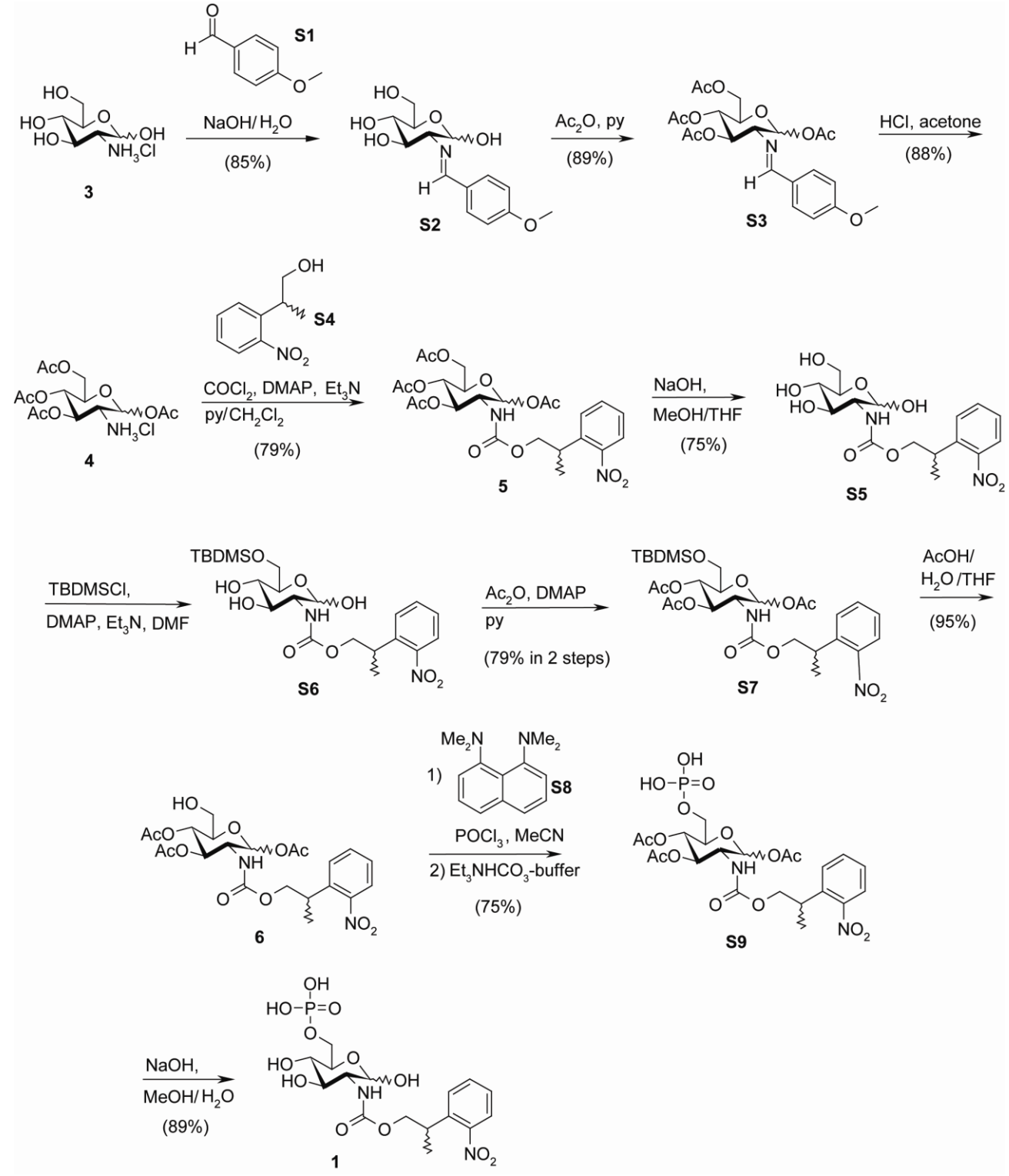
Electronic Supplementary Material (ESI) for Photochemical & Photobiological Science

SUPPORTING INFORMATION

Caged glucosamine-6-phosphate for the light-control of riboswitch activity

Bernhard Wulffen, Maximilian Clemens Rudolf Buff, Monika Pofahl, Günter Mayer* and Alexander Heckel*

1. Synthesis of the caged GlcN6P-derivative 1



Compounds **S2**, **S3** and **4** were obtained according to literature procedures.[1] All reactions were performed under argon atmosphere using dry solvents except for reactions were water was used as solvent.

Protected glucosamine derivative S2: In a slight variation of a literature procedure[1] D-(+)-Glucosamine hydrochloride (**3**) (10.0 g, 46.4 mmol, 1 equiv) was dissolved in aqueous NaOH (47 mL, 1 M). Anisaldehyde (**S1**) (5.72 mL, 47.0 mmol, 1 equiv) was added. The solution was shaken till a white precipitate appeared. Then the mixture was stored in a freezer (-20 °C) for 1 hour. The content of the flask had become a thick, white slurry. It was diluted with water and filtered. The residue was washed with ice-cold water and ice-cold Et_2O/EtOH (1:1). After drying in vacuum the product was obtained as a white, crystalline powder (11.68 g, 39.3 mmol, 85 %). ^{1}H-NMR (DMSO, 400 MHz): δ = 2.77-2.81 (m, 1H), 3.11-3.17 (m, 1H), 3.21-3.26 (m, 1H), 3.39-3.51 (m, 2H), 3.70-3.75 (m, 1H), 3.80 (s, 3H; OMe), 4.53 (t, J=5.8 Hz, 1H), 4.69 (t, J=7.3 Hz, 1H), 4.79 (d, J=5.7 Hz, 1H), 4.90 (d, J=5.4 Hz, 1H), 6.51 (d, J=6.8 Hz, 1H; 1'-H), 6.99 (d, J=8.8 Hz, 2H; arom. H), 7.68 (d, J=8.8 Hz, 2H; arom. H), 8.11 (s, 1H; $MeOC_6H_4CHN$).

Protected glucosamine derivative S3: In a slight variation of a literature procedure[1] compound **S2** (5.0 g, 16.8 mmol, 1 equiv) was dissolved in pyridine (50 mL) and acetic anhydride (12.6 mL, 135 mmol, 8 equiv) was added. The mixture was stirred at room temperature over night. Then the reaction mixture was poured in ice-water (1.4 L) and stirred until the ice had melted. The white precipitate was collected by filtration and washed first with ice-cold water, then carefully with ice-cold EtOH. The product was dried in vacuum to give **S3** as a white solid (6.97 g, 15.0 mmol, 89 %). ^{1}H-NMR (DMSO, 400 MHz): δ = 1.82 (s, 3H; OAc), 1.98 (s, 6H; OAc), 2.02 (s, 3H; OAc), 3.42-3.47 (m, 1H), 3.80 (s, 3H; OMe), 3.99-4.03 (m, 1H), 4.20-4.28 (m, 2H), 4.97 (t, J=9.6 Hz, 1H), 5.44 (t, J=9.6 Hz, 1H), 6.06 (d, J=4.1 Hz, 1H; 1'-H), 6.99 (d, J=8.9 Hz, 2H; arom. H), 7.65 (d, J=8.8 Hz, 2H; arom. H), 8.28 (s, 1H; $MeOC_6H_4CHN$).

Protected glucosamine derivative 4: In a slight variation of a literature procedure[1] compound **S3** (6.97 g, 15.0 mmol, 1 equiv) was dissolved in acetone (60 mL) and heated to boiling. When hydrochloric acid (4.75 mL, 19.0 mmol, 1.27 equiv, c=4 mol/l) was added, the product formed immediately and precipitated. Heating was continued for 1 minute. After the mixture had cooled down to room temperature, it was diluted with acetone. The precipitate was collected by filtration and washed three times with ice-cold acetone. After drying in vacuum the product was obtained as a white solid (5.09 g, 13.3 mmol, 88 %). ^{1}H-NMR (DMSO, 400 MHz): δ = 1.97 (s, 3H; OAc), 2.00 (s, 3H; OAc), 2.03 (s, 3H; OAc), 2.17 (s, 3H; OAc), 3.55-3.60 (m, 1H), 3.98-4.01 (m, 1H), 4.03-4.07 (m, 1H), 4.17-4.21 (m, 1H), 4.91-4.96 (m, 1H), 5.33-5.38 (m, 1H), 5.90 (d, J=8.8 Hz, 1H; 1'-H), 8.78 (bs; NH).

[1] a) M. Bergmann, L. Zervas, *Chem. Ber.*, 1931, **64**, 975.
b) A. C. Cunha, L. O. R. Pereira, M. C. B. V. de Souza, V. F. Ferreira, J. Chem. Edu. 1999, **76**, 79.

NPP-alcohol S4: Compound **S4** was synthesized according to a literature procedure[2]: 1-Nitro-2-ethylbenzene (45.4 g, 300 mmol, 2.4 equiv), paraformaldehyde (3.7 g, 123 mmol, 1 equiv) and Triton B (benzyltrimethylammonium hydroxide, 40% solution in MeOH, 3.6 mL, 0.06 equiv) were dissolved in DMSO (40 mL). The mixture was stirred over night at 90 °C. The product (**S4**) was isolated by two consecutive distillations (130 °C/3.7 hPa) as viscous, yellow liquid (14.1 g, 77.8 mmol, 63 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.33 (d, J=6.9 Hz, 3H; CH_3), 1.68 (bs, 1H; OH), 3.47-3.56 (m, 1H; CH), 3.75-3.83 (m, 2H; CH_2), 7.36 (t, J=7.7 Hz, 1H; arom. H), 7.49 (d, J=8.0 Hz, 1H; arom. H), 7.57 (t, J=7.6 Hz, 1H; arom. H), 7.74 (d, J=8.2 Hz, 1H; arom. H).

Protected glucosamine derivative 5: 4-(Dimethylamino)-pyridine (16 mg, 0.13 mmol, 0.1 equiv) was dissolved in a mixture of CH_2Cl_2 (5 mL) and pyridine (5 mL). A solution of 20 % phosgene in toluene (1.03 mL, 1.95 mmol, 1.5 equiv) was added. This resulted in a yellowish precipitate. A solution of compound **4** (500 mg, 1.3 mmol, 1 equiv) and Et_3N (180 µL, 1.3 mmol, 1 equiv) in CH_2Cl_2 (8 mL) was added with a syringe over 15 min at room temperature. The mixture was stirred for additional 15 min. A solution of NPPOH (**S4**) (707 mg, 3.9 mmol, 3 equiv) and Et_3N (721 µL, 4.2 mmol, 3 equiv) in CH_2Cl_2 (4 mL) was added and the solution was stirred at room temperature over night. The reaction mixture was diluted with CH_2Cl_2, washed with aqueous citric acid (10 %) and sat. aq. $NaHCO_3$ and was dried with $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography (SiO_2, cyclohexane/EtOAc 7:3 → 6:4 → 1:1) to give the diastereomeric mixture **5** as light yellow foam (571 mg, 1.03 mmol, 79 %). R_f (SiO_2, cyclohexane/EtOAc 1:1) = 0.37; ^{1}H-NMR (DMSO, 300 MHz): δ = 1.23 (d, J=6.6 Hz, 3H; NPP-Me), 1.90-2.05 (m, 12H; 4x Ac), 3.32-3.44 (m, 1H; CH-NPP), 3.56-3.68 (m, 1H), 3.85-4.01 (m, 2H), 4.13-4.21 (m, 3H), 4.87 (t, J=9.9 Hz, 1H), 5.13 (q, J=9.4, 1H), 5.65 (d, J=8.4 Hz, 1H), 7.24-7.31 (m, 1H), 7.45-7.51 (m, 1H, arom. H), 7.65-7.70 (m, 2H, arom. H), 7.80-7.85 (m, 1H, arom. H); ^{13}C-NMR (DMSO, 75 MHz): δ = 17.86, 20.25, 20.39, 20.48, 33.36, 54.06, 61.43, 67.50, 68.09, 71.50, 72.11, 72.41, 91.80, 123.74, 127.75, 128.40, 132.89, 136.73, 150.04, 155.68, 168.76, 169.23, 169.46, 170.00; HR-MS (ESI): calcd. for $C_{24}H_{30}N_2O_{13}$ ($M+Na^+$) 577.1645; found 577.1637.

Protected glucosamine derivative S5: Compound **5** (1.86 g, 3.35 mmol, 1 equiv) was dissolved in a mixture of THF (50 mL) and MeOH (50 mL). After the solution had been cooled to 0 °C with an ice bath, a solution of NaOH (33.5 mL, 67.1 mmol, 20 equiv, c=2 mol/l) was added. The solution was stirred for 10 min at 0 °C and was then neutralised with AcOH. The solvent was evaporated and the residue was dissolved in saturated NaCl-solution. The solution was extracted four times with CH_2Cl_2/iPrOH (9:1). The combined extracts were dried with $MgSO_4$ and the solvent was evaporated. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 88:12) to give the diastereomeric mixture **S5** as light yellow foam (975 mg, 2.52 mmol, 75 %). R_f (SiO_2, CH_2Cl_2/MeOH 88:12) = 0.29; ^{1}H-NMR (DMSO, 300 MHz): δ = 1.28 (d, J=7.0 Hz, 3H; NPP-Me), 2.98-3.70 (m, 7H),

[2] Y. Tsuji, S. Kotachi, K.-T. Huh, Y. Watanabe *J. Org. Chem.*, 1990, **55**, 580-584.

4.05-4.17 (m, 2H), 4.33-4.93 (m, 4H), 6.35-6.92 (m, 2H), 7.45-7.51 (m, 1H; arom. H), 7.65-7.73 (m, 2H; arom. H), 7.81-7.85 (m, 1H; arom. H); ^{13}C-NMR (100 MHz, DMSO): δ = 18.07; 18.36; 33.31; 33.51; 48.62; 56.29; 58.67; 61.17; 67.14; 67.46; 70.22; 70.99; 72.05; 74.14; 76.77; 90.63; 95.45; 123.72; 127.73; 128.69; 132.95; 136.91; 150.04; 155.96 ppm. HR-MS (ESI): calcd. for $C_{16}H_{22}N_2O_9$ ($M+Na^+$) 409.1223; found 409.1221.

Protected glucosamine derivatives S6 and S7: A solution of compound **S5** (975 mg, 2.52 mmol, 1 equiv), 4-(dimethylamino)-pyridine (15.4 mg, 126 µmol, 0.05 equiv) and Et_3N (524 µL, 3.78 mmol, 1.5 equiv) was cooled to -15 °C. *t*-Butyldimethylsilyl chloride (458 mg, 2.77 mmol, 1.1 equiv) was added and the solution was stirred at 0 °C. After 3.5 h TLC showed that the reaction was complete. The DMF was evaporated and the residue was diluted with CH_2Cl_2/iPrOH (9:1), washed with sat. aq. NaCl followed by two extractions of the aqueous layer with CH_2Cl_2/iPrOH (9:1). The combined organic phases were dried with $MgSO_4$ and the solvent was evaporated. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 92:8) to give the diastereomeric mixture **S6** as thick oil that contained still some DMF. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.08 (s, 6H; Me(TBDMS)), 0.89 (s, 9H; *t*-Bu(TBDMS)), 1.32-1.36 (m, 3H; NPP-Me), 3.29-5.22 (m, 10H), 7.34-7.39 (m, 1H, arom. H), 7.44-7.48 (m, 1H, arom. H), 7.55-7.59 (m, 1H, arom. H), 7.71-7.75 (m, 1H, arom. H). This crude product was used without further purification: Compound **S6** and 4-(dimethylamino)-pyridine (6 mg, 49.1 µmol, 0.02 equiv) were dissolved in pyridine (15 mL). Acetic anhydride (1.42 mL, 15.1 mmol, 6 equiv) was added and the solution was stirred at room temperature over night. TLC showed that the conversion was complete. The pyridine was evaporated and the residue was coevaporated twice with toluene. The residue was diluted with CH_2Cl_2 and washed with sat. aq. $NaHCO_3$, followed by two extractions of the aqueous layer with CH_2Cl_2. The combined organic layers were dried with $MgSO_4$ and the solvent was evaporated. The product (**S7**) was obtained as mixture of diastereomers by flash chromatography (SiO_2, cyclohexane/EtOAc 6:4) as light yellow foam (1.26 g, 2.01 mmol, 79% over the two steps). R_f (SiO_2, cyclohexane/EtOAc 1:1) = 0.71; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.00-0.08 (m, 6H; Me(TBDMS)), 0.85-0.88 (m, 9H; *t*-Bu(TBDMS)), 1.30-1.33 (m, 3H; NPP-Me), 1.95-2.18 (m, 9H; 3x Ac), 3.63-3.83 (m, 4H), 3.99-4.33 (m, 3H), 4.54-4.73 (m, 1H), 5.04-5.22 (m, 2H), 5.62-6.19 (m, 1H), 7.34-7.74 (m, 4H; arom. H); 13C NMR (100 MHz, $CDCl_3$): δ = -5.30; 17.00; 17.14; 18.38; 20.82; 20.89; 21.07; 25.93; 33.21; 52.79; 52.95; 62.06; 68.37; 69.30; 69.47; 71.06; 71.13; 72.51; 75.47; 90.83; 124.07; 124.25; 127.58; 127.63; 127.90; 127.96; 132.69; 132.78; 136.94; 137.24; 151.01; 155.42; 168.95; 169.18; 171.39 ppm. HR-MS (ESI): calcd. for $C_{28}H_{42}N_2O_{12}Si$ ($M+Na^+$) 649.2404; found 649.2400; elemental analysis calcd. (%) for $C_{28}H_{42}N_2O_{12}Si$: C 53.66, H 6.75, N 4.47; found C 53.75, H 6.87, N 4.40.

Protected glucosamine derivative 6: Compound **S7** (1.18 g, 1.88 mmol, 1 equiv) was dissolved in a mixture of THF (5 mL), AcOH (15 mL) and H_2O (5 mL). The solution was stirred 90 min at 65 °C. TLC showed that the reaction was complete. The THF was evaporated and the residue was diluted with CH_2Cl_2. The organic layer was washed with H_2O and with sat. aq. $NaHCO_3$. Each of the aqueous layers

was extracted two more times with CH_2Cl_2. The combined organic extracts were dried with $MgSO_4$ and the solvent was evaporated. The residue was purified by flash chromatography (SiO_2, cyclohexane/EtOAc 2:8) to give the diastereomeric mixture **6** as light yellow foam (923 mg, 1.80 mmol, 95 %). R_f (SiO_2, EtOAc) = 0.67; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ = 1.31-1.33 (m, 3H; NPP-Me), 1.97-2.21 (m, 9H; 3x Ac), 3.53-3.81 (m, 4H), 3.98-4.34 (m, 3H), 4.72-4.78 (m, 1H), 5.01-5.12 (m, 1H), 5.22-5.27 (m, 1H), 5.99-6.19 (m, 1H), 7.34-7.74 (m, 4H; arom. H); ^{13}C-NMR (DMSO, 126 MHz): δ = 14.33, 16.99, 17.10, 20.71, 20.76, 20.84, 20.97, 21.06, 21.18, 33.15, 52.82, 52.98, 60.53, 61.01, 61.09, 68.23, 68.27, 68.49, 69.37, 69.54, 70.27, 70.37, 72.00, 75.00, 90.81, 92.45, 124.04, 124.26, 127.59, 127.63, 127.88, 127.98, 132.74, 132.79, 136.88, 137.19, 155.31, 155.40, 169.08, 170.21, 170.26, 171.26, 171.30; HR-MS (ESI): calcd. for $C_{22}H_{28}N_2O_{12}$ ($M+Na^+$) 535.1540; found 535.1530.

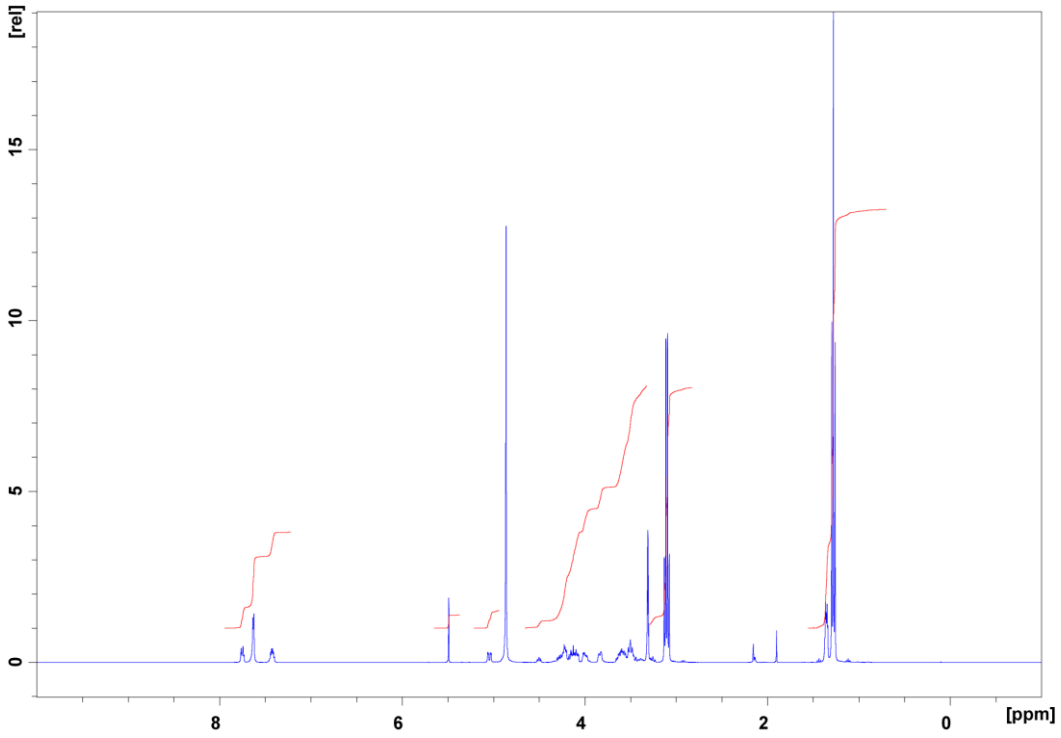
Triethylammonium hydrogen carbonate buffer (TEAB): TEAB-buffer was prepared by adding dry ice to a solution of Et_3N in water (1 L, c=1 mol/l) until pH = 7.4.

Protected glucosamine derivative S9: Compound **6** (793 mg, 1.55 mmol, 1 equiv) and proton sponge (**S8**; N,N,N',N'-tetramethyl-1,8-naphtalenediamine, 1.99 g, 9.3 mmol, 6 equiv) were dissolved in acetonitrile (40 mL) and the solution was stirred 30 min at 0 °C. Phosphorus oxide chloride (568 µL, 6.2 mmol, 4 equiv) was added and the reaction mixture was stirred at 0 °C. After 3 h no more starting material could be detected by TLC. The reaction mixture was poured into ice cold TEAB-buffer (400 mL, c=0.2 mol/l). The aqueous solution was stored at 4 °C over night, washed four times with EtOAc and concentrated under reduced pressure to a volume of 10 mL. The resulting solution was purified in 2 portions by MPLC (stationary phase: Merck Lobar LiChroprep RP-18 resin 310-25, equilibrated with 0.1M TEAB buffer; gradient: 0–100% MeCN against 0.1M TEAB over 30 min; flow rate: 20 mL/min). The product-containing fractions were combined and evaporated to dryness to give the diastereomeric mixture **S9** as light brown glassy solid (929 mg, 1.17 mmol, 75 %). R_f (RP-18, MeCN/TEAB 0.1 M 3:7) = 0.30; ^{1}H-NMR (DMSO, 400 MHz): δ = 1.21-1.27 (m, 3H; NPP-Me), 1.78-2.14 (m, 9H; 3x Ac), 3.33-4.31 (m, 7H), 4.59-5.14 (m, 2H), 5.61-5.91 (m, 1H), 7.45-7.84 (m, 4H; arom. H); ^{13}C NMR (100 MHz, DMSO-d6): δ = 9.68; 17.94; 20.40; 20.80; 33.31; 33.44; 45.19; 52.21; 54.08; 62.53; 63.03; 67.59; 68.75; 70.25; 70.45; 70.78; 73.35; 89.69; 123.70; 127.79; 128.55; 132.88; 136.74; 149.95; 156.04; 169.15; 169.75 ppm; ^{31}P NMR (DMSO, 162 MHz): δ = 0.11; HR-MS (ESI): calcd. for $C_{22}H_{29}N_2O_{15}P$ ($M+Na^+$) 615.1203; found 615.1183.

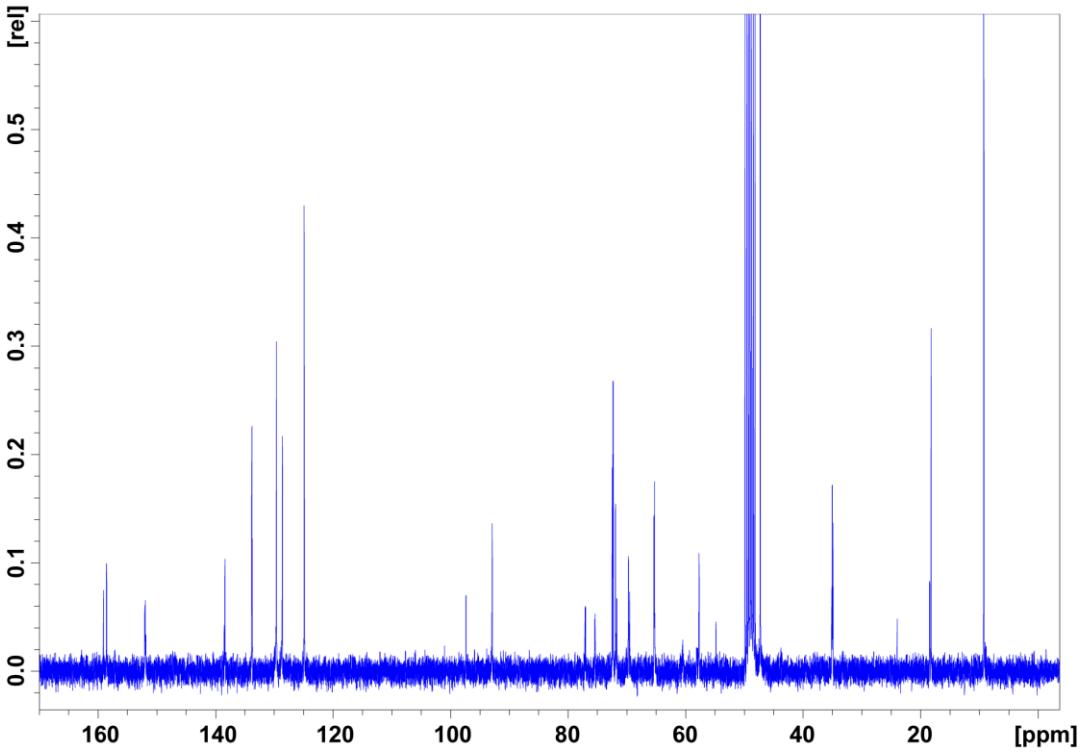
Protected glucosamine derivative 1: Compound **S9** (877 mg, 1.10 mmol, 1 equiv) was dissolved in a mixture of THF, MeOH and water (80 mL; THF/MeOH/H_2O 1:1:2). The solution was cooled in an ice bath to 0 °C and aq. sodium hydroxide solution (11 mL, c=2 mol/l, 20 equiv) was added. After stirring for 10 min at 0 °C the reaction mixture was neutralized by addition of acetic acid. The solvents were evaporated and the residue was dissolved in 0.1 M TEAB-Buffer (5 mL). The resulting solution was purified in one portion by MPLC (stationary phase: Merck Lobar LiChroprep RP-18 resin 310-25, equilibrated with 0.1M TEAB buffer; gradient: 0–20% MeCN against 0.1M TEAB over 30 min; flow

rate: 20 mL/min). The product-containing fractions were combined and evaporated to dryness to give the diastereomeric mixture **1** as light yellow glassy solid (559 mg, 986 µmol, 89 %). R_f (RP-18, MeCN/TEAB 0.1M 3:7) = 0.61; ^{1}H NMR (CD_3OD, 400 MHz): δ = 1.33-1.37 (m, 3H; NPP-Me), 3.34-3.66 (m, 4H), 3.81-3.86 (m, 1H), 3.97-4.02 (m, 1H), 4.06-4.31 (m, 4H), 5.02-5.07 (m, 1H), 7.40-7.49 (m, 1H; arom. H), 7.61-7.65 (m, 2H; arom. H), 7.73-7.77 (m, 1H; arom. H); ^{13}C NMR (75 MHz, D_2O): δ = 9.25; 18.15; 18.50; 35.06; 47.29; 57.67; 65.36; 69.47; 69.76; 71.64; 71.92; 72.38; 75.43; 77.02; 92.94; 97.34; 124.91; 128.63; 129.59; 133.81; 138.41; 151.99; 158.55; 159.25 ppm; ^{31}P NMR (CD_3OD, 162 MHz): δ = 3.58; HR-MS (ESI): calcd. for $C_{16}H_{23}N_2O_{12}P$ ($M\text{-}H^+$) 465.0910; found 465.0907.

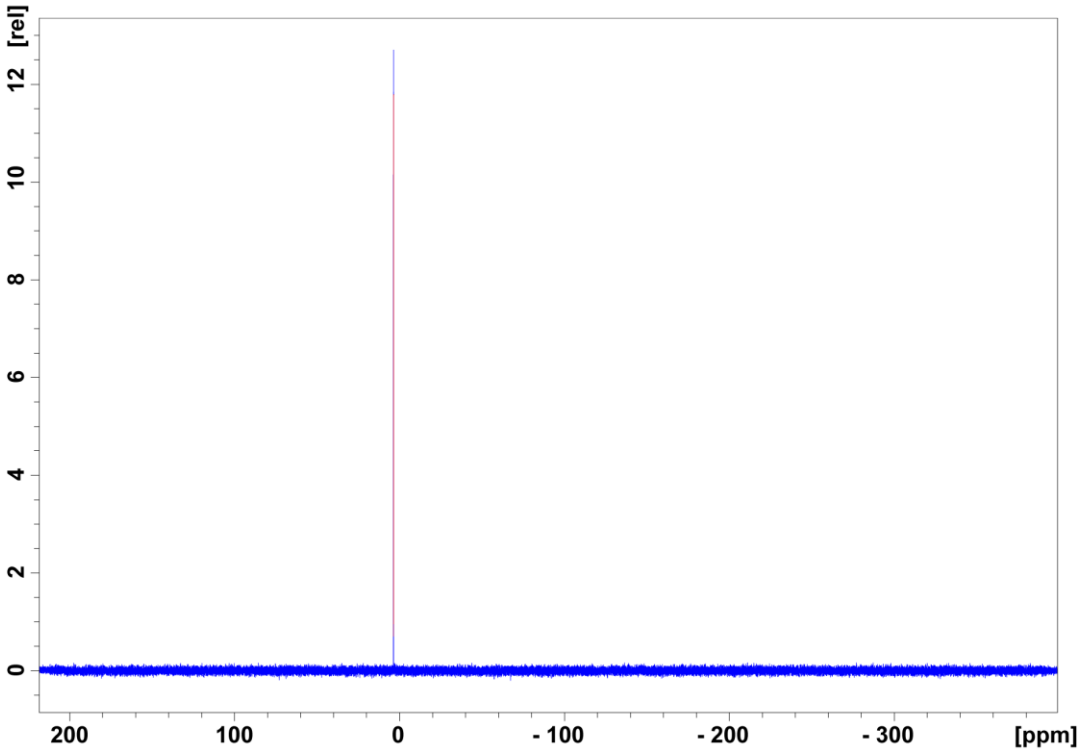
2. NMR-spectra of the caged GlcN6P (1):



^{1}H-NMR spectrum (400 MHz) of compound **1** in CD_3OD

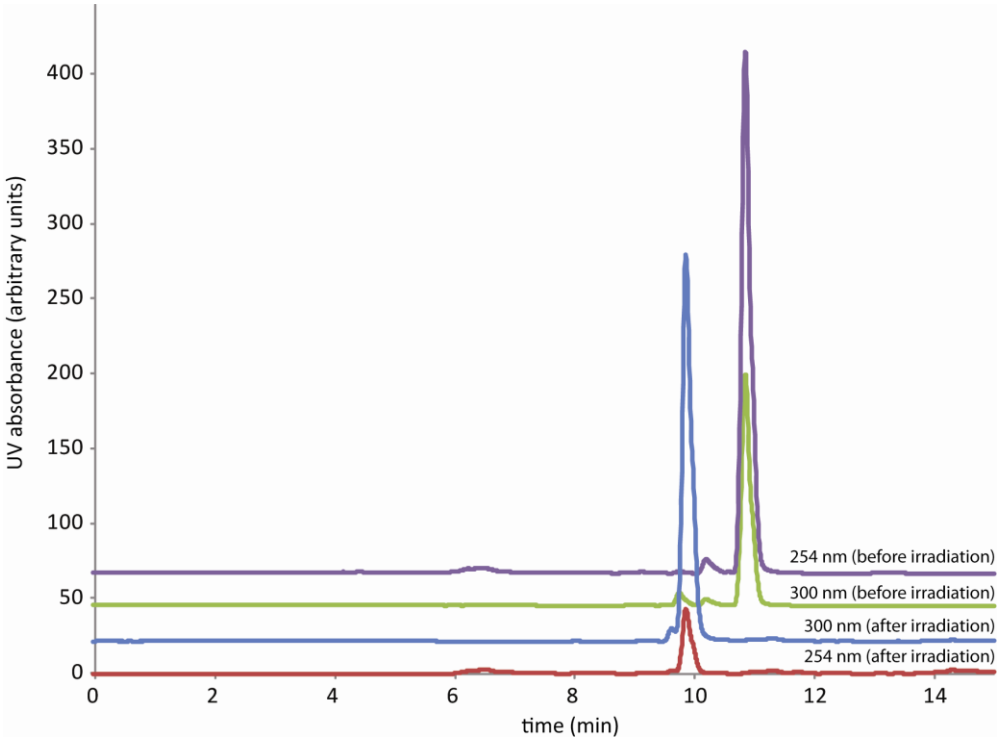


^{13}C-NMR spectrum (75 MHz) of compound **1** in CD_3OD



^{31}P-NMR spectrum (162 MHz) of compound **1** in CD_3OD

3. HPLC deprotection studies of compound 1:



For this study a sample of compound **1** (1 mg) was dissolved in PBS buffer. Then an aliquot was analyzed by RP-HPLC (Nucleosil 100 C18 5 µm, 250 mm x 4.6 mm, 0.01 M aqueous triethylammonium acetate/MeCN 0%→50% in 15 min), monitoring traces both at 254 nm and 300 nm. Another aliquot was first irradiated (366 nm, 3 min, 3 x UV-LED Nichia NCCU033, 0.5 A, 100mW) and then analyzed by HPLC. The shifted peak after irradiation arises from the cleavage product of the caging group and has a significantly different UV absorbance characteristic. The formation of the (UV-inactive) uncaging product GlcN6P was for example proven by TLC analysis.

4. RNA synthesis and labeling

dsDNA templates for the in vitro transcription were prepared by PCR using the template 5'-GATAGCGCCCGAACTAAGCGCCCGGAAAAAGGCTTAGTTGACGAGGATGGAGGTT-ATCGAATTTTCGGCGGATGCCTCCCG-3' and the primer sequences
5'-glmS.mini 5'-TAATACGACTCACTATAGATAGCGCCCGAACTAAG-3' and
3'-glmS.mini 5'-CGGGAGGCATCCGCCGAAAATTC-3'.
For fluorescein labeling of RNA the dsDNA was transcribed in the presence of 10mM GMPS (emp biotech, Germany) o/n at 37 ºC. After GMPS transcription the reaction was treated with DNase I for 15 min at 37ºC and subsequently phenol/chloroform extracted. After ethanol precipitation, the RNA was dissolved in 100µl H_2O and gel filtered twice using microspin columns (G25, GE Amersham).

Labeling with fluorescein was accomplished by incubation of the GMPS-RNA with a 200 fold molar excess of 5-(iodoacetamido)-fluorescein (Sigma) in 50mM Tris pH 8.0, 50mM EDTA and 2M Urea for 2h at 40ºC. The labeled RNA was purified by denaturing polyacrylamide gel electrophoresis (10%), followed by UV-shadowing and eluted from the gel by the crush and soak method.

5. Fluorescence polarization assays

Fluorescence polarization assays were performed by incubating the fluorescein labeled *glmS* ribozyme [100nM] in cleavage buffer (50mM Tris pH 7.9, 200mM KCl, 10mM $MgCl_2$, 0.001% Tween-20) for 30 min at 25ºC in the presence or absence of the metabolites as indicated (GlcN6P, GlcN were purchased from Sigma.). Irradiation with UV light (λ = 366 nm) was achieved as indicated by employing a commercially available UV hand lamp. The final reaction volume was 25µl and all reactions were incubated in black 96well plates (Corning) in the dark. After incubation the samples were read by a Tecan Ultra fluorescence reader (Tecan, Crailsheim, Germany). All assays were performed in duplicates.

6. Cloning and synthesis of mRNA with the *glmS* riboswitch in its 3'-UTR

The glmS *B. subtilis* sequence was inserted into the NotI site of pEGFP-N1 (Clontech). Cleavage with NotI restriction enzyme (NEB) and ligation with T4 DNA ligase (NEB) was performed according to the manufacturer instructions. Prior to ligation, the linearized vector was treated with CIAP (Promega) to minimize religation. Super competent XL 10 Gold *E. coli* bacteria were incubated with 10 µl of the ligation reaction for 30 min. on ice. After a 30 sec. heat-pulse at 42 °C the bacteria were incubated for 2 min on ice and 500 ml SOC medium was added. The suspension was incubated for 60 min. at 37 °C at 1000 rpm in a thermomixer (Eppendorf). Bacteria were plated on LB agar plates with ampicillin and incubated o.n. at 37°C. Colonies were picked and grown o.n. at 37 °C in LB media with ampicillin. Subsequently, plasmids were isolated and analysed by sequencing for correct introduction of the *glmS* ribozyme sequence.

The synthesis of mRNA was achieved through amplification of the corresponding dsDNA template using PCR and the following primer molecules:

FP: 5'-TCTAATACGACTCACTATAGGGTCAGATCCGCTAGCGCTACC-3' and

RV: 5'-GCTTTATTTGTAACCATTATAAGCTGC-3' or

RV polyA(30) : 5'-A(30)GCTTTATTTGTAACCATTATAAGCTGC-3' .

PCR was done using Pfu DNA Polymerase 0.5 ng/ µl DNA plasmid template and the following PCR program: initial denaturation 60 sec at 98 °C; denaturation 30 sec at 98 °C, annealing 30 sec at 58 °C (using RV-primer) or 64 °C (using RV polyA(30)-primer), extension 72 °C 120 sec, 20 cycles; final extension 180 sec 72 °C. After cycling the reaction product was purified with NucleoSpin Extract II (Macherey-Nagel) according to the manufacturer's instructions. mRNA molecules were prepared as follows. The transcription reaction was incubated at 37 °C for 120 min in a final volume of 100 µl with DNA Template (5 nM), 40 mM HEPES pH 7,9, 25 mM $MgCl_2$, 10 mM DTT, 2 U/ µl RNasin

(Promega), 2 ng/µl IPP, 4 mM NTP and 0,8 U/ µl T7 RNA Polymerase (Stratagene). Capped-mRNA transcripts were obtained as described above including 2 mM NTP and a four fold molar excess of the cap-analogue [m^7G(5')ppp(5')G] (Ambion). Before performing a phenol and chloroform extraction the transcription was stopped in a final volume of 750 µl with 0,5 M ammonium acetate pH 7 and 10 mM EDTA pH 8. An equal volume of isopropanol was added, thoroughly mixed and incubated o.n. at -20°C. After centrifugation (20 min., 20000 rpm) the pellet was washed with 70% ethanol, dissolved in DEPC water and finally gel filtrated through a G25 column (GE Healthcare).

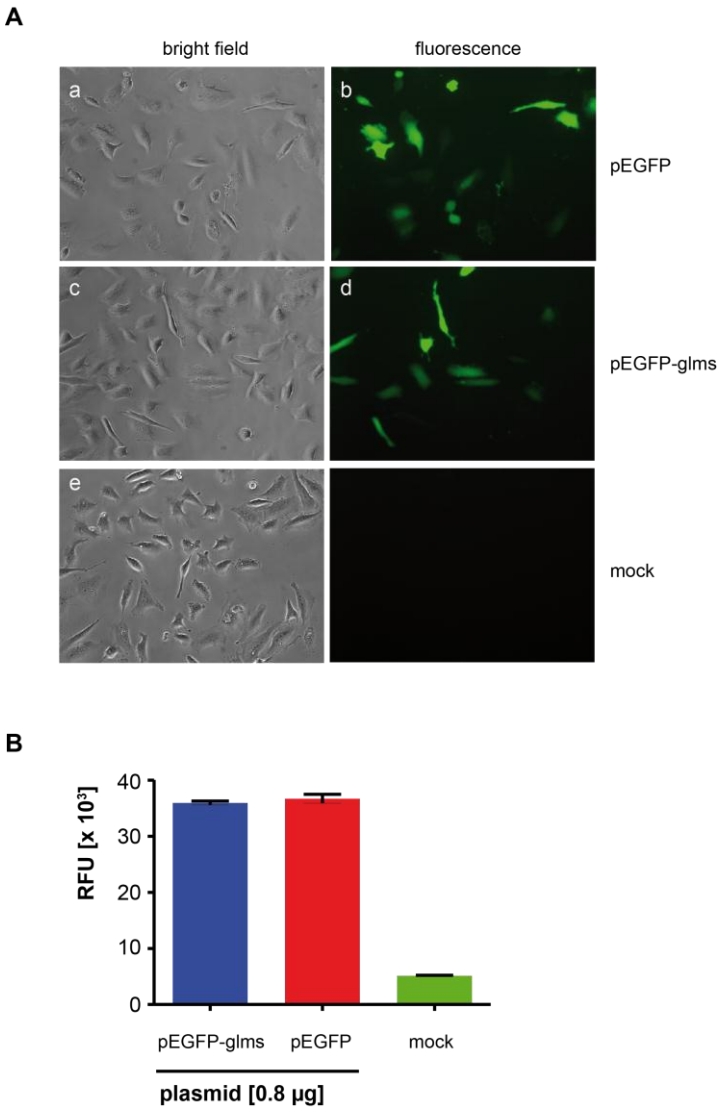
7. In vitro mRNA cleavage and translation

GlcN6P and caged GlcN6P dependent cleavage of corresponding mRNA molecules was performed in a final volume of 16.5 µl at 30 °C for 1.5 h using cleavage buffer (10 mM HEPES pH 7.4, 100 mM KCl, 2.1 mM magnesium acetate, 100 nM RNA and 50 µM GlcN6P or caged GlcN6P). Prior to assaying, mRNA molecules were heated at 80 °C for 2 min and subsequently cooled on ice. Irradiation of caged GlcN6P with UV light (λ = 365 nm) was performed for 3 minutes. After incubation 8.5 µl of 2x RNA loading buffer (Peqlab) was added to each sample and the RNA fragments were separated using a 1 % agarose gel.

In vitro translation was carried out using Flexi Rabbit Reticulocyte Lysate (Promega) according to the manufacturer instruction with the following modifications. In a final volume of 33 µl the in vitro translation was performed with 10 µM amino acid mixture without leucine, 10 µM amino acid mixture without methionine, 100 nM RNA, 50 µM GlcN6P or caged GlcN6P, and 16.5 µl reticulocyte lysate. Magnesium acetate was supplemented to a final magnesium ion concentration of 2.1 mM. Irradiation of caged GlcN6P with UV light (λ = 365 nm) was performed for 3 minutes. After incubation for 90 min 13 µl water was added and the samples were transferred to a 96 well plate (Greiner). Fluorescence emission was detected using a TECAN Ultra (Tecan) plate reader: λ ex.: 485nm, λ em.: 535 nm. Data analysis was performed with the XFLUOR4 (Version: V 4.51) Software.

8. Cellular expression of the EGFP under *glmS* riboswitch control

To analyze whether the cloned construct, bearing the *glmS* riboswitch in the 3'-UTR, can produce EGFP in eukaryotic cells at the same level as in the absence of the *glmS* riboswitch element we transfected HeLa cells (confluency 50%) using Lipofectamine 2000 (Invitrogene) with the corresponding plasmids [0.8 µg]. After 18h EGFP expression was analyzed by fluorescence microscopy (A) and the amount EGFP produced was quantified by fluorescence spectroscopy (B).



A) EGFP-expression in HeLa cells, which have been transfected with the plasmid encoding for EGFP without (top) or with (middle) the *glmS* riboswitch RNA element. Bottom: mock control without plasmid. Left: bright field, right: fluorescence microscopy (λ_{ab} = 488 nm, λ_{em} = 507 nm). B) Quantification of EGFP expression in HeLa cells after transfection with the indicated plasmids.