

ExciTides: NTP-derived probes for monitoring pyrophosphatase activity based on excimer-to-monomer transitions

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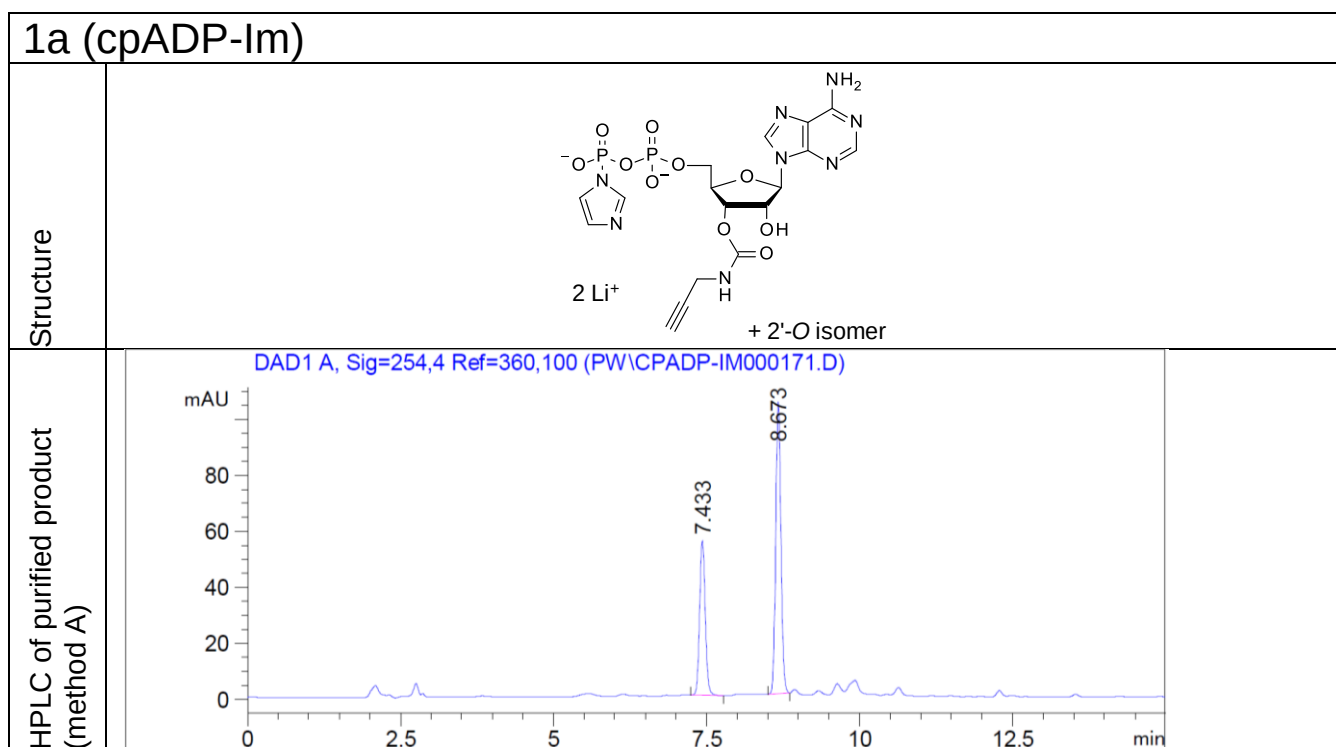
^a. Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland.

^b. Centre of New Technologies, University of Warsaw, Banacha 2c, 02-097 Warsaw, Poland Poland

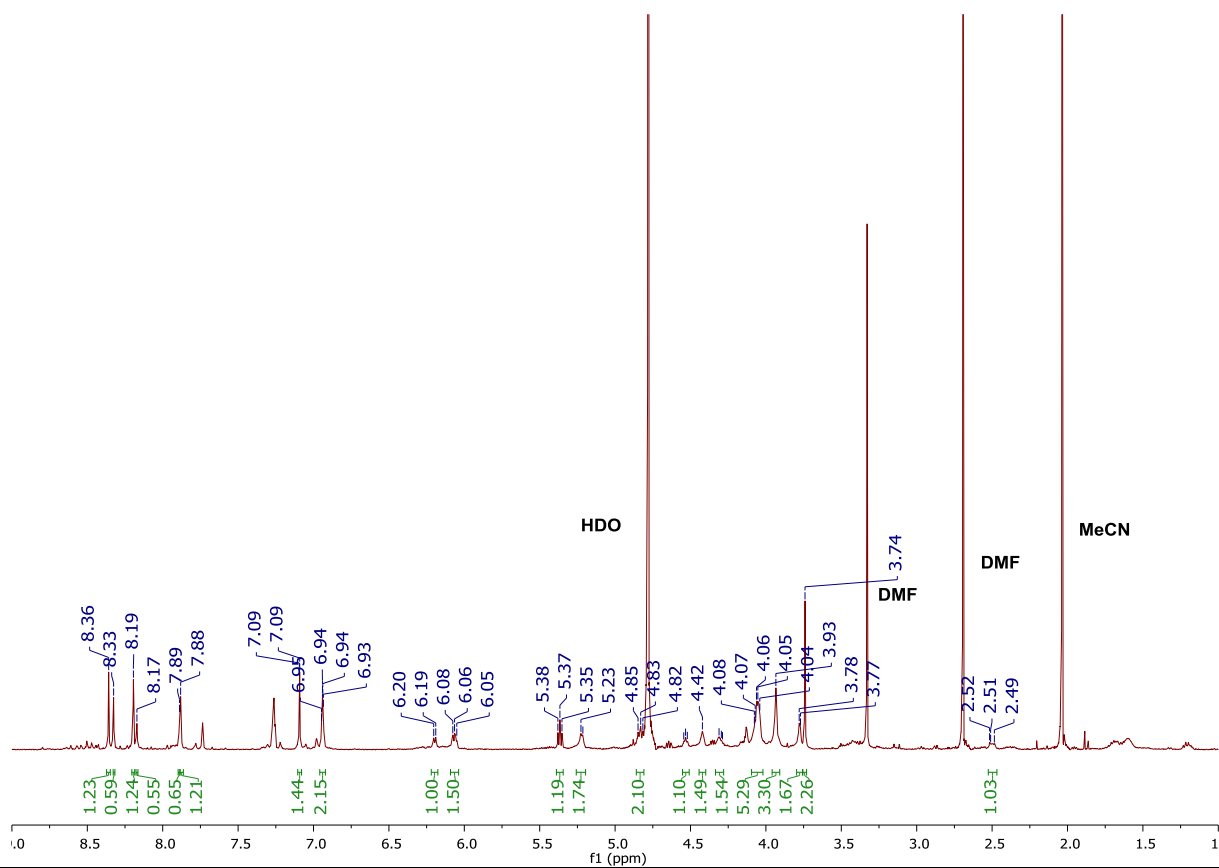
^c. College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Banacha 2c, 02-097 Warsaw

Supporting Information (SI2)

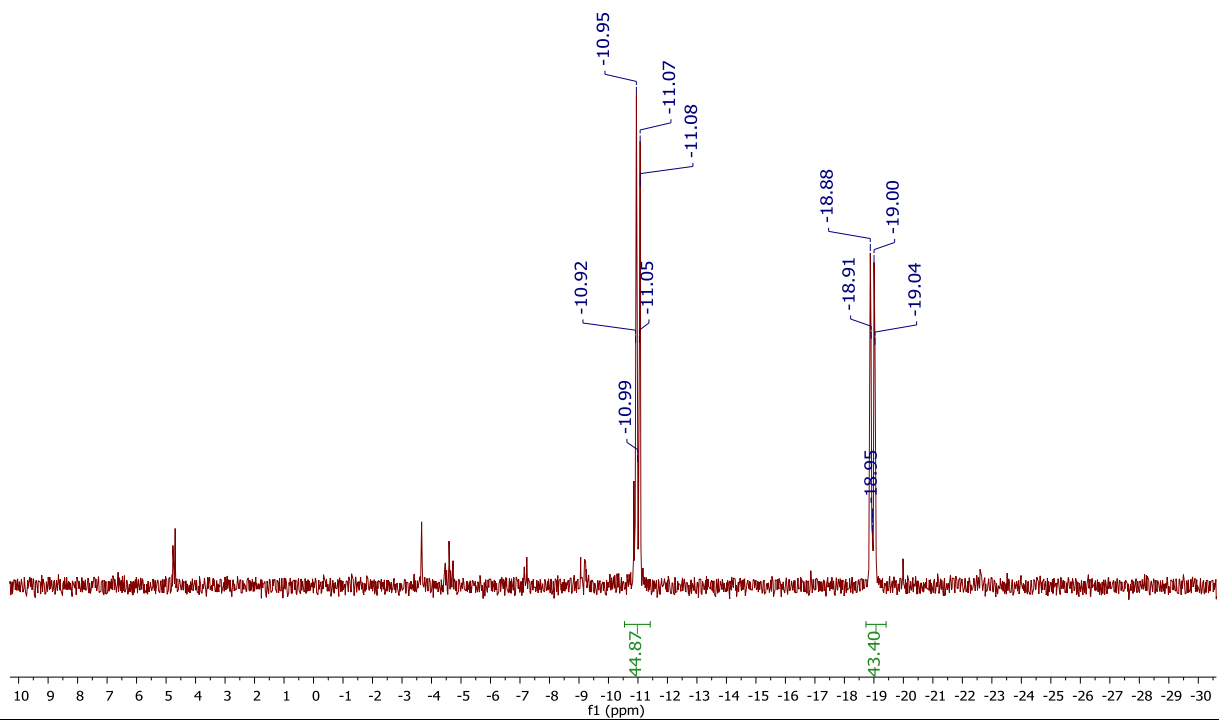
Supporting Information (SI2)



¹H NMR (500 MHz, D₂O)

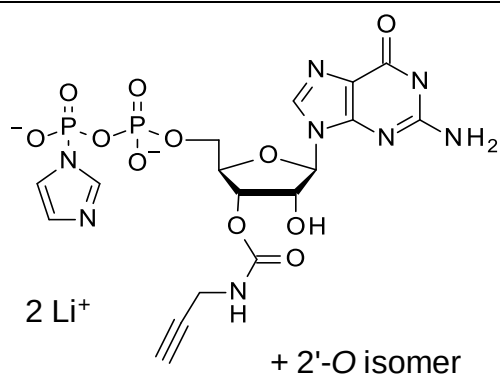


³¹P NMR (202 MHz, D₂O)

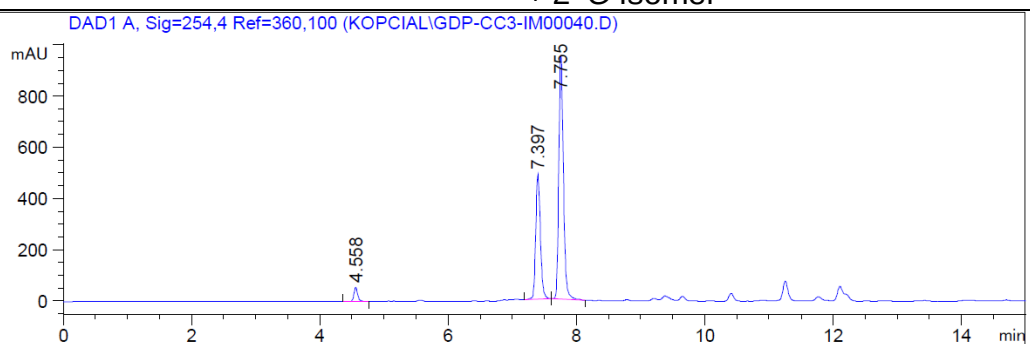


1b cpGDP-Im

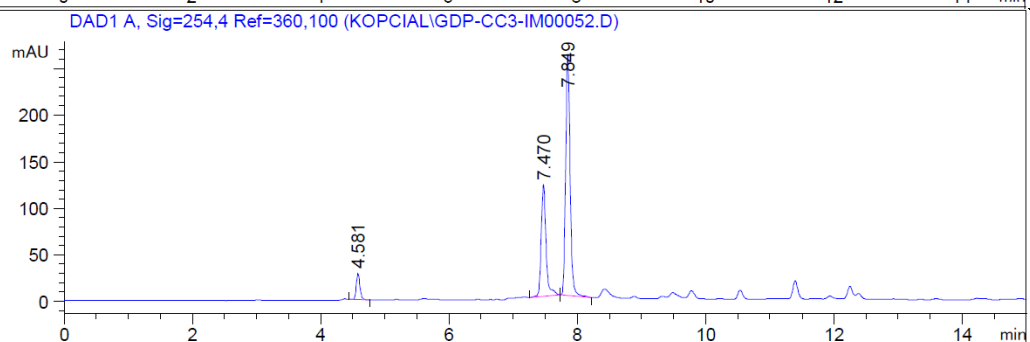
Structure



Reaction HPLC

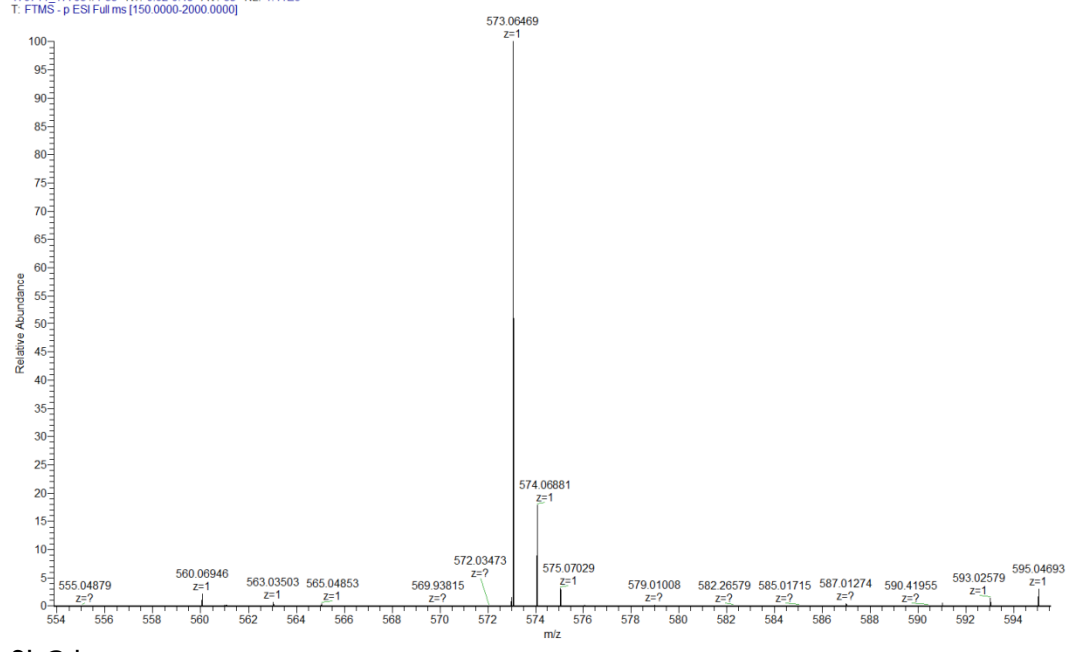


Purified HPLC



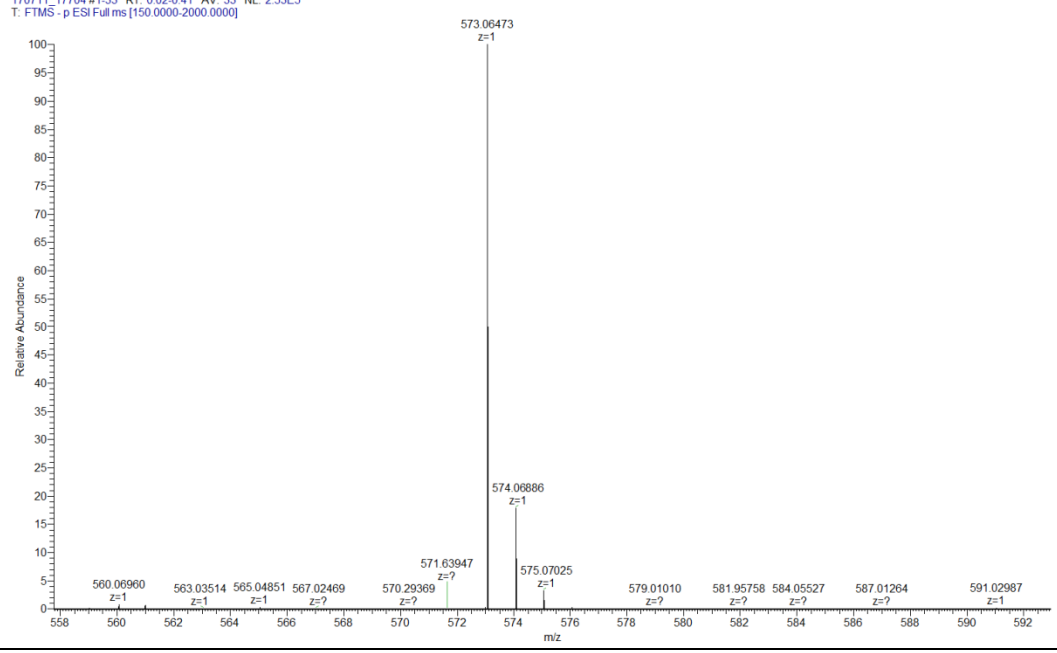
2'-O isomer:

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T: FTMS - p ESI Full ms [150.0000-2000.0000]

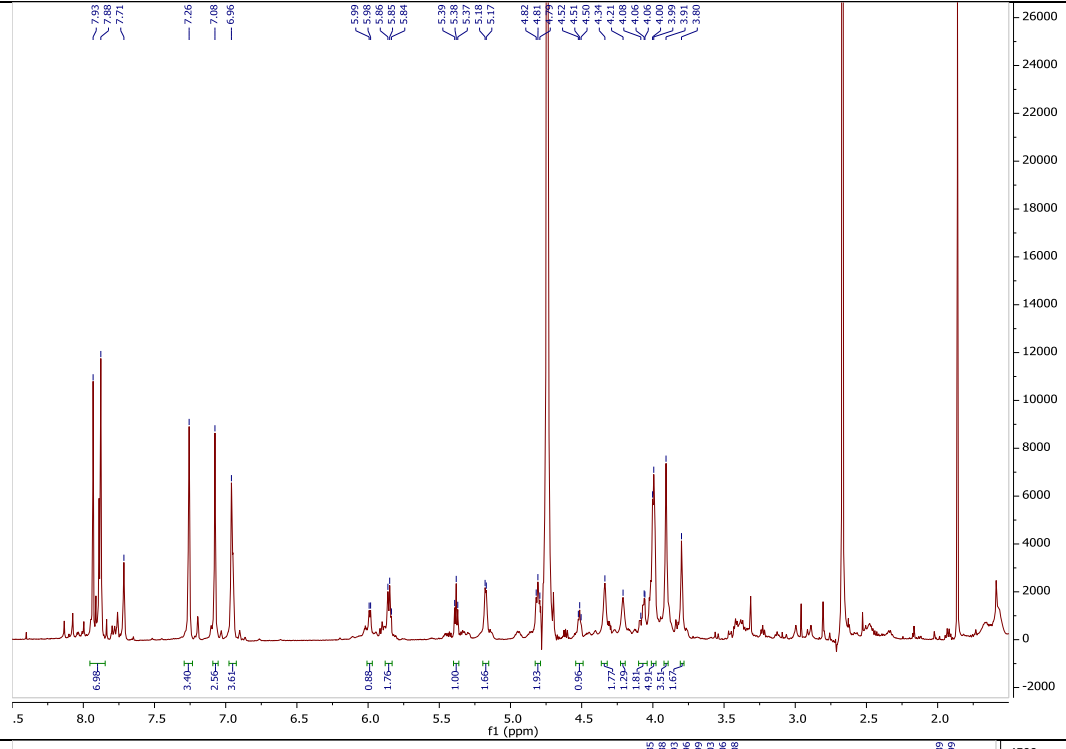


3'-O isomer:

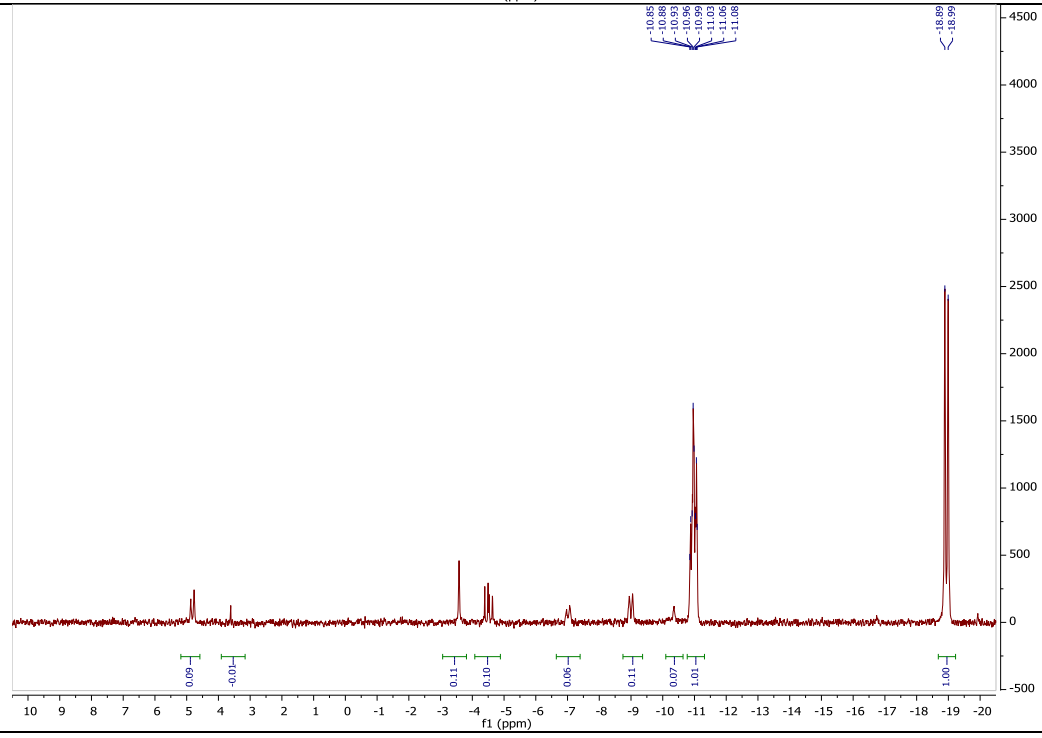
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T: FTMS - p ESI Full ms [150.0000-2000.0000]



1H NMR

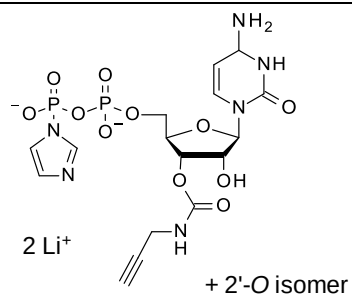


31P NMR (202 MHz)

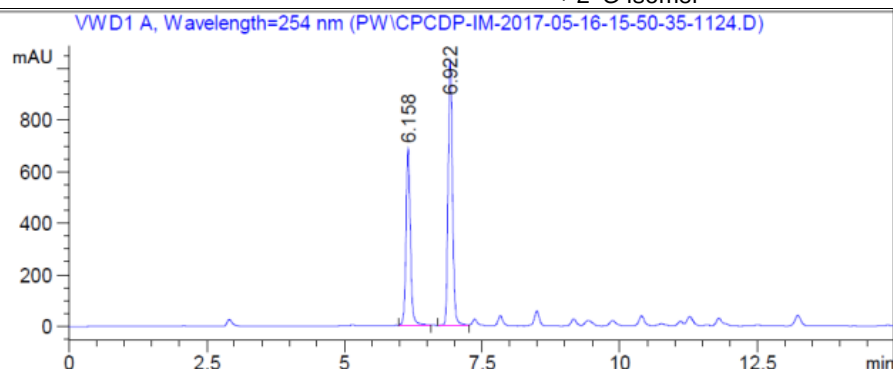


1c cpCDP-Im

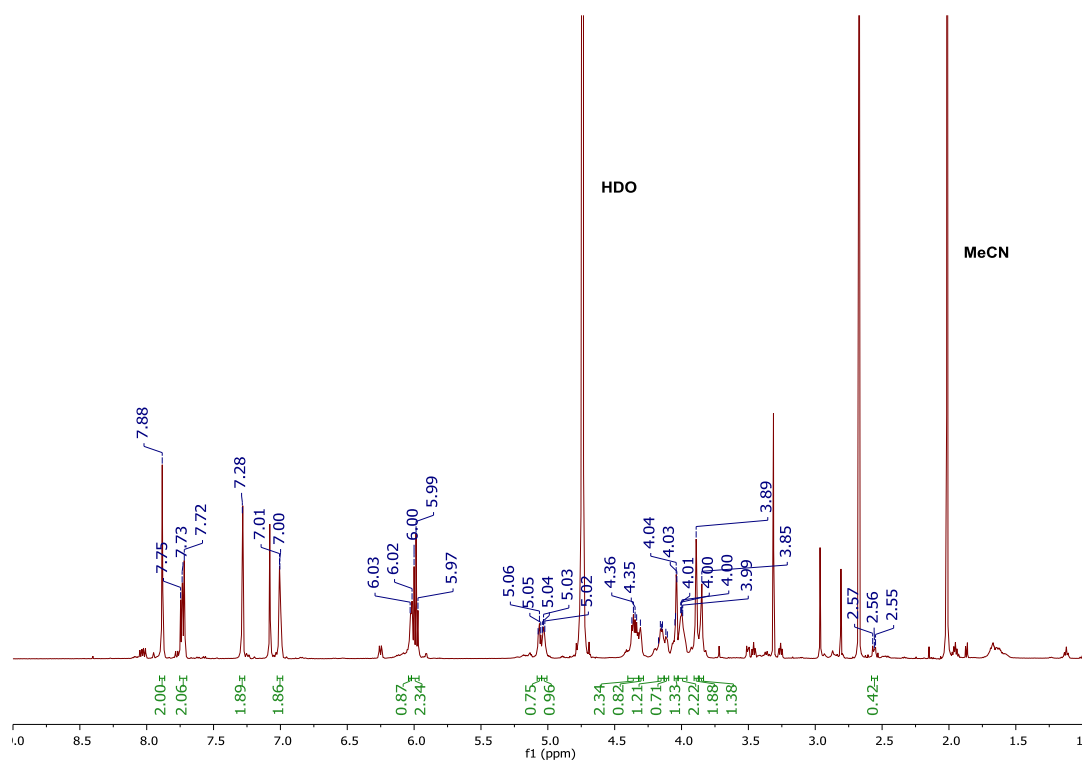
Structure

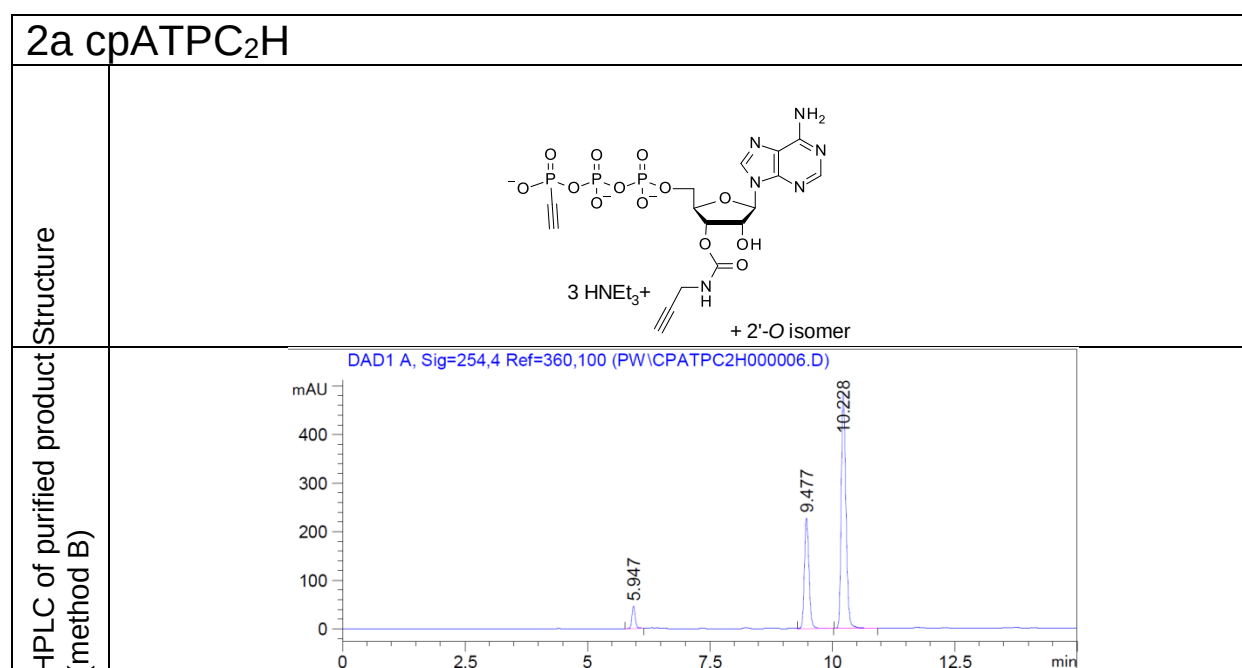
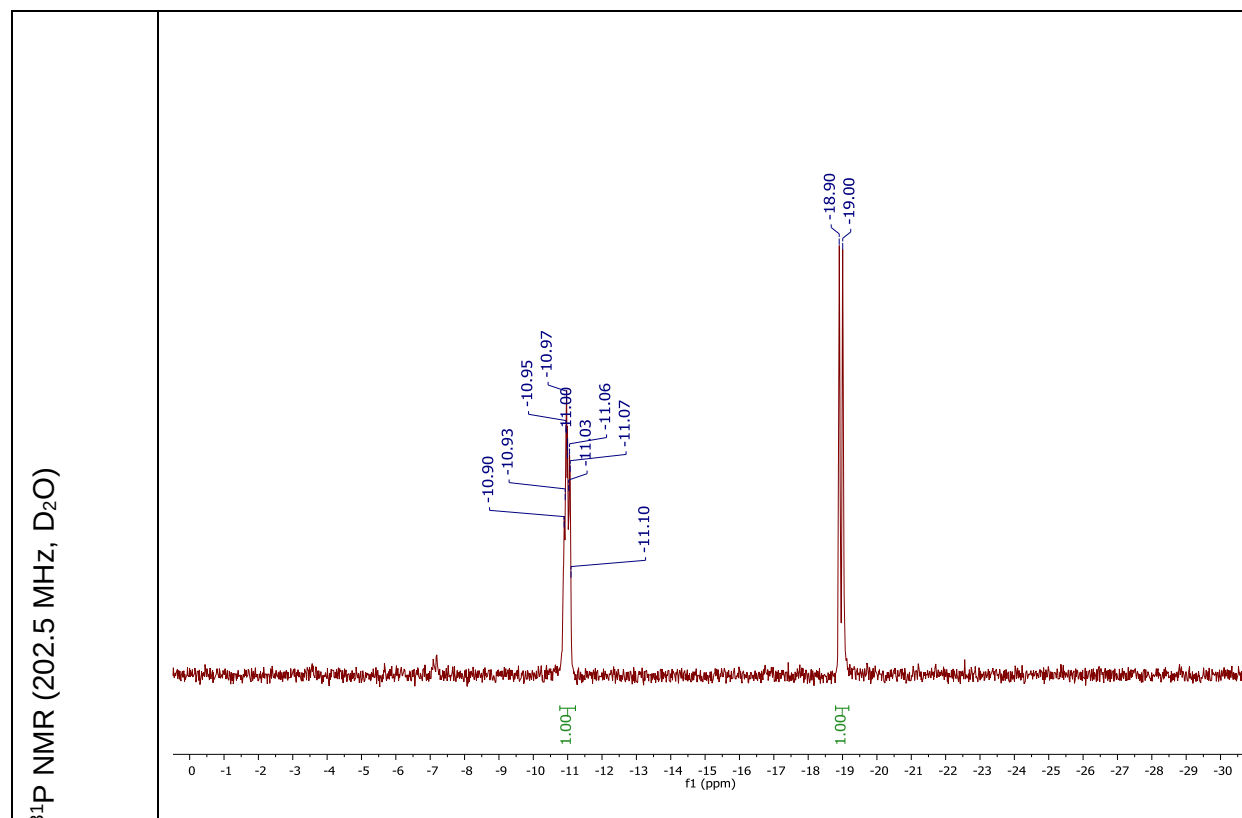


HPLC of purified product
(Method A)

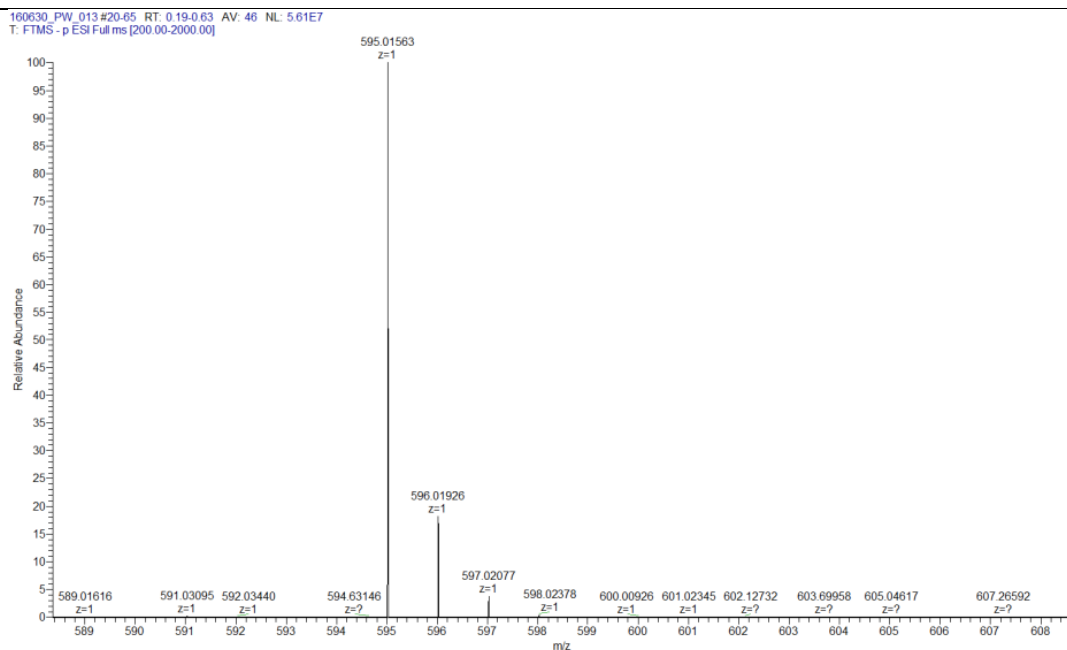


¹H NMR (500 MHz, D₂O)

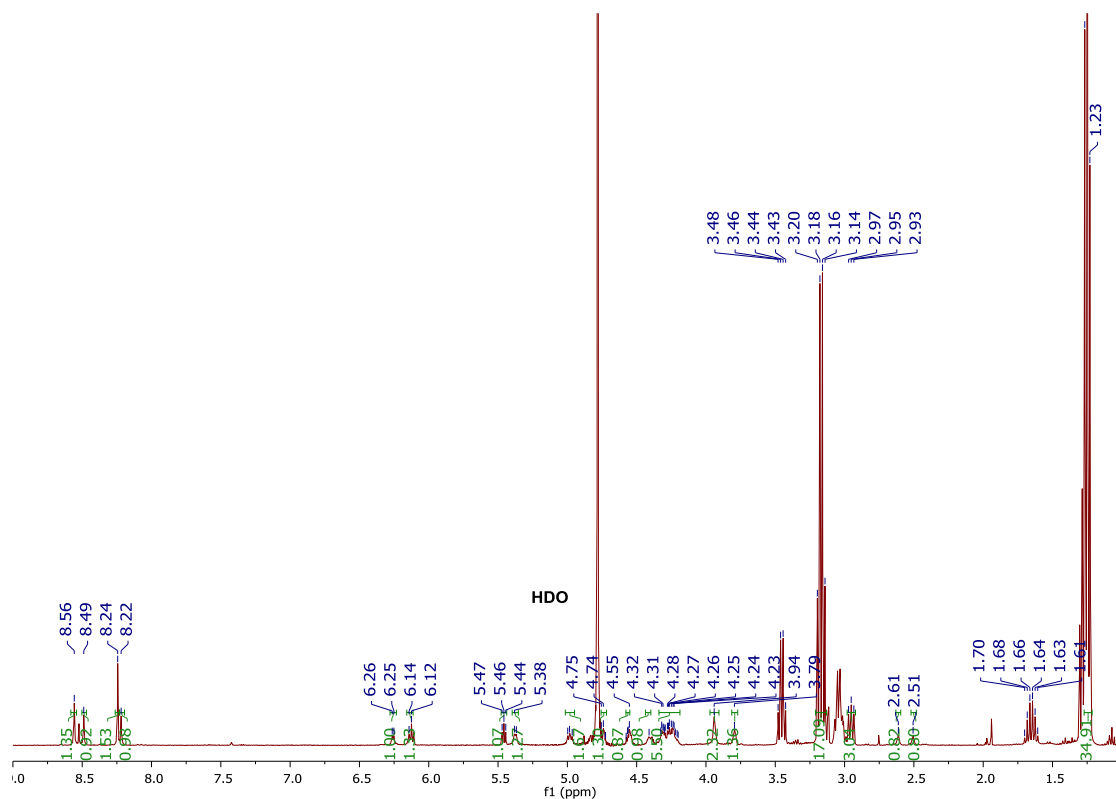


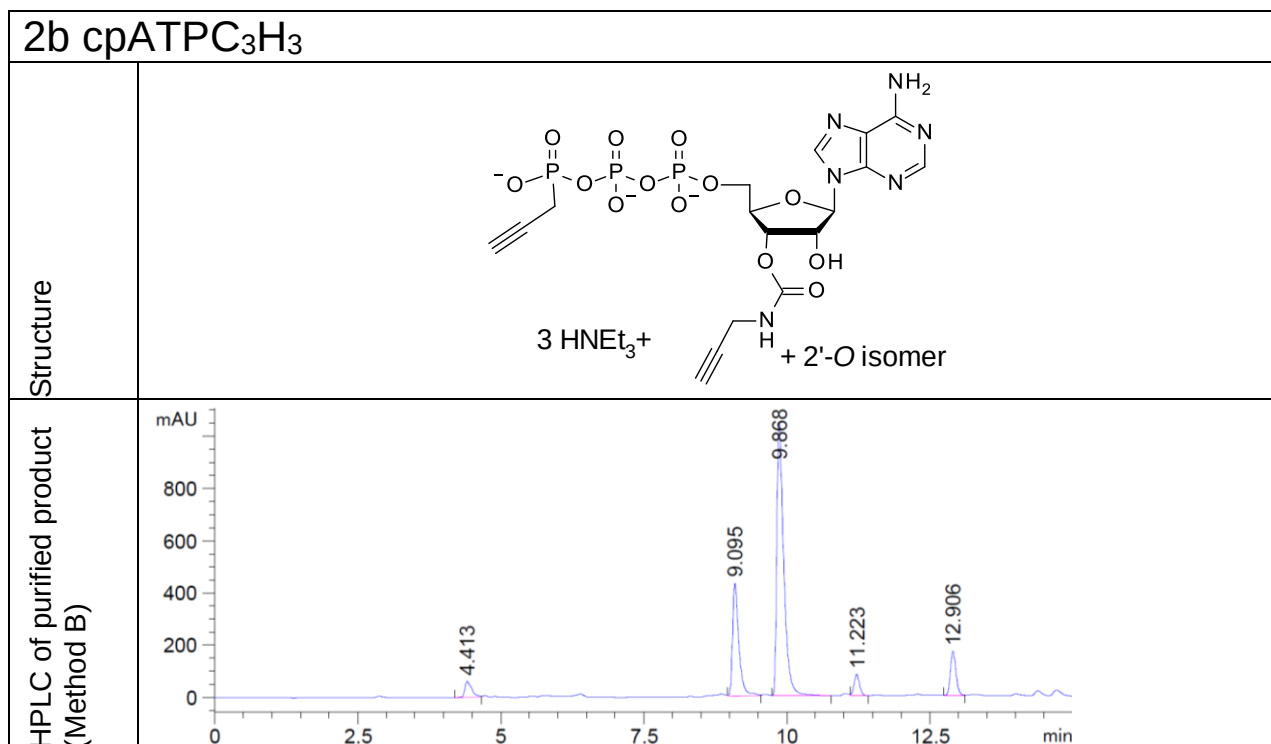
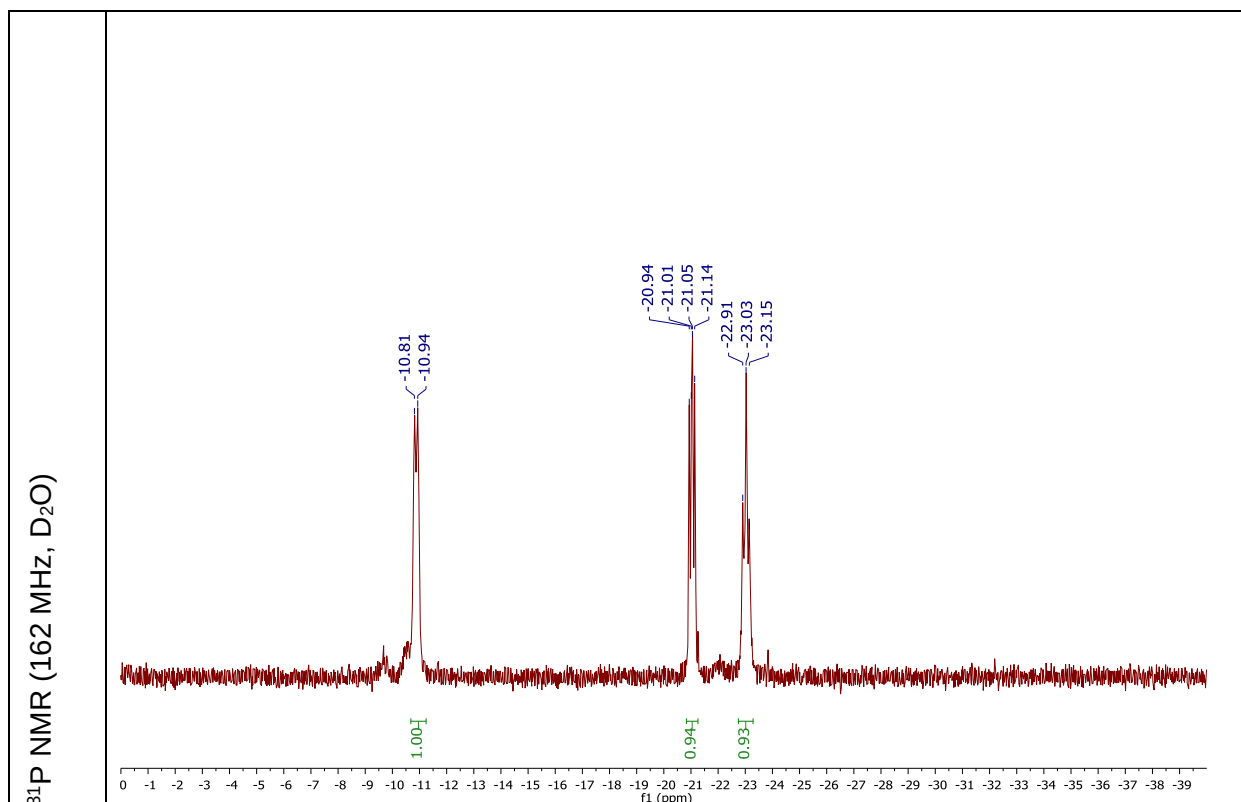


HR MS

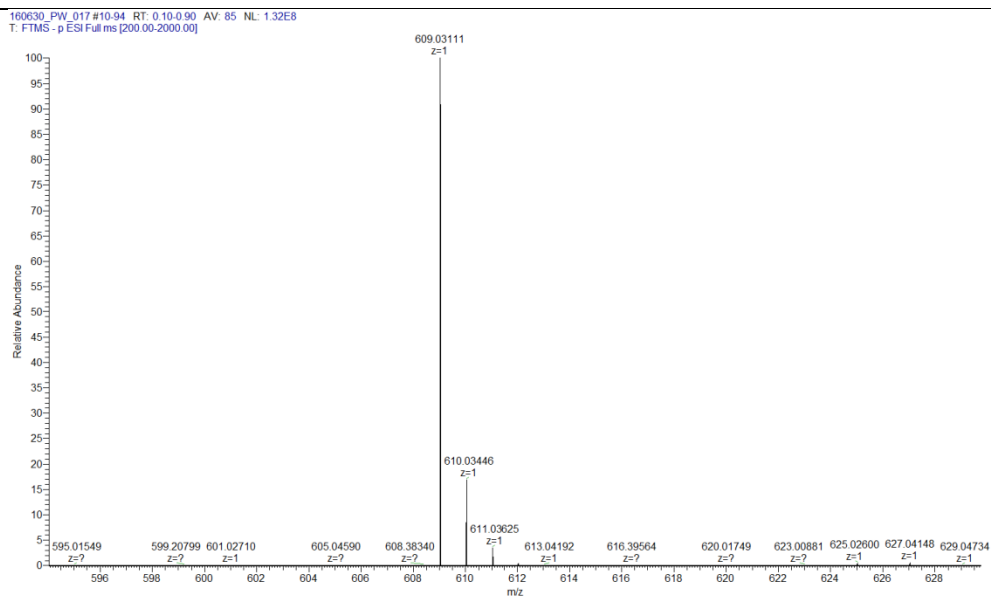


¹H NMR (400 MHz, D₂O)

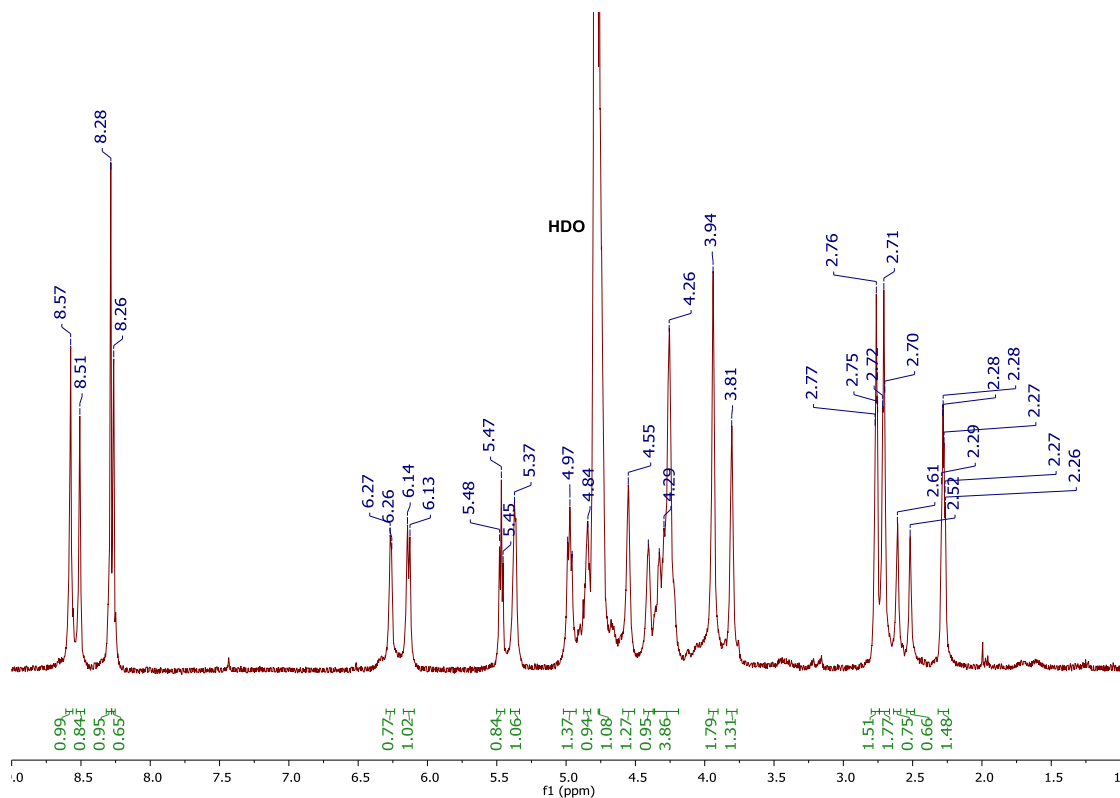


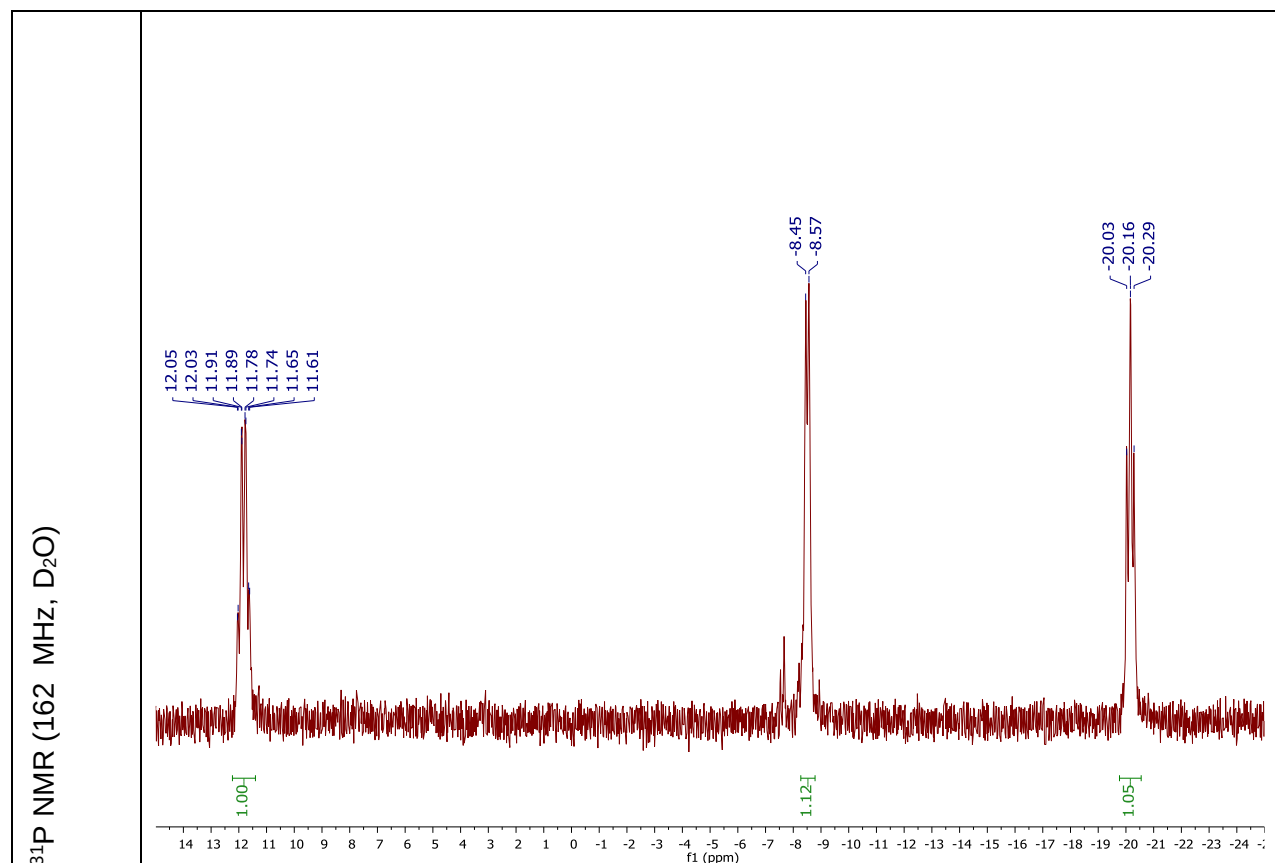


HR MS



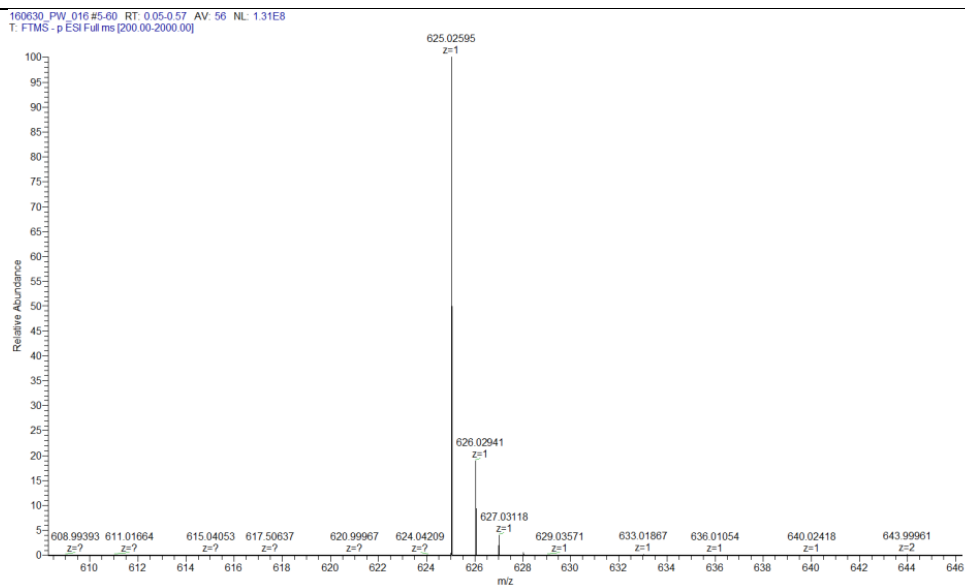
¹H NMR (400 MHz, D₂O)



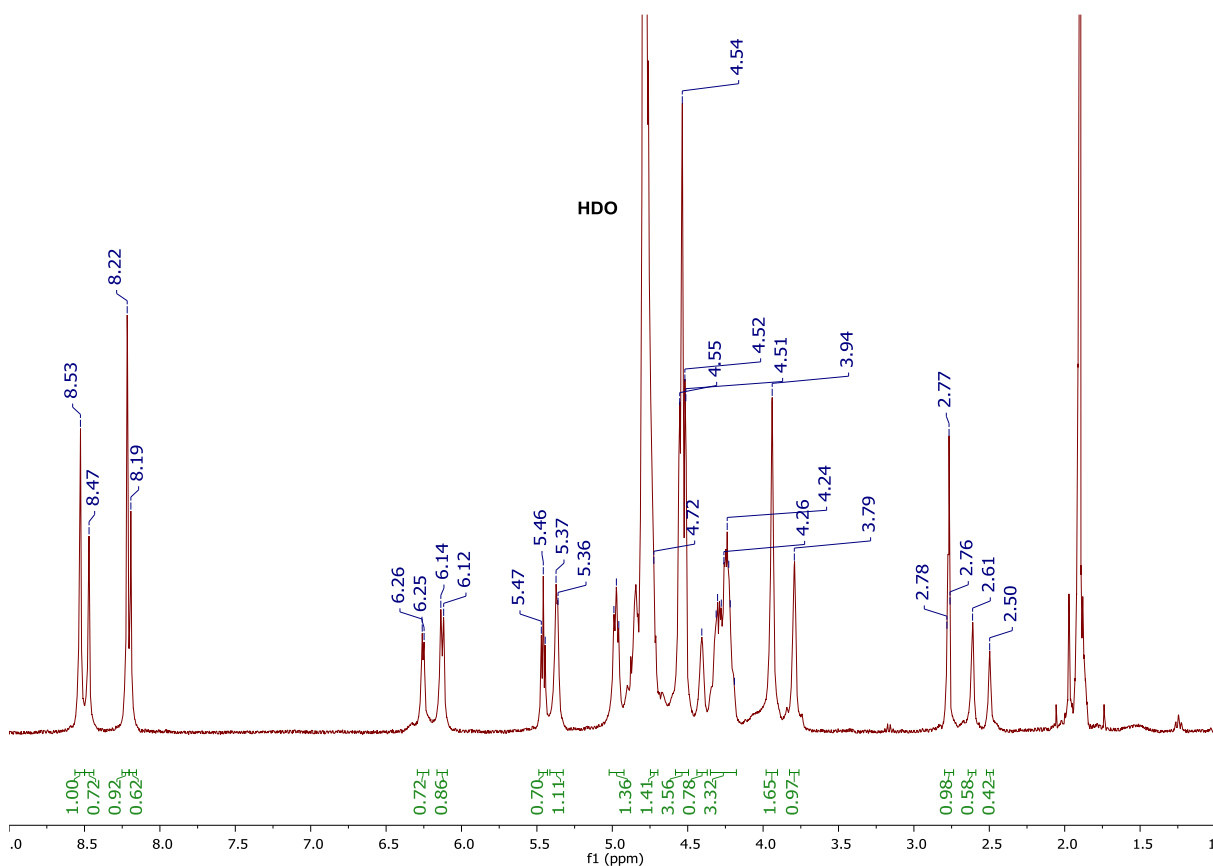


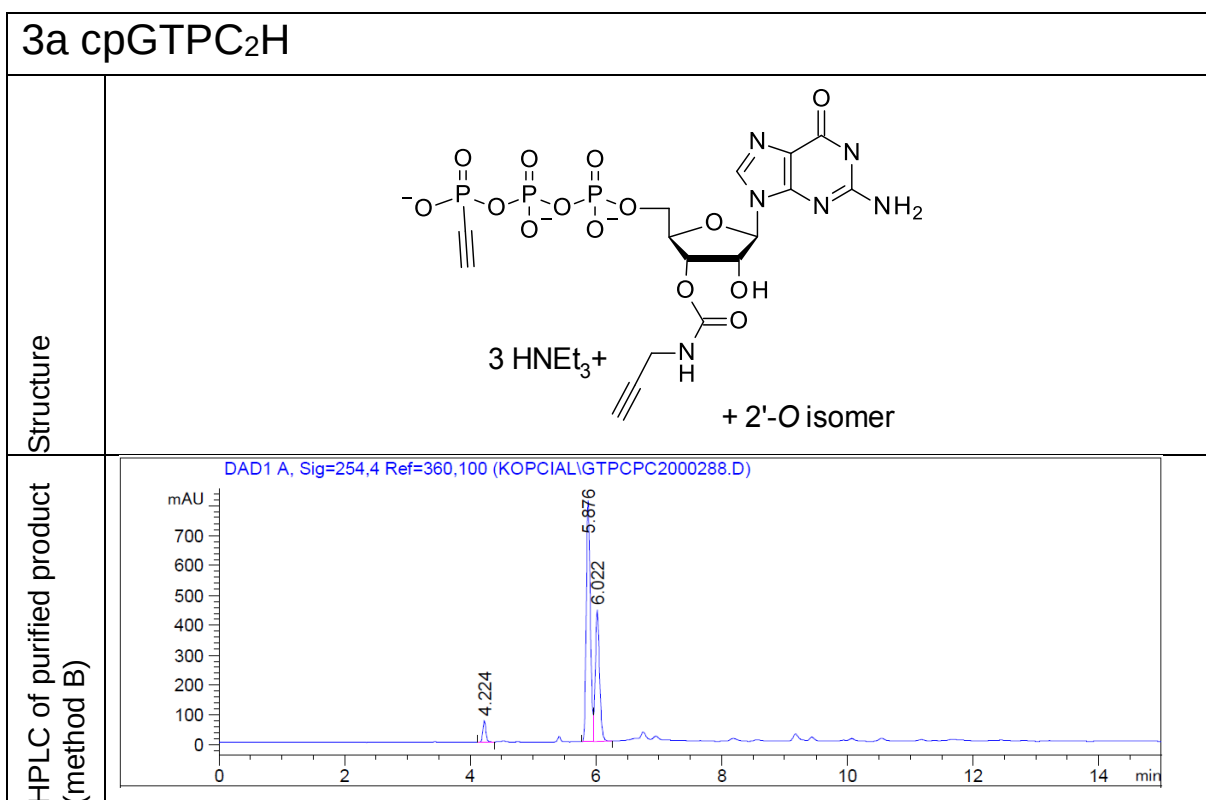
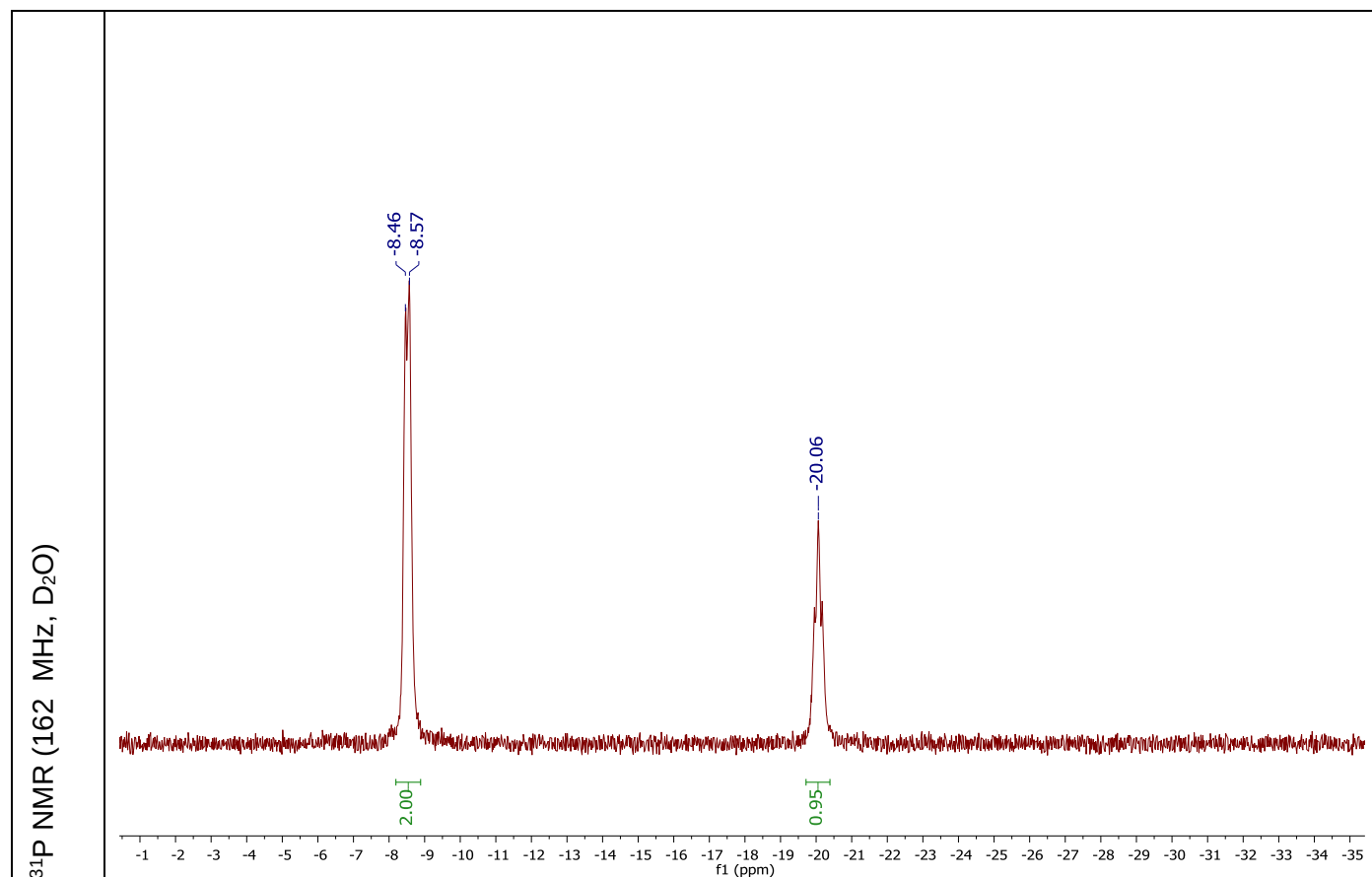
2c cpATPOC ₃ H ₃	
Structure	3 HNEt₃⁺ + 2'-O isomer
HPLC of purified product (Method B)	DAD1 A, Sig=254,4 Ref=360,100 (PW\CPATPOC3H300132.D) Peak 1: 10.395 min Peak 2: 11.475 min

HR MS

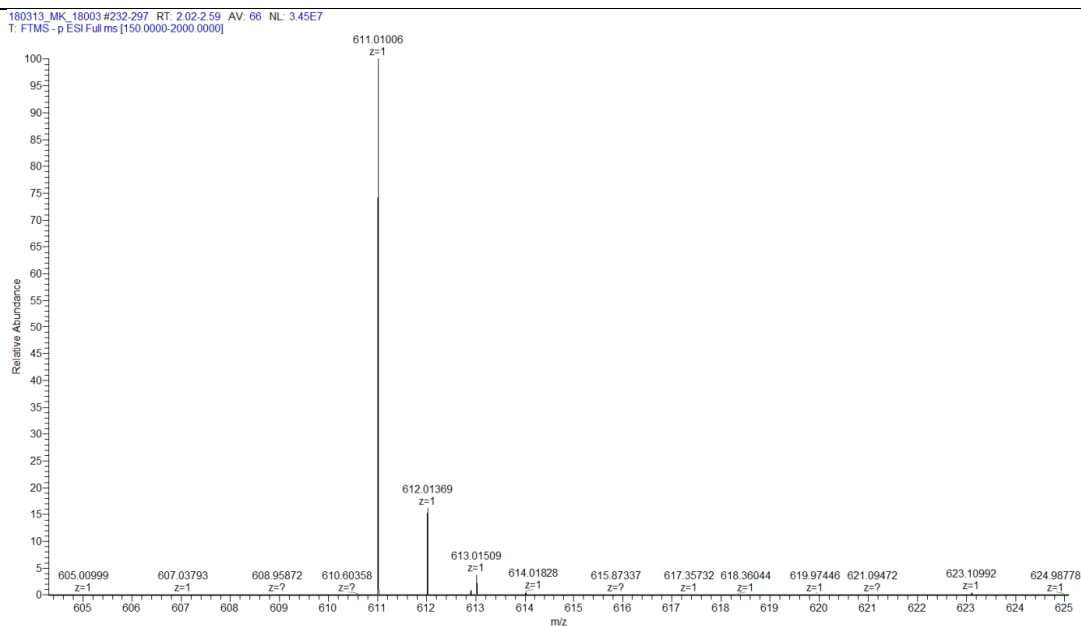


¹H NMR (400 MHz, D₂O)

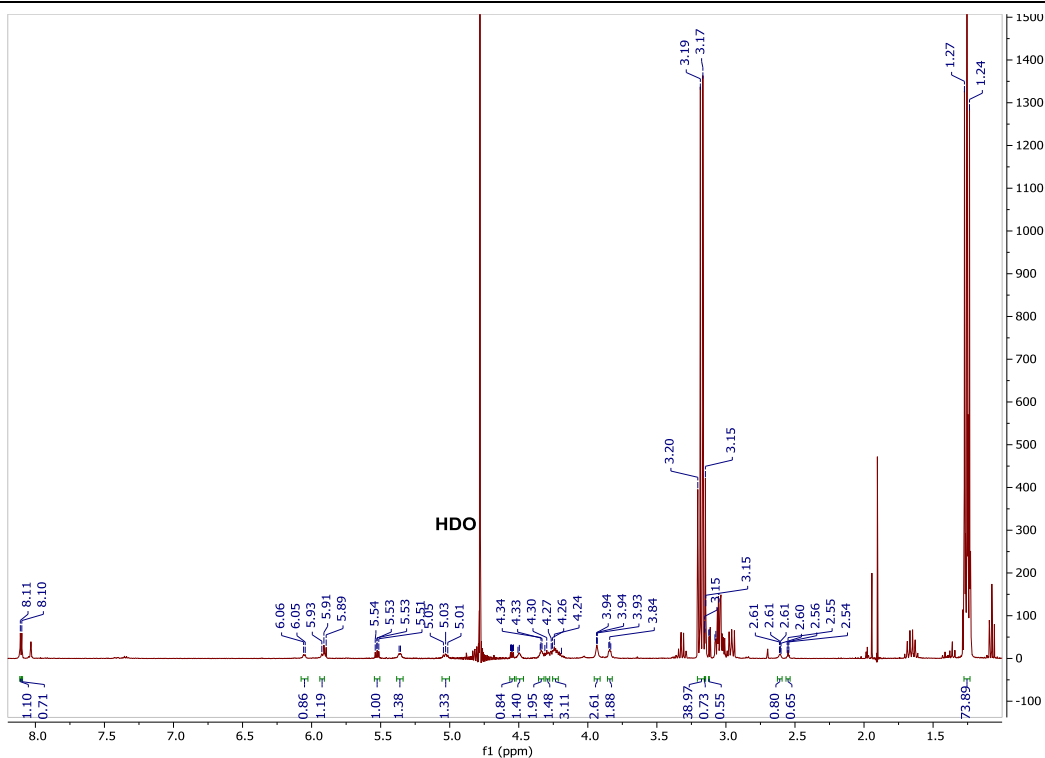


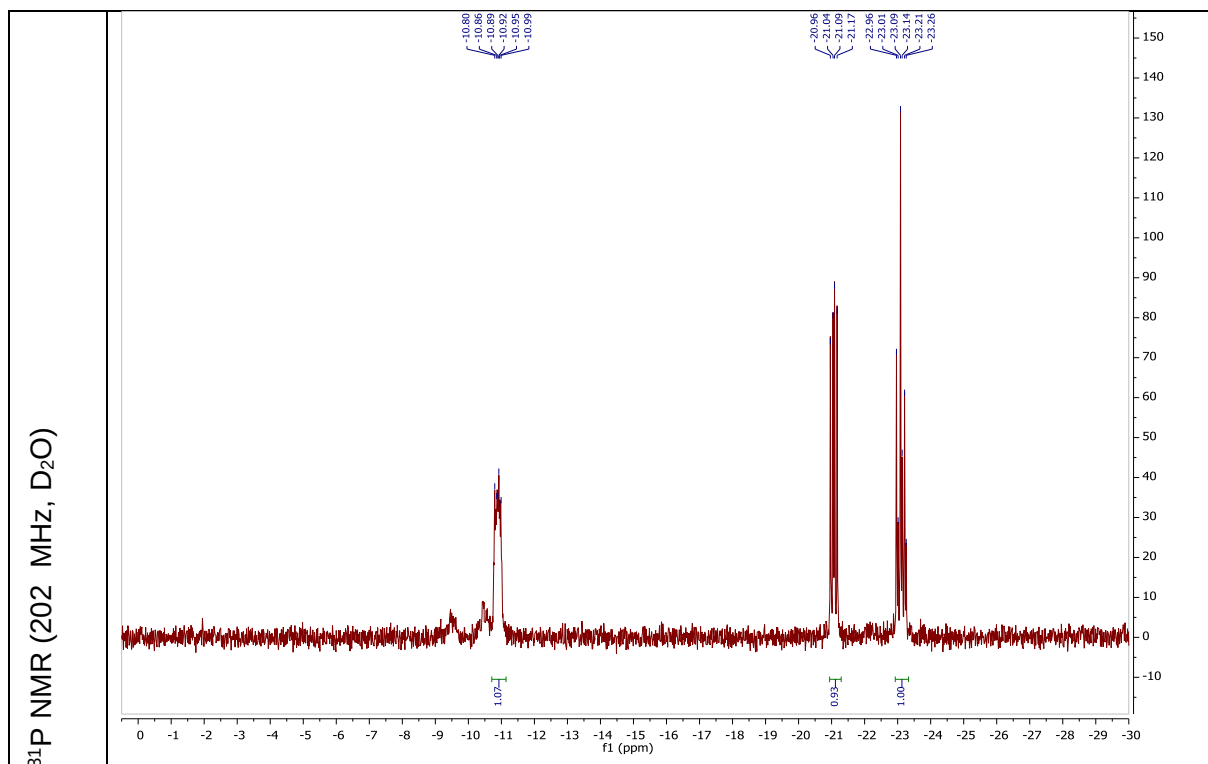


HR MS



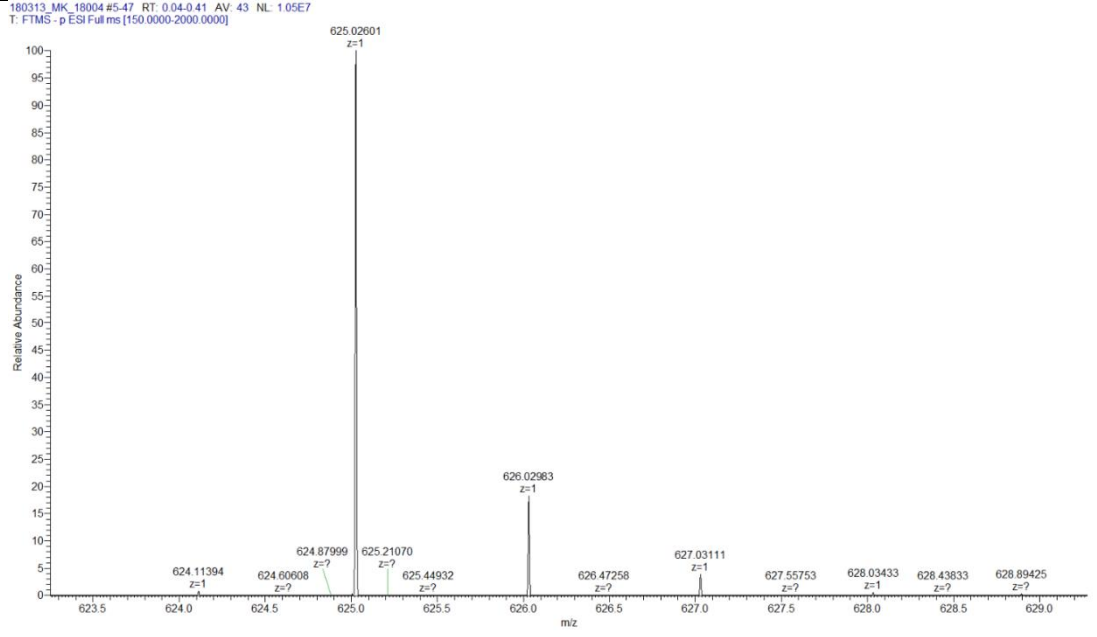
¹H NMR (400 MHz, D₂O)



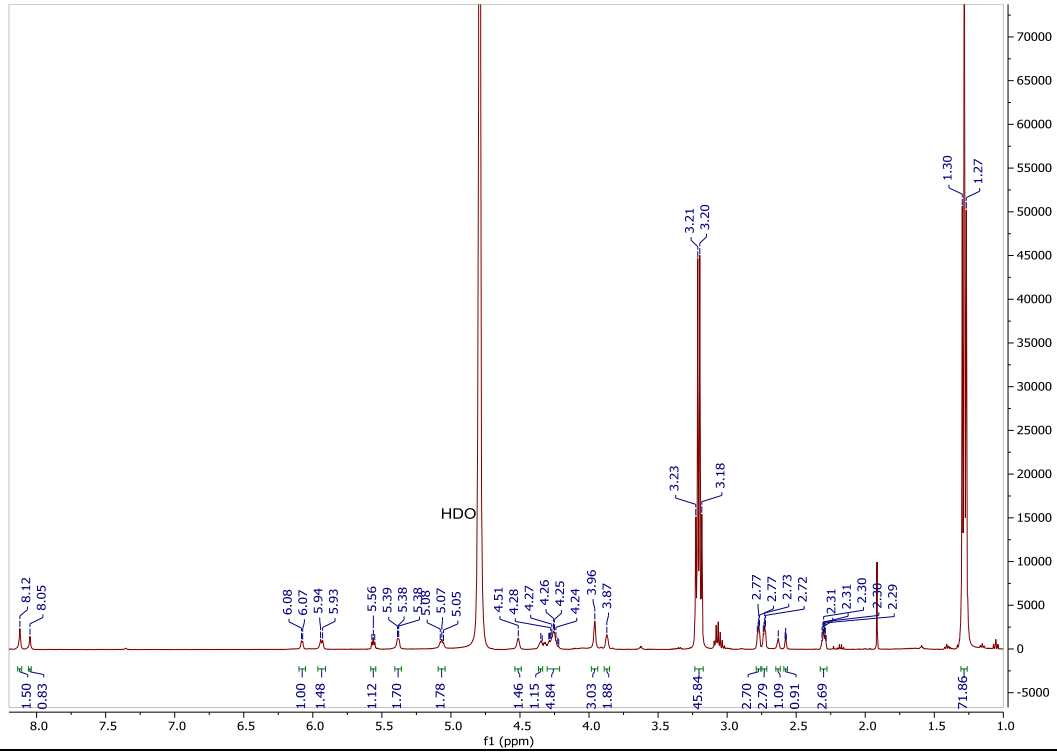


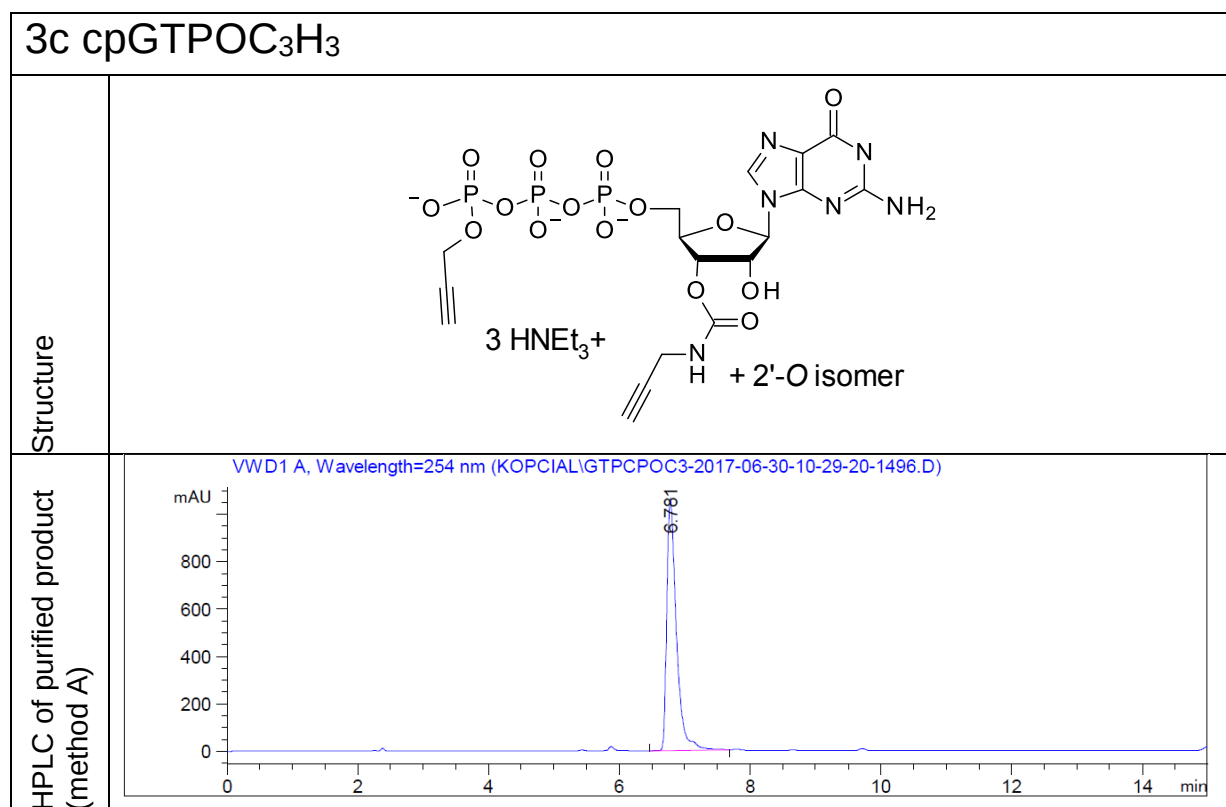
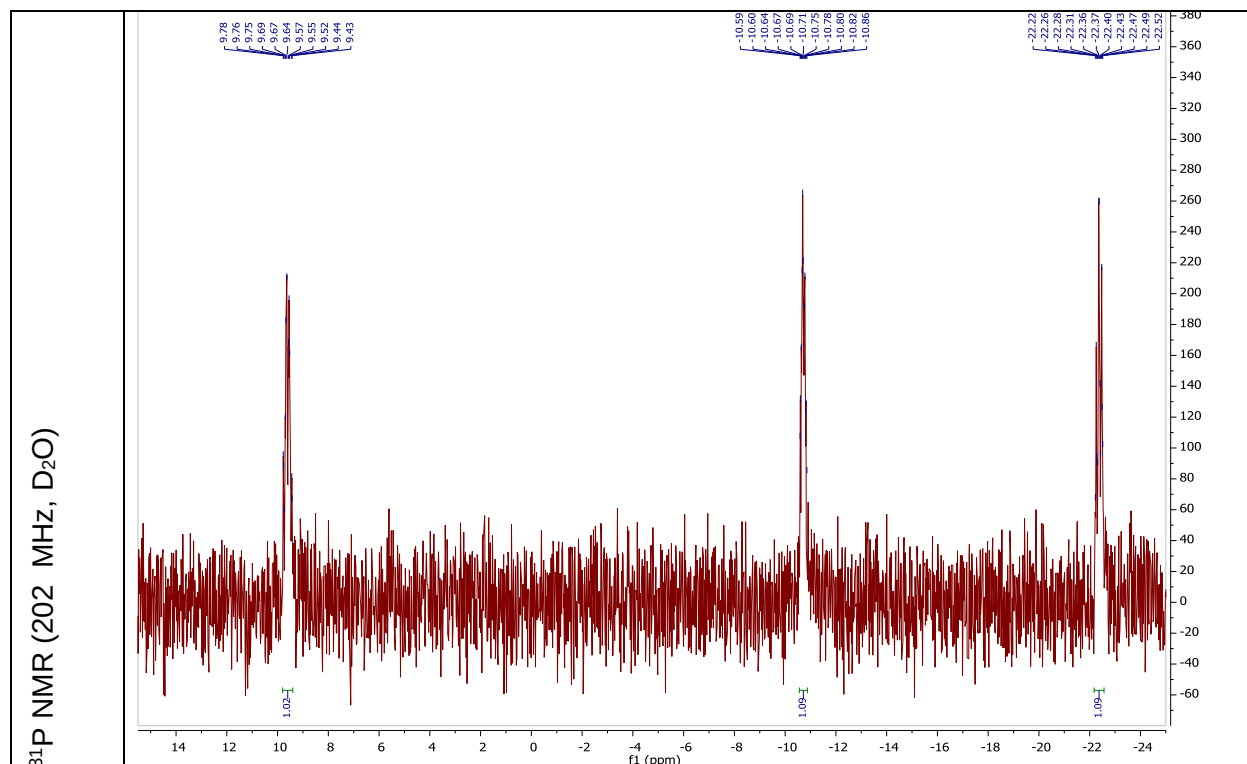
3b cpGTPC ₃ H ₃	
Structure	3 HNEt₃⁺ + 2'-O isomer
HPLC of purified product (method A)	DAD1 A, Sig=254,4 Ref=360,100 (KOPCIAL\GTPCPC3000242.D) 6.047

HR MS

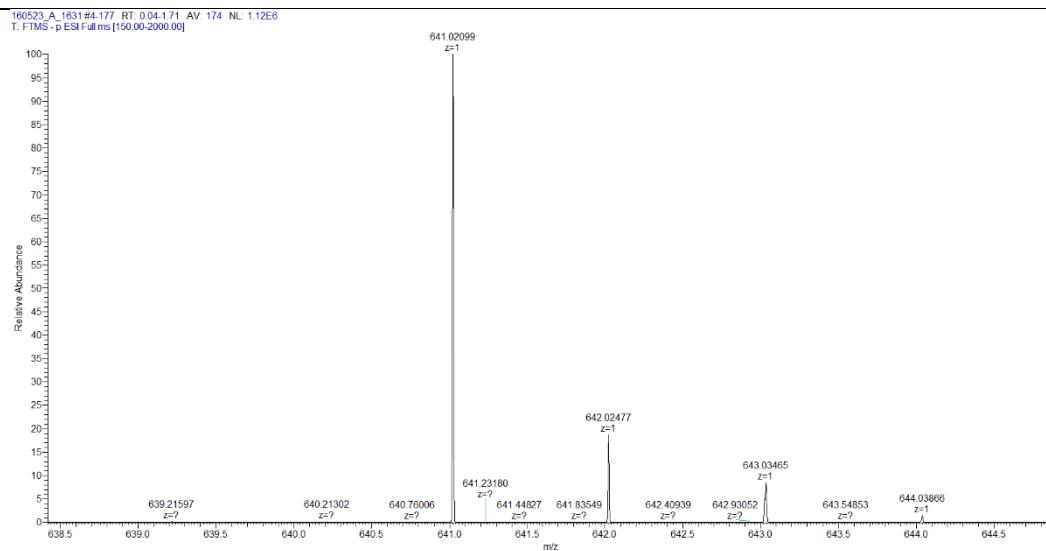


¹H NMR (500 MHz, D₂O)

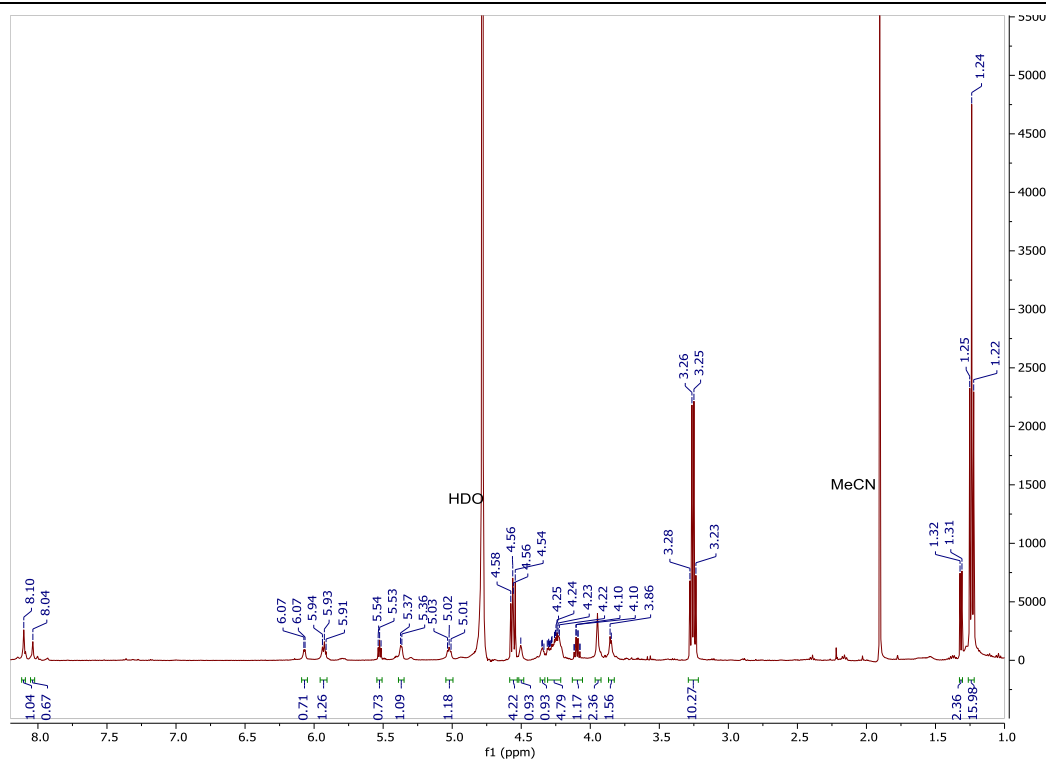


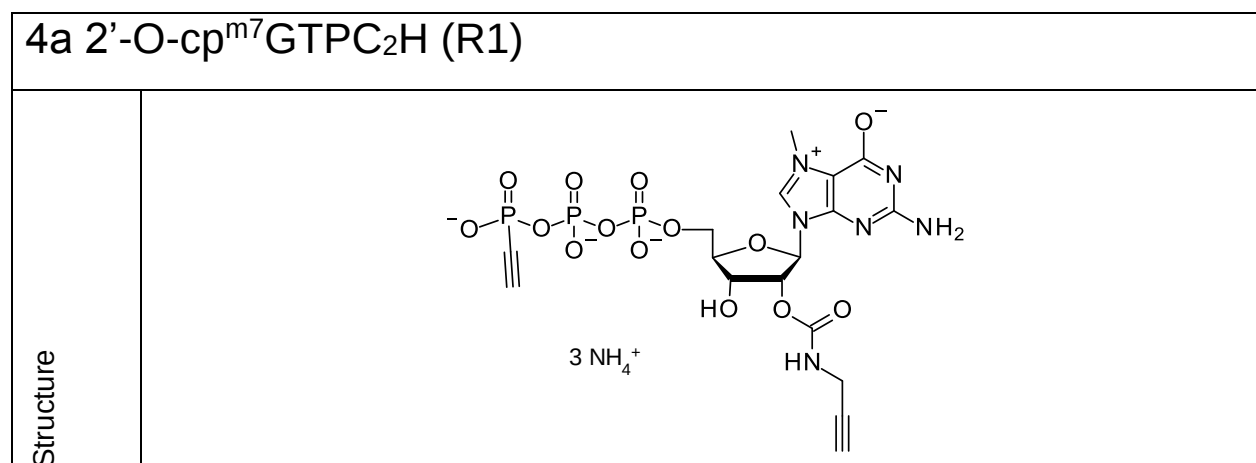
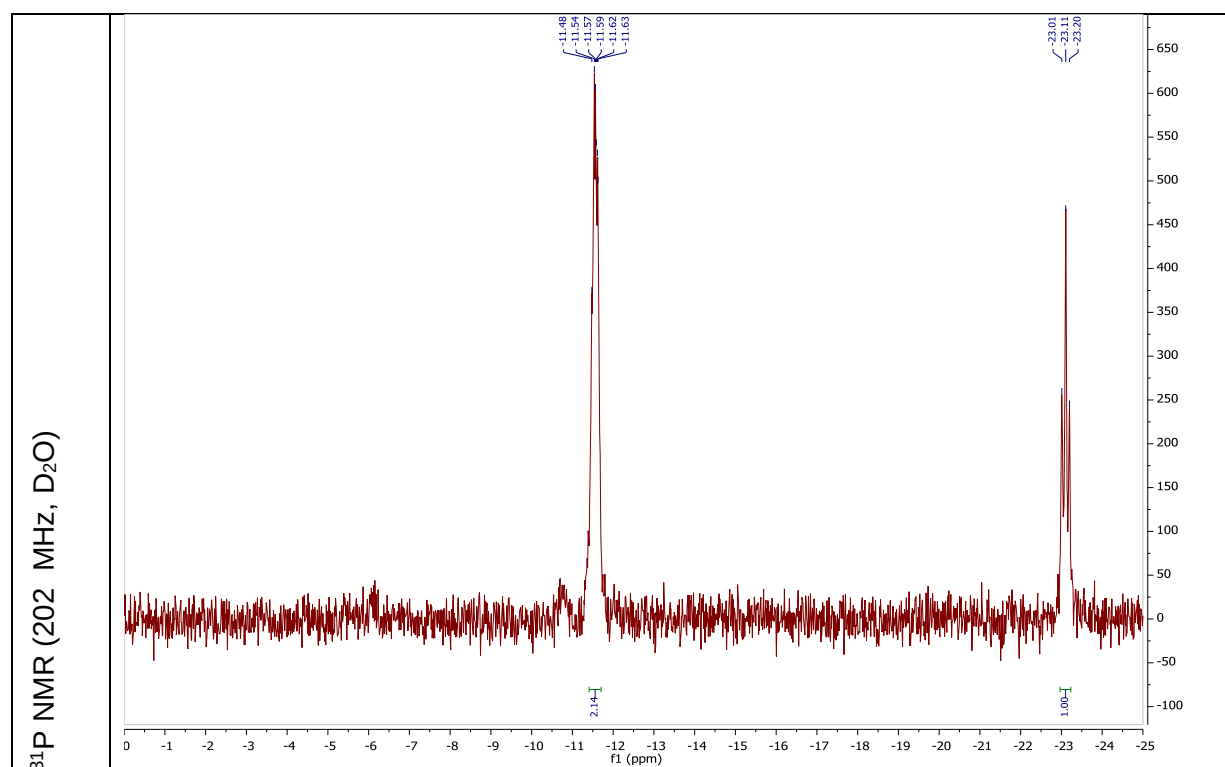


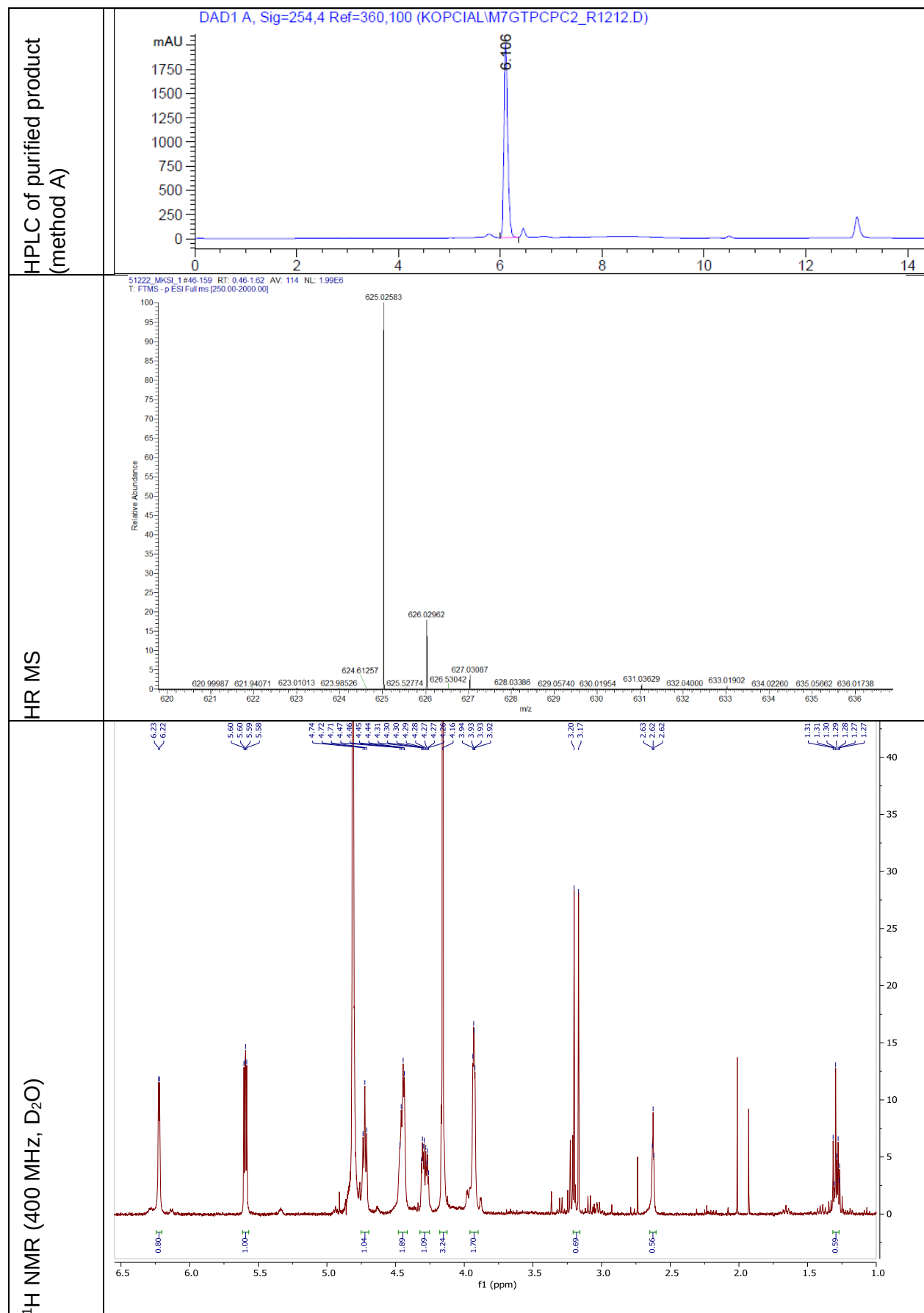
HR MS



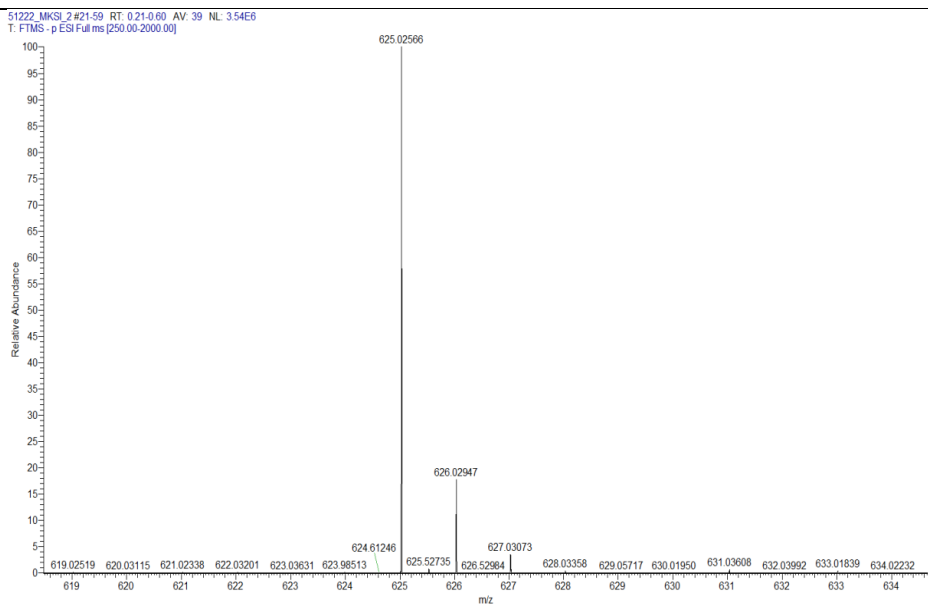
¹H NMR (500 MHz, D₂O)



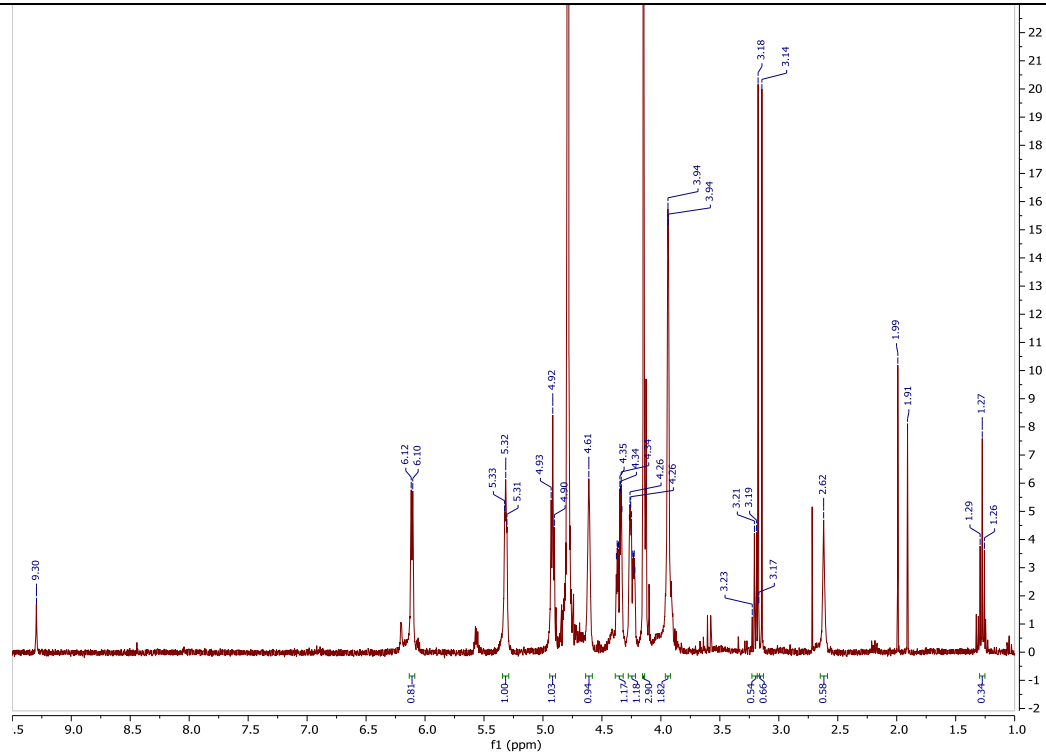


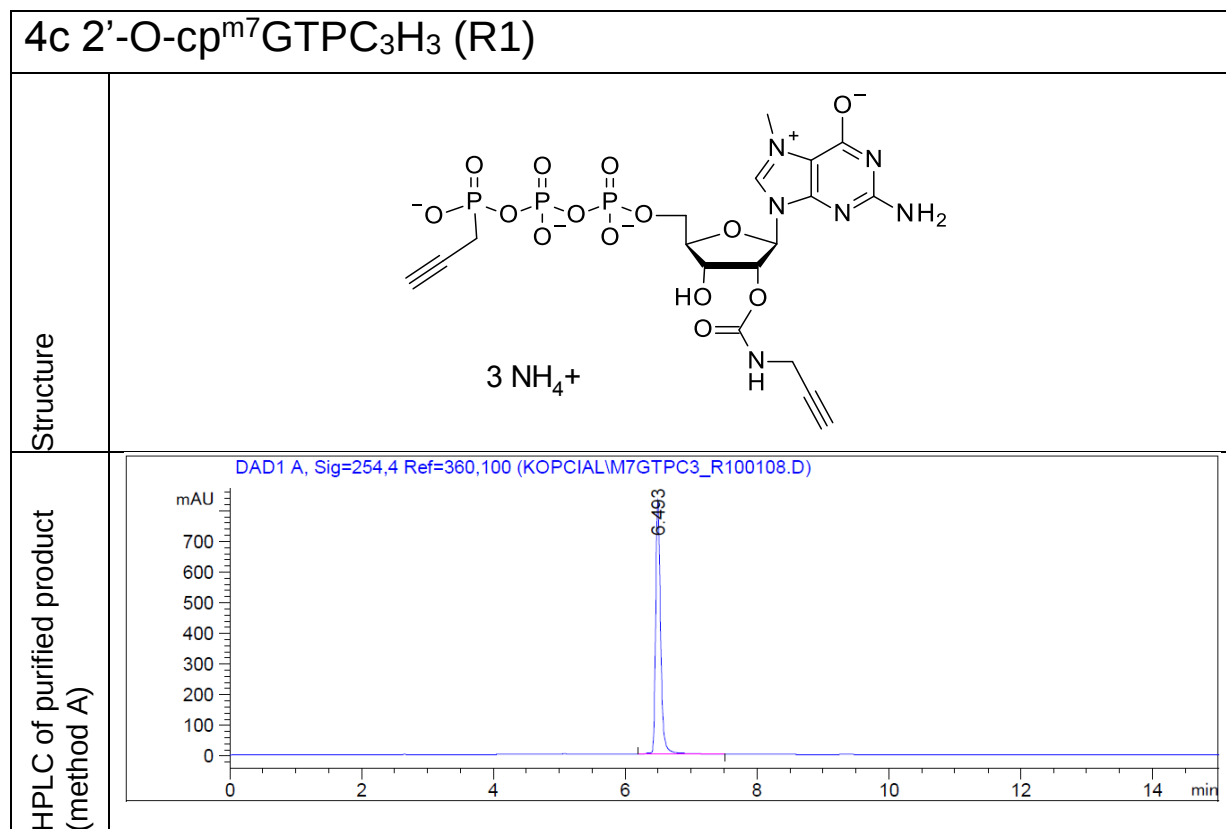
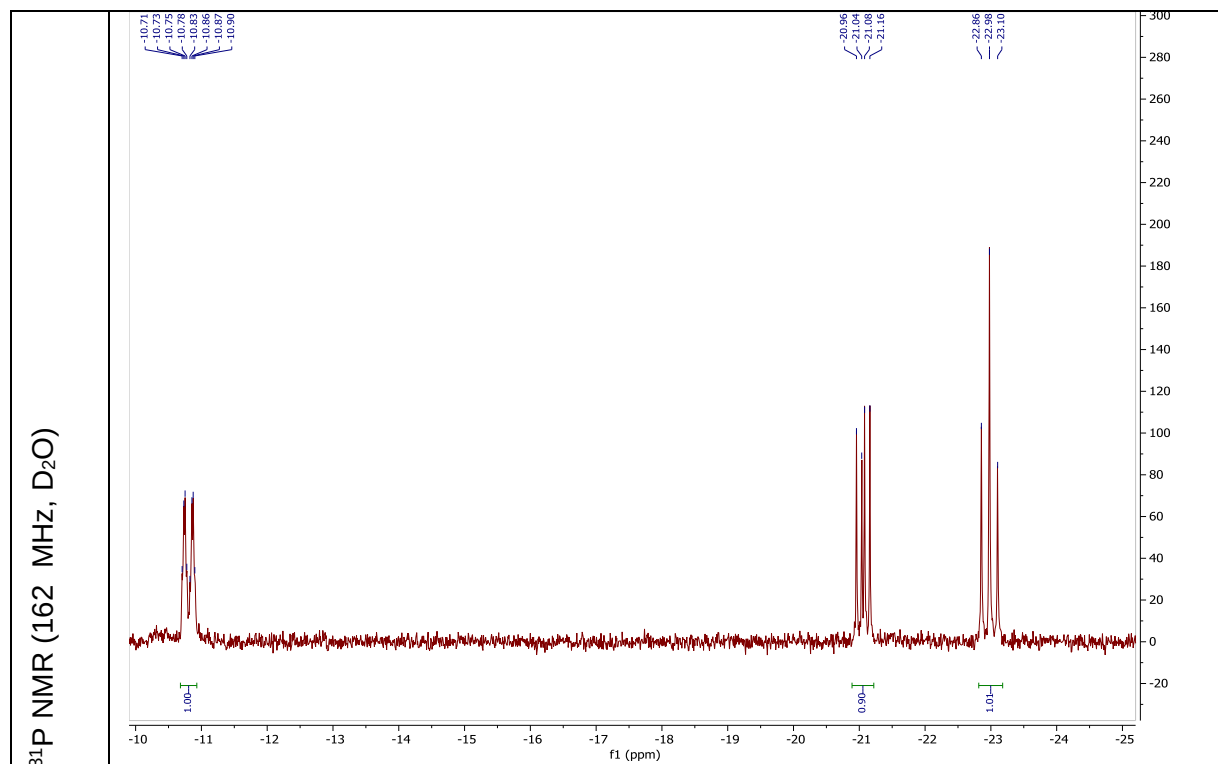


HR MS

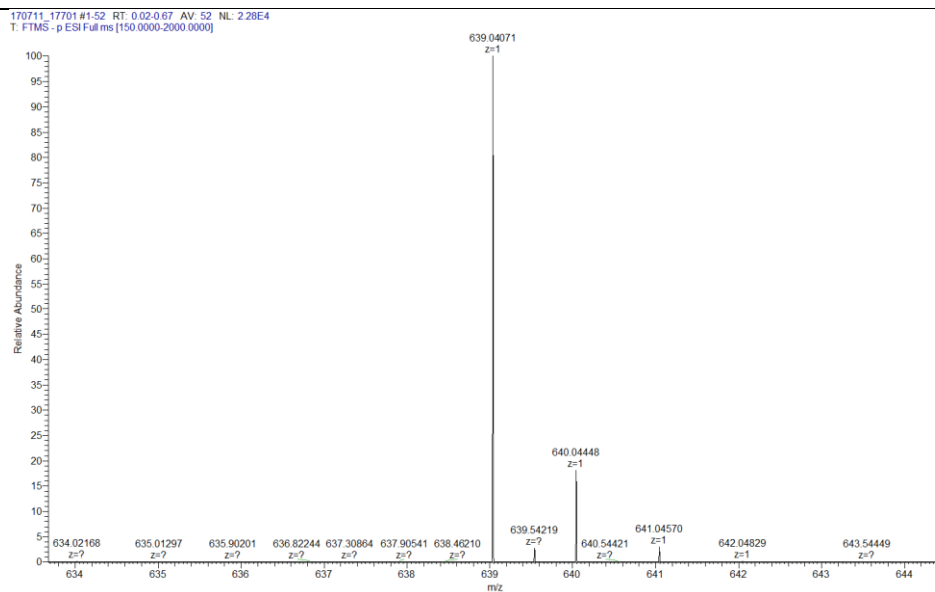


¹H NMR (400 MHz, D₂O)

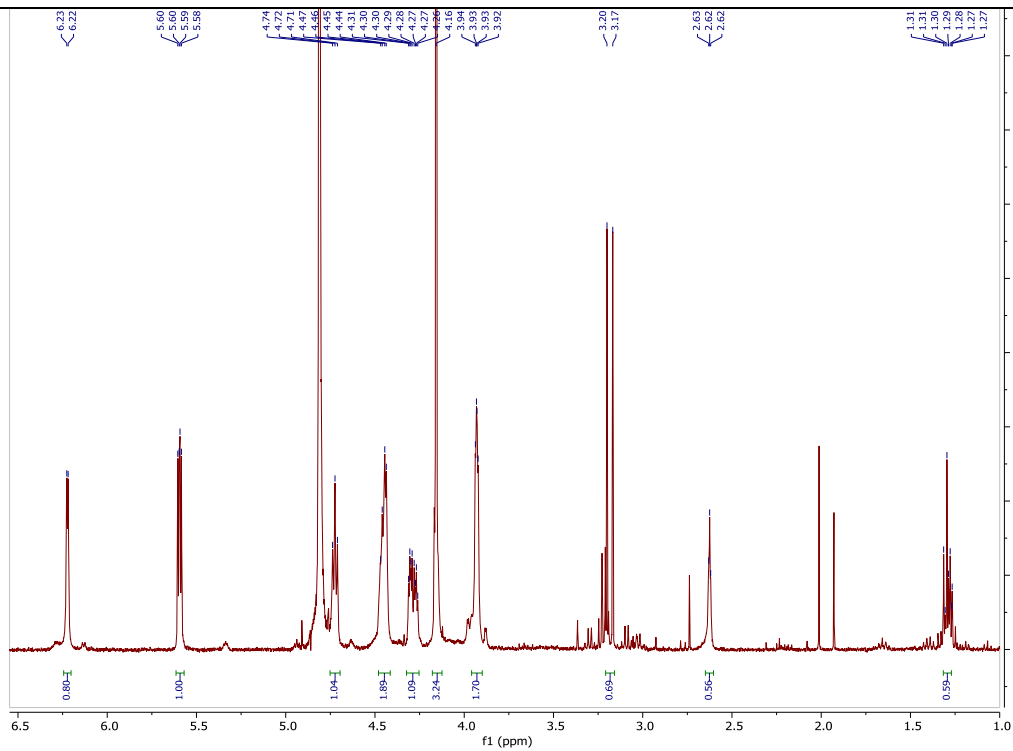


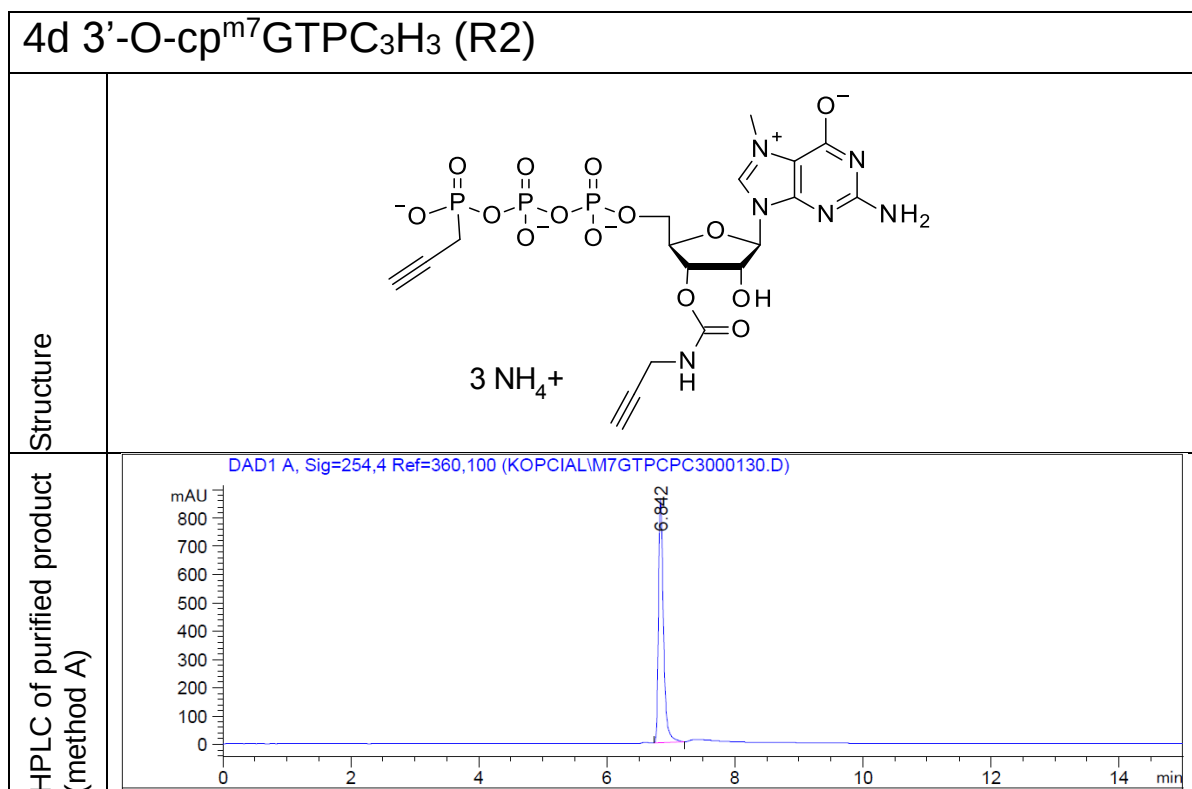
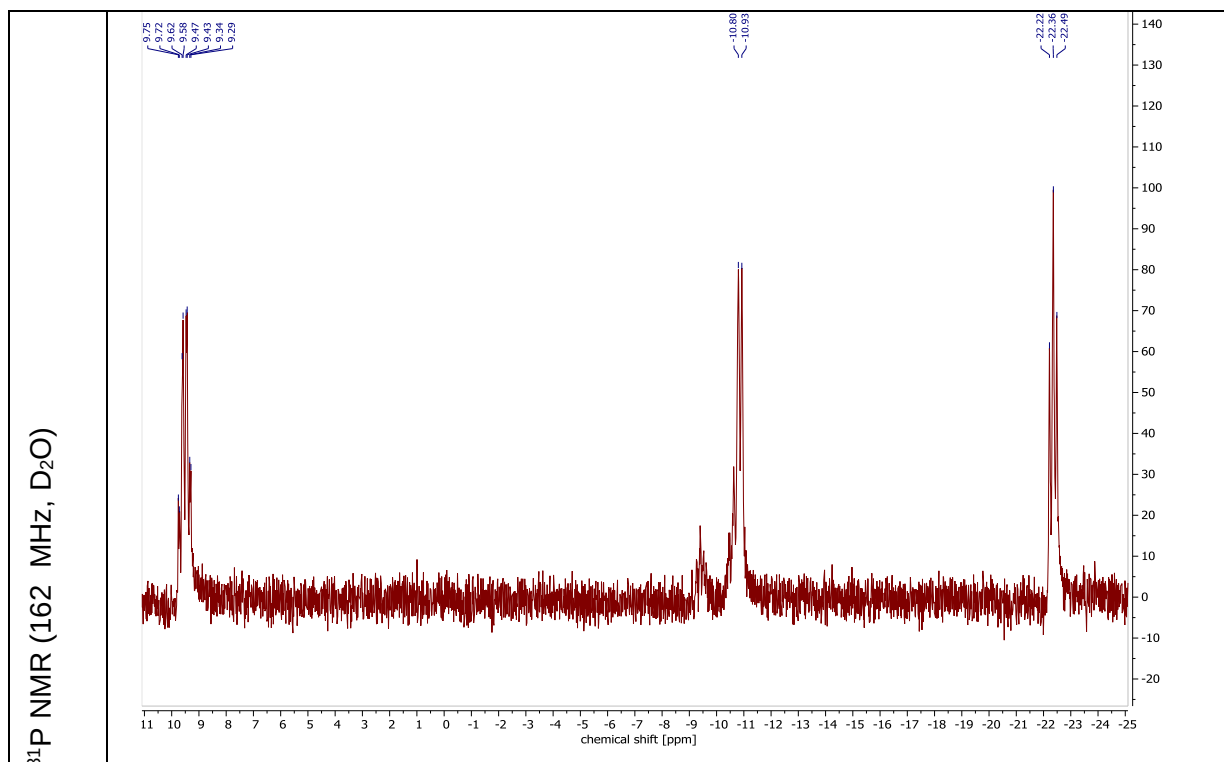


HR MS

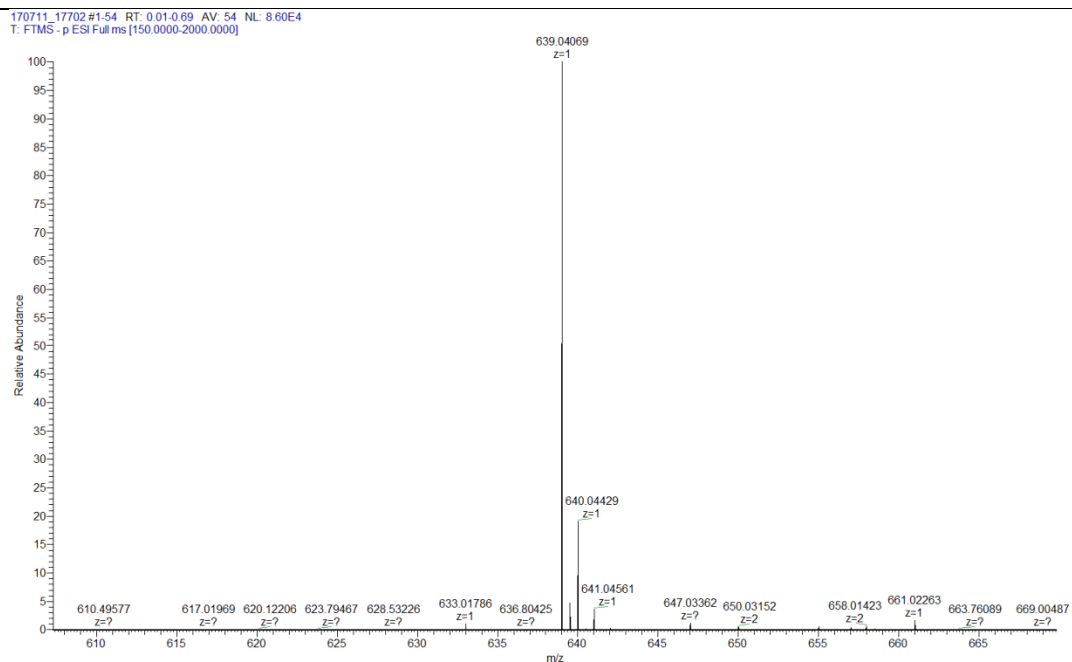


¹H NMR (400 MHz, D₂O)

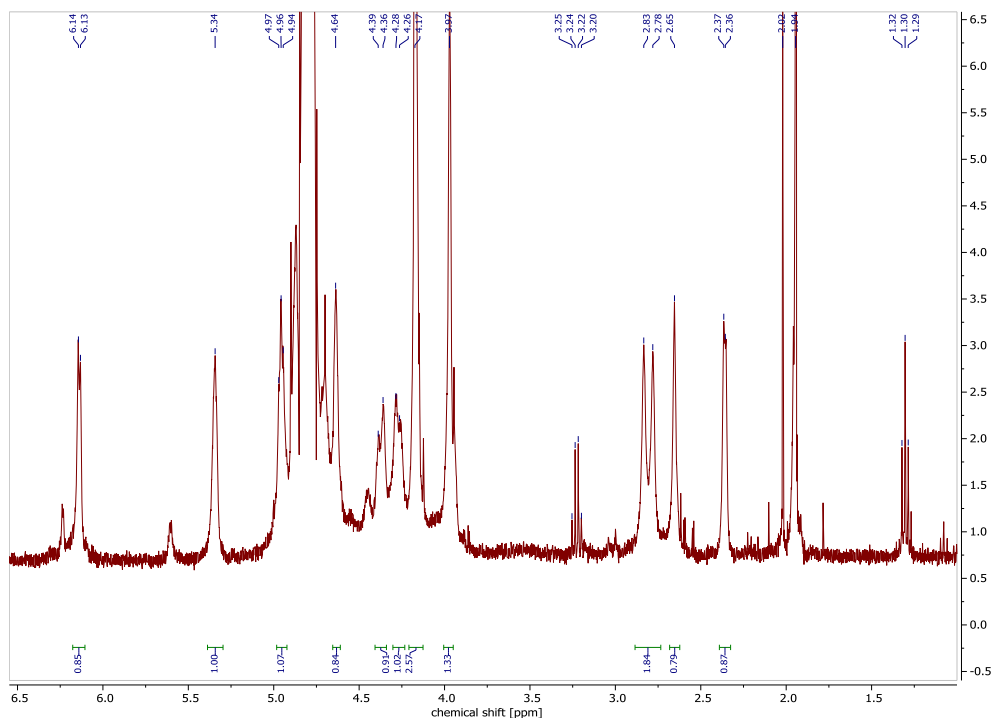


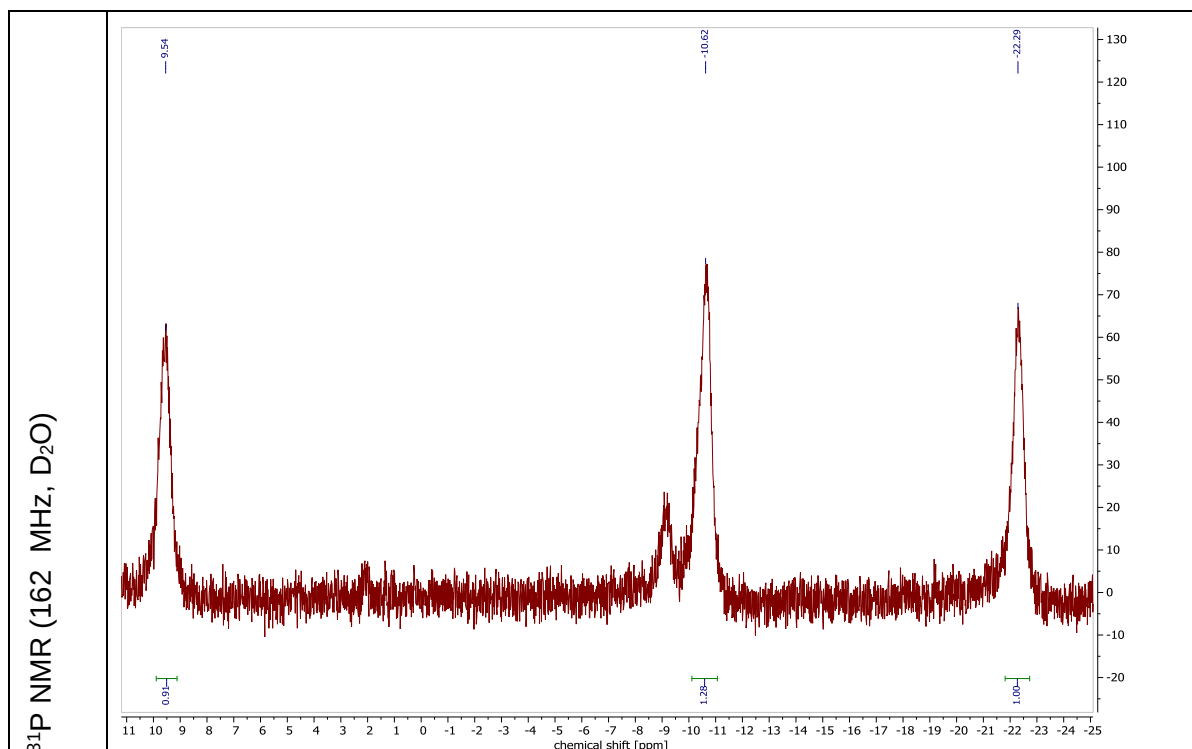


HR MS

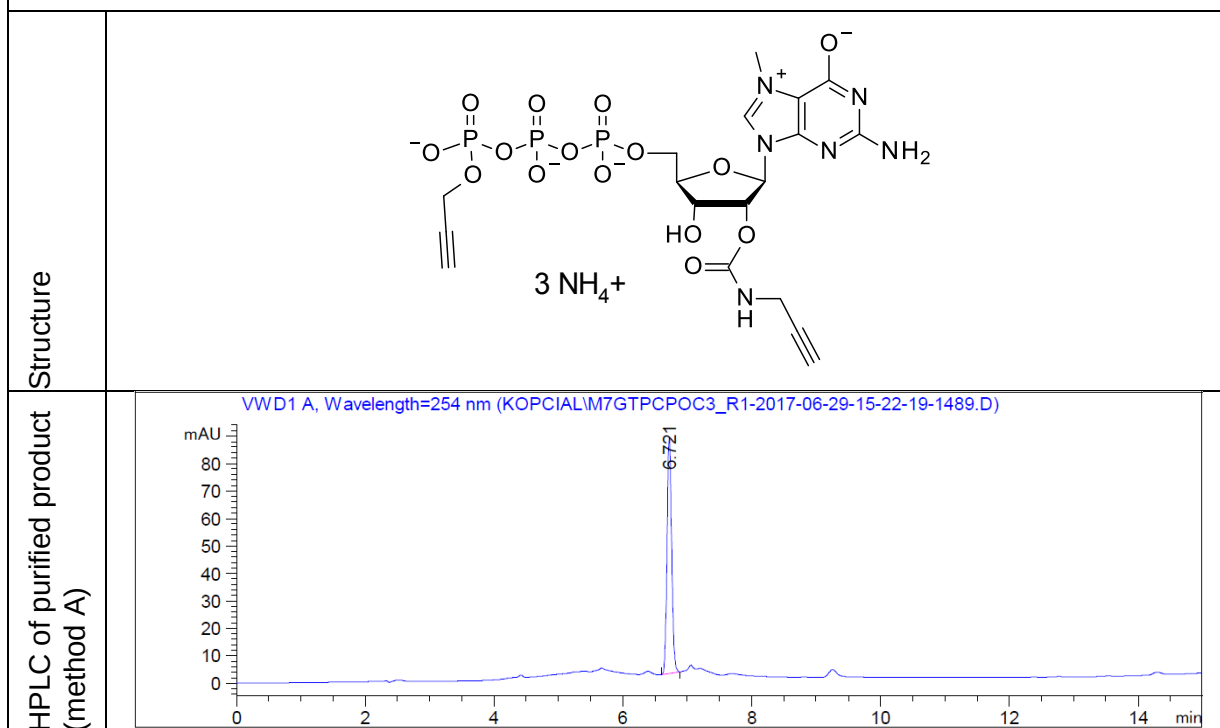


¹H NMR (400 MHz, D₂O)

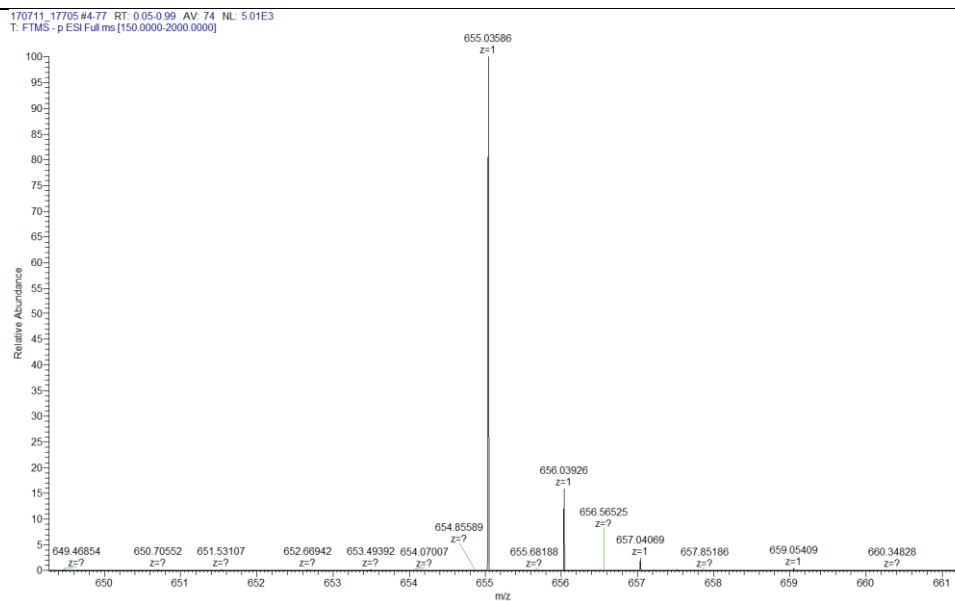




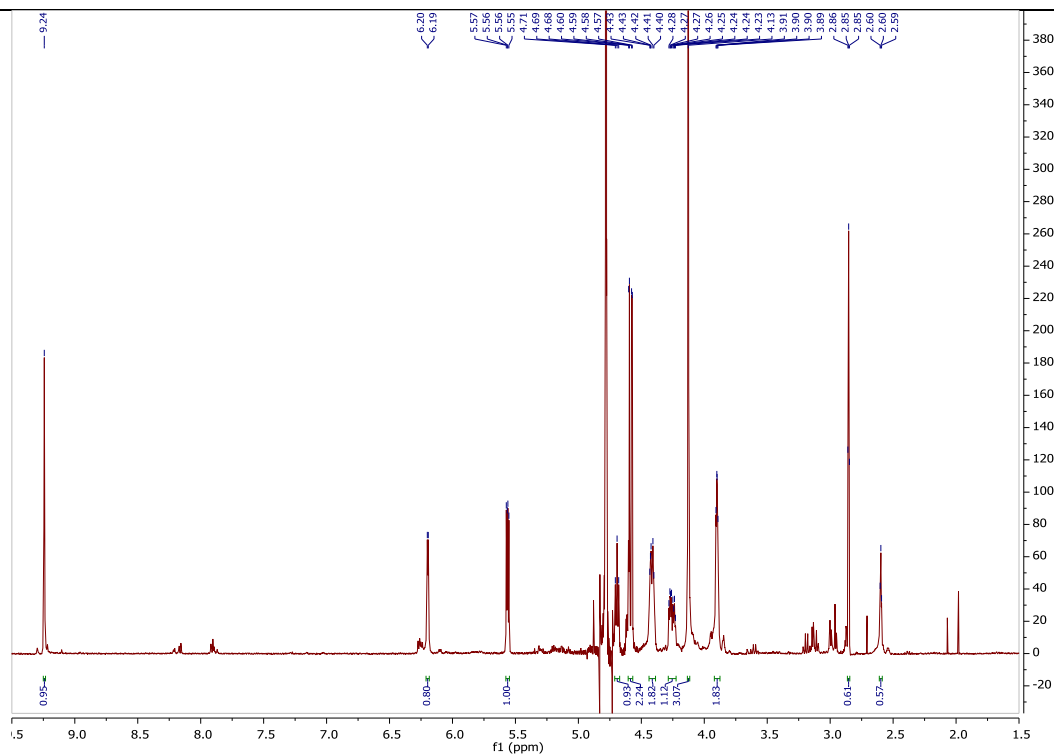
4e 2'-O-cp^{m7}GTPOC₃H₃ (R1)

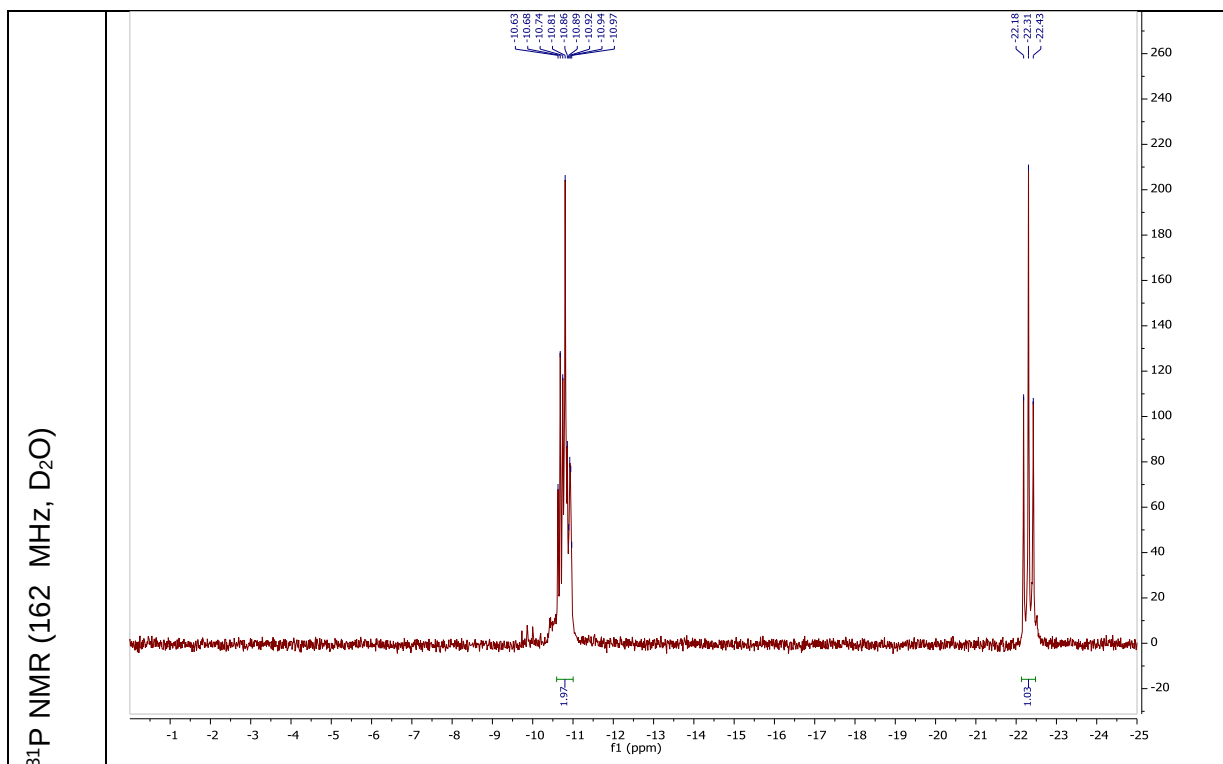


HR MS



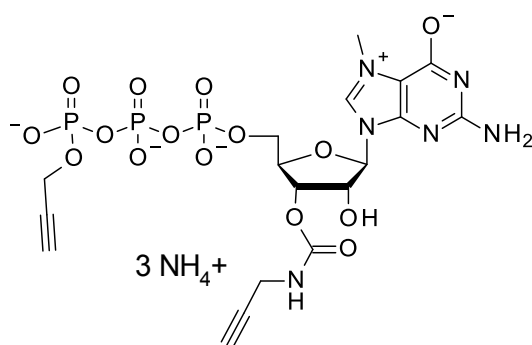
¹H NMR (400 MHz, D₂O)

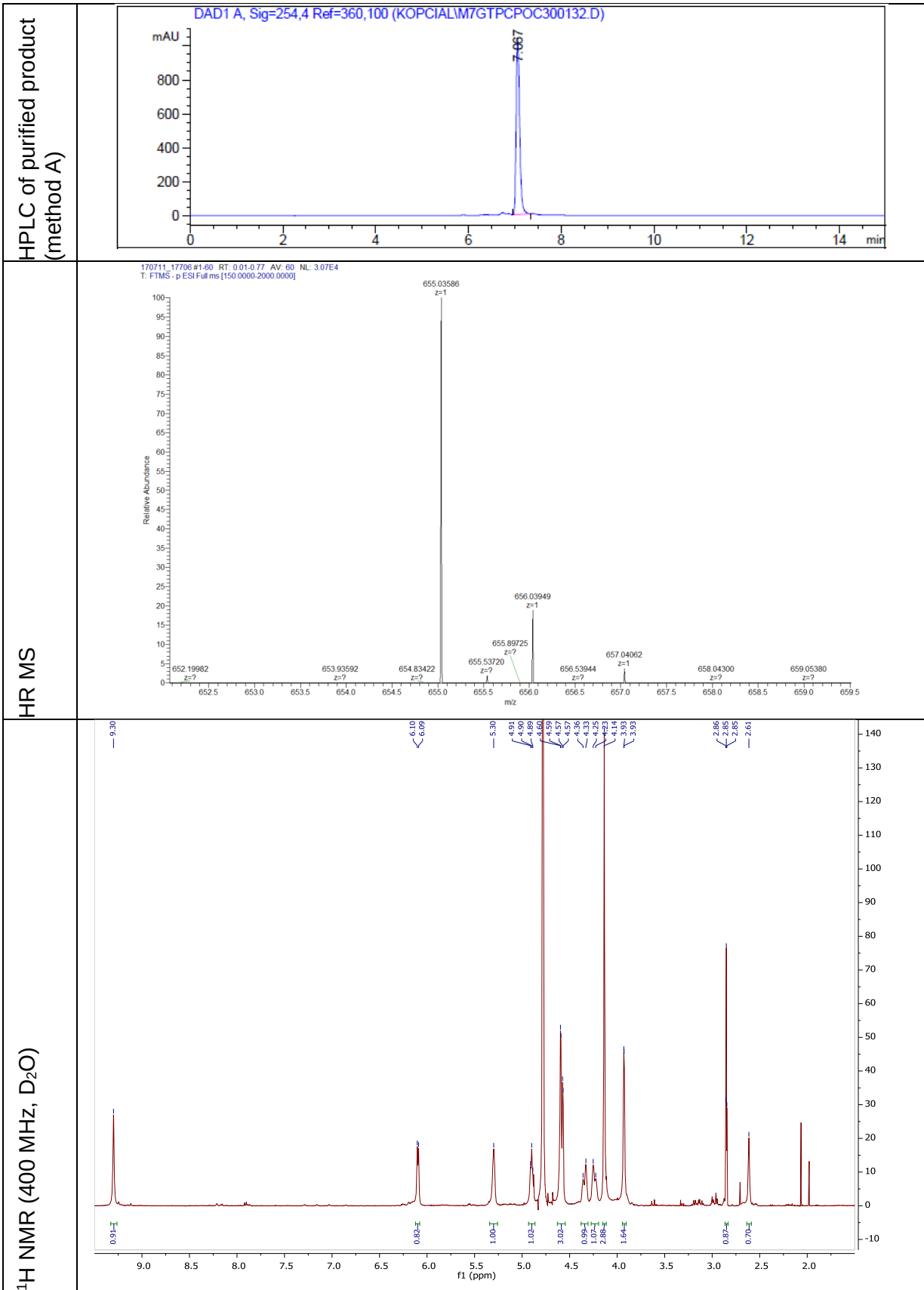


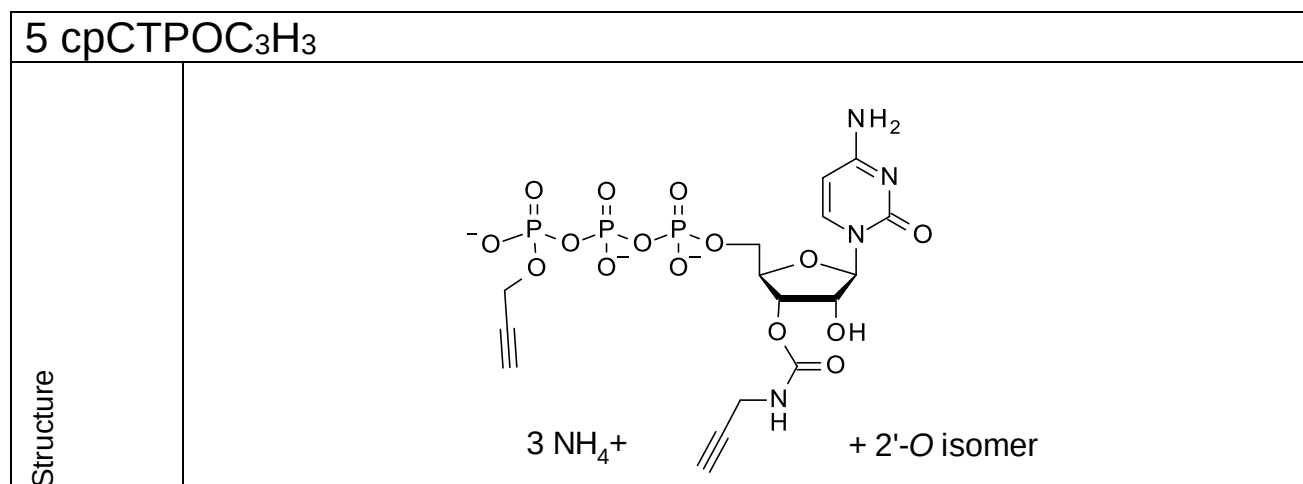
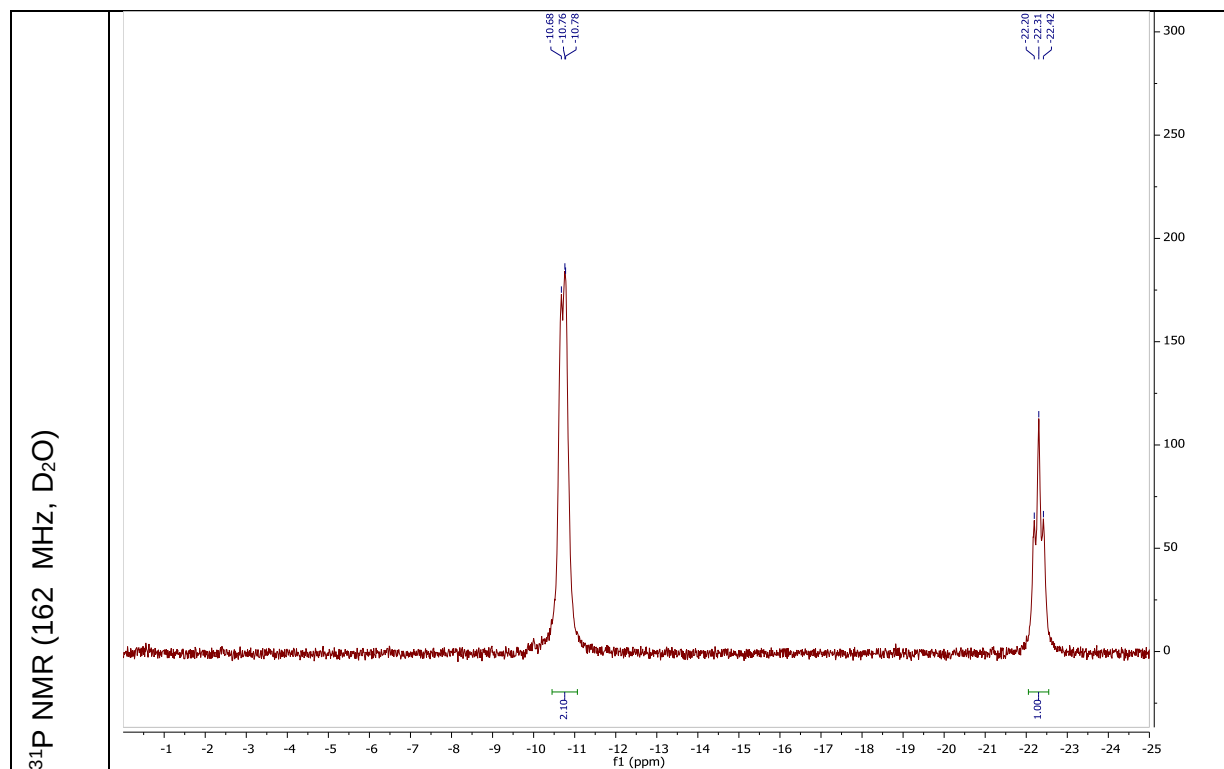


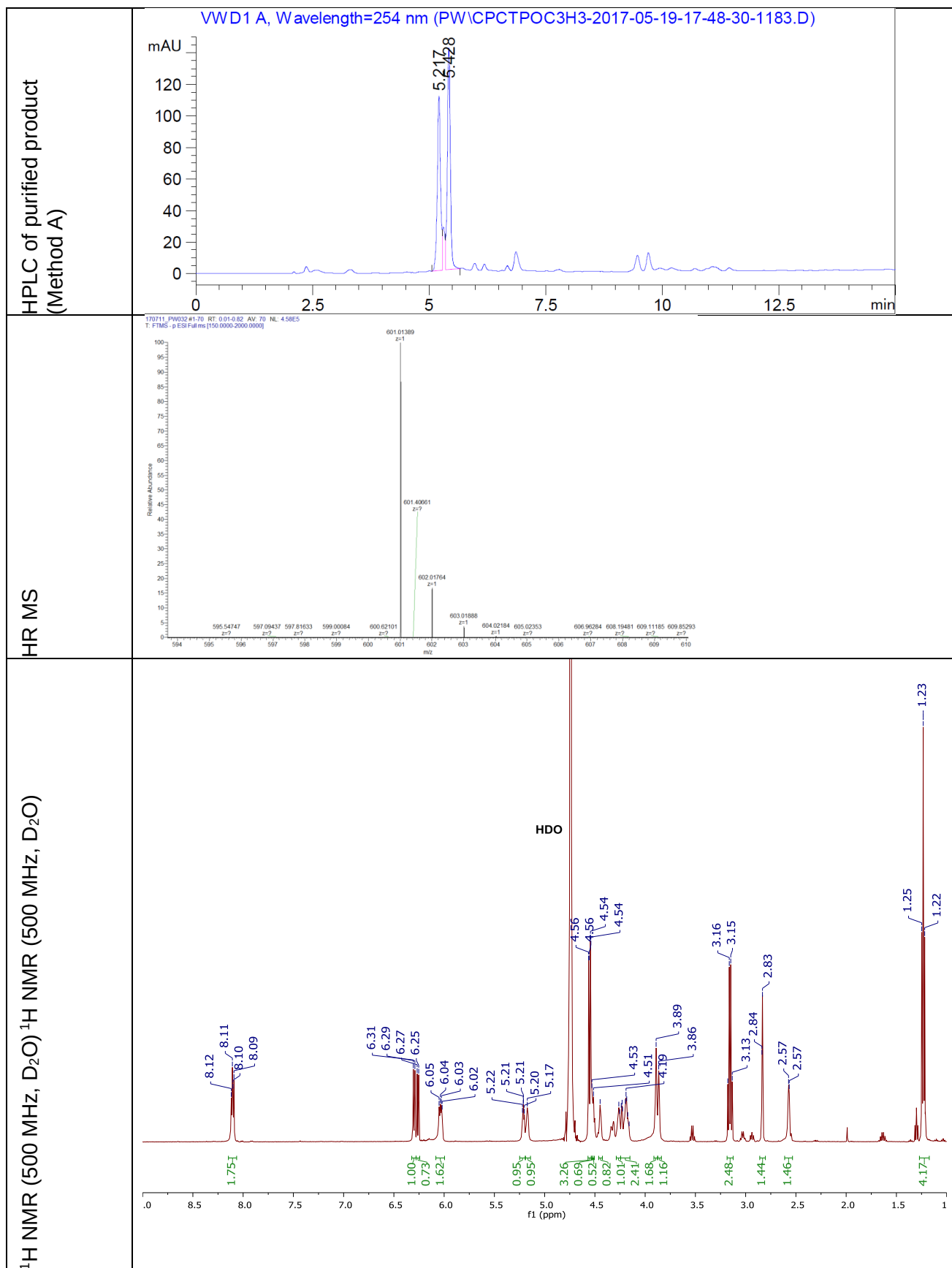
4f 3'-O-cp^{m7}GTPOC₃H₃ (R2)

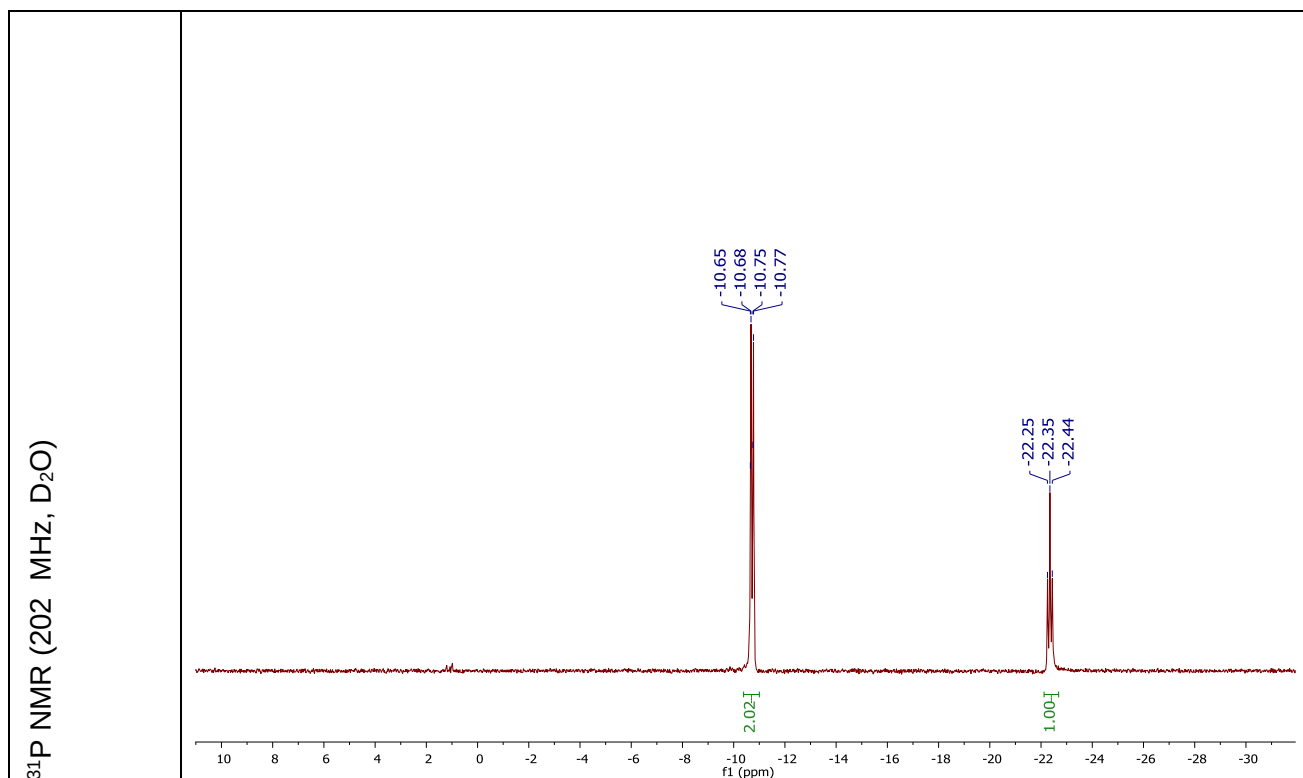
Structure





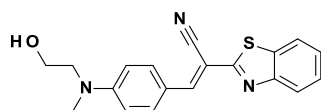




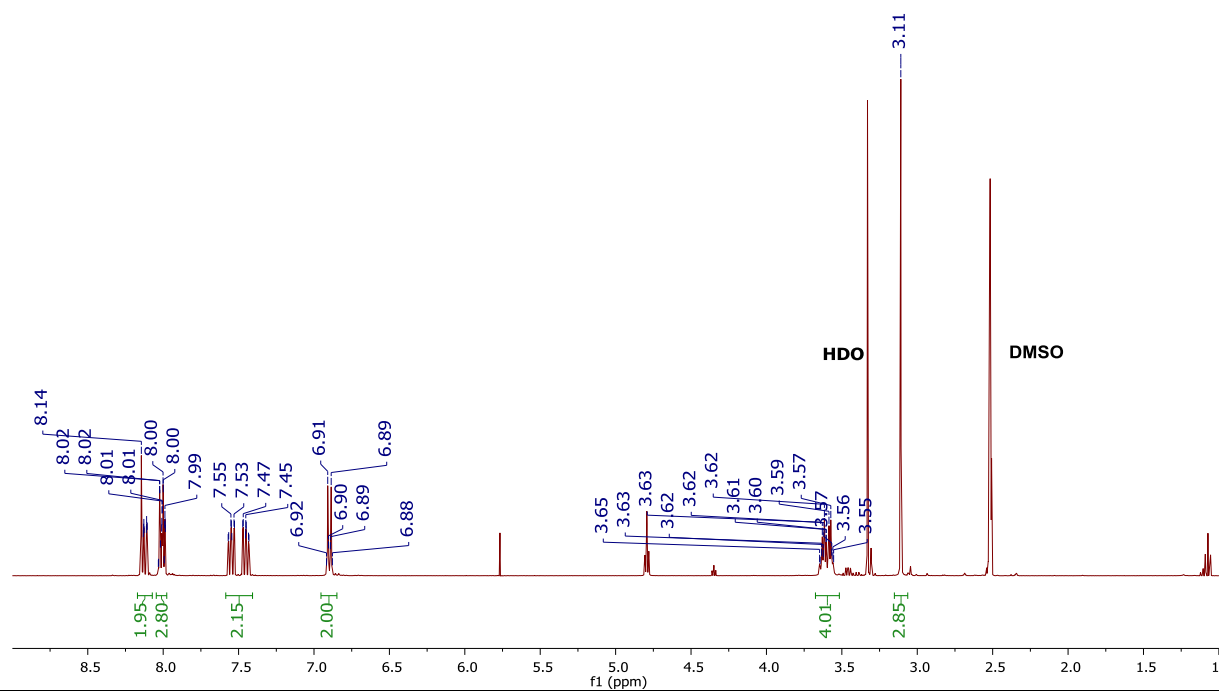


Cyanovinylene Dye - OH

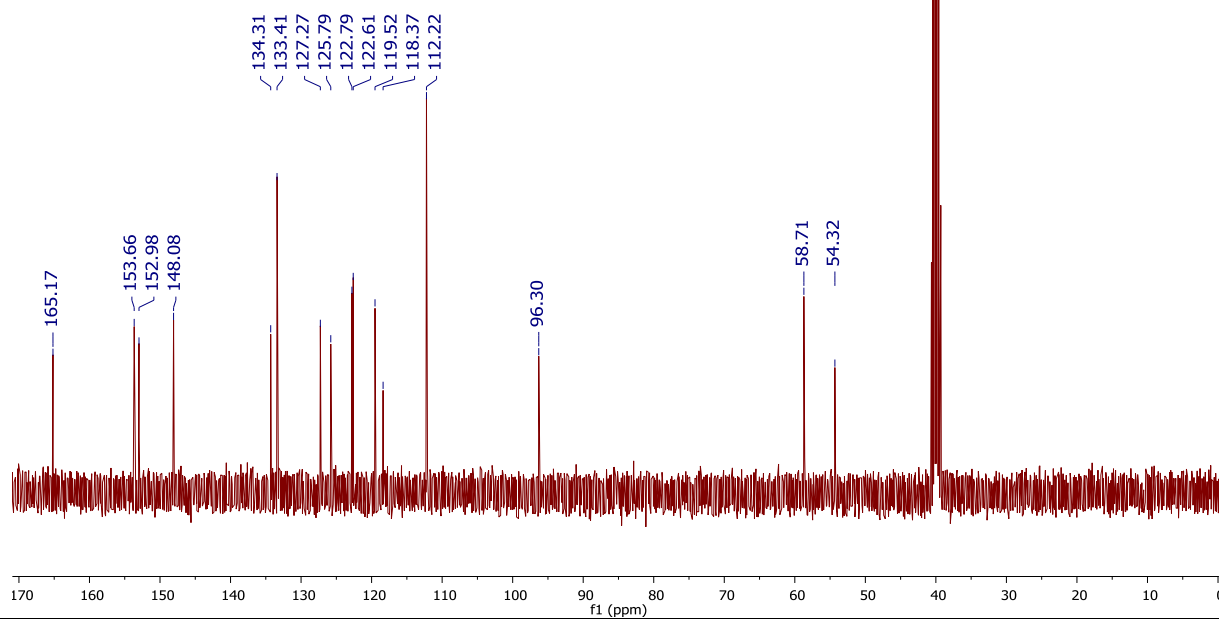
Structure



¹H NMR (400 MHz, DMSO)

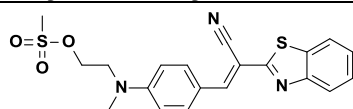


¹³C NMR (101 MHz, DMSO)

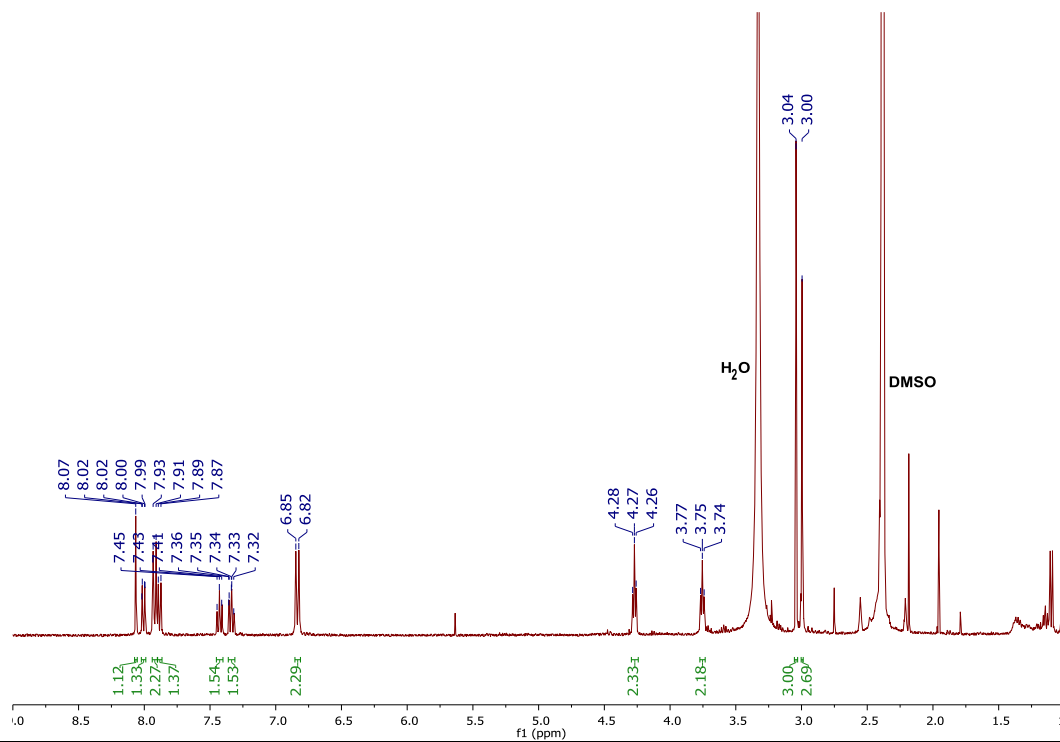


Cyanovinylene Dye - Ms

Structure

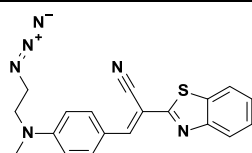


¹H NMR (400 MHz, DMSO)

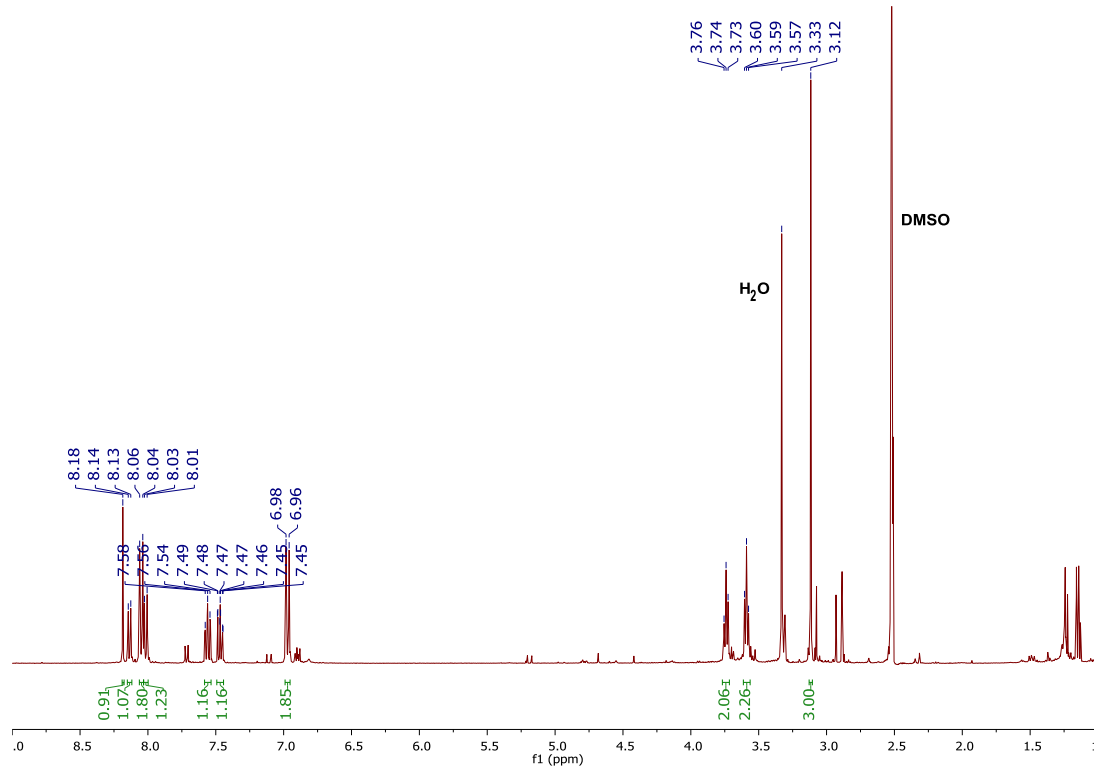


6a Cyanovinylene Dye - N₃

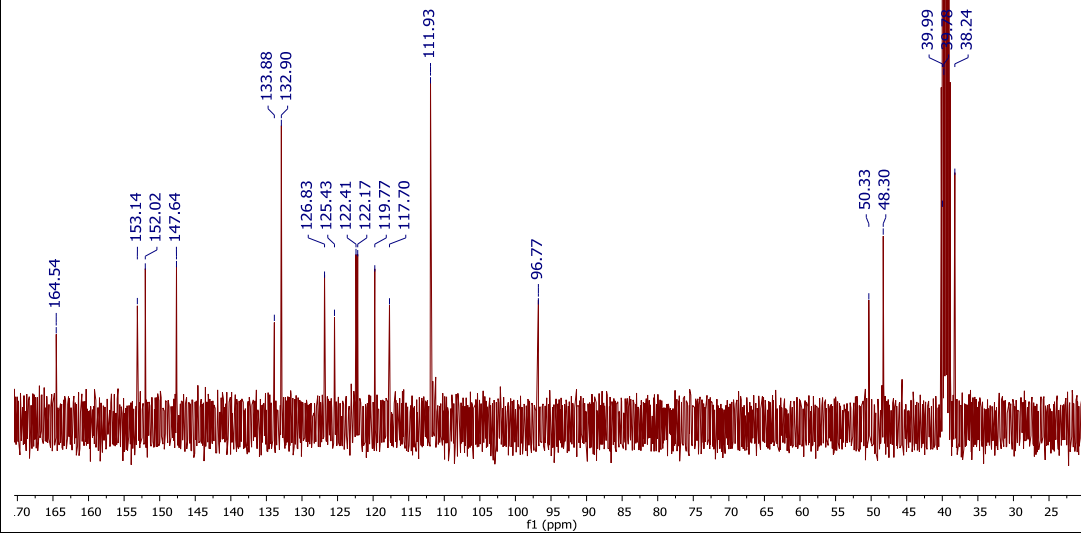
Structure



¹H NMR (400 MHz, DMSO)

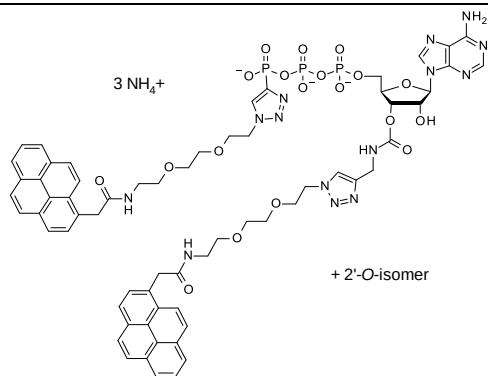


¹³C NMR (101 MHz, DMSO)



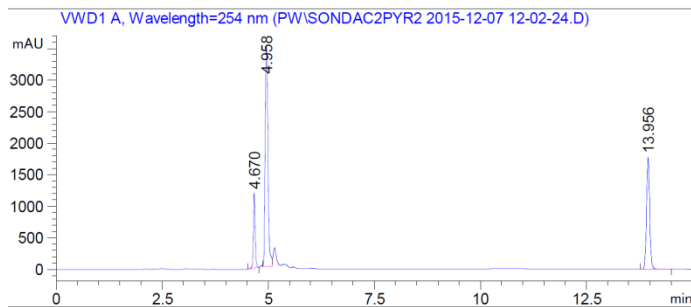
7a

Structure

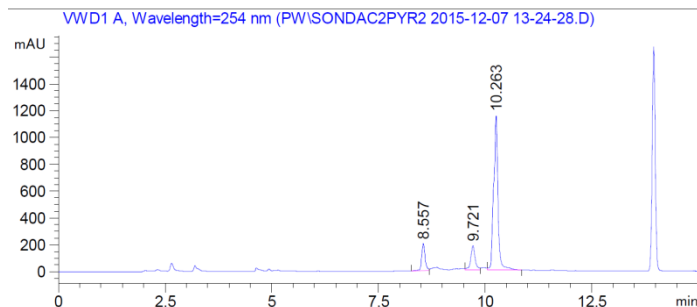


Reaction HPLC (Method C)

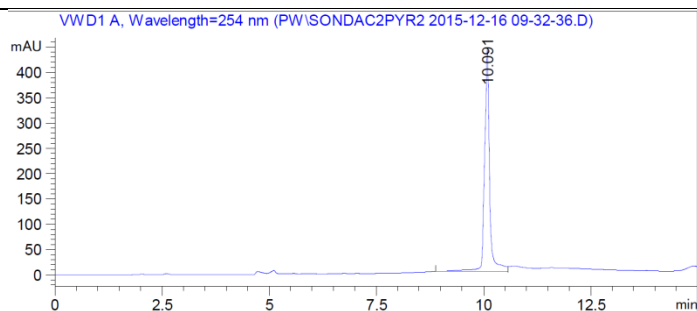
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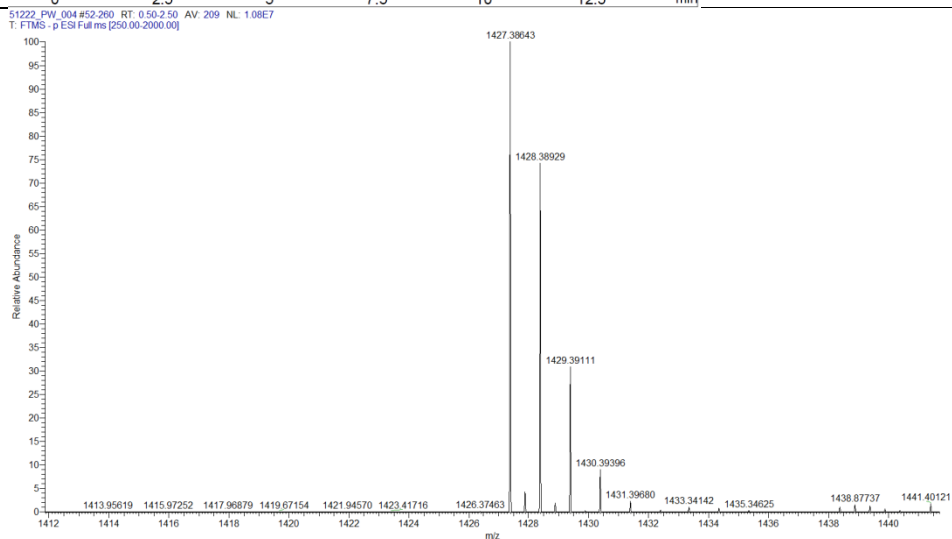
t = 1 h



HPLC of purified product (method C)

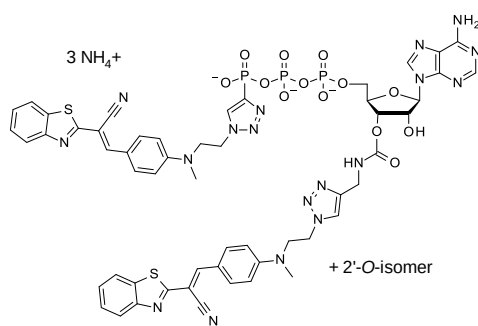


HR MS



7b

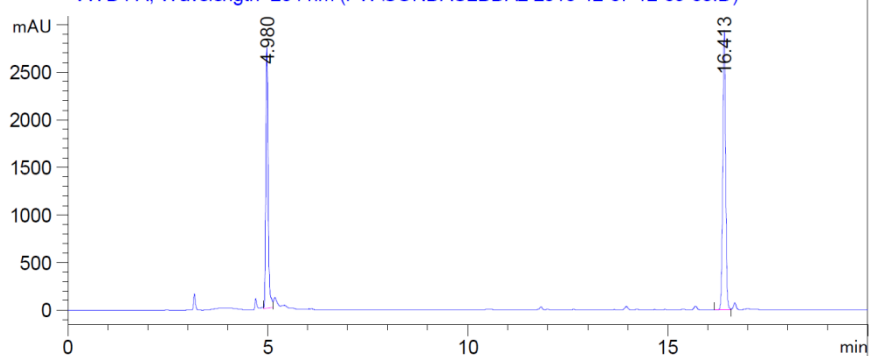
Structure



Reaction HPLC (method C)

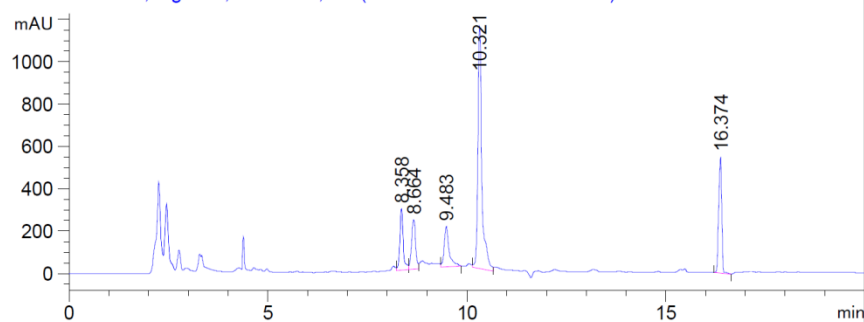
t = 0

VWD1 A, Wavelength=254 nm (PW\SONDAC2BDA2 2015-12-07 12-59-38.D)



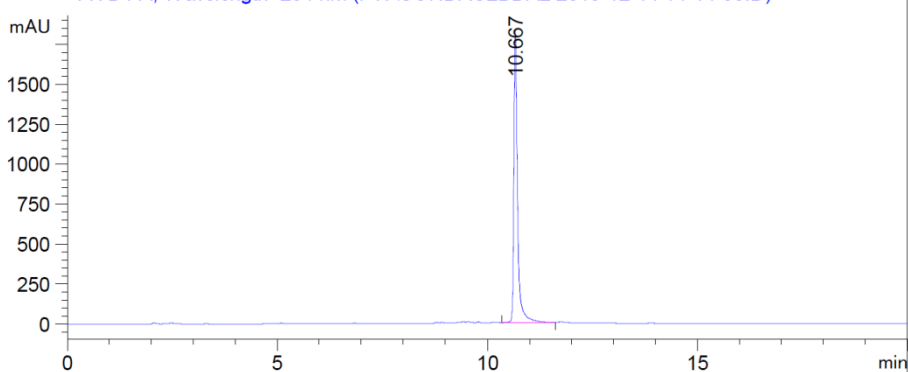
t = 2 h

DAD1 A, Sig=254,4 Ref=360,100 (PW\SONDAC2BDA00126.D)

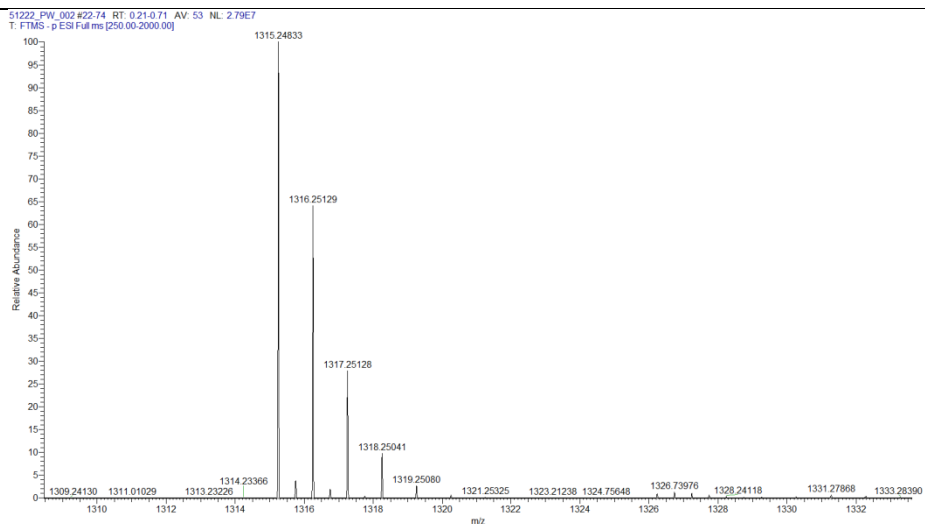


HPLC of purified product (method C)

VWD1 A, Wavelength=254 nm (PW\SONDAC2BDA2 2015-12-14 14-14-30.D)

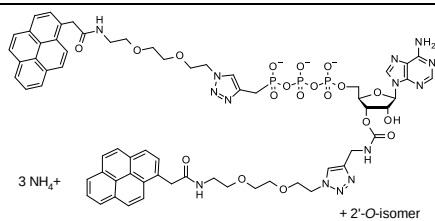


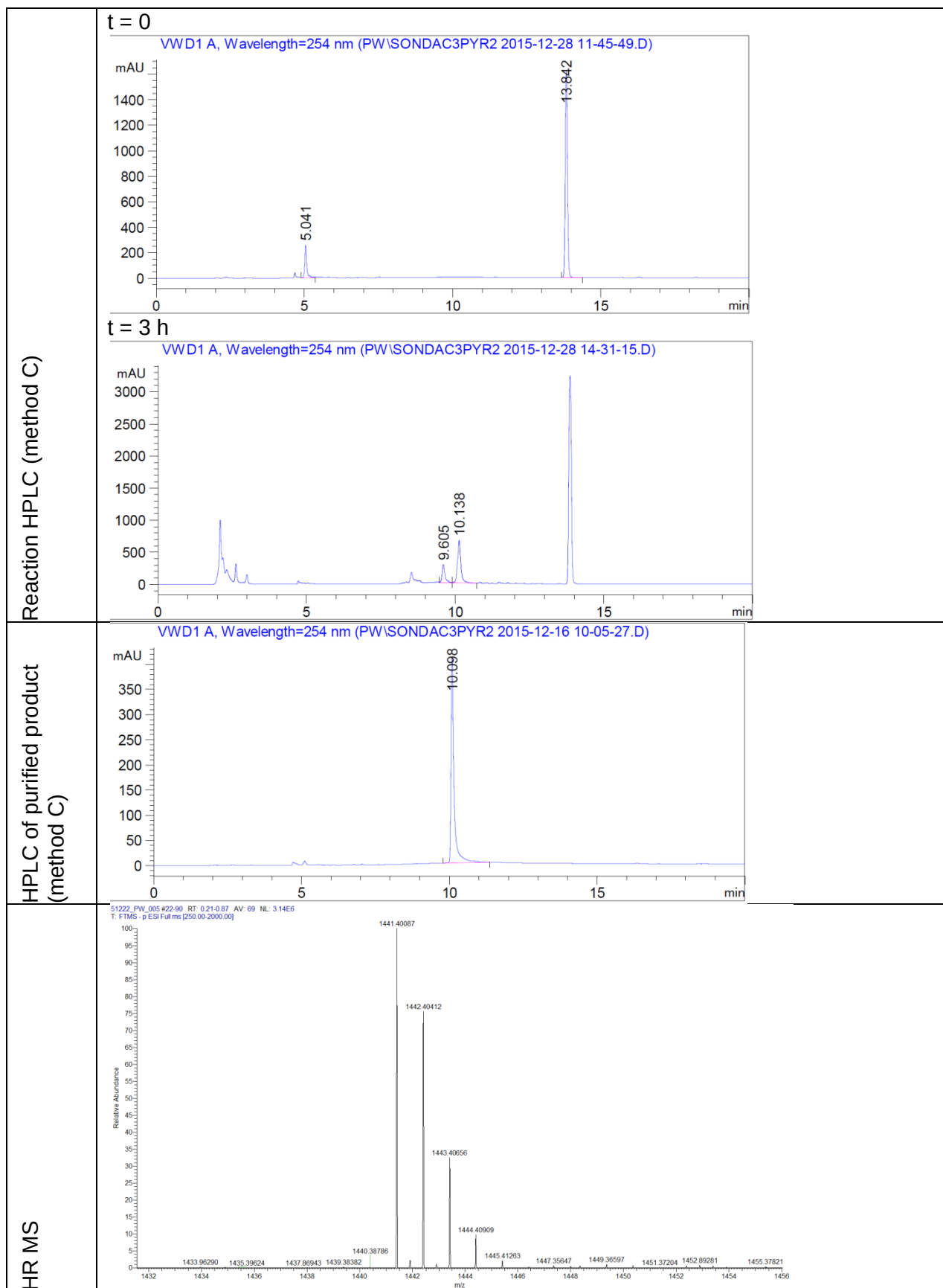
HR MS



7c

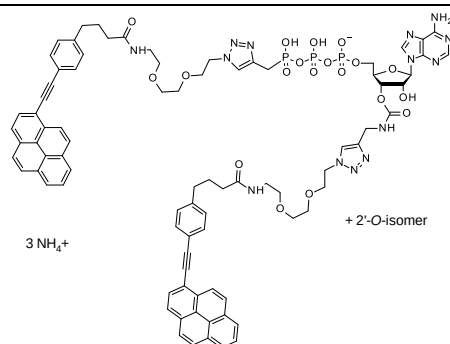
Structure





7d

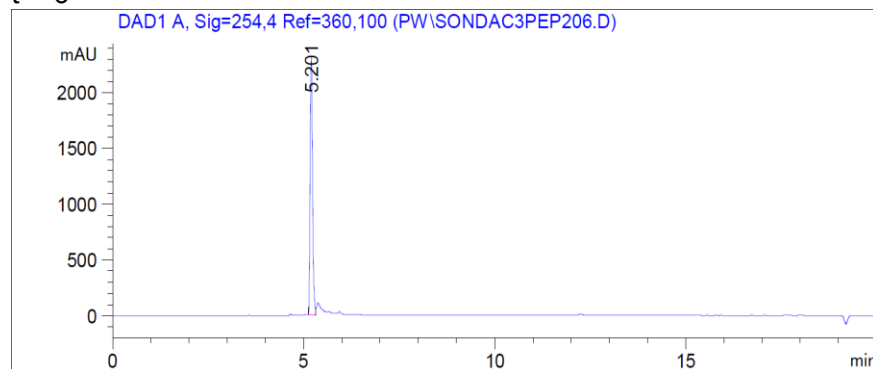
Structure



Reaction HPLC (method C)

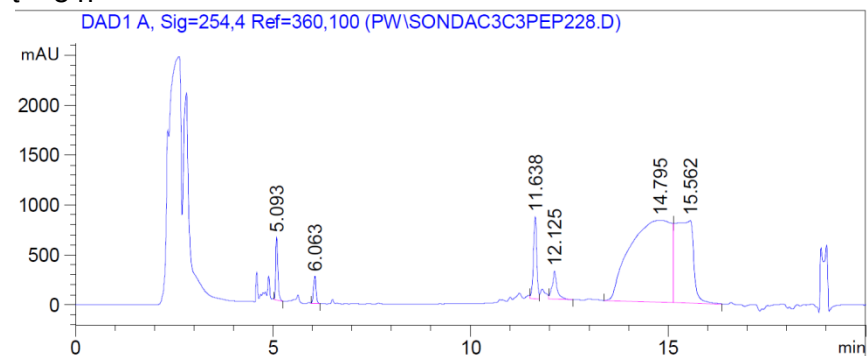
t = 0

DAD1 A, Sig=254,4 Ref=360,100 (PW\SONDAC3PEP206.D)



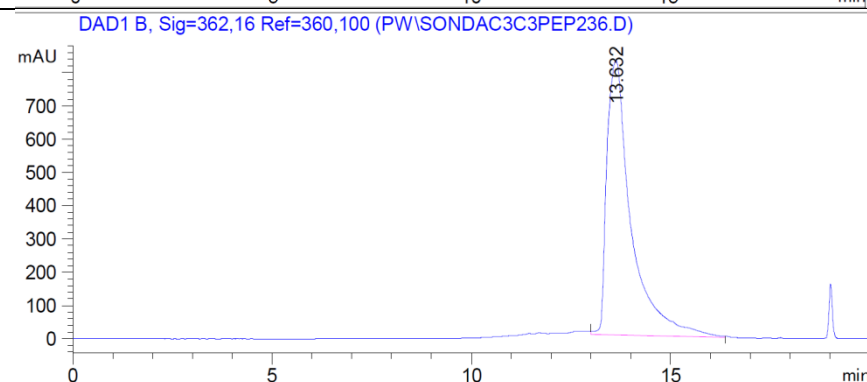
t = 5 h

DAD1 A, Sig=254,4 Ref=360,100 (PW\SONDAC3C3PEP228.D)



HPLC of purified product (method C)

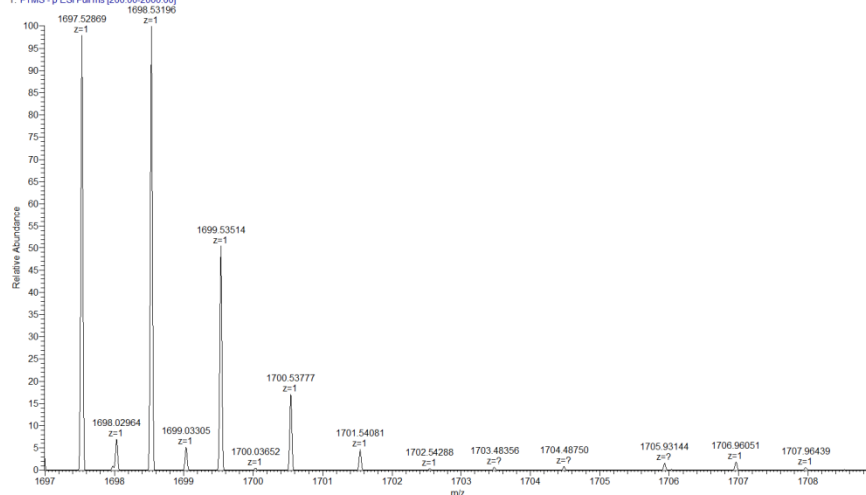
DAD1 B, Sig=362,16 Ref=360,100 (PW\SONDAC3C3PEP236.D)



HR MS

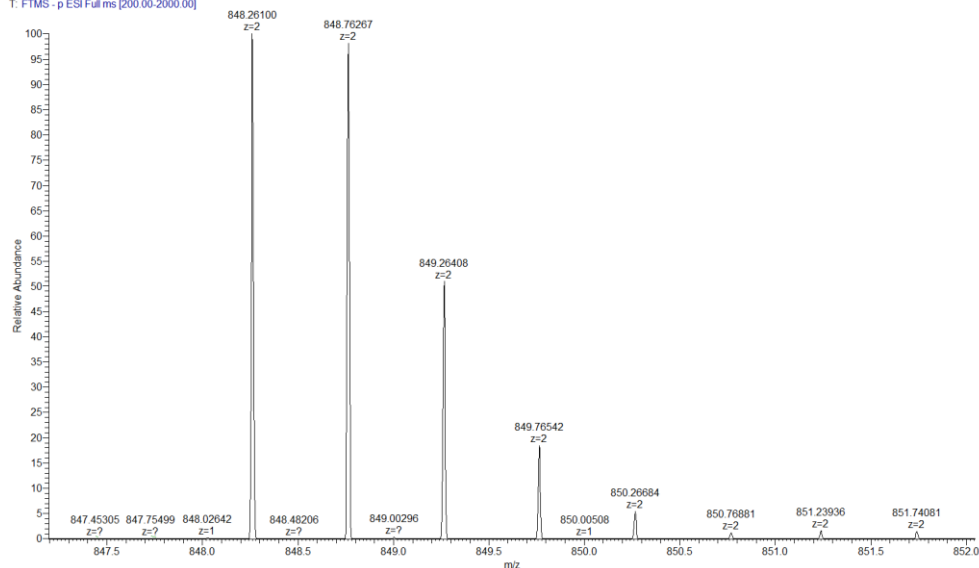
$m/z = 1$:

160630_PW_010 #92-199 RT: 0.91-1.94 AV: 108 NL: 8.78E5
T: FTMS - p ESI Full ms [200.00-2000.00]



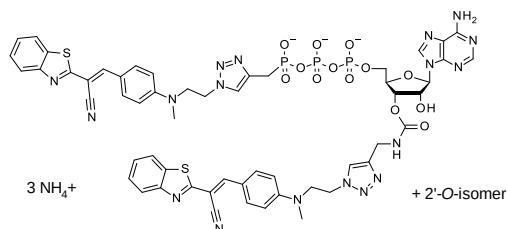
$m/z = 2$:

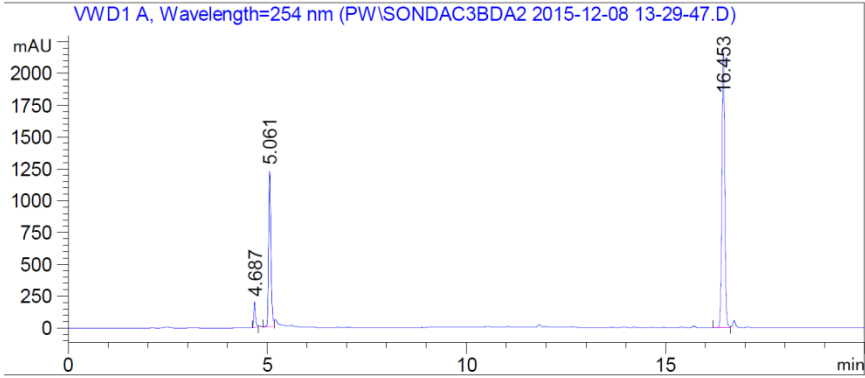
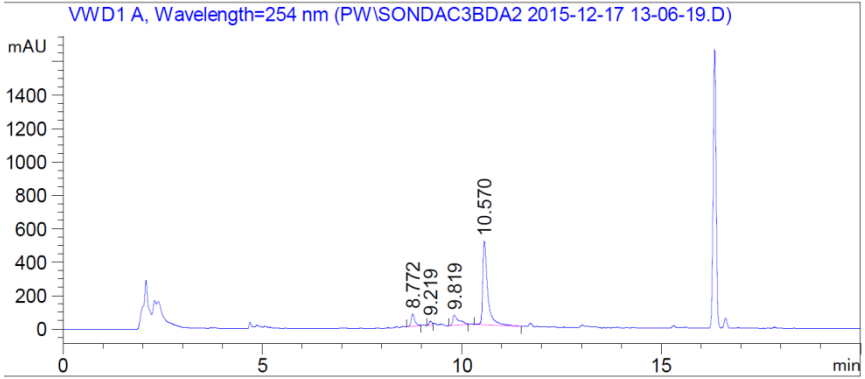
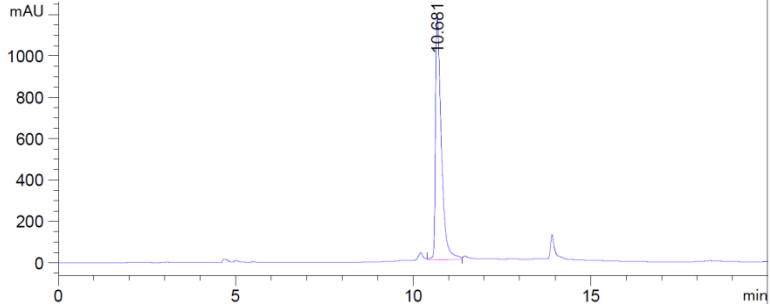
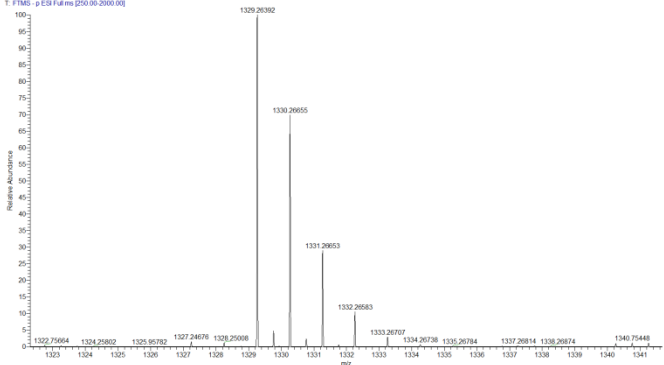
160630_PW_010 #92-199 RT: 0.91-1.94 AV: 108 NL: 2.09E6
T: FTMS - p ESI Full ms [200.00-2000.00]



7e

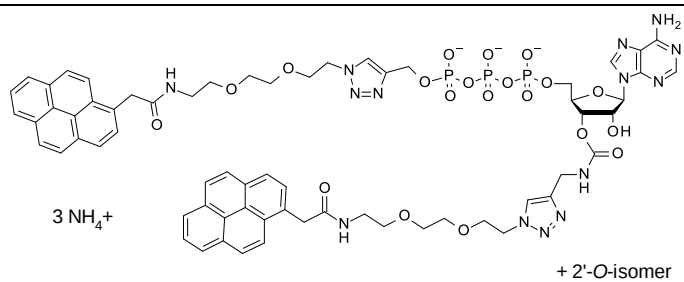
Structure



Reaction HPLC (method C)	t = 0 VWD1 A, Wavelength=254 nm (PW\SONDAC3BDA2 2015-12-08 13-29-47.D)  t = 2 h VWD1 A, Wavelength=254 nm (PW\SONDAC3BDA2 2015-12-17 13-06-19.D) 
HPLC of purified product (method C)	VWD1 A, Wavelength=254 nm (PW\SONDAC3(BDA)22016-06-1416-15-42.D) 
HR MS	

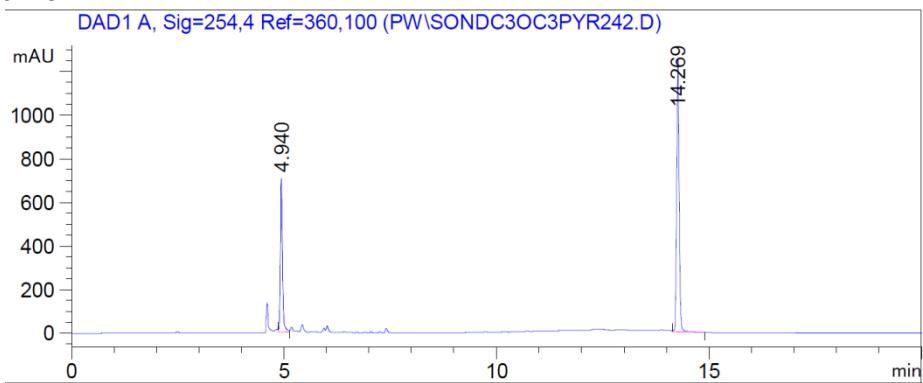
7f

Structure

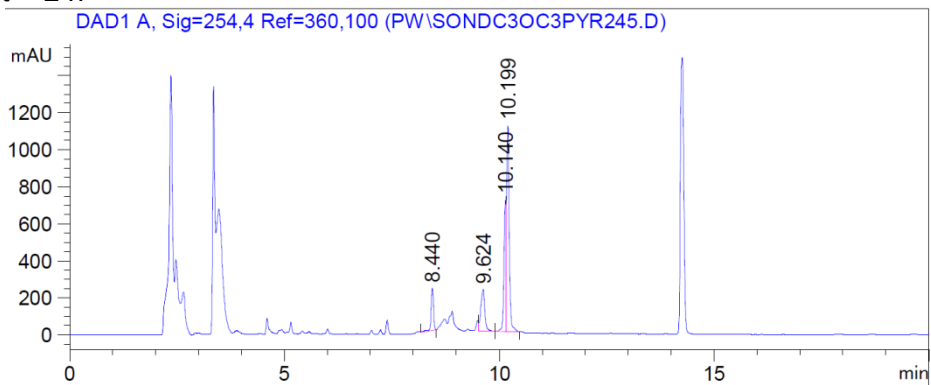
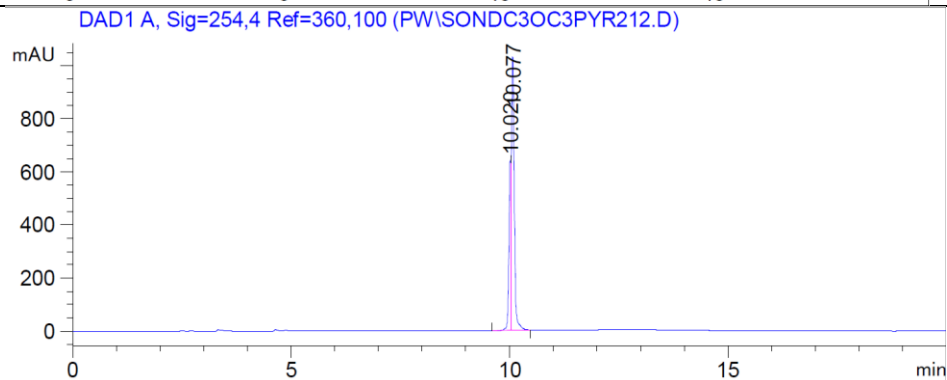


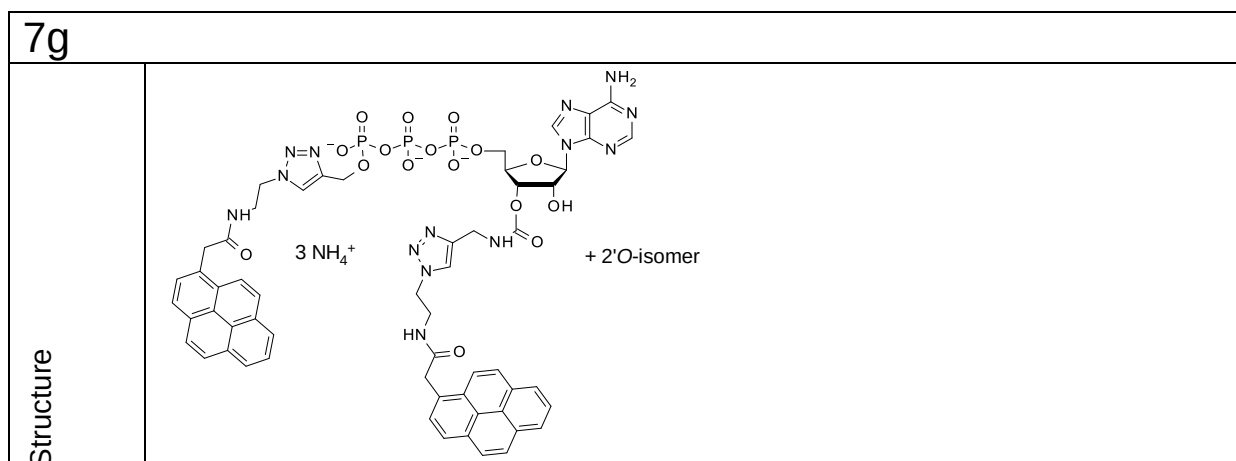
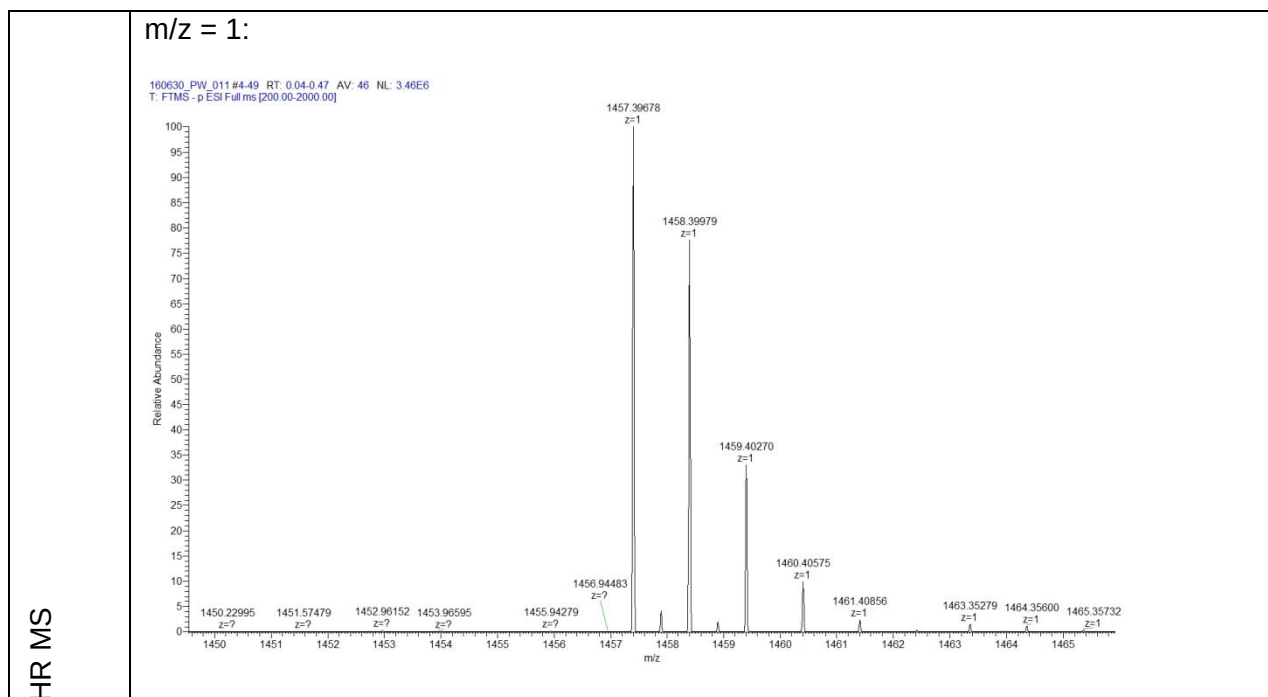
Reaction HPLC (method C)

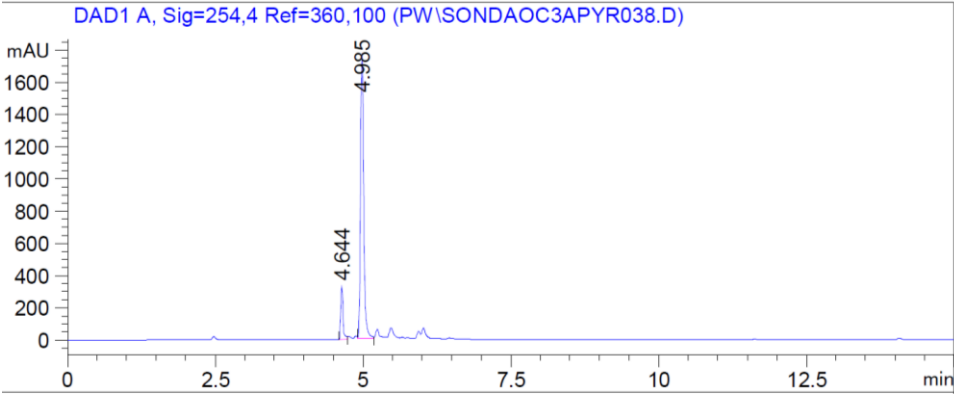
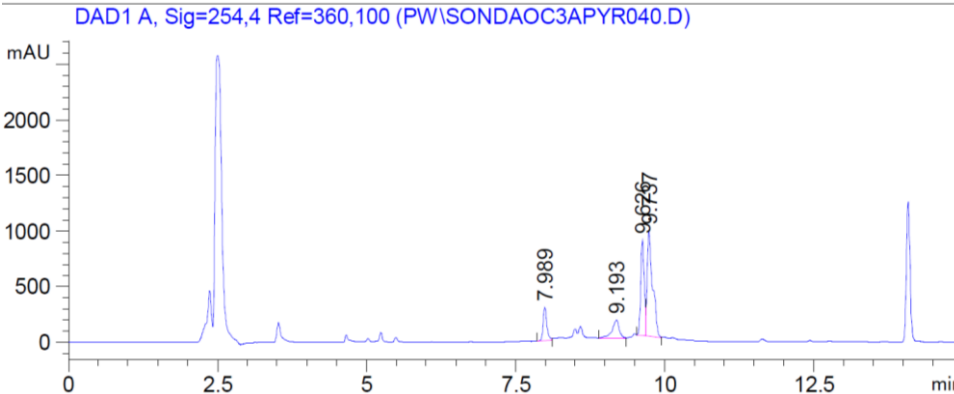
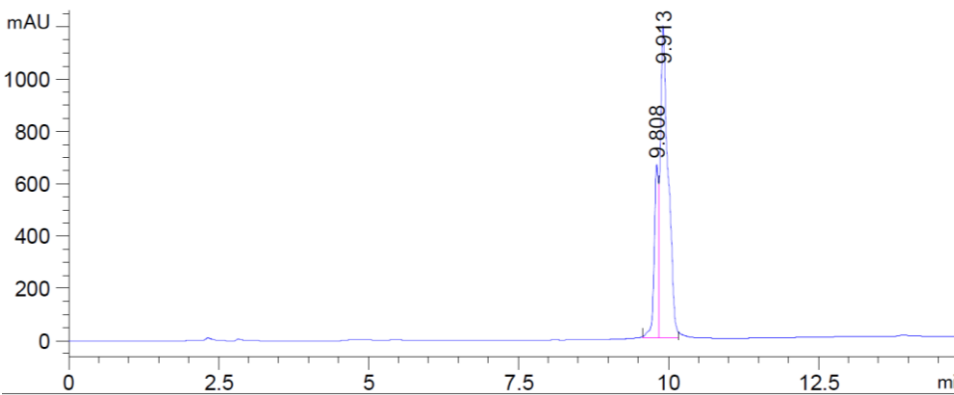
t = 0

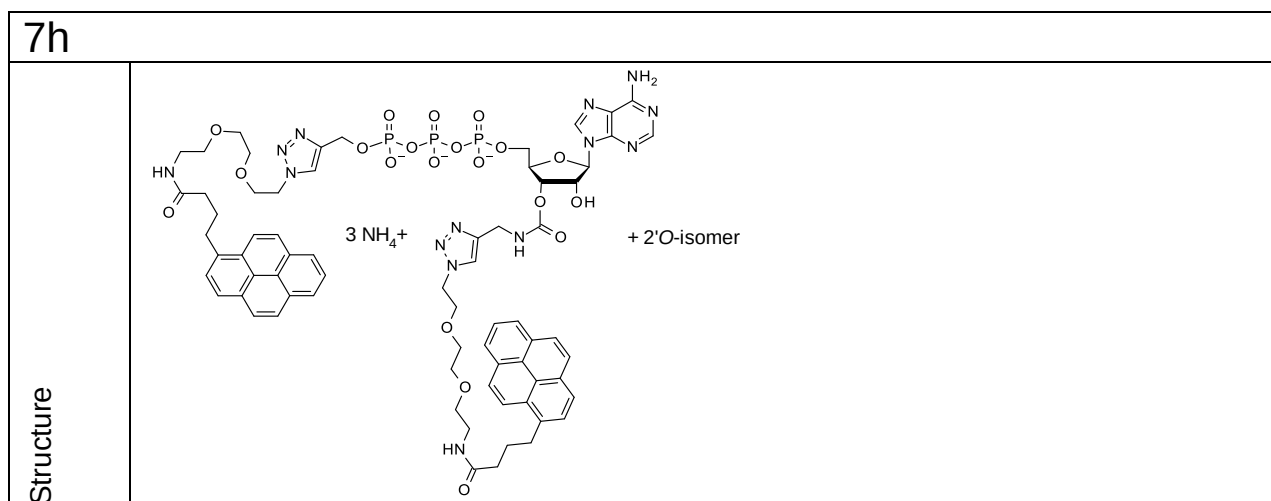
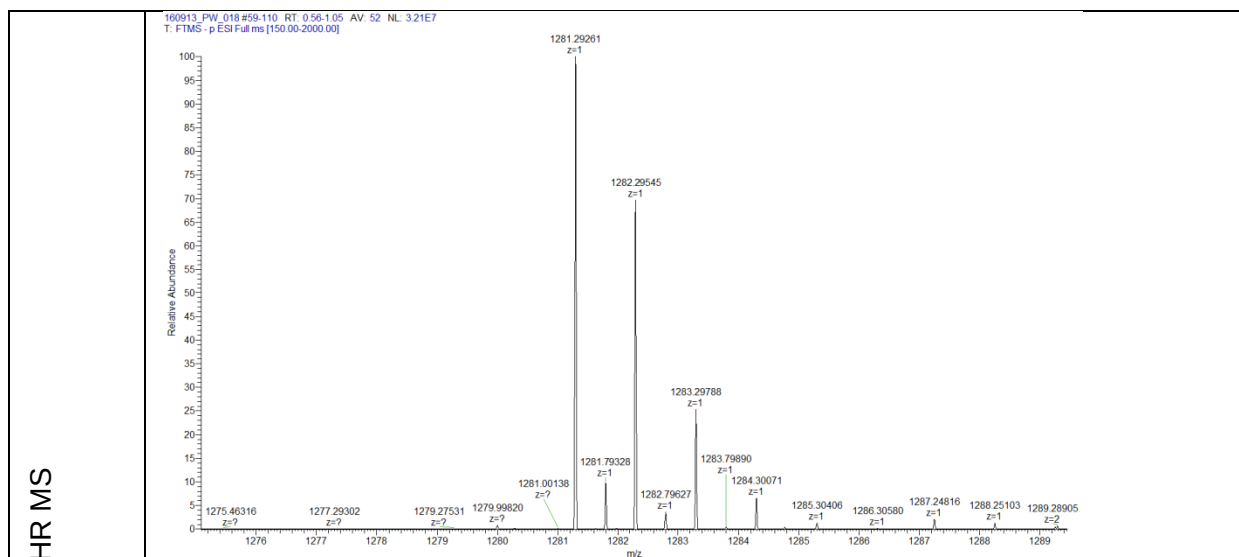


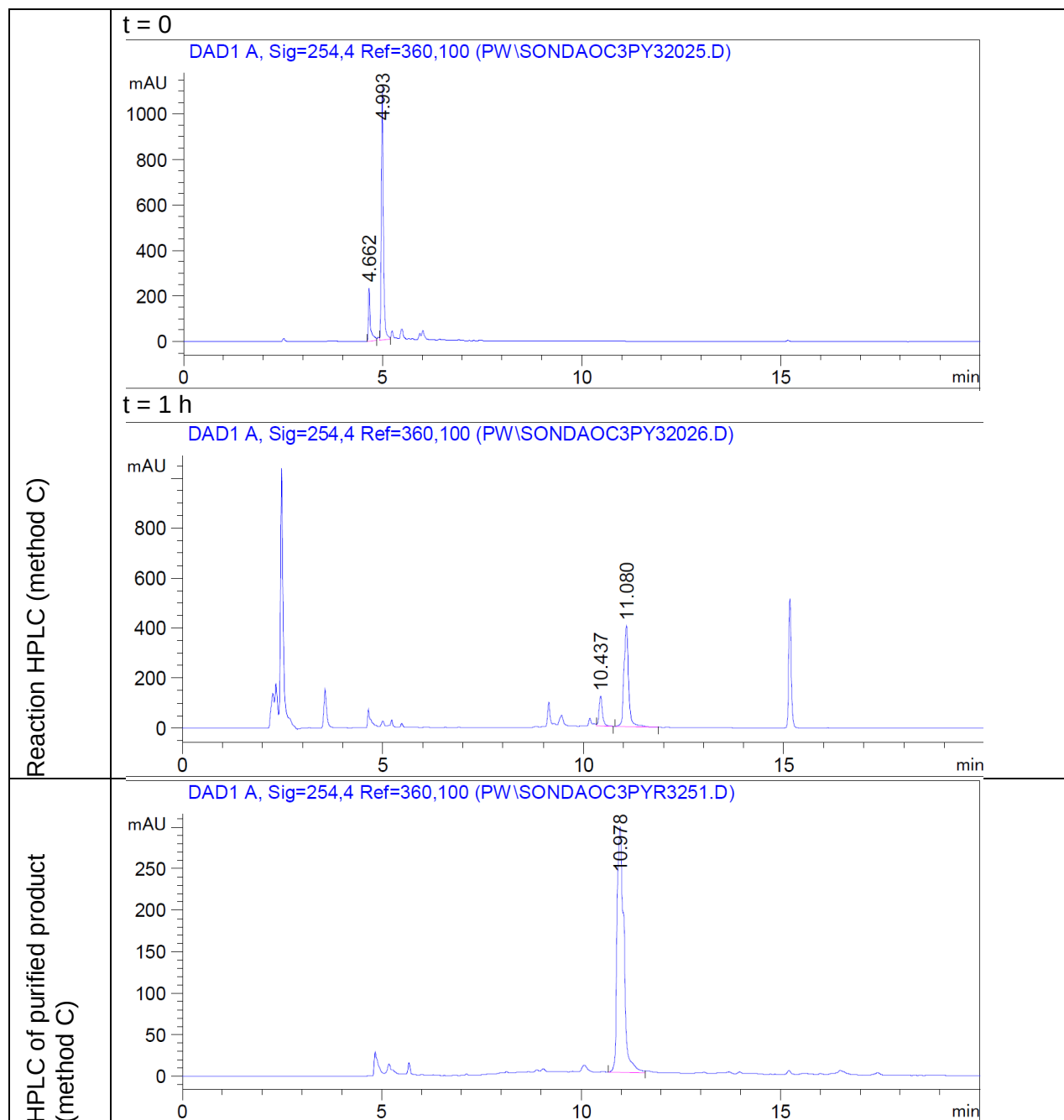
t = 2 h

HPLC of purified product
(method C)



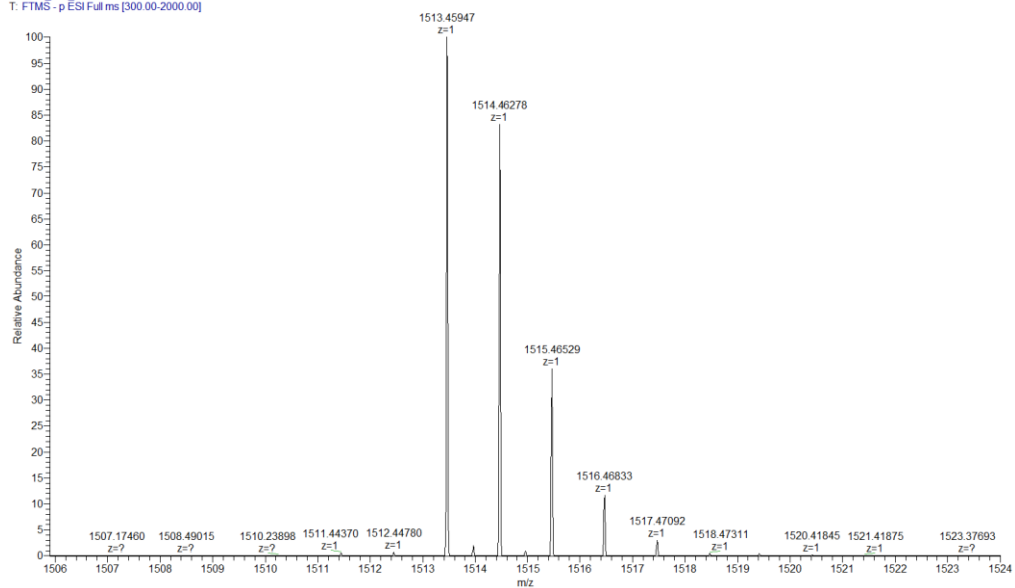
Reaction HPLC (Method C)	t = 0 DAD1 A, Sig=254,4 Ref=360,100 (PW\SONDAOC3APYR038.D)  t = 1 h DAD1 A, Sig=254,4 Ref=360,100 (PW\SONDAOC3APYR040.D) 
HPLC of purified product (method C)	VWD1 A, Wavelength=254 nm (PW\SONDAOC3(AAEPYR)212-50-022016-08-1988.D) 





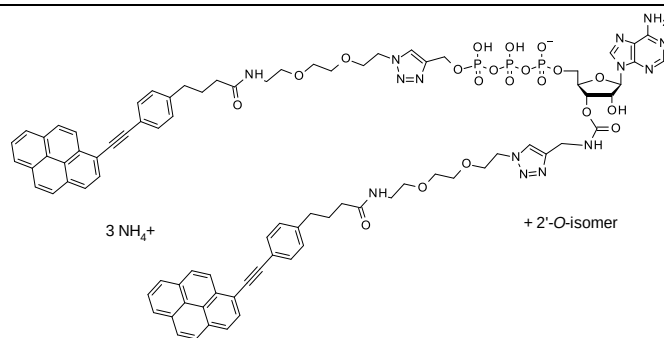
HR MS

160913_PW_019 #33-178 RT: 0.32-1.71 AV: 146 NL: 2.28E6
T: FTMS - p ESI Full ms [300.00-2000.00]



7i

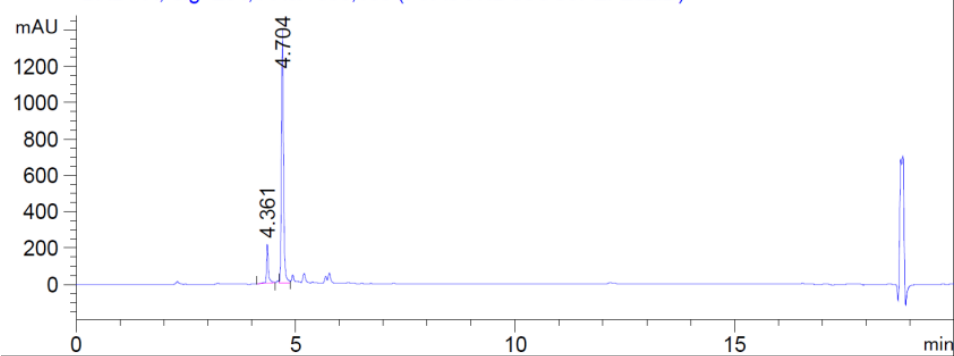
Structure



Reaction HPLC (method C + additional absorbance detection at 362 nm)

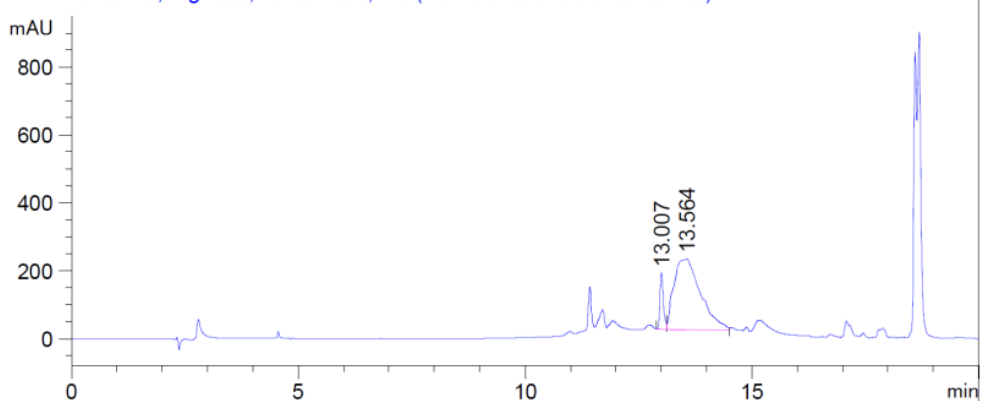
t = 0

DAD1 A, Sig=254,4 Ref=360,100 (PW\SONDC3OC3PEP202.D)



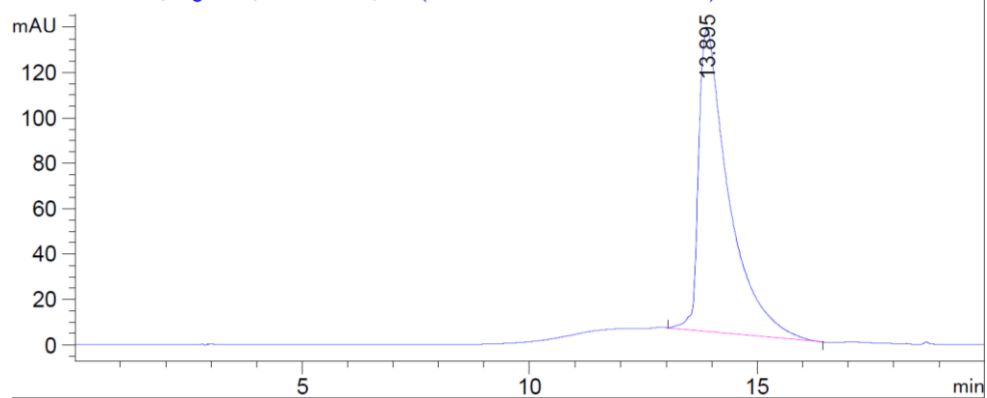
t = 5 h

DAD1 B, Sig=362,16 Ref=360,100 (PW\SONDC3OC3PEP211.D)



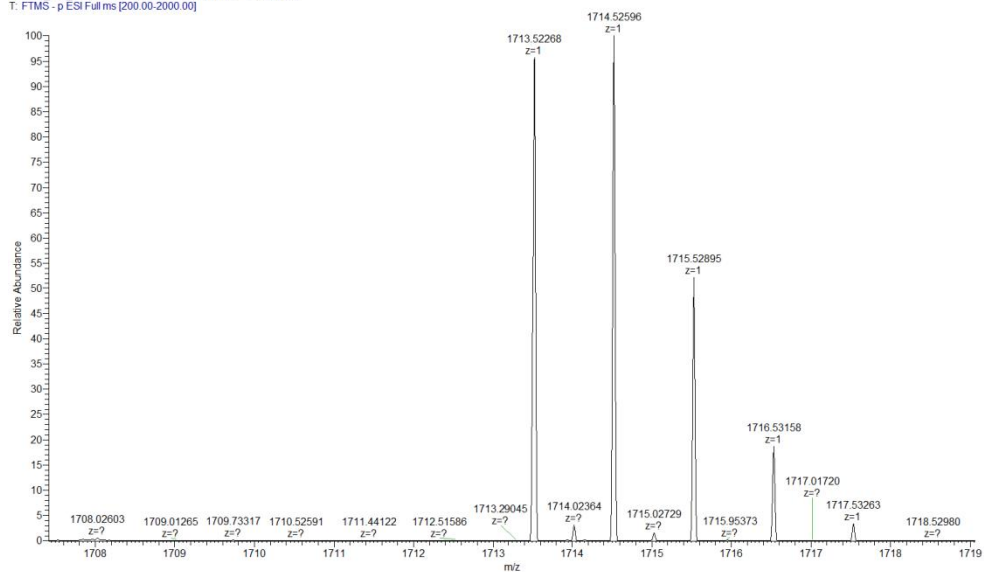
HPLC of purified product (method C + additional absorbance detection at 362 nm)

DAD1 B, Sig=362,16 Ref=360,100 (PW\SONDAOC3PEP2013.D)

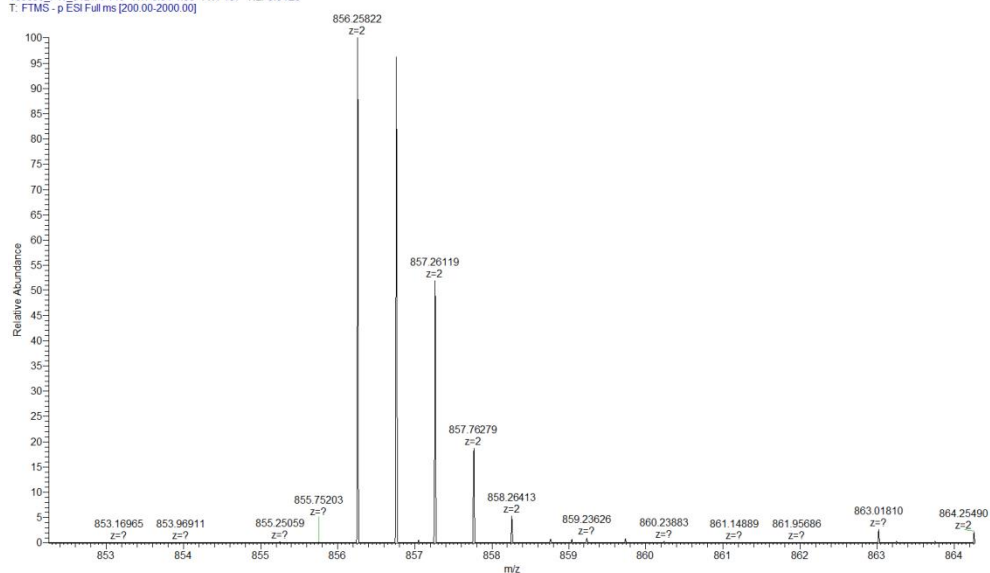


$m/z = 1:$

160630_PW_012 #4-140 RT: 0.04-1.35 AV: 137 NL: 3.48E5
T: FTMS - p ESI Full ms [200.00-2000.00]

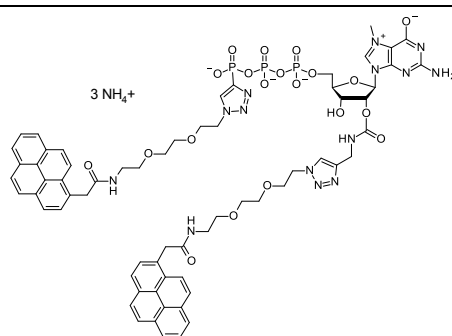
 $m/z = 2:$

160630_PW_012 #4-140 RT: 0.04-1.35 AV: 137 NL: 9.64E5
T: FTMS - p ESI Full ms [200.00-2000.00]



8a

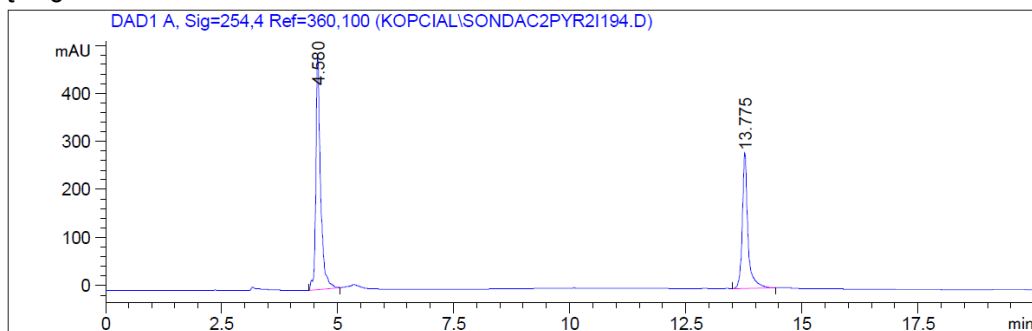
Structure



Reaction HPLC (method C)

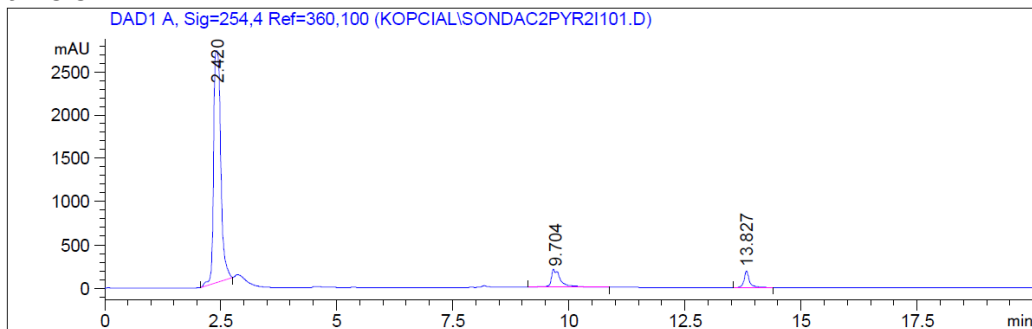
t = 0

DAD1 A, Sig=254,4 Ref=360,100 (KOPCIAL\SONDAC2PYR21194.D)



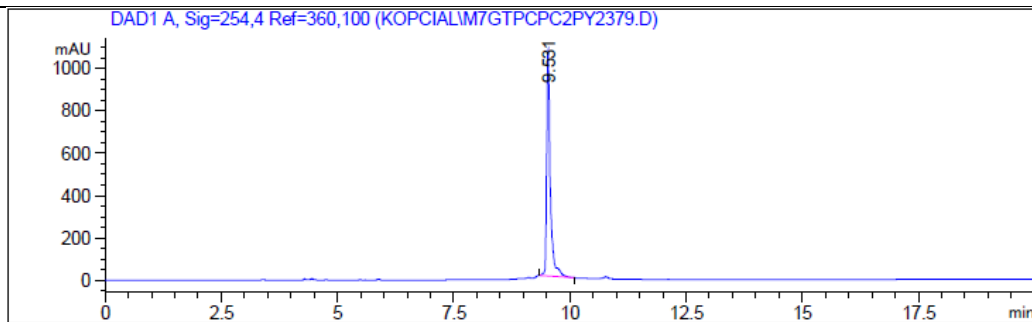
t = 5.5 h

DAD1 A, Sig=254,4 Ref=360,100 (KOPCIAL\SONDAC2PYR21101.D)



HPLC of purified product (method C)

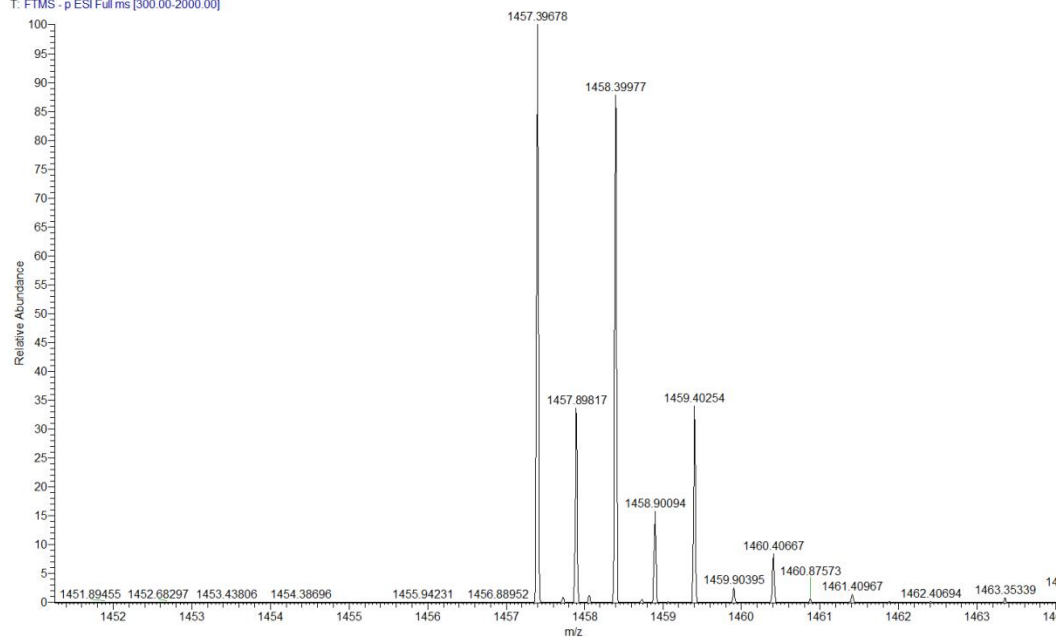
DAD1 A, Sig=254,4 Ref=360,100 (KOPCIAL\M7GTPCPC2PY2379.D)



HR MS

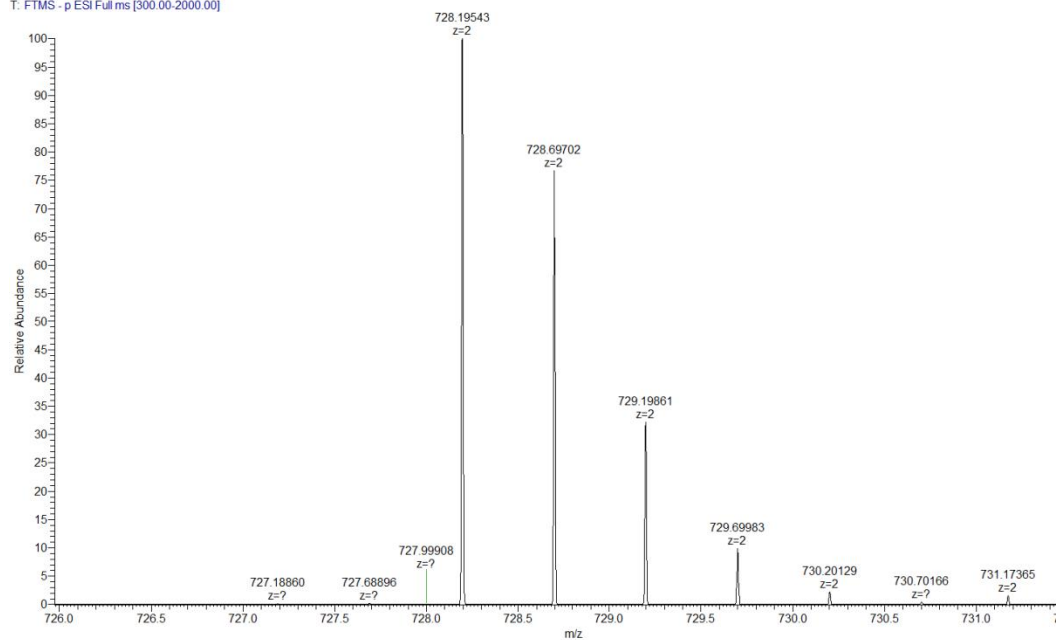
Z=1

160913_MK_A1685 #26-168 RT: 0.25-1.60 AV: 143 NL: 3.14E6
T: FTMS - p ESI Full ms [300.00-2000.00]



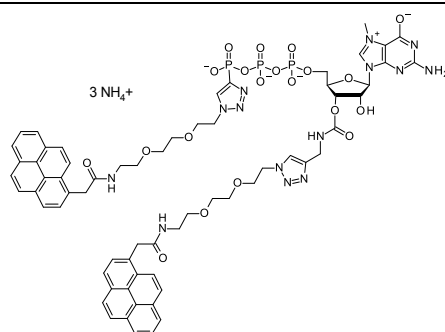
Z=2

160913_MK_A1685 #168 RT: 1.60 AV: 1 NL: 2.91E7
T: FTMS - p ESI Full ms [300.00-2000.00]



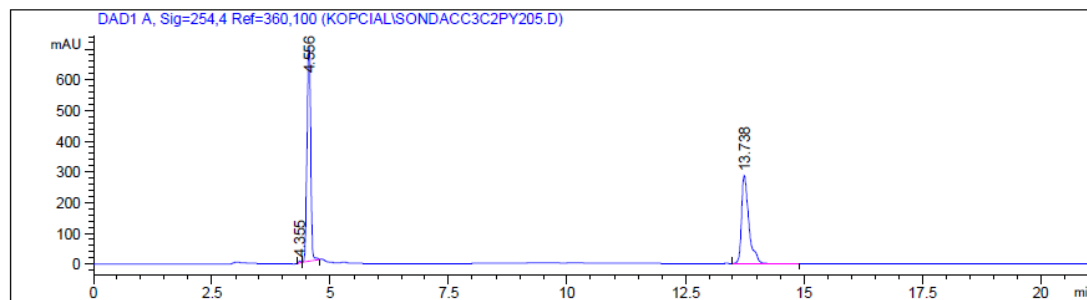
8b

Structure

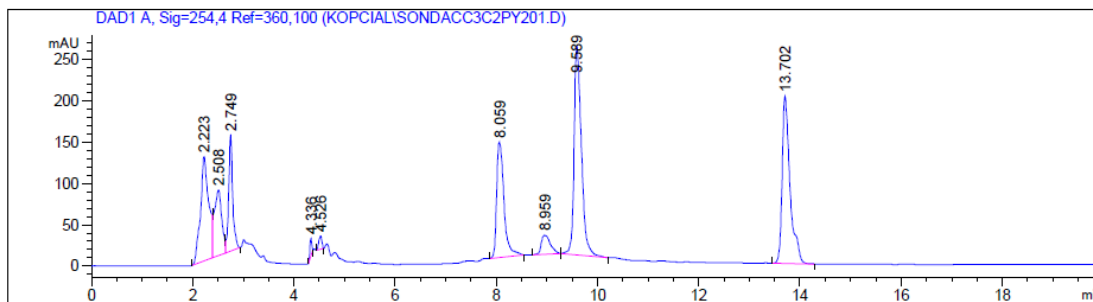


Reaction HPLC (method C)

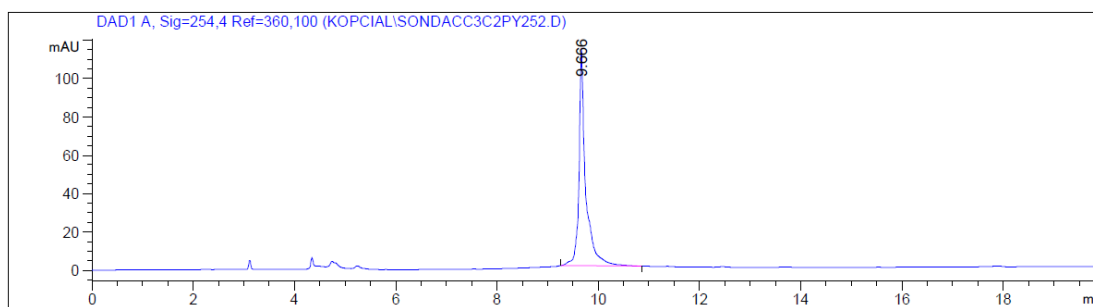
t = 0



t = 5 h



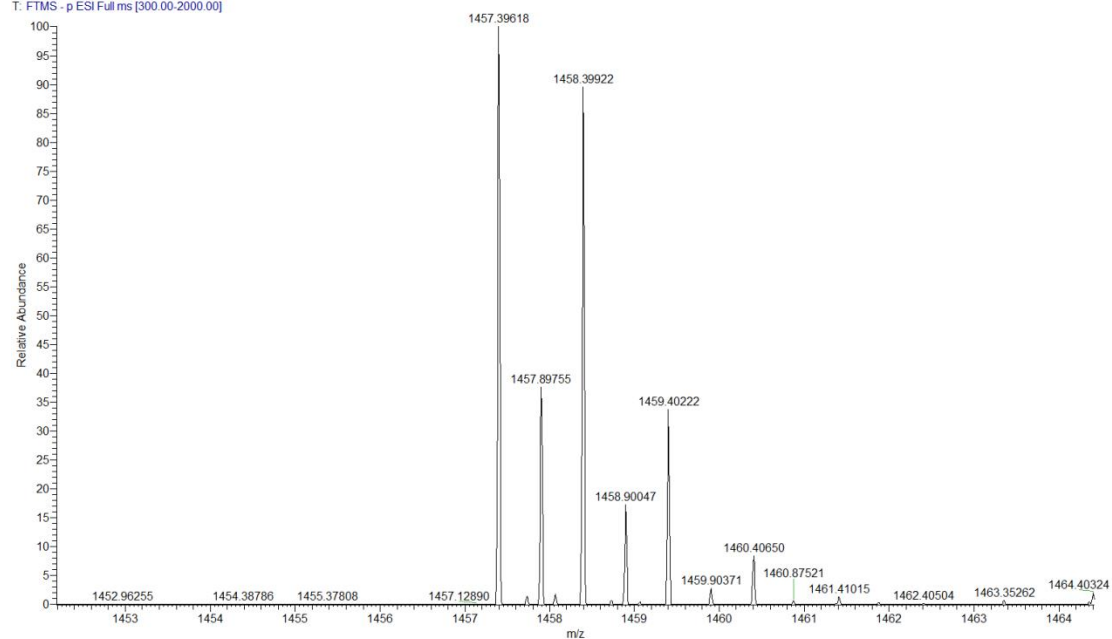
HPLC of purified product (method C)



HR MS

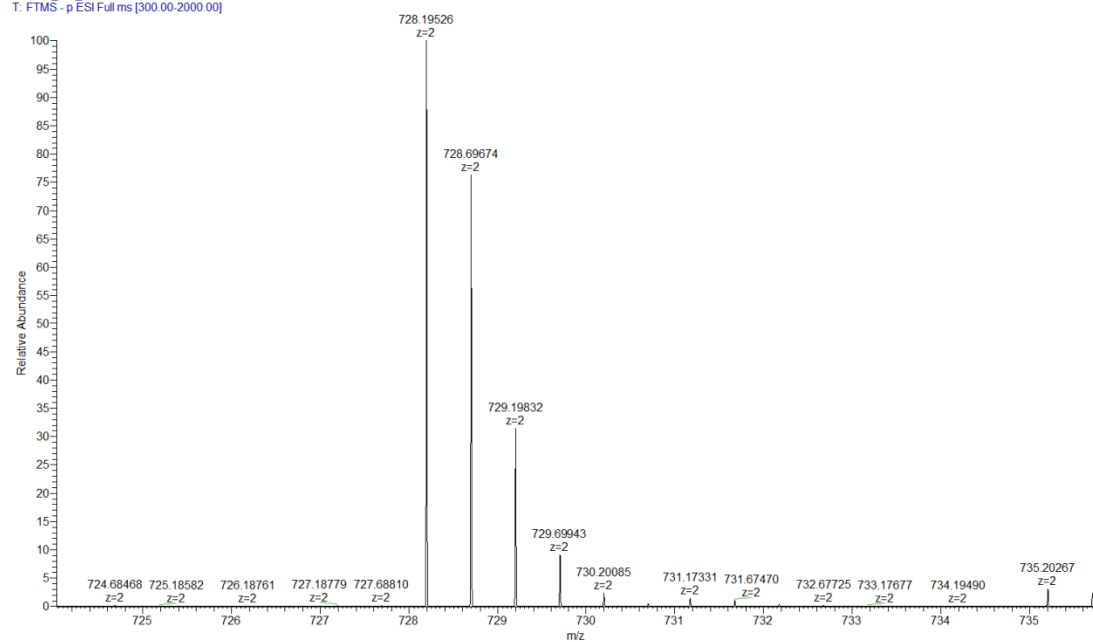
Z=1

160913_MK_A1686 #4-61 RT: 0.04-0.58 AV: 58 NL: 2.69E6
T: FTMS -p ESI Full ms [300.00-2000.00]



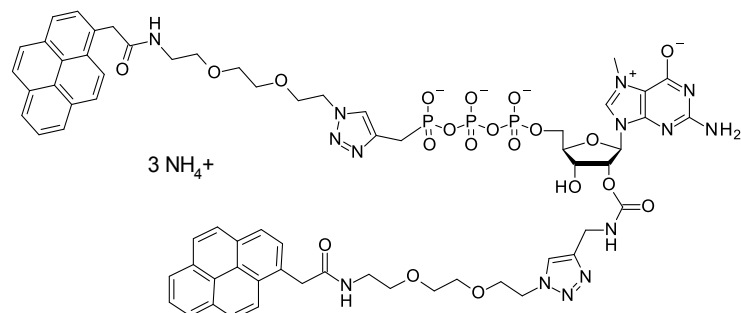
Z=2

160913_MK_A1686 #4-61 RT: 0.04-0.58 AV: 58 NL: 3.65E7
T: FTMS -p ESI Full ms [300.00-2000.00]



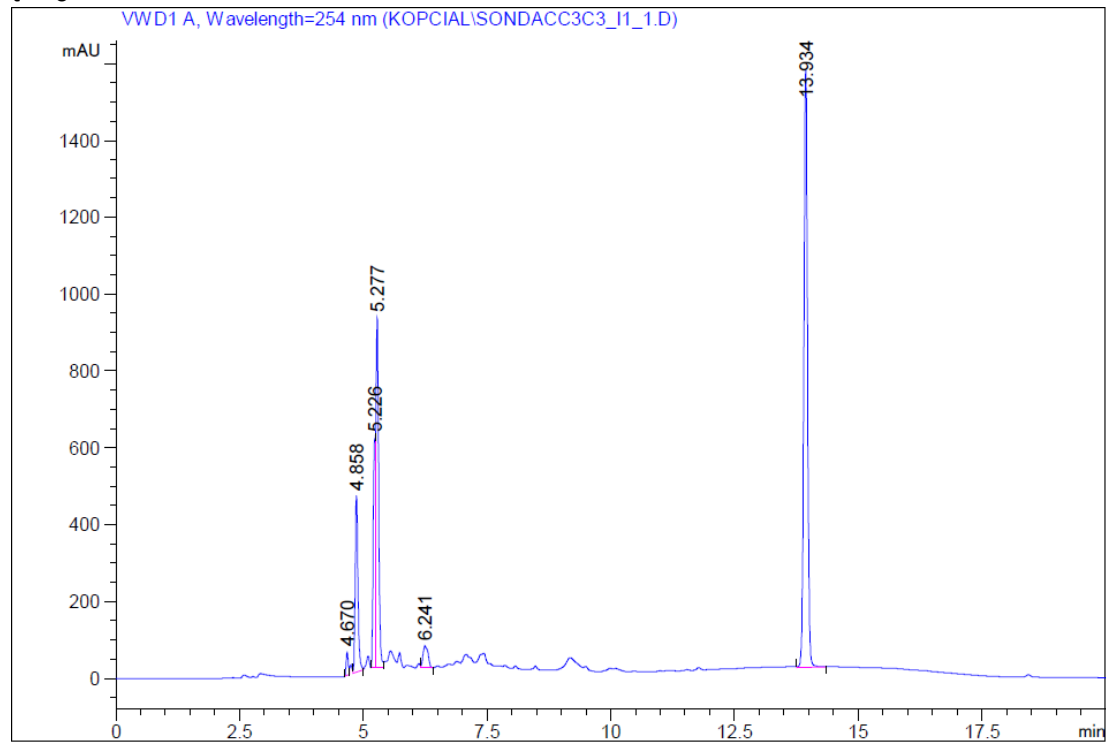
8c

Structure

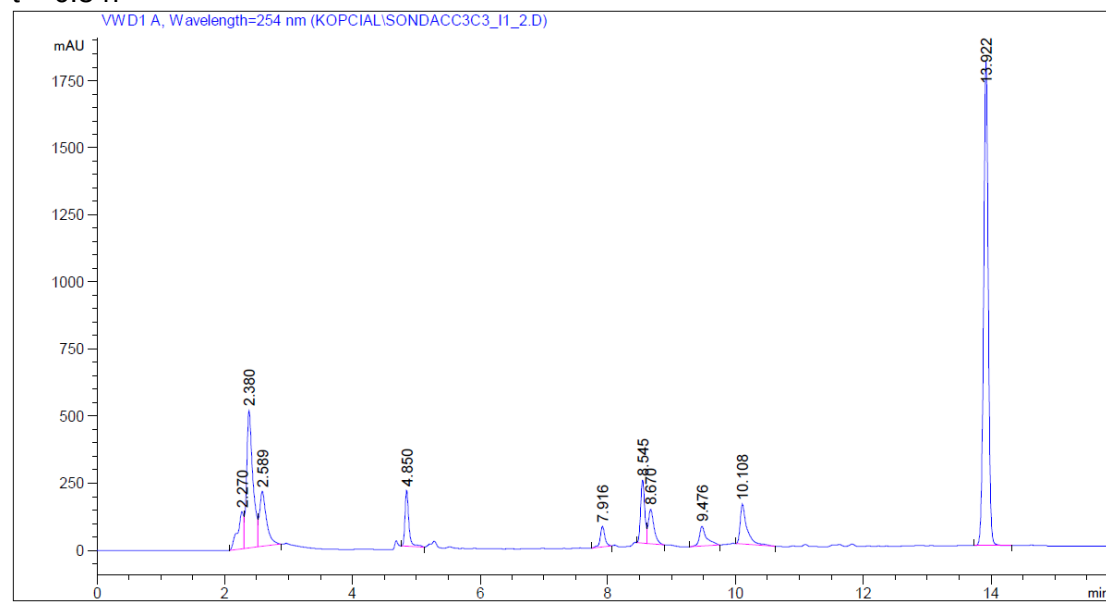


Reaction HPLC (method C)

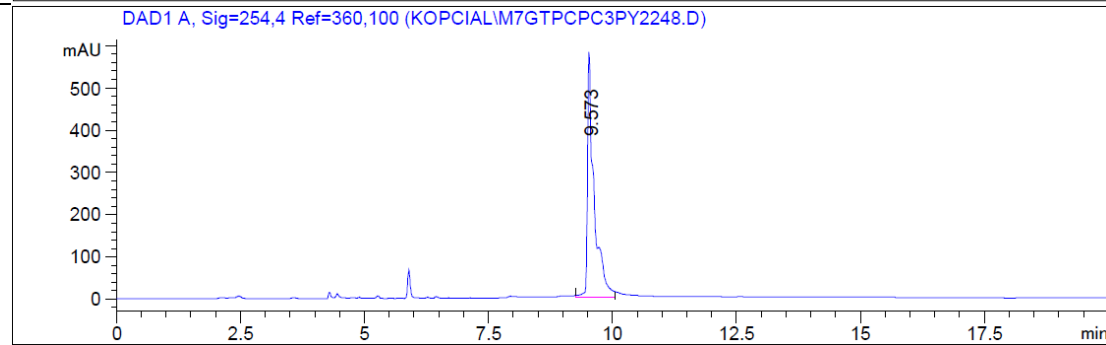
t = 0



t = 0.5 h



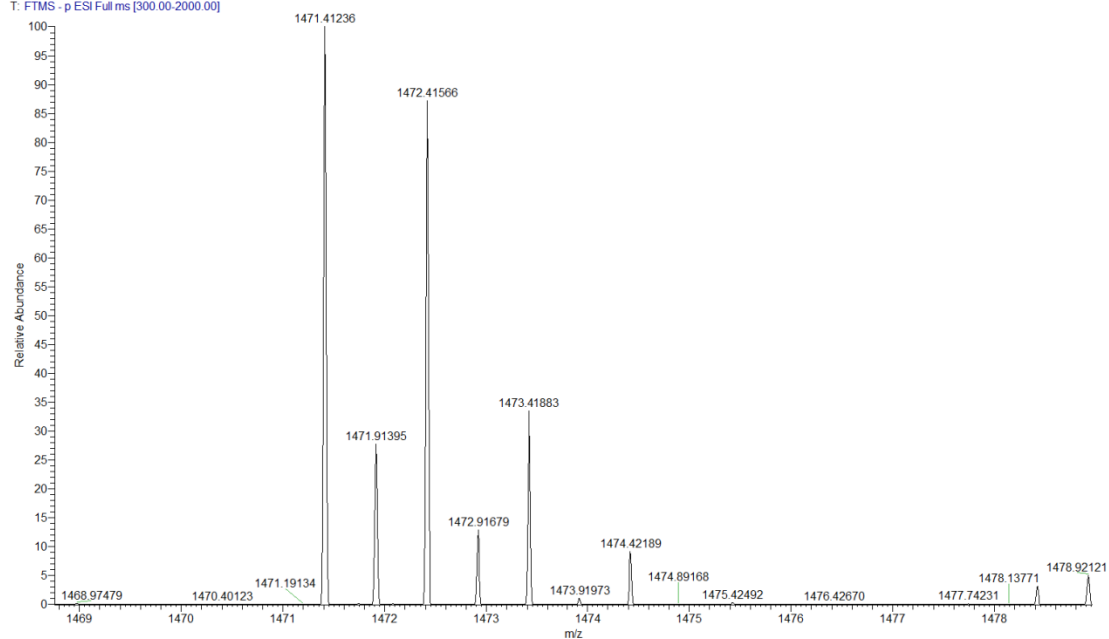
HPLC of purified product (method C)



HR MS

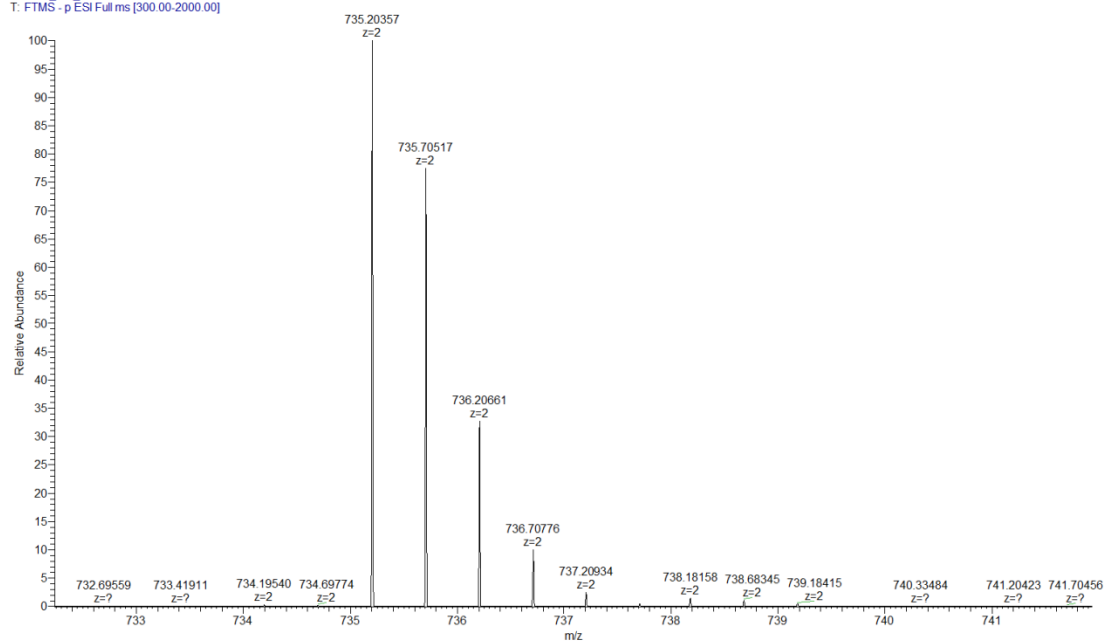
Z=1

160913_MK_A1684 #13-157 RT: 0.12-1.50 AV: 145 NL: 5.64E5
T: FTMS -p ESI Full ms [300.00-2000.00]



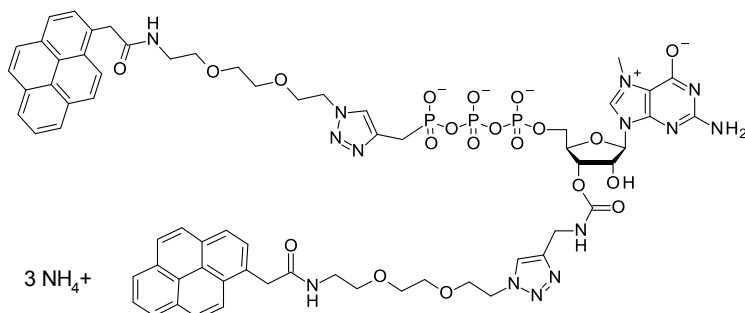
Z=2

160913_MK_A1684 #10-157 RT: 0.10-1.50 AV: 148 NL: 1.35E7
T: FTMS -p ESI Full ms [300.00-2000.00]



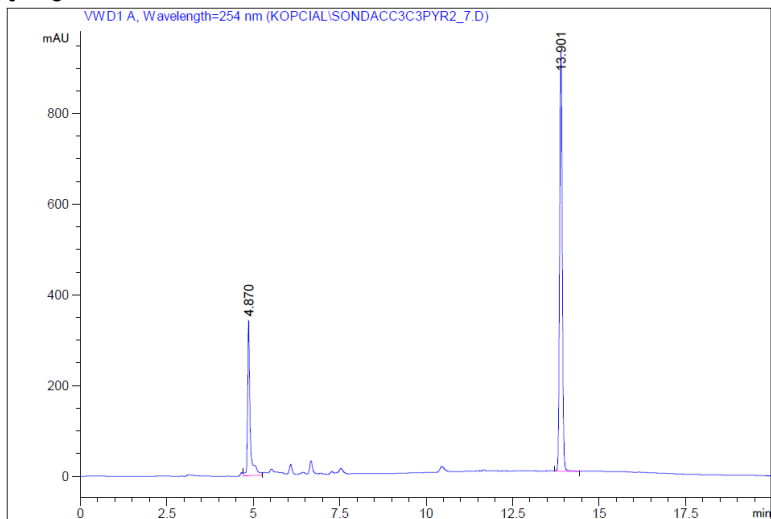
8d

Structure

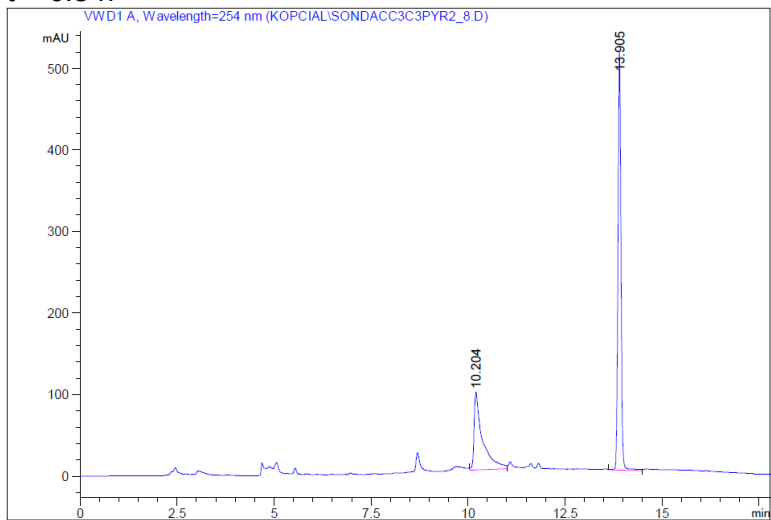


Reaction HPLC (method C)

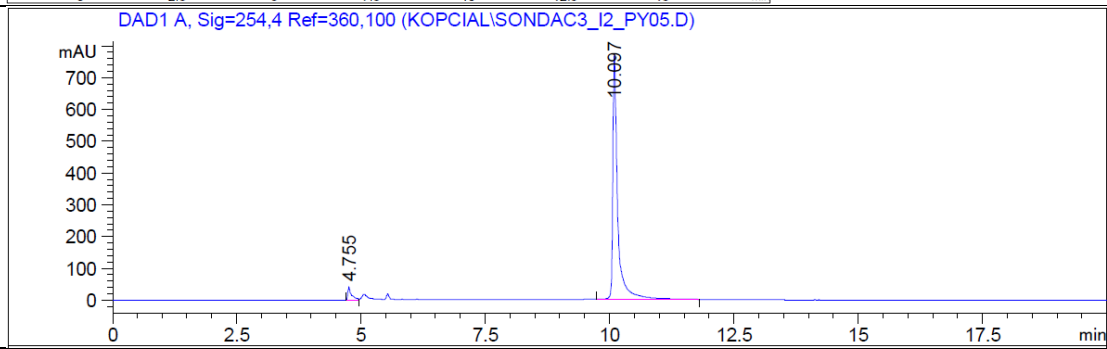
t = 0



t = 0.5 h



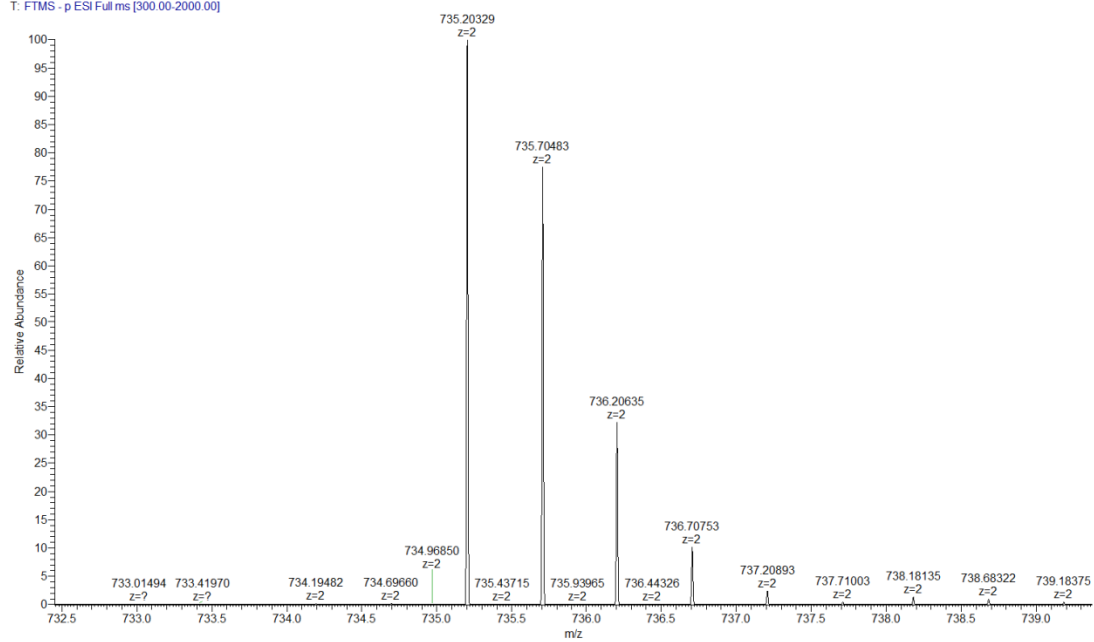
HPLC of purified product (method C)



HR MS

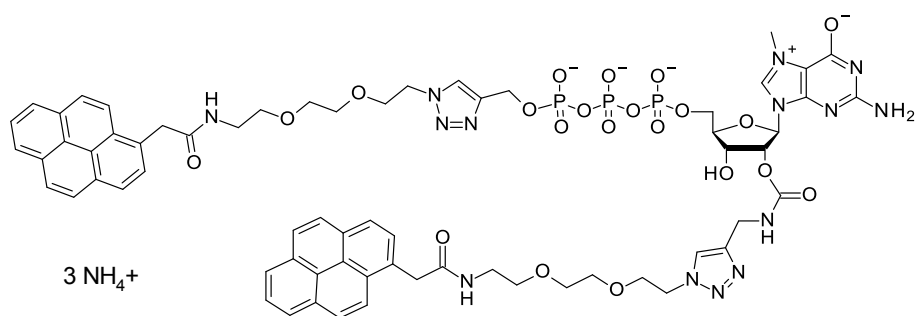
Z=2

160913_MK_A1683 #33-175 RT: 0.31-1.66 AV: 143 NL: 5.37E7
T: FTMS - p ESI Full ms [300.00-2000.00]



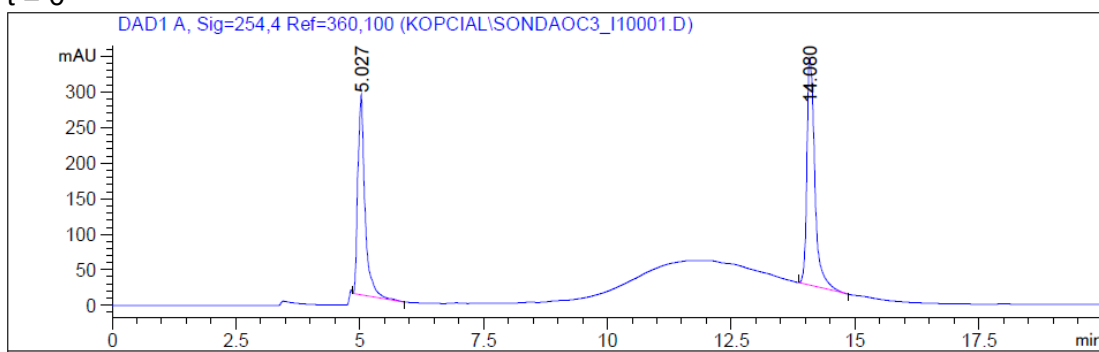
8e

Structure

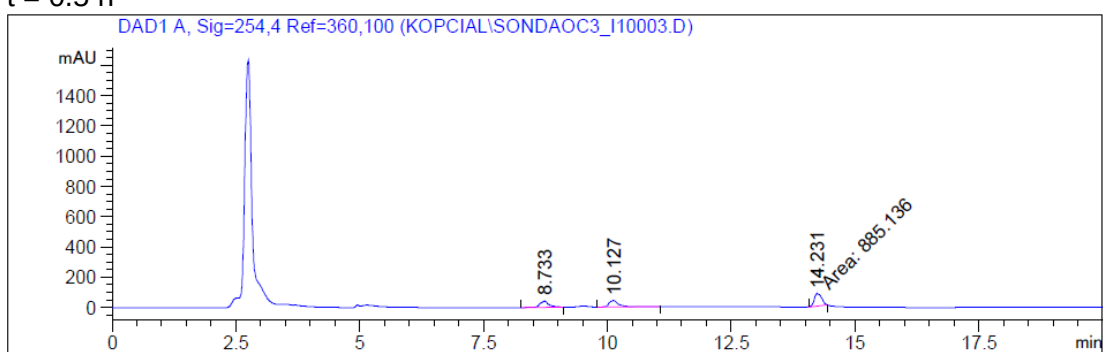


Reaction HPLC (method C)

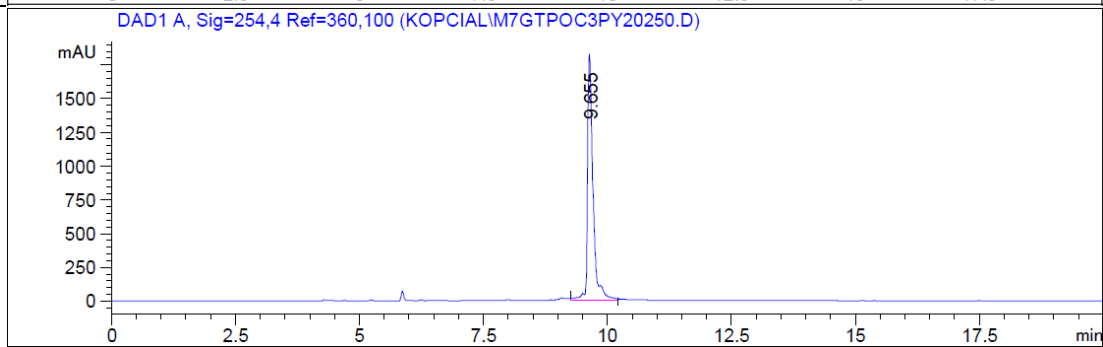
t = 0



t = 0.5 h



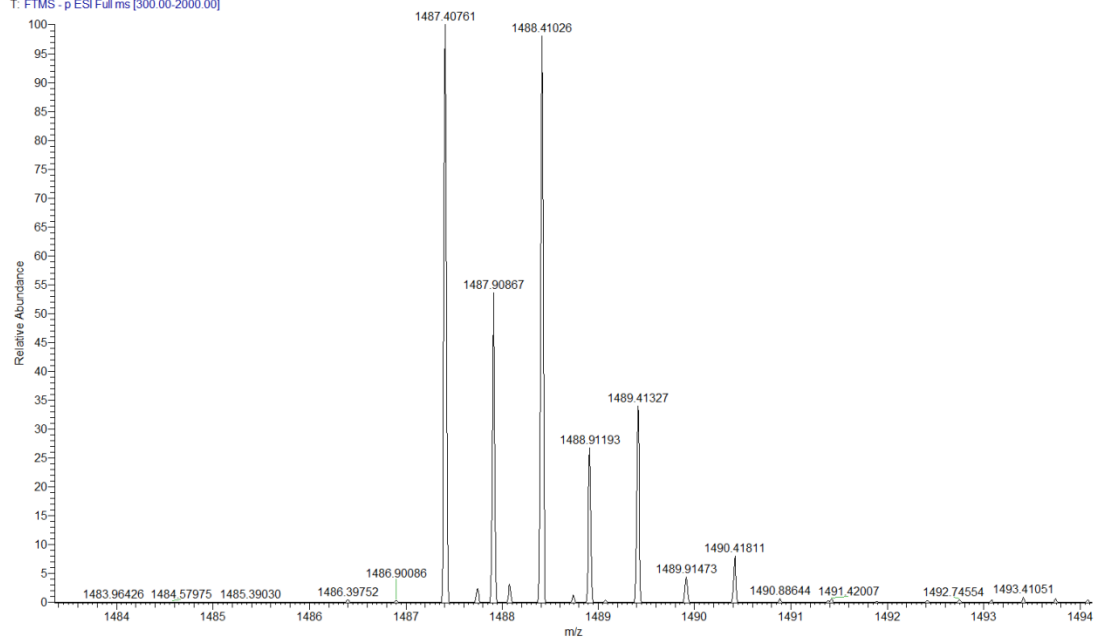
HPLC of purified product (method C)



HR MS

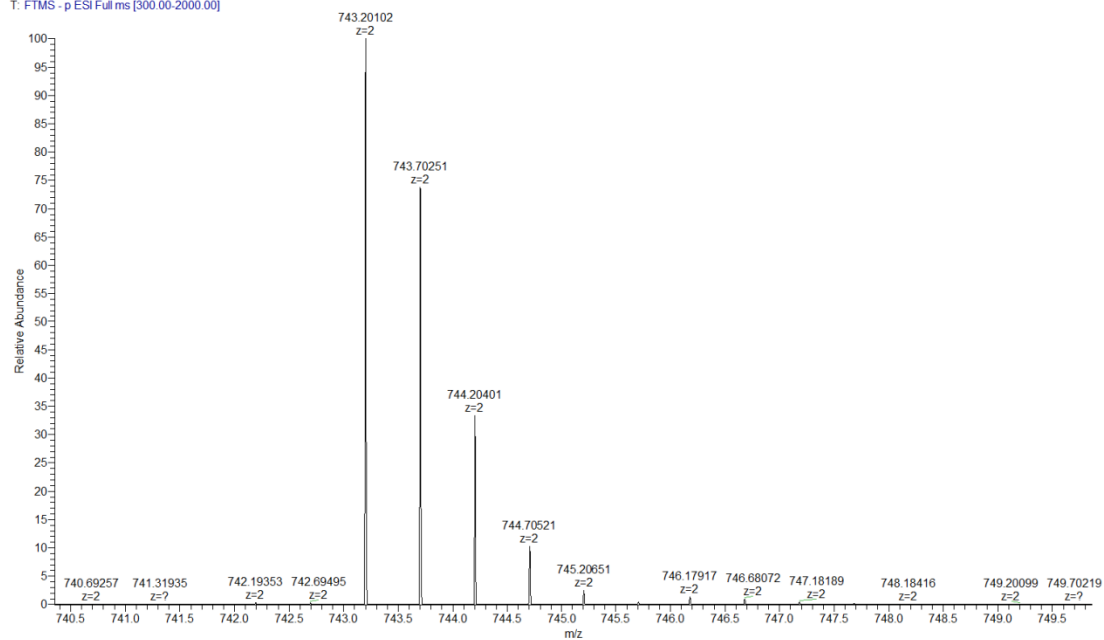
Z=1

160913_MK_A1681 #23-176 RT: 0.22-1.68 AV: 154 NL: 1.28E6
T: FTMS -p ESI Full ms [300.00-2000.00]



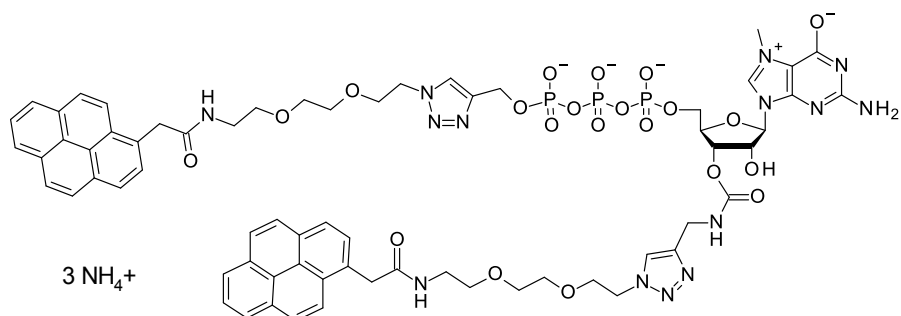
Z=2

160913_MK_A1681 #23-176 RT: 0.22-1.68 AV: 154 NL: 2.97E7
T: FTMS -p ESI Full ms [300.00-2000.00]



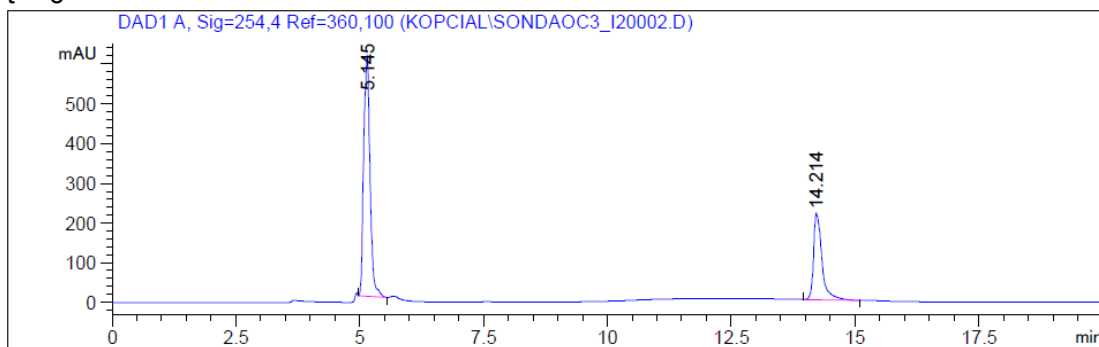
8f

Structure

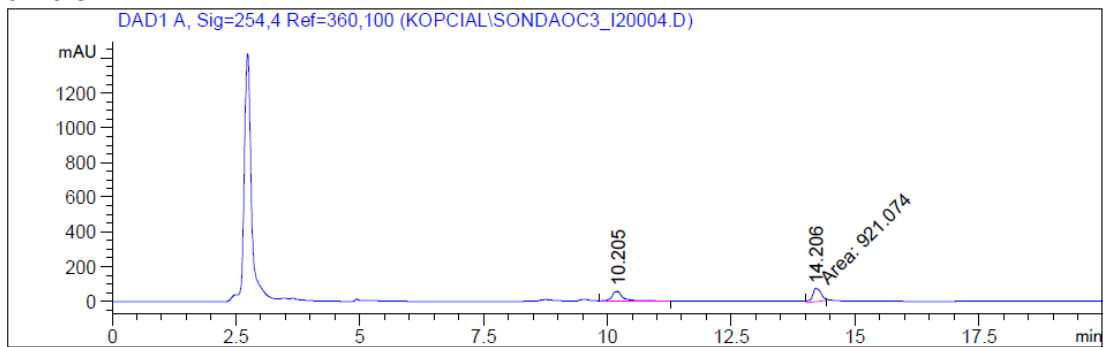


Reaction HPLC (method C)

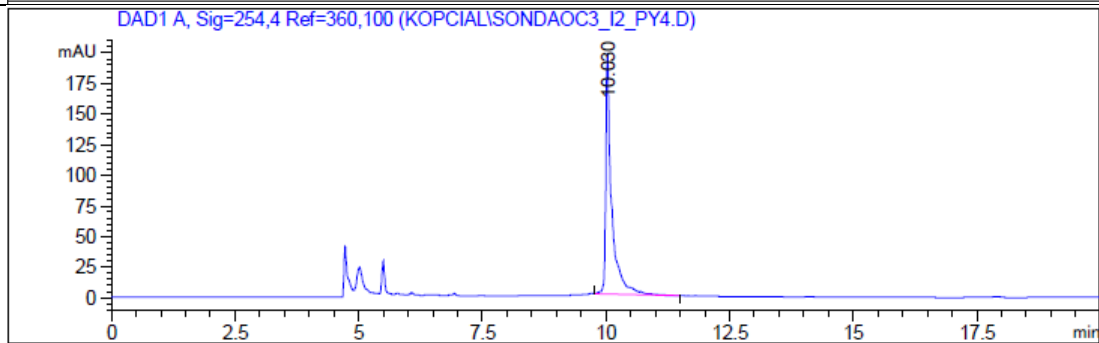
t = 0



t = 0.5 h



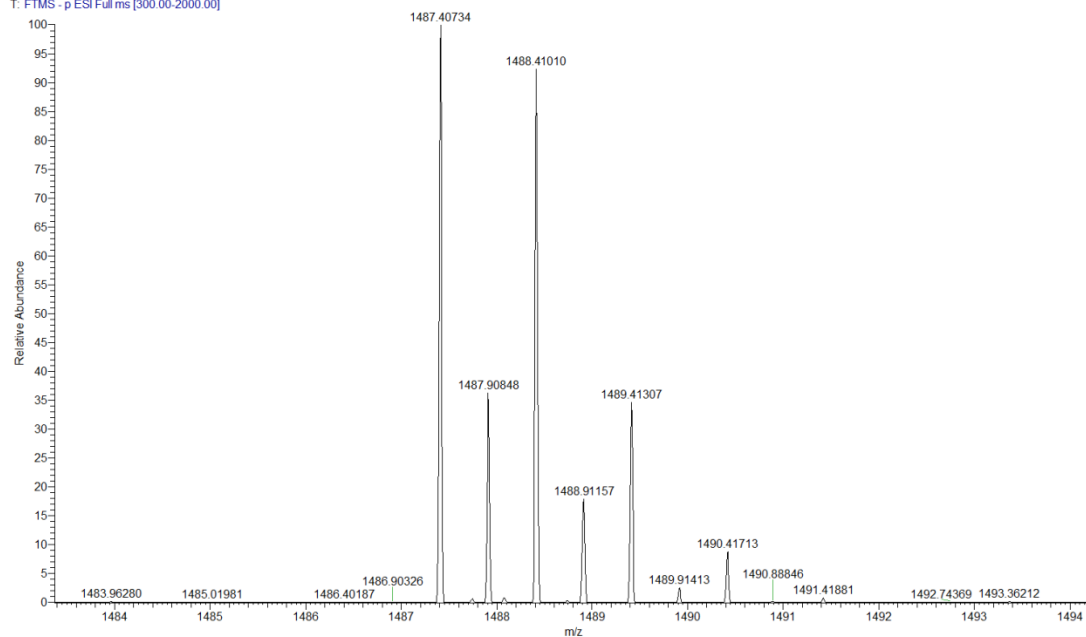
HPLC of purified product (method C)



HR MS

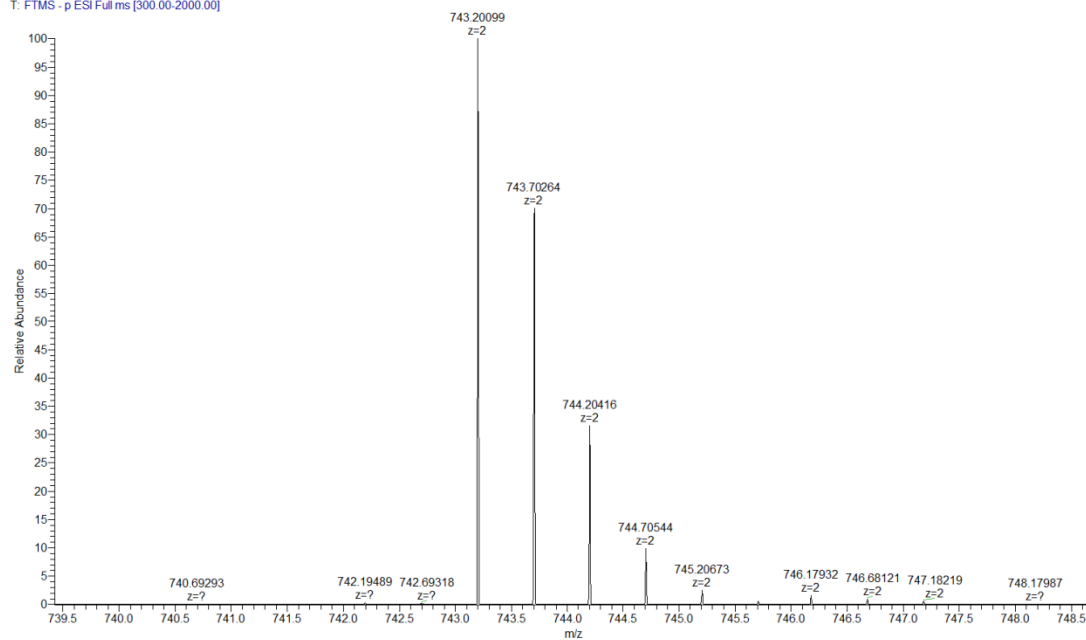
Z=1

160913_MK_A1682 #29-179 RT: 0.28-1.71 AV: 151 NL: 9.31E5
T: FTMS -p ESI Full ms [300.00-2000.00]



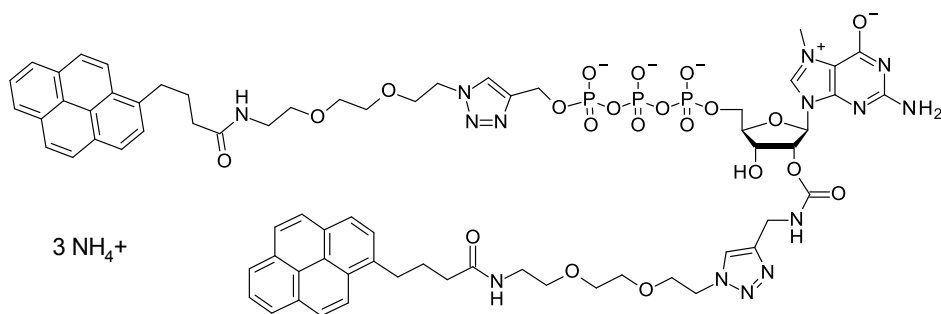
Z=2

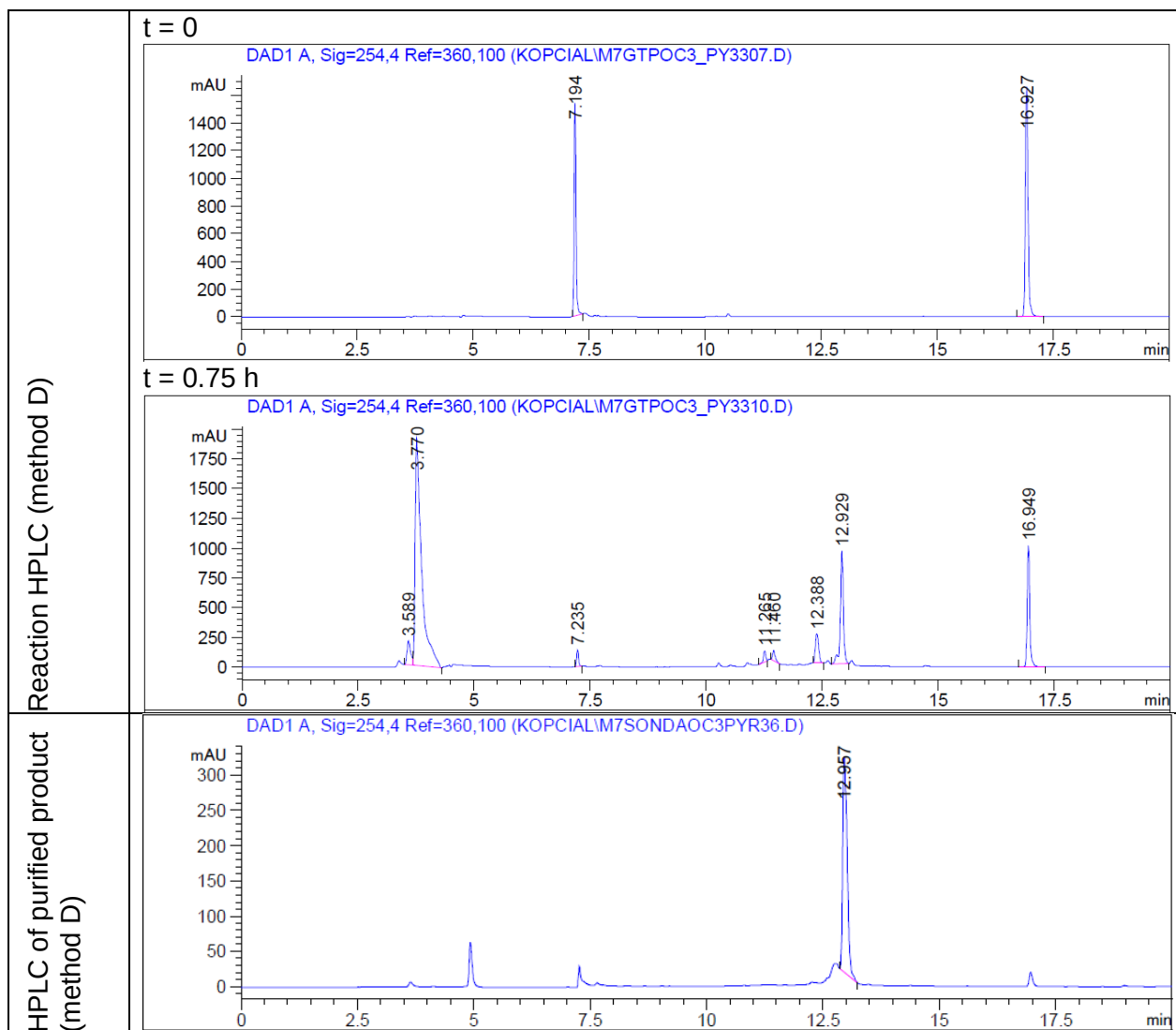
160913_MK_A1682 #179 RT: 1.71 AV: 1 NL: 2.54E7
T: FTMS -p ESI Full ms [300.00-2000.00]



8g

Structure

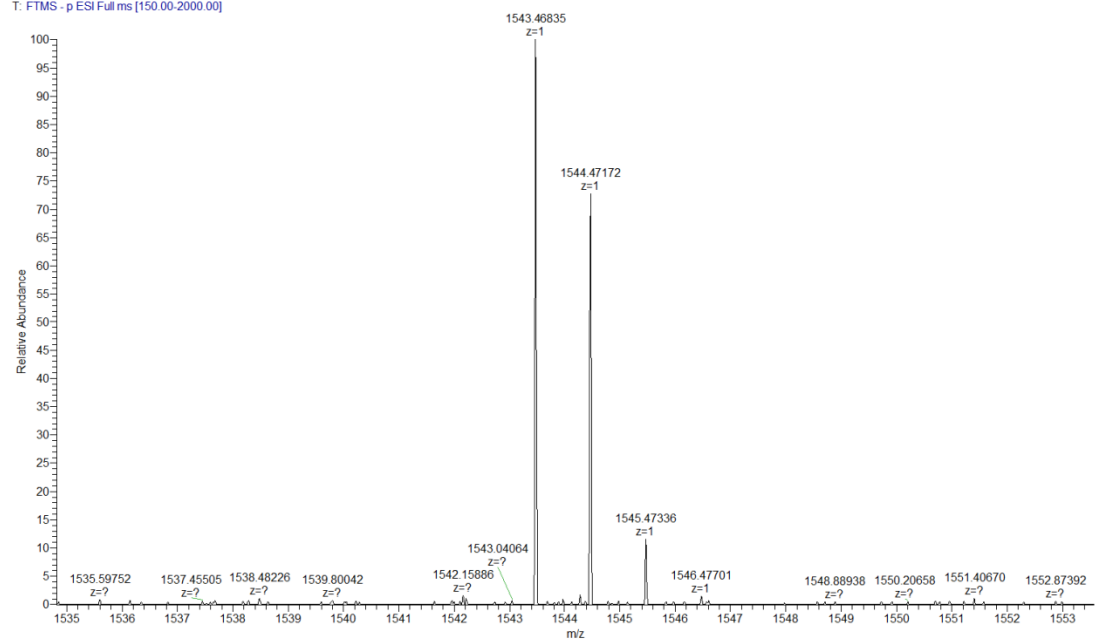




HR MS

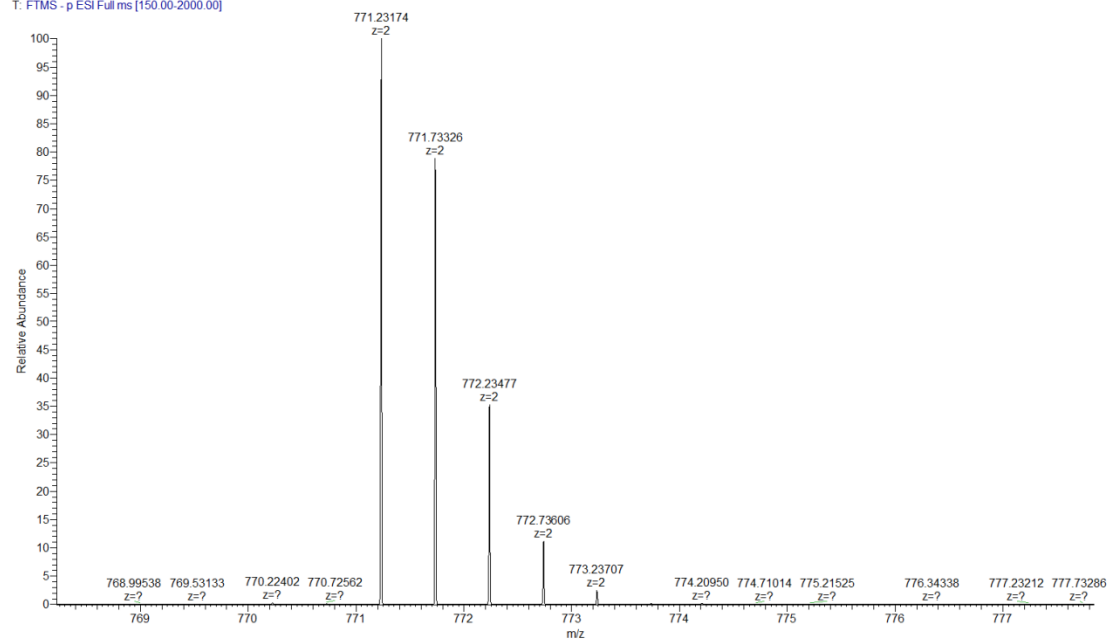
Z=1

161115_mkpa_2 #13-164 RT: 0.12-1.56 AV: 152 NL: 3.70E4
T: FTMS - p ESI Full ms [150.00-2000.00]



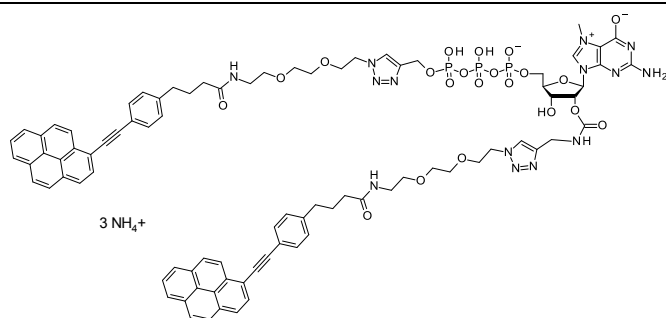
Z=2

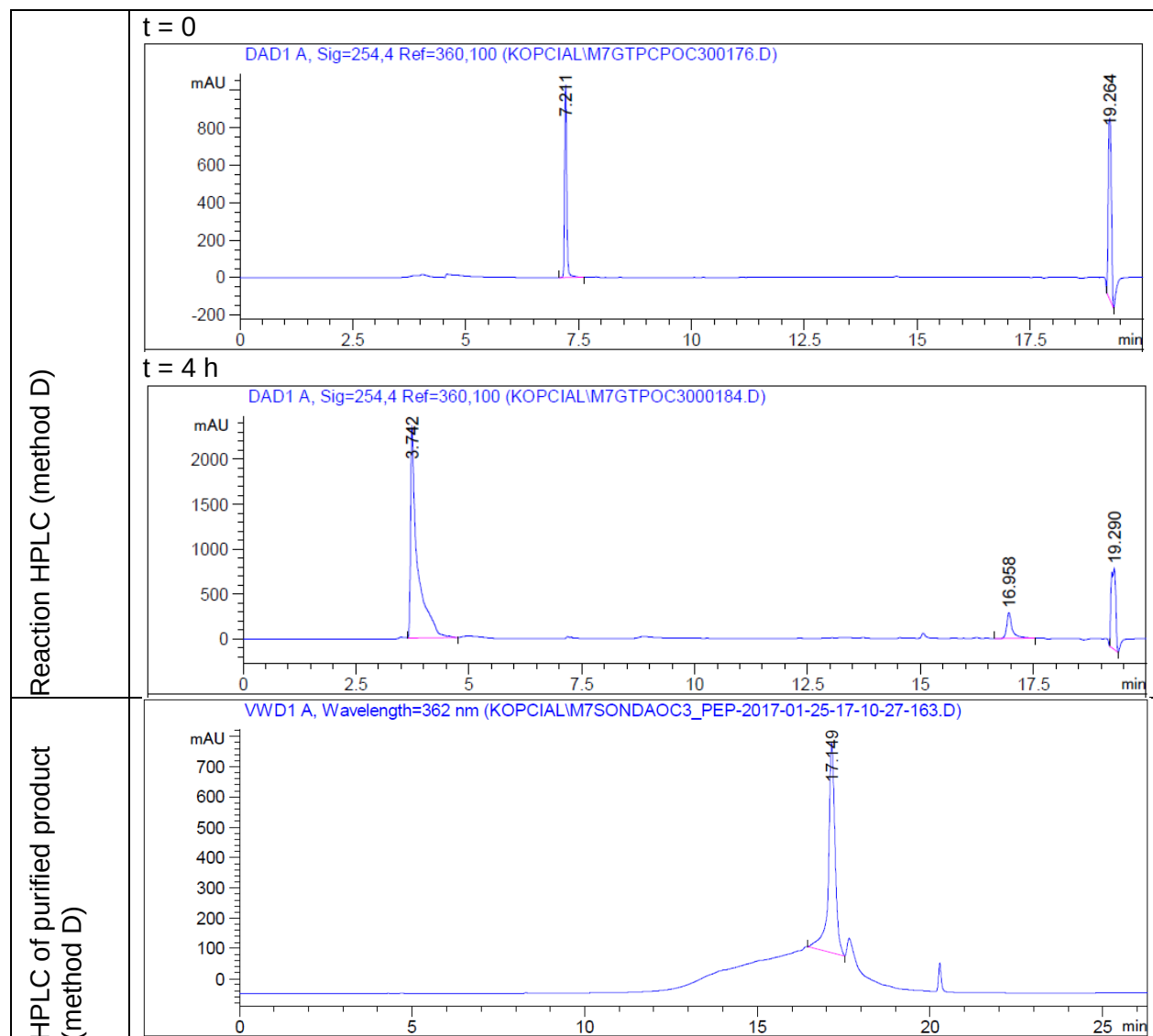
161115_mkpa_2 #13-164 RT: 0.12-1.56 AV: 152 NL: 2.53E6
T: FTMS - p ESI Full ms [150.00-2000.00]

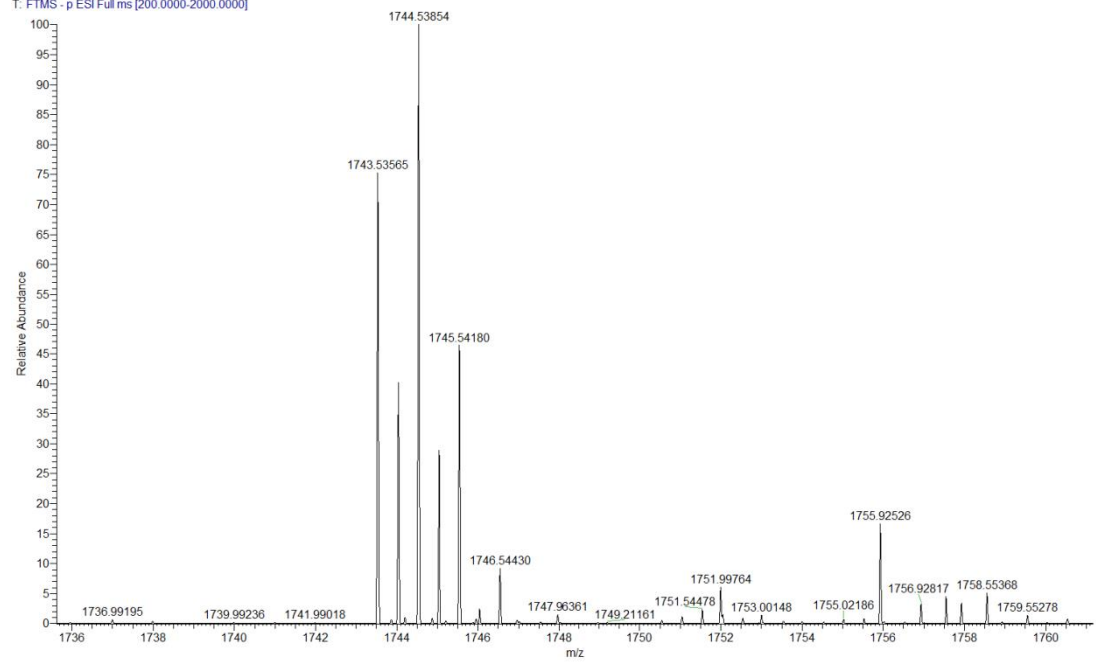
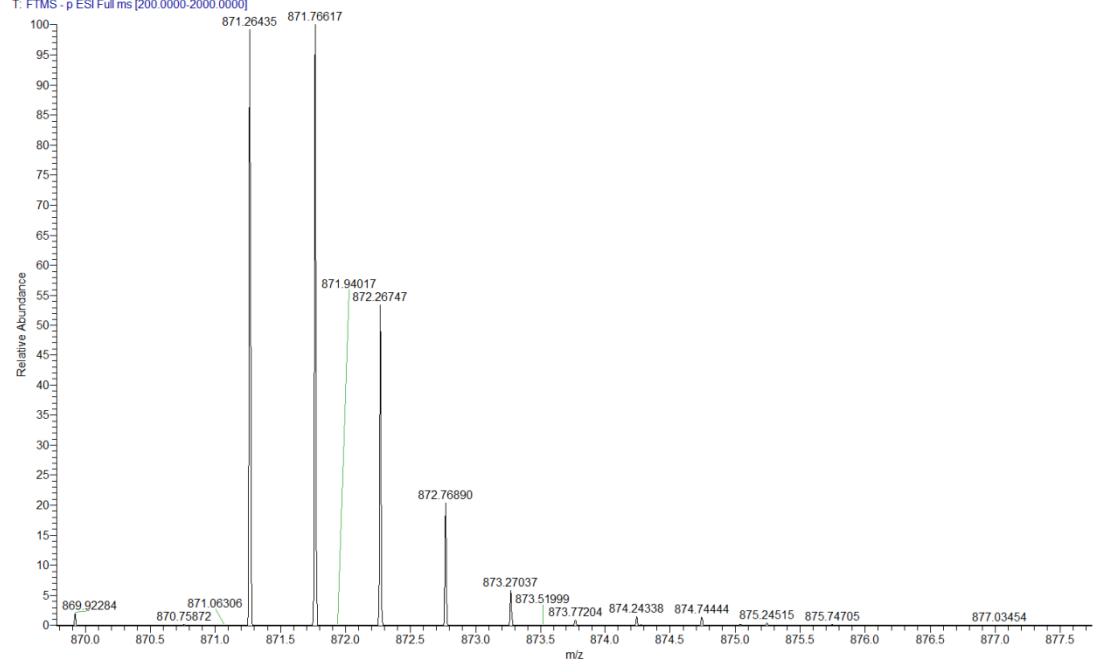


8h

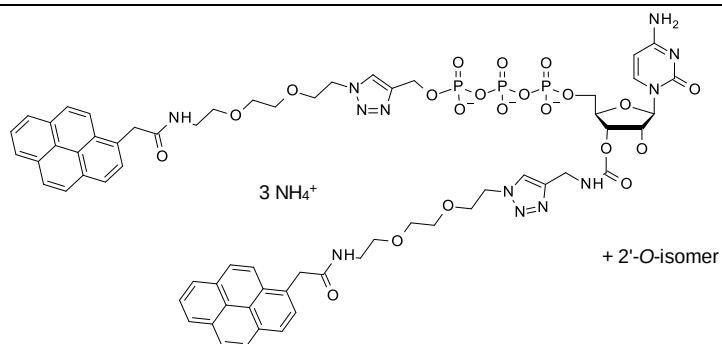
Structure





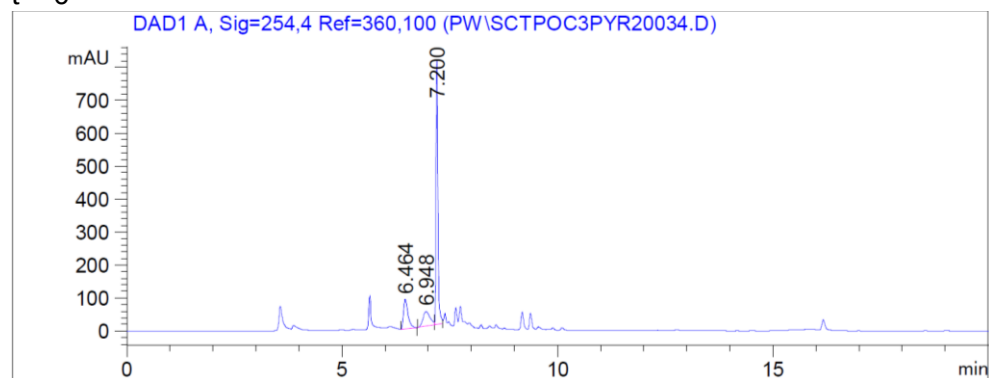
Z=1170215_MK_003 #136-348 RT: 1.29-3.31 AV: 213 NL: 2.98E5
T: FTMS - p ESI Full ms [200.0000-2000.0000]**Z=2**170215_MK_003 #8-44 RT: 0.08-0.42 AV: 37 NL: 3.06E6
T: FTMS - p ESI Full ms [200.0000-2000.0000]

Structure

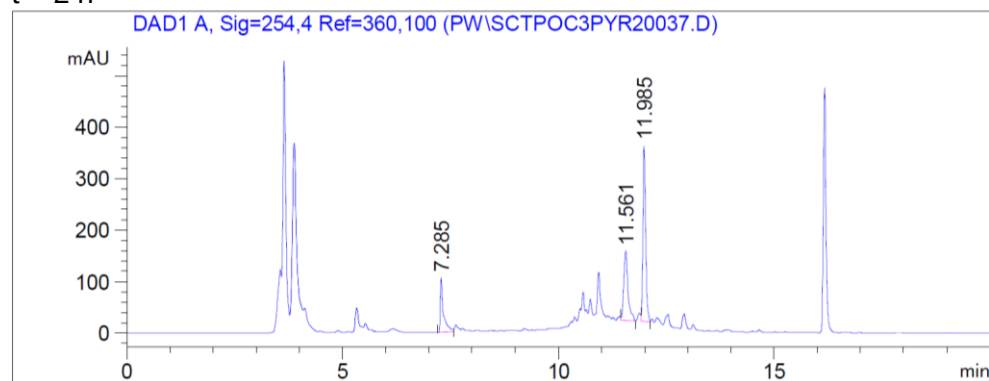


Reaction HPLC (method D)

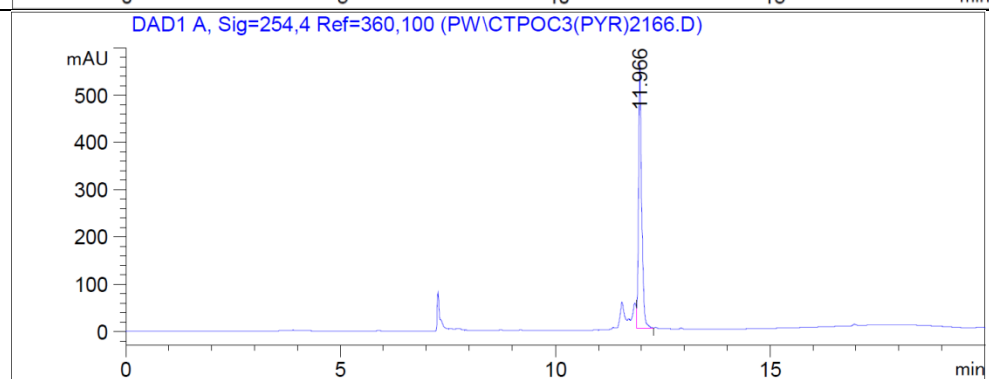
t = 0



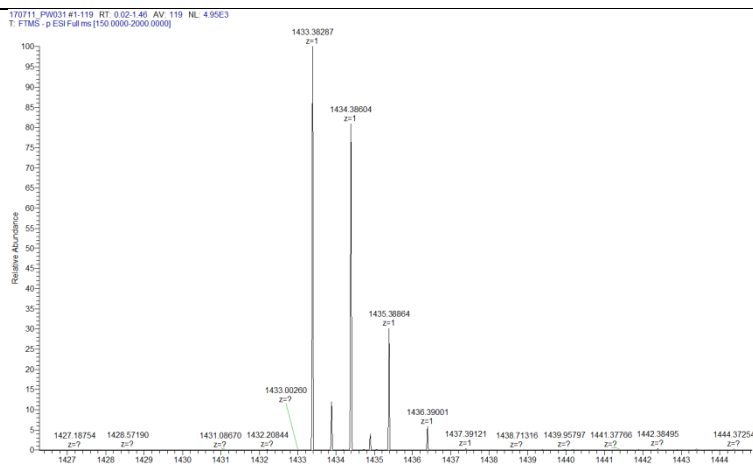
t = 2 h



HPLC of purified product (method D)

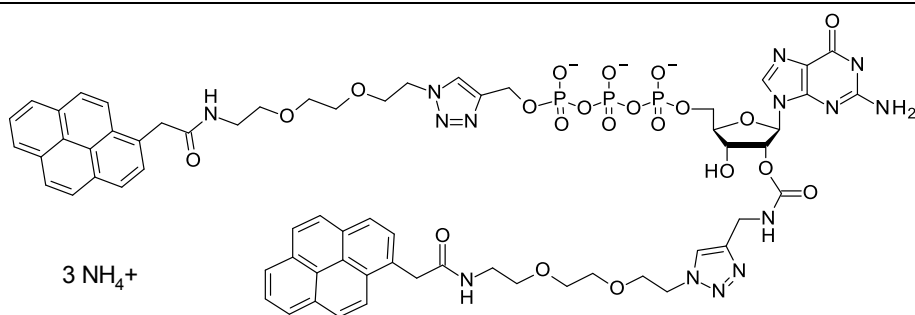


HR MS



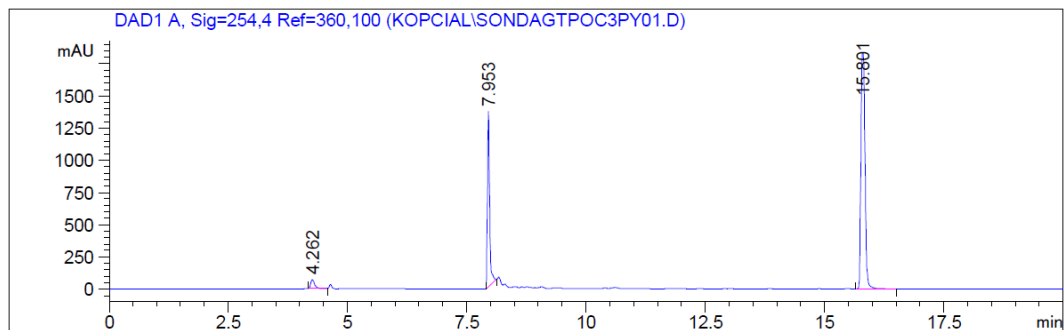
10

Structure

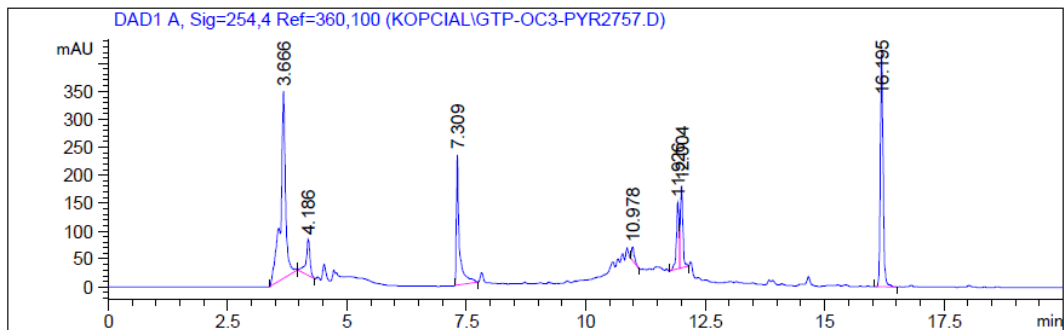


Reaction HPLC (method D)

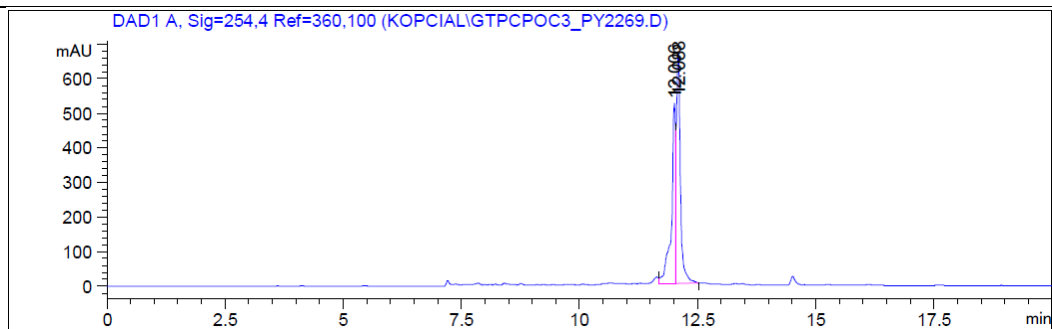
t = 0



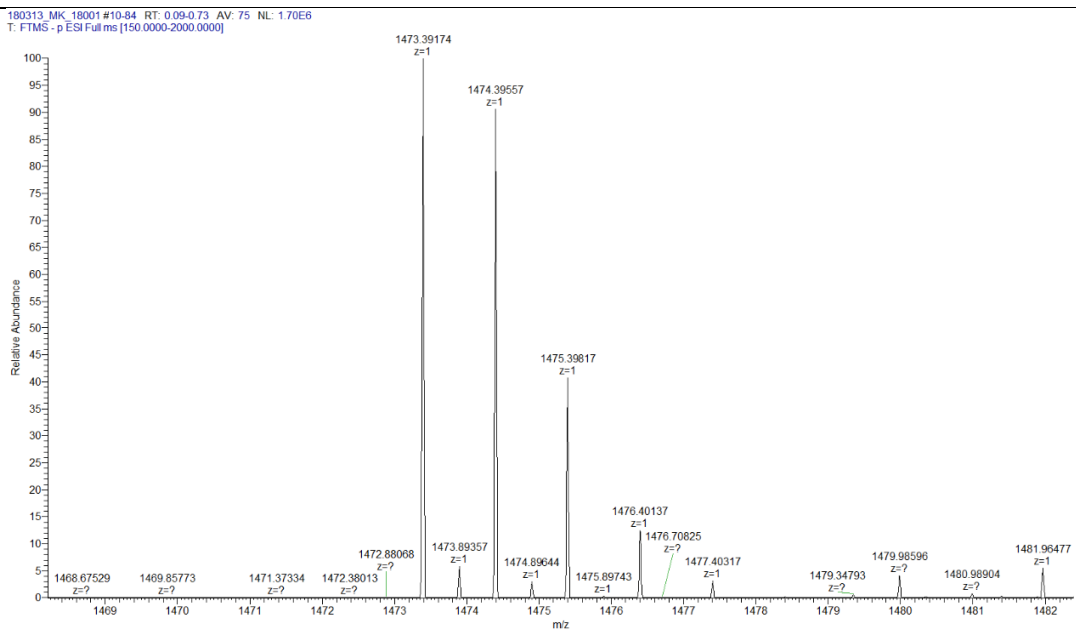
t = 0.5 h



HPLC of purified product
(method D)

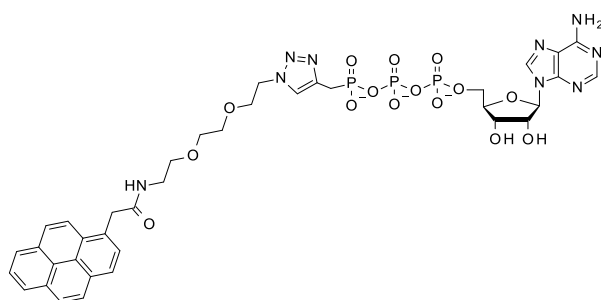


HR MS



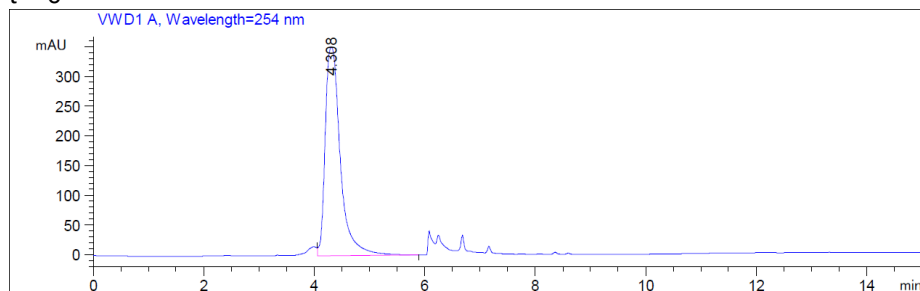
11a

Structure

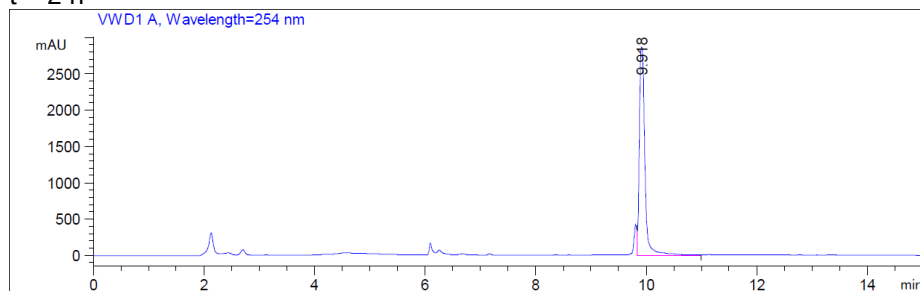


Reaction HPLC (method C)

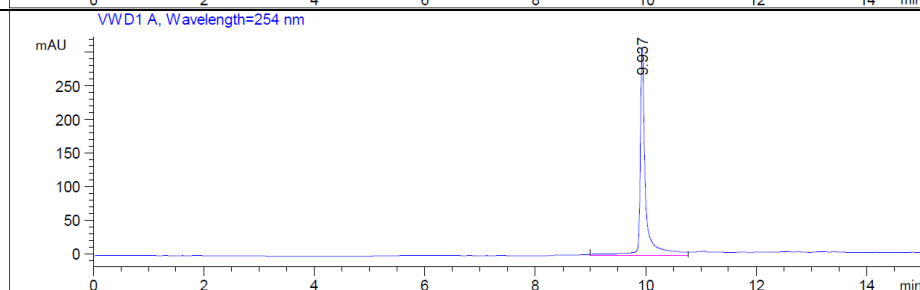
t = 0



t = 2 h

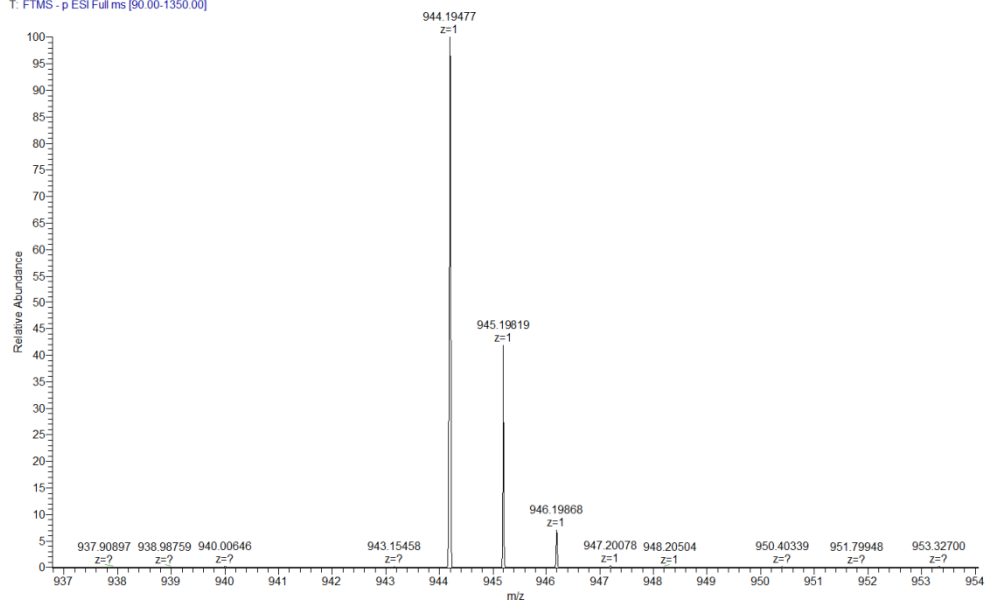


HPLC of purified product (method C)

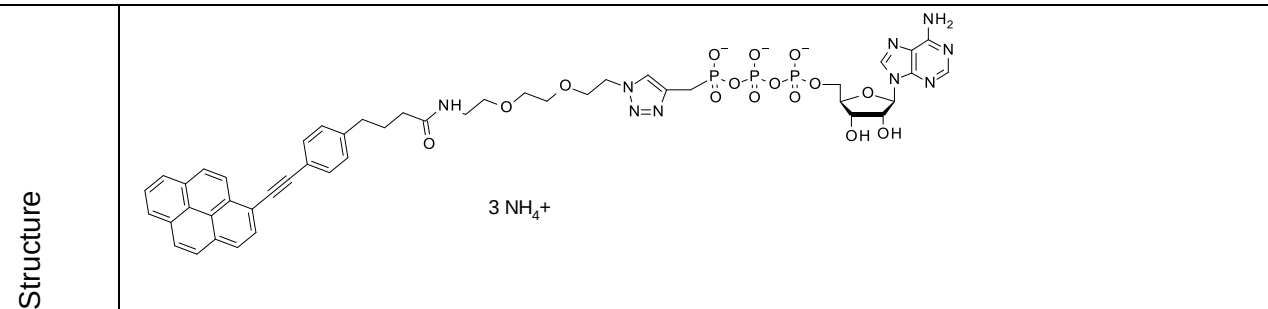


HRMS

140731_PW_026 #1-127 RT: 0.00-0.43 AV: 96 NL: 5.33E5
T: FTMS - p ESI Full ms [90.00-1350.00]



11b



Reaction HPLC (method C)

t = 0

DAD1 A, Sig=254,4 Ref=360,100 (PW\ATPC3H3000082.D)

Chromatogram showing absorbance (mAU) versus time (min). The y-axis ranges from 0 to 1750 mAU. The x-axis ranges from 0 to 18 minutes. A major peak is labeled at 4.228 min, and a minor peak is labeled at 18.172 min.

t = 1 h

VWD1 A, Wavelength=254 nm (PW\ATPCTPEP2016-06-1012-07-54.D)

Chromatogram showing absorbance (mAU) versus time (min). The y-axis ranges from 0 to 1400 mAU. The x-axis ranges from 0 to 18 minutes. Peaks are labeled at 4.659, 10.253, 11.425, 13.916, and 18.426 min.

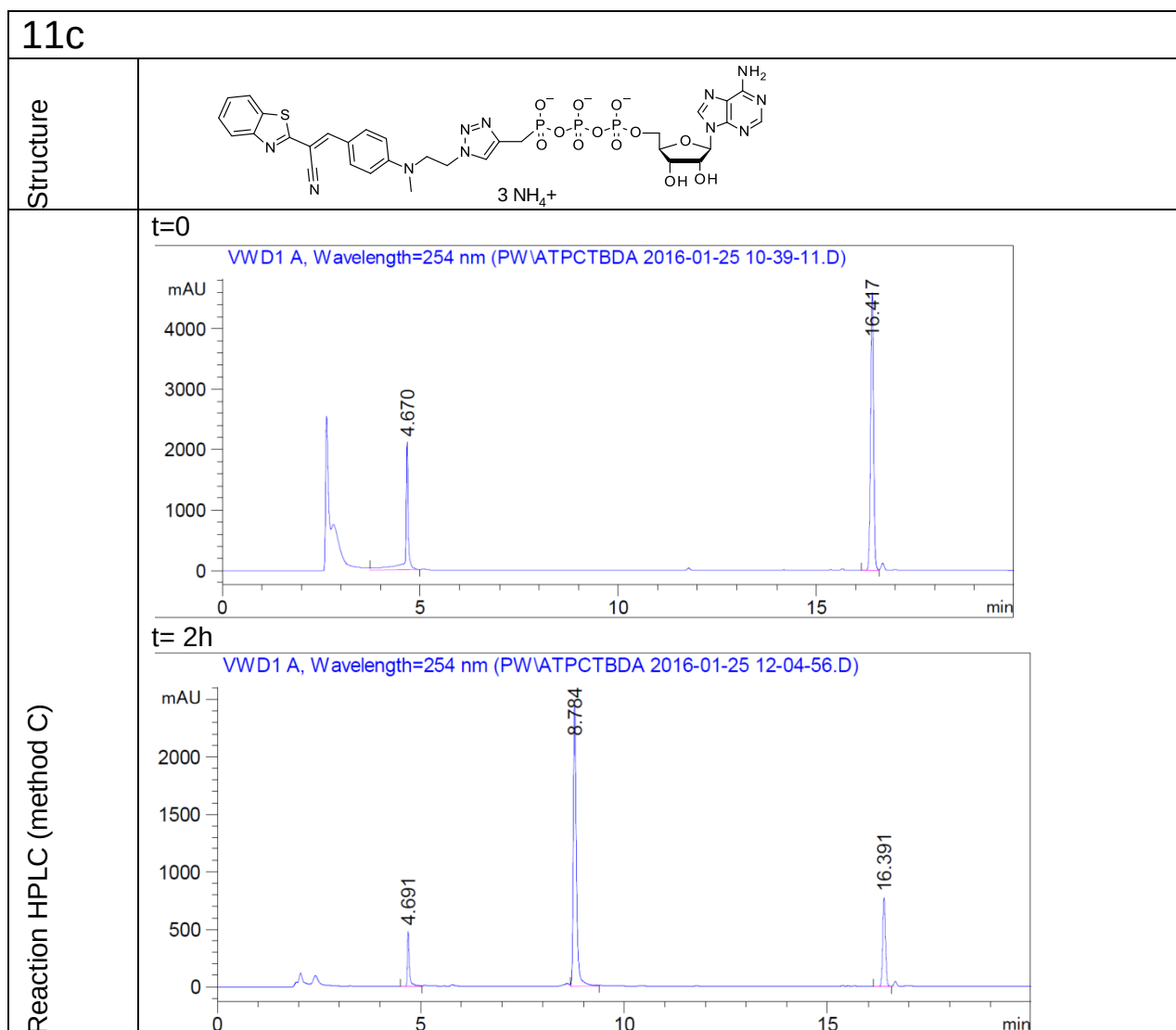
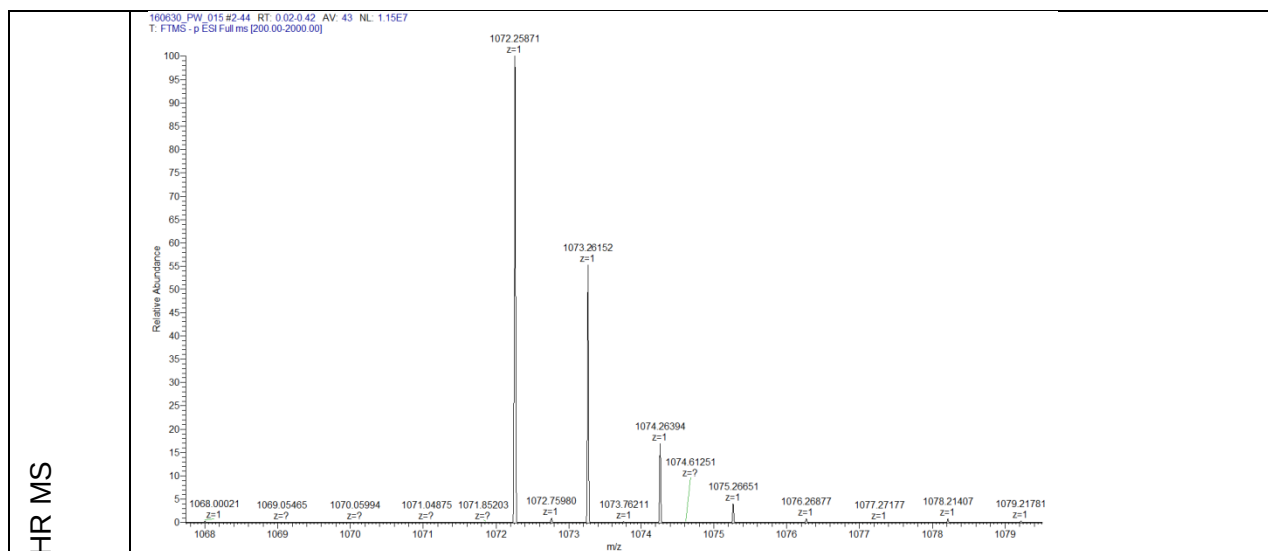
HPLC of purified product (method C)

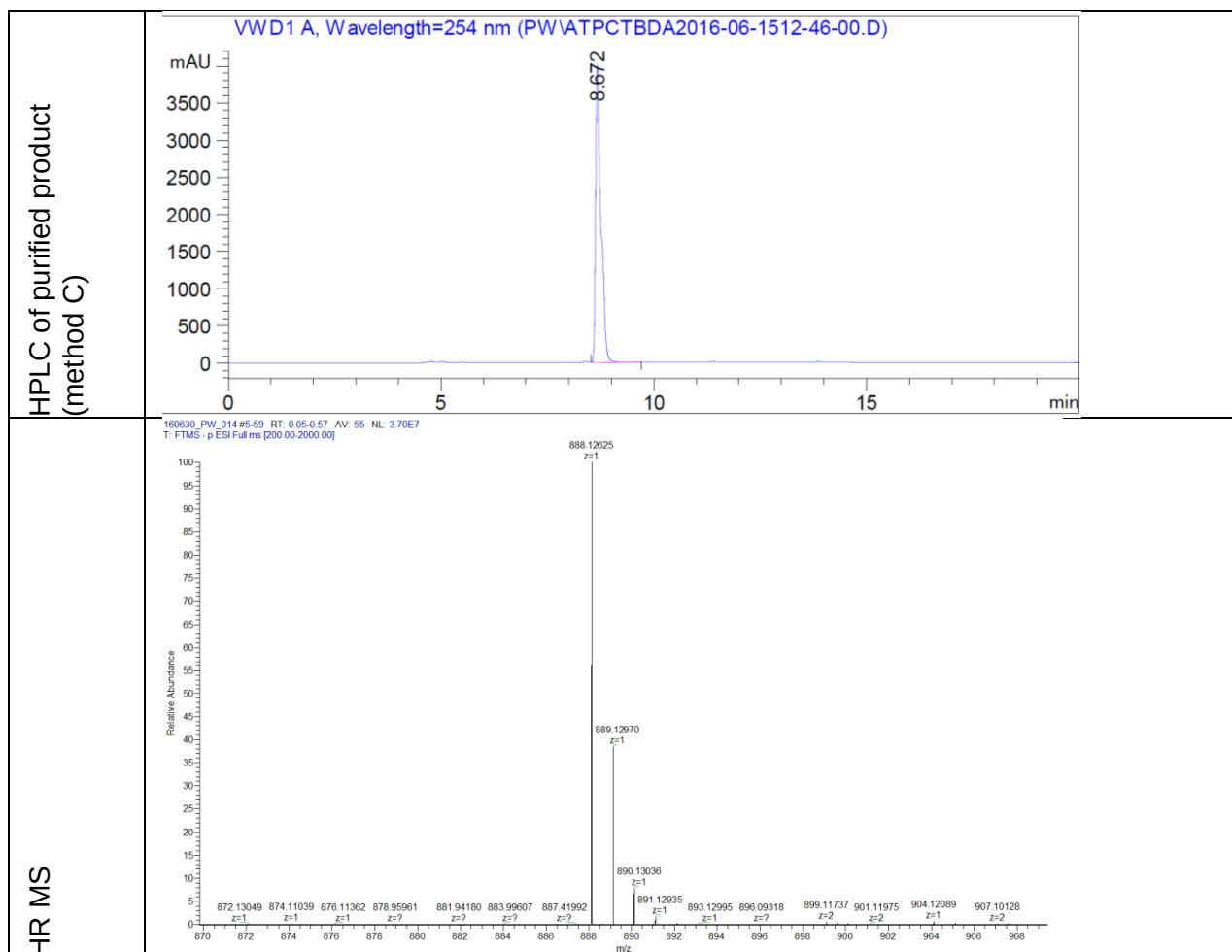
DAD1 A, Sig=254,4 Ref=360,100 (PW\ATPCTPEP000018.D)

11.52429

mAU

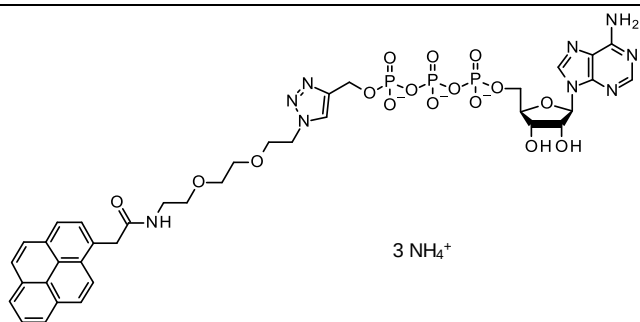
min





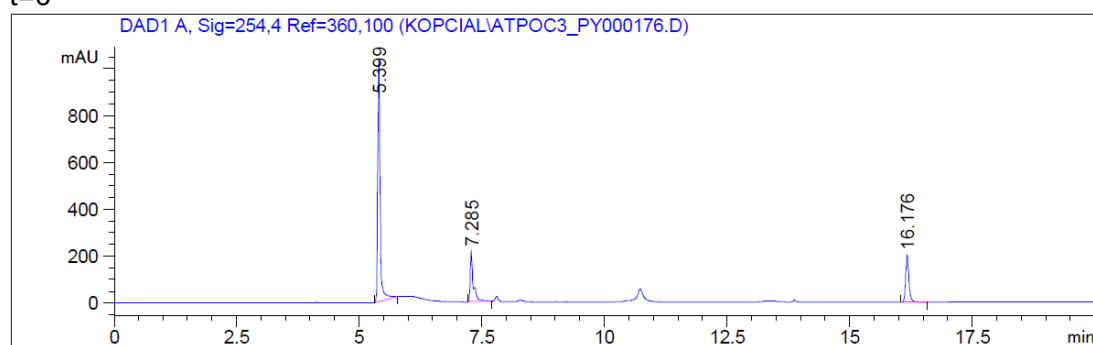
11d

Structure

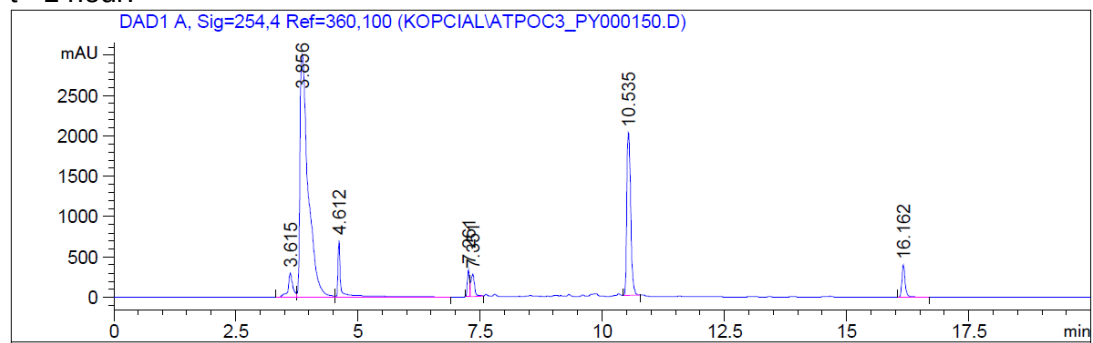
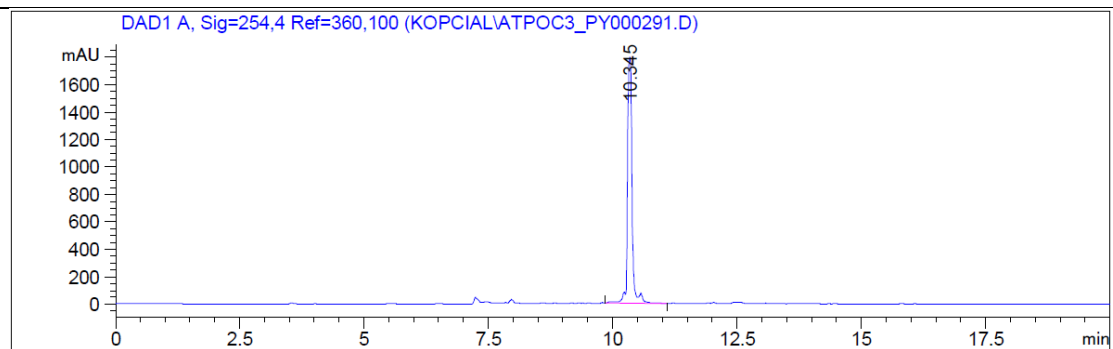


Reaction HPLC (method D)

t=0



t= 1 hour:

HPLC of purified product
(method D)

HR MS

180514_MK_18006 #35-83 RT: 0.35-0.81 AV: 49 NL: 3.78E7
T: FTMS -p ESI Full ms [150.0000-2000.0000]

