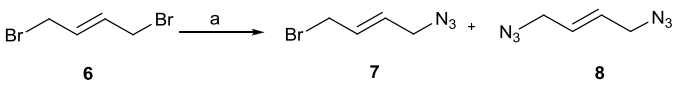
Experimental Section

Materials

1,4-Dibromobut-2-ene, *S*-adenosyl-L-homocysteine and m^7GTP were purchased from Sigma-Aldrich and sodium azide from Roth. m^7GpppA 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® Xtra SIL G aluminium sheets with a fluorescence indicator (F_{254}). Visualisation on the TLC plates was achieved using UV light (254 nm) or by staining with potassium permanganate ($KMnO_4$). Analytical HPLC was carried out on an Agilent 1260 Infinity HPLC equipped with a Diode Array Detector (190-640 nm) using a Nucelodur® C_{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® C_{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 (^1H NMR) were recorded on a Bruker 300 MHz instrument. The chemical shifts (δ) were reported in ppm relative to TMS or deuturated solvents as internal standard (δ_H: $CDCl_3$ 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™ Hybrid Ion Trap-Orbitrap Mass Spectrometer from ThermoScientific and MALDI-TOF-MS on a Bruker UltrafleXtreme Smartbeam II laser spectrometer.

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



Scheme 1 Synthesis of (*E*)-1-azido-4-bromobut-2-ene 7. a) NaN_3, DMF, 50 °C, 15 h.

The synthesis of (*E*)-1-azido-4-bromobut-2-ene **7** was performed according to Agnew *et al.*[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_2SO_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, $CDCl_3$): δ = 6.03-5.97 (m, 1H, =CH), 5.86-5.82 (m, 1H, =CH), 4.0 (d, 2H, ^3J = 4.0 Hz), 3.82 (d, 2H).

Synthesis and characterization of 5'-[(*R*/*S*)-[(3*S*)-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® 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 ε_{260} = 15,400 $L \cdot mol^{-1} \cdot cm^{-1}$. The compound was obtained in ~11 % yield. t_R = 9.9 min. The molecular weight was confirmed by ESI-MS (calculated for $C_{18}H_{26}N_9O_5S^+$: 480.1778 $[M]^+$; found 480.1785).

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

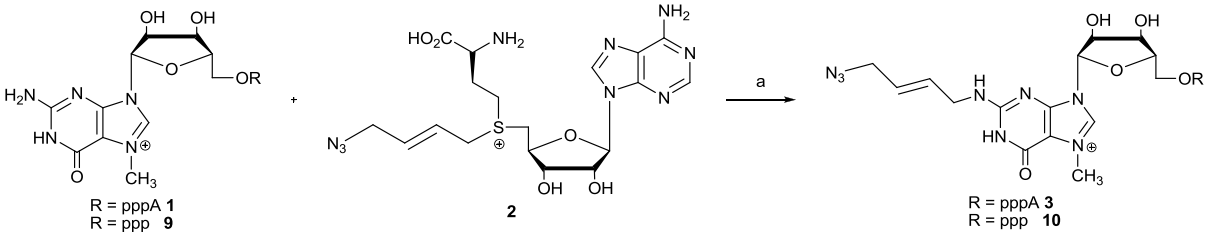
For recombinant production of GlaTgs2-Var1, *E. coli* Tuner DE3 pLacI cells were transformed with pRSET-A-GlaTgs2-Var1. Overnight cultures were grown in 2YT-medium in presence of ampicillin (100 µg/mL) and glucose (7.3 g/L). After inoculation with 2.5 % (v/v) overnight culture, the cells were cultivated 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™ 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.[3] 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^{7}GpppA and m^{7}GTP

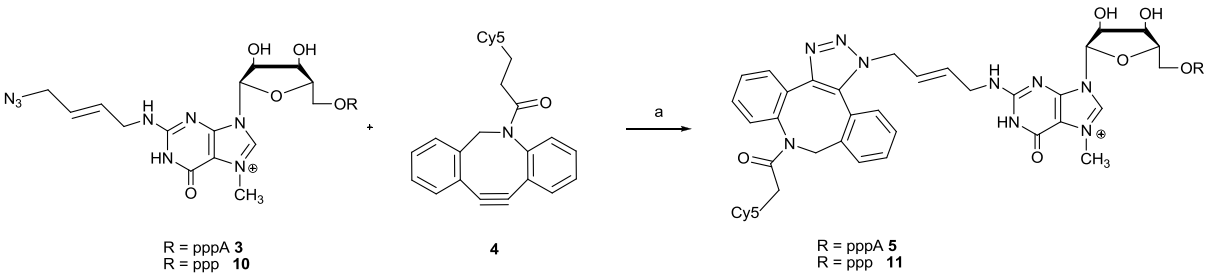
Synthesis of P^1-adenosine(5')-P^3-[N^2-4-azidobut-2-enyl,7-methylguanosine(5')] triphosphate 3



Scheme 2 Synthesis of 3 and 10. a) 90 µM GlaTgs2-Var1, 4 µM MTAN, 3 µM LuxS, PBS or reaction buffer, 37 °C, 3 h.

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 $MgCl_2$, 100 mM NH_4OAc, 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 $HClO_4$ 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



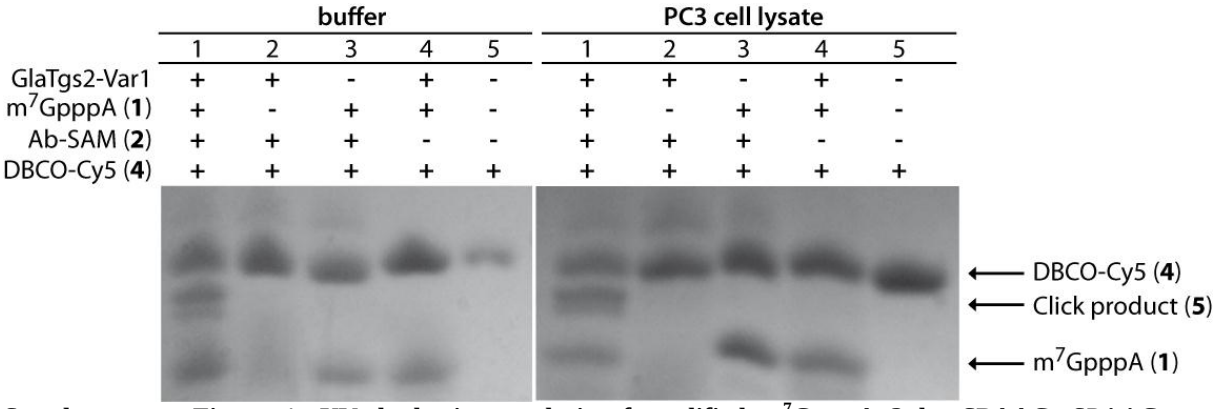
Scheme 3 Synthesis of 5 and 11. a) PBS, 24 °C, 1 h, darkness.

The *in situ* generated N^2-4-azidobut-2-enyl-m^7GpppA **3** or -m^7GTP 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.

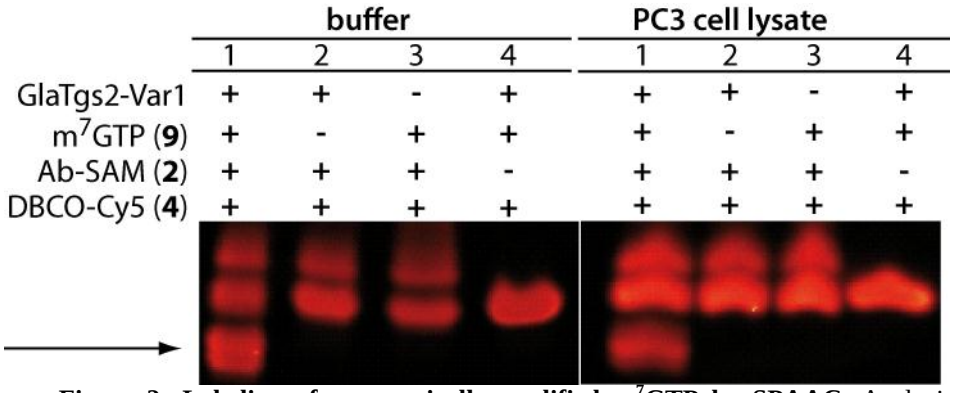
Strain-promoted azide-alkyne cycloaddition with enzymatically modified RNA

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.

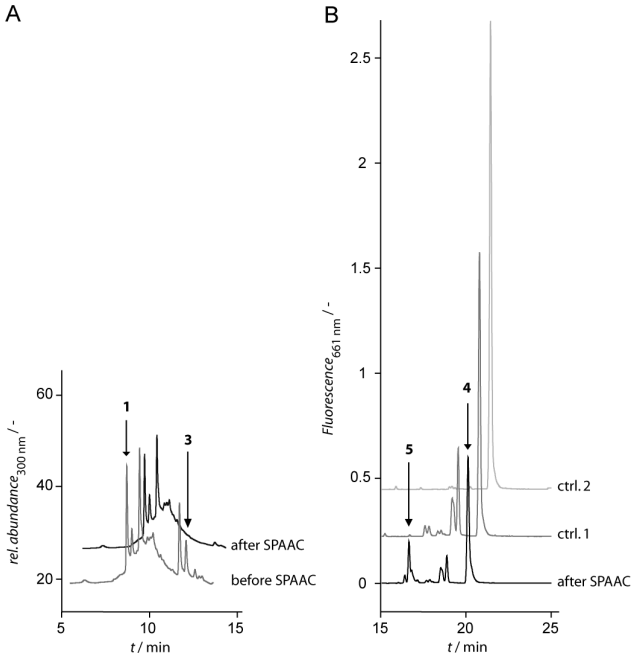
Supplementary Figures



Supplementary Figure 1: UV-shadowing analysis of modified m^{7}GpppA 3 by SPAAC. SPAAC was performed in PBS buffer as well as in PC3 cell lysate using DBCO-Cy5^{TM}646/661 **4**. Next to the fluorescent bands of the DBCO-Cy5 **4** reagent (lanes 1-5) and the SPAAC product **5** (two bands, lanes 1), m^{7}GpppA **1** can be detected, which was left out in lanes 2.



Supplementary Figure 2: Labeling of enzymatically modified m^{7}GTP by SPAAC. Analysis of chemo-enzymatic modification of m^{7}GTP using in-gel fluorescence and PAGE. SPAAC was performed in PBS-buffer as well as in PC3 cell lysate using DBCO-Cy5^{TM}646/661. Two new fluorescent bands (arrow) were only detected in samples containing N^2-azidobutenyl-m^{7}GTP and DBCO-Cy5 but in none of the controls, lacking either m^{7}GTP, enzyme or Ab-SAM. Fluorescent bands in controls result from DBCO-Cy5 alone and probably a reaction product of **2** and **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) N^2-4-azidobut-2-enyl-m^7GpppA **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

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atgagcacctggctgctggatagcaaatgtgttgaacgtatgaaatggctgtttagcgat
 M  S  T  W  L  L  D  S  K  C  V  E  R  M  K  W  L  F  S  D
ctgccggaagaaaaacgtgtgatgatcaaaatgaatgaagcggccttttttagcgttaca
 L  P  E  E  K  R  V  M  I  K  M  N  E  A  A  F  F  S  V  T
ccggcagtttatgcagatgaagttgcacgtatgatgcgtaccgttctggcactgctgggt
 P  A  V  Y  A  D  E  V  A  R  M  M  R  T  V  L  A  L  L  G
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 K  P  P  Y  A  V  I  D  G  T  A  C  V  G  G  D  T  R  L  L
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 K  A  R  S  E  E  T  K  E  D  G  E  T  R  G  S  G  E
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Supplementary Figure 4: Nucleic and amino acid sequence of GlaTgs2-Var1.

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