Electronic Supplementary Material (ESI) for RSC Advances.

Clickable trimethylguanosine cap analogs modified within the triphosphate bridge: synthesis, conjugation to RNA and susceptibility to degradation

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Supporting information

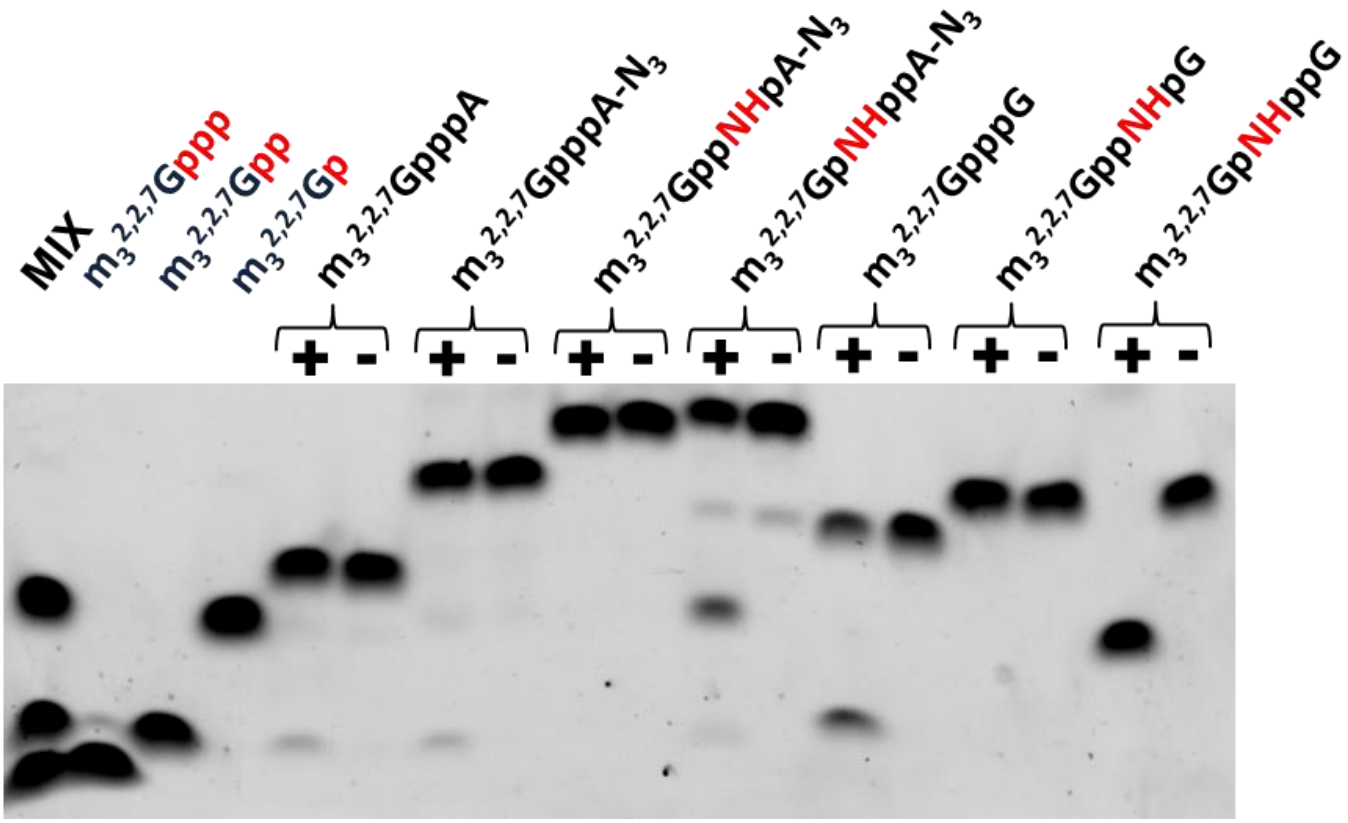


Figure S1. *Susceptibility of TMG analogues to the Nudt16 decapping enzyme. Electrophoretic mobilities of TMG cap analogues and TMG species (visible under UV 305nm). Active hNudt16 enzyme (lanes indicated with [+]), but not thermally inactivated hNudt16 (lanes indicated with [−]), shows low decapping activity on TMG caps: $m^{2,2,7}$GpppA, $m^{2,2,7}$GpppG, $m^{2,2,7}$GpppA-N_3, high decapping activity on: $m^{2,2,7}$GpNHppA-N_3, $m^{2,2,7}$GpNHppG. TMG caps $m^{2,2,7}$GppNHpG and $m^{2,2,7}$GppNHpA-N_3 are stable against hNudt16 decapping activity under the same conditions.*

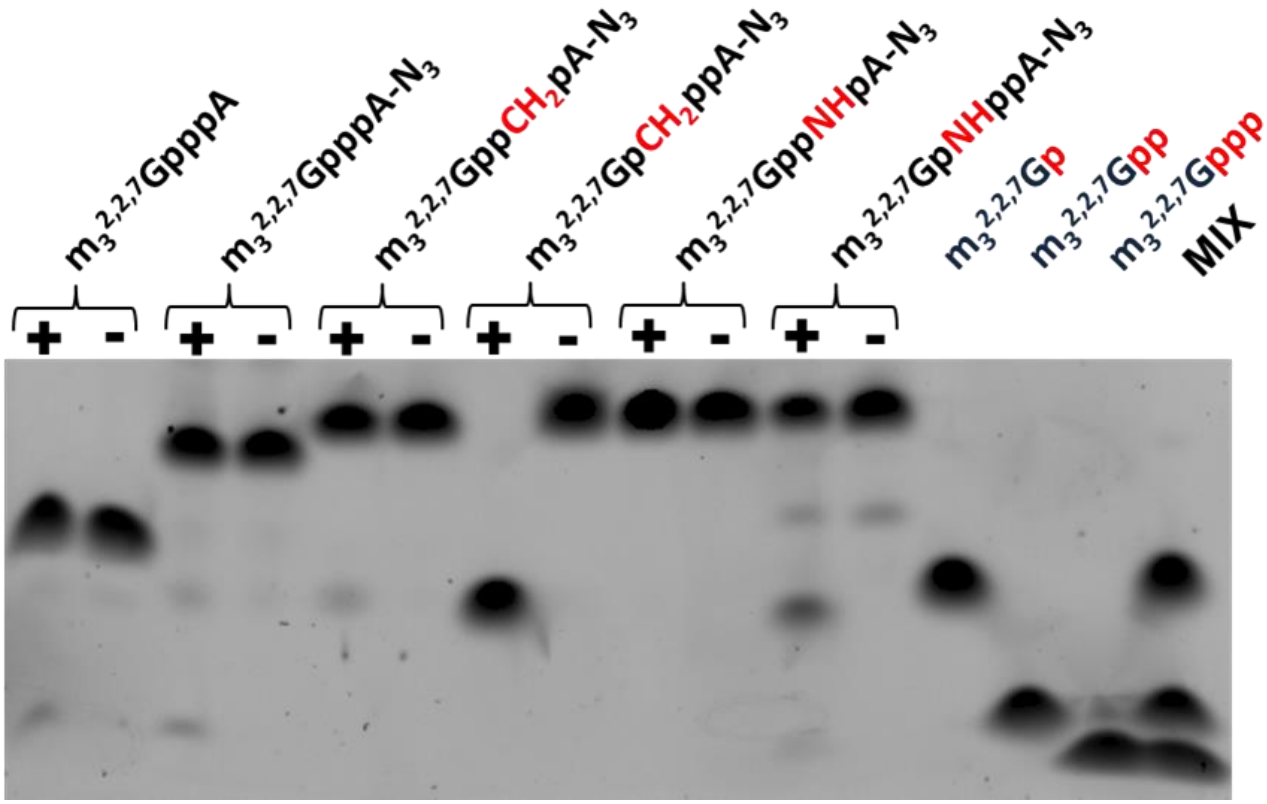


Figure S2. *Susceptibility of TMG analogues to the Nudt16 decapping enzyme. Electrophoretic mobilities of TMG cap analogues and TMG species (visible under UV 305nm). Active hNudt16 enzyme (lanes indicated with [+]), but not thermally inactivated hNudt16 (lanes indicated with [−]), shows low decapping activity on TMG caps: $m^{2,2,7}$GpppA, $m^{2,2,7}$GpppA-N_3, $m^{2,2,7}$GppCH$_2$pA-N_3, high decapping activity on: $m^{2,2,7}$GpCH$_2$ppA-N_3,. TMG cap $m^{2,2,7}$GppNHpA-N_3 is stable against hNudt16 decapping activity under the same conditions.*

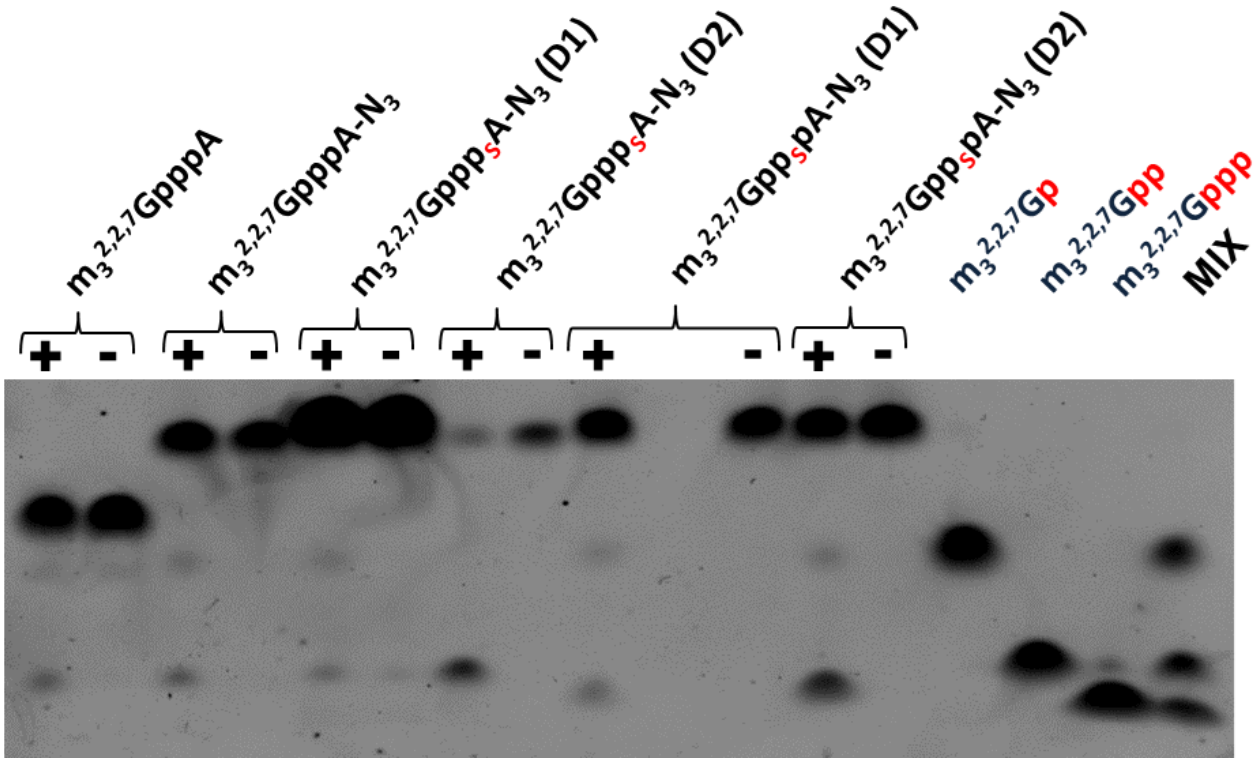
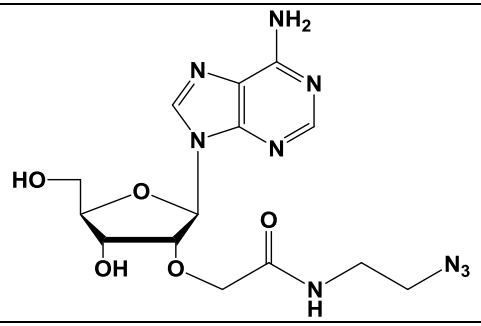
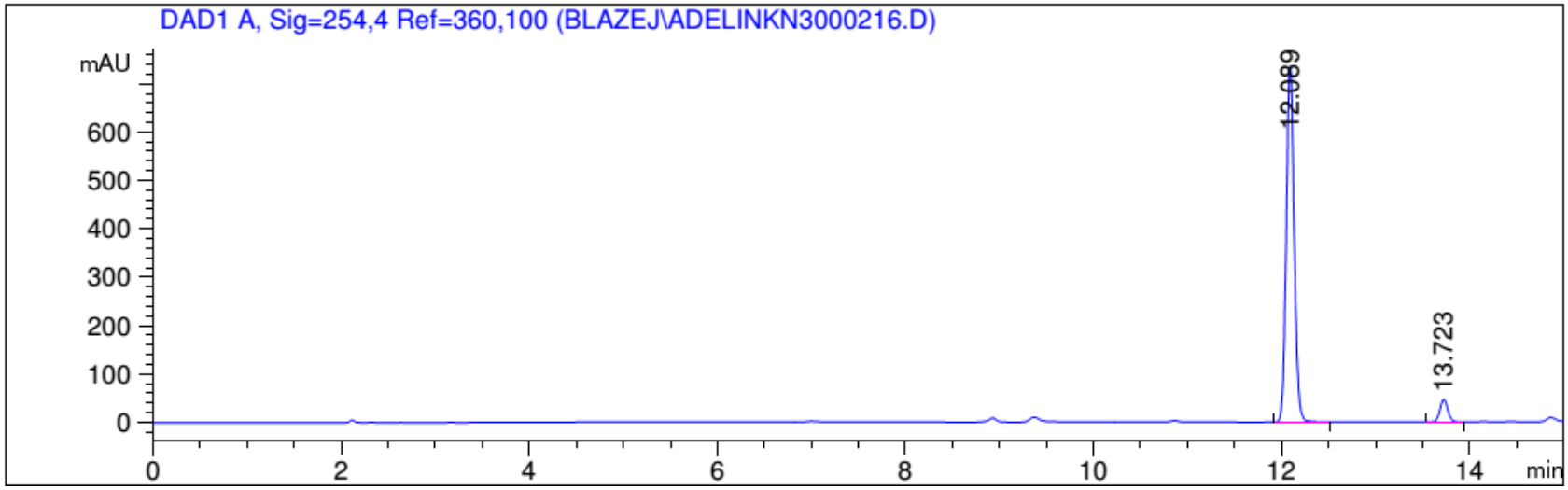


Figure S3. *Susceptibility of TMG analogues to the Nudt16 decapping enzyme. Electrophoretic mobilities of TMG cap analogues and TMG species (visible under UV 305nm). Active hNudt16 enzyme (lanes indicated with [+]), but not thermally inactivated hNudt16 (lanes indicated with [−]), shows low decapping activity on TMG caps: $m^{2,2,7}GpppA$, $m^{2,2,7}GpppA\text{-}N_3$, high decapping activity on: $m^{2,2,7}Ggpp_sA\text{-}N_3$ D2 and $m^{2,2,7}Gpp_spA\text{-}N_3$ D2,. TMG cap $m^{2,2,7}Ggpp_sA\text{-}N_3$ D1 and $m^{2,2,7}Gpp_spA\text{-}N_3$ are stable against hNudt16 decapping activity under the same conditions.*

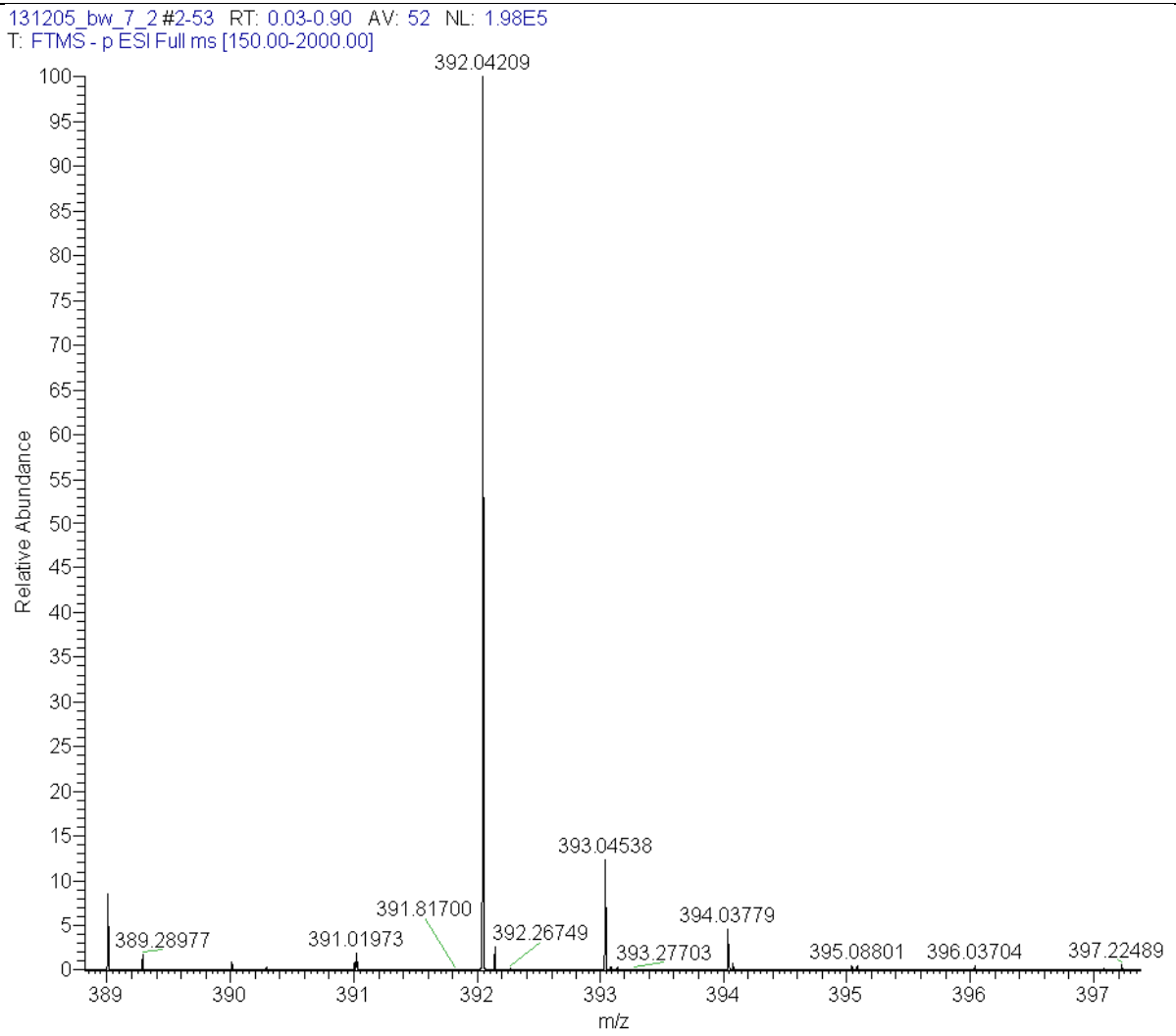
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine (9)





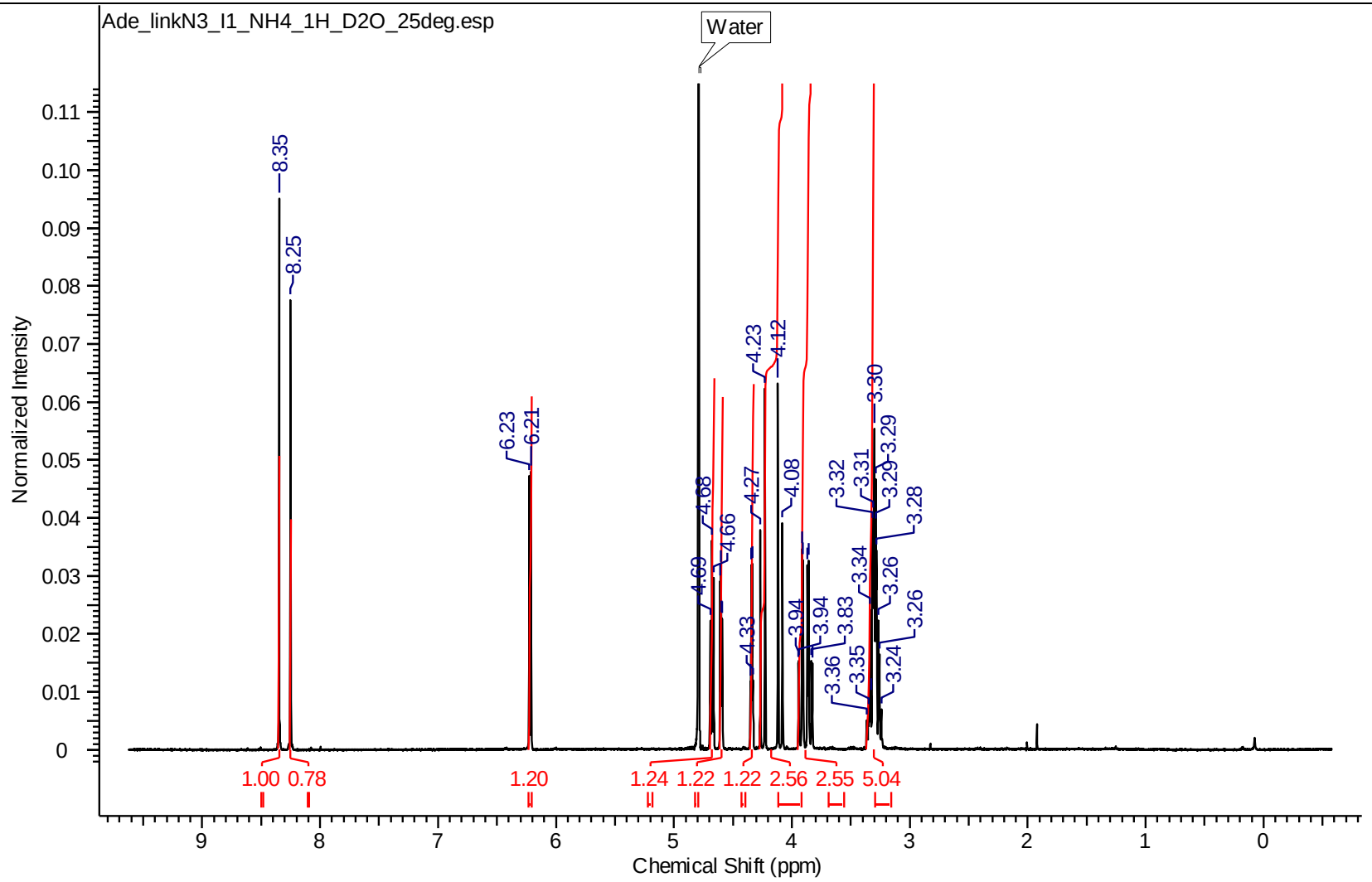
2'-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine (9)

Ado-link-N_3 /NH_4^+ HRMS (-)ES $C_{14}H_{19}N_9O_5$ 392,04209, $[M-H]^-$ Exact Mass: 392,14309

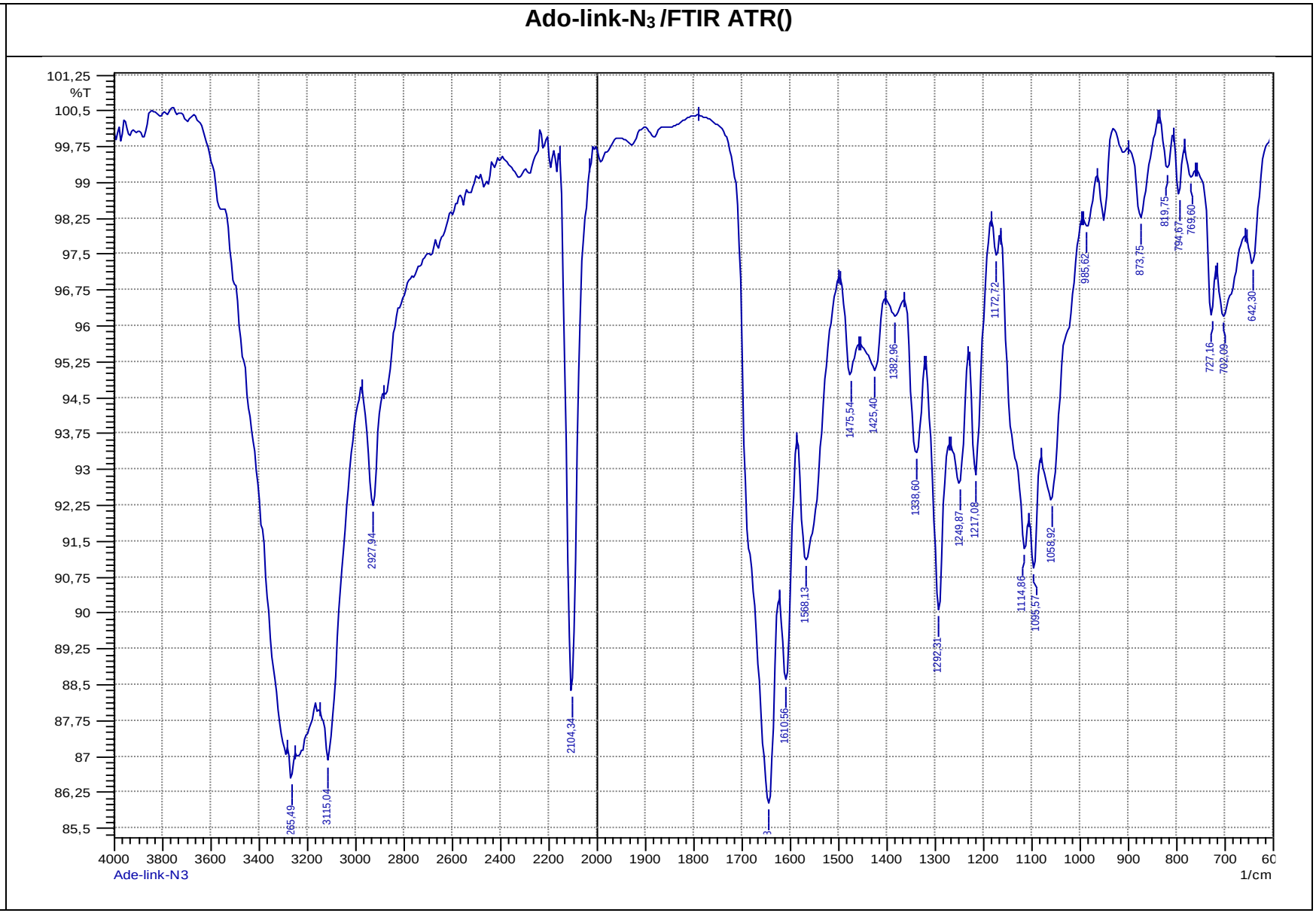


2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine (9)

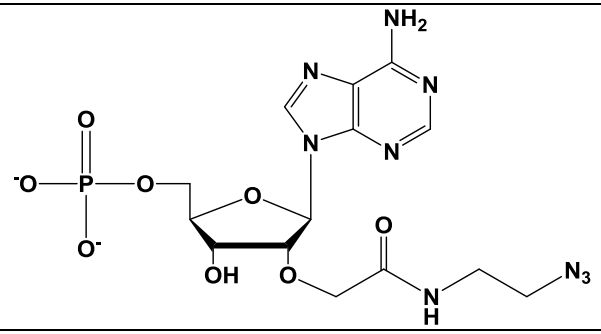
Ado-link-N_3 /^{1}H NMR ()

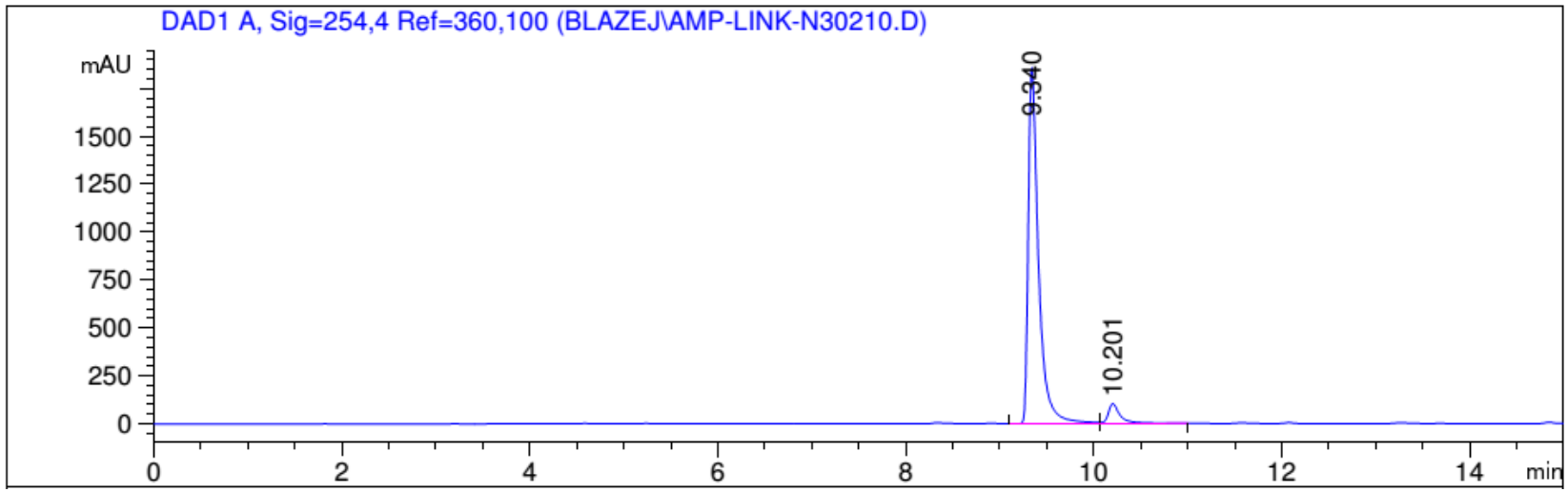


2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine (9)



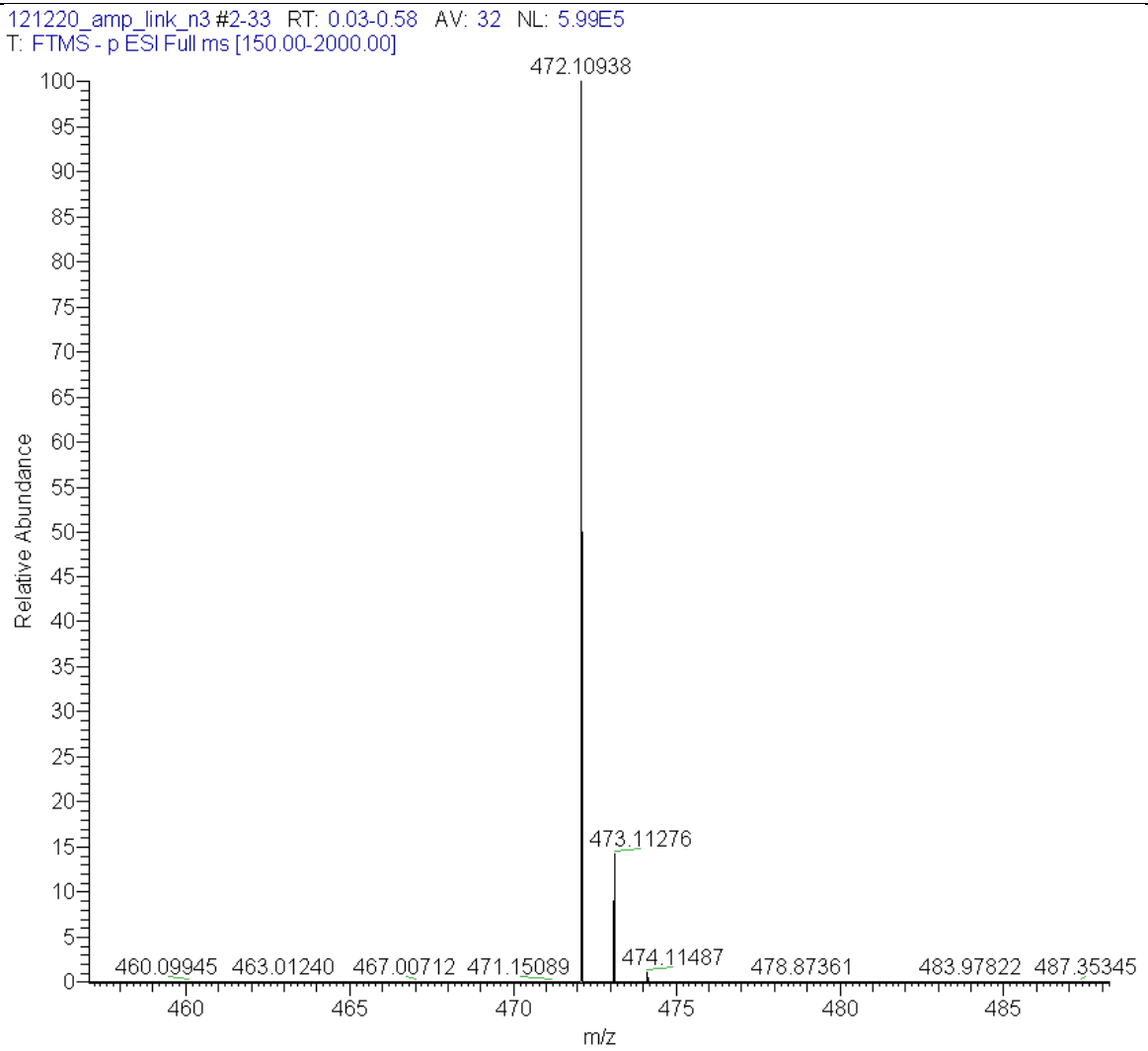
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-monophosphate (10)





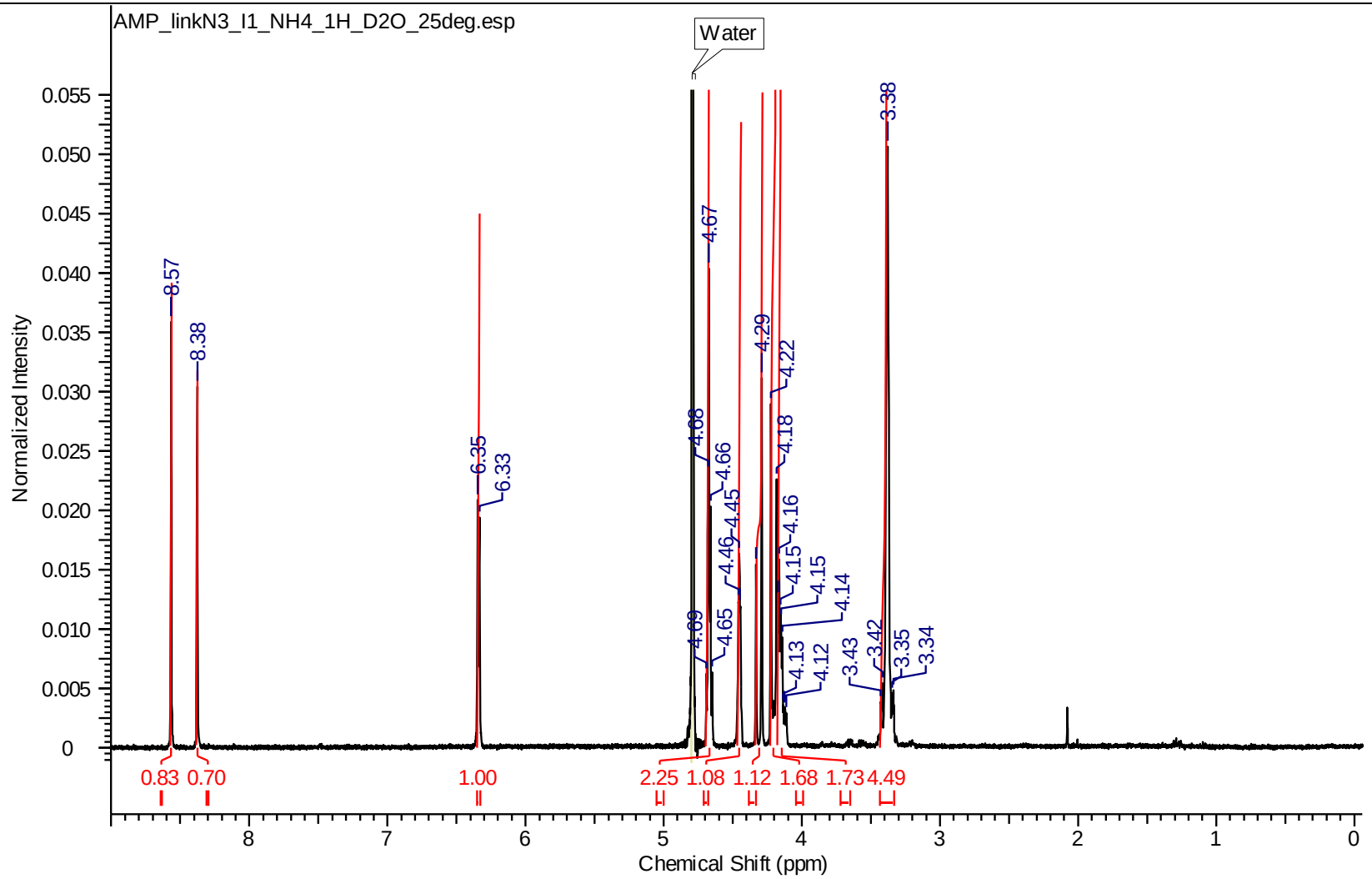
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-monophosphate (10)

AMP-link-N_3/NH_4^+ HRMS (-)ES $C_{14}H_{20}N_9O_8P$, 472.10938 $[M-H]^-$ Exact Mass: 473,117249

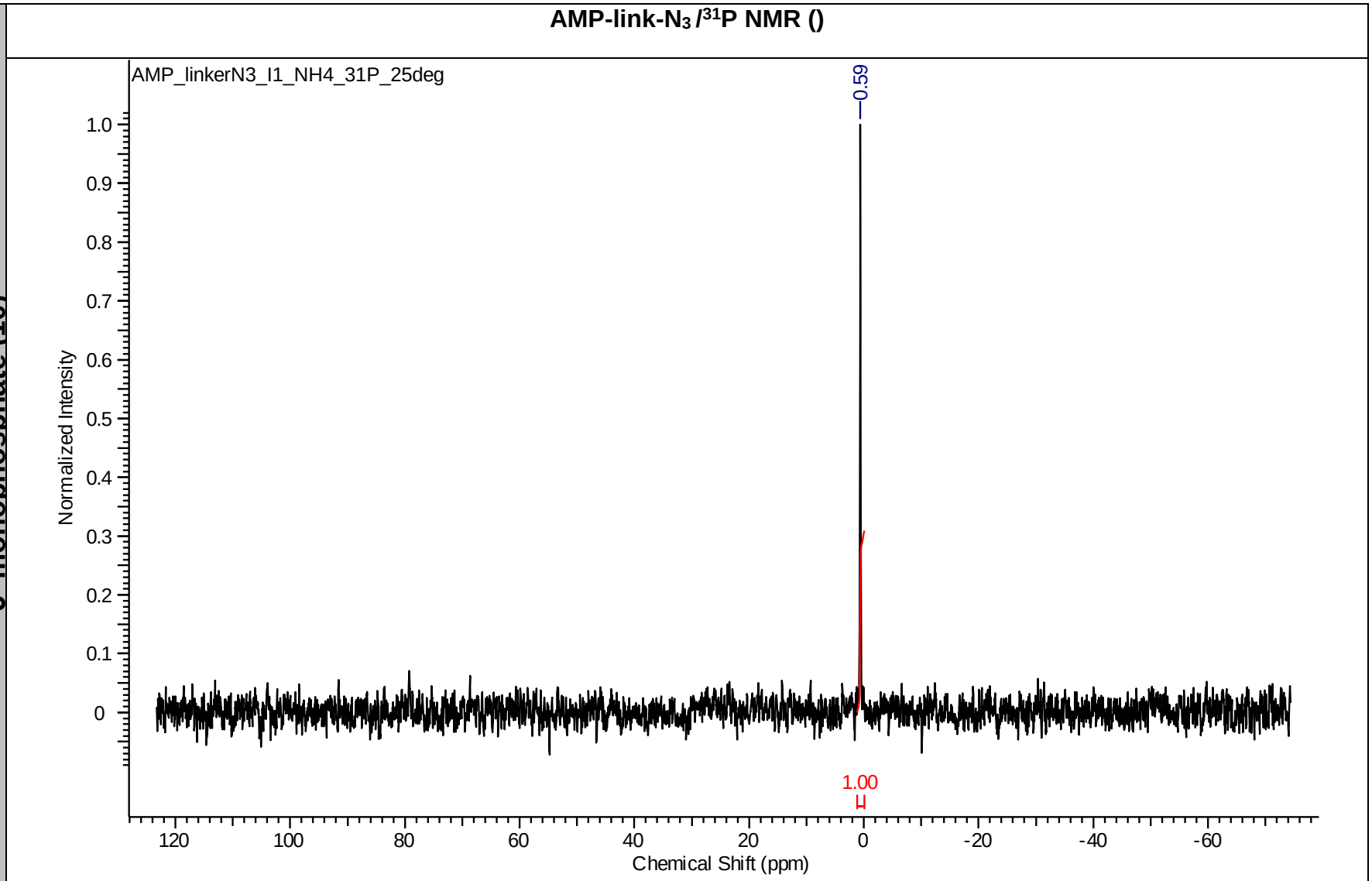


2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-monophosphate (10)

AMP-link-N_3 /^{1}H NMR ()

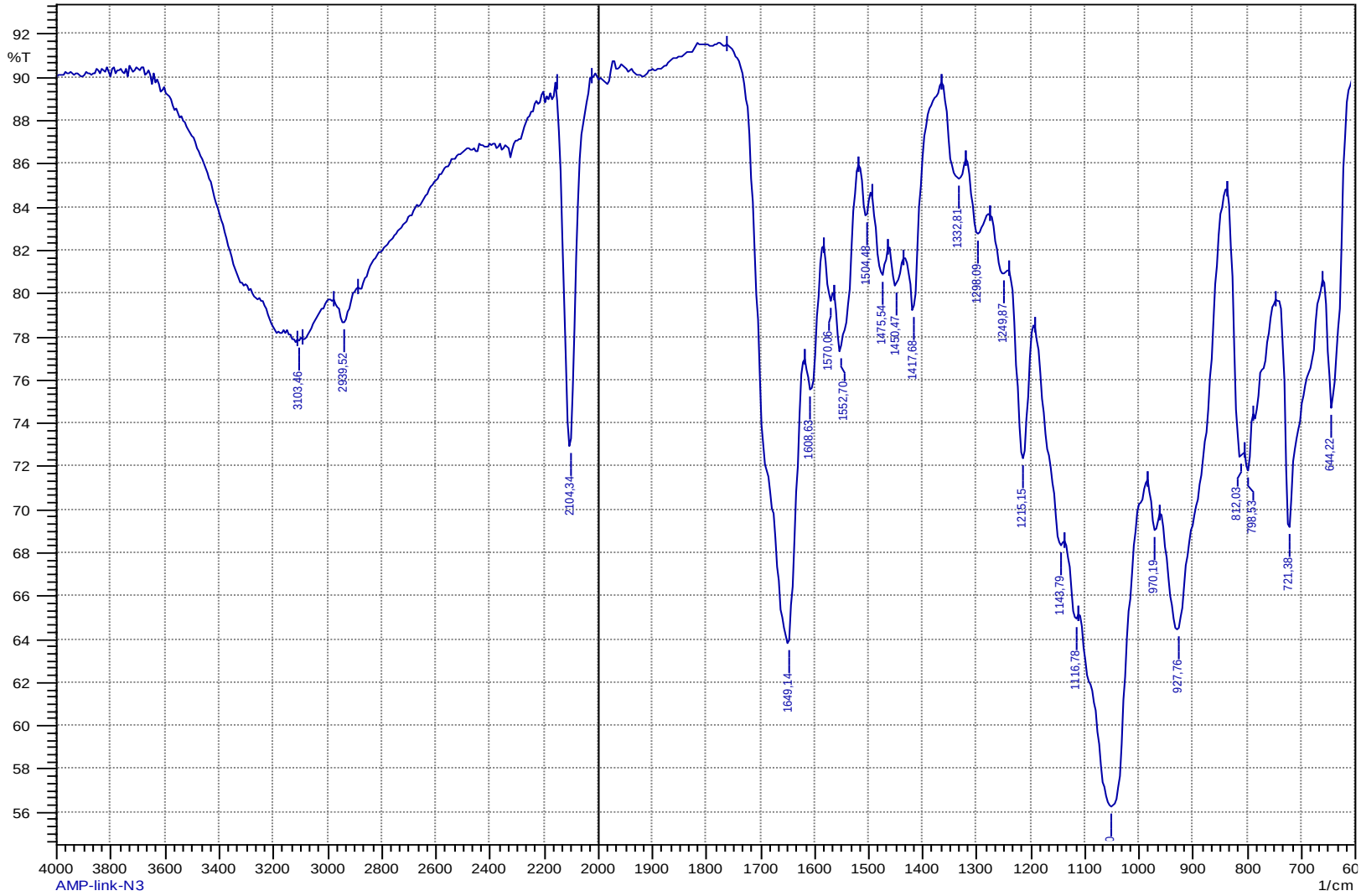


2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-monophosphate (10)

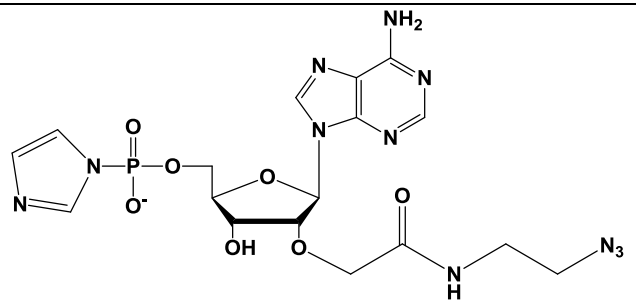


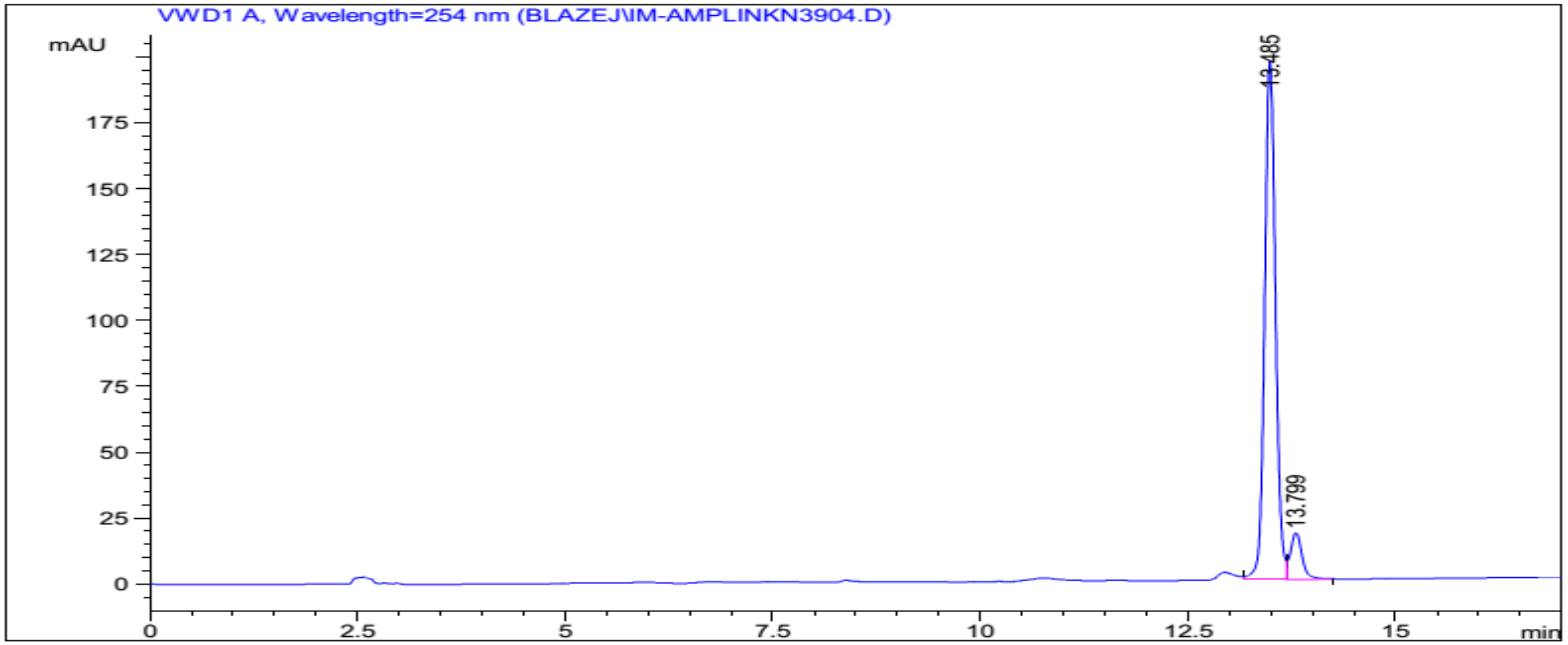
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-monophosphate (10)

AMP-link-N_3 /FTIR ATR ()



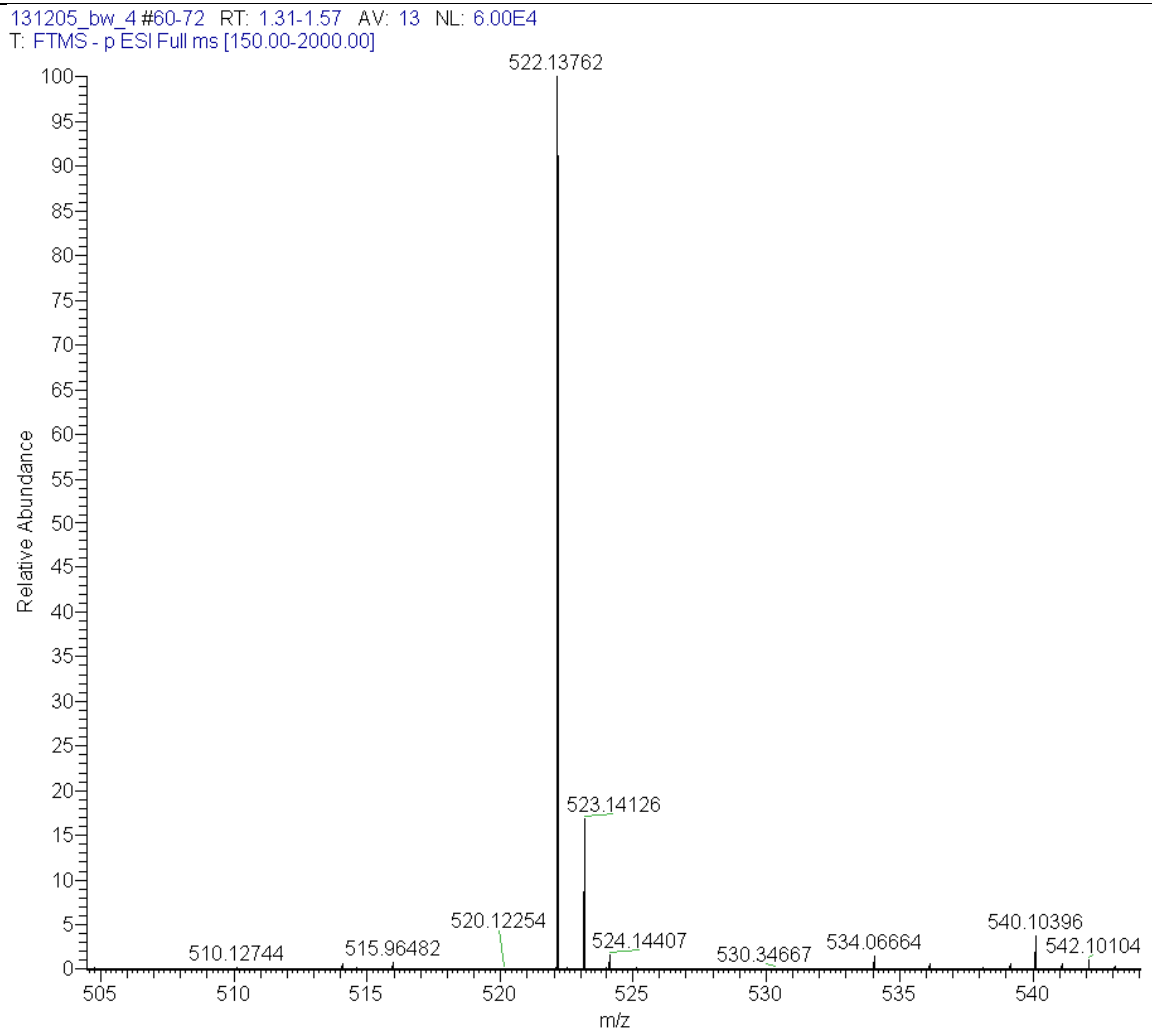
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-imidazolidemonophosphate (12)





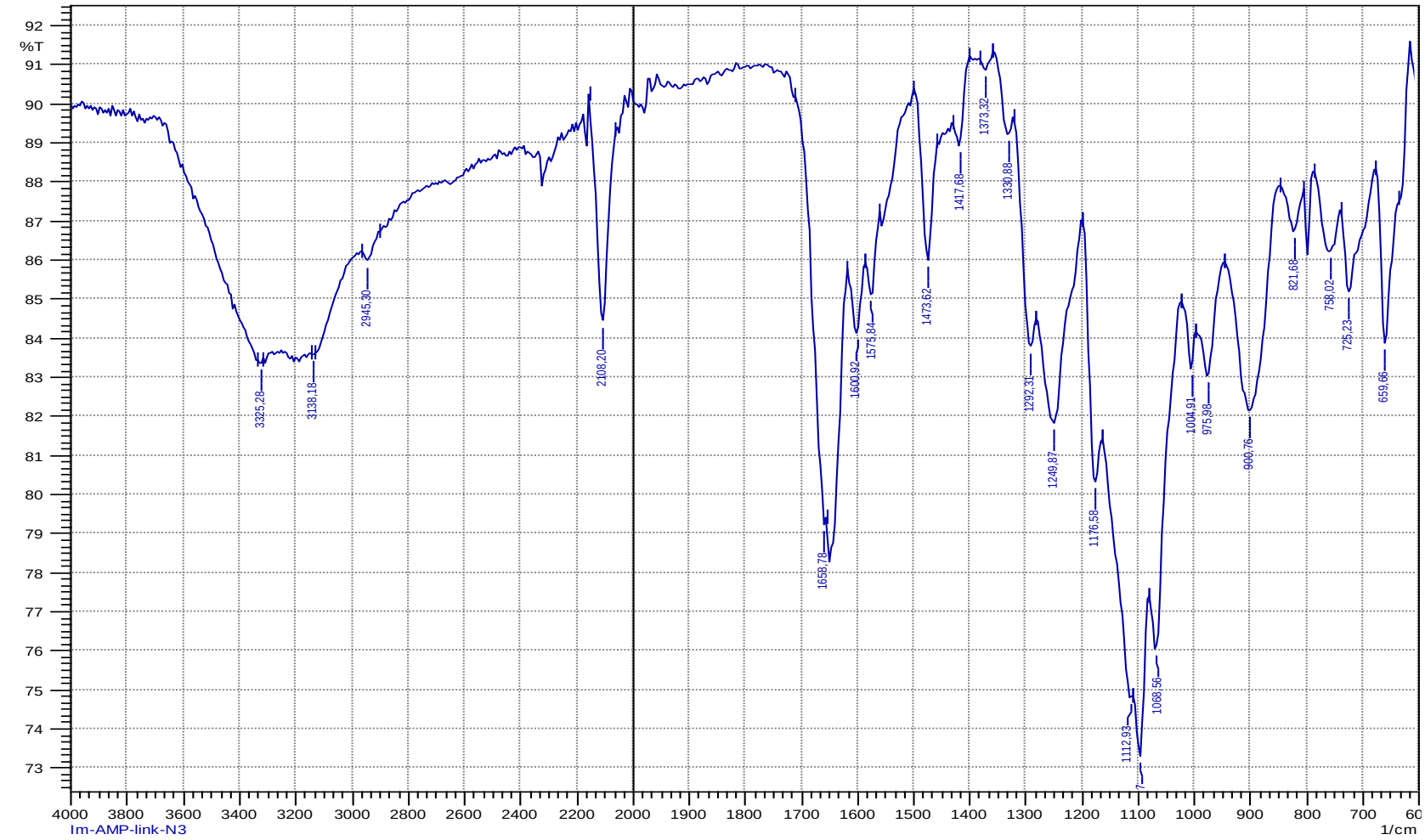
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-imidazolidemonophosphate (12)

Im-AMP-link-N_3/Na^+ HRMS (-)ES $C_{17}H_{22}N_{11}O_7P$, 522.13762, $[M-H]^-$ Exact Mass: 523,144132

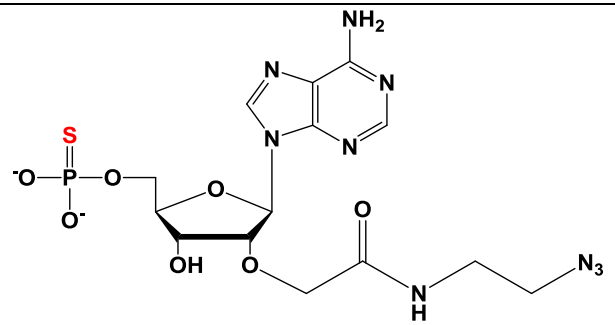


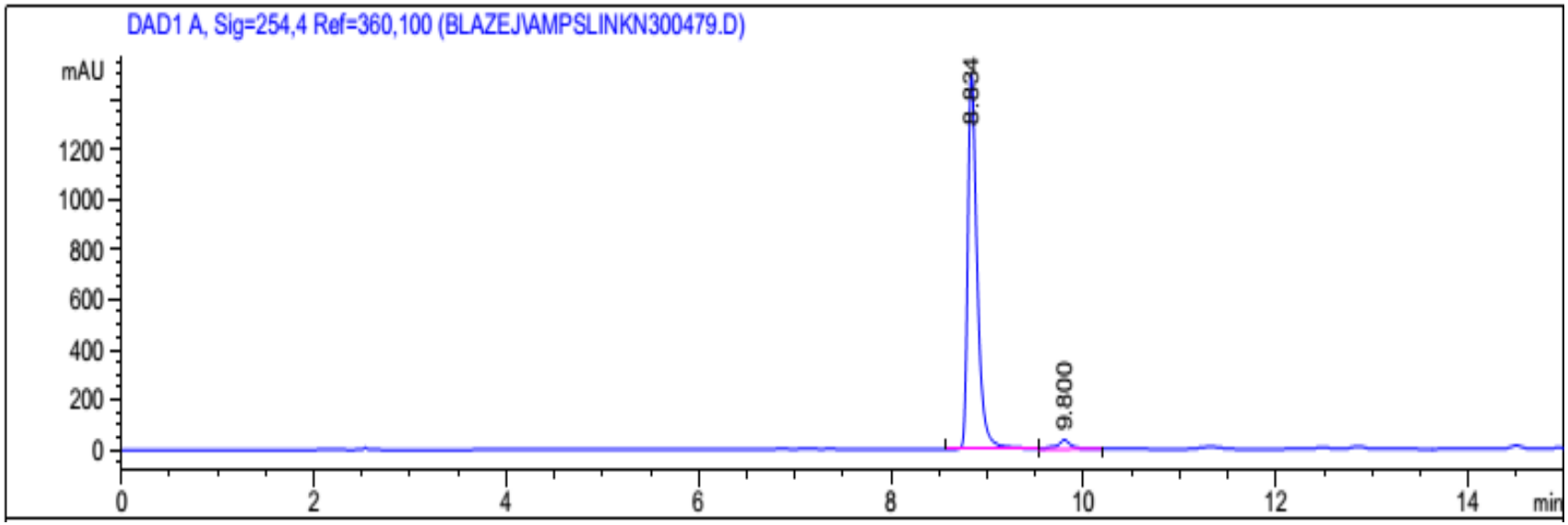
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-imidazolidemonophosphate (12)

Im-AMP-link-N_3 /FTIR ATR ()



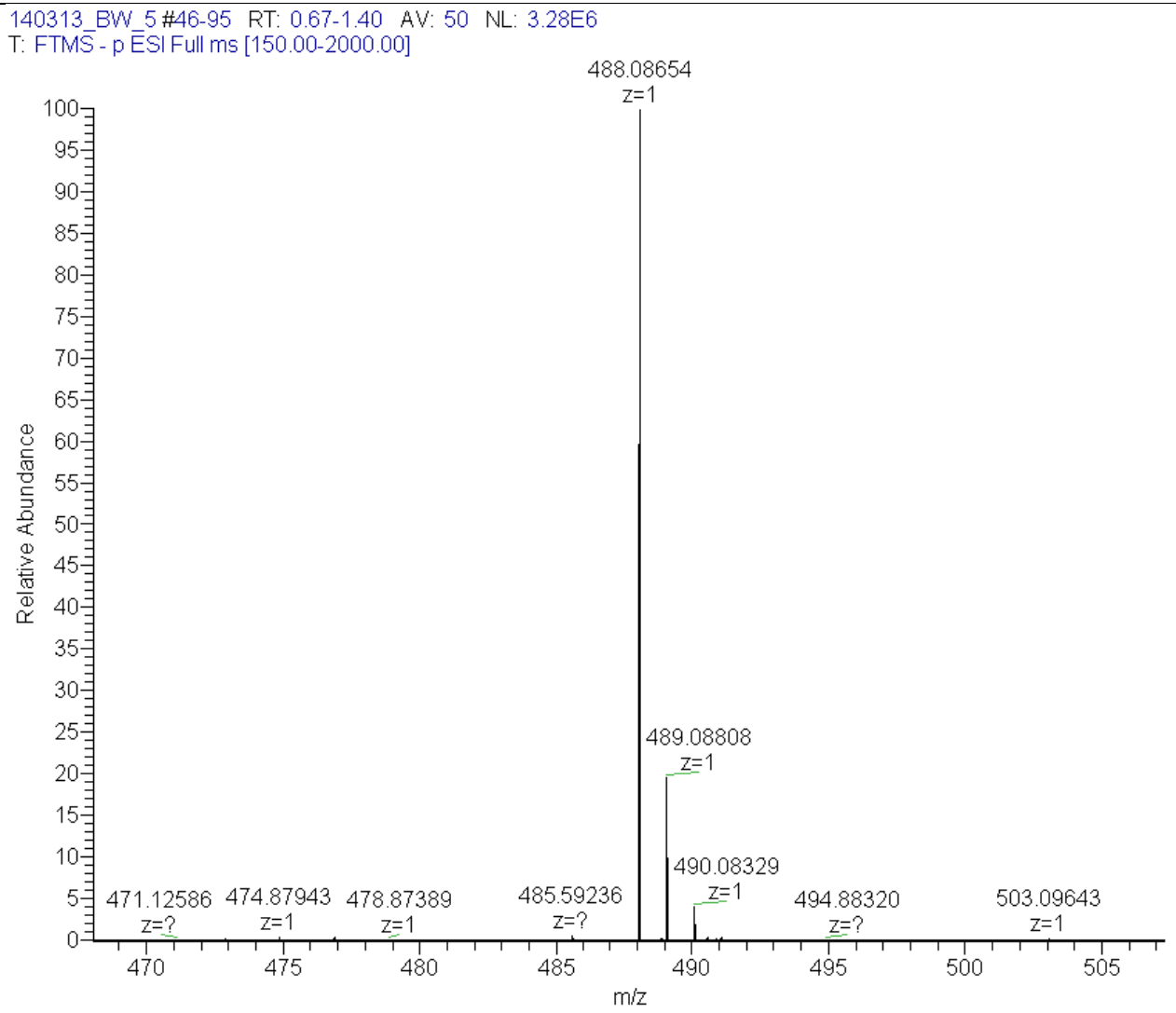
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-monothiophosphate (11)

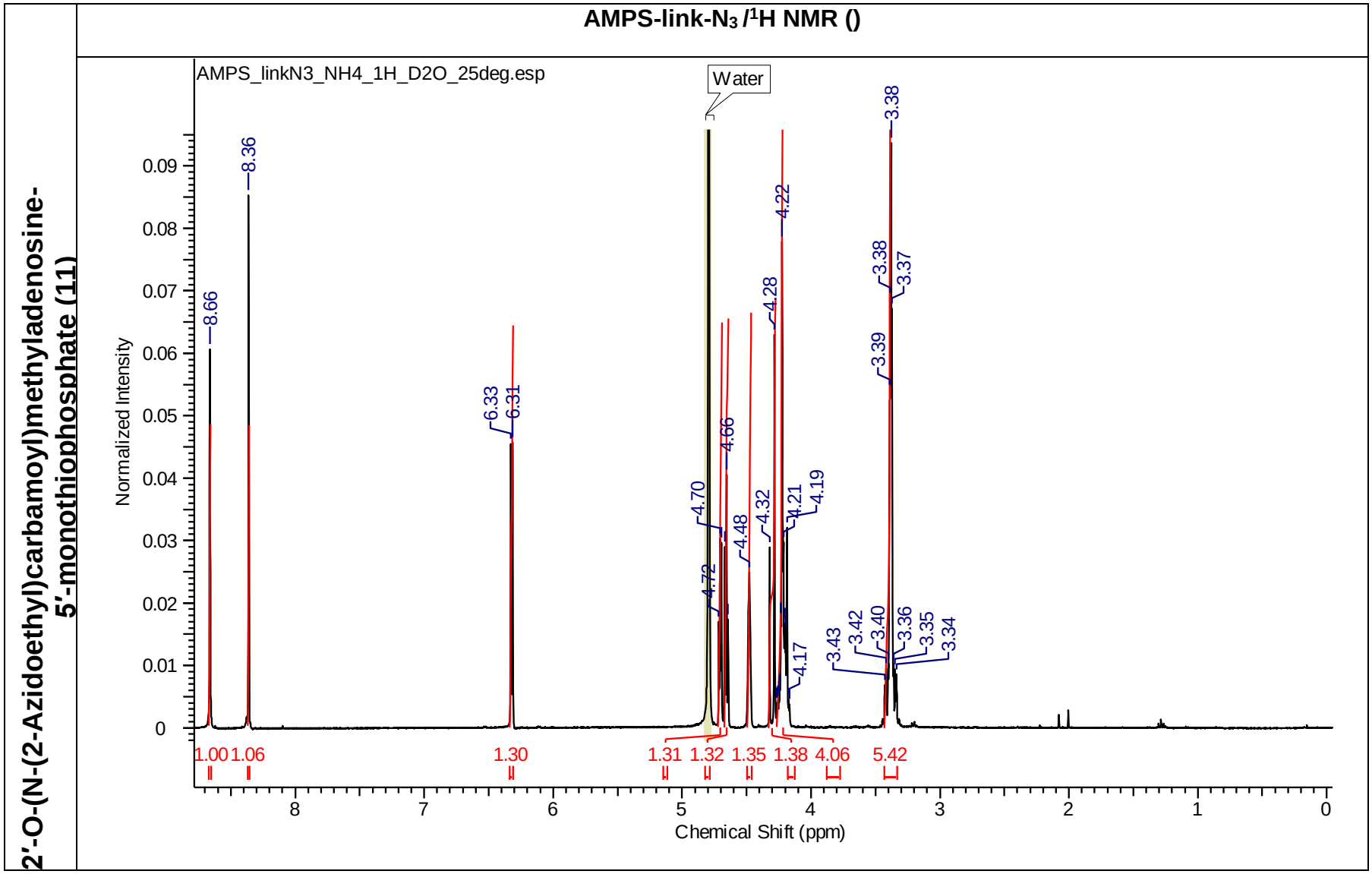




2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-monothiophosphate (11)

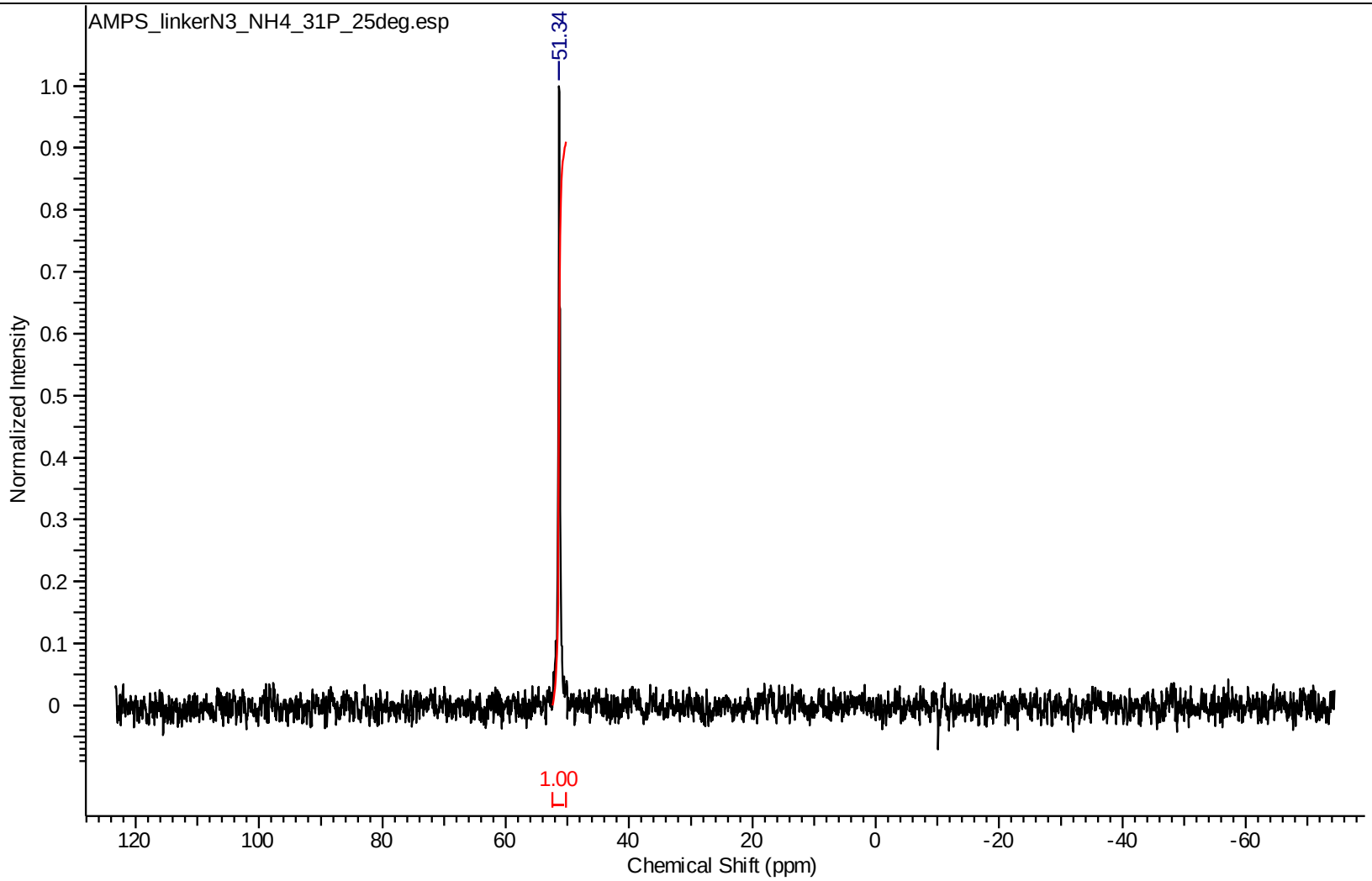
AMPS-link-N_3 /NH_4^+ HRMS (-)ES $C_{14}H_{20}N_9O_7PS$, 488.08654 $[M-H]^-$ Exact Mass: 489,094406




2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-monothiophosphate (11)
AMPS-link-N3 /1H NMR ()
AMPS_linkN3_NH4_1H_D2O_25deg.esp
Water
Normalized Intensity
Chemical Shift (ppm)
8.66
8.36
6.33
6.31
4.70
4.72
4.66
4.48
4.32
4.28
4.22
4.21
4.19
4.17
3.43
3.42
3.40
3.38
3.38
3.37
3.39
3.36
3.35
3.34
1.00 1.06
1.30
1.31 1.32 1.35 1.38 4.06
5.42

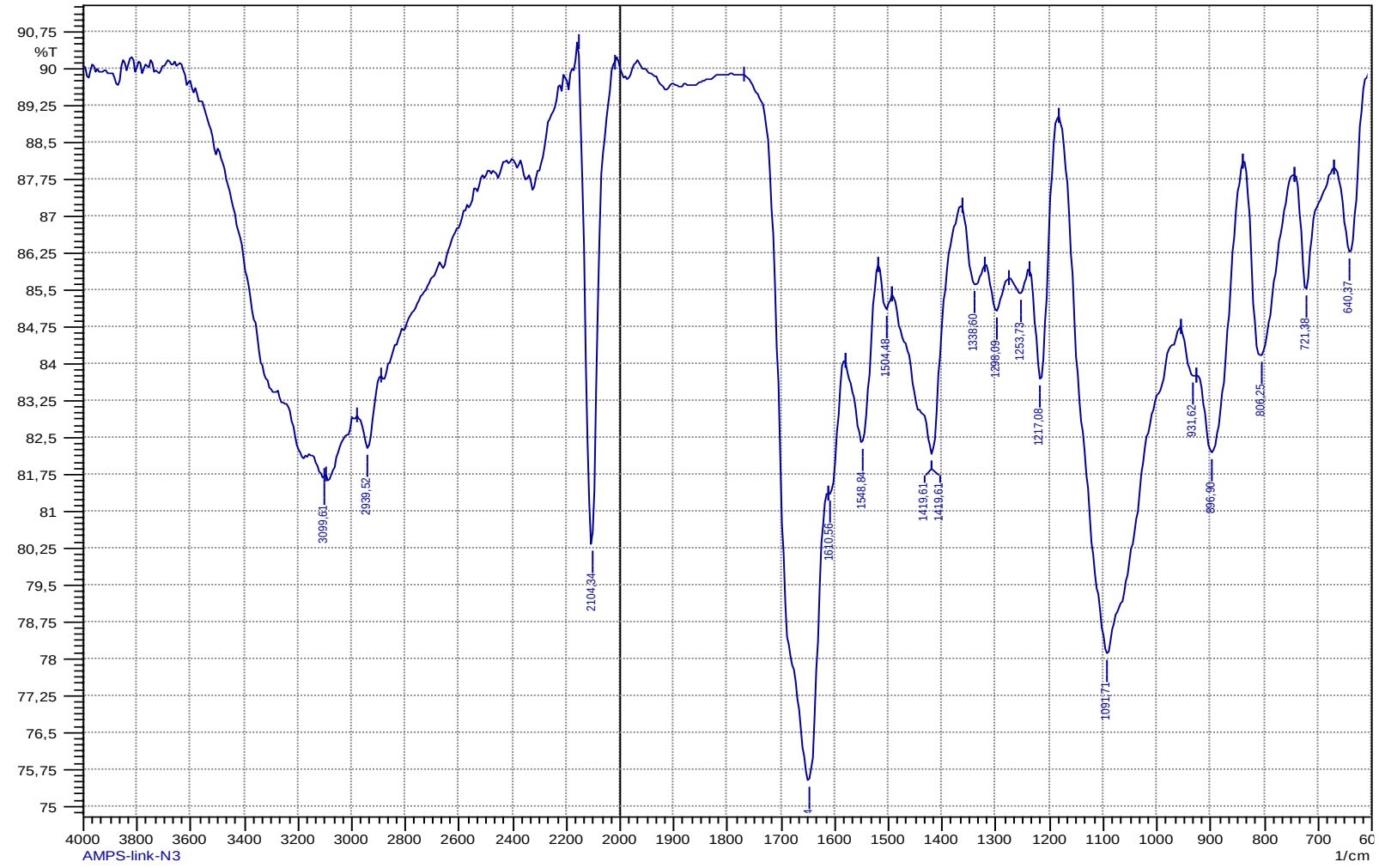
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-monothiophosphate (11)

AMPS-link-N_3 /^{31}P NMR ()

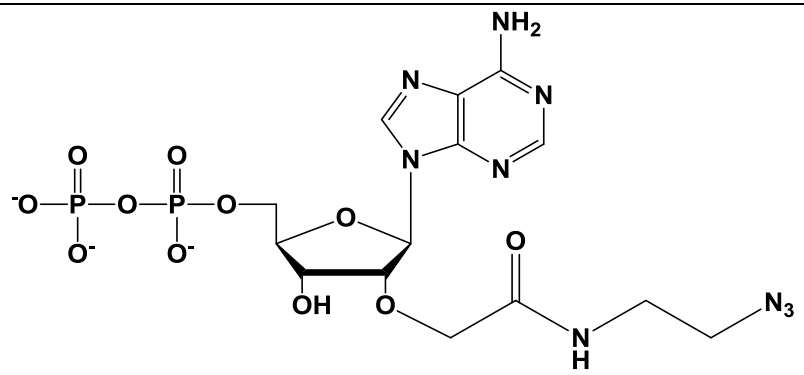


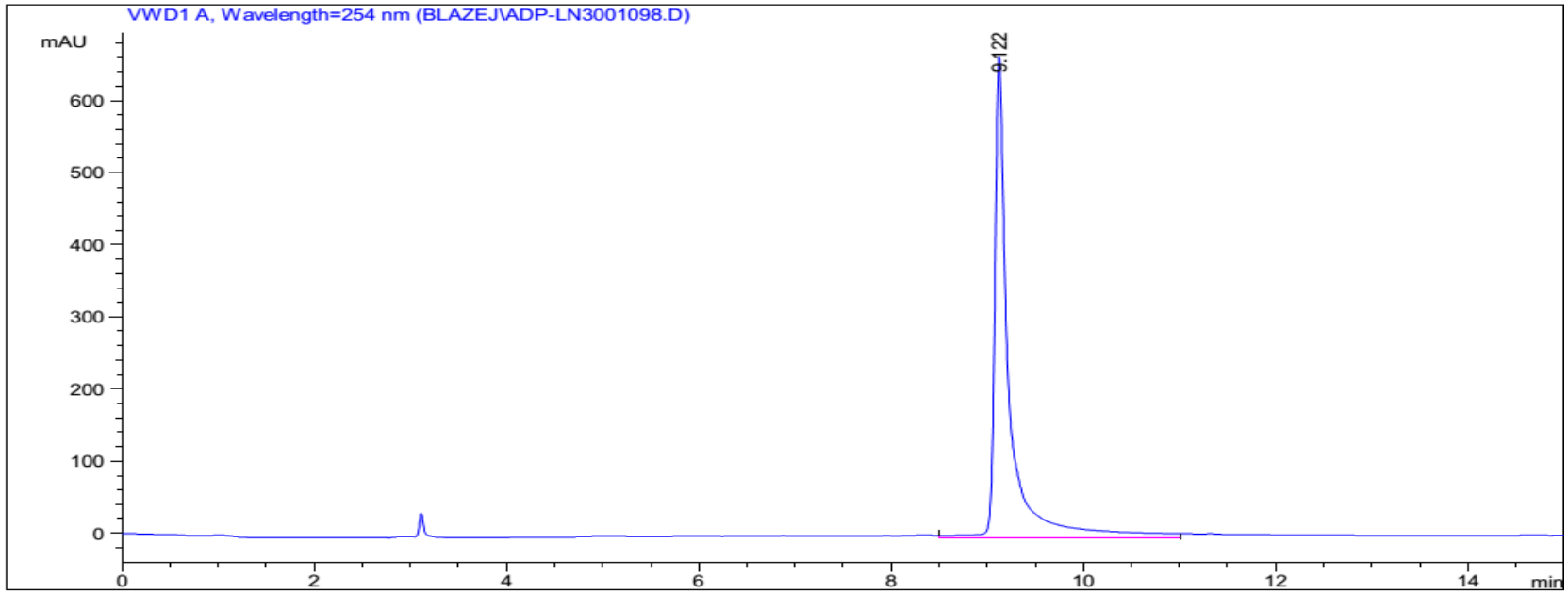
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-monothiophosphate (11)

AMPS-link-N_3 /FTIR ATR

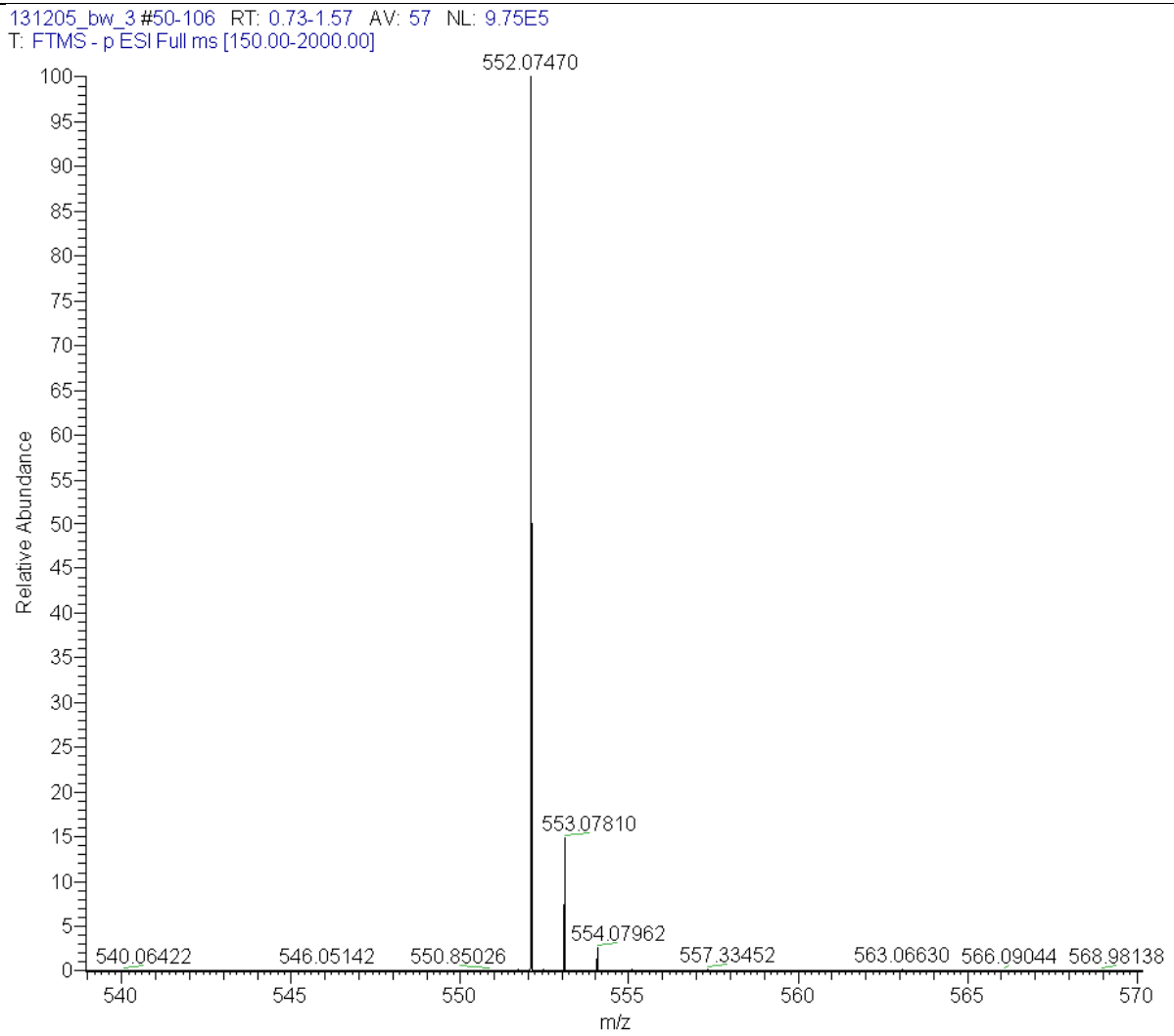


2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-diphosphate (13)



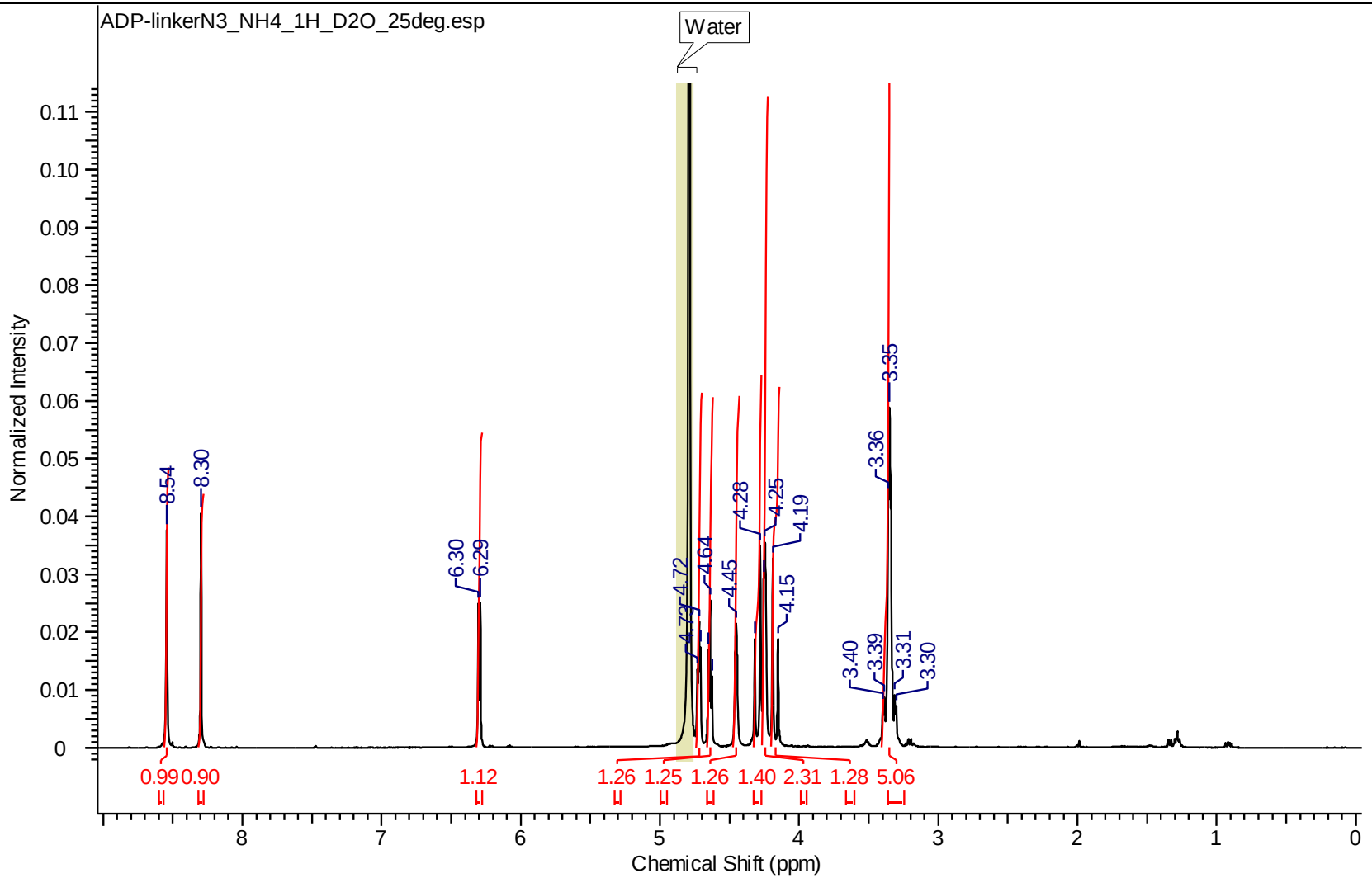


2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-diphosphate (13)	**ADP-link-N_3 /NH_4^+ HRMS (-)ES $C_{14}H_{21}N_9O_{11}P_2$, 552.07470 $[M-H]^-$ Exact Mass: 553.083582**



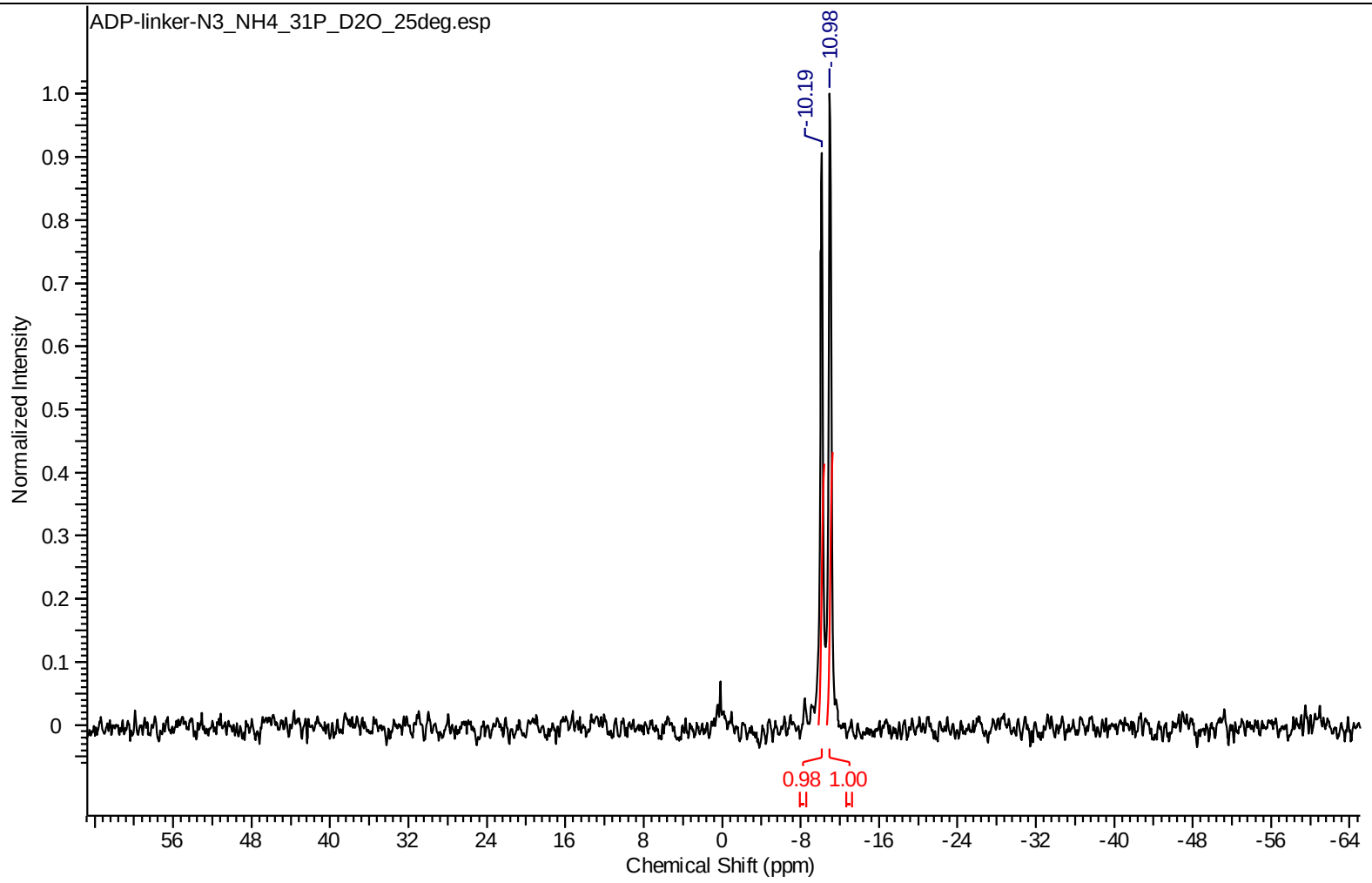
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-diphosphate (13)

ADP-link-N_3/NH_4^+ ^{1}H NMR ()



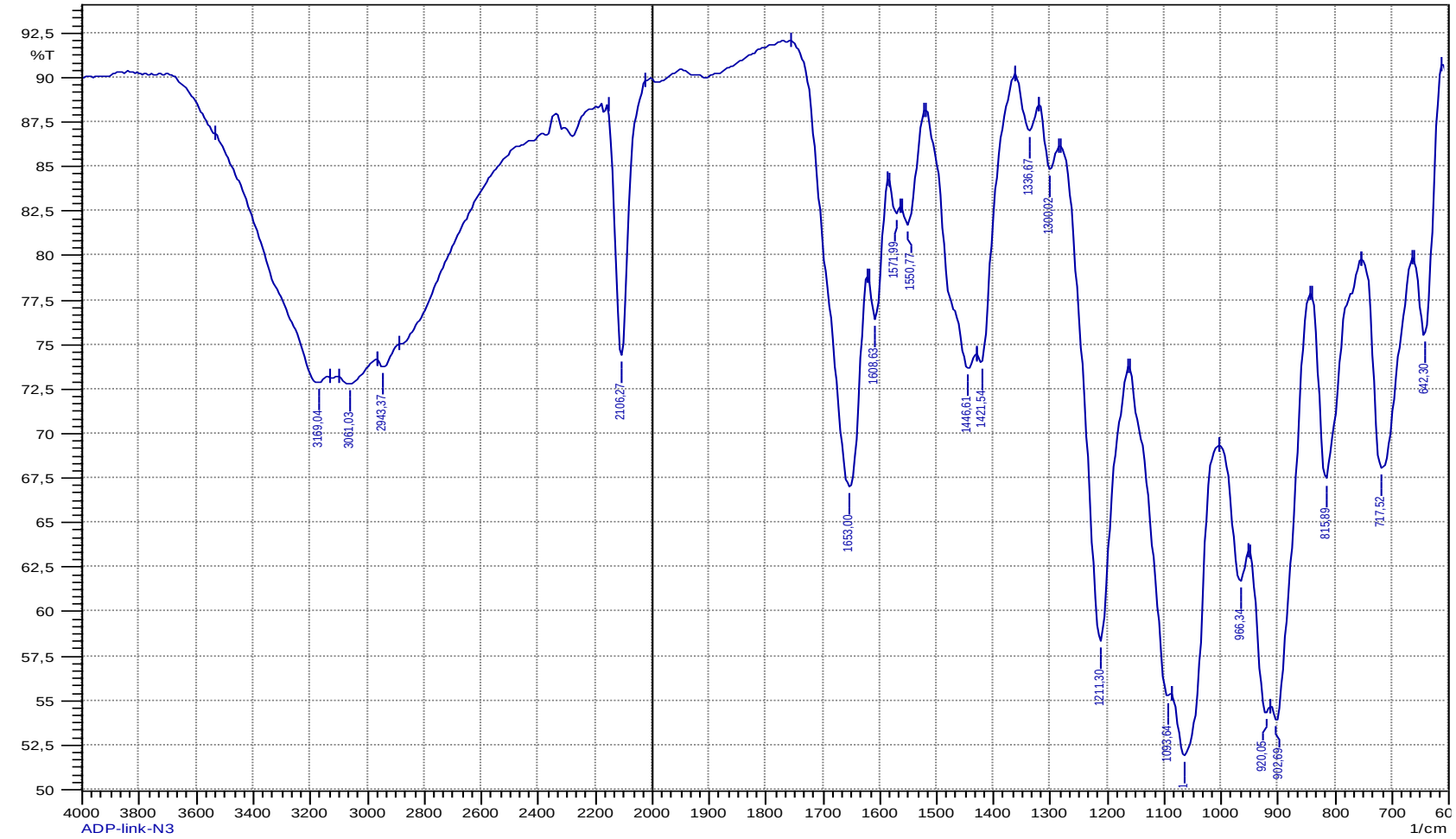
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-diphosphate (13)

ADP-link-N_3 /NH_4^+ ^{31}P NMR ()

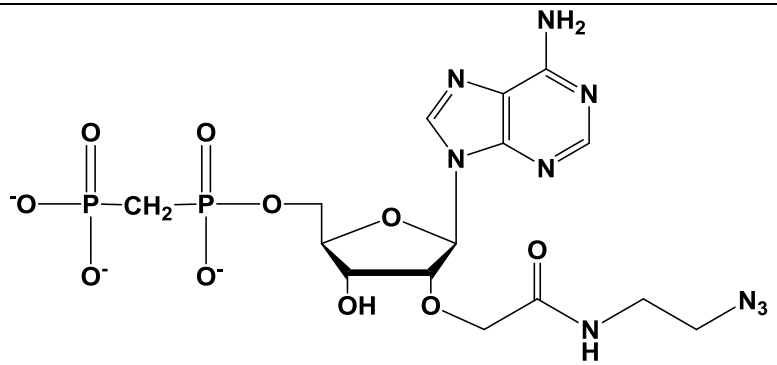


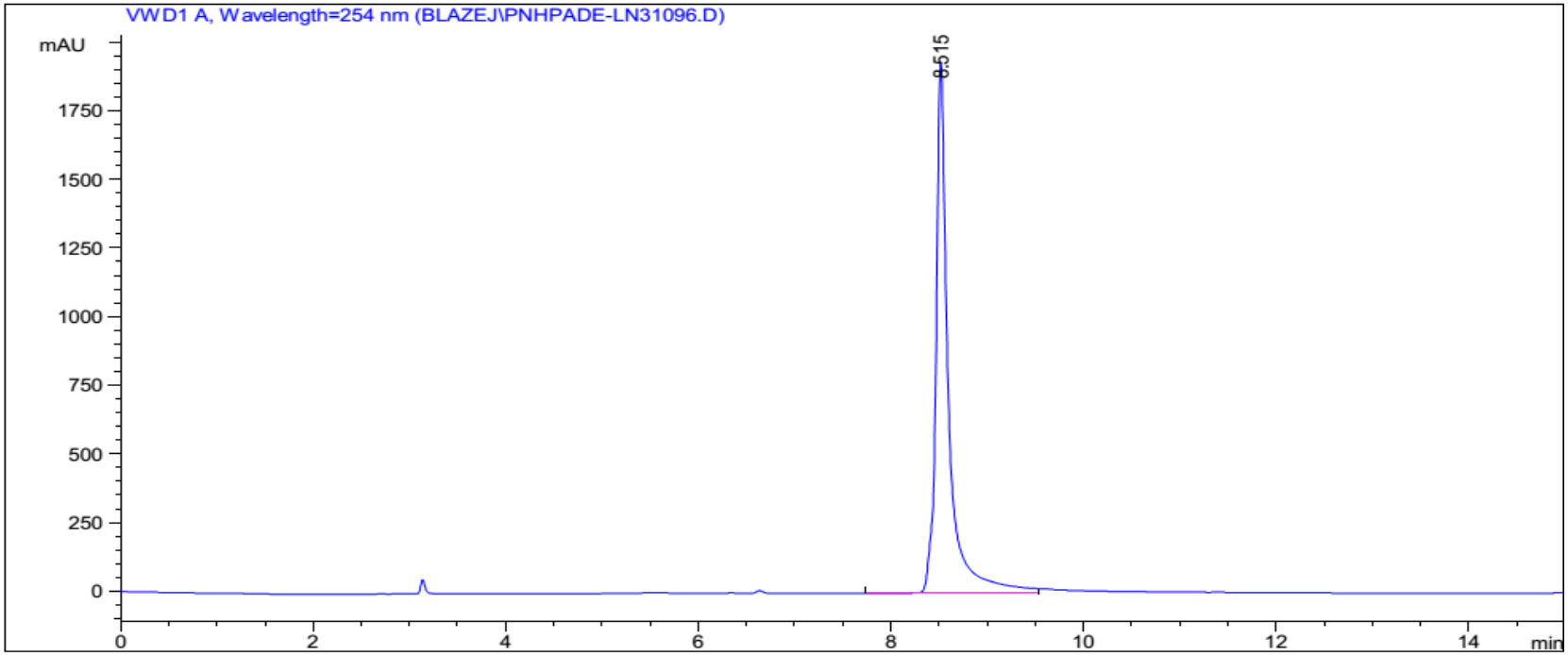
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-diphosphate (13)

ADP-link-N_3 /FTIR ATR



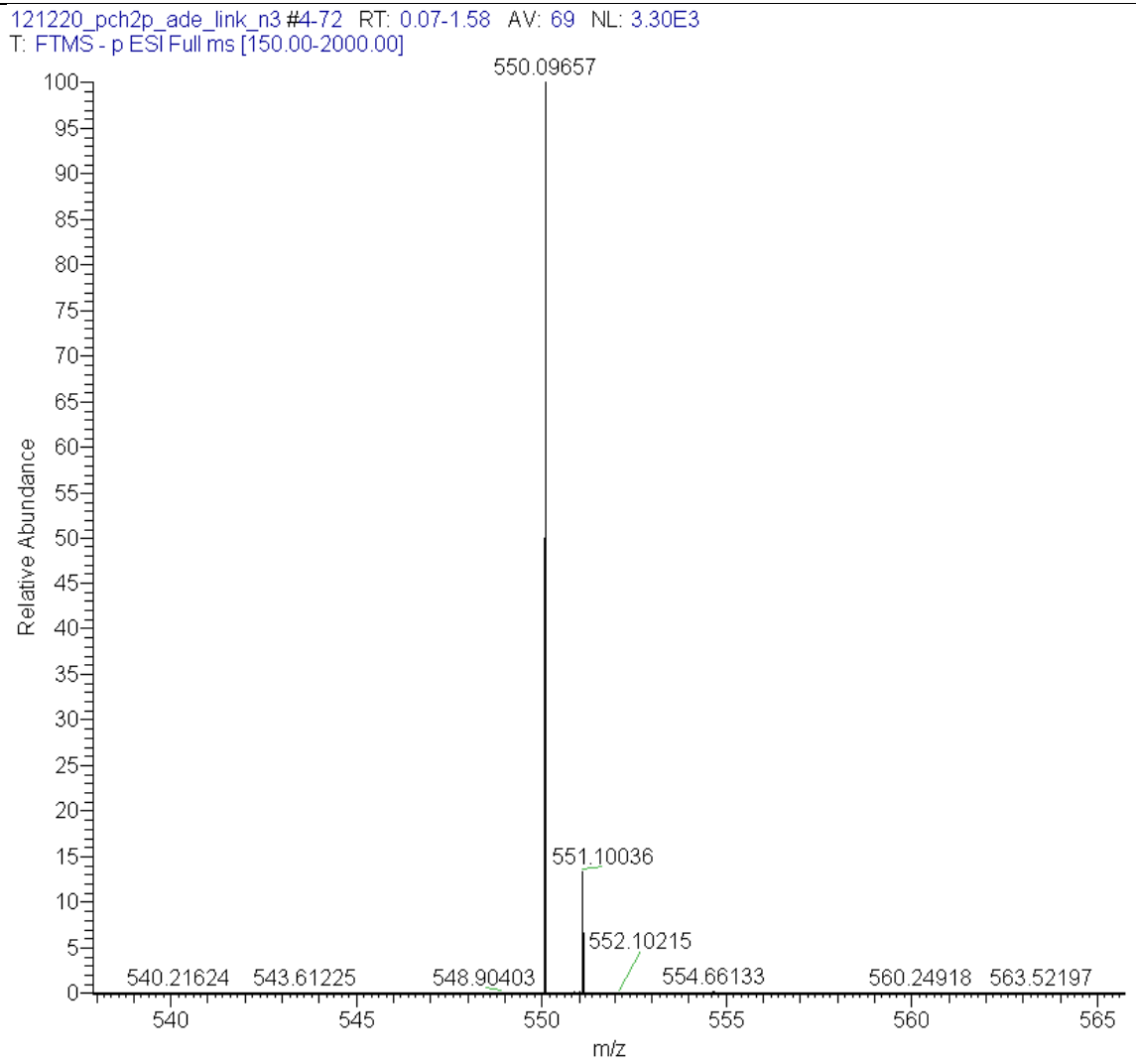
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-methylene(bisphosphonate) (14)





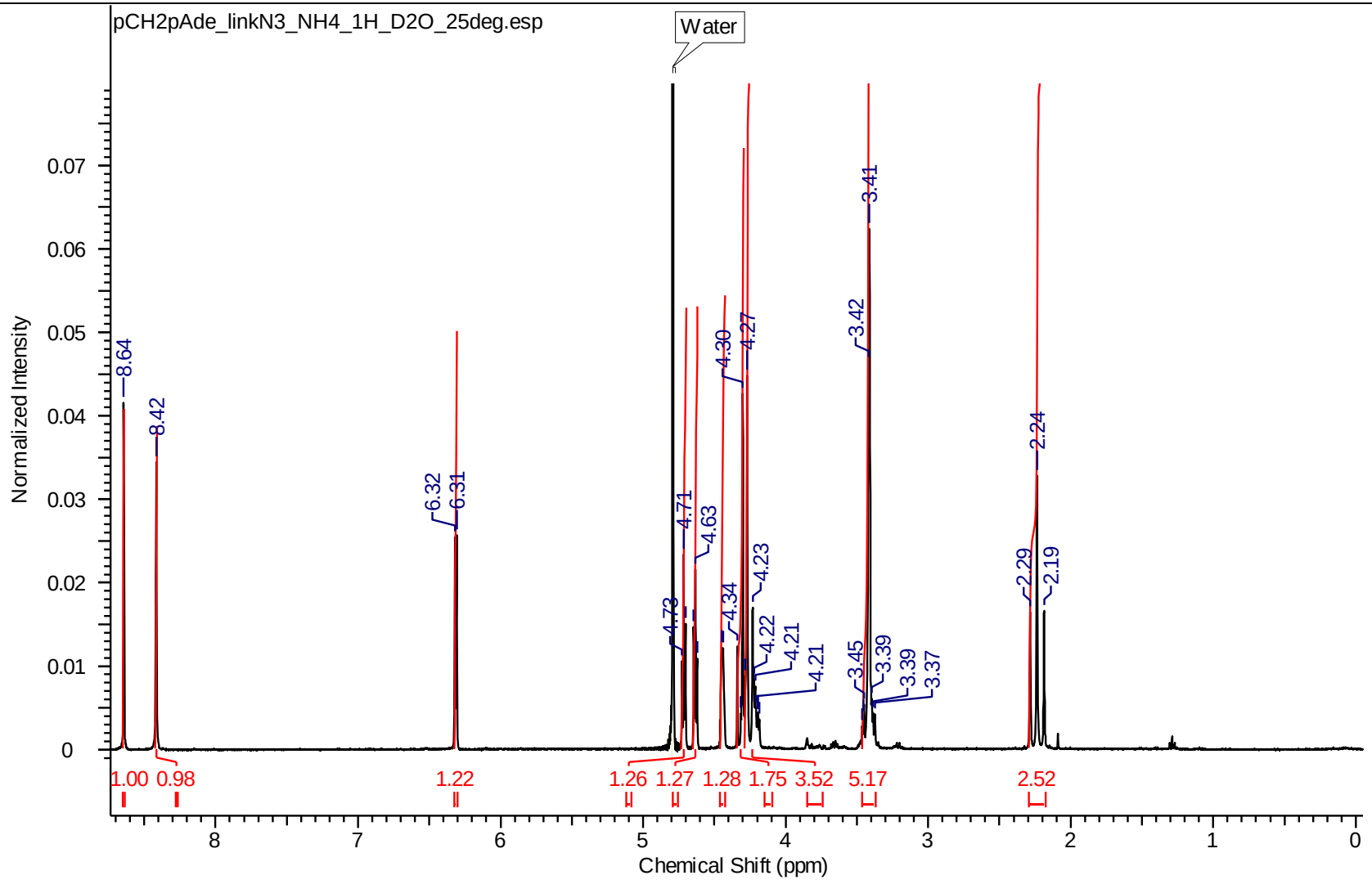
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-methylene(bisphosphonate) (14)

pCH_2p-Ado-link-N_3 /NH_4^+ HRMS (-)ES $C_{15}H_{23}N_9O_{10}P_2$, 550.09657 $[M-H]^-$ Exact Mass: 551,104317



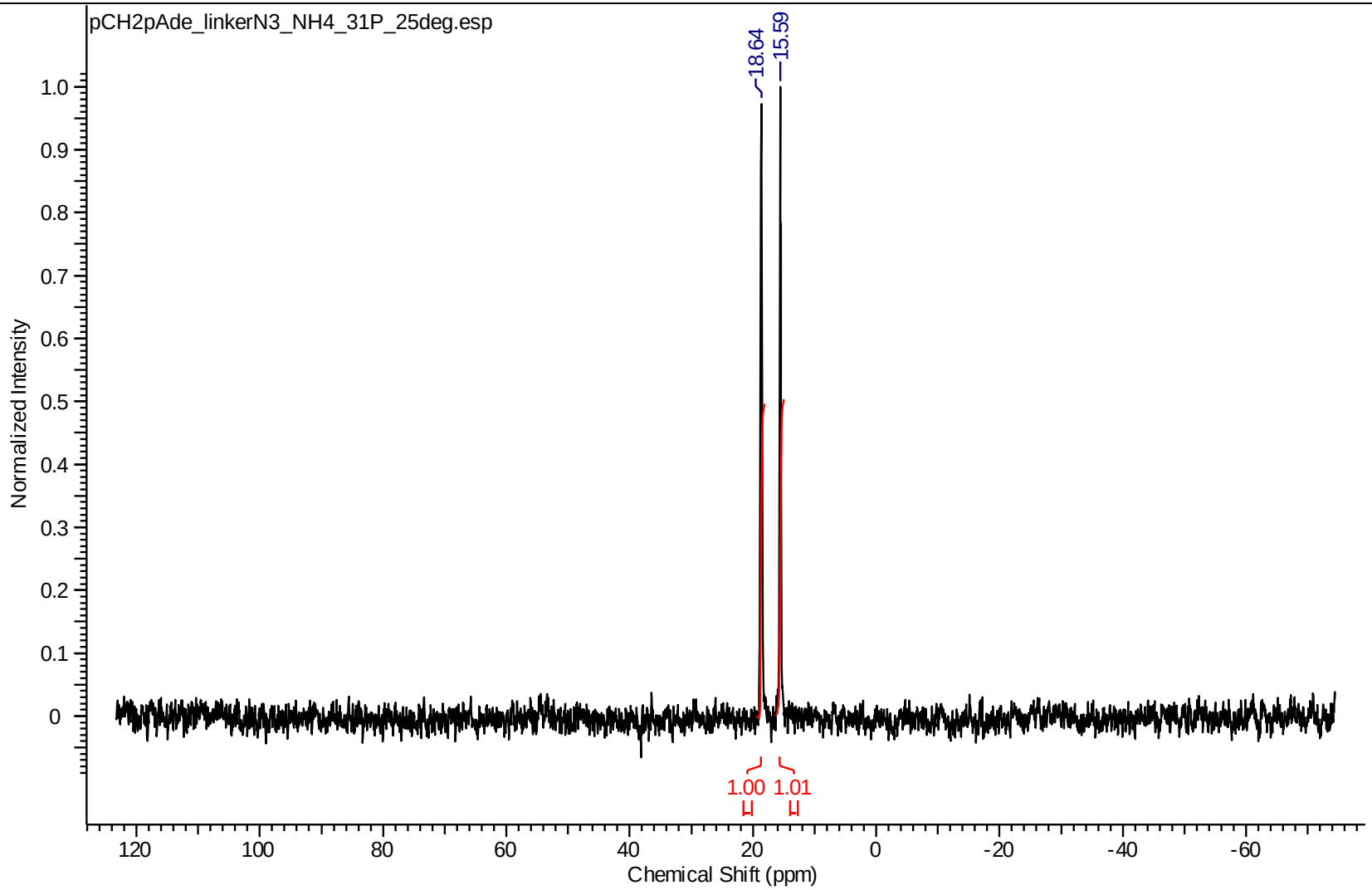
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-methylene(bisphosphonate) (14)

pCH₂p-Ado-link-N₃ /NH₄⁺ ¹H NMR ()



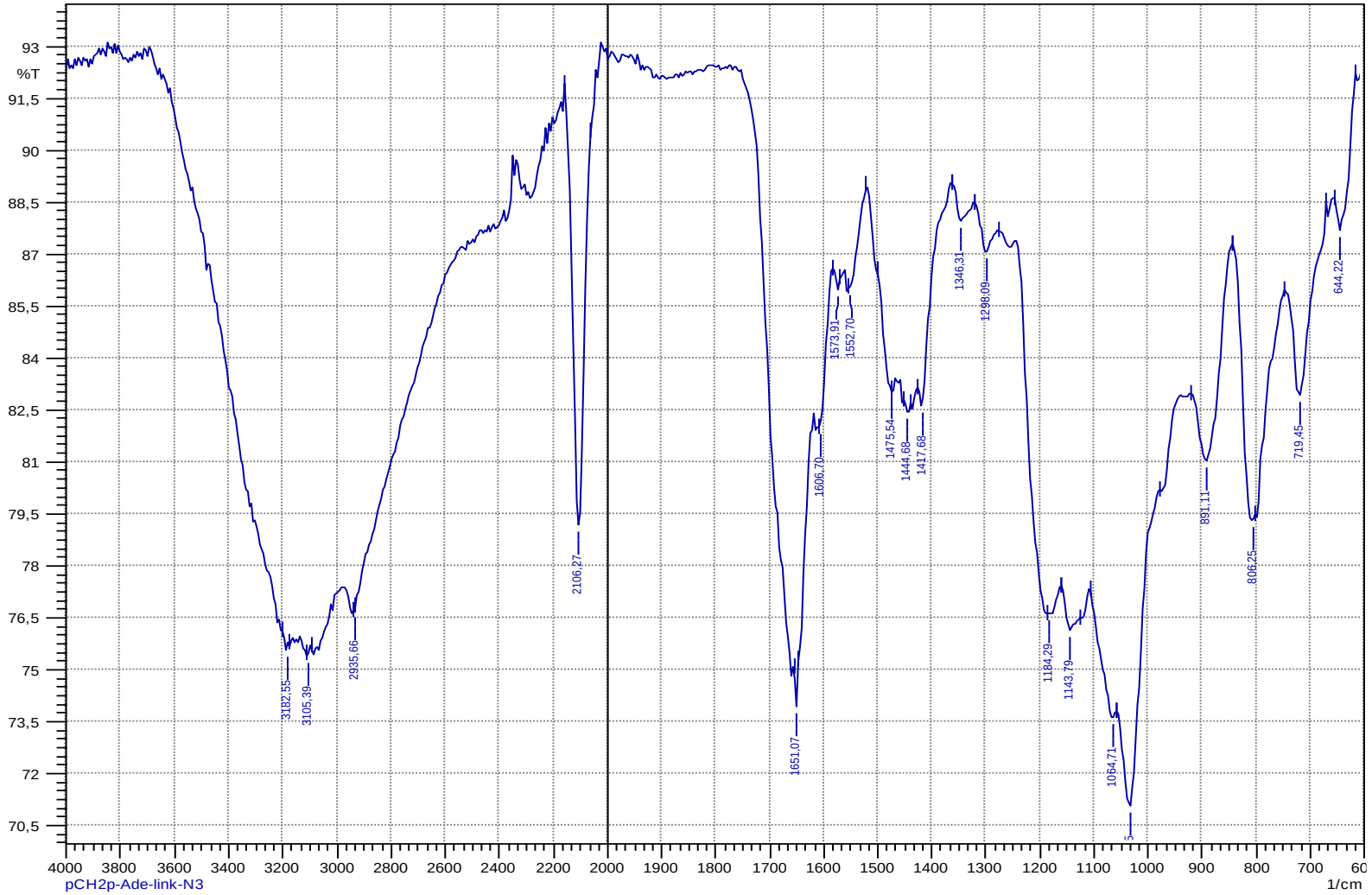
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-methylene(bisphosphonate) (14)

pCH_2p-Ado-link-N_3 /NH_4^+ ^{31}P NMR ()

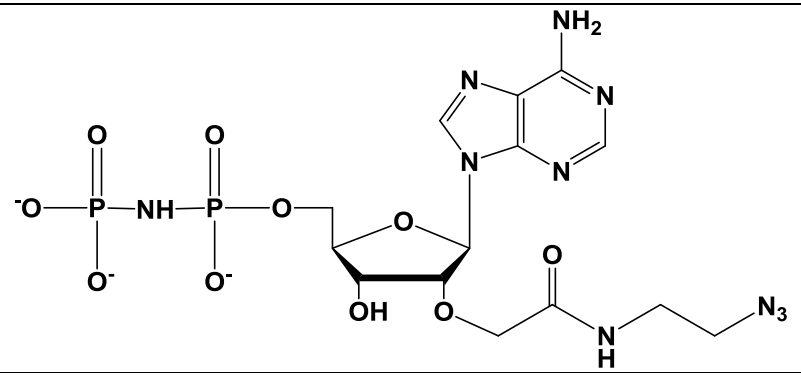


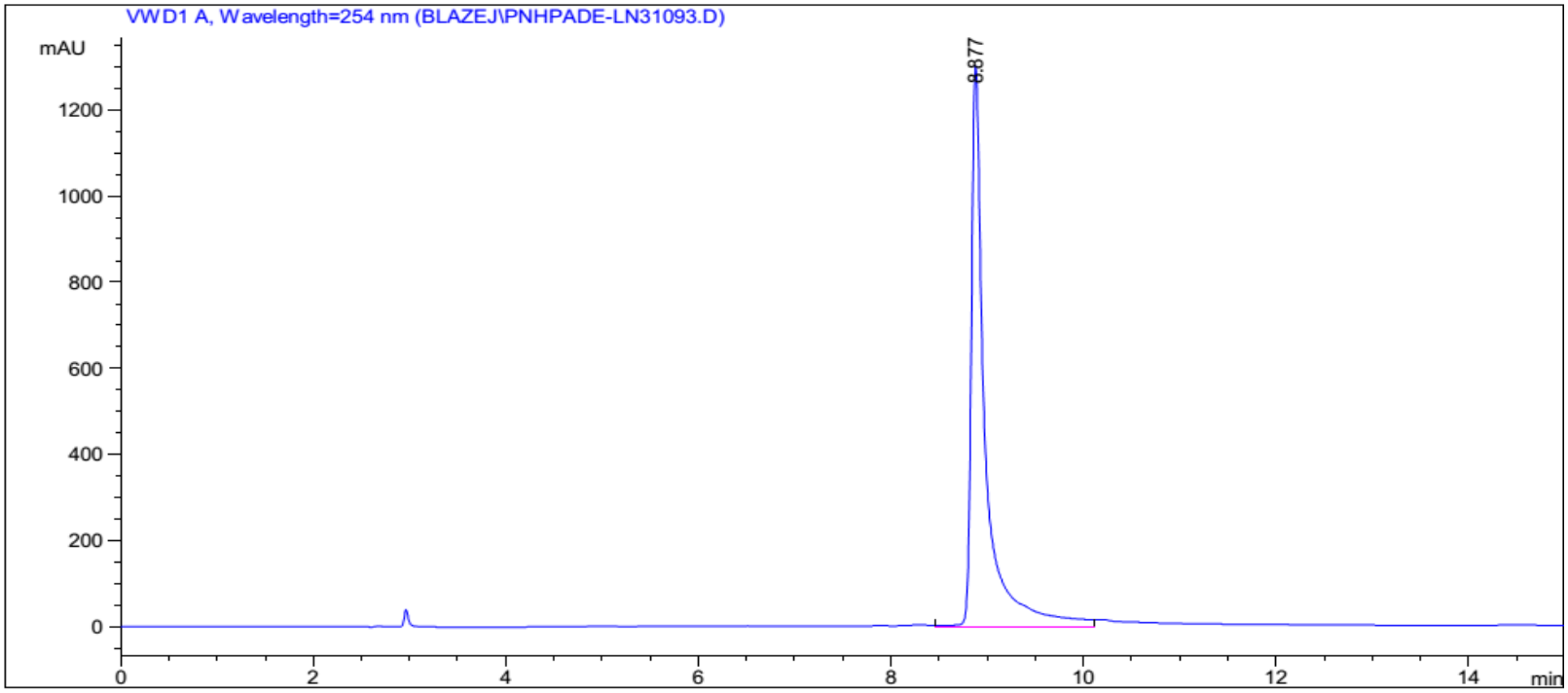
2'-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5'-methylene(bisphosphonate) (14)

pCH_2p-Ado-link-N_3 /FTIR ATR

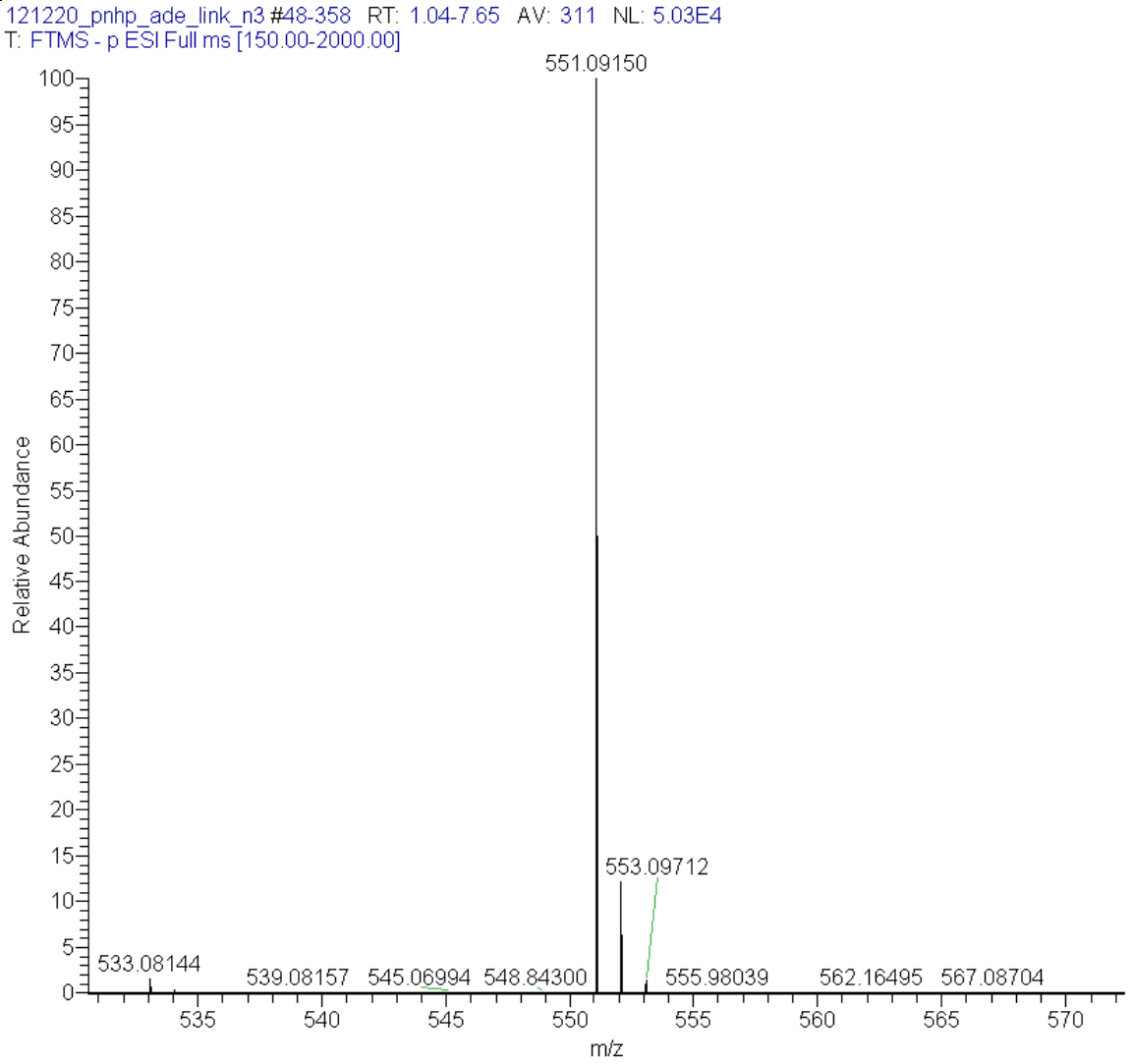


2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-imidodiphosphate (15)



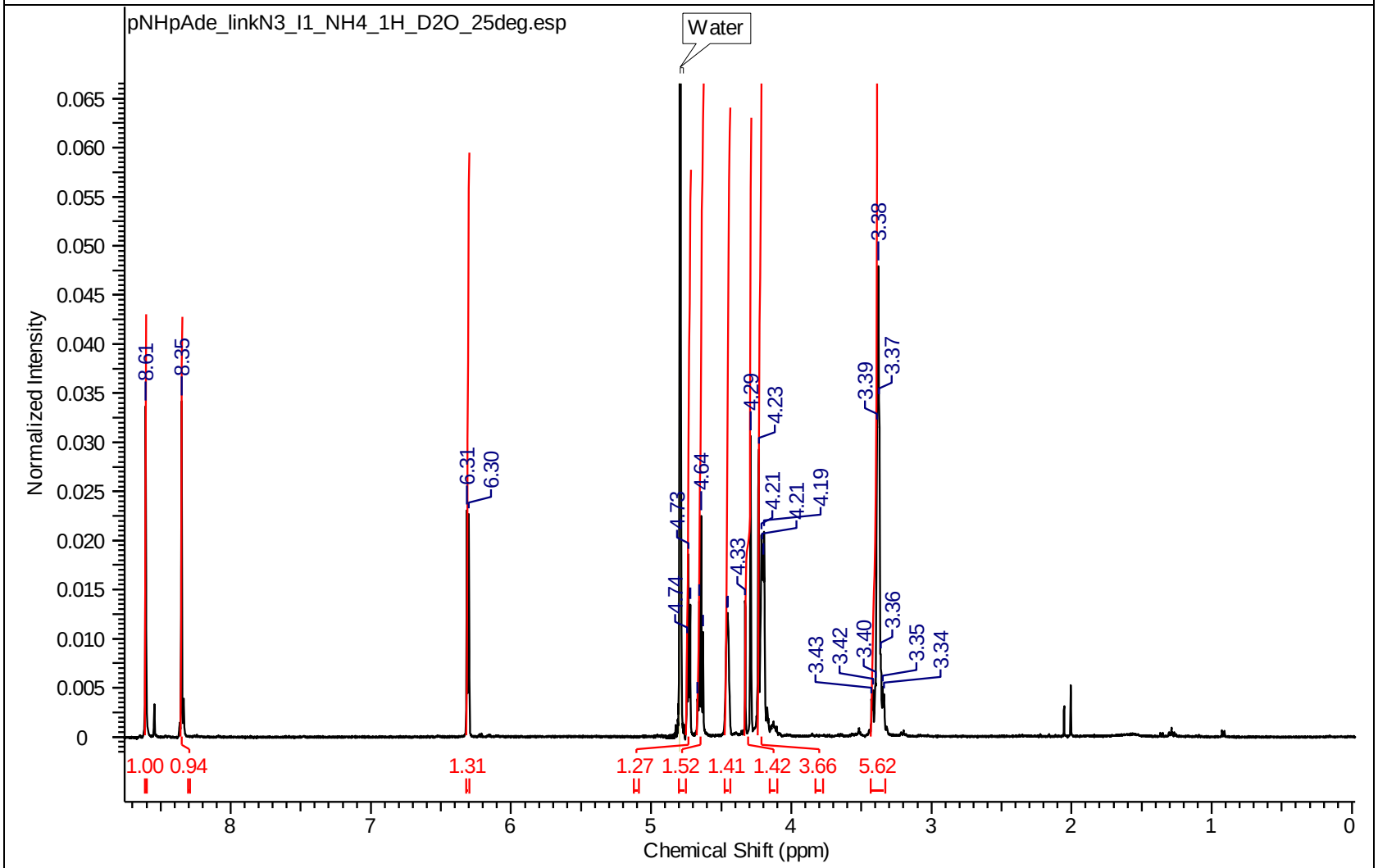


2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-imidodiphosphate (15)	**pNHp-Ado-link-N_3 /NH_4^+ HRMS (-)ES $C_{14}H_{22}N_{10}O_{10}P_2$, 551.09150 $[M-H]^-$ Exact Mass: 552,099556**



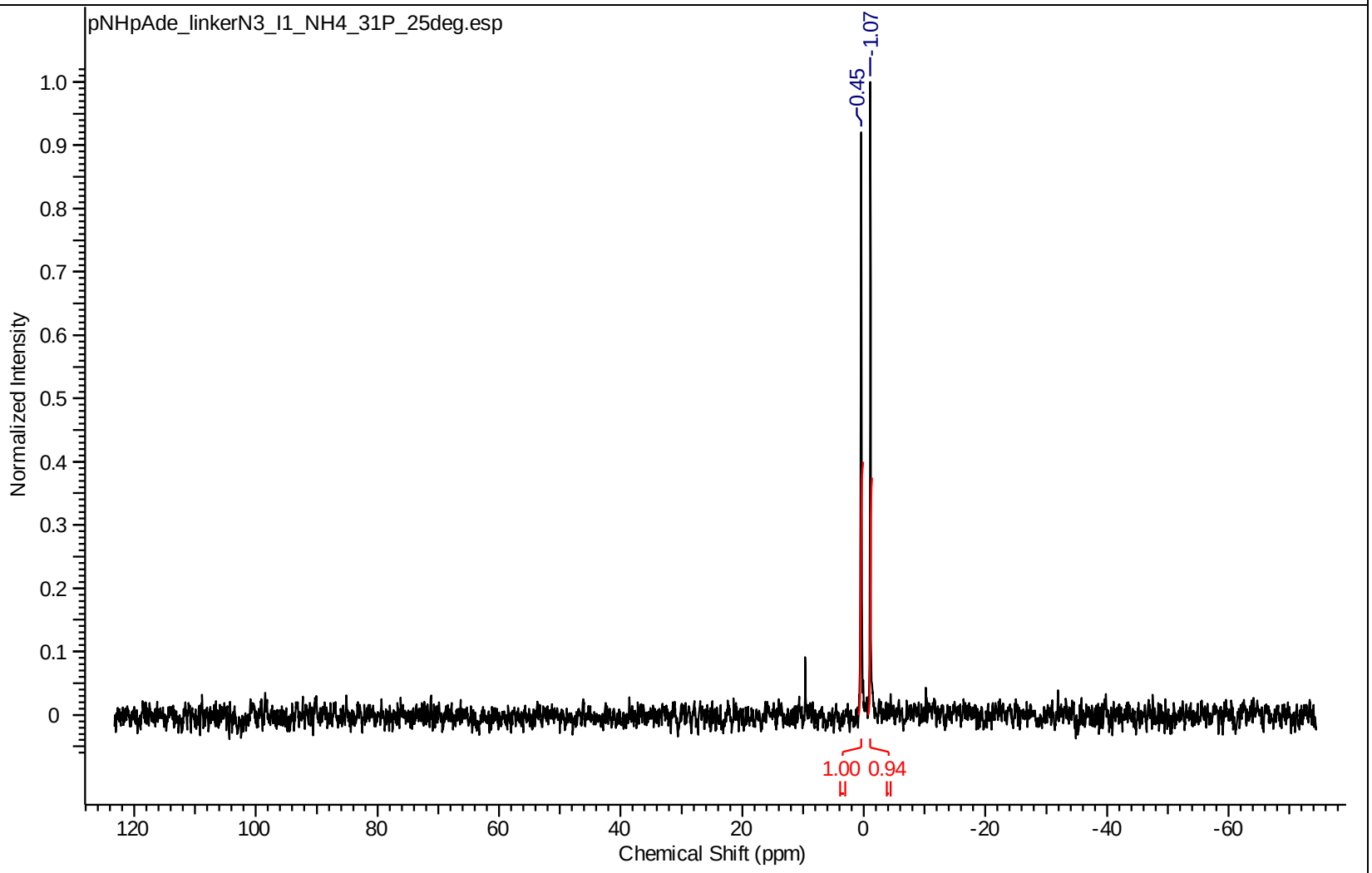
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-imidodiphosphate (15)

pNHp-Ado-link-N_3 /NH_4^+ ^{1}H NMR ()



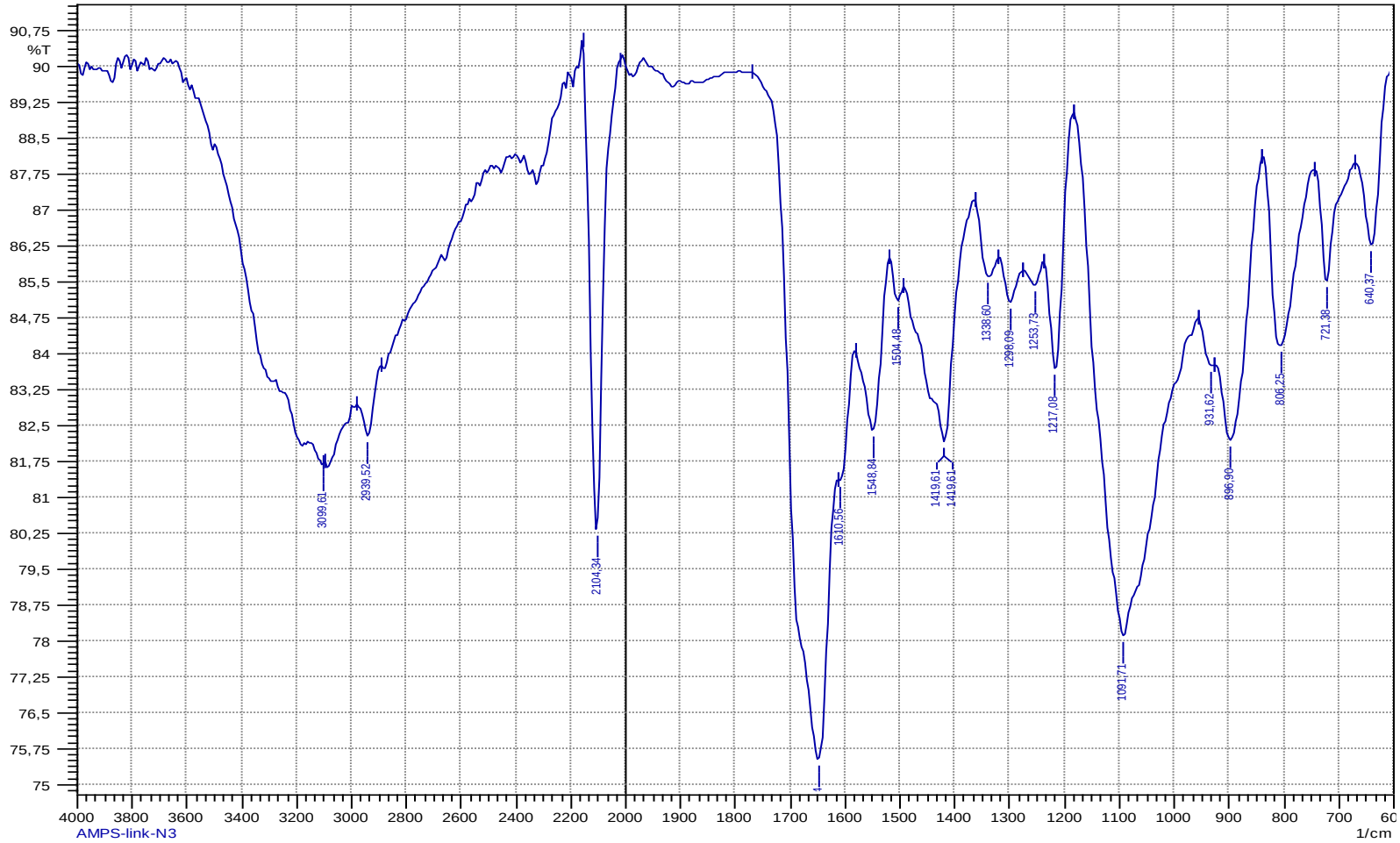
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-imidodiphosphate (15)

pNHp-Ado-link-N_3 /NH_4^+ ^{31}P NMR ()

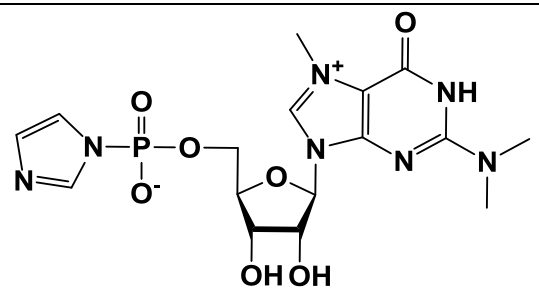


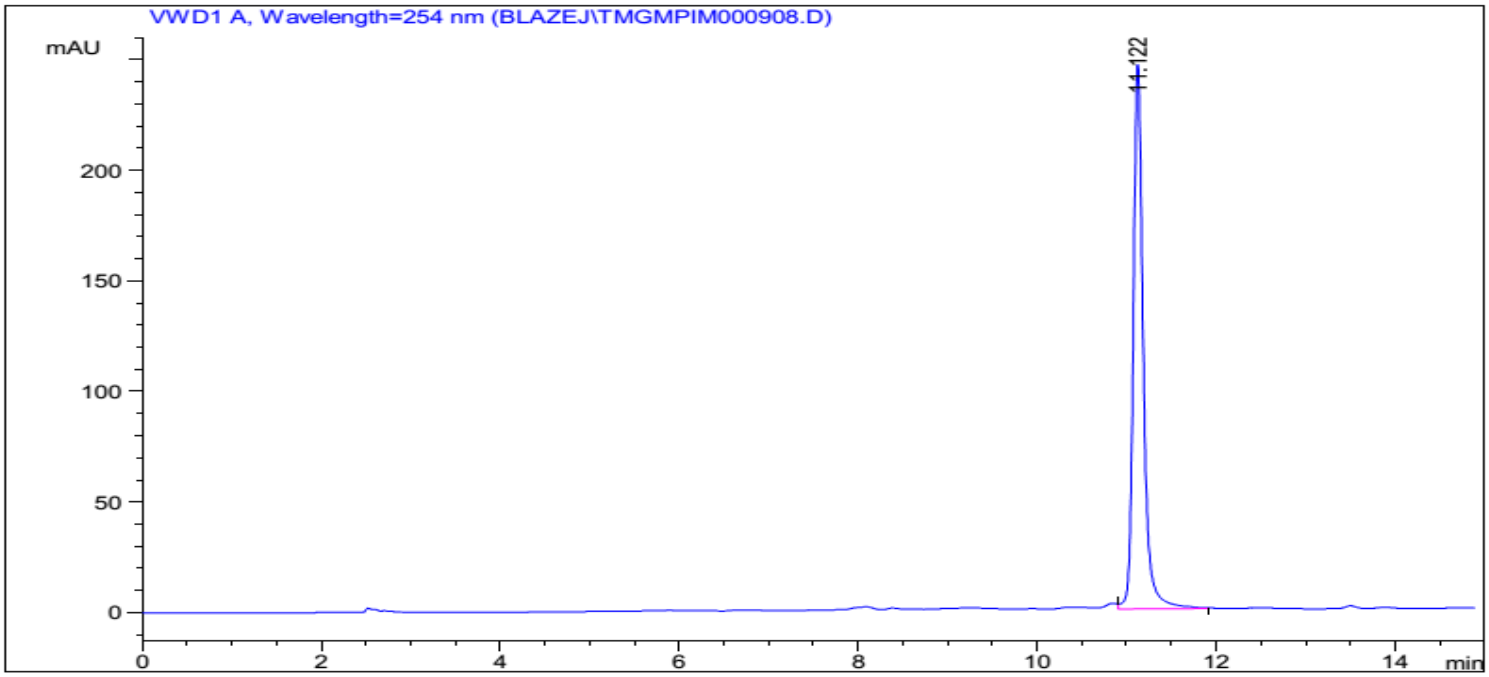
2′-O-(N-(2-Azidoethyl)carbamoyl)methyladenosine-5′-imidodiphosphate (15)

pNHp-Ado-link-N_3 /FTIR ATR



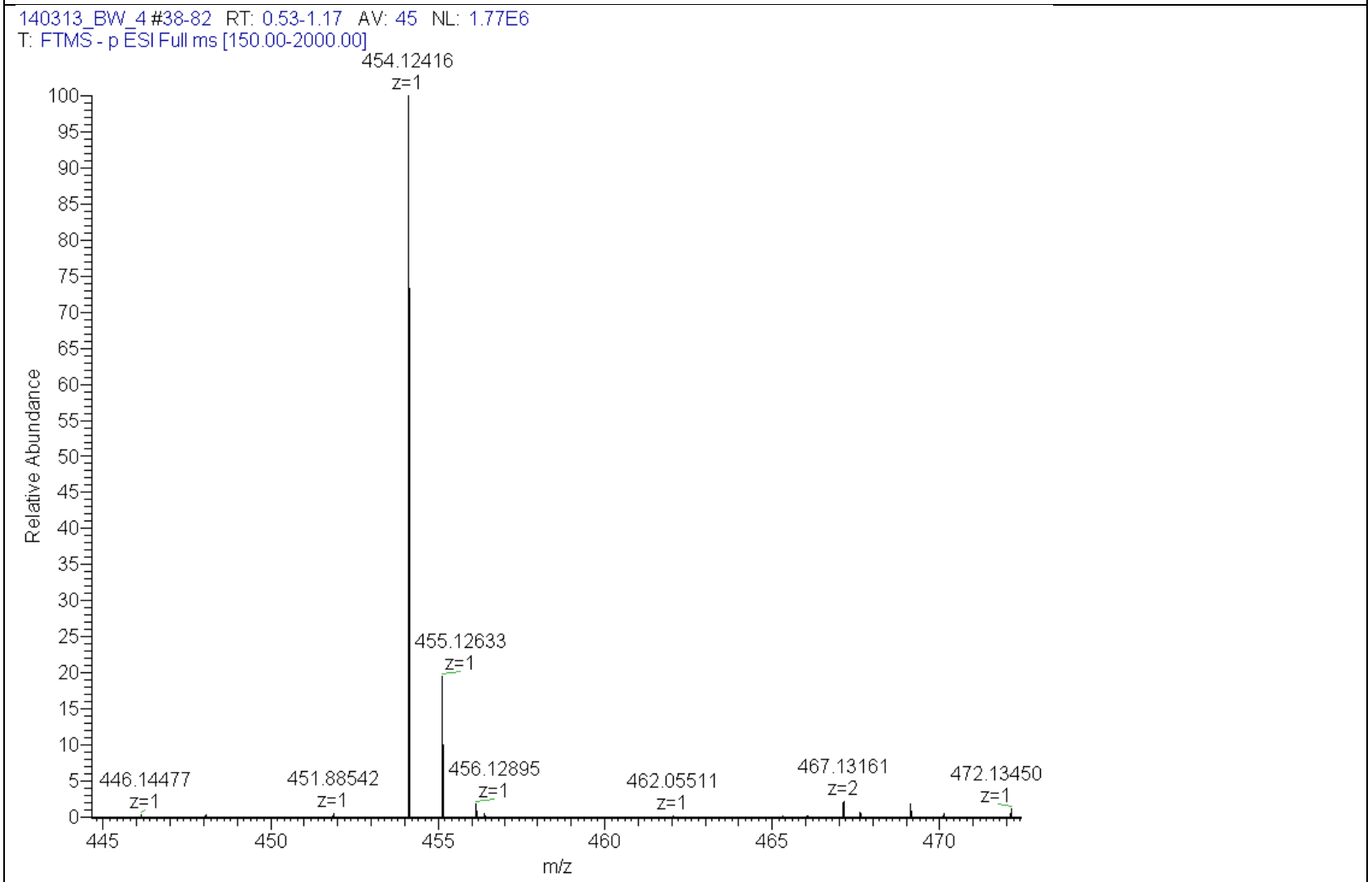
TMGMP-Im (16)

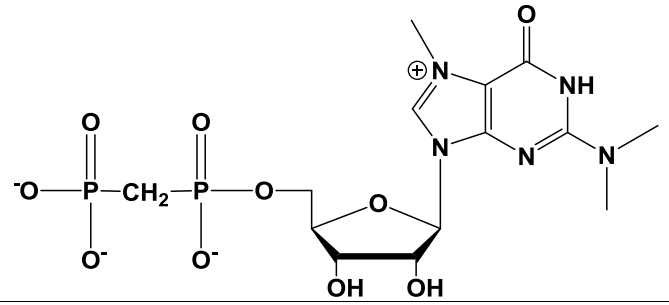
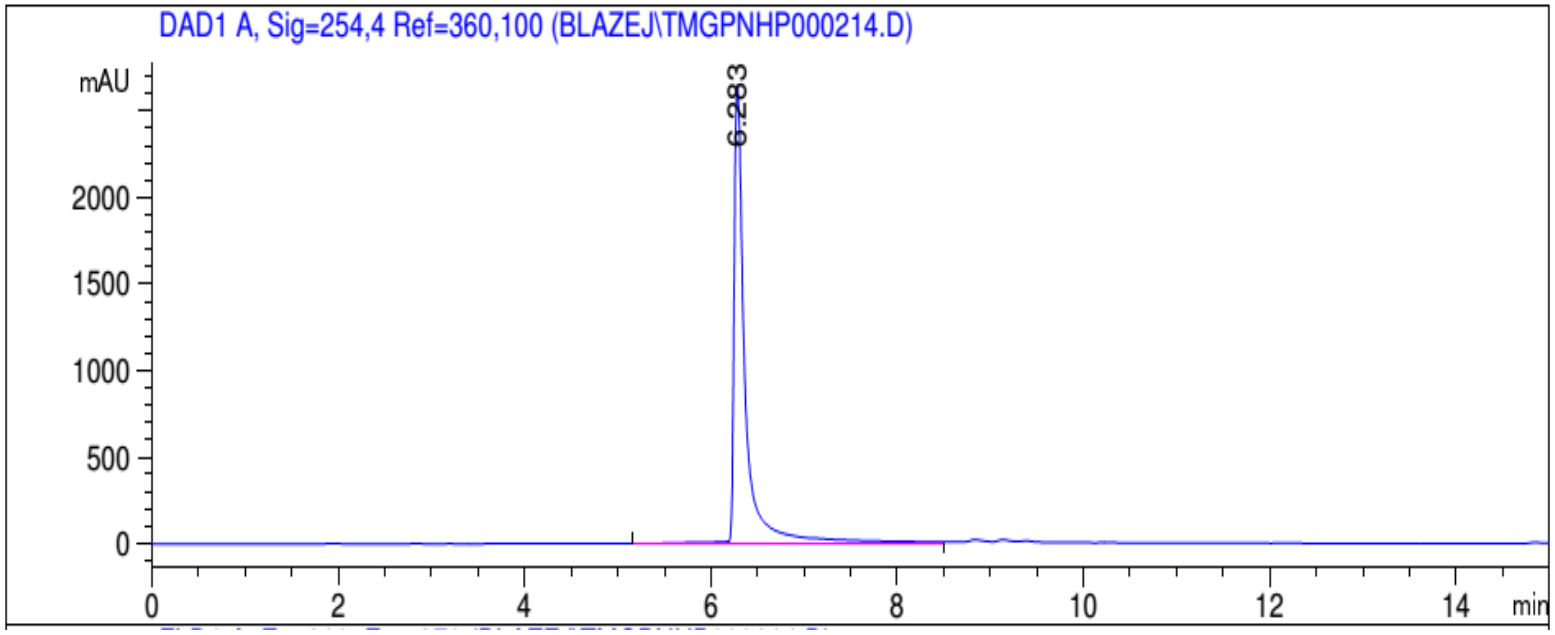

O
N+
NH
O
N
P
O
N
N
N
O
O-
OH OH


VWD1 A, Wavelength=254 nm (BLAZEJ\TMGMPIM000908.D)
mAU
200
150
100
50
0
11.122
0
2
4
6
8
10
12
14
min

TMGMP-Im (16)

TMGMP-Im /Na^+ HRMS (-)ES $C_{16}H_{21}N_7O_7P^-$, 454.12416 $[M-2H]^-$ Exact Mass: 454,12456

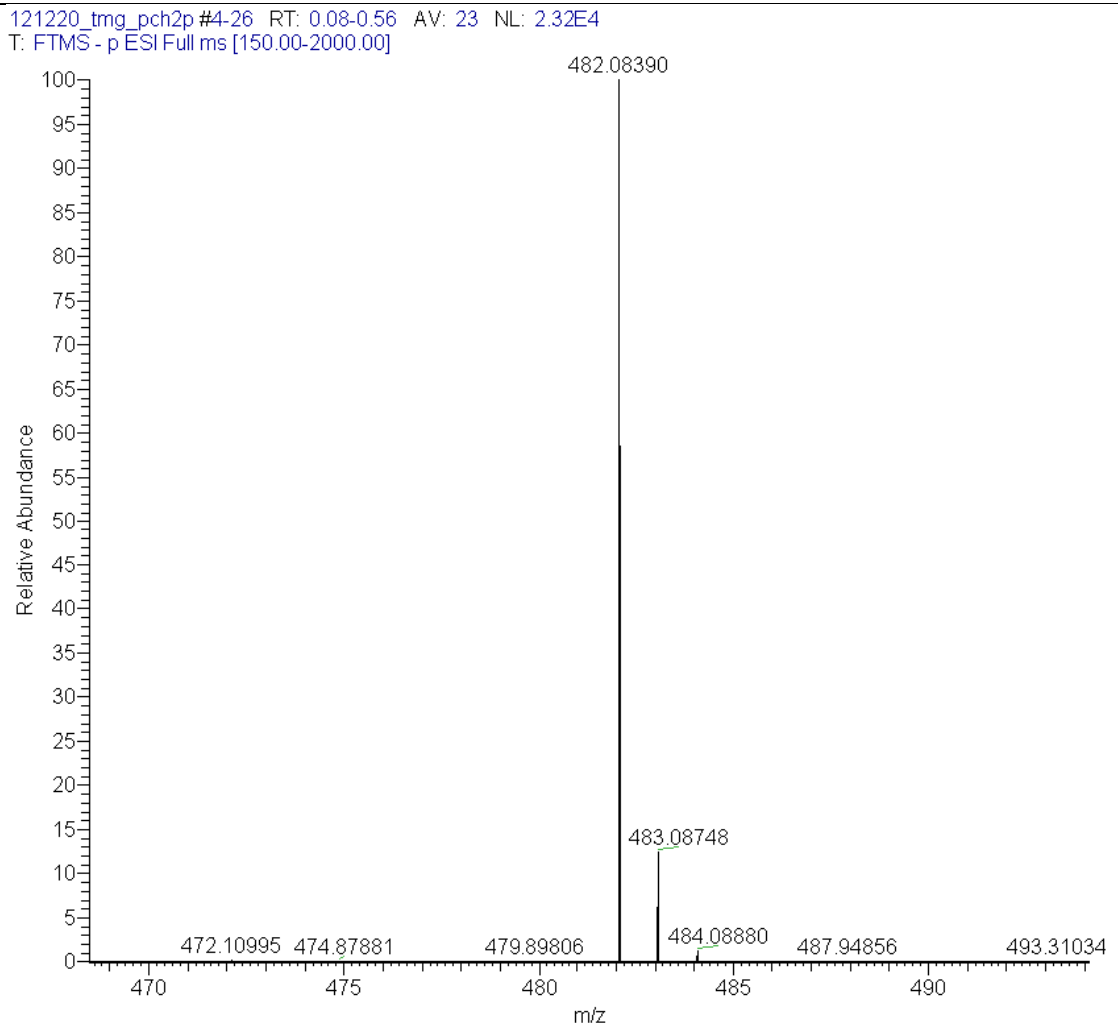


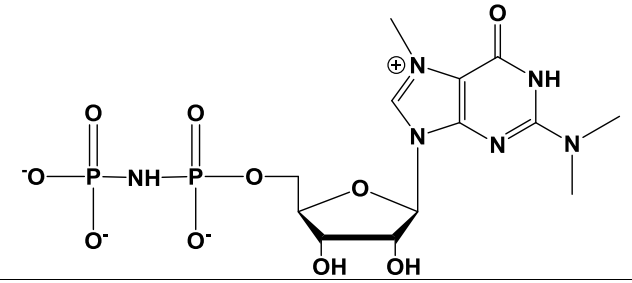
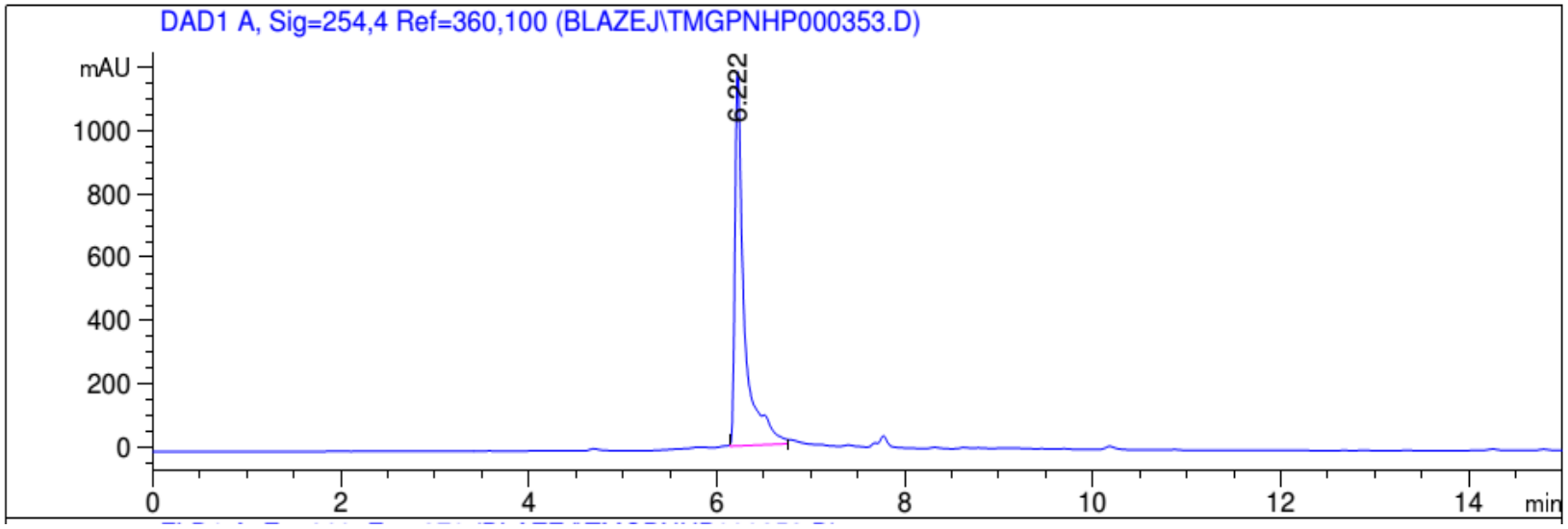
TMG-pCH$_2$p (18)	
	

DAD1 A, Sig=254,4 Ref=360,100 (BLAZEJ\TMGPNHP000214.D)
mAU
6.283
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
min

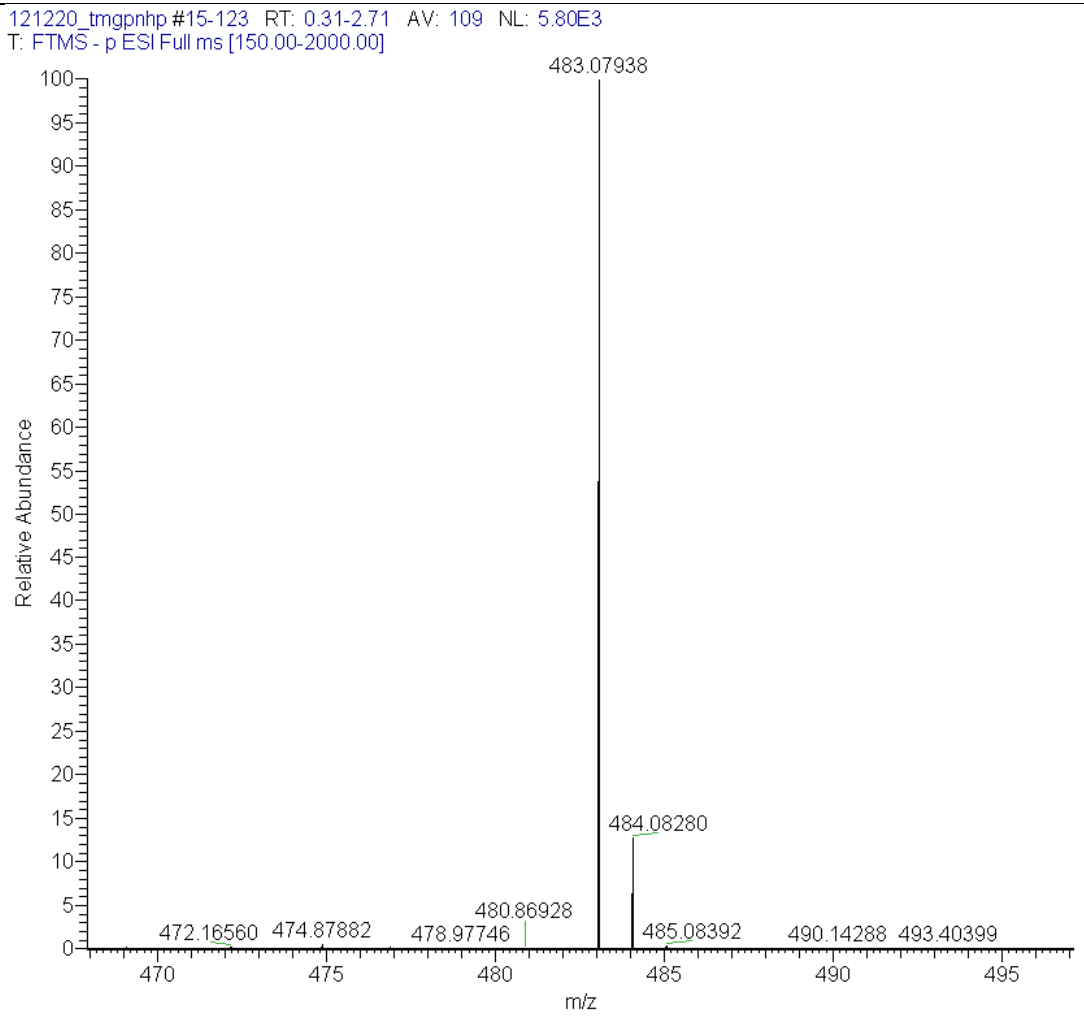
TMG-pCH$_2$p (18)

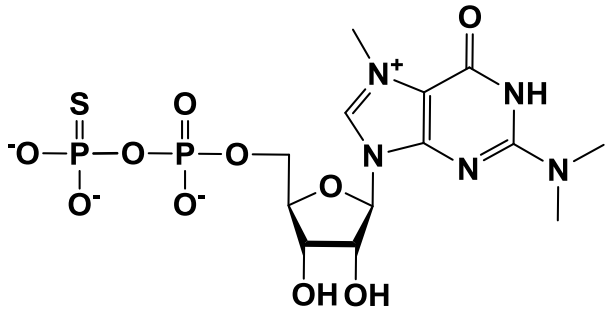
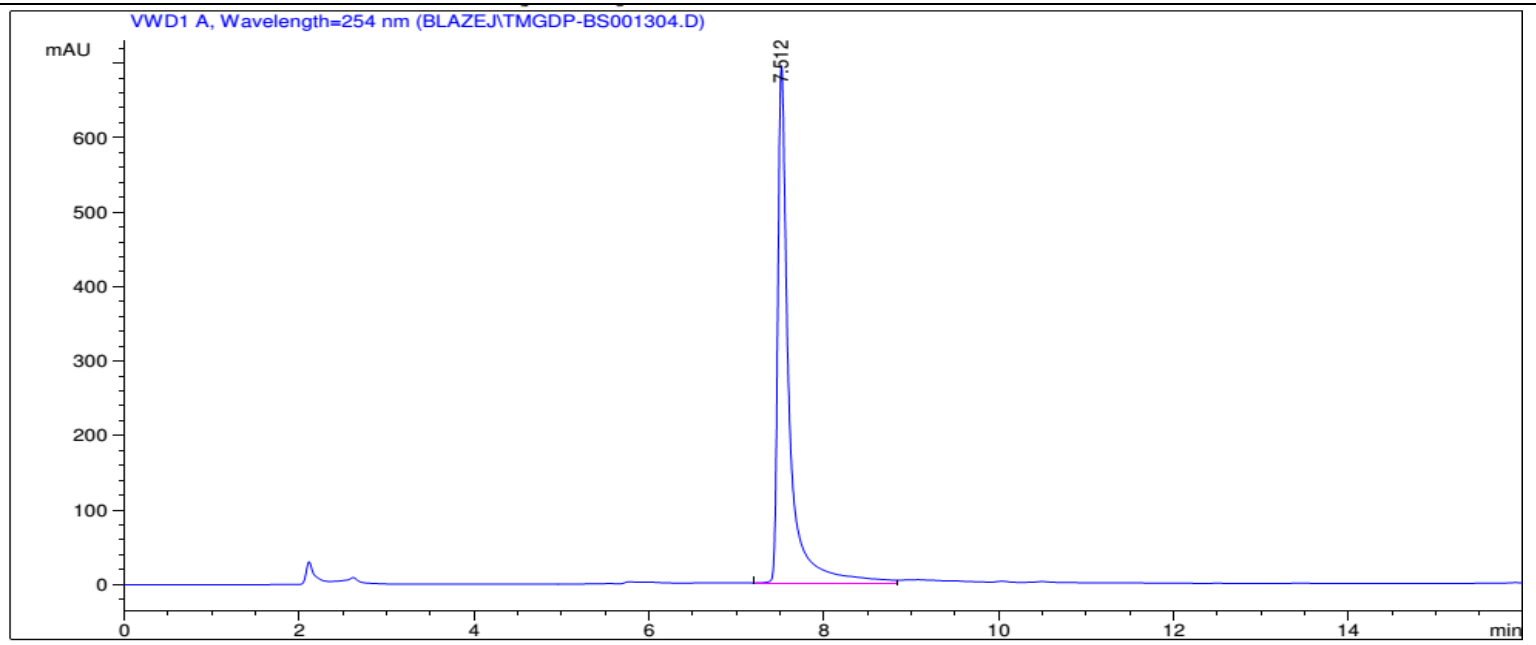
TMGpCH$_2$p /TEA$^+$ HRMS (-)ES C$_{14}$H$_{22}$N$_5$O$_{10}$P$_2$, 482.08390 [M-2H]$^-$ Exact Mass: 482,08419



TMG-pNHp (19)	  DAD1 A, Sig=254,4 Ref=360,100 (BLAZEJ\TMGPNHP000353.D) mAU 1000 800 600 400 200 0 6.222 0 2 4 6 8 10 12 14 min

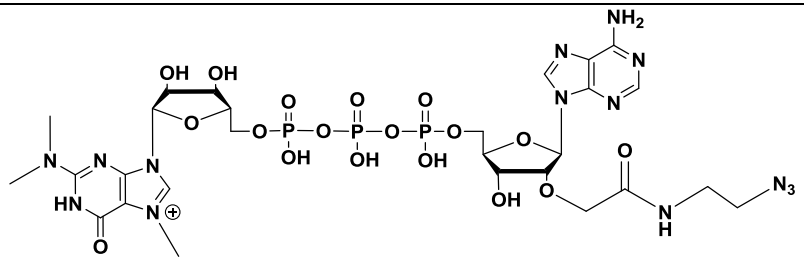
TMG-pNHp (19)	**TMGpNHp /TEA^{+} HRMS (-)ES $C_{13}H_{21}N_6O_{10}P_2$, 483.07938 [M-2H]$^{-}$ Exact Mass: 483,07944**

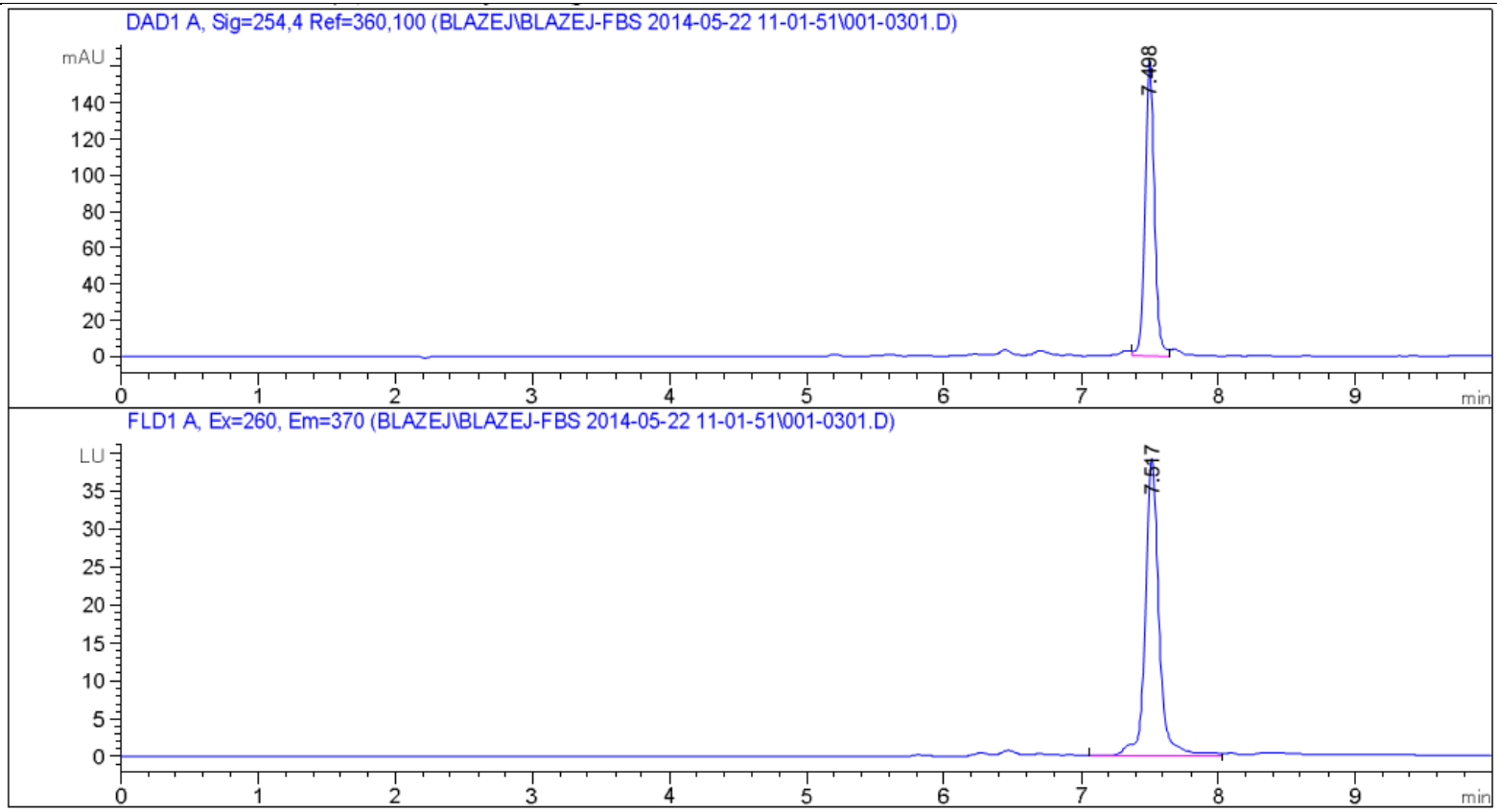


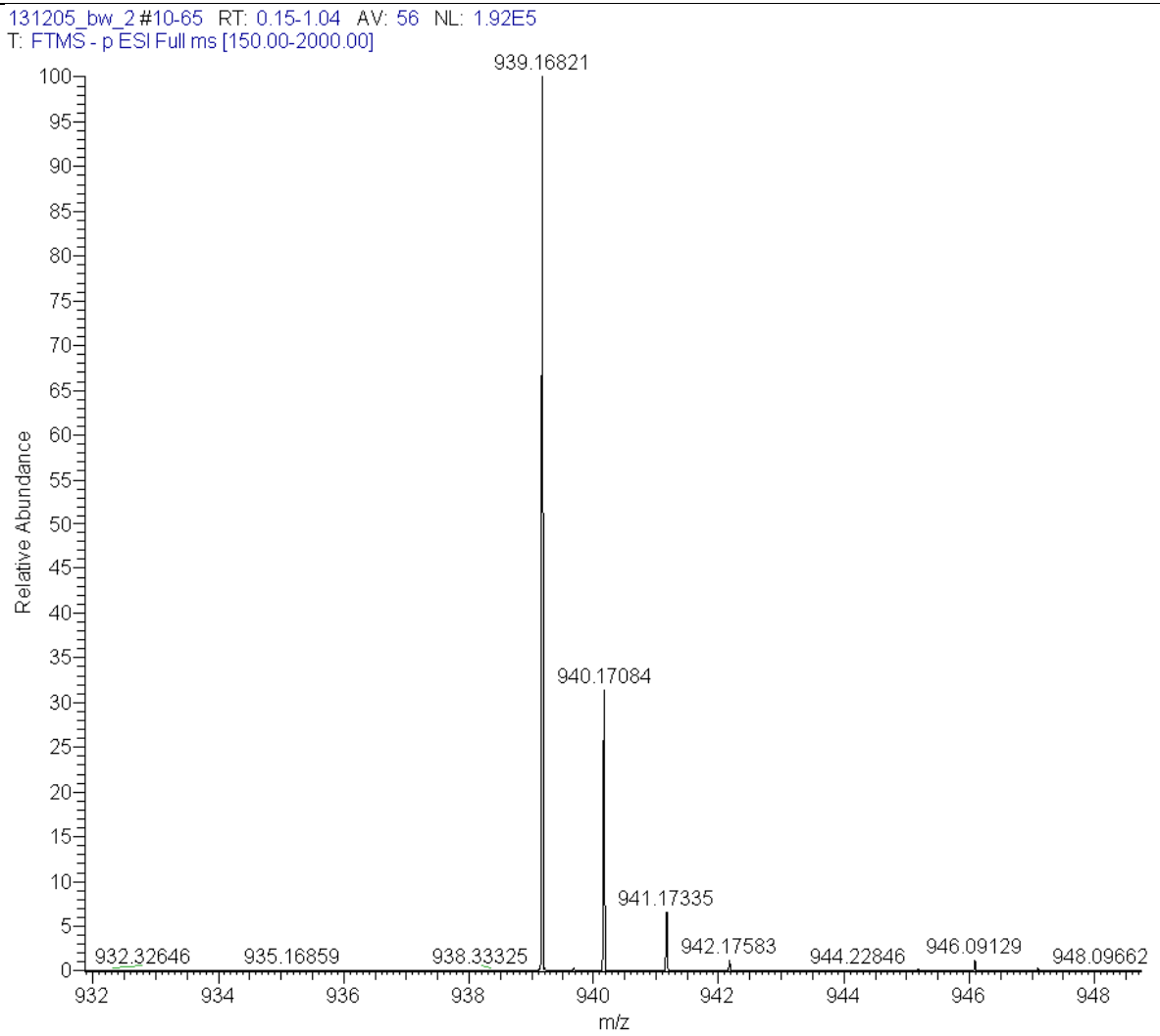
TMGDP-βS (20)	
	

VWD1 A, Wavelength=254 nm (BLAZEJ\TMGDP-BS001304.D)
mAU
600
500
400
300
200
100
0
7.512
0
2
4
6
8
10
12
14
min

TMG-ppp-Ado-link-N_3 (1)

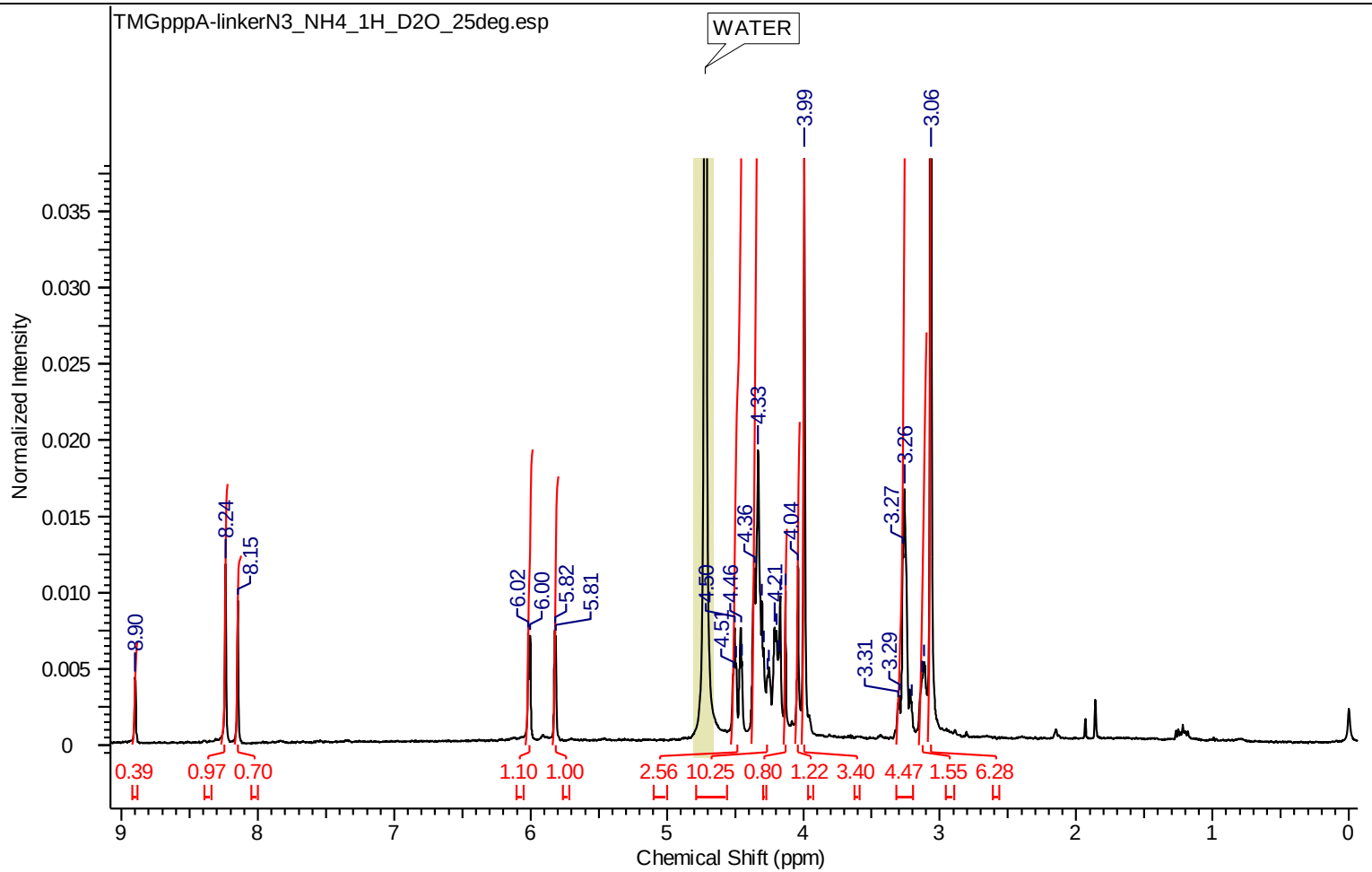




TMG-ppp-Ado-link-N_3 (1)	**TMG-ppp-Ado-link-N_3/NH_4^+ HRMS (-)ES $C_{27}H_{38}N_{14}O_{18}P_3$, 939.16821 $[M-2H]^-$ Exact Mass: 939.17014** 

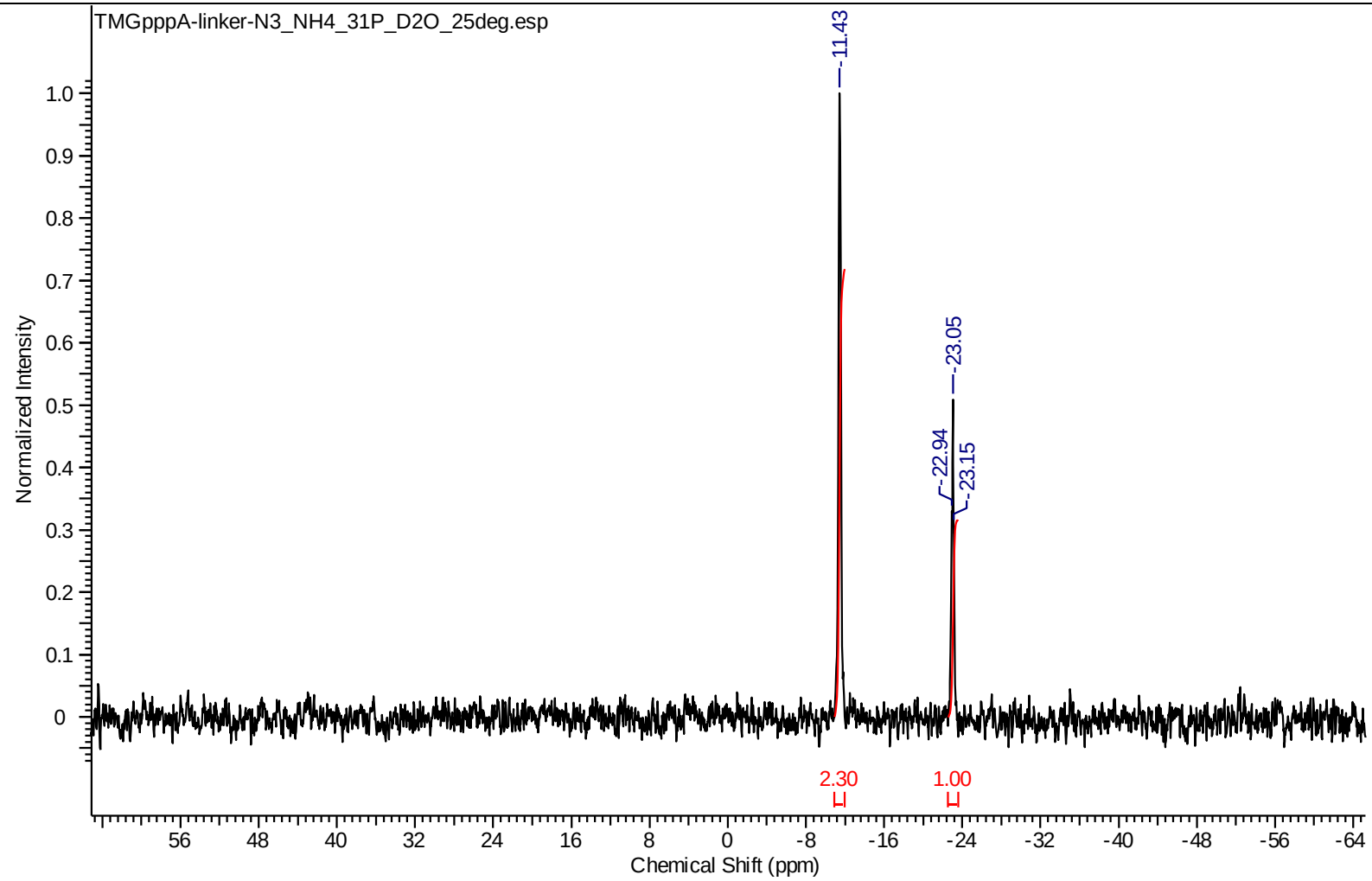
TMG-ppp-Ado-link-N_3 (1)

TMG-ppp-Ado-link-N_3 ^{1}H NMR



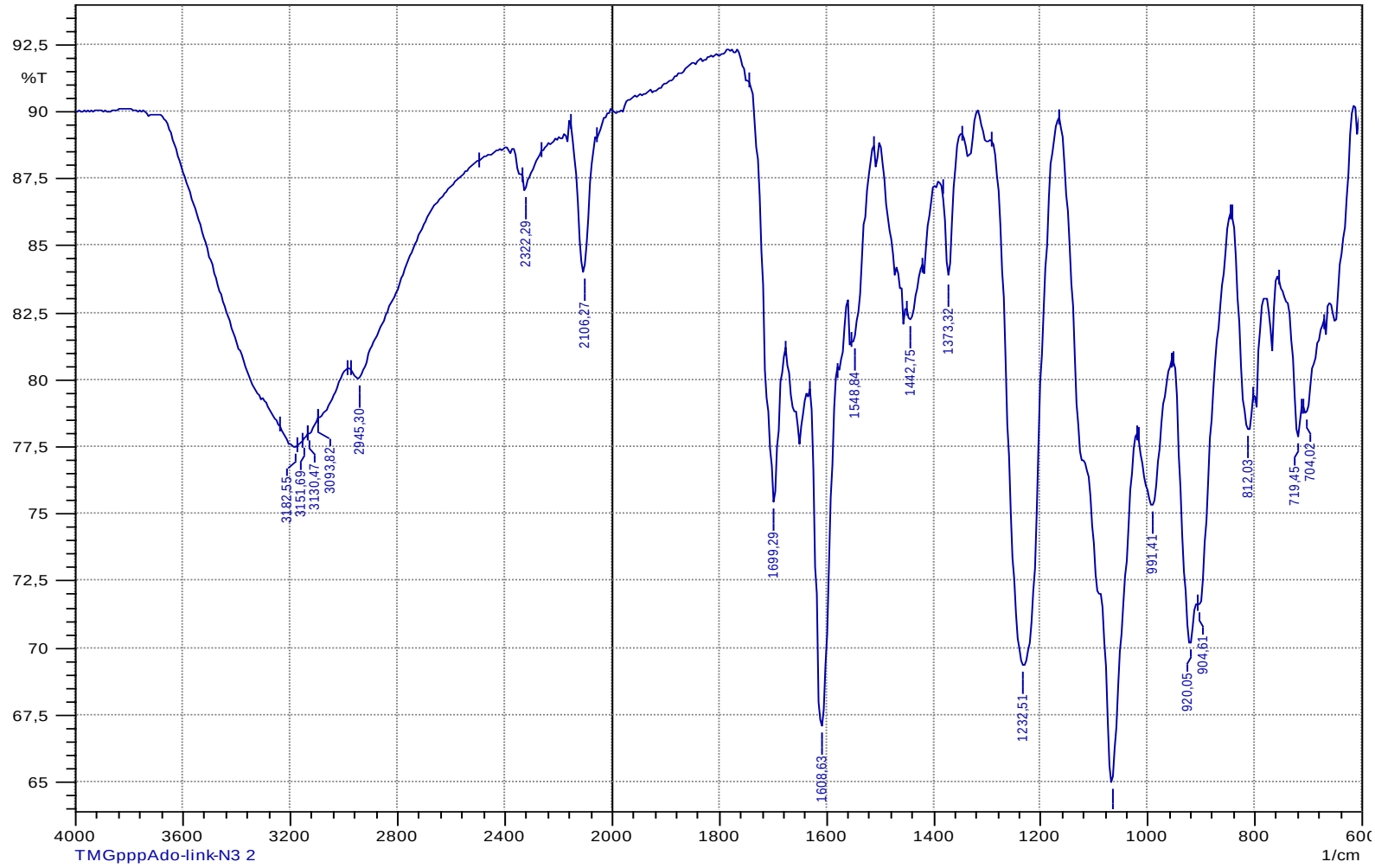
TMG-ppp-Ado-link-N_3 (1)

TMG-ppp-Ado-link-N_3 ^{31}P NMR

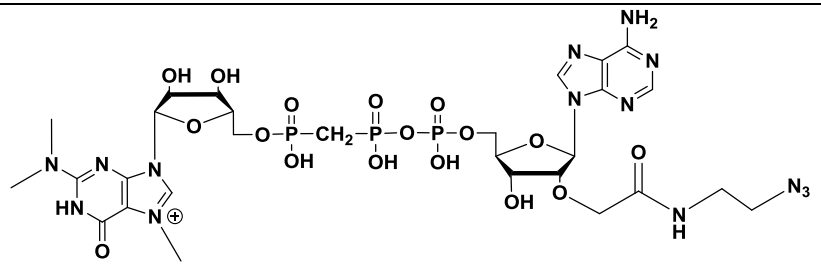


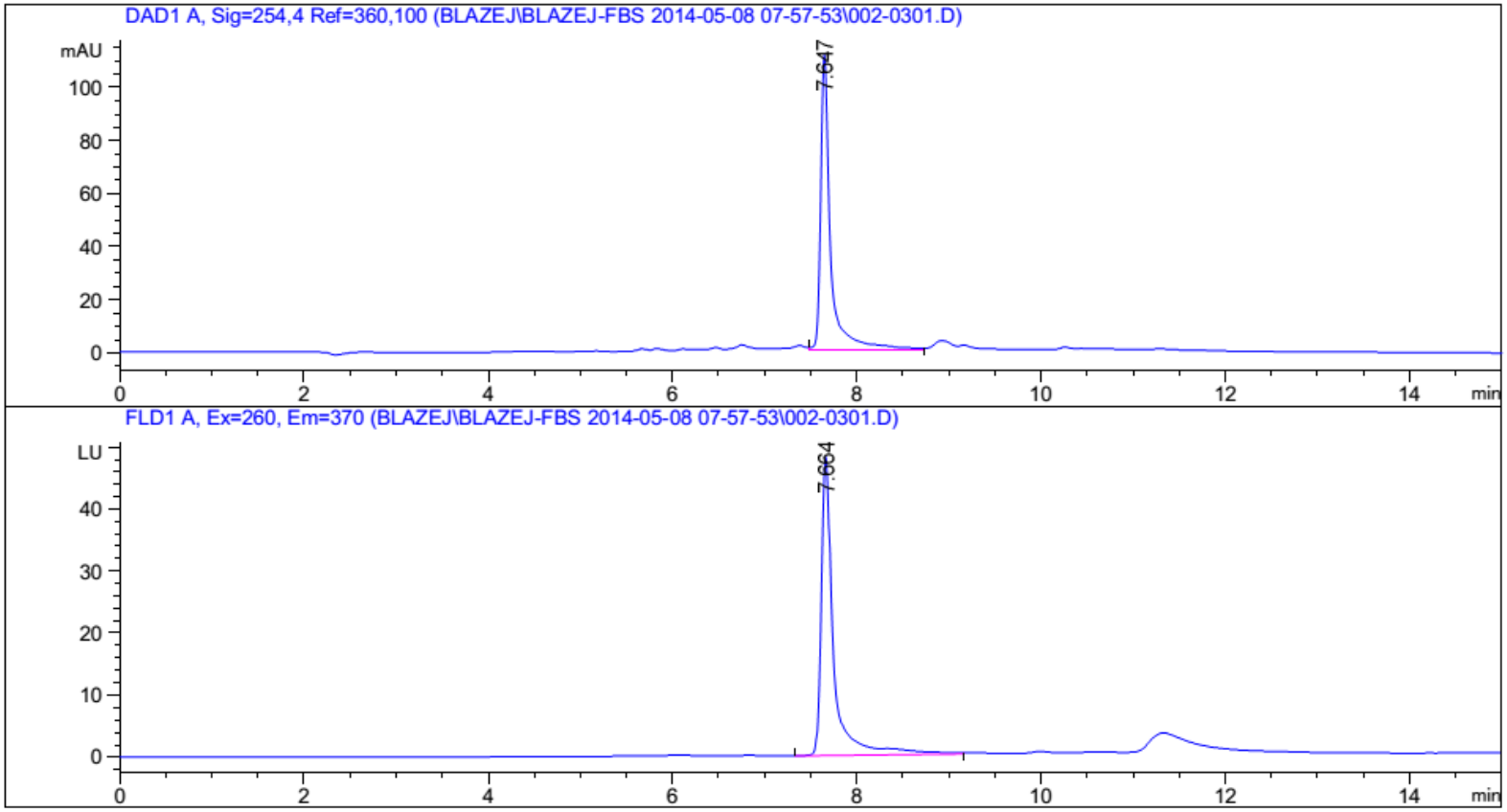
TMG-ppCH$_2$p-Ado-link-N$_3$ (1)

TMG-ppp-Ado-link-N$_3$ FTIR ATR

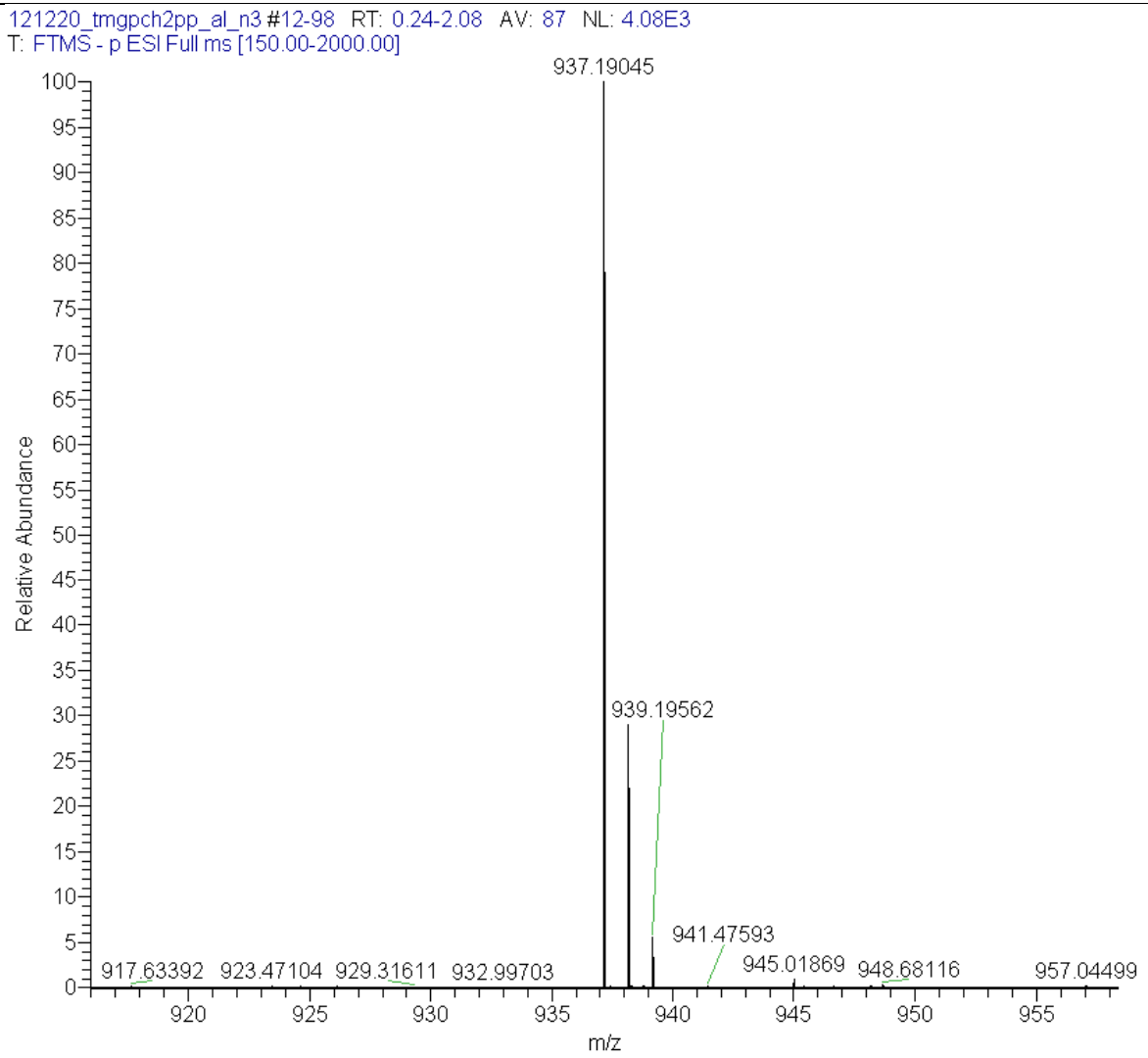


TMG-pCH$_2$pp-Ado-link-N$_3$ (4)



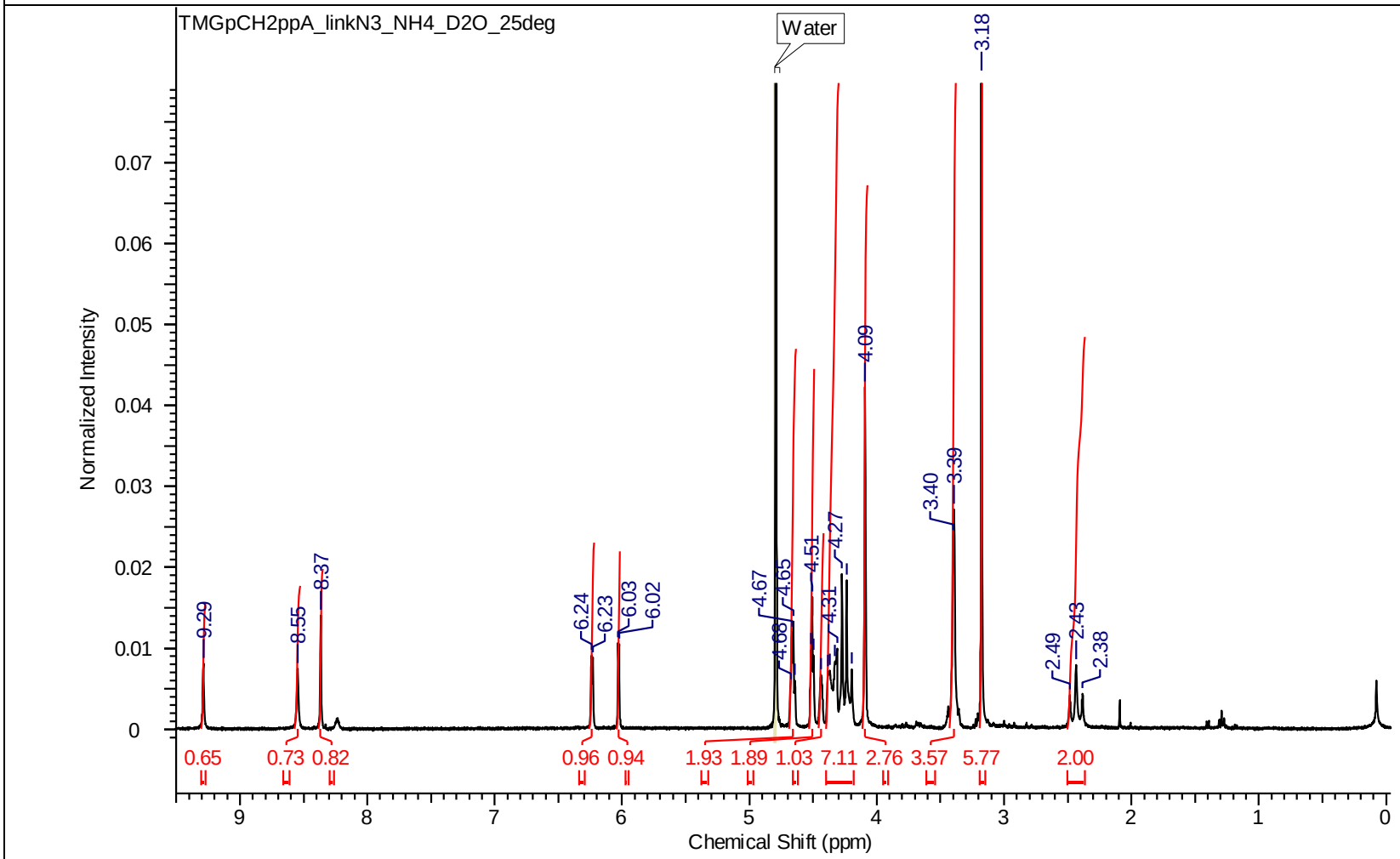


TMG-pCH$_2$pp-Ado-link-N$_3$ (4)	**TMG-pCH$_2$pp-Ado-link-N$_3$/NH$_4^+$ HRMS (-)ES C$_{28}$H$_{40}$N$_{14}$O$_{17}$P$_3$, 937.19045 [M-2H]$^-$ Exact Mass: 937,19087**



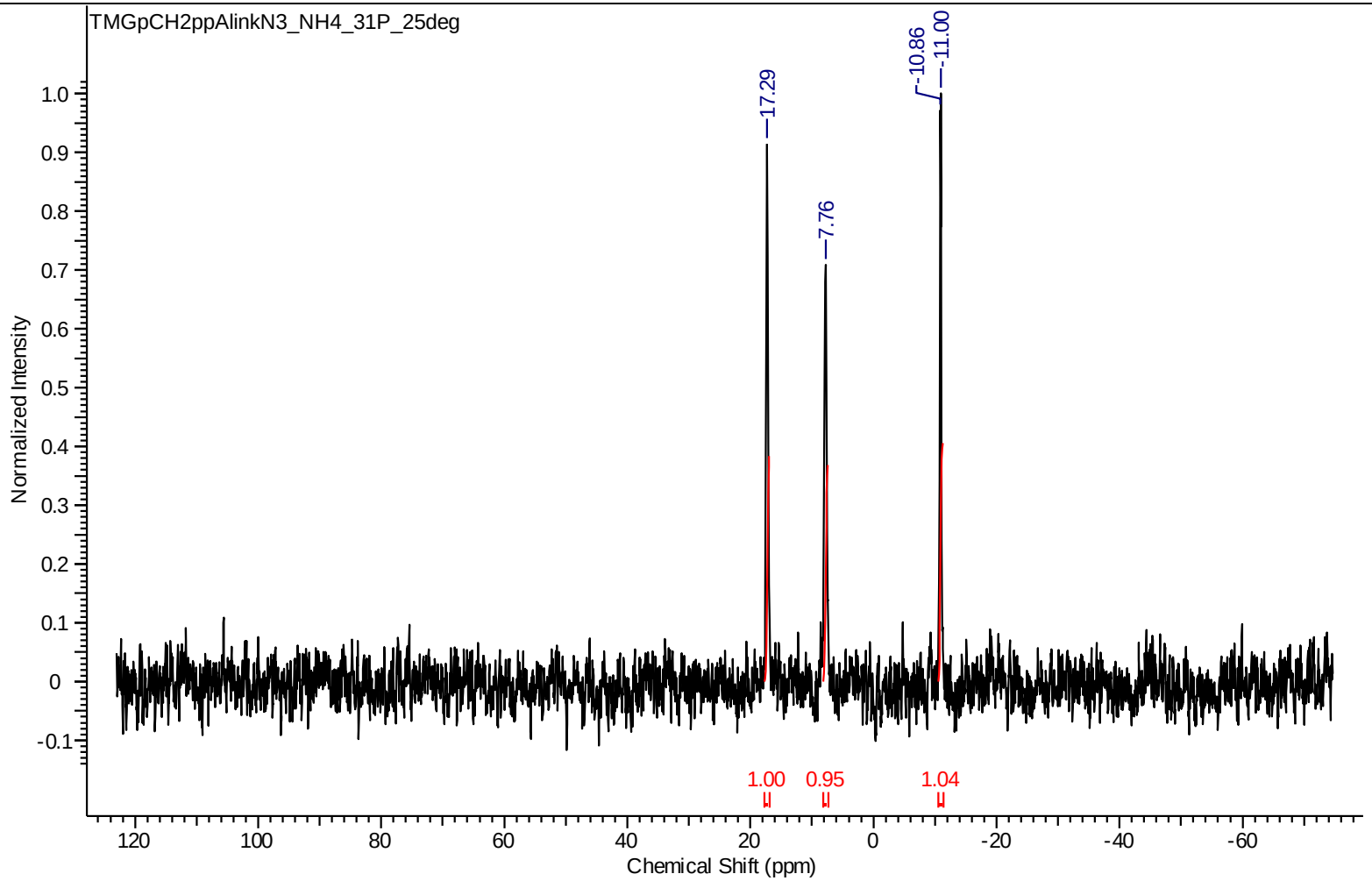
TMG-pCH$_2$pp-Ado-link-N$_3$ (4)

TMG-pCH$_2$pp-Ado-link-N$_3$ ^{1}H NMR



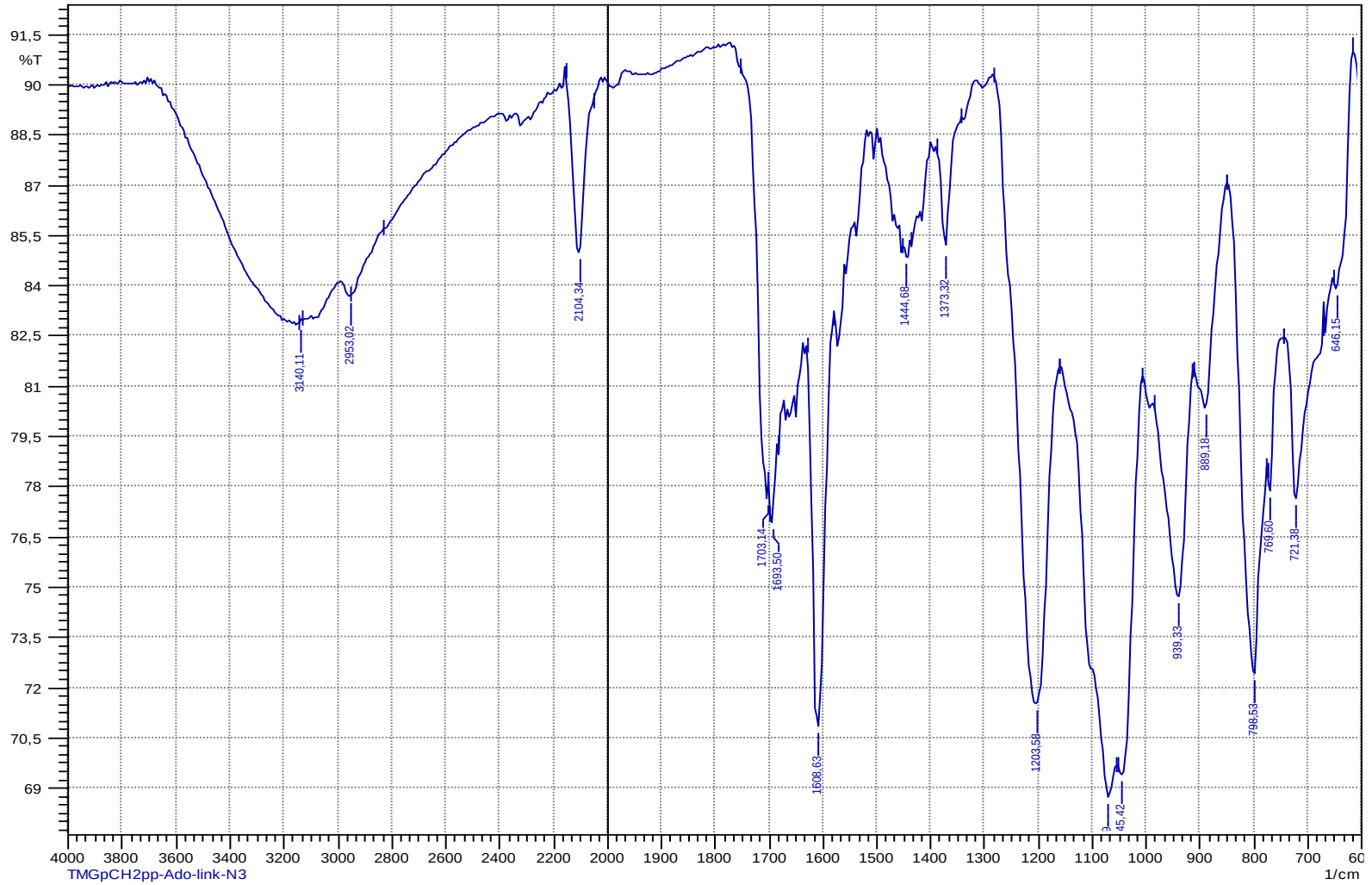
TMG-pCH$_2$pp-Ado-link-N$_3$ (4)

TMG-pCH$_2$pp-Ado-link-N$_3$ ^{31}P NMR

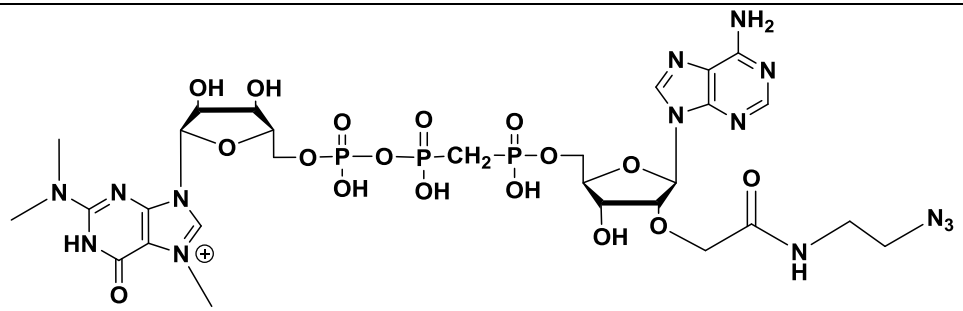


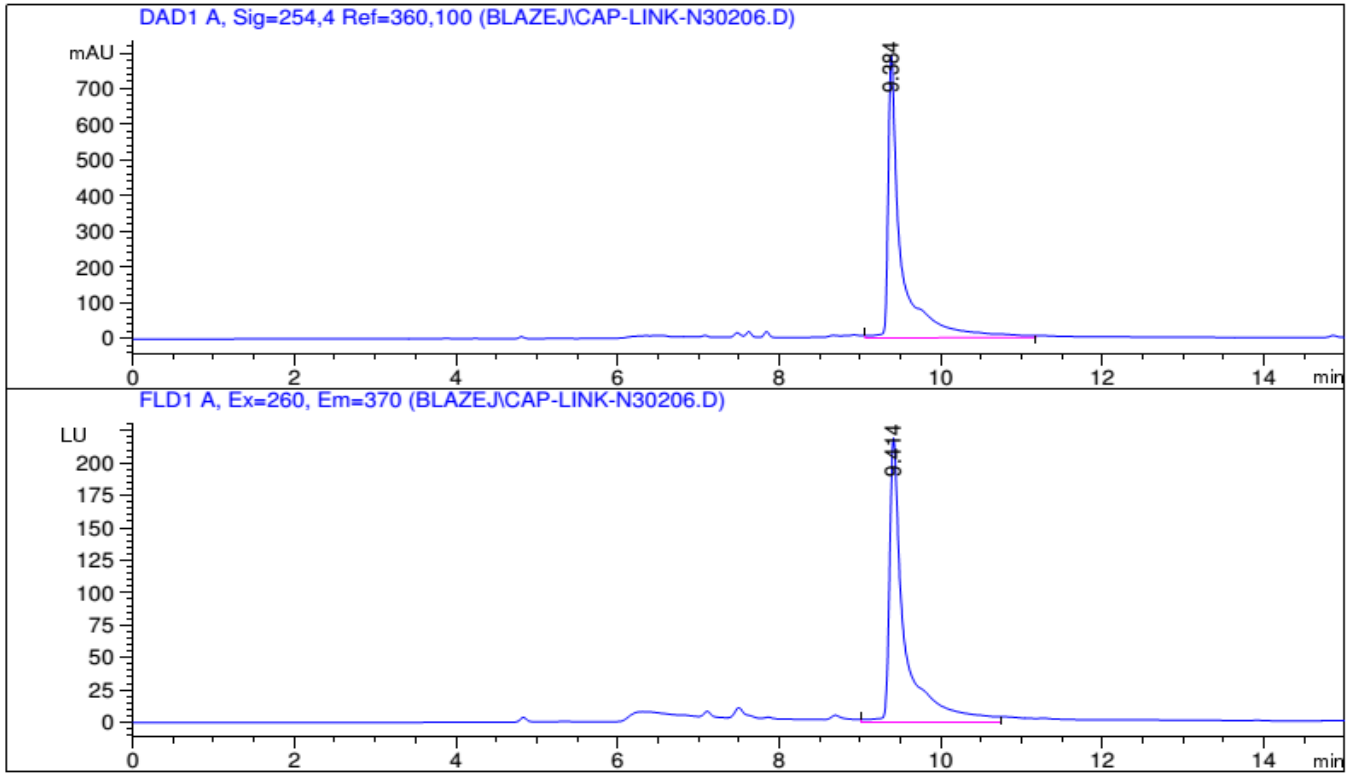
TMG-pCH$_2$pp-Ado-link-N$_3$ (4)

TMG-pCH$_2$pp-Ado-link-N$_3$ FTIR ATR

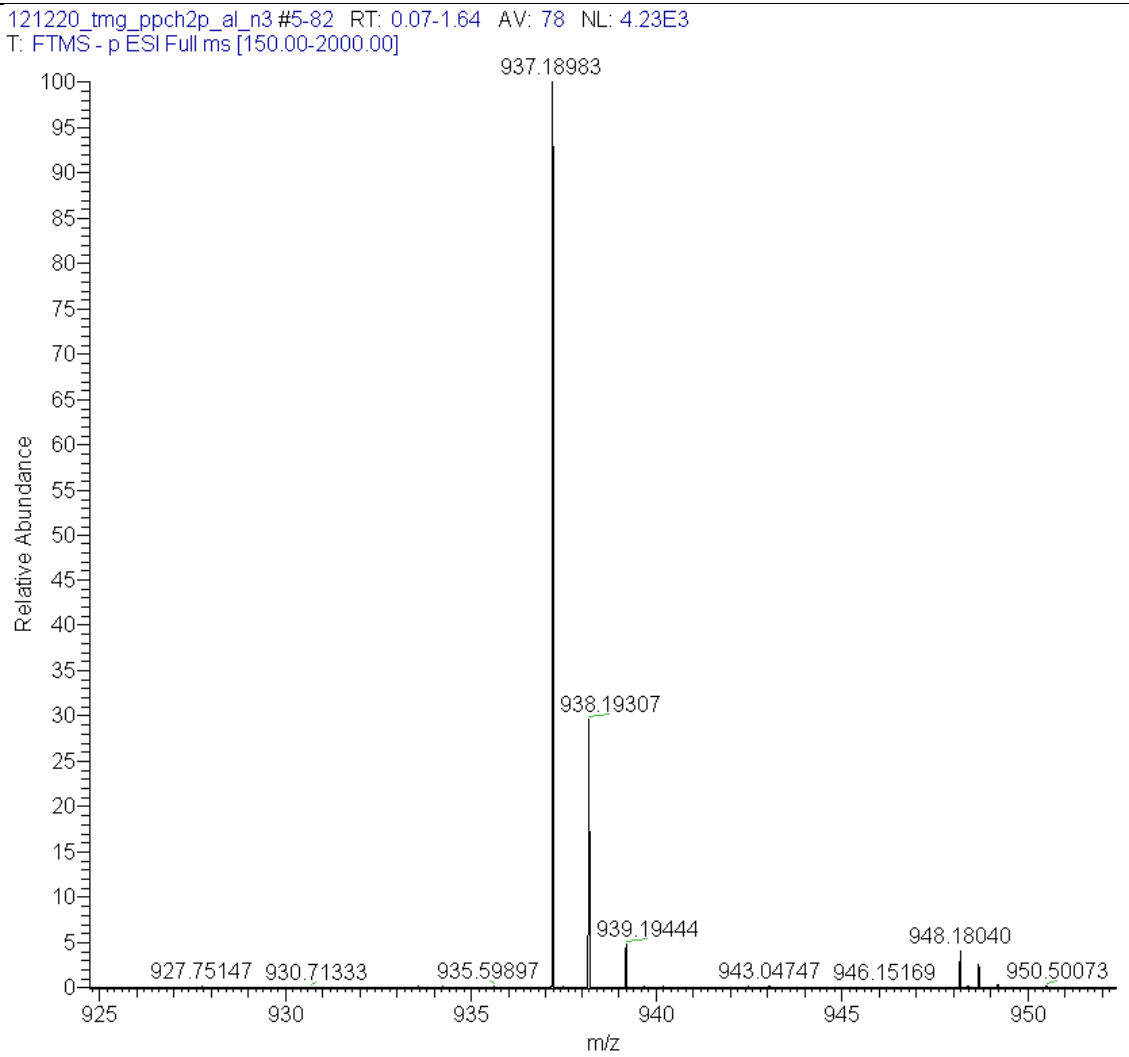


TMG-ppCH_2p-Ado-link-N_3 (2)



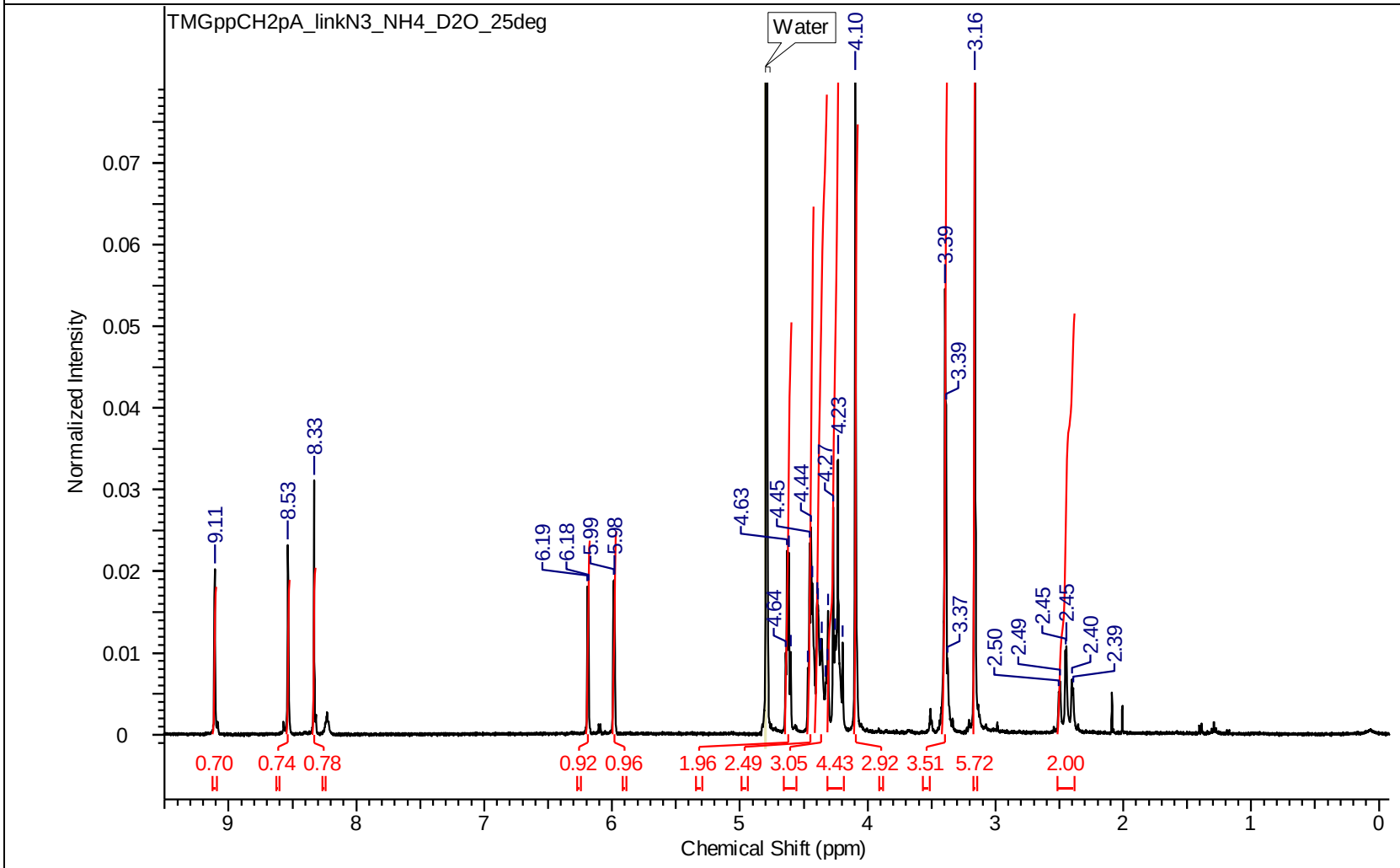


TMG-ppCH$_2$p-Ado-link-N$_3$ (2)	**TMG-ppCH$_2$p-Ado-link-N$_3$/NH$_4^+$ HRMS (-)ES C$_{28}$H$_{40}$N$_{14}$O$_{17}$P$_3$, 937.18983 [M-2H]$^-$ Exact Mass: 937,19870**



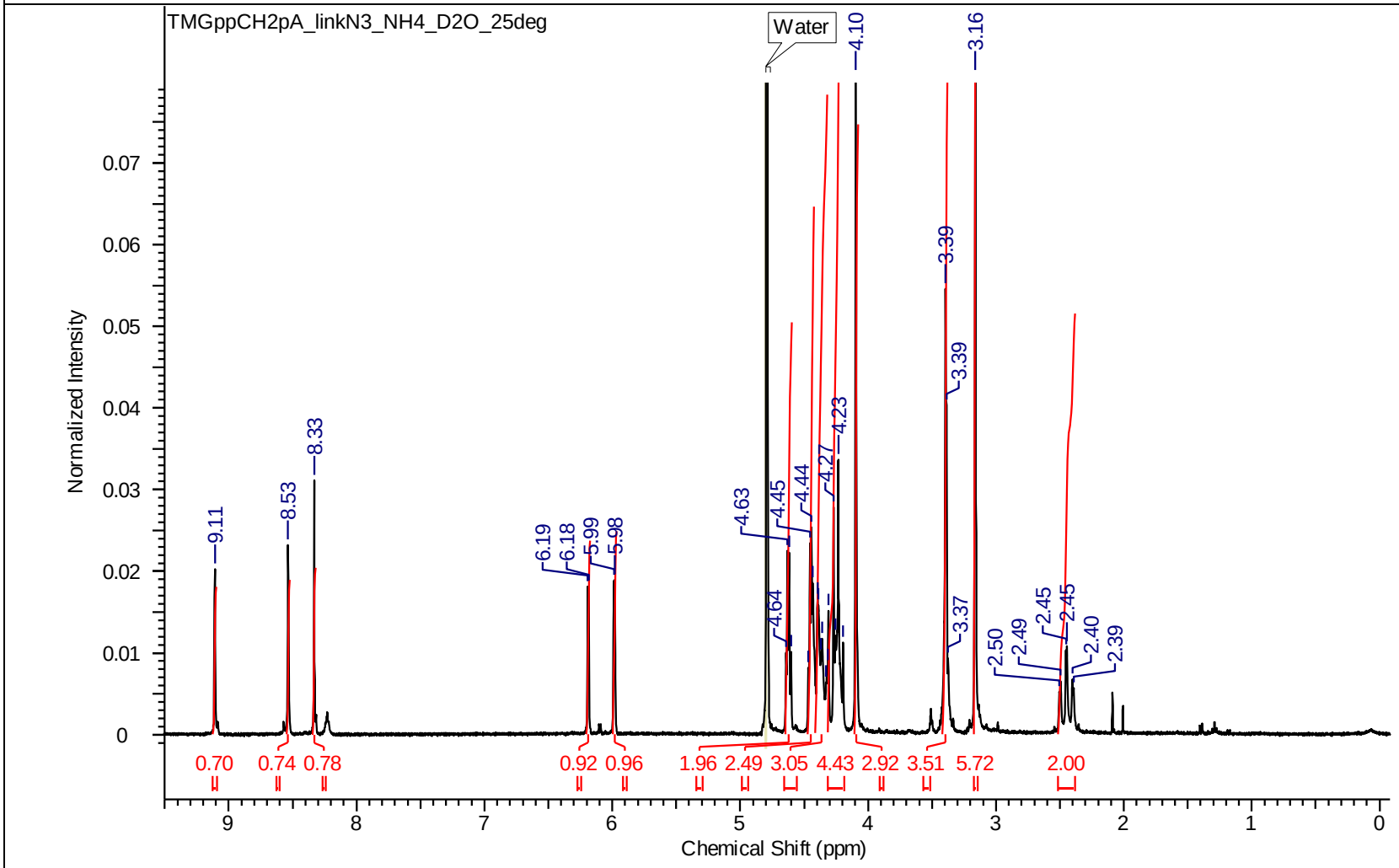
TMG-ppCH_2p-Ado-link-N_3 (2)

TMG-ppCH_2p-Ado-link-N_3 ^{1}H NMR



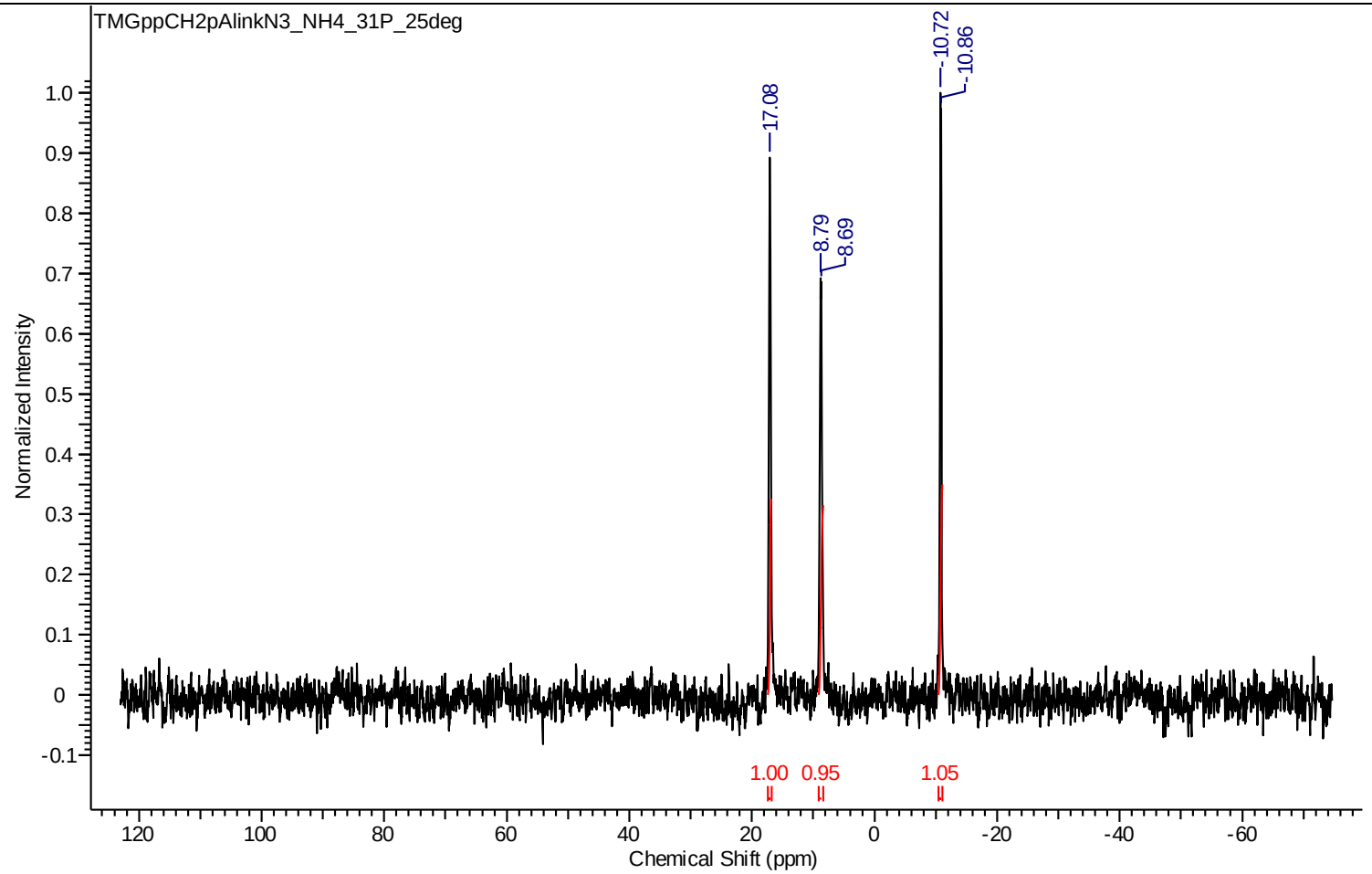
TMG-ppCH_2p-Ado-link-N_3 (2)

TMG-ppCH_2p-Ado-link-N_3 ^{1}H NMR



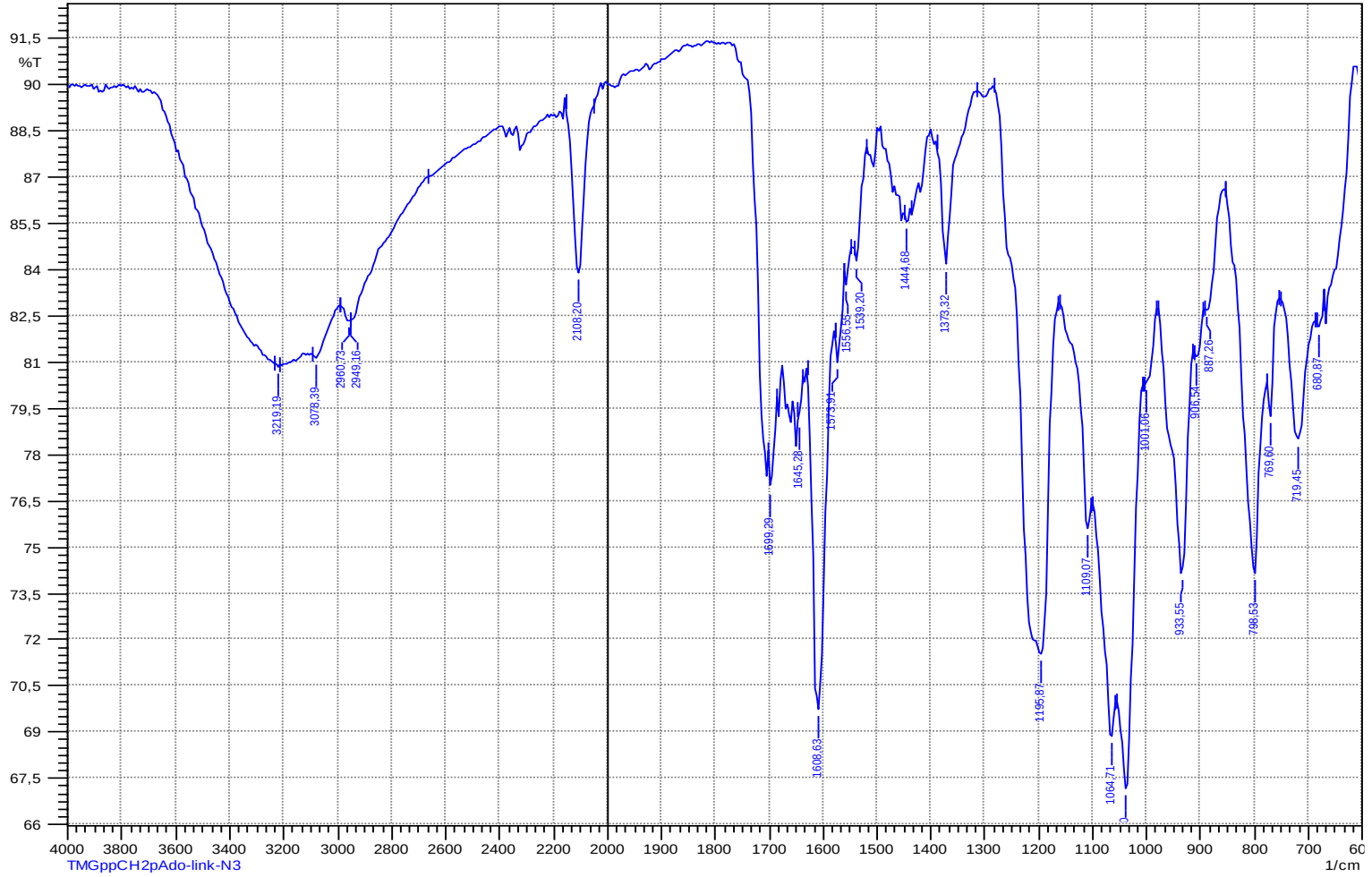
TMG-ppCH_2p-Ado-link-N_3 (2)

TMG-ppCH_2p-Ado-link-N_3 ^{31}P NMR

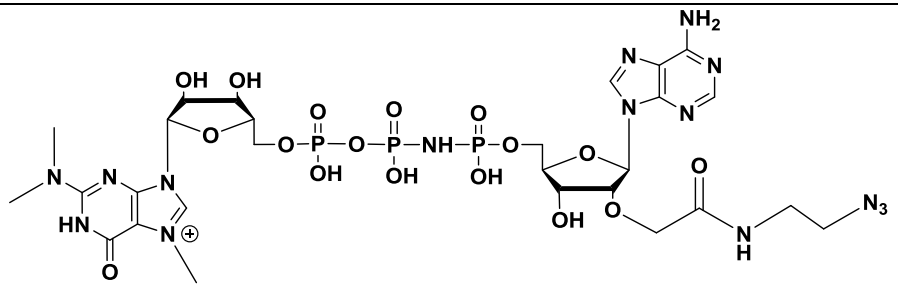


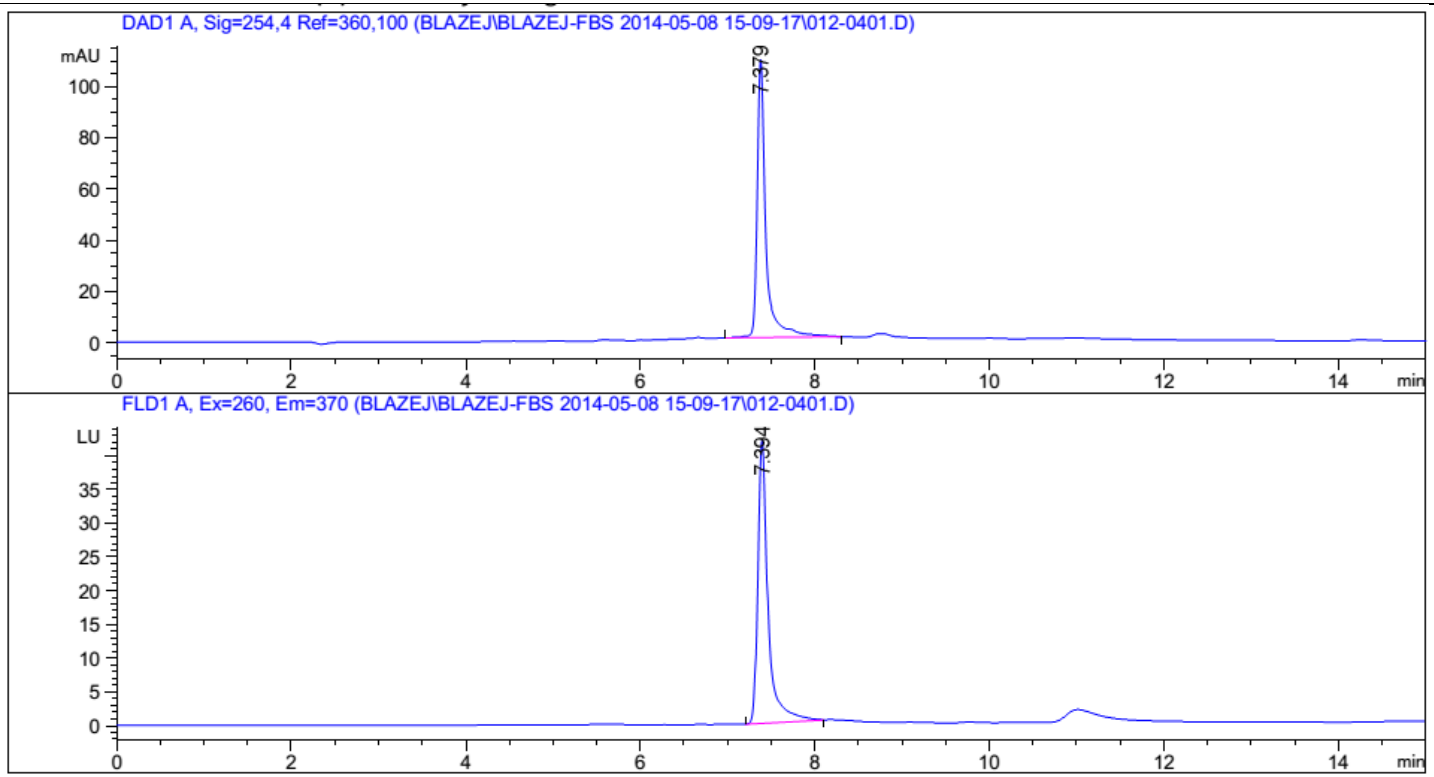
TMG-ppCH$_2$p-Ado-link-N$_3$ (2)

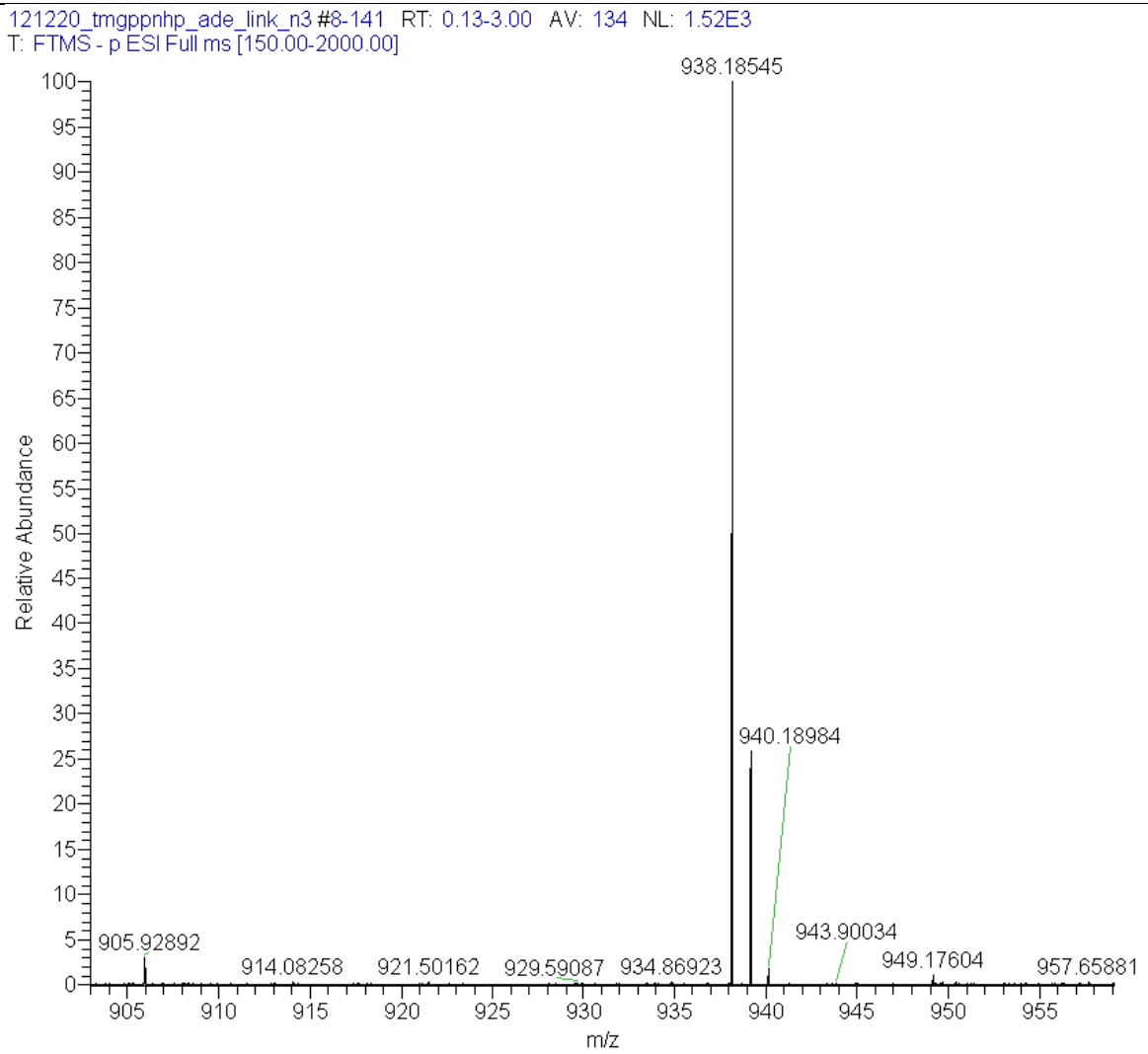
TMG-ppCH$_2$p-Ado-link-N$_3$ FTIR ATR



TMG-ppNHp-Ado-link-N_3 (3)

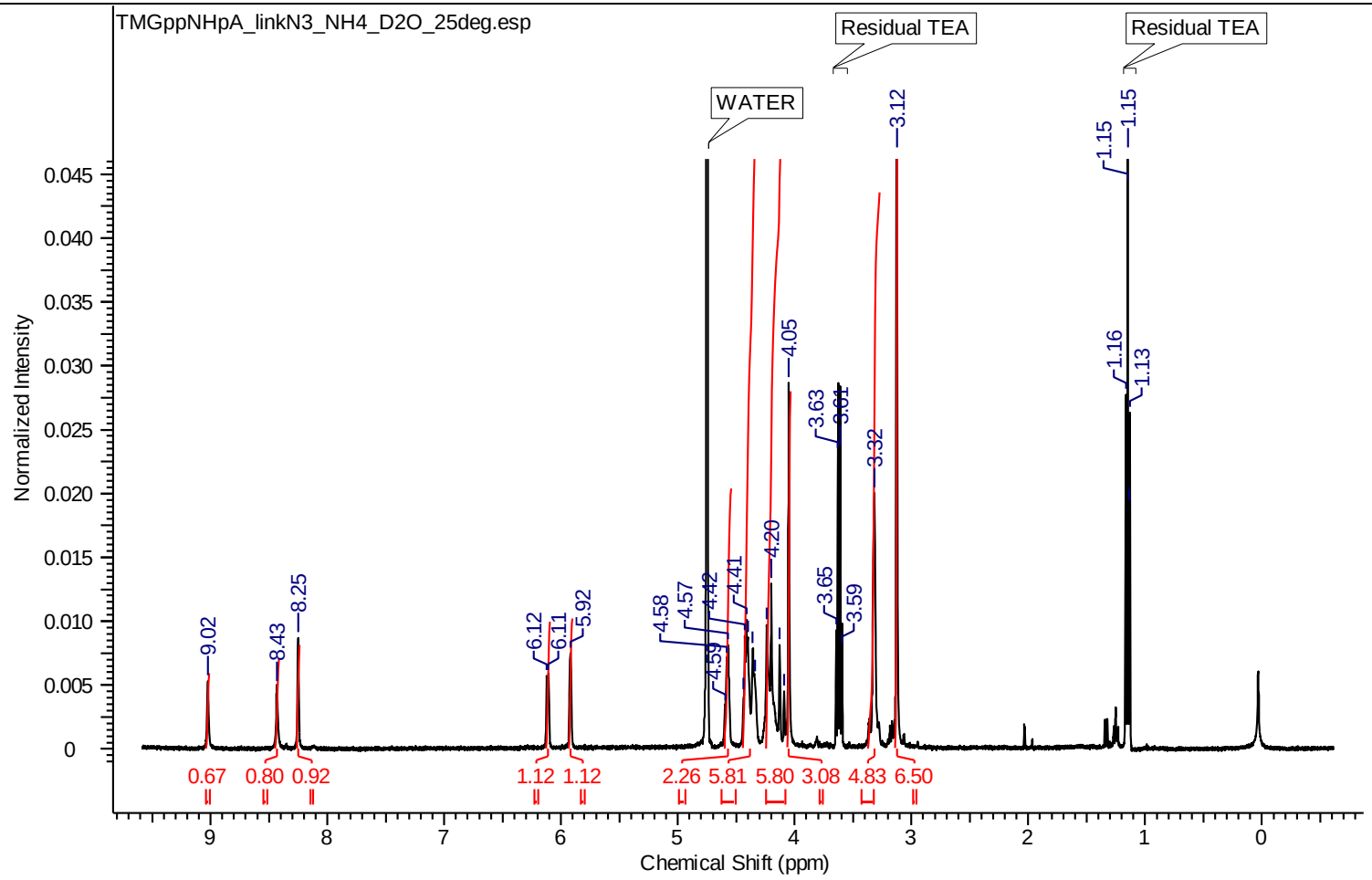




TMG-ppNHp-Ado-link-N_3 (3)	**TMG-ppNHp-Ado-link-N_3/NH_4^+ HRMS (-)ES $C_{27}H_{39}N_{15}O_{17}P_3$, 938.18545 $[M-2H]^-$ Exact Mass: 938,80612** 

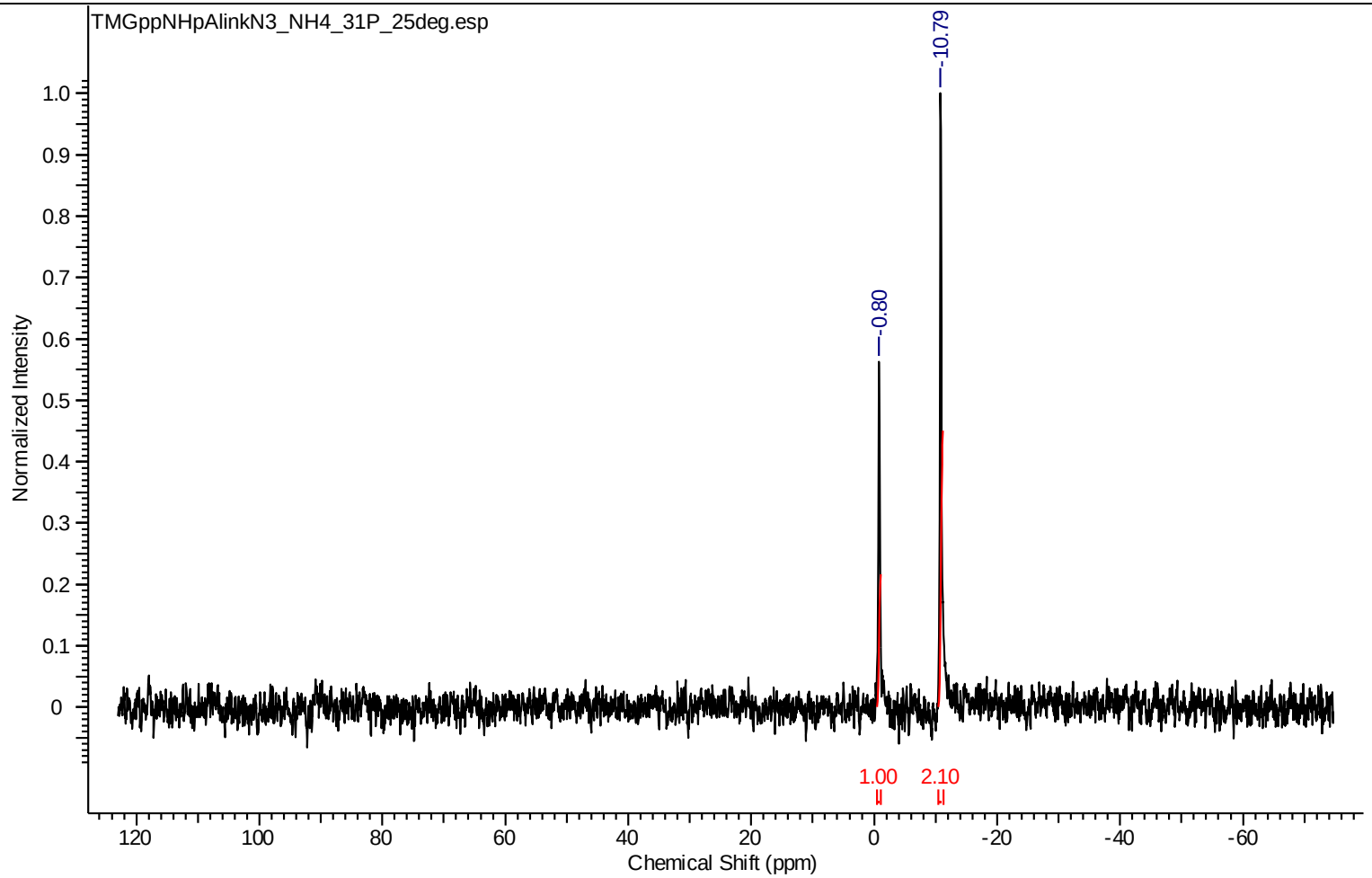
TMG-ppNHp-Ado-link-N_3 (3)

TMG-ppNHp-Ado-link-N_3 ^{1}H NMR



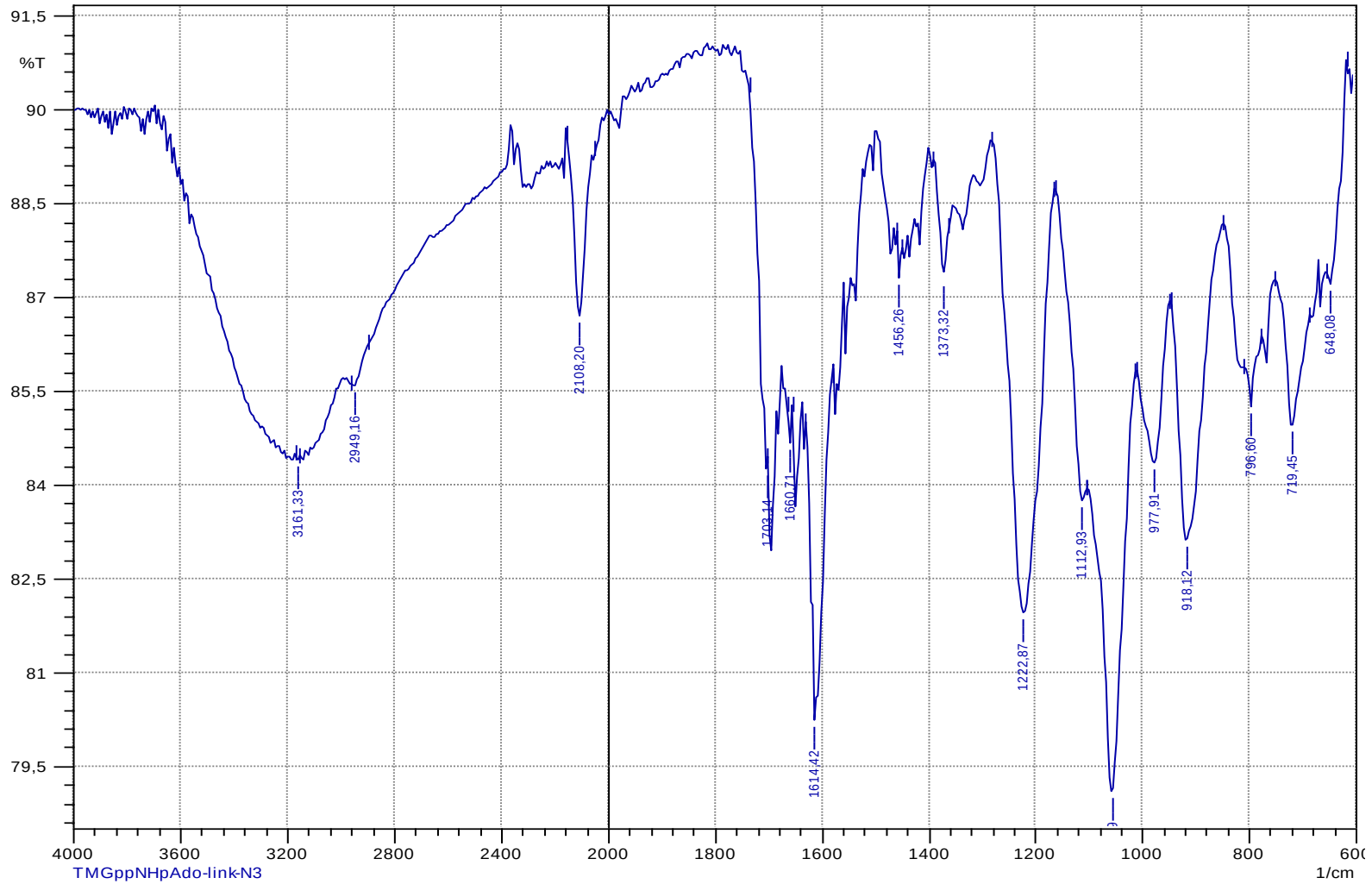
TMG-ppNHp-Ado-link-N_3 (3)

TMG-ppNHp-Ado-link-N_3 ^{31}P NMR

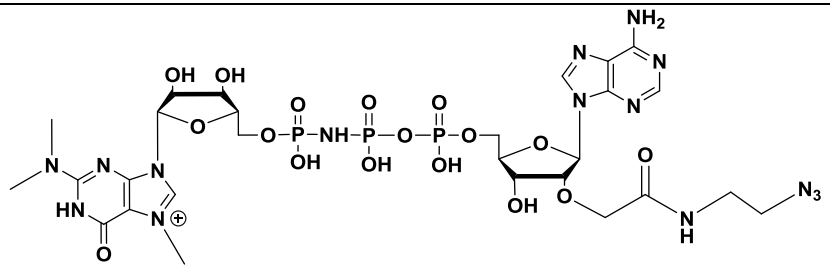


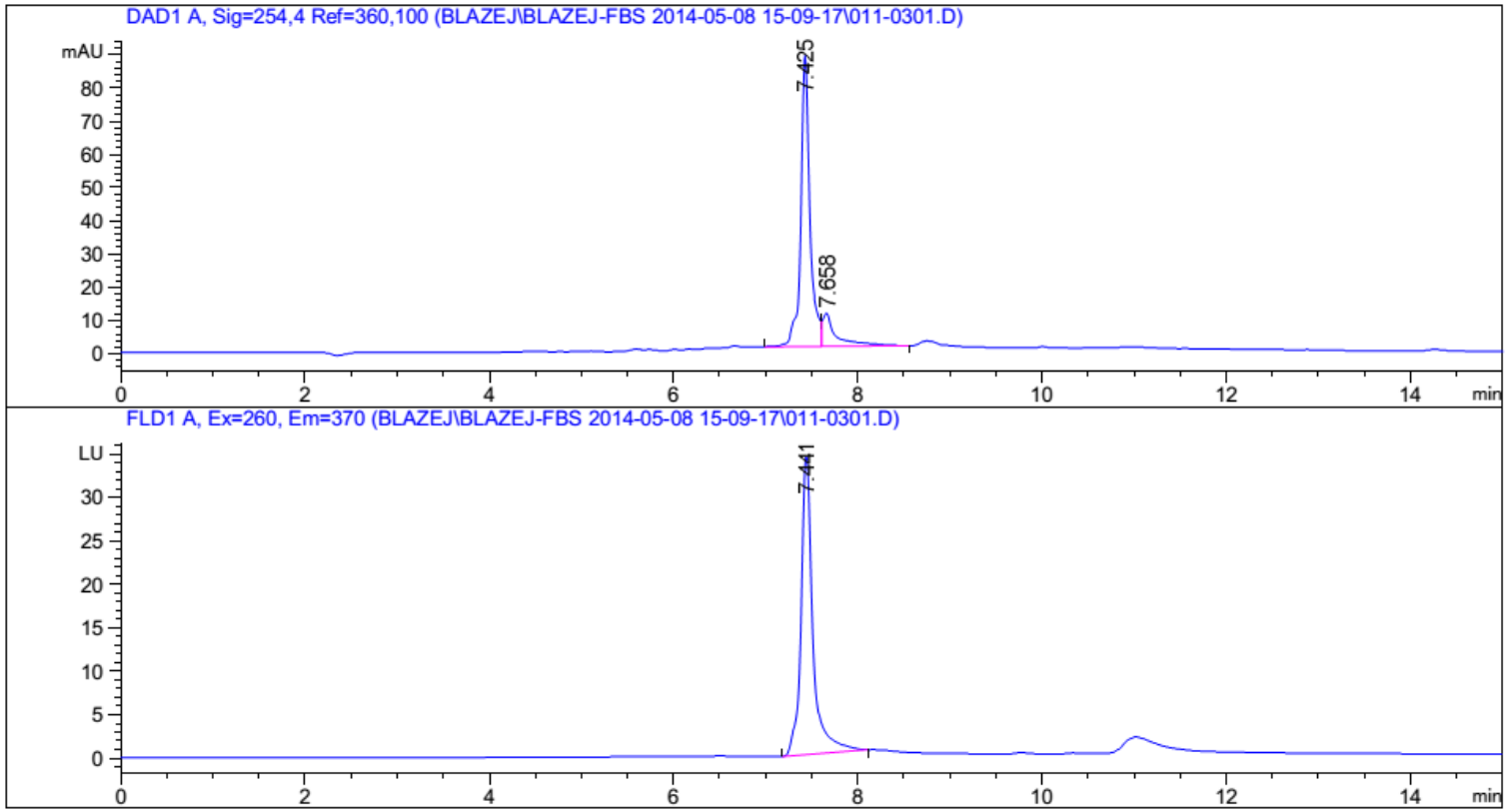
TMG-ppNHp-Ado-link-N_3 (3)

TMG-ppNHp-Ado-link-N_3 FTIR ATR

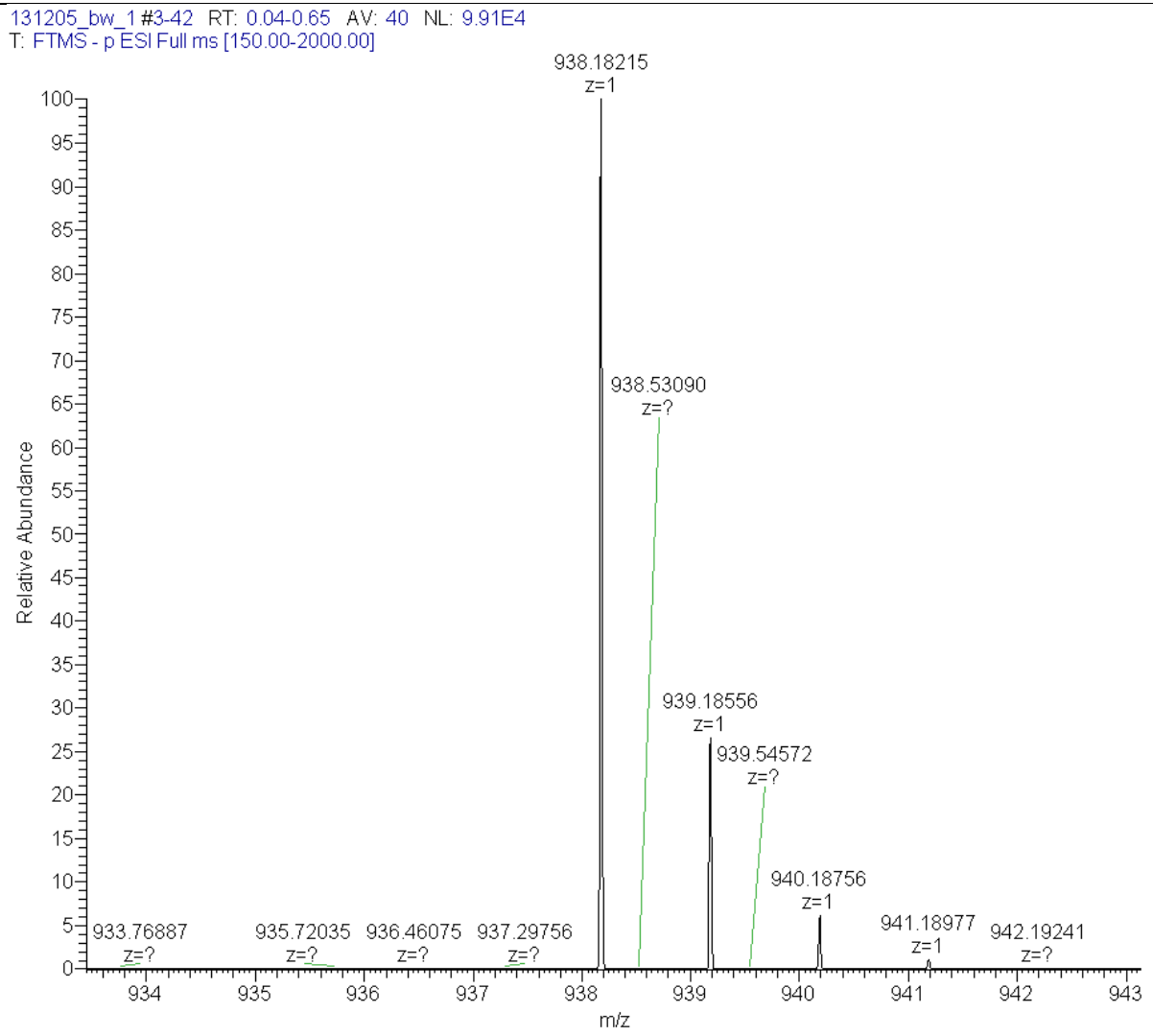


TMG-pNHpp-Ade-link-N_3 (5)



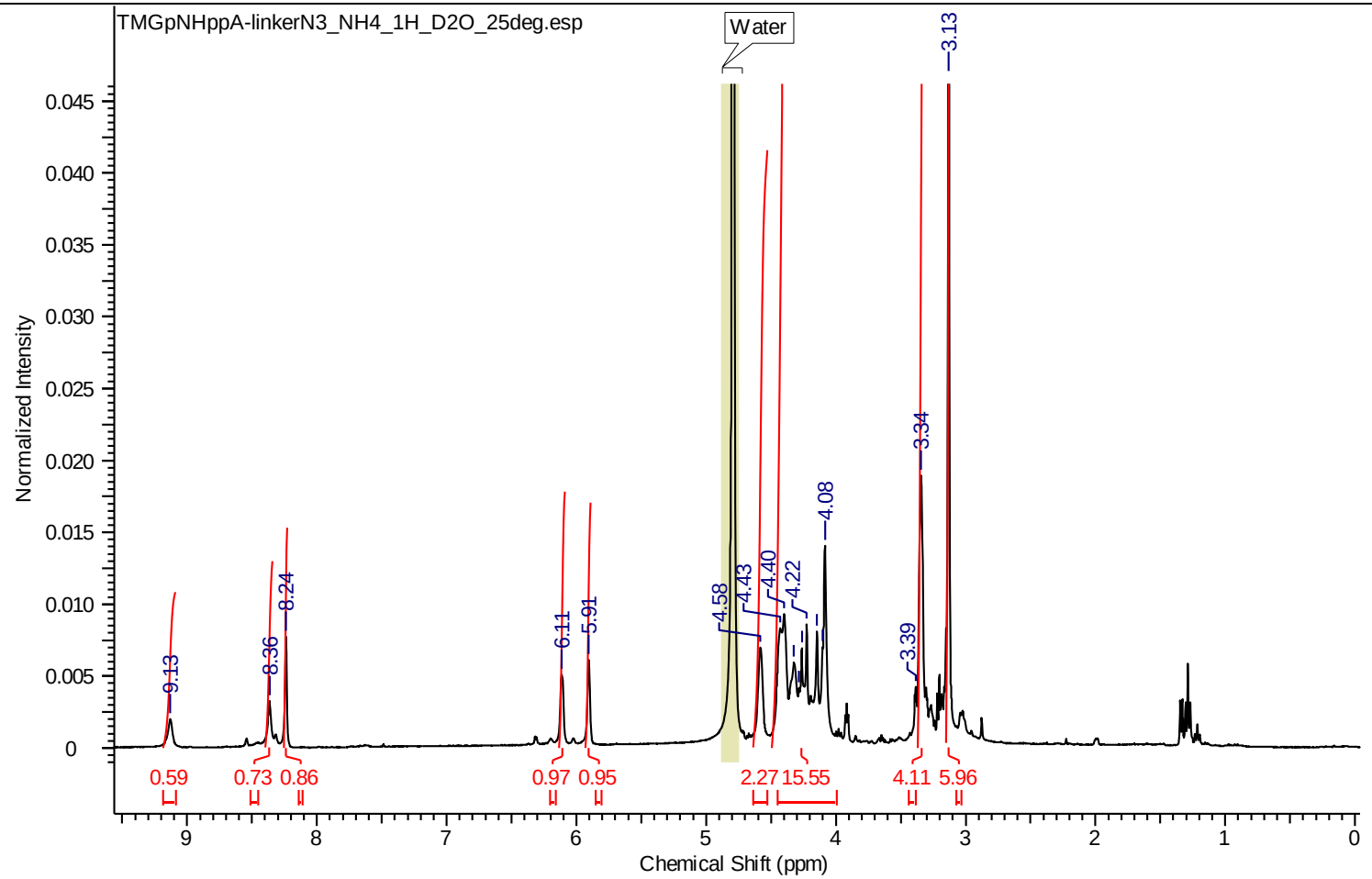


TMG-pNHpp-Ado-link-N$_3$ (5)	**TMG-pNHpp-Ado-link-N3/NH_4^+ HRMS (-)ES $C_{27}H_{39}N_{15}O_{17}P_3$, 938.18215 $[M-2H]^-$ Exact Mass: 938.18612**



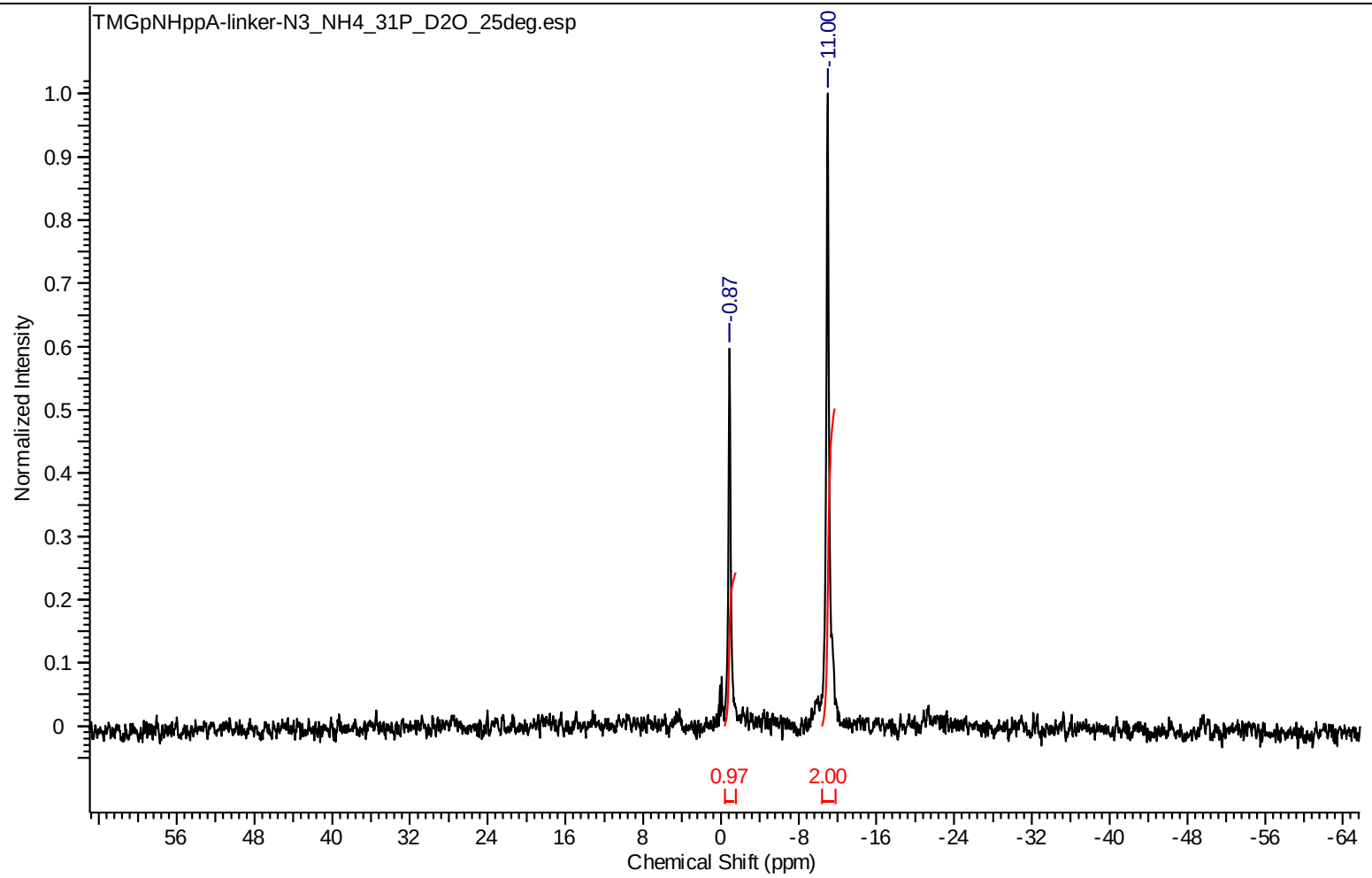
TMG-pNHpp-Ado-link-N_3 (5)

TMG-pNHpp-Ado-link-N_3 ^{1}H NMR



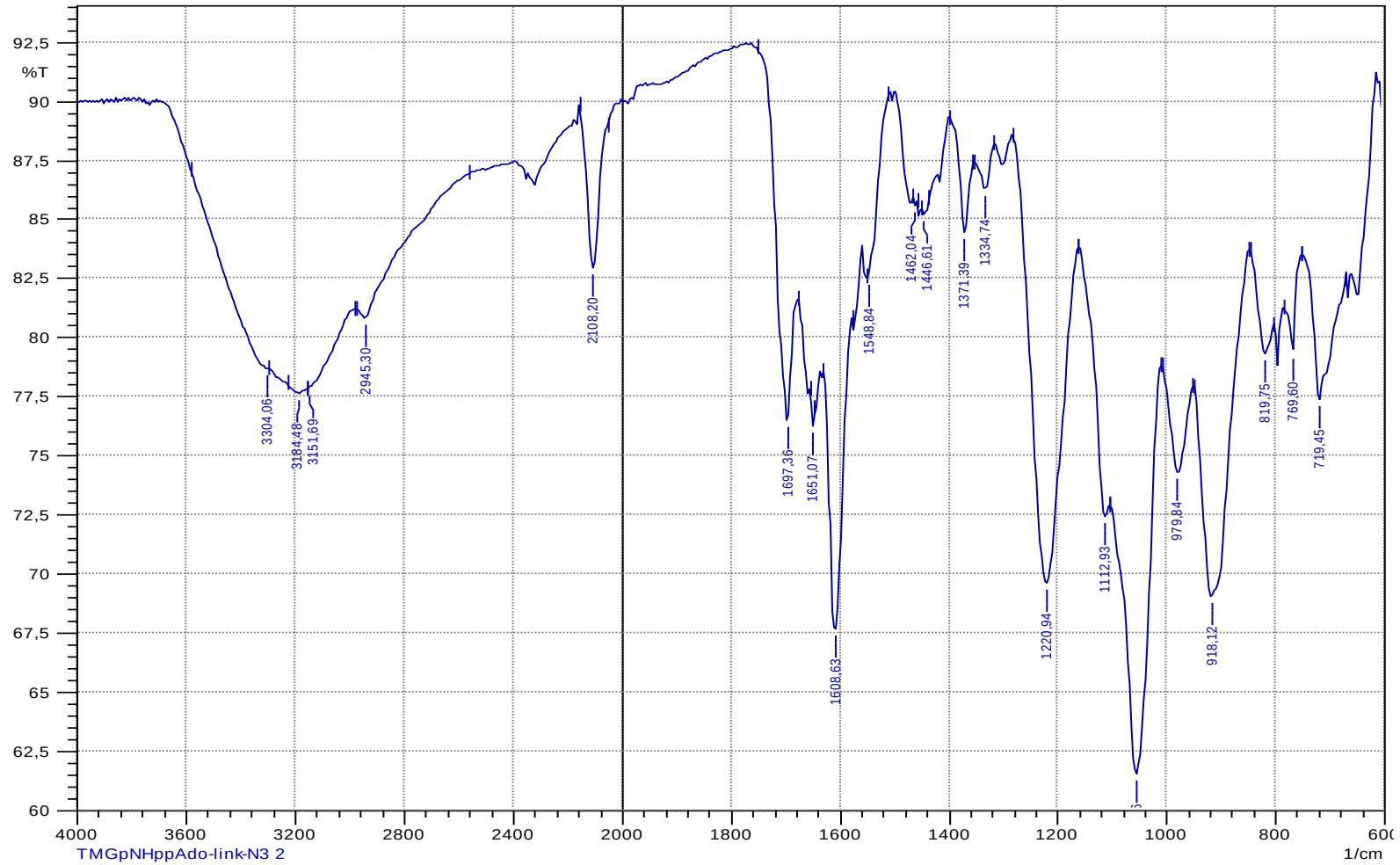
TMG-pNHpp-Ado-link-N_3 (5)

TMG-pNHpp-Ado-link-N_3 ^{31}P NMR

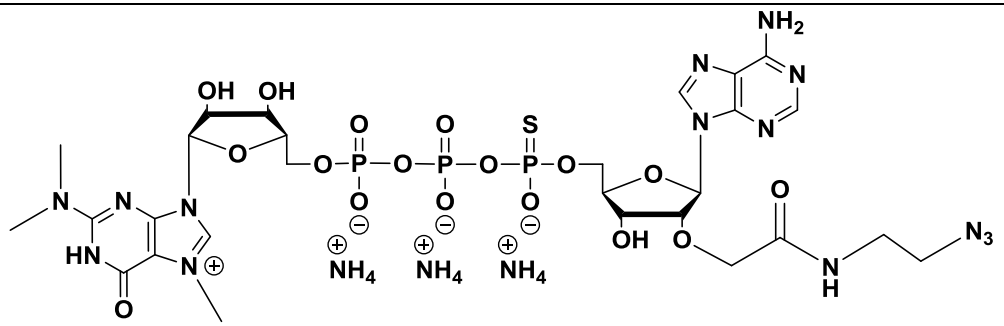


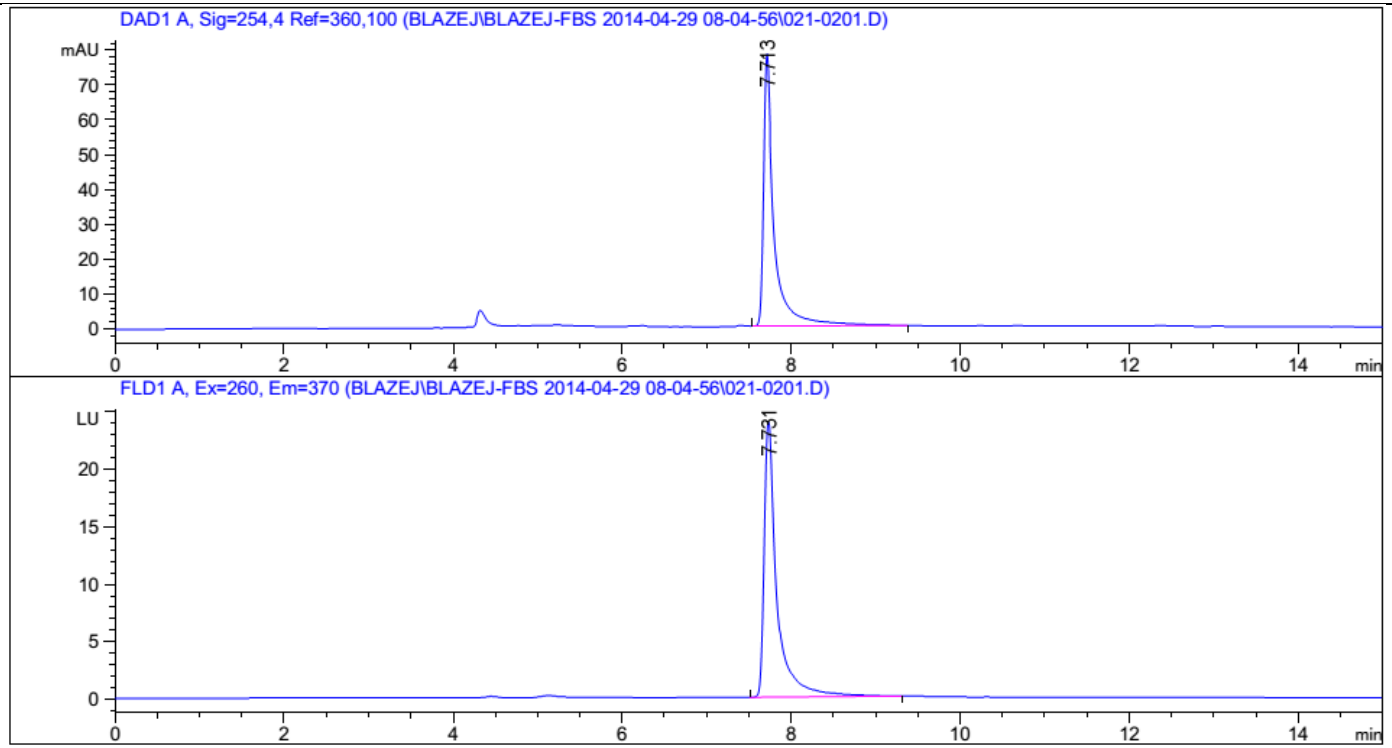
TMG-ppNHp-Ado-link-N_3 (5)

TMG-pNHpp-Ado-link-N_3 FTIR ATR



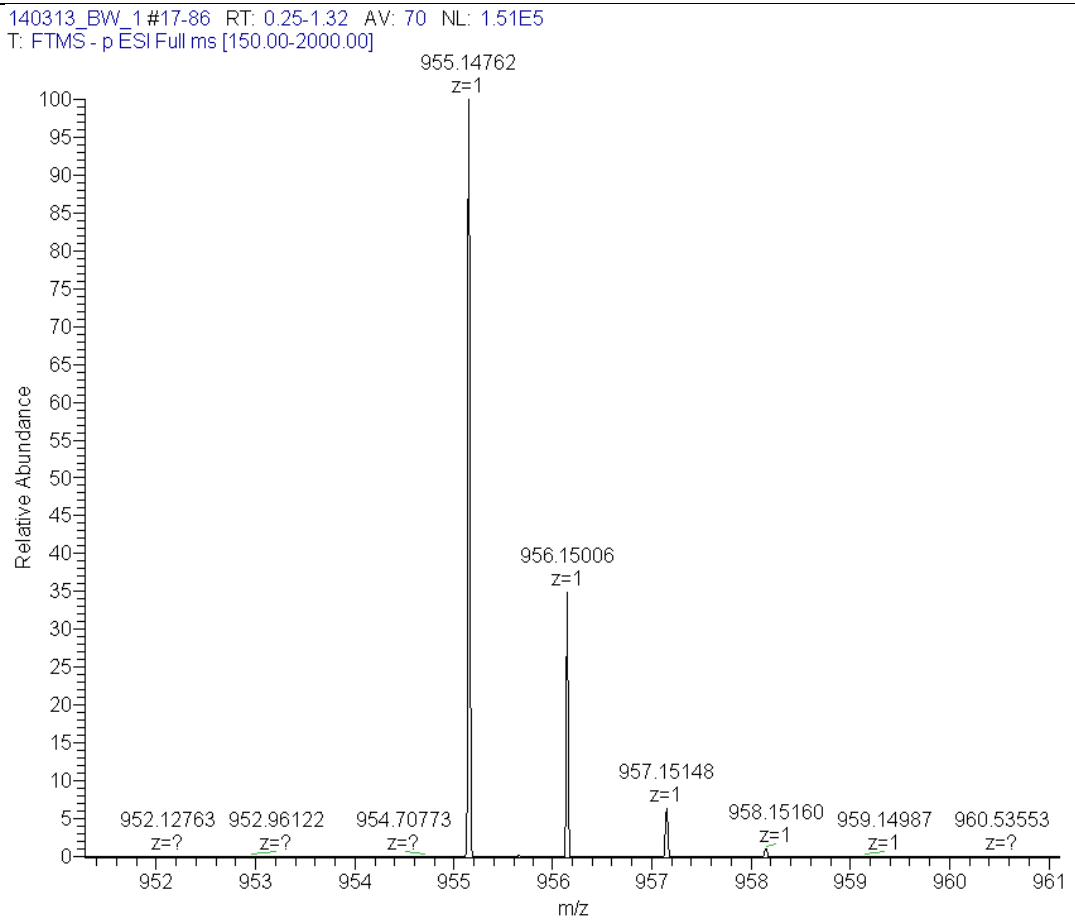
TMG-ppp$_S$-Ado-link-N$_3$ D1 (7a)





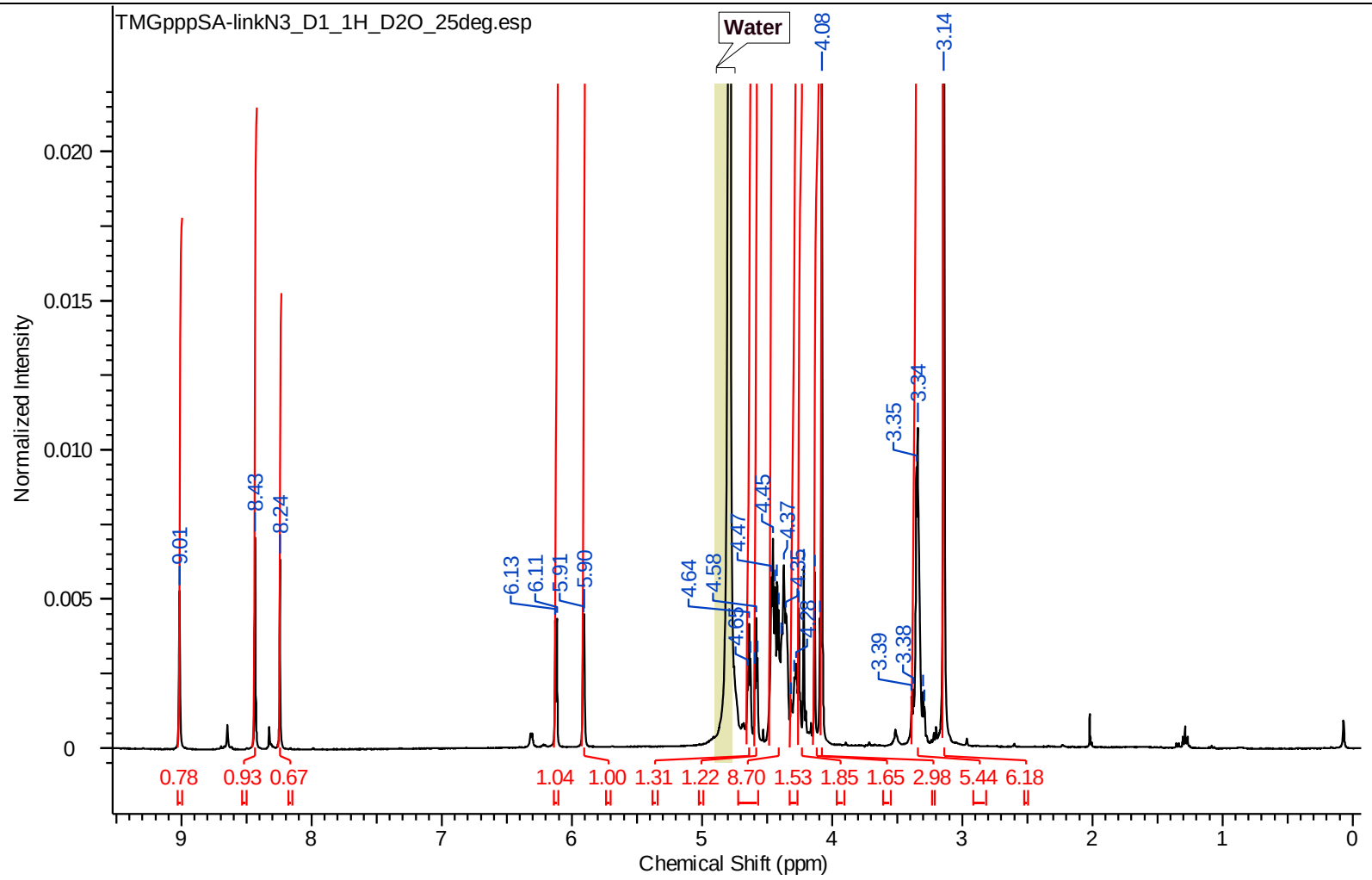
TMG-ppps-Ado-link-N_3 D1/NH_4^+ HRMS (-)ES $C_{27}H_{38}N_{14}O_{17}P_3S$, 955.14762 $[M-2H]^-$ Exact Mass: 955.14729

TMG-ppp_s-Ado-link-N_3 D1 (7a)



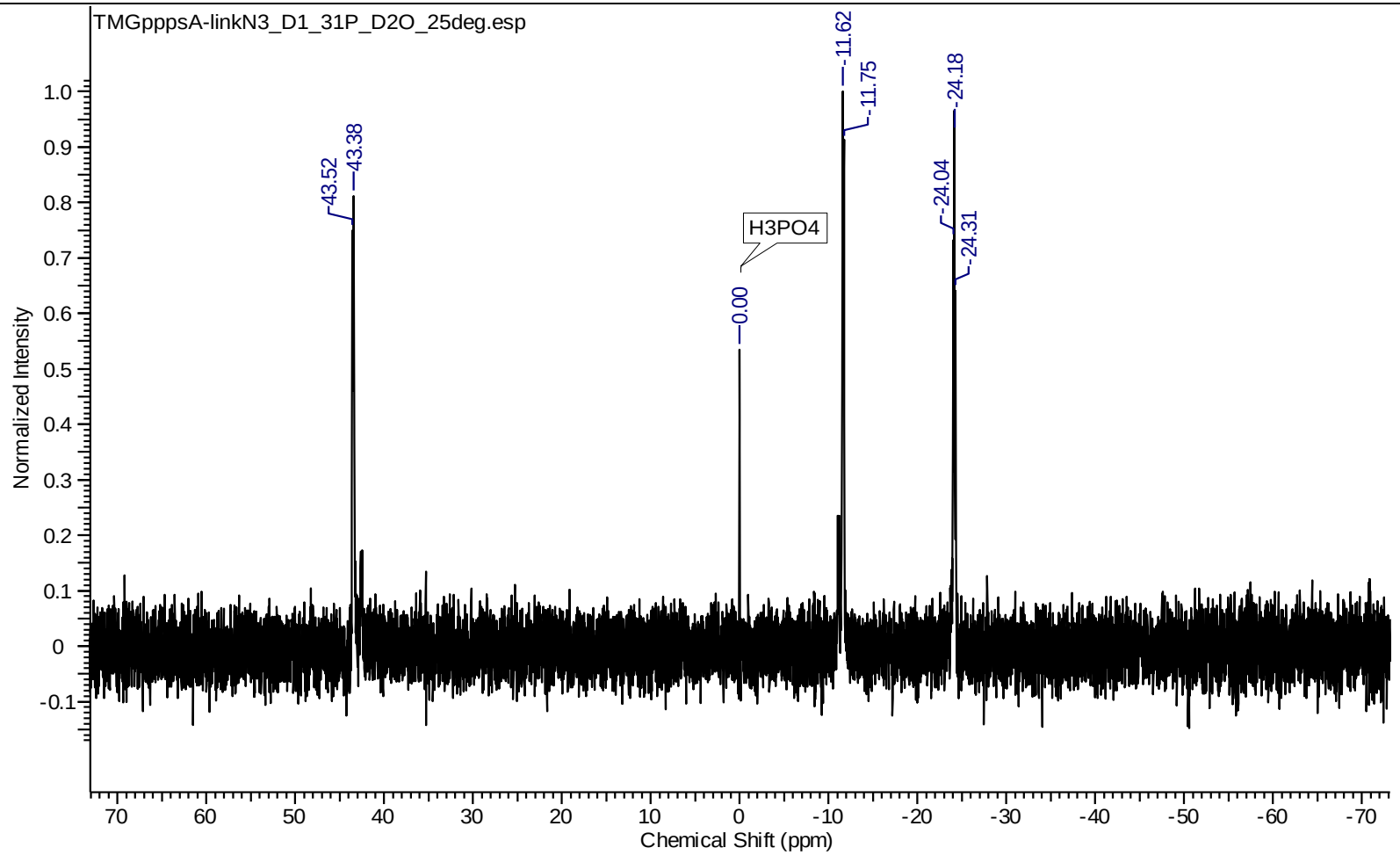
TMG-ppp$_S$-Ado-link-N$_3$ D1 (7a)

TMG-ppp$_S$-Ado-link-N$_3$ D1 ^{1}H NMR



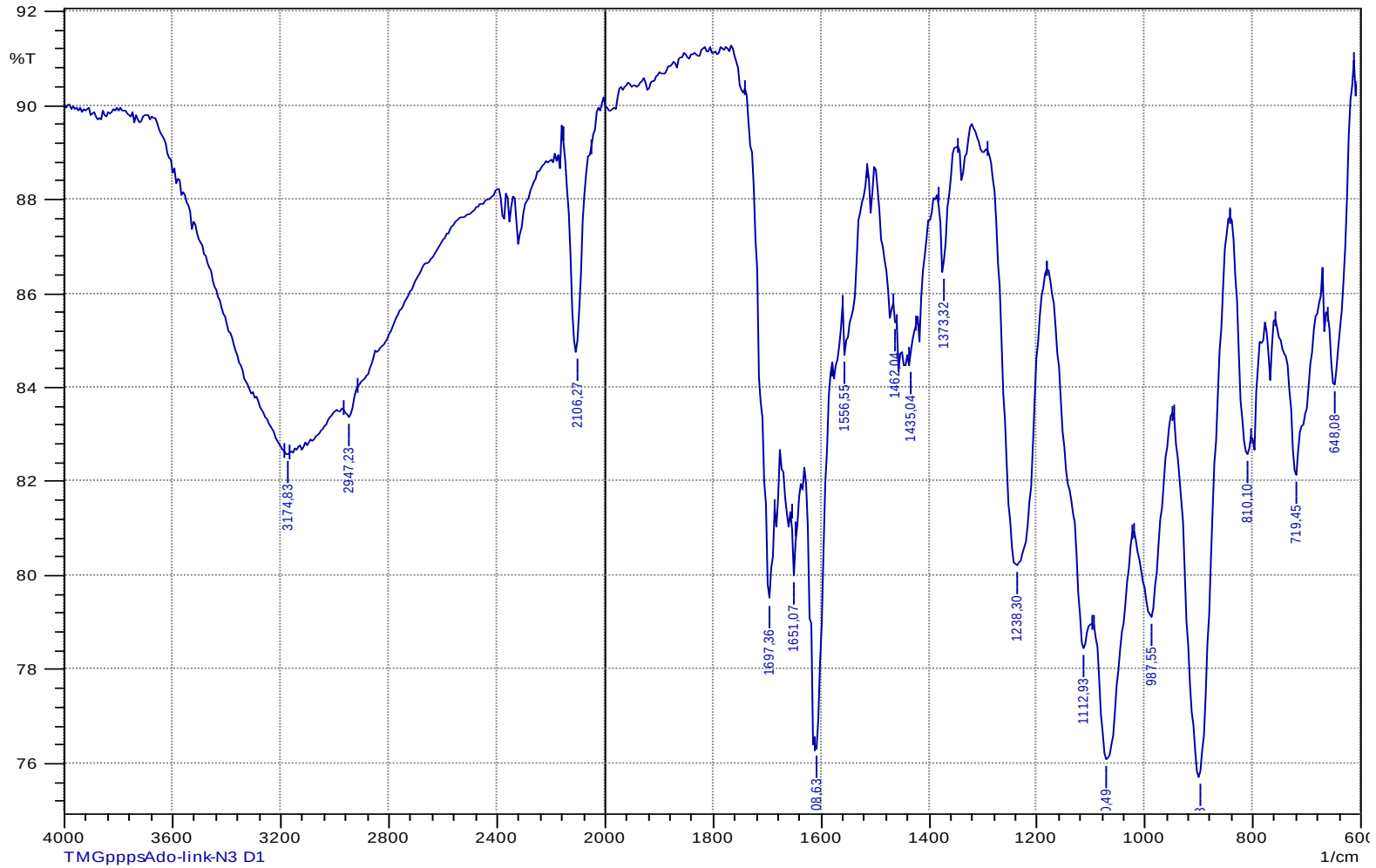
TMG-ppp$_s$-Ado-link-N$_3$ D1 (7a)

TMG-ppp$_s$-Ado-link-N$_3$ D1 ^{31}P NMR

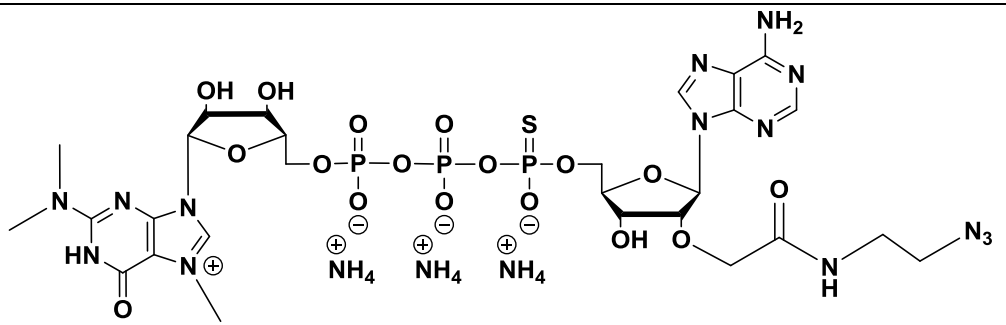


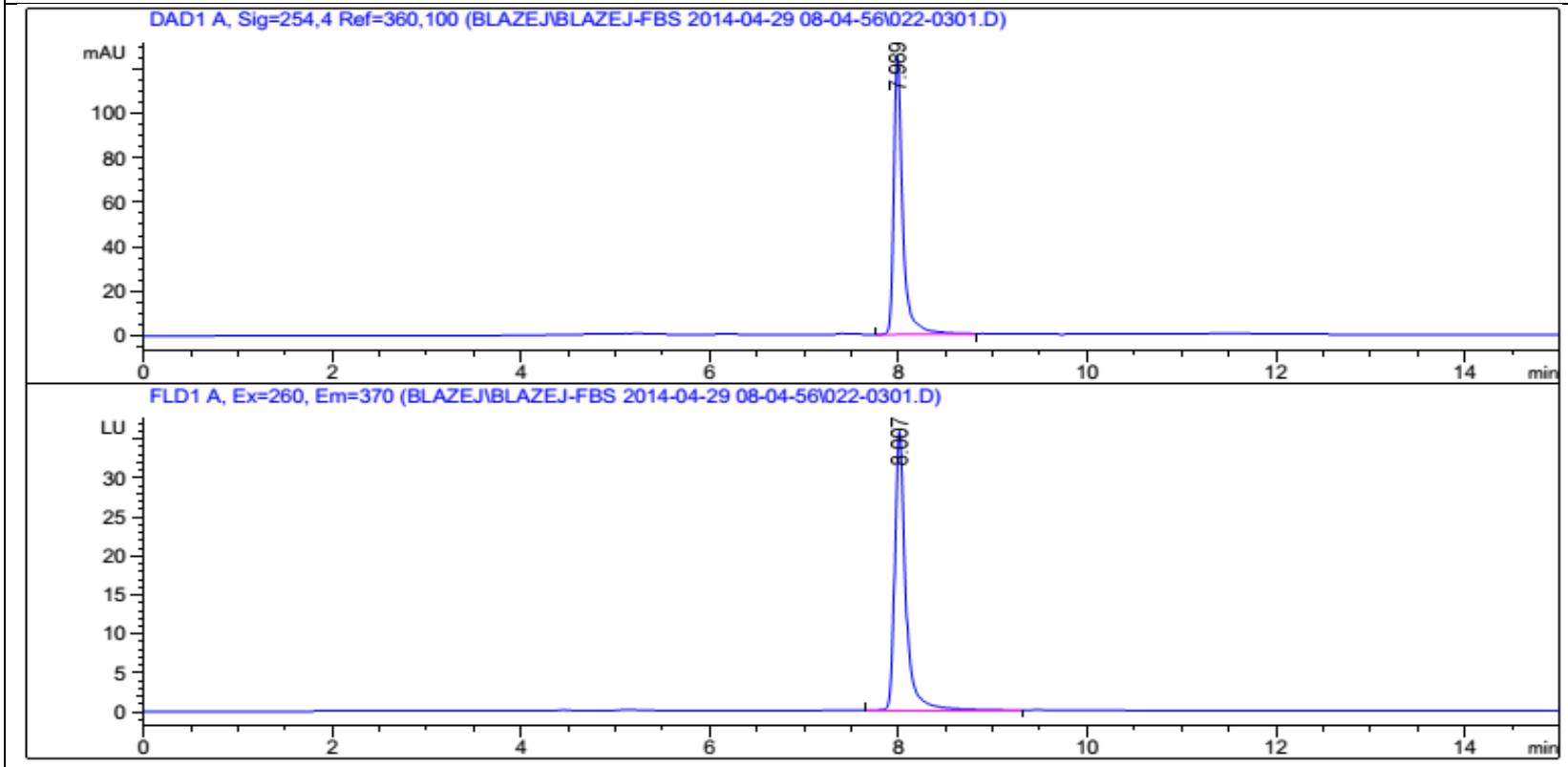
TMG-ppp$_s$-Ado-link-N$_3$ D1 (7a)

TMG-ppps-Ado-link-N$_3$ D1, FTIR ATR



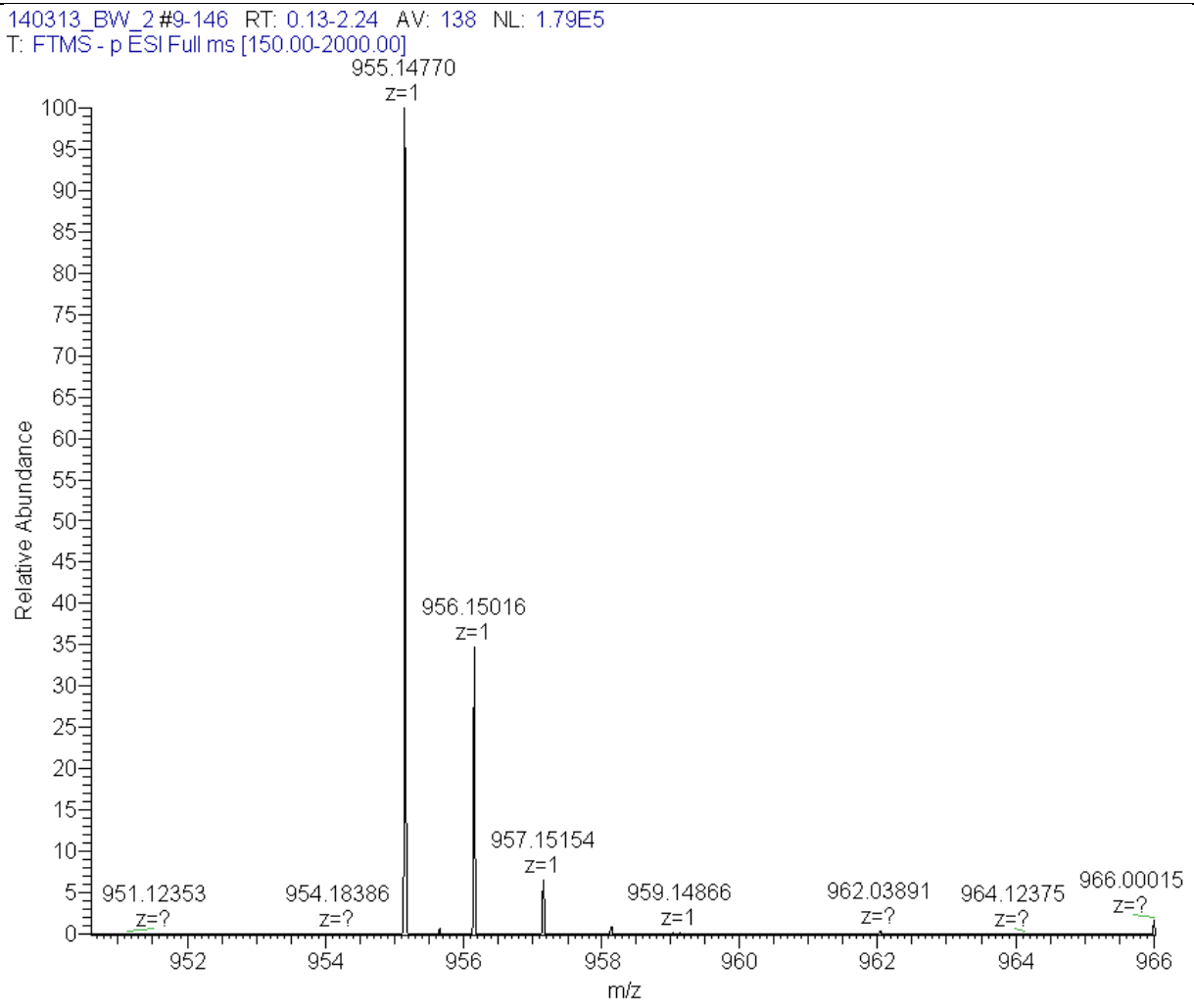
TMG-ppp$_S$-Ado-link-N$_3$ D2 (7b)





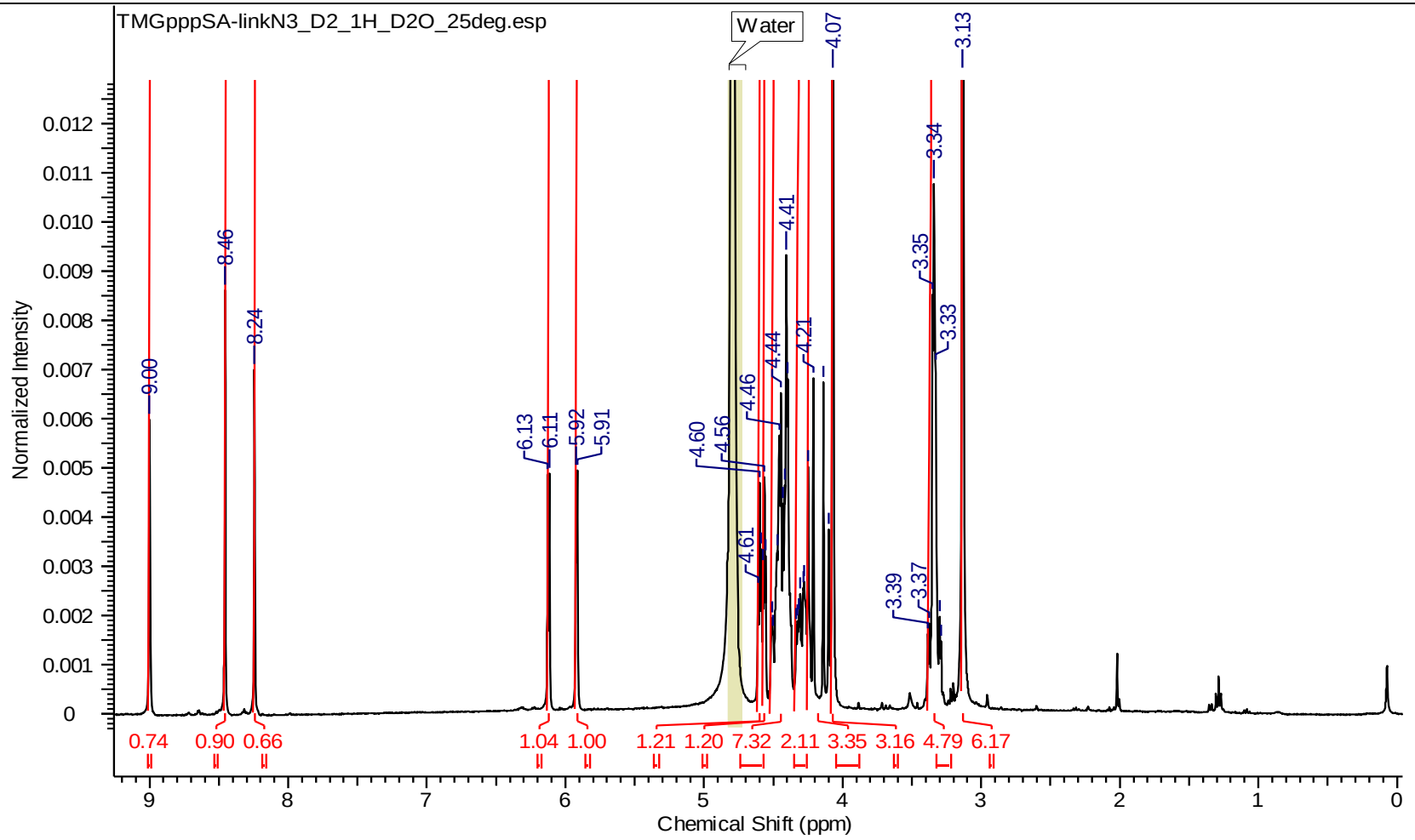
TMG-ppp$_s$-Ado-link-N$_3$ D2 (7b)

TMG-ppp$_s$-Ado-link-N$_3$ D2/NH$_4^+$ HRMS (-)ES C$_{27}$H$_{38}$N$_{14}$O$_{17}$P$_3$S, 955.14770 [M-2H]$^-$ Exact Mass: 955.14729



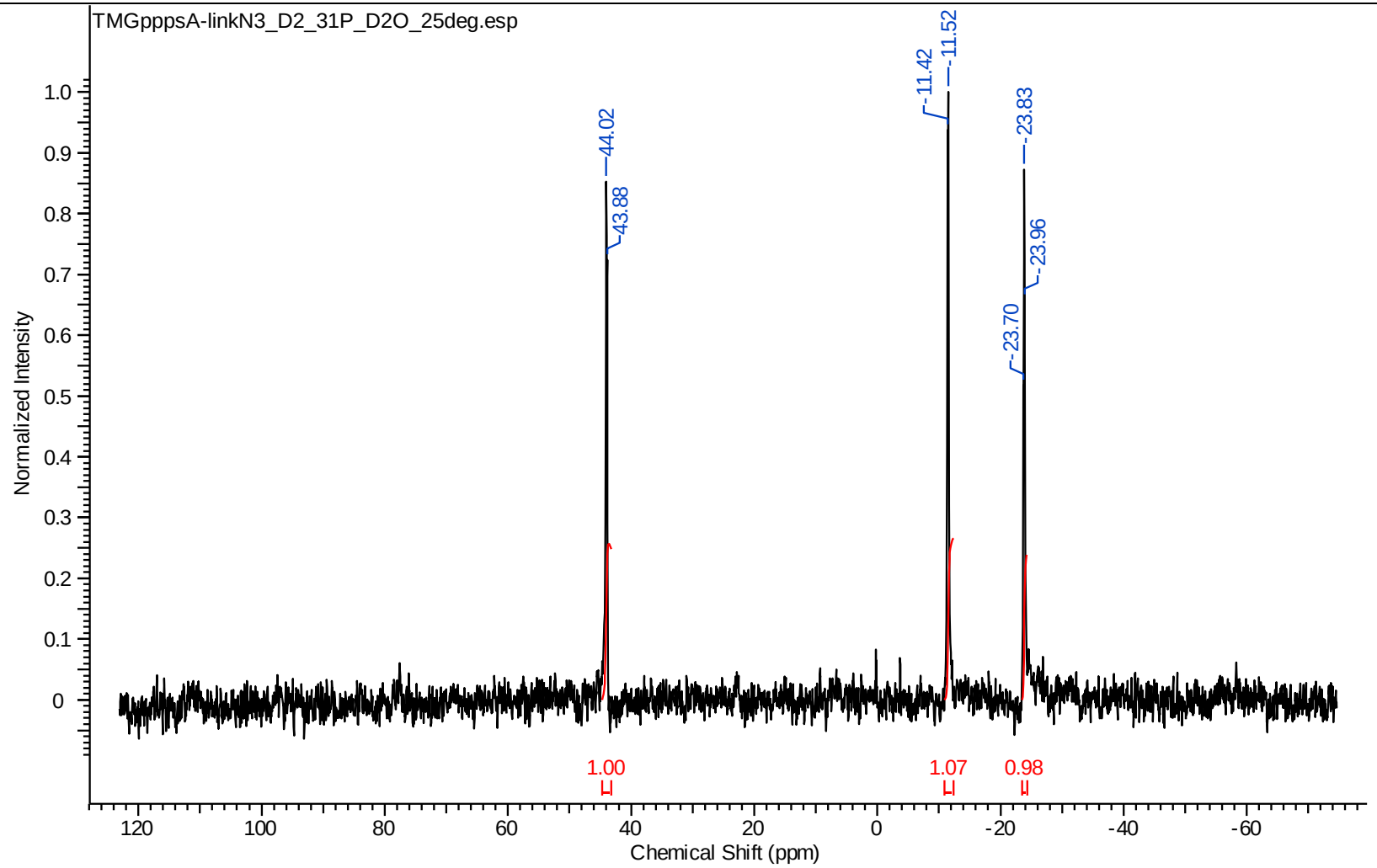
TMG-ppp$_s$-Ado-link-N$_3$ D2 (7b)

TMG-ppp$_s$-Ado-link-N$_3$ D2 ^{1}H NMR



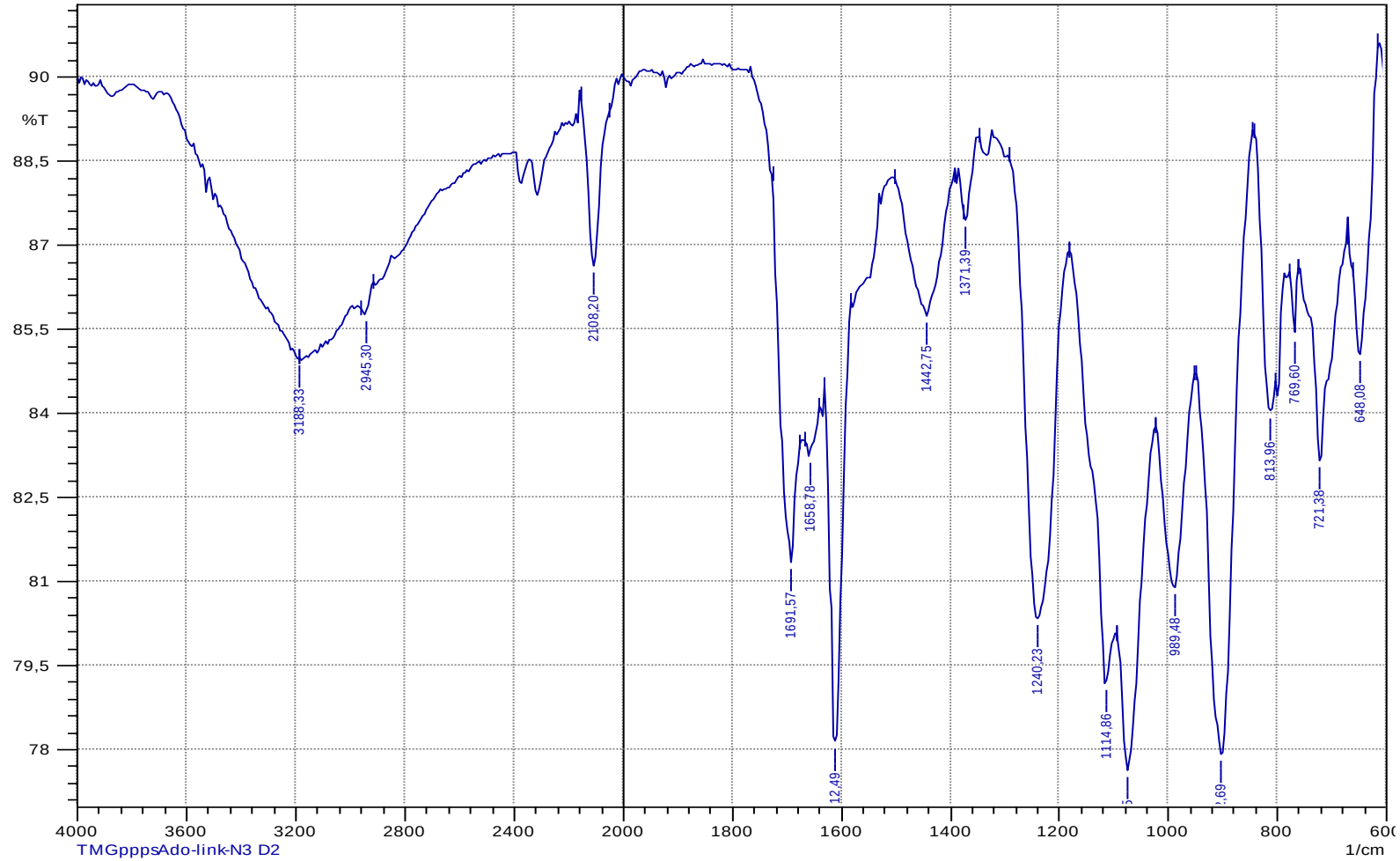
TMG-ppp$_s$-Ado-link-N$_3$ D2 (7b)

TMG-ppp$_s$-Ado-link-N$_3$ D2 ^{31}P NMR

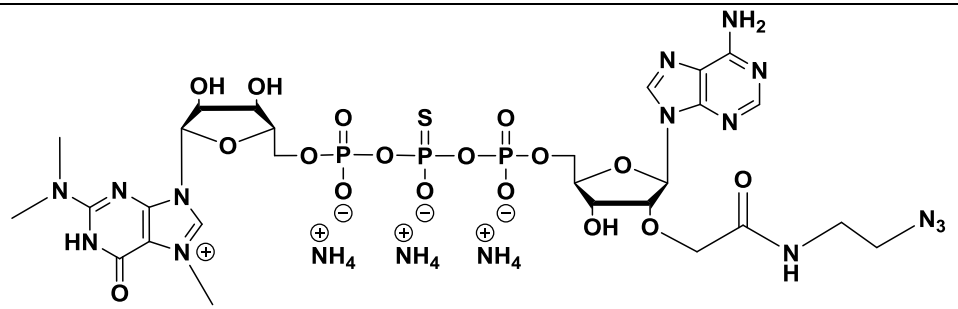


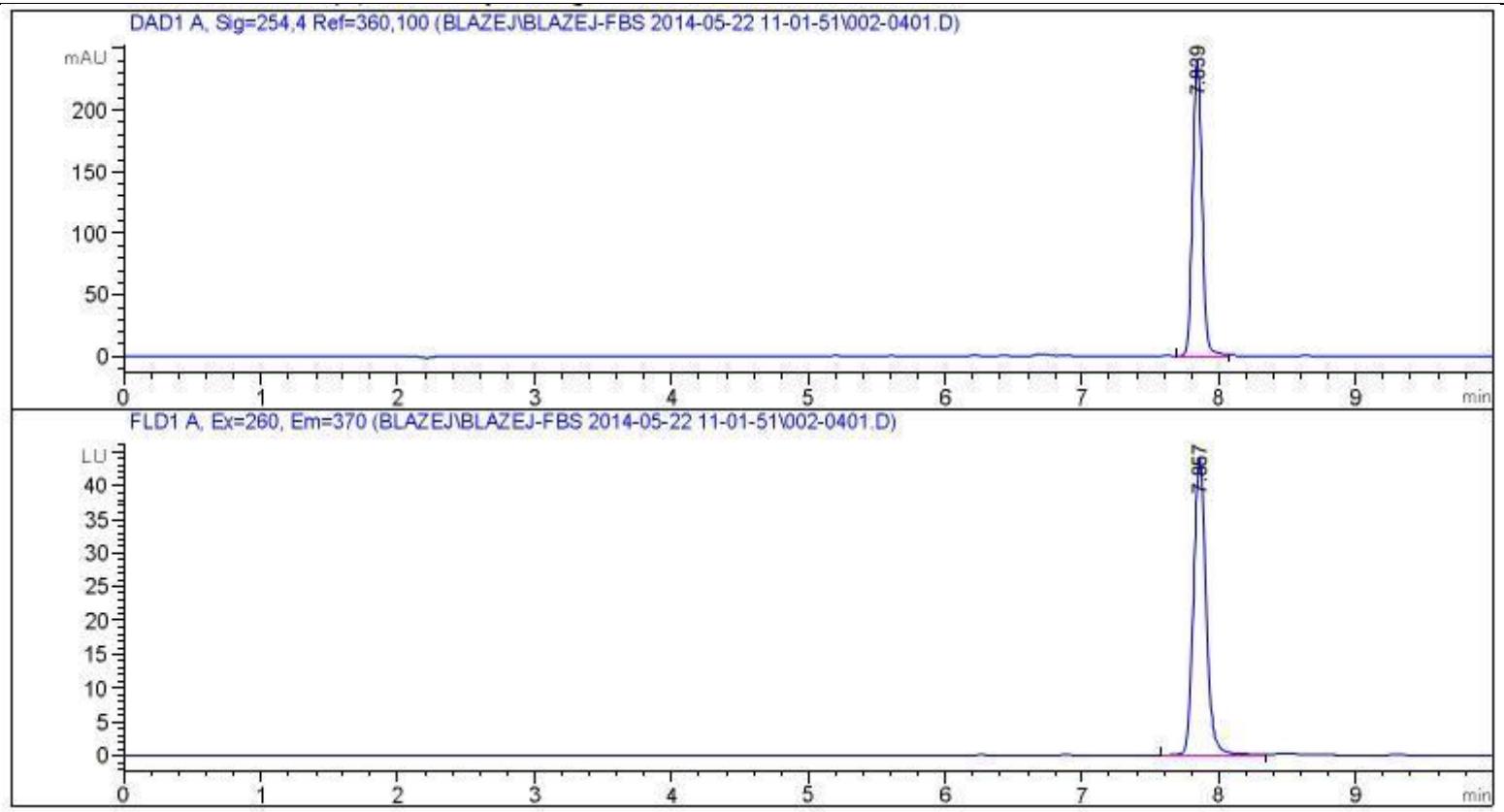
TMG-ppp$_s$-Ado-link-N$_3$ D2 (7b)

TMG-ppp$_s$-Ado-link-N$_3$ D2, FTIR ATR



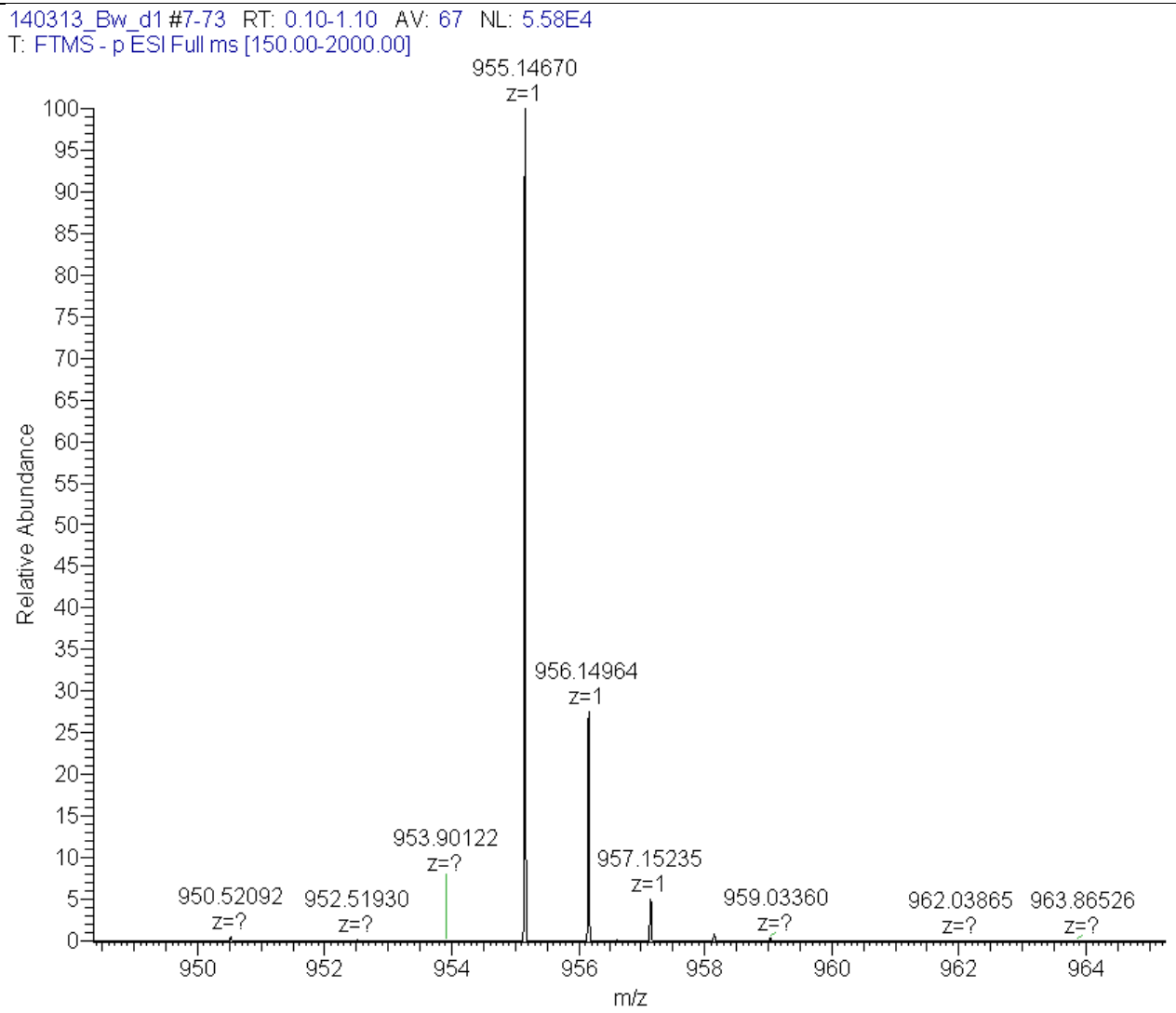
TMG-pp$_S$p-Ado-link-N$_3$ D1 (6a)





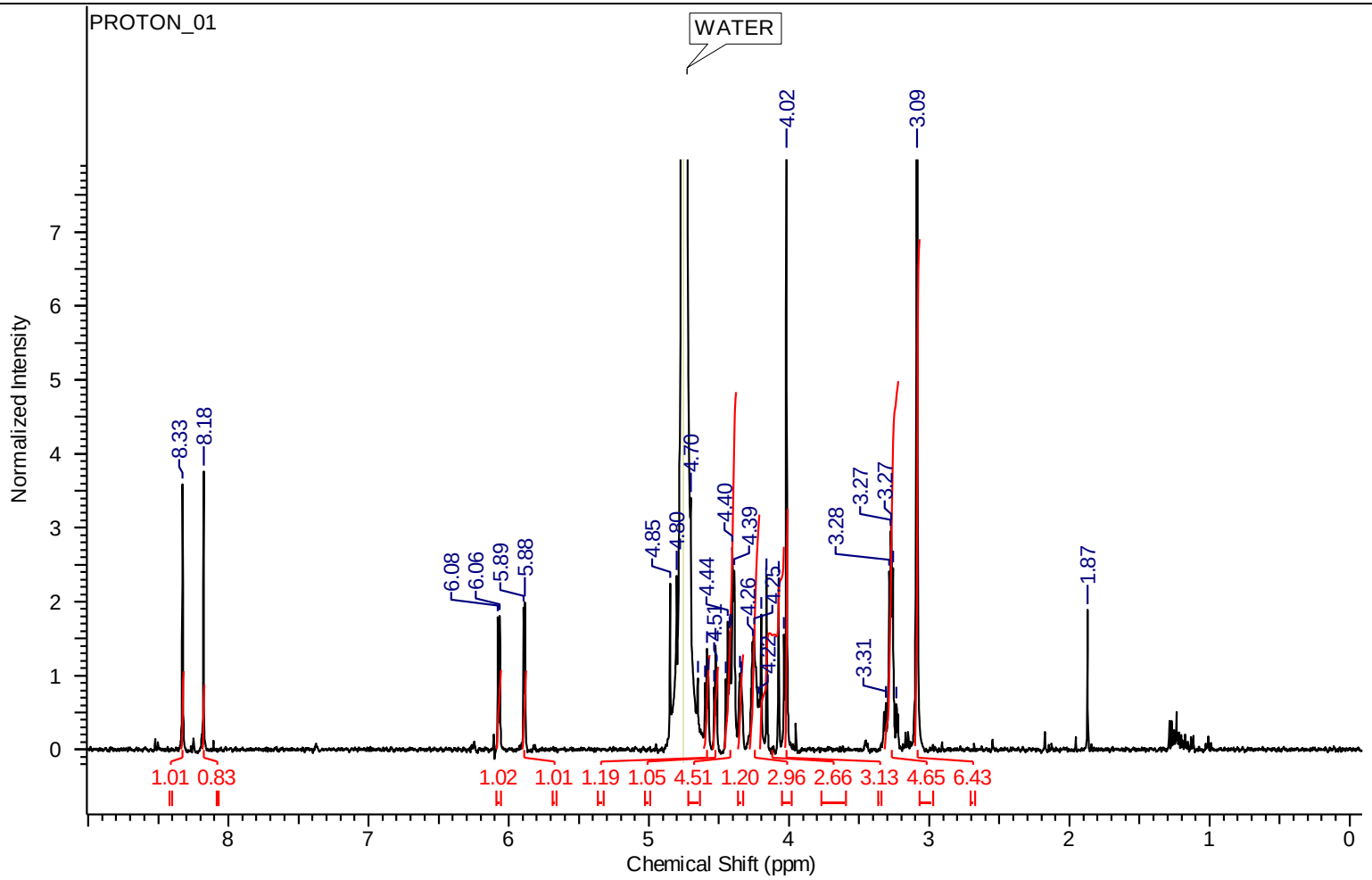
TMG-pp$_s$p-Ado-link-N$_3$ D1 (6a)

TMG-pp$_s$p-Ado-link-N$_3$ D1/NH$_4^+$ HRMS (-)ES C$_{27}$H$_{38}$N$_{14}$O$_{17}$P$_3$S, 955.14670 [M-2H]$^-$ Exact Mass: 955.14729



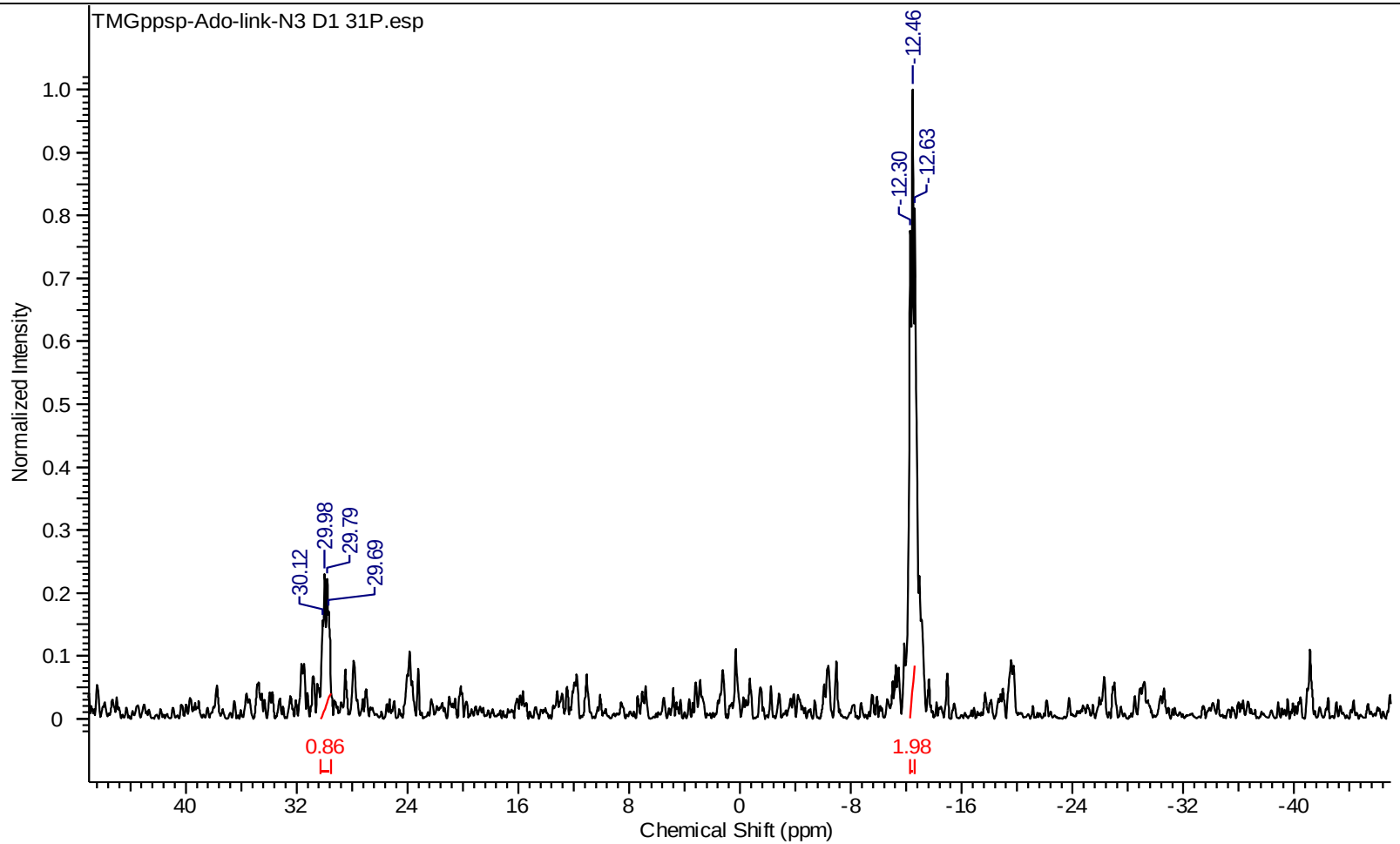
TMG-pp$_s$p-Ado-link-N$_3$ D1 (6a)

TMG-pp$_s$p-Ado-link-N$_3$ D1 ^{1}H NMR



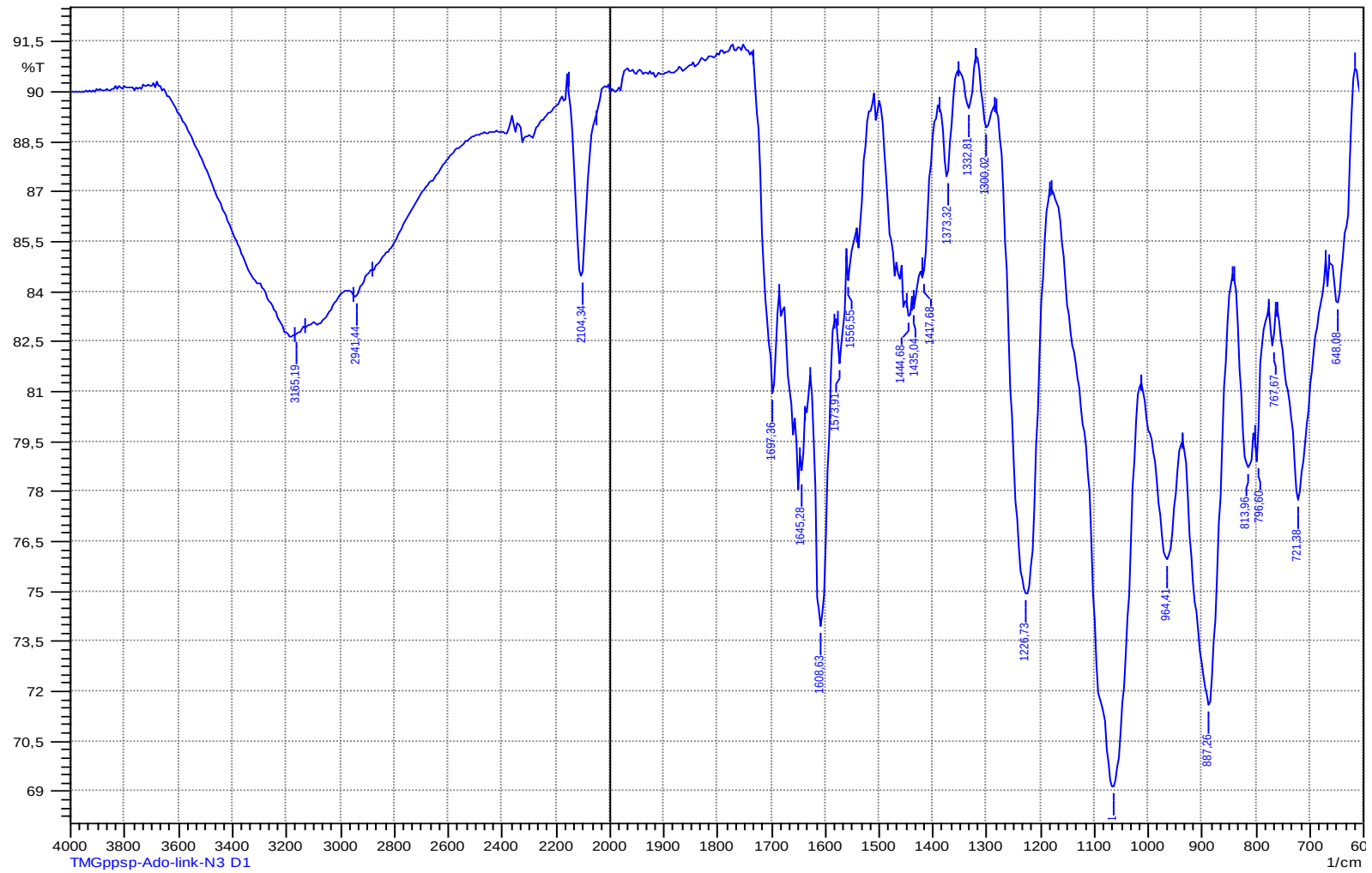
TMG-pp$_s$p-Ado-link-N$_3$ D1 (6a)

TMG-pp$_s$p-Ado-link-N$_3$ D1 ^{31}P NMR

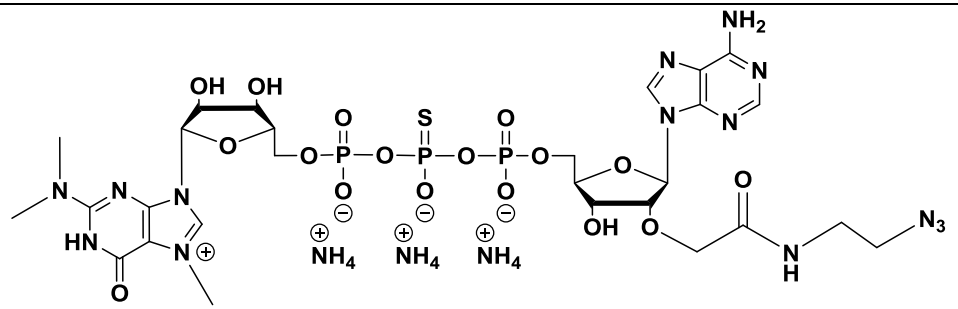


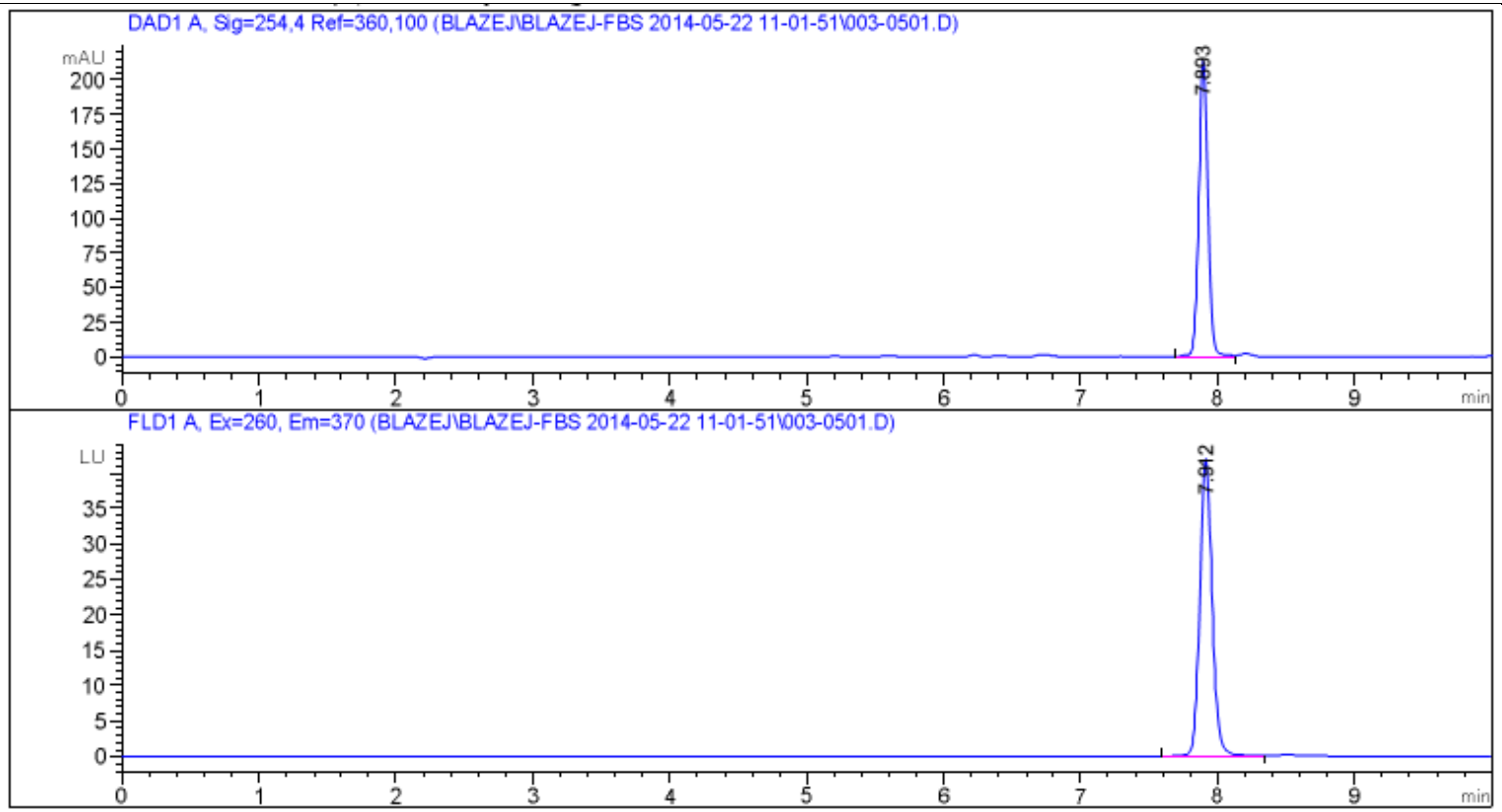
TMG-pp$_s$p-Ado-link-N$_3$ D1 (6a)

TMG-pp$_s$p-Ado-link-N$_3$ D1, FTIR ATR



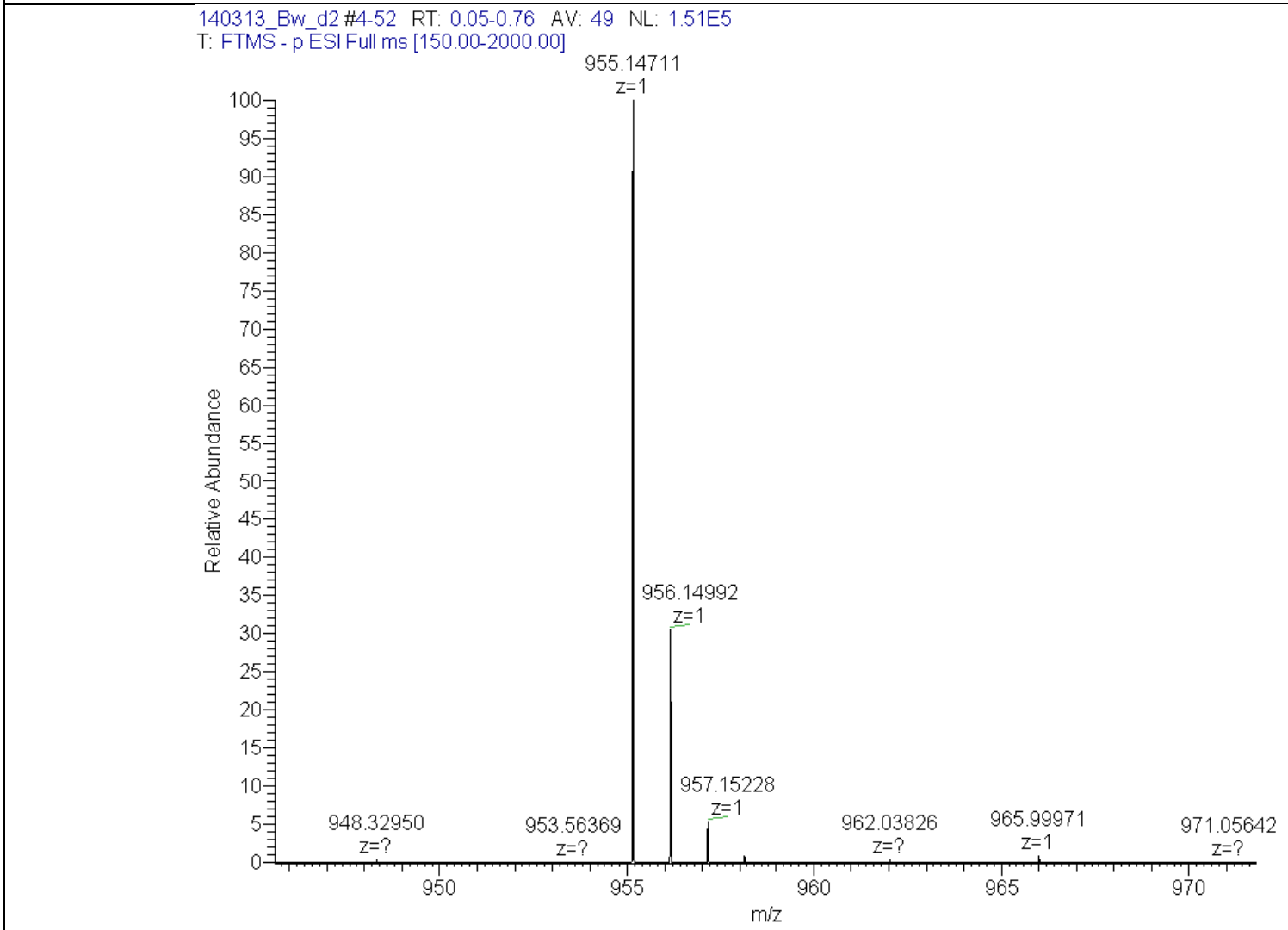
TMG-pp$_S$p-Ado-link-N$_3$ D2 (6b)





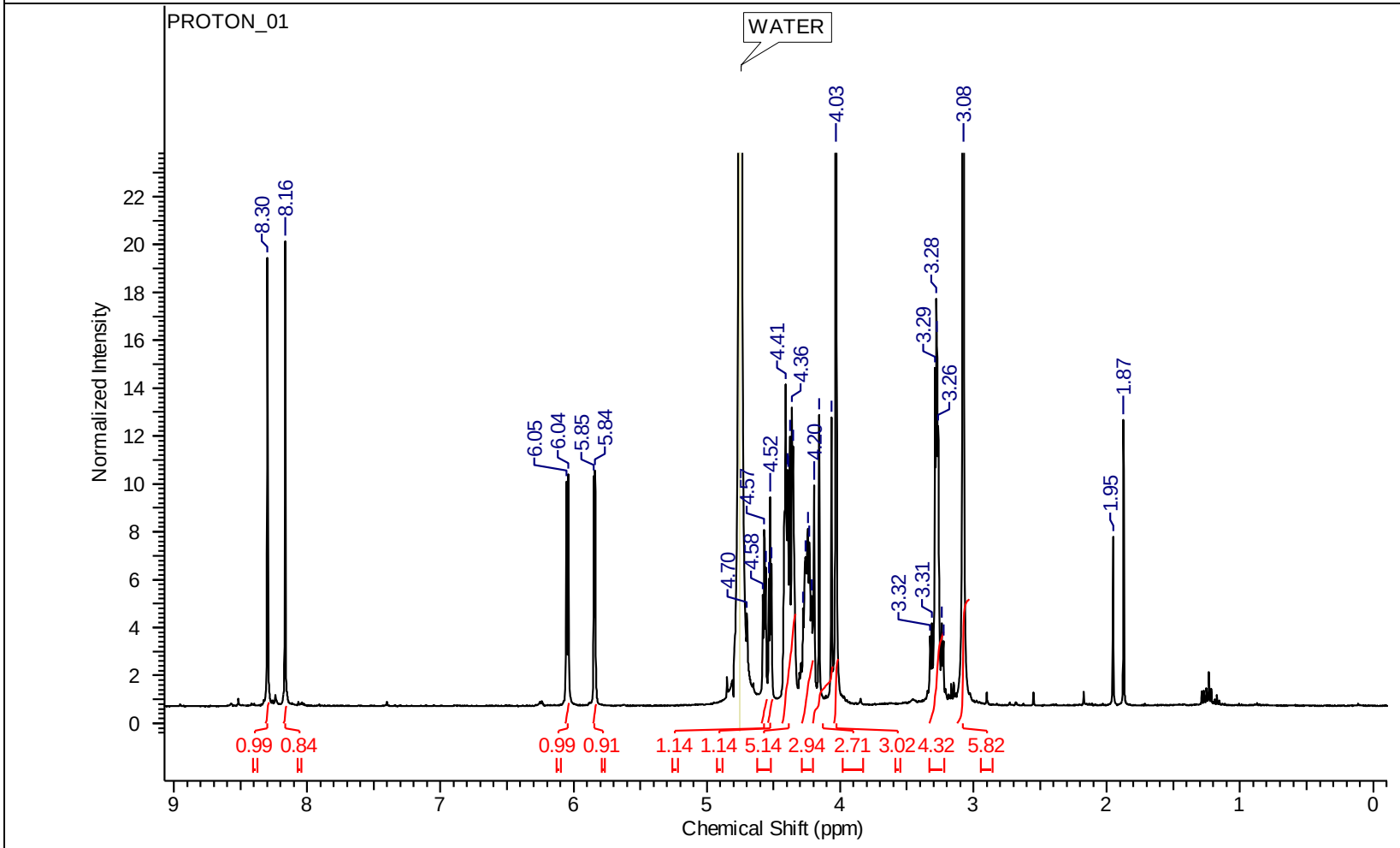
TMG-pp$_s$p-Ado-link-N$_3$ D2 (6b)

TMG-pp$_s$p-Ado-link-N$_3$ D2/NH$_4^+$ HRMS (-)ES C$_{27}$H$_{38}$N$_{14}$O$_{17}$P$_3$S, 955.14711 [M-2H]$^-$ Exact Mass: 955.14729



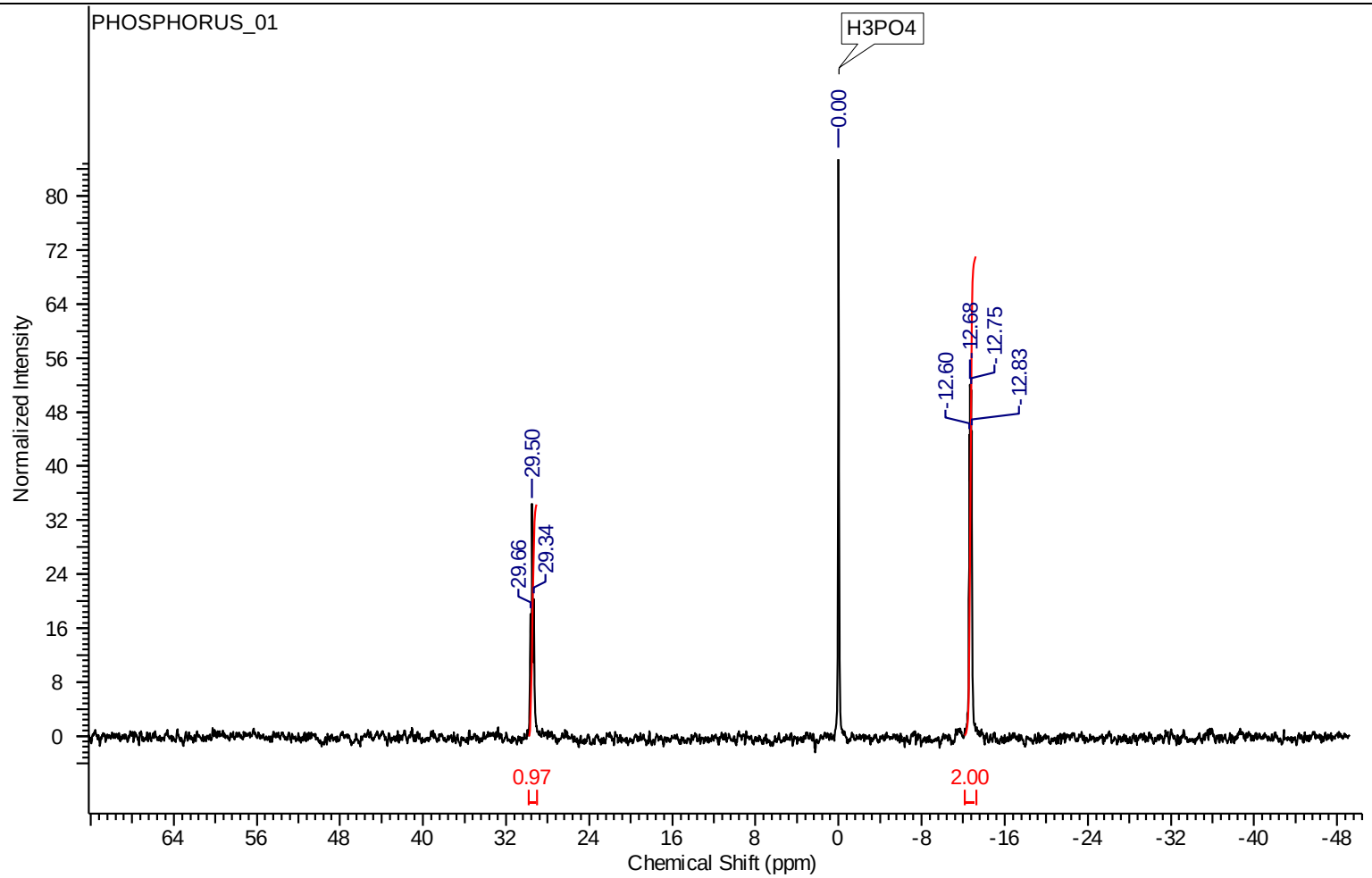
TMG-pp$_s$p-Ado-link-N$_3$ D2 (6b)

TMG-pp$_s$p-Ado-link-N$_3$ D2 ^{1}H NMR

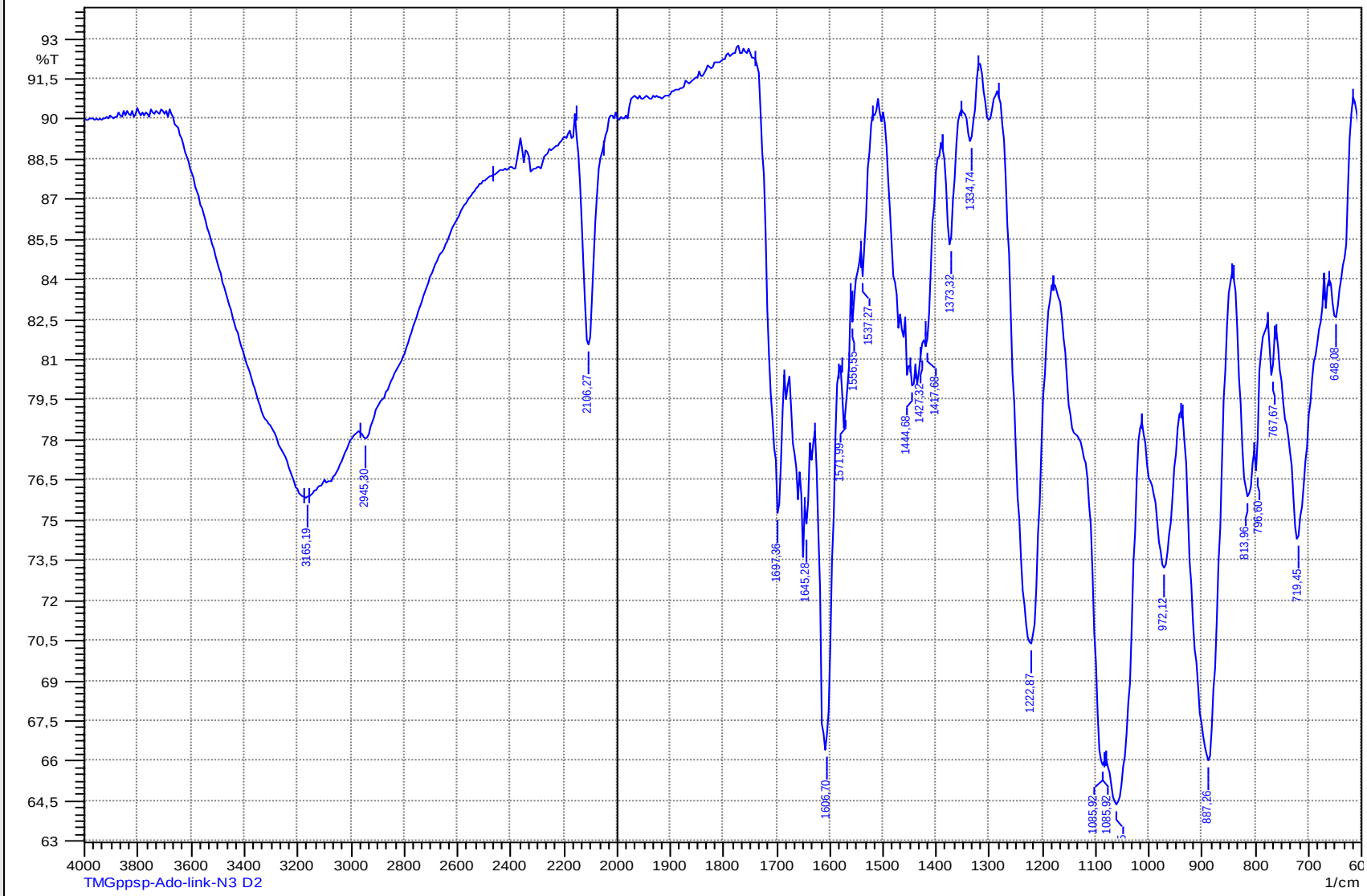


TMG-pp$_s$p-Ado-link-N$_3$ D2 (6b)

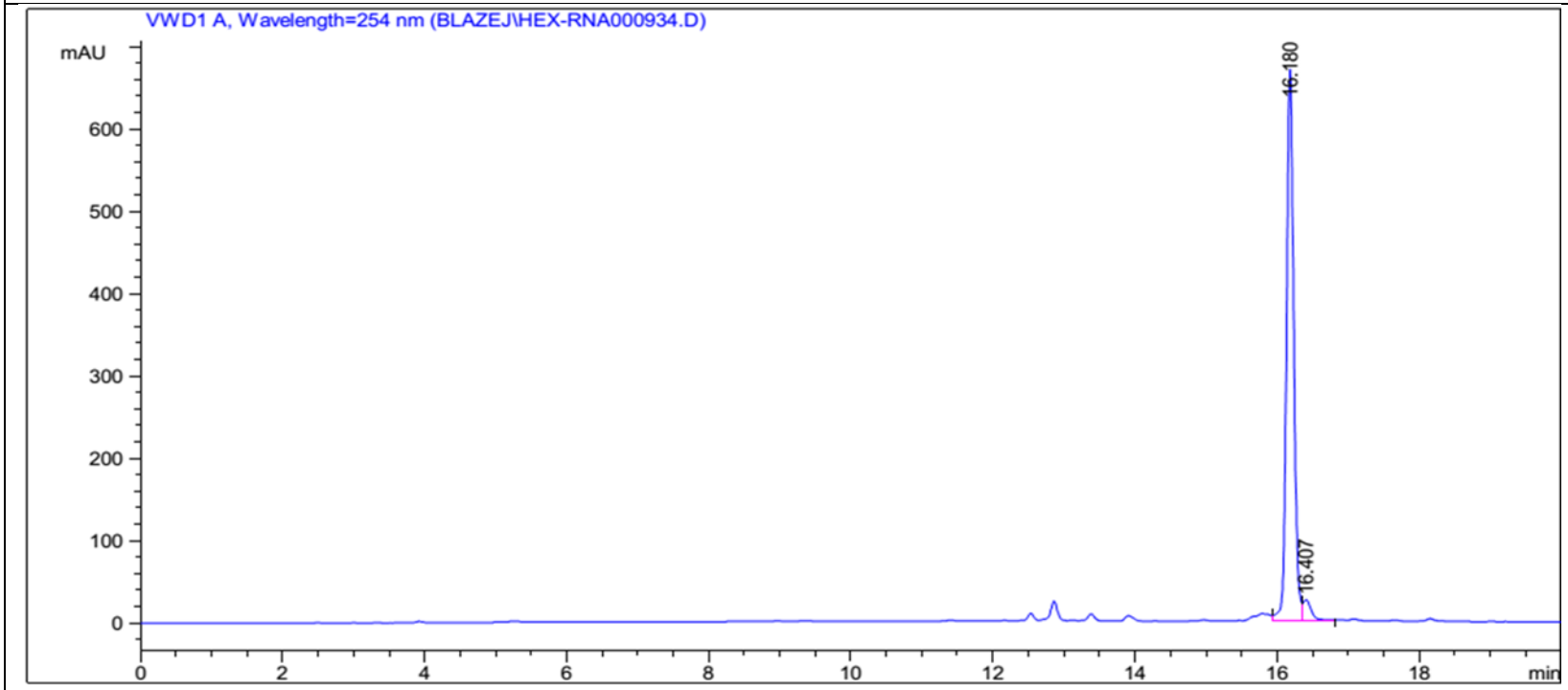
TMG-pp$_s$p-Ado-link-N$_3$ D2 ^{31}P NMR

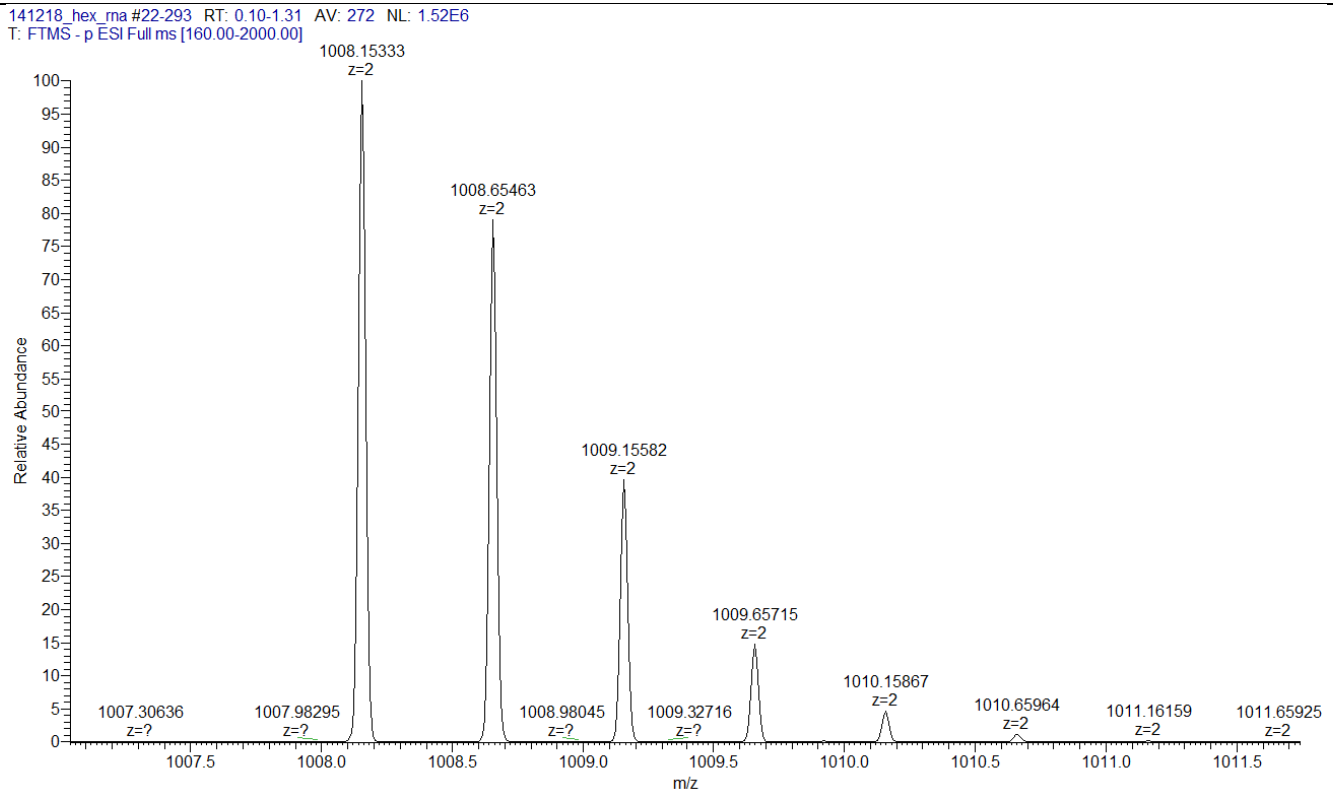


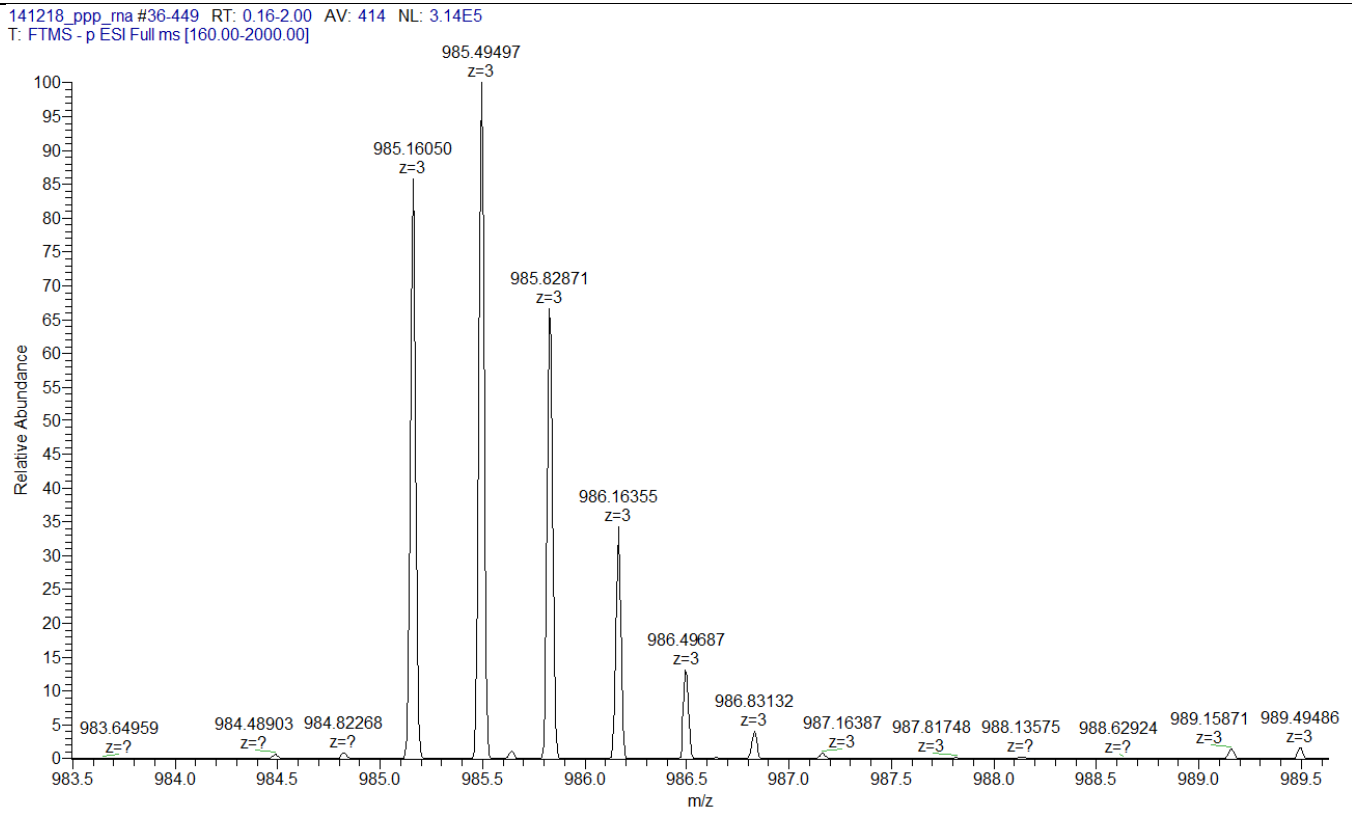
TMG-pp$_s$p-Ado-link-N$_3$ D2 (6b)

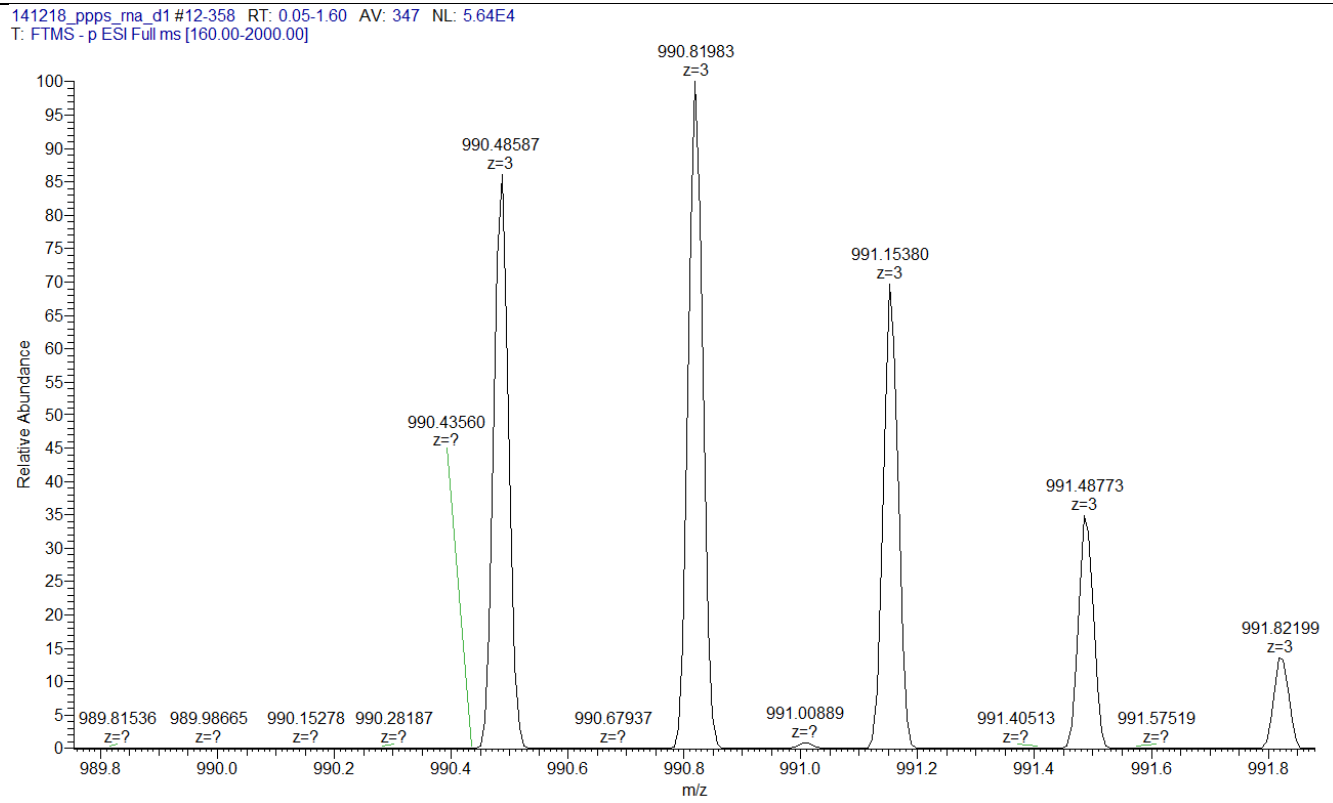


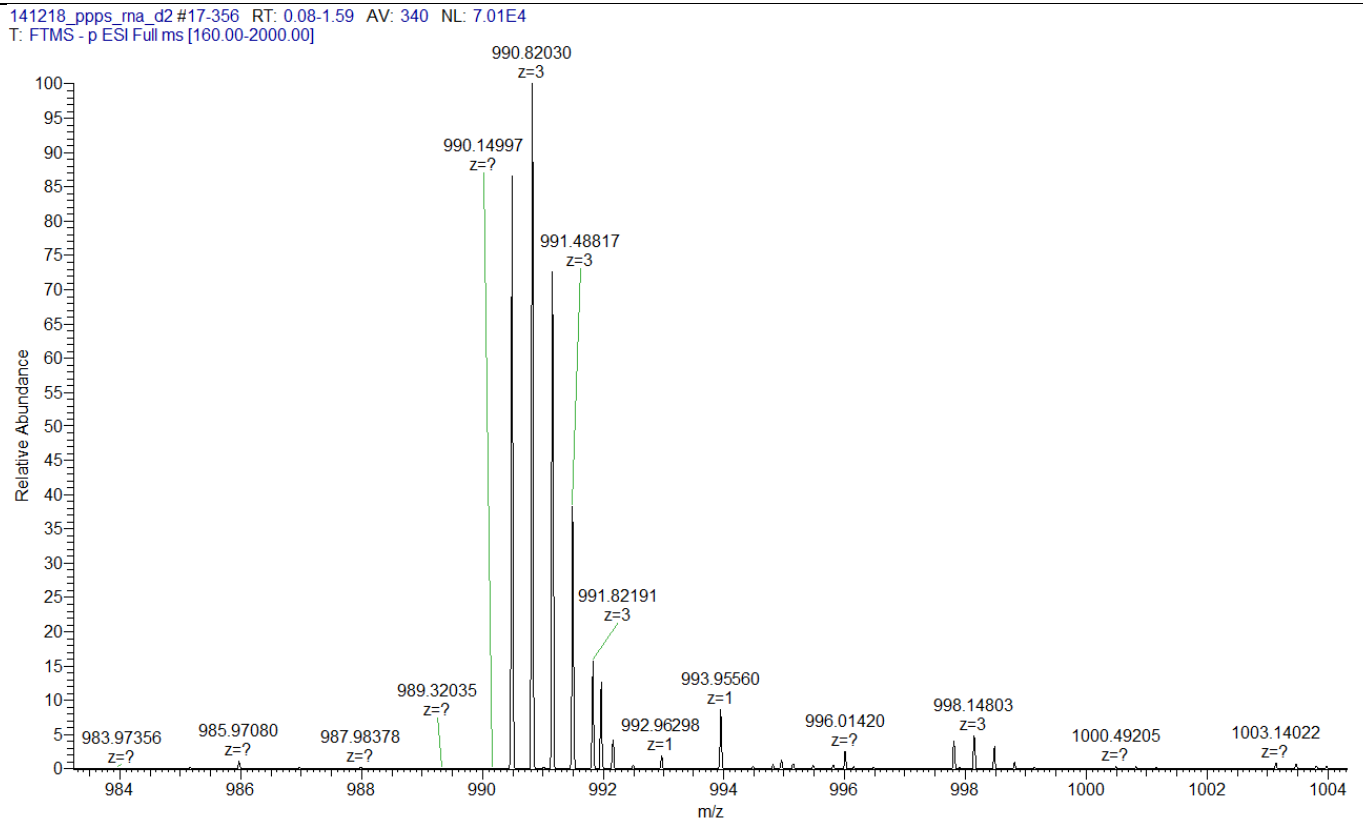
hex-GCUAAU (21)	**RP-HPLC profile of crude hex-GCUAAU (21)**

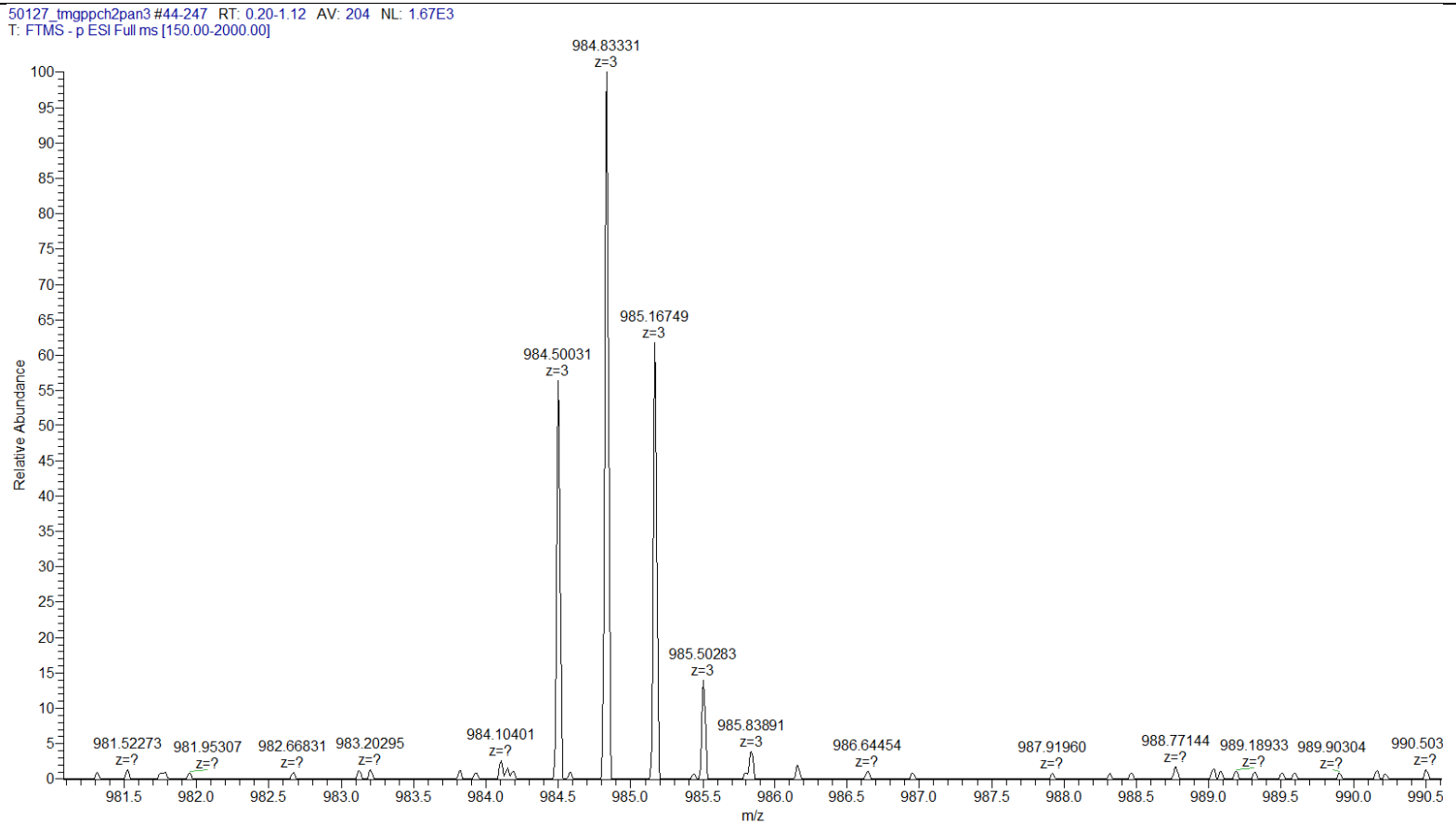


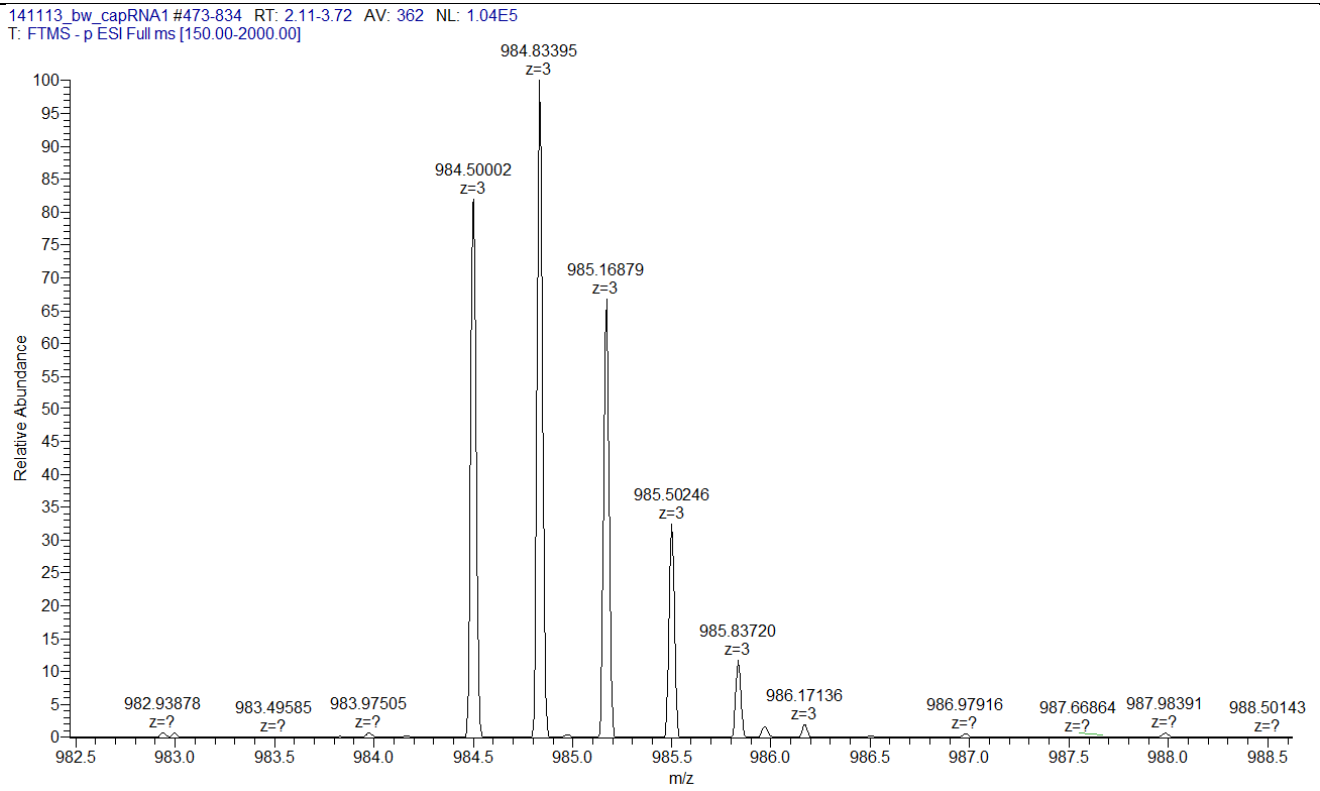
HRMS ESI (-)of TMG capped oligonucleotides		
No.		**Monoisotopic mass**
21		*hex*-RNA $C_{63}H_{78}N_{22}O_{43}P_6^{-2}$ **1008.15149**

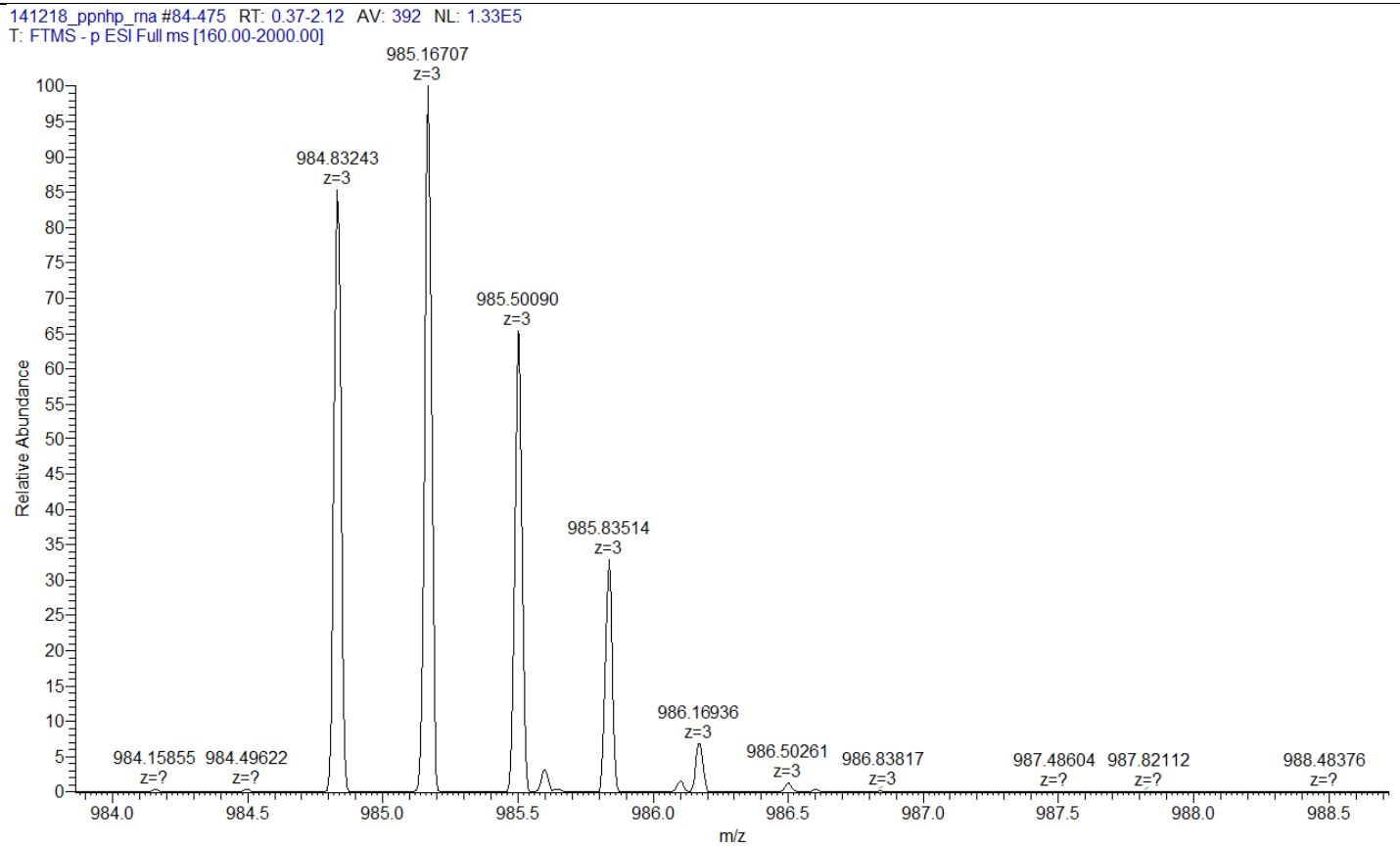
22		TMGpppA-linker-GCUAAU $C_{90}H_{116}N_{36}O_{61}P_9^{-3}$ **985.15789**

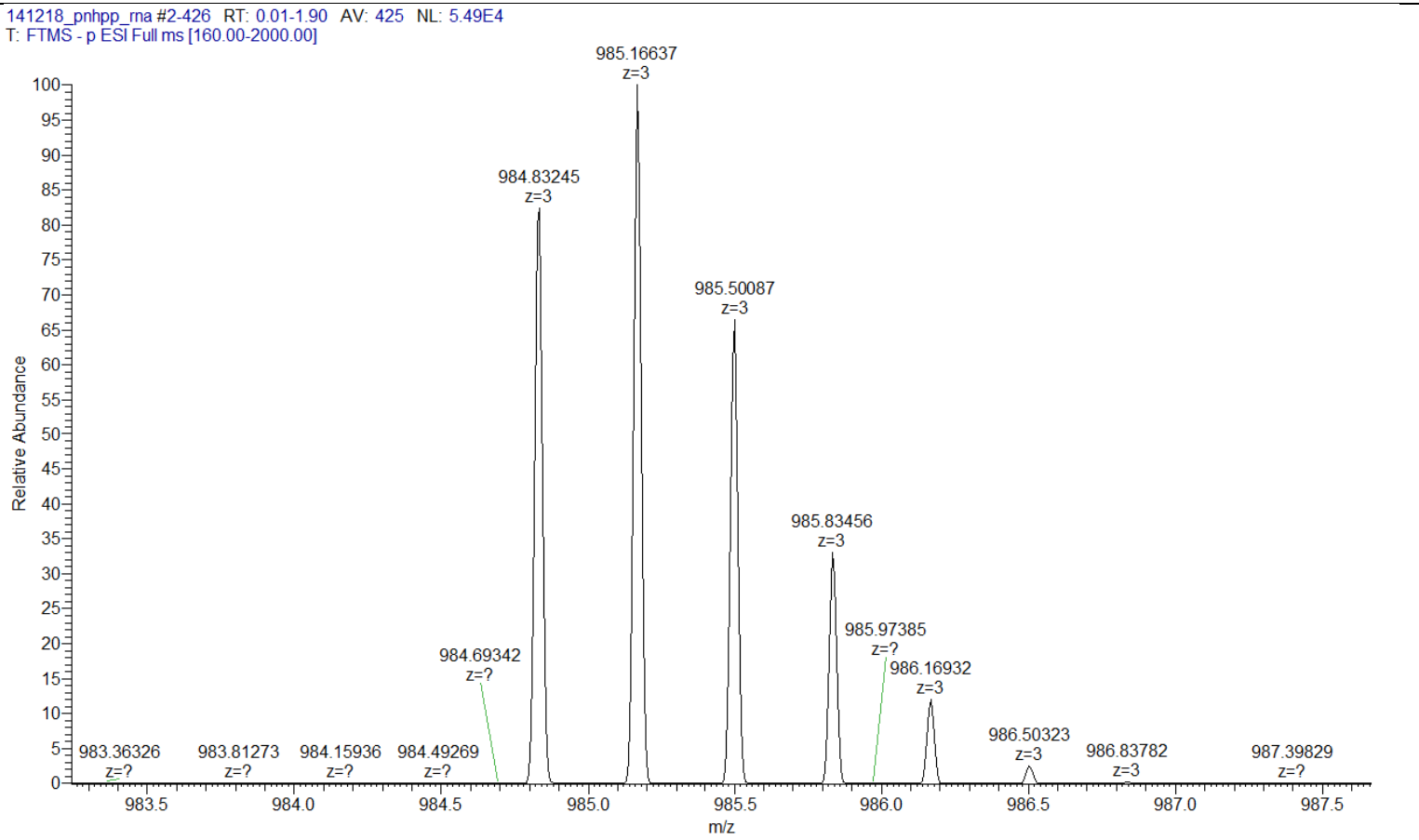
23a		TMGpp**p$_s$**A-linker-GCUAAU D1 $C_{90}H_{116}N_{36}O_{60}P_9S^{-3}$ **990.48361**

23b		TMGpp**p$_s$**A-linker-GCUAAU D2 $C_{90}H_{116}N_{36}O_{60}P_9S^{-3}$ **990.48361**

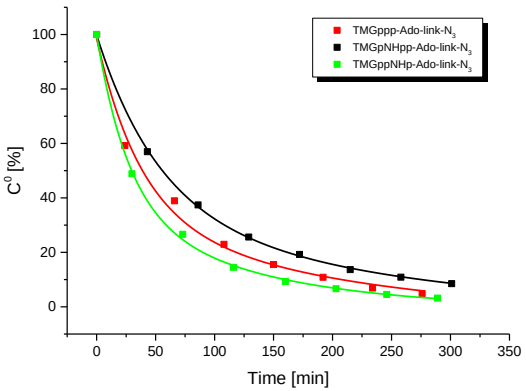
25		TMGpp**CH$_2$**pA-linker-GCUAAU $C_{91}H_{118}N_{36}O_{60}P_9^{-3}$ **984.49813**

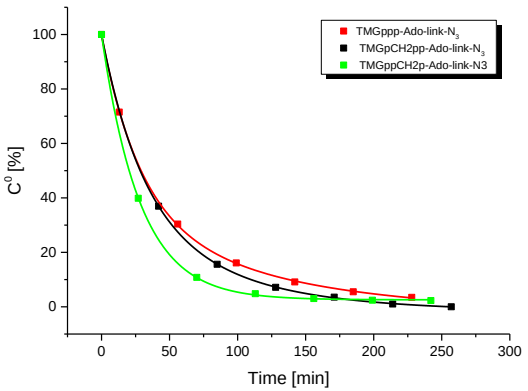
24		TMGp**CH$_2$**ppA-linker-GCUAAU $C_{91}H_{118}N_{36}O_{60}P_9^{-3}$ **984.49813**

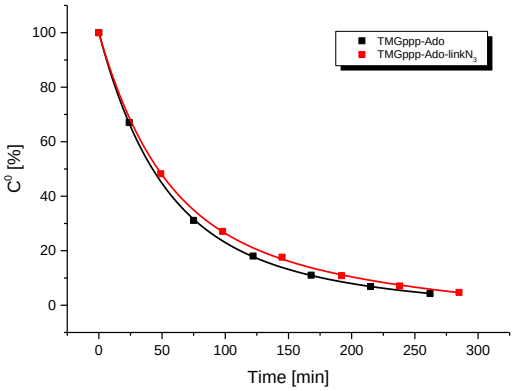
27		TMGpp**NH**pA-linker-GCUAAU $C_{90}H_{117}N_{37}O_{60}P_9^{-3}$ **984.82988**

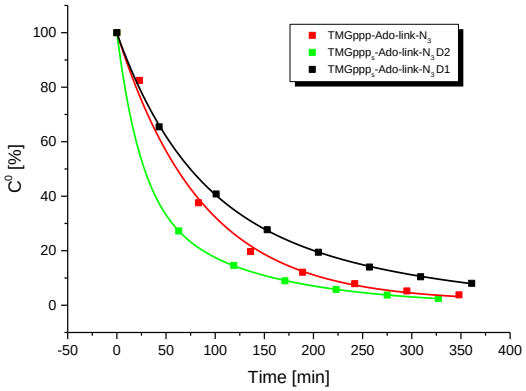
26		TMGp**NH**ppA-linker-GCUAAU $C_{90}H_{117}N_{37}O_{60}P_9^{-3}$ **984.82988**

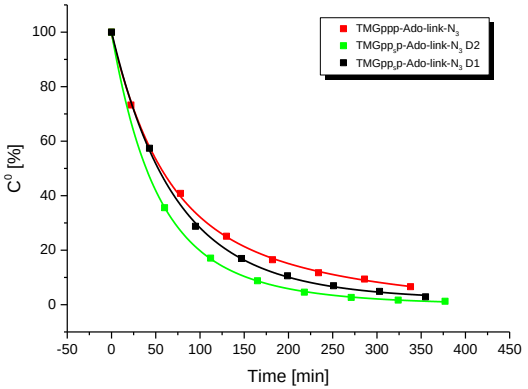
Stability studies of phosphate-modified m3G-cap analogs in FBS.






TMGppp-Ado
TMGppp-Ado-linkN3
C0 [%]
Time [min]


TMGppp-Ado-link-N3
TMGppp2-Ado-link-N3 D2
TMGppp5-Ado-link-N3 D1
C0 [%]
Time [min]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TMGpp5p-Ado-link-N3 D2
TMGpp2p-Ado-link-N3 D1
C0 [%]
Time [min]