Supplementary information

A light-responsive RNA aptamer for an azobenzene derivative

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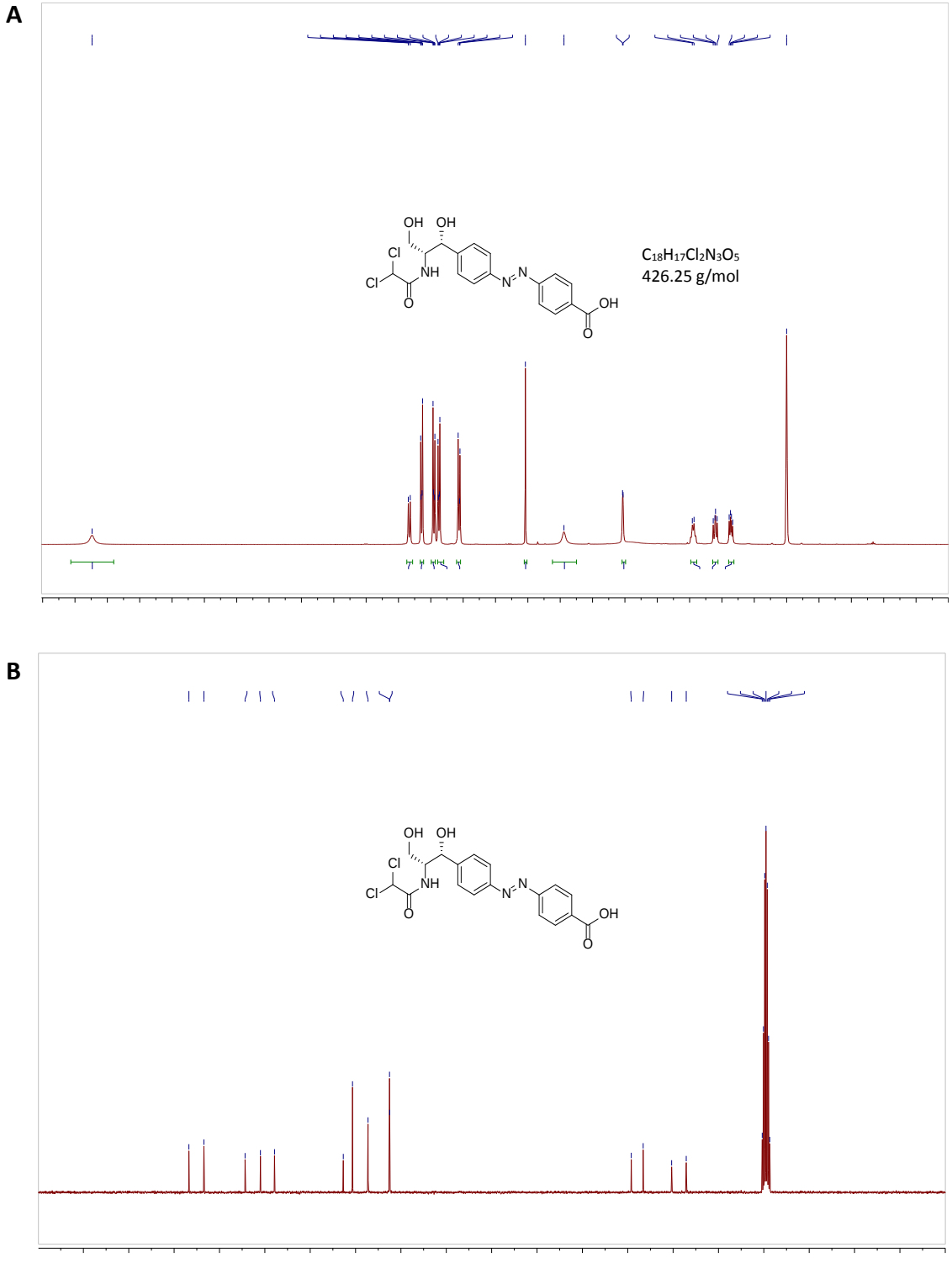
Supplementary Figure S1



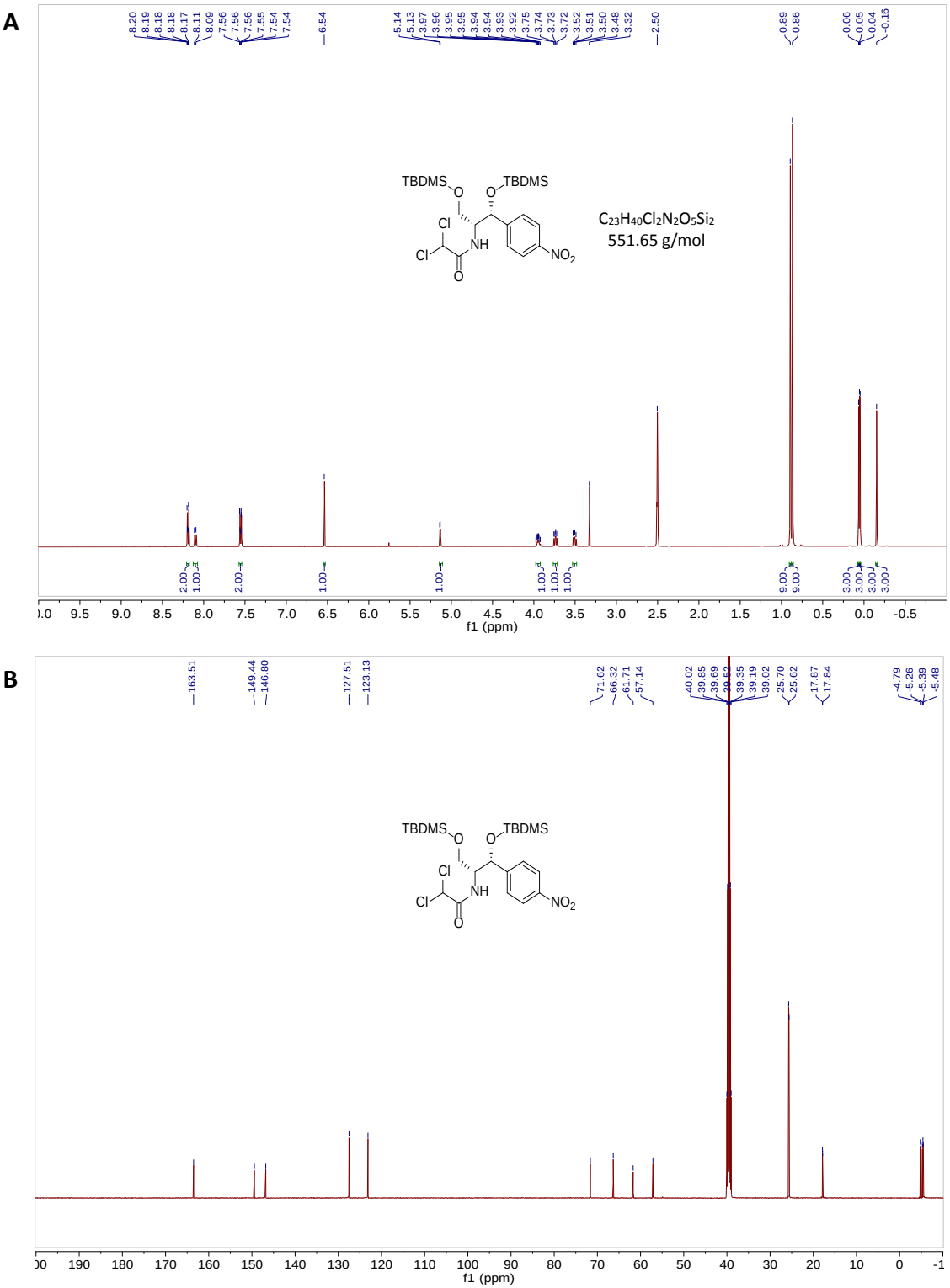
Supplementary Figure S1: NMR of 4-((*E*)-(4-((1*R*)-2-(2,2-Dichloroacetamido)-1,3-dihydroxypropyl) phenyl) diazenyl) benzoic acid [3]. A) ^{1}H-NMR of compound **3. B)** ^{13}C-NMR of compound **3**.

Chloramphenicol (1.6 g, 5.0 mmol) was dissolved in EtOH (25 mL) and NH_4Cl (0.6 g, 11.2 mmol, solution in 4 mL H_2O) was added. Tin powder (2.5 g, 37.0 mmol) was slowly added and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was filtered and transferred to a dropping funnel and slowly added to $FeCl_3 \cdot 6\ H_2O$ (2.0 g, 9.2 mmol) in EtOH (6.0 mL) at -5 °C and stirred for 2 h. Brine was added to the cold mixture and the green product was extracted with CH_2Cl_2 (100 mL). The organic layer was washed with brine (2x 100 mL) and H_2O (2x 100 mL) to remove remaining $FeCl_3$ and dried over Na_2SO_4. The solvent was removed under reduced pressure.

4-Aminobenzoic acid (0.7 g, 5.0 mmol) and acetic acid (60 mL) were added and the reaction mixture was stirred at 60 °C over night. The solvent was removed under reduced pressure. Purification via flash chromatography (EtOAc → EtOAc:MeOH 9:1) afforded 4-((*E*)-(4-((1*R*)-2-(2,2-dichloroacetamido)-1,3-dihydroxypropyl)phenyl)-diazenyl)benzoic acid as an orange solid (1.1 g, 2.6 mmol, 52%).

^{1}H-NMR:	(300 MHz, DMSO-d_6) δ = 13.24 (s, 1H), 8.33 (d, *J* = 9.0 Hz, 1H), 8.14 (d, *J* = 8.6 Hz, 2H), 7.95 (d, *J* = 8.6 Hz, 2H), 7.87 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 6.54 (s, 1H), 5.94 (s, 1H), 5.03 (s, 1H), 3.99-3.90 (m, 1H), 3.63-3.57 (m, 1H), 3.39-3.33 (m, 1H) ppm.
^{13}C-NMR:	(75 MHz, DMSO-d_6) δ = 166.7, 163.4, 154.4, 151.0, 147.9, 132.7, 130.7, 127.3, 122.5, 122.5, 69.2, 66.6, 60.3, 57.1 ppm.
ESI-MS (+):	*m/z*: $[M-H]^+$ 424.04
HRMS:	*m/z* calcd. for $C_{18}H_{17}Cl_2N_3O_5$ 426.06180 $[M+H]^+$, found 426.06078 (Δm = 0.00102, error 2.4 ppm).

Supplementary Figure S2



Supplementary Figure S2: 2,2-Dichloro-*N*-((5*R*)-2,2,3,3,9,9,10,10-octamethyl-5-(4-nitrophenyl)-4,8-dioxa-3,9-disilaundecan-6-yl)acetamide [2]. A) ^{1}H-NMR of compound **2**. **B)** ^{13}C-NMR of compound **2**.

Chloramphenicol (5.00 g, 15.47 mmol, 1.0 eq) and imidazole (9.48 g, 139.20 mmol, 9.0 eq) were dissolved in dry DMF (40 mL) and TBDMS-Cl (17.24 g, 112.90 mmol, 7.3 eq) was slowly added. The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure. CH_2Cl_2 (100 mL) was added and the organic layer was washed with citric acid (5x400 mL, 5% wt. in H_2O). The combined aqueous solutions were extracted with CH_2Cl_2 (3x 50 mL) and the combined organic layers were washed with brine (2x 50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure. Purification via flash chromatography (cyclohexane:EtOAc 9:1) afforded 2,2-dichloro-*N*-((5*R*)-2,2,3,3,9,9,10,10-octamethyl-5-(4-nitrophenyl)-4,8-dioxa-3,9-disilaundecan-6-yl)acetamide as a white solid (6.83 g, 12.37 mmol, 80%).

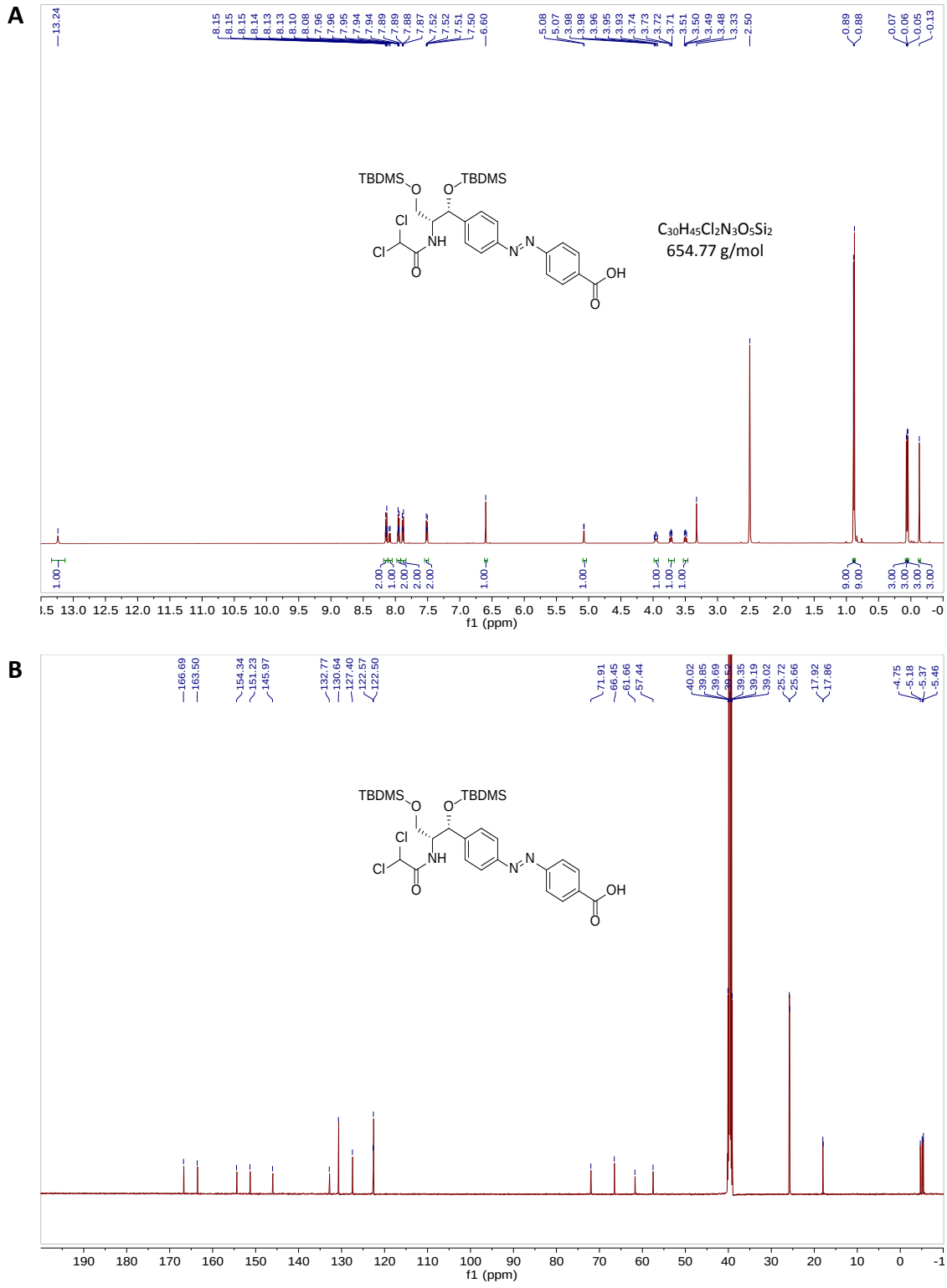
^{1}H-NMR: (500 MHz, DMSO-d_6) δ = 8.19 (d, *J* = 8.8 Hz, 2H), 8.10 (d, *J* = 9.3 Hz, 1H), 7.55 (d, *J* = 8.7 Hz, 2H), 6.54 (s, 1H), 5.13 (d, *J* = 2.9 Hz, 1H), 3.97-3.92 (m, 1H), 3.75-3.72 (m, 1H), 3.52-3.48 (m, 1H), 0.89 (s, 9H), 0.86 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H), -0.16 (s, 3H).

^{13}C-NMR: (125 MHz, DMSO-d_6) δ = 163.5, 149.4, 146.8, 127.5, 123.1, 71.6, 66.3, 61.7, 57.1, 25.7, 25.6, 17.9, 17.8, -4.8, -5.3, -5.4, -5.5 ppm.

ESI-MS (+): *m/z*: $[M+H]^+$ 551.23

HRMS: *m/z* calcd. for $C_{23}H_{40}Cl_2N_2O_5Si_2$ 551.19256 $[M+H]^+$, found 551.19160 (Δm = 0.00096, error 1.7 ppm).

Supplementary Figure S3



Supplementary Figure S3: 4-((*E*)-(4-((5*R*)-6-(2,2-Dichloroacetamido)-2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-5-yl)phenyl)diazenyl)benzoic acid [4]. A) ^{1}H-NMR of compound **4. B)** ^{13}C-NMR of compound **4**.

2,2-Dichloro-*N*-((5*R*)-2,2,3,3,9,9,10,10-octamethyl-5-(4-nitrophenyl)-4,8-dioxa-3,9-disilaundecan-6-yl) acetamide (2.00 g, 3.63 mmol, 1.0 eq) was dissolved in EtOH (20 mL) and ammonium chloride (0.44 g, 8.30 mmol, 2.3 eq solution in 3 mL water) was added. Tin powder (1.83 g, 27.95 mmol, 7.7 eq) was slowly added and the reaction mixture was stirred at room temperature for 30 min. The reaction mixture was filtered and transferred to a dropping funnel. The reaction mixture was slowly added to $FeCl_3 \cdot 6\ H_2O$ (2.42 g, 5.26 mmol, 1.5 eq) in EtOH (3 mL) and water (9 mL) at -5 °C and stirred for 2 h. Brine was added and the green reaction mixture was extracted with CH_2Cl_2 (100 mL). The organic layer was washed with brine (2x 100 mL) and H_2O (2x 100 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure.

4-Aminobenzoic acid (1.90 g, 3.63 mmol, 1.0 eq) and acetic acid (50 mL) were added and stirred at 60 °C over night. The solvent was removed under reduced pressure. Purification via flash chromatography (cyclohexane:EtOAc 9:1 → EtOAc) afforded 4-((*E*)-(4-((5*R*)-6-(2,2-dichloroacetamido)-2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-5-yl)phenyl)diazenyl)benzoic acid as an orange foam (1.5 g, 2.32 mmol, 64%).

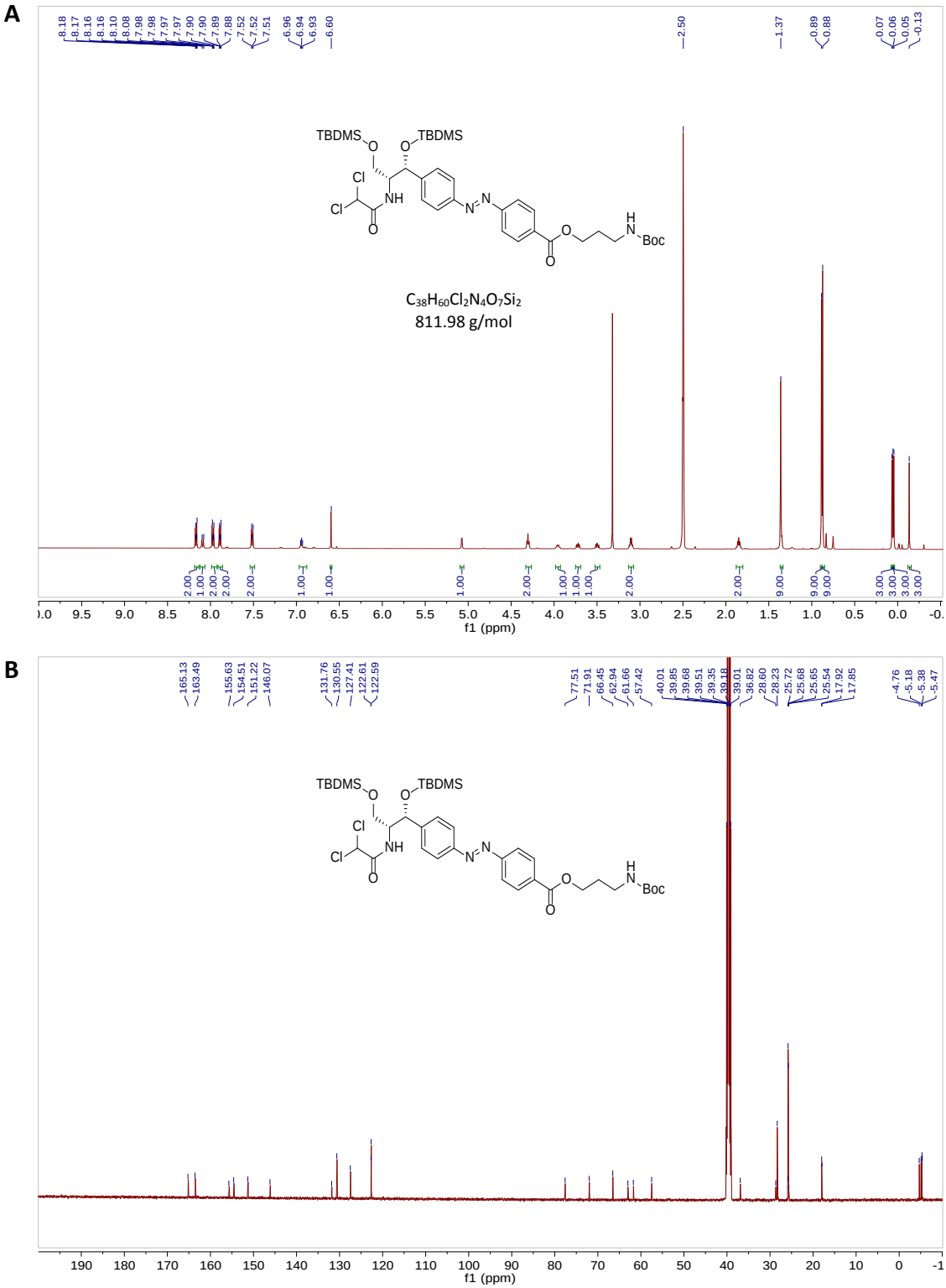
^{1}H-NMR: (500 MHz, DMSO-d_6) δ = 13.24 (s, 1H), 8.14 (d, *J* = 8.6 Hz, 2H), 8.09 (d, *J* = 9.2 Hz, 1H), 7.95 (d, *J* = 8.6 Hz, 2H), 7.88 (d, *J* = 8.5 Hz, 2H), 7.51 (d, *J* = 8.5 Hz, 2H), 6.60 (s, 1H), 5.07 (d, *J* = 3.2 Hz, 1H), 3.98-3.93 (m, 1H), 3.74-3.70 (m, 1H), 3.51-3.48 (m, 1H), 0.89 (s, 9H), 0.88 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H), -0.13 (s, 3H) ppm.

^{13}C-NMR: (125 MHz, DMSO-d_6) δ = 166.7, 163.5, 154.3, 151.2, 146.0, 132.8, 130.6, 127.4, 122.6, 122.5, 71.9, 66.5, 61.7, 57.4, 25.7, 25.7, 17.9, 17.9, -4.7, -5.2, -5.4, -5.5 ppm.

ESI-MS (+): *m/z*: $[M-H]^+$ 652.30

HRMS: *m/z* calcd. for $C_{30}H_{45}Cl_2N_3O_5Si_2$ 654.23476 $[M+H]^+$, found 654.23462 (Δm = 0.00014, error 0.2 ppm).

Supplementary Figure S4



Supplementary Figure S4: 3- ((*tert*-Butoxycarbonyl) amino) propyl 4-((*E*)- (4-((5*R*) -6-(2,2-dichloroacetamido) -2,2,3,3,9,9,10,10- octamethyl -4,8-dioxa-3,9-disilaundecan-5-yl) phenyl) diazenyl) benzoate [5]. A) ^{1}H-NMR of compound **5**. **B)** ^{13}C-NMR of compound **5**.

4-((*E*)-(4-((5*R*)-6-(2,2-Dichloroacetamido)-2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-5-yl)phenyl)diazenyl)benzoic acid (0.53 g, 0.81 mmol, 1.0 eq), EDC (0.34 g, 2.43 mmol, 3.0 eq), 3-(boc-amino)-1-propanol (0.17 g, 0.97 mmol, 1.2 eq) and 4-DMAP (0.30 g, 2.43 mmol, 3.0 eq) were dissolved in dry DMF (50 mL) and stirred at room temperature for 48 h. H_2O (50 mL) was added and the reaction mixture was extracted with EtOAc (3x 50 mL). The combined organic layers were washed with brine (2x 100 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification via flash chromatography (cyclohexane:EtOAc 9:1) afforded 3-((*tert*-butoxycarbonyl)amino)propyl 4-((E)-(4-((5R)-6-(2,2-dichloroacetamido)-2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-5-yl)phenyl)diazenyl)benzoate as an orange foam (0.56 g, 0.70 mmol, 86%).

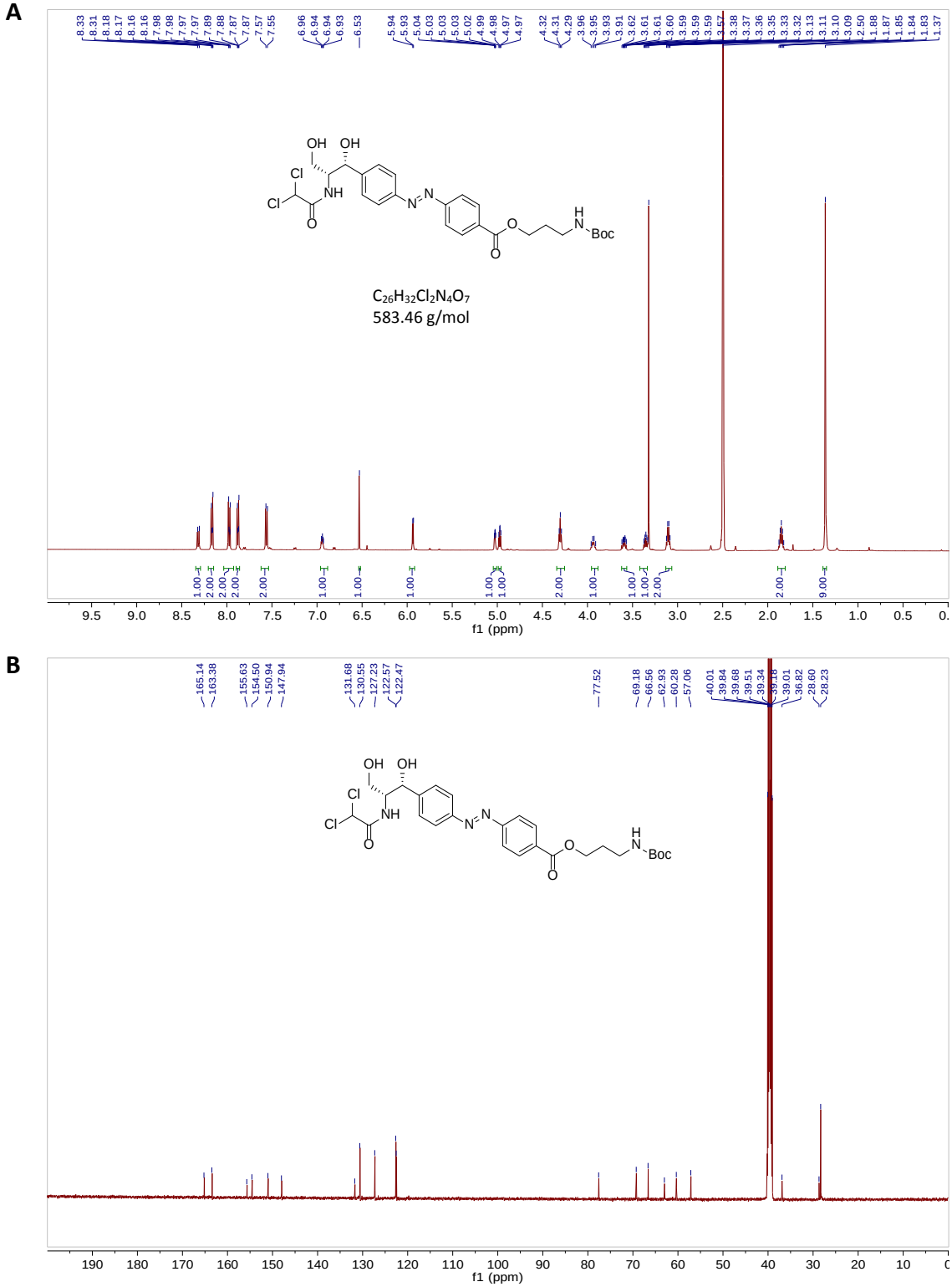
^{1}H-NMR: (500 MHz, DMSO-d_6) δ = 8.17 (d, *J* = 8.6 Hz, 2H), 8.09 (d, *J* = 9.2 Hz, 1H), 7.97 (d, *J* = 8.6 Hz, 2H), 7.89 (d, *J* = 8.5 Hz, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 6.94 (t, *J* = 5.6 Hz, 1H), 6.60 (s, 1H), 5.08 (d, *J* = 3.1 Hz, 1H), 4.31 (t, *J* = 6.2 Hz, 2H), 3.98-3.93 (m, 1H), 3.74-3.71 (m, 1H), 3.52-3.48 (m, 1H), 3.13-3.09 (m, 2H), 1.88-1.83 (m, 2H), 1.37 (s, 9H), 0.89 (s, 9H), 0.88 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H), -0.13 (s, 3H) ppm.

^{13}C-NMR: (125 MHz, DMSO-d_6) δ = 165.1, 163.5, 155.6, 154.5, 151.2, 146.1, 131.8, 130.5, 127.4, 122.6, 77.5, 71.9, 66.4, 62.9, 61.7, 57.4, 36.8, 28.6, 28.2, 25.7, 25.7, 17.9, 17.9, -4.8, -5.2, -5.4, -5.5 ppm.

ESI-MS (+): *m/z*: $[M-H]^+$ 811.45

HRMS: *m/z* calcd. for $C_{38}H_{60}Cl_2N_4O_7Si_2$ 833.32698 $[M+Na]^+$, found 833.32712 (Δm = 0.00014, error 0.2 ppm).

Supplementary Figure S5



Supplementary Figure S5: 3-((*tert*-Butoxycarbonyl) amino) propyl 4-((*E*) -(4-((1*R*)-2-(2,2-dichloroacetamido)-1,3-dihydroxypropyl) phenyl) diazenyl) benzoate [6]. A) ^{1}H-NMR of compound **6**. **B)** ^{13}C-NMR of compound **6**.

3- ((*tert*-Butoxycarbonyl) amino) propyl 4-((*E*) -(4- ((5*R*) -6- (2,2-dichloroacetamido)- 2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-5-yl)phenyl)diazenyl)benzoate (0.56 g, 0.70 mmol, 1.0 eq) was dissolved in dry THF (20 mL) and TBAF (6.25 mL, 6.25 mmol, 1 M solution in THF, 9.0 eq) was added. The reaction mixture was stirred at room temperature for 2 h. H_2O (30 mL) was added and the reaction mixture was extracted with EtOAc (3x 30 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification via flash chromatography (CH_2Cl_2:MeOH 9:1) afforded 3-((*tert*-butoxycarbonyl) amino) propyl 4-((*E*) -(4- ((1*R*) -2- (2,2-dichloroacetamido)-1,3-dihydroxypropyl) phenyl) diazenyl)-benzoate as an orange foam (0.16 g, 0.27 mmol, 39%).

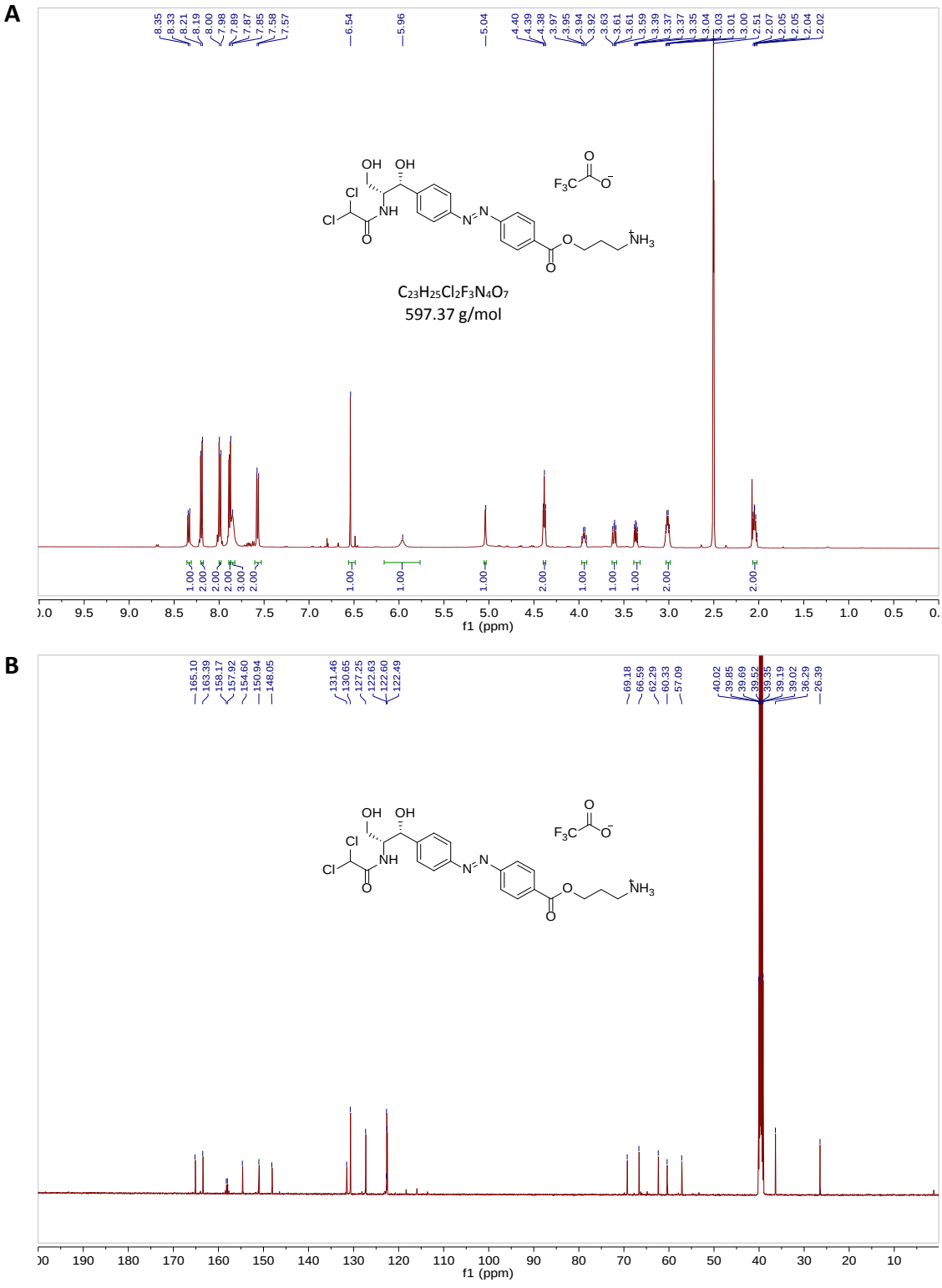
^{1}H-NMR: (500 MHz, DMSO-d_6) δ = 8.32 (d, *J* = 9.1 Hz, 1H), 8.17 (d, *J* = 8.6 Hz, 2H), 7.98 (d, *J* = 8.6 Hz, 2H), 7.88 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 6.96-6.93 (m, 1H), 6.53 (s, 1H), 5.94 (d, *J* = 4.3 Hz, 1H), 5.03 (t, *J* = 3.3 Hz, 1H), 4.98 (t, *J* = 5.5 Hz, 1H), 4.30 (t, *J* = 6.7 Hz, 1H), 3.96-3.91 (m, 1H), 3.62-3.57 (m, 1H), 3.38-3.35 (m, 1H), 3.13-3.09 (m, 2H), 1.88-1.83 (m, *2*H), 1.37 (s, 9H) ppm.

^{13}C-NMR: (125 MHz, DMSO-d_6) δ = 165.1, 163.4, 155.6, 154.5, 150.9, 147.9, 131.7, 130.5, 127.2, 122.6, 122.5, 77.5, 69.2, 66.6, 62.9, 60.3, 57.1, 36.8, 28.6, 28.2 ppm.

ESI-MS (+): *m/z*: $[M-H]^+$ 581.21

HRMS: *m/z* calcd. for $C_{26}H_{32}Cl_2N_4O_7$ 605.15403 $[M+Na]^+$, found 605.15281 (Δm = 0.00122, error 2.0 ppm).

Supplementary Figure S6



$C_{23}H_{25}Cl_2F_3N_4O_7$
597.37 g/mol

Supplementary Figure S6: 3-((4-((*E*)-(4-((1*R*)-2-(2,2-Dichloroacetamido)-1,3-dihydroxypropyl) phenyl) diazenyl)benzoyl)oxy)propan-1-aminium 2,2,2-trifluoroacetate [7]. A) ^{1}H-NMR of compound **7. B)** ^{13}C-NMR of compound **7**.

4-((*E*)-(4-((1*R*)-2-(2,2-Dichloroacetamido)-1,3-dihydroxypropyl)phenyl)diazenyl)benzoate (0.16 g, 0.27 mmol, 1.0 eq) was dissolved in dry CH_2Cl_2 (10 mL) and TFA (0.2 mL, 2.59 mmol, 9.6 eq) was added. The reaction mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure. Purification via RP-HPLC (C18) afforded 3-((4-((*E*)-(4-((1*R*)-2-(2,2-dichloroacetamido)-1,3-dihydroxypropyl)phenyl)diazenyl)-benzoyl)oxy)propan-1-aminium 2,2,2-trifluoroacetate as an orange solid (0.09 g, 0.15 mmol, 54%).

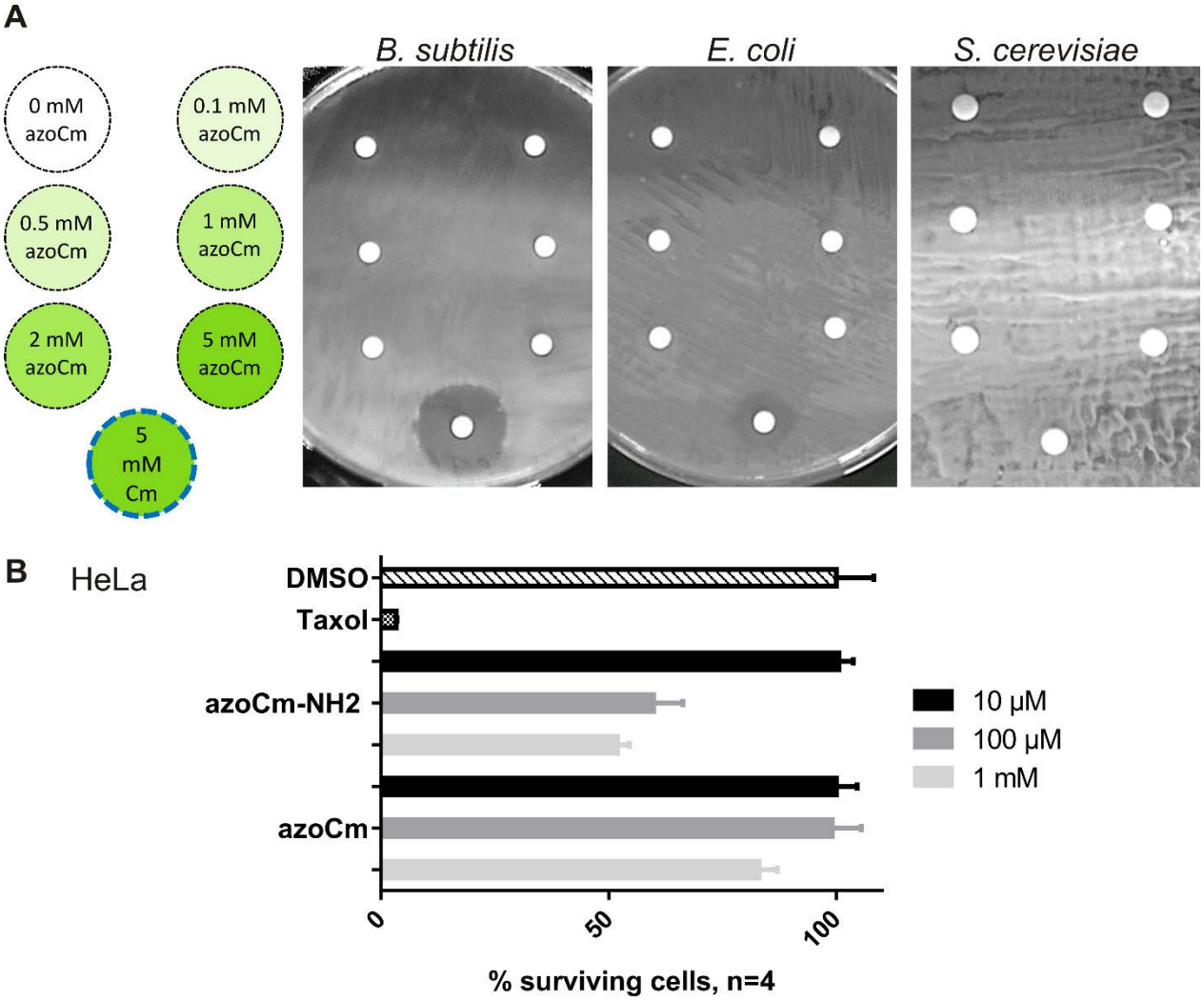
^{1}H-NMR: (500 MHz, DMSO-d_6) δ = 8.34 (d, *J* = 9.1 Hz, 1H), 8.20 (d, *J* = 8.7 Hz, 2H), 7.99 (d, *J* = 8.7 Hz, 2H), 7.88 (d, *J* = 8.5 Hz, 2H), 7.85 (s, 3H), 7.57 (d, *J* = 8.5 Hz, 2H), 6.54 (s, 1H), 5.96 (s, 1H), 5.04 (d, *J* = 1.5 Hz, 1H), 4.39 (t, *J* = 6.1 Hz, 2H), 3.97-3.92 (m, 1H), 3.63-3.59 (m, 1H), 3.39-3.35 (m, 1H), 3.04-3.00 (m, 2H), 2.08-2.03 (m, 2H) ppm.

^{13}C-NMR: (125 MHz, DMSO-d_6) δ = 165.1, 163.4, 158.2, 157.9, 154.6, 150.9, 148.0, 131.4, 130.6, 127.2, 122.6, 122.5, 69.2, 66.6, 62.3, 60.3, 57.1,, 36.3,26.4 ppm.

ESI-MS (+): *m/z*: $[M+H]^+$ 483.14

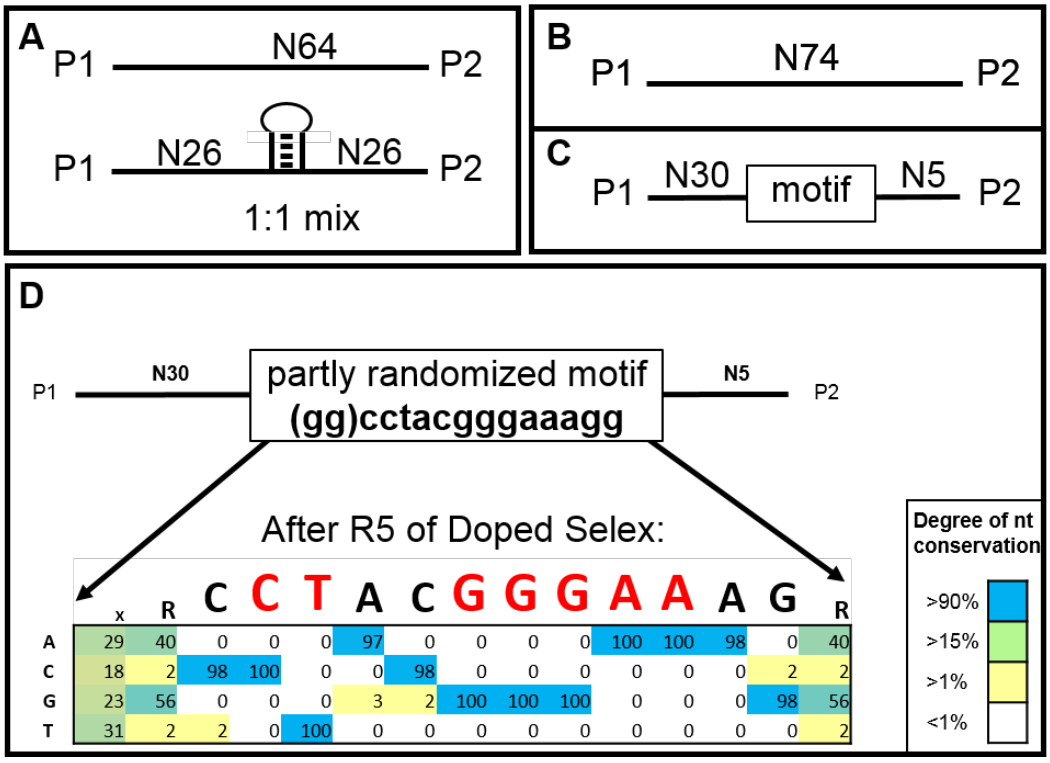
HRMS: *m/z* calcd. for $C_{21}H_{25}Cl_2N_4O_5$ 483.11965 $[M+H]^+$, found 483.11855 (Δm = 0.00110, error 2.3 ppm).

Supplementary Figure S7



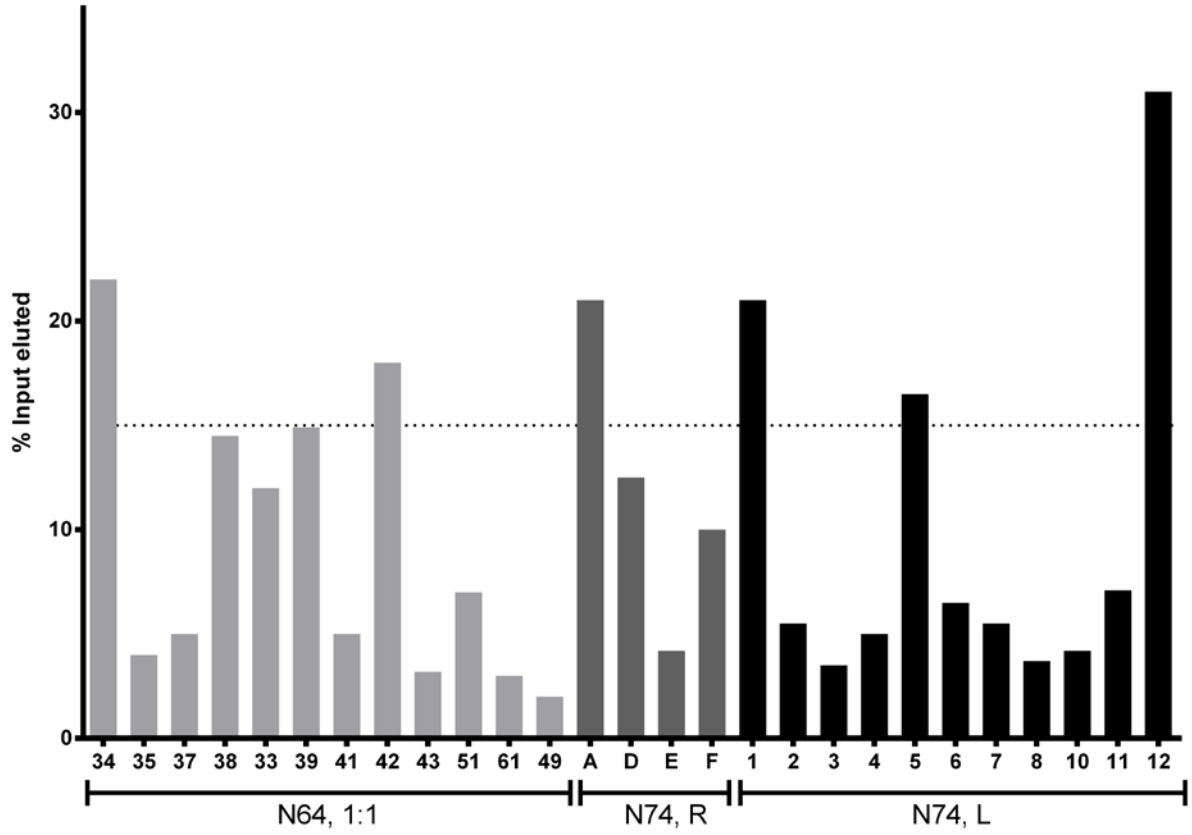
Supplementary Figure S7: Toxicity tests of azoCm. A) Inhibition zone test with *B. subtilis*, *E. coli* and *S. cerevisiae* cultures. Sterile filter discs were placed on agar plates densely covered in the respective model organism. Different concentrations of ligand solution, distributed as shown in the layout on the left, were pipetted onto the filter discs to diffuse into the agar. The cultures were grown at appropriate conditions. A circle of agar without any grown cells around a filter disc indicates a toxic effect of the ligand on cell growth, exemplarily seen for *B. subtilis* and *E. coli* on the bottom most filter discs suffused with 5 mM chloramphenicol (Cm) solution. **B)** Toxicity test of azoCm on HeLa cells. Cultures of HeLa cells were treated with varying concentrations of azoCm. As positive and negative controls, HeLa cells were treated with 0.2% DMSO (cell survival normalized to 100%) and Taxol [10 µM] (cell survival of 3.3%) added to the media. Adding azoCm shows a 100% (±4.5/6.5) survival rate of HeLa cells for 10 and 100 µM of azoCm. Addition of 1 mM of azoCm leads to a drop in cell survival to 83% (±4). While addition of 10 µM of azoCm-NH_2 show a 100% (±3.1) survival rate in HeLa cells, addition of 100 µM and 1 mM lead to a drop to 59.8% (±6.4) and 51.8% (±2.6), respectively.

Supplementary Figure S8



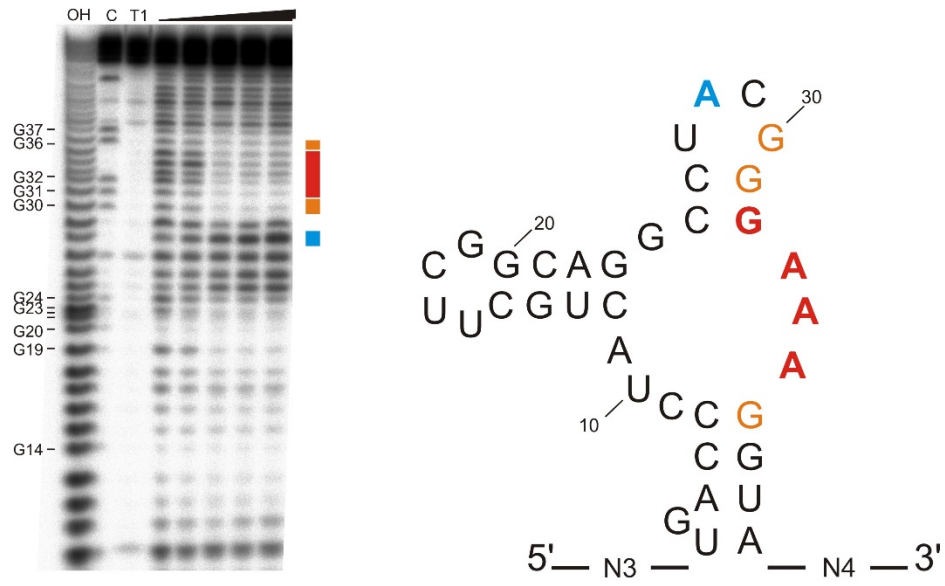
Supplementary Figure S8: RNA libraries used in this study. A) The RNA pool of the first selection consists of a 1:1 mix of a 64 nucleotides randomized region (N64) flanked by constant regions (P1, P2) with a pool containing a defined stem loop in the middle of the random region. **B)** The pool of the second selection consists of 74 randomized nucleotides (N74) flanked by constant regions P1 and P2. **C)** The third selection was carried out with a partially structured library containing a 15 nucleotide sequence motif (GGC CTA CGG GAA AGG) derived from aptamers of the first and second selection flanked by a 30 nucleotide random region on the motif's 5' end, and 5 random nucleotides on its 3' end. The motif was partially randomized as well, with a 50% chance of a nucleotide exchange for the first two nt (GG), and a 2% chance for the remaining 13 nt. This partially structured library is flanked by the constant regions P1 and P2. **D)** Enrichment analysis of the sequence motif of the doped selection. Over the course of the doped SELEX, the initially partly randomized motif re-evolved mostly back to its original sequence, thus highlighting the nucleotides relevant to ligand binding. Some positions show a 97 to 100% probability of recovering their original nucleotide after round 5 of the doped SELEX (blue markings). Other nucleotides, like the very first position (originally a G) don't show a preference for one particular nucleotide in this position, leading to the conclusion that this position does not play a role in ligand binding (yellow and white markings).

Supplementary Figure S9



Supplementary Figure S9: Elution studies of light SELEX aptamers. Radioactively labelled aptamers were folded and pipetted on azoCm derivatized columns using the same conditions as during SELEX (100.000 counts). Four elution steps with 50 µM azoCm *trans* were carried out and the percentage of eluted RNA versus total RNA was calculated. Several aptamers from different SELEX experiments (34, 42, A, 1, 5, 12) showed more than 15% RNA elution (dotted line). Die aptamers are from different SELEX pools indicated by N64 (first SELEX with N64 pool, and of the N74 pool from the regular (N74-R) and the light branch (N74-L), respectively.

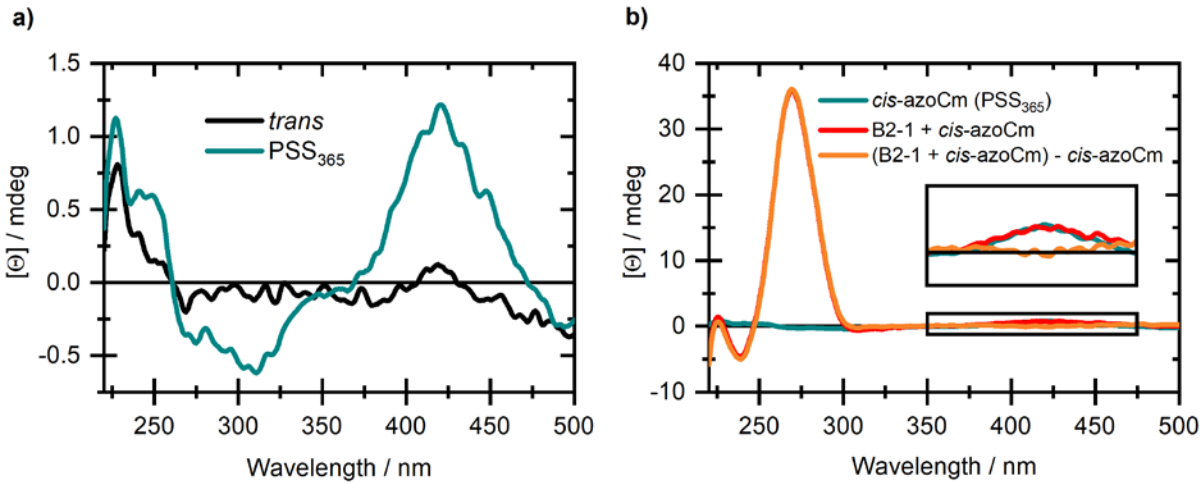
Supplementary Figure S10



Supplementary Figure S10: In-line probing analysis of aptamer 42. Shown is the cleavage pattern with increasing concentration of azoCm (0, 0.1, 1, 10, 100 µM, respectively). As references and for nucleotide position assignment, hydroxyl reaction (OH), non-reacted RNA (C) and nuclease T1 digestion (T1) were loaded onto the gel. G nucleotides are marked. The colour coded bar indicates nucleotide positions with changes in the cleavage pattern (orange/red: weak/strong decrease, blue: increase). The corresponding nucleotides are marked with the same colour code in the secondary structure on the right side.

For in-line probing the RNA was dephosphorylated and 5' ^{32}P-labeled. After PAGE purification, 35 kcpm of each 5' ^{32}P-labeled RNA were incubated for 68 h at 22°C in in-line reaction buffer (10 mM Tris-Cl pH 8.3 @ 20°C, 10 mM $MgCl_2$, 100 mM KCl). To generate a size marker, the 5' ^{32}P-labeled RNAs were subjected to alkaline hydroxylation by incubation for 3 min at 96°C in 50 mM Na_2CO_3 (pH 9.0), or incubated for 3 min at 55°C with 20 U RNase T1 at denaturing conditions to identify guanine. After in-line reaction, alkaline hydroxylation or RNase T1 treatment, samples were ethanol precipitated and the pellet was dissolved in 5 M urea. All samples were separated by denaturing polyacrylamide gel electrophoresis. Afterwards, gels were dried and analyzed using phosphoimaging (GE Healthcare). The experiment was repeated three times, a representative gel is shown.

Supplementary Figure 11



Supplementary Figure S11: CD spectra of azoCm. A) CD spectra of azoCm in its *trans*-form and in its PSS after irradiation with 365 nm. **B)** CD spectra of B2-1 in presence of azoCm and the corresponding spectrum corrected for the contribution of the ligand.

Supplementary Table S1

Supplementary Table S1: Detailed summary of the azoCm selection process. Pre-elution steps, buffer washes and elution steps are shown in column volumes (CV)

Regular SELEX								
Round	Negative selection	Pre-elution steps [CV]	Buffer washes [CV]	Binding time [min]	Specific elution	Eluent	Elution steps [CV]	% Input eluted
1	yes	-	10	30	-	20 mM EDTA	4	0.4
2	yes	-	10	30	-	20 mM EDTA	4	1
3	yes	-	10	30	-	20 mM EDTA	4	0.9
4	-	-	10	30	-	20 mM EDTA	4	1.2
5	-	-	10	30	-	20 mM EDTA	4	2.9
6	-	-	10	30	yes	5 mM azoCm	4	0.9
7	-	-	10	30	yes	5 mM azoCm	4	6.4
8	-	2	25	45	yes	5 mM azoCm	4	7.1
9	-	3	25	45	yes	5 mM azoCm	4	6.7
Light SELEX								
Round	Negative selection	Pre-elution steps [CV]	Buffer washes [CV]	Binding time [min]	Specific elution	Eluent	Elution steps [CV]	% Input eluted
1	yes	-	10	30	-	20 mM EDTA	4	2
2	yes	-	10	30	yes	5 mM azoCm	4	0.3
3	yes	-	10	30	yes	5 mM azoCm	4	0.4
4	yes	-	10	30	yes	5 mM azoCm	4	0.6
5	-	-	10	30	yes	5 mM azoCm	4	0.9
6	-	-	10	30	yes	5 mM azoCm	4	4.8
7	-	2	20	30	yes	5 mM azoCm	4	1
8	-	2	20	30	yes	5 mM azoCm	4	2.3
9	-	2	20	15	yes	5 mM azoCm	4	6.3
Light 7	-	-	10	30	yes	UV-light+buffer	6	1.8
Light 8	-	-	10	30	yes	UV-light+buffer	6	1.9
Light 9	-	-	10	30	yes	UV-light+buffer	6	3.3
Light 10	-	-	10	30	yes	UV-light+buffer	6	6.7

Doped SELEX								
Round	Negative selection	Pre-elution steps [CV]	Buffer washes [CV]	Binding time [min]	Specific elution	Eluent	Elution steps [CV]	% Input eluted
1	-	-	10	30	yes	1 mM azoCm	4	0.8
2	-	-	10	30	yes	1 mM azoCm	4	0.5
3	-	-	10	30	yes	1 mM azoCm	4	2
4	-	-	10	30	yes	1 mM azoCm	4	11
5	-	-	10	30	yes	1 mM azoCm	4	38.1

Supplementary Table S2

Supplementary Table S2: Aptamer sequences

Aptamer	Sequence
33	AACAAUAGGAGCCAGAGUUGUUUUCUCUGCUUCGGCAGAAACGGAUGUAAGGGACCUAUAACCA
34	CUGUGCAAAAGCAAAAAUUCGGCGUUCCUGCUUCGGCAGAGACGUAACACGACACAGGGGUAGAA
35	CGCUCUGGUAGCGUCAACACCCUAAGUGGAGGGGUGGCCCGAAGACCCGGGAUAUAUGGUACUA
37	CGUGGUCGAACUUCAACCACGUCAACUGCUUCGGCAGGCCCUAGGCUAGUAUUCGAGUAAUGA
38	UACUGCAAUGGGCAGUUUGGAAACCUCUGCUUCGGCAGCCGCCCGGGUUCACACGGGAGAGCUU
39	GCUAGGCUAAAAAGCUGCGAAGUCUACUGCUUCGGCAGCGCCCGUAAGAAACUGUAACGGUUAA
41	UUGUAGAGAAAUGCCUCUGCAAUCAACUGCUUCGGCAGGCCCAAGGACCAGAUACA
42	GGUUGACCCUACUGCUUCGGCAGGCCUACGGGAAAGGUAACA
49	CUGACCAUAAAAAUCAGUCAACCACGCCUGCUUCGGCAGCCGUAGGGCCCAAGAACUAGUGACUG
51	AUCGGCGAACAAAAGAAAACAUUUAUCUGCUUCGGCAGGGUCGGCAGGCAAGUAGGCUGGAGCC
61	CCAUUCCGCGCUGCGGAAGGUCAACCCUGCUUCGGCAGAUGGGCCCGGGCUAGCCGGACAAAAA
A	AGUUAAUACACGACGCAGUGAUCGACGUAGGUAGAAAAGCGAUAAGUAUGGGCCUAAUCCGAUACCGCGAUCAC
D	GUUAAUACACGACGCAGUGAUCGACGUAGGUAGAAAAGCGAUAAGUAUGGGCCUAAUCCGAUACCGCGAUCA
E	GCGACUAGAACCCAUGUUGCGAAGUCGACCUGUGACCCGAGUGAAGCGGGAAAGCAAGGACGAGCCUUCAAUCC
F	GGUCGGUCGAGGACCUUAGACCGUCAGCUUCUUCUCCGGUGUAAAGGCUGUGAGACCAGGAGGCCAAGAACCAA
1	UAGGGACCCUAAAGCAGGGCUUCAACGGAUACGGCCAUGAGGCGUUCUAGGAACCAAAGAGCGUAAUAAGCAAUC
2	GUGUUCCGACACGUGAACUCCAGCCCCCUUAAUAACGCUGUCGACCCUUGCGCUGUUACCUACGGGAAAGGGGU
3	GAAUGCUAACAUCCGAUUGUCCAGUACUGCCUUGGUAUAAGUCUGAAUACGUGGAUCCGACCGUGGUGCC
4	UUCCAUCCAUUCUCACCCGUGGAUGAAAAGGGACCCGUAUAAGCACAGAGGCCGCCGAGGAGCCAAGGAACCAAU
5	AGUUAAUGCACGACGCAGUGAUCGACGUAGGUAGAAAAGCGAUAAGUAUGGGCCUAAUCCGAUACCGCGAUCAC
6	AAAACCGAUUUAAAGGCUACCCAGUGAUCCAUGGAGUAGGAAAAGCUGCUAACGAGCCGCUCCAUCCAGCCGA
7	CCCGCAACCGCGACCCCUUGCCGCCUGAGGCUAAAUGCCCCUCUACGGAAAAGCGGAAGACUCGUGAGUGGGA
8	AGUUAAUACACGACGCAGUGAUCGACGUAGGUAGAAAAGCGAUAAGUAUGGGCCUAAUCC
9	AGUUAAUACACGACGCAGUGAUCGACGUAGGUAGAAAAGCGAUAAGUAUGGGCCUAAUCCGAUACCGCGAUCAC
10	GACGGGCUCCCGCACCUGAGGUGUCUGCCAGAAACGACAAACGUCGCCUCGGCCACCACUCUGGUUACAAGAGG
11	AGUUAAUACACGACGCAGUGAUCGACGUAGGUAGAAAAGCGAUAAGUAUGGGCCUAAUCCGAUACCGCGAUCGC
12	CCCCCUCCUACUGUGACUGAACGAACCCACAGUAGGGCCUACGGGAAAGGGGAGUGACCUGGCCCAAAGCCACC
B2-1	CCAAGCUAGAUCUACCGGUGCUCCCUUUAGAGGUUUGUCGAAGACCUCUAACCUACGGGAAAGGAGACCAAAAUGGCU AGCAAAGGAGAAGAACUUUUCACU

Supplementary Table S3

Supplementary Table S3: Analysis of ligand binding of the aptamers 42 and B2-1 with the corresponding photoisomers of azoCm by isothermal titration calorimetry (ITC).

Aptamer	Ligand	K_D	ΔG [kJ/mol]	ΔH [kJ/mol]	-TΔS [kJ/mol]	Stoichio-metry
42	azoCm ***trans***	1.9 ± 0.6 µM	-32.7 ± 0.4	-70.3 ± 8.5	38.0 ± 9.2	0.9 ± 0.1
42	azoCm ***cis***	no binding detectable				
B2-1	azoCm ***trans***	0.54 ± 0.1 µM	-35.8 ± 0.1	-76.6 ± 2.5	40.8 ± 1.5	1.1 ± 0.1
B2-1	azoCm ***cis***	no binding detectable				

Shown are the mean and standard deviations of two independent measurements. All samples were measured on a MicroCal PEAQ-ITC (Malvern Instruments) @ T = 25°C and constant stirring @ 750 rpm. Fitting was performed with Malvern MicroCal PEAQ-ITC Analysis Software (v.1.1.0.1262) to a one site binding model (‘one set of sites’).