mRNAs biotinylated within the 5’-cap and protected against decapping – new tools to capture RNA-protein complexes.

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Supplementary material

1.1. Supplementary Figures

Bednarek et al. “mRNAs biotinylated within the 5’-cap and protected against decapping – new tools to capture RNA-protein complexes” in theme issue of *Philosophical Transactions B* “RNA modifications and degradation”

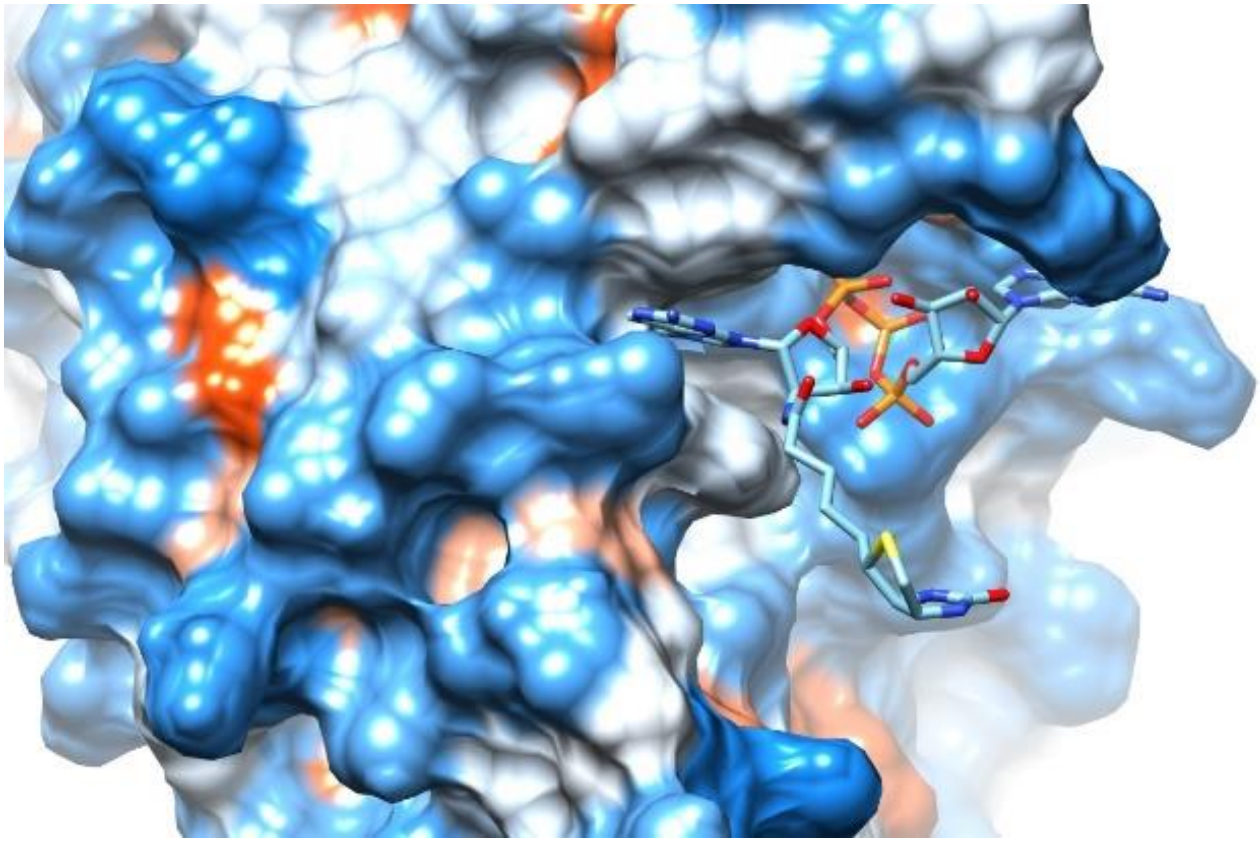

Fig. S1. Visualization of biotinylates cap analog in cap-binding pocket of eIF4E (pdb: 1ap8) *via* ligand-based active site alignment [1]. The surface colors reflect amino acid hydrophobicity in the Kyte-Doolittle scale with colors ranging from dodger blue for the most hydrophilic to white at 0.0 to orange red for the most hydrophobic [2].

[1] A. Heifets and R. H. Lilien, “LigAlign: Flexible ligand-based active site alignment and analysis,” *J. Mol. Graph. Model.*, vol. 29, no. 1, pp. 93–101, 2010.

[2] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, and T. E. Ferrin, “UCSF Chimera — A Visualization System for Exploratory Research and Analysis,” *J. Comput. Chem.*, no. 25, pp. 1605–1612, 2004.

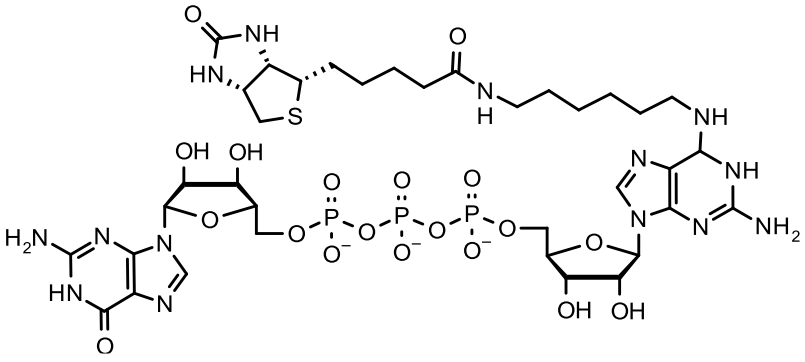

Fig. S2. Structure of biotin-hexamethylenediamine-ApppG (Biotin-ApppG)

Bednarek et al. “mRNAs biotinylated within the 5’-cap and protected against decapping – new tools to capture RNA-protein complexes” in theme issue of *Philosophical Transactions B* “RNA modifications and degradation”

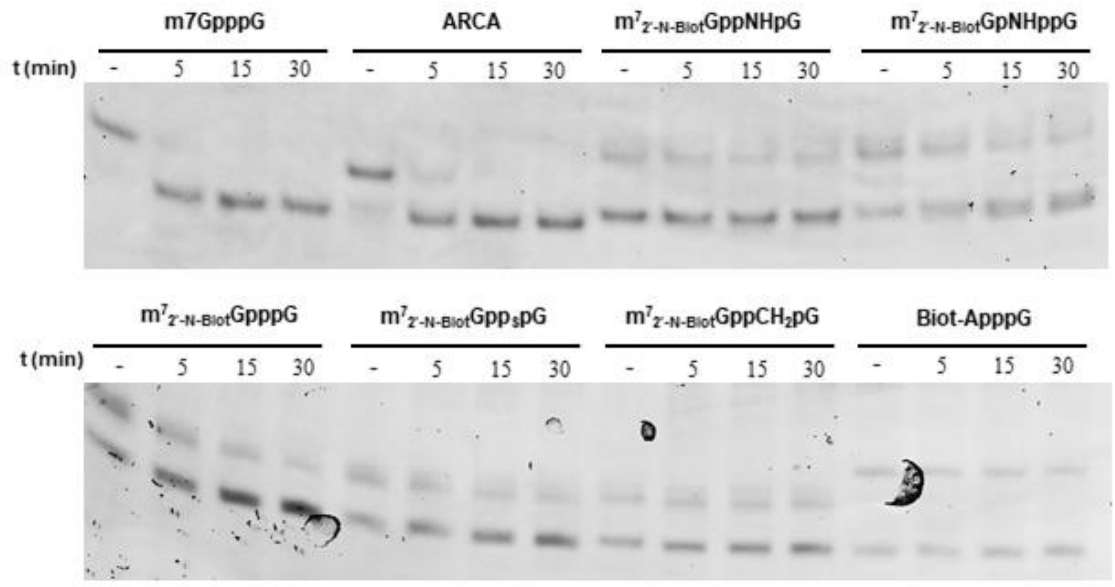

Fig. S3. Electrophoretic resolution of reaction products of short capped RNAs incubated with hDcp2 enzyme for indicated time periods.

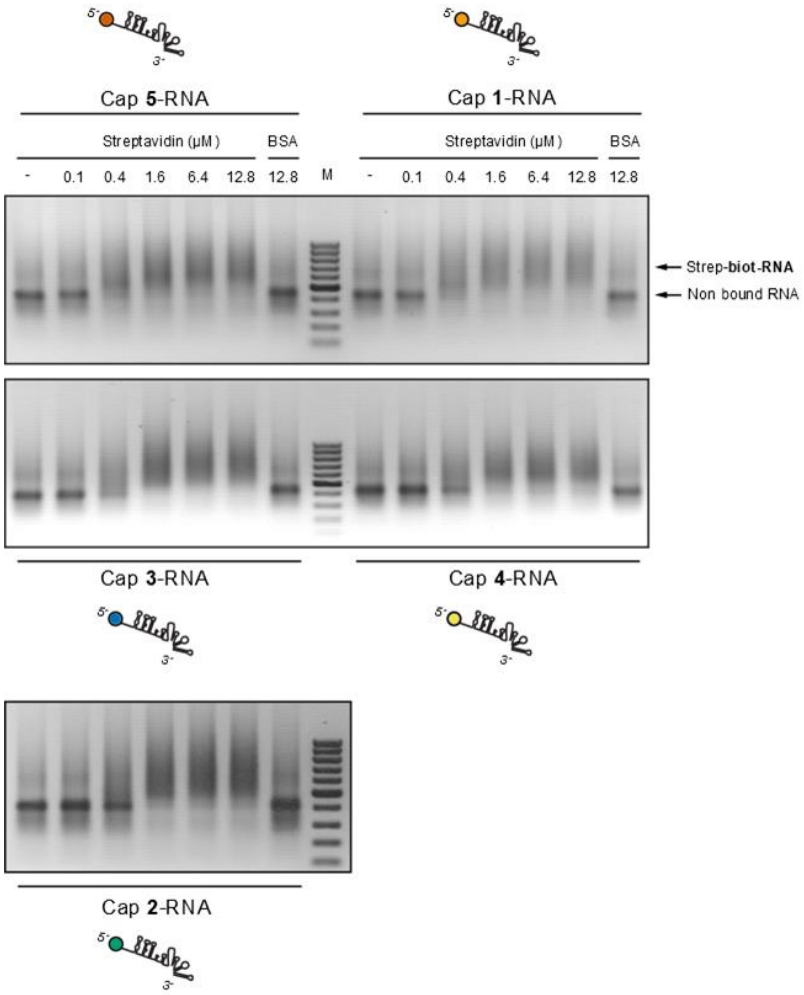

Fig. S4 Electrophoretic mobility shift assay of unbound RNA (non-biotinylated RNA) and biotinylated RNA-streptavidin complexes in 1 % agarose gels under non-denaturing conditions. Bands corresponding to unbound RNA and to RNA-streptavidin complexes are indicated by arrows. Capped RNAs without streptavidin, or incubated with BSA (bovine serum albumin) served as reference controls of RNA mobility.

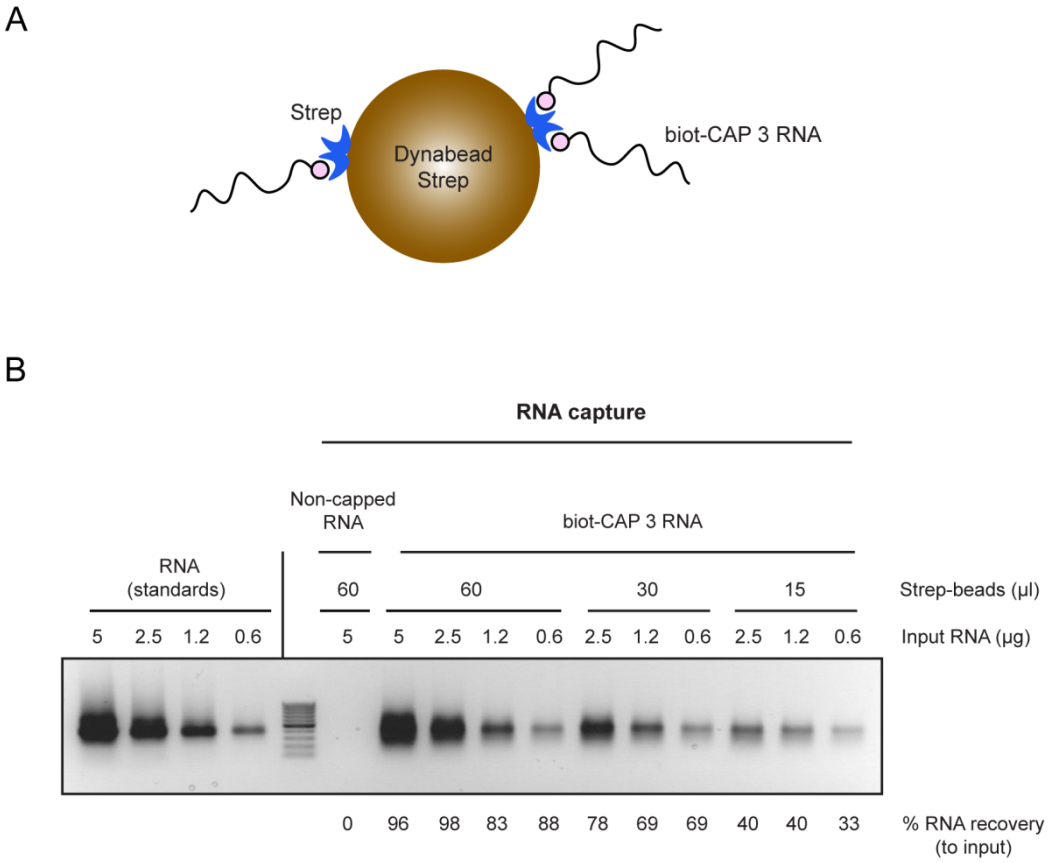

Fig. S5. Affinity capture of single-biotin capped 3´NTR of HCV. Schematic of biotinylated RNA-streptavidin binding on the surface of a magnetic dynabead (A). Affinity capture of a short HCV-derived RNA via biotin-cap analog **3** was established by titration of increasing amounts of RNA and streptavidin dynabeads. Different amounts of RNAs standards (on the left) were employed to measure the intensity of the bands by densitometry and generate a standard curve. The percentage of biotinylated RNA recovered upon affinity capture (numbers below the gel), under each experimental condition, was calculated by extrapolation of the corresponding densitometry values from the standard curve.

1.2. Chemical syntheses

GENERAL REMARKS

I. Solvents and reagents

Experiments were carried out using commercially available solvents of high purity (DMF, DMSO, $(MeO)_3PO$). Anhydrous solvents were stored over 4Å molecular sieves. Water was deionized and purified by Millipore Milli-Q system. Chemical reagents were purchased from Sigma-Aldrich (2’NH_2 G?) and used without any pre-treatment unless indicated otherwise.

II. Reaction monitoring

Reaction progress was assessed based on RP-HPLC profiles (Agilent Tech. Series 1200, Supelcosil LC-18-T 4.6x250 mm column, flow rate 1.3 ml/min, UV-detection at 260 nm, fluorescence excitation at 260, fluorescence detection at 337 nm). For analytical purposes linear gradient (0-25%) of methanol in 0.05 M ammonium acetate buffer (pH 5.9) was used.

III. Product purification

Nucleotides obtained as intermediate products were separated by ion-exchange on DEAE-Sephadex A-25 (HCO_3^- form) column. After loading the column with reaction mixture, it was rinsed with water to wash out EDTA/Zinc (ll) salt complexes. Then, linear gradient of triethylammonium bicarbonate (TEAB) in deionized water was applied. Four different gradients were used, depending on overall charge of target compound: 0–0.5 M for 2’-amine-2’-dexoyguanosine 5’-O-monphosphate, 0–0.7 M for

other monophosphate nucleosides and 2’-amine-2’-deoxyguanosine 5’-O-imidodiphosphate, 0–1.0 M for other diphosphate nucleosides, and 0–1.2 M for triphosphate nucleosides. Eluted fraction was evaporated under reduced pressure, with several additions of ethanol and acetonitrile to decompose TEAB. Compounds were isolated as triethylammonium (TEA) salts. Final products (cap analogues) were separated by semi-preparative HPLC (Agilent Tech. Series 1200, Discovery RP Amide C-16 (250x21.2 mm, 5 µm) column, flow rate 5 ml/min) with linear gradient (0-50%) of acetonitrile in 0.05 M ammonium acetate buffer (pH 5.9) with UV detection at 260 nm.

IV. Structure confirmation methods

^{1}H-NMR spectra were recorded at 399.94 MHz on a Varian UNITY-plus spectrometer spectrometer. Chemical shifts are reported in ppm as δ values from tetramethylsilane. Mass spectra (electrospray ionization) were recorded on a Thermo Scientific LTQ Orbitrap Velos (high resolution).

V. General procedures

GENERAL PROCEDURE I: SYNTHESIS OF PHOSPHORIMIDAZOLIDES

To a well-mixed suspension of an appropriate nucleotide (TEA salt, 1 equiv.), 10 equiv. of imidazole and 3 equiv. of 2-2’-dithiodipiridine in DMF, 3 equiv. of triphenylphosphine were added. The reaction mixture was stirred for 6-8 h. The product was precipitated with anhydrous $NaClO_4$ solution in dry acetone (10 volumes). The precipitate was cooled to 4 ºC and repeatedly centrifuged and rinsed with anhydrous acetone. The product was then dried over P_2O_5.

GENERAL PROCEDURE II: BIOTINYLATION

To a suspension of 1.5 equiv. of D-Biotin in 0.4 ml of DMSO 1.5 equiv. of TEA and 1.5 equiv. of O-(N-succinimidyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TSTU) were added. The solution was then shaken for 30 min at room temperature. Over the course of 1 h this mixture was then added portion-wise to a solution of an appropriate nucleotide in 0.5 borate buffer (pH 8.5). As the reaction proceeded, the pH of reaction mixture was adjusted to 8.5 with 10% aqueous solution of KOH. Once full conversion of substrate was attained, the reaction was neutralized with a few drops of 50% acetic acid.

2’-AMINO-2’-DEOXYGUANOSINE 5’-O-MONOPHOSPHATE (10)

330 mg (1.18 mmol) of 2’-amino-2’-deoxyguanosine **(1)** was suspended in 3 ml of trimethyl phosphate. The mixture was placed on ice-bath and cooled down to 0 ºC, then freshly distilled $POCl_3$ (6 equiv., 660 µl) was added. The reaction mixture was stirred for 8 h at 0 ºC, until full conversion of substrate was attained, as observed on HPLC profile. The reaction was stopped by dilution with 30 ml of 0.7 M TEAB (pH 7.0). Yield: 460 mg, 0.82 mmol, 70%

7-METHYL-2’-AMINO-2’-DEOXYGUANOSINE 5’-O-MONOPHOSPHATE (11)

460 mg (0.82 mmol) of 2’-amino-2’-deoxyguanosine monophosphate **(10)** (TEA salt) was suspended in 6 ml of DMSO. Methyl iodide (620 µl, 9.8 mmol) was then added to the reaction, and the mixture was stirred for 10 h at room temperature. The solution

was poured into water (60 ml) and extracted 3 times with diethyl ether (10 ml). Retained aqueous layer was brought to pH 7.0 with $NaHCO_3$. Yield: 155 mg, 0.27 mmol, 33%

7-METHYL-2’-AMINO-2’-DEOXYGUANOSINE 5’-O-MONOPHOSPHATE (11)

460 mg (0.82 mmol) of 2’-amino-2’-deoxyguanosine monophosphate **(7)** (TEA salt) was dissolved in 40 ml of deionized water. The solution was brought to pH 4.0 with dropwise addition of 50% acetic acid. Over the course of 2 hours 1.2 ml of dimethyl sulfate was added to the reaction mixture (12.4 mmol, 15 equiv.), which was then stirred for 8 h at room temperature. As the reaction proceeded, pH 4.0 was maintained with 10% aqueous solution of NaOH. Once the reaction completed, the solution was neutralized with $NaHCO_3$ and extracted 3 times with diethyl ether. Yield: 252 mg, 0.44 mmol, 54%

2’-AMINO-2’-DEOXYGUANOSINE 5’-O-IMIDODIPHOSPHATE (6)

190 mg (0.68 mmol) of 2’-amino-2’-deoxyguanosine **(commercially available)** was suspended in 7 ml of trimethyl phosphate. The solution was cooled down to -8 ºC. $Cl_3PNP(O)Cl_2$ was then added (1 mmol, 3 equiv.) under vigorous stirring. The reaction mixture was stirred for 6 h at -8 ºC. The reaction was then stopped by dilution with 70 ml of 0.7 M TEAB. Yield: 247 mg, 0.33 mmol, 49%

7-METHYL-2’-AMINO-2’-DEOXYGUANOSINE 5’-O-IMIDODIPHOSPHATE (7)

247 mg (0.33 mmol) of 2’-amiono-2’-deoxy 5’-O-imidodiphosphate **(6)** was dissolved in 3 ml of deionized water. The solution was brought down to pH 4.0 with dropwise addition of 50% acetic acid. Over 1 h period 157 µl of dimethyl sulfate (1.65 mmol, 5

equiv.) was added to the reaction mixture, which was then stirred for 5 h at room temperature. As the reaction proceeded, pH 4.0 was maintained with 10% aqueous solution of NaOH. Once completed, the reaction was neutralized with $NaHCO_3$ and extracted 3 times with diethyl ether. Yield: 117.5 mg, 0.156 mmol, 47%

GUANOSINE 5’-O-IMIDODIPHOSPHATE (15)

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1 g (3.5 mmol) of guanosine was suspended in 37.5 ml of trimethyl phosphate. The reaction mixture was cooled down to -8 ºC, then $Cl_3PNP(O)Cl_2$ (10.5 mmol, 3 equiv.) was added under vigorous stirring. The solution was stirred for 2 h at -8 ºC. The reaction was stopped by dilution with 100 ml of 0.7 TEAB. Yield: 860 mg, 1.16 mmol, 33%

GUANOSINE 5’-O-METHYLENEBISPHOSPHONATE (16)

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1 g (3.5 mmol) of guanosine was suspended in 15 ml of trimethyl phosphate. The reaction mixture was placed on ice-bath and cooled down to 0 ºC, then 2.6 g of methylenebis(phosphonic dichloride) (10.5 mmol, 3 equiv.) was added. The solution was stirred at 0 ºC. After 2 h, the reaction was stopped by dilution with 40 ml of 0.7 TEAB. Yield: 1037 mg, 1.4 mmol, 40%

7-METHYL-2’-N-BIOTIN-GUANOSINE 5’-O-MONOPHOSPHATE (12)

Applying general procedure II 252 mg of 7-methyl-2’-amino-2’-deoxyguanosine **(11)** (0.44 mmol) was conjugated with biotin. Yield: 225 mg,0.28 mmol, 64%

7-METHYL-2’-N-BIOTIN-GUANOSINE 5’-O-IMIDODIPHOSPHATE (8)

Applying general procedure II 117.5 mg of 7-methyl-2’-amino-2’-deoxyguanosine 5’-O-imidodiphosphate **(7)** (0.16 mmol) was conjugated with biotin. Yield: 112 mg, 0.114 mmol, 73%

7-METHYL-2’-N-BIOTIN-GUANOSINE 5’-O-MONOPHOSPHATE P-IMIDAZOLIDE (13)

Applying general procedure I 140 mg (TEA salt, 0.175 mmol) of 7-methyl-2’-N-Biotin-Guanosine 5’-O-monophosphate **(12)** was converted to its respective P-Imidazolide. Yield: 105 mg, 0.155 mmol, 89%

GUANOSINE 5’-O-METHYLENEBISPHOSPHONATE P-IMIDAZOLIDE (18)

Applying general procedure I 518 mg (TEA salt, 0.7 mmol) of Guanosine 5’-O-methylenebisphosphonate **(16)** was converted to its respective P-Imidazolide. Yield: 321 mg, 0.6 mmol, 86%

GUANOSINE 5’-O-MONOPHOSPHATE P-IMIDAZOLIDE (9)

Applying general procedure I GMP (TEA salt, obtained from 6 g of GMP disodium salt hydrate) was converted to its respective P-Imidazolide. Yield: 5815 mg

7-METHYL-2’-N-BIOTIN-GUANOSINE 5’-O-(2-THIODIPHOSPHATE) (14)

To a well-mixed suspension of **(13)** (Na salt, 60 mg, 0.09 mmol) and thiophosphate triethylammonium salt (0.21 mmol) in 1 ml of DMF, anhydrous $ZnCl_2$ (114 mg, 8 equiv.) was added. The solution was then stirred at room temperature. After 40 min, the

reaction was quenched with an aqueous solution (10 volumes) of disodium EDTA (313 mg) and $NaHCO_3$ (157 mg). Yield: 47 mg, 0.047 mmol, 53%

$M^7{}_{2'N\text{-}BIOT}GPP_SPG$ (3)

To a well-mixed suspension of **(14)** (TEA salt, 38 mg, 0.04 mmol) and **(9)** (Na salt, 43 mg, 0.09 mmol) in 0.7 DMF, anhydrous $ZnCl_2$ (102 mg, 16 equiv.) was added. The solution was then stirred at room temperature for 14 h. The reaction was quenched with an aqueous solution (10 volumes) of disodium EDTA (281 mg) and $NaHCO_3$ (140 mg). Yield: 12 mg, 0.011 mmol, 29%

^{1}H NMR (400MHz, D_2O) δ ppm: 9,18 (s, *slow exchange*, 1 H, H8 m^7G_{D1}), 9,15 (s *slow exchange*, 1 H, H8 m^7G_{D2}), 8,05 (s, 1 H, H8 G_{D1}), 8,00 (s, 1 H, H8 G_{D2}), 5,99 (d, J = 7,6 Hz, 1 H, H1’ m^7G_{D1}), 5,95 (d, J = 7,6 Hz, 1 H, H1’ m^7G_{D2}), 5,83 (d, J = 6,2 Hz, 1 H, H1’ G_{D1}), 5,79 (d, J = 6,2 Hz, 1 H, H1’ G_{D2}), 4,73 (dd, *overlaps with 4,70,* J = 5,5, 6,2 Hz, 1 H, H2’ G_{D1}), 4,70 (dd, , *overlaps with 4,73*, J = 5,5, 6,2 Hz, 1 H, H2’ G_{D2}), 4,64 - 4,53 (m, 6 H, H10 $Biot_{D1/D2}$, H3’ $m^7G_{D1/D2}$, H4’ $m^7G_{D1/D2}$), 4,53 - 4,45 (m, 3 H, H2’ m^7G_{D1}, H3’ $G_{D1/D2}$), 4,38 - 4,27 (m, 13 H, H2’ m^7G_{D2} H4’ $G_{D1/D2}$, H5’ H5’’ $G_{D1/D2}$, H7 $Biot_{D1/D2}$, H5’ H5’’ $m^7G_{D1/D2}$), 4,13 (s, 6 H, N^7-CH_3 $m^7G_{D1/D2}$), 3,22 (dt, J = 4,9, 9,6 Hz, 2 H, H6 $Biot_{D1/D2}$), 2,96 (dd, J = 4,9, 13,1 Hz, 2 H, H9a $Biot_{D1/D2}$), 2,75 (d, J = 13,1 Hz, 2 H, H9b $Biot_{D1/D2}$), 2,32 - 2,23 (m, 4 H, 2xH2 $Biot_{D1/D2}$), 1,73 - 1,38 (m, 8 H, H5a 2xH3 H5b $Biot_{D1/D2}$), 1,27 - 1,05 (m, 4 H, H4a H4b $Biot_{D1/D2}$); ^{31}P NMR (160 MHz, D_2O) δ ppm: 30,73 (t, *overlaps with 30,44*, J = 26 Hz, 1 P, P_β D1), 30,44 (t, *overlaps with 30,73*, J = 26 Hz, 1 P, P_β D2), -11,71 (m, 4 P, P_α and P_γ D1/D2);

$M^7_{2'N\text{-}BIOT}GPPNHPG$ (4)

To a well-mixed suspension of **(13)** (Na salt, 18 mg, 0.026 mmol) and **(15)** (TEA salt, 19 mg, 0.026 mmol) in 1.5 ml of DMF, anhydrous $ZnCl_2$ (90 mg) was added. The solution was stirred at room temperature. After 24 h, the reaction was quenched with an aqueous solution (10 volumes) of disodium EDTA (247 mg) and $NaHCO_3$ (123 mg). Yield: 4.3 mg, 0.004 mmol, 15%

^{1}H NMR (400MHz, D_2O) δ ppm: 5,98 (d, J = 7,8 Hz, 1 H, H1’ m^7G), 5,83 (d, J = 5,6 Hz, 1 H, H1’ G), 4,71 – 4,66 (m, *overlaps with D_2O*, 1 H, H2’ G), 4,59 (dd, J = 4,9, 8,0 Hz, 1 H, H10 Biot), 4,53 (dd, J = 1,2, 5,8 Hz, 1 H, H3’ m^7 G), 4,50 - 4,45 (m, 2 H, H4’ m^7G, H3’ G), 4,38 - 4,14 (m, 7 H, H2’ m^7G, H4’ G, H5’ H5’’ G, H7 Biot, H5’ H5’’ m^7G), 4,12 (s, 3 H, N^7-CH_3), 3,25 - 3,16 (m, 2 H, H6 Biot, TEA), 2,96 (dd, J = 4,9, 13,1 Hz, 1 H, H9a Biot), 2,74 (d, J = 13,1 Hz, 1 H, H9b Biot), 2,35 - 2,21 (m, 2 H, 2xH2 Biot), 1,69 - 1,40 (m, 4 H, H5a 2xH3 H5b Biot), 1,33 - 1,20 (m, 2 H, H4a Biot, TEA), 1,16 - 1,03 (m, 1 H, H4b Biot); ^{31}P NMR (160 MHz, D_2O) δ ppm: -1,05 (m,1 P, P_α), -10,71 (m, 2 P, P_γ , P_β);

$M^7_{2'N\text{-}BIOT}GPNHPPG$ (2)

To a well-mixed suspension of **(8)** (TEA salt, 98 mg, 0.1 mmol) and **(9)** (Na salt, 112 mg, 0.25 mmol) in 1 ml of DMF, anhydrous $ZnCl_2$ (320 mg) was added. The solution was stirred at room temperature. After 9 h the reaction was quenched with an aqueous solution (10 volumes) of disodium EDTA (874 mg) and $NaHCO_3$ (437 mg). Yield: 9 mg, 0.008 mmol, 8%

^{1}H NMR (400 MHz, D_2O) δ ppm: 8,02 (s, 1 H, H8 G), 5,96 (d, J = 7,4 Hz, 1 H, H1’ m^7G), 5,8 (d, J = 5,9 Hz, 1 H, H1’ G), 4,67 (dd, J = 5,5, 5,9 Hz, 1 H, H2’ G), 4,58 (dd, J = 8,0, 4,9 Hz, 1 H, H10 Biot), 4,53 (dd, J = 5,8, 1,2 Hz, 1 H, H3’ m^7G), 4,46 – 4,49 (m, 2 H, H4’ m^7G, H3’ G), 4,19 - 4.37 (m, 7 H, H2’ m^7G, H4’ G, H5’ H5’’ G, H7 Biot, H5’ H5’’ m^7G), 4,11 (s, 3 H, N^7-CH_3), 3,21 (dt, J = 4,9, 9,4 Hz, 1 H, H6 Biot), 2,95 (dd, J = 4,9, 13,1 Hz, 1 H, H9a Biot), 2,74 (d, J = 13,1 Hz, 1 H, H9b Biot), 2,24 – 2,31 (m, 2 H, 2xH2 Biot), 1,43 – 1,67 (m, 4 H, H5a 2xH3 H5b Biot), 1,20 – 1,30 (m, 1 H, H4a Biot), 1,05 – 1,14 (m, 1 H, H4b Biot); ^{31}P NMR (160 MHz, D_2O) δ ppm: -0,86 (m,1 P, P_γ), -11,25 (m, 1 P, P_α), -11,59 (m, 1 P, P_β);

$M^7_{2'N\text{-}BIOT}GPPCH_2PG$ (5)

I. To a well-mixed suspension of **(16)** (TEA salt, 13 mg, 0.022 mmol) and **(13)** (Na salt, 15 mg, 0.022 mmol) in 1.3 ml DMF, anhydrous $ZnCl_2$ (195 mg) was added. The solution was stirred at room temperature. After 9 h the reaction was quenched with an aqueous solution (10 volumes) of disodium EDTA (538 mg) and $NaHCO_3$ (270 mg). Yield: 4.6 mg, 0.0034 mmol, 15%

II. To a well-mixed suspension of **(12)** (TEA salt, 62 mg, 0.1 mmol) and **$GpCH_2p$-Im (18)** (Na salt, 71 mg, 0.14 mmol) in 1.2 ml DMF, anhydrous $ZnCl_2$ (65 mg) was added. The solution was stirred at room temperature. After 5 h the reaction was quenched with an aqueous solution (10 volumes) of disodium EDTA (178 mg) and $NaHCO_3$ (89 mg). Yield: 7 mg, 0.006 mmol, 6%

^{1}H NMR (400MHz, D_2O) δ ppm: 8,05 (s, 1 H, H8 G), 6,00 (d, J = 7,6 Hz, 1 H, H1’ m^7G), 5,82 (d, J = 6,0 Hz, 1 H, H1’ G), 4,72 (dd, J = 5,5, 6,0 Hz, 1 H, H2’ G), 4,60 (dd, J = 5,0, 8,0 Hz, 1 H, H10 Biot), 4,54 (dd, J = 1,2, 5,7 Hz, 1 H, H3’ m^7G), 4,52 - 4,47 (m, 2

H, H4’ m^7G, H3’ G), 4,39 - 4,15 (m, 7 H, H2’ m^7G, H4’ G, H5’ H5’’ G, H7 Biot, H5’ H5’’ m^7G), 4,13 (s, 3 H, N^7-CH_3), 3,22 (m, 1 H, H6 Biot), 2,97 (dd, J = 4,9, 13,1 Hz, 1 H, H9a Biot), 2,76 (d, J = 13,1 Hz, 1 H, H9b Biot), 2,39 (d, J = 20,5 Hz, 2 H, CH_2), 2,32 - 2,25 (m, 2 H, 2xH2 Biot), 1,69 - 1,43 (m, 4 H, H5a 2xH3 H5b Biot), 1,28 - 1,23 (m, 1 H, H4a Biot), 1,17 - 1,06 (m, 1 H, H4b Biot); ^{31}P NMR (160 MHz, D_2O) δ ppm: 17,11 (m,1 P, P_α), 7,67 (m, 1 P, P_β), -11,4 (m, 1 P, P_γ);

$M^7_{2'N\text{-}BIOT}GPPPG$ (1)

To a well-mixed suspension of **(12)** (TEA salt, 62 mg, 0.1 mmol) and **GDP-Im (17)** (Na salt, 55 mg, 0.1 mmol) in 1.2 ml DMF, anhydrous $ZnCl_2$ (235 mg) was added. The solution was stirred at room temperature. After 9 h the reaction was quenched with an aqueous solution (10 volumes) of disodium EDTA (470 mg) and $NaHCO_3$ (235 mg). Yield: 15 mg, 0.014 mmol, 14%

1.3. Supplementary methods

Experimental details for the EMSA assay.

RNA transcripts (3.75 pmol) were thermally denatured for 8 min at 98°C and then cool down on ice prior incubation with increasing amounts of tetrameric streptavidin (Promega). The electrophoretic mobility of unbound RNA (non-biotinylated RNA) and biotinylated RNA-streptavidin complexes was analyzed in 1% agarose gels under non-denaturing conditions. Bovine serum albumin (BSA) was used as negative control. Bands corresponding with unbound RNA and slower mobility bands, corresponding with RNA-streptavidin complexes, are indicated by arrows (Fig. S3). Less intense and slower migrating bands in absence of strep correspond with a small proportion of

refolded RNA. The proportion of unbound RNA observed in presence of high excess of streptavidin corresponds with non-capped RNA synthesized during *in vitro* transcription. The proportion of non-capped RNA was calculated by dividing the densitometry values obtained from the band corresponding to unbound RNA (at the highest concentration of streptavidin) to the value of the band representing total RNA (i.e. in absence of streptavidin). The percentage of RNA capped with different cap analogs in independent *in vitro* transcription reactions was 80-99% and 61% for RNA containing cap analog with $–CH_2$ group.