DNA-Templated Release of Functional Molecules with Azide-Reduction-Triggered Immolative Linker

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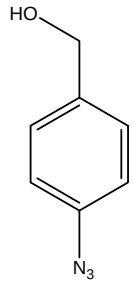
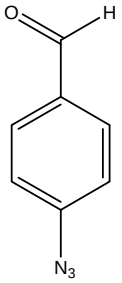
SUPPORTING INFORMATION

General techniques: All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Anhydrous solvents were obtained by passing them through a commercially available alumina column (Innovative technology, MA). Reactions were monitored by TLC carried out on 0.25 mm E. Merck silica-gel plates (60F-254) by using UV light as visualizing agent and ninhydrin or vanillin solution and heat as developing agents. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash-column chromatography. PNA were prepared in a fully automated fashion using a MultiPep RS Intavis instrument. NMR spectra were recorded on Bruker Avance-400 instrument and calibrated by using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, and br=broad. IR spectra were measured using a FT-IR Nicolet Magna 200 spectrophotometer, in reflective mode (ATR) directly from solid or liquid samples. LC-MS were recorded by using an Agilent 1100 HPLC with a Surveyor MSQ spectrometer equipped with a Supelco C18 (5 cm x 2.1 mm, 5 μm particles) column (method A, linear elution gradient from 95% H_2O 0.01% TFA to 100% MeCN 0.01% TFA in 8 minutes at a flow rate of 0,7 $mLmin^{-1}$) or by using a Thermo Scientific Accela HPLC with a Surveyor MSQ Plus spectrometer equipped with a Thermo C18 (5 cm x 2.1 mm, 1.9 μm particles) Hypersil gold column (method B, linear elution gradient for 95% H_2O 0.01% TFA to 90% MeCN 0.01% TFA in 3.6 minutes at a flow rate of 1.0 $mLmin^{-1}$; method C, linear elution gradient for 95% H_2O 0.01% TFA to 90% MeCN 0.01% TFA in 2.2 minutes at a flow rate of 1.0 $mLmin^{-1}$). If mass spectrum was not obtained in LCMS conditions compounds were analysed by direct infusion into LCQ Fleet three-dimensional ion trap mass spectrometer (Thermo Scientific).The MALDI spectra were measured using Bruker Daltonics Autoflex TOF/TOF spectrometer. All solid phase chemistry was carried on NovaPEG Rink Amide resin (Novabiochem) loaded at 0.2 mmol/g. PNA oligomers were purified by reverse-phase chromatography using a Biotage Isolera ONE equipped with a Biotage SNAP Cartridge KP-C18-HS 12g (linear gradient from 100% H_2O 0.01% TFA to 30% MeCN 0.01% TFA in 22.5 minutes with a flow rate of 10 $mLmin^{-1}$) or using an Agilent 1100 series HPLC equipped with DAD and with a Agilent ZORBAX Eclipse XDB-C18 (4.6 x 250 mm, 5μm) column (linear gradient from 100% H_2O 0.1% TFA to 50% MeCN 0.1% TFA in 35 minutes with a flow rate of 1 $mLmin^{-1}$).

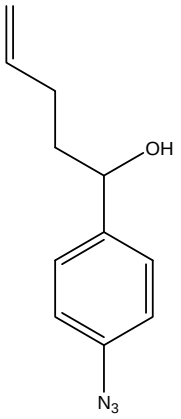
Synthesis leading to aldehyde **3**



S1 (4-azidobenzyl alcohol): Solution of 4-aminobenzyl alcohol (2.15 g, 17.5 mmol, 1.0 equiv.) in 60 mL of water and 25 mL of THF was cooled to 0^{o}C and 4.8 mL of concentrated sulfuric acid was added slowly. Then solution of sodium nitrite (1.45 g, 21.0 mmol, 1.2 equiv) in 10 mL of water was added drop-wise. After next 1h at 0^{o}C, solution of sodium azide (1.82 mg, 28.9 mmol, 1.2 equiv) in 5 mL of water was added drop-wise and the reaction mixture was stirred overnight while warming to room temperature. The reaction was quenched with brine and extracted with CH_2Cl_2 (3x100 mL). Combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. Crude product was purified by flash chromatography (hexanes:EtOAc 4:1) to give **S1** as yellow oil (2.33 g, 89.6% yield). **IR ν(cm^{-1}):** 3539.5, 2113.1, 1610.6, 1508.4, 1417.7, 1291.4, 1204.6, 1181.4, 1129.4, 1042.6, 1013.6, 822.7; **^{1}H NMR ($CDCl_3$) δ(ppm):** 7.33 (2H, d, *J* = 8.4 Hz), 7.00 (2H, d, *J* = 8.4 Hz), 4.64 (2H, s); **^{13}C NMR ($CDCl_3$) δ(ppm):** 139.6, 137.8, 128.8, 119.4, 64.9; **LCMS (ESI):** RT = 4.05 min (Method A); **MS (ESI-ION TRAP)** *m/z* calcd for $C_7H_8N_3O$ $[M+H]^+$: 150.07, found: 122.08 $[M+H-N_2]$+

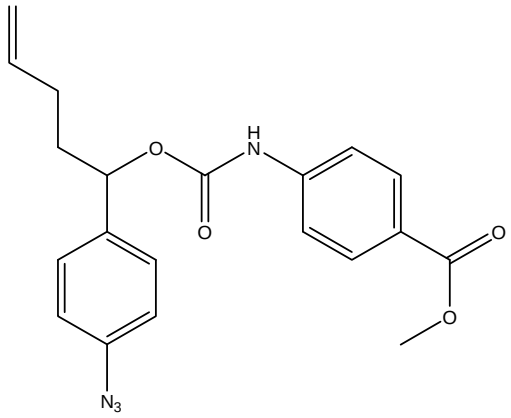


S2 (4-azidobenzyl aldehyde): To solution of alcohol **S1** (2.33 g, 15.6 mmol, 1.0 equiv.) in CH_2Cl_2 (200 mL) was added pyridinium dichromate (7.05 g, 18.7 mmol, 1.2 equiv.) and the reaction was stirred for 4h at room temperature. After the reaction mixture was filtered through 130 g of silica and silica was washed with additional 1.5 L of CH_2Cl_2. Evaporation of solvent under reduced pressure and drying under high vacuum yielded **S2** as pale yellow oil (2.03 g, 88%). **IR ν(cm^{-1}):** 2116.0, 1693.6, 1598.1, 1502.6, 1422.6, 1391.7, 1285.6, 1212.3, 1667.0, 1126.5, 826.5, 787.0; **^{1}H NMR ($CDCl_3$) δ(ppm):** 9.94 (1H, s), 7.88 (2H, d, *J* = 8.4 Hz), 7.16 (2H, d, *J* = 8.4 Hz); **^{13}C NMR ($CDCl_3$) δ(ppm):** 190.5, 146.2, 133.2, 131.5, 119.4; **LCMS (ESI):** RT = 3.31 min (Method A); **MS (ESI-ION TRAP)** *m/z* calcd for $C_7H_5N_3O$ $[M+H]^+$: 148.05, found: 147.86 $[M+H]^+$.

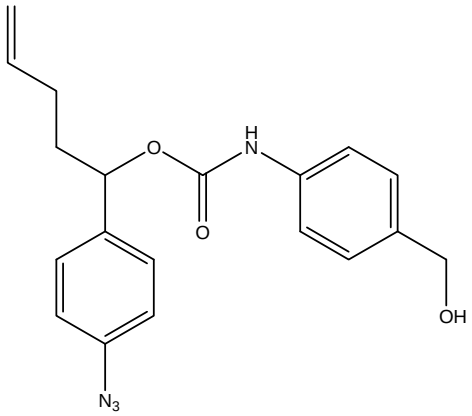


1: Magnesium turnings (0.48 g, 19.8 mmol, 1.4 equiv.) were suspended in THF (22 ml) in a flask equipped with vacuum condenser and 4-bromo-1-butene (1.6 mL, 16.5 mmol, 1.2 equiv.) was added drop-wise. The mixture was stirred for 12 h at room temperature. The Grignard reagent was next added dropwise (20 mL/h) to the solution of aldehyde **S2** (2.03 g, 13.8 mmol, 1.0 equiv.) in THF (50 ml) at -78^{o}C. When addition was finished, the reaction mixture was stirred next 2h at -78^{o}C, after quenched with saturated ammonium chloride solution and extracted with CH_2Cl_2 (3x100 mL). Combined organic layers were washed with saturated ammonium chloride and brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*. Purification of residue by flash chromatography (hexanes:EtOAc 10:1) gave **1** as yellow solid (2.1 g, 75% yield). **IR ν(cm^{-1}):** 3730.5, 2937.7, 2121.8, 1741.8, 1699.3, 1645.3, 1605.8, 1507.4, 1290.4, 1128.4, 1067.6, 1010.7, 918.2, 838.1; **^{1}H NMR ($CDCl_3$) δ(ppm):** 7.31 (2H, d, *J* = 8.6 Hz), 6.99 (2H, d, *J* = 8.1 Hz), 5.86-5.79 (1H, m), 5.06-4.98 (1H, m), 4.67-4.62 (2H, m), 2.47 (1H, s), 2.13-2.08 (2H, m), 1.89-1.59 (2H, m); **^{13}C**

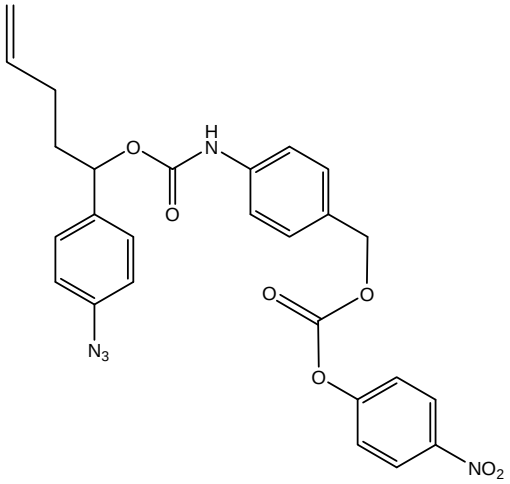
NMR ($CDCl_3$) δ(ppm): 141.4, 139.0, 137.9, 127.3, 118.9, 115.0, 73.2, 38.0, 29.9; **LCMS (ESI):** RT = 3.66 min (Method A); **MS (ESI-ION TRAP)** *m/z* calcd for $C_{11}H_{14}N_3O$ $[M+H]^+$: 204.1138, found: 176.13 $[M+H-N2]^+$, 204.12 $[M+H]^+$.



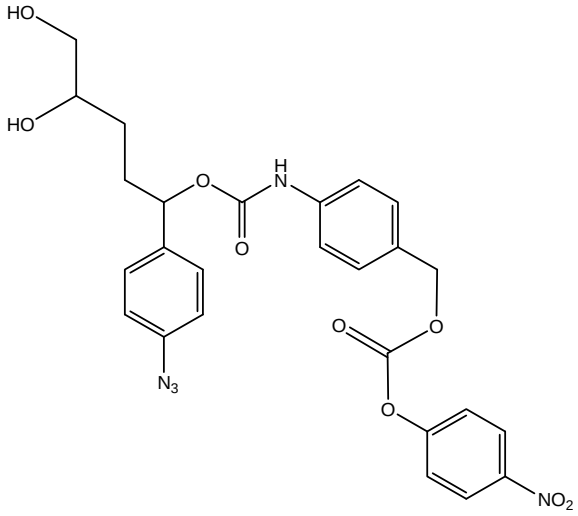
S3: 4-aminobenzoic acid methyl ester (93 mg, 0.61 mmol, 1.0 equiv.) and triphosgene (93 mg, 0.31 mmol, 0.5 equiv.) were dissolved in THF (5 mL) and DIPEA (310 μL, 1.84 mmol, 3.0 equiv.) was added drop-wise. The reaction mixture was stirred 40 minutes at room temperature. Then solution of alcohol **1** (500 mg, 2.46 mmol, 1.05 equiv.) in THF (2 mL) was added and the reaction was stirred 18h at 60°C. After the reaction mixture was cooled to ambient temperature, quenched with saturated ammonium chloride solution and extracted with CH_2Cl_2 (3x10 mL). Combined organic layers were washed with saturated ammonium chloride, dried over sodium sulfate nad concentrated under reduced pressure. Flash chromatography (hexanes:EtOAc 5:1) yielded **S3** as yellow oil (101.2 mg, 43.4% yield). **IR ν(cm^{-1}):** 3625.3, 2122.7, 1723.5, 1708.0, 1652.1, 1646.3, 1549.9, 1542.1, 1515.1, 1364.7, 1289.5, 1210.4; **^{1}H NMR (acetone) δ(ppm):** 7.96 (2H, d, *J* = 8.6 Hz), 7.46 (2H, d, *J* = 9.1 Hz), 7.32 (2H, d, *J* = 8.6 Hz), 6.98 (2H, d, *J* = 8.0 Hz), 5.83-5.76 (1H, m), 5.72-5.70 (1H, m), 5.04-4.99 (2H, m), 3.91 (3H, s), 2.09-2.03 (3H, m), 1.89-1.84 (1H, m); **^{13}C NMR ($CDCl_3$) δ(ppm):** 166.7, 152.4, 142.2, 139.8, 137.0, 136.9, 130.8, 128.0, 124.6, 119.1, 117.5, 115.5, 76.2, 51.9, 35.2, 29.6; **LCMS (ESI):** RT = 3.55 min (Method A); **HRMS (MALDI-TOF)** could not be measured due to molecule fragmentation



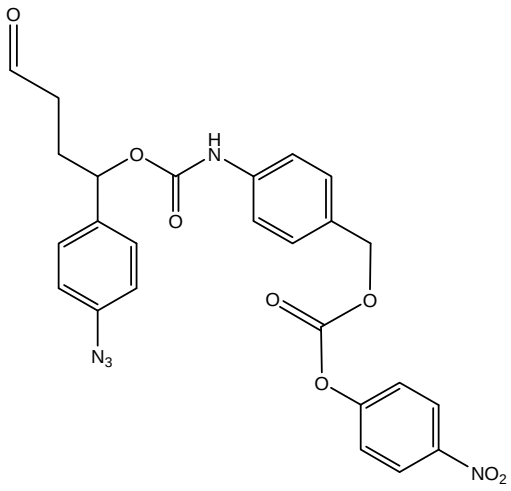
2: Solution of **S3** (101.2 mg, 0.266 mmol, 1.0 equiv.) in CH_2Cl_2 was cooled to -78^{o}C and DIBAL (1.0 M solution in toluene, 910 μL, 0.91 mmol, 3.4 equiv.) was added drop-wise (1h). After next 1h at -78^{o}C supplementary portion of DIBAL (266mL, 0.266 mmol, 1equiv.) was added drop-wise (20 minutes) to complete the reaction. The reaction mixture was stirred for 40 minutes more, quenched with ice-cold 20% NaOH solution (10 mL) when still at -78^{o}C and after allowed to warmed up to ambient temperature. Then layers where separated, aqueous phase was extracted with CH_2Cl_2 (4x10 mL), combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. Residue was redissolved in CH_2Cl_2 and evaporated with silica. Flash chromatography (hexanes:EtOAc 8:1, then 3:1) yielded **2** as pale yellow oil (69.2 mg, 77.3% yield). **IR ν(cm^{-1}):** 3730.5, 2123.7, 1731.2, 1652.1, 1605.8, 1533.5, 1364.7, 1291.4, 1221.0, 1046.2, 920.1, 835.2; **^{1}H NMR ($CDCl_3$) δ(ppm):** 7.33 (4H, d, *J* = 8.0 Hz), 7.23 (2H, d, *J* = 8.6 Hz), 7.09 (1H, br s), 6.98 (2H, d, *J* = 8.6 Hz), 5.84-5.74 (1H, m), 5.71-5.68 (1H, m), 5.03-4.97 (2H, m), 4.58 (2H, s), 3.85 (1H, s), 2.11-1.99 (3H, m), 1.88-1.83 (1H, m); **^{13}C NMR ($CDCl_3$) δ(ppm):** 152.8, 139.6, 137.3, 137.2, 137.1, 136.0, 128.0, 127.7 (x2), 119.0, 115.3, 75.8, 64.6, 35.3, 29.6; **LCMS (ESI):** RT = 4.19 min (Method A); (**HRMS (MALDI-TOF)** could not be measured due to molecule fragmentation



S4: To a solution of p-nitrophenyl chloroformate (55.3 mg, 0.274 mmol, 1.33 equiv.) and TEA (72 μL, 0.512 mmol, 2.1 equiv.) in CH_2Cl_2 (4 mL) cooled to 0^oC **2** (69.2 mg, 0.206 mmol, 1.0 equiv.) dissolved in 2 mL of CH_2Cl_2 was added dropwise during 1h. Then reaction mixture was allowed to warm up to room temperature. After 22 h brine was added to quench the reaction. Layers were separated and aqueous fraction was extracted with CH_2Cl_2 (3x10mL). Combined organic layers after washing with saturated $NaHCO_3$ and water were dried over anhydrous sodium sulfate and concentrated under reduced pressure to give **S4** as orange oil (88.2 mg, 82.7%). NMR spectra of crude product indicated no need for chromatographic purification before proceeding to next step. **IR ν(cm^{-1}):** 3732.4, 2122.7, 1740.8, 1717.7, 1652.1, 1538.3, 1516.1, 1364.7, 1291.4, 1217.1, 922.0, 862.2; **^{1}H NMR ($CDCl_3$) δ(ppm):** 8.26 (2H, d, *J*=9.2 Hz), 7.43-7.35 (8H, m), 7.02 (2H, d, *J* = 8.4 Hz), 6.82 (1H, br s), 5.87-5.77 (1H, m), 5.74-5.71 (1H, m), 5.24 (2H, s), 5.06-5.00 (2H, m), 2.15-2.03 (3H, m), 1.94-1.85 (1H, m); **^{13}C NMR ($CDCl_3$) δ(ppm):** 155.5, 152.6, 152.4, 145.3, 144.1, 139.8, 138.6, 137.1, 129.9, 129.0, 128.1, 125.2, 121.7 (x2), 119.1, 115.5, 76.2, 70.6, 35.3, 29.6; **LCMS (ESI):** RT = 2.25 min (Method B); **HRMS (MALDI-TOF)** could not be measured due to molecule fragmentation

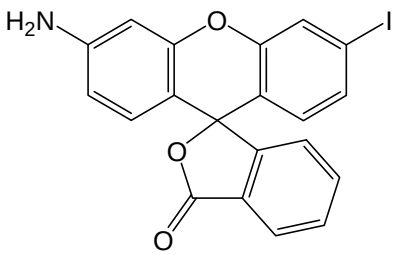


S5: To a solution of alkene **S4** (44.0 mg, 0.085 mmol, 1.0 equiv) in acetone (2.5 mL) N-methyl morpholine oxide monohydrate (34.5 mg, 0.255 mmol, 3.0 equiv) and water (0.5 mL) were added followed by OsO_4 (4% solution in H_2O, 27 μL, 0.0043 mmol, 0.05 equiv). After stirring for overnigth at room temperature in absence of light the reaction mixture was cooled down to 0^oC and saturated $Na_2S_2O_5$ solution was added to quench remaining OsO_4. During 30 minutes of stirring reaction mixture was allowed to warm up to ambient temperature and then brine (10mL) was added. Extraction with CH_2Cl_2 (3 x 15 mL), drying over anhydrous Na_2SO_4 and solvent removal yielded crude diol. Later purification by flash chromatography (dry packing, elution with 50% EtOAc in hexanes and then 100% EtOAc) provided 38.2 mg of **S5** as brown oil (81.5 % yield). **IR ν(cm^{-1}):** 3625.3, 3544.3, 3484.5, 2926.1, 2122.7, 1768.8, 1731.2, 1602.9, 1532.5, 1413.9, 1350.2, 1216.2, 1050.3, 864.2; **^{1}H NMR ($CDCl_3$) δ(ppm):** 8.27 (2H, d, *J*=9.2 Hz), 7.43-7.34 (8H, m), 7.01 (2H, d, *J* = 8.0 Hz), 5.73 (1H, t, *J* = 6.8 Hz), 5.26 (2H, s), 4.02-3.97 (1H, m), 3.89 (1H, t, *J* = 14.4 Hz), 3.78-3.74 (1H, m), 3.66 (1H, d, *J* = 8.8 Hz), 3.50-3.43 (1H, m), 2.89 (1H, br s), 2.21-2.10 (0.5H, m), 2.00 (1H, q, *J* = 7.6 Hz), 1.89-1.80 (0.5H, m), 1.59-1.52 (1H, m), 1.47-1.41 (1H, m); **^{13}C NMR ($CDCl_3$) δ(ppm):** 155.5, 152.9, 152.4, 145.3, 139.8, 138.7, 137.0, 129.8, 129.0, 127.9, 125.4(x2), 121.7, 119.1, 77.2, 71.8, 70.6, 66.5, 32.4, 28.9 ; **LCMS (ESI):** RT = 1.82 min (Method B) *m/z* calcd for $C_{26}H_{26}N_5O_9$ $[M+H]^+$: 552.17 found: 552.15 $[M+H]^+$; **HRMS (MALDI-TOF)** could not be measured due to molecule fragmentation

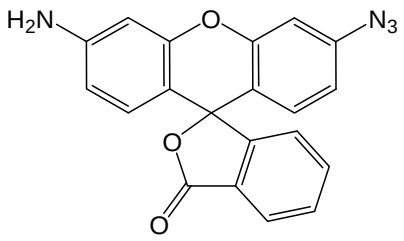


3 Solution of $NaIO_4$ (40 mg, 0.202 mmol, 2.9 equiv.) in water (300 μL) was added drop-wise to suspension of silica (450 mg) in Et_2O (1.4 mL). The resulting mixture was added to the solution of the diol **S5** (38.2 mg, 0.069 mmol, 1.0 equiv.) in THF (1 mL). After 30 minutes of stirring at room temperature the reaction mixture was filtered and silica washed extensively with Et_2O. Filtrate was concentrated to yield aldehyde **3** as brown oil (20.0 mg). Crude product was used without further purification for next step. **IR ν(cm^{-1}):** 2965.7, 2940.6, 2123.7, 1768.8, 1732.1, 1692.6, 1532.5, 1460.2, 1417.7, 1215.2, 1049.3, 862.2; **^{1}H NMR ($CDCl_3$) δ(ppm):** 9.78 (1H, s), 8.28 (2H, d, *J*=9.2 Hz), 7.41-7.36 (8H, m), 7.04 (2H, d, *J* = 8.4 Hz), 6.73 (1H, br s), 5.77-5.74 (1H, m), 5.24 (2H, s), 2.57-2.54 (2H, m), 2.34-2.25 (1H, m), 2.22-2.14 (1H, m); **^{13}C NMR ($CDCl_3$) δ(ppm):** 200.8, 155.4, 152.4 (x2), 145.4, 140.1, 138.4, 136.3, 129.9, 129.2, 127.9, 125.5 (x2), 121.7, 119.3, 75.7, 70.6, 39.9, 28.6; **LCMS (ESI):** RT= 2.03 min (Method B), RT= 1.42 min (Method C) ; **HRMS (MALDI-TOF)** could not be measured due to molecule fragmentation

Preparation of rhodamine derivative **4**

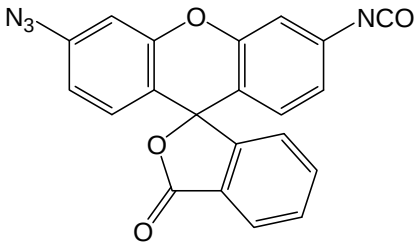


S6: 3-Aminophenol (2.00 g, 18.32 mmol, 1.0 eq.), 3-iodophenol (4.03 g, 18.32 mmol, 1.0 eq.) and phtalic anhydride (2.71 g, 18.32 mmol, 1.0 eq.) were disolved in methanosulfonic acid (16 mL) at room temperature. The reaction mixture was stirred for 40 h at 140^{o}C. After cooling to ambient temperature, the reaction mixture was poured onto 400 g of ice, the resulting solution was neutralized with 10% NaOH and extracted with CH_2Cl_2. The combined organic fractions were washed with 10% NaOH, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure in the presence of silica to be loaded directly on a flash column. Flash chromatography (hexanes:EtOAc 5:1, then 3:1) yielded **S6** as orange solid (1.95 g, 24% yield). **IR ν(cm^{-1}):** 3457.3, 3371.6, 2915.2, 2850.8, 1761.5, 1633.0, 1594.1, 1448.5, 1403.5, 1285.2, 1255.8, 1228.7, 1107.7, 1083.0; **^{1}H NMR (DMSO) δ(ppm):** 8.00 (1H, d, *J* = 7.6 Hz), 7.80 (1H, t, *J* = 7.2 Hz), 7.78 (1H, d, *J* = 1.6 Hz), 7.48 (1H, t, *J* = 7.6 Hz), 7.43 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz), 7.31 (1H, d, *J* = 7.6 Hz), 6.51 (1H, d, *J* = 8.4 Hz), 6.46 (1H, d, *J* = 2.0 Hz), 6.40-6.36 (2H, m), 5.73 (2H, br s); **^{13}C NMR (DMSO) δ(ppm):** 168.6, 152.2, 151.6, 151.5, 151.3, 135.7, 132.5, 130.2, 129.6, 128.5, 126.0, 125.4, 124.7, 124.0, 119.1, 111.6, 104.7, 99.1, 96.2, 82.7; **LCMS (ESI):** RT = 4.29 min (Method A) *m/z* calcd for $C_{20}H_{13}INO_3$ $[M+H]^+$: 441.99, found: 442.39; **HRMS (MALDI-TOF)** *m/z* calcd for $C_{20}H_{13}INO_3$ $[M+H]^+$: 441.9941, $C_{20}H_{12}INO_3Na$ $[M+Na]^+$: 463.9760, found: 441.9979 $[M+H]^+$, 463.9757 $[M+Na]^+$.

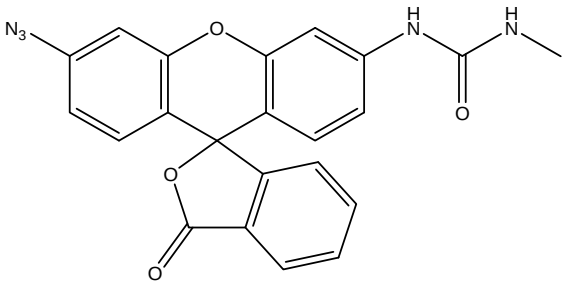


S7: The iodide **S6** (140 mg, 0.317 mmol, 1.0 eq.), sodium azide (42 mg, 0.634 mmol, 2.0 eq.) and sodium ascorbate (3.2 mg, 0.016 mmol, 0.05 eq.) were dissolved in DMSO/H_2O mixture (2 mL and 0,4 mL) at room temperature and degassed under vacuum. Copper iodide (11.5 mg, 0.064 mmol, 0.2 eq.) and N, N'- dimethylethylenediamine (5.5 μL, 0.048 mmol, 0.15 eq.) were added and reaction mixture was stirred until complete conversion of the starting material as judged by LCMS analysis (8h). The reaction was dilute with brine and extracted with EtOAc. The combined organic fractions were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (dry pack, hexanes:EtOAc 5:1, then 3:1 and 1:1) afforded **S7** as red solid (74.0 mg, 65% yield). **IR**

ν(cm^{-1}): 3465.8, 3363.2, 2973.9, 2866.9, 2115.8, 1763.0, 1634.5, 1610.7, 1513.3, 1497.6, 1452.9, 1427.7, 1284.6, 1255.1, 1225.3, 1106.9, 1066.1; **^{1}H NMR (MeOD) δ(ppm):** 8.07 (1H, d, *J* = 7.9 Hz), 7.84 (1H, t, *J* = 7.4 Hz), 7.77 (1H, t, *J* = 7.4 Hz), 7.28 (1H, d, *J* = 7.6 Hz), 7.05 (1H, br s), 6.85 (2H, br s), 6.63 (1H, br s), 6.53-6.48 (2H, m); **^{13}C NMR (acetone) δ(ppm):** 169.4, 153.8, 153.3, 153.1, 152.1, 143.1, 136.0, 130.7, 130.6, 129.6, 127.8, 125.4, 124.9, 117.5, 115.5, 112.6, 107.8, 107.4, 100.8, 83.5; **LCMS (ESI):** RT = 3.95 min (Method A) *m/z* calcd for $C_{20}H_{13}N_4O_3$ $[M+H]^+$: 357.10, found: 357.49 $[M+H]^+$, 329.37 $[M+H-N_2]^+$; **HRMS (MALDI-TOF)** *m/z* calcd for $C_{20}H_{13}N_4O_3$ $[M+H]^+$: 357.0988, $C_{20}H_{12}N_4O_3Na$ $[M+Na]^+$: 379.0807, found: , 379.1012 $[M+Na]^+$.

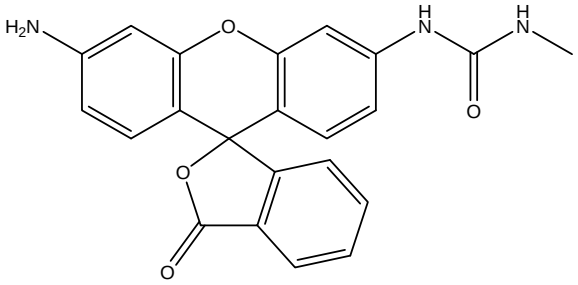


S8: The amine **S7** (74 mg, 0.208 mmol, 1.0 eq.) and triphosgene (31.3 mg, 0.104 mmol, 0.5 eq.) were dissolved in THF (5 ml) at room temperature and DIPEA (105 μL, 0.624 mmol, 3.0 eq.) was added dropwise. The reaction mixture was stirred 1h after which, the starting material was cleanly converted to the isocyanate (conversion was monitored by quenching an aliquot of the reaction with EtOH and analyzing the product by LCMS). The solution of **S8** in THF was used immediately for subsequent reaction without further purification. **LCMS (ESI):** for an aliquot quenched with ethanol RT = 4.97 min (Method A) *m/z* calcd for $C_{23}H_{17}N_4O_5$ $[M+EtOH+H]^+$: 429.40, found: 429.56 $[M+EtOH+H]^+$, 401.47 $[M+EtOH-Et+H]^+$;



S9: To the isocyanate **S8** (solution of 0.047 mmol in 1.5 ml THF, 1.0 equiv.), methylamine hydrochloride (9.5 mg, 0.141 mmol, 3.0 equiv.) and DIPEA (23.3 μL, 0.141 mmol, 3.0

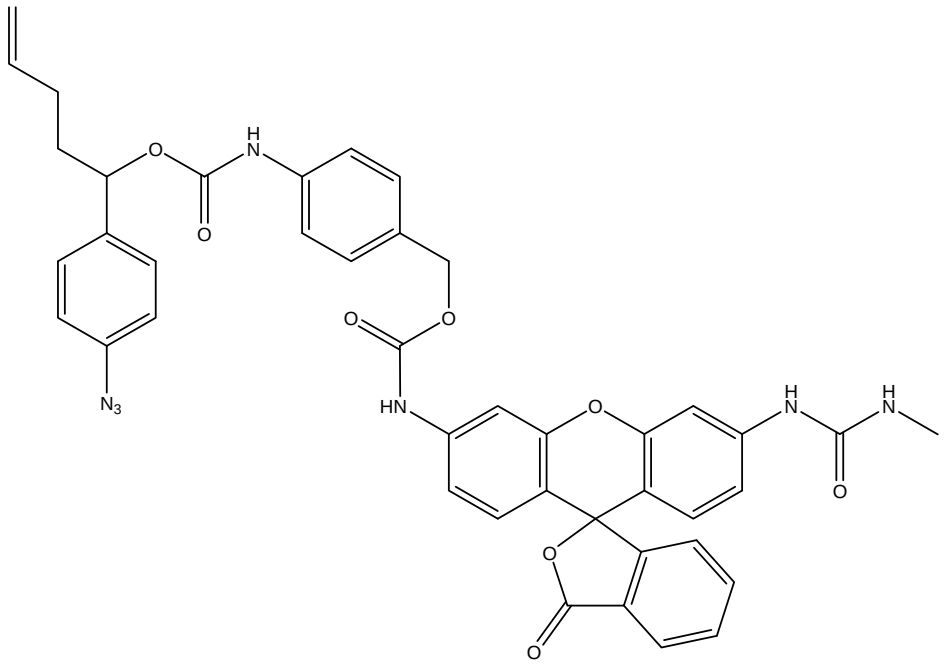
equiv.) were added and resulting mixture was stirred for 17 h. After reaction was quenched with brine, aqueous phase extracted 3x CH_2Cl_2, combined organic fractions dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Residue was redissolved in CH_2Cl_2 and evaporated with silica. Flash chromatography (hexanes:EtOAc 2:1, then EtOAc) yielded **S9** as orange solid (14.9 mg, 75.5% yield). **IR ν(cm^{-1}):** 2119.8, 1764.9, 1741.8, 1701.3, 1614.5, 1538.3, 1501.6, 1411.0, 1284.6, 1220.0, 1105.3, 891.1; **^{1}H NMR (MeOD) δ(ppm):** 8.08 (1H, d, *J* = 7.6 Hz), 7.81 (1H, t, *J* = 7.6 Hz), 7.77 (1H, t, *J* = 7.2 Hz), 7.66 (1H, s), 7.25 (1H, d, *J* = 7.6 Hz), 7.06 (1H, s), 7.01 (1H, d, *J* = 8,4 Hz), 6.86 (2H, s), 6.60 (1H, d, *J* = 8.0 Hz), 2.84 (3H, s); **^{13}C NMR (MeOD) δ(ppm):** 171.2, 158.3, 154.3, 153.6, 152.8, 144.2, 143.9, 136.7, 131.3, 130.7, 129.2, 127.6, 125.9, 125.1, 117.0, 116.0, 115.8, 112.9, 108.2, 106.7, 84.2, 26.8; **LCMS (ESI):** RT = 3.87 min (Method A) *m/z* calcd for $C_{22}H_{16}N_5O_4$ $[M+H]^+$: 414.12, found: 414.56 $[M+H]^+$, 386.46 $[M+H\text{-}N_2]^+$; **HRMS (MALDI-TOF)** *m/z* calcd for $C_{22}H_{16}N_5O_4$ $[M+H]^+$: 414.1203, found: 414.1179 $[M+H]^+$.



4: To the azide **S9** (14.9 mg, 0.036 mmol, 1.0 equiv.) dissolved in 3 mL of THF trimethylphospine (72 μL of 1.0M solution in THF, 0.072 mmol, 2.0 eq) was added. After 1h of stirring at room temperature, water (0.5 mL) was added and the reaction mixture was stirred next 30 minutes. Then brine and CH_2Cl_2 were added, layers were separated, aqueous phase was extracted with CH_2Cl_2 (3x10 mL), combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield **4** as red solid (20.0 mg crude) which was used for next step without further purification. **IR ν(cm^{-1}):** 2928.0, 2855.7, 1692.6, 1601.9, 1552.8, 1500.7, 1458.2, 1411.9, 1336.7, 1289.5, 1177.6, 1136.1; **^{1}H NMR (acetone) δ(ppm):** 7.94 (1H, d, *J* = 7.2 Hz), 7.79 (1H, s), 7.77 (1H, t, *J* = 8.4 Hz), 7.70 (1H, t, *J* = 7.6 Hz), 7.25 (1H, d, *J* = 7.6 Hz), 6.92 (1H, dd, J_1= 8.4 Hz, J_2= 2 Hz), 6.60-6.55 (2H, m), 6.52-6.40 (2H, m), 5.90 (1H, br s), 5.15 (1H, br s), 2.73 (3H, s); **^{13}C NMR (acetone) δ(ppm):** 171.7, 156.4, 154.1, 152.9, 151.8, 143.9, 135.9, 131.9, 130.4, 129.5, 128.8, 128.1, 125.1, 124.8, 117.9, 114.3, 112.4, 112.0, 105.5, 100.8, 85.2, 26.8; **LCMS (ESI):**

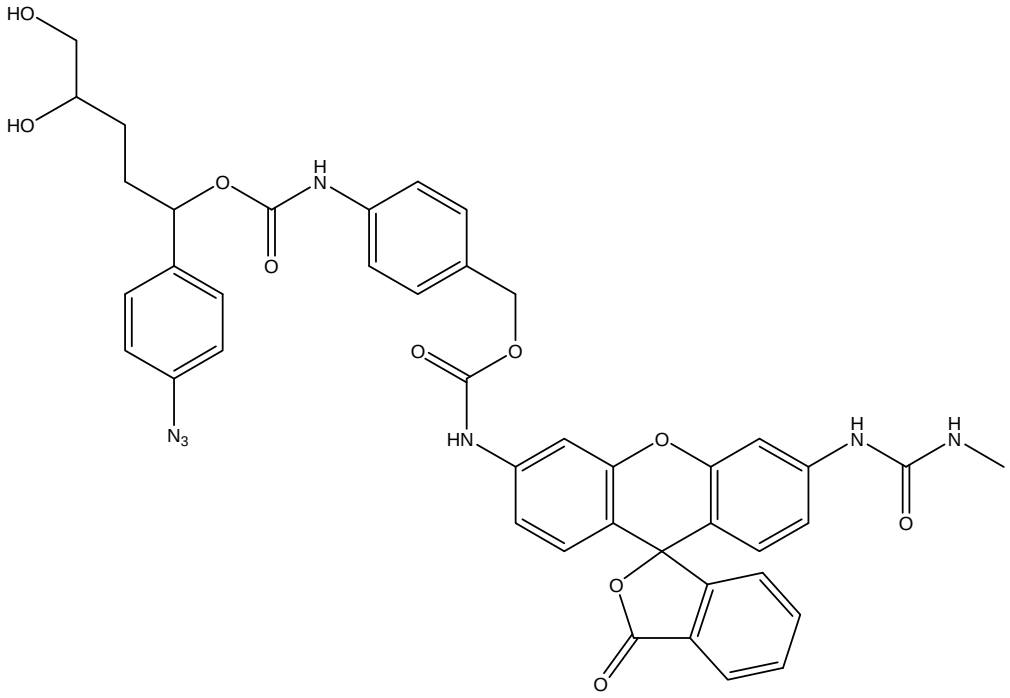
RT = 2.90 min (Method A) *m/z* calcd for $C_{22}H_{18}N_3O_4$ $[M+H]^+$: 388.13, found: 388.40 $[M+H]^+$; **HRMS (MALDI-TOF)** *m/z* calcd for $C_{22}H_{18}N_3O_4$ $[M+H]^+$: 388.1298, found: 388.1308 $[M+H]^+$.

Preparation of pro-functional conjugates **7**



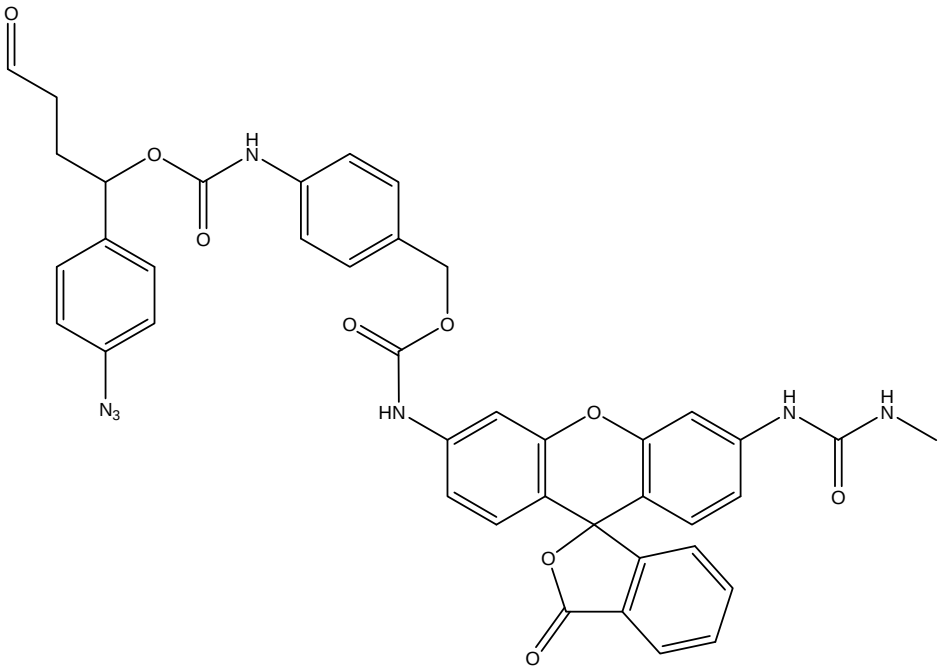
S10a: The amine **4** (11.5 mg, 29.3 μmol, 1.0 equiv.) and triphosgene (4.4 mg, 14.6 μmol, 0.5 equiv.) were dissolved in THF (1 mL) and DIPEA (15 μL, 87.9 mmol, 3.0 equiv.) was added drop-wise. The reaction mixture was stirred 30 minutes at room temperature. Then solution of alcohol **2** (10.5 mg, 3.12 mmol, 1.05 equiv.) in THF (1 mL) was added and the reaction was stirred 20 h at 60°C. After the reaction mixture was cooled to ambient temperature, quenched with saturated ammonium chloride solution and extracted with CH_2Cl_2 (3x10 mL). Combined organic layers were washed with saturated ammonium chloride, dried over sodium sulfate nad concentrated under reduced pressure. Residue was redissolved in acetone, evaporated with silica and purified by flash chromatography (hexanes:EtOAc 3:1, then 1:1 and pure EtOAc) which gave 5 mg of mixture of S**10a** with a side product (propyl carbamate). That mixture was used without further purification for next step. **IR ν(cm^{-1}):** 2957.9, 2928.0, 2853.8, 2118.9, 1692.6, 1602.9, 1546.0, 1508.4, 1412.9, 1287.5, 1231.6, 1110.7; **LCMS (ESI):** propyl carbamate: RT= 1.75 min *m/z* calcd for $C_{26}H_{24}N_3O_6$ $[M+H]^+$: 474.14, found: 474.06

$[M+H]^+$; desired product: RT=2.13 min – *m/z* calcd for $C_{42}H_{36}N_7O_8$ $[M+H]^+$: 766.26, found: 766.04 $[M+H]^+$; (Method B); **HRMS (MALDI-TOF)** propyl carbamate: *m/z* calcd for $C_{26}H_{23}N_3O_6Na$ $[M+Na]^+$: 496.1485, found: 496.1534 $[M+Na]^+$; desired product: $C_{42}H_{35}N_7O_8Na$ $[M+Na]^+$: 788.2545, found: 788.2470 $[M+Na]^+$.



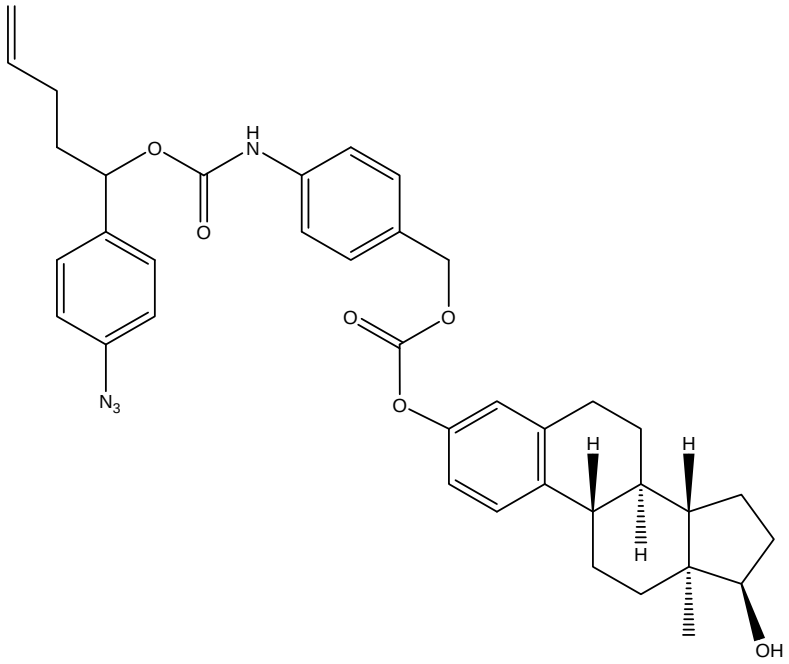
S11a: The mixture containing **S10a** (5mg, max. 6.5 μmol, 1.0 equiv.) was dissolved in acetone (1 mL). Water (0.5 mL), N-methyl morpholine oxide monohydrate (3 mg, 19.6 μmol, 3.0 equiv.), osmium tetroxide (4% solution in water, 2.1 μL, 0.33 μmol, 0.05 equiv.) and the resulting mixture was stirred at room temperature for 44 h. Then the reaction mixture was cooled to 0°C, quenched with saturated $Na_2S_2O_5$ solution (2 mL) and stirred for next 30 minutes. After the reaction mixture was extracted with CH_2Cl_2 (4x5 mL), combined organic fractions washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Residue was redissolved in acetone and evaporated with silica. Flash chromatography (hexanes:EtOAc 1:1, then 9:1 EtOAc:MeOH) yielded **S11a** as orange oil (2.5 mg, 10.7% for two steps). **IR ν(cm^{-1}):** 3350.5, 2968.6, 2928.0, 2858.6, 2117.9, 1694.5, 1602.9, 1546.0, 1412.9, 1287.5, 1233.5, 1049.3; **^{1}H NMR (MeOD) δ(ppm):** 8.08 (1H, d, *J* = 7.2 Hz), 7.84 (1H, t, *J* = 7.2 Hz), 7.78 (1H, t, *J* = 7.2 Hz), 7.66 (2H, d, *J* = 2.0 Hz), 7.51-7.48 (4H, m), 7.41 (2H, d, *J* = 8.4), 7.28 (1H, d, , *J* = 7.6 Hz); 7.14-7.12 (3H, m), 7.02 (1H, dd, J_1=

8.4 Hz, J_2= 2.0 Hz); 6.73-6.69 (2H, m), 5.80-5.74 (1H, m), 5.20 (2H, s), 3.68-3.65 (2H, m), 3.51-3.49 (1H, m), 2.85 (3H, s), 1.78-1.62 (2H, m), 1.56-1.49 (2H, m); **^{13}C NMR (acetone) δ(ppm):** 169.3, 154.2, 154.0, 153.8, 152.9, 152.7, 152.6, 142.4, 140.2, 140.1, 139.5, 136.1, 131.5, 130.7, 130.5, 130.0 (x4), 129.4, 129.0, 128.9 (x2), 127.6, 125.4, 124.9, 119.8 (x2), 115.0, 114.8, (x2), 114.3, 106.3, 105.6, 83.2, 74.8, 72.3, 70.0, 67.0, 27.8, 26.7, 26.3; **LCMS (ESI):** RT = 3.89 min; (Method A) *m/z* calcd for $C_{42}H_{39}N_7O_{10}$ $[M+H]^+$: 800.27, found: 800.80 $[M+H]^+$; **HRMS (MALDI-TOF)** *m/z* calcd for $C_{42}H_{38}N_7O_{10}$ $[M+H]^+$: 800.2681, found: 800.2743 $[M+H]^+$.



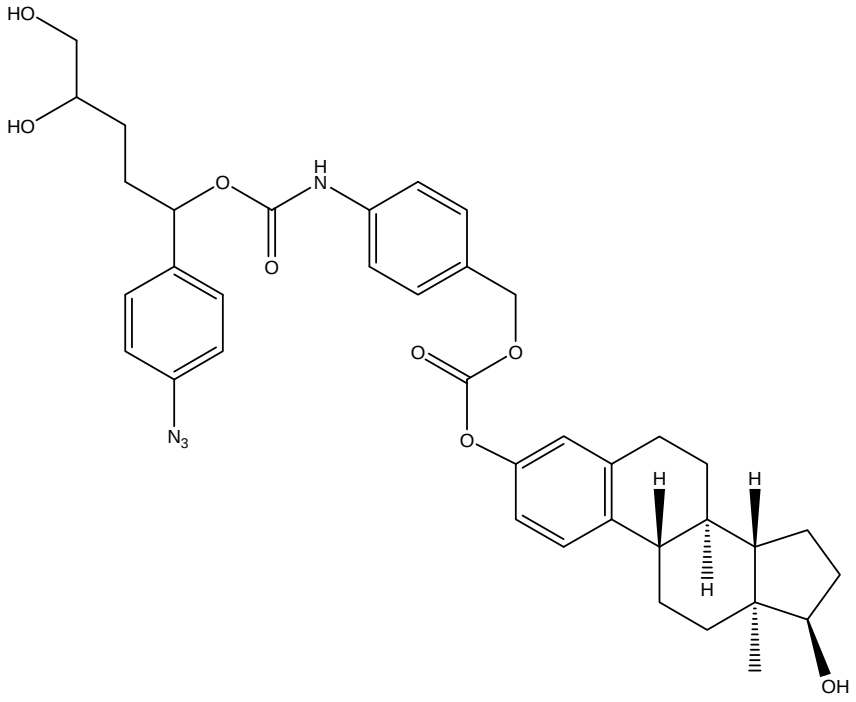
7a: To a solution of diol **S11a** (13.2 mg, 16.5 μmol, 1.0 eq) in MeOH (3 mL) was added a solution of $NaIO_4$ (70.6 mg, 330.0 μmol, 20 eq) in water (1 mL). After 30 minutes the organic solvent was evaporated under reduced pressure and the resulting solution was diluted with 5 mL brine and 5 mL water, then extracted with CH_2Cl_2 (5x10 mL) dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield **7a** (11.2 mg, 88.4%) as a pink/red solid. **IR ν(cm^{-1}):** 2971.4, 2930.0, 2119.8, 1729.2, 1609.7, 1546.0, 1530.6, 1411.9, 1288.5, 1230.6, 1049.3, 876.7; **^{1}H NMR (acetone) δ(ppm):** 9.72 (1H, d), 9.02 (1H, s), 8.90 (1H, s), 8.25 (1H, s), 7.78 (1H, d, J = 7.6 Hz), 7.81 (1H, d, J = 2.0 Hz), 7.78 (1H, dd, J_1 = 7.6 Hz, J_2 = 0.8 Hz), 7.74-7.72 (2H, m), 7.54 (2H, d, J = 8.4), 7.45 (2H, d, J = 8.4 Hz), 7.36 (2H, d, J =

8.4 Hz), 7.29 (1H, d, J = 7.6 Hz), 7.19 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz), 7.09 (2H, d, J = 8.4 Hz), 7.00 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 6.95 (1H, s), 6.74 (2H, d, J = 8.8 Hz), 6.65 (2H, d, J = 8.4 Hz), 5.78-5.75 (1H, m), 5.12 (2H, s), 2.74 (3H, s), 2.55 (2H, t, J = 6.8 Hz), 2.32-2.25 (1H, m), 2.19-2.13 (1H, m); **^{13}C NMR (acetone) δ(ppm):** 201.9, 169.5, 154.2, 154.0, 153.6, 152.7, 152.6, 151.6, 144.0, 142.4, 140.4, 138.7, 136.1, 131.7, 130.7, 130 (x4), 129.7, 129.3, 129.0, 128.8 (x2), 127.6, 125.4, 124.9, 119.9 (x2), 115.0, 114.9, 114.3, 112.6, 106.3, 105.7, 83.2, 75.6, 67.0, 40.4, 29.7, 26.7**; LCMS (ESI):** RT = 4.14 min (Method A) *m/z* calcd for $C_{41}H_{34}N_7O_9$ $[M+H]^+$: 768.24, found: 768.97 $[M+H]^+$; **HRMS (MALDI-TOF)** *m/z* calcd for $C_{41}H_{34}N_7O_9$ $[M+H]^+$: 768.2419, found: 768.2390 $[M+H]^+$.



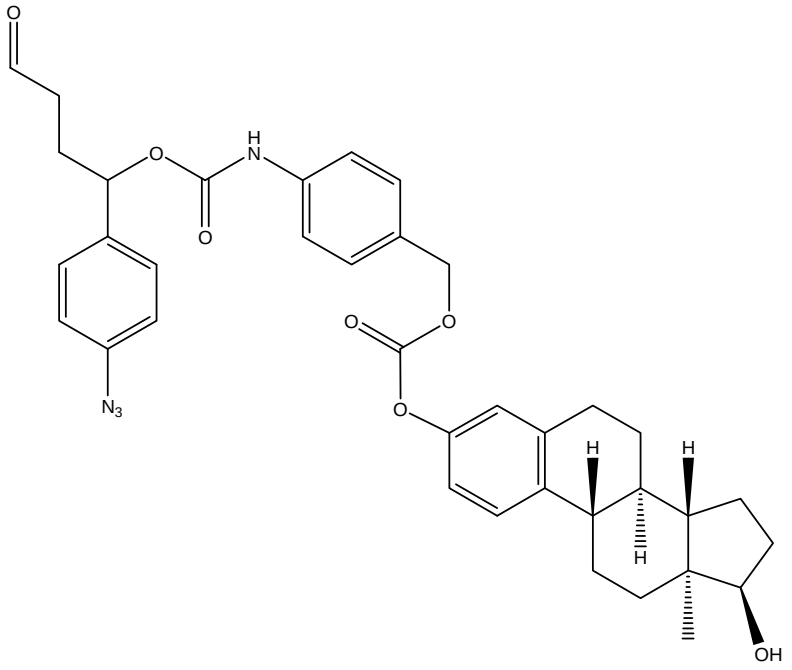
S10b: α-estradiol **5** (30.0 mg, 0.11 mmol, 1.0equiv) was dissolved in CH_2Cl_2 (2 mL) together with mixed carbonate **S4** (57.0 mg, 0.11 mmol, 1.0 equiv) and TEA (20μL, 0.22 mmol, 2.0 equiv.) was added. Reaction mixture was stirred at room temperature for 72h and then diluted with 10 mL of CH_2Cl_2 washed three times with saturated $NaHCO_3$ solution to remove *p*-nitrofenol. Organic layer was later washed with water until neutral pH, dried over anhydrous Na_2SO_4 and evaporated. Residue was dissolved in ethyl acetate and after dry-packing purified by flash chromatography (hexanes:EtOAc 5:1, then 3:1) yielding **S10b** as pale yellow oil (43.0 mg, 85% yield based on 30% recovery of starting material). Significant shift downfield

of 1H NMR signal of only aromatic and benzyl protons in estradiol moiety suggested that coupling occurred as desired on phenol group rather than on secondary alcohol. **IR ν(cm^{-1}):** 3372.6, 2970.5, 2929.0, 2858.6, 2119.8, 1758.2, 1729.2, 1602.9, 1591.3, 1409.1, 1285.6, 1235.5, 1048.4 ; **^{1}H NMR ($CDCl_3$) δ(ppm):** 7.41-7.35 (6H, m), 7.30 (1H, d, , *J*=8.4 Hz), 7.03 (2H, d, *J*=8.8 Hz), 6.93 (1H, dd, J_1=8.4 Hz, J_2=2.4 Hz), 6.87 (1H, d, *J*=2.4 Hz), 6.76 (1H, bs), 5.88-5.78 (1H, m), 5.75-5.71 (1H, m), 5.20 (2H, s), 5.07-5.01 (2H, m), 3.82 (1H, d, *J*=5.6 Hz), 2.87-2.85 (m, 2H), 2.40-2.38 (1H, m), 2.26-2.21 (m, 2H), 2.11-2.06 (3H, m), 1.95-1.86 (3H, m), 1.78-1.74 (m, 1H), 1.65-1.60 (m, 2H), 1.58-1.52 (2H, m), 1.50-1.40 (2H, m), 1.31-1.26 (1H,m), 0.71 (3H, s); **^{13}C NMR ($CDCl_3$) δ(ppm):** 154.1(x2), 149.0, 139.9, 139.8, 138.5 (x2) 137.2, 129.8 (x2), 128.1, 126.5, 120.9, 119.2, 118.0, 115.5, 112.7, 80.0, 76.1, 69.9, 47.8, 45.5, 43.8, 38.7, 35.3, 32.4, 31.5, 29.7, 27.8, 26.9, 26.1, 24.3, 17.0, **LCMS (ESI):** RT= 2.46 min (Method B); *m/z* calcd for $C_{38}H_{43}N_4O_6$ $[M+H]^+$: 651.32, found: 633.40 $[M+H^+-H_2O]$ **HRMS (MALDI-TOF)** could not be measured due to molecule fragmentation

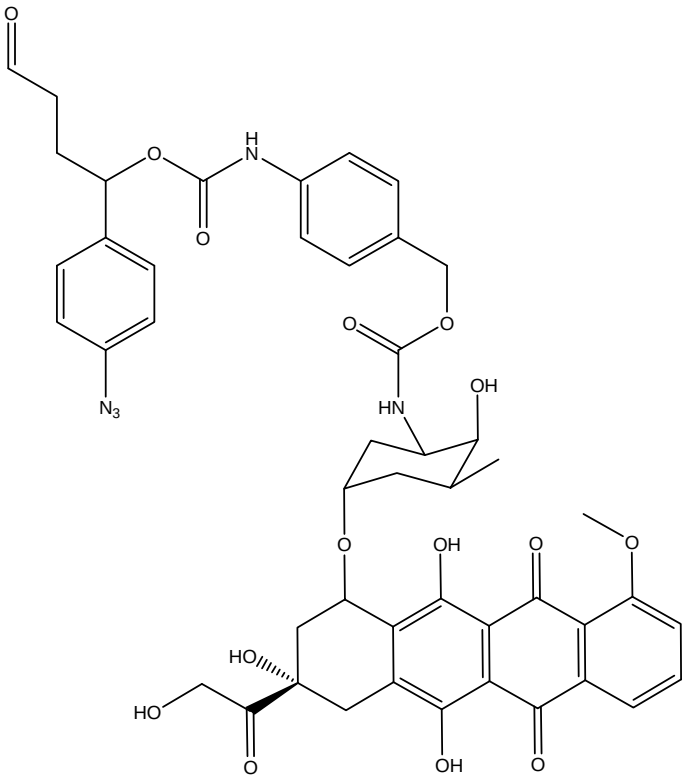


S11b: To a solution of alkene **S10b** (43.0 mg, 0.066 mmol, 1.0 equiv) in acetone (5 mL) N-methyl morpholine oxide monohydrate (26.8 mg, 0.198 mmol, 3.0 equiv) and water (1 mL) were added followed by OsO_4 (4% solution in H_2O, 21 μL, 0.0033mmol, 0.05 equiv). After stirring for 24h at room temperature in absence of light the reaction mixture was cooled down

to 0^{o}C and saturated $Na_2S_2O_5$ solution was added to quench remaining OsO_4. During 30 minutes of stirring reaction mixture was allowed to warm up to ambient temperature and then brine (10mL) was added. Extraction with CH_2Cl_2 (3 x 15 mL), drying over anhydrous Na_2SO_4 and solvent removal yielded crude diol. Later purification by flash chromatography (dry packing, elution with hexanes:EtOAc 3:1, 1:1 and then 100% EtOAc) provided 42.6 mg of **S11b** as white powder (94.3 % yield). **IR ν(cm^{-1}):** 3406.4, 3370.7, 2119.8, 1758.2, 1729.2, 1666.6, 1597.1, 1527.7, 1229.7, 1113.0, 1008.8, 870.9; **^{1}H NMR ($CDCl_3$) δ(ppm):** 7.38-7.35 (6H, m), 7.29 (1H, d , *J*=8.8 Hz), 7.07 (1H, bs), 7.00 (2H, d, *J*=8.8 Hz), 6.91 (1H, dd, J_1=8.8 Hz, J_2=2.4 Hz), 6.86 (1H, d, *J*=2.0 Hz), 5.72 (1H, t, *J*=6.8 Hz), 5.18 (2H, s), 3.94-3.91 (1H, m), 3.88-3.86 (1H, m), 3.81 (1H, d, *J*=6.0 Hz), 3.77-3.73 (1H, m), 3.64 (1H, d, *J*=11.2 Hz), 3.46-3.41 (1H, m), 2.86-2.83 (2H, m), 2.39-2.35 (1H, m), 2.29-2.22 (4H, m), 2.05-1.99 (1H, m), 1.92-1.85 (3H, m), 1.75 (1H, dd, J_1=13.2 Hz, J_2=4.2 Hz), 1.67-1.57 (2H, m), 1.57-1.51 (2H, m), 1.46-1.39 (2H, m), 1.33-1.30 (1H,m), 0.70 (3H, s); **^{13}C NMR ($CDCl_3$) δ(ppm):** 153.9 (x2), 148.8, 139.8 (x2), 138.4 (x2), 138.3 (x2), 129,6 (x2) 127.9, 126.3, 120.8, 119.1, 117.9, 79.9, 76.3, 71.6, 69.8, 66.6, 47.7, 45.4, 43.7, 38.6, 32.3 (x2), 31.4, 29.5, 28.9, 27.7, 26.0, 24.2, 16.9; **LCMS (ESI):** RT = 4.21 min (Method A) *m/z* calcd for $C_{38}H_{44}N_4O_8Na$ $[M+Na]^+$: 707.30, found: 707.39 $[M+Na]^+$; RT= 2.04 min, 2.07 min (Method B) – 2 diasteroisomers; **HRMS (MALDI-TOF)** could not be measured due to molecule fragmentation



7b: To a solution of diol **S11b** (20.0 mg, 31.1 µmol, 1.0 eq) in MeOH (3 mL) was added a solution of $NaIO_4$ (133.1 mg, 0.622 mmol, 20 eq) in water (1 mL). After 1 hour the organic solvent was evaporated under reduced pressure and the resulting solution was diluted with 5 mL brine and 5 mL water, then extracted with CH_2Cl_2 (3x10 mL) dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield **7b** (12.5 mg, 61.6%) as a pale yellow oil. **IR ν(cm^{-1}):** 3515.4, 3023.5, 2121.8, 1758.2, 1747.6, 1730.2, 1602.9, 1590.4, 1443.8, 1374.3, 1228.7, 1008.8; **^{1}H NMR ($CDCl_3$) δ(ppm):** 9.80 (1H, s), 7.41-7.38 (6H, m), 7.30 (1H, d, *J*=8.4 Hz), 7.05 (2H, d, *J*=8.4 Hz), 6.94 (1H, dd, J_1=9.6 Hz, J_2=2.4 Hz), 6.90 (1H, d, *J*=2.0 Hz), 6.71 (1H, bs), 5.22 (2H, s), 3.85 (1H, d, *J*=6.0 Hz), 2.89-2.87 (m, 2H), 2.60-2.56 (1H, m), 2.40-2.35 (1H, m), 2.32-2.25 (2H, m), 2.23-2.18 (2H, m), 1.95-1.85 (2H, m), 1.76 (1H, dd, J_1=12.8 Hz, J_2=4.2 Hz), 1.68-1.60 (4H, m), 1.58-1.49 (2H, m), 1.47-1.45 (1H, m), 0.71 (3H, s); **^{13}C NMR ($CDCl_3$) δ(ppm):** 199.4, 153.9 (x2), 148.8, 138.3 (x4), 137.0, 136.9, 129.7 (x2), 127.9, 126.4, 120.9, 119.1, 118.5, 117.9, 80.1, 77.2, 69.8, 47.7, 45.4, 43.7, 38.6, 32.4 (x2), 31.4, 29.6, 29.2, 27,8, 26.0, 24.2, 16.9; **LCMS (ESI):** RT= 2.22 min, 2.31 min (Method B) – 2 diasteroisomers; *m/z* calcd for $C_{37}H_{41}N_4O_7$ $[M+H]^+$: 653.41, found: 635.40 $[M+H^+-H_2O]$ **HRMS (MALDI-TOF)** could not be measured due to molecule fragmentation



7c: Doxorubicin hydrochloride **6** (3.0 mg, 5.17 μmol, 1.0 eq) was combined in DMF (0.5mL) with mixed carbonate **3** and TEA (2.2 μL, 16 μmol, 3.1 equiv) was added. After 48h at room temperature solvent was evaporated (30^{o}C, high vacuum). Residue was taken into CH_2Cl_2 and loaded on small patch of silica. After washing with CH_2Cl_2 and 1% MeOH in CH_2Cl_2 pure product was eluted with 5% MeOH in CH_2Cl_2 (3.3 mg, 69%, red powder). **IR ν(cm^{-1}):** 3446.9, 2127.6, 1662.7, 1420.6, 1369.5, 1324.2, 1263.4, 1026.2, 1005.9, 939.4, 907.6, 822.7; **^{1}H NMR (MeOD) δ(ppm):** 8.00 (1H, d, *J*=7.6 Hz), 7.92-7.88 (1H, m), 7.65 (1H, d, *J*=9.2 Hz), 7.50-7.40 (4H, m), 7.35-7.28 (2H, m), 7.15-7.11 (2H, m), 5.78-5.72 (1H, m), 5.49-5.45 (1H, m) 5.26-5.22 (1H, m), 5.02 (2H, s), 4.80 (2H, s), 4.36-4.29 (1H, m), 4.10 (3H, s), 3.98-3.92 (1H, m), 3.67 (1H, s), 3.24-3.16 (2H, m), 2.47-2.43 (1H, m), 2.28-2.24 (2H, m), 2.11-2.08 (2H, m), 2.00-1.95 (2H, m), 1.85-1.78 (1H, m), 1.32 (3H, d, *J*=6.4 Hz; **LCMS (ESI):** RT= 1.34 min (Method C) *m/z* calcd for $C_{46}H_{45}N_5O_{16}Na$ $[M+Na]^+$: 946.28, found: 946.46 $[M+Na]^+$; **HRMS (MALDI-TOF)** *m/z* calcd for $C_{46}H_{45}N_5O_{16}Na$ $[M+Na]^+$: 946.2759, for $C_{46}H_{45}N_5O_{16}K$ $[M+K]^+$: 962.3844, found: 946.2680 $[M+K]^+$, 962.3840.

Kinetics of small molecule release upon TCEP treatment

Aldehydes **9a**-**9c** dissolved in acetonitrile were added to PBS and release of functional molecule after addition of TCEP (final concentration of aldehyde: 500μM, TCEP 25mM, 100mM PBS, pH=7.4) was followed by LCMS (5μL injections).

Time of reaction before injection [min]	Total UV absorbance – peak area **7a**	Total UV absorbance – peak area **8a**	Total UV absorbance – peak area **9a**
0	118985	-	-
1	52386	-	49312
6	7703	-	81115
11	-	-	85377

Time of reaction before injection [min]	Total UV absorbance – peak area **7b**	Total UV absorbance – peak area **8b**	Total UV absorbance – peak area **9b**
0	330548	-	-
1	200995	-	49823
6	85496	-	302269
11	37040	-	516143
16	18766	-	652717
23	-	-	688557
31	-	-	783900

Time of reaction before injection [min]	Total UV absorbance – peak area **7c**	Total UV absorbance – peak area **8c**	Total UV absorbance – peak area **9c**
0	55208	-	-
1	42687	14332	16475
6	30885	46178	33258
11	3816	40064	42663
21	-	45508	95019
26	-	44247	103863
31	-	41027	112045
48	-	32894	147665
96	-	10927	124318

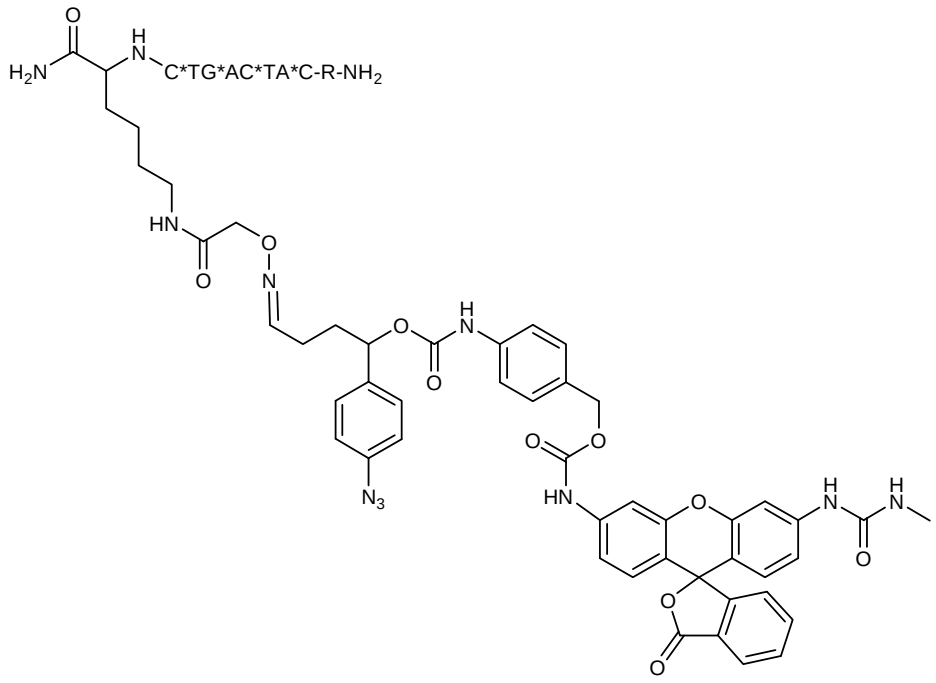
Preparation of PNA-tagged conjugates **11**

10 (AOOA-PNA): PNA synthesis was performed by standard Fmoc chemistry as previously described on NovaPEG (NovaBiochem) Rink amide resin loaded with Fmoc-Lys(Mtt)-OH (0,2 mmol/g). Mtt from side chain of lysine was deprotected before removal of terminal Fmoc from PNA strand and additional coupling to introduce aminooxyacetyl moiety on liberated amino group was done without capping at the end. Then terminal Fmoc was removed and product was cleaved from resin with 100% TFA for 3h. The TFA solution was precipitated in Et_2O (10 x TFA volume) and centrifuged to recover the PNA as a pellet. The PNA was redissolved in water, purified on C18 using Biotage ISOLERA flash chromatography system and characterized by MALDI:

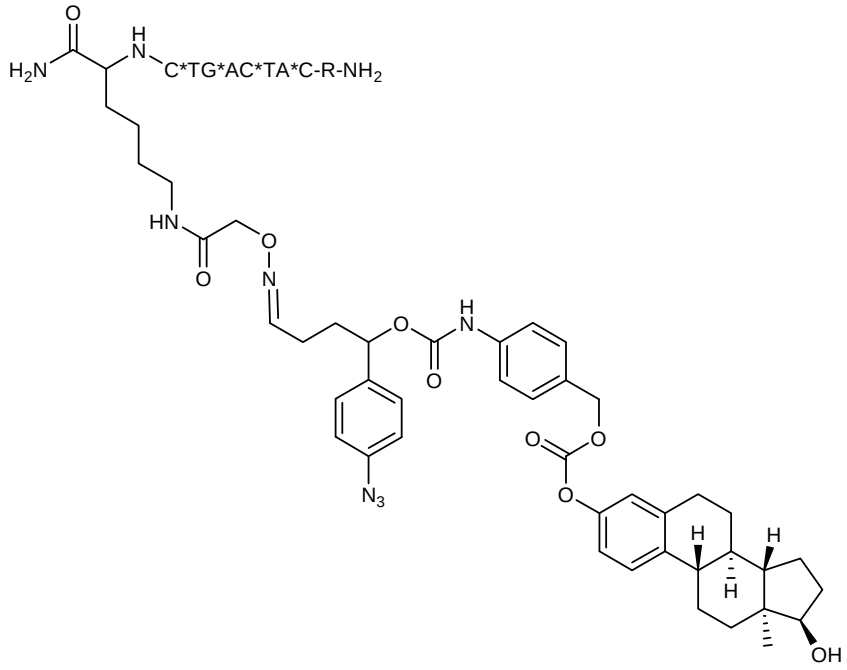
K(AOOA) C*TG*AC*TA*C-R MW: 2897.53 MALDI-TOF *m/z* found: 2898.57 $[M+H]^+$

General Procedure for the aldehyde coupling: 50 µL of a 5 mM solution of AOOA-PNA (250 nmol, 1.0 equiv) in H_2O/MeCN 1:1 were diluted with 125 µL of MeCN, then 75 µL of a 10 mM solution of aldehyde (750 nmol, 3.0 equiv) in MeCN was added. After 5 hours the reaction was stopped adding 400 µL of H_2O and lyophilizing. The product was then isolated by HPLC purification.

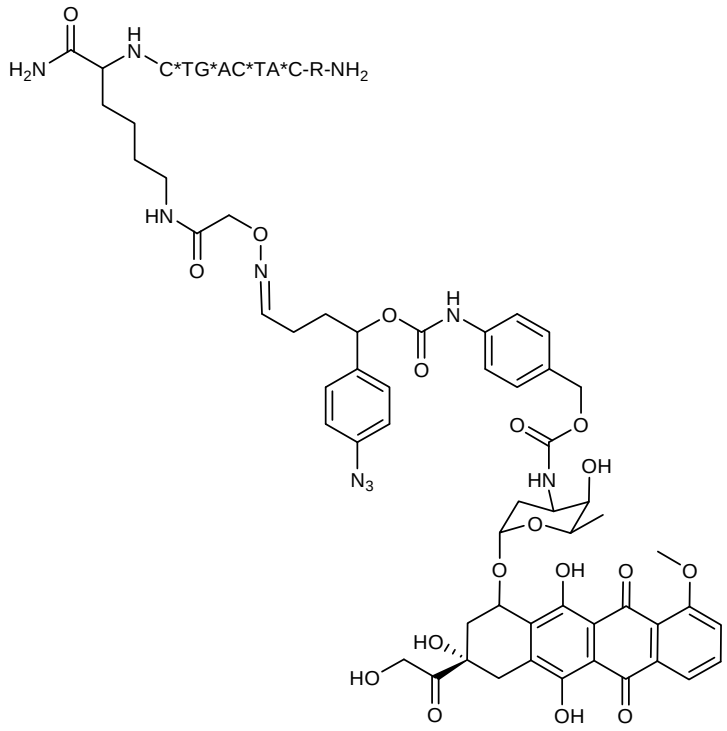
Characterization of PNA-pro-functional molecule conjugates:



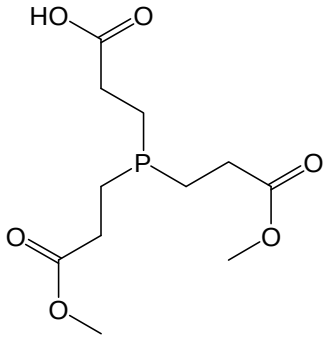
11a MW: 3646.76 MALDI-TOF *m/z* found: 3647.93 $[M+H]^+$



11b Yield after purification: 79% MW: 3531.82 MALDI-TOF *m/z* found: 3532.48 $[M+H]^+$



11c MW: 3802.82 LC-MS (ESI) RT= 1.06 min (Method B) *m/z* found: 544.15 $[M+7H]^{7+}$, 761.97 $[M+5H]^{5+}$, 952.25 $[M+4H]^{4+}$, 1269.75 $[M+3H]^{3+}$ MALDI-TOF *m/z* found: 3804.63 $[M+H]^{+}$



dmTCEP: Tricarboxyethylphosphine hydrochloride (TCEP-HCl, 200 mg, 0.208 mmol.) was stirred with 200 mg of sulfonic acid resin (Amberlyst) in 3 mL of methanol at room temperature for 40 minutes. The resin was then removed by filtatration and the filtrates, containing a mixture of mono-, di-and tri-methylester, were fractionated by flash chromatography (dry packing, 0, 5, 10, 15% of MeOH in DCM) to give dmTCEP as colorless oil (67mg, 34% yield). **LCMS (ESI):** RT = 0.71 min (Method B) *m/z* calcd for $C_{11}H_{19}O_6P$ $[M+H]^{+}$: 279.10, found: 279.16 $[M+H]^{+}$.

PNA-dmTCEP: Polymer-bound PNA prepared by standard Fmoc chemistry, deprotected at the N-terminus, was treated with dmTCEP **7** (6.0 equiv) in DMF previously activated for one minute with DIC (24 equiv) and HOBt (12 equiv) for 30 min. Then resin was washed first with 5% AcOH in CH_2Cl_2 then with CH_2Cl_2 and DMF and cleaved with 100% TFA for 3h. The TFA solution was precipitated in Et_2O (10 x TFA volume) and centrifuged to recover the PNA as a pellet. The PNA was redissolved in water, purified on C18 using Biotage ISOLERA flash chromatography system and characterized by MALDI.

Characterisation of PNA-dmTCEP:

RAT*CG*AA*TdmTCEP MW: 2630.33 MALDI-TOF *m/z* found: 2632.03 $[M+H]^{+}$

Templated reactions – rhodamine release

Black 96-well plates (Nunc) (500 μL/well) were used to perform the templated reactions. Stock PNA and DNA solutions at 10 μM and 1 μM were prepared in deionized water and stored at 4°C. They were diluted to their final concentrations with buffer containing 10 mM PBS, 154 mM NaCl, 25 mM $MgCl_2$, 0.1 mg/mL Bovine γ-Globuline (BGG) (pH=7,4; equivalent to physiological buffer, prepared as follow: 25 ml of 0,2 M PBS pH=7.4, 15.4 ml of 5 M NaCl, 25 ml of 1 M $MgCl_2$ and 50 mg of BGG was added to a 447 ml of deionized water, buffer was filtered through Millipore ExpressPLUS 0 and stored at 4°C; BGG added freshly before each experiment). First wells were filled with 240 μL of the PNA-dmTCEP solution containing appropriate DNA. After 240 μL of N_3-Rh-PNA solutions was added using multichannel pipette and the plate was placed in a fluorometer (SpectraMax GeminiXS, Molecular Devices) pre-heated to 37°C and the fluorescence readout was recorded immediately. All experiments were performed in duplicates and each individual experiment included a positive control (phosphine-PNA solution was replace with 240 μL of 20 mM TCEP solution in buffer), two negative control (no DNA template and random DNA template), and a background florescence measurement (reaction without PNA-dmTCEP). The fluorescence level corresponding to 100% conversion was measured by treating all reactions with large excess of TCEP. The fluorescence measurements of the templated reactions was performed using 495 nm (excitation) and 525 nm(emission) at 37°C. The curves shown represent the average of the duplicate measurements.

Templated reactions – estradiol release

Stock solutions and buffer were prepared identically as for fluorescence experiments. 250 μL of the PNA-dmTCEP solution containing appropriate DNA template was added to eppendorfs. After 250 μL of estradiol-PNA conjugate solutions was added using multichannel pipette and the rack with tubes was shaken for 30 minutes at 37°C. Then all reaction mixtures were quenched by addition of 100μL of 35% H_2O_2 solution. After 10 minutes at room temperatures resulting solutions were lyophilized. Released estradiol was extracted from dry residue with 1:1 CH_2Cl_2:MeCN. Tubes were sonicated 10 minutes after addition of 1mL of sucj mixture. Then samples were spun down, supernatant was transferred to fresh tubes and

evaporated to dryness in speedvac. Residue was then re-dissolved in 50μL of MeCN and 10μL portions of samples were injected to HPLC (Thermo Scientific Accela HPLC with a Thermo C18 (5 cm x 2.1 mm, 1.9 μm particles) hypersil gold column, linear elution gradient for 95% H_2O 0.01% TFA to 90% MeCN 0.01% TFA in 3.6 minutes at a flow rate of 1.0 $mLmin^{-1}$) equipped with a fluorescence detector (Jasco X-LC^{TM} 312 OFP). All experiments were performed in duplicates or triplicates and each samples was injected twice. The fluorescence measurements were performed using 290 nm (excitation) and 310 nm(emission) wavelength. Each individual experiment included a positive control (reaction mixture with random DNA template spiked with known amount of estradiol) and two negative controls (no DNA template and random DNA template), and a background florescence measurement (reaction without PNA-dmTCEP). The convertion of measured peak areas to estradiol concentrations was done using a calibration curve. Calibration curve was recorded following identical preparation, incubation, quenching and extraction procedures but estradiol-PNA conjugate was replaced by the same amount of non-labeled PNA oligomer and known amount of estradiol was added before starting incubation.

Synthetic DNA templates used:

Matching template (PM):	**TAGCTTA**TCA**GACTGATG**TTGA
Mutated template (2xMM):	**TA<u>CT</u>TTAT**CA**GAC<u>GT</u>AT**GTTGA
Random template:	CTGTGCGTGTGACAGCGGCTGA

Purchased from Eurogentec as desalted 100 μM solutions in deionized water. Diluted with deionized water to the concentrations of 10 μM and 1 μM.