REPORT

Triazole-containing monophosphate mRNA cap analogs as effective translation inhibitors

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ABSTRACT

Synthetic analogs of the 5' end of mRNA (cap structure) are widely used in molecular studies on mechanisms of cellular processes such as translation, intracellular transport, splicing, and turnover. The best-characterized cap binding protein is translation initiation factor 4E (eIF4E). Recognition of the mRNA cap by eIF4E is a critical, rate-limiting step for efficient translation initiation and is considered a major target for anticancer therapy. Here, we report a facile methodology for the preparation of N2-triazole-containing monophosphate cap analogs and present their biological evaluation as inhibitors of protein synthesis. Five analogs possessing this unique hetero-cyclic ring spaced from the m7-guanine of the cap structure at a distance of one or three carbon atoms and/or additionally substituted by various groups containing the benzene ring were synthesized. All obtained compounds turned out to be effective translation inhibitors with IC₅₀ similar to dinucleotide triphosphate m⁷GpppG. As these compounds possess a reduced number of phosphate groups and, thereby, a negative charge, which may support their cell penetration, this type of cap analog might be promising in terms of designing new potential therapeutic molecules. In addition, an exemplary dinucleotide from a corresponding mononucleotide containing benzyl substituted 1,2,3-triazole was prepared and examined. The superior inhibitory properties of this analog (10-fold vs. m⁷GpppG) suggest the usefulness of such compounds for the preparation of mRNA transcripts with high translational activity.

Keywords: mRNA; cap-dependent translation; translation inhibitor; cap analog; click chemistry; 1,2,3-triazole

INTRODUCTION

All cellular eukaryotic mRNAs have at their 5' end a unique m⁷GpppN cap structure (Fig. 1) composed of a 7-methylguanosine connected with the first transcribed nucleotide (N) via a 5',5'-triphosphate bridge (Furuichi and Shatkin 2000). The cap plays a crucial role in several cellular processes, such as pre-mRNA splicing (Sharp 1994), transport of RNA from the nucleus to the cytoplasm (Lewis and Izaurralde 1997), protection of mRNA against degradation (Rhoads 1985), and translation initiation (Gingras et al. 1999). Over the years, chemically modified cap analogs have been used for biochemical, biophysical, and structural studies on mechanisms of the aforementioned processes (Niedzwiecka et al. 2002; Grudzien et al. 2004; Jankowska-Anyszka et al. 2011; Ziemniak et al. 2013). So far, some of the most intense research directed toward the application of synthetic cap analogs has been focused on designing translation inhibitors that prevent binding of mRNA to eIF4E. Interaction of the eukaryotic initiation factor 4E (eIF4E) with the mRNA cap

structure is crucial and a rate-limiting step of protein synthesis (Sonenberg 1996; Rhoads 2009). Under physiological conditions, the cellular level of eIF4E is low, which constitutes one of the mechanisms enabling regulation of gene expression at the translational level. The idea of using cap analogs for therapeutic purposes emerged with reports that an elevated level of eIF4E is associated with tumor formation and progression in human malignancies and cancers of the breast, colon, bladder, prostate, lung, head, and neck (Clemens and Bommer 1999; De Benedetti and Graff 2004; Mamane et al. 2004). As the cap is essential for efficient binding with the eIF4E protein, to date several structural features for the effectiveness of this interaction have been determined (Darzynkiewicz et al. 1985; Carberry et al. 1990; Marcotrigiano et al. 1997; Matsuo et al. 1997; Cai et al. 1999; Shen et al. 2001). It was shown that elements such as (1) the positive charge of the imidazole ring of guanosine, (2) the presence of at least two negative charges (or a negative charge and

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FIGURE 1. mRNA cap structure.

the free electron pair) within the phosphate chain, and (3) the presence of at least one hydrogen atom on the amino group of the 7-methylguanosine should be preserved in order to synthesize an efficient translation inhibitor. To date, numerous modified cap analogs have been tested as inhibitors of cap-dependent translation in vitro by competing with mRNA for the binding site of eIF4E (Cai et al. 1999; Grudzien et al. 2004; Ghosh et al. 2005; Jia et al. 2010; Piecyk et al. 2012; Ziemniak et al. 2013). Unfortunately, due to their charged nature, in vivo application of such analogs is strictly limited. The first step to circumvent this problem might be designing nucleotide analogs with the least phosphate groups possible (mononucleotides monophosphates) that possess other, e.g., base modifications that provide an efficient level of inhibition. It was previously shown that a single substitution at the N2 position of the first (methylated) guanine moiety leads to increased translation inhibition (Cai et al. 1999). Based on these findings, we decided to introduce a new type of N2 substituents and evaluate the ability of obtained monophosphate analogs to inhibit the cap-dependent translation. We have chosen triazole-based groups as they not only exhibit excellent biological properties in the context of medicinal chemistry (Tron et al. 2008; Hein and Fokin 2010) but are also stable to oxidation and reduction and resistant to metabolic degradation (De las Heras et al. 1979). In this report, we describe the synthetic route for the preparation of N2-triazole-containing 7-methylguanosine-5'-monophosphates (mononucleotide cap analogs), and their biological evaluation as effective inhibitors of a cap-dependent translation. These types of compounds have not been described so far in the chemistry of mRNA cap analogs and have not been evaluated in vitro.

RESULTS AND DISCUSSION

Chemical synthesis

The triazole ring that was planned to be introduced within cap analogs can be easily generated by a copper (I) catalyzed

version of the Huisgen 1,3 dipolar cycloaddition (CuAAC) between azides and terminal alkynes, which is one of the most popular “click chemistry” methodologies. This highly versatile reaction requiring mild conditions has gained over the past years enormous attention in pharmaceutical sciences and drug discovery (Soltis et al. 1996; Johnson et al. 2008; Huang et al. 2013). During the past few years, a burst in the number of publications on the topic has occurred; likewise, in the area of nucleic acids, the CuAAC chemistry has been used to synthesize a number of modified nucleosides, nucleotides, and oligonucleotides for a broad range of applications (Ami and Fujimoto 2008; Seela and Sirivolu 2008; Seela et al. 2008; Amblard et al. 2009; El-Sagheer and Brown 2010). The widespread use of the CuAAC reaction is associated with the fact that both alkyne and azide functional groups can be incorporated into a wide range of compounds by several general methods. As a result of this, various strategies for the preparation of cap analogs containing 1,2,3-triazole substituents at the N2 position may be theoretically applied. Analyzing all technical aspects for the synthesis of such derivatives having this unique hetero-cyclic triazole ring (1) directly adjacent to the guanine ring, (2) spaced from the purine ring at a distance of one or three carbon atoms, or (3) additionally substituted by another aromatic ring such as benzene, we decided to explore whether a reliable efficient method(s) for their preparation could be found. Therefore, to synthesize guanosine analogs containing a triazole ring directly adjacent to the purine ring, we planned to introduce an azide group at the guanine N2 position through direct substitution of an appropriate intermediate (2) and subsequently perform the CuAAC reaction (Supplemental Scheme S1). Thus, guanosine was converted to its fully (ribose and O6 guanine) protected analog (1) through O-acylation and the Mitsunobu reaction, followed by introduction of the fluorine atom under anhydrous conditions with *t*-butyl nitrite (*t*BuONO) as the diazotizing agent and HF as the fluoride source (Scheme 1; Piecyk et al. 2012). The obtained 2-fluorinosine (2) was subjected to nucleophilic substitution with sodium azide. Unfortunately, the preparation of 2-azidinosine was unsatisfactory, so we postulated that it might have been a consequence of spontaneous cyclization of azide substituted π -deficient nitrogen hetero-cycles to the corresponding fused tetrazole (Supplemental Fig. S1; Johnson et al. 1958; Temple et al. 1966a,b). It was previously speculated that some of the lower-yielding azide-alkyne ligations involving a C-2 azidopurine nucleoside could be due to the presence of the tetrazolyl tautomer (Amblard et al. 2009), and it was shown that the tetrazole-azide equilibrium depends on the temperature and the solvent polarity (Lakshman et al. 2010). Given these unsatisfactory results of obtaining 2-azidinosine, we considered another route to triazole-containing cap analogs using the methodology shown in Scheme 2. We decided to perform a 1,3-dipolar cycloaddition reaction at the final stage of the synthesis using as substrates the methylated nucleotides possessing pent-4-ynyl

SCHEME 1. Synthesis of the N²-fluoro-2',3',5'-O-triacetyl-O⁶-[2-(4-nitrophenyl)ethyl]inosine.

or propargyl substituent at the N2 position and one of five selected azide derivatives (Fig. 2). For this, we used 2-fluoroinosine (2) as a starting material and performed a substitution reaction with propargyl amine or pent-4-yn-1-amine, leading to **3a** and **3b**, respectively and their selective deprotection (Scheme 2). Subsequently, the N2-modified guanosine derivatives (**3**) were 5'-phosphorylated (Scheme 3) using the Yoshikawa method (Yoshikawa et al. 1967) with phosphorus oxide trichloride in trimethyl phosphate at 4°C (giving **4a–b**) and further methylated at the N⁷ position of the guanine ring with CH₃I in DMSO at RT, leading to compounds **5a–b**. Purification of the analogs was done using ion-exchange chromatography on DEAE–Sephadex A-25 (HCO₃[−] form). The obtained mononucleotides and selected azides (Fig. 2) were converted into triazole-containing analogs via a “click” reaction (Scheme 3). Due to the fact that the substrates (**5a–b**) and reaction products (**6a–e**) had exactly the same negative net charge, separation of the final products from the reaction mixtures using ion-exchange chromatography may have become problematic. In order to assure quantitative conversion of substrates into desired products, several trials were conducted to determine optimal reaction conditions such as solvent, copper ion concentration, reaction time, and temperature. Among all tested reaction parameters, DMF with a small amount of H₂O ensuring complete dissolution of the reagents, a temperature of 50°C, and the concentration of copper ions 1.3 eq. in relation to the nucleotide turned out to be the most effective. Another important factor that allowed us to obtain efficiently triazole-containing cap analogs was usage as a coupling reaction catalyst Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (Chan et al. 2004). This polytriazolylamine ligand stabilized Cu(I) toward disproportionation and oxidation and, thus, enhanced its catalytic effect. The conducted experiments have shown that,

regardless of the azide used, the reaction proceeded quantitatively when suitable coupling conditions, argon atmosphere, and TBTA were applied. In this manner, five new cap analogs containing the triazole ring at the N2 position (**6a–e**) (Table 1) were obtained and purified on ion-exchange DEAE–Sephadex resin. The structure and homogeneity of final products were confirmed by HPLC, MS, ¹H NMR, and ³¹P NMR. For biological studies, compounds **6a–e** were subsequently rechromatographed on a semipreparative reversed-phase HPLC column and isolated as ammonium salts.

Given these satisfactory results, we managed to develop a methodology that simplifies the synthesis, assures higher solubility of intermediates in solvents used in the “click” reaction, and eases product isolation and purification. Based on biological data described in the next paragraph, one of the mononucleotide cap analogs (**6b**) was converted into its dinucleotide counterpart (**7**). For the preparation of the desired compound, we used the well-known strategy in which the triphosphate bridge is created via a reaction between two mononucleotide units, one of which has been converted to a reactive imidazolide derivative (Kadokura et al. 1997). For this, in the first step, the imidazolide derivative of GDP (imGDP) was prepared through reaction with imidazole in the presence of 2,2'-dithiodipyridine and triphenylphosphine. Subsequently, the resultant imGDP was coupled with compound **6b** in anhydrous conditions in the presence of zinc ions, giving dinucleotide **7** (Supplemental Scheme S2).

Biology

To evaluate the ability of newly synthesized triazole-containing monophosphates to inhibit cap-dependent translation in a cell-free translation system, the previously reported procedure was used (Kowalska et al. 2009). The chosen method,

SCHEME 2. Preparation of N2 modified guanosine derivatives containing alkynyl substituents.

FIGURE 2. Azides used for the “click” reaction.

although less direct than binding in a purified system, is preferred as it mimics competition between the cap analog and mRNA in a system containing all natural components. Briefly, experiments were performed in a micrococcal nuclease-treated rabbit reticulocyte lysate using an *in vitro*-transcribed, ARCA-capped, β -globin 5' UTR containing mRNA encoding firefly luciferase to allow determination of protein synthesis by luminometry. Three standard cap analogs were used as controls in the experiments. m^7 GTP and m^7 GpppG were positive controls, as both are known to be effective translation inhibitors in various cell-free systems (Cai et al. 1999; Grudzien et al. 2004), and m^7 GMP was a negative control that has very little or no inhibitory capacity (Cai et al. 1999). It is well known that the largest determinants of inhibitory activity for m^7 G cap analogs are the phosphate groups (Cai et al. 1999). The strength of translation inhibition increases ~ 20 -fold for m^7 GpppG and ~ 50 -fold for m^7 GTP as compared to m^7 GMP.

In the present work, we reveal that synthesized triazole-containing monophosphate cap analogs efficiently inhibit translation similarly to m^7 GpppG. These results are promising in the context of designing new cap analogs that can be used as novel inhibitors of protein synthesis. We have observed that, in general, presence of a triazole ring within the N2 position of a guanine ring is sufficient to make a simple 5'-monophosphate cap analog a good translation inhibitor (Fig. 3). The determined IC_{50} values (Table 1) are relatively similar for all tested analogs; nevertheless, some trends can be definitely outlined.

The first explored factor was the distance between the triazole ring and the purine base and its influence on the ability

of cap analogs to inhibit cap-dependent translation. Two (**6b** and **6e**) out of five analogs were designed to differ only in the number of carbon atoms spacing both rings (one and three, respectively). Comparing IC_{50} values for compounds **6b** and **6e**, only a slight increase in inhibitory properties of the analog **6b** vs. **6e** was observed. This may suggest that the spacer length does not have a great impact on the effectiveness of cap analogs as translation inhibitors. A clear improvement of inhibitory properties was observed for compounds with the substituted triazole ring (**6b–d**) as compared to compound **6a**. Interestingly, introduction of an additional oxygen hetero-atom close to the benzene ring affected considerably the ability of the cap analog to inhibit translation (about twofold for **6c** and **6d** vs. **6b**). Introduction of further electro-donor substituents (e.g., methoxy) into the benzene ring did not decrease the IC_{50} value. Out of all tested monophosphate analogs, the best properties were observed for compounds **6c** and **6d**. Designated IC_{50} values for these compounds indicated that the strength of inhibition is similar to m^7 GTP which is known and used as a good translation inhibitor, and it is at least 10 times higher than for the corresponding 7-methylated monophosphate (m^7 GMP). The performed studies suggest that an appropriate substituent at the N2 position of guanine compensates for the absence of the two phosphate groups. As a consequence, the net negative charge of the cap analogs—a key factor related to the inability of compounds to cross the cell membrane—is decreased.

Taking all this into account, we decided to explore the effect of the N2 modification within the guanine ring on the inhibitory properties of the dinucleotide cap analog. This idea has appeared due to the fact that, in the case of analogs having various modifications at the N7 position, it was shown that, while the presence of a suitable substituent (e.g., benzyl or *p*-chlorobenzyl) caused increased (several fold) inhibitory properties of mononucleotide monophosphates, this effect was no longer visible for the corresponding dinucleotide triphosphates (Cai et al. 1999). To perform these studies, we

SCHEME 3. Synthesis of triazole-containing cap analogs.

TABLE 1. Inhibition of translation in rabbit reticulocyte lysate extract by N2-modified cap analogs

N°	Cap analogue structure	IC ₅₀ (μM)
6a		14.4 +/- 1.1
6b		10.5 +/- 1.3
6c		2.0 +/- 0.1
6d		5.0 +/- 0.5
6e		8.5 +/- 1.6
7		0.8 +/- 0.2
	m ⁷ GTP	2.3 +/- 0.2
	m ⁷ GpppG	8.6 +/- 0.7
	m ⁷ GMP	>50

decided to convert compound **6b** into a dinucleotide (**7**) and test it analogously as monophosphates. It turned out that the obtained analog exhibited superior inhibitory properties (~ 10 -fold vs. **6c** and threefold vs. $m^7\text{GTP}$). This observation emphasizes that the character of the modifications at the N7 and N2 positions is diverse and the influence of the N2 substituents on the ability of the cap analog to inhibit protein synthesis, as opposed to analogs modified at the N7 position, can still be beneficial for both mono- and dinucleotides.

In conclusion, the obtained analogs are good translation inhibitors and can be used as potential therapeutics in the future due to the fact that they possess a reduced number of phosphate groups, which may support their cell penetration. In addition to that, superior inhibitory properties for the examined dinucleotide analog suggest that this type of compound (N2 modified) may also be useful for a preparation of mRNA transcripts with high translational activity.

MATERIALS AND METHODS

Inhibition of cap-dependent translation in vitro in RRL

An inhibition assay of the cap-dependent translation in vitro in a micrococcal nuclease-treated RRL lysate (Flexi Rabbit Reticulocyte Lysate System, Promega) was performed as previously described (Kowalska et al. 2009).

A typical translation reaction contained: 56% of RRL lysate, 0.01 mM amino acids mixture, 0.32 units/ μL RiboLock Ribonuclease Inhibitor (Fementas), magnesium acetate, potassium acetate, and $m^2,3'\text{OGpppG}$ -capped (Stepinski et al. 2001) luciferase mRNA transcript (in 12.5 μL final volume). Capped and polyadenylated luciferase mRNAs were obtained via transcription in vitro with SP6 RNA polymerase on the dsDNA template encoding: 5' UTR sequence of rabbit β -globin mRNA following by the firefly luciferase ORF and a stretch of 31 adenines. The obtained RNA transcripts, after removal of DNA template with RQ1 DNase (Promega), were purified using

NucAway Spin columns (Ambion) and used in the translation reactions in RRL at a final concentration 5 ng/ μL . Optimal conditions for cap-dependent translation in vitro in the used batch of Flexi RRL lysate (3.2 mM concentration of endogenous Mg^{2+}) were achieved at 0.6 mM concentration of magnesium acetate and 210 mM of potassium acetate. At these conditions, ApppG-capped luciferase RNA showed minimal translational activity that was insensitive to inhibition with the $m^7\text{GpppG}$ cap analog (Supplemental Fig. 2). Translation reactions were pre-incubated at 30°C for 60 min prior to addition of luciferase mRNA and appropriate dilution of a tested cap analog, and incubated further at 30°C. Reactions were stopped after 60 min after addition of mRNA by chilling on ice. The pre-incubation step was described in Kowalska et al. (2009) and was used in all of the further experiments that were performed in our lab. Using the same conditions allows comparison of data obtained for various cap analogs. To measure the luciferase activity, 2.5 μL of the translation reaction was mixed with 40 μL of Luciferase Assay Reagent (Promega), and a luminescence signal was detected in a luminometer (Glomax, Promega). The obtained data were used to determine IC_{50} values for inhibition of cap-dependent translation by cap analogs as previously described (Kowalska et al. 2009).

Chemical synthesis

All used reagents were purchased in the highest available purity from Sigma-Aldrich Chemical Co. and were used without any further treatment, except for the benzyl azides, which were prepared by treating the corresponding benzyl bromides with NaN_3 in DMF. TLC analysis for N2 modified nucleosides was performed on TLC plates coated with silicagel 60 F-254 (Merck), developed in methylene chloride-methanol-triethylamine 9:1:0.1 (v/v/v). Triethylammonium bicarbonate (TEAB) buffer was prepared by bubbling CO_2 through an ice-cold aqueous solution of redistilled triethylamine. Intermediate nucleotides and final products were separated by ion-exchange chromatography on a DEAE-Sephadex A-25 (HCO_3^- form) using a linear gradient of TEAB buffer (pH 7.6). Fractions containing products were combined, evaporated under reduced pressure with several additions of ethanol, and isolated as

FIGURE 3. Inhibition of $m^2,3'\text{OGpppG}$ -capped-luciferase mRNA translation in RRL lysate by novel N²-substituted $m^7\text{GMP}$ analogs: (A,B) **6b** (●), **6a** (○), **6e** (△), and **6d** (□). An example of inhibition by the dinucleotide cap analog **7** (★) derived from compound **6b** is also shown (B). Standard unmodified compounds $m^7\text{GTP}$ (■) and $m^7\text{GpppG}$ (▲) are included as controls. The figure shows fitted curves to the experimental data points (as described in Materials and Methods). The unmodified $m^7\text{GMP}$ (up to 200 μM) does not inhibit cap-dependent translation in the same experimental set-up.

triethylammonium salts (TEA salts). HPLC was performed on a Knauer instrument using a Supelcosil LC-18-T RP column (4.6 × 250 mm, flow rate 1.0 mL min⁻¹) with a linear gradient of methanol from 0% to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9). UV detection was performed at 254 nm. Exact mass measurements were recorded on a Micromass QToF spectrometer using electrospray ionization. ¹H NMR spectra were recorded on a Varian INOVA 200 and 400 MHz spectrometers using tetramethylsilane (TMS) as the internal standard in CDCl₃ and sodium 3-trimethylsilyl-[2,2,3,3-D₄]-propionate (TSP) in D₂O. ³¹P NMR spectra were recorded on a Varian INOVA 200 MHz.

N²-Fluoro-2',3',5'-O-triacetyl-O⁶-[2-(4-nitrophenyl)ethyl]inosine (2)

2',3',5'-tri-O-acetyl-O⁶-[2-(4-nitrophenyl)ethyl]guanosine (1.0 g, 1.79 mmol) was dissolved in anhydrous pyridine (6.75 mL, 0.082 mol) in a polypropylene tube under nitrogen atmosphere. The tube was cooled in a dry ice/acetonitrile bath (−35°C) and 70% HF/pyridine solution (12 mL, 0.42 mol) was added dropwise. The reaction mixture was stirred for 15 min, and *t*-butyl nitrite (0.54 mL, 4.5 mmol) was added. After 1 h, the reaction was quenched at 0°C by pouring the reaction mixture into an aqueous K₂CO₃ solution (28.5 g in 25 mL of water) and extracted three times with ethyl acetate. The organic layers were collected, dried over anhydrous Na₂SO₄, and evaporated to dryness. Purification by column chromatography on silica gel using as eluent 60:1 (v/v) CH₂Cl₂:MeOH gave 0.85 g (85%) of product; TLC silica gel, CH₂Cl₂:MeOH, 60:1 R_F = 0.4; ¹H NMR (700 MHz, CDCl₃) δ 8.21–8.15 (m, 2H, Ph), 8.08 (s, 1H, H-8), 7.50 (d, 2H, Ph), 6.13 (d, *J* = 5.6 Hz, 1H, H-1'), 5.82 (t, *J* = 4.9 Hz, 1H, H-2'), 5.60–5.55 (m, 1H, H-3'), 4.84 (t, *J* = 6.7 Hz, 2H, O-CH₂, NPE), 4.46–4.42 (m, 2H, H-4', H-5'), 4.38–4.36 (m, 1H, H-5''), 3.32 (t, *J* = 6.7 Hz, 2H, CH₂Ph, NPE), 2.15 (s, 6H, CH₃, acetyl), 2.08 (s, 3H, CH₃, acetyl). ¹³C NMR (200 MHz, CDCl₃) δ 170.2 (C=O), 169.5 (C=O), 169.3 (C=O), 160.3, 159.3 (C-6), 154.2 (C-2), 147.1, 145.1, 140.8, 129.9 (Ph), 123.8 (Ph), 86.4 (C-1'), 80.5 (C-4'), 73.0 (C-2'), 72.4 (C-3'), 67.7 (OCH₂, NPE), 62.8 (C-5'), 34.9 (CH₂Ph), 20.7 (C, acetyl), 20.5 (C, acetyl), 20.3 (C, acetyl); m/z: calcd for C₂₄H₂₄FN₅O₁₀ (M + H)⁺: 562.1579, found: 562.1581.

Procedure for the synthesis of N2-substituted derivatives 3a–3b

N²-fluoro-2',3',5'-O-triacetyl-O⁶-[2-(4-nitrophenyl)ethyl]inosine (2) (250 mg, 0.45 mmol) was dissolved in 2 mL of anhydrous dimethylsulfoxide (DMSO) and propargylamine (0.9 mmol, 58 μL) or pent-4-yn-1-amine (0.9 mmol, 87 μL) was added. The reaction mixture was stirred at 60°C until the fluoronucleoside completely disappeared (TLC). Next, 0.5 M NaOH in THF/MeOH/H₂O (5:4:2) was added and stirred at room temperature until the full deprotection of nucleoside was observed based on TLC. The solvent was removed under high vacuum, and the resulting oily residue was purified by column chromatography on Diaion HP-20 using for elution water with a linear gradient of methanol from 0% to 40% (v/v). Fractions containing product were collected, evaporated, and dried over P₄O₁₀ to yield:

N²-(prop-2-ynyl)guanosine (3a); 70%, 0.315 mmol, 102 mg; (200 MHz, D₂O) δ 7.80 (s, 1H, H-8), 6.13 (d, *J* = 4.8 Hz, 1H,

H-1'), 4.75–4.73 (m, 1H, H-2'), 4.48–4.45 (m, 1H, H-3'), 4.24–4.19 (m, 2H, NH-CH₂), 4.39–4.35 (m, 1H, H-4'), 4.24–4.19 (m, 1H, H-5'), 4.06–4.04 (m, 1H, H-5''), 4.09 (s, 3H, N7-CH₃), 2.64–2.63 (t, 1H, CHC); m/z: (M+H)⁺: 322.1608.

N²-(pent-4-yn-1-yl)guanosine (3b); 68%, 0.306 mmol, 107 mg; (200 MHz, D₂O) δ 7.76 (s, 1H, H-8), 6.12 (d, *J* = 4.7 Hz, 1H, H-1'), 4.69–4.66 (m, 1H, H-2'), 4.45–4.42 (m, 1H, H-3'), 4.37–4.34 (m, 1H, H-4'), 4.23–4.18 (m, 1H, H-5'), 4.08–4.03 (m, 1H, H-5''), 4.06 (s, 3H, N7-CH₃), 3.55–3.51 (t, 2H, NH-CH₂), 2.34–2.35 (t, 1H, CHC), 2.30–2.26 (m, 2H, NH-CH₂-CH₂-CH₂), 1.85–1.79 (quin, 2H, NH-CH₂-CH₂-CH₂); m/z: (M+H)⁺: 350.0777.

Procedure for the synthesis of the N7 methylated N2 modified 5'-monophosphates 5a–5b

Phosphorus oxide trichloride (POCl₃) (1.1 mmol, 103 μL) and trimethyl phosphate (0.018 mol, 2.15 mL) were cooled to 4°C and added to the dried compounds 3a (0.31 mmol, 100 mg) or 3b (0.31 mmol, 109 mg). The reaction mixtures were stirred at 4°C for 3 h. The solutions were allowed to warm to RT, and 1.0 M aqueous TEAB was added to maintain the pH as neutral. Products were purified by ion-exchange chromatography on a DEAE-Sephadex A-25 column using a linear 0–1 M TEAB gradient to produce products 4a–4b as TEA salts. Compounds 4a and 4b were used directly for the methylation reaction. To the suspension of 0.28 mmol of mononucleotide derivatives 4a (112 mg) or 4b (120 mg) in 1.5 mL anhydrous dimethylsulfoxide, methyl iodide (1.4 mmol, 87 μL) was added. The reaction mixtures were stirred at room temperature for 2 h and then were poured into the water (6 mL) and extracted several times with diethyl ether (~5 × 6 mL). The aqueous phases were purified on DEAE-Sephadex using a linear 0–0.8 M gradient of TEAB. Additionally, both nucleotides were purified on reversed-phase HPLC column. Products were lyophilized to yield:

N²-(prop-2-ynyl)-7-methylguanosine-5'-monophosphate (5a); 0.185 mmol 59%, 78 mg; ¹H NMR (400 MHz, D₂O) δ 6.13 (d, *J* = 3.2 Hz, 1H, H-1'), 4.75–4.73 (m, 1H, H-2'), 4.48–4.45 (m, 1H, H-3'), 4.39–4.35 (m, 1H, H-4'), 4.24–4.19 (m, 3H, NHCH₂, H-5'), 4.09 (s, 3H, N7-CH₃), 4.06–4.04 (m, 1H, H-5''), 2.63 (t, *J* = 2.5 Hz, 1H, CCH); ³¹P NMR (283 MHz, D₂O) δ 1.527; m/z: (M+H)⁺: 416.0937; t_R 13 min.

N²-(pent-4-yn-1-yl)-7-methylguanosine-5'-monophosphate (5b); 0.162 mmol, 53%, 72 mg; ¹H NMR (400 MHz, D₂O) δ 6.12 (d, *J* = 3.2 Hz, 1H, H-1'), 4.69–4.66 (m, 1H, H-2'), 4.45–4.42 (m, 1H, H-3'), 4.37–4.34 (m, 1H, H-4'), 4.23–4.18 (m, 1H, H-5'), 4.08–4.03 (m, 1H, H-5''), 4.06 (s, 3H, N7-CH₃), 3.54 (t, *J* = 8.0 Hz, 2H, NHCH₂), 2.35 (t, *J* = 2.6 Hz, 1H, CCH), 2.30–2.26 (m, 2H, NHCH₂CH₂CH₂), 1.83 (quin, *J* = 6.8 Hz, 2H, NHCH₂CH₂CH₂); ³¹P NMR (283 MHz, D₂O) δ 1.742; m/z: (M+H)⁺: 444.0418; t_R 15 min.

Procedure for the synthesis of mononucleotide cap analogs containing triazole ring ("click" reaction)

Solution 1: 100 mg CuSO₄ × 5 H₂O in 10 mL of water; Solution 2: 10 mg of sodium ascorbate in 200 μL of water.

To the suspension of mononucleotide derivatives 5a (0.0625 mmol, 26 mg) or 5b (0.0625 mmol, 27 mg) in anhydrous DMF (2 mL), solution 1 (80 μL), TBTA (0.00416 mmol, 2.2 mg), solution 2 (38 μL), and appropriate azide (0.125 mmol) (see Fig. 2) were

added and stirred under argon atmosphere overnight at 60°C. The next day reaction mixtures were terminated by the addition of EDTA (0.01 mmol, 4 mL) in 0.1 mL of water. Final products were purified by ion-exchange chromatography on a DEAE-Sephadex A-25 column using a linear 0–1 M TEAB gradient to produce compounds **6a–6e** as TEA salts. Final nucleotides were additionally purified on a reversed-phase HPLC column. Compounds **6a–6e** were lyophilized to yield:

N²-((1H-1,2,3-triazol-4-yl)methyl)-7-methylguanosine-5'-monophosphate (**6a**); 24 mg, 86%, ammonium salt; ¹H NMR (400 MHz, D₂O) δ 7.89 (s, 1H, triazole), 6.07 (d, *J* = 3.3 Hz, 1H, H-1'), 4.73 (s, 2H, NHCH₂), 4.58–4.56 (m, 1H, H-2'), 4.41–4.38 (m, 1H, H-3'), 4.35–4.32 (m, 1H, H-4'), 4.20–4.15 (m, *J* = 2.4 Hz, *J* = 4.3 Hz, *J* = 11.9 Hz, 1H, H-5'), 4.04–3.99 (m, *J* = 2.4 Hz, *J* = 5.0 Hz, *J* = 11.9 Hz, 1H, H-5''), 4.07 (s, 3H, N7-CH₃); ³¹P NMR (162 MHz, D₂O) δ 1.17; m/z: (M+H)⁺: 459.0882, t_R 11 min.

N²-((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-7-methylguanosine-5'-monophosphate (**6b**); 31 mg, 92%, ammonium salt; ¹H NMR (400 MHz, D₂O) δ 7.99 (s, 1H, triazole), 7.37–7.34 (m, 3H, Ph), 7.28–7.26 (m, 2H, Ph), 5.94 (d, *J* = 3.2 Hz, 1H, H-1'), 5.56 (s, 2H, NCH₂Ph), 4.66 (s, 2H, NHCH₂), 4.54–4.52 (m, 1H, H-2'), 4.38–4.36 (m, 1H, H-3'), 4.34–4.31 (m, 1H, H-4'), 4.21–4.17 (m, *J* = 2.4 Hz, *J* = 4.4 Hz, *J* = 12.0 Hz, 1H, H-5'), 4.02–4.05 (m overlapped with N7-CH₃, 1H, H-5''), 4.03 (s, 3H, N7-CH₃); ³¹P NMR (162 MHz, D₂O) δ 0.61; m/z: (M+H)⁺: 549.1570, t_R 16 min.

N²-((1-(3-(2,6-dimethoxyphenoxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)-7-methylguanosine-5'-monophosphate (**6c**); 33 mg, 82%, ammonium salt; ¹H NMR (400 MHz, D₂O) δ 8.10 (s, 1H, triazole), 6.95 (t, *J* = 8.4 Hz, 1H, Ph), 6.47 (d, *J* = 8.4 Hz, 2H, Ph), 5.86 (d, *J* = 3.3 Hz, 1H, H-1'), 4.67–4.61 (m, 3H, H-2', NCH₂), 4.55 (m, 2H, NHCH₂), 4.37–4.24 (m, 2H, H-3', H-4'), 4.28–4.23 (m, 1H, H-5'), 4.09–4.05 (m, 1H, H-5''), 3.95 (s, 3H, N7-CH₃), 3.87–3.72 (m, 2H, CH₂O), 3.66 (s, 6H, OCH₃), 2.38–2.32 (m, 2H, CH₂CH₂CH₂); ³¹P NMR (162 MHz, D₂O) δ 0.17; m/z: (M+H)⁺: 653.0789, t_R 13 min.

N²-((1-(3-phenoxypropyl)-1H-1,2,3-triazol-4-yl)methyl)-7-methylguanosine-5'-monophosphate (**6d**); 32 mg, 88%, ammonium salt; ¹H NMR (400 MHz, D₂O) δ 8.00 (s, 1H, triazole), 7.05–7.01 (m, 2H, Ph), 6.80–6.75 (m, 1H, Ph), 6.51–6.48 (m, 2H, Ph), 5.88 (d, *J* = 2.9 Hz, 1H, H-1'), 4.65–4.62 (t, *J* = 6.1 Hz, 2H, NCH₂), 4.55 (s, 2H, NHCH₂), 4.45–4.43 (m, 1H, H-2'), 4.38–4.36 (m, 2H, H-3', H-4'), 4.25–4.21 (m, 1H, H-5'), 4.09–4.04 (m, 1H, H-5''), 4.02 (s, 3H, N7-CH₃), 3.87–3.72 (m, 2H, CH₂O), 2.31–2.26 (m, 2H, CH₂CH₂CH₂); ³¹P NMR (162 MHz, D₂O) δ 0.70; m/z: (M+H)⁺: 593.1526, t_R 13.5 min.

N²-(3-(1-benzyl-1H-1,2,3-triazol-4-yl)propyl)-7-methylguanosine-5'-monophosphate (**6e**); 32 mg, 91%, ammonium salt; ¹H NMR (400 MHz, D₂O) δ 7.77 (s, 1H, triazole), 7.39–7.35 (m, 3H, Ph), 7.29–7.27 (m, 2H, Ph), 6.00 (d, *J* = 3.3 Hz, 1H, H-1'), 5.52 (s, 2H, CH₂Ph), 4.61–4.58 (m, 1H, H-2'), 4.43–4.40 (m, 1H, H-3'), 4.35–4.33 (m, 1H, H-4'), 4.23–4.18 (m, 1H, H-5'), 4.08–4.03 (m, 1H, H-5''), 4.06 (s, 3H, N7-CH₃), 3.43–3.39 (t, *J* = 6.5 Hz, 2H, NHCH₂), 2.78–2.75 (t, *J* = 7.1 Hz, 2H, NHCH₂CH₂CH₂), 2.04–1.92 (m, 2H, NHCH₂CH₂CH₂); ³¹P NMR (162 MHz, D₂O) δ 0.68; m/z: (M+H)⁺: 577.1842; t_R 13 min.

Procedure for the synthesis of a dinucleotide cap analog (7)

P¹-N²-((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-7-methylguanosine-5'-P³-guanosine-5'-triphosphate (7):

7-Methylguanosine 5'-diphosphate imidazolidide (25.8 mg, 0.05 mmol) and ZnCl₂ (33 mg, 0.24 mmol) were stirred in anhydrous DMF (0.7 mL) with compound **6b** (33 mg, 0.06 mmol, TEA salt) at room temperature for 24 h. The reaction mixture was poured into a solution of EDTA (114 mg, 0.3 mmol) in water (1.5 mL) and neutralized to pH 7 by addition of 1 M TEAB. The product was separated from the reaction mixture by chromatography on DEAE-Sephadex using a 0–1.0 M gradient of TEAB. Compound **7** was obtained as colorless crystals (26 mg, 0.025 mmol, 42%, TEA salt). The final dinucleotide cap analogs were additionally purified on a reversed-phase HPLC column. Compound **7** was lyophilized to yield: 26 mg (0.025 mmol), 42%, ammonium salt; ¹H NMR (400 MHz, D₂O) m⁷G: δ 8.00 (s, 1H, triazole) 7.92 (s, 1H, H8 [G]), 7.33–7.29 (m, 3H, Ph), 7.24–7.22 (m, 2H, Ph), 5.78 (d, 1H, *J* = 3.0 Hz, H-1' [m⁷G]), 5.72 (d, *J* = 6.1 Hz, 1H, H-1' [G]), 5.52 (s, 2H, PhCH₂), 4.66–4.55 (m, 4H, H-2' [G], H-2' [m⁷G], NHCH₂), 4.42–4.20 (m, 8H, H-3' [G], H-3' [m⁷G], H-4' [G], H-4' [m⁷G], H-5' [G], H-5' [m⁷G], H-5'' [G], H-5'' [m⁷G]), 3.99 (s, 3H, N7-CH₃); ³¹P NMR (162 MHz, D₂O) δ −11.64 (2P, P_{α,γ}), −23.23 (1P, P_β) m/z: (M+H)⁺: 974.0634.

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article.

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REFERENCES

- Amblard F, Cho JH, Schinazi RF. 2009. Cu(I)-catalyzed Huisgen azide-alkyne 1,3-dipolar cycloaddition reaction in nucleoside, nucleotide, and oligonucleotide chemistry. *Chem Rev* **109**: 4207–4220.
- Ami T, Fujimoto K. 2008. Click chemistry as an efficient method for preparing a sensitive DNA probe for photochemical ligation. *ChemBioChem* **9**: 2071–2074.
- Cai A, Jankowska-Anyszka M, Centers A, Chlebicka L, Stepinski J, Stolarski R, Darzynkiewicz E, Rhoads RE. 1999. Quantitative assessment of mRNA cap analogues as inhibitors of in vitro translation. *Biochemistry* **38**: 8538–8547.
- Carberry SE, Darzynkiewicz E, Stepinski J, Tahara SM, Rhoads RE, Goss DJ. 1990. A spectroscopic study of the binding of N-7-substituted cap analogues to human protein synthesis initiation factor 4E. *Biochemistry* **29**: 3337–3341.
- Chan TR, Hilgraf R, Sharpless KB, Fokin W. 2004. Polytriazoles as copper(I)-stabilizing ligands in catalysis. *Org Lett* **6**: 2853–2855.
- Clemens MJ, Bommer UA. 1999. Translational control: the cancer connection. *Int J Biochem Cell Biol* **31**: 1–23.
- Darzynkiewicz E, Ekiel I, Tahara SM, Seliger LS, Shatkin AJ. 1985. Chemical synthesis and characterization of 7-methylguanosine cap analogs. *Biochemistry* **24**: 1701–1707.
- De Benedetti A, Graff JR. 2004. eIF-4E expression and its role in malignancies and metastases. *Oncogene* **23**: 3189–3199.
- De las Heras FG, Alonso R, Alonso GJ. 1979. Alkylating nucleosides 1. Synthesis and cytostatic activity of N-glycosyl(halomethyl)-

- 1,2,3-triazoles. A new type of alkylating agent. *J Med Chem* **22**: 496–501.
- El-Sagheer AH, Brown T. 2010. Click chemistry with DNA. *Chem Soc Rev* **39**: 1388–1405.
- Furuichi Y, Shatkin AJ. 2000. Viral and cellular mRNA capping: past and prospects. *Adv Virus Res* **55**: 135–184.
- Ghosh P, Park C, Peterson MS, Bitterman PB, Polunovsky VA, Wagner CR. 2005. Synthesis and evaluation of potential inhibitors of eIF4E cap binding to 7-methyl GTP. *Bioorg Med Chem Lett* **15**: 2177–2180.
- Gingras AC, Raught B, Sonenberg N. 1999. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu Rev Biochem* **68**: 913–963.
- Grudzien E, Stepinski J, Jankowska-Anyszka M, Stolarski R, Darzynkiewicz E, Rhoads RE. 2004. Novel cap analogs for in vitro synthesis of mRNAs with high translational efficiency. *RNA* **10**: 1479–1487.
- Hein JE, Fokin VV. 2010. Copper-catalyzed azide-alkyne cycloaddition (CuAAC) and beyond: new reactivity of copper(I) acetylides. *Chem Soc Rev* **39**: 1302–1315.
- Huang ZH, Zhuo ST, Li CY, Xie HT, Li D, Tan JH, Ou TM, Huang ZH, Gu LQ, Huang SL. 2013. Design, synthesis and biological evaluation of novel mansonone E derivatives prepared via CuAAC click chemistry as topoisomerase II inhibitors. *Eur J Med Chem* **68**: 58–71.
- Jankowska-Anyszka M, Piecyk K, Šamonina-Kosicka J. 2011. Synthesis of a new class of ribose functionalized dinucleotide cap analogues for biophysical studies on interaction of cap-binding proteins with the 5' end of mRNA. *Org Biomol Chem* **9**: 5564–5572.
- Jia Y, Chiu TL, Amin EA, Polunovsky V, Bitterman PB, Wagner CR. 2010. Design, synthesis and evaluation of analogs of initiation factor 4E (eIF4E) cap-binding antagonist Bn7-GMP. *Eur J Med Chem* **45**: 1304–1313.
- Johnson JA Jr, Thomas HJ, Schaeffer HJ. 1958. Synthesis of potential anticancer agents. XIII. Ribosides of 6-substituted purines. *J Am Chem Soc* **80**: 699–702.
- Johnson EA, Marks RS, Mandrekar SJ, Hillman SL, Hauge MD, Bauman MD, Wos EJ, Moore DF, Kugler JW, Windschitl HE, et al. 2008. Phase III randomized, double-blind study of maintenance CAI or placebo in patients with advanced non-small cell lung cancer (NSCLC) after completion of initial therapy (NCCTG 97-24-51). *Lung Cancer* **60**: 200–207.
- Kadokura M, Wada T, Urashima C, Sekine M. 1997. Efficient synthesis of γ -methyl-capped guanosine 5'-triphosphate as a 5'-terminal unique structure of U6 RNA via a new triphosphate bond formation involving activation of methyl phosphorimidazolide using $ZnCl_2$ as catalyst in DMF under anhydrous conditions. *Tetrahedron Lett* **38**: 8359–8362.
- Kowalska J, Lukaszewicz M, Zuberek J, Ziemniak M, Darzynkiewicz E, Jemielity J. 2009. Phosphorothioate analogs of m⁷GTP are enzymatically stable inhibitors of cap-dependent translation. *Bioorg Med Chem Lett* **19**: 1921–1925.
- Lakshman MK, Singh MK, Parrish MK, Balachandran R, Day BW. 2010. Azide-tetrazole equilibrium of C-6 azidopurine nucleosides and their ligation reactions with alkynes. *J Org Chem* **75**: 2461–2473.
- Lewis JD, Izaurralde E. 1997. The role of the cap structure in RNA processing and nuclear export. *Eur J Biochem* **247**: 461–469.
- Mamane Y, Petroulakis E, Rong L, Yoshida K, Ler LW, Sonenberg N. 2004. eIF4E – from translation to transformation. *Oncogene* **23**: 3172–3179.
- Marcotrigiano J, Gingras AC, Sonenberg N, Burley SK. 1997. Cocystal structure of the messenger RNA 5' cap-binding protein (eIF4E) bound to 7-methyl-GDP. *Cell* **89**: 951–961.
- Matsuo H, Li H, McGuire AM, Fletcher CM, Gingras AC, Sonenberg N, Wagner G. 1997. Structure of translation factor eIF4E bound to m⁷GDP and interaction with 4E-binding protein. *Nat Struct Biol* **4**: 717–724.
- Niedzwiecka A, Marcotrigiano J, Stepinski J, Jankowska-Anyszka M, Wyslouch-Cieszyńska A, Dadlez M, Gingras AC, Mak P, Darzynkiewicz E, Sonenberg N, et al. 2002. Biophysical studies of eIF4E cap-binding protein: recognition of mRNA 5' cap structure and synthetic fragments of eIF4G and 4E-BP1 proteins. *J Mol Biol* **319**: 615–635.
- Piecyk K, Davis RE, Jankowska-Anyszka M. 2012. Synthesis of N²-modified 7-methylguanosine 5'-monophosphates as nematode translation inhibitors. *Bioorg Med Chem* **20**: 4781–4789.
- Rhoads RE. 1985. The cap structure of eukaryotic messenger RNA and its interaction with cap-binding protein. *Prog Mol Subcell Biol* **9**: 104–155.
- Rhoads RE. 2009. eIF4E: new family members, new binding partners, new roles. *J Biol Chem* **284**: 16711–16715.
- Seela F, Sirivolu VR. 2008. Pyrrolo-dC oligonucleotides bearing alkynyl side chains with terminal triple bonds: synthesis, base pairing and fluorescent dye conjugates prepared by the azide-alkyne “click” reaction. *Org Biomol Chem* **6**: 1674–1687.
- Seela F, Sirivolu VR, Chittepu P. 2008. Modification of DNA with octadiynyl side chains: synthesis, base pairing, and formation of fluorescent coumarin dye conjugates of four nucleobases by the alkyne-azide “click” reaction. *Bioconjug Chem* **19**: 211–224.
- Sharp PA. 1994. Split genes and RNA splicing. *Cell* **77**: 805–815.
- Shen X, Tomoo K, Uchiyama S, Kobayashi Y, Ishida T. 2001. Structural and thermodynamic behavior of eukaryotic initiation factor 4E in supramolecular formation with 4E-binding protein 1 and mRNA cap analogue, studied by spectroscopic methods. *Chem Pharm Bull (Tokyo)* **49**: 1299–1303.
- Soltis MJ, Yeh HJ, Cole KA, Whittaker N, Wersto RP, Kohn EC. 1996. Identification and characterization of human metabolites of CAI [5-amino-1-(4'-chlorobenzoyl-3,5-dichlorobenzyl)-1,2,3-triazole-4-carboxamide]. *Drug Metab Dispos* **24**: 799–806.
- Sonenberg N. 1996. mRNA 5' cap-binding protein eIF4E and control of cell growth. In *Translational control* (ed. Hershey JWB, et al.), pp. 245–269. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Stepinski J, Waddell C, Stolarski R, Darzynkiewicz E, Rhoads RE. 2001. Synthesis and properties of mRNAs containing the novel “anti-reverse” cap analogs 7-methyl(3'-O-methyl)GpppG and 7-methyl(3'-deoxy)GpppG. *RNA* **7**: 1486–1495.
- Temple C Jr, Thorpe MC, Coburn WC Jr, Montgomery JA. 1966a. Studies on the azidoazomethine–tetrazole equilibrium. IV. Azidopurines. *J Org Chem* **31**: 935–938.
- Temple C Jr, Kussner CL, Montgomery JA. 1966b. Studies on the azidoazomethine–tetrazole equilibrium. V. 2- and 6-azidopurines. *J Org Chem* **31**: 2210–2215.
- Tron GC, Piralì T, Billington RA, Canonico PL, Sorba G, Genazzani AA. 2008. Click chemistry reactions in medicinal chemistry: applications of the 1,3-dipolar cycloaddition between azides and alkynes. *Med Res Rev* **28**: 278–308.
- Yoshikawa M, Kato T, Takenishi T. 1967. A novel method for phosphorylation of nucleosides to 5'-nucleotides. *Tetrahedron Lett* **8**: 5065–5068.
- Ziemniak M, Strenkowska M, Kowalska J, Jemielity J. 2013. Potential therapeutic applications of RNA cap analogs. *Future Med Chem* **5**: 1141–1172.

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