Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting information

Fluorescent SAM analogues for methyltransferase based DNA labeling

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Experimental section

General. All reactions were carried out in a flame dried reaction flask equipped with a stirring bar and under argon atmosphere. Commercial chemicals were obtained from ACROS, TCI, Fluorochem or Sigma-Aldrich and used without further purification. Reaction solvents were used as received from commercial sources. MilliQ water was obtained using a Synergy® UV water purification system from Merck-Millipore.

Column chromatography was performed using 70-230 mesh silica 60 (E. M. Merck) as the stationary phase. Reverse phase column chromatography was performed using C18-reversed phase silica gel, fully end capped, 230-400 mesh, 90 Å pore size.

^{1}H NMR spectra were acquired on a Bruker Avance 300, 400 or 600 spectrometer, operating at 300 MHz, 400 MHz or 600 MHz, respectively with trimethylsilane (TMS) as the internal standard and $CDCl_3$ or d^6-DMSO as the solvent. The chemical shifts (δ values) are expressed in parts per million (ppm). ^{13}C NMR spectra were recorded on the same instruments, operating at 75 MHz, 100 MHz or 150 MHz respectively, with the deuterated solvent as an internal standard ($CDCl_3$ = 77.16 ppm, d^6-DMSO = 39.52 ppm).

LC-MS were measured using a Shimadzu LC-MS 2020 Liquid Chromatograph Mass Spectrometer (Shim-Pack Gist C18 2µm, 2.1x100 mm). Compounds were eluted with methanol/aqueous formic acid (0.1%), linear gradient from 20% methanol to 100% over 30 min, then isocratic methanol for 5 minutes, unless specified otherwise. A flow rate of 0.3 mL/min was used. Purity of measured compounds was determined by automatic integration using the LabSolutions software.

HRMS Spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3µL/min and spectra were obtained in positive (or: negative) ionization mode with a resolution of 15000 (FWHM) using leucine enkephalin as lock mass.

General procedures

(A) NHS coupling. Carboxylic acid (1 equiv.) and *N*,*N*′-disuccinimidyl carbonate (DSC, 1.1 equiv.) were dissolved in dry DMF and triethylamine (3 equiv.) was added. The reaction was stirred at rt for 1 hour. A solution of amine (1.2 equiv.) in dry DMF was added and the reaction was stirred for an additional 1 hour at rt. The solvent was removed by rotary evaporation and the target compound was purified by column chromatography (silica, DCM/MeOH mixtures).

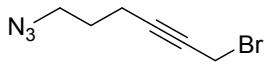
(B) NHS coupling after Boc removal. Carboxylic acid (1 equiv.) and *N*,*N*′-disuccinimidyl carbonate (1.1 equiv.) were dissolved in dry DMF and triethylamine (3 equiv.) was added. In parallel, Boc-protected amine (1.2 equiv.) was dissolved in DCM/TFA (1/1). Both reactions were stirred at rt for 1 hour. Upon completion, the crude amine was evaporated, dissolved in dry DMF and neutralized with triethylamine. Both solutions were combined and the reaction was stirred for 1 hour at rt. After 1 hour, the solvent was removed by rotary evaporation and the target compound was purified by column chromatography (silica, DCM/MeOH mixtures).

(C) Synthesis of cofactors. Thioether (0.05 mmol, 1 equiv.) and β-lactone (37.5 mg, 0.2 mmol, 4 equiv.) were dissolved in formic acid (30 µL). The reaction was stirred at rt for 7 hours, after which a conversion of 70-90% was observed. Formic acid was removed and dry TFA (30 µL) was added. The reaction was stirred for 1 hour at rt. After removal of the solvent, the cofactor was purified through reverse phase silica gel filtration (0.1% formic acid in MilliQ/MeOH mixtures). Fractions containing the cofactor were combined and evaporated (<30°C), and stored in buffered solution.

Synthesis of Ado-6-azide

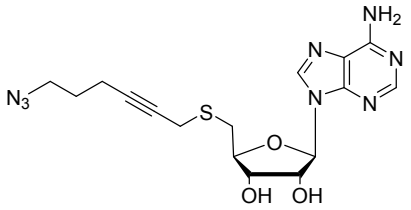
Ado-6-azide was prepared according to the method reported by *Lukinavičius et al.*[1]

Synthesis of 6-azido-1-bromohex-2-yne



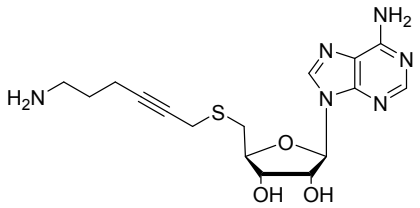
N-Bromosuccinimide (5.59 g, 31.40 mmol, 1.57 equiv.) was dissolved in dry DCM (20 mL). At 0°C, a solution of triphenylphosphine (7.87 g, 30.00 mmol, 1.50 equiv.) in dry DCM (20 mL) was added slowly. The reaction was stirred for 10 minutes and a solution of 6-azido-2-hexyn-1-ol[1] (2.78 g, 20.00 mmol, 1 equiv.) in dry DCM (20 mL) was slowly added. The reaction was stirred at 0°C for 1 hour, followed by an additional 1 hour at rt. After evaporation of the solvent, 6-azido-1-bromohex-2-yne was obtained after flash column chromatography (silica, DCM/Hept, 1/1) as a pale yellow oil (2.44 g) in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (t, *J* = 2.2 Hz, 2H), 3.41 (t, *J* = 6.6 Hz, 2H), 2.38 (tt, *J* = 6.8, 2.1 Hz, 2H), 1.79 (quintet, *J* = 6.8 Hz, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 86.2, 76.6, 50.3, 27.7, 16.4, 15.3.

Synthesis of 1a



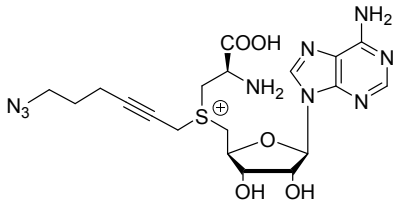
5'-Thioadenosine[2] (2.12 g, 7.50 mmol) was dissolved in dry DMF (22.5 mL). At 0°C, a solution of 6-azido-1-bromohex-2-yne (1.82 g, 9.00 mmol, 1.2 equiv.) in dry DMF (15 mL) was added, followed by the slow addition of DBU (2.10 mL, 15.00 mmol, 2 equiv.). The reaction was stirred at rt. After evaporation of the solvent, compound **1a** was obtained after purification by column chromatography (silica, DCM/MeOH, 96/4) as a white foam (2.18 g) in 72% yield. ^{1}H NMR (600 MHz, DMSO) δ 8.33 (s, 1H), 8.15 (s, 1H), 7.28 (br s, 2H), 5.88 (d, *J* = 5.6 Hz, 1H), 5.51 (d, *J* = 5.8 Hz, 1H), 5.32 (d, *J* = 5.0 Hz, 1H), 4.75 (q, *J* = 5.4 Hz, 1H), 4.17 (dd, *J* = 9.0, 4.8 Hz, 1H), 4.06 (td, *J* = 6.3, 3.9 Hz, 1H), 3.39 (t, *J* = 6.7 Hz, 2H), 3.35 (q, *J* = 2.4 Hz, 2H), 3.02 (dd, *J* = 13.9, 5.8 Hz, 1H), 2.92 (dd, *J* = 13.9, 7.0 Hz, 1H), 2.26 (tt, *J* = 7.0, 2.3 Hz, 2H), 1.66 (quintet, *J* = 6.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ 156.1, 152.6, 149.4, 139.8, 119.2, 87.5, 83.6, 81.8, 77.1, 72.6, 72.6, 49.6, 39.9, 39.8, 39.7, 39.5, 39.4, 39.2, 39.1, 33.6, 27.5, 19.6, 15.4. HRMS (ES+): calculated for $C_{16}H_{20}N_8O_3S$ [M+H]+: 405.1452 Found: 405.1435. LC-MS: 22.36 min.

Synthesis of 1b



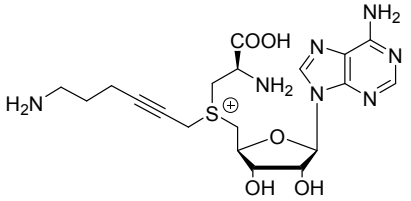
Compound **1a** (2.02 g, 5.00 mmol) and triphenylphosphine (1.44 g, 5.50 mmol, 1.1 equiv.) were dissolved in THF/H_2O (4/1, 25 mL). The reaction was stirred at 60°C for 2 hours. After evaporation of the solvents, compound **1b** was obtained after purification by column chromatography (silica, DCM/MeOH/NH_4OH, 75/20/5) as a pale yellow foam (1.38 g) in 73% yield. ^{1}H NMR (300 MHz, DMSO) δ 8.35 (s, 1H), 8.17 (s, 1H), 7.27 (s, 2H), 5.90 (d, *J* = 5.5 Hz, 1H), 4.76 (t, *J* = 5.3 Hz, 1H), 4.35 (br s, 4H), 4.22 – 4.16 (m, 1H), 4.08 (dd, *J* = 10.0, 6.0 Hz, 1H), 3.35 (s, 2H), 3.03 (dd, *J* = 14.0, 5.7 Hz, 1H), 2.92 (dd, *J* = 13.8, 6.9 Hz, 1H), 2.69 (t, *J* = 7.0 Hz, 2H), 2.25 (t, *J* = 7.0 Hz, 2H), 1.59 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 156.1, 152.7, 149.5, 139.9, 119.2, 87.5, 83.7, 82.6, 76.6, 72.6, 72.6, 39.5, 33.6, 29.6, 19.6, 15.5. HRMS (ES+): calculated for $C_{16}H_{22}N_6O_3S$ [M+H]+: 379.1547 Found: 379.1543. LC-MS: 1.89 min.

Synthesis of 2a



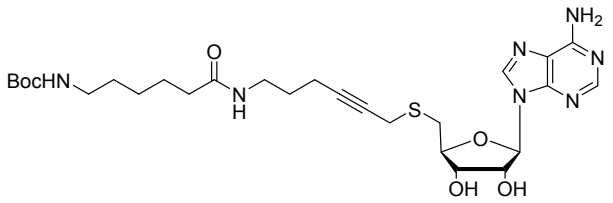
Prepared according to **general procedure C**, from product **1a** (20.2 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **2a** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 97/3. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: isocratic 2.5% methanol for 10 minutes, then linear gradient 100% methanol over 20 min, then isocratic methanol for 5 minutes; epimer 1 at 9.90 min, epimer 2 at 11.94 min, combined purity of 91%. MS (ESI+): 492.00 (M^+).

Synthesis of 2b



Prepared according to **general procedure C**, from product **2b** (18.9 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **2b** was obtained after purification through reverse phase silica gel filtration, eluting with pure 0.1% formic acid in MilliQ. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: isocratic 0.5% methanol for 10 minutes, then linear gradient 100% methanol over 20 min, then isocratic methanol for 5 minutes; 1.39 min, purity of 90%. MS (ESI+): 465.95 (M^+).

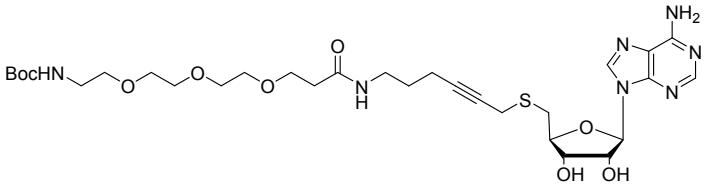
Synthesis of S1



Prepared according to **general procedure A**, from Boc-6-Ahx-OH (0.28 g, 1.20 mmol), DSC (0.34 g, 1.32 mmol), triethylamine (0.50 mL, 3.60 mmol) and compound **1b** (0.55 g, 1.44 mmol). Total volume of DMF was 6 mL. Compound **S1** was obtained after purification (silica, DCM/MeOH, 85/15) as a white foam (0.60 g) in 85% yield. ^{1}H NMR (600 MHz, DMSO) δ 8.37 (s, 1H), 8.17 (s, 1H), 7.82 (t, *J* = 5.3 Hz, 1H), 7.34 (br s, 2H), 6.78 (t, *J* = 5.4 Hz, 1H), 5.90 (d, *J* = 5.7 Hz, 1H), 5.55 (d, *J* = 6.0 Hz, 1H), 5.38 (d, *J* = 4.9 Hz, 1H), 4.76 (q, *J* = 5.5 Hz, 1H), 4.18 (dd, *J* = 8.8, 4.5 Hz, 1H), 4.07 (dd, *J* = 10.1, 6.3 Hz, 1H), 3.36 – 3.33 (m, 2H), 3.11 – 3.05 (m, 3H), 3.03 (dd, *J* = 14.0, 5.8 Hz, 1H), 2.93 (dd, *J* = 13.9, 7.0 Hz, 1H), 2.88 (dd, *J* = 13.1, 6.6 Hz, 2H), 2.18 (t, *J* = 7.0 Hz, 2H), 2.03 (t, *J* = 7.4 Hz, 2H), 1.54 (quintet, *J* = 7.0 Hz, 2H), 1.49 – 1.42 (m, 2H), 1.40 – 1.31 (m, 11H), 1.22 – 1.15 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 172.1, 156.1, 155.6, 152.7,

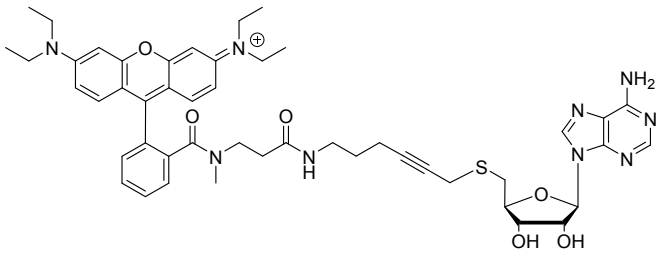
149.5, 139.8, 119.2, 87.4, 83.7, 82.7, 77.3, 76.5, 72.6, 45.5, 37.7, 35.4, 33.6, 29.3, 28.4, 28.3, 26.0, 25.0, 19.6, 15.8, 8.5. HRMS (ES+): calculated for $C_{27}H_{41}N_7O_6S$ [M+H]+: 592.2912 Found: 592.2905. LC-MS: 19.13 min.

Synthesis of S2



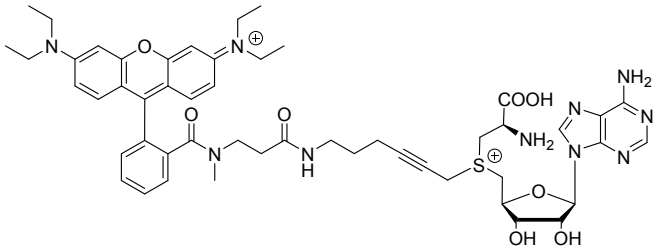
Prepared according to adapted **general procedure A**, from tBoc-N-amido-PEG3-acid (1.00 g, 3.11 mmol), DSC (0.96 g, 3.73 mmol, 1.2 equiv.), triethylamine (1.30 mL, 9.34 mmol, 3 equiv.) and compound **1b** (1.65 g, 4.36 mmol, 1.4 equiv.). Total volume of DMF was 30 mL. Compound **S2** was obtained after purification by column chromatography (silica, DCM/MeOH, 85/15) as a white foam (1.77 g) in 83% yield. ^{1}H NMR (600 MHz, DMSO) δ 8.34 (s, 1H), 8.15 (s, 1H), 7.83 (t, *J* = 5.5 Hz, 1H), 7.28 (br s, 2H), 6.75 (t, *J* = 5.5 Hz, 1H), 5.88 (d, *J* = 5.7 Hz, 1H), 5.50 (d, *J* = 6.1 Hz, 1H), 5.32 (d, *J* = 5.1 Hz, 1H), 4.74 (dd, *J* = 11.2, 5.7 Hz, 1H), 4.17 (dd, *J* = 9.0, 4.9 Hz, 1H), 4.06 (td, *J* = 6.4, 3.9 Hz, 1H), 3.58 (t, *J* = 6.5 Hz, 2H), 3.50 – 3.44 (m, 8H), 3.38 – 3.33 (m, 4H), 3.10 – 3.04 (m, 4H), 3.01 (dd, *J* = 13.9, 5.8 Hz, 1H), 2.91 (dd, *J* = 13.9, 7.0 Hz, 1H), 2.29 (t, *J* = 6.5 Hz, 2H), 2.18 (ddd, *J* = 7.2, 4.9, 2.3 Hz, 2H), 1.54 (quintet, *J* = 7.1 Hz, 2H), 1.36 (s, 9H). ^{13}C NMR (151 MHz, DMSO) δ 170.0, 156.1, 155.8, 152.7, 149.4, 139.8, 119.2, 87.4, 83.6, 82.7, 77.6, 76.4, 72.6, 69.8, 69.7, 69.7, 69.5, 69.2, 66.8, 39.9, 39.8, 39.7, 39.5, 39.4, 39.2, 39.1, 37.7, 36.1, 33.5, 28.4, 28.2, 19.6, 15.7. HRMS (ES+): calculated for $C_{30}H_{47}N_7O_9S$ [M+H]+: 682.3228 Found: 682.3240. LC-MS: 24.04 min.

Synthesis of S3



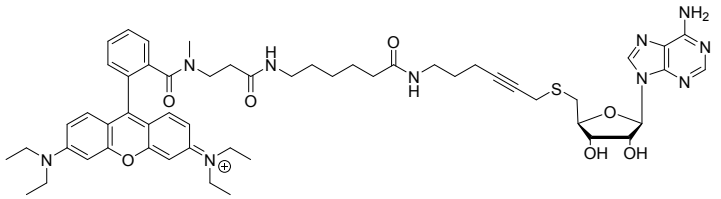
Prepared according to **general procedure A**, from extended rhodamine B[3] (56.4 mg, 0.1 mmol), DSC (28.2 mg, 0.11 mmol), triethylamine (41.8 µL, 0.3 mmol) and compound **1b** (45.4 mg, 0.12 mmol). Total volume of DMF was 1 mL. Compound **S3** was obtained after purification by column chromatography (Silica, DCM/MeOH, 85/15) as a deep purple foam with gold metallic luster (82.0 mg) in 89% yield. LC-MS: 22.13 min. HRMS (ES+): calculated for $C_{48}H_{58}N_9O_6S^+$ M+: 888.4225 Found: 888.4173.

Synthesis of 3



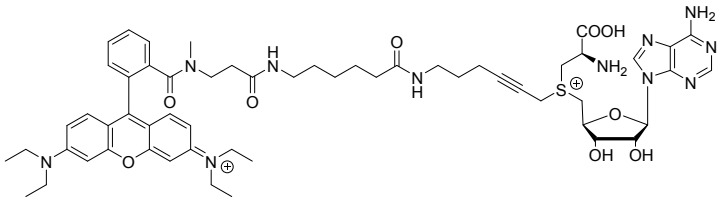
Prepared according to **general procedure C**, from product **S3** (46.2 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **3** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 60/40. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 16.13 min, purity of 87%. MS (ESI+): 488.80 (M^{2+}).

Synthesis of S4



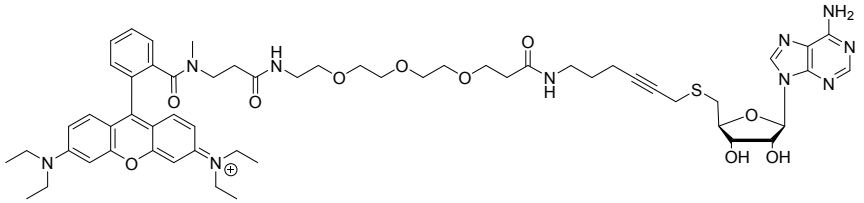
Prepared according to **general procedure B**, from extended rhodamine B[3] (56.4 mg, 0.1 mmol), DSC (28.2 mg, 0.11 mmol) and triethylamine (41.8 µL, 0.3 mmol). Total volume of DMF was 1 mL. Compound **S1** (71.0 mg, 0.12 mmol) was dissolved in DCM/TFA (50/50, 0.4 mL). The crude amine was dissolved in 1 mL DMF and neutralized with 0.25 mL triethylamine. Compound **S4** was obtained after purification by column chromatography (silica, DCM/MeOH, 8/2) as a deep purple foam with gold metallic luster (67.6 mg) in 65% yield. LC-MS: 18.83 min. HRMS (ES+): calculated for $C_{54}H_{69}N_{10}O_7S^+$ M+: 1001.5066 Found: 1001.5053.

Synthesis of 4



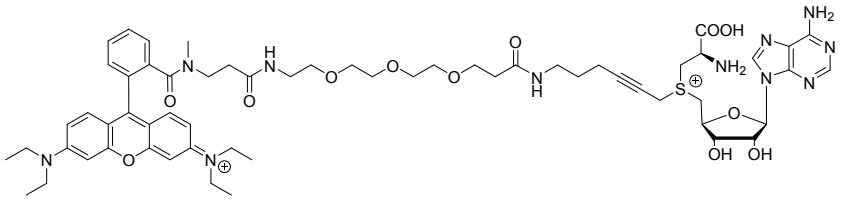
Prepared according to **general procedure C**, from product **S4** (51.9 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **4** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 65/35. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 14.90 min, purity of 96%. MS (ESI+): 545.40 (M^{2+}).

Synthesis of S5



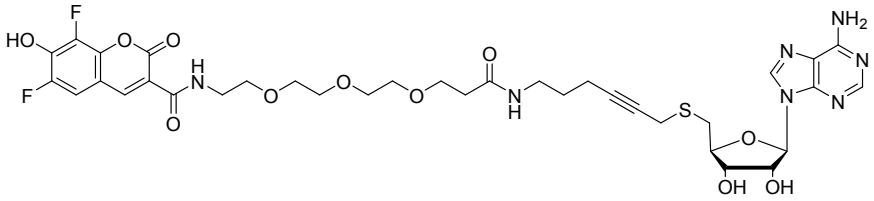
Prepared according to **general procedure B**, from extended rhodamine B[3] (112.8mg, 0.2 mmol), DSC (56.4 mg, 0.22 mmol) and triethylamine (83.6 µL, 0.6 mmol). Total volume of DMF was 2 mL. Compound **S2** (163.6 mg, 0.24 mmol) was dissolved in DCM/TFA (50/50, 0.8 mL). The crude amine was dissolved in 1.5 mL DMF and neutralized with 0.5 mL triethylamine. Compound **S5** was obtained after purification by column chromatography (silica, DCM/MeOH, 8/2) as a deep purple foam with gold metallic luster (138.5 mg) in 61% yield. LC-MS: 21.10 min. HRMS (ES+): calculated for $C_{57}H_{75}N_{10}O_{10}S^+$ M+: 1091.5382 Found: 1091.5353.

Synthesis of 5a



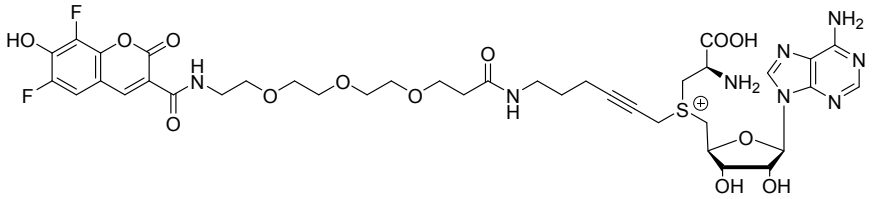
Prepared according to **general procedure C**, from product **S5** (56.4 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **5a** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 60/40. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 19.80 min, purity of 96%. MS (ESI+): 589.95 (M^{2+}).

Synthesis of S6



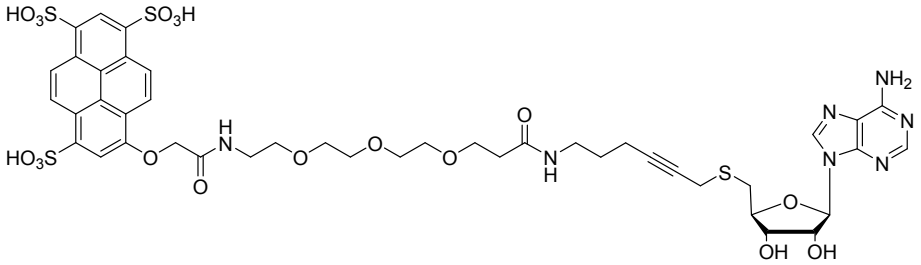
Prepared according to **general procedure B**, from Pacific Blue[4] (48.4 mg, 0.2 mmol), DSC (56.4 mg, 0.22 mmol) and triethylamine (83.6 µL, 0.6 mmol). Total volume of DMF was 1 mL. Compound **S2** (163.6 mg, 0.24 mmol) was dissolved in DCM/TFA (50/50, 0.8 mL). The crude amine was dissolved in 1.5 mL DMF and neutralized with 0.5 mL triethylamine. Compound **S6** was obtained after purification by column chromatography (silica, DCM/MeOH, 85/15) as a yellow/orange solid (99.4 mg) in 49% yield. LC-MS: 18.52 min. HRMS (ES+): calculated for $C_{35}H_{41}F_2N_7O_{11}S$ [M+H]+: 806.2625 Found: 806.2629.

Synthesis of 5b



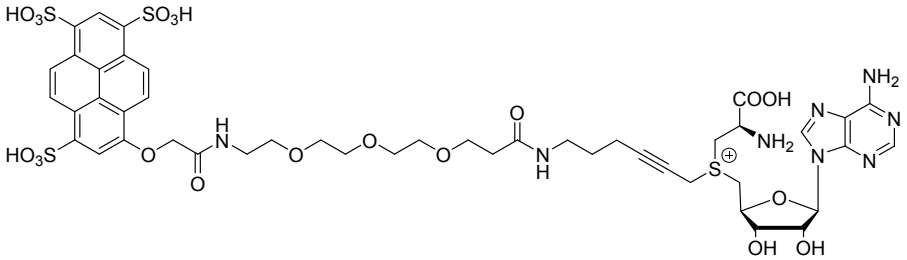
Prepared according to **general procedure C**, from product **S6** (40.3 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **5b** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 75/25. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 12.36 min, purity of 96%. MS (ESI+): 893.10 (M^+).

Synthesis of S7



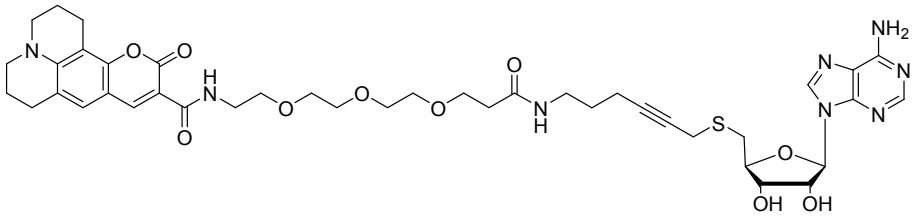
Prepared according to adapted **general procedure B**, from Cascade blue[5] (87.4 mg, 0.15 mmol), EDC (31.6 mg, 0.165 mmol), NHS (20.7 mg, 0.18 mmol) and triethylamine (62.7 µL, 0.45 mmol). Total volume of DMF was 1.5 mL. Compound **S2** (122.7 mg, 0.18 mmol) was dissolved in DCM/TFA (50/50, 0.6 mL). The crude amine was dissolved in 1.0 mL DMF and neutralized with 0.4 mL triethylamine. Compound **S7** was obtained after precipitation out of isopropanol as an off white solid (132.4 mg) in 82% yield. LC-MS: 8.75 min. HRMS (ES-): calculated for $C_{43}H_{49}N_7O_{18}S_4$ [M-H]-: 1078.1944 Found: 1078.1960.

Synthesis of 5c



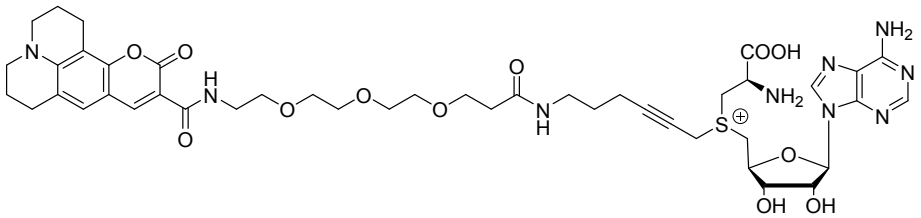
Prepared according to **general procedure C**, from product **S7** (54.0 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **5c** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 80/20. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 2.54 min, purity of 91%. MS (ESI-): 582.35 (M^{2-}).

Synthesis of S8



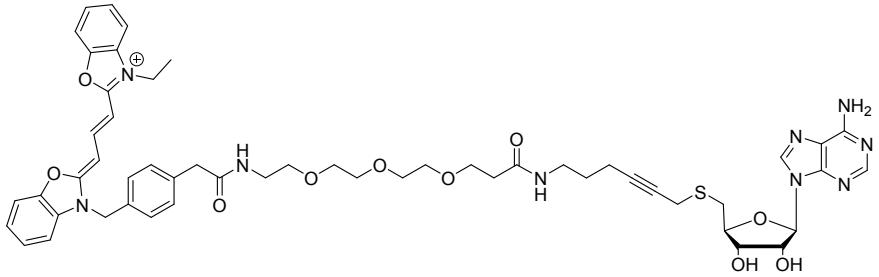
Prepared according to adapted **general procedure B**, from Coumarin 343[6] (57.1 mg, 0.2 mmol), DSC (56.4 mg, 0.22 mmol), DMAP (5 mg, cat.) and triethylamine (83.6 µL, 0.6 mmol). Total volume of DMF was 4 mL. Compound **S2** (163.6 mg, 0.24 mmol) was dissolved in DCM/TFA (50/50, 0.8 mL). The crude amine was dissolved in 1.5 mL DMF and neutralized with 0.5 mL triethylamine. Compound **S8** was obtained after purification by column chromatography (silica, DCM/MeOH, 95/5, gradual increase to 8/2) as a yellow solid (113.2 mg) in 67% yield. LC-MS: 31.84 min. HRMS (ES+): calculated for $C_{41}H_{52}N_8O_{10}S$ [M+H]+: 849.3600 Found: 849.3615.

Synthesis of 5d



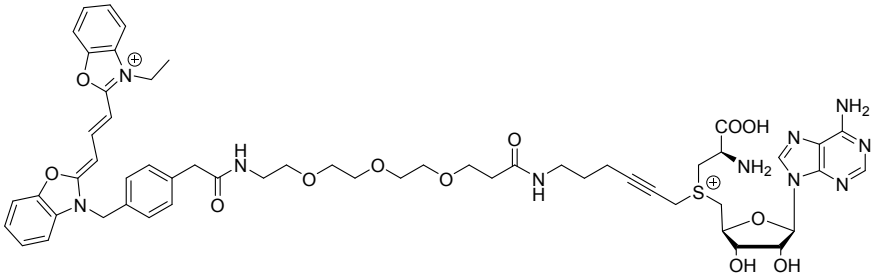
Prepared according to **general procedure C**, from product **S8** (42.4 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **5d** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 60/40. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 19.78 min, purity of 95%. MS (ESI+): 963.35 (M^+).

Synthesis of S9



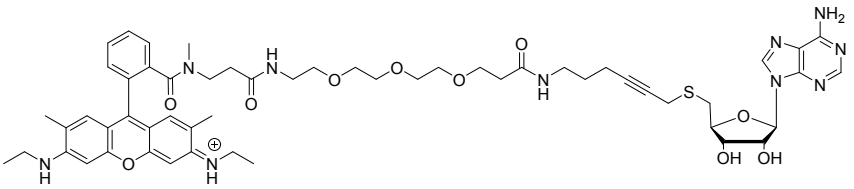
Prepared according to **general procedure B**, from Cy2[7] (58.0 mg, 0.1 mmol), DSC (28.2 mg, 0.11 mmol) and triethylamine (41.8 µL, 0.3 mmol). Total volume of DMF was 1.0 mL. Compound **S2** (85.2 mg, 0.125 mmol) was dissolved in DCM/TFA (50/50, 0.4 mL). The crude amine was dissolved in 0.5 mL DMF and neutralized with 0.25 mL triethylamine. Compound **S9** was obtained after purification by column chromatography (silica, DCM/MeOH, 9/1, gradual increase to 8/2) as an orange solid (45.6 mg) in 40% yield. LC-MS: 22.60 min. HRMS (ES+): calculated for $C_{53}H_{62}N_9O_{10}S^+$ M+: 1016.4335 Found: 1016.4323.

Synthesis of 5e



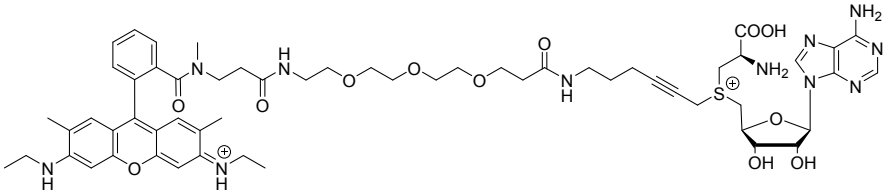
Prepared according to **general procedure C**, from product **S9** (57.2 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **5e** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 55/45. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 17.47 min, purity of 95%. MS (ESI+): 552.60 (M^{2+}).

Synthesis of S10



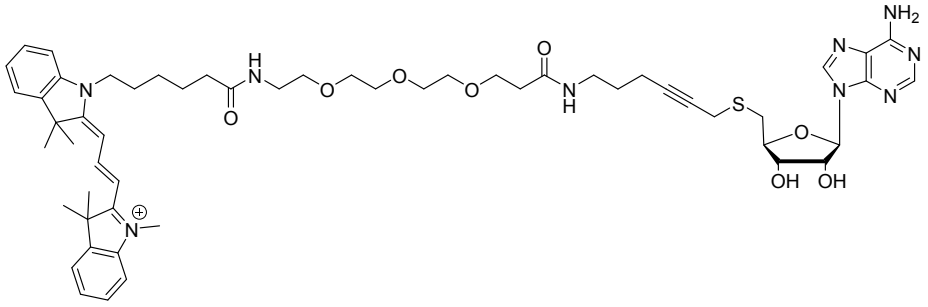
Prepared according to **general procedure B**, from extended rhodamine 6G[3] (107.2 mg, 0.2 mmol), DSC (56.4 mg, 0.22 mmol) and triethylamine (83.6 µL, 0.6 mmol). Total volume of DMF was 2 mL. Compound **S2** (163.6 mg, 0.24 mmol) was dissolved in DCM/TFA (50/50, 0.8 mL). The crude amine was dissolved in 1.5 mL DMF and neutralized with 0.5 mL triethylamine. Compound **S10** was obtained after purification by column chromatography (silica, DCM/MeOH, 8/2) as a bordeaux solid (121.5 mg) in 55% yield. LC-MS: 22.20 min. HRMS (ES+): calculated for $C_{55}H_{71}N_{10}O_{10}S^+$ M+: 1063.5070 Found: 1063.5085.

Synthesis of 5f



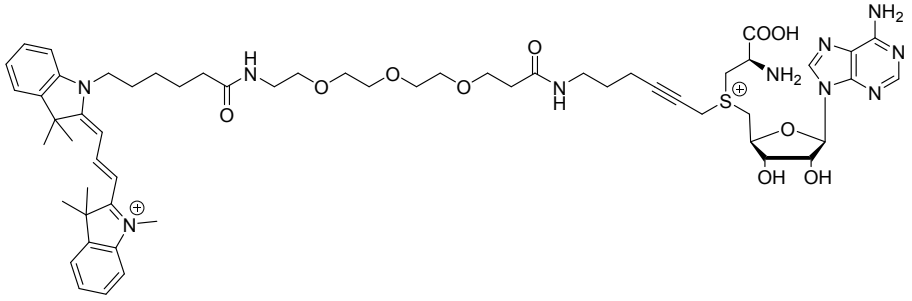
Prepared according to **general procedure C**, from product **S10** (55.0 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **5f** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 65/35. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 17.96 min, purity of 90%. MS (ESI+): 576.00 (M^{2+}).

Synthesis of S11



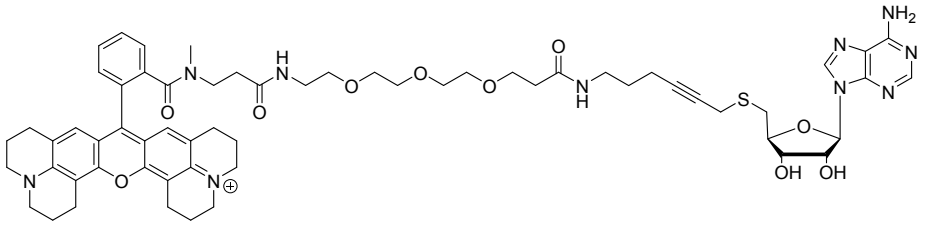
Prepared according to **general procedure B**, from Cy3[8] (116.9 mg, 0.2 mmol), DSC (56.4 mg, 0.22 mmol) and triethylamine (83.6 µL, 0.6 mmol). Total volume of DMF was 1.5 mL. Compound **S2** (163.6 mg, 0.24 mmol) was dissolved in DCM/TFA (50/50, 0.8 mL). The crude amine was dissolved in 1.5 mL DMF and neutralized with 0.5 mL triethylamine. Compound **S11** was obtained after purification by column chromatography (silica, DCM/MeOH, 9/1, gradual increase to 8/2) as a deep red solid (146.9 mg) in 64% yield. LC-MS: 20.74 min. HRMS (ES+): calculated for $C_{55}H_{74}N_9O_8S^+$ M+: 1020.5375 Found: 1020.5419.

Synthesis of 5g



Prepared according to **general procedure C**, from product **S11** (57.4 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **5g** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 60/40. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 18.53 min, purity of 96%. MS (ESI+): 554.45 (M^{2+}).

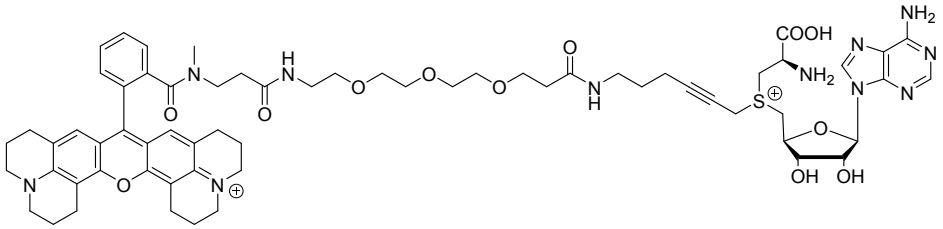
Synthesis of S12



Prepared according to **general procedure B**, from extended Rhodamine 101[3] (61.2 mg, 0.1 mmol), DSC (28.2 mg, 0.11 mmol) and triethylamine (41.8 µL, 0.3 mmol). Total volume of DMF was 1.0 mL. Compound **S2** (81.8 mg, 0.12 mmol) was dissolved in DCM/TFA (50/50, 0.4 mL). The crude amine was dissolved in 0.5 mL DMF and neutralized with 0.25 mL triethylamine. Compound **S12** was obtained after

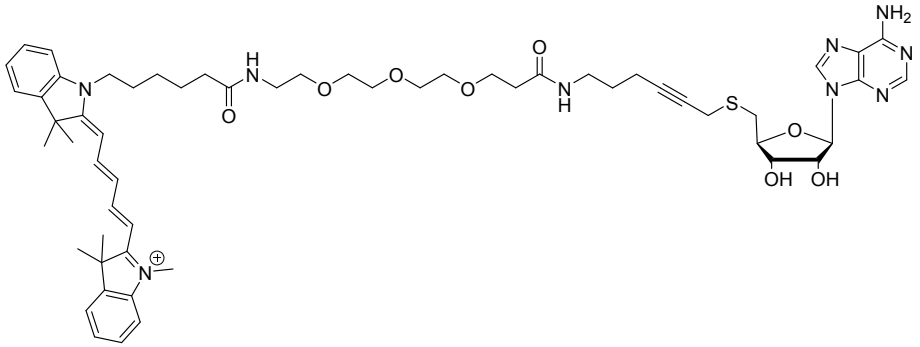
purification by column chromatography (silica, DCM/MeOH, 95/5, gradual increase to 8/2) as a deep purple solid (52.7 mg) in 45% yield. LC-MS: 27.53 min. HRMS (ES+): calculated for $C_{61}H_{75}N_{10}O_{10}S^+$ M+: 1139.5382 Found: 1139.5455.

Synthesis of 5h



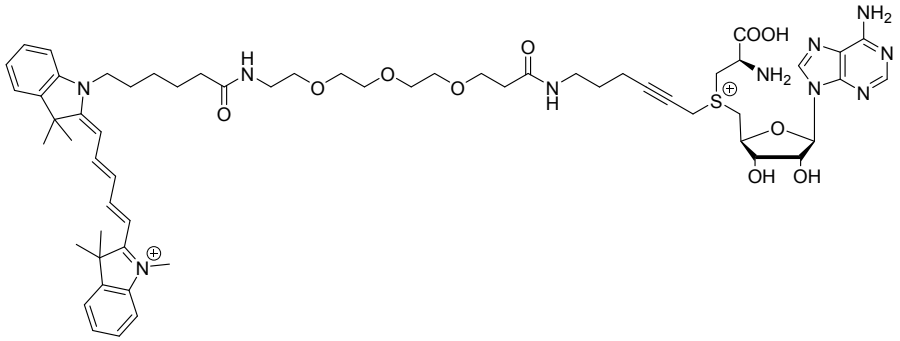
Prepared according to **general procedure C**, from product **S12** (58.8 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **5h** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 55/45. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 19.06 min, purity of 93%. MS (ESI+): 614.15 (M^{2+}).

Synthesis of S13



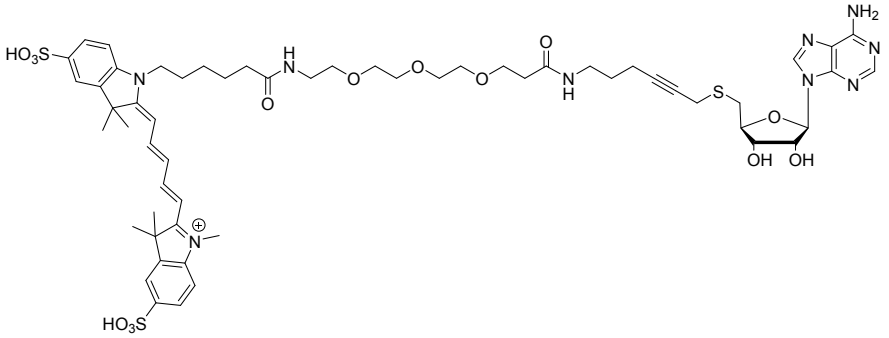
Prepared according to **general procedure B**, from Cy5[8] (122.1 mg, 0.2 mmol), DSC (56.4 mg, 0.22 mmol) and triethylamine (83.6 µL, 0.6 mmol). Total volume of DMF was 1.5 mL. Compound **S2** (163.6 mg, 0.24 mmol) was dissolved in DCM/TFA (50/50, 0.8 mL). The crude amine was dissolved in 1.5 mL DMF and neutralized with 0.5 mL triethylamine. Compound **S13** was obtained after purification by column chromatography (silica, DCM/MeOH, 9/1, gradual increase to 8/2) as a deep blue solid (101.1 mg) in 43% yield. LC-MS: 24.35 min. HRMS (ES+): calculated for $C_{57}H_{76}N_9O_8S^+$ M+: 1046.5532 Found: 1046.5535.

Synthesis of 5i



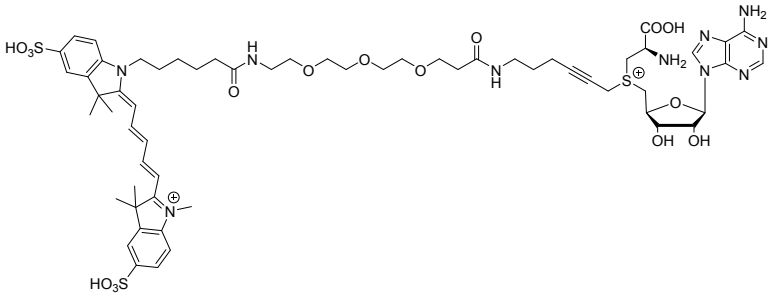
Prepared according to **general procedure C**, from product **S13** (58.7 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **5i** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 60/40. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 19.75 min, purity of 96%. MS (ESI+): 567.70 (M^{2+}).

Synthesis of S14



Prepared according to **general procedure B**, from sCy5[9] (156.4 mg, 0.2 mmol), DSC (56.4 mg, 0.22 mmol) and triethylamine (83.6 µL, 0.6 mmol). Total volume of DMF was 2 mL. Compound **S2** (163.6 mg, 0.24 mmol) was dissolved in DCM/TFA (50/50, 0.8 mL). The crude amine was dissolved in 1.5 mL DMF and neutralized with 0.5 mL triethylamine. Compound **S14** was obtained after purification by column chromatography (silica, DCM/MeOH, 7/3) as a deep blue solid (148.7 mg) in 48% yield. LC-MS: 17.43 min. HRMS (ES+): calculated for $C_{57}H_{76}N_9O_{14}S_3^+$ M+: 1206.4668 Found: 1206.4725.

Synthesis of 5j



Prepared according to **general procedure C**, from product **S14** (62.2 mg, 0.05 mmol). After complete reaction, as observed from HPLC analysis, compound **5j** was obtained after purification through reverse phase silica gel filtration, eluting with 0.1% formic acid in MilliQ/MeOH 70/30. Fractions containing cofactor were combined and concentrated to a final concentration of 10 mM. LC-MS: 13.31 min, purity of 97%. MS (ESI+): 647.80 (M^{2+}).

Gel based restriction assay

The compatibly of the various synthesized cofactors with DNA methyltransferases was examined through a gel based restriction assay. The principle of this assay takes advantage of the methylation-sensitive restriction enzymes. Briefly, if the DNA methyltransferase is compatible with the synthetic cofactor, the successful labeling will block the subsequent digestion of DNA by a restriction enzyme that recognizes the same sequence. In case of digestion, the DNA is cut into smaller fragments which will migrate faster through the gel. Thus, the digestion patterns from electrophoresis gels indicate the compatibility between the enzymes and cofactors of interest.

When a non-digested plasmid is loaded into an agarose gel and exposed to the applied voltage, naturally three bands can be expected corresponding to the three plasmid conformations (from high to low: nicked, linear and supercoiled DNA). Any band lower than the natural conformations can be considered as the result of plasmid digestion, indicating incomplete protection.

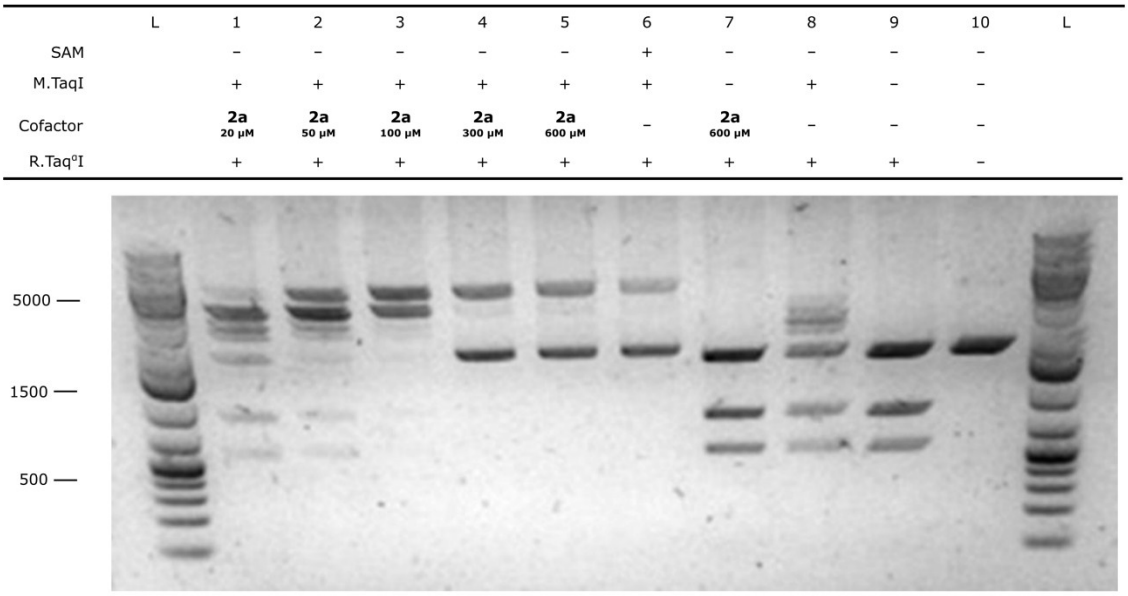


Fig. S1: TaqI restriction assay on pUC19 DNA using oligonucleotide-treated M.TaqI (0.03 µg/µl) with **2a** at various concentrations. All samples were reacted with TaqI restriction enzyme unless stated otherwise. **From left to right**: GeneRuler 1 kb plus (ladder); 1. M.TaqI with 20 µM **2a**, 2. M.TaqI with 50 µM **2a**, 3. M.TaqI with 100 µM **2a**, 4. M.TaqI with 300 µM **2a**, 5. M.TaqI with 600 µM **2a**, 6. control sample with 50 µM natural SAM cofactor, 7. control sample without M.TaqI enzyme, 8. control sample without cofactor, 9. control sample without M.TaqI enzyme and without cofactor, 10. pUC19 DNA, GeneRuler 1 kb plus (ladder). Full protection is observed for samples containing more than 100 µM **2a**.

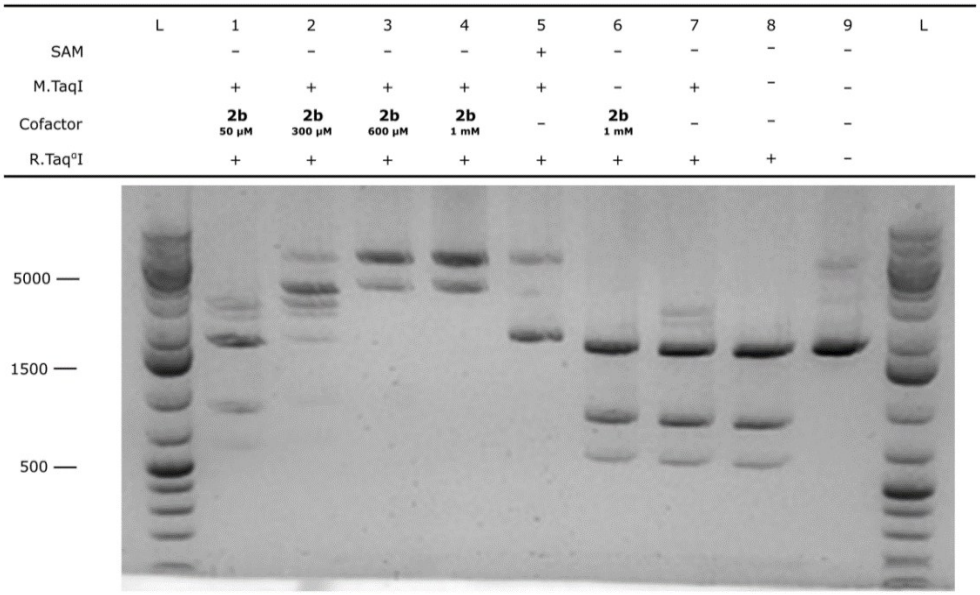


Fig. S2: TaqI restriction assay on pUC19 DNA using oligonucleotide-treated M.TaqI (0.03 µg/µl) with **2b**. All samples were reacted with TaqI restriction enzyme unless stated otherwise. **From left to right**: GeneRuler 1 kb plus (ladder); 1. M.TaqI with 50 µM **2b**, 2. M.TaqI with 300 µM **2b**, 3. M.TaqI with 600 µM **2b**, 4. M.TaqI with 1 mM **2b,** 5. control sample with 50 µM natural SAM cofactor, 6. control sample without M.TaqI enzyme and with 1 mM **2b**, 7. control sample without cofactor, 8. control sample without M.TaqI enzyme and without cofactor, 9. pUC19 DNA, GeneRuler 1 kb plus (ladder). Full protection is observed for samples containing more than 600 µM **2b**.

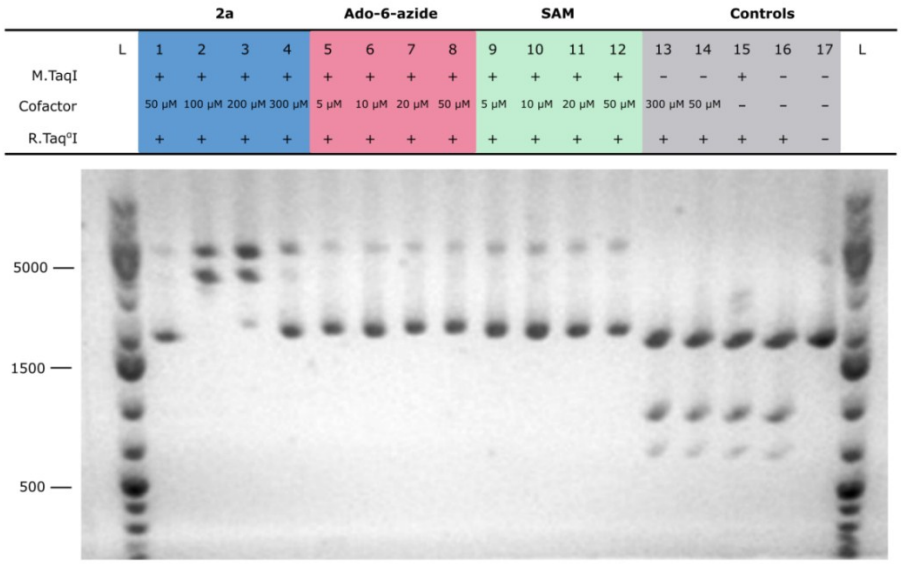


Fig. S3: TaqI restriction assay based comparison of the cysteine cofactor **2a** and its homocysteine counterpart **Ado-6-azide** on pUC19 DNA using oligonucleotide-treated M.TaqI (0.03 µg/µl). All samples were reacted with TaqI restriction enzyme unless stated otherwise. **From left to right**: GeneRuler 1 kb plus (ladder); lanes 1-4. cofactor **2a** concentration gradient, lanes 5-8. cofactor **Ado-6-azide** concentration gradient, lanes 9-12. cofactor SAM concentration gradient, 13. control sample without M.TaqI enzyme and with 300 µM **2a**, 14. control sample without M.TaqI enzyme and with 50 µM **Ado-6-azide**, 15. control sample without cofactor, 16. control sample without M.TaqI enzyme and without cofactor, 17. pUC19 DNA, GeneRuler 1 kb plus (ladder). Full protection can be achieved for all cofactor types, although at increased concentrations for the cysteine based cofactor.

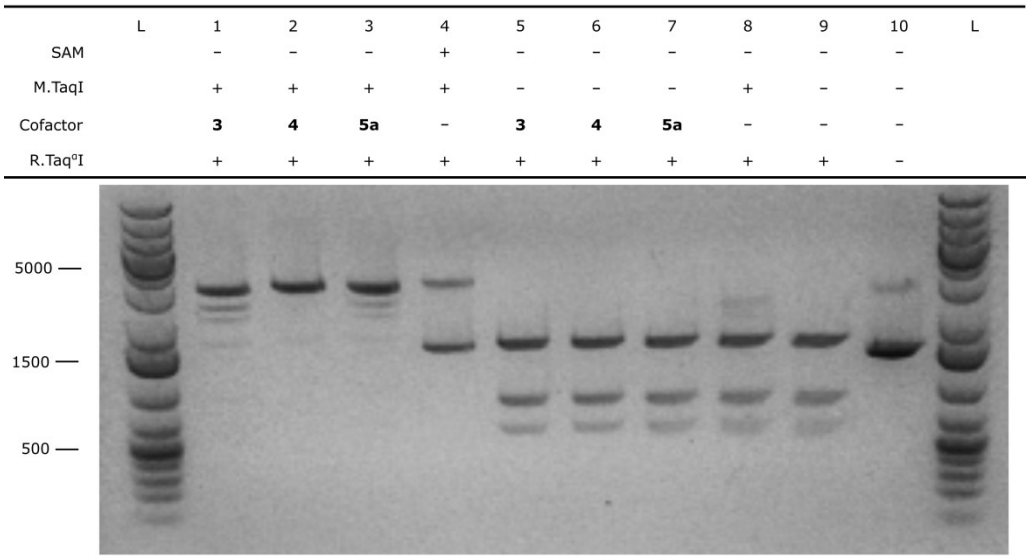


Fig. S4: TaqI restriction assay on pUC19 DNA using oligonucleotide-treated M.TaqI (0.03 µg/µl) with **3**, **4** and **5a**. All samples were reacted with TaqI restriction enzyme unless stated otherwise. **From left to right**: GeneRuler 1 kb plus (ladder); 1. M.TaqI with 50 µM **3**, 2. M.TaqI with 50 µM **4**, 3. M.TaqI with 50 µM **5a**, 4. control sample with 50 µM natural SAM cofactor, 5. control sample without M.TaqI enzyme and with 50 µM **3**, 6. control sample without M.TaqI enzyme and with 50 µM **4**, 7. control sample without M.TaqI enzyme and with 50 µM **5a**, 8. control sample without cofactor, 9. control sample without M.TaqI enzyme and without cofactor, 10. pUC19 DNA, GeneRuler 1 kb plus (ladder). Full protection is observed for all cofactors.

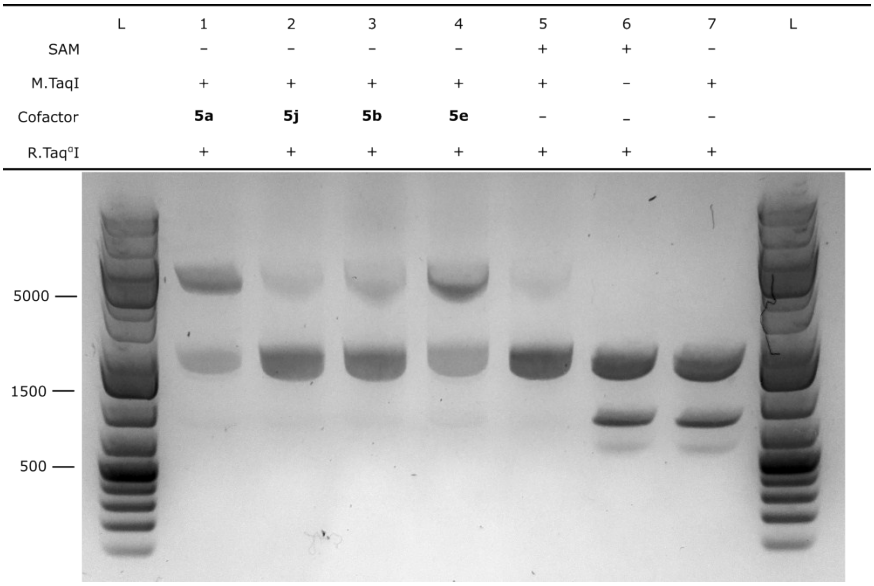


Fig. S5: TaqI restriction assay on pUC19 DNA using oligonucleotide-treated M.TaqI (0.03 µg/µl) with **5a**, **5b**, **5e** and **5j**. All samples were reacted with TaqI restriction enzyme unless stated otherwise. **From left to right**: GeneRuler 1 kb plus (ladder); 1. M.TaqI with 240 µM **5a**, 2. M.TaqI with 240 µM **5j**, 3. M.TaqI with 240 µM **5b**, 4. M.TaqI with 240 µM **5e**, 5. control sample with 160 µM natural SAM cofactor, 6. control sample without M.TaqI enzyme and with SAM, 7. control sample without cofactor, GeneRuler 1 kb plus (ladder). Full protection is observed for all cofactors.

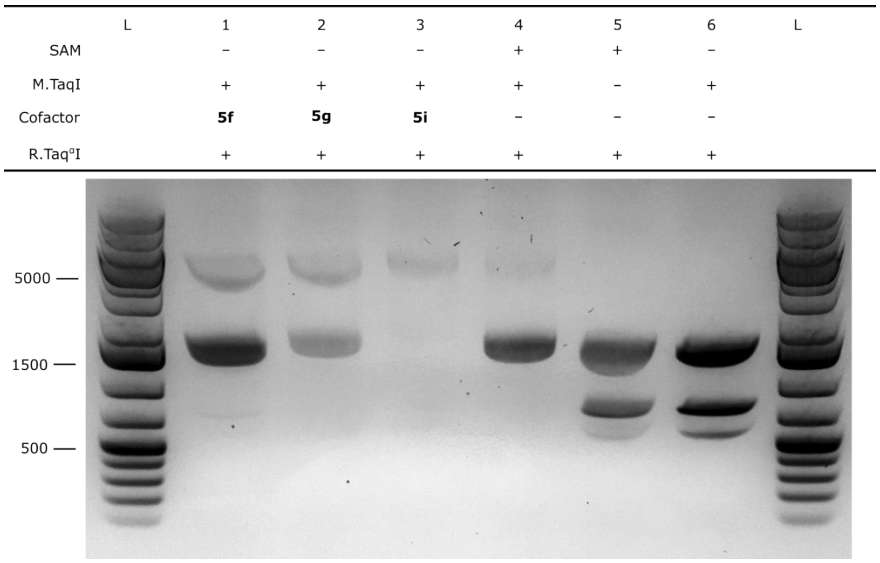


Fig. S6: TaqI restriction assay on pUC19 DNA using oligonucleotide-treated M.TaqI (0.03 µg/µl) with **5f**, **5g**, and **5i**. All samples were reacted with TaqI restriction enzyme unless stated otherwise. **From left to right**: GeneRuler 1 kb plus (ladder); 1. M.TaqI with 240 µM **5f**, 2. M.TaqI with 240 µM **5g**, 3. M.TaqI with 240 µM **5i**, 4. control sample with 160 µM natural SAM cofactor, 5. control sample without M.TaqI enzyme and with SAM, 6. control sample without cofactor, GeneRuler 1 kb plus (ladder). Full protection is observed for all cofactors.

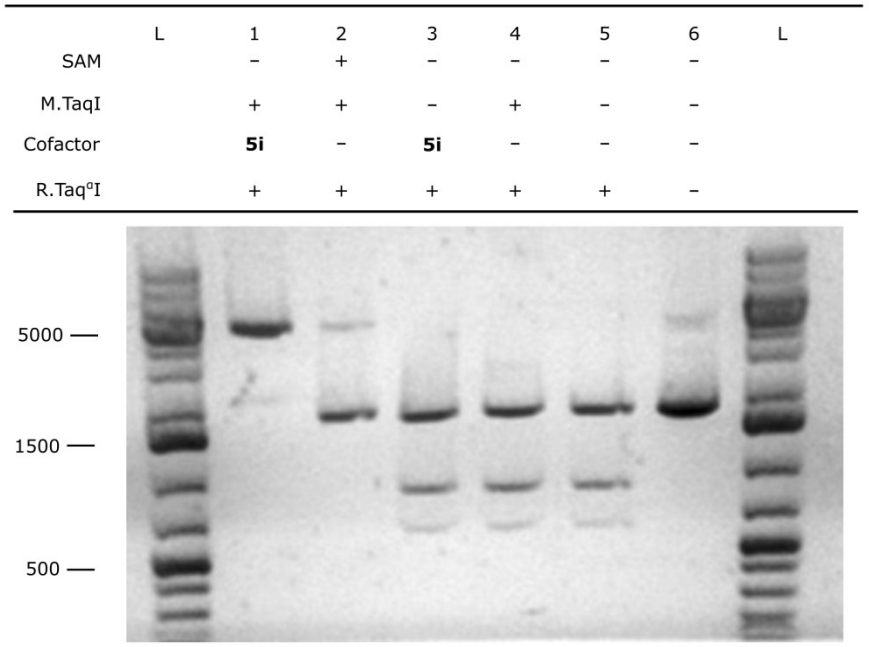


Fig. S7: TaqI restriction assay on pUC19 DNA using oligonucleotide-treated M.TaqI (0.03 µg/µl) with **5i**. All samples were reacted with TaqI restriction enzyme unless stated otherwise. **From left to right**: GeneRuler 1 kb plus (ladder); 1. M.TaqI with 50 µM **5i**, 2. control sample with 50 µM natural SAM cofactor, 3. control sample without M.TaqI enzyme and with 50 µM **5i**, 4. control sample without cofactor, 5. control sample without M.TaqI enzyme and without cofactor, 6. pUC19 DNA, GeneRuler 1 kb plus (ladder). Full protection is observed for cofactor **5i**.

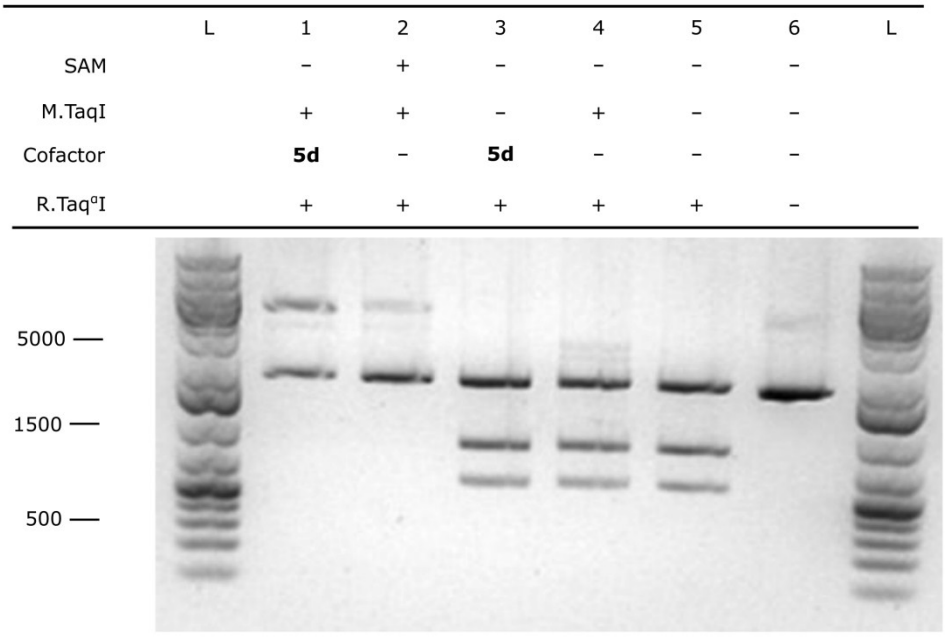


Fig. S8: TaqI restriction assay on pUC19 DNA using oligonucleotide-treated M.TaqI (0.03 µg/µl) with **5d**. All samples were reacted with TaqI restriction enzyme unless stated otherwise. **From left to right**: GeneRuler 1 kb plus (ladder); 1. M.TaqI with 260 µM **5d**, 2. control sample with 50 µM natural SAM cofactor, 3. control sample without M.TaqI enzyme and with 260 µM **5d**, 4. control sample without cofactor, 5. control sample without M.TaqI enzyme and without cofactor, 6. pUC19 DNA, GeneRuler 1 kb plus (ladder). Full protection is observed for cofactor **5d**.

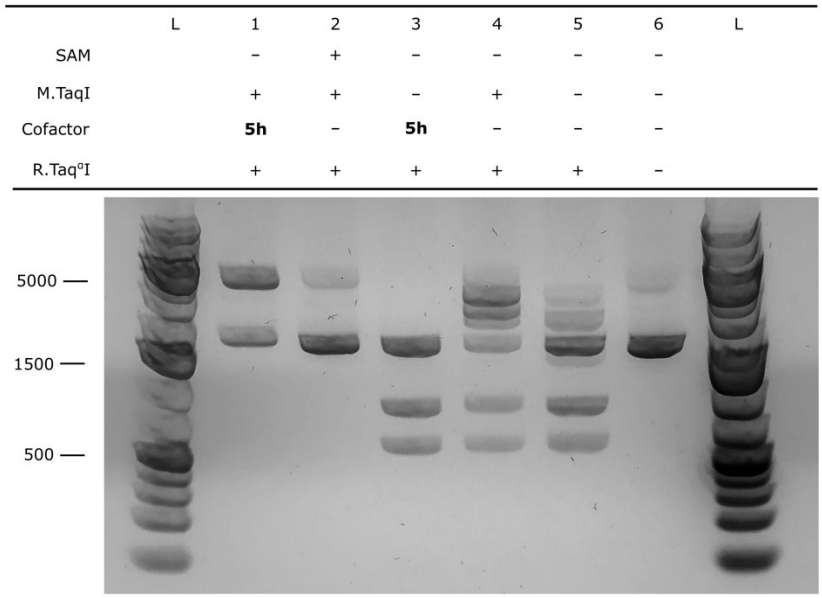


Fig. S9: TaqI restriction assay on pUC19 DNA using oligonucleotide-treated M.TaqI (0.03 µg/µl) with **5h**. All samples were reacted with TaqI restriction enzyme unless stated otherwise. **From left to right**: GeneRuler 1 kb plus (ladder); 1. M.TaqI with 240 µM **5h**, 2. control sample with 50 µM natural SAM cofactor, 3. control sample without M.TaqI enzyme and with 240 µM **5h**, 4. control sample without cofactor, 5. control sample without M.TaqI enzyme and without cofactor, 6. pUC19 DNA, GeneRuler 1 kb plus (ladder). Full protection is observed for cofactor **5h**.

Counting assay

Fluorescence counting assay was performed as described in literature[10] in order to assess the DNA labeling efficiency. Briefly, 10 samples of labeled pUC19 DNA were prepared using the direct cofactors **5a-5j**. Next, for each sample, the labeled molecules were deposited on a poly-L-lysine (PLL) (0.01% w/v in H_2O) coated coverslip and visualized using fluorescent microscopy. After long excitation the reporter fluorophores will be photobleached. Each bleaching step can be counted, yielding the number of dyes bound to a single DNA molecule. Up to 20000 DNA molecules per sample were measured and the average dye content was calculated. These averages are presented in Fig. S10 for all 10 samples.

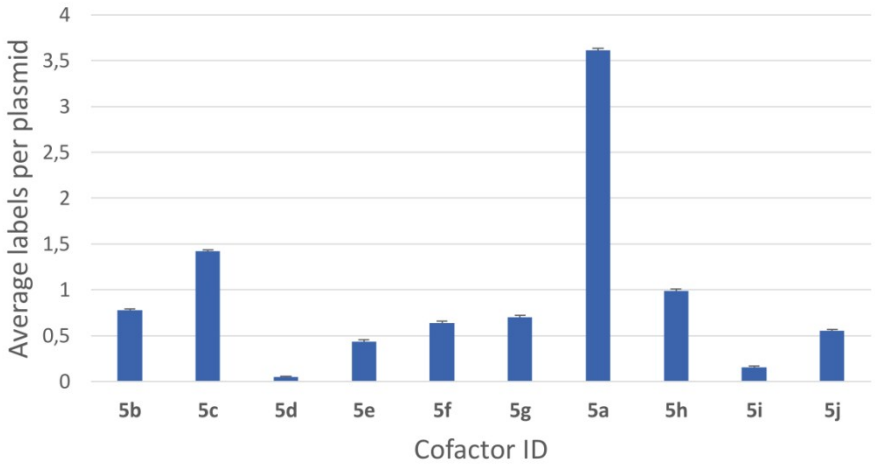


Fig. S10: DNA labeling efficiency for cofactors **5a-5j**. Each blue bar represents the ratio between the total number of fluorescent labels localized on all the single DNA molecules analyzed and the total number of examined DNA molecules. Every error bar delimits an interval of ± the standard deviation of the corresponding ratio over the 10 samples under study.

M.TaqI directed labeling using a rhodamine B-tagged SAM analogue

A similar labeling protocol[11] was used as described by Bouwens *et al*. In brief, Lambda DNA (Thermo Scientific) was enzymatically labeled using 35 µM of the fluorescent analogue **4** or **5a** and 0.14 mg/ml M.TaqI methyltransferase enzyme (recognition sequence 5'-TCGA-3'), in a final concentration of 50 ng/µl. The reaction was carried out at 60°C for 2 hours in a custom labeling buffer. Next, proteinase k was added and reacted for 1 hour at 50°C. Finally, the reaction product was purified using CHROMA SPIN+TE-1000 columns (Clontech, Takara Bio).

Purified DNA was dissolved in 50 mM MES (pH 5.6), and deposited in stretched conformation by mechanically dragging a 2 µl droplet over the surface of a Zeonex-coated coverslip at a speed of 4.4 mm/min, as described earlier[12]. Stretched samples were vacuum dried overnight prior to imaging.

Imaging was performed with a Zeiss SIM Elyra microscope with a Zeiss Plan-APOCHROMAT 63× oil immersion objective (numerical aperture 1.4) and an EMCCD camera. For each field of view, 25 frames were recorded for 5 SIM modulation angles and 5 phases/angle. The illumination patterns for SR-SIM were created by a grating with a period of 34 µm. A wide-field image was calculated by averaging over the 25 frames. SR-SIM reconstruction was done with the open-source fairSIM plugin for ImageJ. DNA fragments were segmented manually on the SR-SIM images using ImageJ. For each imaged DNA fragment, both widefield and SR-SIM signals were extracted.

NMR spectra

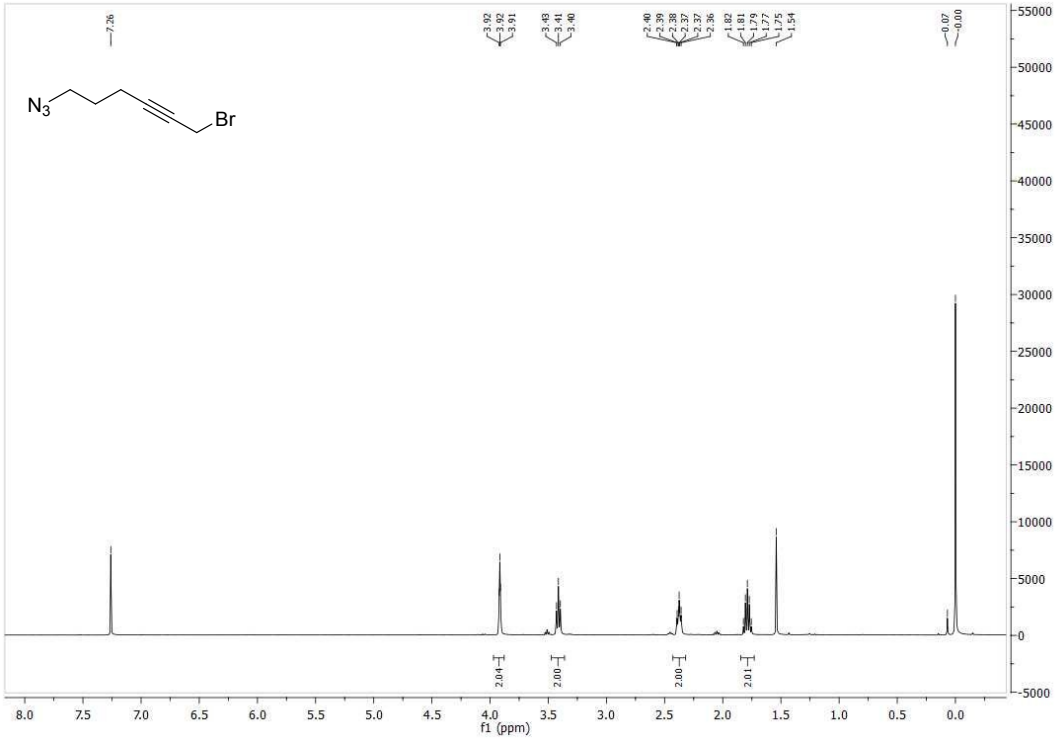


Fig. S11: ^{1}H-NMR (400 MHz) of 6-azido-1-bromohex-2-yne.

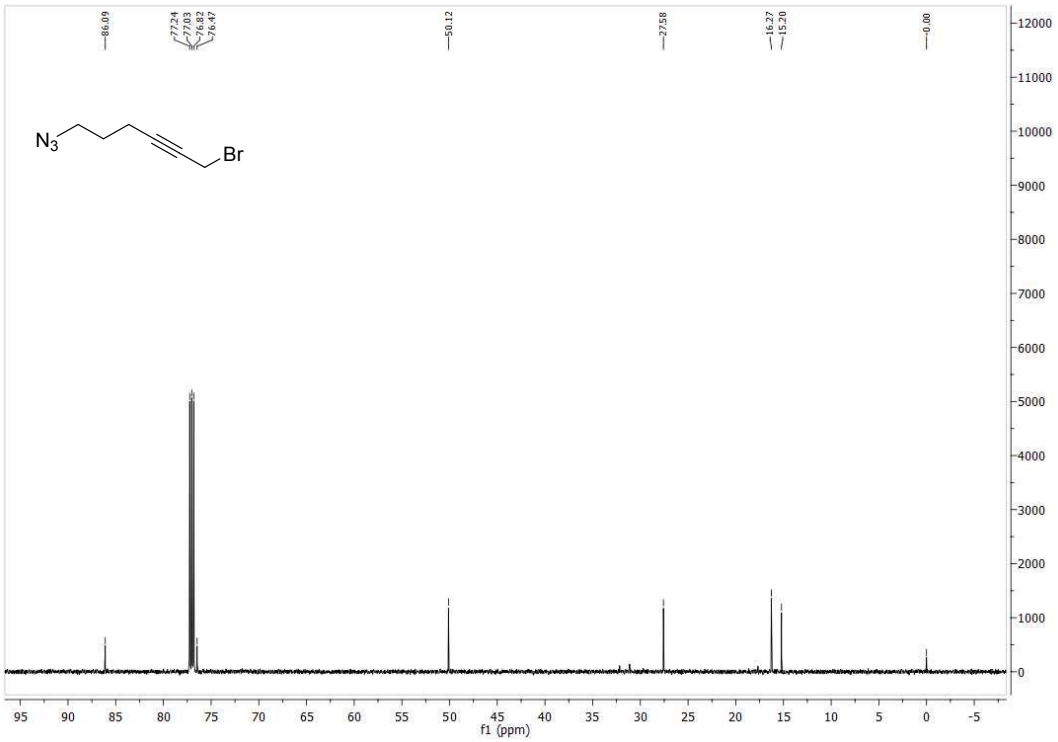


Fig. S12: ^{13}C-NMR (151 MHz) of 6-azido-1-bromohex-2-yne.

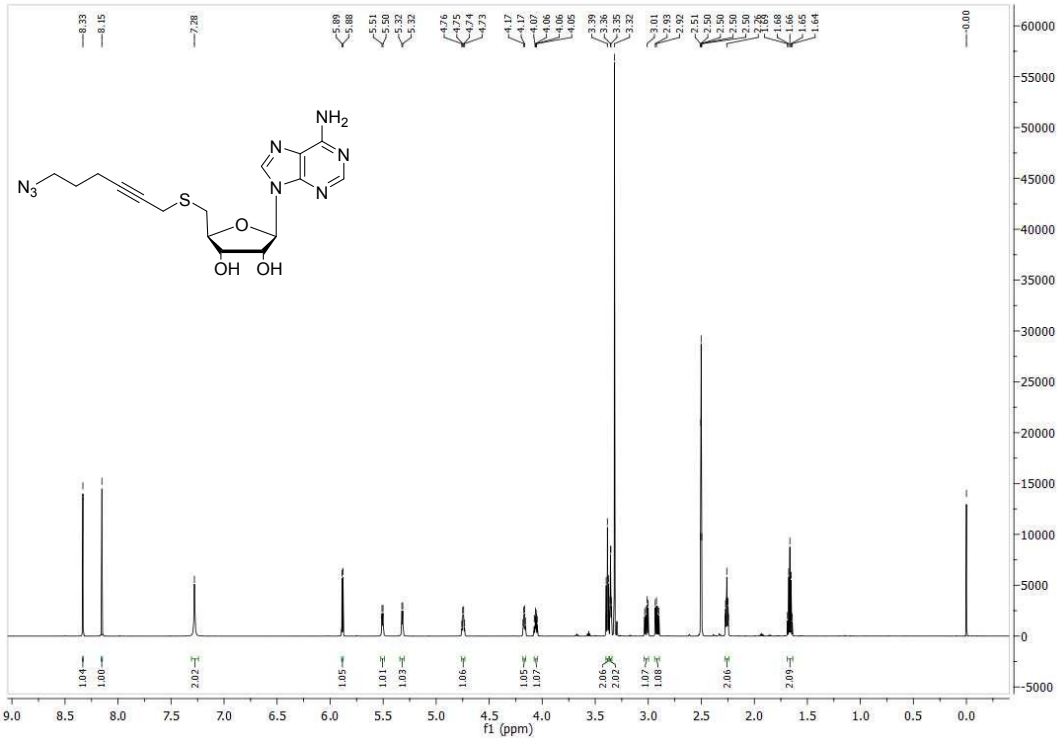


Fig. S13: ^{1}H-NMR (600 MHz) of **1a**.

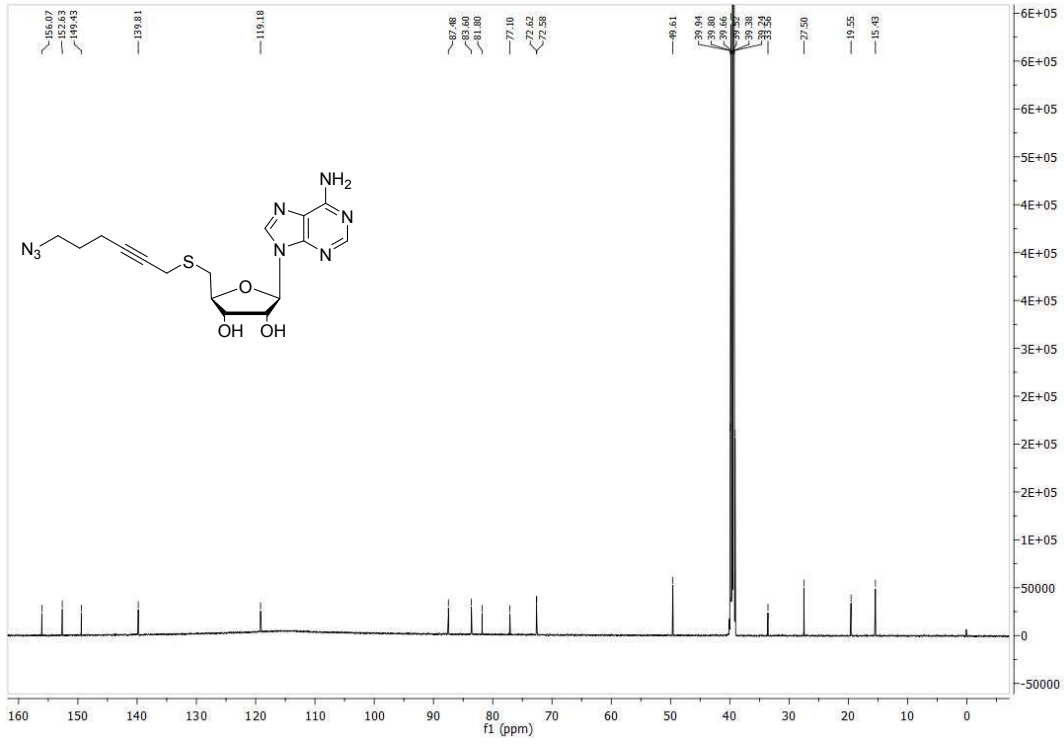


Fig. S14: ^{13}C-NMR (151 MHz) of **1a**.

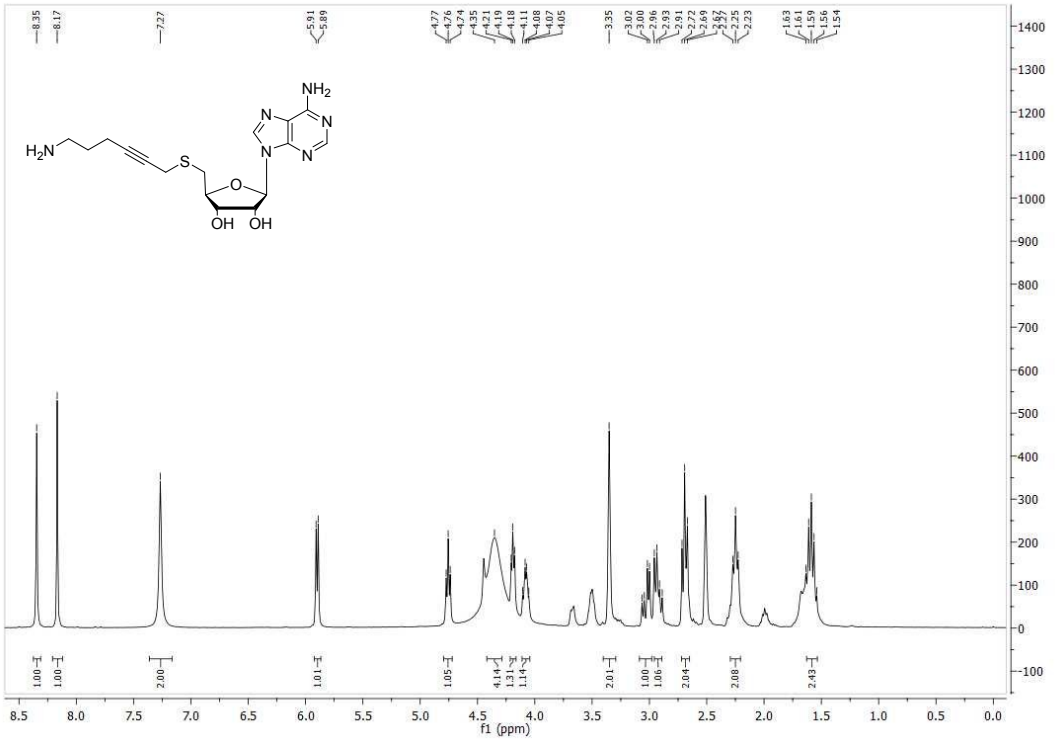


Fig. S15: ^{1}H-NMR (300 MHz) of **1b**.

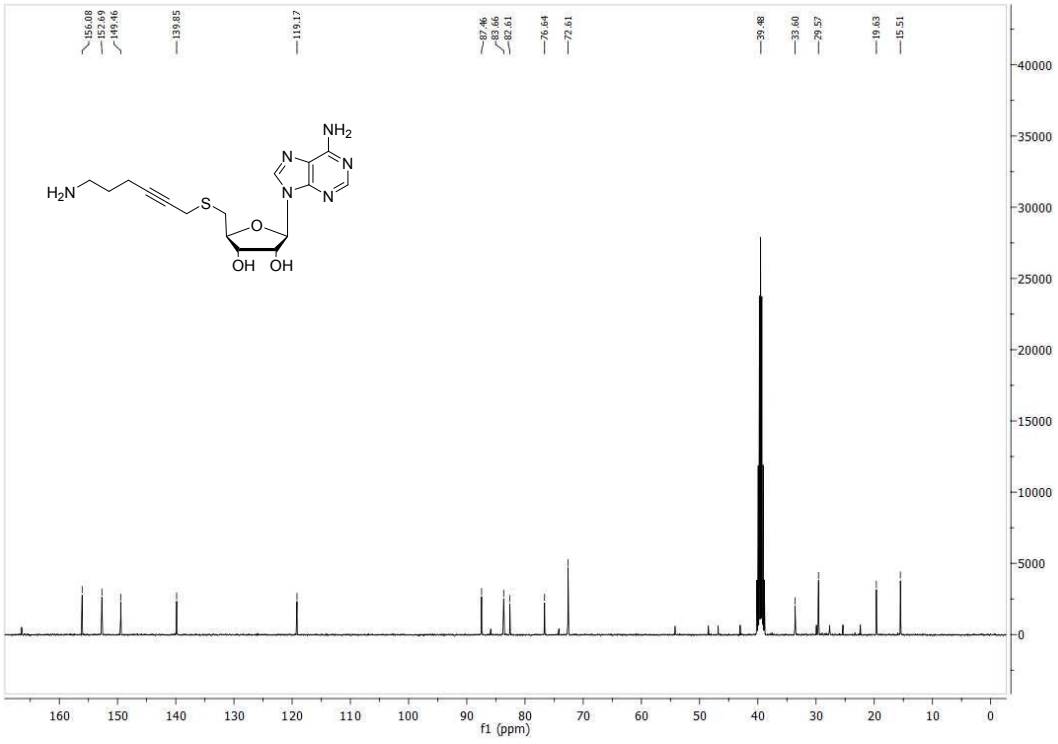


Fig. S16: ^{13}C-NMR (101 MHz) of **1b**.

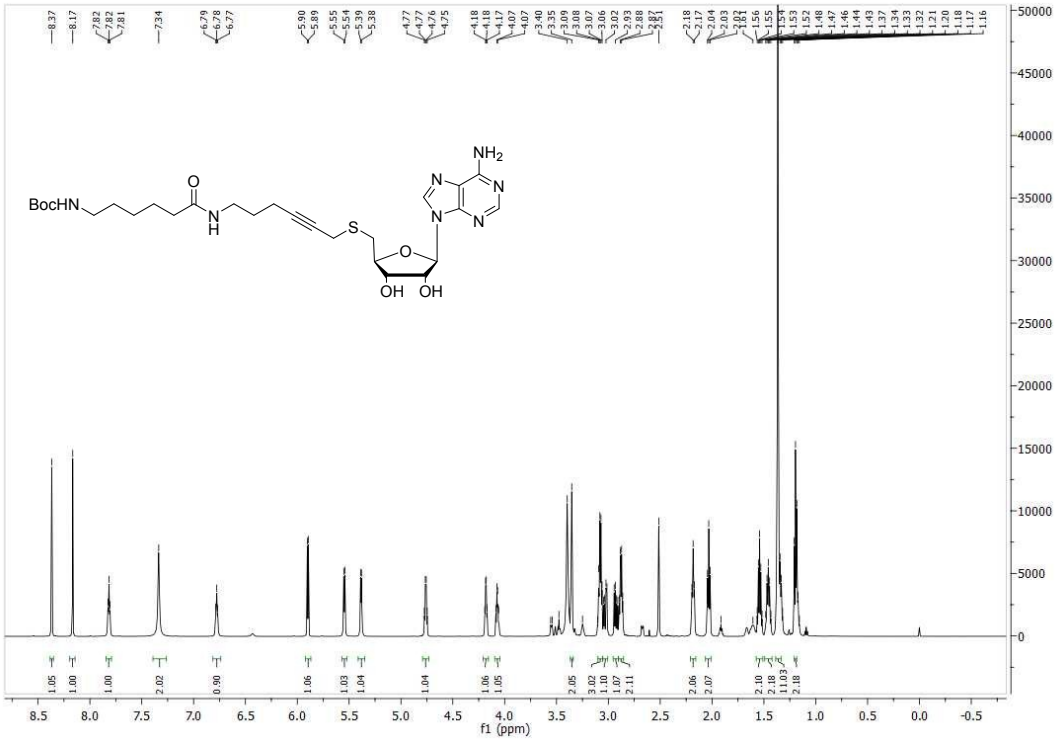


Fig. S17: ^{1}H-NMR (600 MHz) of **S1**.

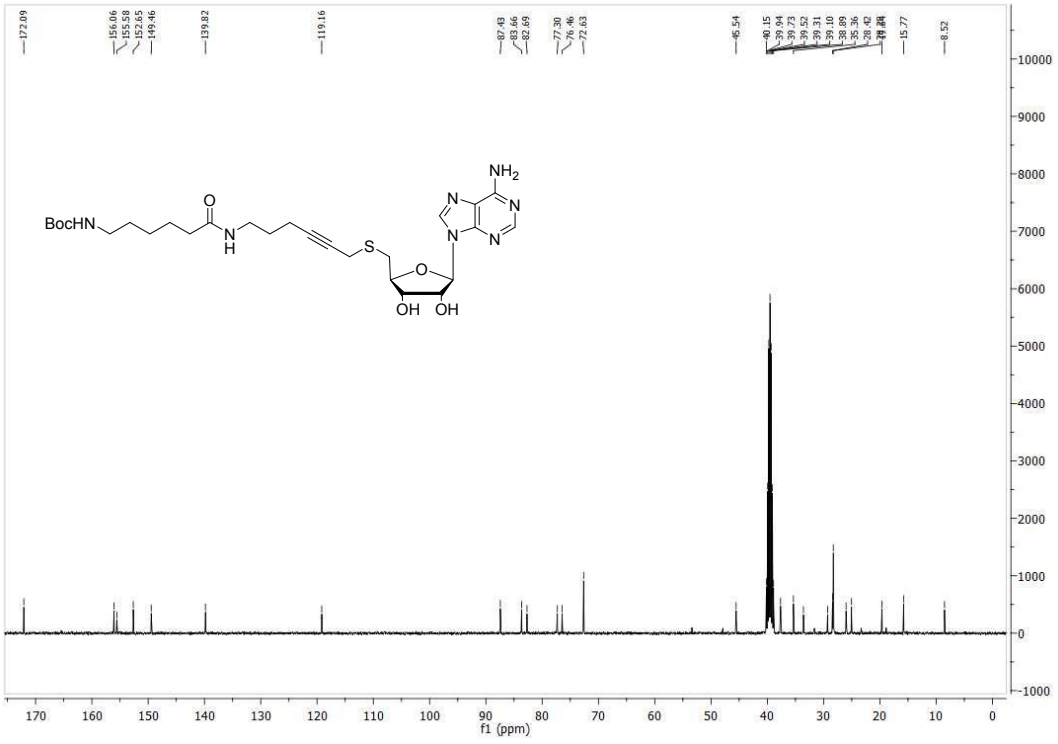


Fig. S18: ^{13}C-NMR (101 MHz) of **S1**.

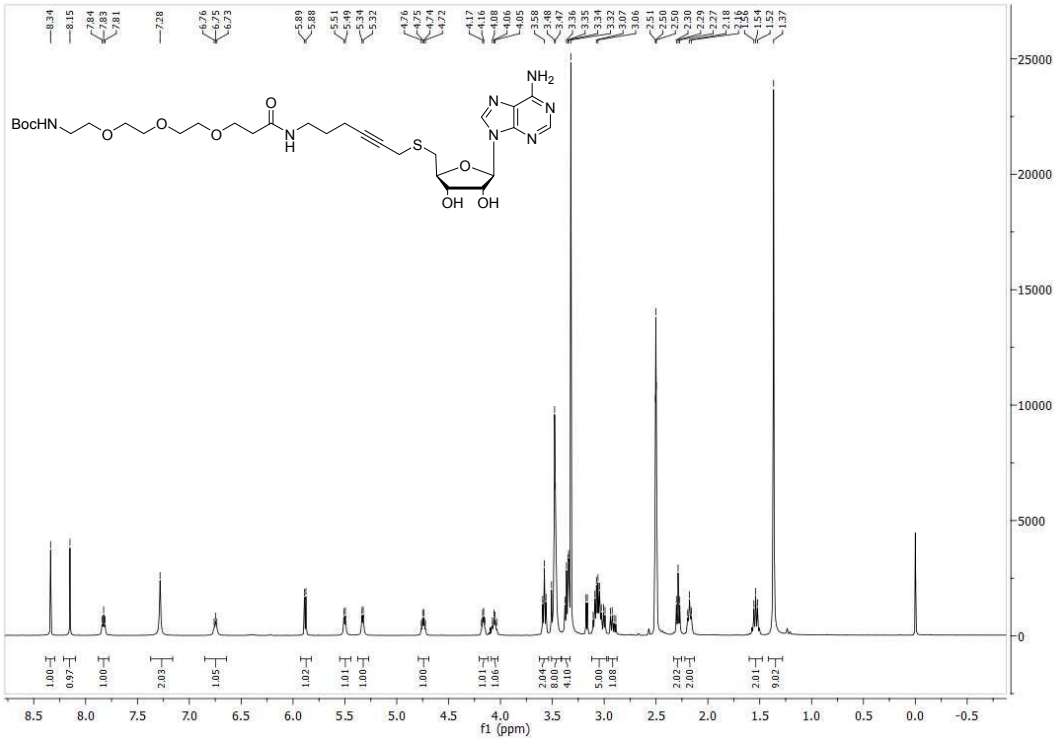


Fig. S19: ^{1}H-NMR (600 MHz) of **S2**.

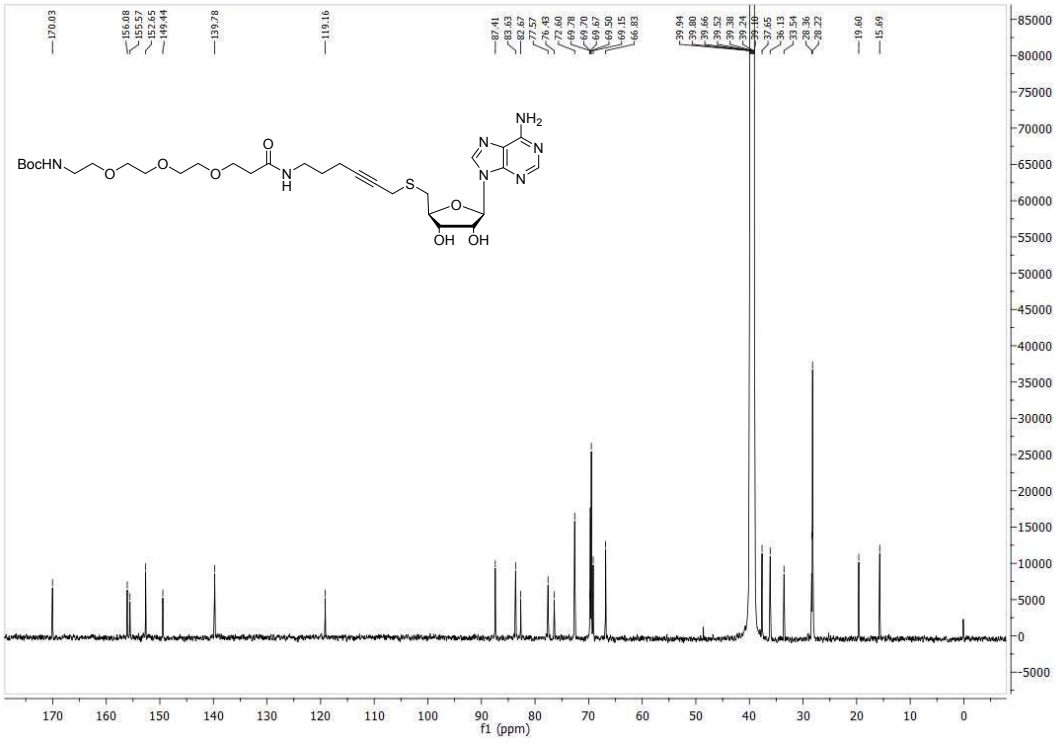


Fig. S20: ^{13}C-NMR (151 MHz) of **S1.**

LCMS spectra

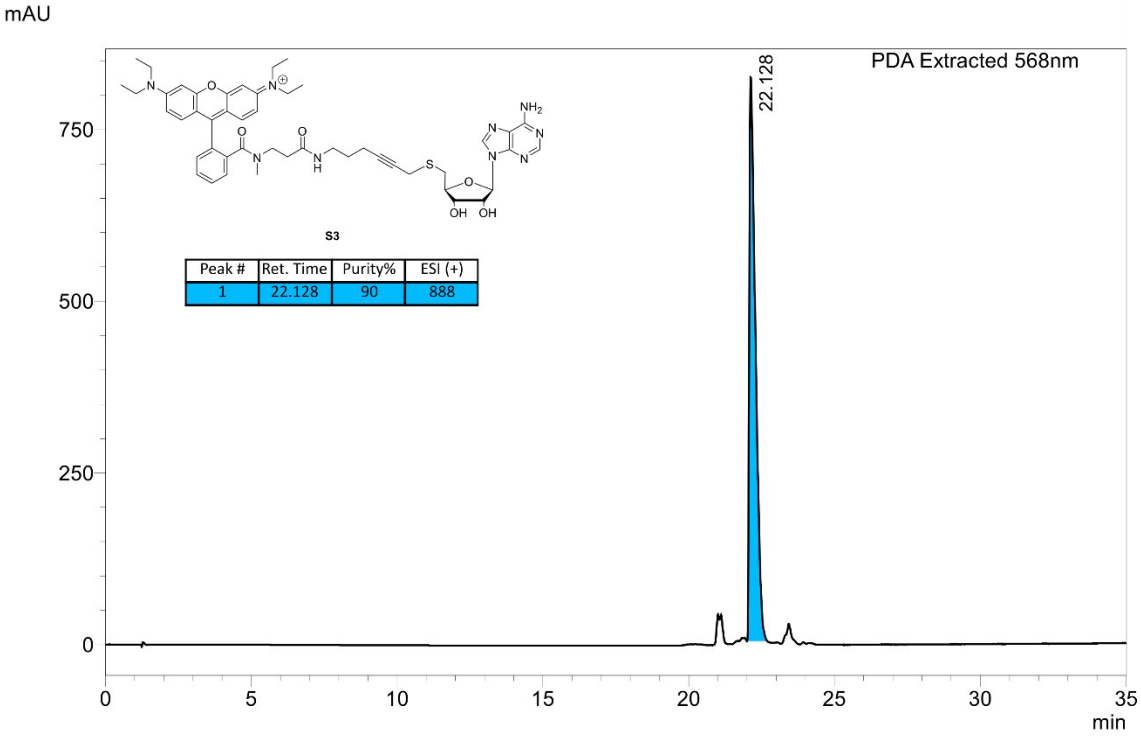


Fig. S21: Reverse phase LCMS chromatogram monitored at λ = 568 nm of purified **S3**.

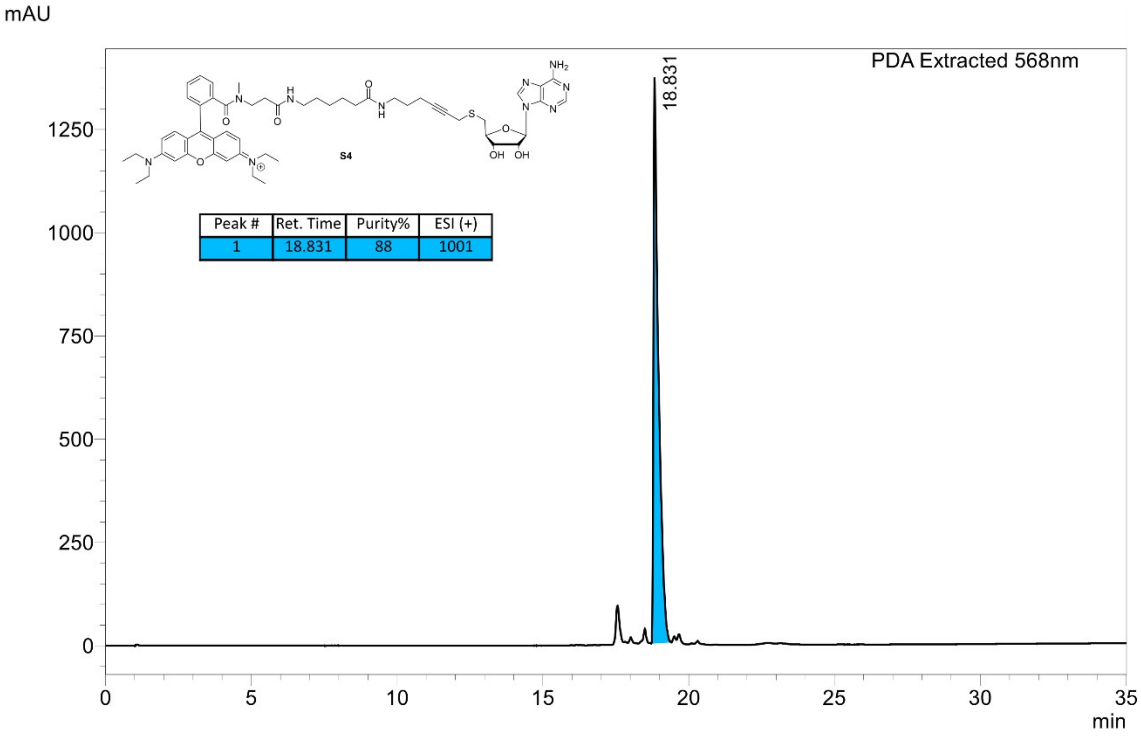


Fig. S22: Reverse phase LCMS chromatogram monitored at λ = 568 nm of purified **S4**.

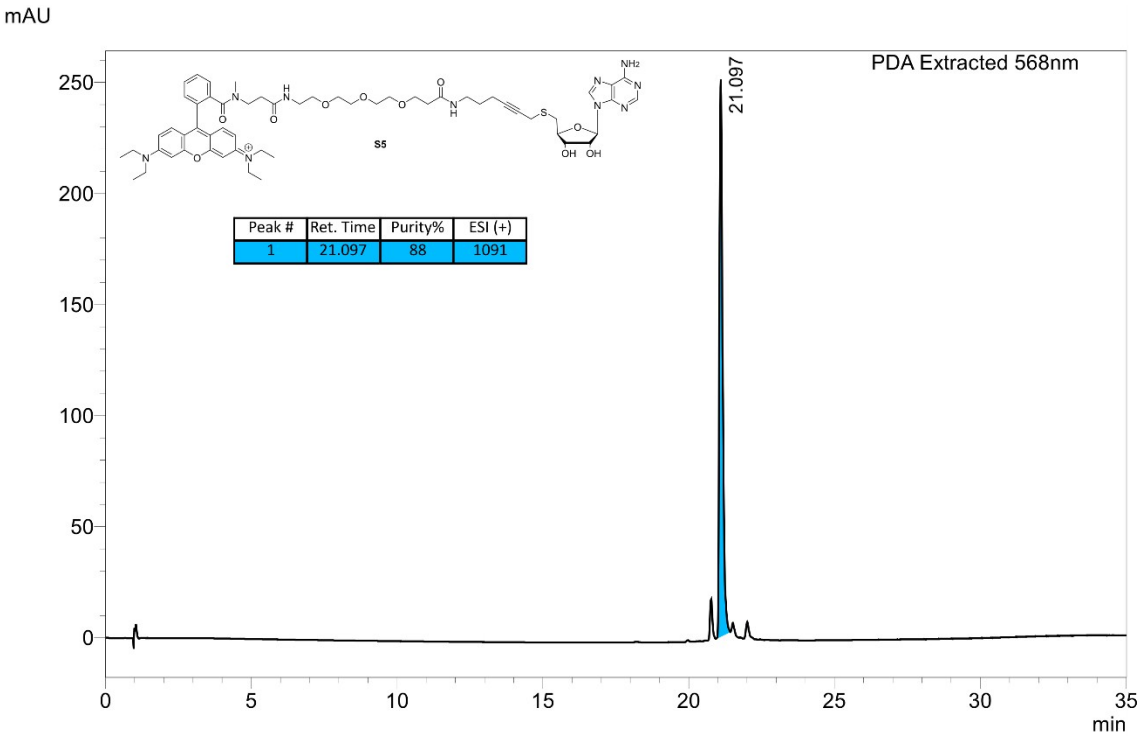


Fig. S23: Reverse phase LCMS chromatogram monitored at λ = 568 nm of purified **S5**.

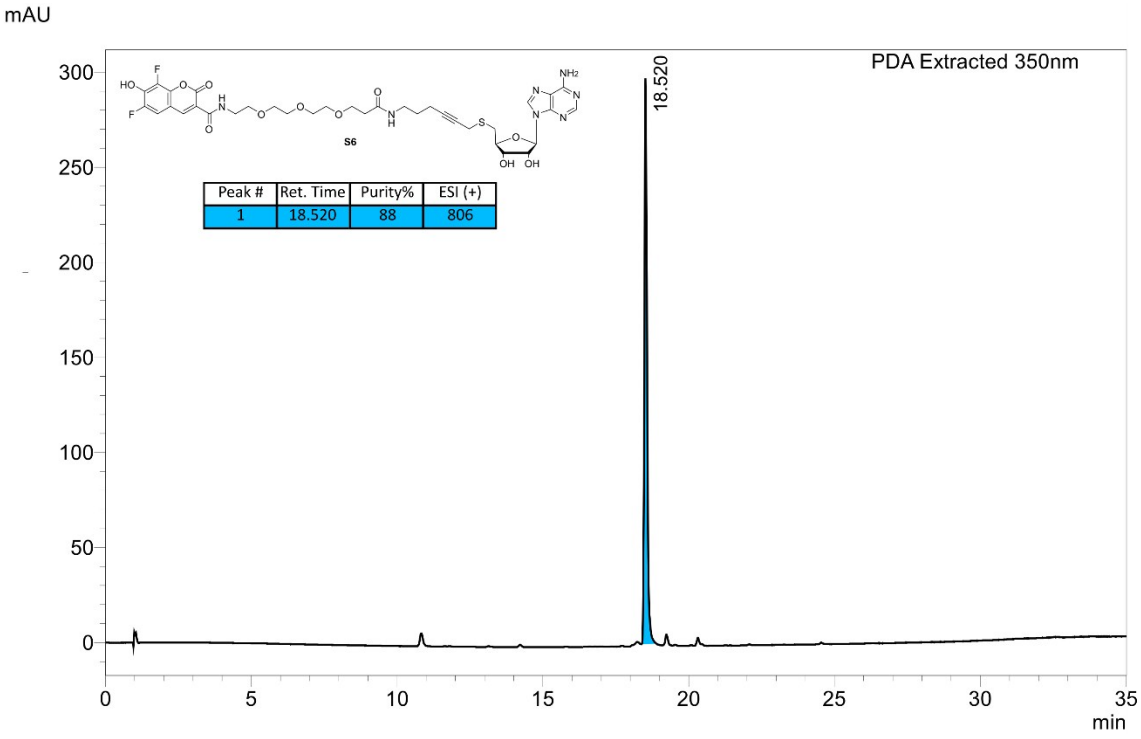


Fig. S24: Reverse phase LCMS chromatogram monitored at λ = 350 nm of purified **S6**.

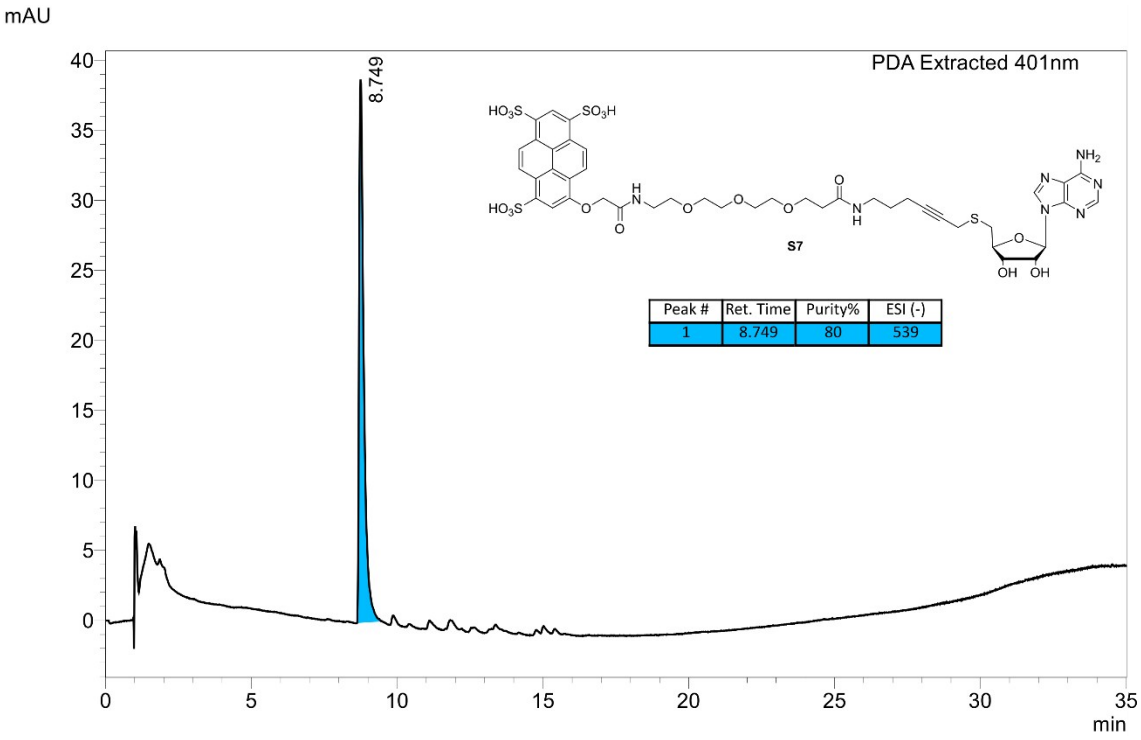


Fig. S25: Reverse phase LCMS chromatogram monitored at λ = 401 nm of purified **S7**.

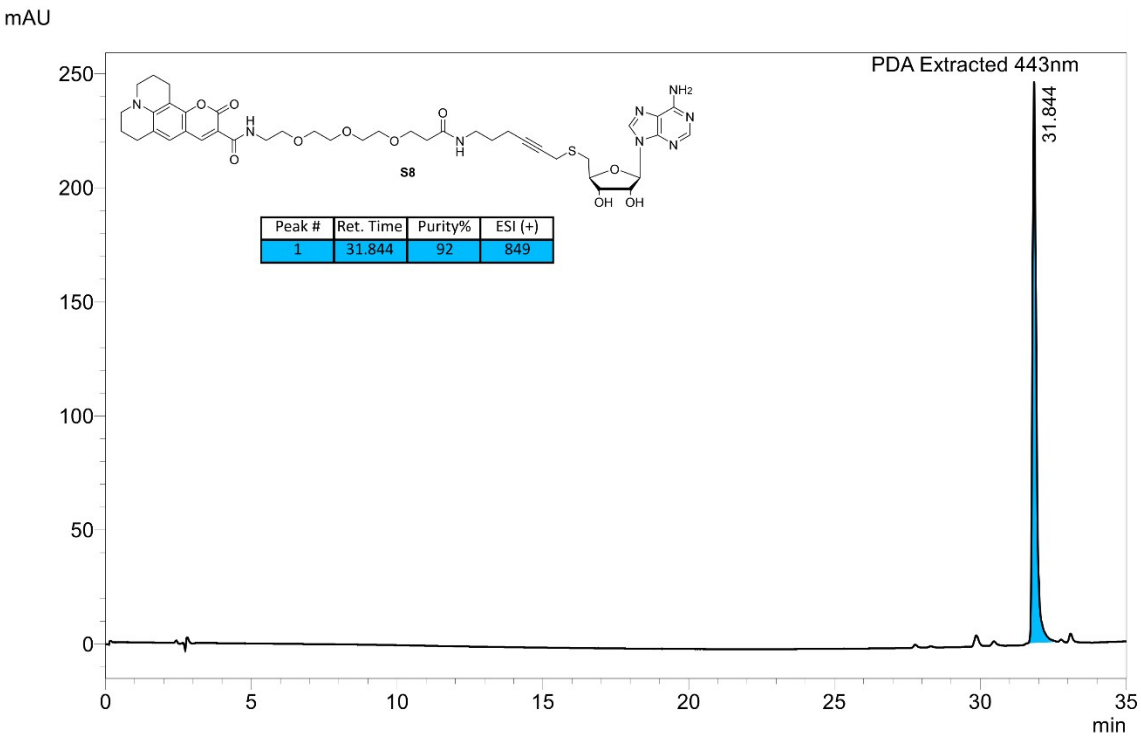


Fig. S26: Reverse phase LCMS chromatogram monitored at λ = 443 nm of purified **S8**.

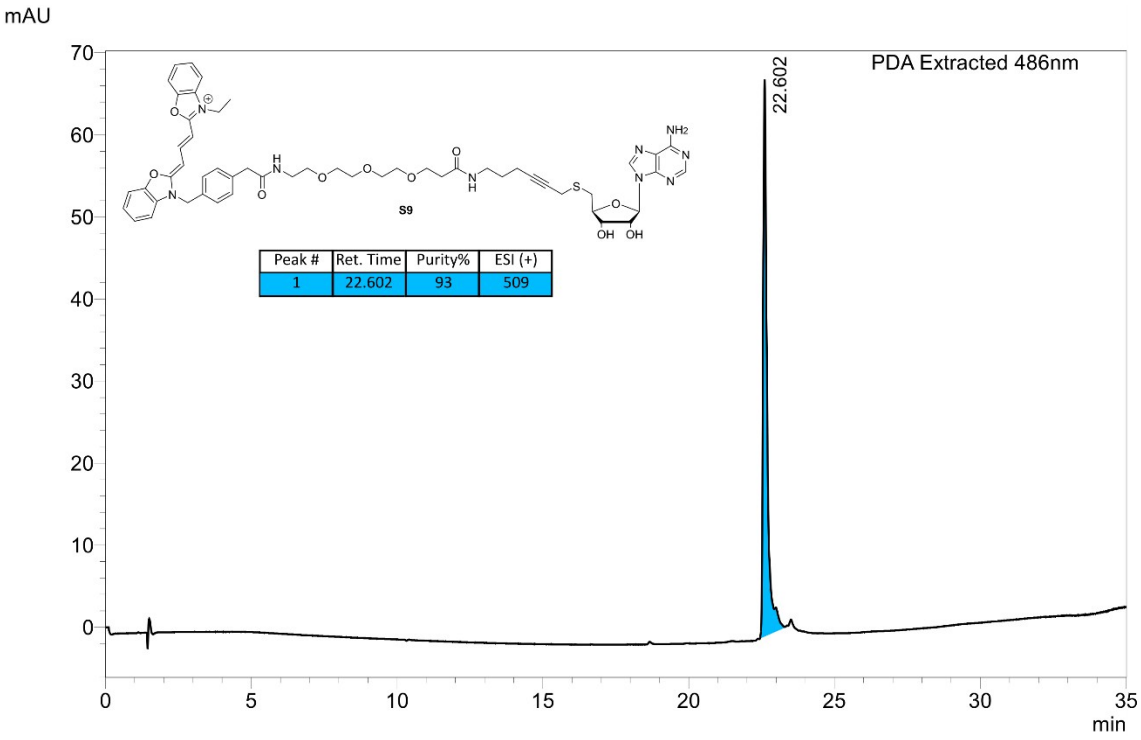


Fig. S27: Reverse phase LCMS chromatogram monitored at λ = 486 nm of purified **S9**.

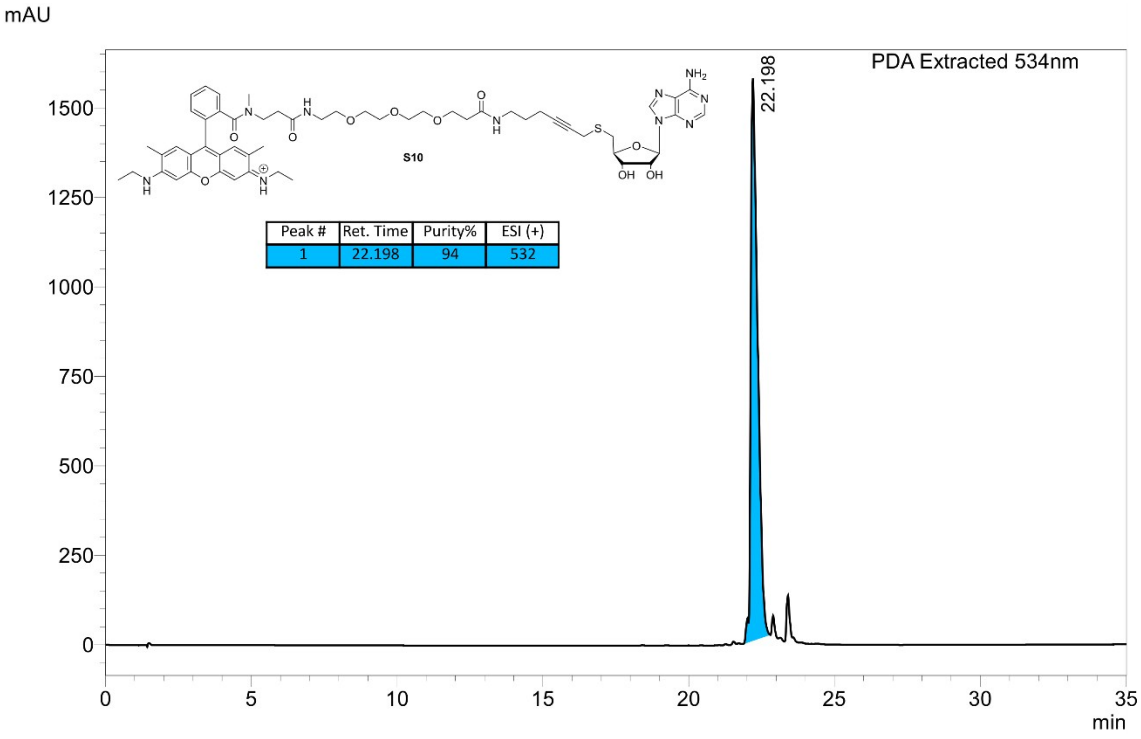


Fig. S28: Reverse phase LCMS chromatogram monitored at λ = 534 nm of purified **S10**.

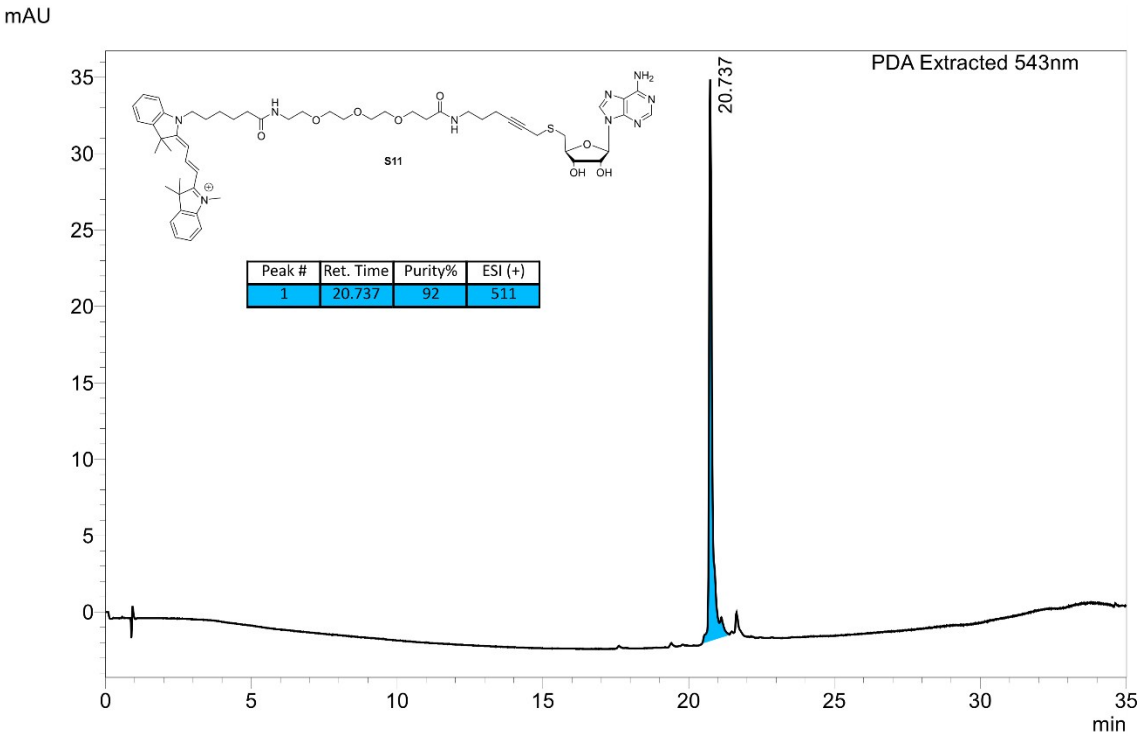


Fig. S29: Reverse phase LCMS chromatogram monitored at λ = 543 nm of purified **S11**.

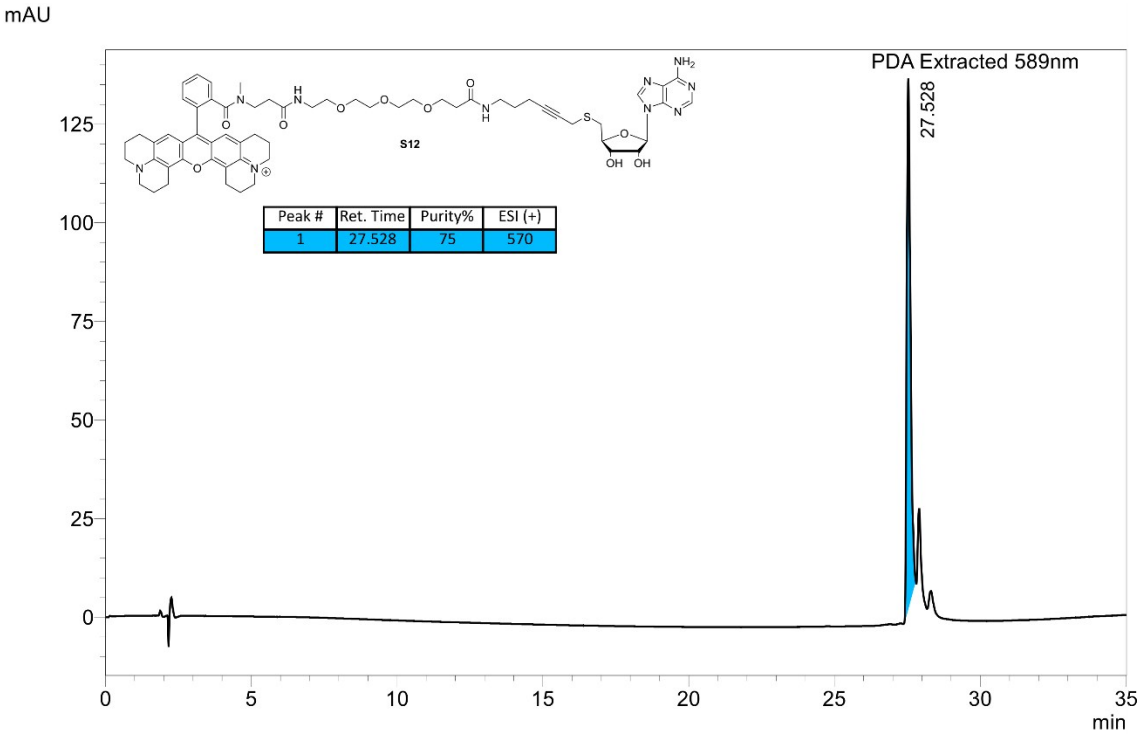


Fig. S30: Reverse phase LCMS chromatogram monitored at λ = 589 nm of purified **S12**.

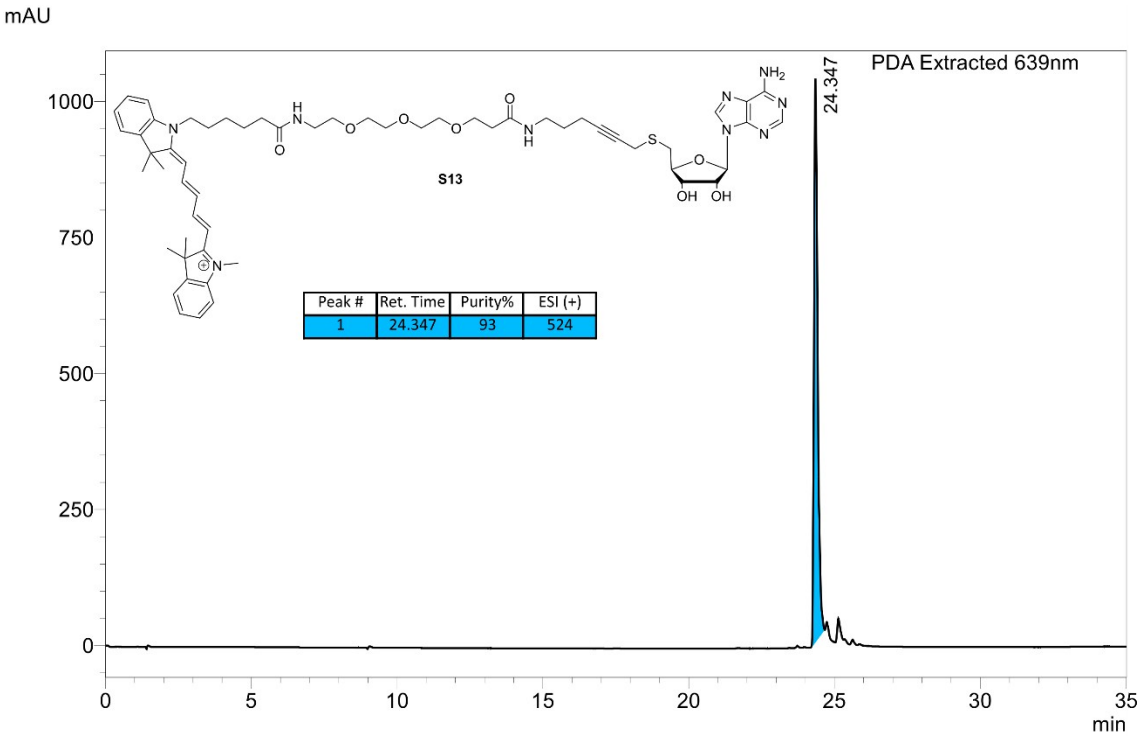


Fig. S31: Reverse phase LCMS chromatogram monitored at λ = 639 nm of purified **S13**.

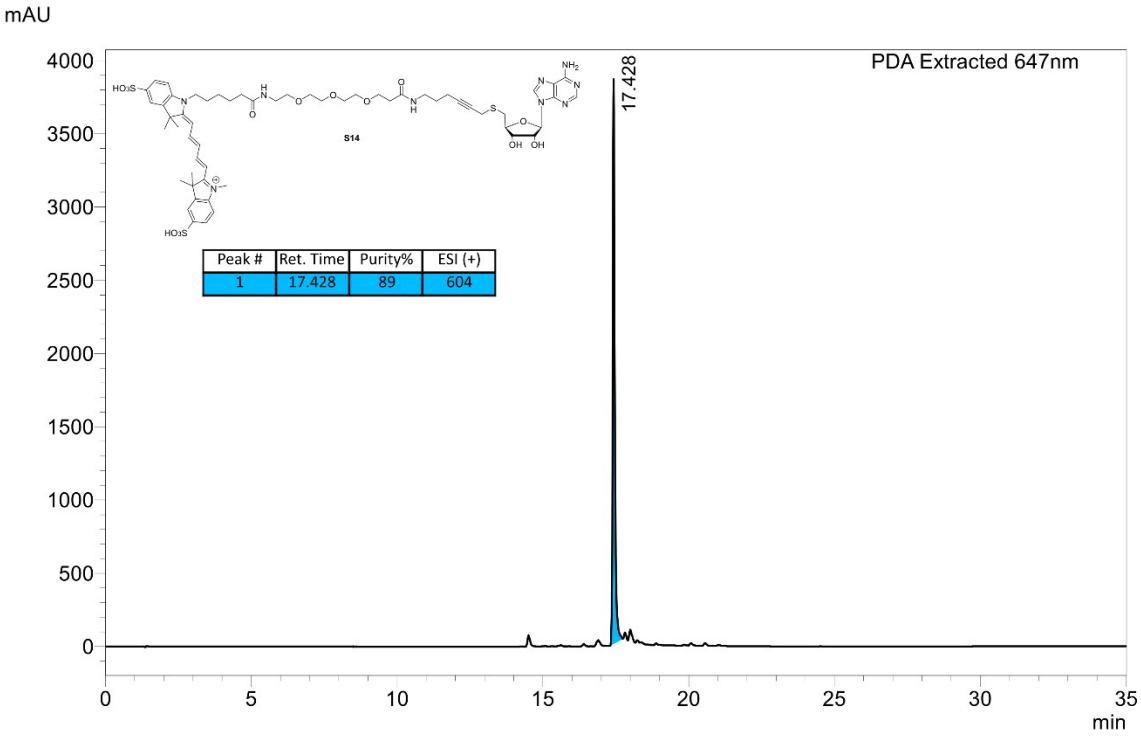


Fig. S32: Reverse phase LCMS chromatogram monitored at λ = 647 nm of purified **S14**.

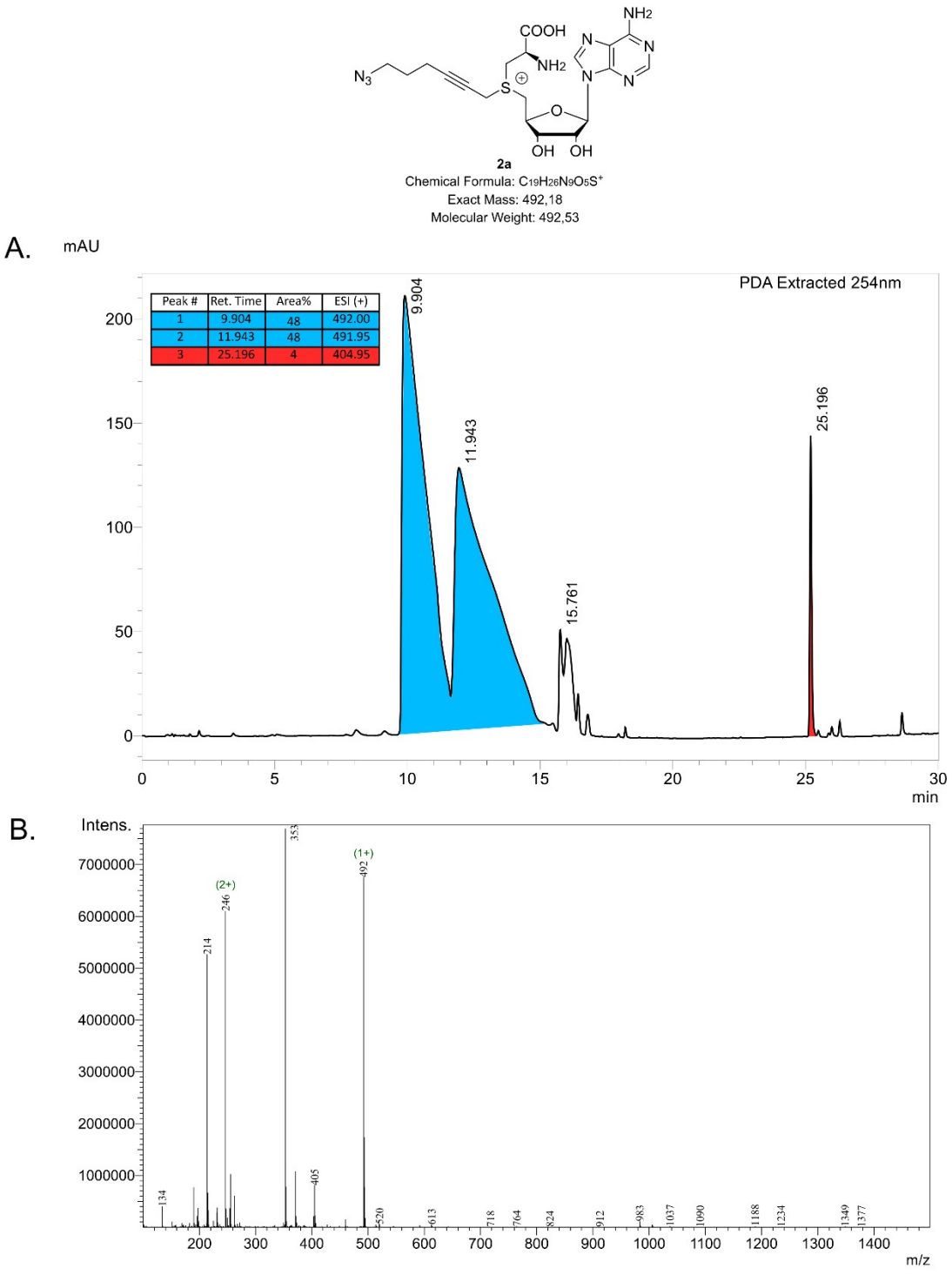


Fig. S33: Reverse phase LCMS chromatogram (A.) monitored at λ = 254 nm as well as ESI analysis in positive mode (B.) of purified **2a**. Blue peaks correspond to cofactor **2a**, red peak corresponds to the starting material **1a** which has formed due to degradation of the cofactor during evaporation.

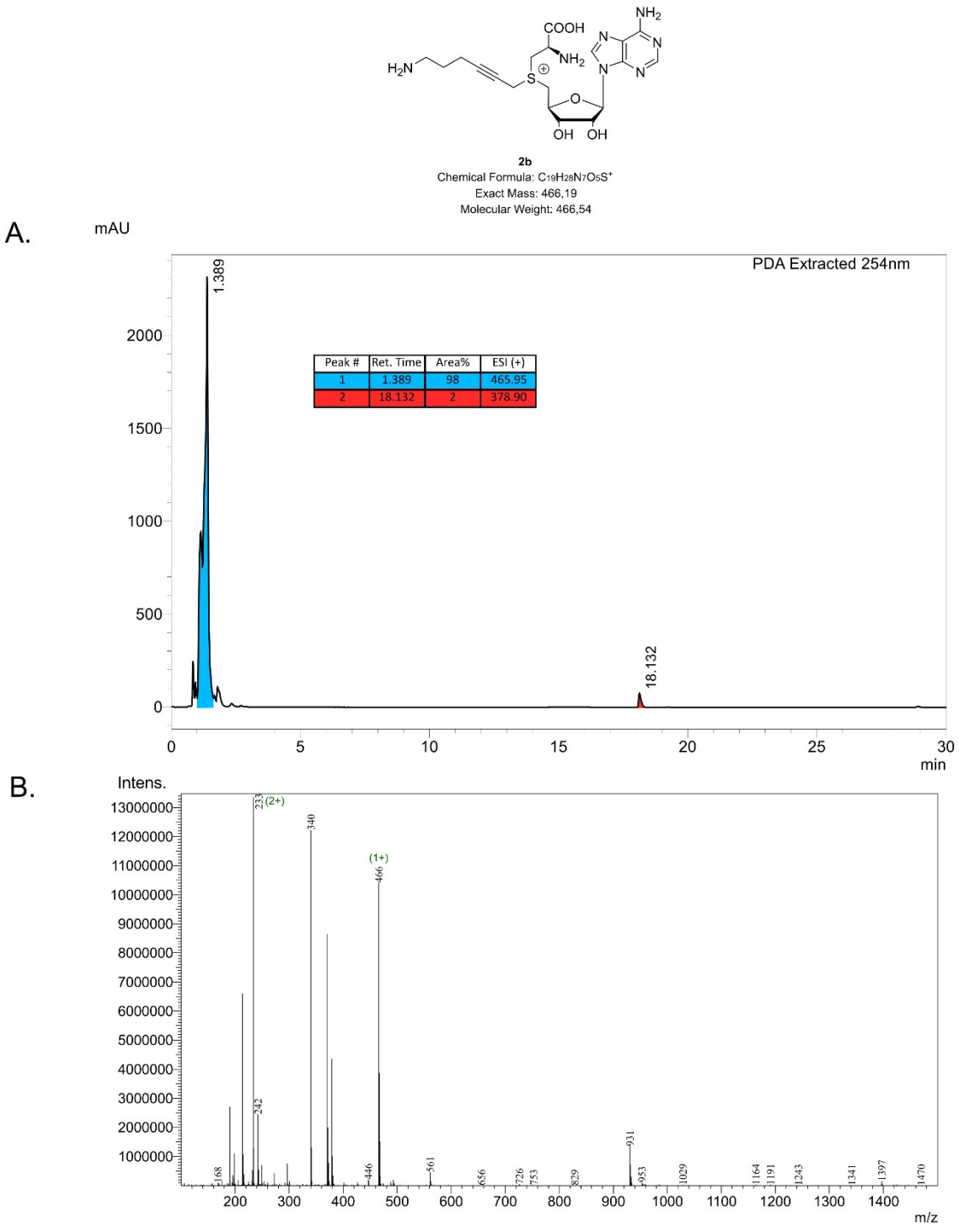
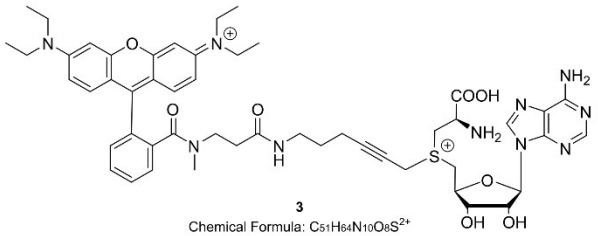
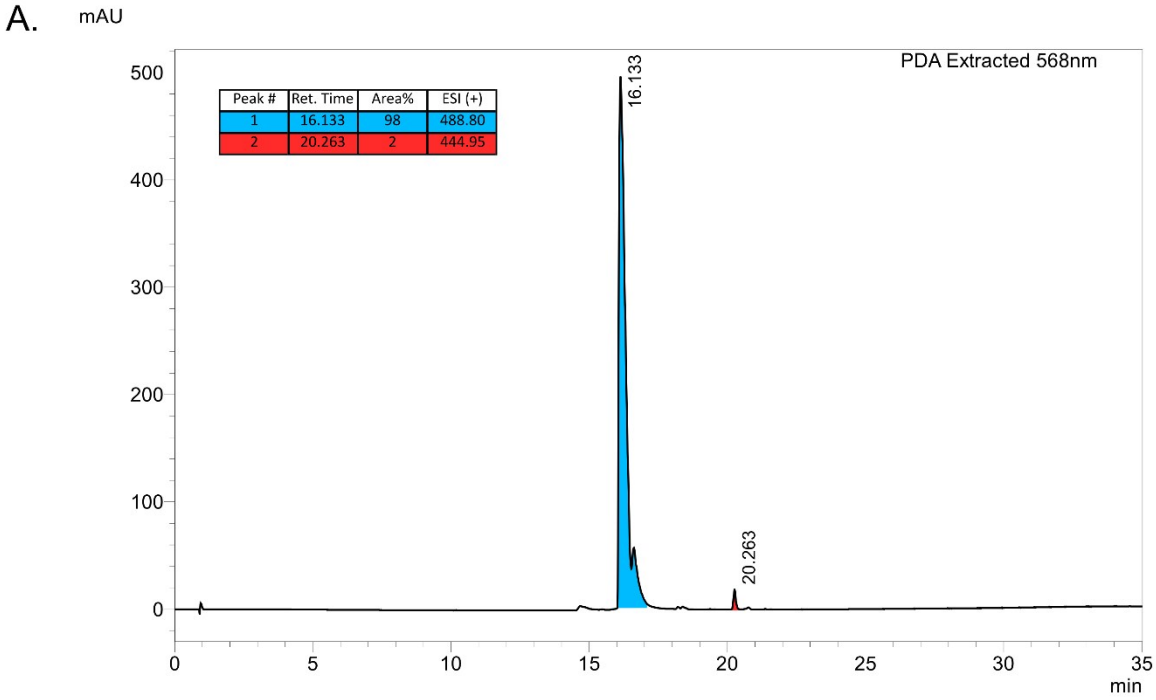


Fig. S34: Reverse phase LCMS chromatogram (A.) monitored at λ = 254 nm as well as ESI analysis in positive mode (B.) of purified **2b**. Blue peak correspond to cofactor **2b**, red peak corresponds to the starting material **1b** which has formed due to degradation of the cofactor during evaporation.



3
Chemical Formula: $C_{51}H_{64}N_{10}O_8S^{2+}$
Exact Mass: 976,46
Molecular Weight: 977,19



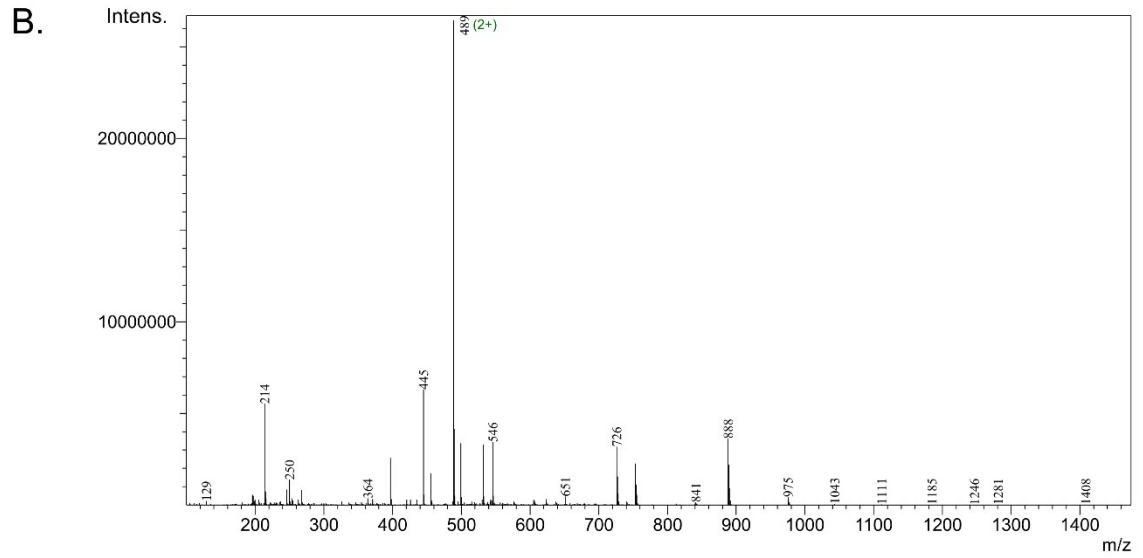
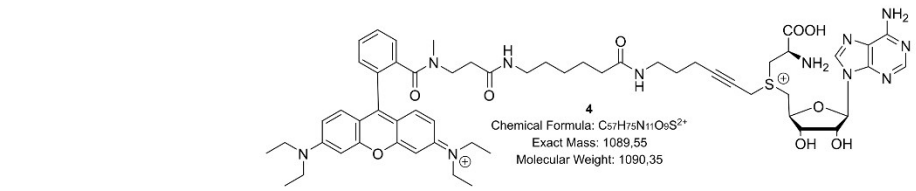
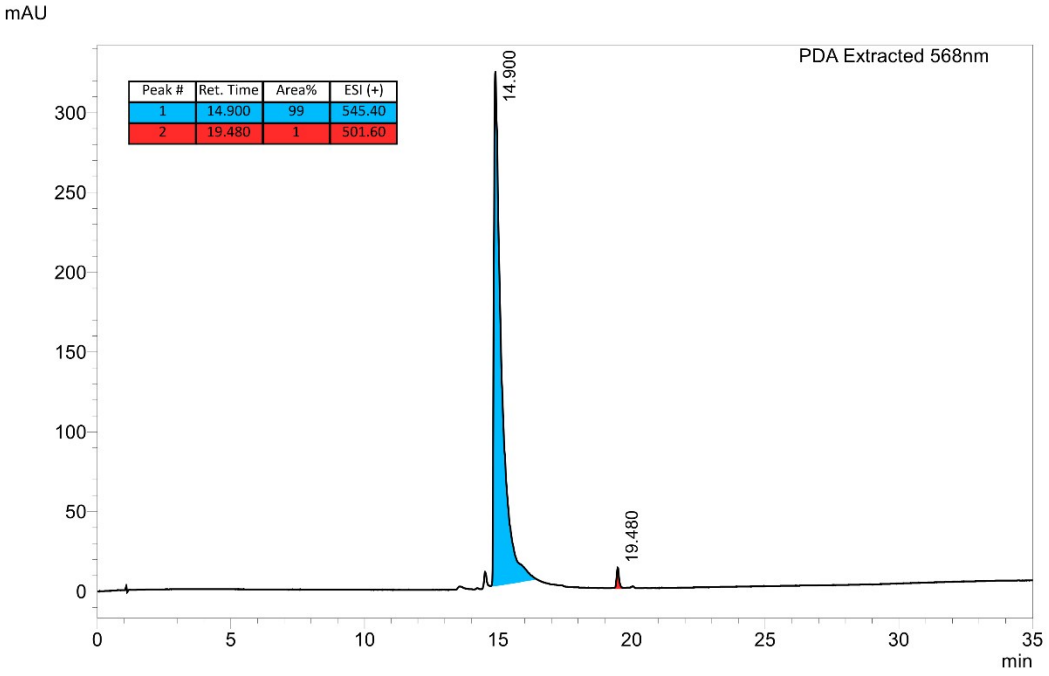


Fig. S35: Reverse phase LCMS chromatogram (A.) monitored at λ = 568 nm as well as ESI analysis in positive mode (B.) of purified **3**. Blue peak correspond to cofactor **3**, red peak corresponds to the starting material **S3** which has formed due to degradation of the cofactor during evaporation.



A.



B.

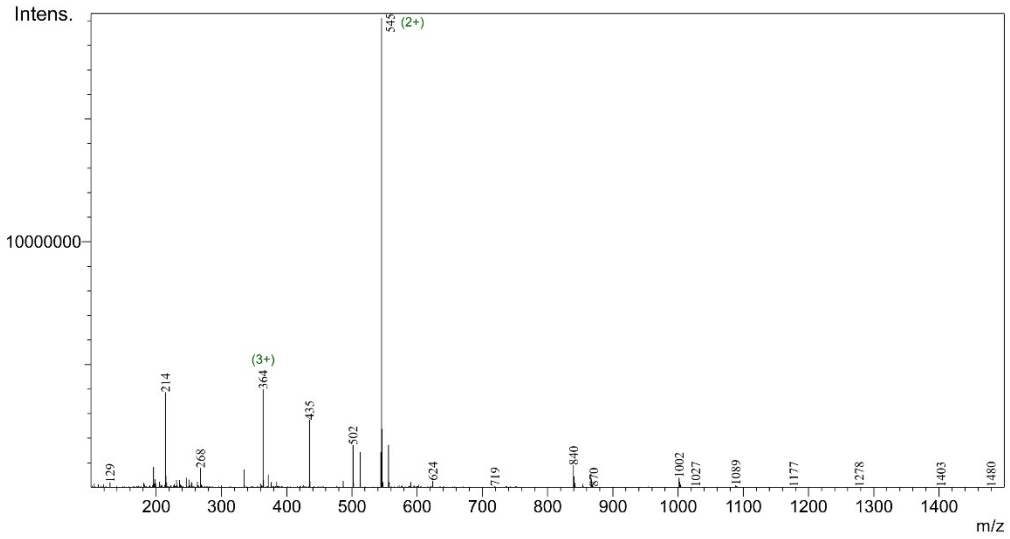
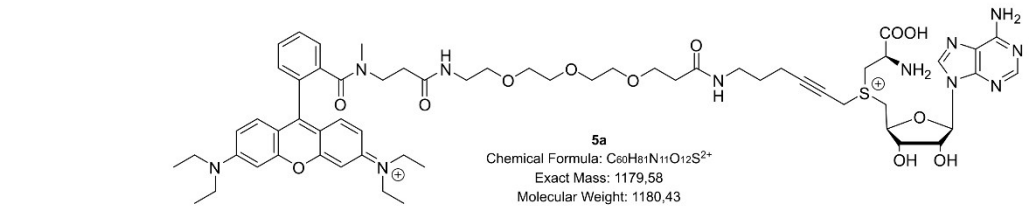
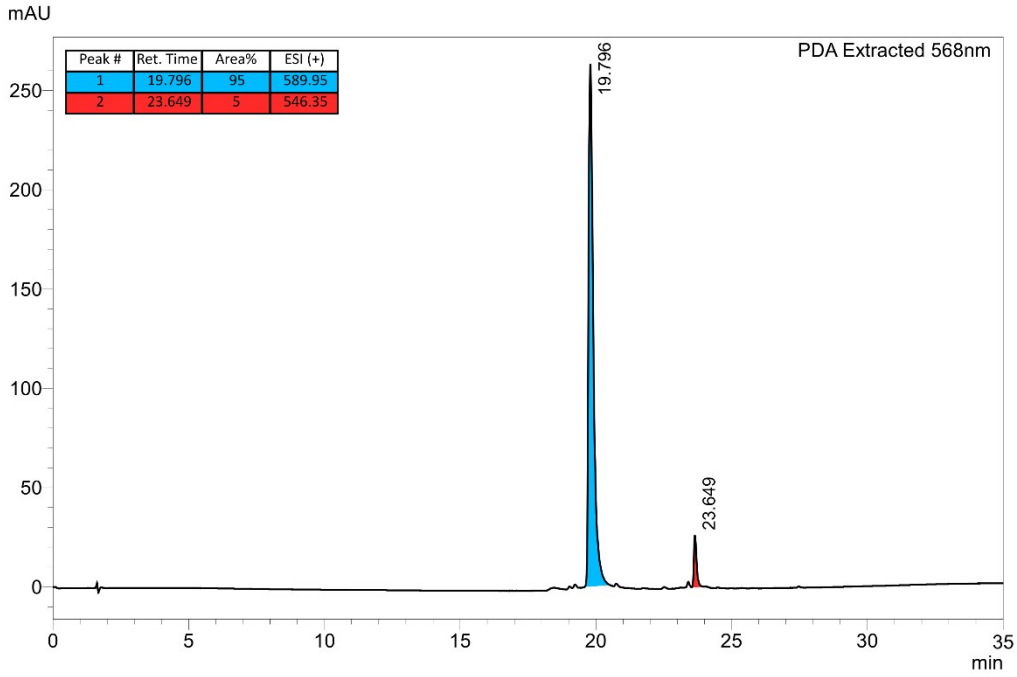


Fig. S36: Reverse phase LCMS chromatogram (A.) monitored at λ = 568 nm as well as ESI analysis in positive mode (B.) of purified **4**. Blue peak correspond to cofactor **4**, red peak corresponds to the starting material **S4** which has formed due to degradation of the cofactor during evaporation.



A.



B.

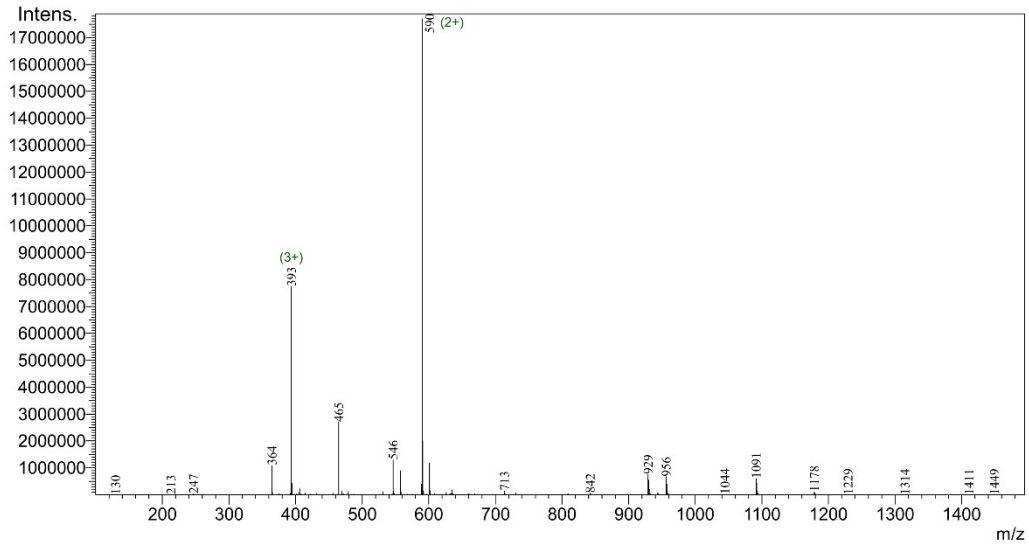


Fig. S37: Reverse phase LCMS chromatogram (A.) monitored at λ = 568 nm as well as ESI analysis in positive mode (B.) of purified **5a**. Blue peak correspond to cofactor **5a**, red peak corresponds to the starting material **S5** which has formed due to degradation of the cofactor during evaporation.

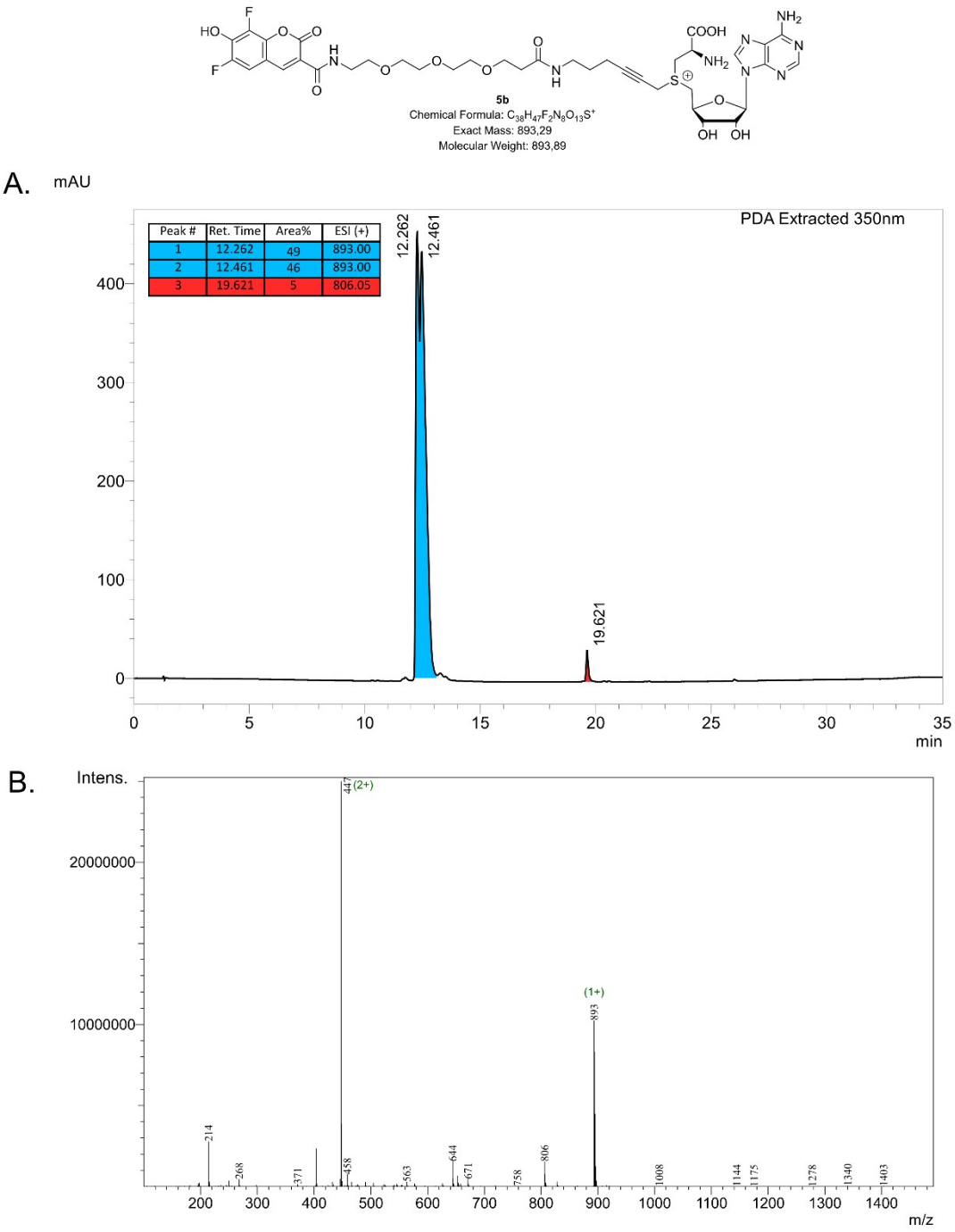
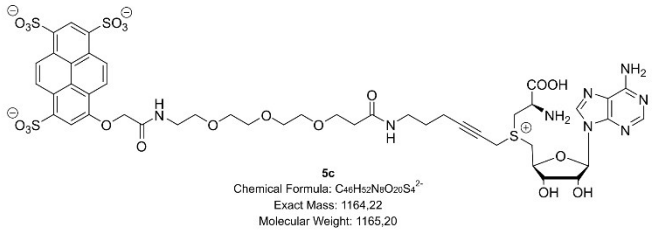
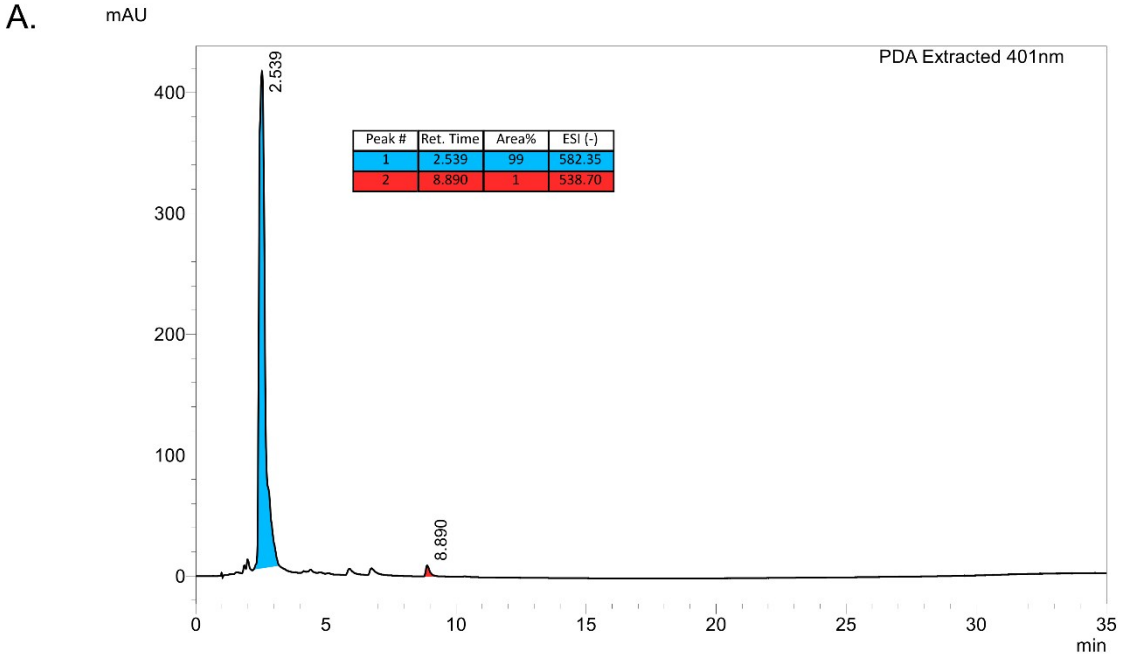


Fig. S38: Reverse phase LCMS chromatogram (A.) monitored at λ = 350 nm as well as ESI analysis in positive mode (B.) of purified **5b**. Blue peaks correspond to cofactor **5b**, red peak corresponds to the starting material **S6** which has formed due to degradation of the cofactor during evaporation.





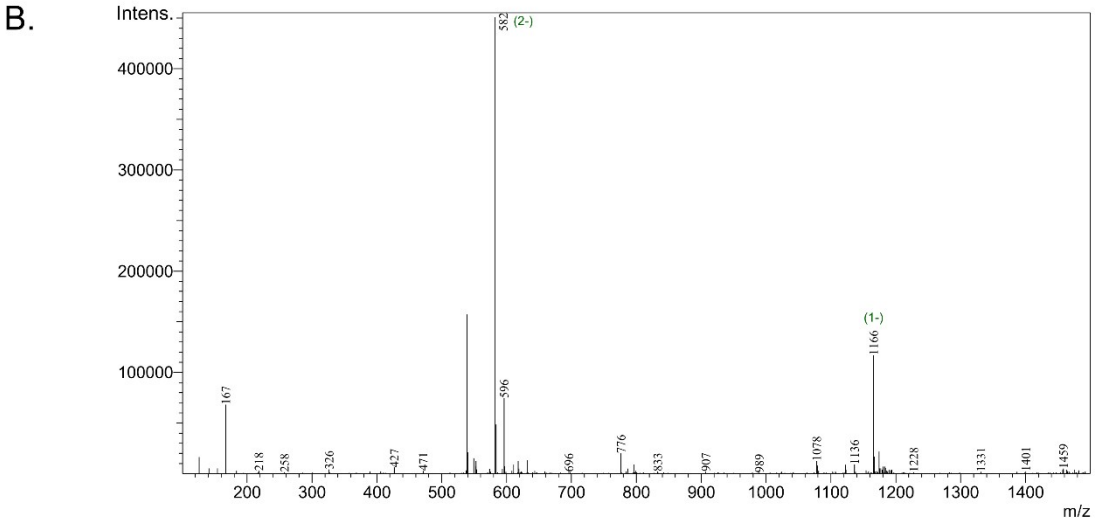


Fig. S39: Reverse phase LCMS chromatogram (A.) monitored at λ = 401 nm as well as ESI analysis in negative mode (B.) of purified **5c**. Blue peak correspond to cofactor **5c**, red peak corresponds to the starting material **S7** which has formed due to degradation of the cofactor during evaporation.

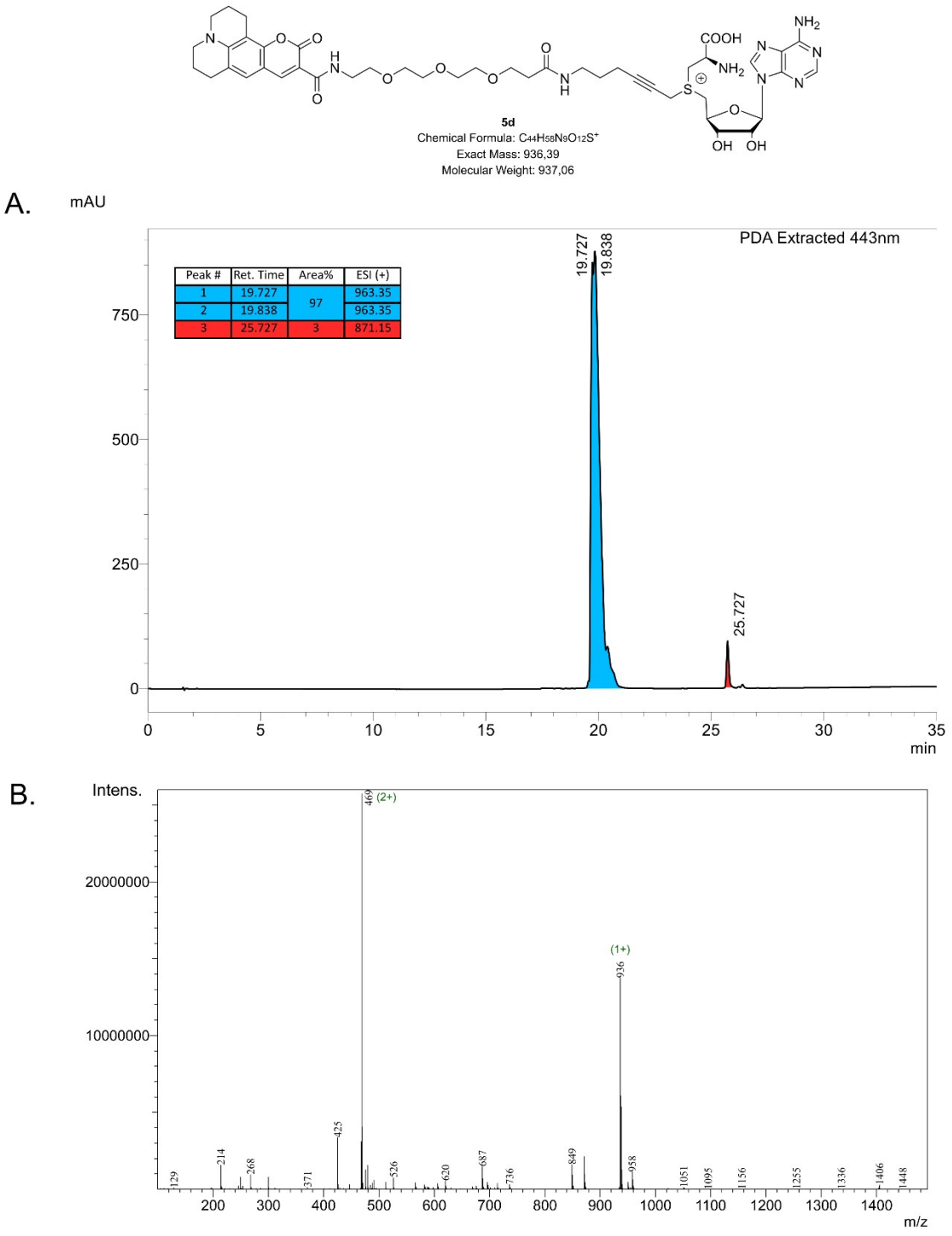


Fig. S40: Reverse phase LCMS chromatogram (A.) monitored at λ = 443 nm as well as ESI analysis in positive mode (B.) of purified **5d**. Blue peak correspond to cofactor **5d**, red peak corresponds to the starting material **S8** which has formed due to degradation of the cofactor during evaporation.

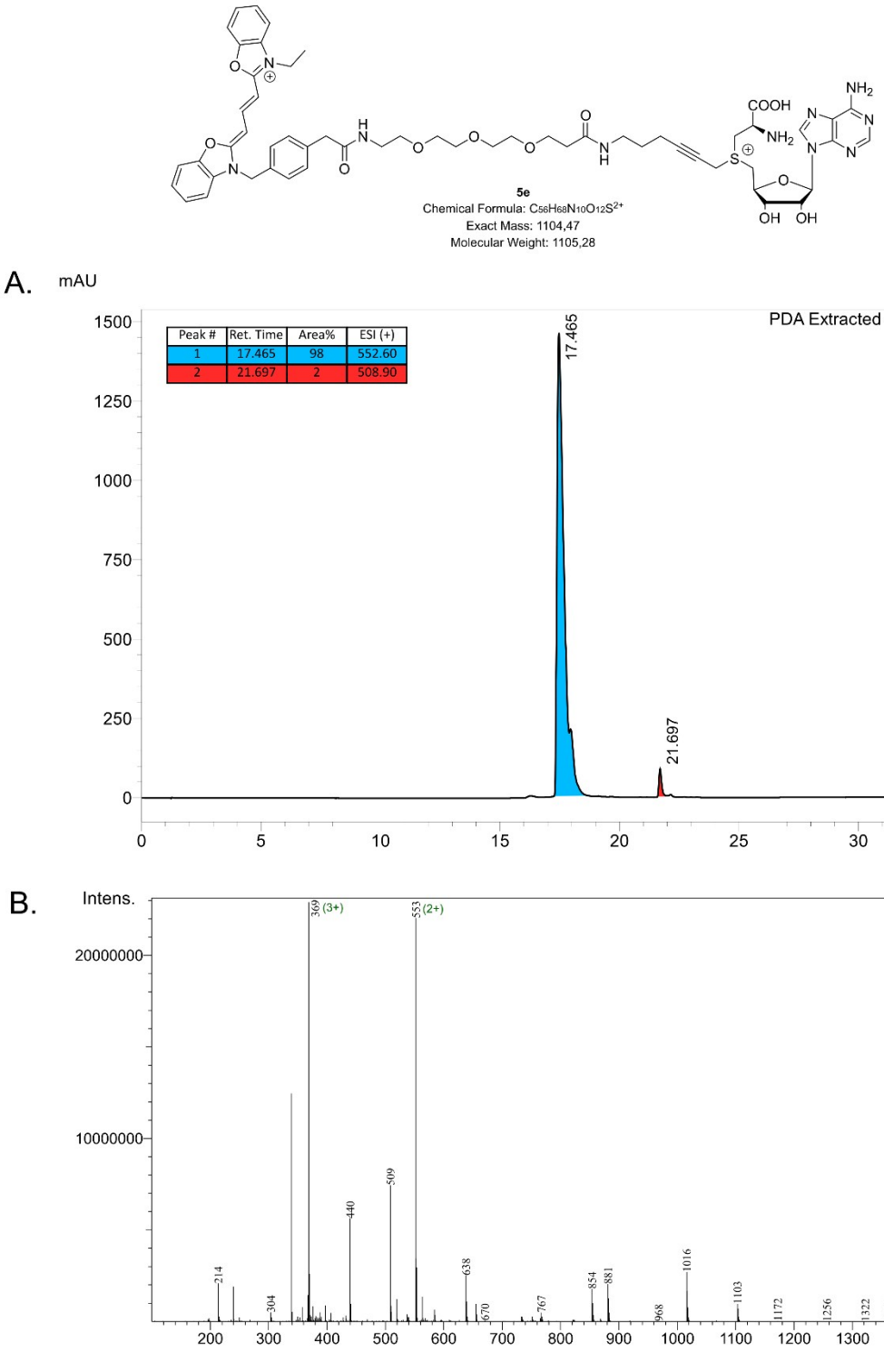


Fig. S41: Reverse phase LCMS chromatogram (A.) monitored at λ = 486 nm as well as ESI analysis in positive mode (B.) of purified **5e**. Blue peak correspond to cofactor **5e**, red peak corresponds to the starting material **S9** which has formed due to degradation of the cofactor during evaporation.

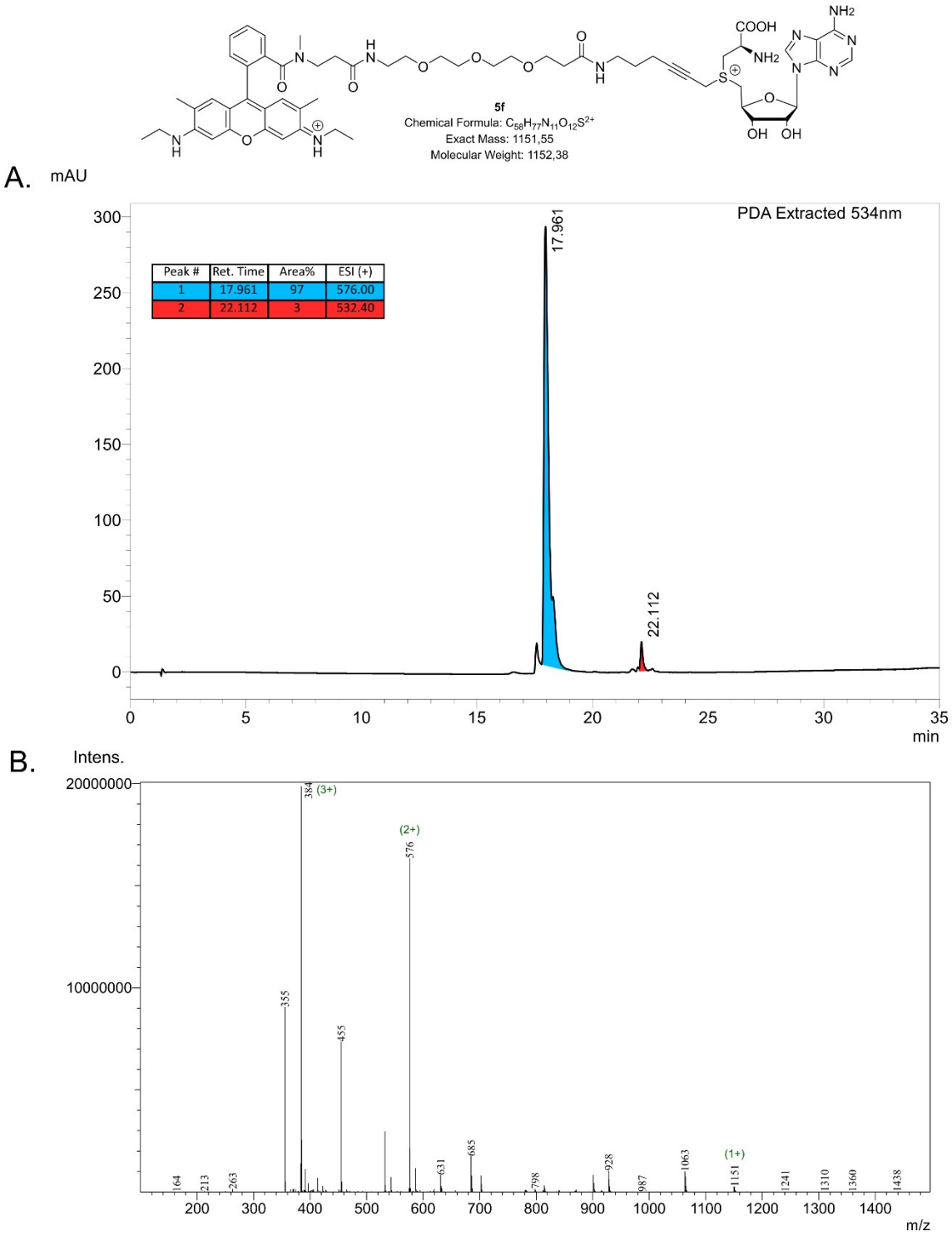
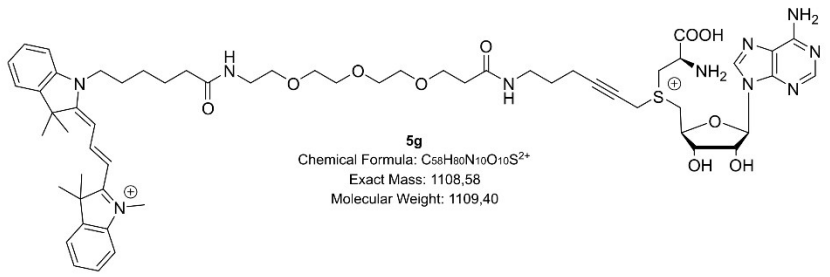
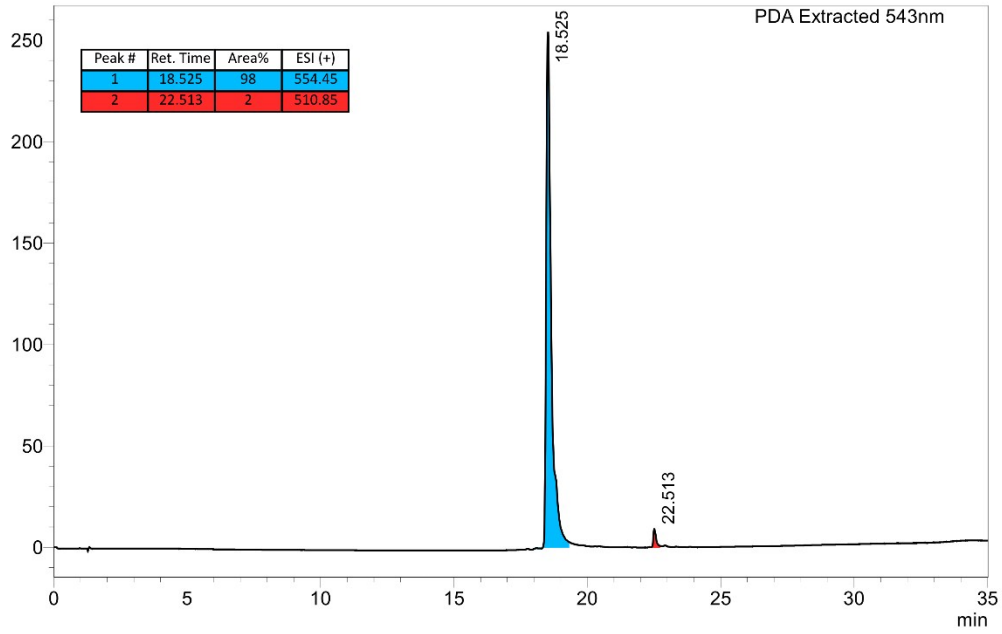


Fig. S42: Reverse phase LCMS chromatogram (A.) monitored at λ = 534 nm as well as ESI analysis in positive mode (B.) of purified **5f**. Blue peak correspond to cofactor **5f**, red peak corresponds to the starting material **S10** which has formed due to degradation of the cofactor during evaporation.



A.



B.

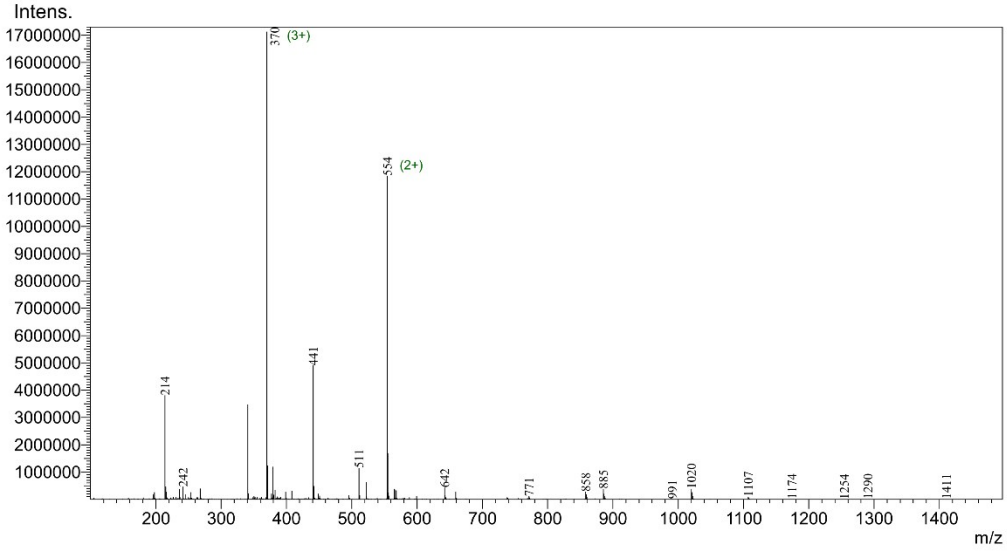


Fig. S43: Reverse phase LCMS chromatogram (A.) monitored at λ = 543 nm as well as ESI analysis in positive mode (B.) of purified **5g**. Blue peak correspond to cofactor **5g**, red peak corresponds to the starting material **S11** which has formed due to degradation of the cofactor during evaporation.

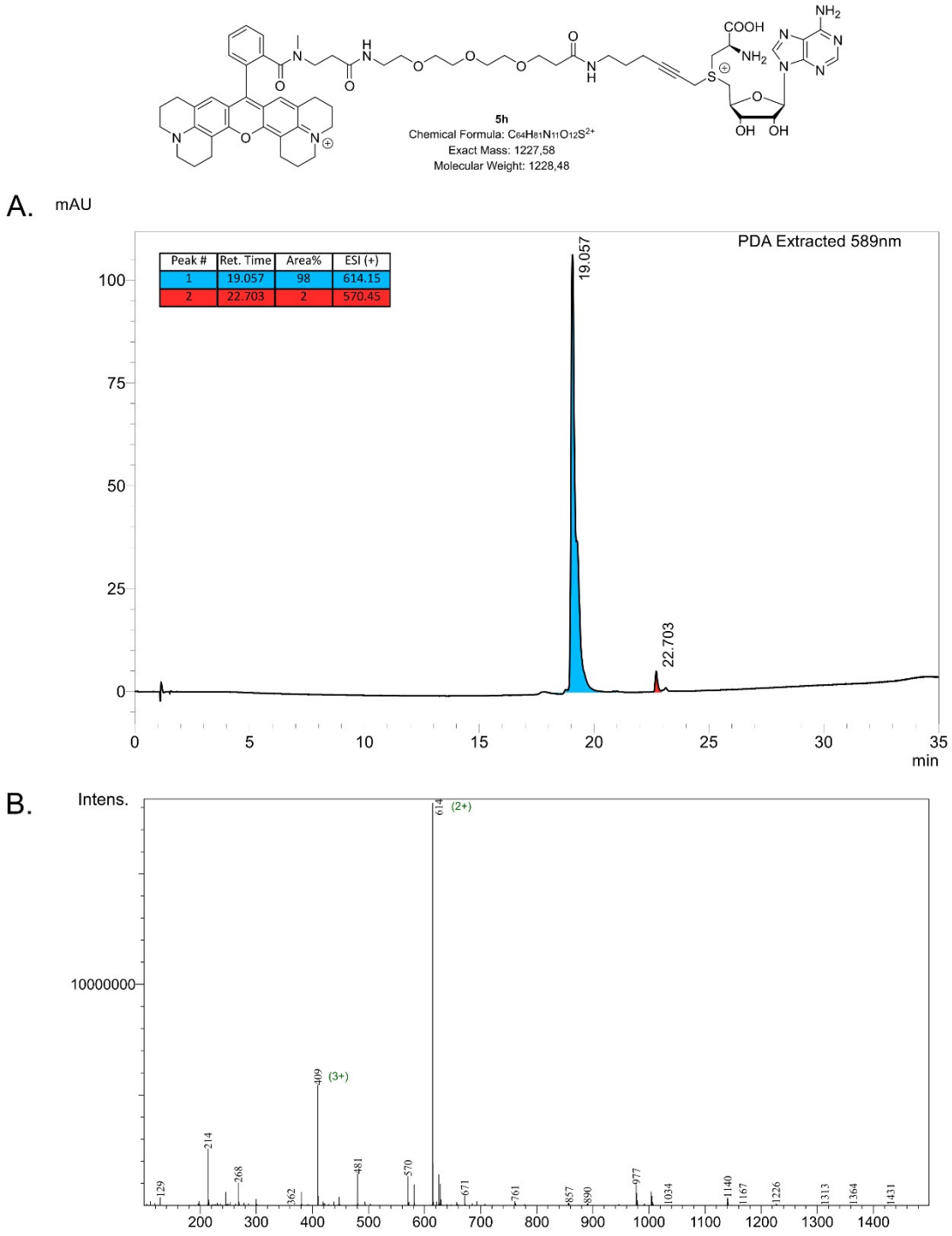


Fig. S44: Reverse phase LCMS chromatogram (A.) monitored at λ = 589 nm as well as ESI analysis in positive mode (B.) of purified **5h**. Blue peak correspond to cofactor **5h**, red peak corresponds to the starting material **S12** which has formed due to degradation of the cofactor during evaporation.

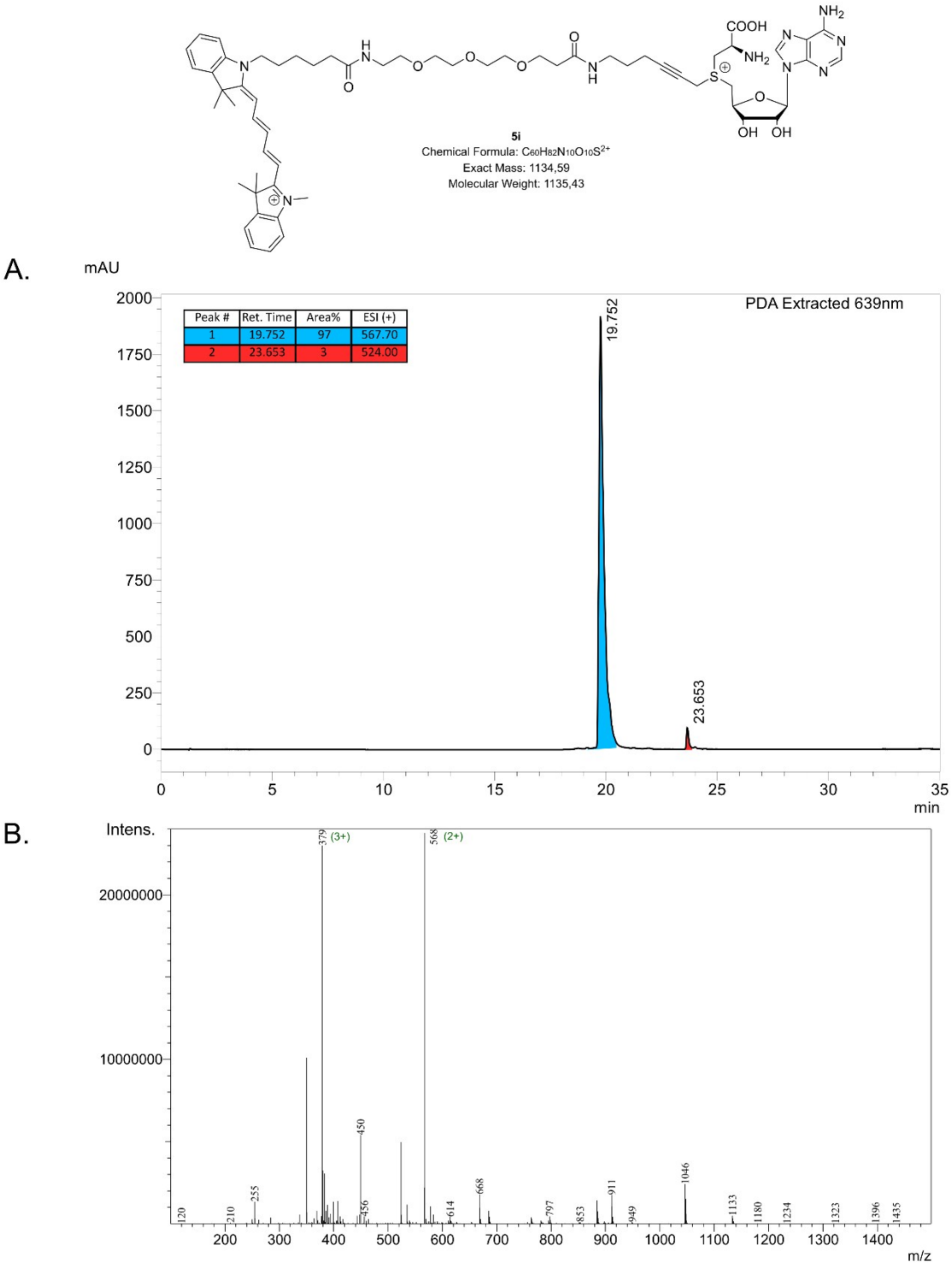


Fig. S45: Reverse phase LCMS chromatogram (A.) monitored at λ = 639 nm as well as ESI analysis in positive mode (B.) of purified **5i**. Blue peak correspond to cofactor **5i**, red peak corresponds to the starting material **S13** which has formed due to degradation of the cofactor during evaporation.

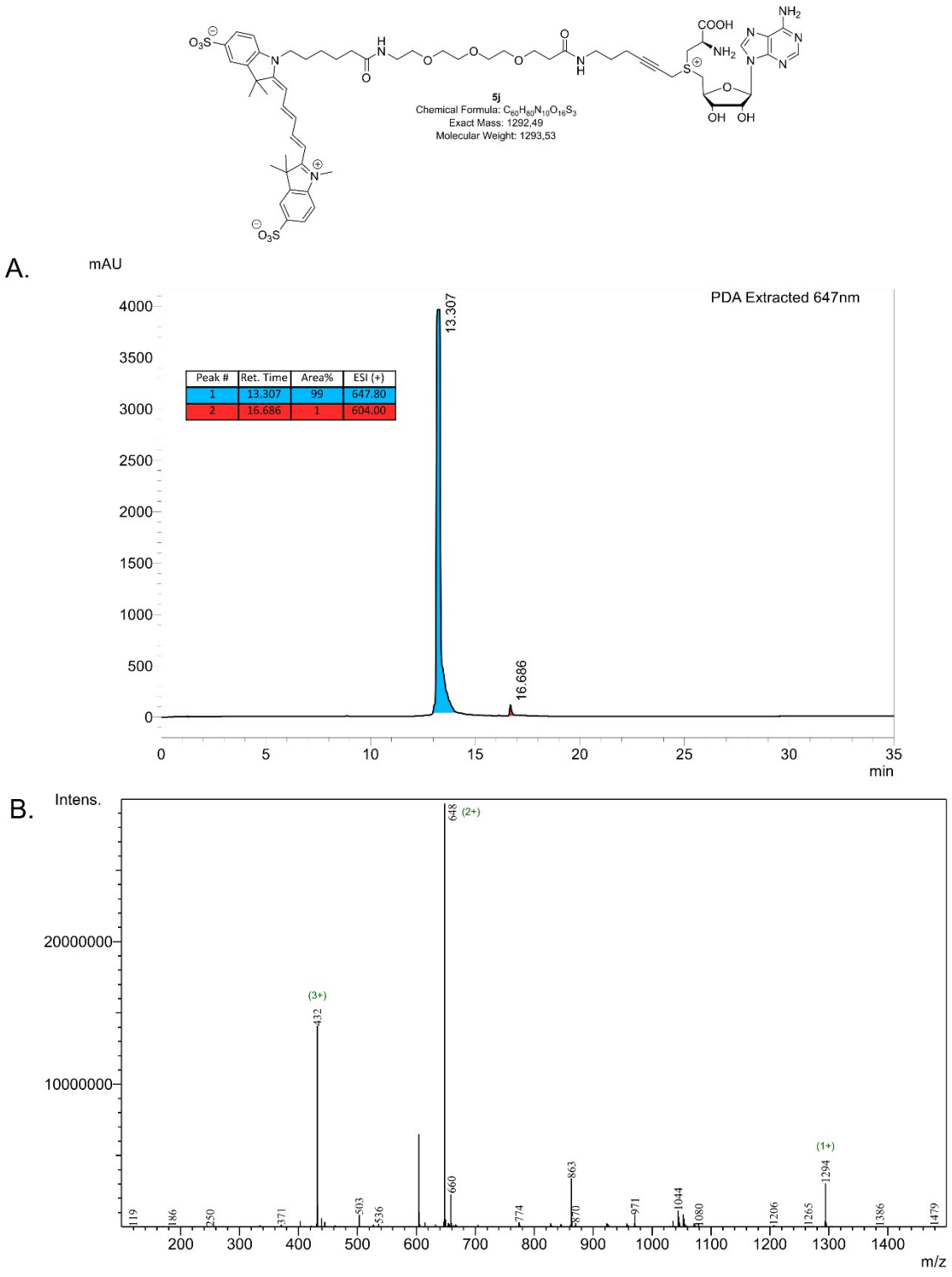


Fig. S46: Reverse phase LCMS chromatogram (A.) monitored at λ = 647 nm as well as ESI analysis in positive mode (B.) of purified **5j**. Blue peak correspond to cofactor **5j**, red peak corresponds to the starting material **S14** which has formed due to degradation of the cofactor during evaporation.

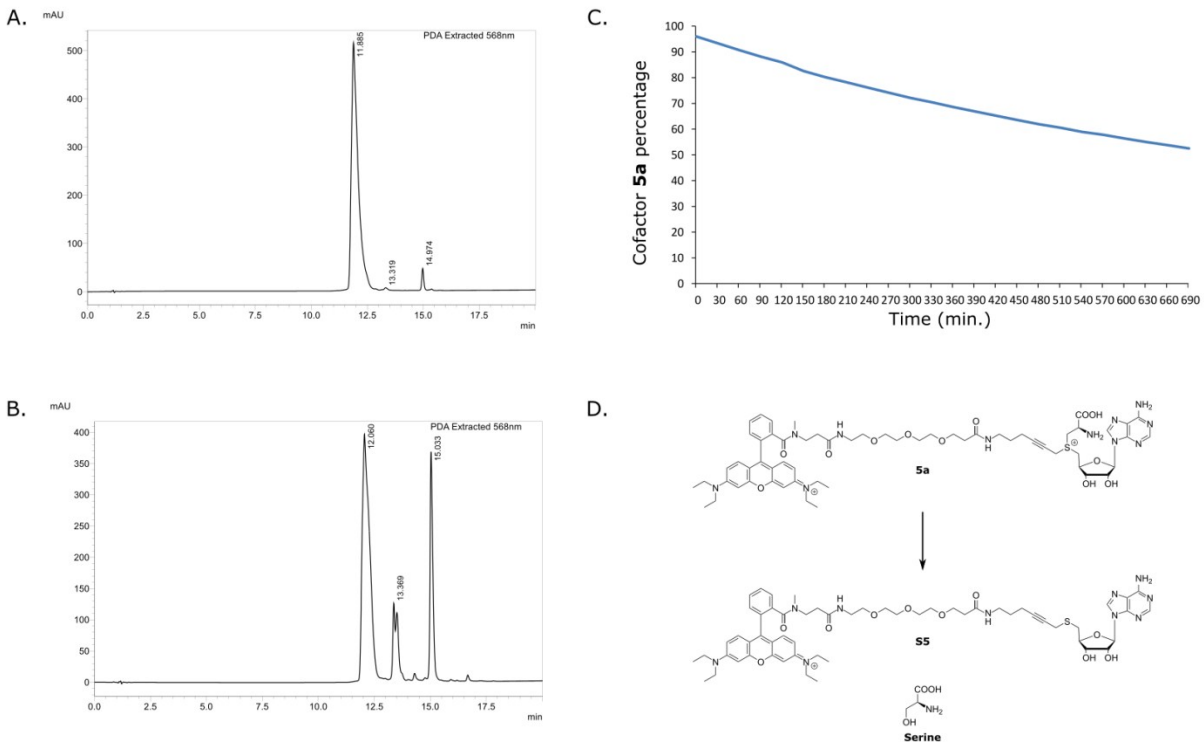


Fig. S47: Stability of the rhodamine B based direct cofactor **5a** under physiological conditions (PBS buffer, pH ~ 7.4): representative LCMS chromatogram of **5a** (absorption at λ = 568 nm) at t = 0 min (A) and at t = 480 min (B). (C) Graph of the decrease of cofactor **5a** concentration over time followed by LCMS. (D) The mainly observed degradation route of cofactor **5a** to the respective starting material **S5** through hydrolysis of the amino acid.

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