Supplementary Information (SI) for RSC Advances.

Supporting Information

One-pot syntheses of fluorescent cap1 mRNAs via labeled trinucleotide m7G-Cap analogs for real-time *in vitro* and *in vivo* mRNA trackings

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Contents

S1. General Chemical syntheses information

All chemicals and solvents were of laboratory grade as obtained from commercial suppliers and were used without further purification. All the nucleotides were purified by ion-exchange chromatography on LX-650 resins from Sunresin New Materials Co.Ltd. NMR spectra were recorded with a Bruker Avance III spectrometer at 500.13 MHz (^{1}H NMR) and 202.45 MHz (^{31}P NMR) or a Bruker Avance IV spectrometer at 400.18 MHz (^{1}H NMR) and 162.00 MHz (^{31}P NMR). The ^{1}H NMR chemical shifts were calibrated to D_2O (4.79 ppm). The ^{31}P NMR chemical shifts were reported directly without calibration. Mass spectra (MS) were recorded with an LTQ XL (Thermo Fisher Scientific) spectrometer.

The HPLC system consisted of an Agilent 1260 Infinity Ⅱ high performance liquid chromatography, a VWD detector (DEACX34582) and a DNAPac PA200 column (8 μm, 4mm * 250 mm).

S2. General procedure for aminomethyl Cap analog 2

The starting material N3 cap analog 1 was synthesized as described in US Pat., 12060385, 2024.

MS : m/z calcd for $C_{33}H_{43}N_{18}O_{30}P_4^-$ [M-H]$^-$: 1183.17, found ：1183.02.

^{1}H NMR (500 MHz, D_2O) δ 9.09 (s, 1H), 8.50 (s, 1H), 8.23 (s, 1H), 7.97 (s, 1H), 6.08 (d, J = 5.0 Hz, 1H), 5.80 - 5.79 (m, 2H), 4.95 - 4.92 (m, 1H), 4.67 (d, J = 4.8 Hz, 1H), 4.52 (s, 1H), 4.49 - 4.46 (m, 2H), 4.43 (t, J = 4.7 Hz, 1H), 4.34 - 4.31 (m, 3H), 4.26 - 4.23 (m, 1H), 4.21 - 4.16 (m, 2H), 4.15 - 4.13 (m, 1H), 4.00 (s, 3H), 3.64 - 3.60 (m, 1H), 3.55 - 3.52 (m, 1H), 3.47 (s, 3H), 2.64 - 2.58 (m, 1H); ^{31}P NMR (202 MHz, D_2O) δ -0.93 (s, 1H), -11.62 (m, 2P), -22.81 (t, J = 18.0 Hz, 1P).

To a solution of cap analog **1** (175 mg, 0.148 mmol) in water (4 mL) and EtOH (1 mL) was added 10% Pd/C (20 mg). The reaction was stirred at room temperature for 6h under hydrogen atmosphere. The Pd/C was filtered off, and the filtrate was diluted with water and purified by ion-exchange chromatography on LX-650 resins (gradient elution from 0 – 1M TEAB) to give the TEA^+ salt form of compound 2 (160 mg, 0.138 mmol, 93%) as white solid after freeze-drying.

MS : m/z calcd for $C_{33}H_{45}N_{16}O_{30}P_4^-$ $[M-H]^-$: 1157.18, found : 1157.09. ^{1}H NMR (500 MHz, D_2O) δ 8.38 (s, 1H), 8.05 (s, 1H), 7.87 (s, 1H), 6.03 (d, J = 5.9 Hz, 1H), 5.87 (s, 1H), 5.77 (d, J = 5.9 Hz, 1H), 4.94 (t, J = 5.6 Hz, 1H), 4.87 - 4.84 (m, 1H), 4.50 – 4.45 (m, 3H), 4.40 - 4.35 (m, 2H), 4.31 - 4.26 (m, 3H), 4.22 - 4.15 (m, 3H), 4.01 (s, 3H), 3.39 (dd, J = 9.2, 13.5 Hz, 1H), 3.31 (s, 3H), 3.23 (dd, J = 3.6, 13.2 Hz,1H), 3.18 (q, J = 7.4 Hz, 14.3H), 2.97 - 2.92 (m, 1H), 1.26 (t, J =

7.4 Hz, 22.1H); ^{31}P NMR (202 MHz, D_2O) δ -0.78 (s, 1H), -11.62 (d, *J* = 17.8 Hz, 2P), -22.85 (t, *J* = 18.5 Hz, 1P).

S3. General procedure for Cy3/Cy5/Cy7 cap analogs

To a solution of Cy3/Cy5/Cy7 (1.0 eq) in DMF was added TEA (5 eq) and TSTU (1.5 eq). After stirring at room temperature for 15min to 2h, a solution of the aminomethyl Cap analog **2** (1.0 eq) and TEA (5 eq) in water was added. The reaction was stirred for 0.5h to 3.5h, diluted with water and purified by ion-exchange chromatography on LX-650 resins (gradient elution from 0 – 1M NH_4HCO_3) to give the $NH4^+$ salt form of Cy3/Cy5/Cy7 cap analogs after freeze-drying.

S4. Characterization data for Cy3 cap analog 8

Cy3 cap analog was prepared starting from the aminomethyl Cap analog **2** (50 mg) yielding 23 mg (30%) of the $NH4^+$ salt form as red solid after freeze-drying with HPLC purity 98%.

MS: m/z calcd for $C_{64}H_{82}N_{18}O_{30}P_4S_2^-$ [M-H]$^-$: 1769.38, found: 1769.45. ^{1}H-NMR (500 MHz, D_2O) δ: 8.37 (s, 1H), 8.32 (t, *J* = 13.3 Hz, 1H), 7.94 (m, 2H), 7.85 – 7.82 (m, 3H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.28 (d, *J* = 8.3 Hz, 1H), 7.16 (d, *J* = 8.4 Hz, 1H), 6.17 (d, *J* = 13.3 Hz, 1H), 6.08 (d, *J* = 13.3 Hz, 1H), 5.91 (d, *J* = 6.0 Hz, 1H), 5.87 (s, 1H), 5.77 (d, *J* = 5.0 Hz, 1H), 4.93 (m, 1H), 4.65 (m, 1H), 4.57 (d, *J*

= 4.2 Hz, 1H), 4.48 – 4.45 (m, 3H), 4.38 (s, 1H), 4.29 – 4.25 (m, 4H), 4.15 (m, 3H), 3.98 (m, 2H), 3.93 (m, 3H), 3.82 – 3.80 (m, 2H), 3.49 – 3.44 (m, 1H), 3.31 (s, 3H), 3.27 – 3.25 (m, 1H), 2.56 (m, 1H), 2.27 (m, 2H), 1.63 – 1.54 (m, 15H), 1.32 – 1.23 (m, 6H). ^{31}P-NMR (202 MHz, D_2O) *δ*: -1.22 (s, 1P), -10.69 (m, 1P), -11.46 (m, 1P), -21.89(m, 1P).

S5. Characterization data for Cy5 cap analog 9

Cy5 cap analog was prepared starting from the aminomethyl Cap analog **2** (100 mg) yielding 33 mg (21%) of the $NH4^+$ salt form as blue solid after freeze-drying with HPLC purity 98%.

MS: m/z calcd for $C_{66}H_{84}N_{18}O_{30}P_4S_2^-$ [M-H]$^-$: 1795.39, found: 1795.12. ^{1}H-NMR (500 MHz, D_2O) *δ*: 9.18 (s, 1H), 8.63 (s, 1H), 8.24 (s, 1H), 8.03 (s, 1H), 7.97 – 7.89 (m, 2H), 7.83 - 7.76 (m, 4H), 7.28 (d, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 6.40 (t, *J* = 12.0 Hz, 1H), 6.21 (d, *J* = 13.5 Hz, 1H), 6.08 (s, 2H), 5.89 (s, 1H), 5.77 (s, 1H), 4.97 (s, 1H), 4.76 – 4.75 (m, 1H), 4.58 (s, 1H), 4.52 - 4.79 (m , 4H), 4.34 - 4.30 (m , 4H), 4.19 (s, 3H), 4.02 (m, 5H), 3.91 (s, 2H), 3.52 – 3.50 (m, 1H), 3.43 (s, 3H), 3.37 - 3.35 (m , 1H), 2.72 (m, 1H), 2.30 (s, 2H), 1.68 – 1.58 (m, 15H), 1.33 – 1.29 (m, 6H). ^{31}P-NMR (202 MHz, D_2O) *δ*: -0.93 (s,1P), -11.46 (d, J = 15.9 Hz, 1P), -11.64 (d, J = 18.3 Hz, 1P), -22.71 (t ,1P).

S6. Characterization data for Cy7 cap analog 10

Cy7 cap analog was prepared starting from the aminomethyl Cap analog **2** (135 mg) yielding 50 mg (23%) of the $NH4^+$ salt form as blue-green solid after freeze-drying with HPLC purity 95%.

MS: m/z calcd for $C_{68}H_{86}N_{18}O_{30}P_4S_2^-$ $[M-H]^-$: 1821.41, found: 1822.20. ^{1}H-NMR (400 MHz, D_2O) δ: 9.05 (s, 1H), 8.42 (s, 1H), 8.00 (s, 1H), 7.86 (s, 1H), 7.74 – 7.44 (m, 6H), 7.18 – 7.13 (m, 2H), 6.99 (d, *J* = 8.5 Hz, 1H), 6.13 (t, *J* = 12.6 Hz, 1H), 6.06 – 6.00 (m, 2H), 5.92 (d, *J* = 5.9 Hz, 1H), 5.82 (s, 1H), 5.77 (d, *J* = 13.8 Hz, 1H), 5.68 (d, *J* = 5.8 Hz, 1H), 4.88 (s, 1H), 4.68 – 4.63 (m, 1H), 4.54 (d, *J* = 3.7 Hz, 1H), 4.41 - 4.37 (m, 4H), 4.31 – 4.04 (m, 7H), 3.95 – 3.93 (m, 2H), 3.88 (s, 3H), 3.71 (s, 2H), 3.49 (dd, *J* = 13.4, 9.9 Hz, 1H), 3.29 (s, 3H), 3.23 (d, *J* = 9.1 Hz, 1H), 2.63 (s, 1H), 2.21 (s, 2H), 1.61 – 1.35 (m, 15H), 1.29 – 1.17 (m, 6H). ^{31}P NMR (162 MHz, D_2O) δ -0.91 (s,1P), -11.32 - -11.60 (m, 2P), -22.76 (t, 1P).

S7. Characterization data for PEG5-FAM cap analog 11

PEG5-FAM was synthesis by condensation of FAM and amino-PEG4-acetic acid. The PEG5-FAM cap analog was prepared starting from the aminomethyl Cap analog **2** (70 mg) according to the procedure as Cy cap analogs using HATU instead of TSTU, yielding 10 mg (9%) of the $NH4^+$ salt form as yellow solid after freeze-drying with HPLC purity 94%.

MS: m/z calcd for $C_{64}H_{75}N_{17}O_{34}P_4^-$ [M-H]$^-$: 1748.35, found: 1748.96. ^{1}H-NMR (500 MHz, D_2O) δ: 9.02 (s, 1H), 8.33 (s, 1H), 8.29 (s, 1H), 8.02 (d, J = 7.2 Hz, 1H), 7.98 (s, 1H), 7.87 (s, 1H), 7.41 (d, J = 7.0 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 6.62 (d, J = 8.6 Hz, 1H), 6.57 – 6.55 (m, 3H), 5.84 (s, 1H), 5.75 (d, J = 4.6 Hz, 1H), 5.72 (s, 1H), 4.89 – 4.82 (m, 2H), 4.68 (s, 1H), 4.52 (s, 1H), 4.46 (m, 3H), 4.36 (s, 1H), 4.31 - 4.24 (m, 4H), 4.17 (m, 3H), 4.01 (s, 3H), 3.97 (d, J = 15.5 Hz, 1H), 3.87 (d, J = 16.3 Hz, 1H), 3.79 (m, 2H), 3.72 (m, 2H), 3.68 (m, 4H), 3.60 – 3.51 (m, 8H), 3.43 (s, 3H), 3.23 (d, J = 12.3 Hz, 1H), 2.58 (m, 1H). ^{31}P-NMR (202 MHz, D_2O) δ: -0.99 (s,1P), -11.27 – -11.94 (m,2P), -22.58 – -23.22 (m,1P).

S8. Characterization data for C6-MANT cap analog 12

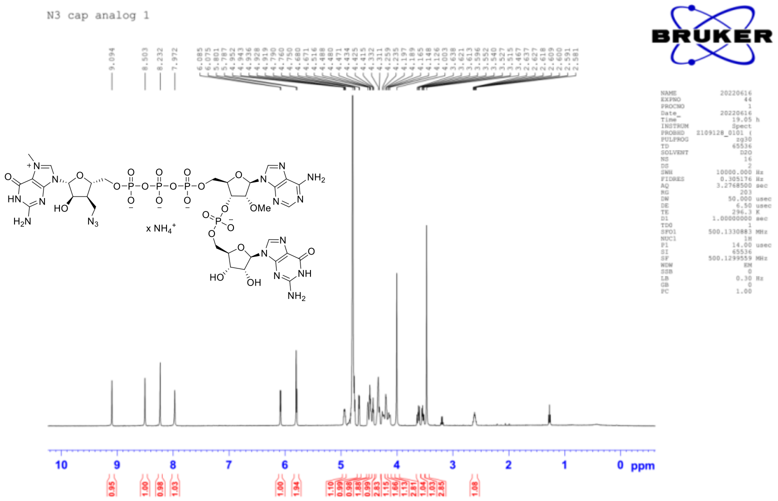
C6-MANT was synthesis by condensation of MANT and 6-aminocaproic acid. To a solution of C6-MANT (36 mg, 0.14mmol) in DMF (1 mL) was added NHS (18 mg, 0.15 mmol) and EDCI (39 mg, 0.2 mmol). After stirring at room temperature for 1h, a solution of the aminomethyl Cap analog **2** (50 mg, 0.034 mmol) in water (0.8 mL) was added. The reaction was stirred for 0.5h, diluted with water and purified by ion-exchange chromatography on LX-650 resins (gradient elution from 0 – 1M NH_4HCO_3) to give the $NH4^+$ salt form of C6-MANT cap analog (15 mg, 0.011 mmol, 31%) as white solid after freeze-drying with HPLC purity 99%.

MS: m/z calcd for $C_{47}H_{64}N_{18}O_{25}P_4^-$ $[M\text{-}H]^-$: 1403.32, found: 1403.18. ^{1}H-NMR (500 MHz, D2O) *δ*: 9.06 (s, 1H), 8.44 (s, 1H), 8.14 (s, 1H), 7.92 (s, 1H), 7.32 (t, *J* = 7.5 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.75 (t, *J* = 7.0 Hz, 1H), 6.02 (d, *J* = 5.5 Hz, 1H), 5.81 (s, 1H), 5.76 (d, J = 6.0 Hz, 1H), 4.91 (s, 1H), 4.58 (d, *J* = 3.5 Hz, 1H), 4.49 – 4.41 (m, 4H), 4.34 (s, 1H), 4.29 – 4.21 (m, 3H), 4.18 (m, 2H), 4.14 – 4.12 (m, 1H), 3.93 (s, 3H), 3.52 (dd, *J* = 13.5, 9.5 Hz, 1H), 3.41 (s, 3H), 3.22 -3.16 (m, 3H), 2.77 (s, 3H), 2.64 (m, 1H), 2.24 (t, *J* = 7.0 Hz, 2H), 1.59 – 1.53 (m, 2H), 1.50 -1.45 (m, 2H), 1.28 -1.22 (m, 2H). ^{31}P-NMR (202 MHz, D_2O) *δ*: -0.98 (s, 1P), -11.18 – -11.84 (m,1P), -22.43 – -23.16 (m,1P).

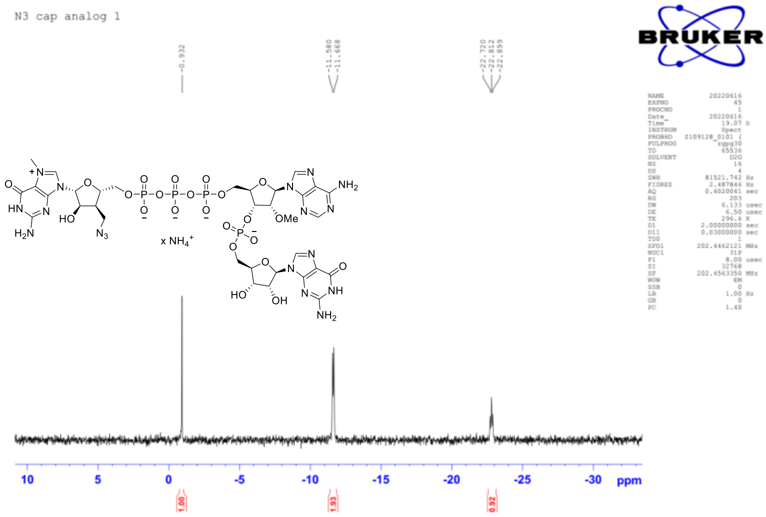
S9. The ^1H NMR and ^31P NMR spectra of cap analog 1, 8, 9, 10, 11 and 12

1. cap analog 1

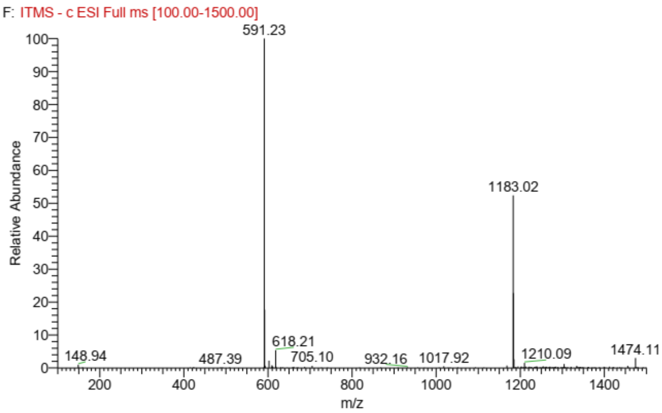
^{1}H NMR (500 MHz, D_2O) of N3 cap analog 1



^{31}P NMR (202 MHz, D_2O) of N3 cap analog 1

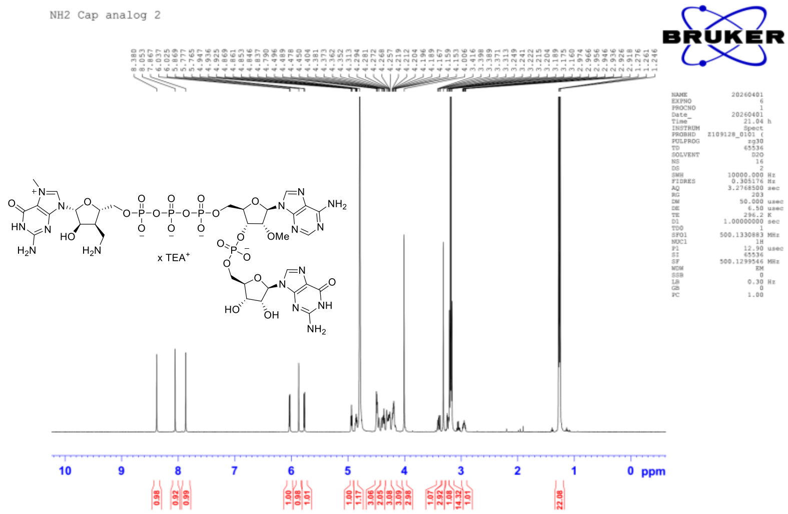


MS of N3 cap analog 1

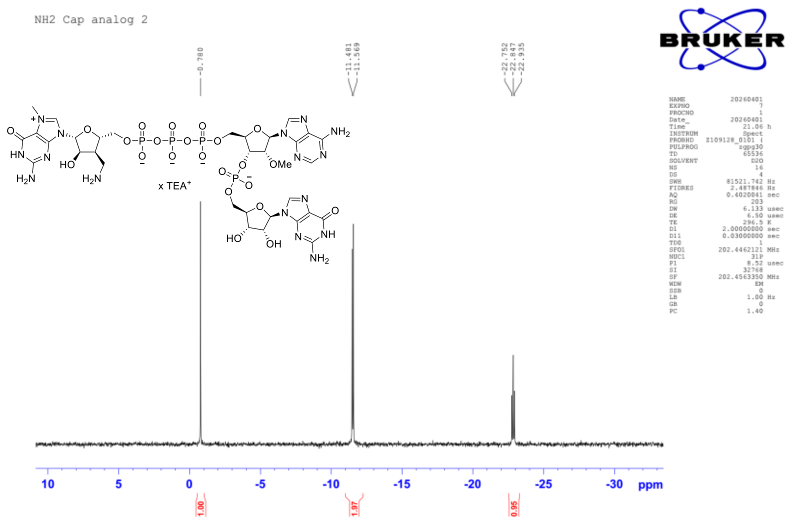


2. cap analog 2

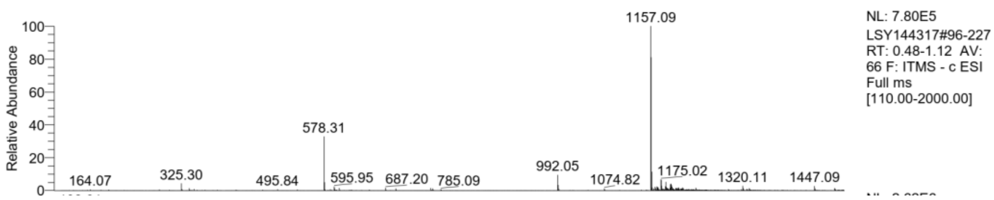
^{1}H NMR (500 MHz, D_2O) of NH2 cap analog 2



^{31}P NMR (202 MHz, D_2O) of NH2 cap analog 2

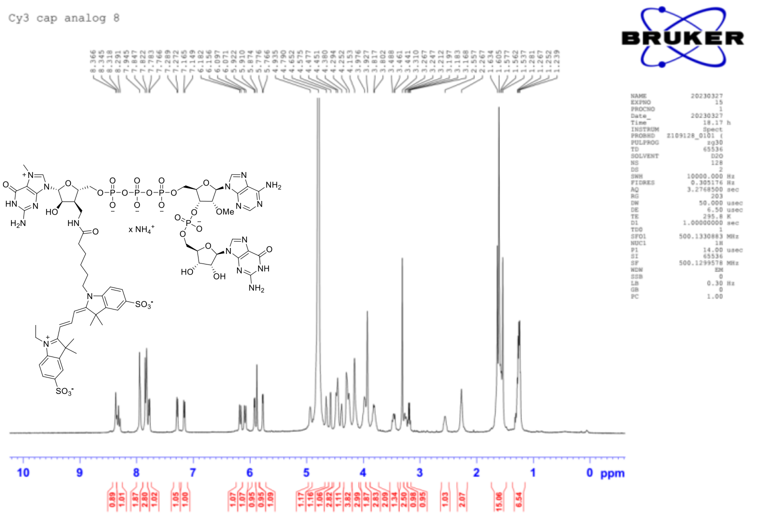


MS of NH2 cap analog 2

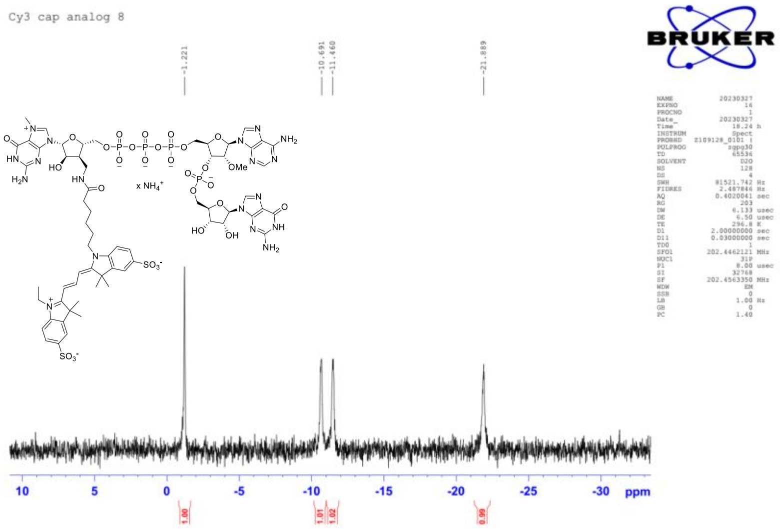


3. Cy3 cap analog 8

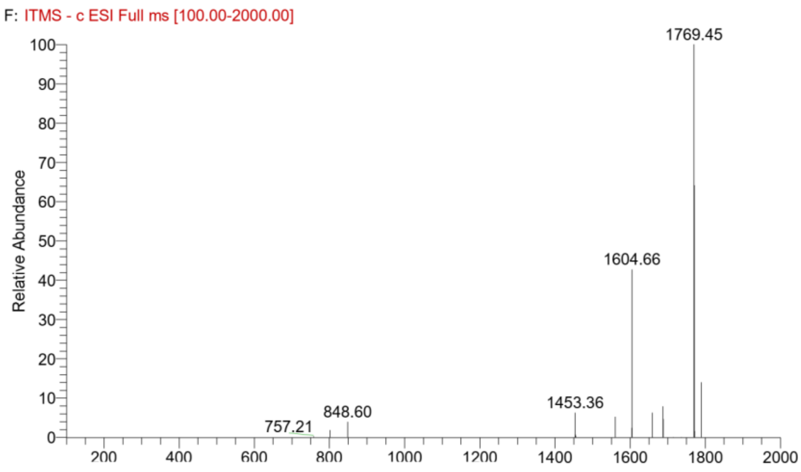
^{1}H NMR (500 MHz, D_2O) of Cy3 cap analog 8



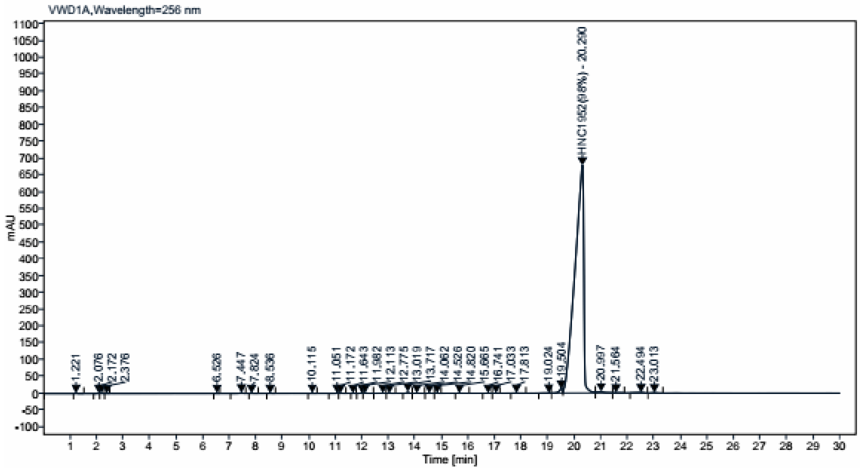
^{31}P NMR (202 MHz, D_2O) of Cy3 cap analog 8



MS of Cy3 cap analog 8

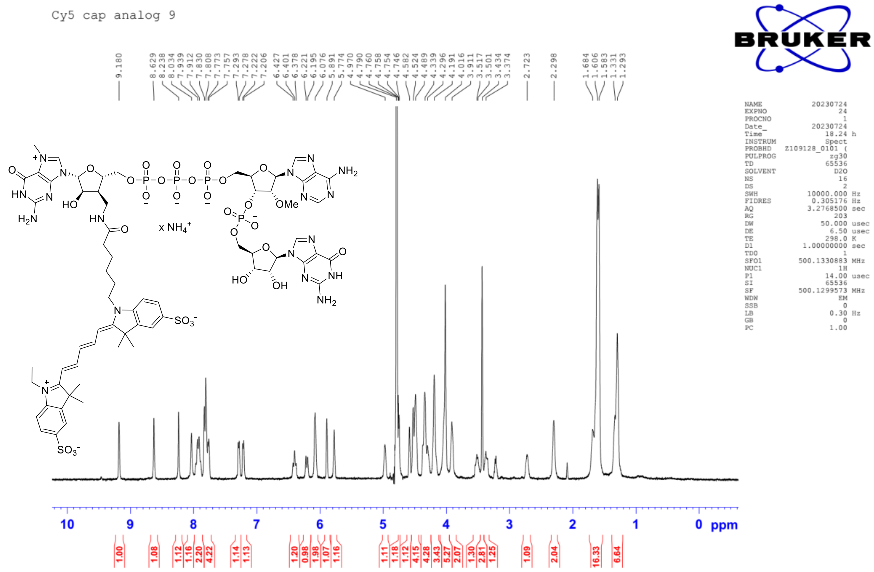


HPLC of Cy3 cap analog 8

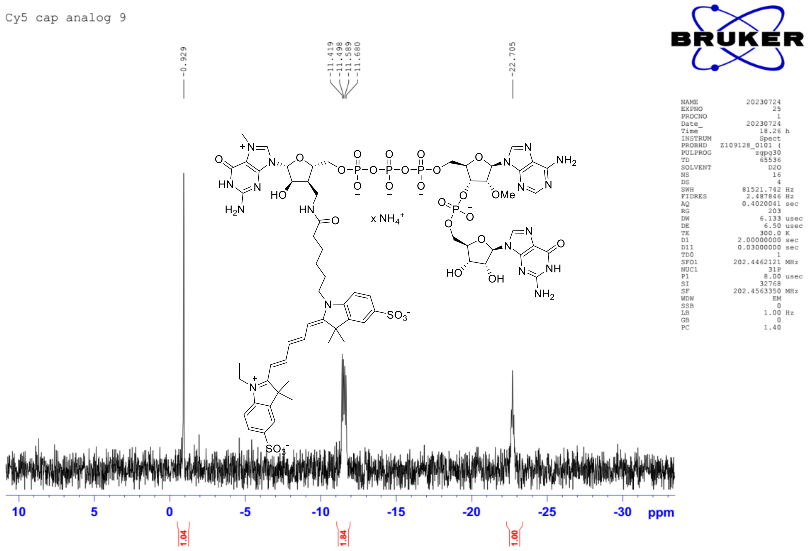


4. Cy5 cap analog 9

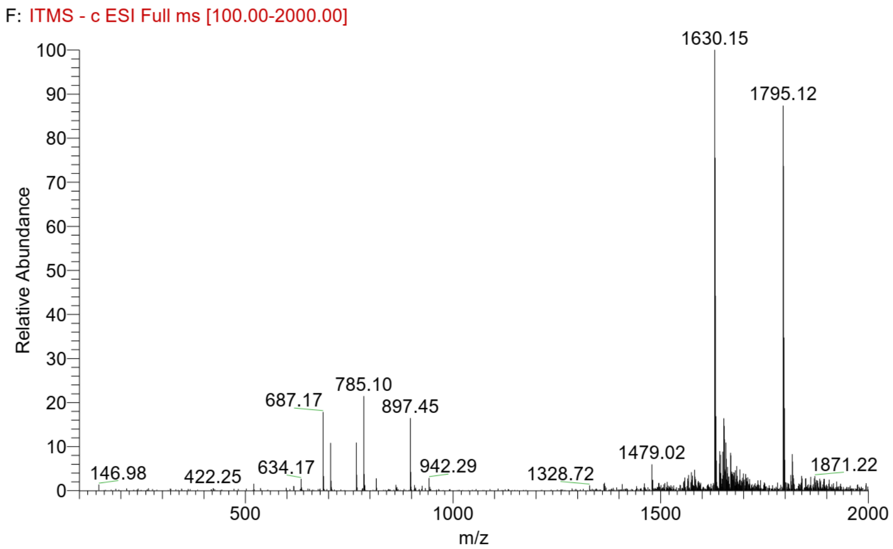
[1]H NMR (500 MHz, D_2O) of Cy5 cap analog 9



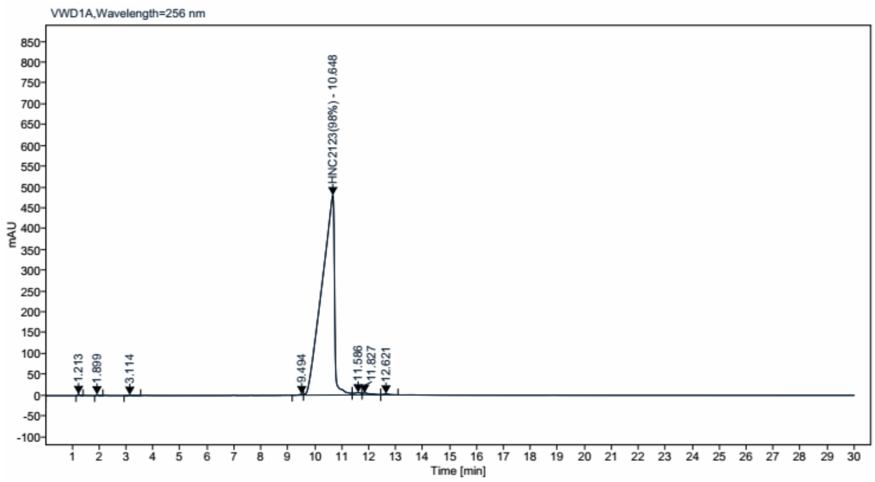
[31]P NMR (202 MHz, D_2O) of Cy5 cap analog 9



MS of Cy5 cap analog 9

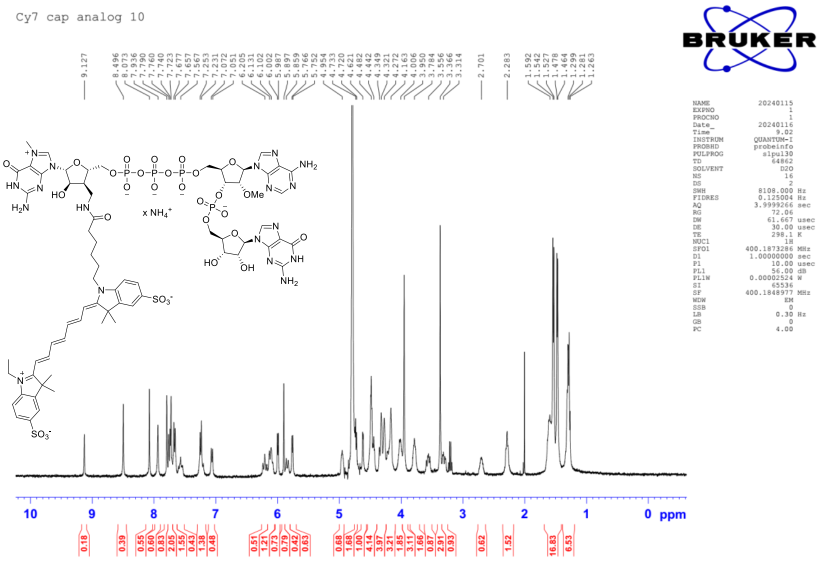


HPLC of Cy5 cap analog 9

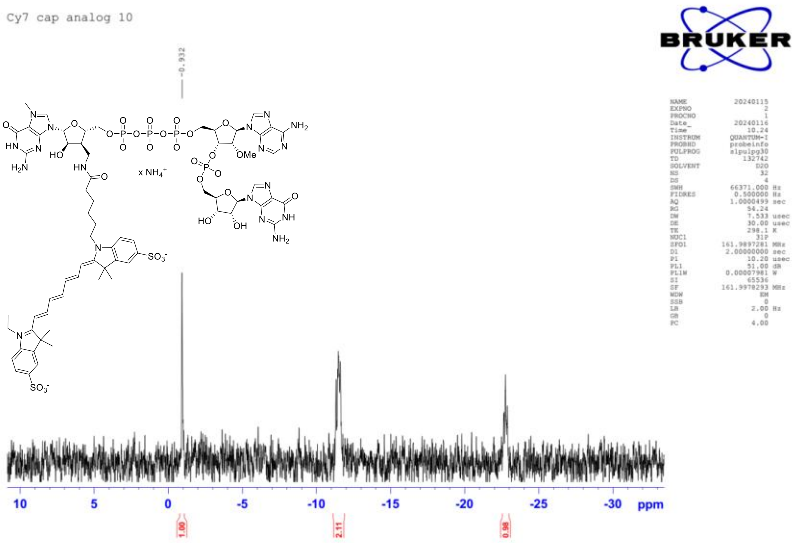


5. Cy7 cap analog 10

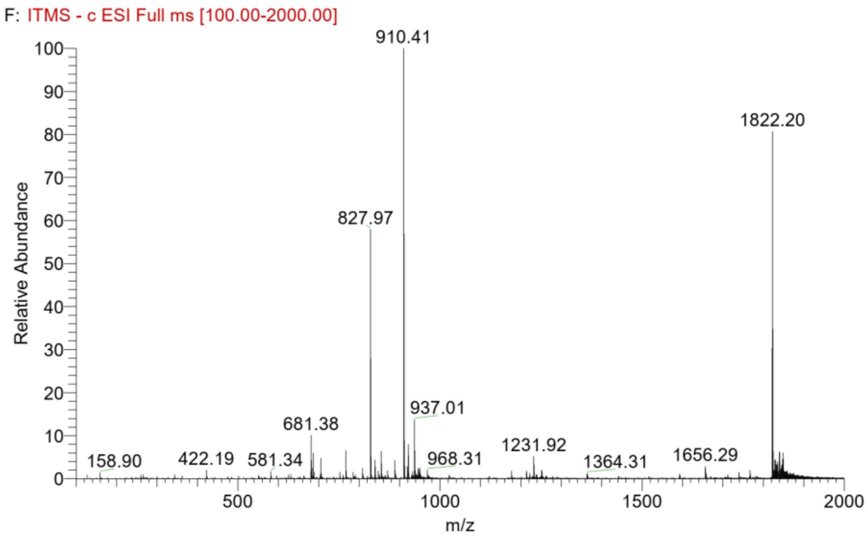
^{1}H NMR (400 MHz, D_2O) of Cy7 cap analog 10



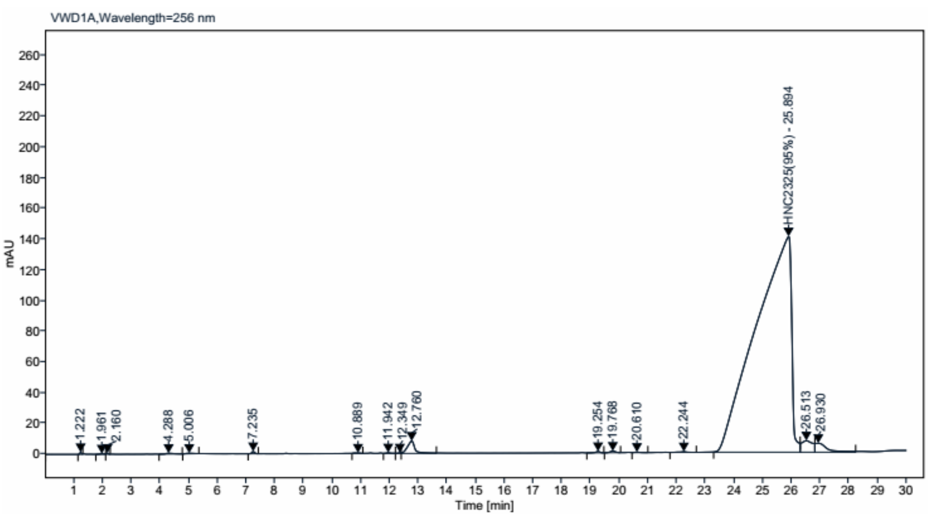
^{31}P NMR (162 MHz, D_2O) of Cy7 cap analog 10



MS of Cy7 cap analog 10

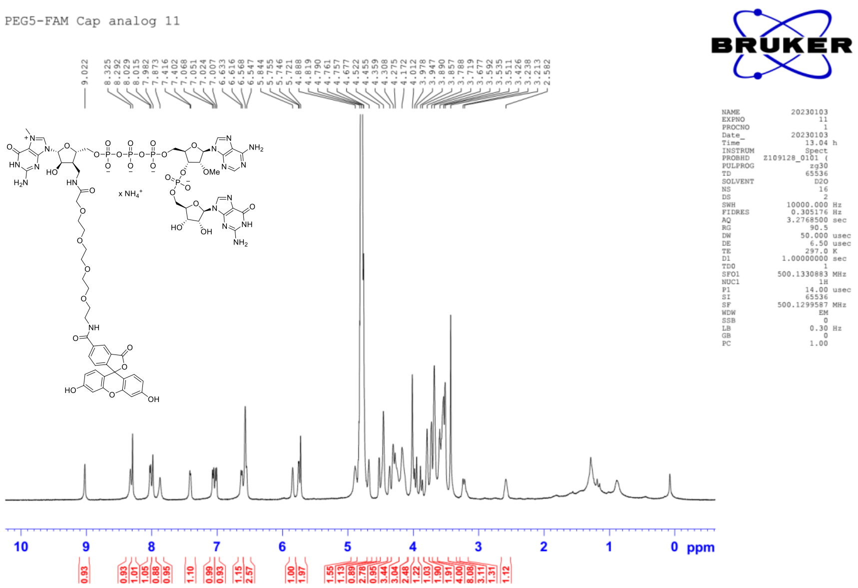


HPLC of Cy7 cap analog 10

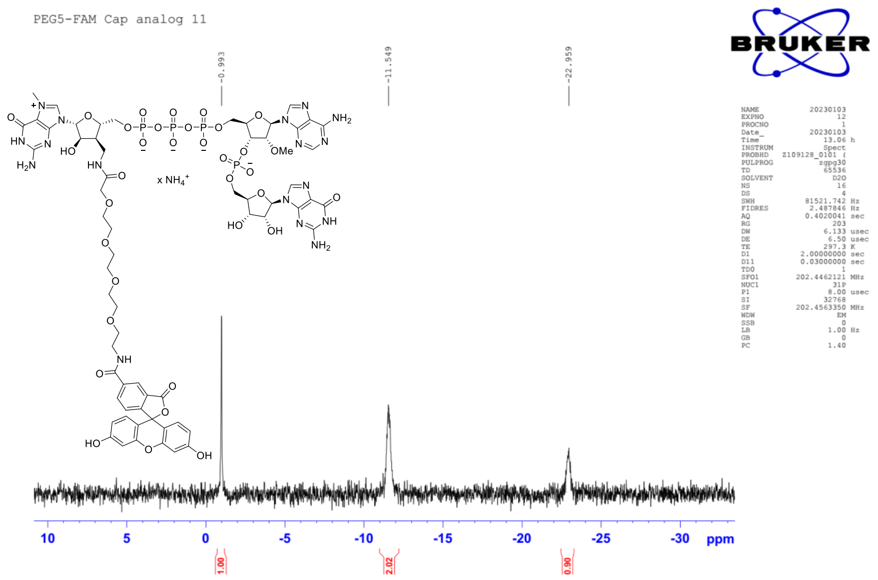


6. PEG5-FAM cap analog 11

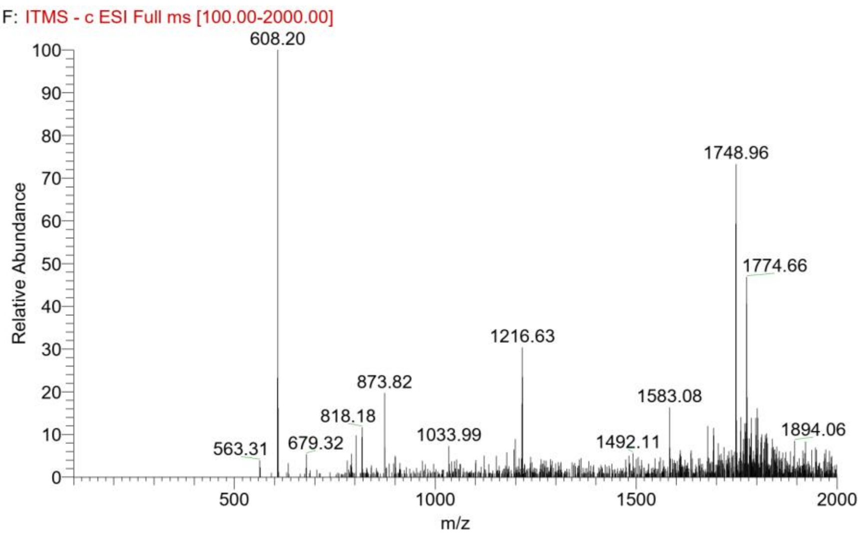
^{1}H NMR (500 MHz, D_2O) of PEG5-FAM cap analog 11



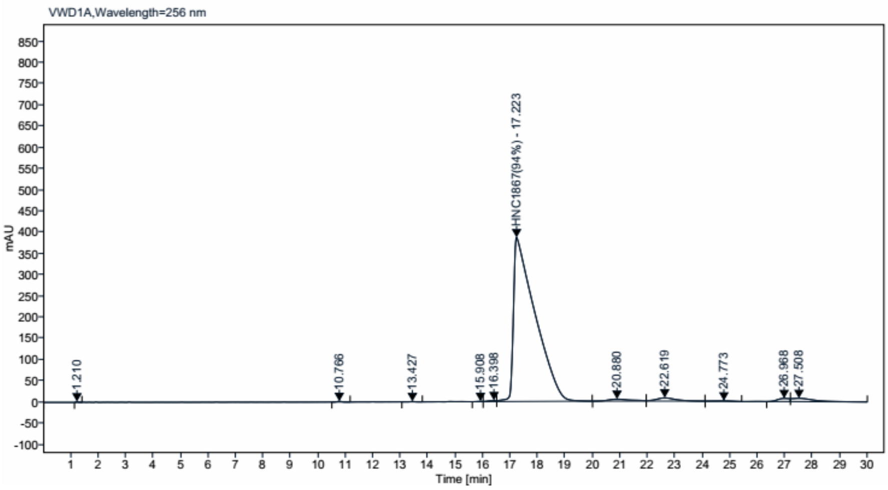
^{31}P NMR (202 MHz, D_2O) of PEG5-FAM cap analog 11



MS of PEG5-FAM cap analog 11

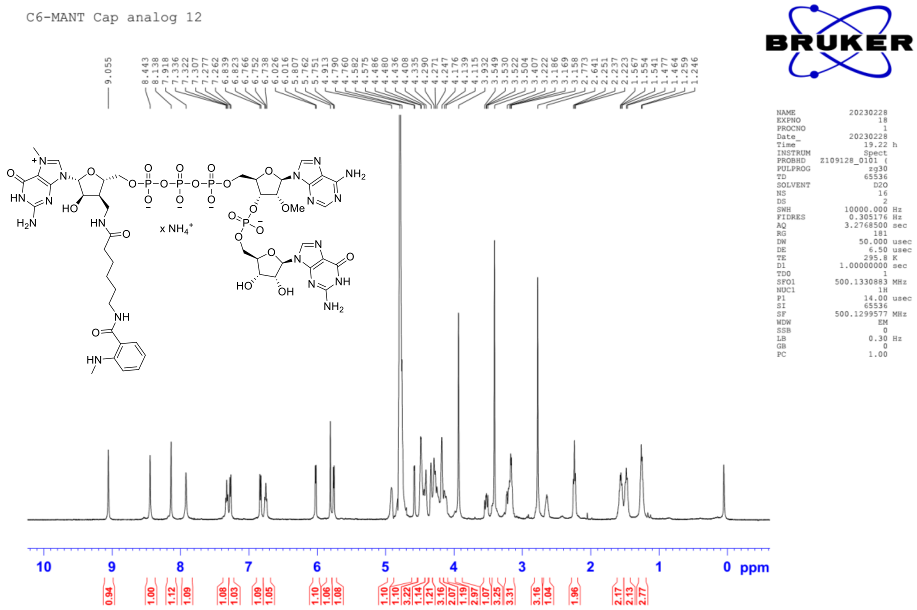


HPLC of PEG5-FAM cap analog 11

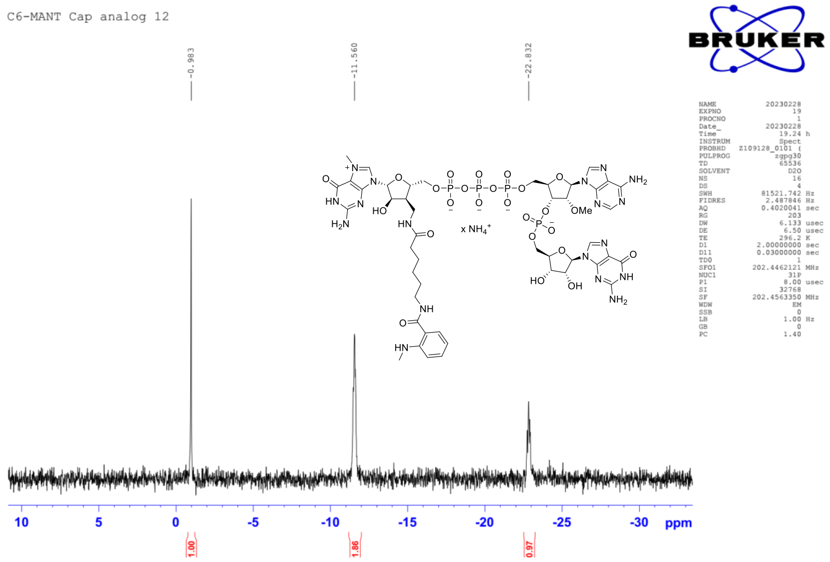


7. C6-MANT cap analog 12

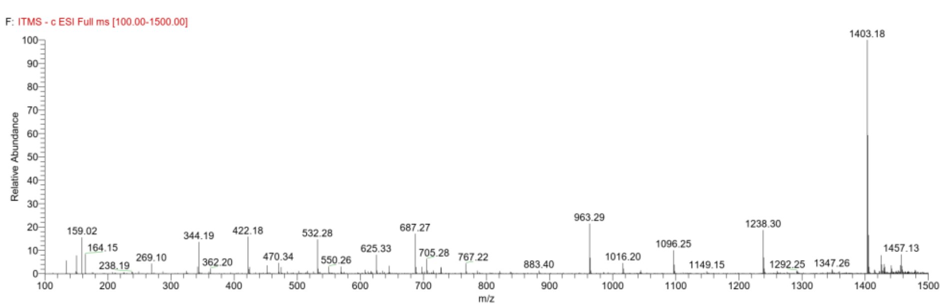
[1]H NMR (500 MHz, D_2O) of C6-MANT cap analog 12



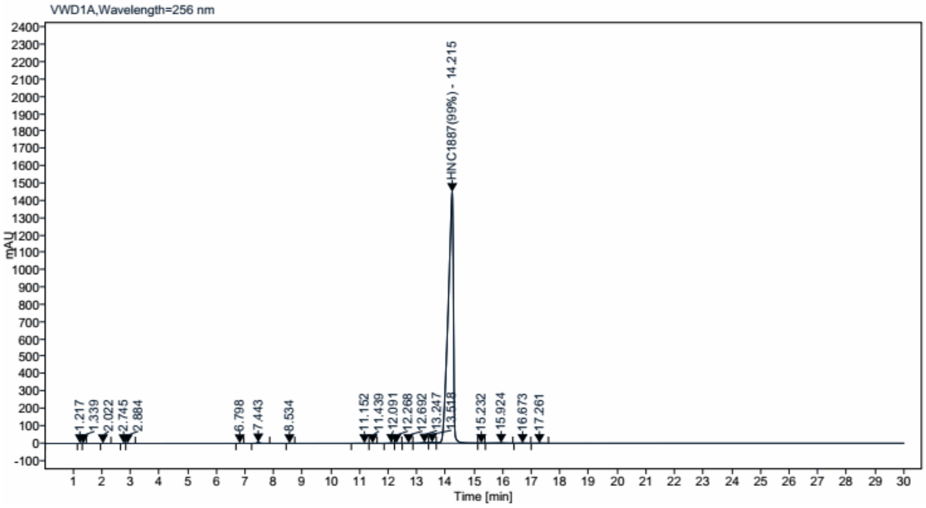
^{31}P NMR (202 MHz, D_2O) of C6-MANT cap analog 12



MS of C6-MANT cap analog 12



HPLC of C6-MANT cap analog 12

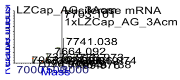


S10. LC-MS analysis of the capping efficiency with the synthesized cap analogs.

Capping efficiency = (Abundance of all capped fragments) / (Abundance of all identified fragments) × 100%

The abundance of each fragment can be calculated using either 'relative abundance' or 'fractional abundance'.

8. LZCap AG (3'Acm)

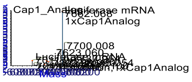


Level	Sequence Name	Modification	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Average Mass	Sum Intensity	Relative Abundance	Fractional Abundance
Aa	= (NonBlanks)	Aa	=	=	=	=	=	=	=
Component	Luciferase mRNA	1xLZCap_AG_3Acm	7703.101	7703.090	1.4	7,706.70	3.37E+07	100.00	33.04
Component	Luciferase mRNA	2x3'Truncation,1xLZCap_AG_3Acm	7053.021	7053.001	2.7	7,056.32	3.15E+06	9.32	3.08
Component	Luciferase mRNA	1x3'Truncation,1xLZCap_AG_3Acm	7358.052	7358.043	1.2	7,361.49	2.82E+06	8.35	2.76
Component	Luciferase mRNA	1xDiphos	7274.966	7274.970	0.6	7,278.37	9.38E+05	2.78	0.92

Capping efficiency

=(100+9.32+8.35)/(100+9.32+8.35+2.78)*100%=98%

9. Cleancap AG (3'OMe)

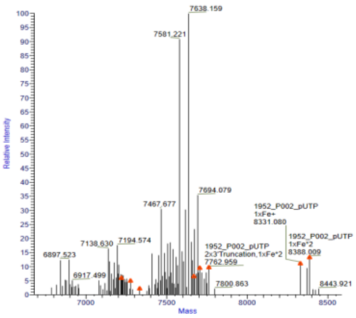


Level	Sequence Name	Modification	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Average Mass	Sum Intensity	Relative Abundance	Fractional Abundance
Component	Luciferase mRNA	1xCap1Analog	7662.068	7662.064	0.5	7,665.65	3.28E+07	100.00	32.69
Component	Luciferase mRNA	2x3'Truncation,1xCap1An...	7011.983	7011.975	1.1	7,015.26	3.13E+06	9.56	3.13
Component	Luciferase mRNA	1x3'Truncation,1xCap1An...	7316.997	7317.017	2.8	7,320.42	3.04E+06	9.28	3.03
Component	Luciferase mRNA	1xTriphos	7354.917	7354.936	2.6	7,358.35	2.53E+06	7.73	2.53
Component	Luciferase mRNA	1xDiphos	7274.956	7274.970	1.9	7,278.36	7.66E+05	2.34	0.76

Capping efficiency

=(100+9.56+9.28)/(100+9.56+9.28+7.73+2.34)*100%=92%

10. LZCap AG (3'Ma-Cy3)



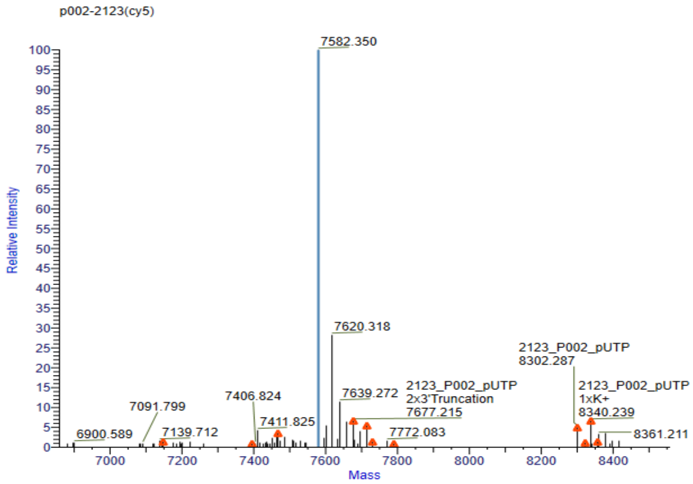
Results

		Level	Sequence Name	Modification	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Average Mass	Sum Intensity	Relative Abundance	Fractional Abundance
1		Component	1952_P002_pUTP	1xFe*2	8388.009	8388.171	19.4	8,393.29	9.48E+04	13.61	1.42
2		Component	1952_P002_pUTP	1xFe+	8331.080	8331.228	17.8	8,336.32	7.92E+04	11.37	1.19
3		Component	1952_P002_pUTP	2x3'Truncation,1xFe*2	7762.959	7763.089	16.8	7,767.80	7.03E+04	10.10	1.06
4		Component	1952_P002_pUTP	2x3'Truncation,1xFe	7707.085	7707.154	8.9	7,711.90	6.83E+04	9.81	1.03
5		Component	1952_P002_pUTP	2x3'Truncation,1xNH4+	7669.128	7669.253	16.3	7,673.92	4.75E+04	6.83	0.71
6		Component	P1_P002_pUTP	1xMg+	7222.151	7222.013	19.0	7,226.68	4.36E+04	6.26	0.65
7		Component	P2_P002_pUTP	1xH	7279.101	7279.003	13.5	7,283.66	3.62E+04	5.20	0.54
8		Component	1952_P002_pUTP	10x3'Truncation	5036.790	5036.860	13.9	5,039.93	2.12E+04	3.05	0.32
9		Component	P2_P002_pUTP	1xFe+	7332.827	7332.922	12.9	7,337.42	1.75E+04	2.52	0.26

Capping efficiency

=(13.61+11.37+10.1+9.81+6.83)/(13.61+11.37+10.1+9.81+6.83+6.26+5.2+2.52) *100%=79%

11. LZCap AG (3'Ma-Cy5)



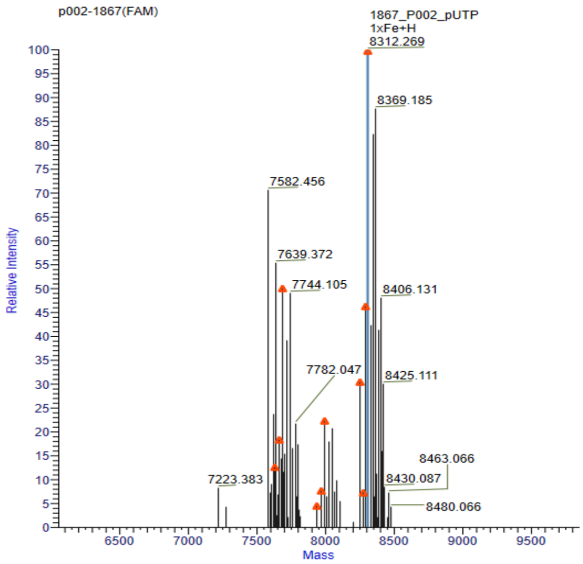
Results

#	Level	Sequence Name	Modification	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Average Mass	Sum Intensity	Relative Abundance	Fractional Abundance
1	Component	P3_P002_pUTP	1x3'Truncation,1xFe*2	7149.721	7149.790	9.7	7,153.06	5.37E+04	1.55	0.51
2	Component	P3_P002_pUTP	1xK+	7395.818	7395.917	13.4	7,399.27	3.67E+04	1.06	0.35
3	Component	P3_P002_pUTP	1xFe*2	7469.762	7469.831	9.3	7,473.25	1.32E+05	3.81	1.25
4	Component	2123_P002_pUTP	2x3'Truncation	7677.215	7677.235	2.6	7,680.80	2.37E+05	6.82	2.23
5	Component	2123_P002_pUTP	2x3'Truncation,1xK+	7715.158	7715.191	4.2	7,718.76	1.91E+05	5.52	1.80
6	Component	2123_P002_pUTP	2x3'Truncation,1xFe+	7732.118	7732.162	5.7	7,735.73	5.67E+04	1.63	0.53
7	Component	2123_P002_pUTP	2x3'Truncation,1xFe*2+H	7790.066	7790.113	6.0	7,793.71	3.64E+04	1.05	0.34
8	Component	2123_P002_pUTP		8302.287	8302.317	3.6	8,306.17	1.78E+05	5.14	1.68
9	Component	2123_P002_pUTP	1xNa+	8324.263	8324.299	4.3	8,328.15	4.80E+04	1.38	0.45
10	Component	2123_P002_pUTP	1xK+	8340.239	8340.273	4.1	8,344.14	2.37E+05	6.84	2.24
11	Component	2123_P002_pUTP	1xFe+	8357.214	8357.244	3.7	8,361.12	5.38E+04	1.55	0.51

Capping efficiency

=(2.23+1.8+0.34+1.68+0.45+2.24+0.51/(2.23+1.8+0.34+1.68+0.45+2.24+0.51+0.51+0.35+1.25)*100%=82%

12. LZCap AG (3'Ma-FAM)



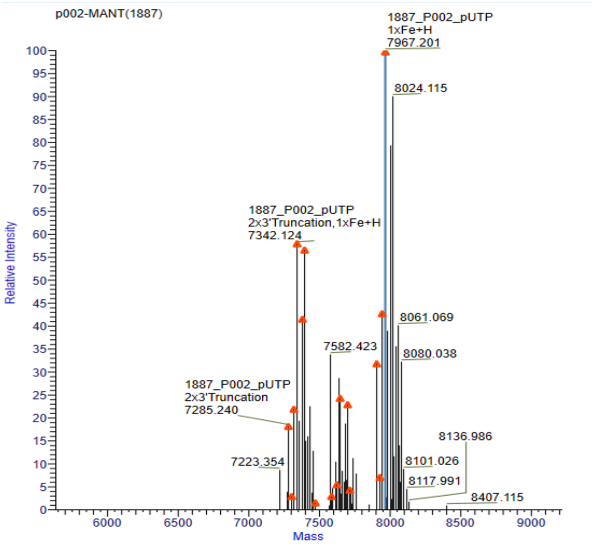
lts

#	Level	Sequence Name	Modification	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Average Mass	Sum Intensity	Relative Abundance	Fractional Abundance
1	Component	1867_P002_pUTP	1xFe+H	8312.269	8312.222	5.7	8,316.15	8.51E+05	100.00	8.10
2	Component	1867_P002_pUTP	2x3'Truncation,1xFe+H	7687.199	7687.140	7.7	7,690.79	4.27E+05	50.11	4.06
3	Component	1867_P002_pUTP	1xK+	8293.296	8293.235	7.3	8,297.17	3.95E+05	46.39	3.76
4	Component	1867_P002_pUTP		8255.342	8255.279	7.6	8,259.20	2.60E+05	30.52	2.47
5	Component	1867_P002_pUTP	1x3'Truncation,1xFe+H	7992.217	7992.181	4.5	7,995.95	1.91E+05	22.39	1.81
6	Component	1867_P002_pUTP	2x3'Truncation,1xK+	7668.236	7668.153	10.8	7,671.82	1.58E+05	18.61	1.51
7	Component	1867_P002_pUTP	2x3'Truncation	7630.281	7630.197	11.0	7,633.84	1.09E+05	12.83	1.04
8	Component	1867_P002_pUTP	1x3'Truncation,1xK+	7973.242	7973.194	6.0	7,976.97	6.60E+04	7.76	0.63
9	Component	1867_P002_pUTP	1xNa+	8277.332	8277.261	8.5	8,281.20	6.27E+04	7.37	0.60
10	Component	1867_P002_pUTP	1x3'Truncation	7935.315	7935.238	9.7	7,939.02	3.90E+04	4.58	0.37

Capping efficiency =

(8.1+4.06+3.16+2.47+1.81+1.51+1.04+0.63+0.6+0.37)/(8.1+4.06+3.16+2.47+1.81+1.51+1.04+0.63+0.6+0.37)*100%=100%

13. LZCap AG (3'Ma-FAM)



Results

	Level	Sequence Name	Modification	Monoisotopic Mass	Theoretical Mass (Da)	Matched Mass Error (ppm)	Average Mass	Sum Intensity	Relative Abundance	Fractional Abundance
1	Component	1887_P002_pUTP	1xFe+H	7967.201	7967.185	2.1	7,970.92	6.78E+05	100.00	8.87
2	Component	1887_P002_pUTP	2x3'Truncation,1xFe+H	7342.124	7342.103	2.8	7,345.55	3.94E+05	58.09	5.16
3	Component	1887_P002_pUTP	2x3'Truncation,1xFe*2+H	7398.032	7398.038	0.7	7,401.49	3.85E+05	56.71	5.03
4	Component	1887_P002_pUTP	1xK+	7948.223	7948.198	3.2	7,951.94	2.90E+05	42.78	3.80
5	Component	P3_P002_pUTP	1xNa+	7380.062	7379.943	16.2	7,383.51	2.82E+05	41.60	3.69
6	Component	1887_P002_pUTP		7910.276	7910.242	4.3	7,913.97	2.18E+05	32.08	2.85
7	Component	1887_P002_pUTP	1x3'Truncation,1xFe+H	7647.203	7647.144	7.7	7,650.77	1.66E+05	24.54	2.18
8	Component	1887_P002_pUTP	1x3'Truncation,1xFe*2+H	7703.098	7703.079	2.4	7,706.70	1.56E+05	23.02	2.04
9	Component	1887_P002_pUTP	2x3'Truncation,1xK+	7323.169	7323.116	7.3	7,326.59	1.50E+05	22.10	1.96
10	Component	1887_P002_pUTP	2x3'Truncation	7285.240	7285.160	11.1	7,288.64	1.24E+05	18.33	1.63
11	Component	1887_P002_pUTP	1xNa+	7932.251	7932.224	3.4	7,935.96	4.89E+04	7.21	0.64
12	Component	1887_P002_pUTP	1x3'Truncation,1xK+	7628.209	7628.157	6.8	7,631.77	3.81E+04	5.62	0.50
13	Component	1887_P002_pUTP	1x3'Truncation,1xDIEPA	7719.317	7719.353	4.6	7,722.92	2.97E+04	4.38	0.39
14	Component	1887_P002_pUTP	2x3'Truncation,1xNa+	7307.182	7307.142	5.6	7,310.60	2.12E+04	3.13	0.28
15	Component	1887_P002_pUTP	1x3'Truncation	7590.230	7590.201	3.8	7,593.77	2.07E+04	3.05	0.27
16	Component	P3_P002_pUTP	1xFe*2+H	7470.943	7470.839	14.0	7,474.43	1.10E+04	1.62	0.14

Capping efficiency

=(8.87+5.16+5.03+3.8+2.85+2.18+2.04+1.96+1.63+0.64+0.5+0.39+0.28+0.2)

/(8.87+5.16+5.03+3.8+2.85+2.18+2.04+1.96+1.63+0.64+0.5+0.39+0.28+0.27

+3.69+0.14)*100%=90%

S11. Transcript length, and template details of luciferase mRNA, eGPF mRNA and HBs$_{183}$-TCR mRNA

Luciferase mRNA (1974nt)

AGGAGACCCAAGCTGGCTAGCTTAAGCTTCTTGTTCTTTTTGCAGAAGCT
CAGAATAAACGCTCAACTTTGGCCTGCAGGGCCACCATGGAAGACGCCA
AAAACATAAAGAAAGGCCCGGCGCCATTCTATCCGCTAGAGGATGGAACC
GCTGGAGAGCAACTGCATAAGGCTATGAAGAGATACGCCCTGGTTCCTG
GAACAATTGCTTTTACAGATGCACATATCGAGGTGAACATCACGTACGCG

GAATACTTCGAAATGTCCGTTCGGTTGGCAGAAGCTATGAAACGATATGG
GCTGAATACAAATCACAGAATCGTCGTATGCAGTGAAAACTCTCTTCAATT
CTTTATGCCGGTGTTGGGCGCGTTATTTATCGGAGTTGCAGTTGCGCCCG
CGAACGACATTTATAATGAACGTGAATTGCTCAACAGTATGAACATTTCGC
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GTGCAAAAAAAATTACCAATAATCCAGAAAATTATTATCATGGATTCTAAAAC
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GTTTCTTCACATTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAA

eGFP mRNA (1210nt)

AGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTTCTTGTTCTTTTTG
CAGAAGCTCAGAATAAACGCTCAACTTTGGCCTGCAGGGCCACCATGGT
GAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGA
GCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGG
CGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACC
ACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACC
TACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACG

ACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCAT
CTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTC
GAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTC
AAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACA
GCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGT
GAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCC
GACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTG
CCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCA
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CAAGAAGGGCGGAAAGTCCAAATTGTAAGTCGACACCAGCCTCAAGAAC
ACCCGAATGGAGTCTCTAAGCTACATAATACCAACTTACACTTTACAAAATG
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TCTTCACATTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAA

HBs$_{183}$-TCR mRNA (2120nt)

AGACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACA
CCATGCTGCTCGCTCTGCTGCCTGTGCTGGGCATCCACTTCCTGCTGAG
AGATGCCCAAGCCCAGAGCGTCACCCAGCCTGACGCTAGAGTGACCGT
GTCCGAGGGCGCTAGCCTGCAGCTGAGATGTAAATACAGCTACTTCGGC
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GCTGCTGAAGTACTACCCGGGAGATCCTGTGGTGCAAGGCGTGAACGG
CTTCGAGGCCGAATTCTCTAAGAGCAACAGCAGCTTCCATCTGCGGAAG
GCCAGCGTGCACTGGTCAGATTGGGCCGTGTACTTTTGCGCCGTGACAA
GTTACGACACCAACGCCTACAAGGTGATCTTTGGAAAGGGCACCCACCT
GCACGTGCTGCCCAACATCCAGAATCCCGAGCCCGCCGTTTACCAGCTC
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ACTCTCAGATCAACGTGCCCAAGACCATGGAATCTGGCACATTCATCACC
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TGACCGAGAAGTCTTTCGAGACAGATATGAACCTGAACTTTCAGAACCTG
CTGGTCATCGTGCTCAGAATCCTGTTACTTAAGGTGGCCGGATTTAACCT
GCTGATGACCCTGCGGCTGTGGAGCTCCCGGGCGAAGAGATCCGGCTC
TGGAGCAACAAACTTCAGCCTGCTTAAACAGGCCGGCGACGTGGAAGAG
AACCCTGGCCCCATGAGCAATACCGTTCTGGCCGACTCTGCCTGGGGAA
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CGACAGCGGAGTGGTGCAGTCTCCAAGACACATCATCAAGGAAAAAGGC
GGCCGGTCTGTGCTTACATGTATCCCCATCAGCGGCCACAGCAATGTGG

TGTGGTACCAGCAGACCCTGGGCAAGGAGCTGAAGTTCCTGATCCAACA
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GAAGTGCATAGCGGCGTGTGCACCGACCCCCAGGCCTATAAAGAGTCTA
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GCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTTCC
TGCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAA

S12 .Characterization of LNP samples.

Sample	Encapsulation %	Size (nm)	PDI	Experiment
Cy5-TCR	97.02%	95.5	0.01809	Detection of mRNA in Jurkat T cells
Anti CD3-LNP-Cy5 TCR	92.53%	183.7	0.0619	Detection of mRNA in Jurkat T cells
Anti CD7-LNP-Cy5-TCR	93.15%	120.6	0.069	Detection of mRNA in Jurkat T cells
Cy5-eGFP-LNP	98.38%	108.3	0.1334	Intracellular localization of mRNA in Huh7 cells
Cy5-Luciferase-LNP	98.98%	108.1	0.04396	Intracellular localization of mRNA in Huh7 cells/ In vivo immaging of mRNA in mice
Cy7-Luciferase-LNP	97.46%	104.8	0.1167	In vivo immaging of mRNA in mice