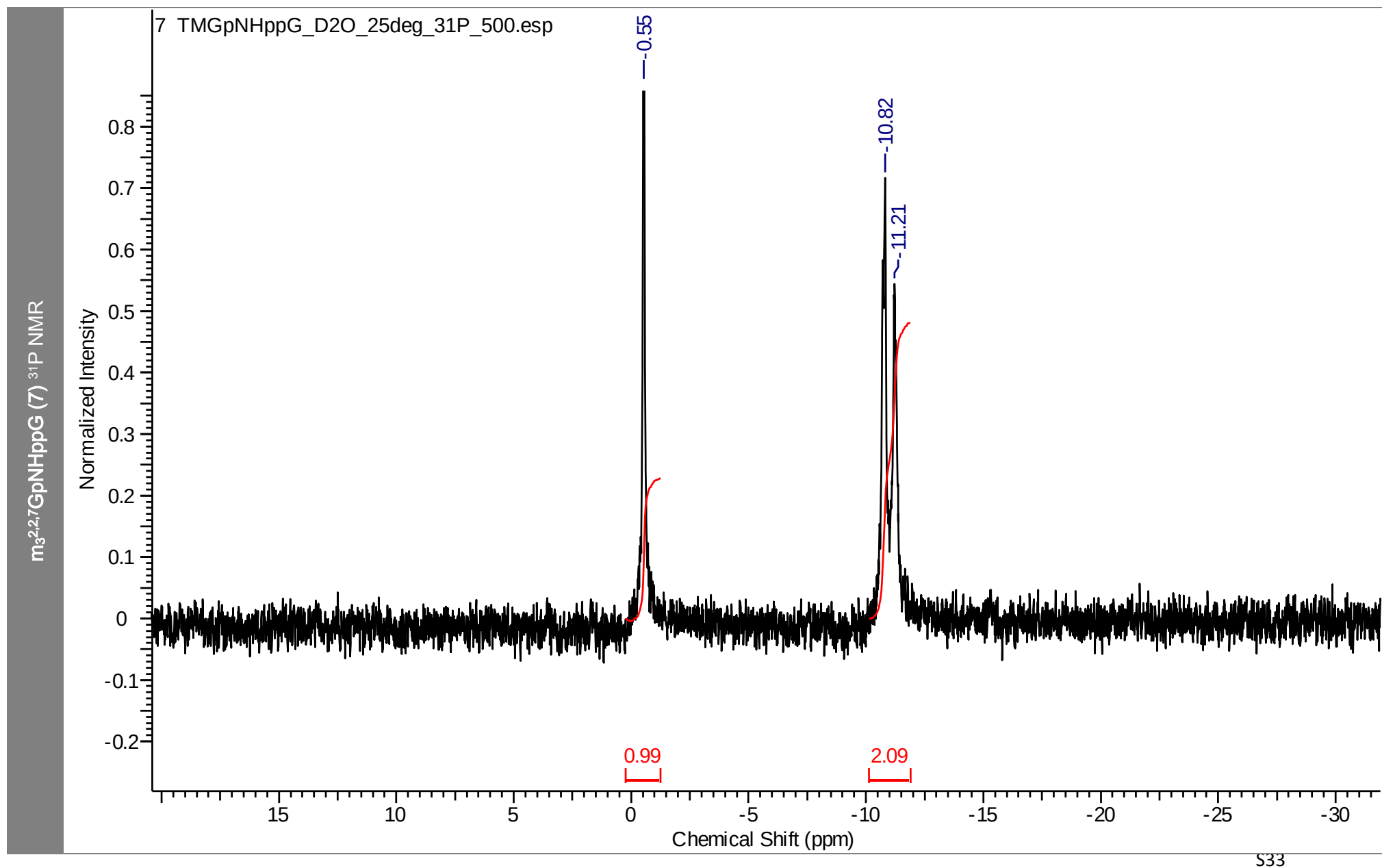


$m_3^{2,2,7}$ GpNHppG (7) MS (+)ES (Calc. [M+H]⁺ 830,1420)

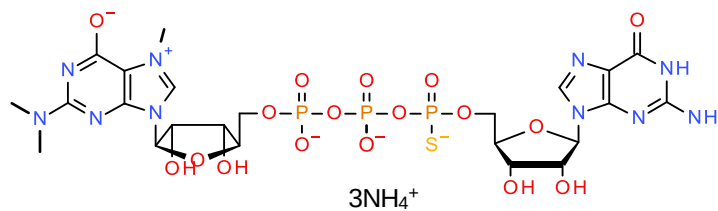
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T: FTMS + p ESI Full ms [150.00-2000.00]



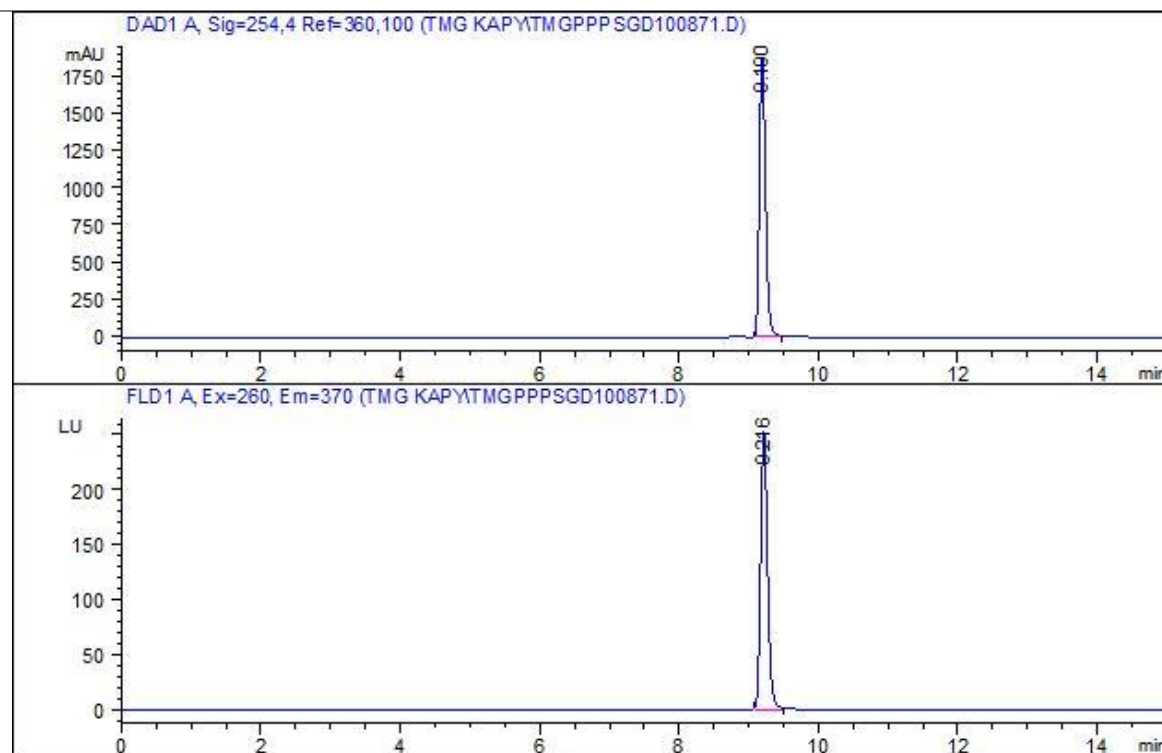




m₃^{2,2,7}GpppSG D1 (8a)

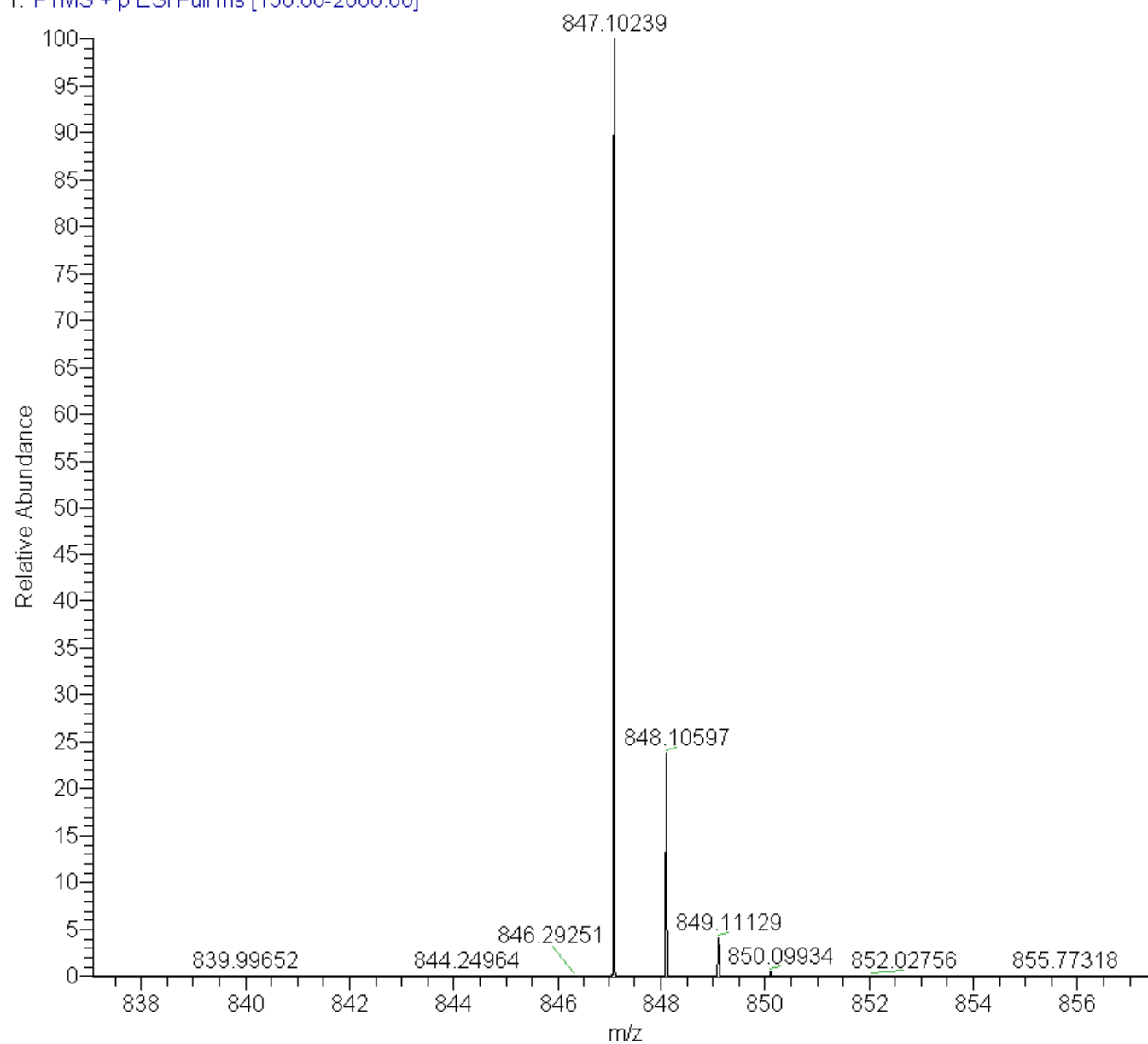


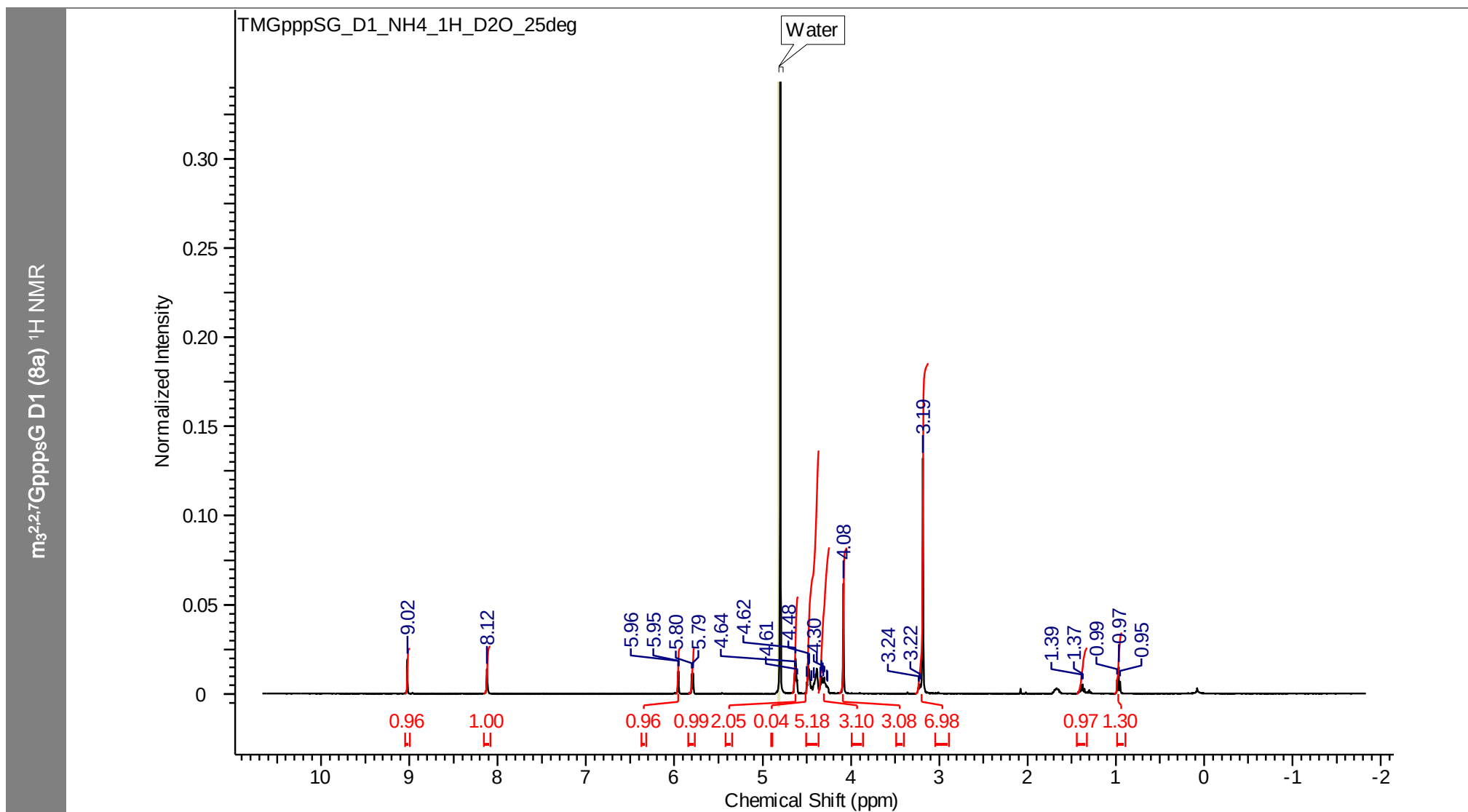
m₃^{2,2,7}GpppsG (8a) RP HPLC

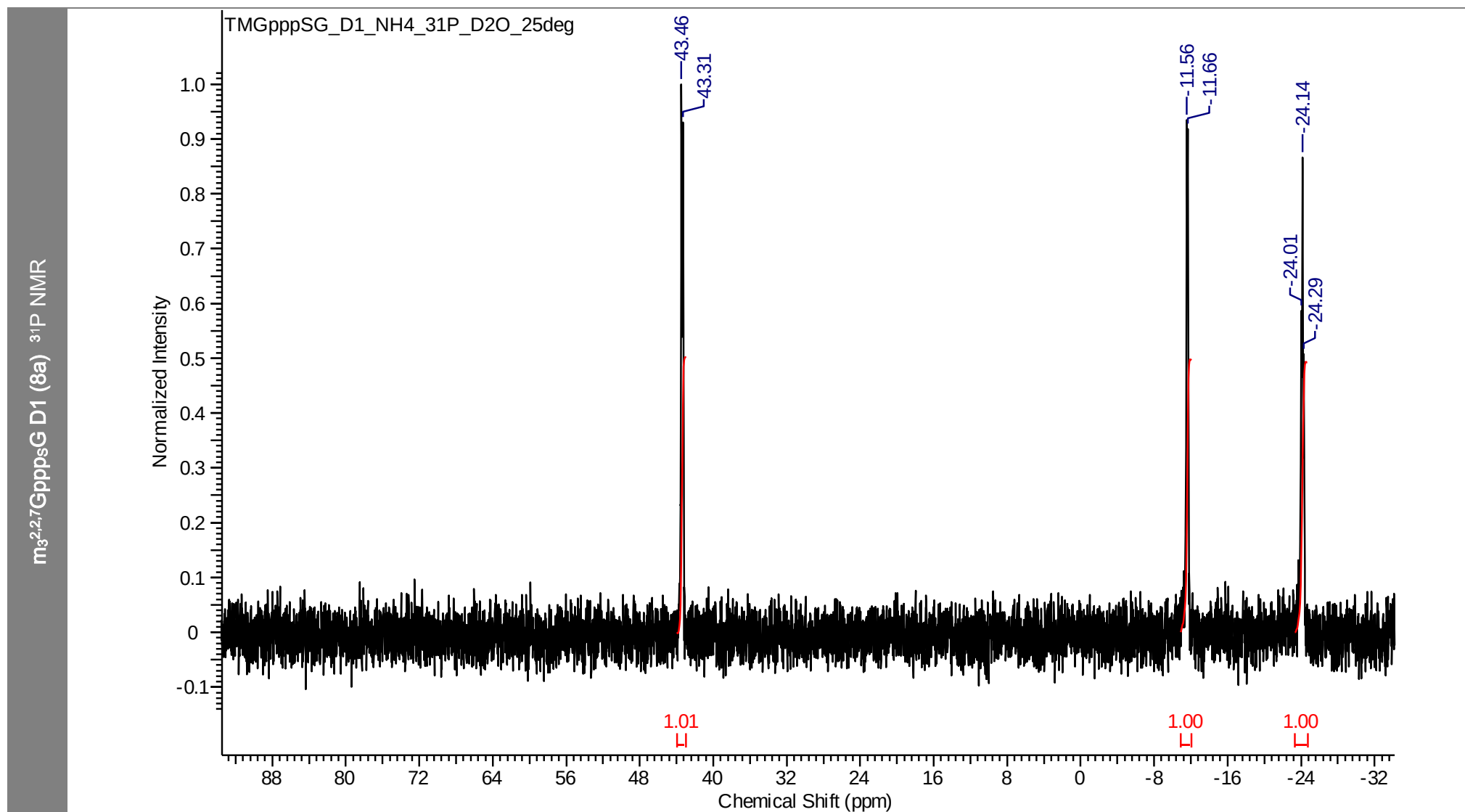


$m_3^{2,2,7}$ GpppSG D1 (8a) M S (+)ES (Calc. [M+H]⁺ 847,1031)

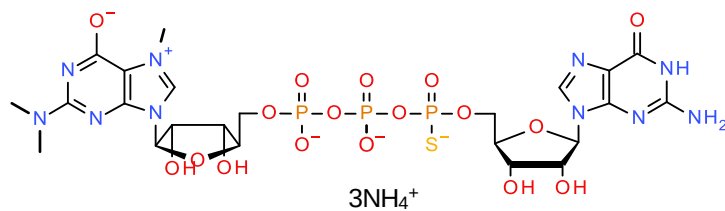
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T: FTMS + p ESI Full ms [150.00-2000.00]



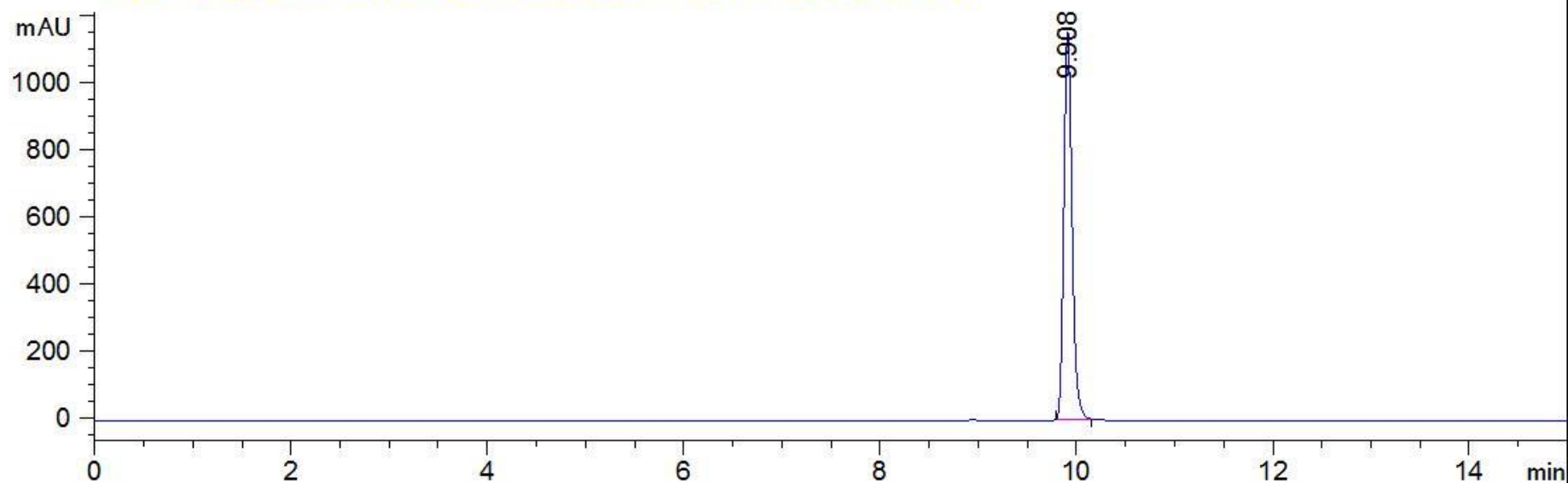




m₃^{2,2,7}GpppSG D2 (8b)

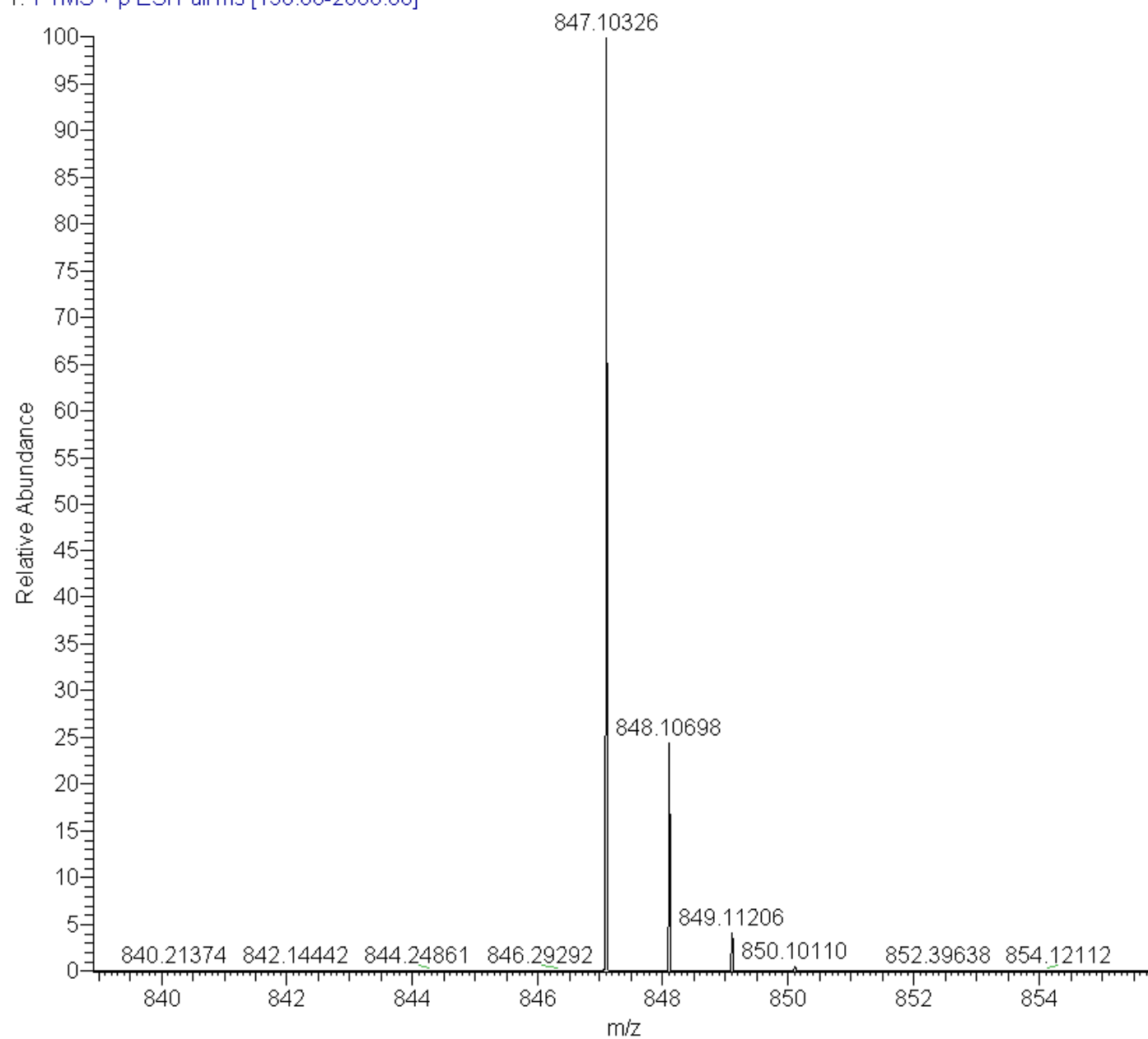


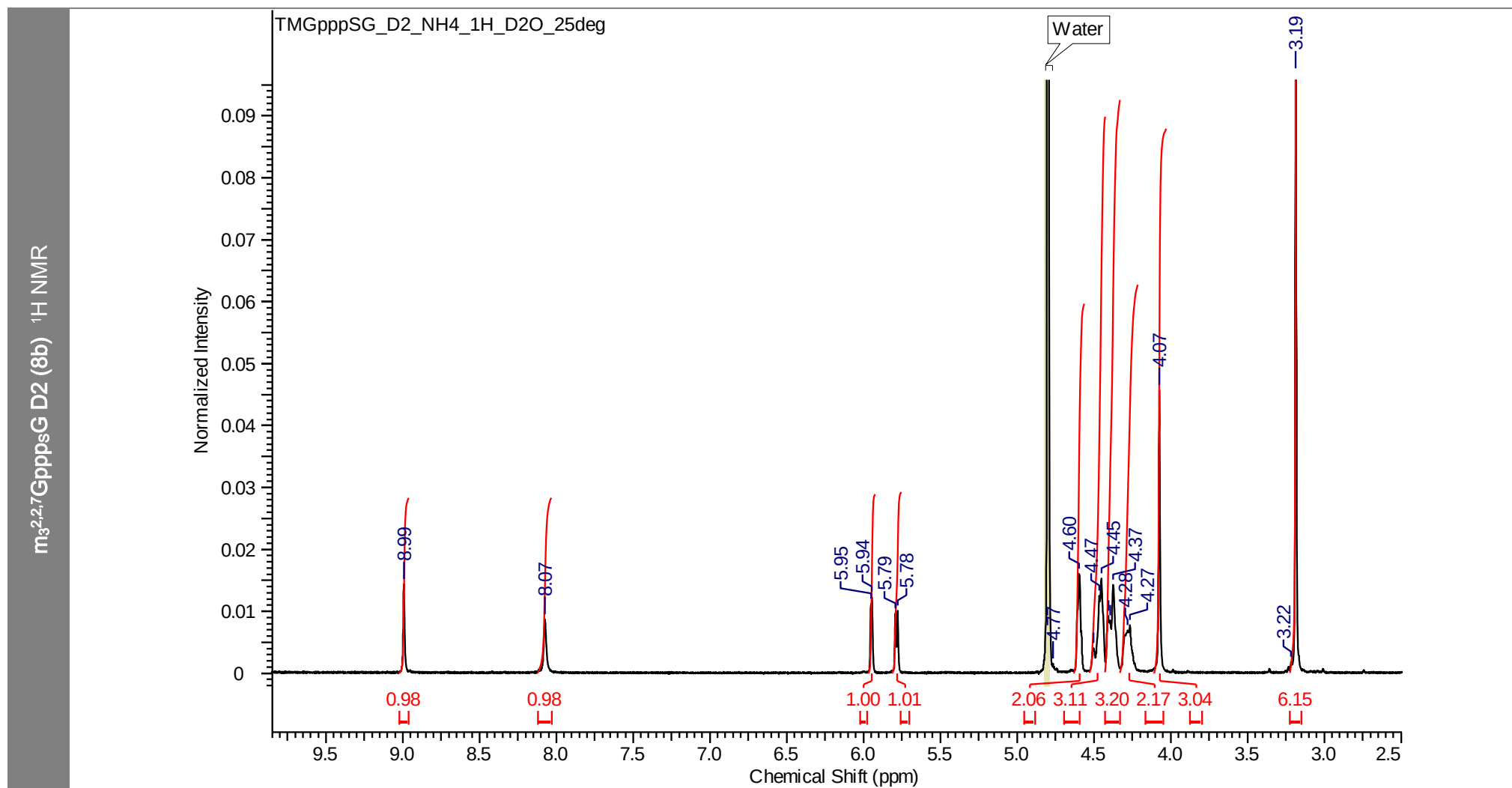
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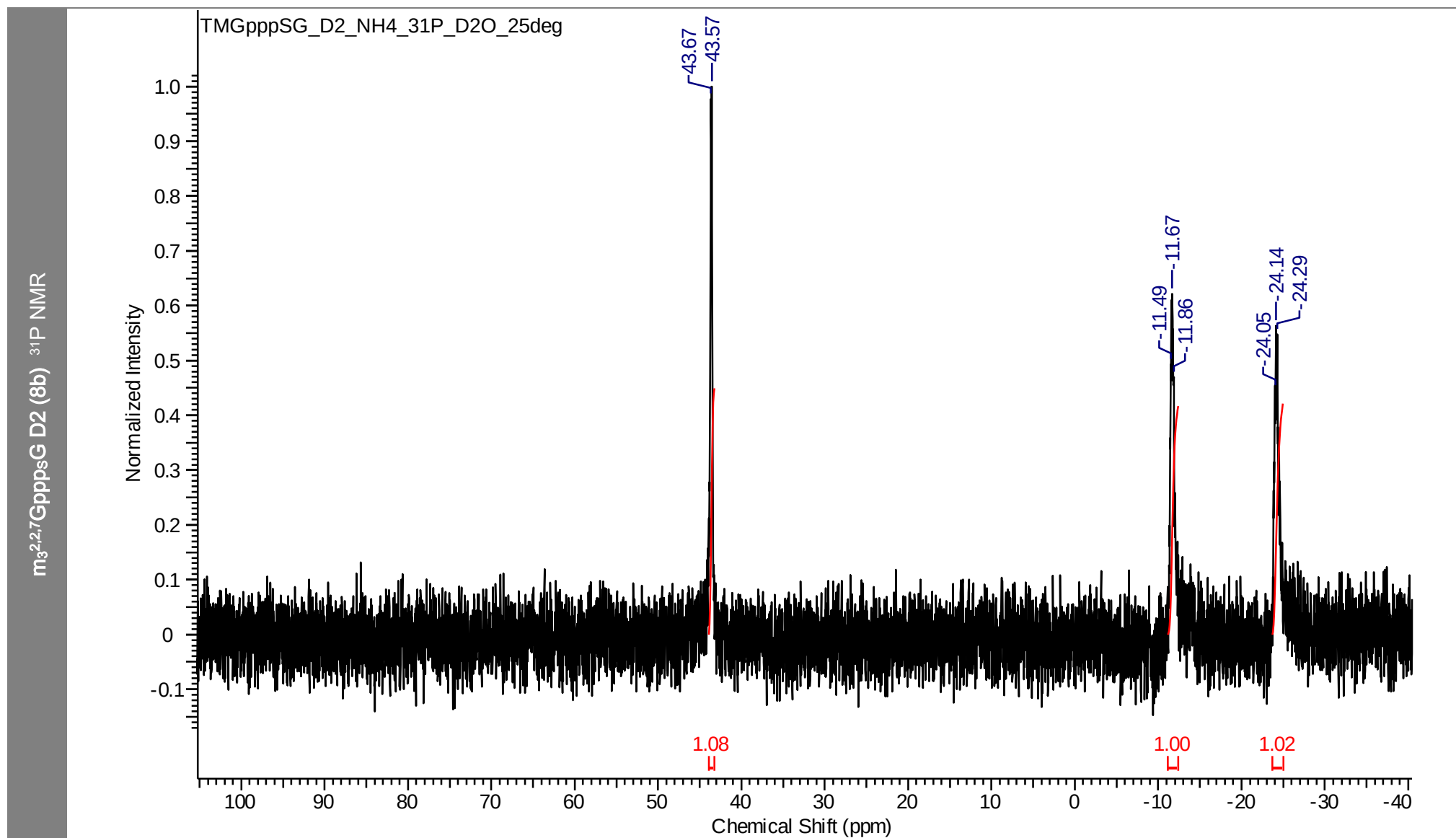


$m_3^{2,7}$ GpppsG D2 (8b) MS (+)ES (Calc. [M+H]⁺ 847,1031)

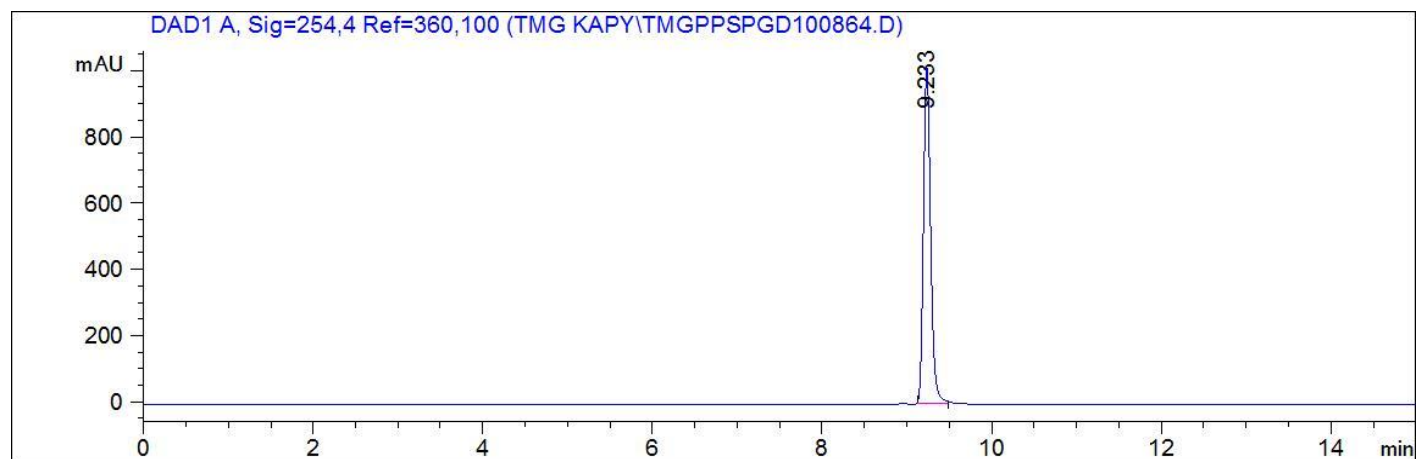
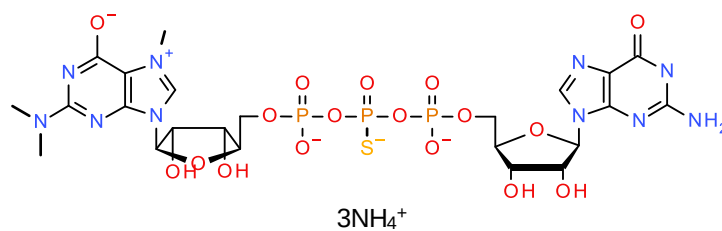
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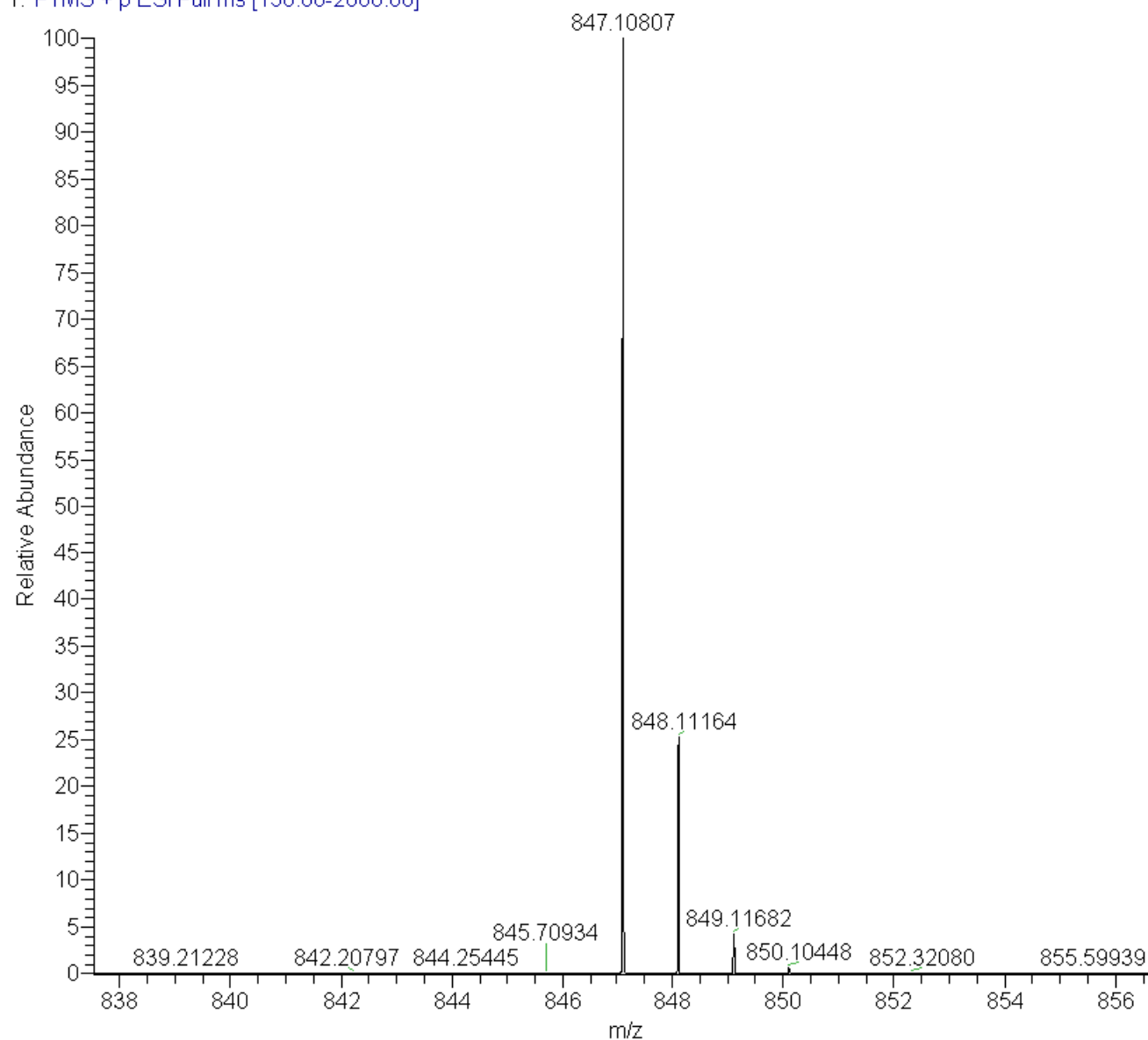


m₃^{2,2,7}GppSpG D1 NH₄⁺ salt (9a)

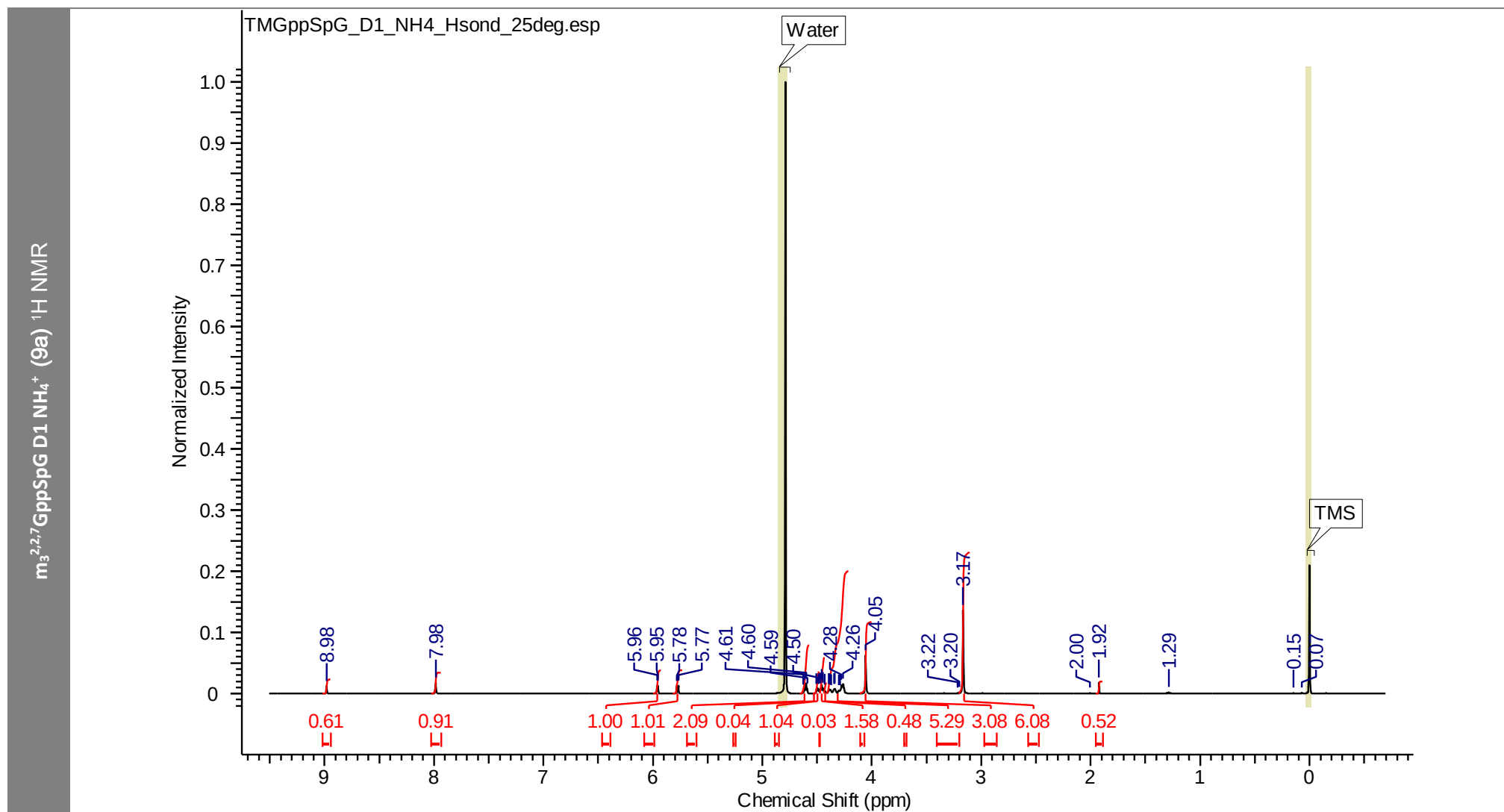


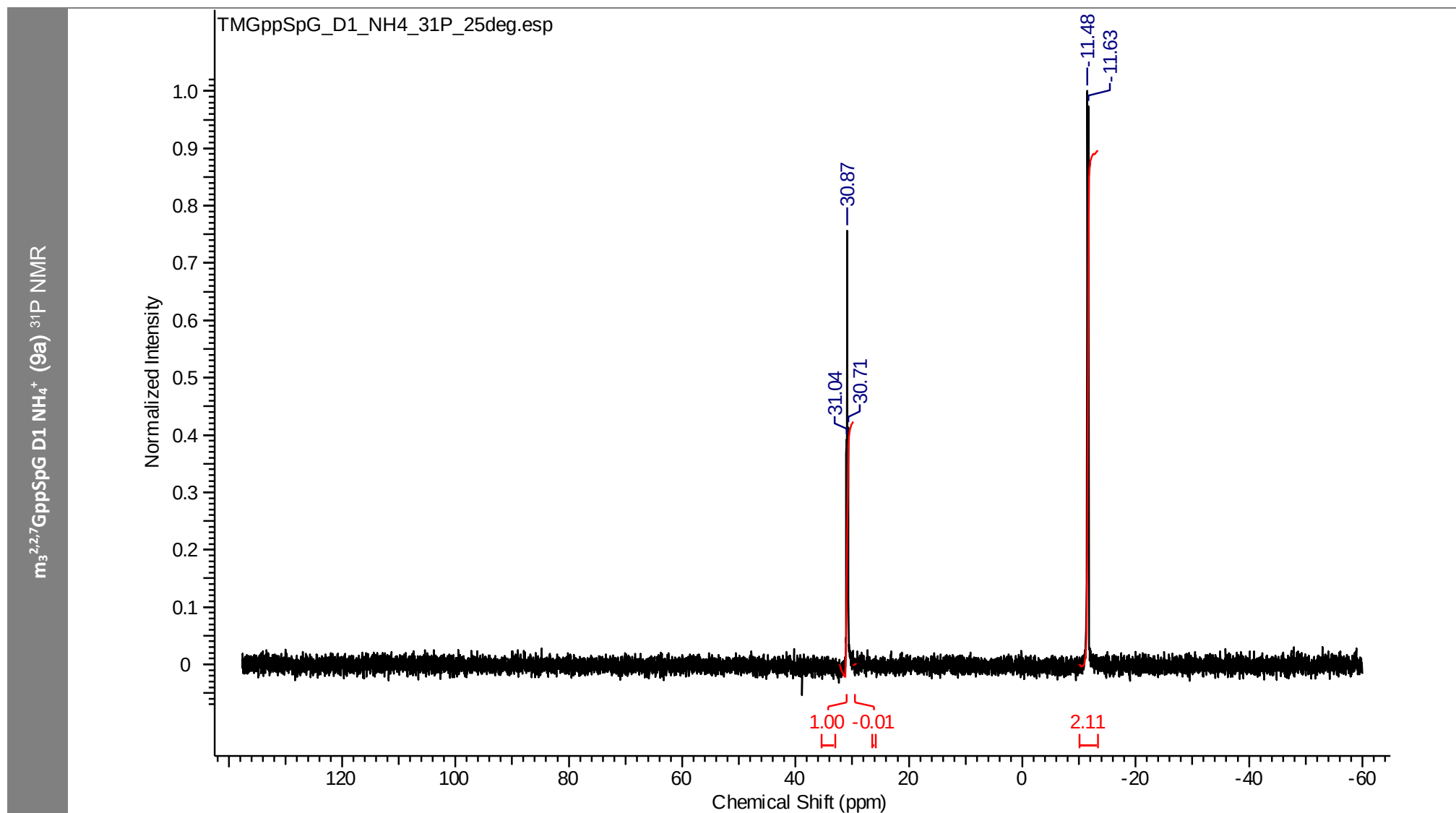
m₃^{2,2,7}GppSpG D1 NH₄⁺ (9a) RP HPLC

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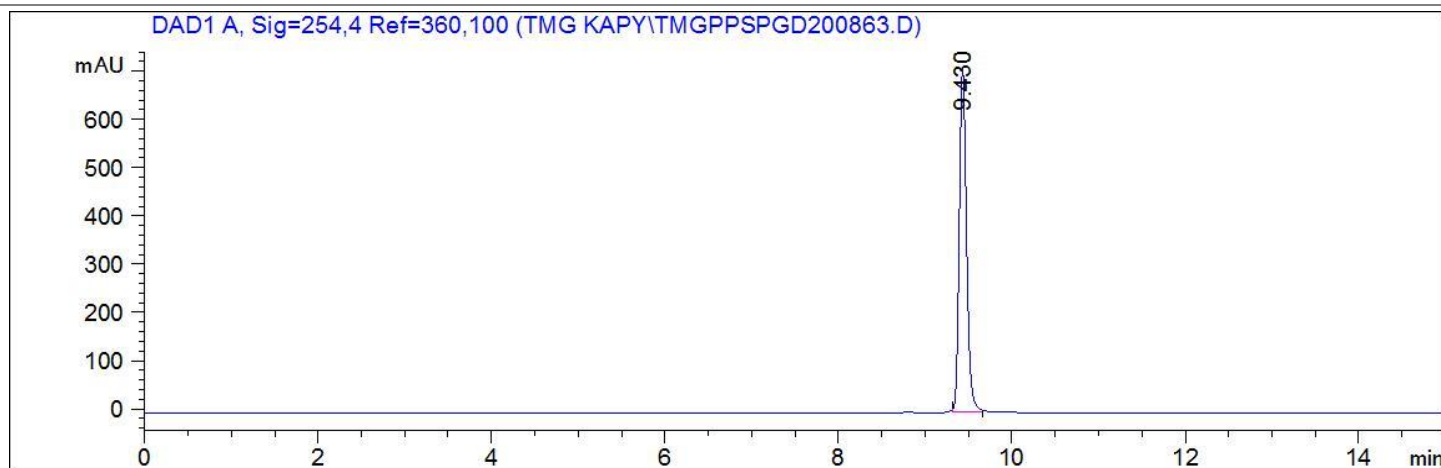
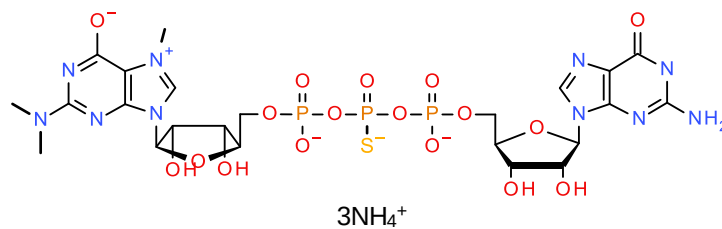


m₃^{2,2,7}GppSpG D1 NH₄⁺ (9a) MS (+)ES (Calc. [M+H]⁺ 847,1031



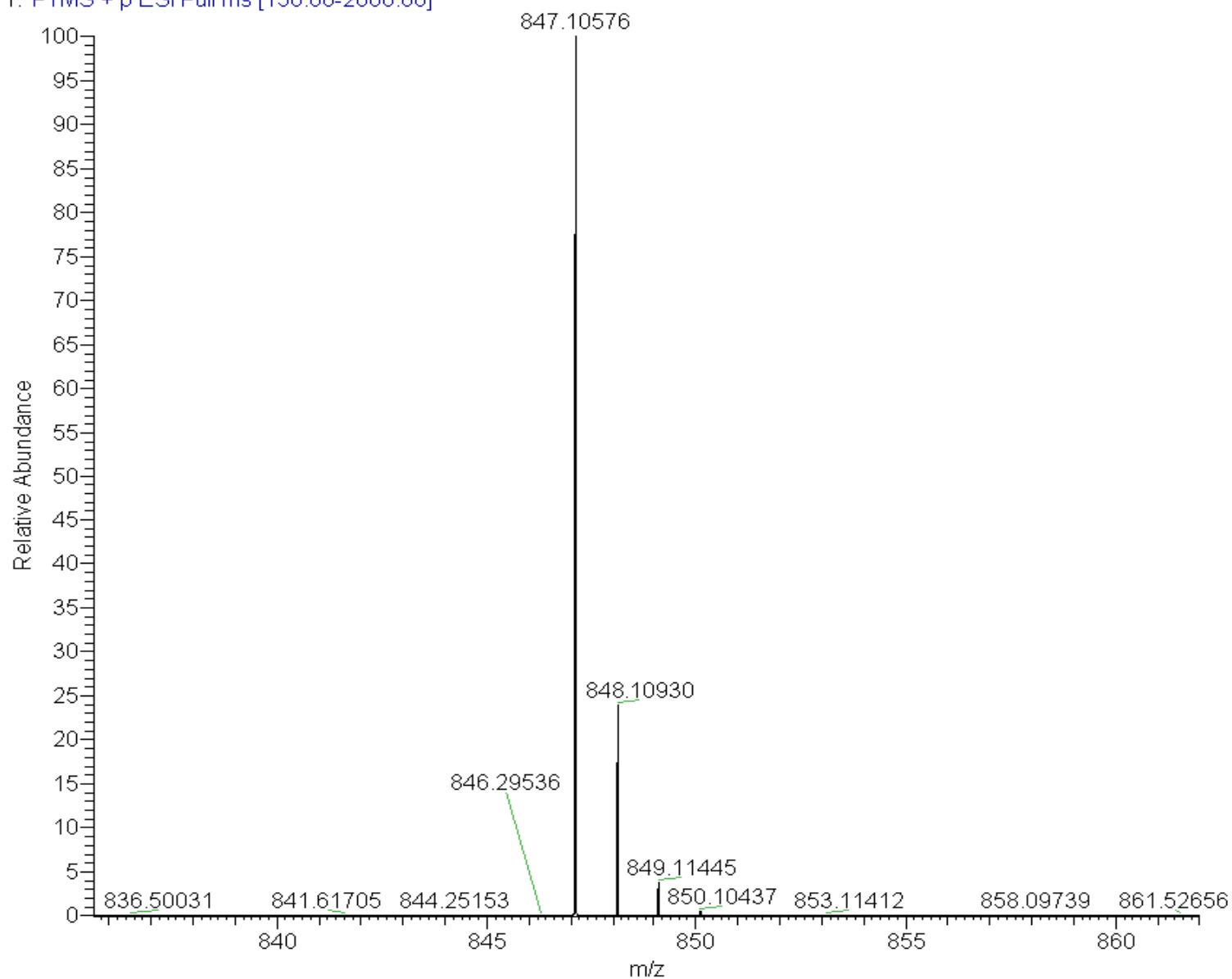


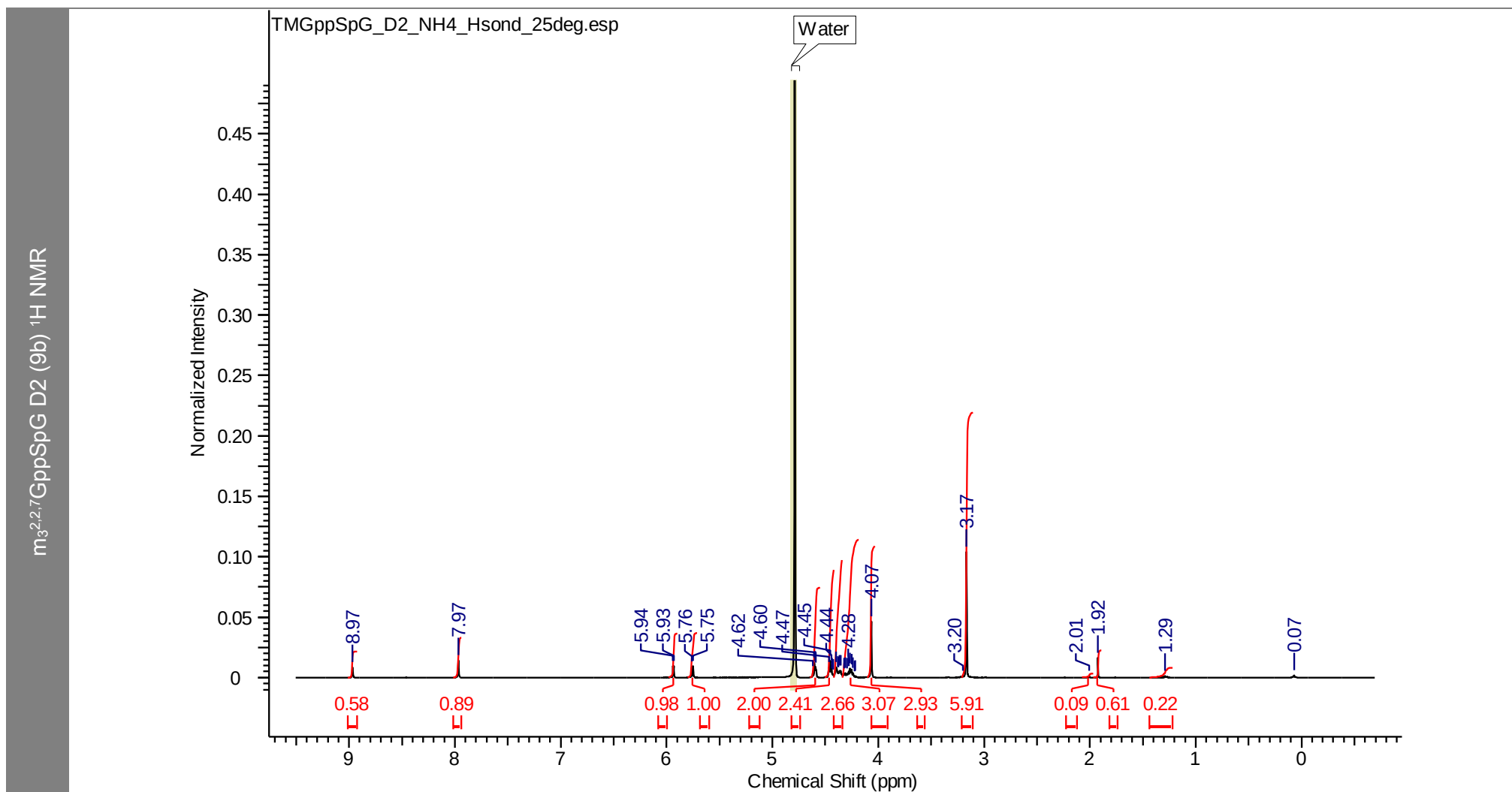
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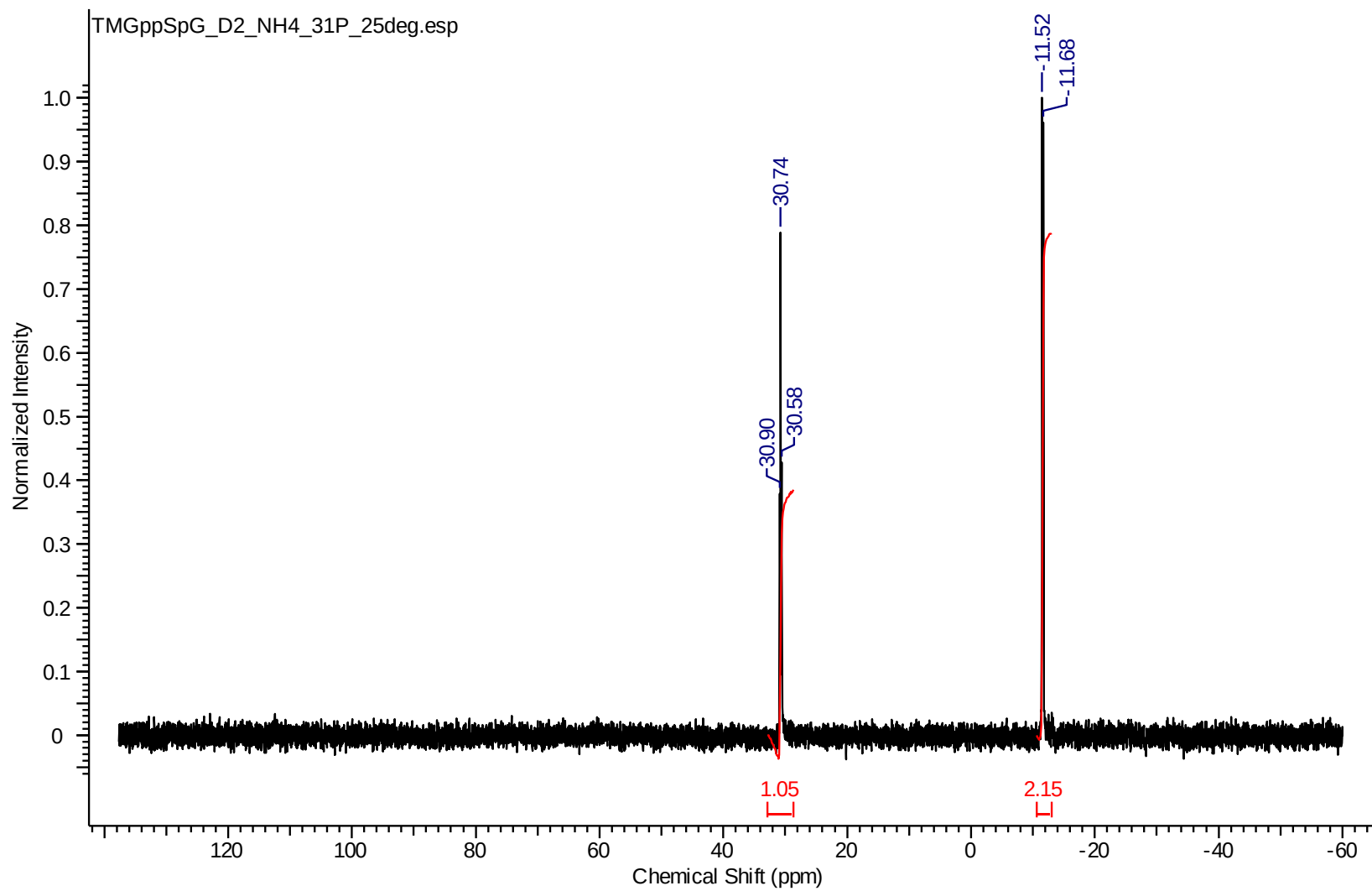
m₃^{2,2,7}GppspG D2 (9b) RP HPLC

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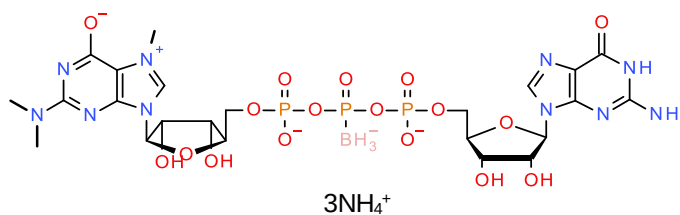




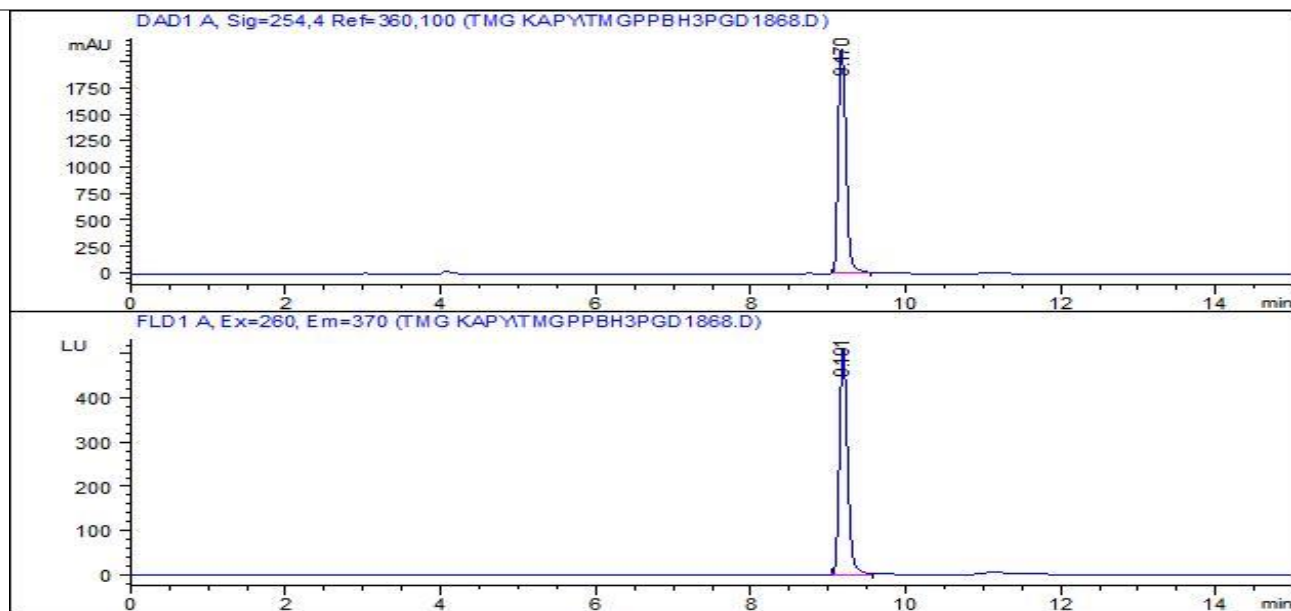
m₃^{2,7}GppSpG D2 (9b) ³¹P NMR

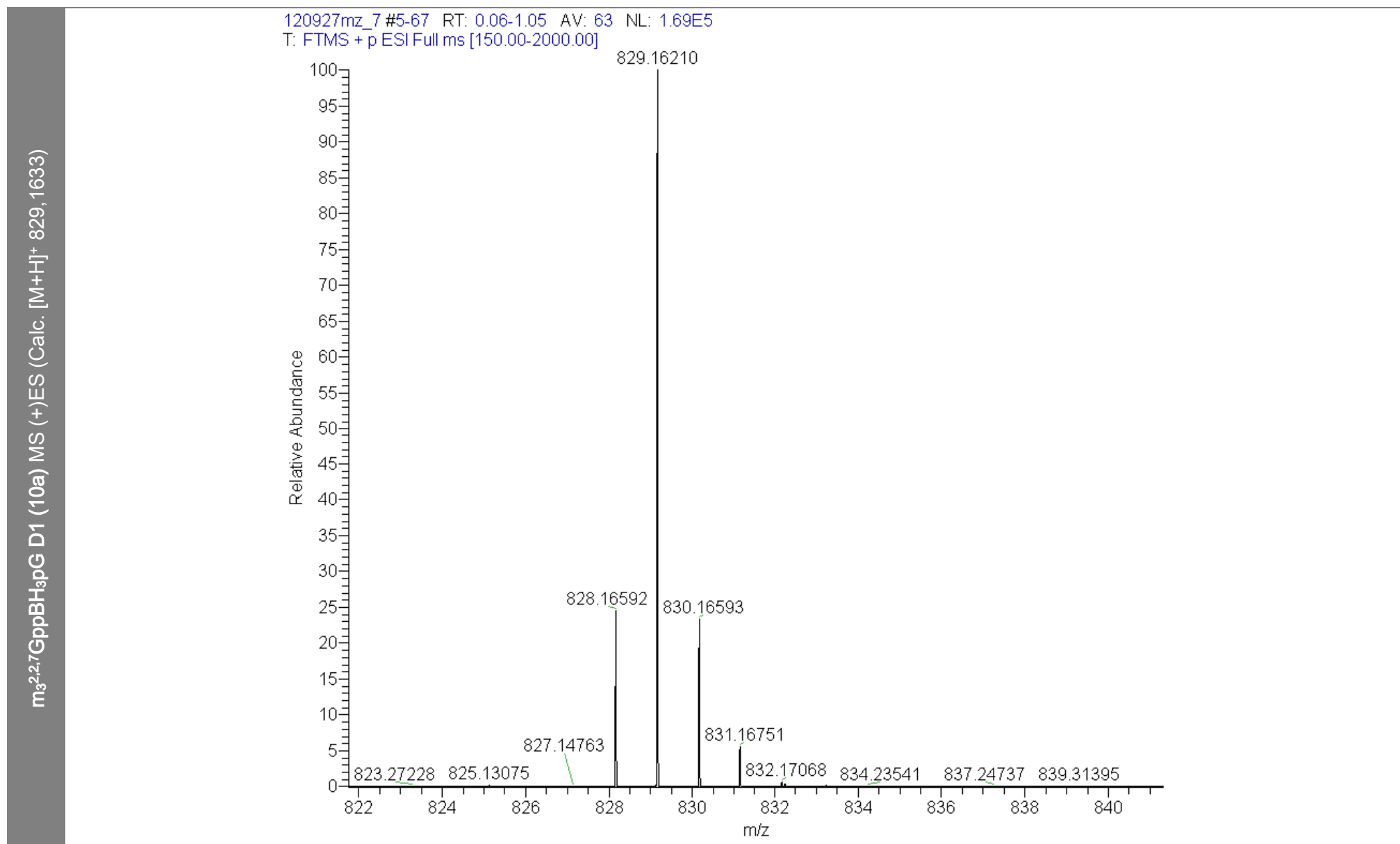


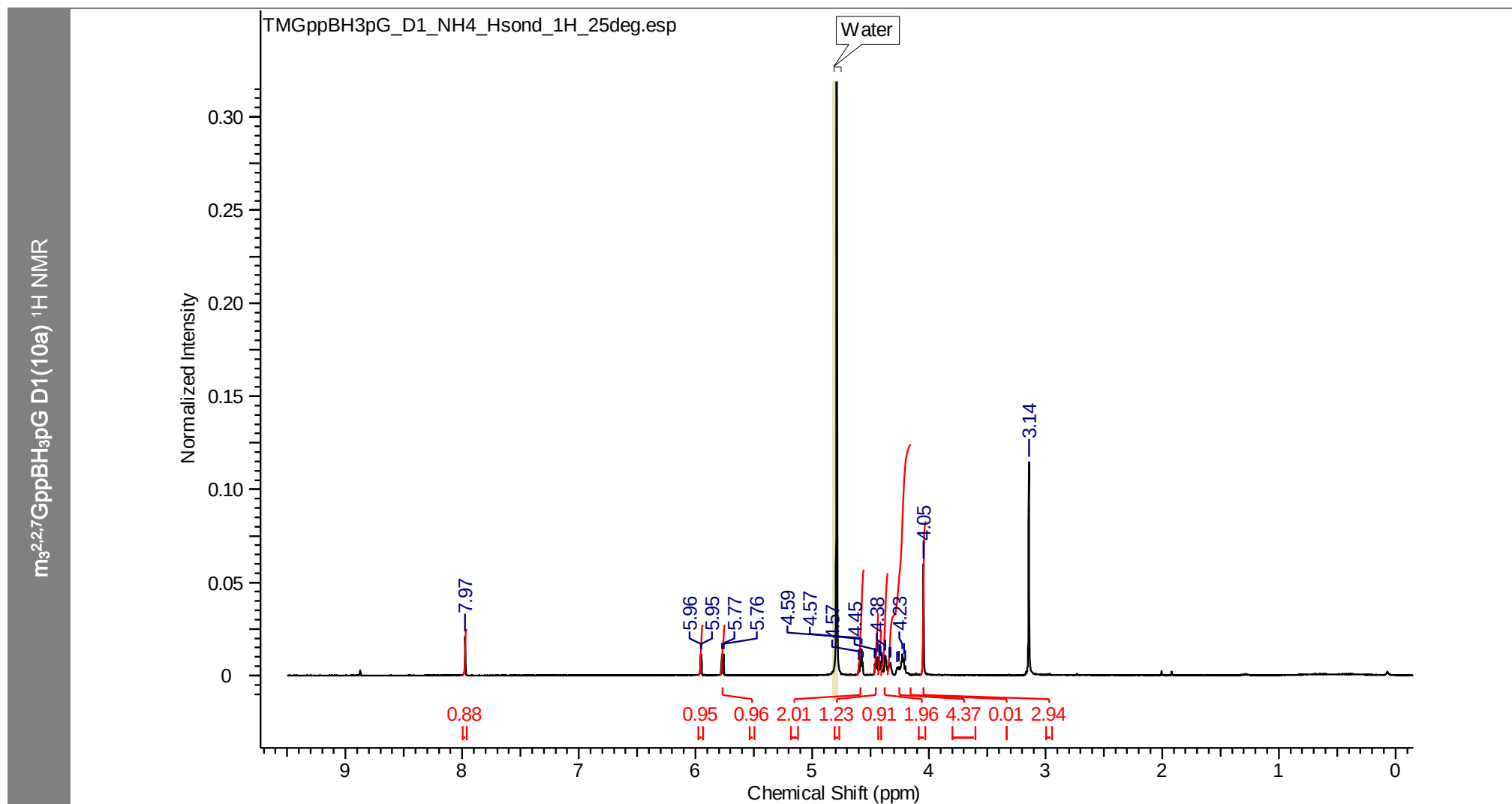
m₃^{2,2,7}GppBH₃pG D1 (10a)

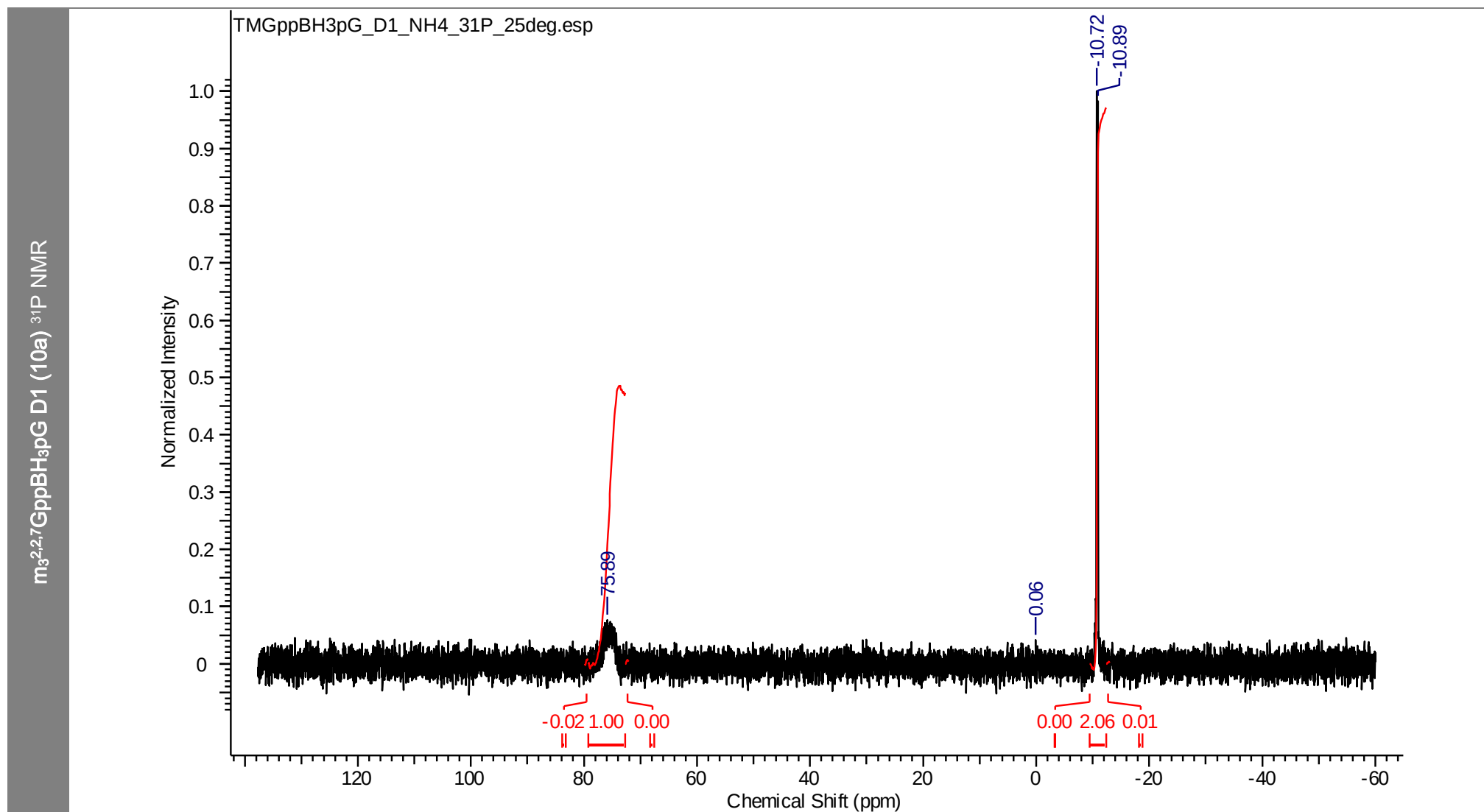


m₃^{2,2,7}GppBH₃pG D1 (10a) RP HPLC

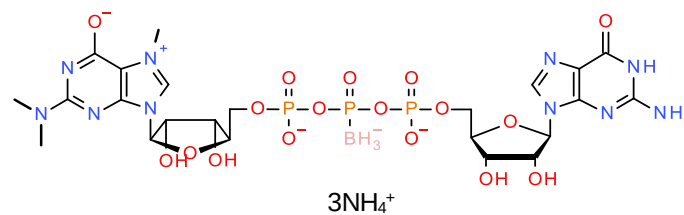




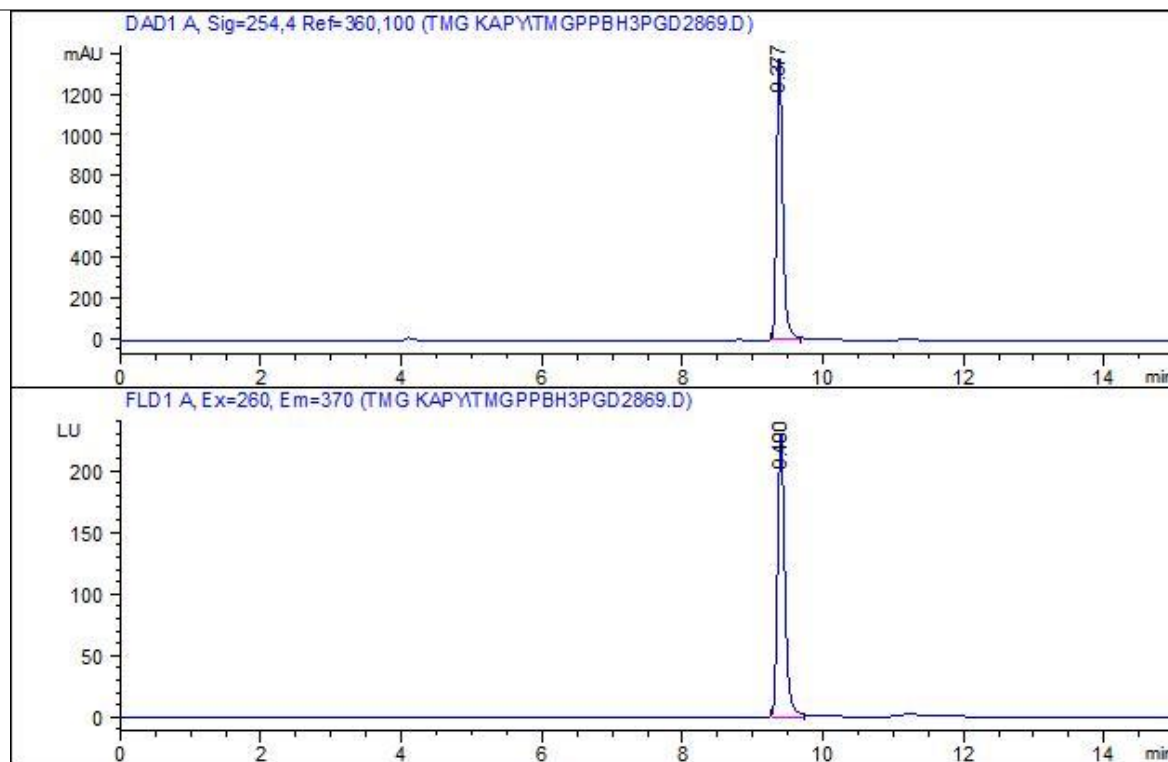




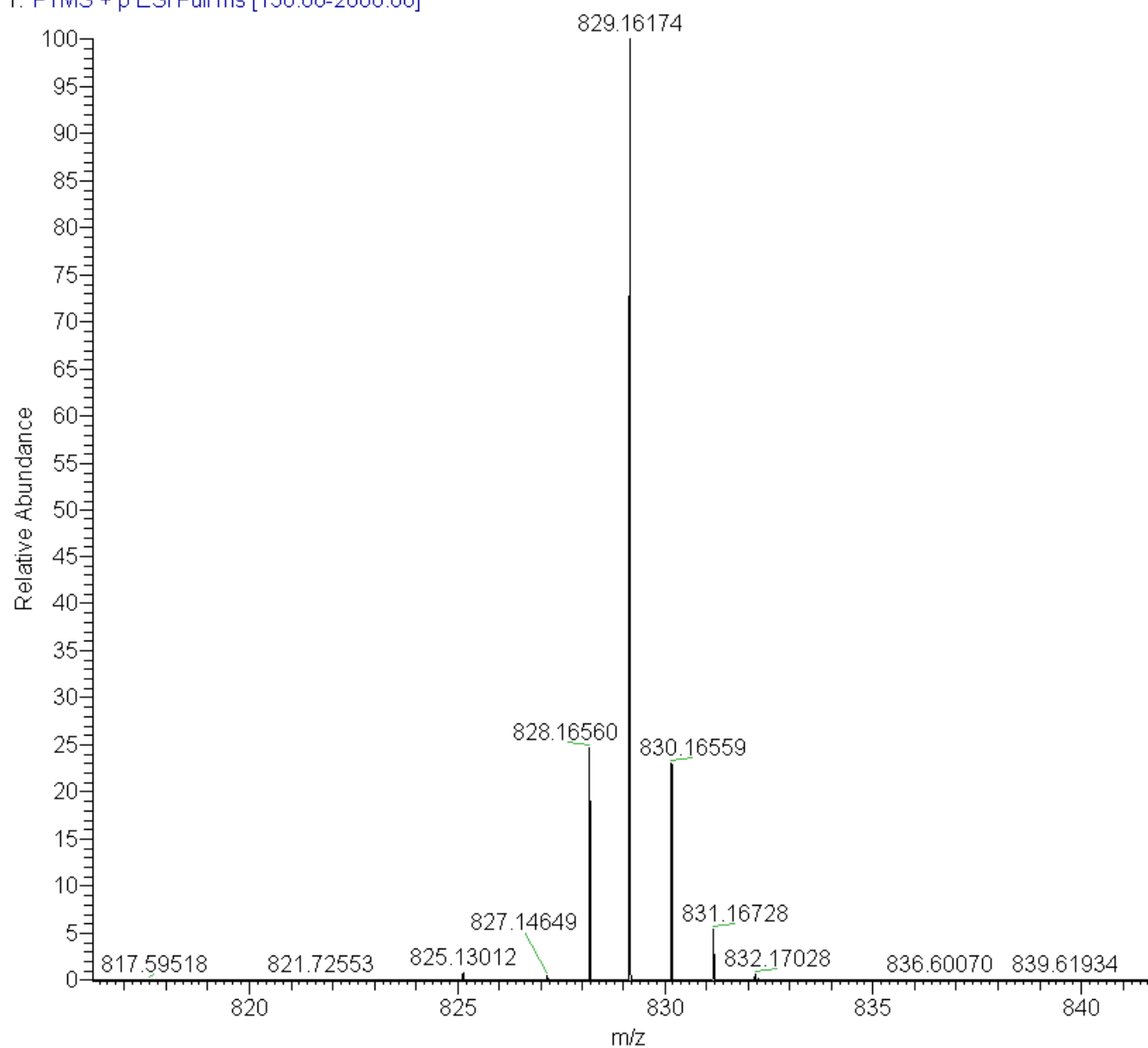
m₃^{2,2,7}GppBH₃pG D2 (10b)

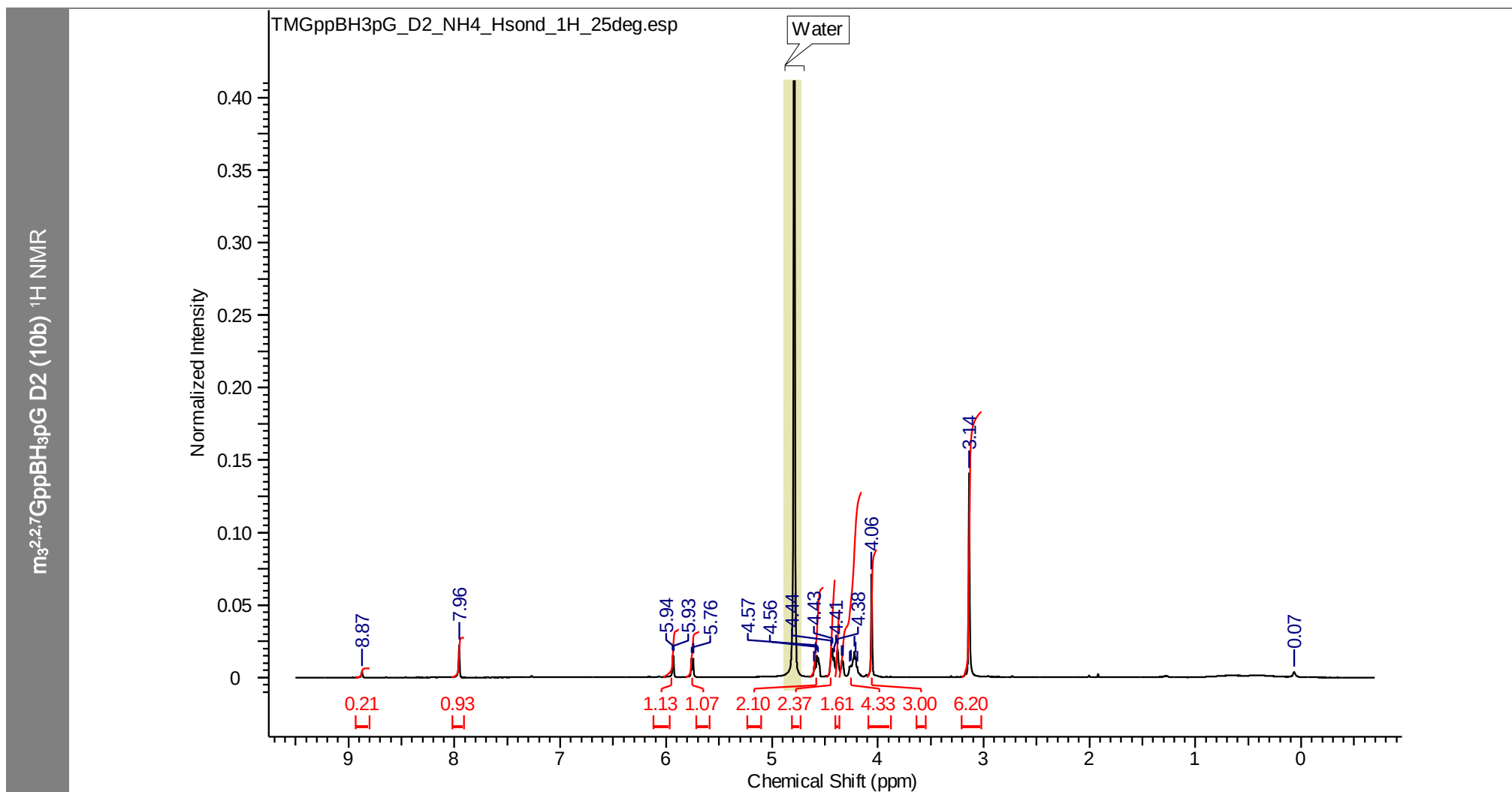


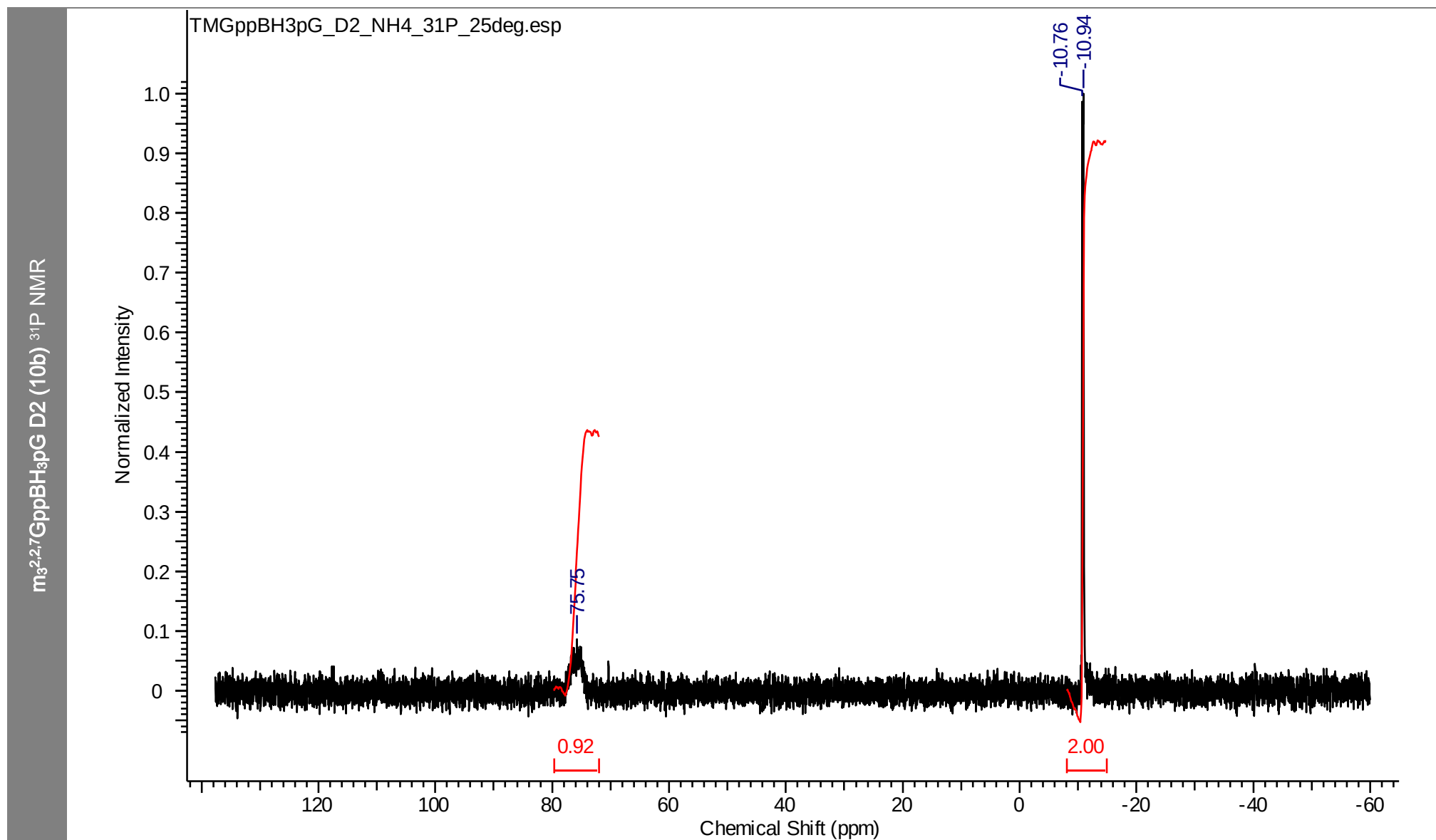
m₃^{2,2,7}GppBH₃pG D2 (10b) RP HPLC



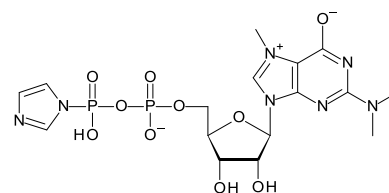
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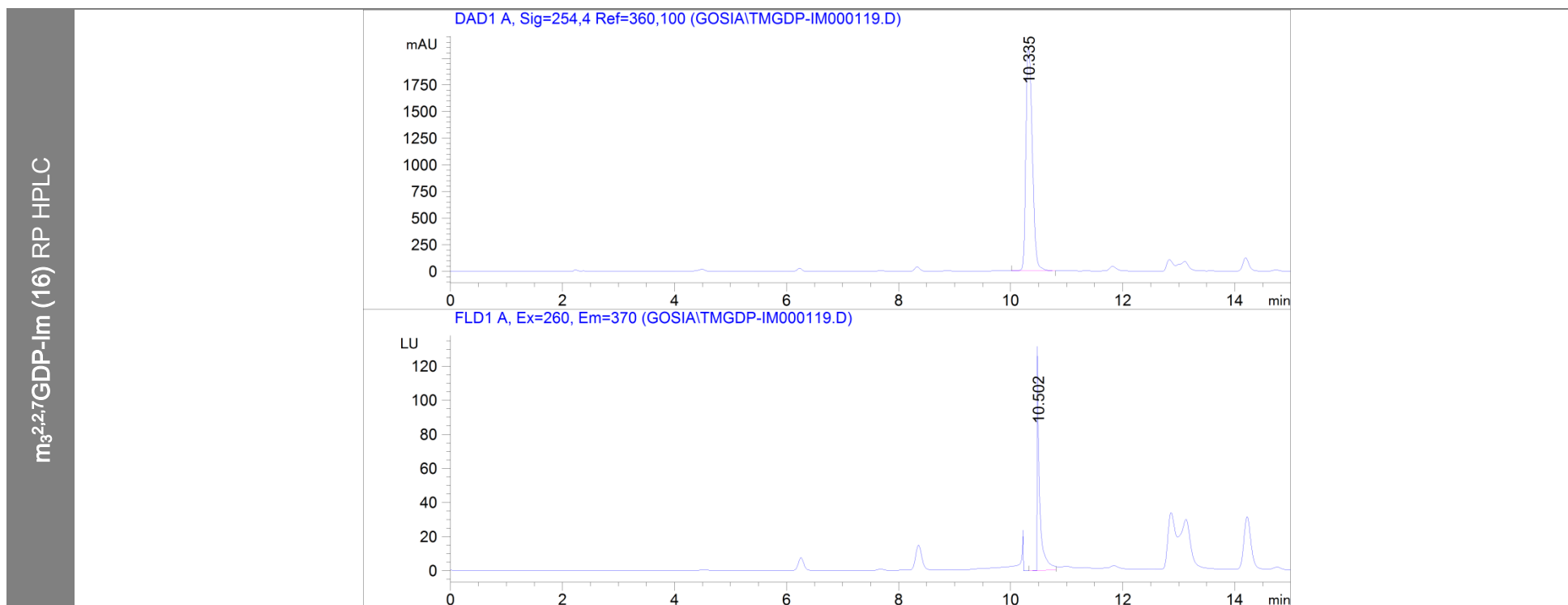






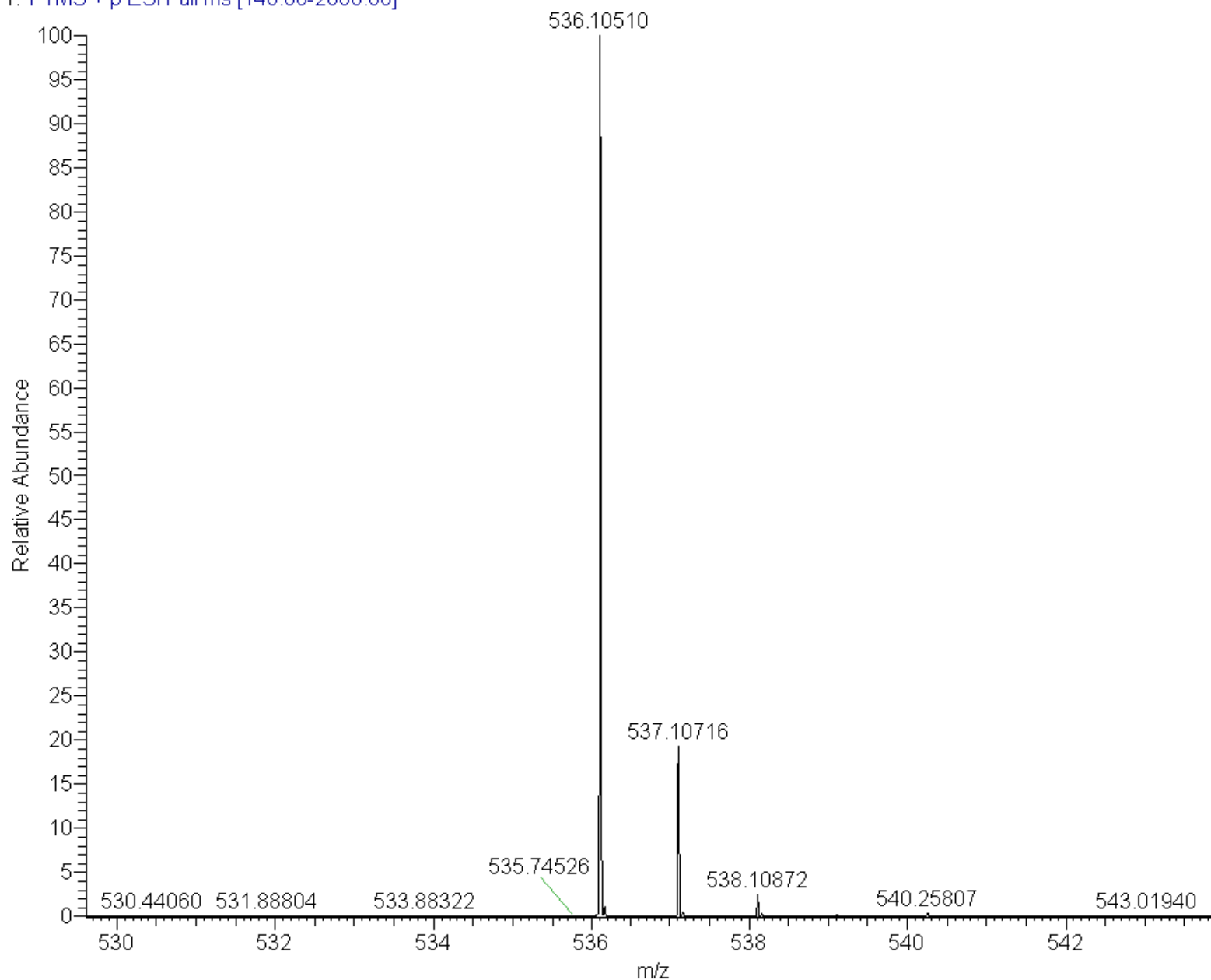
m₃^{2,2,7}GDP-Im (16)

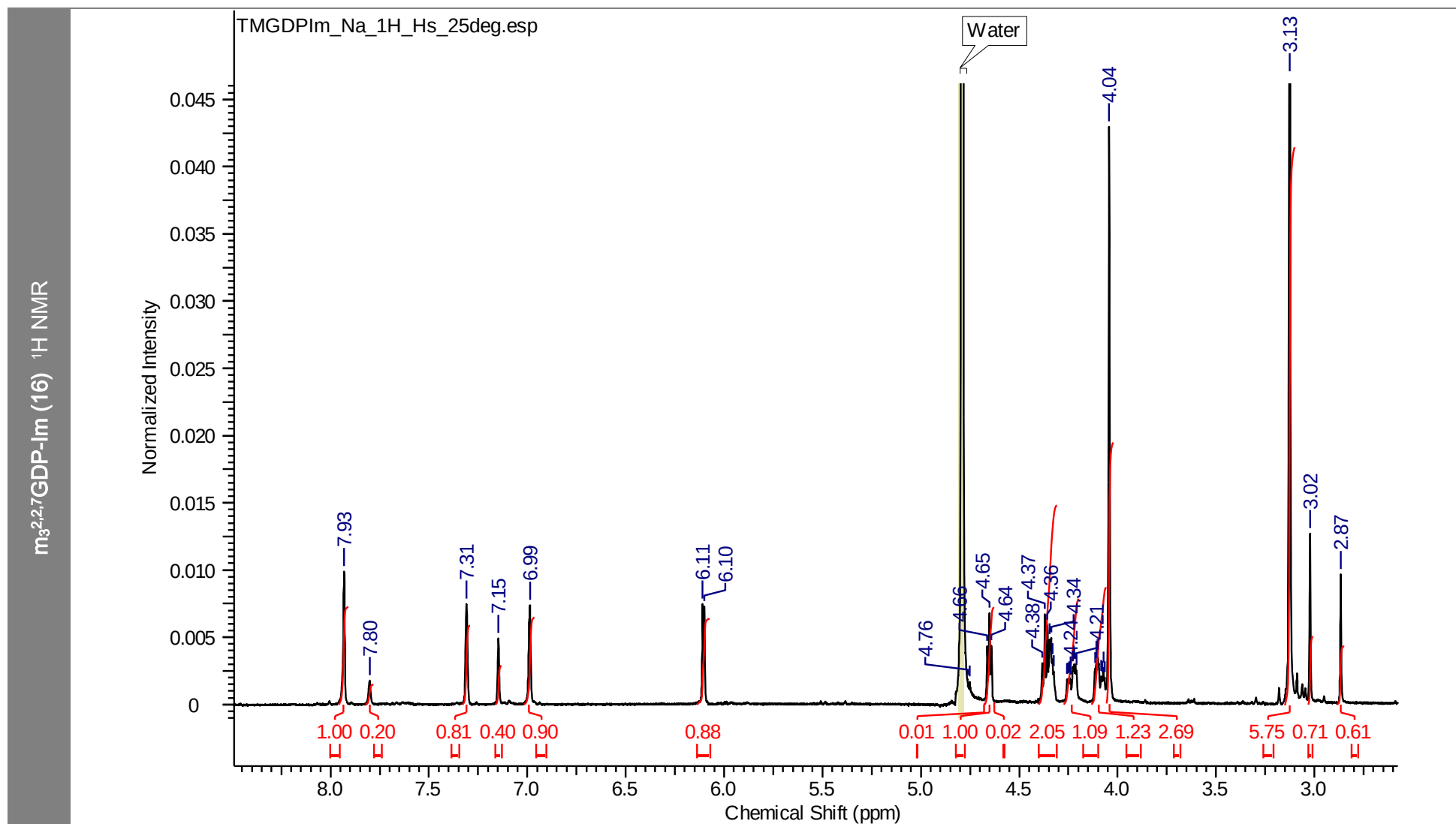


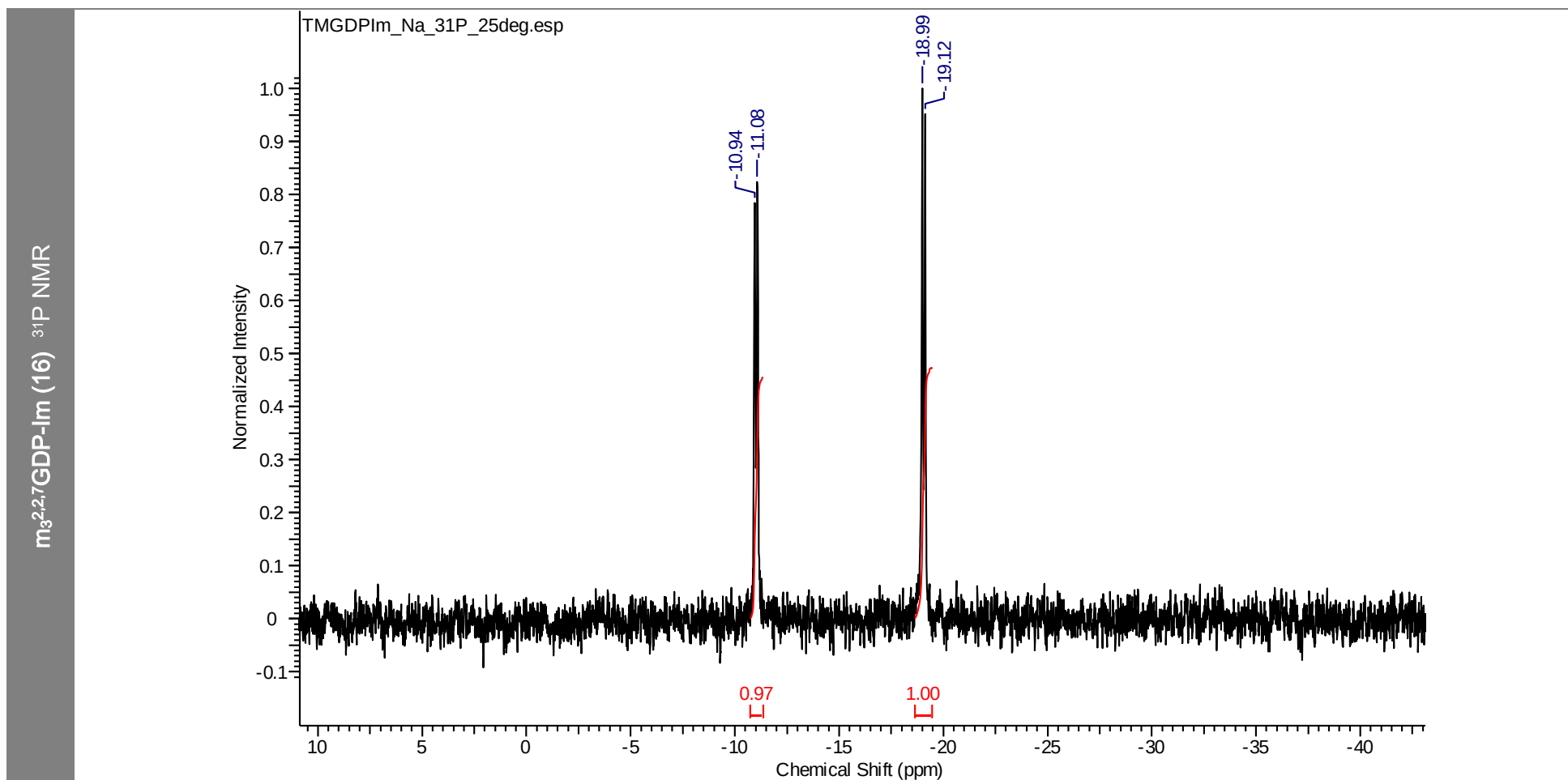


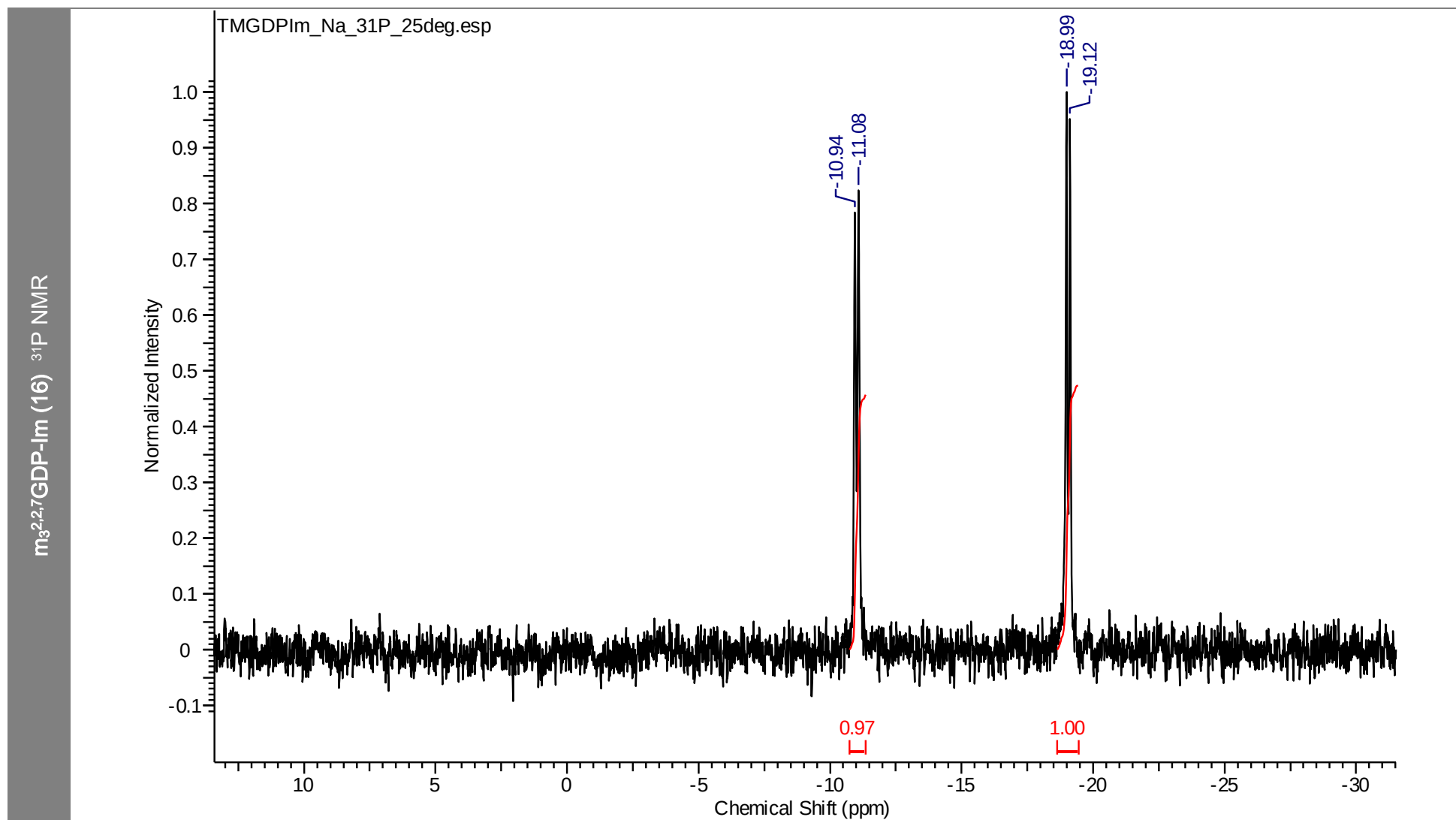
$m_3^{2,2,7}\text{GDP-lm}$ (16) MS (-)ES (Calc. $[\text{M}+\text{H}]^+$ 536.1065)

40714_ma_01 #92-169 RT: 0.94-1.68 AV: 78 NL: 1.92E6
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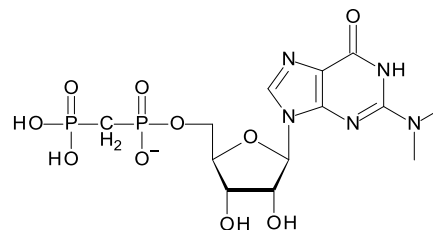




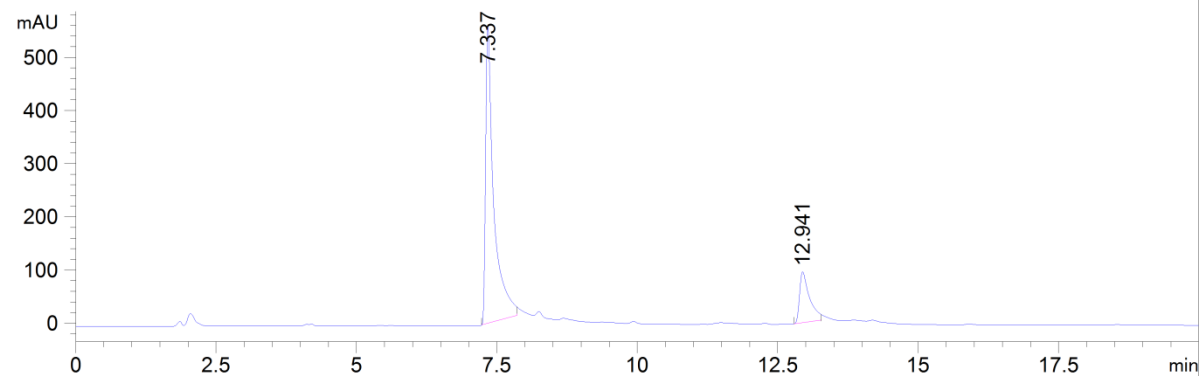




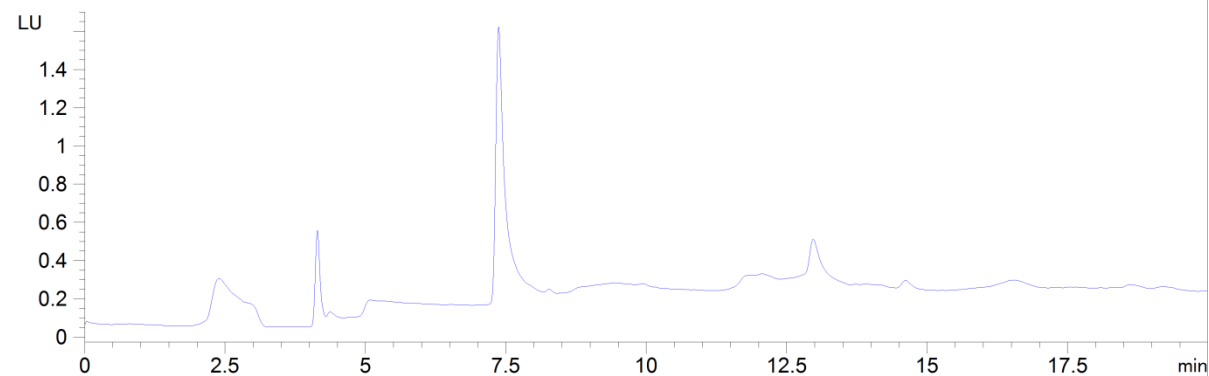
m^{2,2}GpCH₂p (20)



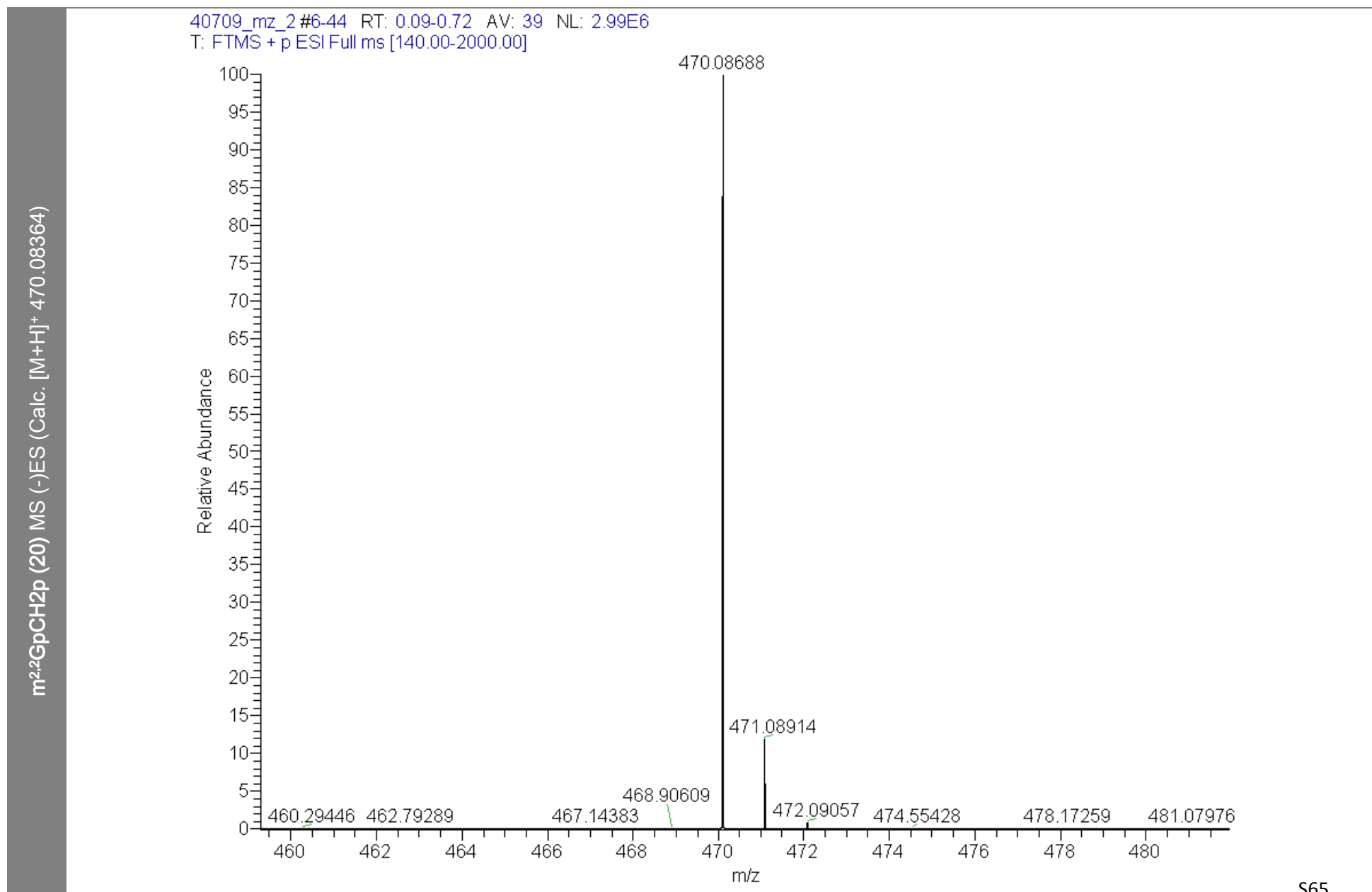
DAD1 A, Sig=254,4 Ref=360,100 (GOSIA\DMGPCH2P000099.D)

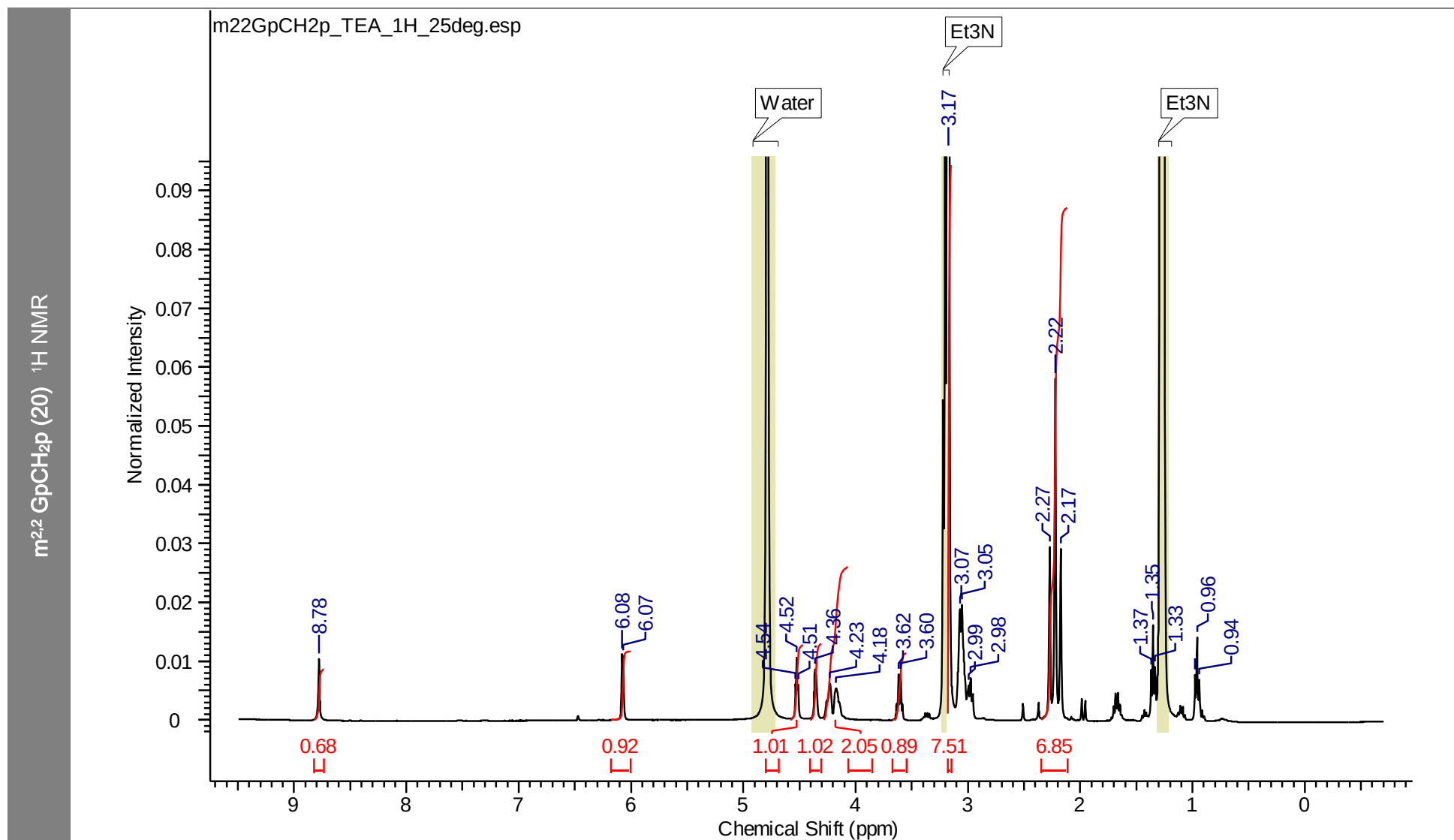


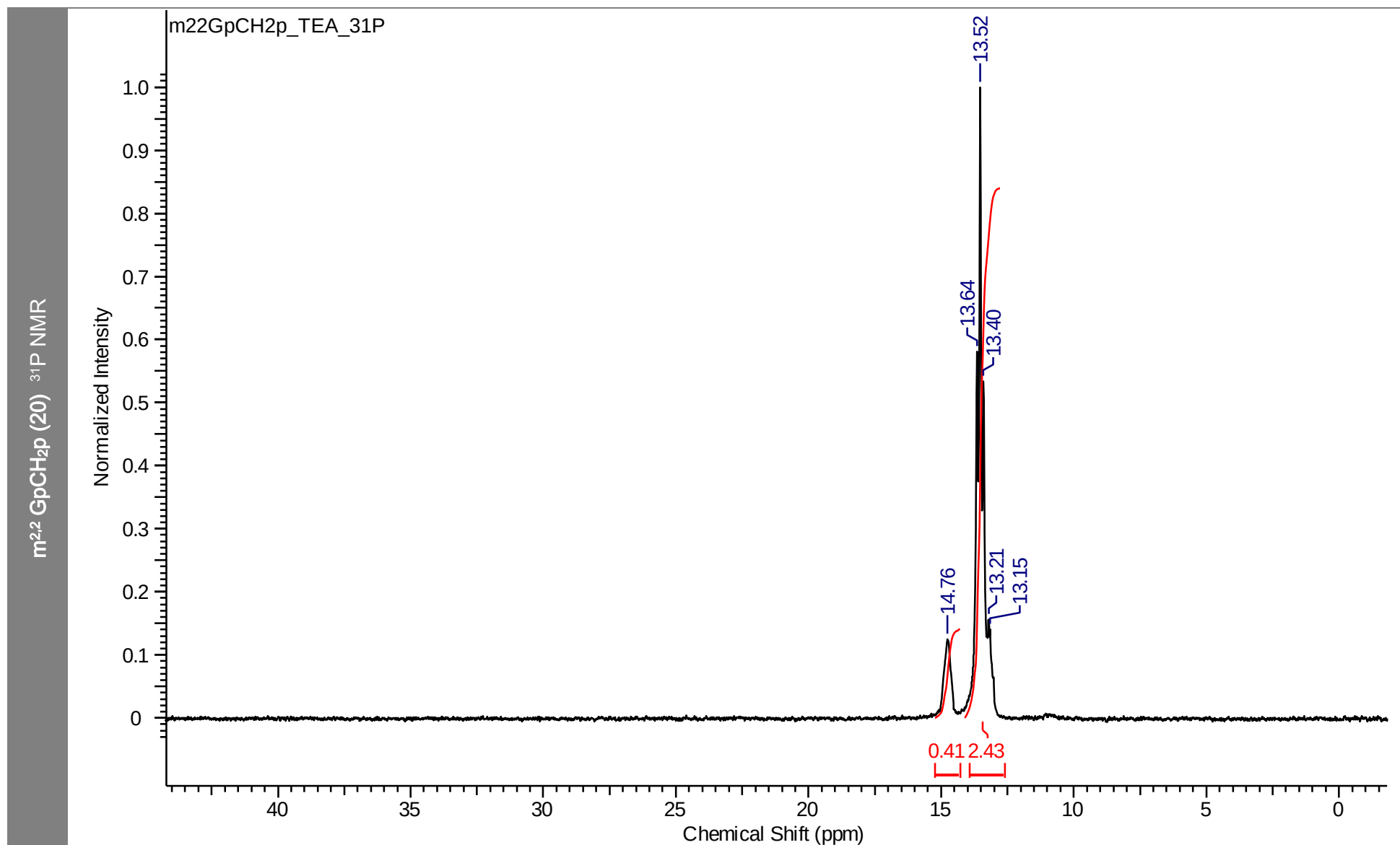
FLD1 A, Ex=260, Em=370 (GOSIA\DMGPCH2P000099.D)



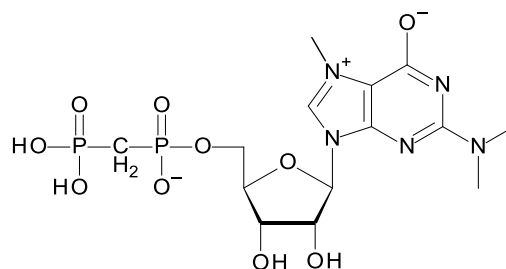
m^{2,2}GpCH₂p (20) RP HPLC



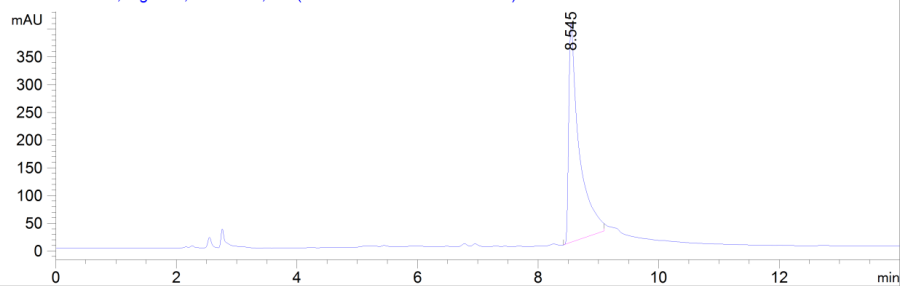




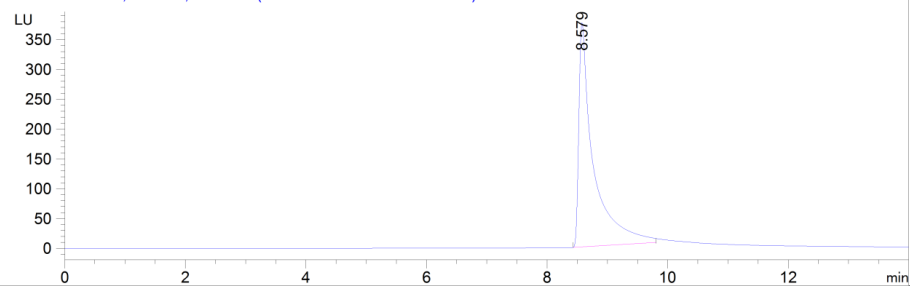
$m_3^{2,2,7}$ GpCH₂p (**22**)



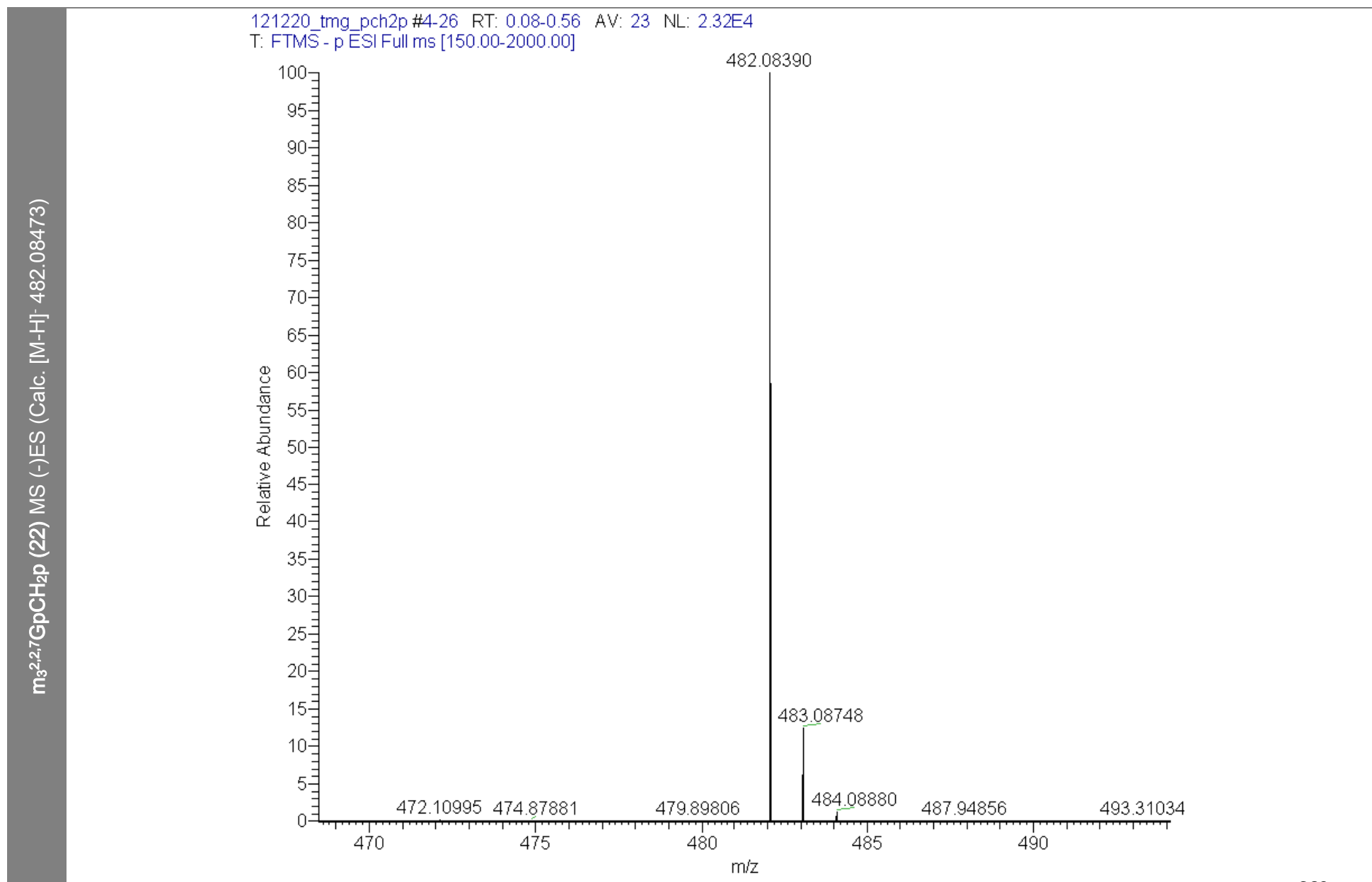
DAD1 A, Sig=254,4 Ref=360,100 (GOSIA\TMGPCH2P000158.D)

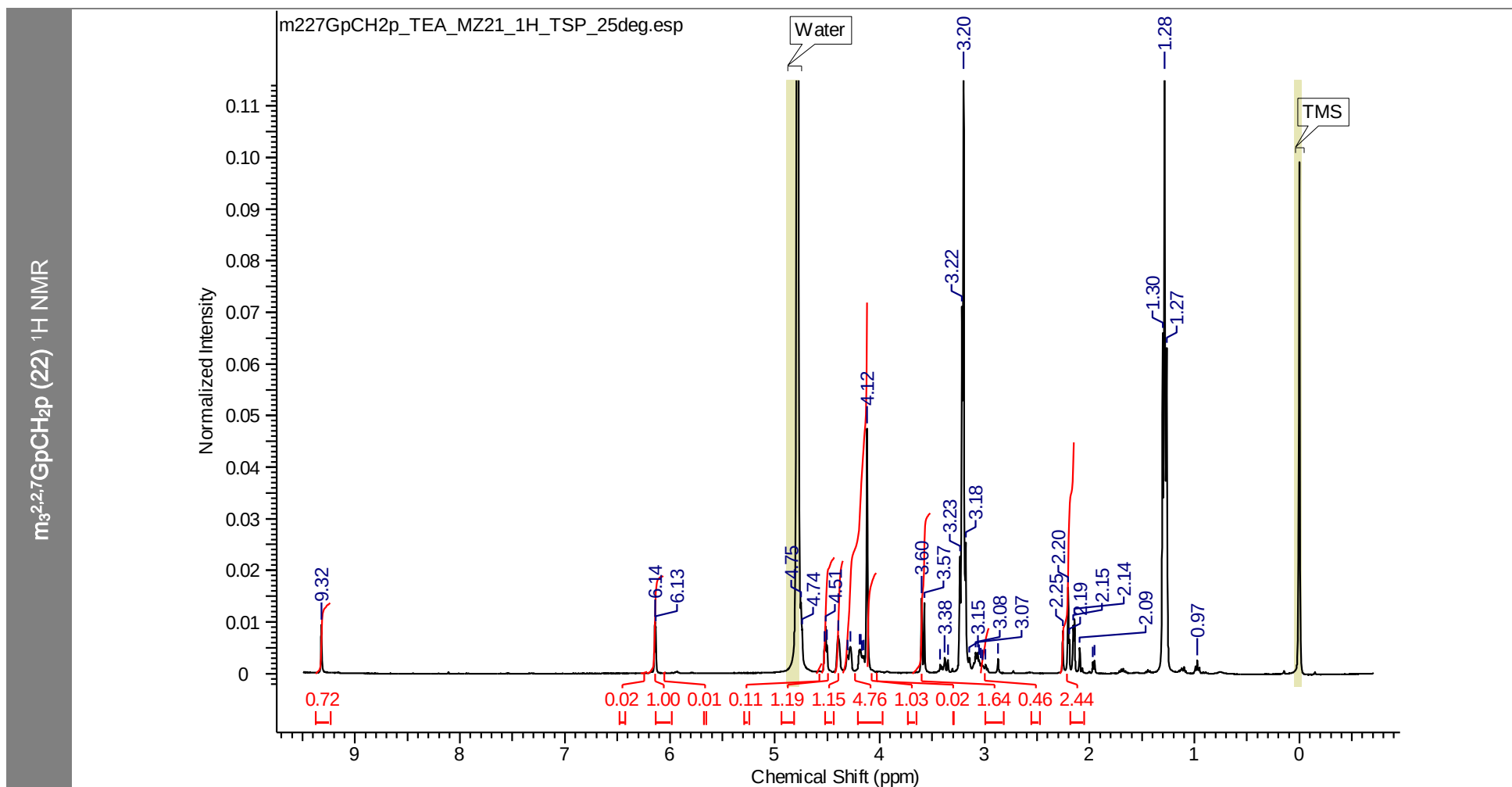


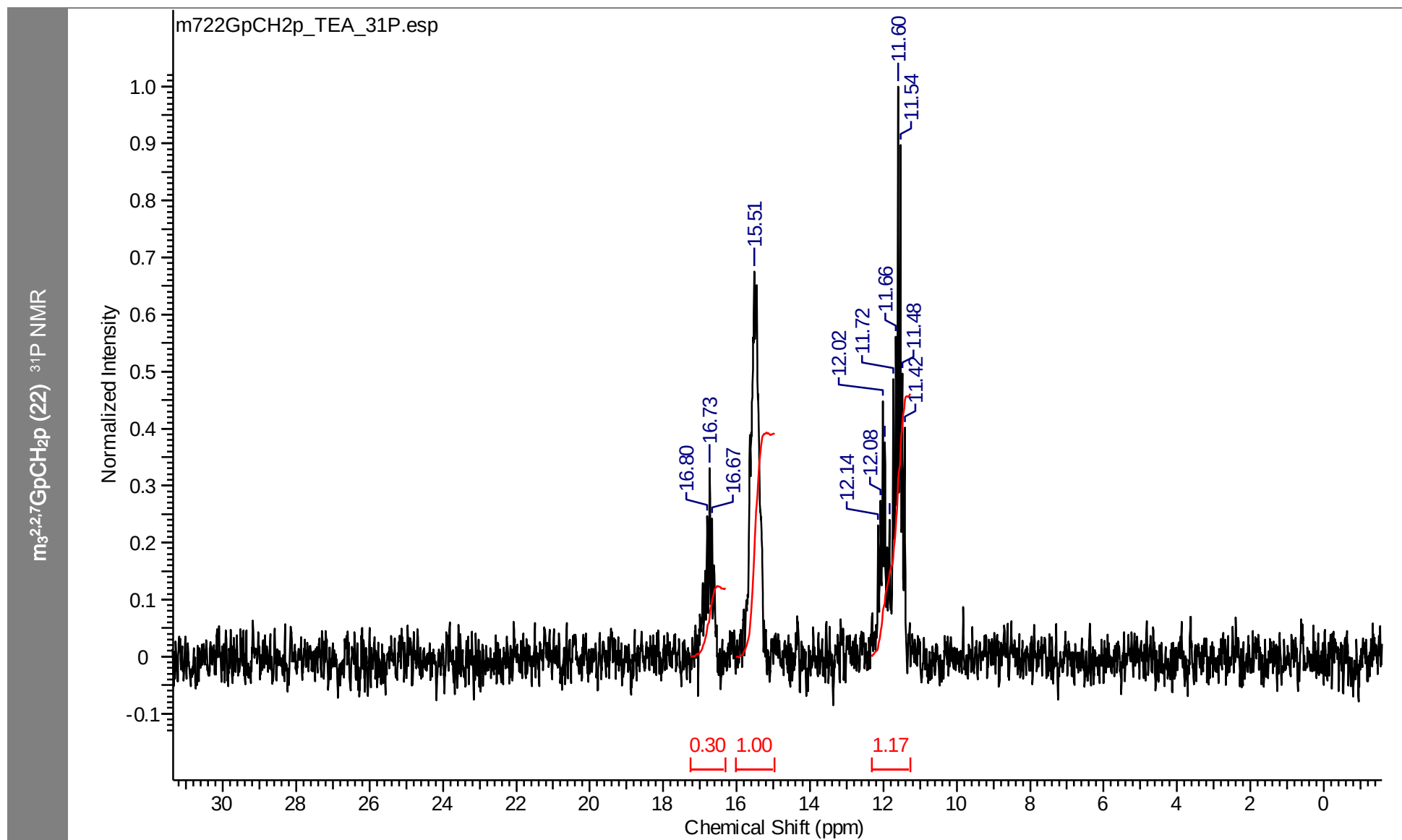
FLD1 A, Ex=260, Em=370 (GOSIA\TMGPCH2P000158.D)



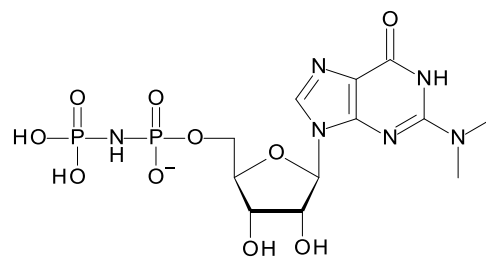
$m_3^{2,2,7}$ GpCH₂p (**22**) RP HPLC

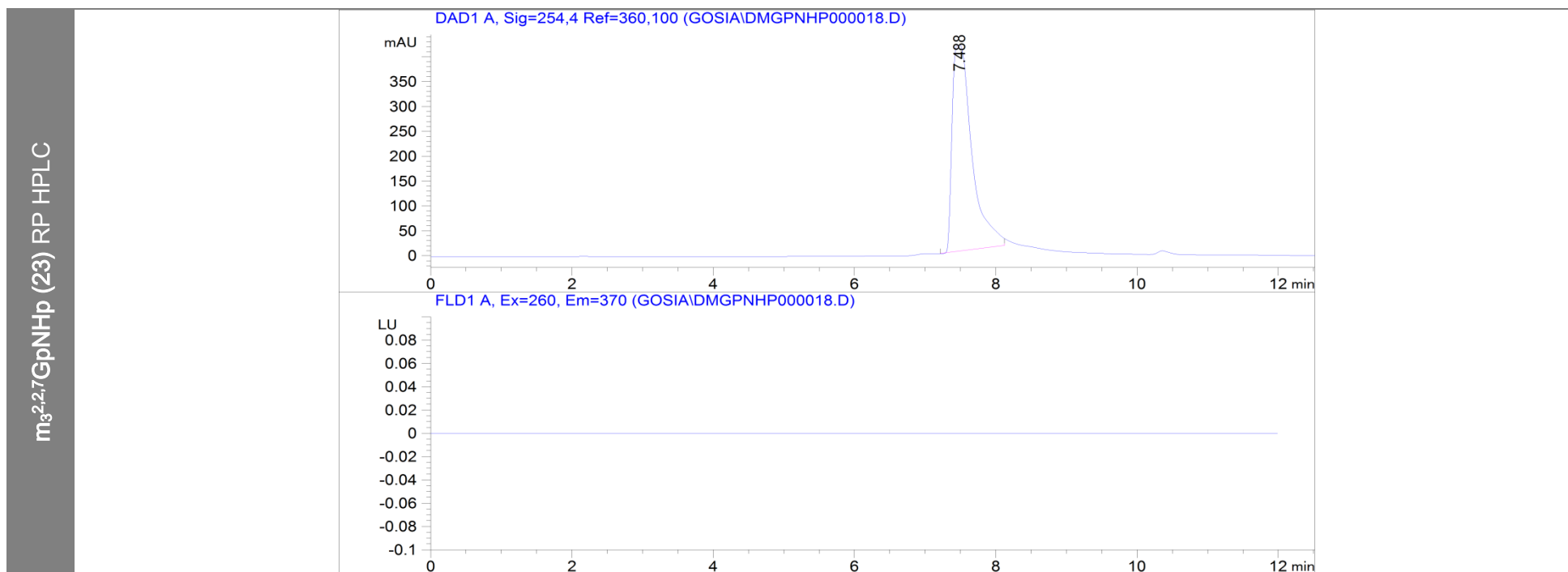






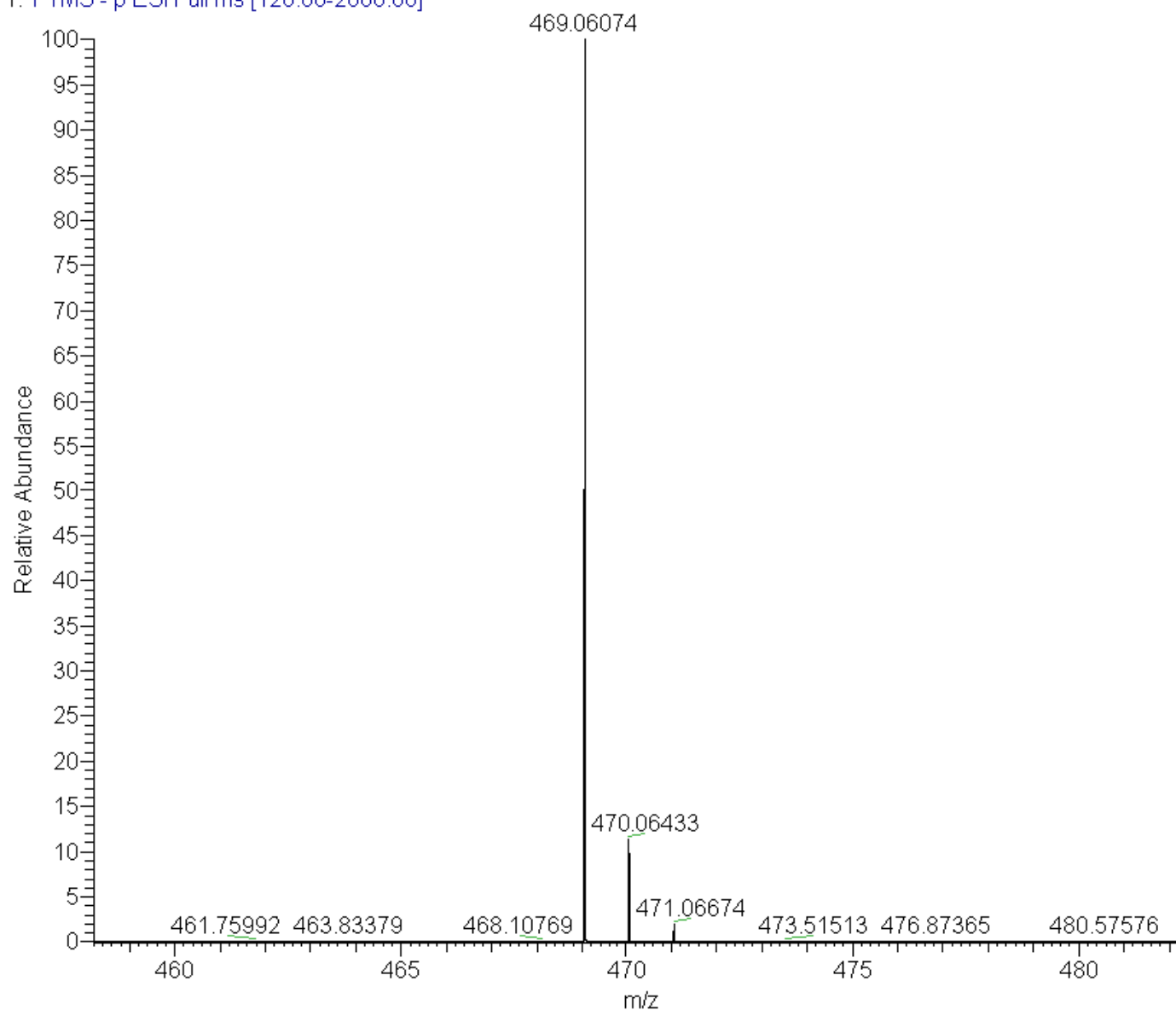
m₂^{2,2}GpNHp (23)

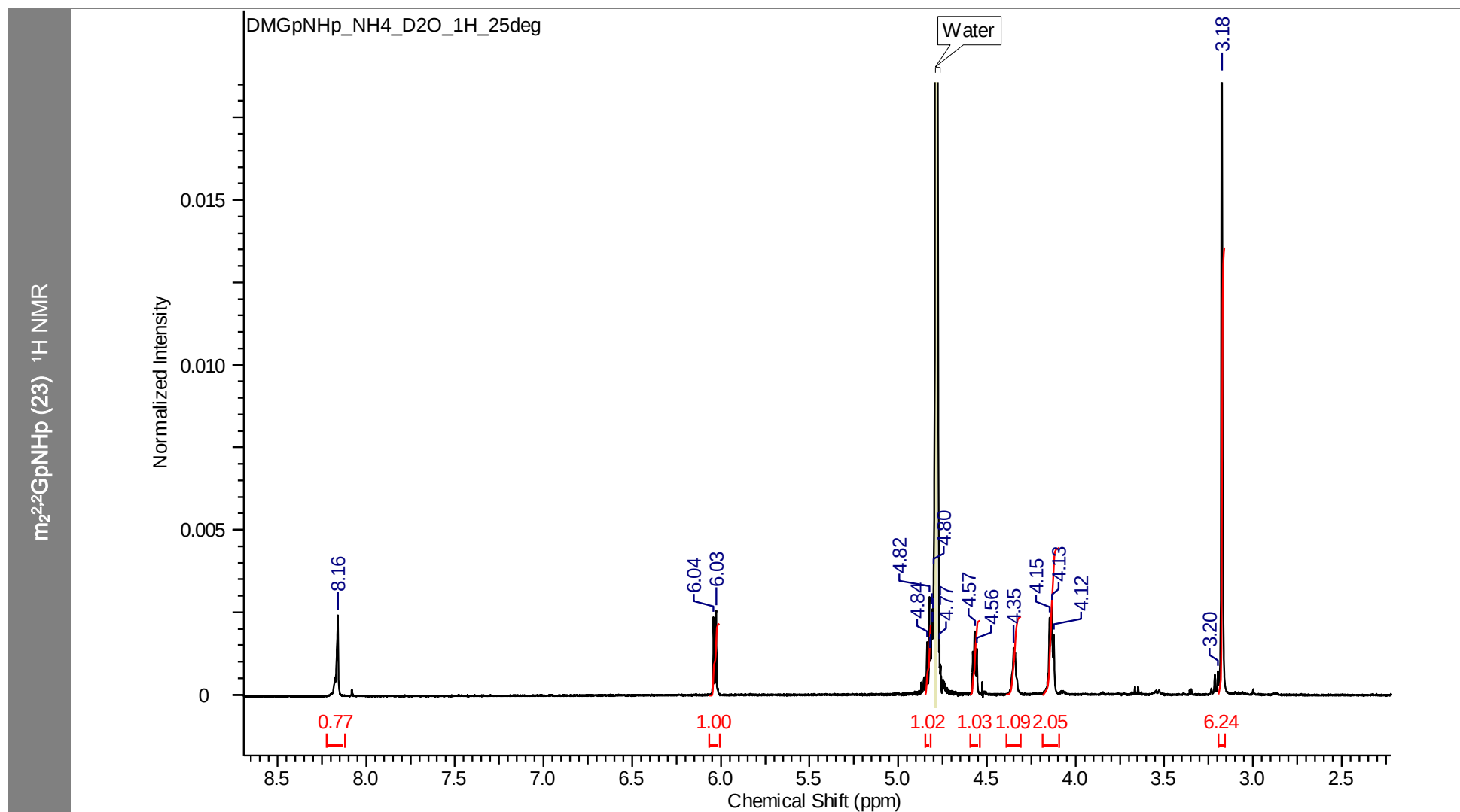


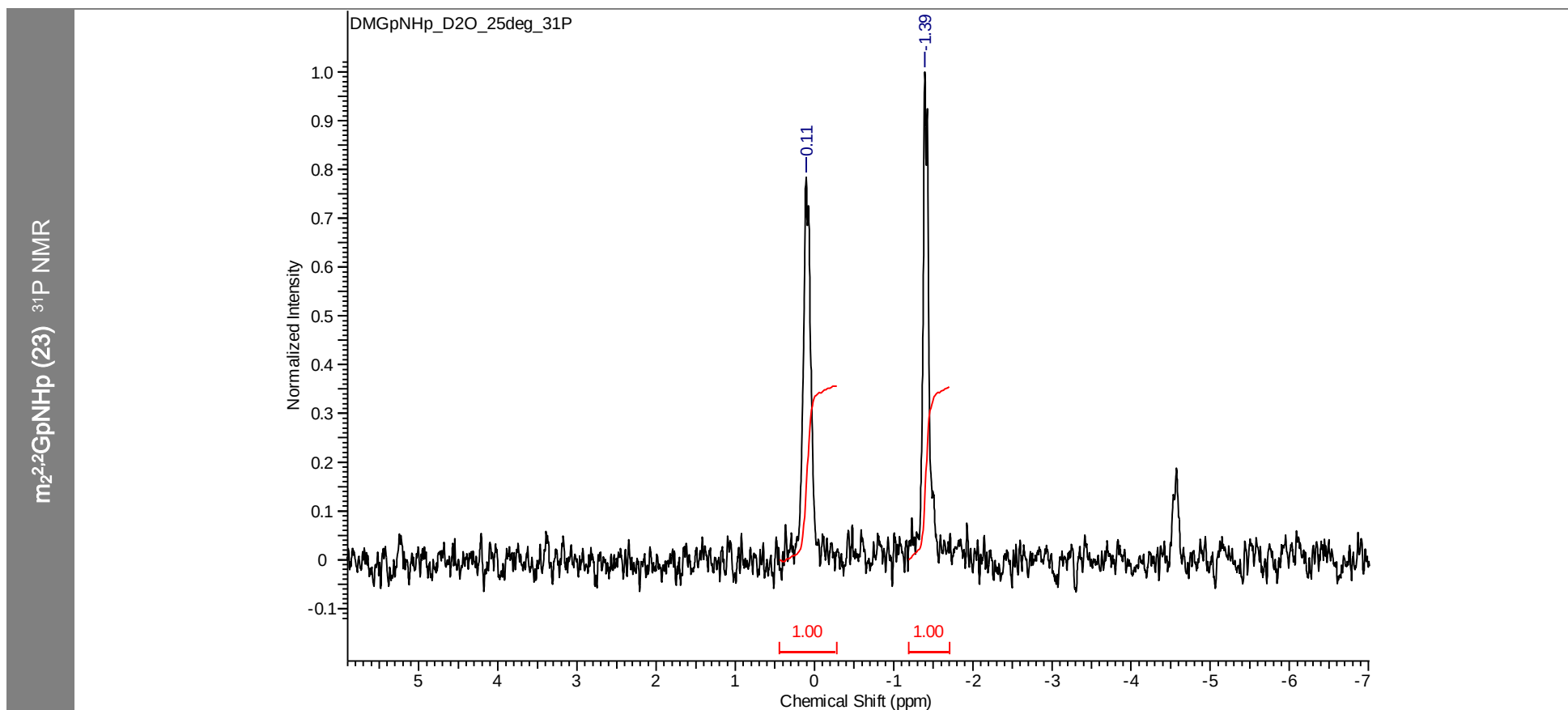


$m_{3^2,2,7}GpNHp$ (23) MS (-)ES (Calc. [M-H]⁻ 469.0643)

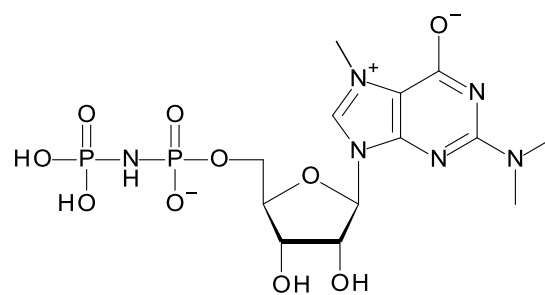
130509_mz4 #3-18 RT: 0.05-0.30 AV: 16 NL: 1.94E6
T: FTMS - p ESI Full ms [120.00-2000.00]

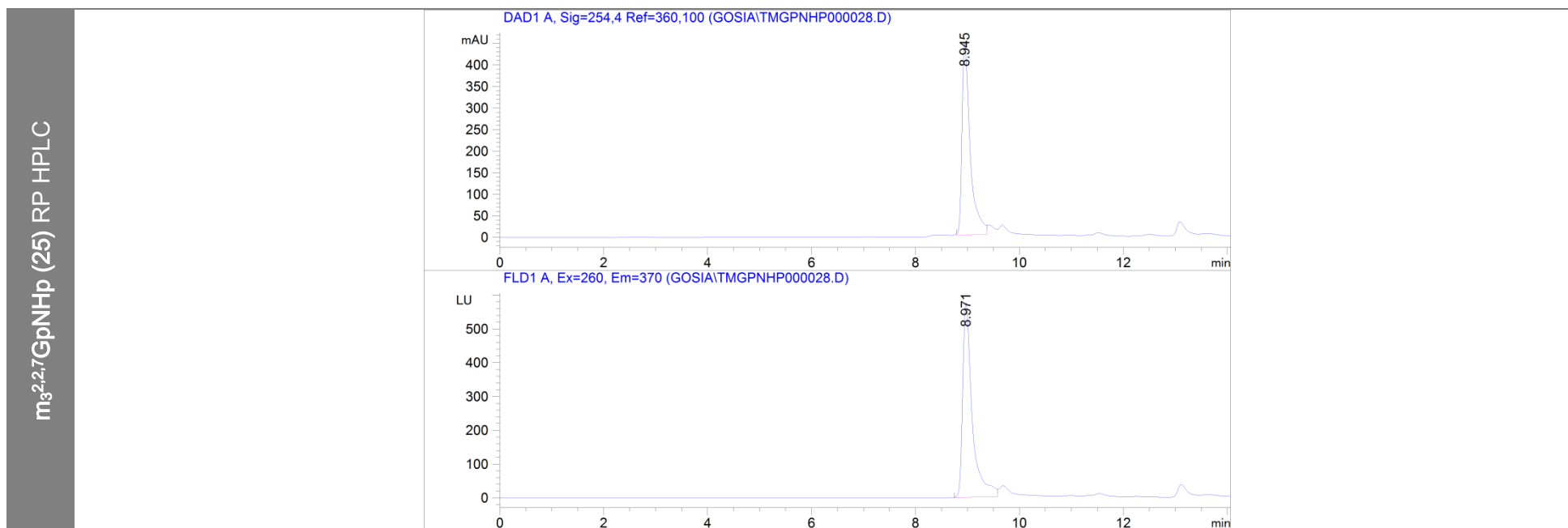






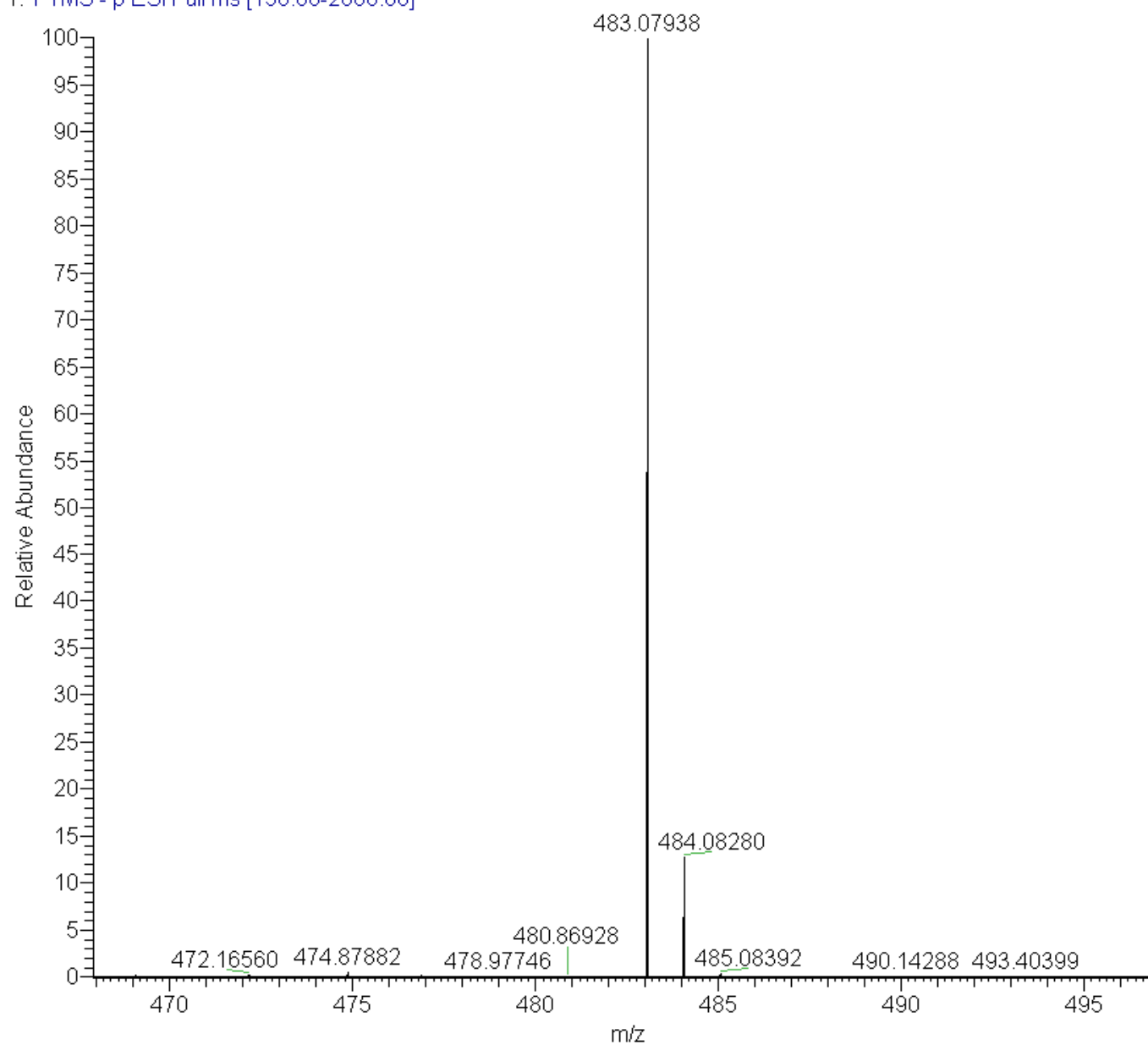
m₃^{2,2,7}GpNHp (25)

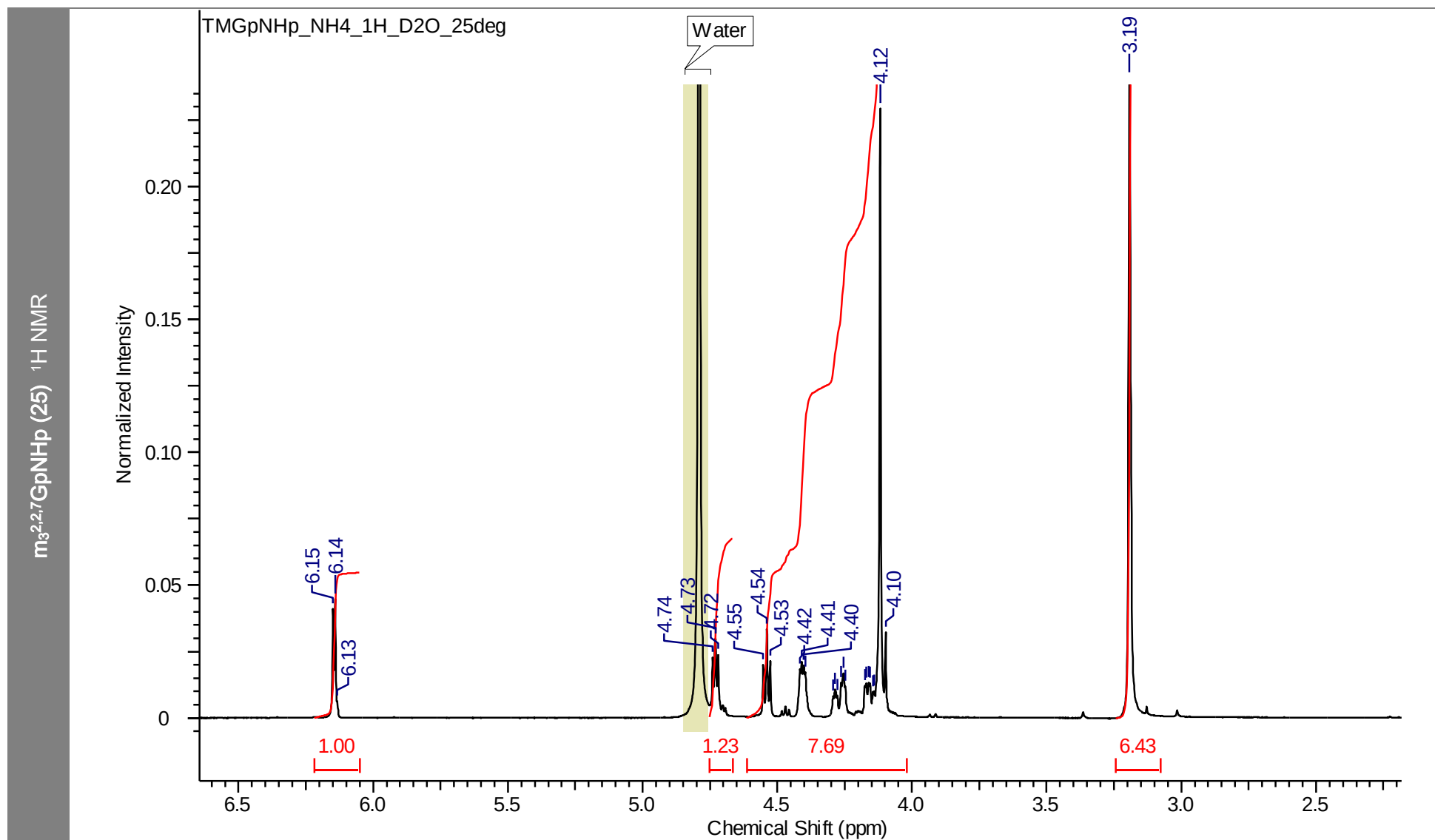


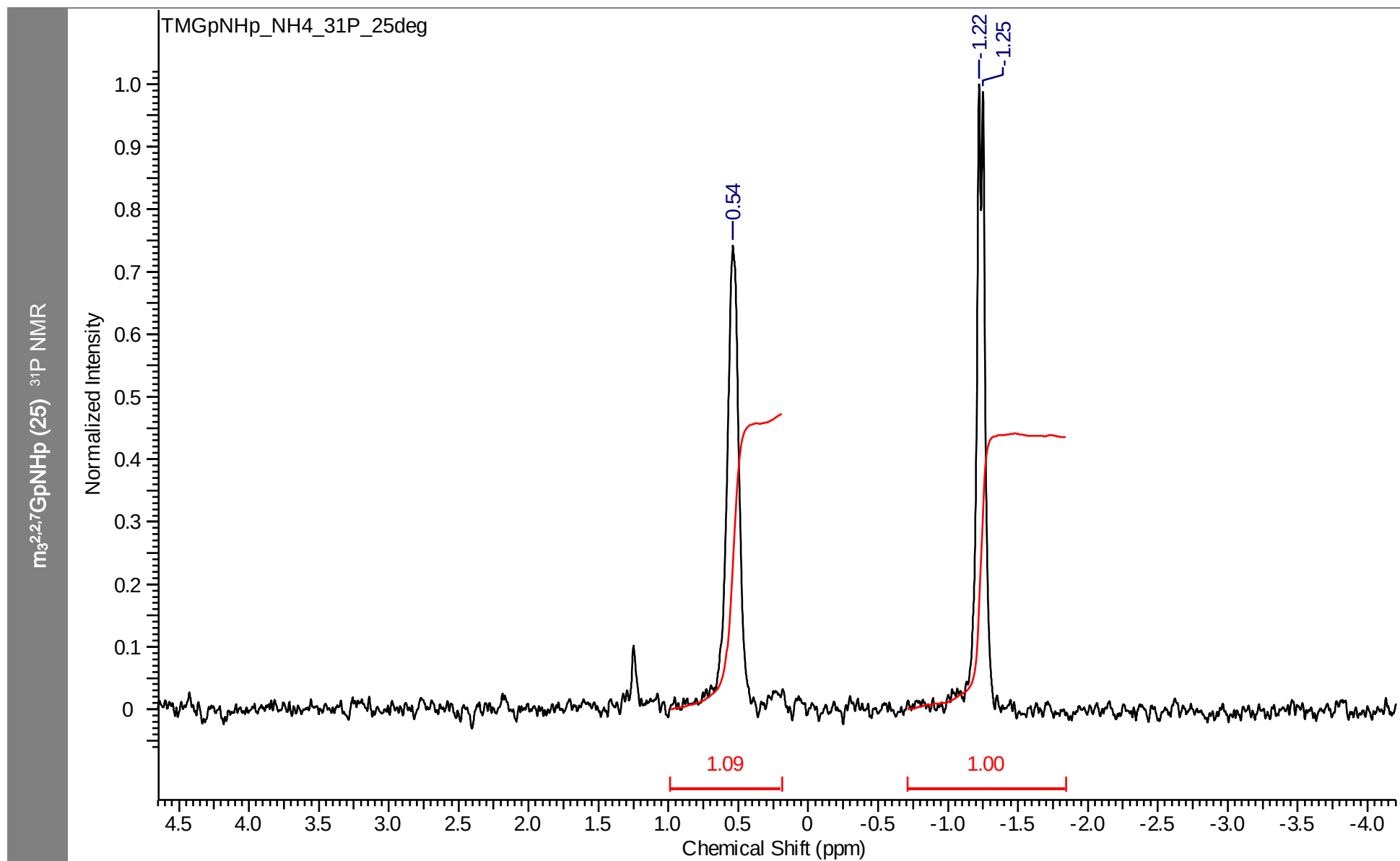


$m_3^{2,2,7}$ GpNHp (25) MS (-)ES (Calc. [M-H]⁻ 483.07998)

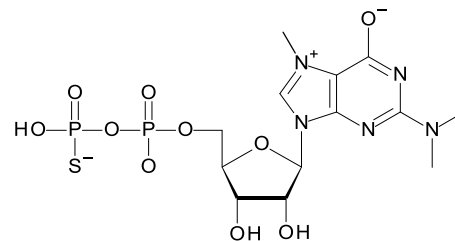
121220_tmgnhp #15-123 RT: 0.31-2.71 AV: 109 NL: 5.80E3
T: FTMS - p ESI Full ms [150.00-2000.00]



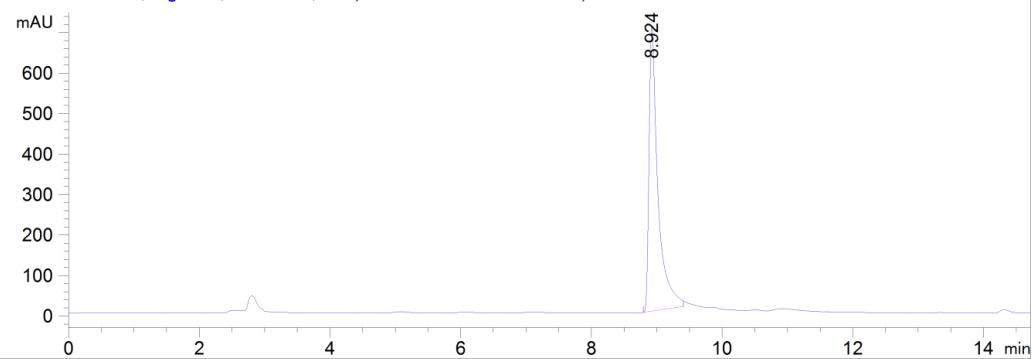




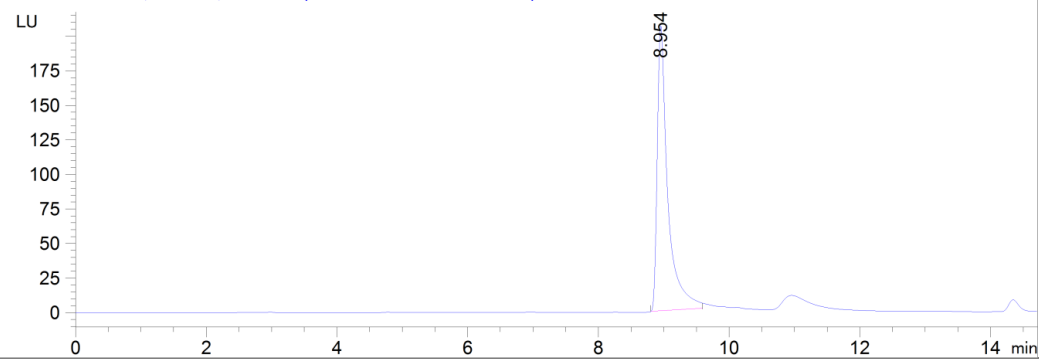
$m_3^{2,2,7}$ GppS (28)



DAD1 A, Sig=254,4 Ref=360,100 (GOSIA\TMGPPS000062.D)



FLD1 A, Ex=260, Em=370 (GOSIA\TMGPPS000062.D)



$m_3^{2,2,7}$ GppS (28) RP HPLC

11:24:52
25-Mar-2011
TOF MS ES-
5.68e3

$m_{3^{2,2,7}}Gpps$ (28) MS (-)ES (Calc. [M-H]⁻ 500.0412)

