



Supplementary Materials: Novel N7-Arylmethyl Substituted Dinucleotide mRNA 5'cap Analogs: Synthesis and Evaluation as Modulators of Translation

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
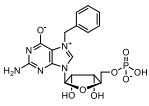

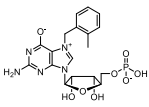

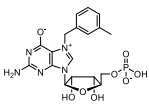

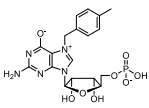

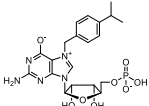

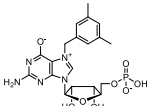

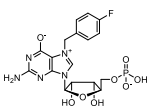

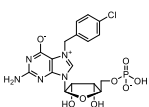
Supplementary Information


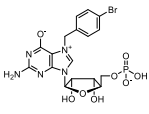

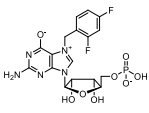

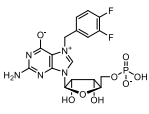

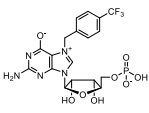

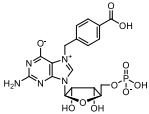

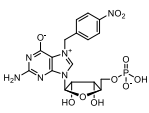

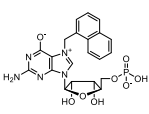

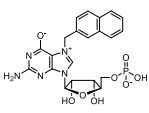
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
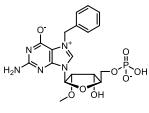
S1. Tables

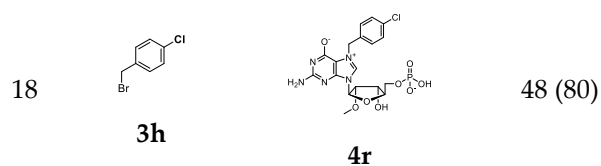
Table S1. Structures and yields for N7-benzyl guanosine monophosphate analogues.

N7-benzylated guanosine monophosphate analogues			
E.	Starting material	Product	Yield % ^a (%) ^b
1			66 (84)
	3a	4a	
2			40 (65)
	3b	4b	
3			49 (86)
	3c	4c	
4			64 (80)
	3d	4d	
5			58 (82)
	3e	4e	
6			47 (88)
	3f	4f	
7			73 (79)
	3g	4g	
8			45 (70)
	3h	4h	

9			42 (83)
	3i	4i	
10			52 (72)
	3j	4j	
11			52 (80)
	3k	4k	
12			44 (83)
	3l	4l	
13			63 (69)
	3m	4m	
14			82 (93)
	3n	4n	
15			71 (88)
	3o	4o	
16			14 (66)
	3p	4p	

N7-benzylated 2'-OMe-guanosine monophosphate analogues

17			51 (74)
	3a	4q	



^aIsolated yield, ^bConversion based on RP HPLC reaction profile

Table S2. Comparison of EC_{50} and K_D values determined by FLINT and Fluorescence Anisotropy methods.

No.	Abbr.	Compound	PyFLINT-B assay		FA assay	
			EC_{50} (μ M)	K_D (nM)	EC_{50} (μ M)	K_D (nM)
1		m ⁷ GpppG	2.7 ± 0.3	229 ± 37	0.41 ± 0.01	127 ± 5
2	1a	Bn ⁷ GpppG	$(2.2 \pm 0.3) \cdot 10^1$	217 ± 36	0.27 ± 0.02	74 ± 23
3	1b	2-MeBn ⁷ GpppG	3.7 ± 0.4	$(191 \pm 36) \cdot 10^1$	3.1 ± 0.1	$(112 \pm 4.9) \cdot 10^1$
4	1c	3-MeBn ⁷ GpppG	5.6 ± 0.6	319 ± 55	0.67 ± 0.02	222 ± 8
5	1d	4-MeBn ⁷ GpppG	6.2 ± 0.8	482 ± 81	0.86 ± 0.03	295 ± 13
6	1e	4- <i>i</i> PrBn ⁷ GpppG	7.5 ± 0.9	534 ± 100	1.3 ± 0.09	444 ± 33
7	1f	3,5-di-MeBn ⁷ GpppG	3.1 ± 0.5	646 ± 114	1.1 ± 0.1	384 ± 41
8	1g	4-F-Bn ⁷ GpppG	2.6 ± 0.3	261 ± 54	0.24 ± 0.08	64 ± 31
9	1h	4-Cl-Bn ⁷ GpppG	2.0 ± 0.2	221 ± 37	0.34 ± 0.14	102 ± 53
10	1i	4-Br-Bn ⁷ GpppG	6.9 ± 0.5	172 ± 26	0.21 ± 0.01	52 ± 3
11	1j	2,4-di-F-Bn ⁷ GpppG	5.7 ± 0.5	598 ± 87	0.87 ± 0.05	298 ± 18
12	1k	3,4-di-F-Bn ⁷ GpppG	3.4 ± 0.2	489 ± 77	0.88 ± 0.08	301 ± 30
13	1l	4-CF ₃ -Bn ⁷ GpppG	$(6.7 \pm 0.8) \cdot 10^2$	293 ± 43	0.59 ± 0.04	194 ± 17
14	1m	4-COOH-Bn ⁷ GpppG	4.3 ± 0.6	$(586 \pm 102) \cdot 10^2$	n.d	n.d
15	1n	4-NO ₂ -Bn ⁷ GpppG	$(3.5 \pm 0.3) \cdot 10^1$	367 ± 71	0.52 ± 0.12	168 ± 43
16	1o	α -Naphm ⁷ GpppG	$(1.2 \pm 0.1) \cdot 10^1$	$(304 \pm 49) \cdot 10^1$	2.1 ± 0.1	739 ± 49
17	1p	β -Naphm ⁷ GpppG	2.0 ± 0.1	$(106 \pm 19) \cdot 10^1$	n.d	n.d
18	1q	Bn ⁷ G _m pppG	1.6 ± 0.1	171 ± 25	0.18 ± 0.03	40 ± 11
19	1r	4-Cl-Bn ⁷ G _m pppG	2.7 ± 0.3	132 ± 20	0.19 ± 0.02	45 ± 7

Table S3. Data collection and refinement statistics for crystal structures.

	bn⁷GpppG (1a)	3Me-bn⁷GpppG (1c)	4-Cl-bn⁷GpppG (1h)
PDB code	6YLR	6YLT	6YLV
Wavelength (Å)	0.9184	0.9184	0.9184
Resolution range (Å)	42.06 - 2.20 (2.27 - 2.20)	49.31 - 2.67 (2.77 - 2.67)	37.4 - 2.66 (2.76 - 2.66)
Space group	P 43	P 1	P 1
Cell dimensions	94.0 94.0 42.4 90 90 90	38.3 38.3 148.8 84.0 87.8 76.8	38.4 38.4 148.1 85.3 88.4 77.2
Total reflections	134095 (13102)	88377 (7780)	103757 (4113)
Unique reflections	19244 (1869)	23009 (2275)	22090 (1453)
Multiplicity	7.0 (7.0)	3.8 (3.4)	4.7 (2.8)
Completeness (%)	99.4 (96.9)	98.9 (96.8)	93.6 (60.4)
Mean I/sigma(I)	11.0 (2.0)	9.8 (2.0)	10.3 (2.0)
Wilson B-factor (Å²)	37.5	33.8	37.7
R-merge	0.147 (0.865)	0.134 (0.616)	0.112 (0.571)
R-meas	0.160 (0.934)	0.155 (0.730)	0.126 (0.680)
CC_{1/2}	0.995 (0.805)	0.99 (0.673)	0.991 (0.764)
CC*	0.999 (0.944)	0.998 (0.897)	0.998 (0.931)
Reflections used in refinement	19182 (1824)	22996 (2274)	22080 (1453)
Reflections used for R-free	961 (92)	849 (84)	814 (54)
R-work	0.224 (0.357)	0.192 (0.240)	0.201 (0.253)
R-free	0.270 (0.359)	0.243 (0.316)	0.255 (0.325)
CC(work)	0.943 (0.724)	0.938 (0.823)	0.936 (0.837)
CC(free)	0.915 (0.682)	0.885 (0.851)	0.906 (0.759)
Number of non-hydrogen atoms	3046	6344	6292
macromolecules	2761	5685	5798
ligands	93	356	252
solvent	192	303	242
Protein residues	354	726	725
RMS(bonds) (Å)	0.003	0.008	0.006
RMS(angles) (deg)	0.76	0.97	0.76
Ramachandran favored (%)	98.0	98.5	98.0
Ramachandran allowed (%)	2.0	1.5	2.0
Ramachandran outliers (%)	0	0	0
Rotamer outliers (%)	0.0	0.2	0.2
Clashscore	3.5	6.4	7.2
Average B-factor	58.3	42.0	44.7
macromolecules	59.2	41.5	44.5
ligands	56.9	53.3	58.5
solvent	47.2	37.9	35.6

Number of TLS groups	12	25	25
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Statistics for the highest-resolution shell are shown in parentheses.

S2. Figures

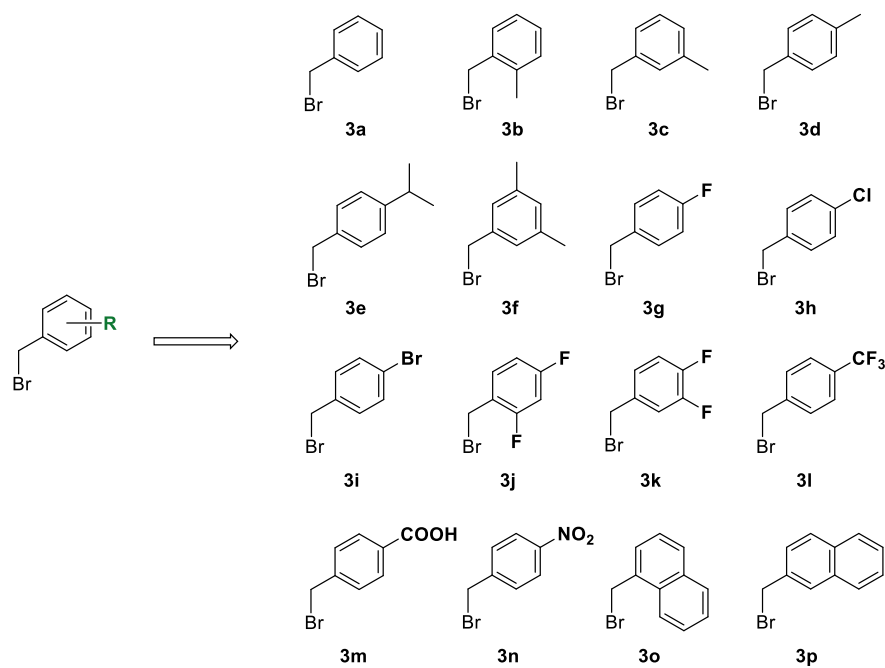


Figure S1. Structures of benzyl bromides used in this work.

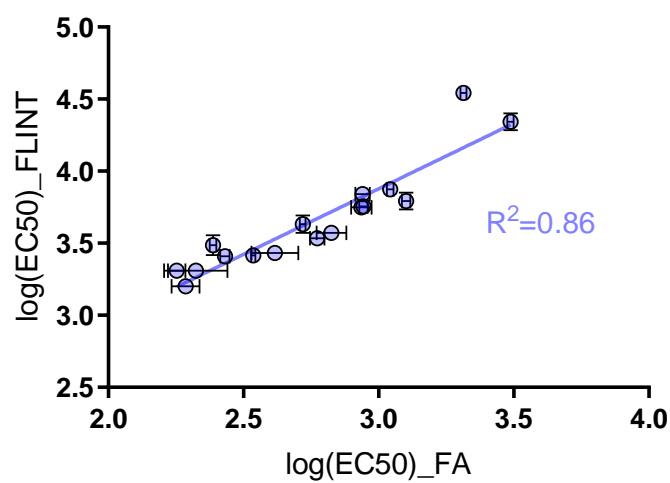
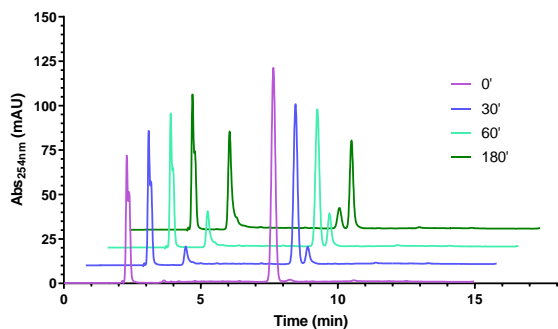
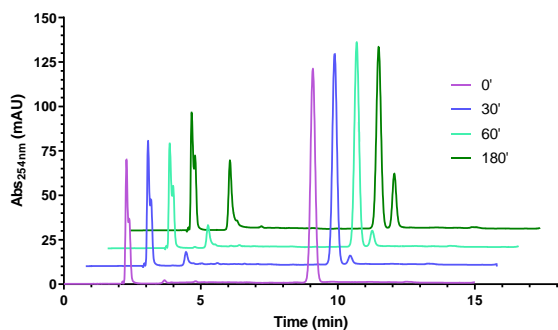
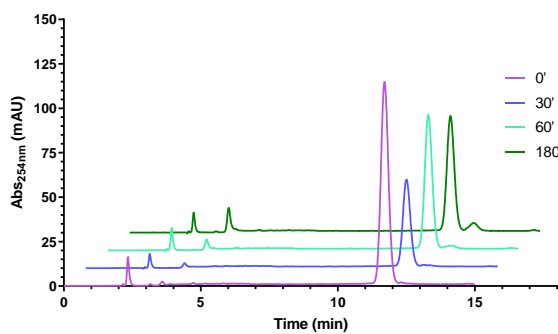
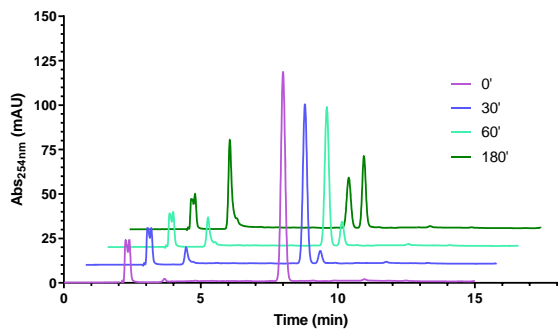
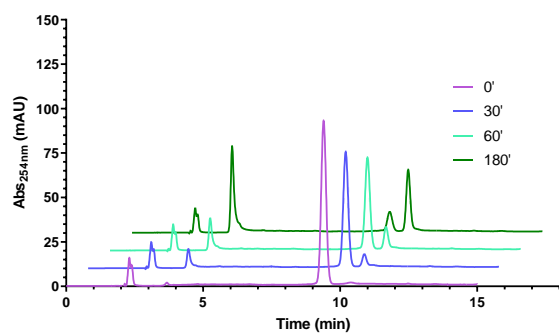
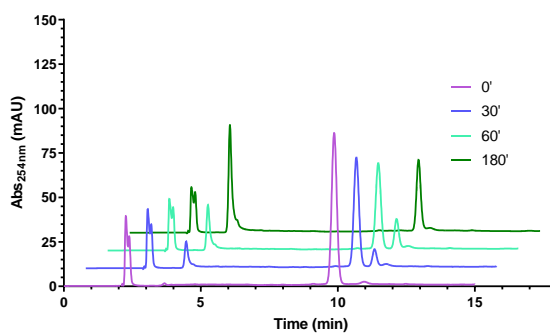
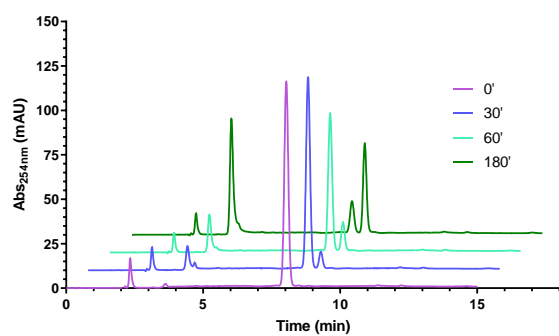
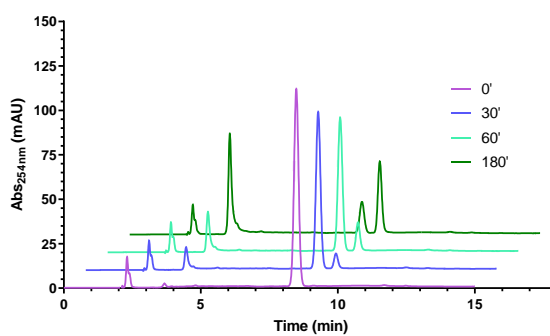
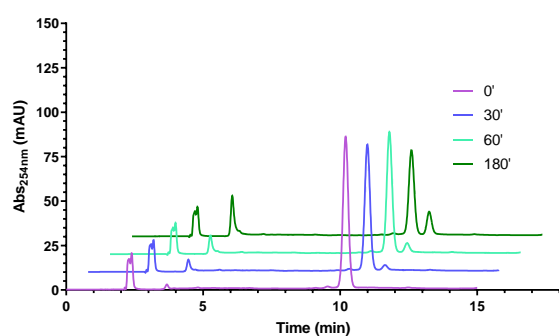
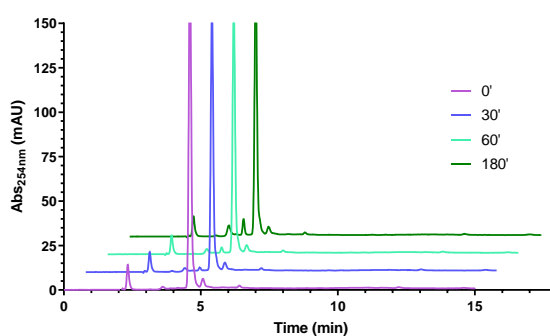


Figure S2. Correlation between the EC_{50} values determined from PyFLINT-B versus fluorescence anisotropy (FA) assay.

Bn⁷GpppG (1a)3-MeBn⁷GpppG (1c)4-*i*PrBn⁷GpppG (1e)

4-F-Bn⁷GpppG (1g)



4-Cl-Bn⁷GpppG (1h)4-Br-Bn⁷GpppG (1i)2,4-di-F-Bn⁷GpppG (1j)3,4-di-F-Bn⁷GpppG (1k)4-CF₃-Bn⁷GpppG (1l)4-COOH-Bn⁷GpppG (1m)4-NO₂-Bn⁷GpppG (1n) α -Naphm⁷GpppG (1o)

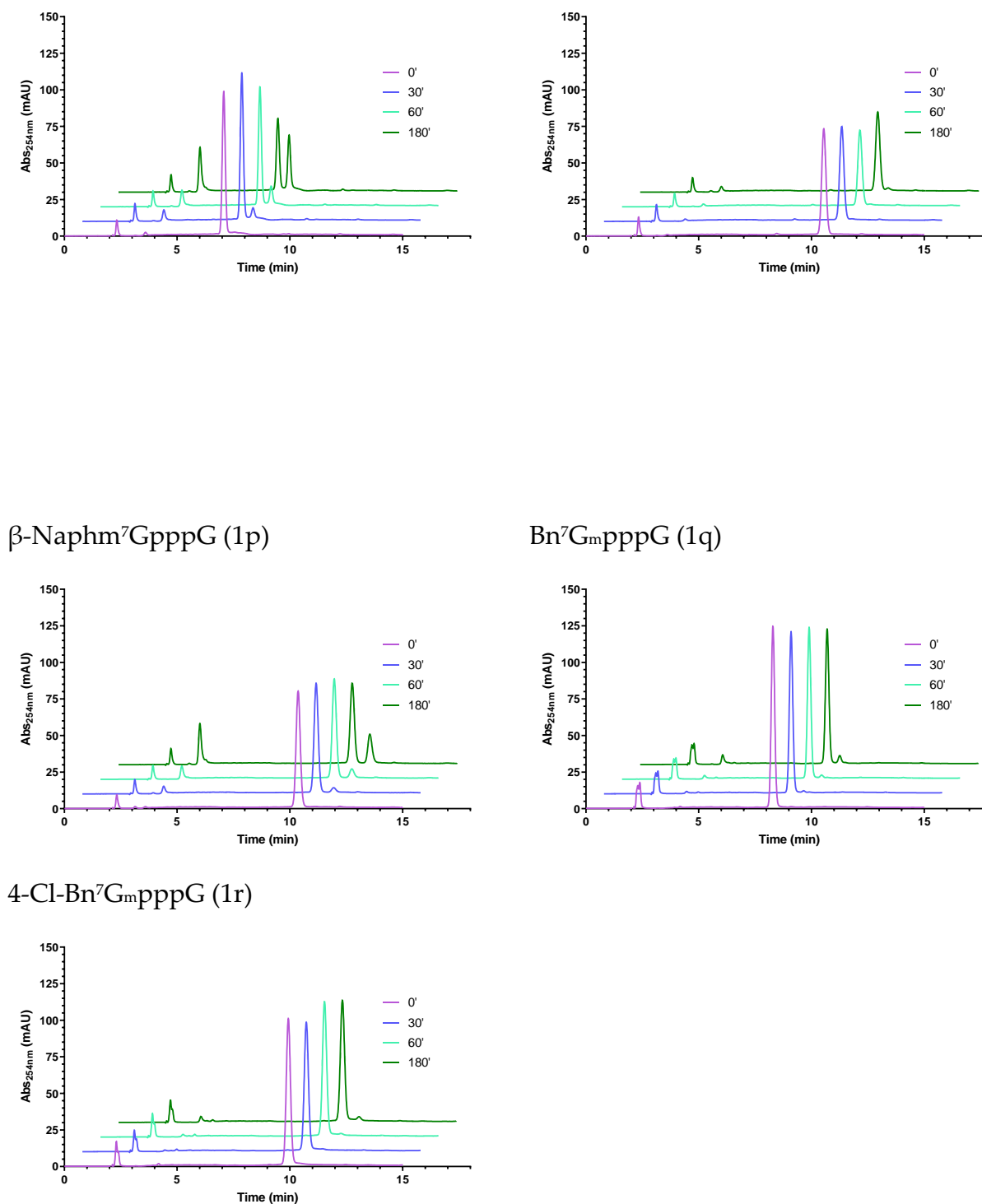


Figure S3. Analysis of cap analog susceptibility to hydrolysis by DcpS using RP-HPLC.

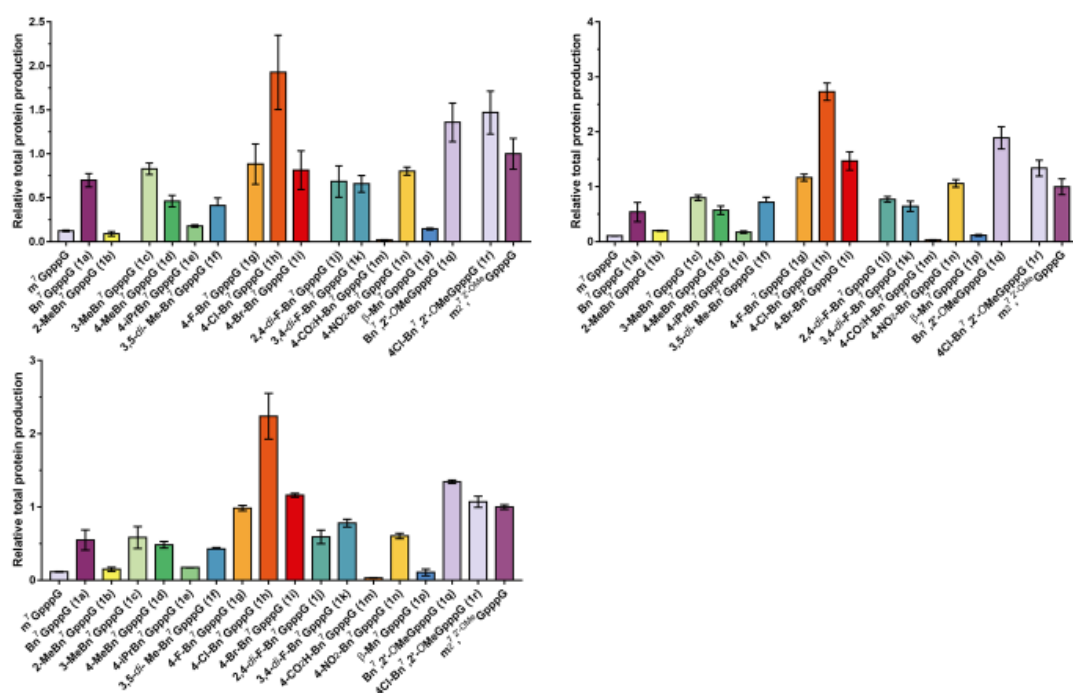


Figure S4. Relative total protein expression in three independent experiments. Data are normalized to m^7GpppG -RNA \pm SD.

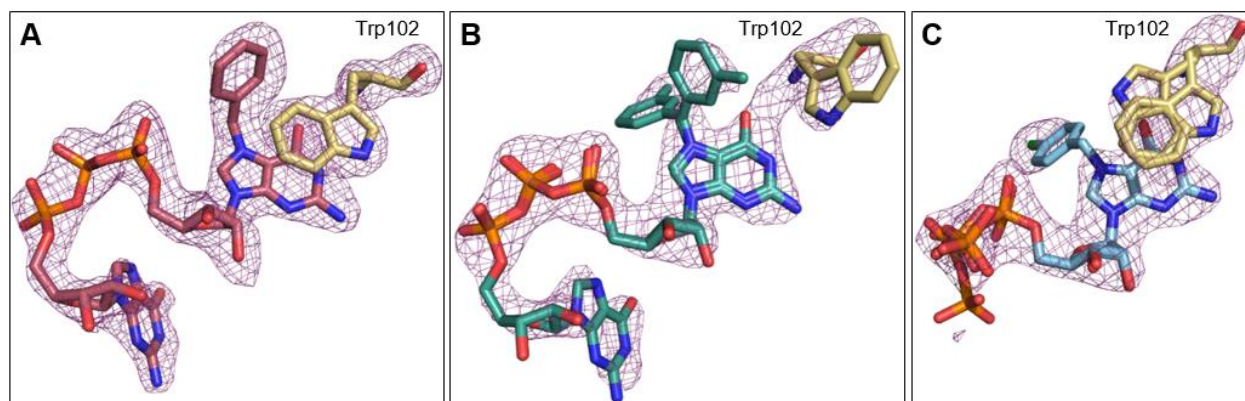
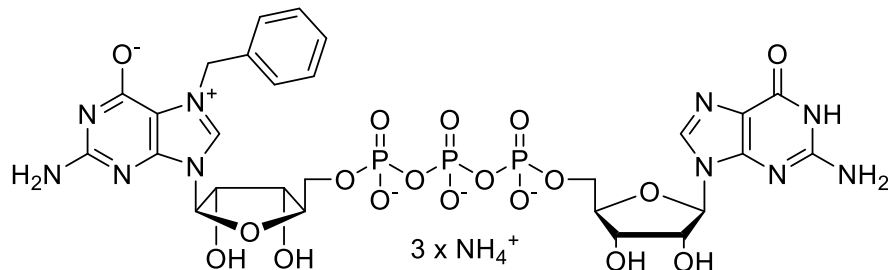


Figure S5. Simulated annealing omit map ($F_o - F_c$) contoured at 3σ around compound **1a** (A), **1c** (B), **1h** (C) and Trp102 from the solved X-ray structures of eIF4E in complex with cap analog

S3. Chemical synthesis of novel Gp₃G cap analogs

1a) Synthesis of Bn⁷GpppG

P1-(7-benzylguanosin-5'-yl) P3-5'-guanosine



Bn⁷GpppG (**1a**) was prepared according to GP-B starting from Bn⁷GMP/TEA⁺ (**4a**) (109 mg, 500 mOD, 0.046 mmol), DMSO (8.0 ml), GDP-Im/Na⁺ (**5a**) (23 mg, 500 mOD, 0.043 mmol) and ZnCl₂ (47 mg, 0.344 mmol) yielding 14.86 mg of **1a** ammonium salt (507 mOD, 0.022 mmol, 51%). Reaction time: 24h.

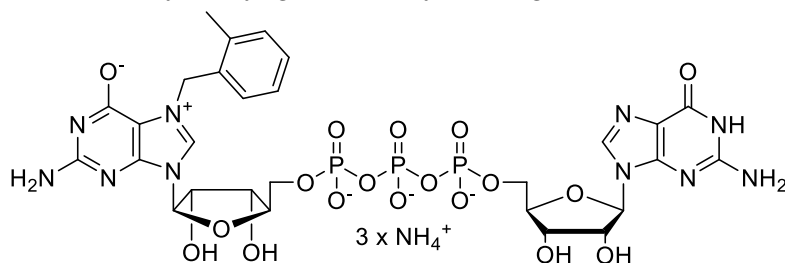
¹H NMR (500 MHz, D₂O) δ = 9.37 (s, 1H), 8.06 (s, 1H), 7.38 – 7.28 (m, 5H), 5.93 (d, J = 3.7 Hz, 1H), 5.78 (d, J = 5.9 Hz, 1H), 5.57 (s, 2H), 4.66 (dd, J = 5.9, 5.2 Hz, 1H), 4.65 (dd, J = 5.0, 3.7 Hz, 1H), 4.49 (t, J = 5.0 Hz, 1H), 4.46 (dd, J = 5.2, 3.3 Hz, 1H), 4.43 – 4.35 (m, 2H), 4.33 – 4.18 (m, 4H);

³¹P NMR (202 MHz, D₂O) δ = -10.29 – -10.73 (m, 2P), -22.15 (t, J = 19.4 Hz, 1P)

HRMS ESI (-) calcd. m/z for C₂₇H₃₂N₁₀O₁₈P₃⁻ [M-H]⁻ : 877.11144, found 877.11251

1b) Synthesis of 2-MeBn⁷GpppG

P1-(7-(2-methylbenzyl)guanosin-5'-yl) P3-5'-guanosine



2-MeBn⁷GpppG (**1b**) was prepared according to GP-B starting from 2-MeBn⁷GMP/TEA⁺ (**4b**) (69 mg, 250 mOD, 0.025 mmol), DMSO (10.0 ml), GDP-Im/Na⁺ (**5a**) (12 mg, 250 mOD, 0.022 mmol) and ZnCl₂ (24 mg, 0.176 mmol) yielding 7.07 mg of **1b** ammonium salt (193 mOD, 0.0086 mmol, 39%). Reaction time: 24h.

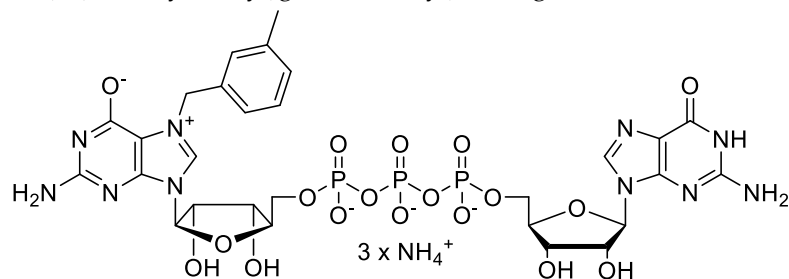
¹H NMR (500 MHz, D₂O) δ = 9.21 (s, 1H), 8.13 (s, 1H), 7.20 – 6.86 (m, 4H), 5.94 (d, J = 3.9 Hz, 1H), 5.81 (d, J = 5.8 Hz, 1H), 5.58 (s, 2H), 4.71 (dd, J = 5.1, 3.9 Hz, 1H), 4.66 (dd, J = 5.8, 5.1 Hz, 1H), 4.51 (t, J = 5.1, 1H), 4.47 (dd, J = 5.1, 3.5 Hz, 1H), 4.42 – 4.32 (m, 2H), 4.32 – 4.17 (m, 4H), 2.31 (s, 3H);

³¹P NMR (202 MHz, D₂O) δ = -10.32 – -10.72 (m, 2P), -22.72 – -22.47 (s, 1P)

HRMS ESI (-) calcd. m/z for C₂₈H₃₄N₁₀O₁₈P₃⁻ [M-H]⁻ : 891.12709, found 891.12841

1c) Synthesis of 3-MeBn⁷GpppG

P1-(7-(3-methylbenzyl)guanosin-5'-yl) P3-5'-guanosine



3-MeBn⁷GpppG (**1c**) was prepared according to GP-B starting from 3-MeBn⁷GMP/TEA⁺ (**4c**) (56 mg, 500 mOD, 0.049 mmol), DMSO (8.0 ml), GDP-Im/Na⁺ (**5a**) (23 mg, 500 mOD, 0.043 mmol) and ZnCl₂ (71 mg, 0.516 mmol) yielding 17.33 mg of **1c** ammonium salt (355 mOD, 0.016 mmol, 36%). Re-

action time: 24h.

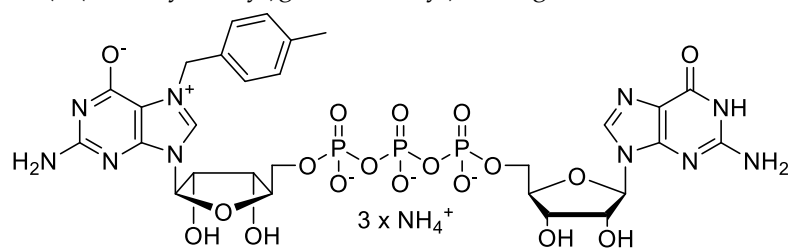
¹H NMR (500 MHz, D₂O) δ = 9.38 (s, 1H), 8.11 (s, 1H), 7.18 – 7.02 (m, 4H), 5.94 (d, *J* = 3.7 Hz, 1H), 5.78 (d, *J* = 5.6 Hz, 1H), 5.50 (s, 2H), 4.67 (dd, *J* = 5.0, 3.7 Hz, 1H), 4.65 (dd, *J* = 5.6, 5.1 Hz, 1H, overlapped with 4.67 (dd)), 4.51 (t, *J* = 5.0 Hz, 1H), 4.47 (dd, *J* = 5.0, 3.5 Hz, 1H), 4.42 – 4.37 (m, 2H), 4.32 – 4.19 (m, 4H), 2.22 (s, 3H);

³¹P NMR (202 MHz, D₂O) δ = -10.25 – -10.65 (m, 2P), -21.66 – -22.55 (m, 1P)

HRMS ESI (-) calcd. *m/z* for C₂₈H₃₄N₁₀O₁₈P₃⁻ [M-H]⁻ : 891.12709, found 891.12828

1d) Synthesis of 4-MeBn⁷GpppG

P1-(7-(4-methylbenzyl)guanosin-5'-yl) P3-5'-guanosine



4-MeBn⁷GpppG (**1d**) was prepared according to GP-B starting from 4-MeBn⁷GMP/TEA⁺ (**4d**) (41 mg, 200 mOD, 0.019 mmol), DMSO (8.0 ml), GDP-Im/Na⁺ (**5a**) (12 mg, 250 mOD, 0.022 mmol) and ZnCl₂ (24 mg, 0.176 mmol) yielding 6.75 mg of **1d**

ammonium salt (243 mOD, 0.011 mmol, 58%). Reaction time: 24h.

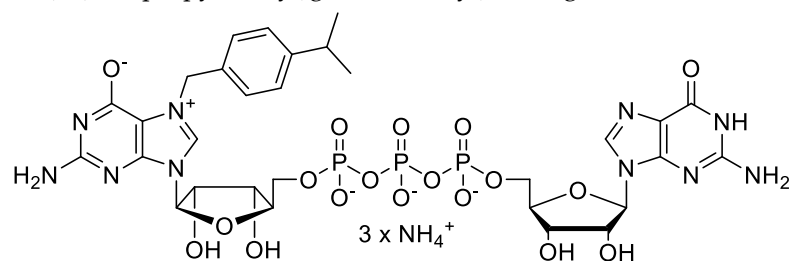
¹H NMR (500 MHz, D₂O) δ = 9.37 (s, 1H), 8.08 (s, 1H), 7.21 (d, *J* = 7.8 Hz, 2H), 7.06 (d, *J* = 7.8 Hz, 2H), 5.93 (d, *J* = 3.7 Hz, 1H), 5.76 (d, *J* = 5.7 Hz, 1H), 5.49 (s, 2H), 4.65 (dd, *J* = 5.0, 3.7 Hz, 1H), 4.64 (dd, *J* = 5.7, 5.1 Hz, 1H, overlapped with 4.65 (dd)), 4.50 (dd, *J* = 5.0, 5.0 Hz, 1H), 4.47 (dd, *J* = 5.1, 3.4 Hz, 1H), 4.41 – 4.37 (m, 2H), 4.32 – 4.19 (m, 4H), 2.20 (s, 3H);

³¹P NMR (202 MHz, D₂O) δ = -10.23 – -10.65 (m, 2P), -21.67 – -22.55 (s, 1P)

HRMS ESI (-) calcd. *m/z* for C₂₈H₃₄N₁₀O₁₈P₃⁻ [M-H]⁻ : 891.12709, found 891.12867

1e) Synthesis of 4-*i*PrBn⁷GpppG

P1-(7-(4-isopropylbenzyl)guanosin-5'-yl) P3-5'-guanosine



4-*i*PrBn⁷GpppG (**1e**) was prepared according to GP-B starting from 4-*i*PrBn⁷GMP/TEA⁺ (**4e**) (37 mg, 250 mOD, 0.023 mmol), DMSO (10.0 ml), GDP-Im/Na⁺ (**5a**) (12 mg, 250 mOD, 0.022 mmol) and ZnCl₂ (24 mg, 0.176 mmol) yielding 8.69 mg of **1e** ammonium salt (206 mOD, 0.0092 mmol, 42%). Reaction time: 24h.

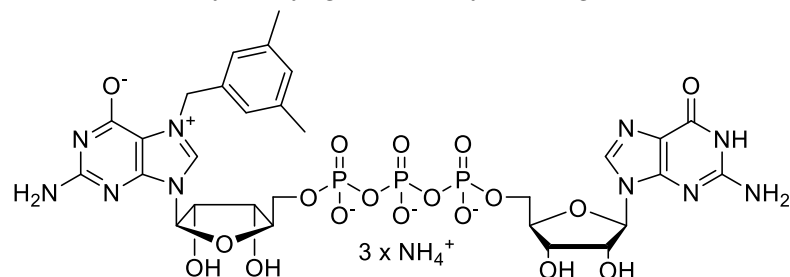
¹H NMR (500 MHz, D₂O) δ = 9.42 (s, 1H), 8.17 (s, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 7.08 (d, *J* = 8.0 Hz, 2H), 5.94 (d, *J* = 3.7 Hz, 1H), 5.80 (d, *J* = 5.4 Hz, 1H), 5.53 (s, 2H), 4.65 (dd, *J* = 5.0, 3.7 Hz, 1H), 4.60 (t, *J* = 5.4, 1H), 4.51 (t, *J* = 5.0 Hz, 1H), 4.46 (dd, *J* = 5.3, 3.6 Hz, 1H), 4.42 – 4.37 (m, 2H), 4.32 – 4.20 (m, 4H), 2.72 (hept, *J* = 6.9 Hz, 1H), 1.06 (d, *J* = 6.9 Hz, 3H), 1.05 (d, *J* = 6.9 Hz, 3H, overlapped with 1.06 (d));

³¹P NMR (202 MHz, D₂O) δ = -10.28 – -10.60 (m, 2P), -22.04 (t, *J* = 19.2 Hz, 1P)

HRMS ESI (-) calcd. *m/z* for C₃₀H₃₈N₁₀O₁₈P₃⁻ [M-H]⁻ : 915.15839, found 919.15983

1f) Synthesis of 3,5-di-MeBn⁷GpppG

P1-(7-(3,5-dimethylbenzyl)guanosin-5'-yl) P3-5'-guanosine



3,5-diMeBn⁷GpppG (**1f**) was prepared according to GP-B starting from 3,5-diMeBn⁷GMP/TEA⁺ (**4f**) (11 mg, 250 mOD, 0.025 mmol), DMSO (10.0 ml), GDP-Im/Na⁺ (**5a**) (12 mg, 250 mOD, 0.022 mmol) and ZnCl₂ (24 mg, 0.176 mmol) yielding 14.61 mg of **1f** ammonium salt (307 mOD, 0.014 mmol,

62%). Reaction time: 24h.

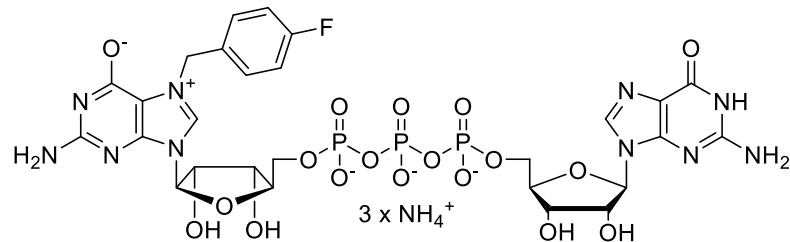
¹H NMR (500 MHz, D₂O) δ = 9.39 (s, 1H), 8.10 (s, 1H), 6.91 (s, 2H), 6.80 (s, 1H), 5.93 (d, *J* = 3.8 Hz, 1H), 5.77 (d, *J* = 5.6 Hz, 1H), 5.45 (s, 2H), 4.66 (dd, *J* = 4.9, 3.8 Hz, 1H), 4.64 (dd, *J* = 5.6, 5.1 Hz, 1H), 4.51 (t, *J* = 5.0, 5.0 Hz, 1H), 4.47 (dd, *J* = 5.1, 3.5 Hz, 1H), 4.42 – 4.37 (m, 2H), 4.32 – 4.20 (m, 4H), 2.14 (s, 6H);

³¹P NMR (202 MHz, D₂O) δ = -10.27 – -10.64 (m, 2P), -22.11 (t, *J* = 19.3 Hz, 1P)

HRMS ESI (-) calcd. *m/z* for C₂₉H₃₆N₁₀O₁₈P₃⁻ [M-H]⁻ : 905.14274, found 905.14393

1g) Synthesis of 4-F-Bn⁷GpppG

P1-(7-(4-fluorobenzyl)guanosin-5'-yl) P3-5'-guanosine



4-F-Bn⁷GpppG (**1g**) was prepared according to GP-B starting from 4-F-Bn⁷GMP/TEA⁺ (**4g**) (77 mg, 500 mOD, 0.046 mmol), DMSO (8.0 ml), GDP-Im/Na⁺ (**5a**) (23 mg, 500 mOD, 0.043 mmol) and ZnCl₂ (47 mg, 0.344 mmol) yielding 16.83 mg of **1g** ammonium salt (432 mOD, 0.019 mmol, 44%). Re-

action time: 24h.

¹H NMR (500 MHz, D₂O) δ = 9.40 (s, 1H), 8.16 (s, 1H), 7.42 – 7.36 (m, 2H), 7.05 – 6.97 (m, 2H), 5.94 (d, *J* = 3.7 Hz, 1H), 5.79 (d, *J* = 5.7 Hz, 1H), 5.54 (s, 2H), 4.67 (dd, *J* = 5.7, 5.3 Hz, 1H), 4.65 (dd, *J* = 4.9, 3.7 Hz, 1H, overlapped with 4.67 (dd)), 4.50 (dd, *J* = 5.0, 4.9 Hz, 1H), 4.47 (dd, *J* = 5.3, 3.5 Hz, 1H), 4.42 – 4.37 (m, 2H), 4.32 – 4.20 (m, 4H);

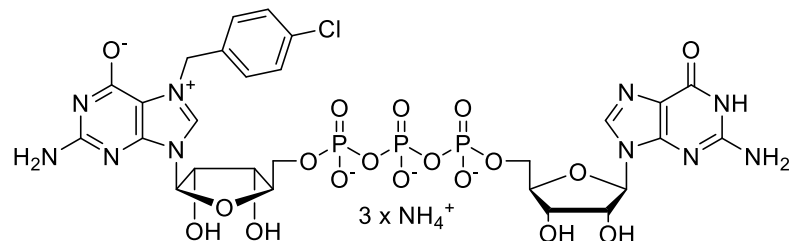
³¹P NMR (202 MHz, D₂O) δ = -10.30 – -10.63 (m, 2P), -22.14 (t, *J* = 19.3 Hz, 1P)

¹⁹F NMR (471 MHz, D₂O) δ = -113.35 – -113.43 (m, 1F)

HRMS ESI (-) calcd. *m/z* for C₂₇H₃₁FN₁₀O₁₈P₃⁻ [M-H]⁻ : 895.10202, found 895.10312

1h) Synthesis of 4-Cl-Bn⁷GpppG

P1-(7-(4-chlorobenzyl)guanosin-5'-yl) P3-5'-guanosine



4-Cl-Bn⁷GpppG (**1h**) was prepared according to GP-B starting from 4-Cl-Bn⁷GMP/TEA⁺ (**4h**) (206 mg, 1000 mOD, 0.095 mmol), DMSO (10.0 ml), GDP-Im/Na⁺ (**5a**) (46 mg, 1000 mOD, 0.086 mmol) and ZnCl₂ (94 mg, 0.688 mmol) yielding 31.27 mg

of **1h** ammonium salt (442 mOD, 0.020 mmol, 22%). Reaction time: 24h.

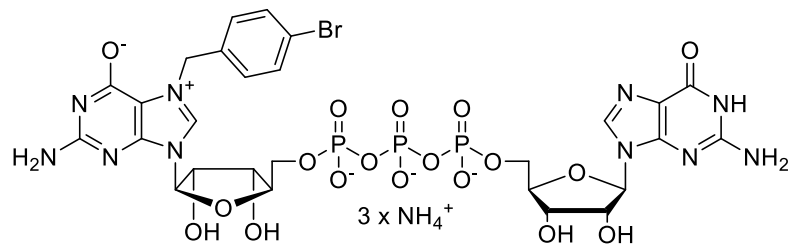
¹H NMR (500 MHz, D₂O) δ = 9.46 (s, 1H), 8.10 (s, 1H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 5.96 (d, *J* = 3.7 Hz, 1H), 5.77 (d, *J* = 5.8 Hz, 1H), 5.54 (s, 2H), 4.67 (dd, *J* = 5.8, 5.1 Hz, 1H), 4.66 (dd, *J* = 5.0, 3.7 Hz, 1H, overlapped with 4.67 (dd)), 4.50 (dd, *J* = 5.0, 5.0 Hz, 1H), 4.46 (dd, *J* = 5.1, 3.4 Hz, 1H), 4.42 – 4.37 (m, 2H), 4.32 – 4.20 (m, 4H);

³¹P NMR (202 MHz, D₂O) δ = -10.21 – -10.64 (m, 2P), -22.73 – 22.61 (m, 1P)

HRMS ESI (-) calcd. *m/z* for C₂₇H₃₁ClN₁₀O₁₈P₃⁻ [M-H]⁻ : 911.07247, found 911.07402

1i) Synthesis of 4-Br-Bn⁷GpppG

P1-(7-(4-bromobenzyl)guanosin-5'-yl) P3-5'-guanosine



4-Br-Bn⁷GpppG (**1i**) was prepared according to GP-B starting from 4-Br-Bn⁷GMP/TEA⁺ (**4i**) (28 mg, 333 mOD, 0.031 mmol), DMSO (8.0 ml), GDP-Im/Na⁺ (**5a**) (23 mg, 500 mOD, 0.043 mmol) and ZnCl₂ (47 mg, 0.344 mmol) yielding 19.93 mg of **1i**

ammonium salt (372 mOD, 0.017 mmol, 56%). Reaction time: 24h.

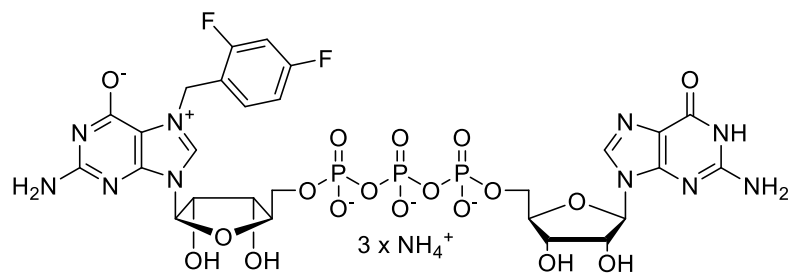
¹H NMR (500 MHz, D₂O) δ = 9.48 (s, 1H), 8.18 (s, 1H), 7.27 (d, *J* = 8.5 Hz, 2H), 7.18 (d, *J* = 8.5 Hz, 2H), 5.97 (d, *J* = 3.5 Hz, 1H), 5.79 (d, *J* = 5.4 Hz, 1H), 5.52 (d, *J* = 15.2 Hz, 1H), 5.48 (d, *J* = 15.2 Hz, 1H), 4.66 (dd, *J* = 4.8, 3.5 Hz, 1H), 4.63 (dd, *J* = 5.4, 5.4 Hz, 1H, overlapped with 4.66 (dd)), 4.51 (t, 4.8 Hz, 1H), 4.46 (dd, *J* = 5.4, 3.8 Hz, 1H), 4.43 – 4.38 (m, 2H), 4.32 – 4.20 (m, 4H);

³¹P NMR (202 MHz, D₂O) δ = -10.06 – -10.72 (m, 2P), -21.59 – -22.58 (m, 1P)

HRMS ESI (-) calcd. *m/z* for C₂₇H₃₁BrN₁₀O₁₈P₃⁻ [M-H]⁻ : 955.02195, found 955.02287

1j) Synthesis of 2,4-di-F-Bn⁷GpppG

P1-(7-(2,4-difluorobenzyl)guanosin-5'-yl) P3-5'-guanosine



2,4-di-F-Bn⁷GpppG (**1j**) was prepared according to GP-B starting from 2,4-di-F-Bn⁷GMP/TEA⁺ (**4j**) (55 mg, 250 mOD, 0.024 mmol), DMSO (8.0 ml), GDP-Im/Na⁺ (**5a**) (12 mg, 250 mOD, 0.022 mmol) and ZnCl₂ (24 mg, 0.176 mmol) yielding 7.05 mg

of **1j** ammonium salt (178 mOD, 0.0079 mmol, 36%). Reaction time: 24h.

¹H NMR (500 MHz, D₂O) δ = 9.34 (s, 1H), 8.11 (s, 1H), 7.42 – 7.35 (m, 1H), 6.93 – 6.86 (m, 2H), 5.95 (d, *J* = 3.8 Hz, 1H), 5.81 (d, *J* = 6.0 Hz, 1H), 5.61 (s, 2H), 4.70 (dd, *J* = 6.0, 5.2 Hz, 1H), 4.68 (dd, *J* = 4.8, 3.8 Hz, 1H, overlapped with 4.70 (dd)), 4.51 – 4.48 (m, 2H), 4.41 – 4.36 (m, 2H), 4.32 – 4.20 (m, 4H);

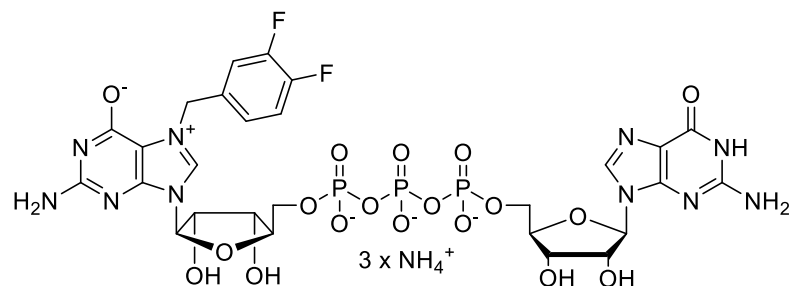
³¹P NMR (202 MHz, D₂O) δ = -10.23 – -10.73 (m, 2P), -22.18 (t, *J* = 19.3 Hz, 1P)

¹⁹F NMR (471 MHz, D₂O) δ = -108.75 – -108.79 (m, 1F), -112.85 – -112.99 (m, 1F)

HRMS ESI (-) calcd. *m/z* for C₂₇H₃₀F₂N₁₀O₁₈P₃⁻ [M-H]⁻ : 913.09259, found 913.09407

1k) Synthesis of 3,4-di-F-Bn⁷GpppG

P1-(7-(3,4-difluorobenzyl)guanosin-5'-yl) P3-5'-guanosine



3,4-di-F-Bn⁷GpppG (**1k**) was prepared according to GP-B starting from 3,4-di-F-Bn⁷GMP/TEA⁺ (**4k**) (49 mg, 250 mOD, 0.025 mmol), DMSO (10.0 ml), GDP-Im/Na⁺ (**5a**) (12 mg, 250 mOD, 0.022 mmol) and ZnCl₂ (24 mg, 0.176 mmol) yielding 6.74 mg

of **1k** ammonium salt (202 mOD, 0.0090 mmol, 41%). Reaction time: 24h.

¹H NMR (500 MHz, D₂O) δ = 9.43 (s, 1H), 8.15 (s, 1H), 7.35 – 7.29 (m, 1H), 7.21 – 7.13 (m, 2H), 5.95 (d, *J* = 3.7 Hz, 1H), 5.79 (d, *J* = 5.7 Hz, 1H), 5.55 (s, 2H), 4.67 (t, *J* = 5.7 Hz, 1H), 4.65 (dd, *J* = 4.9, 3.7 Hz, 1H, overlapped with 4.67 (t)), 4.50 (t, *J* = 4.9 Hz, 1H), 4.47 (dd, *J* = 5.7, 3.5 Hz, 1H, overlapped with 4.50 (t)), 4.42 – 4.37 (m, 2H), 4.31 – 4.20 (m, 4H);

³¹P NMR (202 MHz, D₂O) δ = -10.04 – -10.69 (m, 2P), -21.63 – -22.66 (m, 1P);

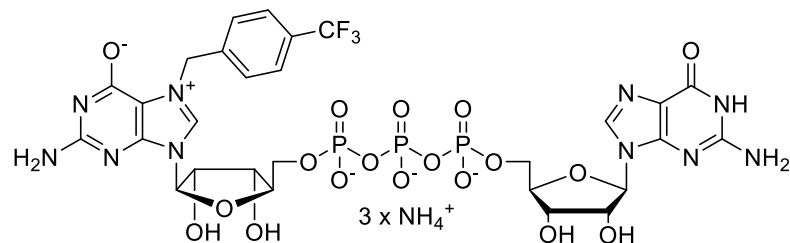
¹⁹F NMR (471 MHz, D₂O) δ = -137.58 – -137.71 (m, 1F), -138.23 – -138.35 (m, 1F);

¹⁹F NMR {1H} (471 MHz, D₂O) δ = -137.65 (d, *J* = 21.4 Hz, 1F), -138.29 (d, *J* = 21.4 Hz, 1F);

HRMS ESI (-) calcd. *m/z* for C₂₇H₃₀F₂N₁₀O₁₈P₃⁻ [M-H]⁻: 913.09259, found 913.09409

1l) Synthesis of 4-CF₃-Bn-GpppG

P1-(7-(4-trifluoromethylbenzyl)guanosin-5'-yl) P3-5'-guanosine



4-CF₃-Bn⁷GpppG (**1l**) was prepared according to GP-B starting from 4-CF₃-Bn⁷GMP/TEA⁺ (**4l**) (10 mg, 54.2 mOD, 0.0048 mmol), DMSO (1.0 ml), GDP-Im/Na⁺ (**5a**) (2 mg, 45.6 mOD, 0.004 mmol) and ZnCl₂ (14 mg, 0.10 mmol) yielding 1.5 mg of

1l ammonium salt (29.4 mOD, 0.0013 mmol, 27%). Reaction time: 24h.

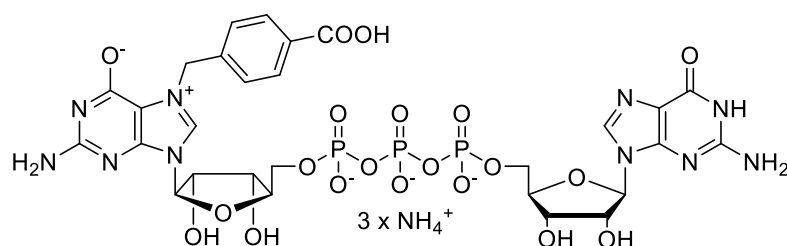
¹H NMR (500 MHz, D₂O) δ 8.01 (s, 1H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 8.2 Hz, 2H), 5.96 (d, *J* = 4.3 Hz, 1H), 5.75 (d, *J* = 5.7 Hz, 1H), 5.70 (d, *J* = 15.4 Hz, 1H), 5.67 (d, *J* = 15.4 Hz, 1H), 4.68 (t, *J* = 5.7 Hz, 1H), 4.67 (t, *J* = 4.3 Hz, 1H, overlapped with 4.68 (t)), 4.50 (t, *J* = 5.0 Hz, 1H), 4.48 – 4.43 (m, 1H), 4.42 – 4.36 (m, 2H), 4.32 – 4.17 (m, 4H);

³¹P NMR (202 MHz, D₂O) δ -10.15 – -10.78 (m), -22.20 (t, *J* = 19.7 Hz); ¹⁹F NMR (471 MHz, D₂O) δ -62.49.

HRMS ESI (-) calcd. *m/z* for C₂₈H₃₁F₃N₁₀O₁₈P₃⁻ [M-H]⁻: 945.09882, found 945.09990

1m) Synthesis of 4-COOH-Bn⁷GpppG

P1-(7-(4-carboxylbenzyl)guanosin-5'-yl) P3-5'-guanosine



4-CO₂H-Bn⁷GpppG (**1m**) was prepared according to GP-B starting from 4-CO₂H-Bn⁷GMP/TEA⁺ (**4m**) (38 mg, 500 mOD, 0.046 mmol), DMSO (10.0 ml), GDP-Im/Na⁺ (**5a**) (23 mg, 500 mOD, 0.043 mmol) and ZnCl₂ (47 mg, 0.344 mmol) yielding

10.68 mg of **1m** ammonium salt (222 mOD, 0.010 mmol, 22%). Reaction time: 24h.

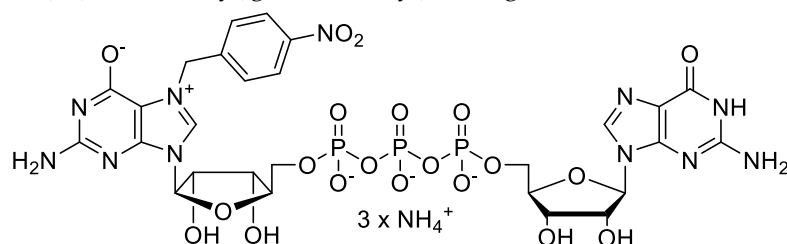
¹H NMR (500 MHz, D₂O) δ = 9.49 (s, 1H), 8.15 (s, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 5.96 (d, *J* = 3.5 Hz, 1H), 5.75 (d, *J* = 5.4 Hz, 1H), 5.64 (d, *J* = 15.3 Hz, 1H), 5.59 (d, *J* = 15.3 Hz, 1H), 4.67 (dd, *J* = 5.0, 3.5 Hz, 1H), 4.61 (t, *J* = 5.4 Hz, 1H), 4.52 (t, *J* = 5.0 Hz, 1H), 4.45 (dd, *J* = 5.4, 3.5 Hz, 1H), 4.43 – 4.39 (m, 2H), 4.33 – 4.20 (m, 4H);

³¹P NMR (202 MHz, D₂O) δ = -10.24 – -10.61 (m, 2P), -22.06 (t, *J* = 19.2 Hz, 1P)

HRMS ESI (-) calcd. *m/z* for C₂₈H₃₂N₁₀O₂₀P₃⁻ [M-H]⁻: 921.10127, found 921.10277

1n) Synthesis of 4-NO₂-Bn⁷GpppG

P1-(7-(4-nitrobenzyl)guanosin-5'-yl) P3-5'-guanosine



4-NO₂H-Bn⁷GpppG (**1n**) was prepared according to GP-B starting from 4-NO₂H-Bn⁷GMP/TEA⁺ (**4n**) (86 mg, 500 mOD, 0.046 mmol), DMSO (8.0 ml), GDP-Im/Na⁺ (**5a**) (23 mg, 500 mOD, 0.043 mmol) and ZnCl₂ (47 mg, 0.344 mmol) yielding 19.46 mg

of **1n** ammonium salt (426 mOD, 0.019 mmol, 43%). Reaction time: 24h.

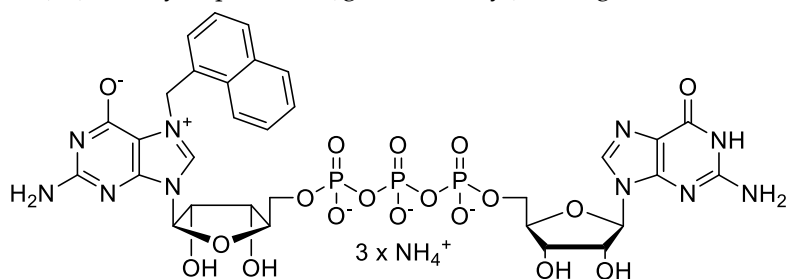
¹H NMR (500 MHz, D₂O) δ = 9.55 (s, 1H), 8.13 (s, 1H), 8.07 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.8 Hz, 2H), 5.97 (d, *J* = 3.6 Hz, 1H), 5.75 (d, *J* = 5.4 Hz, 2H), 5.74 (d, *J* = 15.4 Hz, 1H), 5.70 (d, *J* = 15.4 Hz, 1H), 4.67 (dd, *J* = 4.9, 3.6 Hz, 1H), 4.65 (t, *J* = 5.4 Hz, 2H), 4.51 (t, *J* = 4.9 Hz, 1H), 4.45 (dd, *J* = 5.4, 3.4 Hz, 1H), 4.43 – 4.38 (m, 2H), 4.33 – 4.18 (m, 4H).

³¹P NMR (202 MHz, D₂O) δ = -10.22 – -10.63 (m, 2P), -22.10 (t, *J* = 19.6 Hz, 1P)

HRMS ESI (-) calcd. *m/z* for C₂₇H₃₁N₁₁O₂₀P₃⁻ [M-H]⁻: 922.09652, found 922.09742

1o) Synthesis of α -Naphm⁷GpppG

P1-(7-(1-methylnaphthalene)guanosin-5'-yl) P3-5'-guanosine



α -Mn⁷GpppG (**1o**) was prepared according to GP-B starting from α -Mn⁷GMP/TEA⁺ (**4o**) (40 mg, 1000 mOD, 0.098 mmol), DMSO (10.0 ml), GDP-Im/Na⁺ (**5a**) (46 mg, 1000 mOD, 0.086 mmol) and ZnCl₂ (72 mg, 0.528 mmol) yielding 44.00 mg of **1o** ammonium salt (1129 mOD, 0.050 mmol, 57%).

Reaction time: 24h.

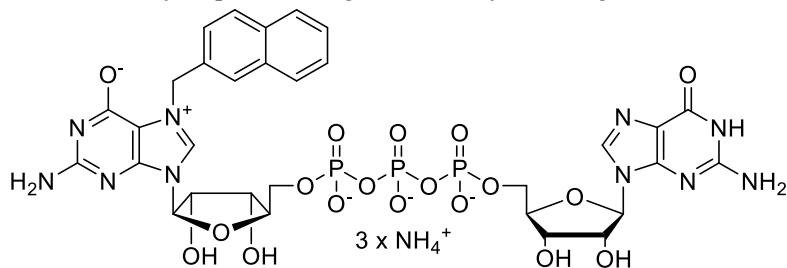
¹H NMR (500 MHz, D₂O) δ = 9.25 (s, 1H), 7.98 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.65 (dd, J = 15.2, 8.0 Hz, 2H), 7.50 (t, J = 7.5 Hz, 1H), 7.41 (t, J = 7.5 Hz, 1H), 7.29 (t, J = 7.5 Hz, 1H), 7.19 (d, J = 7.5 Hz, 1H), 5.85 (q, J = 15.2 Hz, 2H), 5.82 (d, J = 3.7 Hz, 1H), 5.65 (d, J = 5.3 Hz, 1H), 4.58 (t, J = 3.7 Hz, 1H), 4.53 (t, J = 5.3 Hz, 1H), 4.48 (t, J = 5.3 Hz, 1H), 4.46 – 4.42 (m, 1H), 4.40 – 4.18 (m, 6H).

³¹P NMR (202 MHz, D₂O) δ = δ -10.47 (m, 2P), -22.06 (m, 2P).

HRMS ESI (-) calcd. m/z for C₃₁H₃₄N₁₀O₁₈P₃³⁻ [M-H]⁻ : 927.12709, found 927.12841

1p) Synthesis of β -Naphm⁷GpppG

P1-(7-(2-methylnaphthalene)guanosin-5'-yl) P3-5'-guanosine



β -Mn⁷GpppG (**1p**) was prepared according to GP-B starting from β -Mn⁷GMP/TEA⁺ (**4p**) (67 mg, 250 mOD, 0.023 mmol), DMSO (8.0 ml), GDP-Im/Na⁺ (**5a**) (12 mg, 250 mOD, 0.022 mmol) and ZnCl₂ (24 mg, 0.176 mmol) yielding 6.71 mg of **1p** ammonium salt (223 mOD, 0.010 mmol, 45%). Reaction

time: 24h.

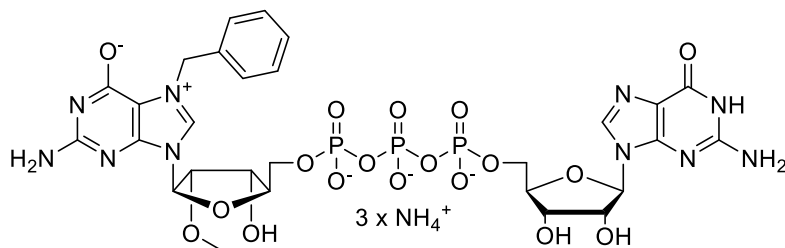
¹H NMR (500 MHz, D₂O) δ = 9.49 (s, 1H), 8.02 (s, 1H), 7.64 (s, 1H), 7.56 – 7.50 (m, 3H), 7.34 – 7.27 (m, 3H), 5.82 (d, J = 3.8 Hz, 1H), 5.62 (d, J = 5.0 Hz, 1H), 5.53 (s, 2H), 4.59 (dd, J = 5.0, 3.7 Hz, 1H), 4.51 (dd, J = 3.8, 5.0 Hz, 1H), 4.48 (dd, J = 5.0, 5.0 Hz, 1H), 4.46 – 4.39 (m, 3H), 4.36 – 4.21 (m, 4H);

³¹P NMR (202 MHz, D₂O) δ = -10.10 – -10.53 (m, 2P), -21.95 (t, J = 19.3 Hz, 1P)

HRMS ESI (-) calcd. m/z for C₃₁H₃₄N₁₀O₁₈P₃³⁻ [M-H]⁻ : 927.12709, found 927.12850

1q) Synthesis of Bn⁷G_{mp}pppG

P1-(2'-O-methyl-7-benzylguanosin-5'-yl) P3-5'-guanosine



Bn^{7,2'}-OMeGpppG (**1q**) was prepared according to GP-B starting from Bn^{7,2'}-OMeGMP/TEA⁺ (**4q**) (250 mg, 500 mOD, 0.044 mmol), DMSO (8.0 ml), GDP-Im/Na⁺ (**5a**) (23 mg, 500 mOD, 0.043 mmol) and ZnCl₂ (71 mg, 0.516 mmol) yielding 16.26 mg of

1q ammonium salt (328 mOD, 0.015 mmol, 33%). Reaction time: 24h.

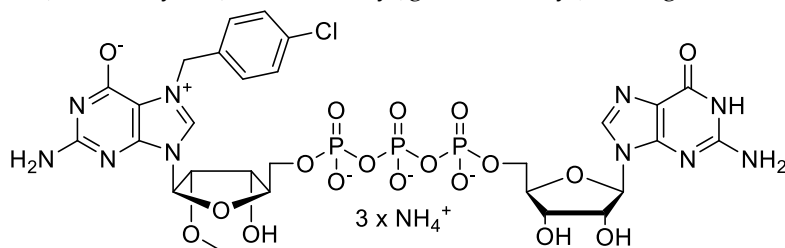
¹H NMR (500 MHz, D₂O) δ = 9.33 (s, 1H), 8.04 (s, 1H), 7.39 – 7.25 (m, 5H), 5.93 (d, *J* = 2.8 Hz, 1H), 5.74 (d, *J* = 5.7 Hz, 1H), 5.56 (s, 2H), 4.64 (t, *J* = 5.7 Hz, 1H), 4.55 (dd, *J* = 6.3, 5.0 Hz, 1H), 4.45 (dd, *J* = 5.0, 3.0 Hz, 1H), 4.43 – 4.40 (m, 1H), 4.27 (dd, *J* = 5.0, 2.8 Hz, 1H, overlapped with 4.35 – 4.19 (m)), 4.35 – 4.19 (m, 5H), 3.59 (s, 3H);

³¹P NMR (202 MHz, D₂O) δ = -10.37 – -10.69 (m, 2P), -22.13 (t, *J* = 19.3 Hz, 1P)

HRMS ESI (-) calcd. *m/z* for C₂₈H₃₄N₁₀O₁₈P₃⁻ [M-H]⁻ : 891.12709, found 891.12836

1r) Synthesis of 4-Cl-Bn⁷G_{mp}pppG

P1-(2'-O-methyl-7-(4-chlorobenzyl)guanosin-5'-yl) P3-5'-guanosine



4-Cl-Bn^{7,2'}-OMeGpppG (**1r**) was prepared according to GP-B starting from 4-Cl-Bn^{7,2'}-OMeGMP/TEA⁺ (**4r**) (104 mg, 500 mOD, 0.046 mmol), DMSO (10.0 ml), GDP-Im/Na⁺ (**5a**) (23 mg, 500 mOD, 0.043 mmol) and ZnCl₂ (47 mg, 0.344 mmol) yielding

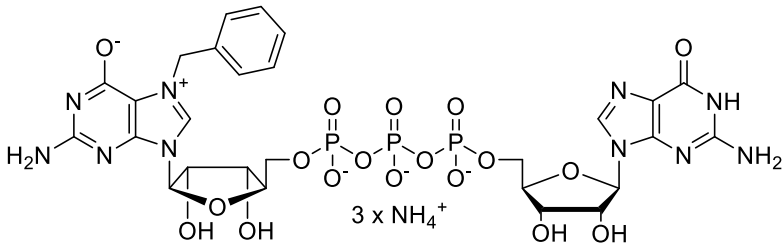
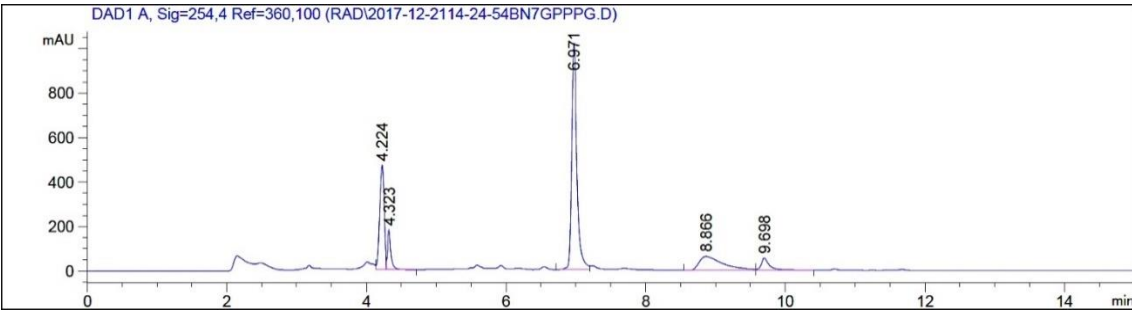
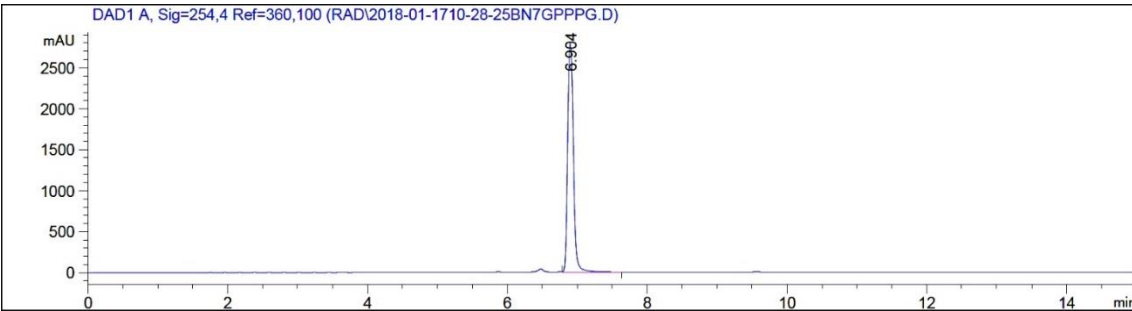
14.75 mg of **1r** ammonium salt (420 mOD, 0.019 mmol, 42%). Reaction time: 24h.

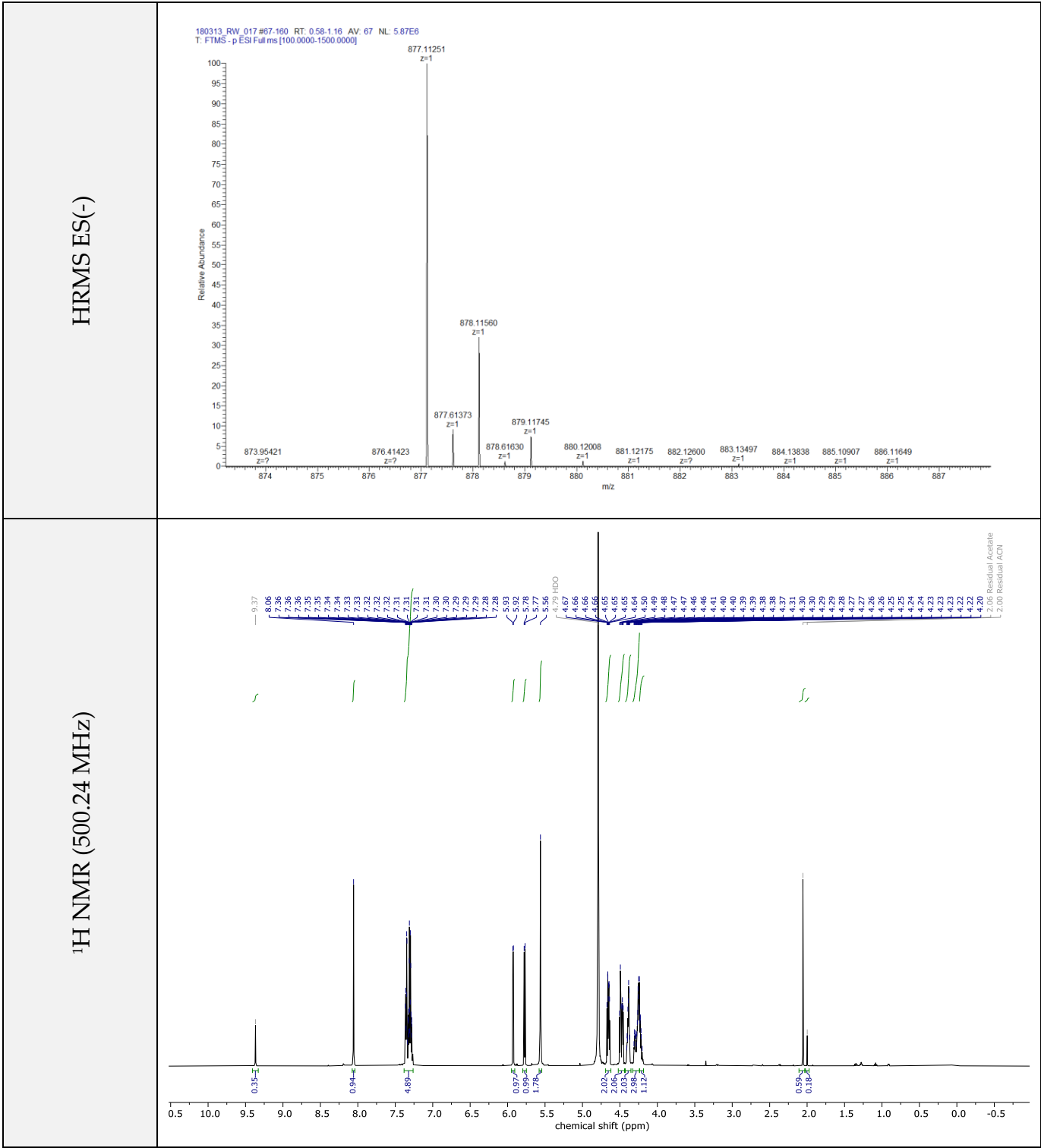
¹H NMR (500 MHz, D₂O) δ = 9.43 (s, 1H), 8.25 (s, 1H), 7.28 (d, *J* = 8.5 Hz, 2H), 7.18 (d, *J* = 8.5 Hz, 2H), 6.00 (d, *J* = 2.7 Hz, 1H), 5.78 (d, *J* = 5.3 Hz, 1H), 5.52 (s, 2H), 4.63 (t, *J* = 5.3 Hz, 1H), 4.58 (dd, *J* = 6.4, 5.0 Hz, 1H), 4.45 (dd, *J* = 5.0, 4.2 Hz, 1H), 4.42 (dd, *J* = 4.2, 2.7 Hz, 1H), 4.37 – 4.19 (m, 6H), 3.59 (s, 3H);

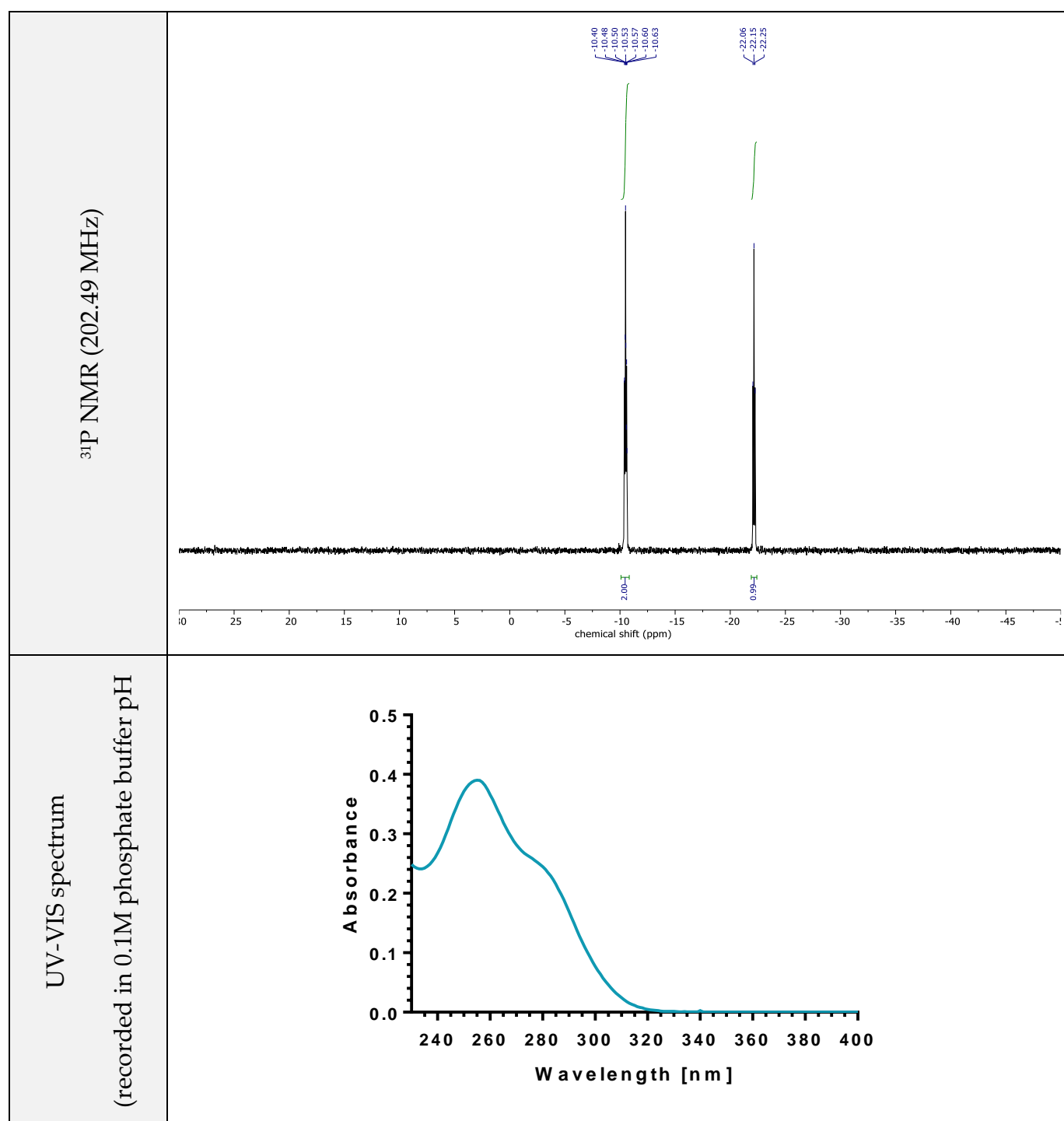
³¹P NMR (202 MHz, D₂O) δ = -10.24 – -10.63 (m, 2P), -22.04 (t, *J* = 19.2 Hz, 1P)

HRMS ESI (-) calcd. *m/z* for C₂₈H₃₃ClN₁₀O₁₈P₃⁻ [M-H]⁻ : 925.08812, found 925.08957

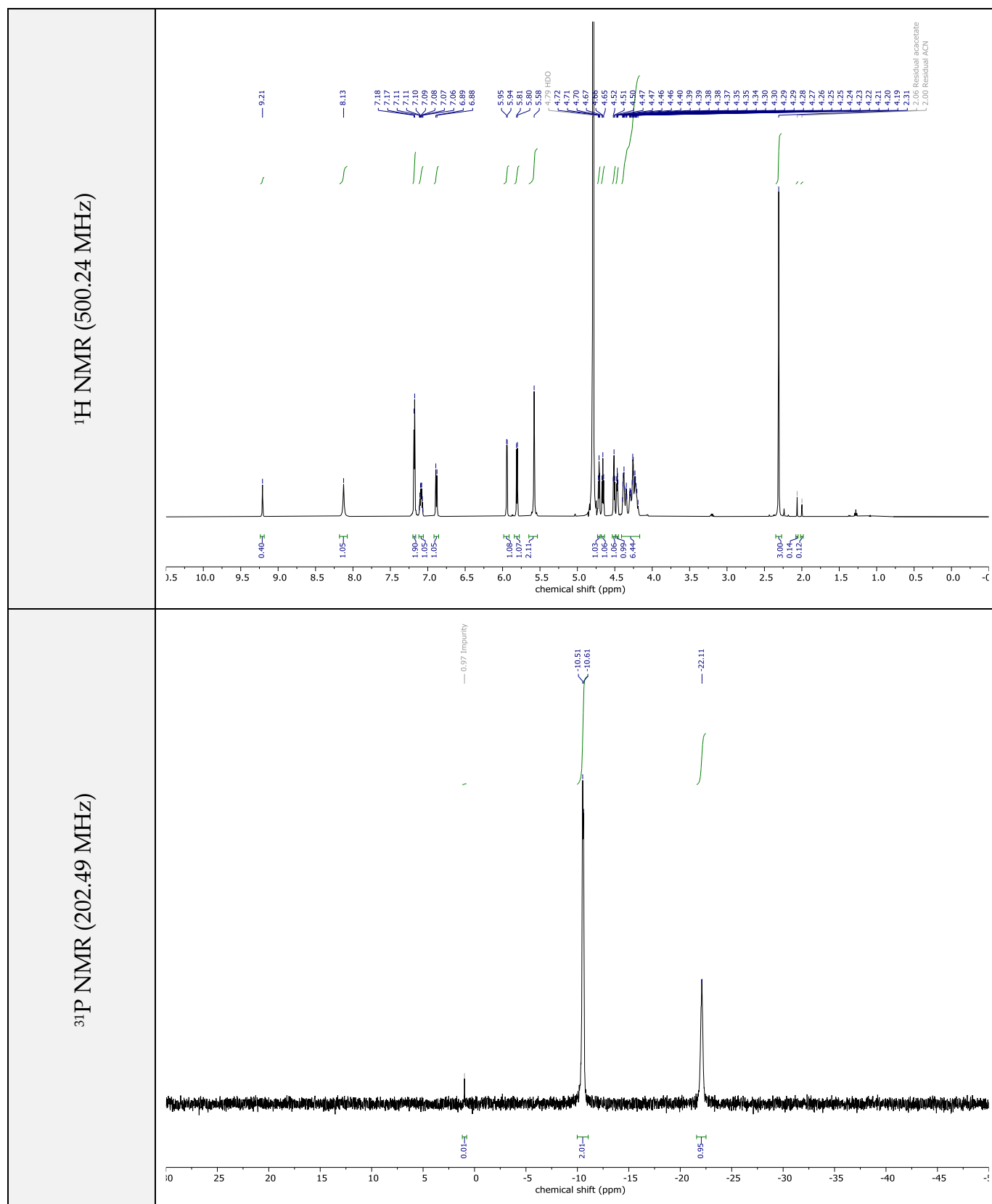
S4. Spectroscopic data: ^1H , ^{31}P , ^{19}F NMR, HRMS and UV/VIS spectra

Bn ⁷ GpppG (1a)	
Structure	
Reaction RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-12-2114-24-54BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-01-1710-28-25BN7GPPPG.D)</p>



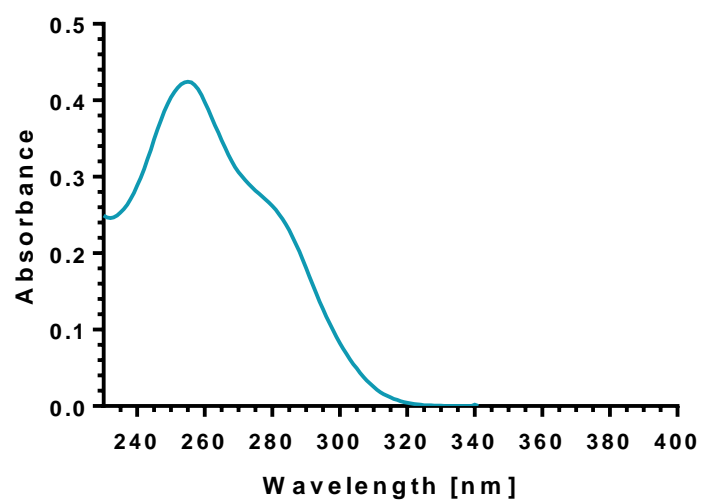


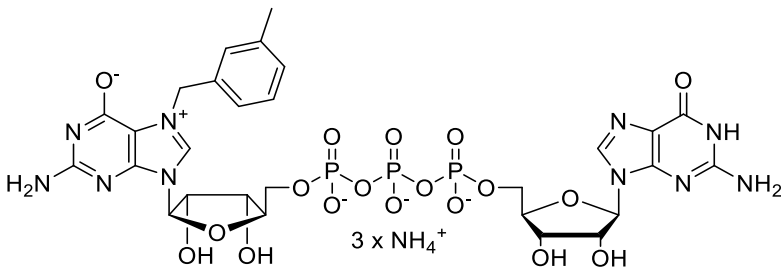
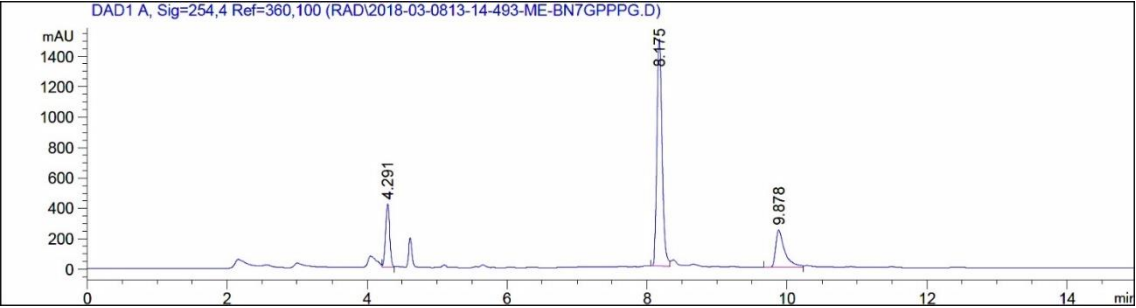
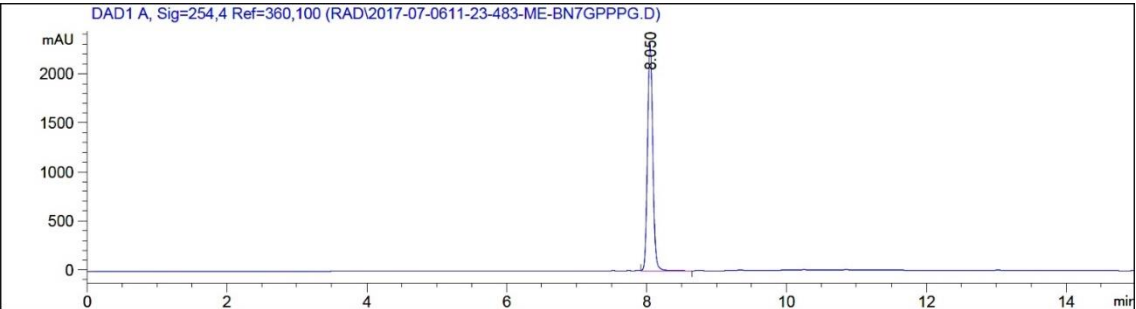
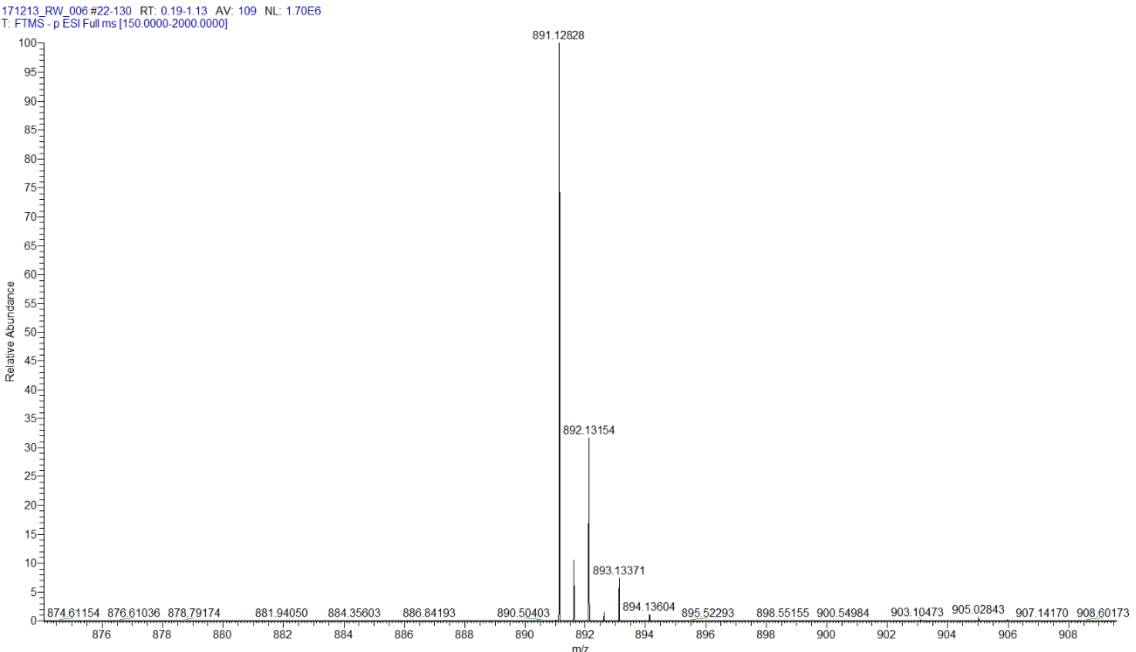
2-MeBn ⁷ GpppG (1b)	
Structure	
Reaction RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-0815-11-492-ME-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-07-0410-43-142-ME-BN7GPPPG.D)</p>
HRMS ES(-)	<p>171213_RW_005 #15-09 RT: 0.13-0.60 AV: 55 NL: 1.66E6 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>

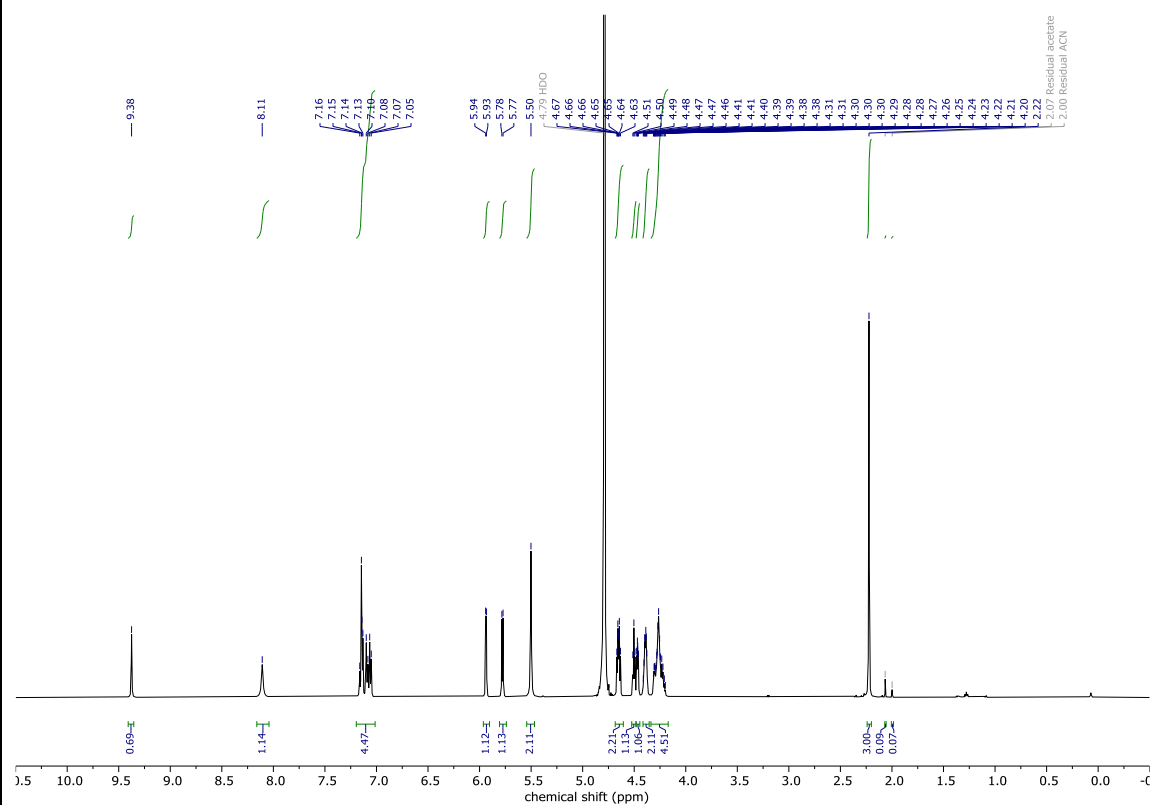
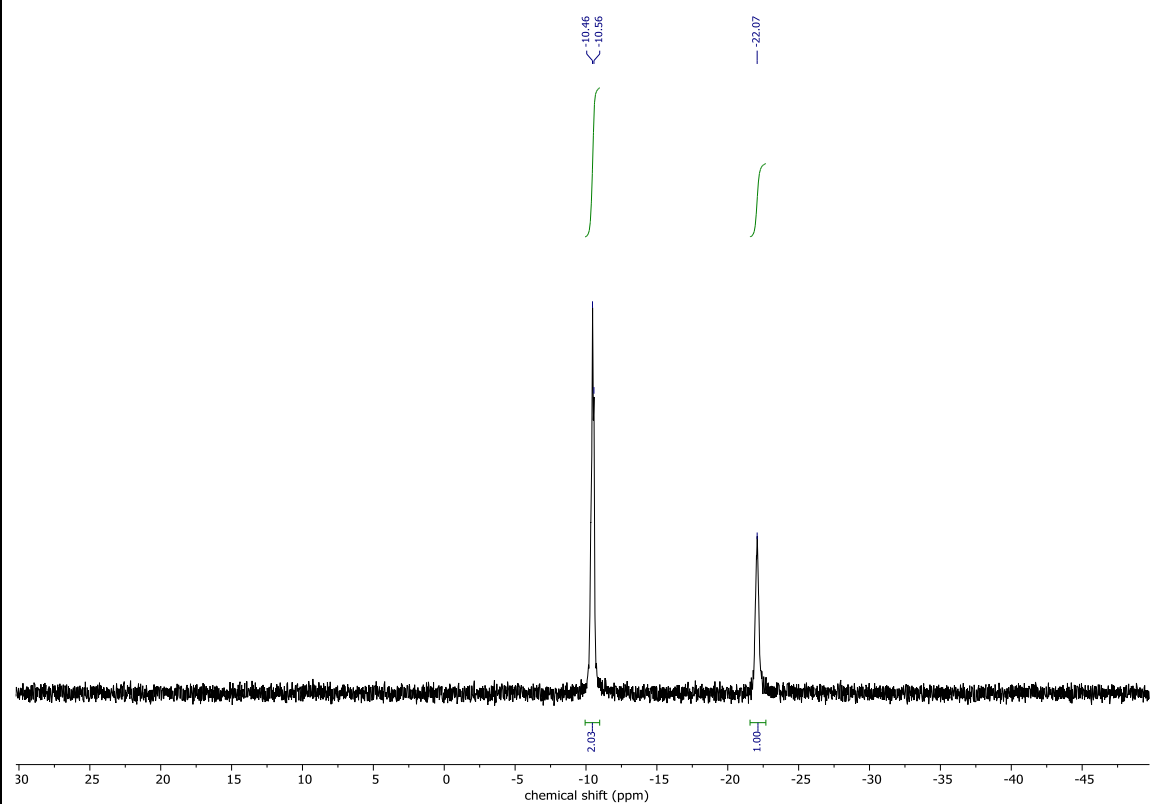


UV-VIS spectrum

(recorded in 0.1M phosphate buffer pH

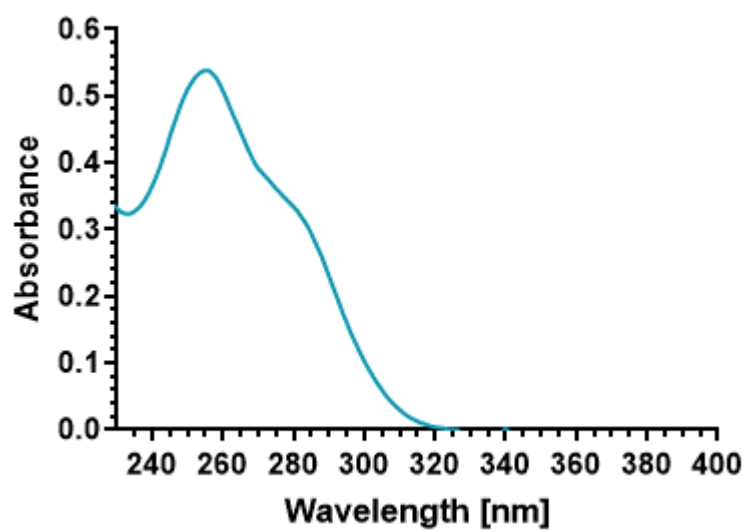


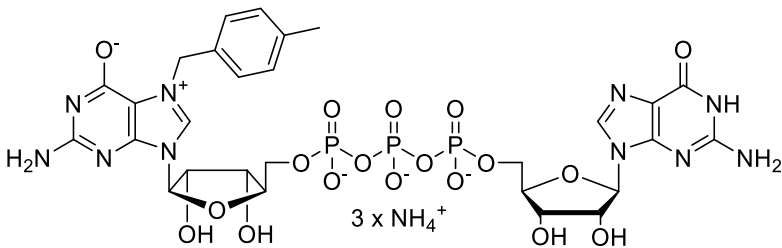
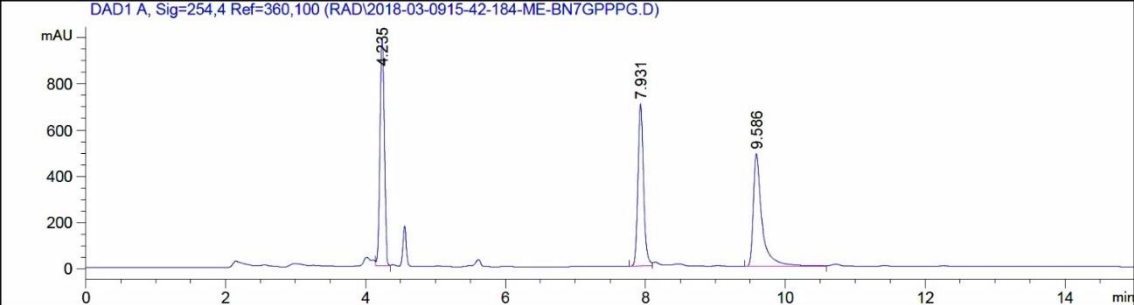
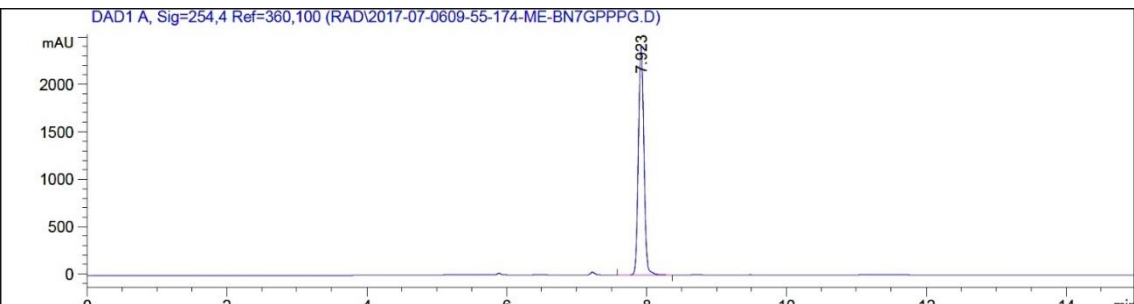
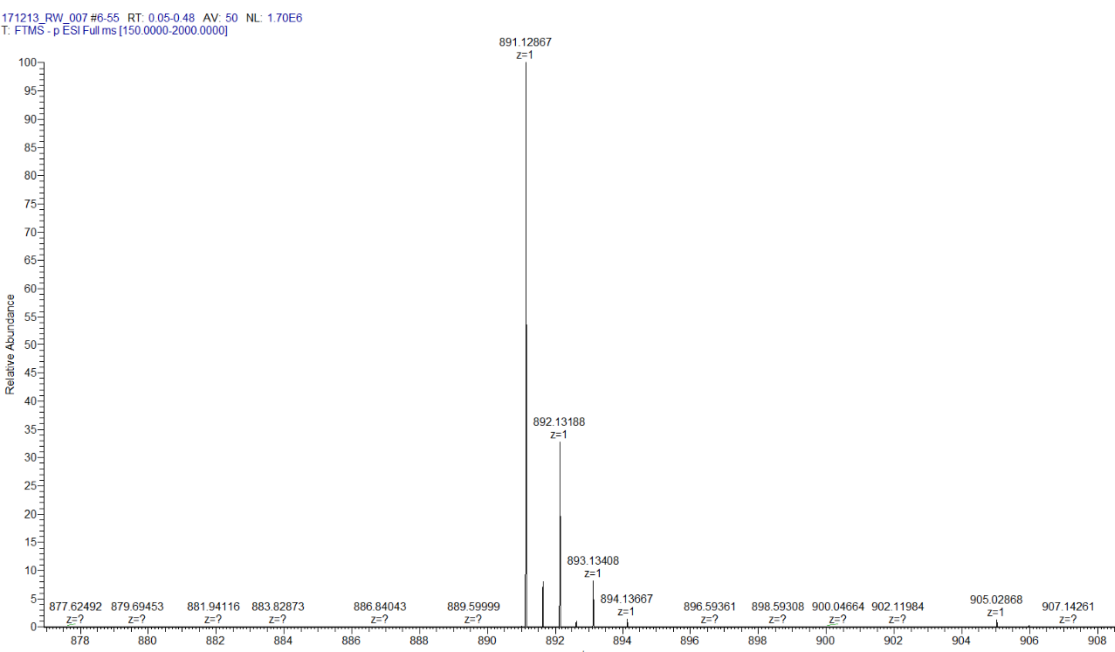
3-MeBn ⁷ GpppG (1c)	
Structure	
Reaction RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-0813-14-493-ME-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-07-0611-23-483-ME-BN7GPPPG.D)</p>
HRMS ES(-)	 <p>171213_RW_006 #22-130 RT: 0.19-1.13 AV: 109 NL: 1.70E6 T: FTMS -p ESI Full ms [150.0000-2000.0000]</p>

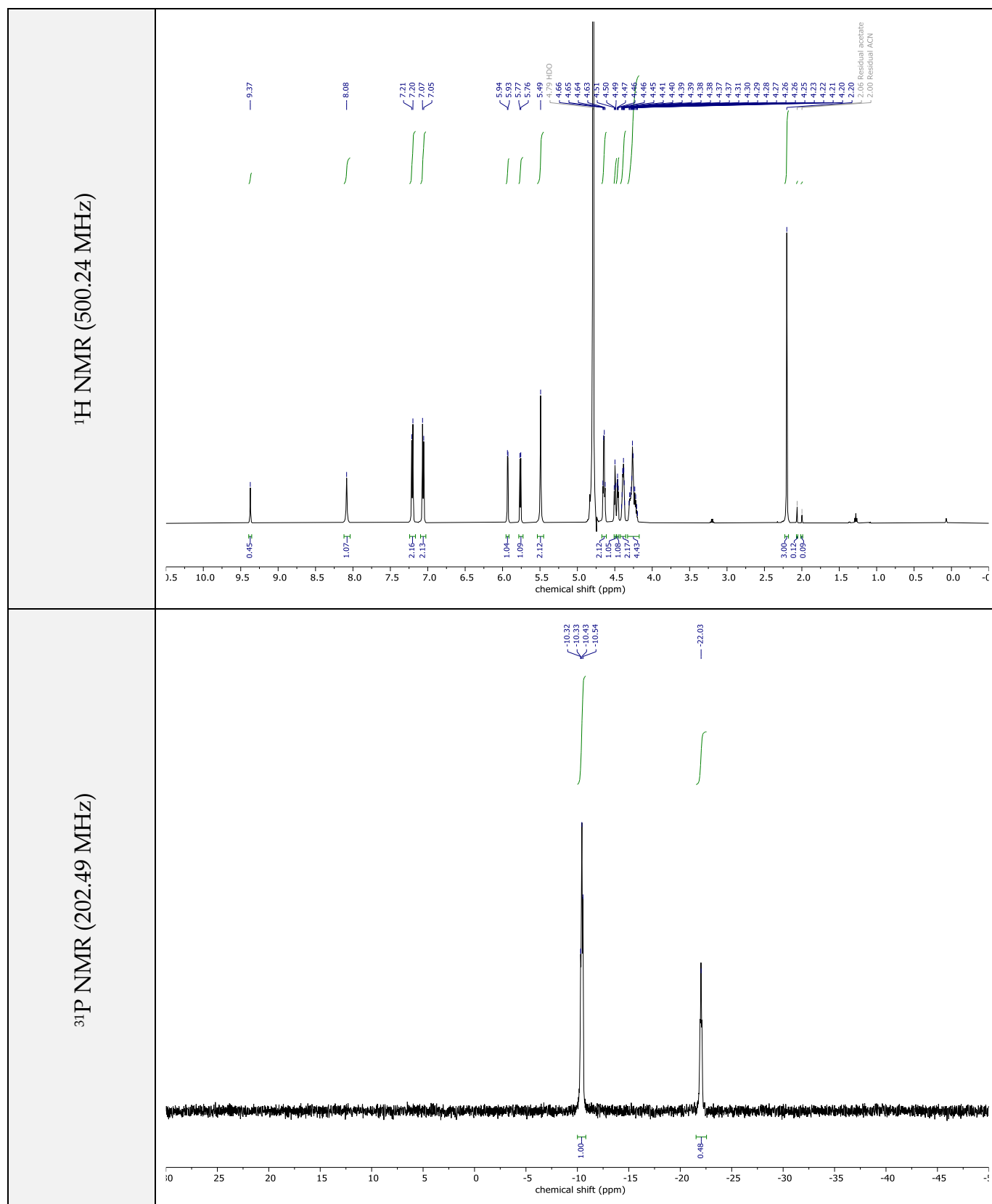
^1H NMR (500.24 MHz) ^{31}P NMR (202.49 MHz)

UV-VIS spectrum

(recorded in 0.1M phosphate buffer pH 7.0)

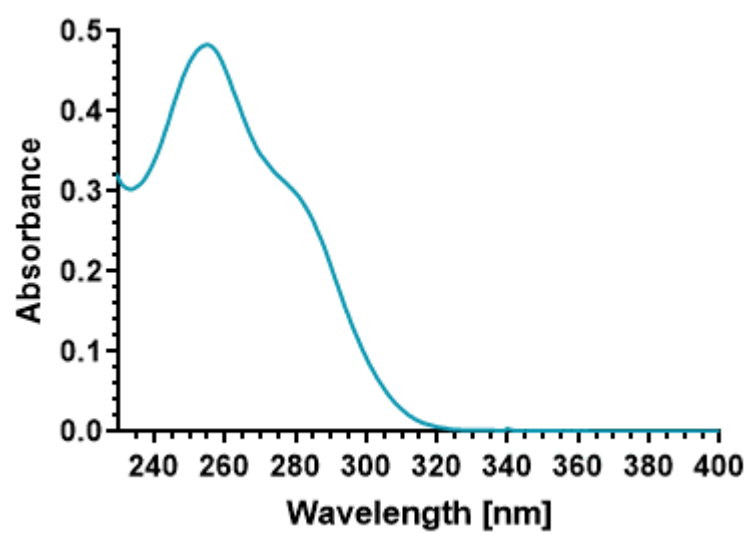


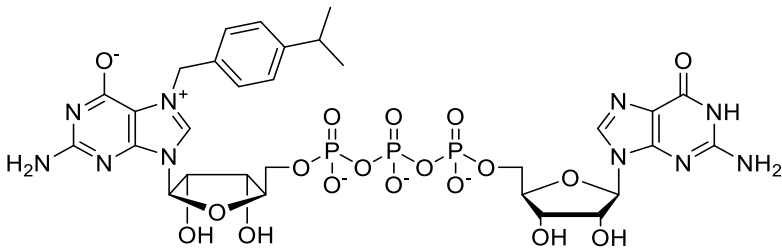
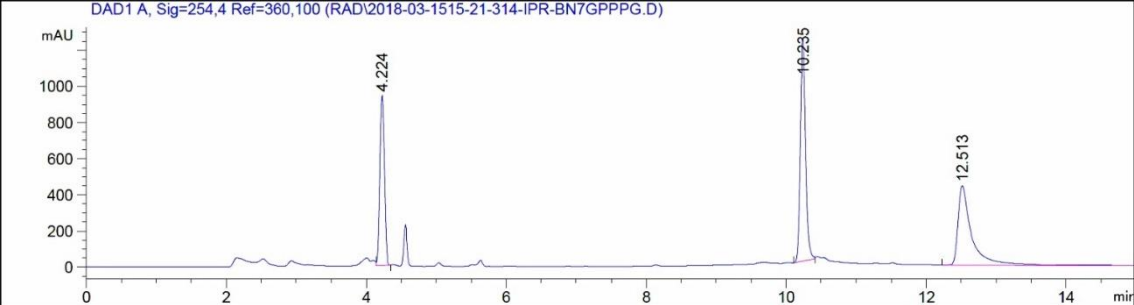
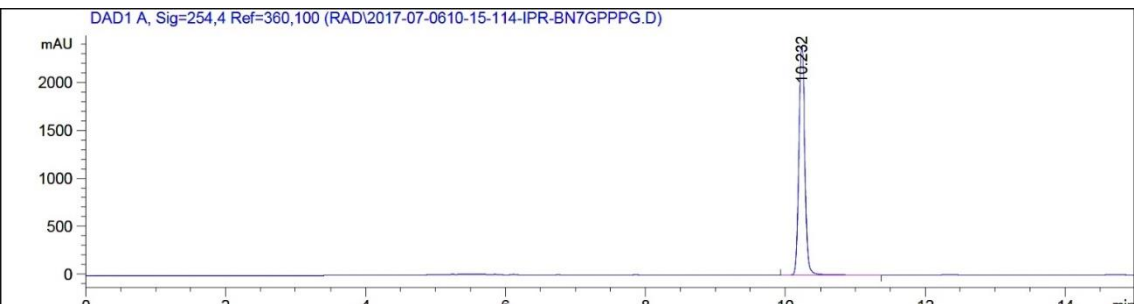
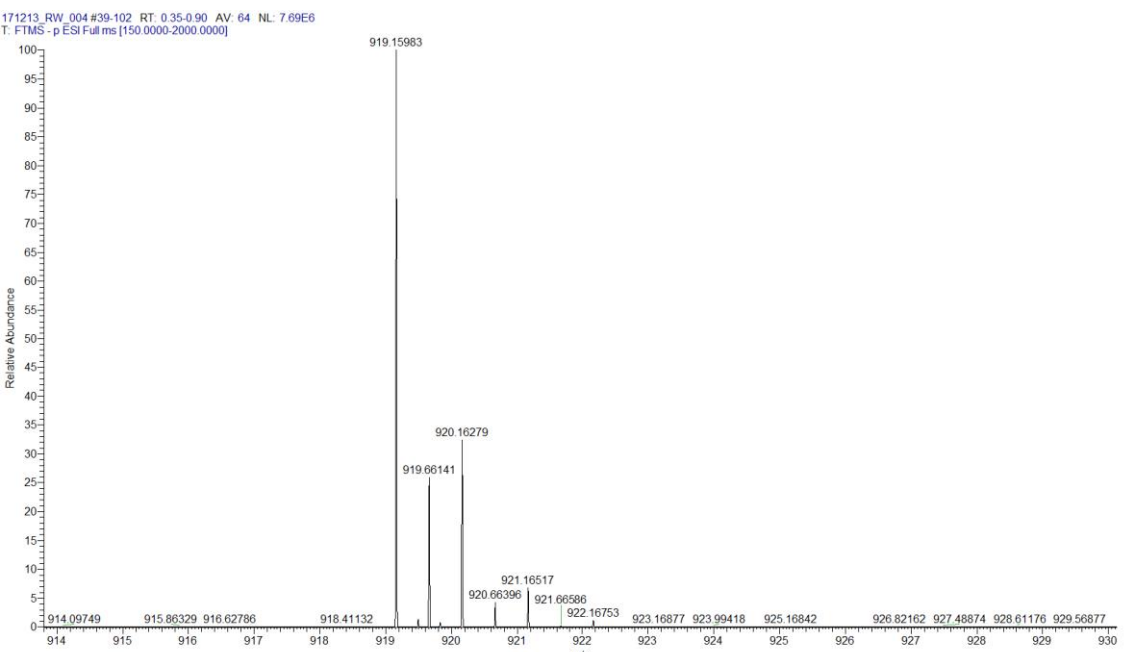
4-MeBn ⁷ GpppG (1d)	
Structure	
Reaction RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-0915-42-184-ME-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-07-0609-55-174-ME-BN7GPPPG.D)</p>
HRMS ES(-)	 <p>171213_RW_007 #6-55 RT: 0.05-0.48 AV: 50 NL: 1.70E6 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>

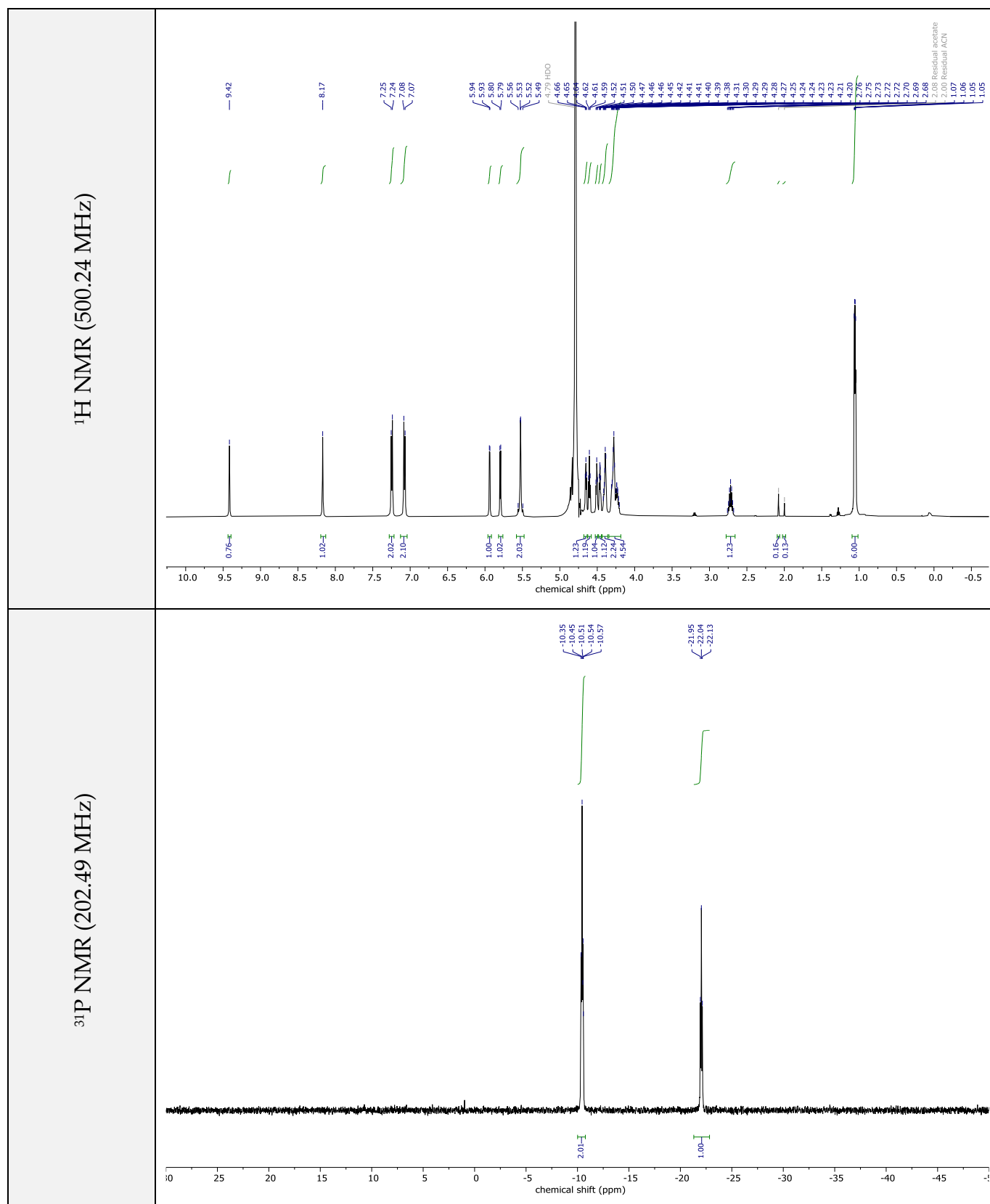


UV-VIS spectrum

(recorded in 0.1M phosphate buffer pH 7.0)

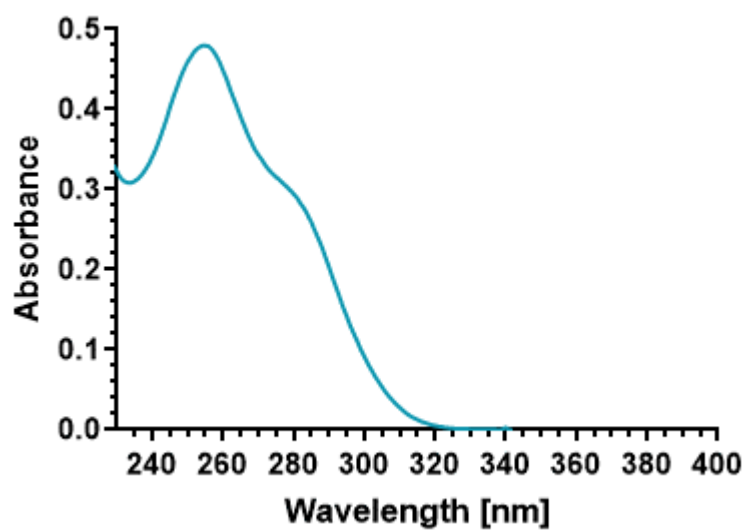


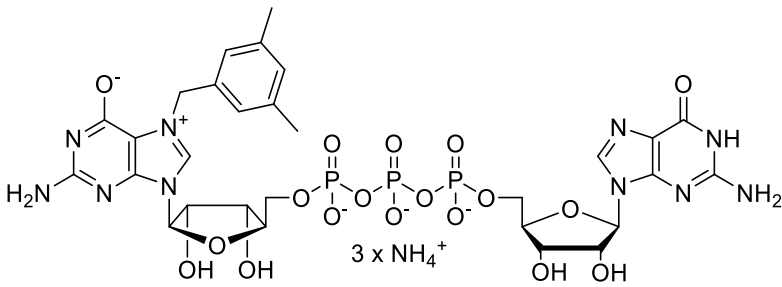
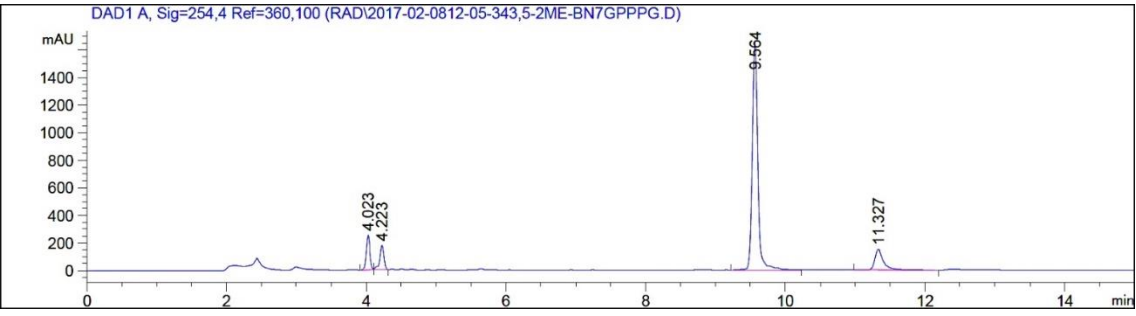
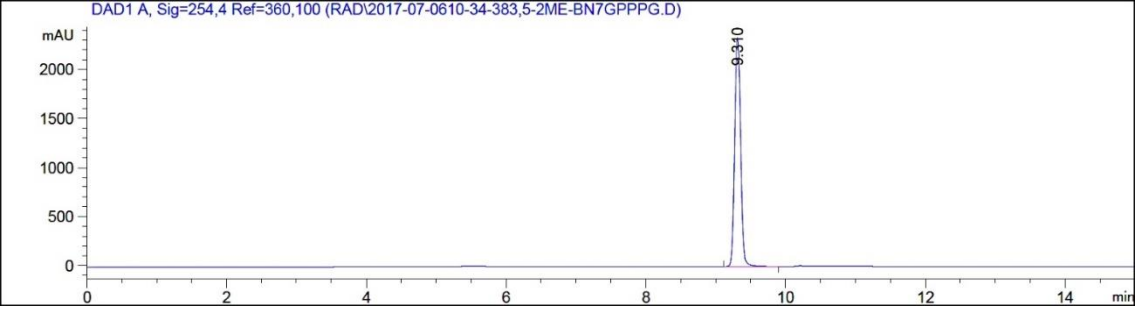
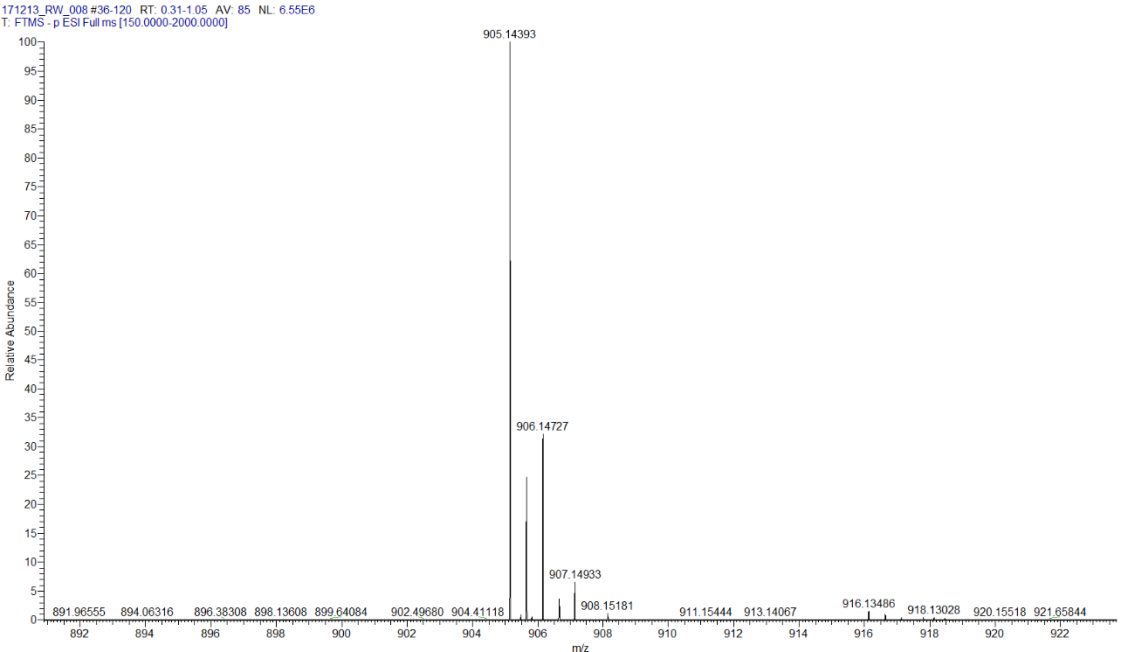
4- <i>i</i> PrBn ⁷ GpppG (1e)	
Structure	
Reaction RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-1515-21-314-IPR-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-07-0610-15-114-IPR-BN7GPPPG.D)</p>
HRMS ES(-)	 <p>171213_RW_004 #39-102 RT: 0.35-0.90 AV: 64 NL: 7.69E6 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>

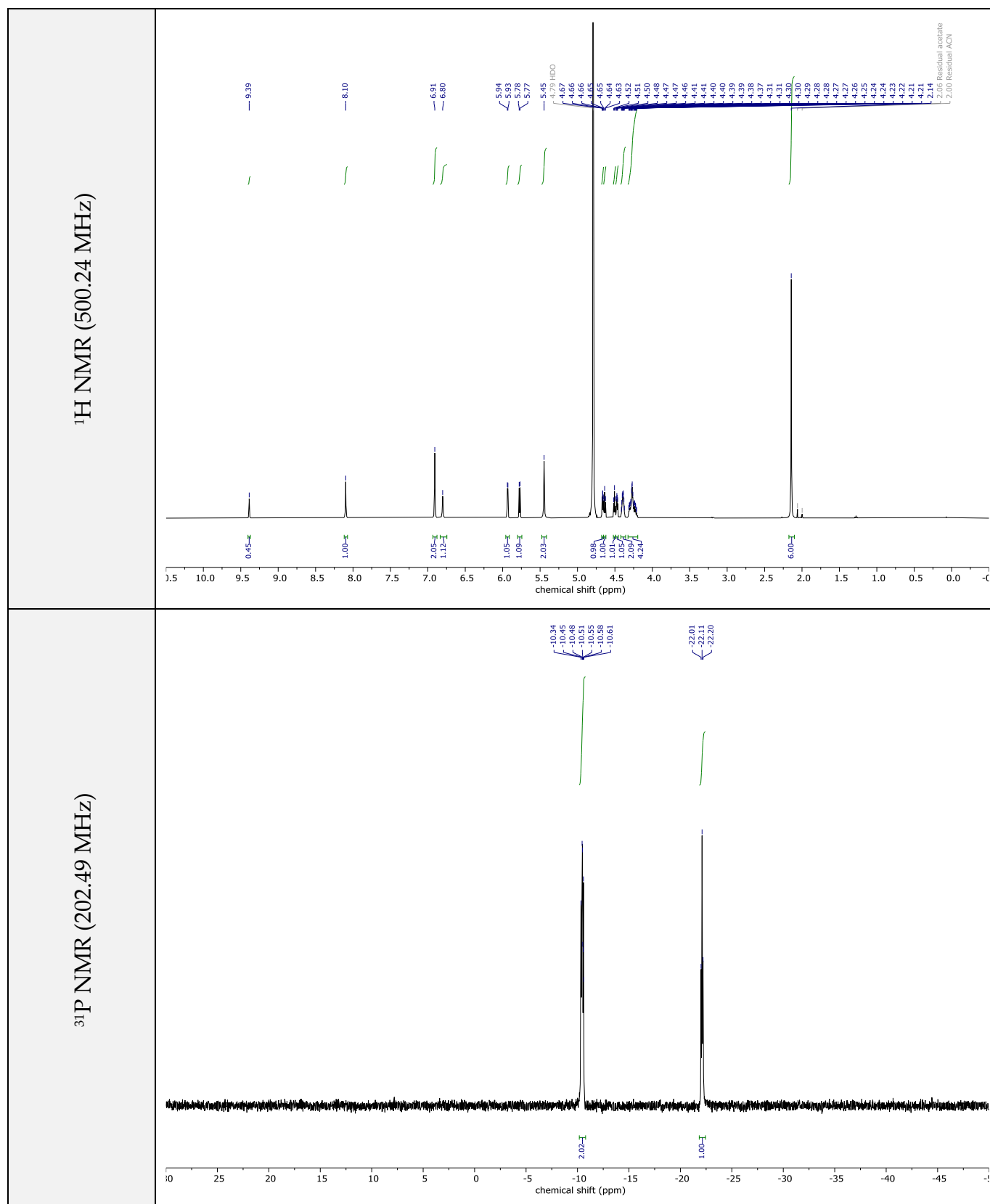


UV-VIS spectrum

(recorded in 0.1M phosphate buffer pH 7.0)

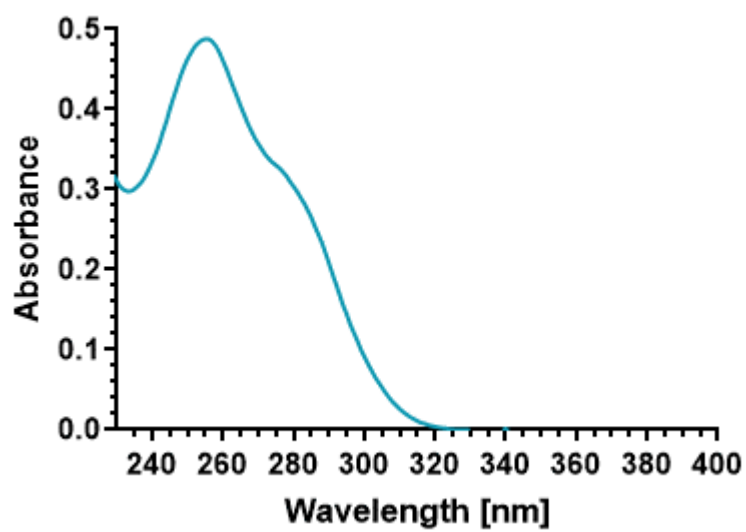


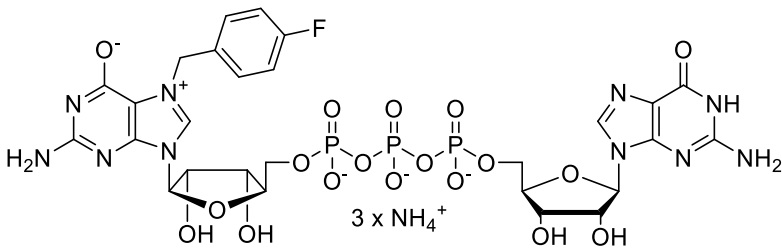
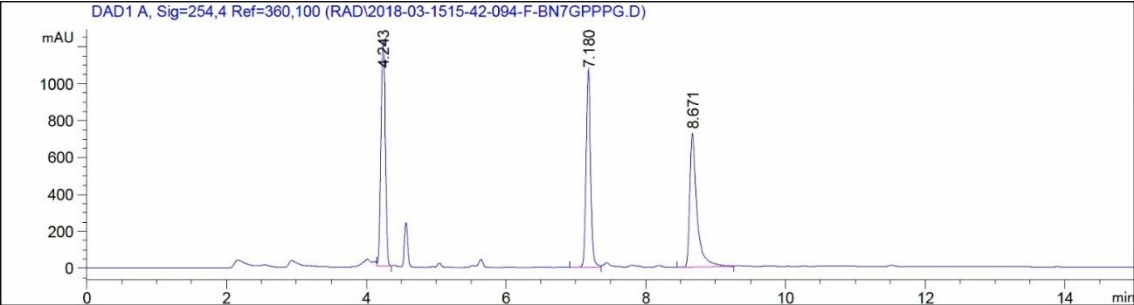
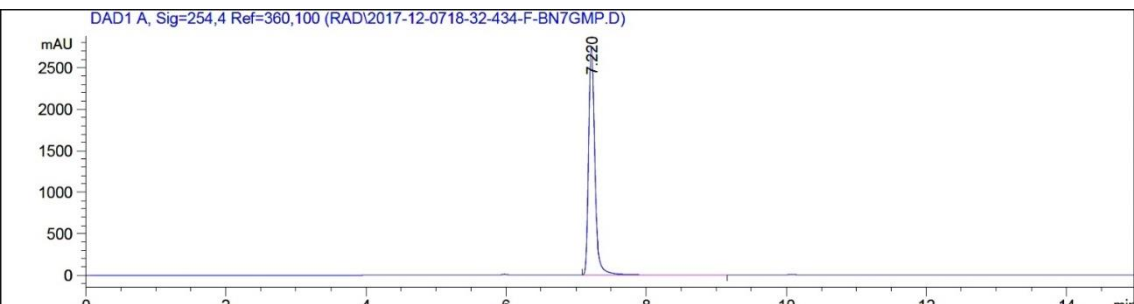
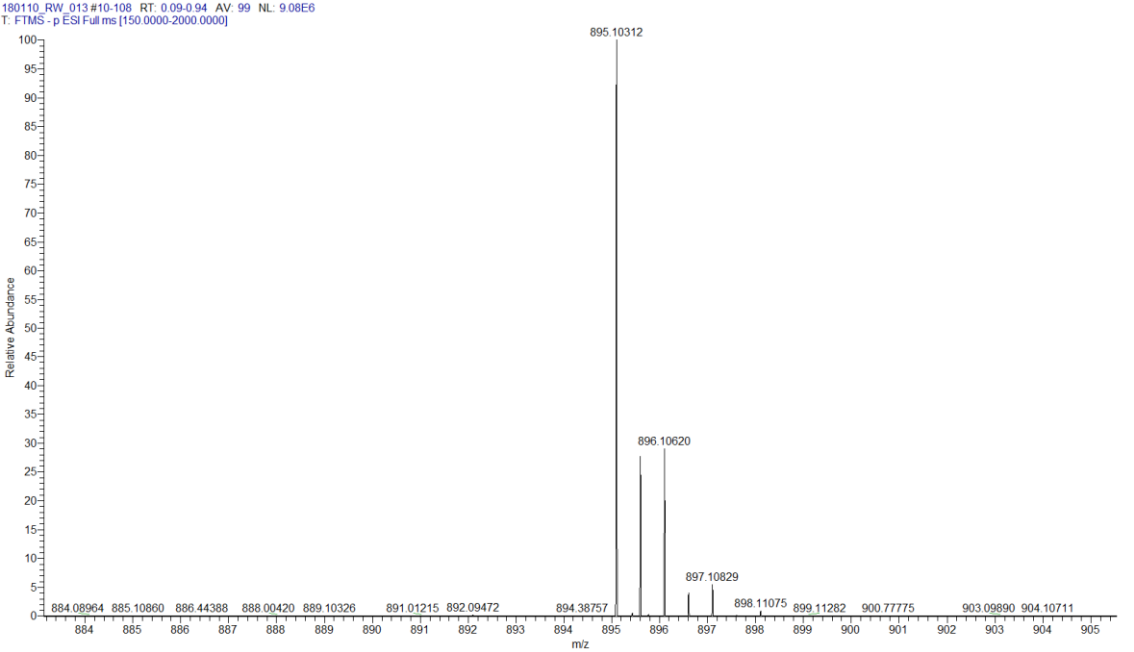
3,5-di-MeBn ⁷ GpppG (1f)	
Structure	
Reaction RP HPLC Profile	
Purified Product RP HPLC Profile	
HRMS ES(-)	

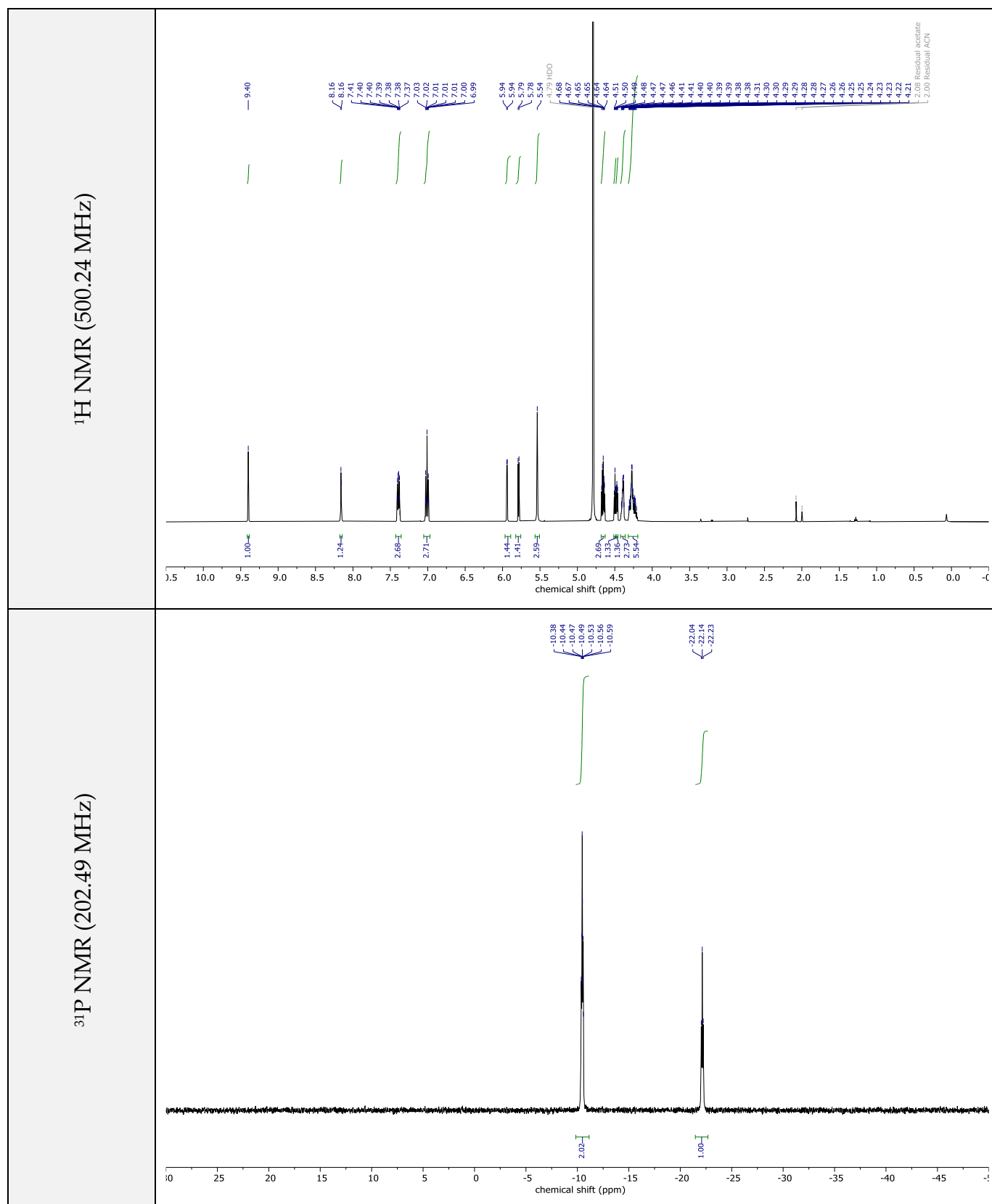


UV-VIS spectrum

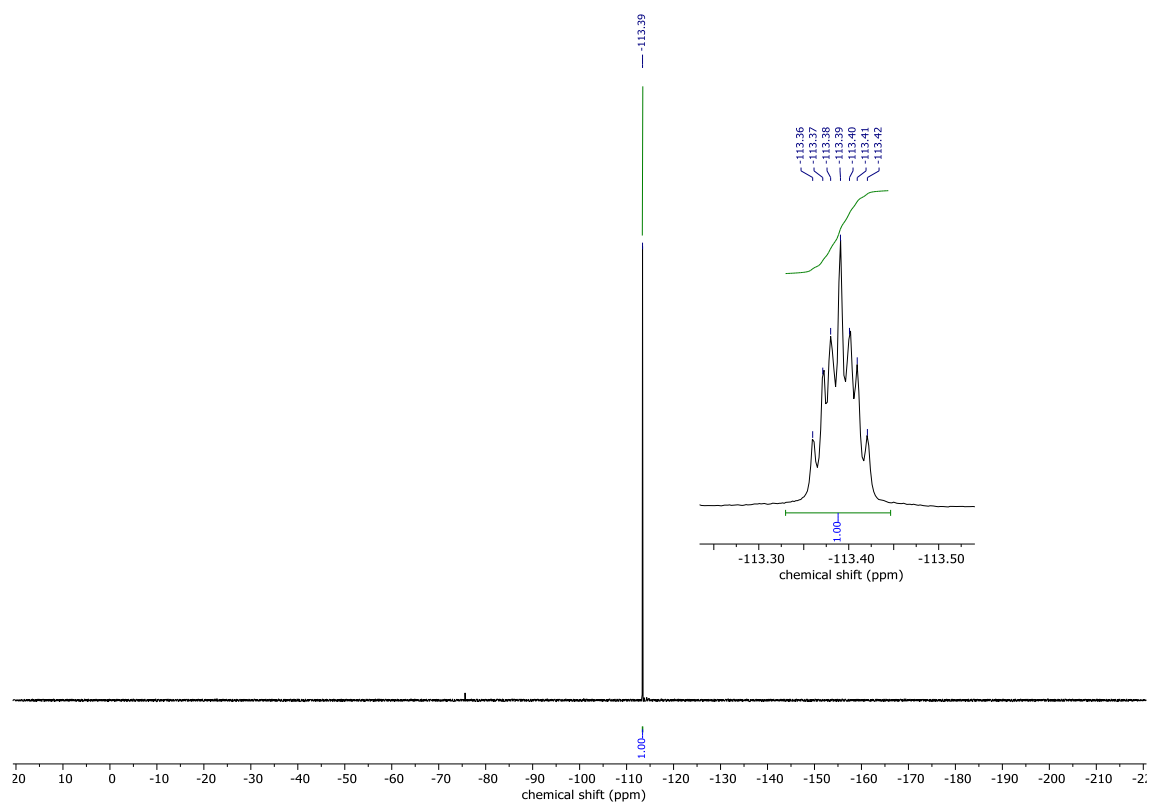
(recorded in 0.1M phosphate buffer pH



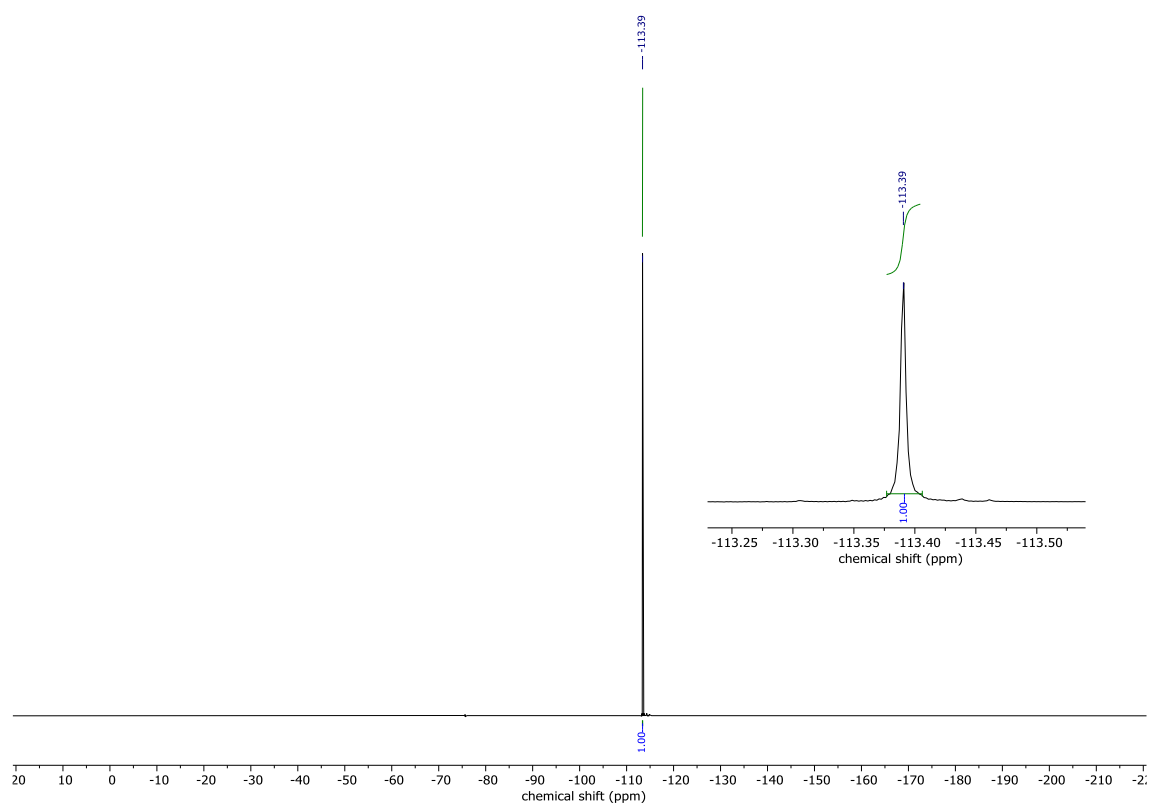
4-F-Bn ⁷ GpppG (1g)	
Structure	
Reaction RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-1515-42-094-F-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-12-0718-32-434-F-BN7GMP.D)</p>
HRMS ES(-)	 <p>180110_RW_013 #10-108 RT: 0.09-0.94 AV: 99 NL: 9.08E6 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>



^{19}F NMR (470.65 MHz)

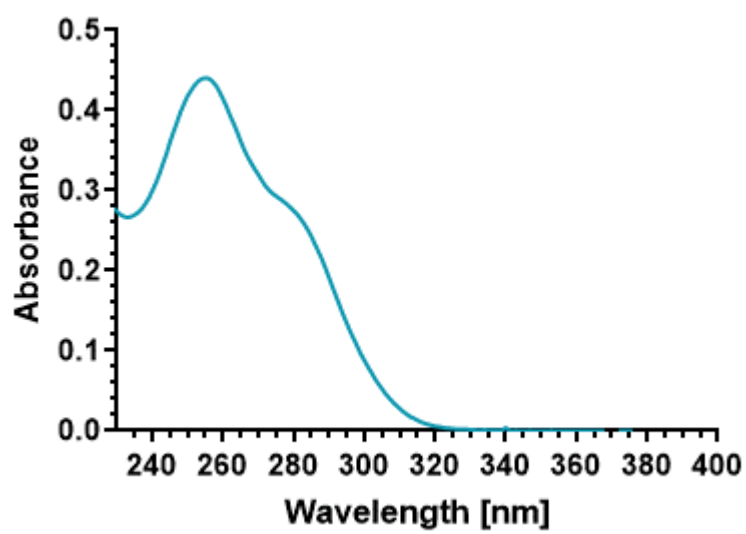


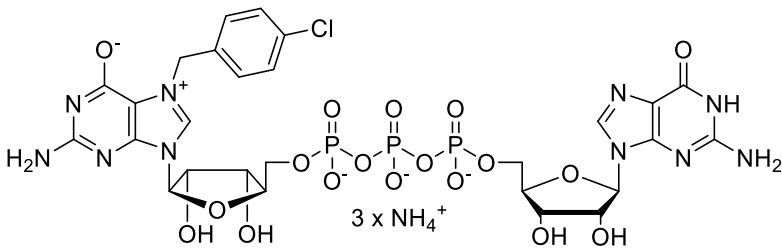
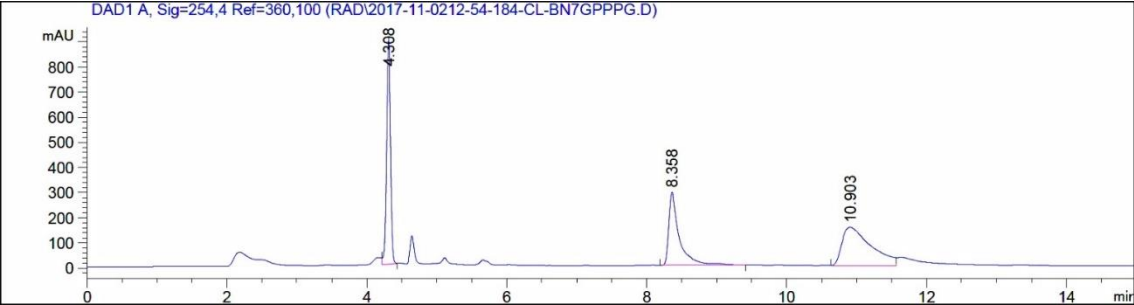
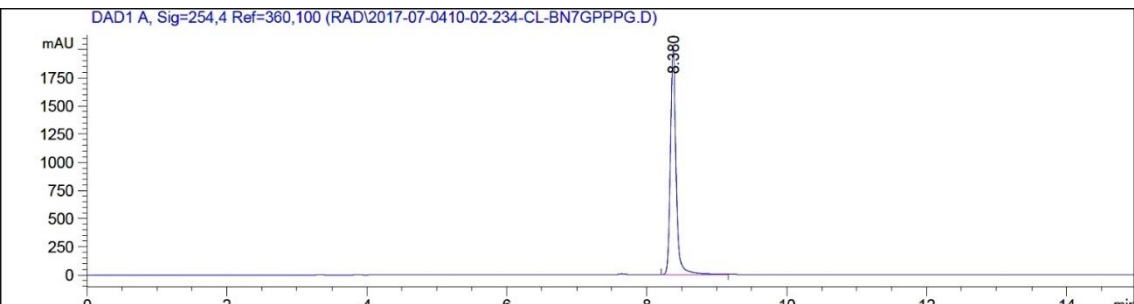
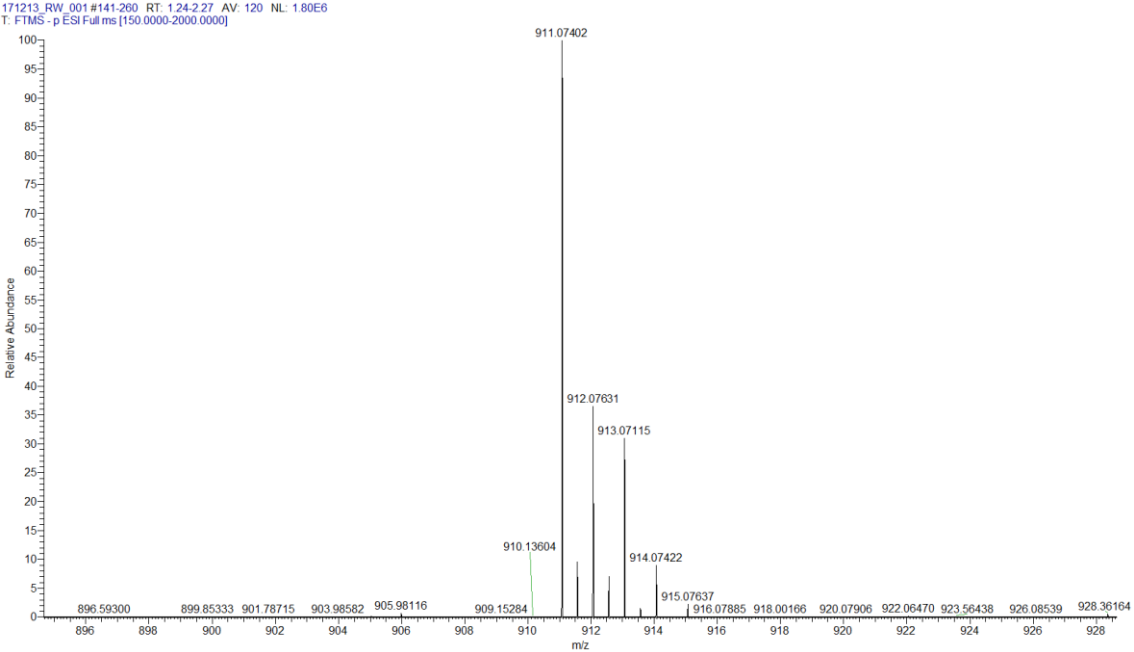
^{19}F { ^1H } NMR (470.65 MHz)

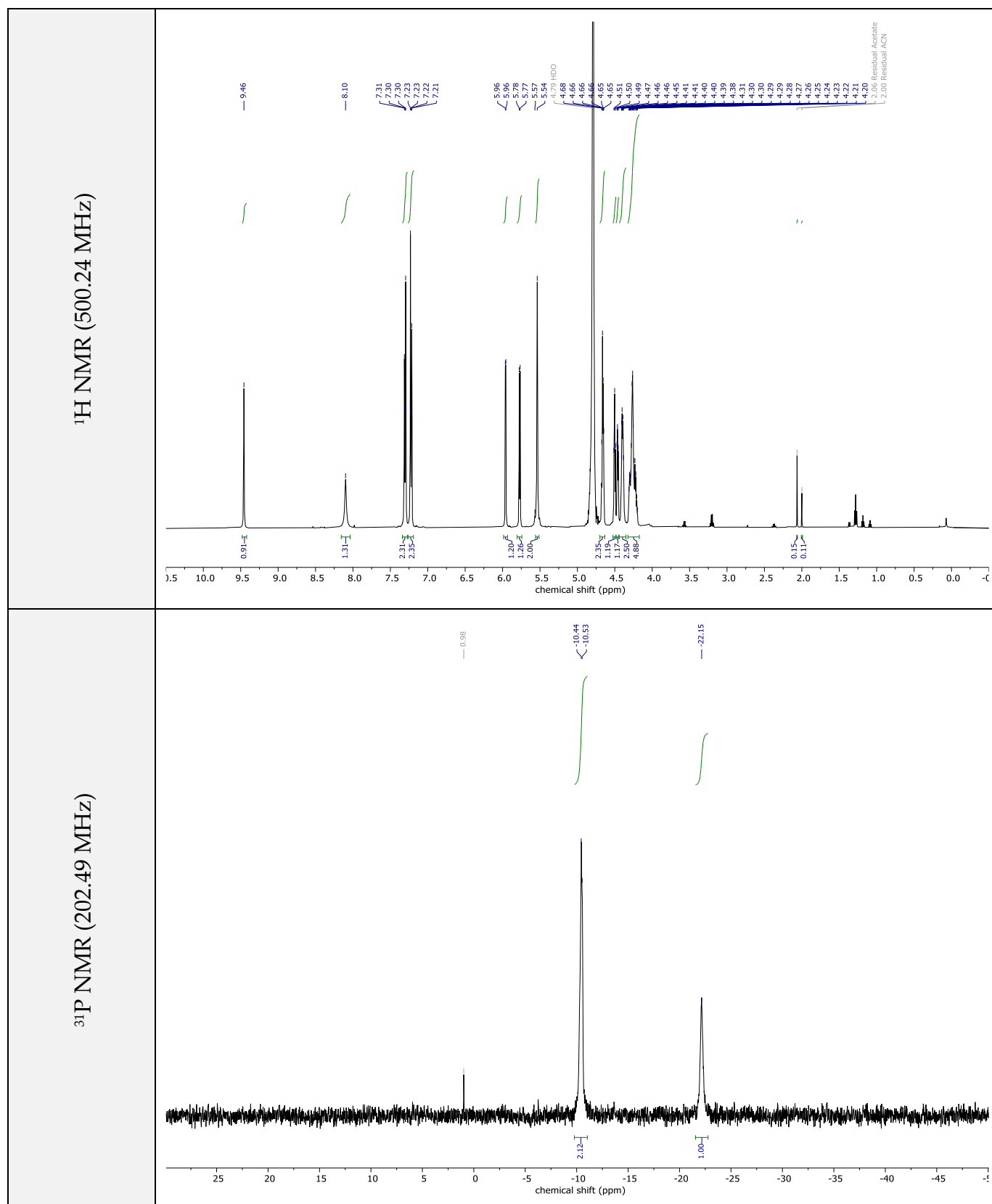


UV-VIS spectrum

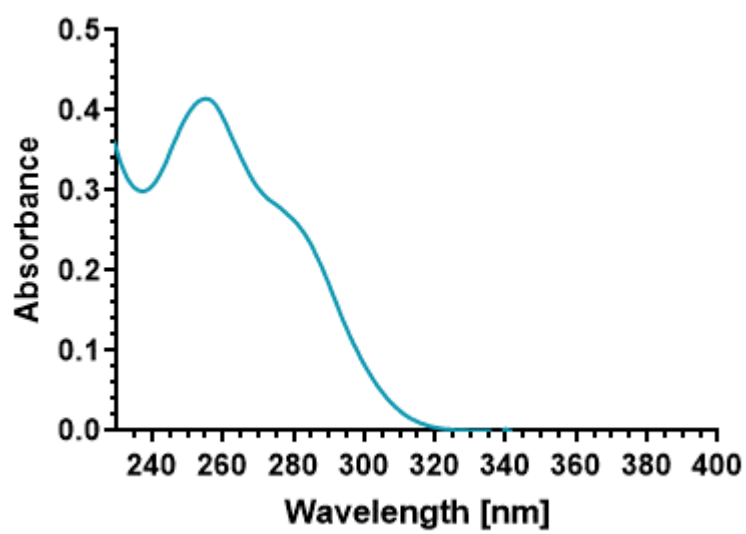
(recorded in 0.1M phosphate buffer pH 7.0)

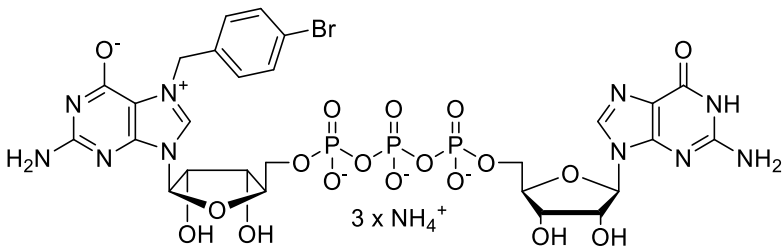
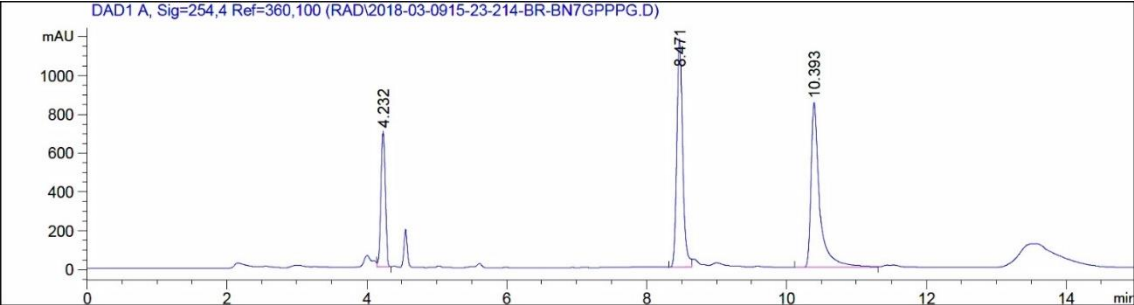
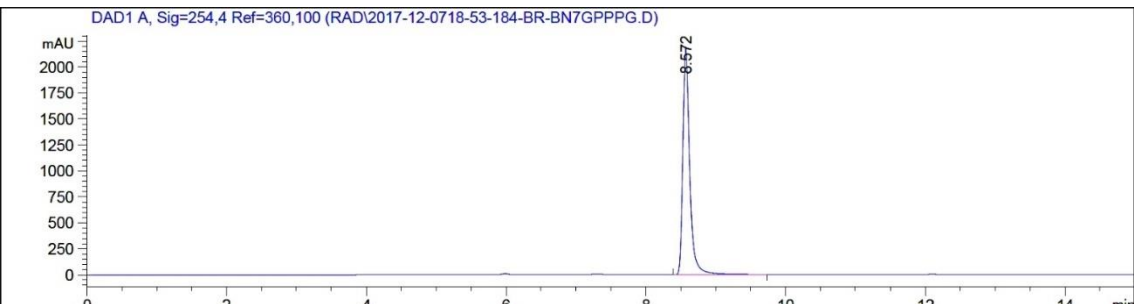
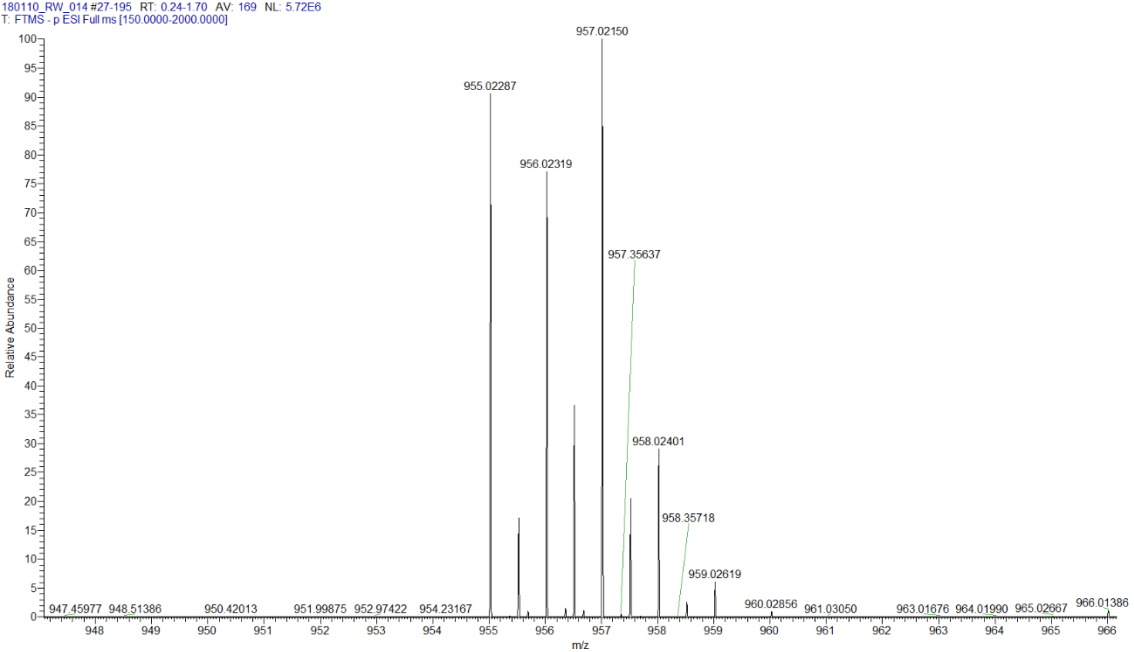


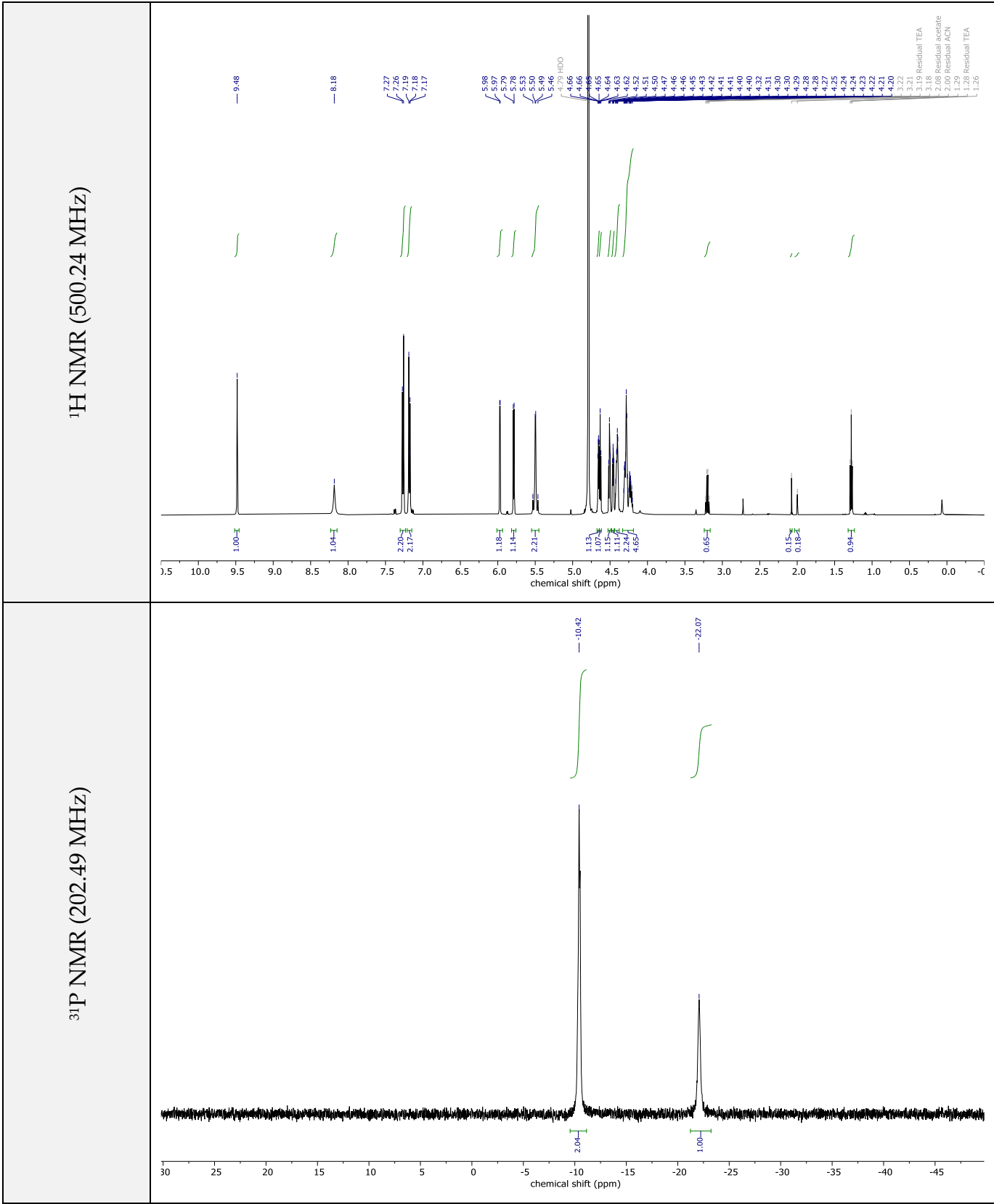
4-Cl-Bn ⁷ GpppG (1h)	
Structure	
Reaction RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-11-02\12-54-184-CL-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-07-04\10-02-234-CL-BN7GPPPG.D)</p>
HRMS ES(-)	 <p>171213_RW_001#141-260 RT: 1.24-2.27 AV: 120 NL: 1.80E6 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>



UV-VIS spectrum
(recorded in 0.1M phosphate buffer pH

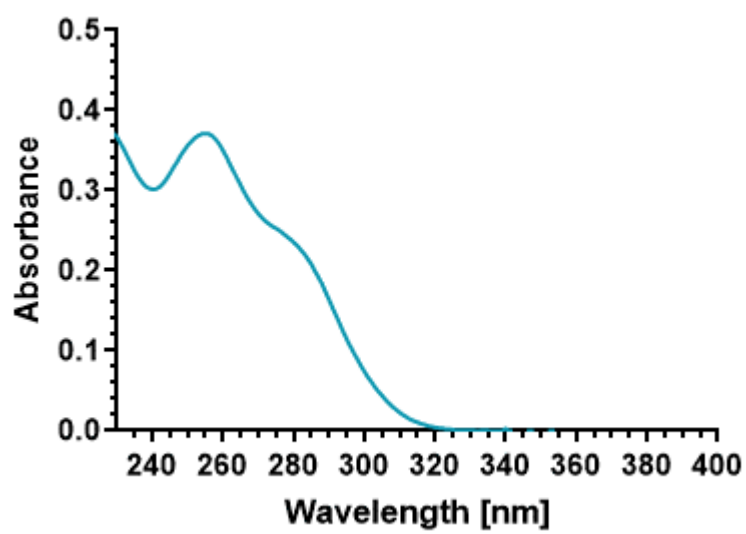


4-Br-Bn ⁷ GpppG (1i)	
Structure	
Reaction RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-0915-23-214-BR-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-12-0718-53-184-BR-BN7GPPPG.D)</p>
HRMS ES(-)	 <p>180110_RW_014 #27-195 RT: 0.24-1.70 AV: 189 NL: 5.72E6 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>

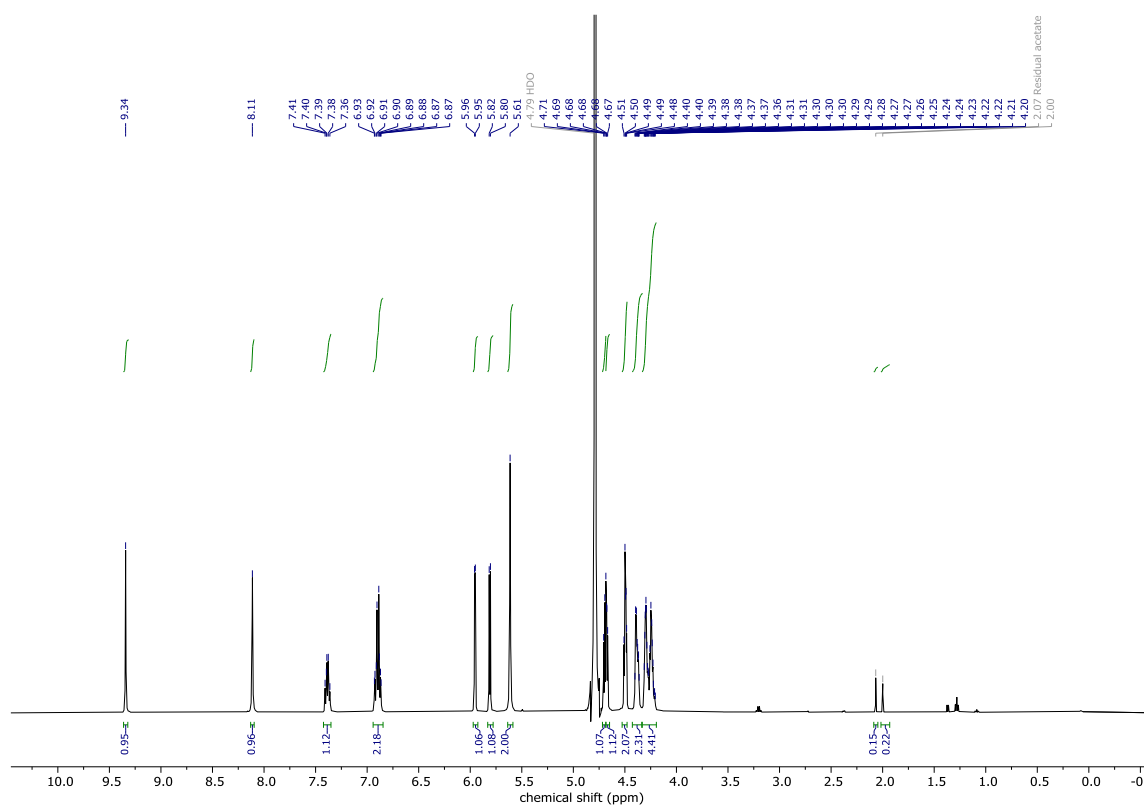
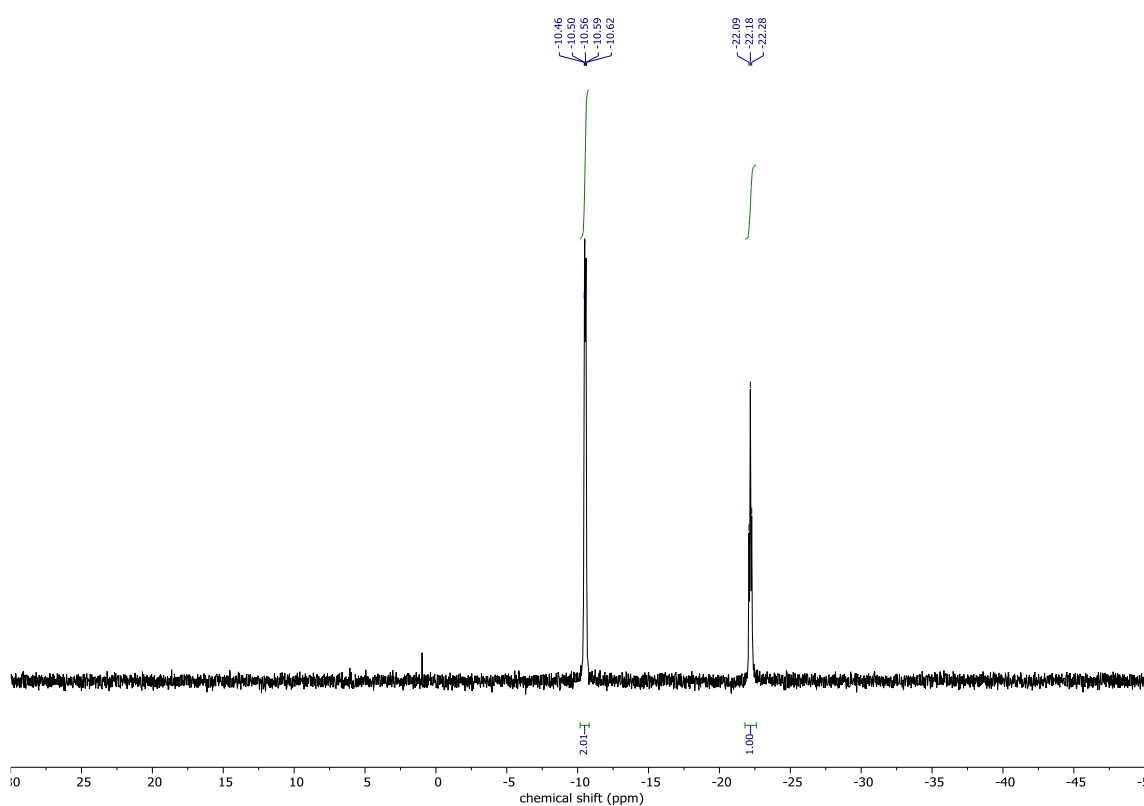


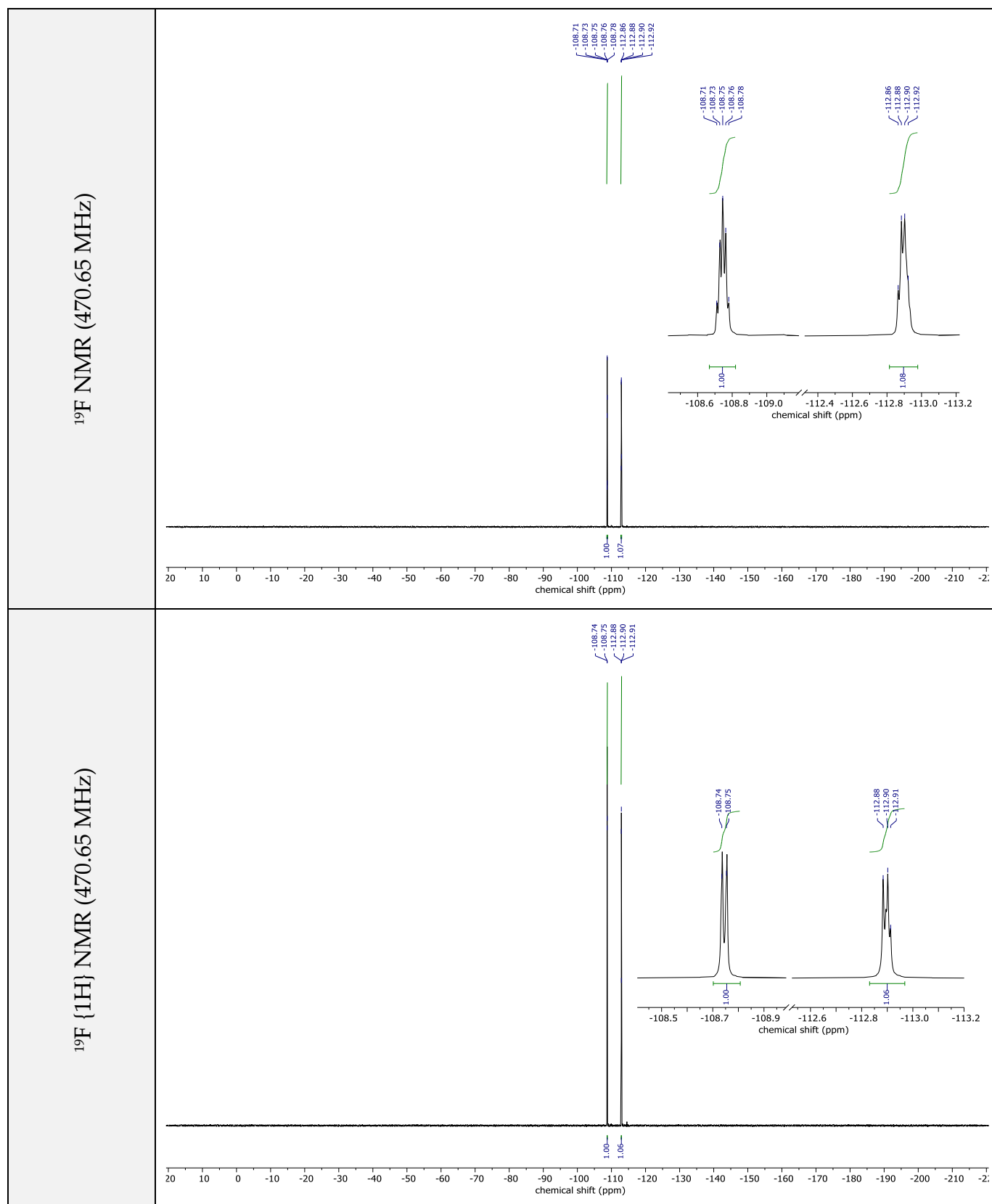
UV-VIS spectrum

(recorded in 0.1M phosphate buffer pH 7.0)



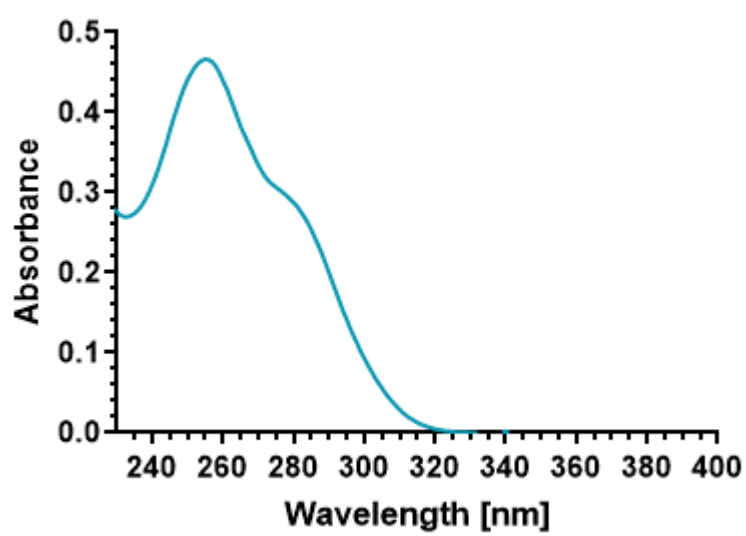
2,4-di-F-Bn ⁷ GpppG (1j)	
Structure	
Reaction RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-0813-34-252,4-2F-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-07-0408-45-302,4-2F-BN7GPPPG.D)</p>
HRMS ES(-)	<p>171213_RW_002 #25-113 RT: 0.22-0.99 AV: 89 NL: 1.52E6 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>

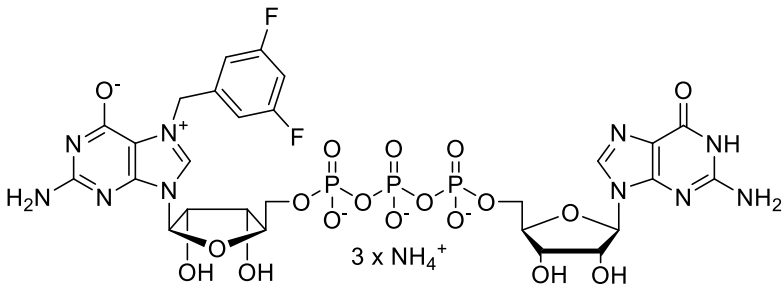
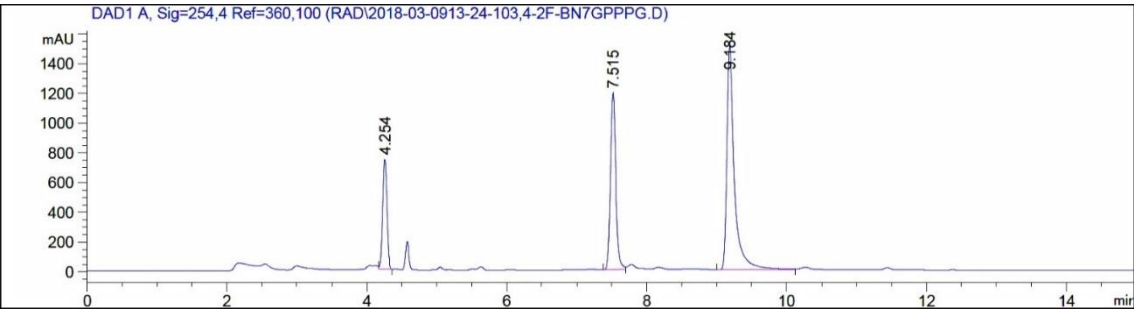
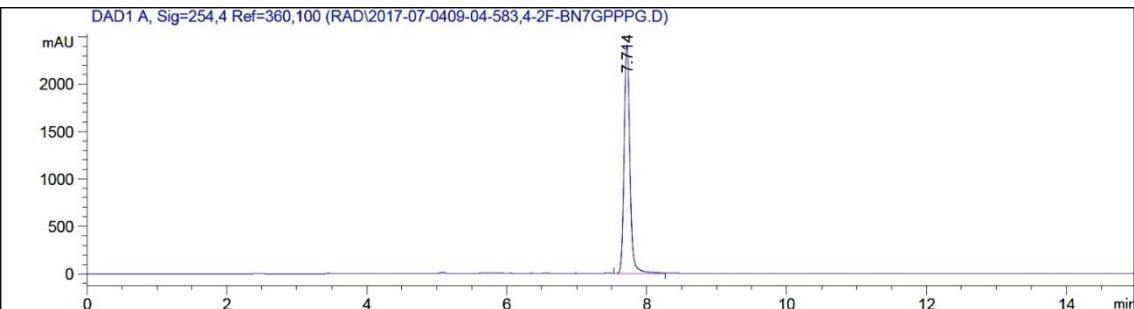
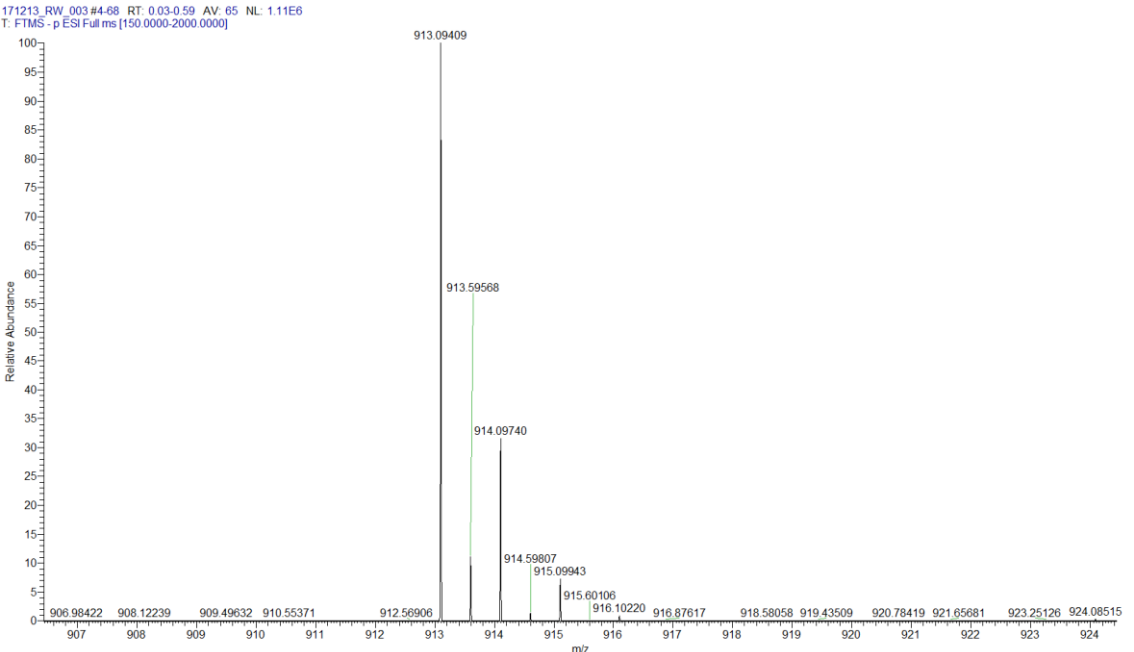
^1H NMR (500.24 MHz) ^{31}P NMR (202.49 MHz)

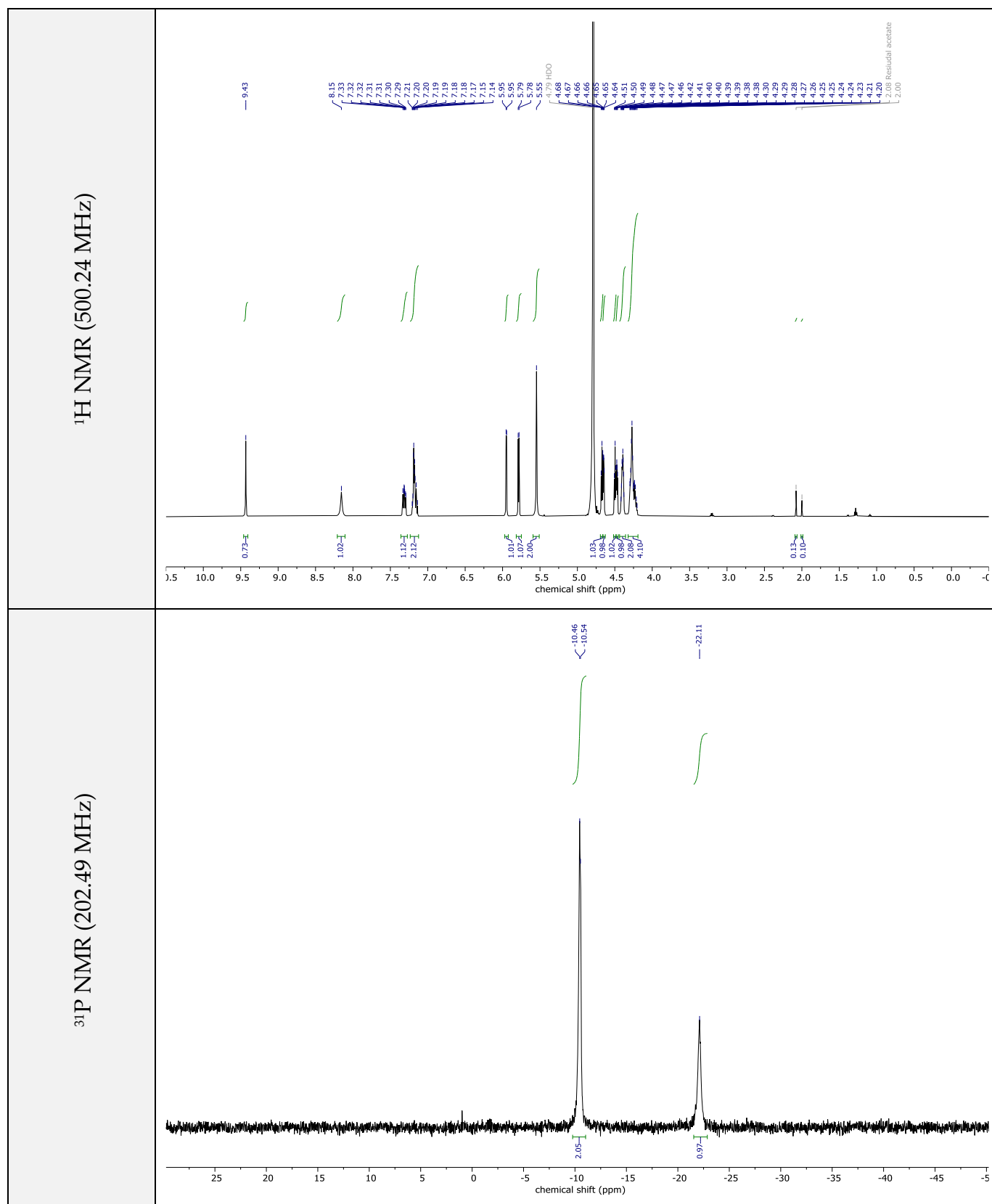


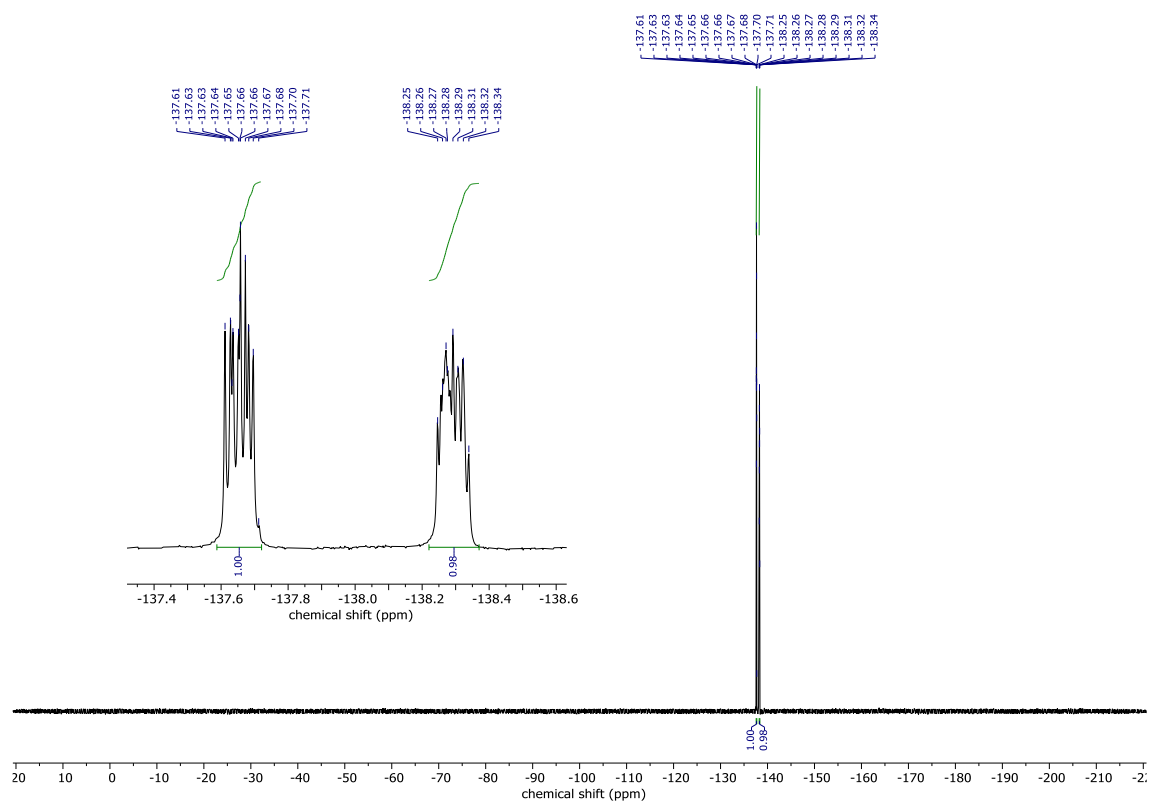
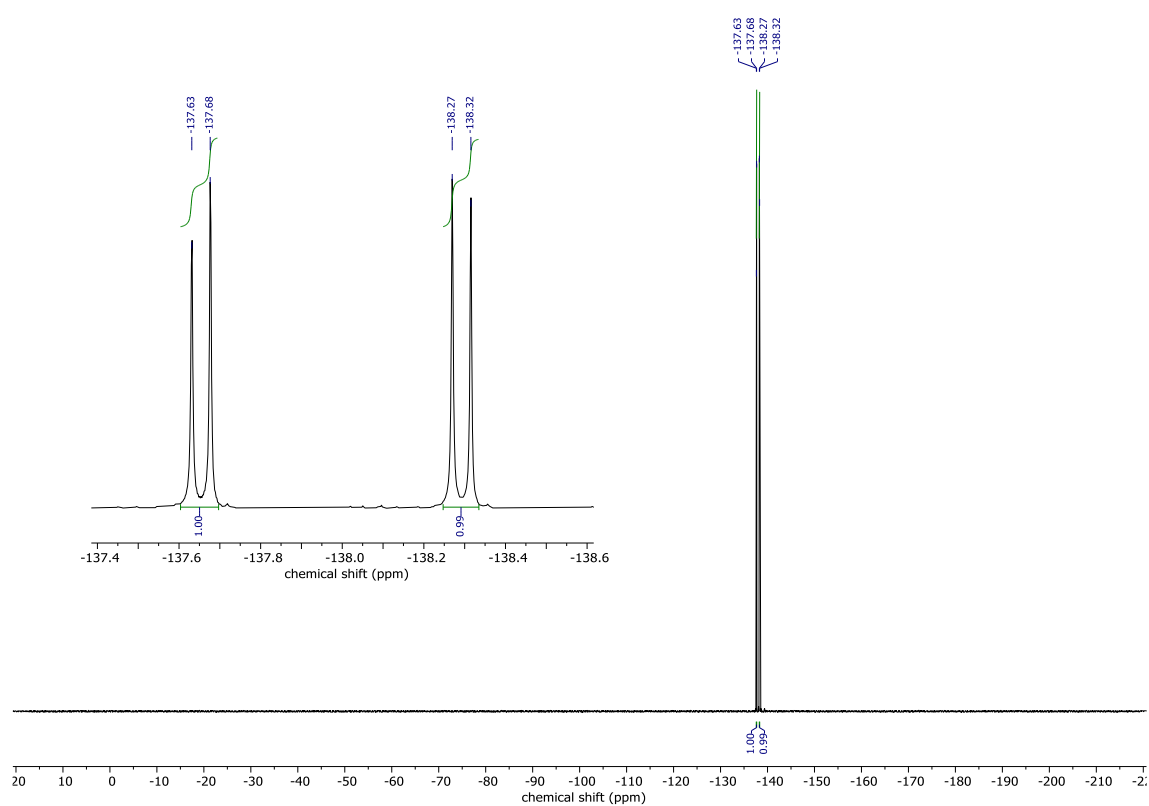
UV-VIS spectrum

(recorded in 0.1M phosphate buffer pH 7.0)



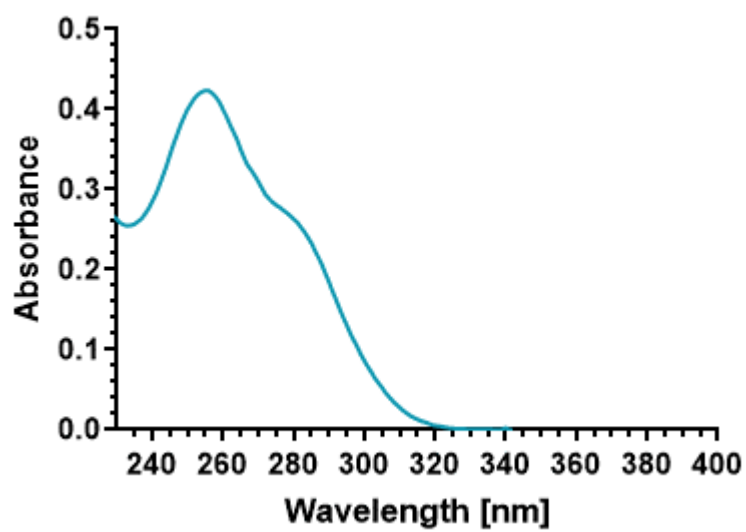
3,4-di-F-Bn ⁷ GpppG (1k)	
Structure	
Reaction RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-0913-24-103,4-2F-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-07-0409-04-583,4-2F-BN7GPPPG.D)</p>
HRMS ES(-)	 <p>171213_RW_003_#4-88 RT: 0.03-0.59 AV: 65 NL: 1.11E6 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>



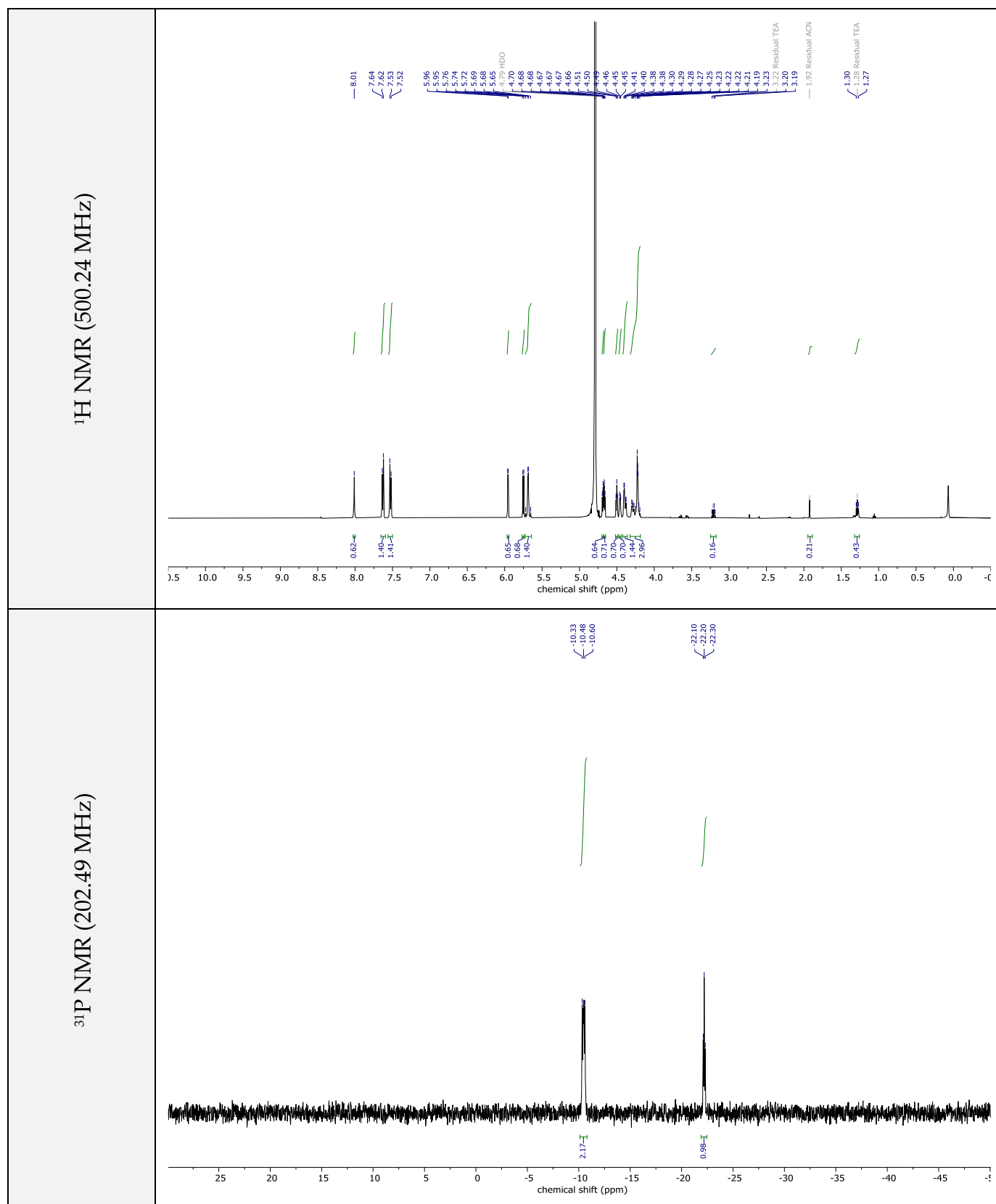
^{19}F NMR (470.65 MHz) ^{19}F { ^1H } NMR (470.65 MHz)

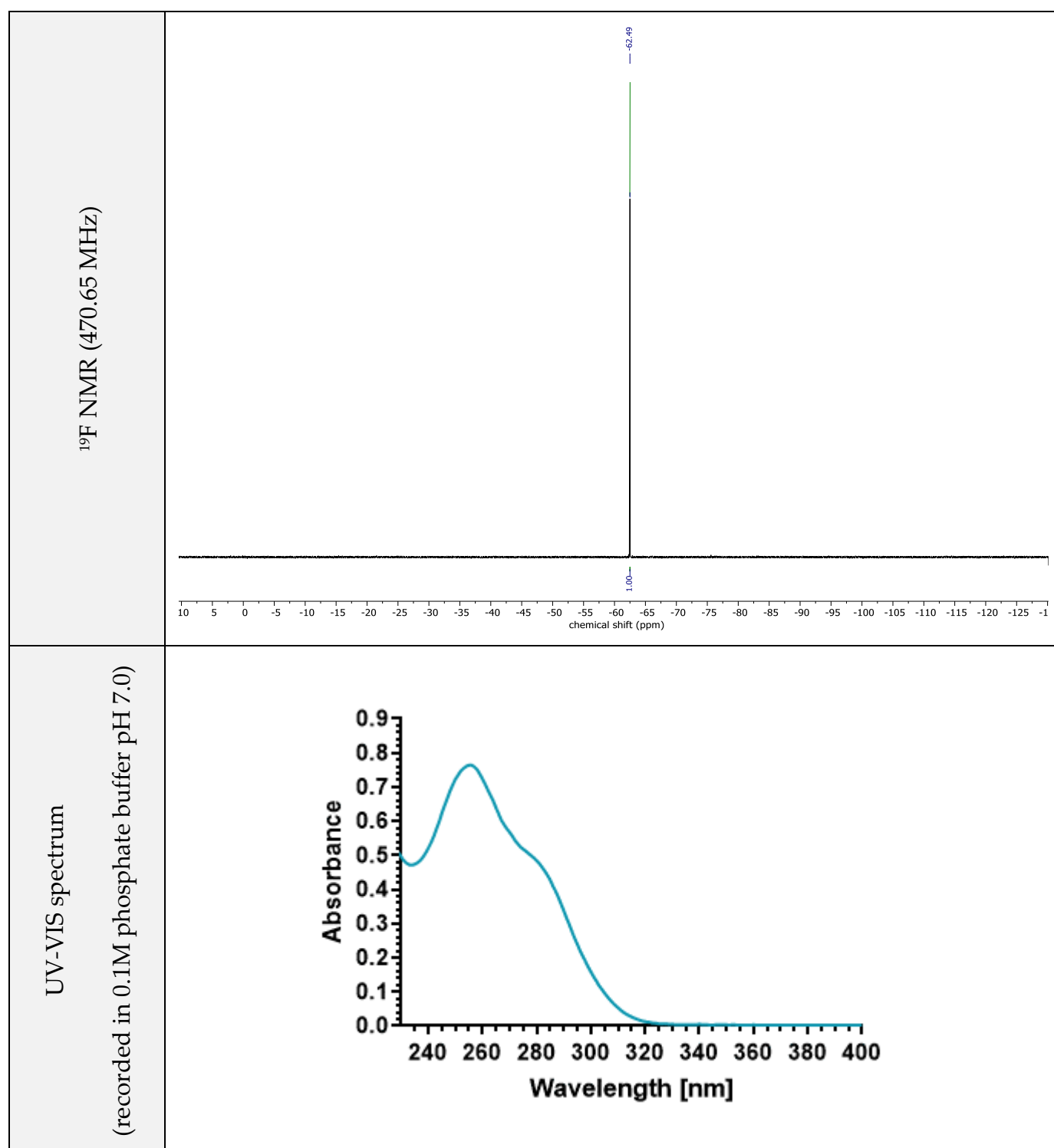
UV-VIS spectrum

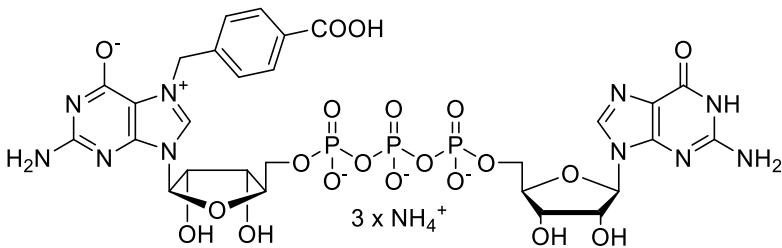
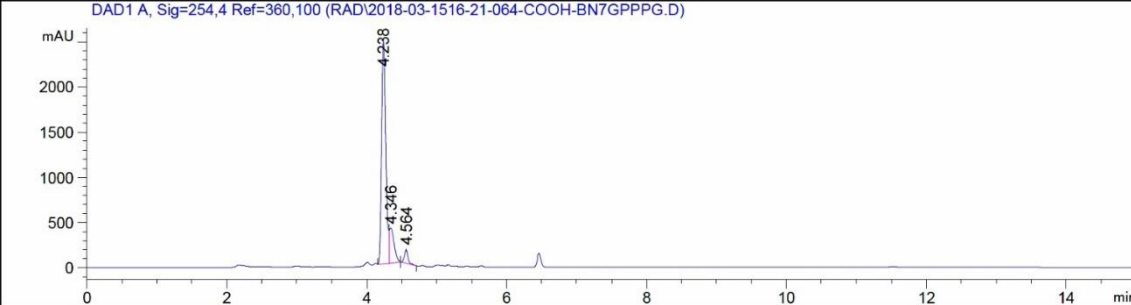
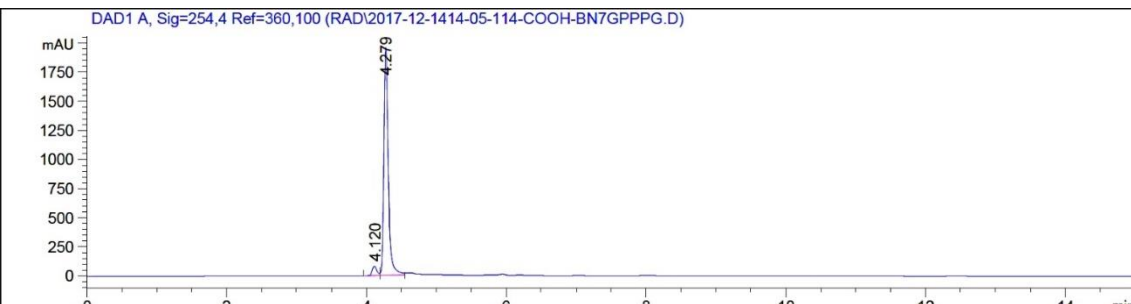
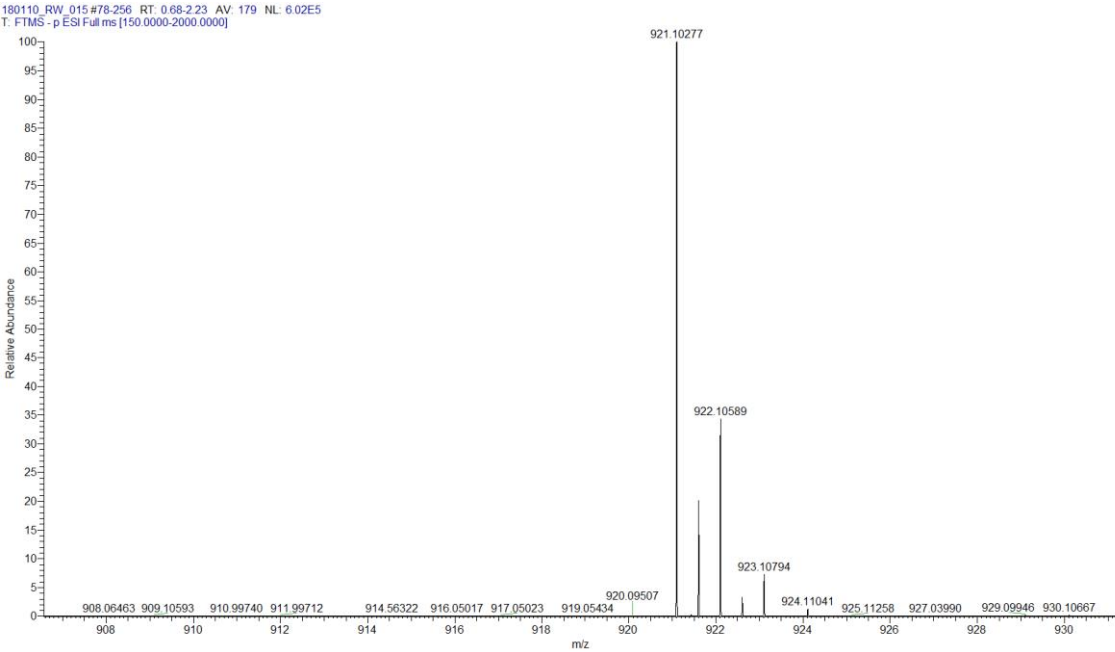
(recorded in 0.1M phosphate buffer pH 7.0)

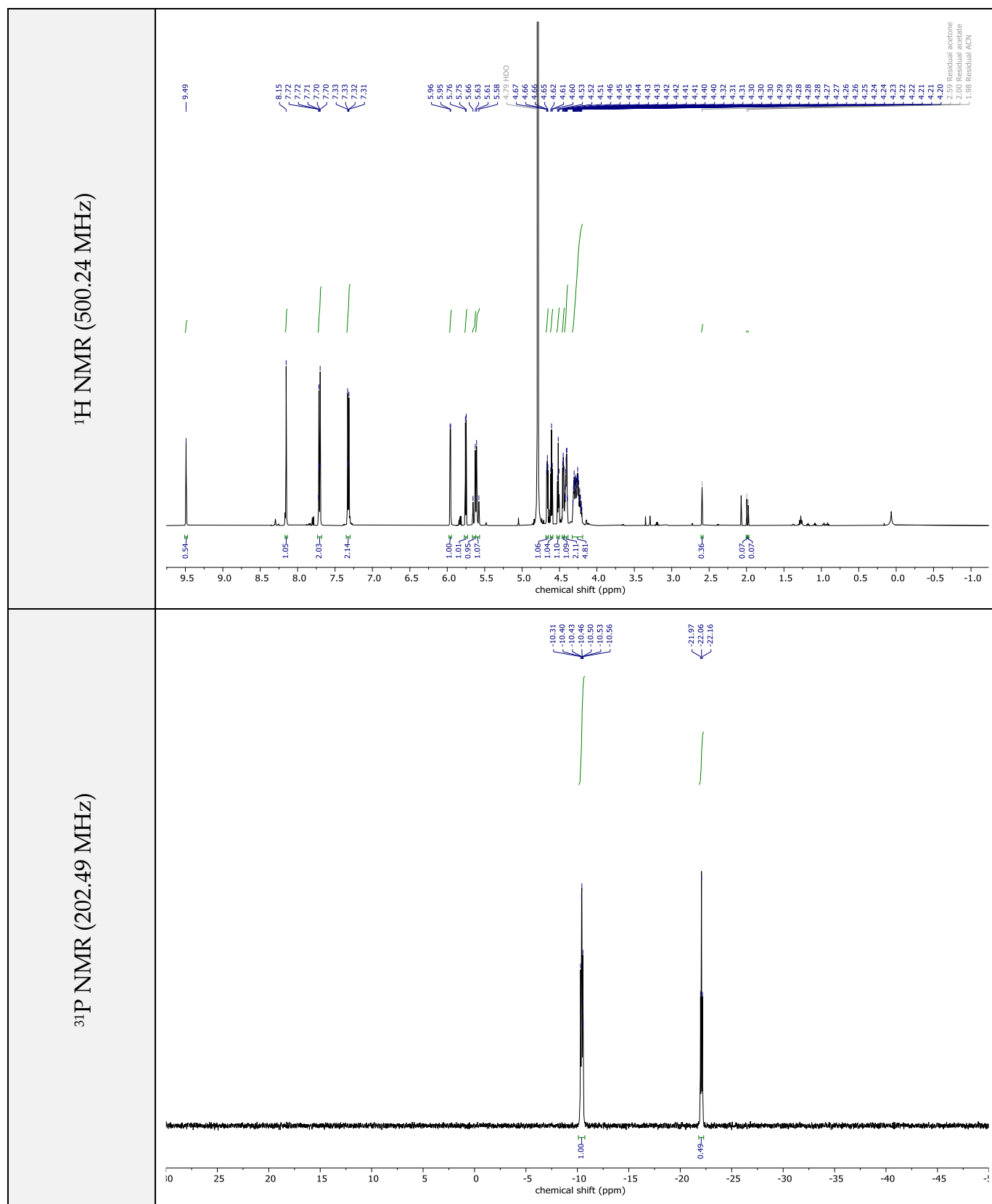


4-CF ₃ -Bn ⁷ GpppG (11)	
Structure	
Reaction RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (MAREKB4CF3BN7GPPPG 2016-11-30 13-26-41.D)</p>
Purified Product RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (MBI2019-12-1610-54-304CF3-BN-GPPPG.D)</p>
HRMS ES(-)	<p>191220_RW_1#194-250 RT: 1.84-2.38 AV: 57 NL: 1.06E7 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>



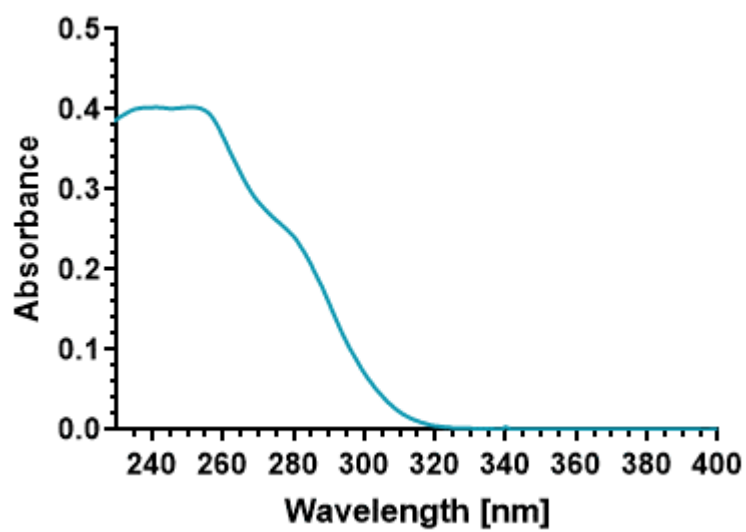


4-COOH-Bn ⁷ GpppG (1m)	
Structure	
Reaction RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-1516-21-064-COOH-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-12-1414-05-114-COOH-BN7GPPPG.D)</p>
HRMS ES(-)	 <p>180110_RW_015 #78-256 RT: 0.68-2.23 AV: 179 NL: 6.02E5 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>

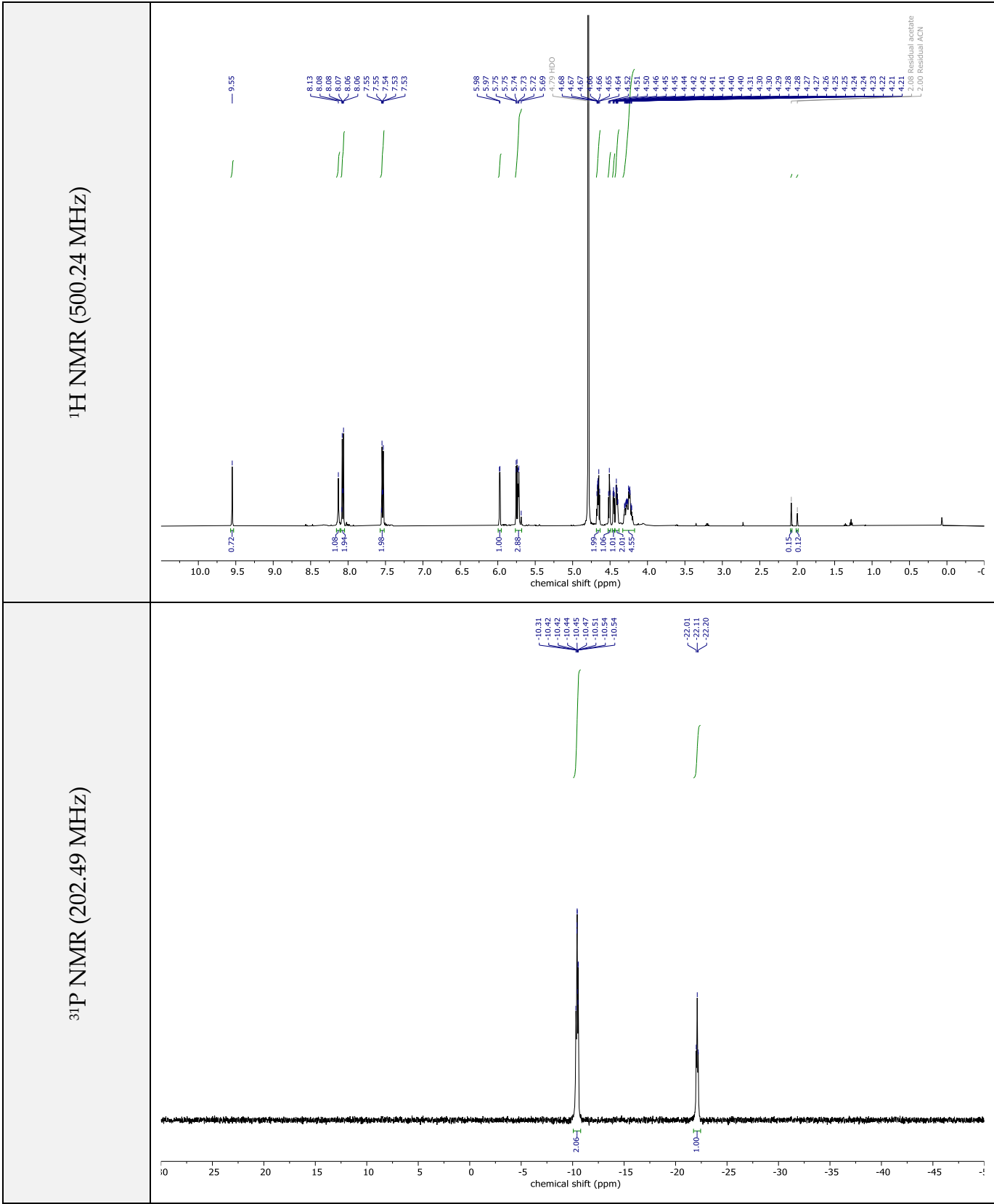


UV-VIS spectrum

(recorded in 0.1M phosphate buffer pH 7.0)

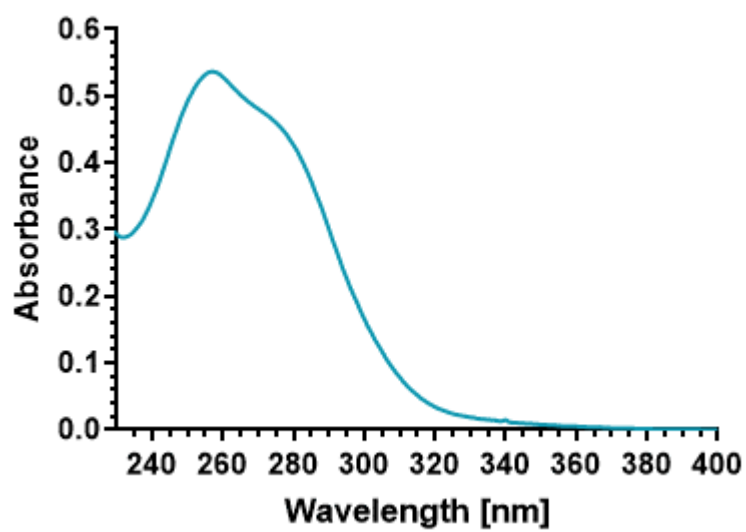


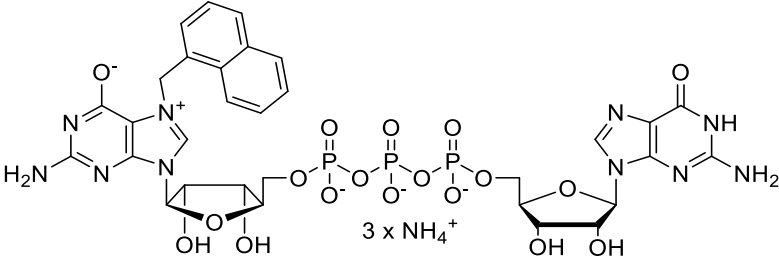
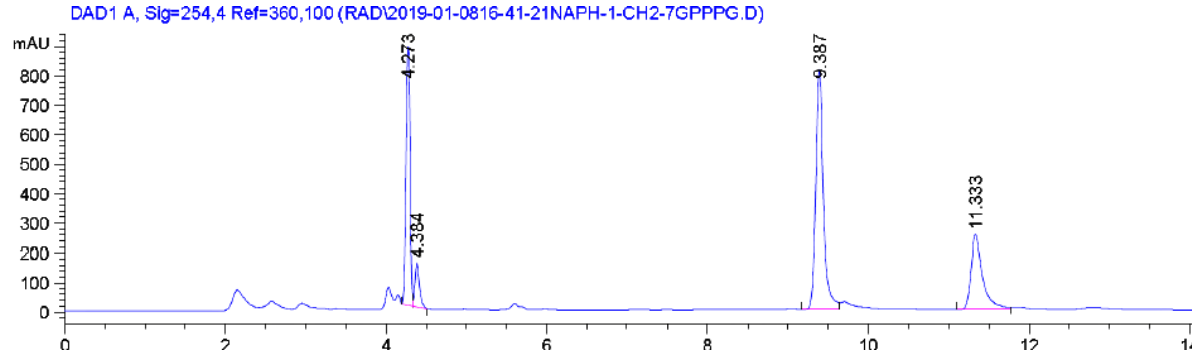
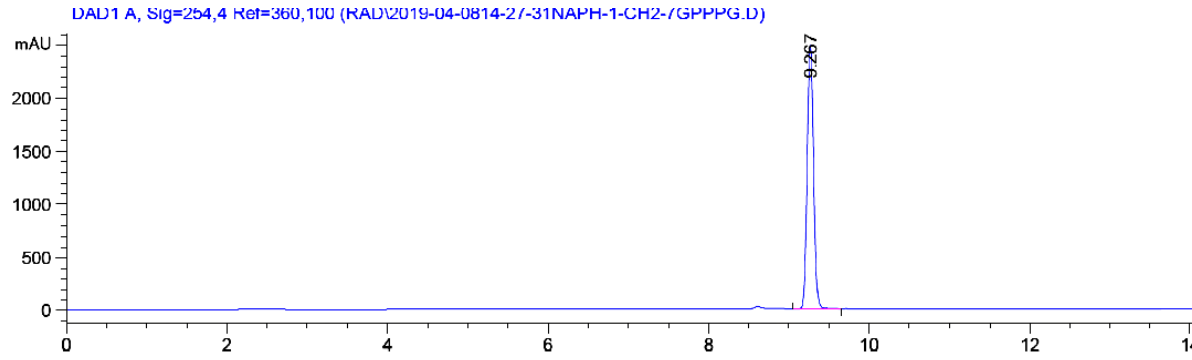
4-NO ₂ -Bn ⁷ GpppG (1n)	
Structure	
Reaction RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-0914-44-594-NO2-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-12-0810-22-324-NO2-BN7GPPPG.D)</p>
HRMS ES(-)	<p>180110_RW_012 #61-251 RT: 0.53-2.19 AV: 191 NL: 1.21E6 T: FTMS - p ESI Full ms [100.0000-1500.0000]</p>



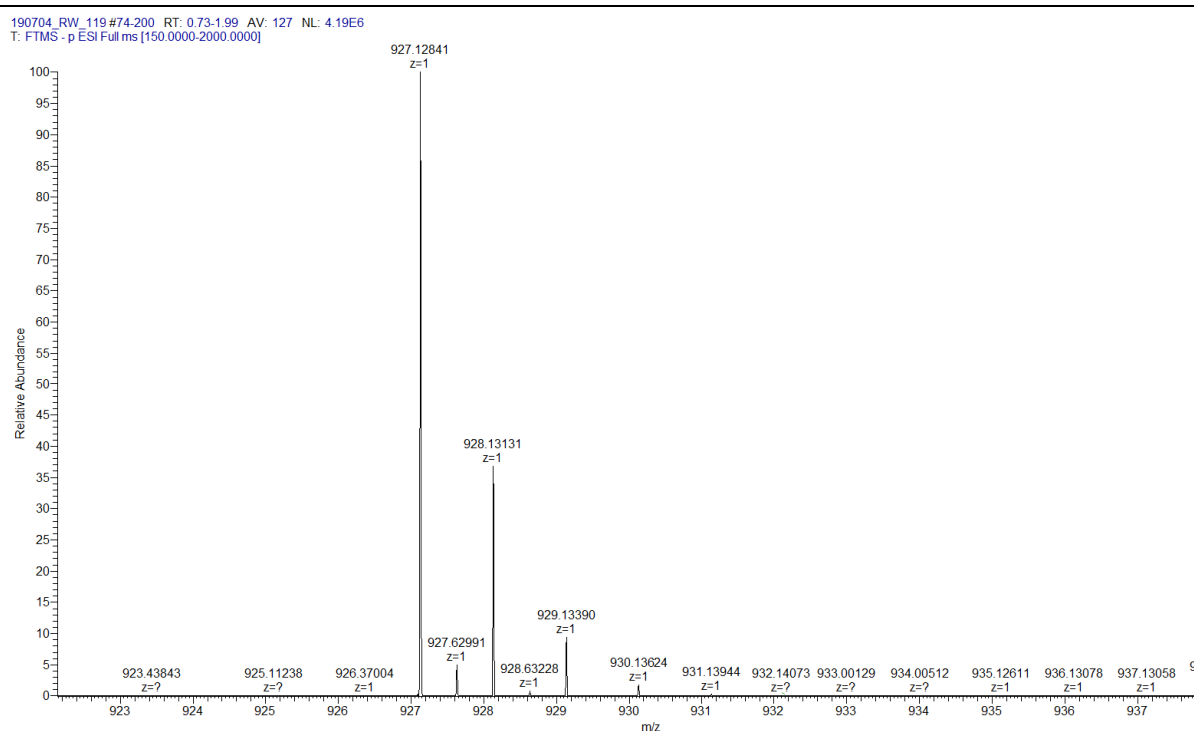
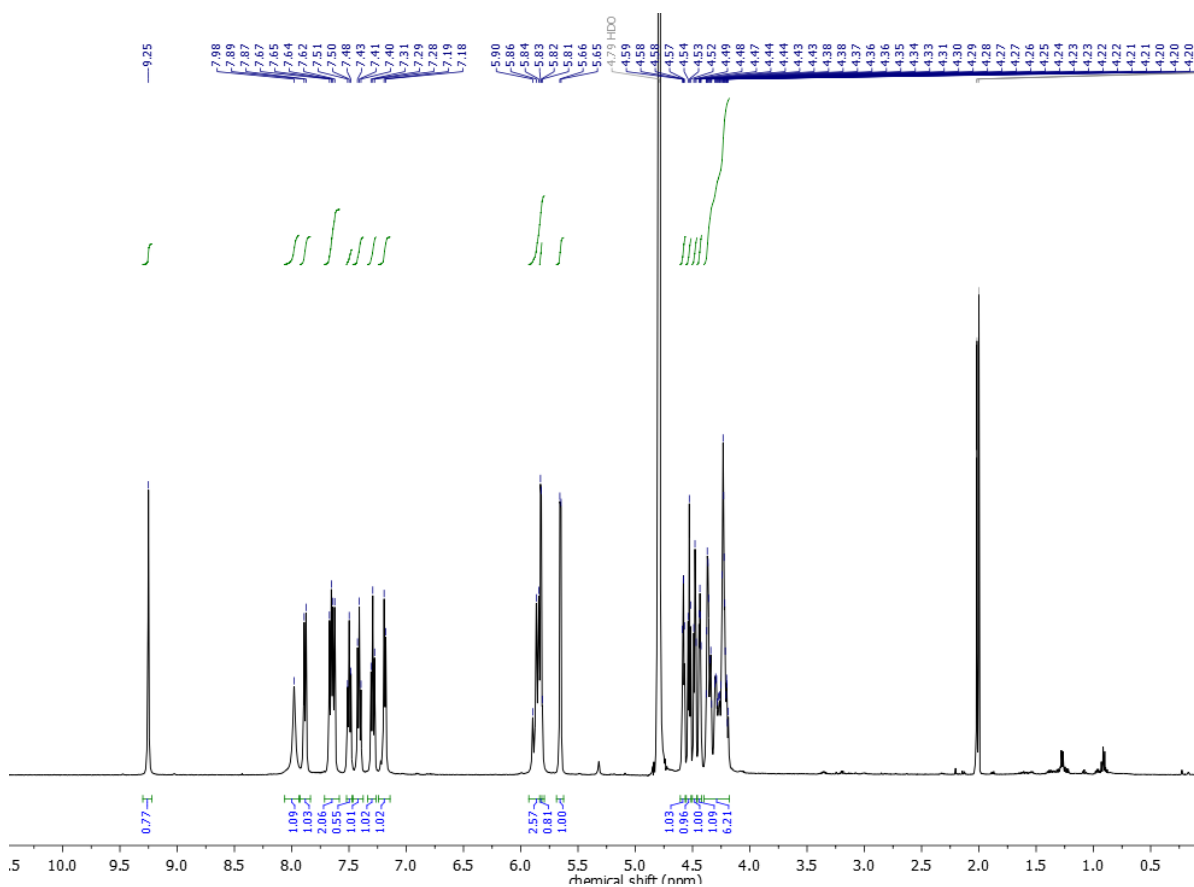
UV-VIS spectrum

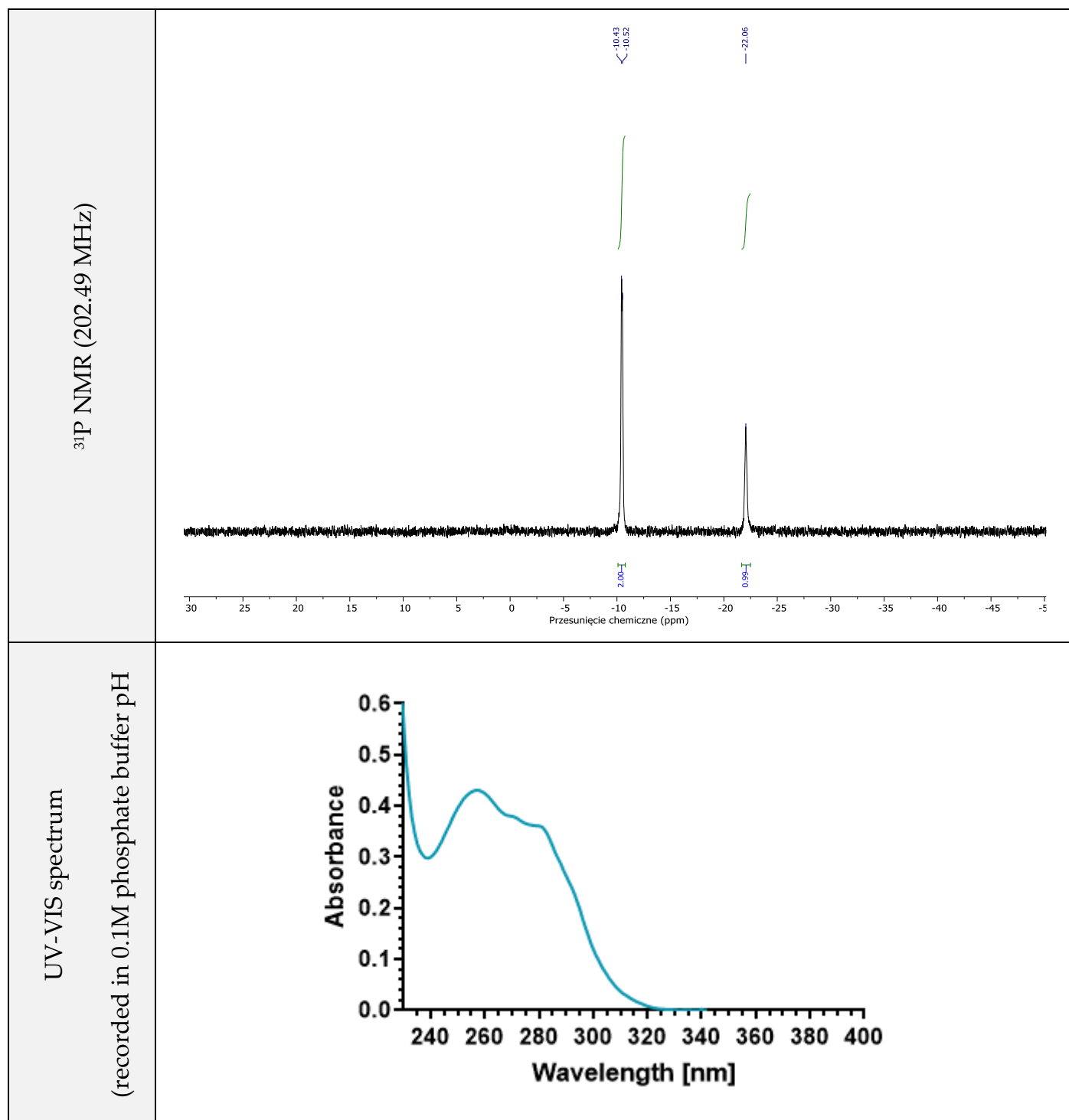
(recorded in 0.1M phosphate buffer pH 7.0)

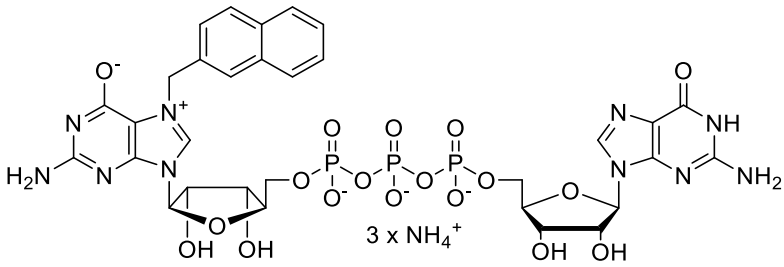
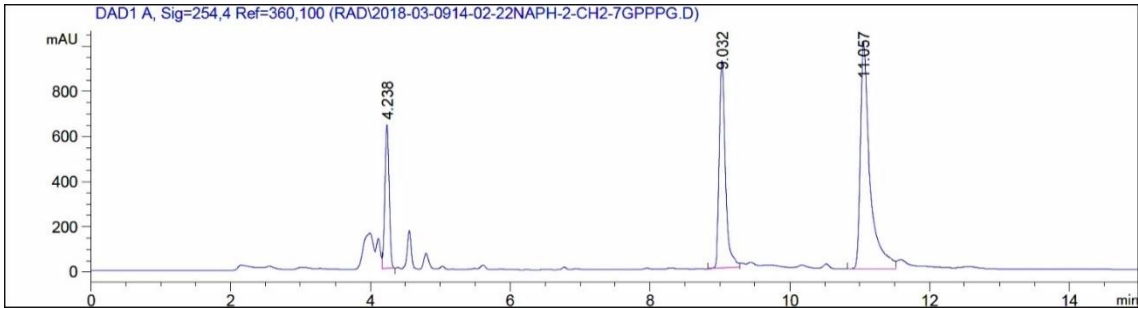
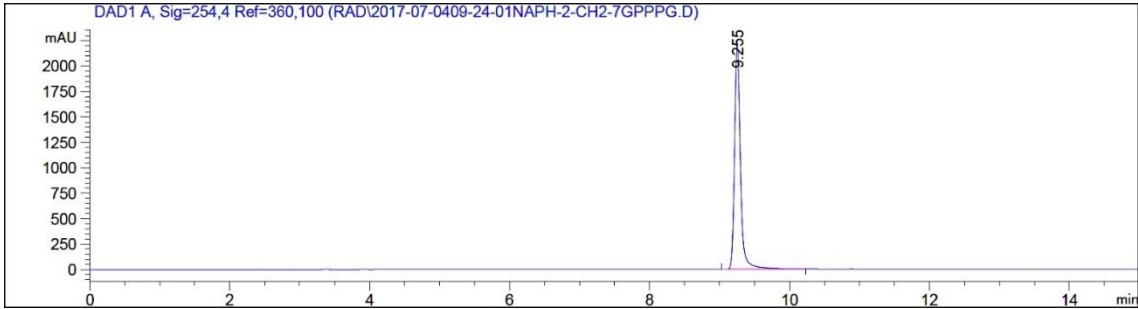
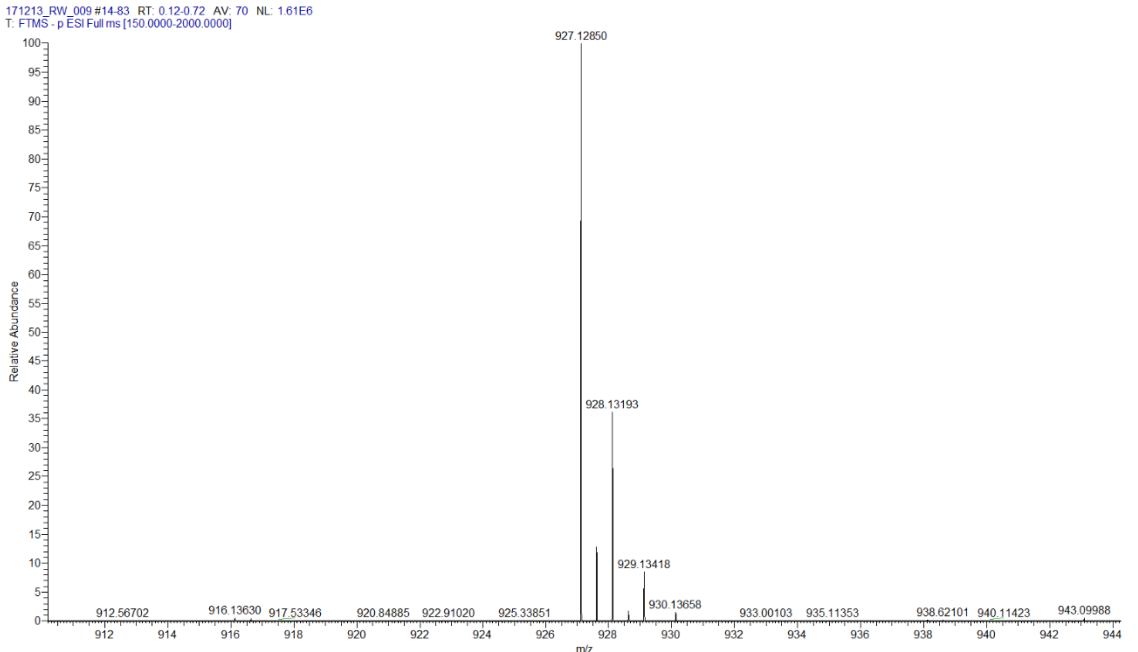


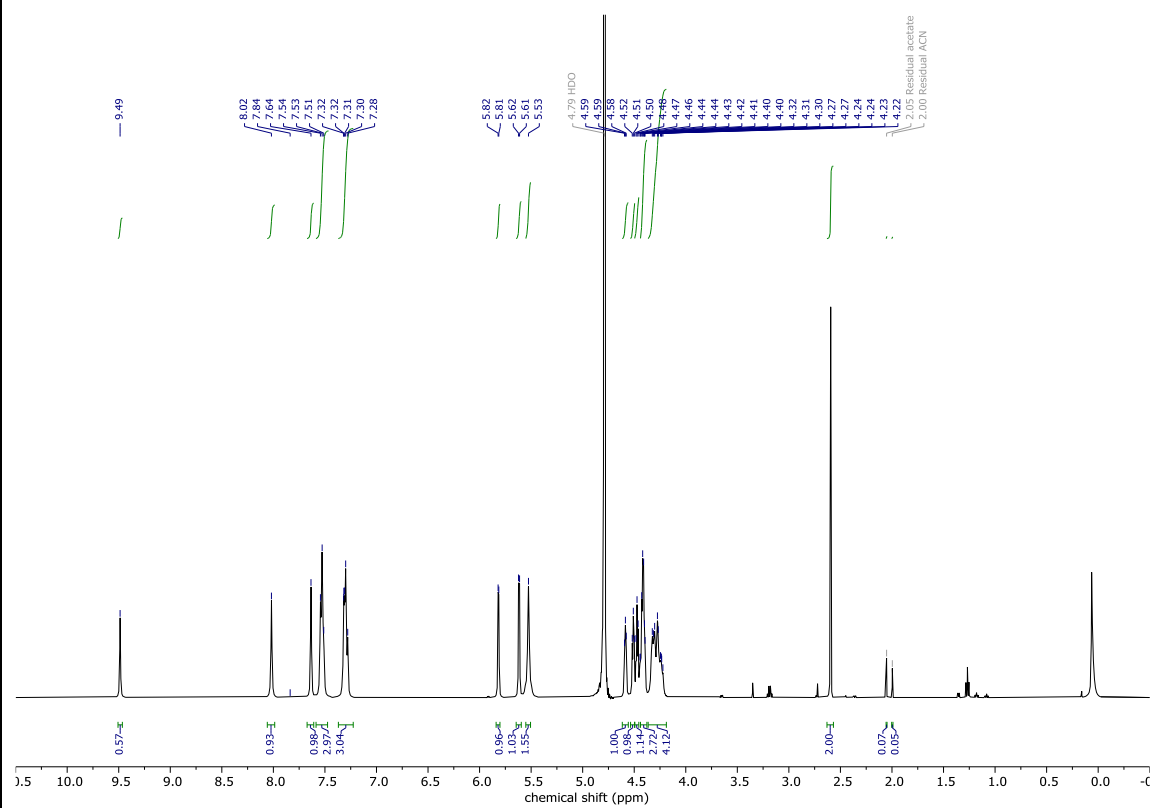
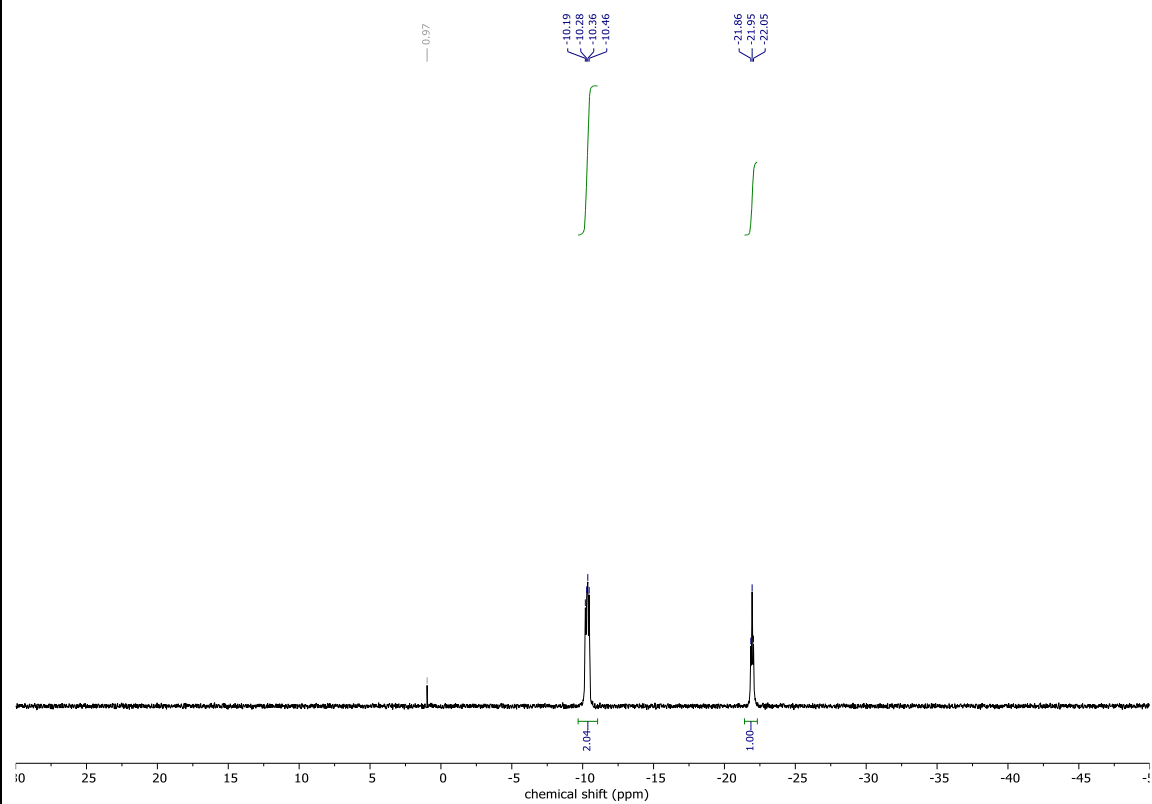
α -Naphm ⁷ GpppG (10)									
Structure									
Reaction RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2019-01-0816-41-21NAPH-1-CH2-7GPPPG.D)</p>  <table border="1"><thead><tr><th>Retention Time (min)</th><th>mAU</th></tr></thead><tbody><tr><td>4.273</td><td>~800</td></tr><tr><td>9.387</td><td>~800</td></tr><tr><td>11.333</td><td>~300</td></tr></tbody></table>	Retention Time (min)	mAU	4.273	~800	9.387	~800	11.333	~300
Retention Time (min)	mAU								
4.273	~800								
9.387	~800								
11.333	~300								
Purified Product RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2019-04-0814-2/-31NAPH-1-CH2-/GPPPG.D)</p>  <table border="1"><thead><tr><th>Retention Time (min)</th><th>mAU</th></tr></thead><tbody><tr><td>9.267</td><td>~2000</td></tr></tbody></table>	Retention Time (min)	mAU	9.267	~2000				
Retention Time (min)	mAU								
9.267	~2000								

HRMS ES(-)

 ^1H NMR (500.24 MHz)

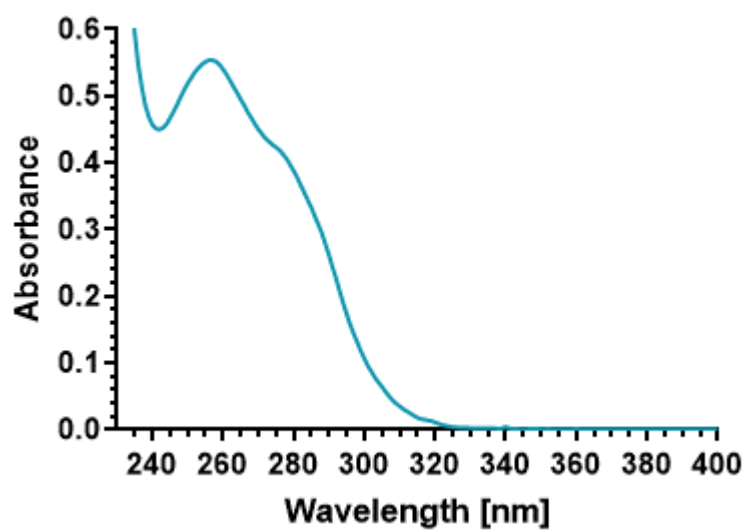


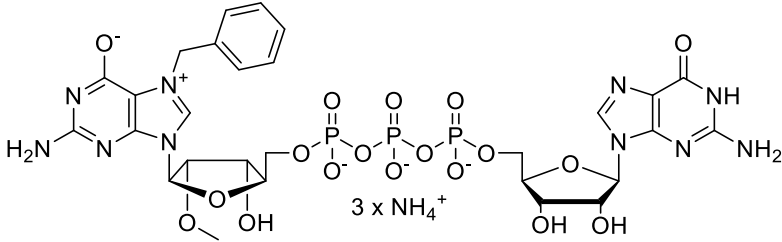
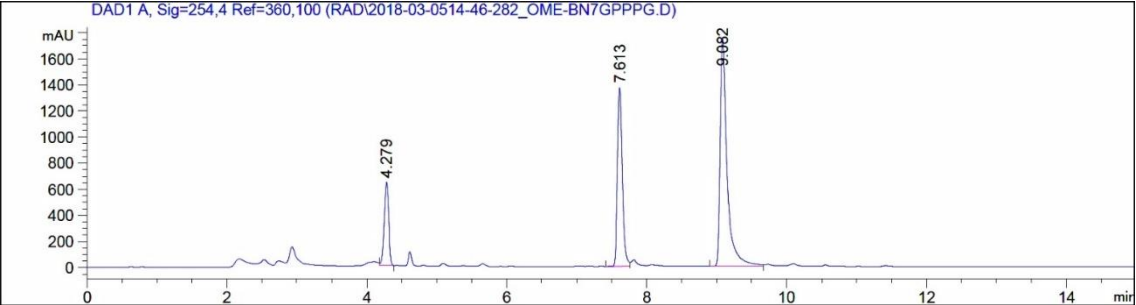
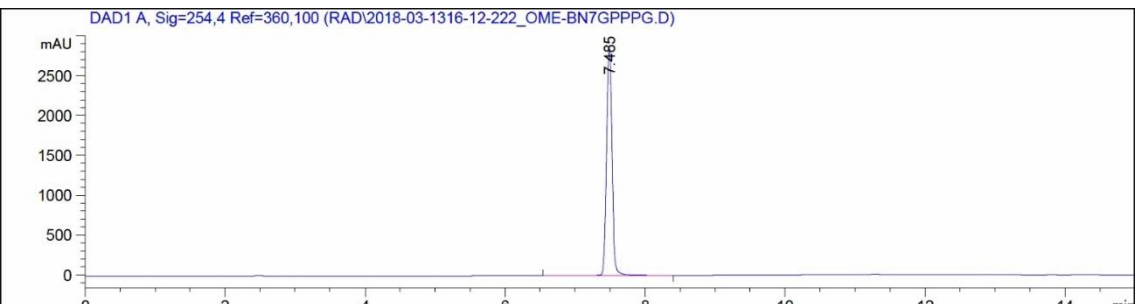
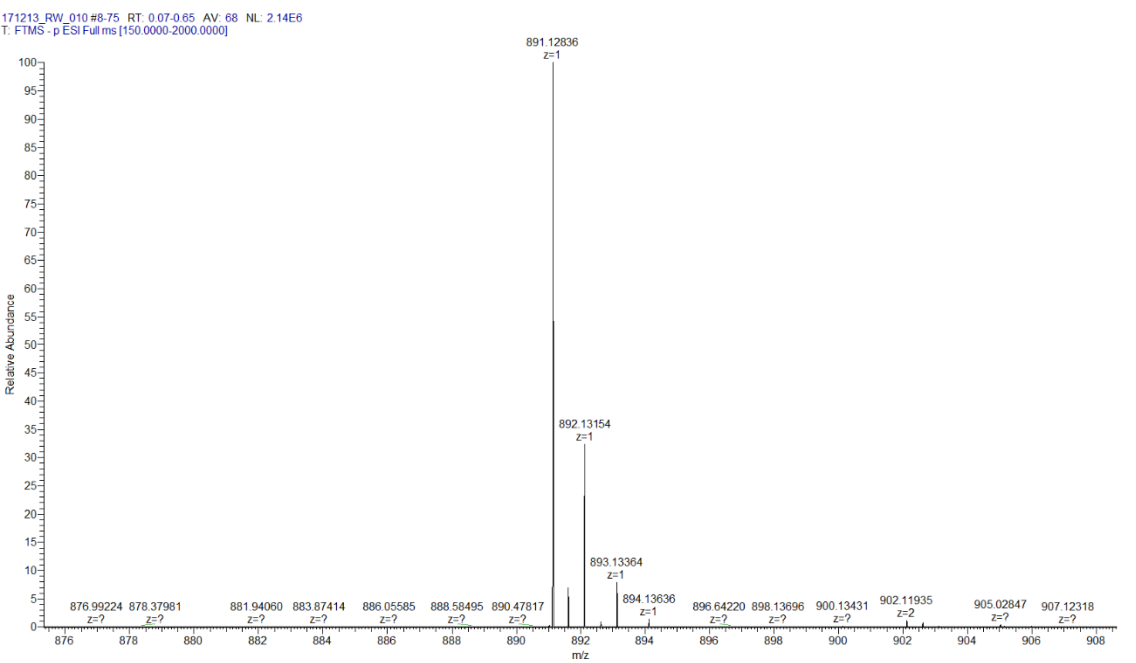
β -Naphm ⁷ GpppG (1p)	
Structure	
Reaction RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-0914-02-22NAPH-2-CH2-7GPPPG.D)</p>
Purified Product RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-07-0409-24-01NAPH-2-CH2-7GPPPG.D)</p>
HRMS ES(-)	 <p>171213_RW_009 #14.93 RT: 0.12-0.72 AV: 70 NL: 1.61E6 T: FTMS -p ESI Full.ms [150.0000-2000.0000]</p>

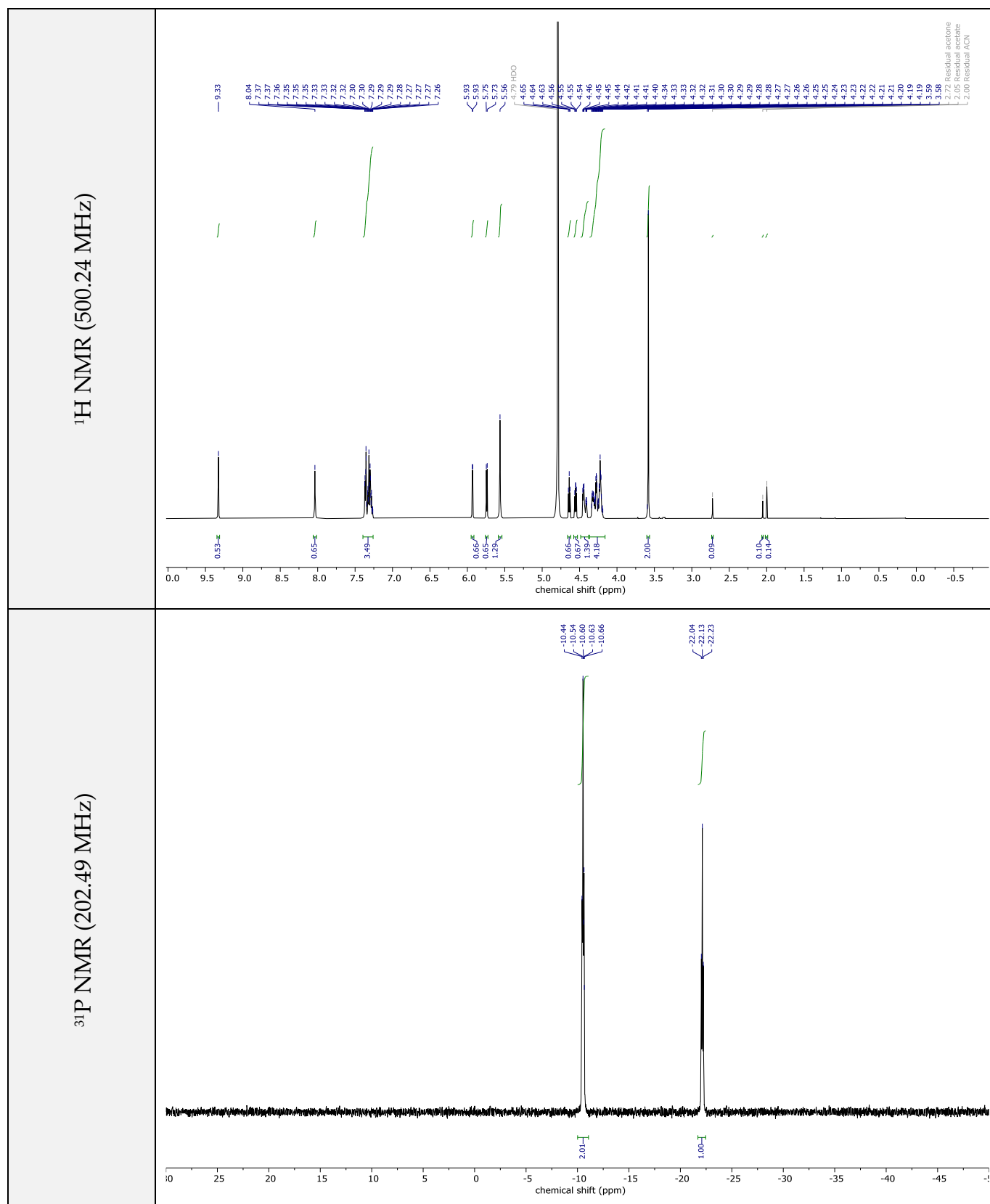
^1H NMR (500.24 MHz) ^{31}P NMR (202.49 MHz)

UV-VIS spectrum

(recorded in 0.1M phosphate buffer pH 7.0)

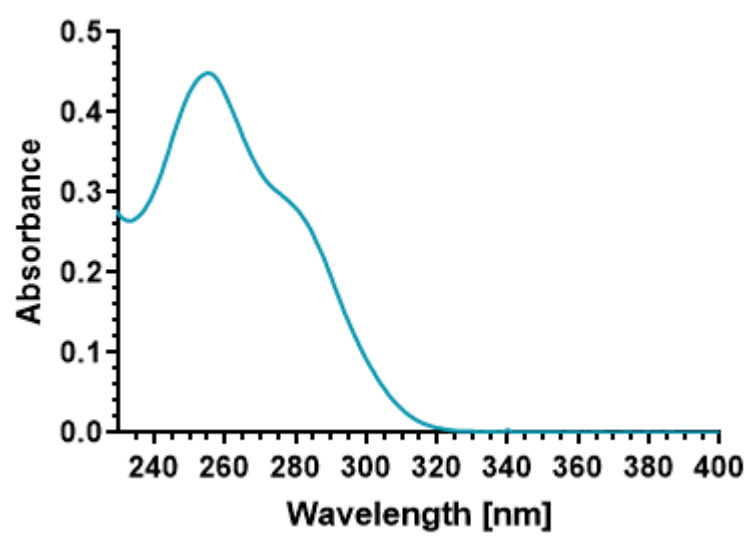


Bn ⁷ G _m pppG (1q)	
Structure	
Reaction RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-0514-46-282_OME-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	 <p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2018-03-1316-12-222_OME-BN7GPPPG.D)</p>
HRMS ES(-)	 <p>171213_RW_010 #8-75 RT: 0.07-0.85 AV: 68 NL: 2.14E6 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>

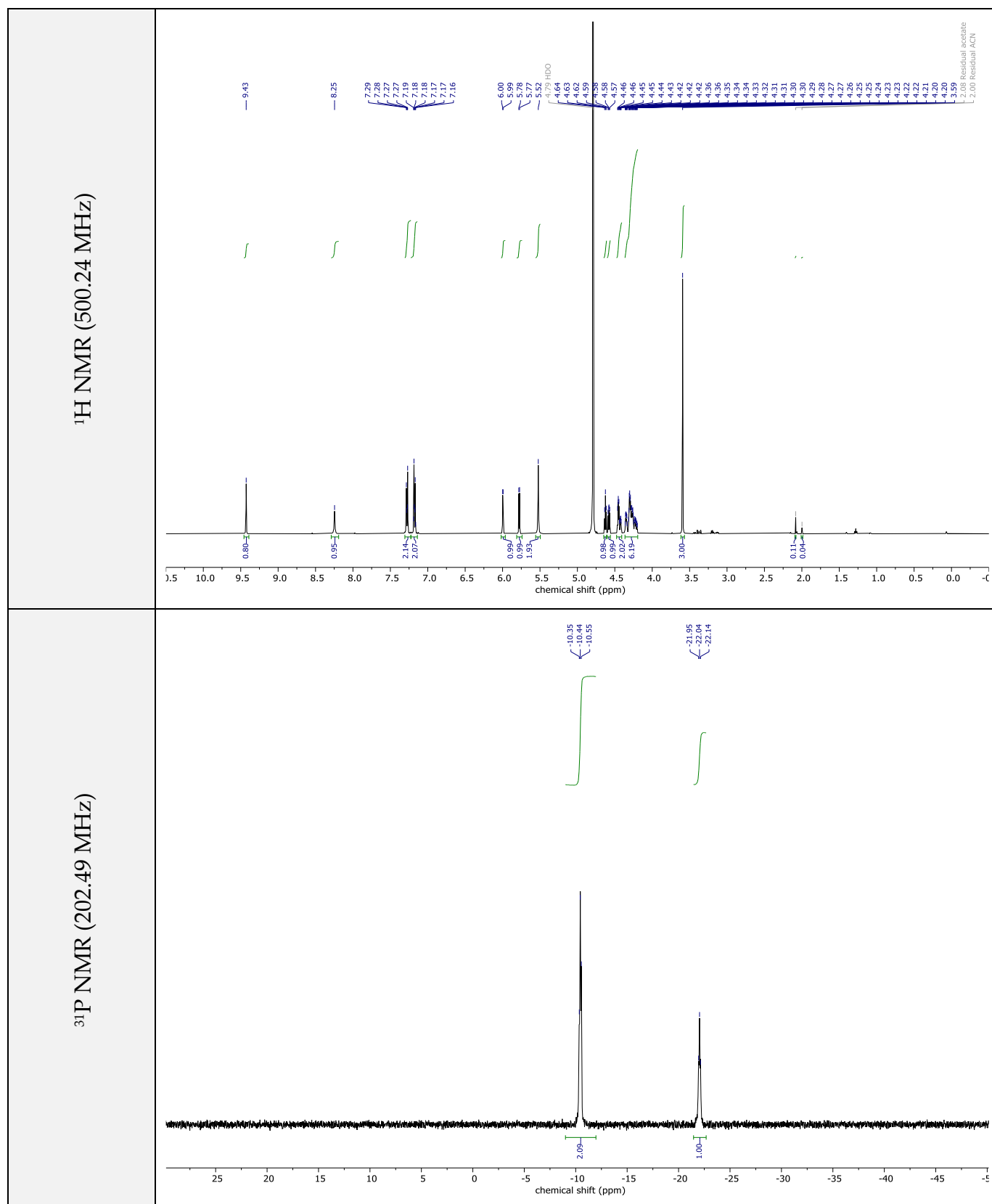


UV-VIS spectrum

(recorded in 0.1M phosphate buffer pH 7.0)



4-Cl-Bn ⁷ G _m pppG (1r)	
Structure	
Reaction RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-11-0213-28-532_OME-4-CL-BN7GPPPG.D)</p>
Purified Product RP HPLC Profile	<p>DAD1 A, Sig=254,4 Ref=360,100 (RAD\2017-11-1515-19-282_OME-4-CL-BN7GPPPG.D)</p>
HRMS ES(-)	<p>171213_RW_011 #3-105 RT: 0.03-0.92 AV: 103 NL: 3.61E6 T: FTMS - p ESI Full ms [150.0000-2000.0000]</p>



UV-VIS spectrum

(recorded in 0.1M phosphate buffer pH 7.0)

