Electronic Supplementary Material (ESI) for RSC Advances.
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Supplementary information

to

Synthetic m_3G-CAP attachment necessitates a minimum trinucleotide constituent to be recognized as Nuclear Import Signals.

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S1. Synthesis of PEG Biotin Linker (13)

The reaction flask was charged with (4-(propiolamidomethyl)benzoic acid) PATA (5 eq., 0.075 mmol, 15 mg) and HBTU (5 eq., 0.075 mmol, 28 mg), evacuated on a pump and then flushed with N_2. The solid substrates were dissolved in DMF (1 mL) and NMM (25 eq., 0.375 mmol, 41 μL) was added with a syringe. The reaction mixture was allowed to stir for 30 min. Biotin-PEG(23)-NH_2 (1 eq, 0.015 mmol, 20 mg) was then added and the solution was stirred for an additional 3 h. After this time MS analysis showed completion of reaction and the mixture was concentrated to dryness. The crude product was purified by RP-HPLC using a linear gradient of buffer B in A from 0%–60% in 20 min, detector at 220nm, t_R = 15.0, ES-MS, calcd (M+H) 1484.8, found 1484.0

S2. Synthesis of 5'-pCH_2p-$A_{(OMe)}U_{(OMe)}$A-*linker*-N_3.

Trinucleotide 5'-HO-$A_{(OMe)}U_{(OMe)}$A-*linker*-N_3 (50mg, 0.035 mmol) [Honcharenko et al., RSC Advances, 2012, 2, 12949–12962] was dissolved in anhydrous trimethyl phosphate (700 μL) and the reaction flask was placed on an ice-bath. Then, tetrachloromethylene(bisphosphonate) was added (26 mg, 0.1 mmol, 3 eq) and the mixture was stirred at 0 °C until the disappearance of the starting material as determined by TLC (iPrOH/NH_{3aq}/H_2O, 7:2:1). Usually, after 1.5-2h, the solvent was evaporated under vacuum and to the glassy remaining, NH_{3aq} was added (1 mL) and stirred for additional 4 h at room temperature. Then, the reaction mixture was neutralized with 1M $NaHCO_{3aq}$, and lyophilized. The crude product was purified by DEAE-Sephadex and isolated as TEA salts. Yield: (285 mOD).

Synthesis of m_3GppCH_2p$A_{(OMe)}U_{(OMe)}$A-*linker*-N_3.

5'-methylene(bis)phosphonylated trinucleotide 5'-pCH_2p$A_{(OMe)}U_{(OMe)}$A-*linker*-N_3 (100 mOD. 0.0025 mmol) was dissolved in morpholine buffer (1.25 mL, pH 7). Then, m_3GMP-Im (11.2 mg, 0.025 mmol, 10 eq) and $MnCl_2$ x $4H_2O$ (5 mg, 0.025 mmol, 10 eq) were added and the reaction mixture was left stirred at 30 °C until the disappearance of the starting material as determined by RP-HPLC. Then, the reaction was stopped by addition of a solution of EDTA in water (0.5 mL) and neutralized with 1 M $NaHCO_3$. The crude product was purified by preparative RP-HPLC and isolated as NH_4^+ salts. Yield: (15 mOD)

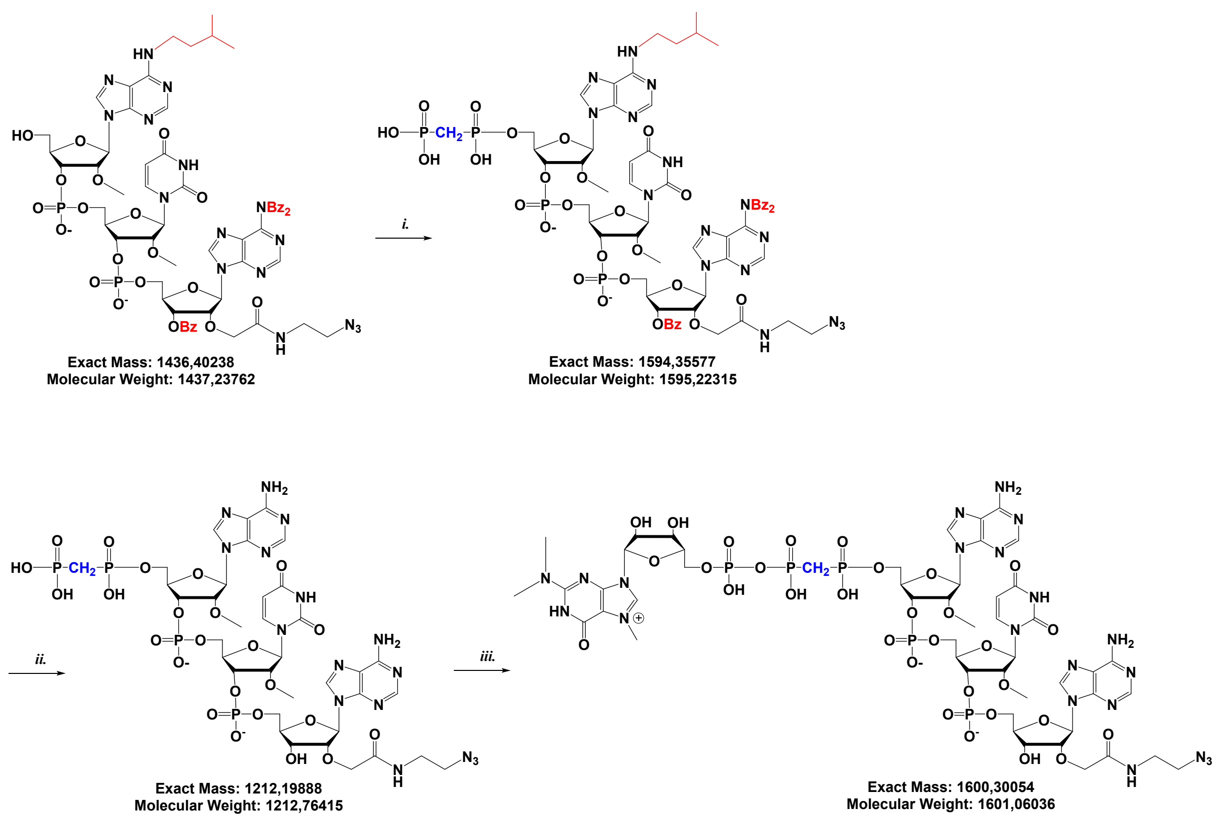


Fig. 1. i.$(POCl_2)_2CH_2$, $P(O)(OMe)_3$, ii. NH_3aq, rt; m_3GMP-Im, $MnCl_2$, morpholine buffer pH 7

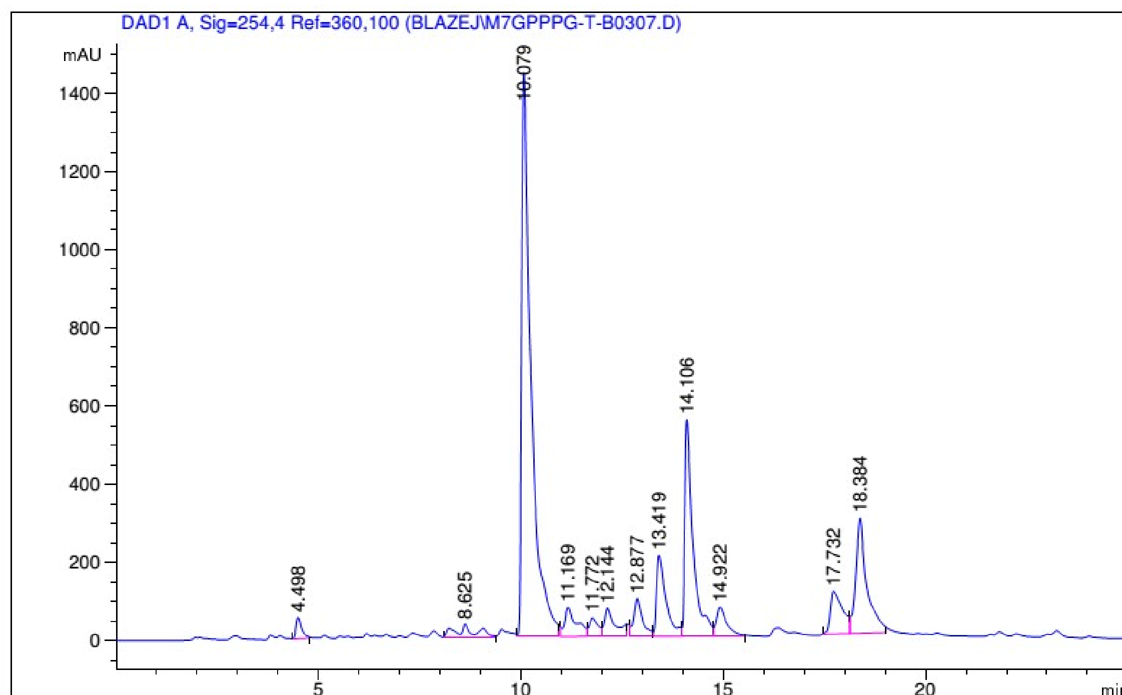


Fig. 2 RP-HPLC profile of the reaction mixture. 18.38 min (m_3GpCH$_2$pp-A$_{2'OMe}$U$_{2'OMe}$A-linker-N$_3$, MW 1601), 14.106 min (m_3GMP-Im), 10.079 min (m_3GMP).

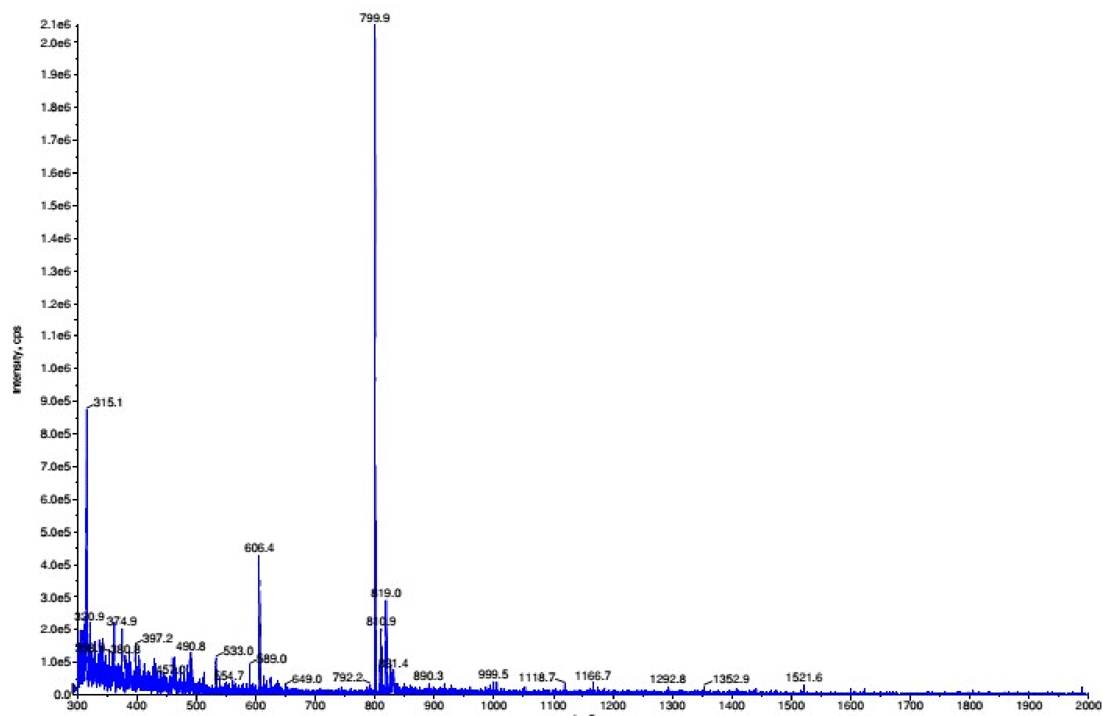
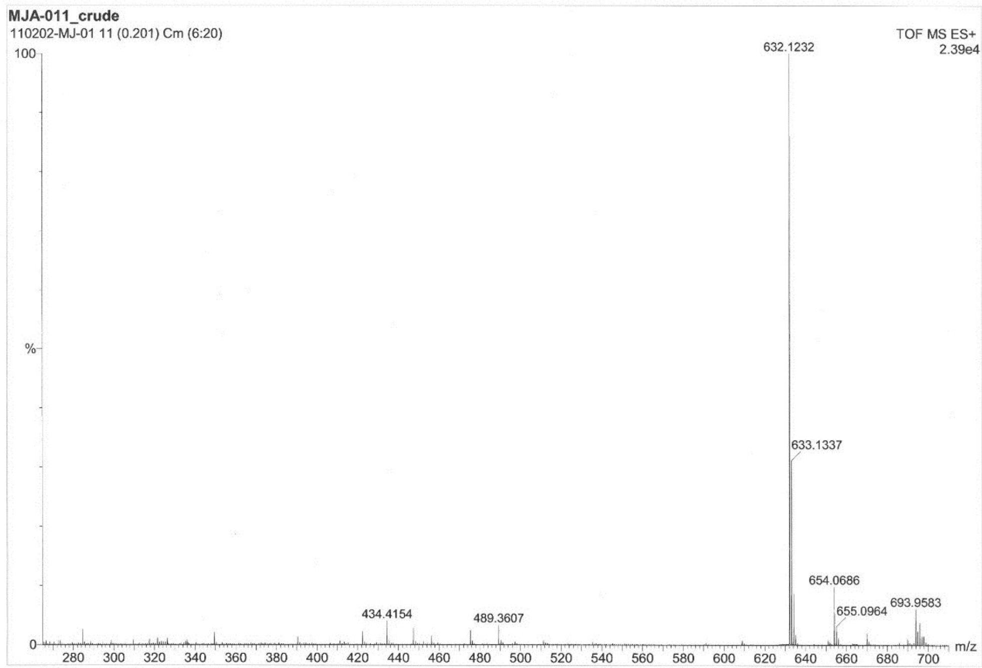


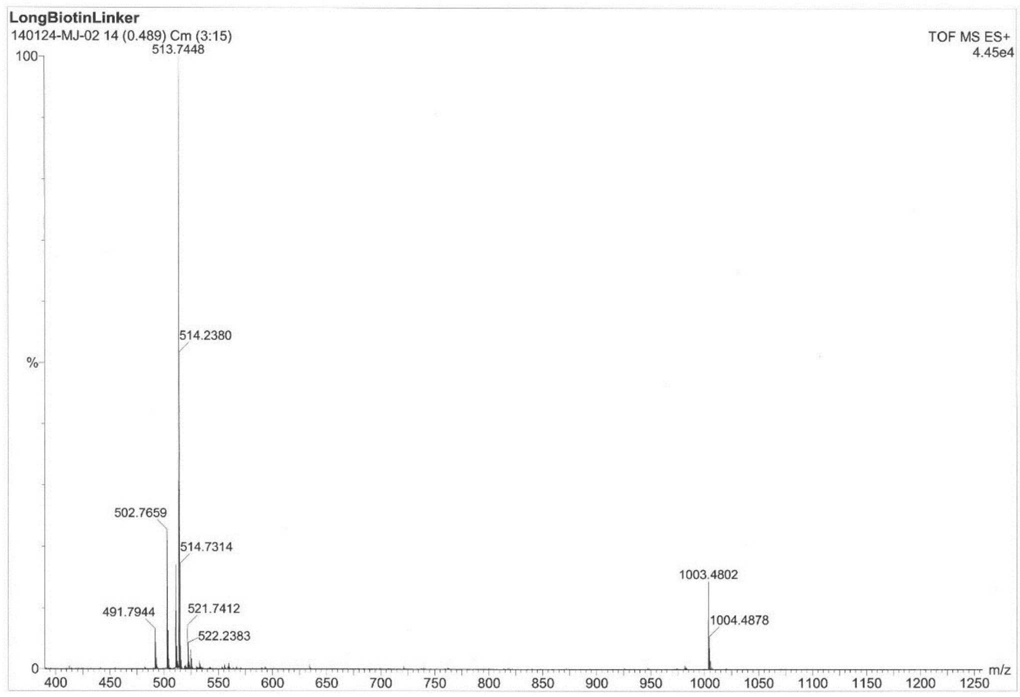
Fig. 3 ESI MS spectrum (negative mode) of the product m_3GpCH_2pp-$A_{2'OMe}U_{2'OMe}A$-linker-N_3, MW 1601

S3. Mass Spectra of Biotin linkers.

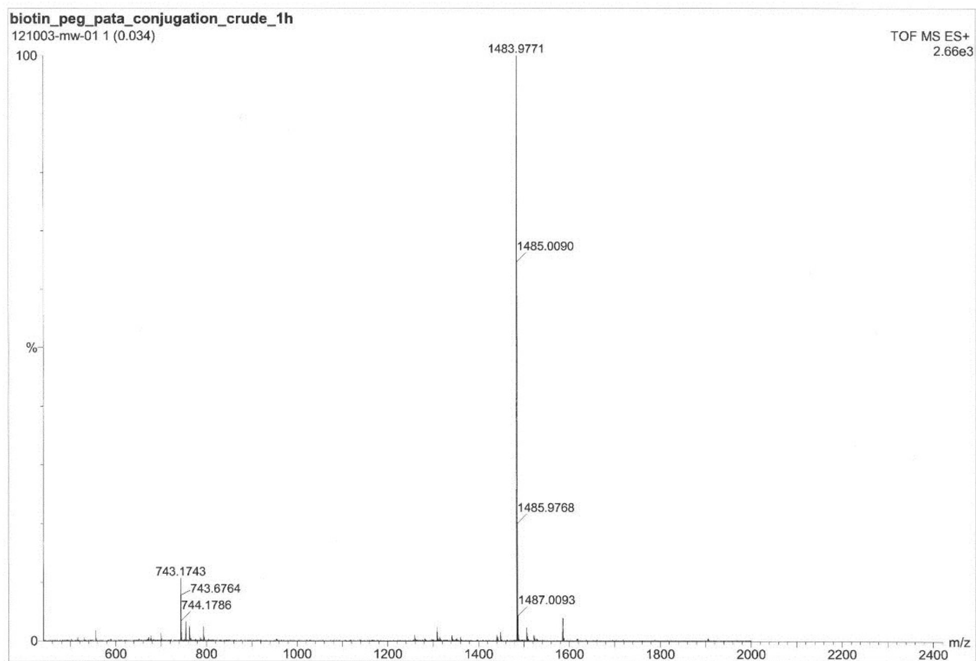
Short Biotin Linker (SBL)



Long Biotin Linker (LBL)

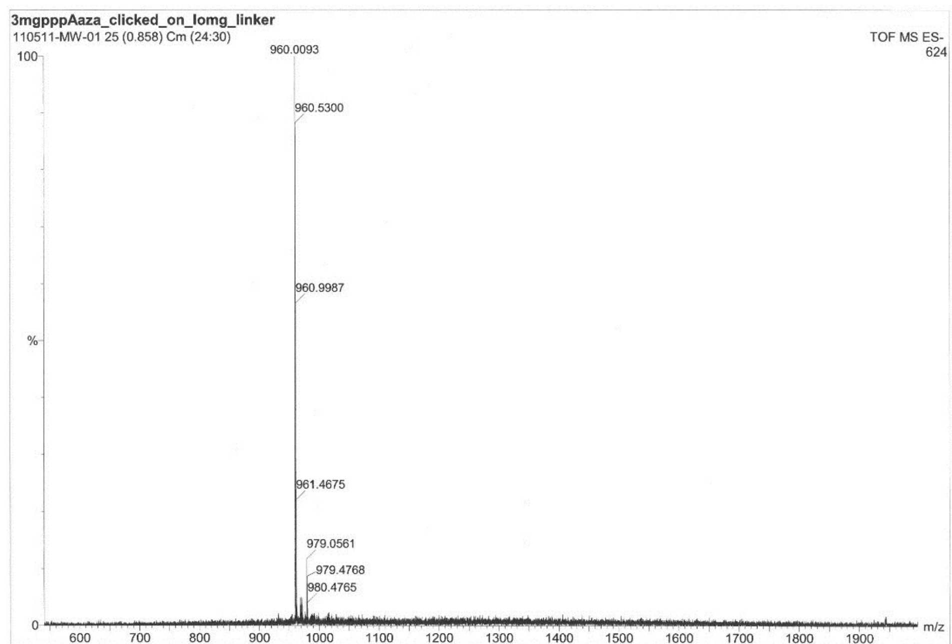


PEG Biotin Linker (PEGBL)

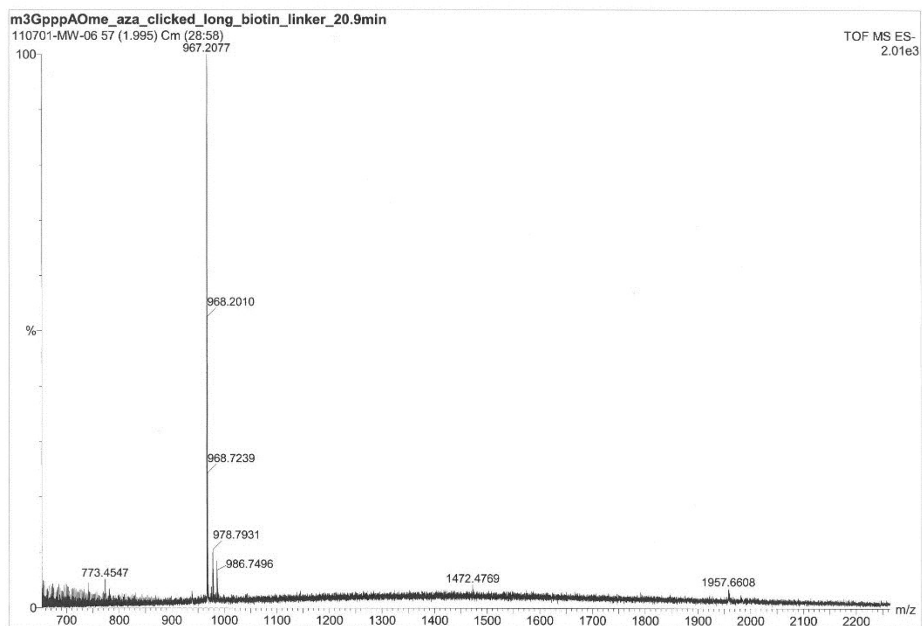


S4. Mass Spectra of purified m_3G-cap analogues.

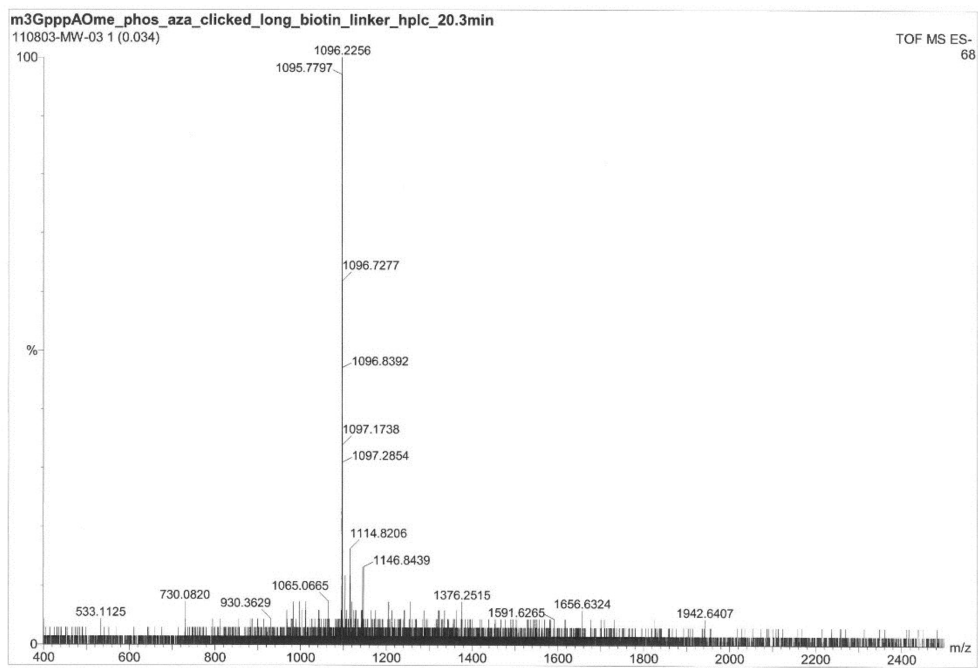
a. **Compound 14:** m_3GpppA-aza-LBL



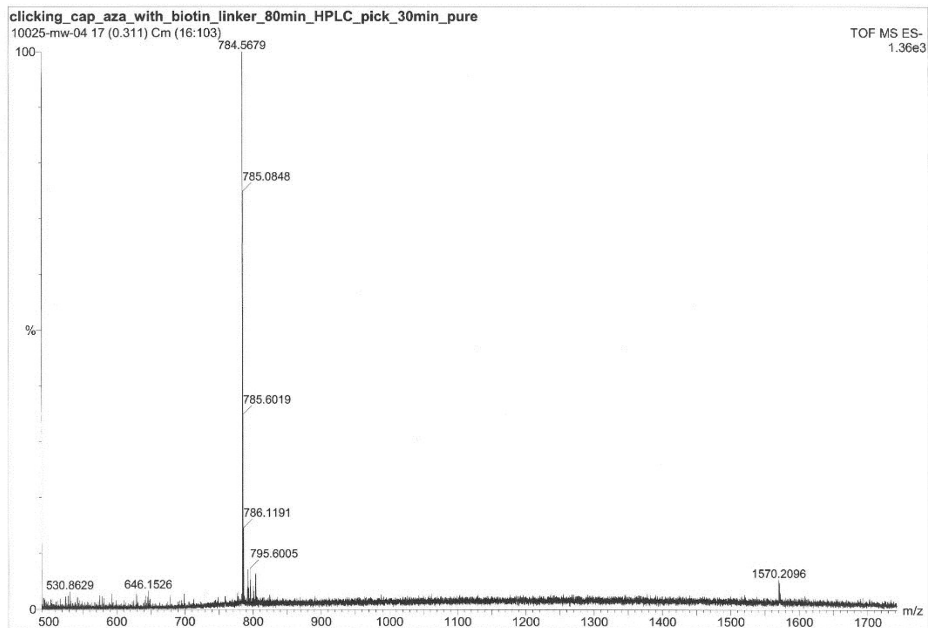
b. Compound 15: m_3GpppAOMe-aza-LBL



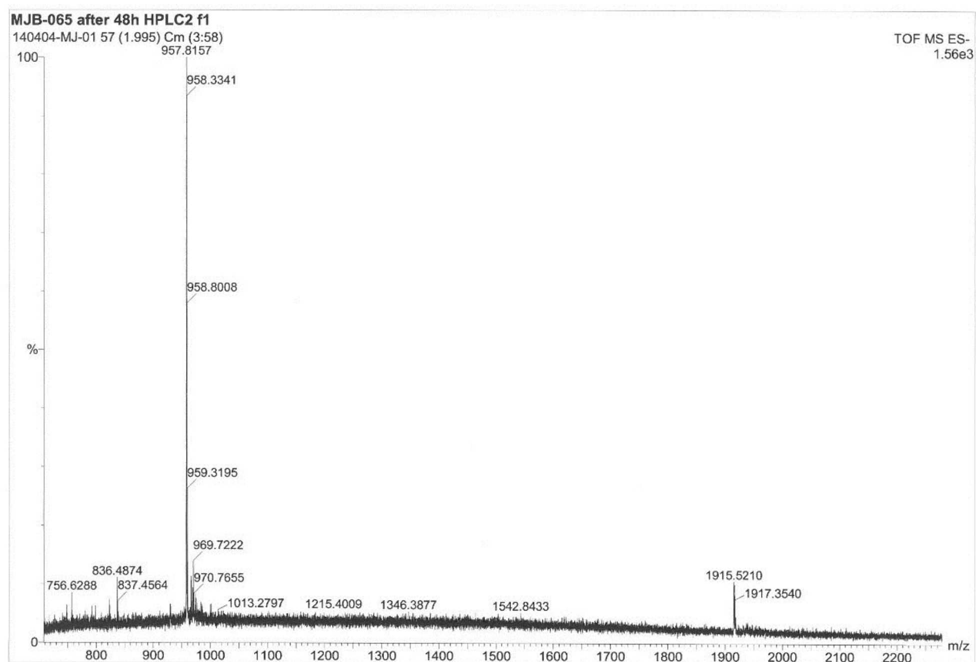
c. Compound 16: m_3GpppAOMe-phos-aza-LBL



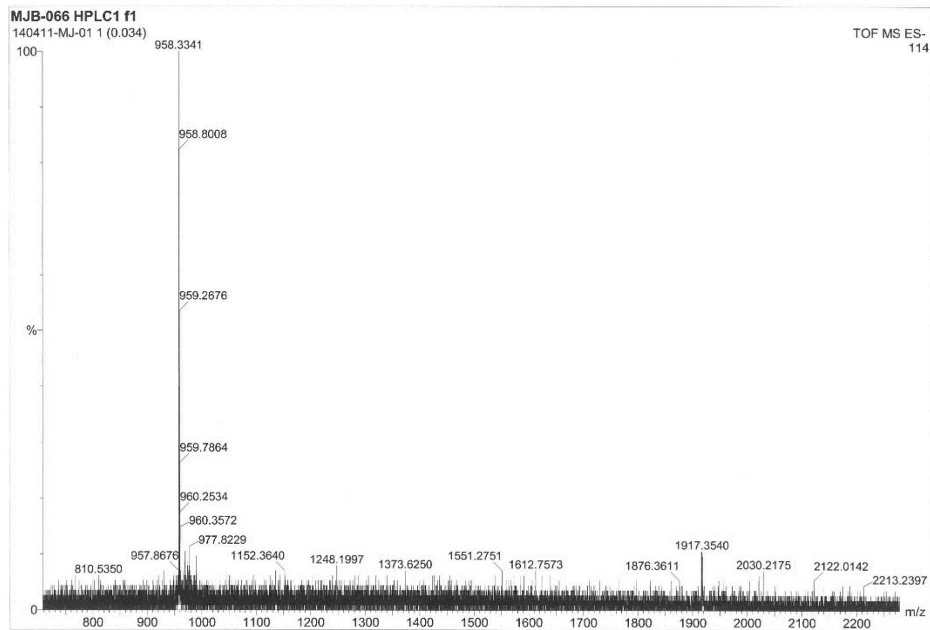
d. **Compound 17:** m_3GpppAOMe-phos-aza-SBL



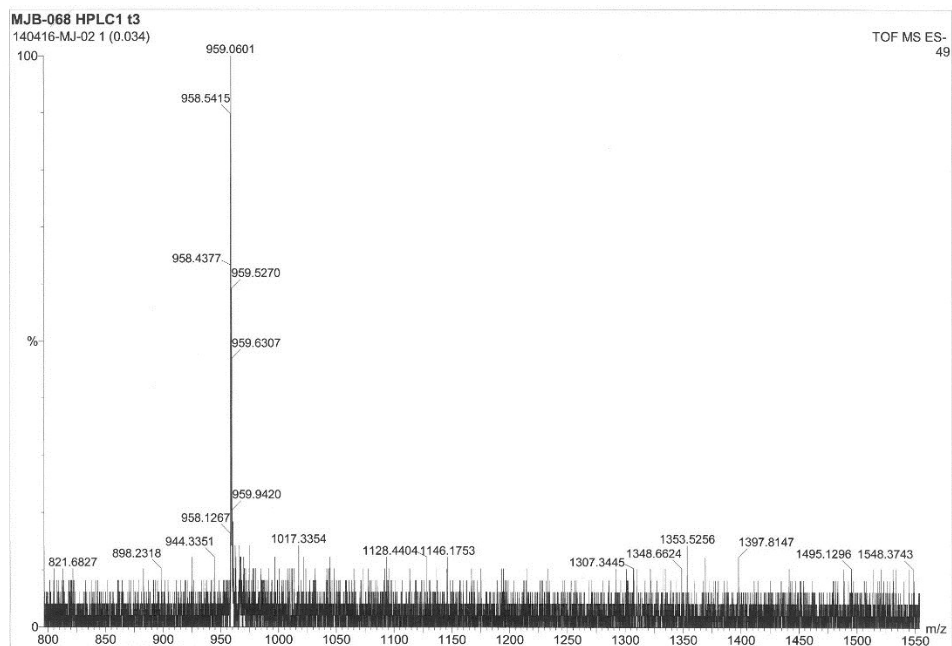
e. **Compound 18:** m_3GppCH_2pA-aza-LBL



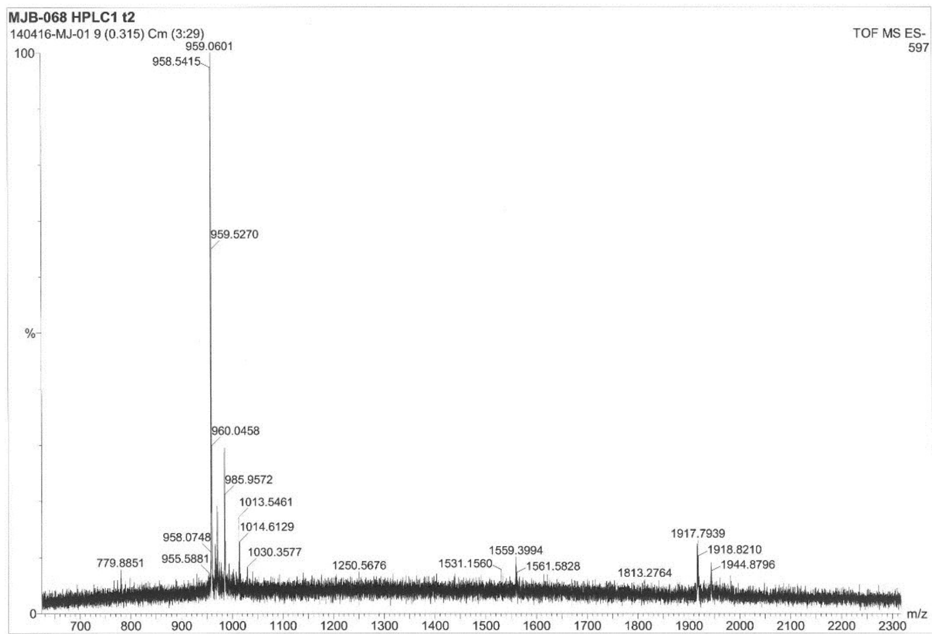
f. Compound 19: m_3GpCH_2ppA-aza-LBL



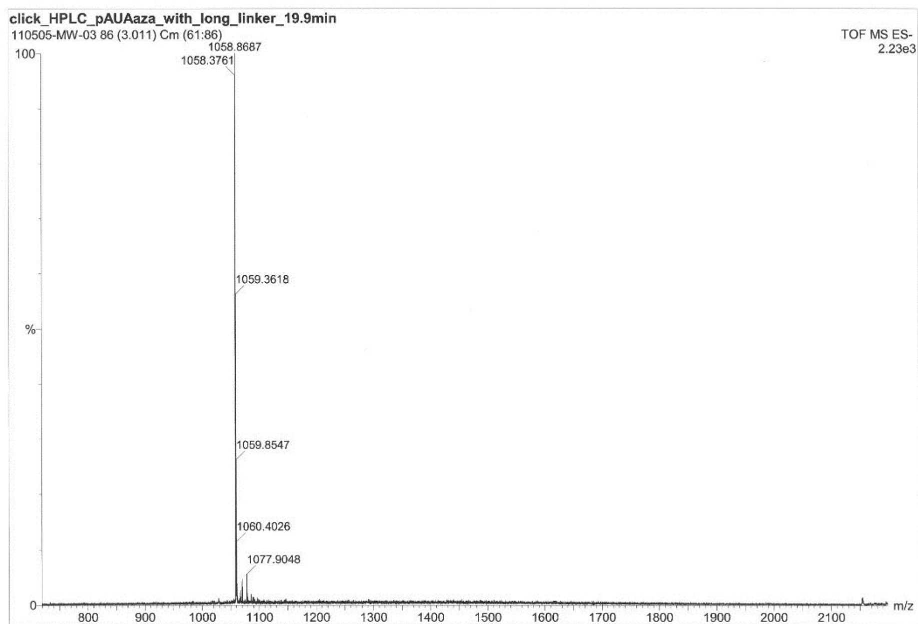
g. Compound 20: m_3GppNHpA-aza-LBL



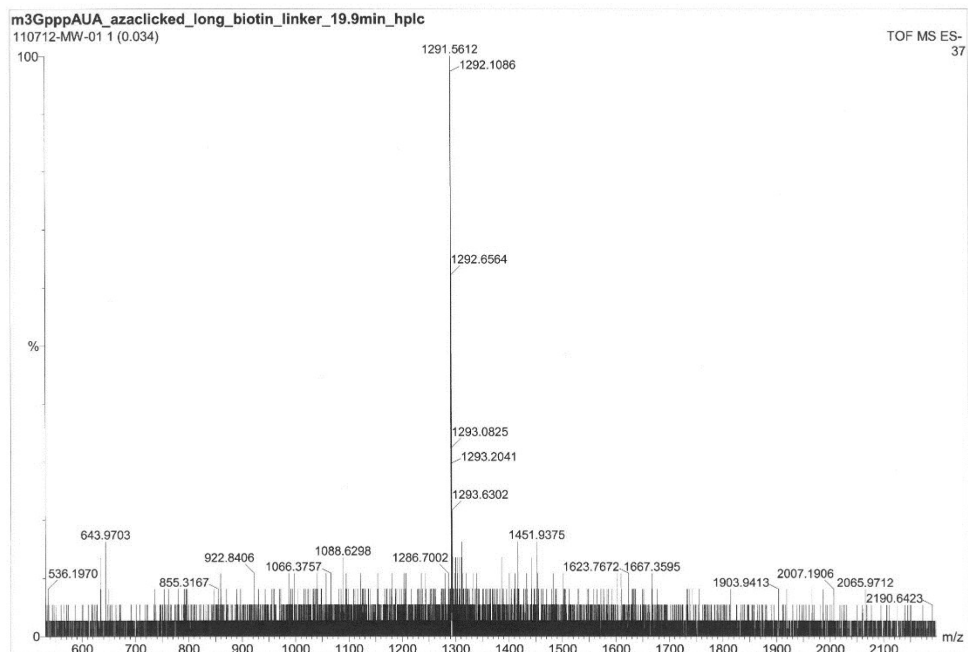
h. Compound 21: m_3GpNHppA-aza-LBL



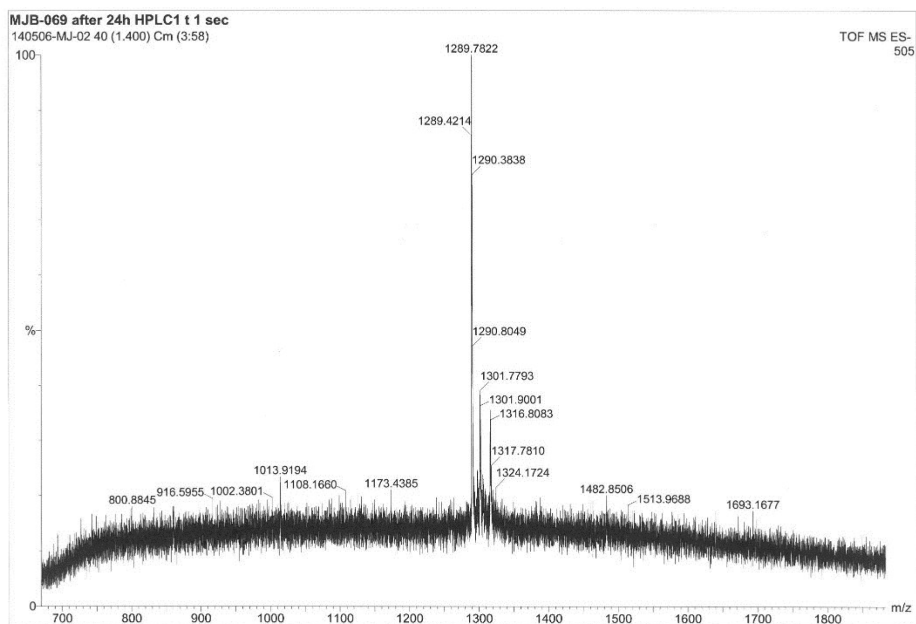
i. **Compound 22:** pAOMeUOMeA-aza-LBL



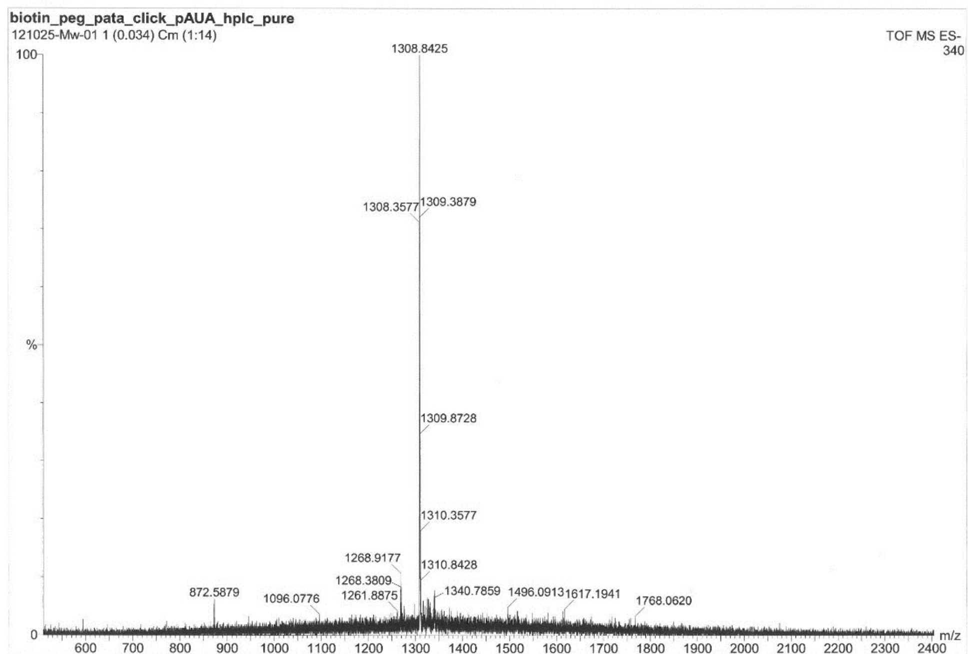
j. Compound 23: m_3GpppAOMeUOMeA-aza-LBL



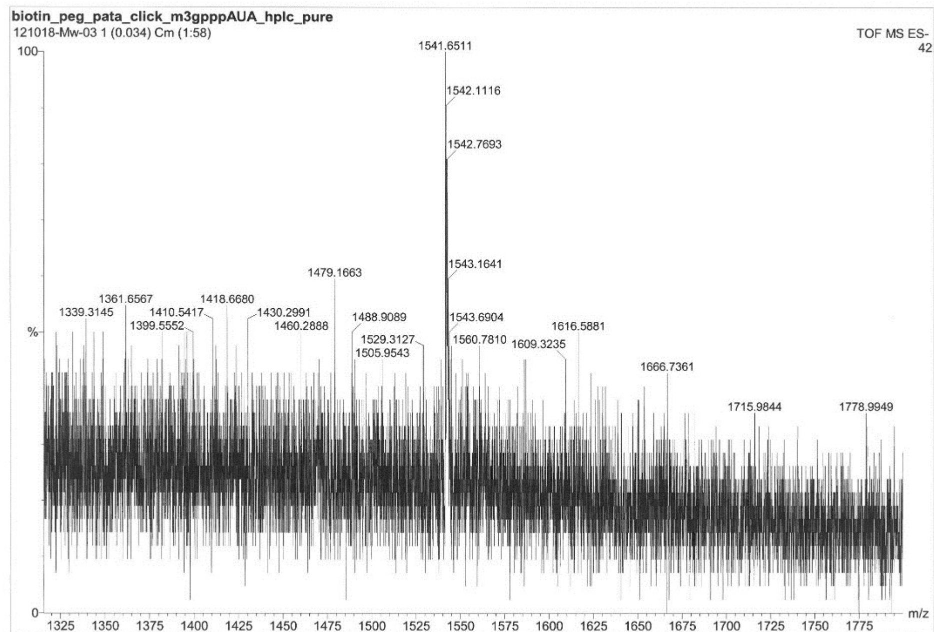
k. Compound 24: $m_3GppCH_2pAOMeUOMeA$-aza-LBL



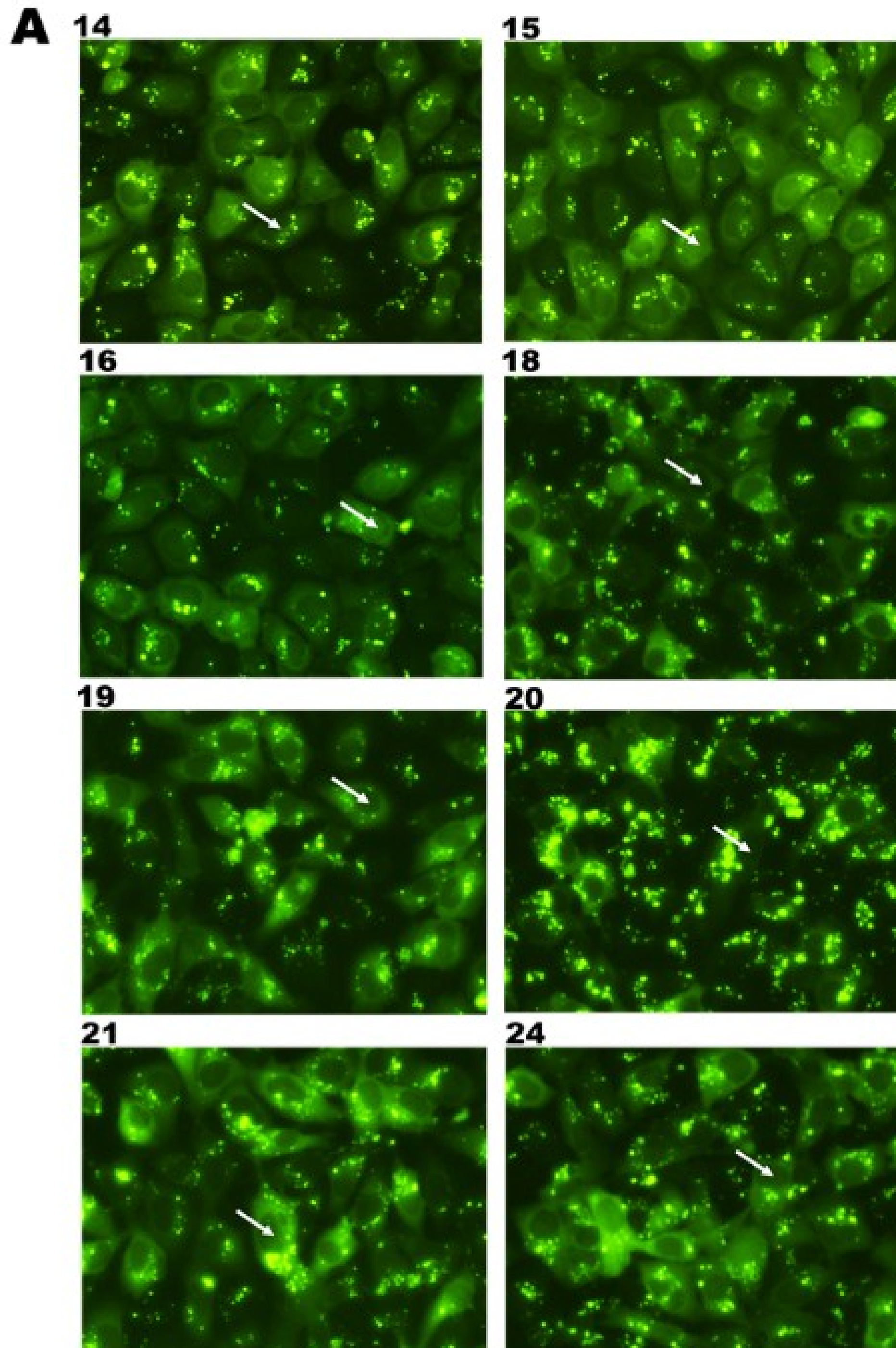
l. Compound 25: pAOMeUOMeA-aza-PEGBL



m. Compound 26: m_3GpppAOMeUOMeA-aza-PEGBL

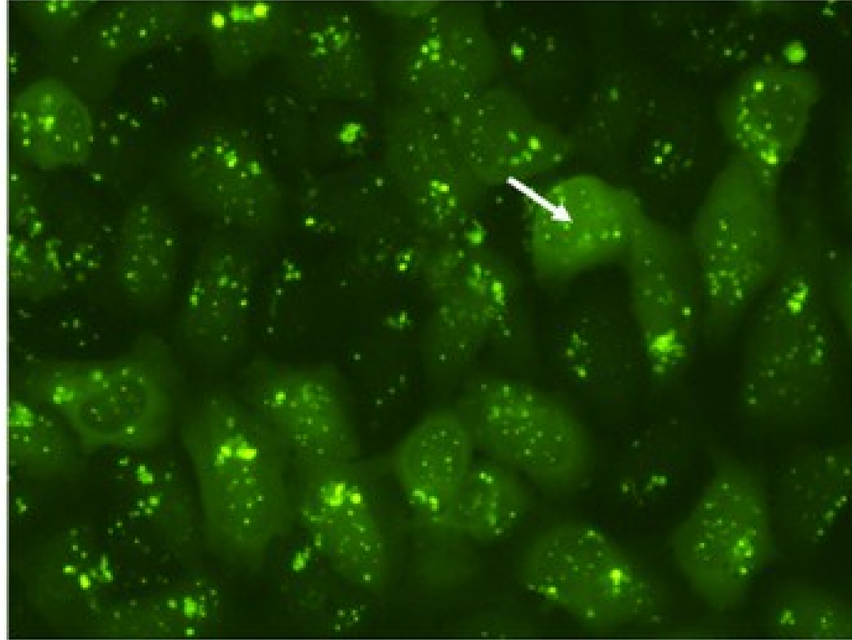


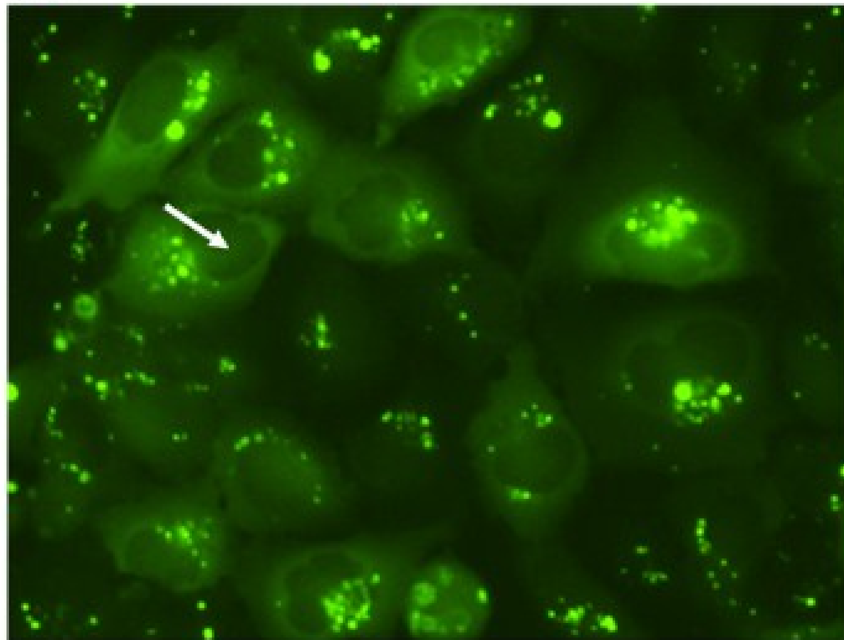
S5. Fluorescence spectroscopy images of cells treated with m_3G-CAP biotin construct complexes with STV-Alexa488 that did not display nuclear uptake.



Fluorescence microscopy images of the cells treated with STV-Alexa488 complexes with constructs **14-15**, **18-21** and **24**. Nuclear region of the cells are marked with a white arrow. Nuclear transport is evaluated by the Alexa-488 signal from the nucleus.

S6. Enlargements of fluorescence spectroscopy images of U2OS cells transfected with STV-Alexa488 complexes of constructs 22 and 23.

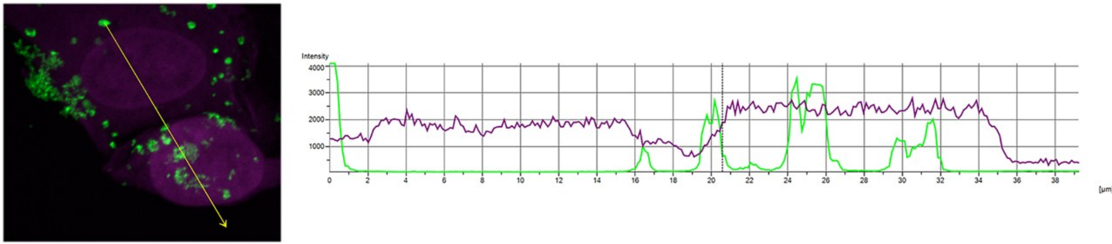




Fluorescence microscopy images of the cells treated with STV-Alexa488 complexes with constructs **22** and**23**. Nuclear region of the cells are marked with a white arrow. Nuclear transport is evaluated by the Alexa-488 signal from the nucleus.

S7. Histogram of fluorescent signal intensities across cells transfected with STV-Alexa488 complex of construct 23.

23



Confocal microcopy analysis of U2OS cells transfected with the complex of STV-Alexa488 with m3G-CAP construct **23**. Left panel shows the confocal image, whereas the right panel shows histograms that demonstrate fluorescent signal intensities along the indicated yellow line traversing the cells using NIS-Elements A1R software. In the histogram, x axis represents the length of the yellow line (left panel) and the y axis represents the signal intensity. For all images, contrast and brightness were changed for visualization purposes throughout the entire image. Purple lines represents **DRAQ5** nuclear stain and green represents Alexa488 signal.