Electronic Supplementary Material (ESI) for Chemical Science.
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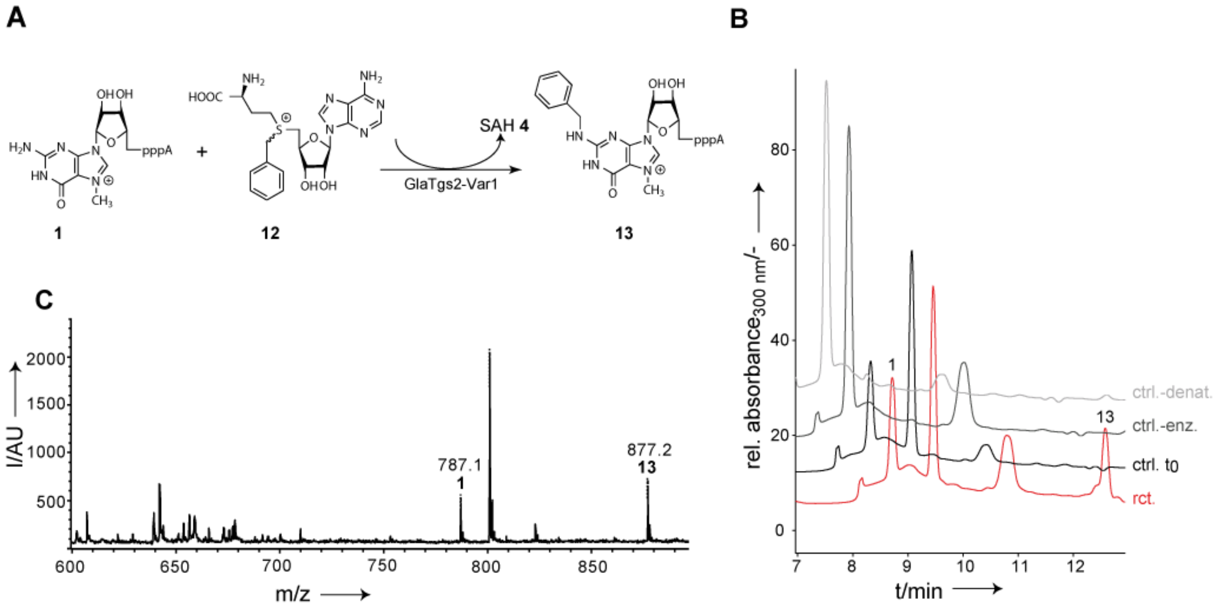
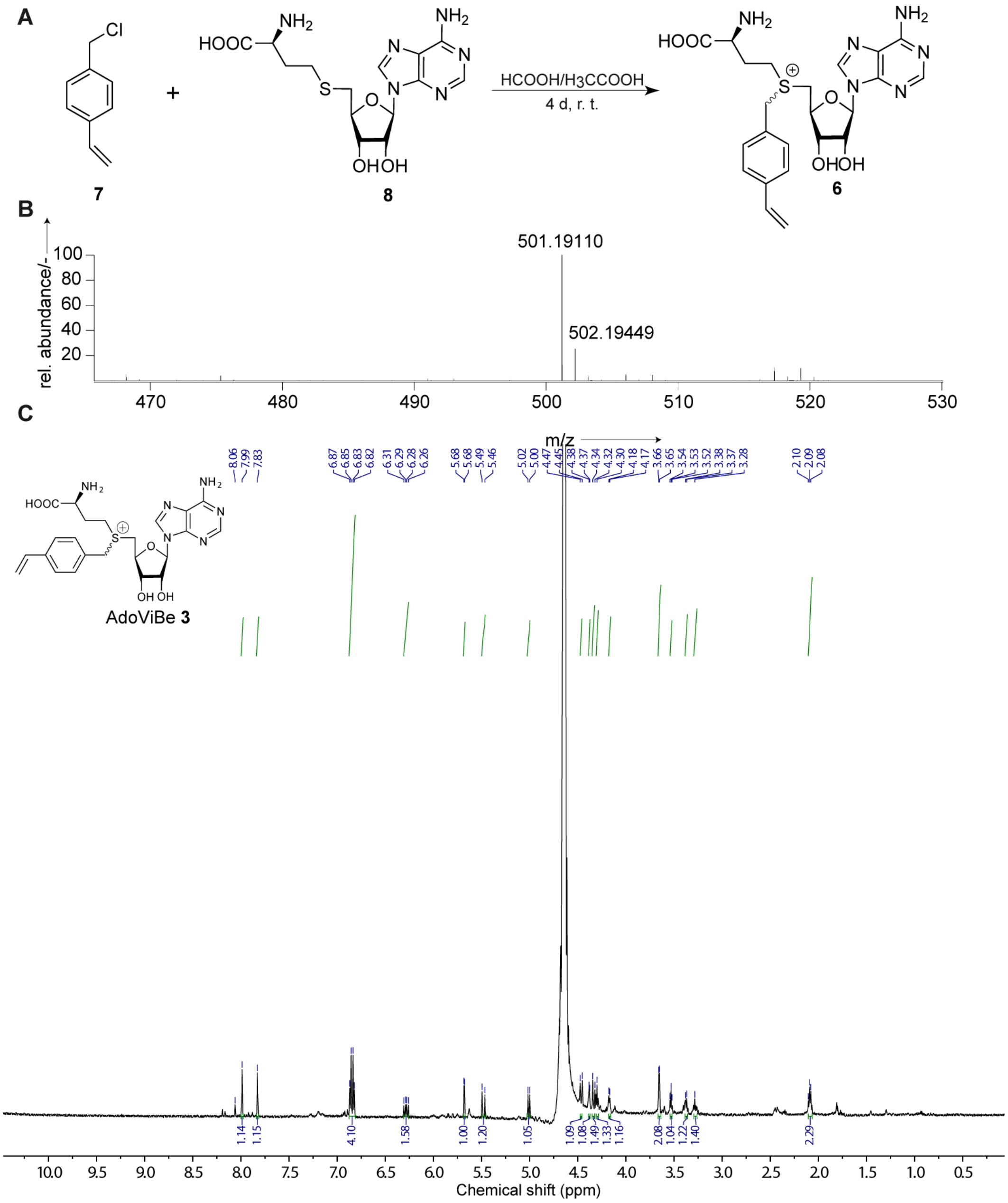
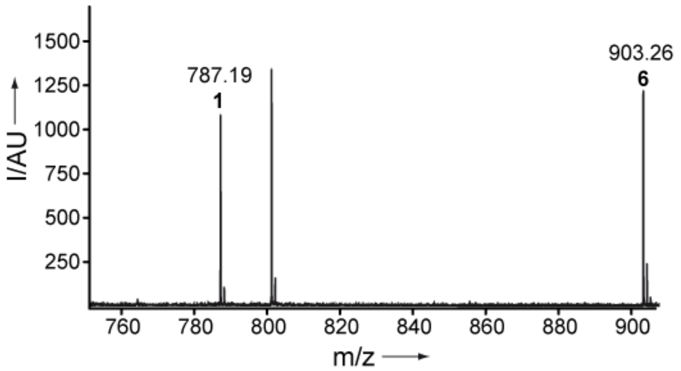
Supplementary Data



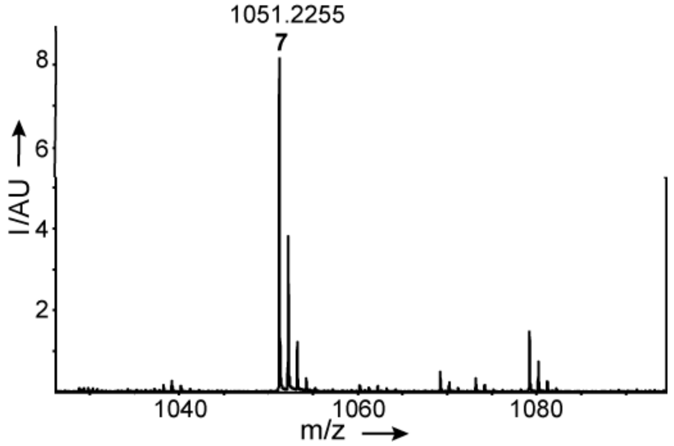
Supplementary Figure 1 **Bioconversion of m^{7}GpppA 1 by GlaTgs2-Var1 with AdoBenz 12 as cosubstrate.** **A**) Reaction scheme of the bioconversion. **B**) HPLC-analysis of bioconversion with AdoBenz **12**. The formation of *N*^{2}-benzyl-m^{7}GpppA **13** was controlled by HPLC-analysis (red) after an incubation period of 3 h at 37 °C. The signal corresponding to **13** was not observed in the negative controls (reaction before incubation, black; without enzyme, grey; with denaturated enzyme, light grey). **C**) MALDI-TOF-MS analysis of rct. shown in **B**). (*m*/*z*): $[M]^+$ calculated for m^{7}GpppA **1** ($C_{21}H_{30}N_{10}O_{17}P_3^+$) 787.10; found 787.1; $[M]^+$ calculated for *N*^{2}-benzyl-m^{7}GpppA **13** ($C_{28}H_{36}N_{10}O_{17}P_3^+$) 877.15; found 877.2.



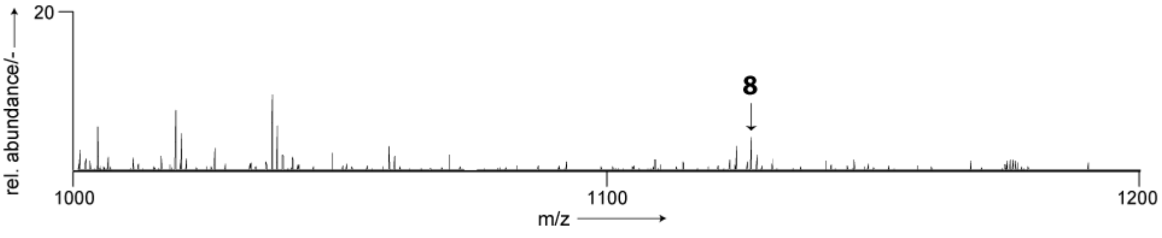
Supplementary Figure 2 **Reaction scheme of AdoViBe-synthesis as well as mass and NMR analysis of the purified product. A)** Reactions scheme. **B)** Mass analysis of purified AdoViBe **3** using ESI-Orbitrap-MS. $[M]^+$ calculated for **3** ($C_{23}H_{29}N_6O_5S^+$): 501.19202; found 501.19110. **C)** ^{1}H NMR spectrum of AdoViBe **3**. Signals could be classified through COSY, TOCSY and HSQC measurements. **^{1}H NMR** (600 MHz, D_2O): δ = 2.09 (q, ^3J = 8.0 Hz, 2H, Hβ), 3.26–3.30 (m, 1H, Hγ), 3.50–3.40 (m, 1H, Hγ), 3.53 (t, ^3J = 6 Hz, 1H, Hα), 3.65-3.66 (m, 2H, H5'), 4.16-4.18 (m, 1H, H4'), 4.3 (t, ^3J = 5.8 Hz, 1H, H3'), 4.33 (dd, ^4J = 12.0 Hz, 1H, H1''), 4.37-4.39 (m, 1H, H2'), 4.46 (dd, ^4J = 12.0 Hz, 1H, H1''), 5.01 (d, ^3J = 10.5 Hz, 1H, vinyl), 5.47 (d, ^3J = 18.2 Hz, 1H, vinyl), 4.69 (d, ^3J = 3.4 Hz, 1H, H1'), 6.26-6.31 (dd, ^3J = 10.5 and 18.2 Hz, 1H, vinyl), 6.82-6.87 (m, 4H, arom. H), 7.83 (s, 1H, arom. H), 7.99 (s, 1H, arom. H).



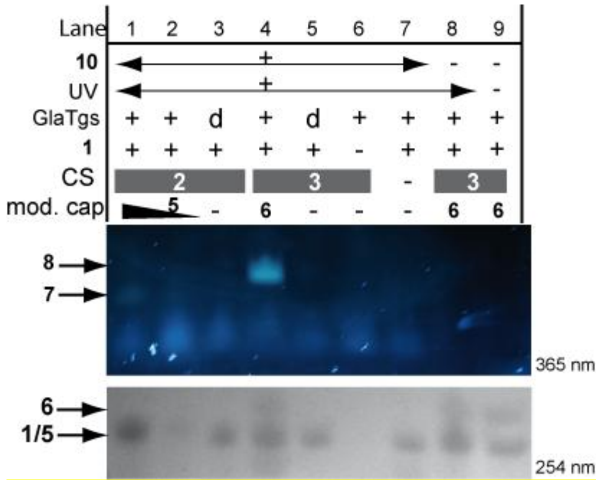
Supplementary Figure 3 **Mass analysis of enzymatically catalyzed reaction to yield *N*^{2}-4-vinylbenzyl-m^{7}GpppA 6.** MALDI-TOF-MS analysis of the transfer reaction showing m^{7}GpppA **1** (*m*/*z* 787) and the formation of **6** (*m*/*z* 903) after three hours.



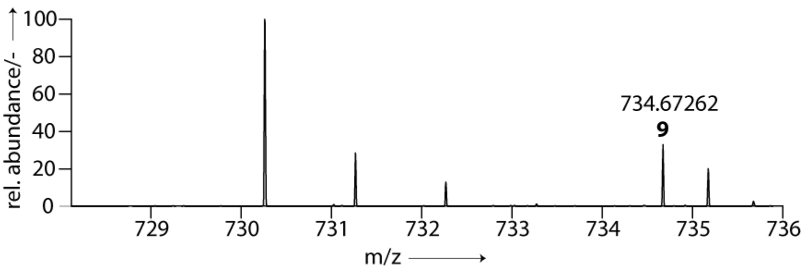
Supplementary Figure 4 **HPLC-ESI-MS analysis of the photoclick reaction of *N*^{2}-allyl-m^{7}GpppA 5 with 10.** The mass of the expected product **7** was detected in the reaction mixture ([M]$^{+}$ calculated for **7** ($C_{38}H_{46}N_{12}O_{18}P_{3}^{+}$): 1051.2266; found 1051.2255).



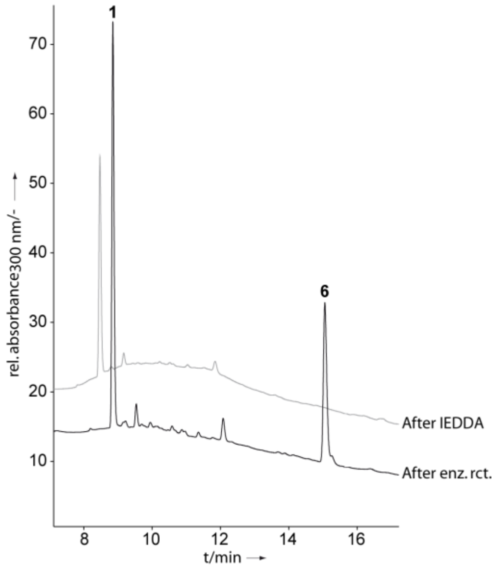
Supplementary Figure 5 **Mass analysis of photoclick reaction between *N*^{2}-4-vinylbenzyl-m^{7}GpppA 6 and 10**. Mass of the expected pyrazoline **8** was detected in the reaction mixture ([M]$^{+}$ calculated for **8** ($C_{44}H_{50}N_{12}O_{18}P_{3}^{+}$): 1127.25790; found 1127.26043).



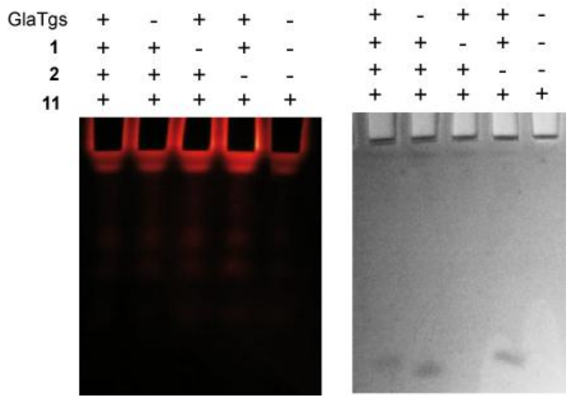
Supplementary Figure 6. **Analysis of photoclick reaction of 5 or 6 with 10 including all negative controls.** The cap-analog m[7]GpppA **1** was used in bioconversions with GlaTgs2-Var1 (GlaTgs) and AdoPropen **2** or AdoViBe **3** yielding **5** or **6**, respectively. To analyze the influence of tetrazole excess, **5** was also diluted to the concentration of **6** (lane 2). The dipolarohiles were subsequently used for photoclick reactions with **10** (lanes 1, 2 and 4). Samples containing enzymatically-modified m[7]GpppA show formation of fluorescing products **7** and **8**, respectively (arrows), as determined after PAGE (20% denat. gel). Controls lacking active enzyme in bioconversion are labeled with “d”. Lower panel: UV-shadowing of the same gel as loading control. The 5’-cap analog **1** and the modified caps **5** and **6** are visible.



Supplementary Figure 7 **Mass analysis of IEDDA between *N*[2]-4-vinylbenzyl-m[7]GpppA 6 and tetrazin-5-TAMRA 11**. Mass of the expected product **9** was detected within the reaction mixture. $[M - 3H]^-$ calculated for **9** ($C_{44}H_{50}N_{12}O_{18}P_3^{2-}$): 734.67342; found 734.67262.



Supplementary Figure 8 **Analyzing the efficiency of the IEDDA of 6 with 11.** Based on reversed-phase HPLC absorbance analyses it was assumed that N^2-4-vinylbenzyl-m^{7}GpppA **6** was completely converted to **9** after IEDDA.



Supplementary Figure 9 **IEDDA with N^2-allyl-modified m^{7}GpppA 5 as dipolarophile.** The cap-analog m^{7}GpppA **1** was used in bioconversion with GlaTgs2-Var1 (GlaTgs) and AdoPropen **2** to give N^2-allyl-m^{7}GpppA **5**, which was subsequently used in IEDDA using tetrazine-5-TAMRA **11** as diene. Samples were analyzed regarding its TAMRA-fluorescence after PAGE (20% denat. gel). No fluorescent product could be detected in samples containing N^2-allyl-m^{7}GpppA **5** (left: fluorescence scan, right: UV-shadowing of the same gel).

GlaTgs2-Var1

```
atgagcacctggctgctggatagcaaatgtgttgaacgtatgaaatggctgtttagcgat
 M  S  T  W  L  L  D  S  K  C  V  E  R  M  K  W  L  F  S  D
ctgccggaagaaaaacgtgtgatgatcaaaatgaatgaagcggcctttttagcgttaca
 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
aaaccgccttatgcagttattgatggcaccgcatgtgttggtggtgatacccgtctgctg
 K  P  P  Y  A  V  I  D  G  T  A  C  V  G  G  D  T  R  L  L
gcaaaacattttgatatgaccgttgccattgaacgtgatccggaaacctatgcactgctg
 A  K  H  F  D  M  T  V  A  I  E  R  D  P  E  T  Y  A  L  L
caggataatctgaccacctggggtgttgatgcaaaaaccattagcggtgataccgcagca
 Q  D  N  L  T  T  W  G  V  D  A  K  T  I  S  G  D  T  A  A
ctgattccgcagttttggaccctgattggtgcagttgcaacctttagcctgtatctggac
 L  I  P  Q  F  W  T  L  I  G  A  V  A  T  F  S  L  Y  L  D
cctccttggggtggtgttgattatcgtagccagaccgatattcagctgaccctgggtagc
 P  P  W  G  G  V  D  Y  R  S  Q  T  D  I  Q  L  T  L  G  S
ctggcagttgaagatgttgttaatcgtgcatttgaagcacatctgagcatgaaactggca
 L  A  V  E  D  V  V  N  R  A  F  E  A  H  L  S  M  K  L  A
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 V  L  K  L  P  R  N  Y  N  C  G  Y  L  F  R  K  L  G  K  H
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 E  V  F  R  I  T  Q  G  N  F  F  V  F  F  V  A  R  R  G  S
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 R  V  K  E  H  G  R  T  A  M  L  Q  L  R  K  A  R  E  E  A
aaagcacgtagcgaagaaaccaaagaagatggcgaaacacgcggtagcggtgaa
 K  A  R  S  E  E  T  K  E  D  G  E  T  R  G  S  G  E
```

Supplementary Figure 10 **Nucleic and amino acid sequence of GlaTgs2-Var1.**