Experimental Section

Materials

1,4-Dibromobut-2-ene, S-adenosyl-L-homocysteine and m\textsuperscript{7}GTP were purchased from Sigma-Aldrich and sodium azide from Roth. m\textsuperscript{7}GpppA as well as the vaccinia capping system were obtained from NEB. DBCO-Cy5 and DBCO-Sulforhodamine B (SRB) were obtained from Click Chemistry Tools. HPLC grade acetonitrile was purchased from Merck. All components were used without further purification.

Thin layer chromatography (TLC) was performed using Alugram\textsuperscript{®} Xtra SIL G aluminium sheets with a fluorescence indicator (F\textsubscript{254}). Visualisation on the TLC plates was achieved using UV light (254 nm) or by staining with potassium permanganate (KMnO\textsubscript{4}). Analytical HPLC was carried out on an Agilent 1260 Infinity HPLC equipped with a Diode Array Detector (190-640 nm) using a Nucelodur\textsuperscript{®} C\textsubscript{18} Pyramid reversed-phase column (5 μm, 125 x 10 mm, 4 mm ID) from Macherey-Nagel. Preparative HPLC purification was carried out on the same HPLC using Nucelodur\textsuperscript{®} C\textsubscript{18} Pyramid reversed phase column (5 μm, 125 x 10 mm, 10 mm ID). Fluorescence images of PAA-gels were recorded on a VersaDoc Gel Imager (Biorad) and on a ChemoCam Imager ECL Type HR 16-3200 (Intas). Proton nuclear magnetic resonance spectra (\textsuperscript{1}H NMR) were recorded on a Bruker 300 MHz instrument. The chemical shifts (\textdelta) were reported in ppm relative to TMS or deuterated solvents as internal standard (\textdelta\textsubscript{H}: CDCl\textsubscript{3} 7.26 ppm). Coupling constants are expressed in Hz. ESI-TOF mass spectra were recorded on an Agilent 6224 instrument, ESI-Orbitrap-MS on a LTQ Orbitrap XL\textsuperscript{™} Hybrid Ion Trap-Orbitrap Mass Spectrometer from ThermoScientific and MALDI-TOF-MS on a Bruker UltraflxeXtreme Smartbeam II laser spectrometer.

Synthesis of (E)-1-azido-4-bromobut-2-ene

\begin{equation}
\text{Br} - \text{Br} \xrightarrow{8} \text{Br} - \text{N}_3 - \text{N}_3 - \text{N}_3
\end{equation}

Scheme 1 Synthesis of (E)-1-azido-4-bromobut-2-ene 7. a) NaN\textsubscript{3}, DMF, 50 °C, 15 h.

The synthesis of (E)-1-azido-4-bromobut-2-ene 7 was performed according to Agnew et al.\textsuperscript{1} (E)-1,4-dibromobut-2-ene 6 (2.0 g, 9.4 mmol) was placed in a round-bottom flask and dissolved in 8 mL DMF. Sodium azide (0.60 g, 9.4 mmol) was added to the solution of 6 and the reaction mixture was stirred for 15 hours at 50 °C. The reaction was diluted with ethyl
acetate (60 mL) and the organic layer was washed with water (15 mL) and a saturated sodium hydrogencarbonate solution. Then the organic layer was dried over anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (100 % petroleum ether) affording (E)-1-azido-4-bromobut-2-ene 7 as colorless liquid.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 6.03-5.97 (m, 1H, =$\text{CH}$), 5.86-5.82 (m, 1H, =$\text{CH}$), 4.0 (d, 2H, $^3J = 4.0$ Hz), 3.82 (d, 2H).

**Synthesis and characterization of 5'-[(R/S)-[(3S)-3-amino-3-carboxypropyl]-4-azidobut-2-enylsulfonio]-5'-deoxyadenosine 2 (Ab-SAM)**

The synthesis of 2 was performed as described by Islam et al.$^2$ Reaction of SAH with (E)-1-azido-4-bromobut-2-ene afforded 2 as a diastereomeric mixture at the sulfonium center (approximately 1:1). The SAM analogs were purified by reversed-phase HPLC using a Nucleodur$^{\textregistered}$ C$_{18}$ Pyramid (5 µm, 125 x 10 mm, 10 mm ID) column. Elution was performed at a flowrate of 5 mL/min using water containing 0.01 % TFA as eluent A and a gradient of acetonitrile containing 0.01 % TFA from 0 to 7 % percent within 15 min. The obtained diastereomeric mixture was concentrated by lyophilization and the dried product was redissolved in water. Aliquots were stored at -20 °C before use. The concentration of 2 was determined by UV absorption with $\varepsilon_{260} = 15,400$ L·mol$^{-1}$·cm$^{-1}$. The compound was obtained in ∼11 % yield. $t_R = 9.9$ min. The molecular weight was confirmed by ESI-MS (calculated for C$_{18}$H$_{26}$N$_9$O$_5$S$: 480.1778 [M]$^+$; found 480.1785).

**Recombinant production and purification of GlaTgs2-Var1, MTAN and LuxS**

For recombinant production of GlaTgs2-Var1, *E. coli* Tuner DE3 pLacI cells were transformed with pRSET-A-GlaTgs2-Var1. Overnight cultures were grown in 2YT-medium in presence of ampicillin (100 µg/mL) and glucose (7.3 g/L). After inoculation with 2.5 % (v/v) overnight culture, the cells were cultivated for 4 h at 37 °C and constant shaking supplemented as described above. Cooled to room temperature, expression was induced with 0.32 mM IPTG and 2 % (v/v) ethanol and performed for 20 h at 17 °C. Cells were harvested by centrifugation and stored at -20 °C. For purification cells were resuspended in lysis buffer (50 mM Tris-HCl, 200 mM NaCl, 10 % glycerol (v/v), pH 8), protease inhibitor cocktail was added (cOmplete, Roche) and the cells were sonicated. The His-tagged enzyme was purified by affinity chromatography using a His-Trap$^{\text{TM}}$ FF 1 mL column and eluted with a gradient
up to 500 mM imidazole in lysis buffer. Fractions containing the enzyme were pooled and concentrated in storage buffer (50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 10 % glycerol (v/v), pH 8) using Amicon Ultra-15 centrifugal filter units (regenerated cellulose, MWCO 10,000). The storage buffer was prepared without DTT due to its azide reducing capabilities. Aliquots were stored at -80 °C.

MTAN and LuxS were recombinantly produced and purified as previously described.4,5

Enzymatic modification of the cap analogs m^7GpppA and m^7GTP

Synthesis of \( P^1\)-adenosine(5')-\( P^3\)-[\( N^2\)-4-azidobut-2-ENyl,7-methylguanosine(5')] triphosphate 3

\[
\begin{align*}
\text{Scheme 2 Synthesis of 3 and 10.} \quad & a) \text{90 µM GlaTgs2-Var1, 4 µM MTAN, 3 µM LuxS, PBS or reaction buffer,} \\
& \text{37 °C, 3 h.}
\end{align*}
\]

Enzymatic conversion of \( m^7\)GpppA 1 or \( m^7\)GTP 9 (275 µM respectively 1 mM) with Ab-SAM 2 (230 µM respectively 833 µM) by GlaTgs2-Var1 (90 µM) was performed in the presence of 4 µM MTAN and 3 µM LuxS in PBS (pH 7.4) or reaction buffer (50 mM Tris-HCl, 100 mM MgCl2, 100 mM NH4OAc, pH 8.4) at 37 °C for 3 h. Transfer reactions were analyzed by reversed-phase HPLC after protein precipitation with 1/10 volume 1 M HClO4 on an analytical Nucleodur® Pyramid 125x4 mm column as described earlier.4-6

Strain-promoted azide-alkyne cycloaddition with modified \( m^7\)GpppA und \( m^7\)GTP

\[
\begin{align*}
\text{Scheme 3 Synthesis of 5 and 11.} \quad & a) \text{PBS, 24 °C, 1 h, darkness.}
\end{align*}
\]
The *in situ* generated N\(^2\)-4-azidobut-2-ethyl-m\(^7\)GpppA 3 or -m\(^7\)GTP was used for SPAAC without further purification. Reactions were incubated with commercially available DBCO-Cy5 (1.1 mM) for 1 h at 24 °C in darkness. For experiments performed in lysate of eukaryotic cells, PC3 cells (ca. 1 x 10\(^7\)) were pelleted, resuspended in 50 μL PBS buffer and lysed by sonication. The supernatant was added to reactions instead of buffer. Samples were analyzed for successful labeling after gelelectrophoresis on a 20 % urea-polyacrylamide-gel using fluorescence scanning on a VersaDoc Gel Imager using red LED and BP (band pass) of 695 nm.

**Strain-promoted azide-alkyne cycloaddition with enzymatically modified RNA**

106 nt RNA was produced by *in vitro* T7 transcription and purified by phenol-chloroform extraction. After precipitation and resuspension in water, the concentration was determined by in-gel quantification. Capping of the obtained RNA was achieved using the vaccinia capping enzyme (NEB) as previously described.\(^5\) To eliminate remaining SAM, samples were heated for 15 min at 65 °C and incubated for 15 min at 4 °C with cation exchanger P11 cellulose phosphate (Whatman). After centrifugation, RNA was precipitated from the supernatant and the pellet was resuspended in GlaTgs2-Var1 (90-110 μM), MTAN (1.2 μM), LuxS (0.6 μM, all purified using an ÄKTA purifier™ system and HisTrap™ FF 1 columns (GE Healthcare)) and Ab-SAM (340-428 μM). Volume was adjusted to 5 μL with PBS buffer and possible RNAses were inhibited by 0.25 μL RiboLock RNase Inhibitor (Thermo Scientific). Samples were incubated for 90 min at 37 °C. After RNA precipitation, DBCO-SRB (214 μM) was added and the solution was adjusted to a final volume of 5 μL with PBS buffer. After an incubation period of 1 hour at 37 °C samples were analyzed on 10 % denaturing PAA gels.
**Supplementary Figures**

**Supplementary Figure 1:** UV-shadowing analysis of modified \( m^7 \text{GpppA} \) by SPAAC. SPAAC was performed in PBS buffer as well as in PC3 cell lysate using DBCO-Cy5\( ^{646/661} \) \( \text{4} \). Next to the fluorescent bands of the DBCO-Cy5 \( \text{4} \) reagent (lanes 1-5) and the SPAAC product \( \text{5} \) (two bands, lanes 1), \( m^7 \text{GpppA} \) \( \text{1} \) can be detected, which was left out in lanes 2.

**Supplementary Figure 2:** Labeling of enzymatically modified \( m^7 \text{GTP} \) by SPAAC. Analysis of chemo-enzymatic modification of \( m^7 \text{GTP} \) using in-gel fluorescence and PAGE. SPAAC was performed in PBS-buffer as well as in PC3 cell lysate using DBCO-Cy5\( ^{646/661} \). Two new fluorescent bands (arrow) were only detected in samples containing \( N^2\)-azidobutenyl-\( m^7 \text{GTP} \) and DBCO-Cy5 but in none of the controls, lacking either \( m^7 \text{GTP} \), enzyme or Ab-SAM. Fluorescent bands in controls result from DBCO-Cy5 alone and probably a reaction product of \( \text{2} \) and \( \text{4} \).
Supplementary Figure 3: Analyzing the efficiency of the SPAAC of 3 with 4. Reversed-phase HPLC separation of the SPAAC reaction with absorbance and fluorescence detection. A) N²-4-azidobut-2-enyl-mGpppA 3 (~2.7 pmol) was completely converted after SPAAC based on absorbance measurements. B) Analysis of 4 and the SPAAC reaction of 3 with 4 by fluorescence detection allows to detect the SPAAC product 5. Assuming that fluorescence properties remain identical in 4 and 5, we calculated the amount of 5 based on the known concentration of 4 to be ~2.6 pmol. Controls show reactions without enzyme (ctrl. 1) or Ab-SAM (ctrl. 2). Fluorescence detector settings were the following: excitation = 646 nm; emission = 661 nm.

GlaTgs2-Var1

atgaccaacctggtcctggatagcaaatgttgtgaacgtatgaaatggctgtttacgtattgcagtaatcgctgtatgaagttgtgatgccactctgtcggtctgtatgtgatgcaagttgtgatccactgctgtgtgtgttgatgtgctgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtg