Supporting information

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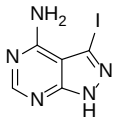
1. Materials and instruments

Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany), Bachem (Bubendorf, Switzerland), Thermo Fisher Scientific (Karlsruhe, Germany), AppliChem (Darmstadt, Germany), and VWR (Langenfeld, Germany). The building blocks shown in tables S1 and S2 were selected and purchased from the Aldrich Market Select building blocks catalogue (version 2013/3). Enzymes were purchased from Thermo Fisher Scientific (PNK), Biozym (T4 DNA ligase rapid), NEB (E.coRI), Roche (FastStart Universal Probe Master (Rox) for qPCR containing SYBR GREEN and Taq polymerase and dNTPs) and Eurogentec (Takyon™ No Rox Probe MasterMix dTTP). 5'-Aminolinker-modified DNA oligonucleotides attached to controlled pore glass solid phase (CPG, 1000 Å) were synthesized by IBA (Goettingen, Germany); unmodified DNA-oligonucleotides were purchased from Integrated DNA Technologies (Integrated DNA Technologies, Leuven, Belgium). Controlled pore glass solid phase was dried on a synthesis column plugged onto a vacuum manifold (Vac-Man®, Promega). Oligonucleotide-small molecule conjugates were purified by ion pair reverse-phase high-pressure liquid chromatography (HPLC, Shimadzu Prominence) using a C18 stationary phase (Phenomenex, Gemini; 5 µm, C18, 110 Å, 100*10.0 mm) and a gradient of 100 mM aqueous triethylammonium acetate/MeOH. The triethylammonium acetate buffer was set to pH 8. Oligonucleotide-small molecule conjugates were analyzed by ion pair reverse phase ultra-pressure liquid chromatography (UPLC, Agilent Technologies 1100) using a C18 stationary phase (Waters, Acquity UPLC® BEH, 1.7 µm, C18, 50*2.1 mm) and a gradient of 100 mM aqueous triethylammonium acetate/MeOH; and by ion pair reverse-phase high-pressure liquid chromatography (HPLC, Shimadzu Prominence) using a C18 stationary phase (Phenomenex, Gemini; 5 µm, C18, 110 Å, 100*4.6 mm) and a gradient of 100 mM aqueous triethylammonium acetate/MeOH. Oligonucleotide concentrations were quantitated by UV spectroscopy using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific). Oligonucleotides were analyzed by MALDI-TOF/TOF-MS (Bruker Daltonics) using THAP or 3-HPA matrix (Dichrom). ^{1}H-NMR-spectra were measured at 400 or 500 MHz on a Bruker DRX400 or Inova 500 spectrometer, respectively. ^{13}C-NMR-spectra were measured at 101 MHz or 126 MHz on a Bruker DRX400 or Inova 500 spectrometer, respectively. The pure substance was dissolved in deuterated chloroform ($CDCl_3$, 99.8 %, VWR) or dimethyl sulfoxide-d_6 (DMSO-d_6, 99.8 %, VWR, Langenfeld, Germany). Chemical shifts are listed relative to the deuterated solvent. Each proton signal was analyzed regarding its multiplicity, coupling constant J [Hz] and the amount of protons. The multiplicity was abbreviated as follows: s = sigulett, d = duplet, t = triplet, q = quartet, quint = quintet, m = multiplet and br = broad signal. Silica gel chromatography was performed on NORMASIL 60 silica gel 40-63 µm (VWR, Langenfeld, Germany); thin layer chromatography was performed on aluminium-backed silica gel 60 F_{254} plates provided by (Merck Millipore, Darmstadt, Germany). LC-MS analysis of low-molecular weight compounds was performed on reverse-phase high-pressure liquid chromatography (HPLC, Shimadzu Prominence) using a C18 column stationary phase (Phenomenex, Luna; 5 µm, C18, 100 Å, 250*4.6 mm) and MeOH/1% aq. formic acid, 50:50 to 100:0 over 13 min.

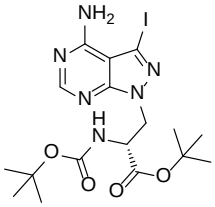
2. Synthesis and characterization of compounds 7 and 8

3-Iodo-1*H*-pyrazolo[3,4-d]pyrimidin-4-amine (**13**)



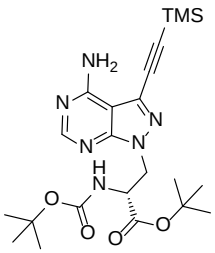
A solution of 3*H*-pyrazolo[3,4-d]pyrimidine-4-amine **12** (3.2 g, 1 eq, 23.68 mmol) and N-iodo-succinimide (8 g, 1.5 eq, 35.5 mmol) in DMF (26 mL) was stirred at 80 °C for 14 hours. The resulting product **13** precipitated from the solution. It was filtered off, rinsed with cold EtOH and dried *in vacuo* (4.7 g, 76 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 13.82 (s, 1H), 8.17 (s, 1H), 7.81 (br. s, 1H), 6.69 (br. s, 1H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ ppm: 157.6, 156.1, 155.0, 102.5, 89.8. MS (ESI): calc. 260.95, found 262.02 ([M+H]+). Purity (HPLC): 97 %.

(*S*)-*tert*-Butyl 3-(4-amino-3-iodo-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)-2-((*tert*-butoxycarbonyl)amino)propanoate (**15**)



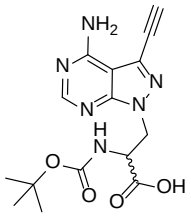
To a solution of **13** (350 mg, 1 eq, 1.3 mmol) in dry THF (83 mL), triphenyl phosphine (703 mg, 2 eq, 2.7 mmol), diisopropyldiazene-1,2-dicarboxylate (528 µL, 2 eq, 2.7 mmol) and the protected (*S*)-serine **14** (700 mg, 2 eq, 2.7 mmol) were added. The reaction mixture was stirred under argon atmosphere for 16 hours at room temperature. The reaction was concentrated *in vacuo* and the product was purified by silica gel column chromatography (solvent system: hexanes/ethyl acetate 60:40 to 20:80) to provide compound **15** (394 mg, 58 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.20 (s, 1H), 7.77 (br. s, 1H), 7.21 (d, ^{3}J= 8.1 Hz, 1H), 6.68 (br. s, 1H), 4.59 (d, ^{3}J= 6.9 Hz, 1H), 4.48 (d, ^{3}J= 6.9 Hz, 1H), 4.39 (q, ^{3}J= 7.3 Hz, 1H), 1.35 (s, 9H), 1.23 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ ppm: 168.2, 158.1, 156.0, 155.4, 153.1, 100.1, 93.3, 82.5, 78.1, 53.3, 48.0, 28.1, 27.2. MS (ESI): calc. 504.10, found 505.05 ([M+H]$^{+}$), and 527.05 ([M+ Na]$^{+}$). Purity (HPLC): 90 %.

(*S*)-*tert*-Butyl 3-(4-amino-3-((trimethylsilyl)ethynyl)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)-2-((*tert*-butoxycarbonyl)amino)propanoate (**16**)



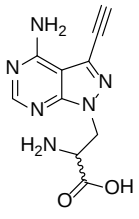
To a solution of compound **15** (394 mg, 1 eq, 0.78 mmol) in dry DMF (10 mL), copper(I) iodide (30 mg, 0.2 eq 0.15 mmol), tetrakis(triphenylphosphine)palladium(0) (135 mg, 0.15 eq, 0.12 mmol), triethylamine (436 µL, 4 eq, 3.12 mmol) and trimethylsilylacetylene (2224 µL, 20 eq, 15.6 mmol) were added. The reaction mixture was stirred under argon for 18 hours at room temperature. The reaction was concentrated *in vacuo* to a residue and the title compound was purified by silica gel column chromatography (solvent system: DCM/MeOH 100:0 to 95:5) to provide the title compound (285 mg, 77 % yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.24 (s, 1H), 7.78 (br. s, 1H), 7.19 (d, ^{3}J= 8.4 Hz, 1H), 6.28 (br. s, 1 H), 4.62 (d, ^{3}J= 6.1 Hz, 1H), 4.47 (d, ^{3}J= 7.7 Hz, 1H), 4.39 (q, ^{3}J= 7.4 Hz, 1H), 1.33 (s, 9H), 1.26 (s, 9H), 0.28 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ ppm: 168.7, 157.6, 156.5, 154.9, 153.4, 128.3, 100.7, 81.0, 78.4, 53.4, 47.3, 28.0, 27.3, -0.5, signals for the ethinyl-carbons were not visible in the spectrum. MS (ESI): calc. 474.24, found 475.25 ([M+H]$^{+}$), and 497.25 ([M+ Na]$^{+}$). Purity (HPLC): 98 %.

3-(4-Amino-3-ethynyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (**16a**)



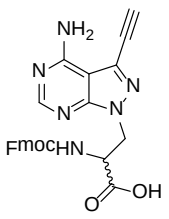
To a solution of compound **16** (270 mg, 1 eq, 0.56 mmol) in dry MeOH (10 mL), potassium carbonate (102 mg, 1.3 eq, 0.72 mmol) was added. The reaction mixture was stirred at room temperature for 5 hours. The consumption of starting material was monitored by TLC. Then, water (30 mL) was added and the solution was acidified to pH 6 with 1N aqueous hydrochloride solution and the product was extracted with EtOAc (3 x 30 mL). The organic layers were combined, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by silica gel column chromatography (solvent system: DCM/MeOH 80:20 to 50:50) yielded compound **16a** (140 mg, 71 % yield). MS (ESI): calc. 346.14, found 346.98 ($[M+H]^+$). Purity (HPLC): 93 %.

2-Amino-3-(4-amino-3-ethynyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)propanoic acid (**16b**)



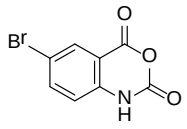
To a solution of compound **16a** (150 mg, 1 eq, 0.43 mmol) in dry DCM (8 mL), 1 mL of TFA was added. The reaction mixture was stirred at the room temperature for 20 hours. The consumption of the starting material was monitored by TLC. Then, the reaction mixture was evaporated *in vacuo* and co-evaporated with acetonitrile until TFA was completely removed. Compound **16b** (100 mg, 94 % yield) was used in the next step without further purification. MS (ESI): calc. 246.09, found 247.10 ($[M+H]^+$). Purity (HPLC): 99 %.

2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-amino-3-ethynyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)propanoic acid (**7**)



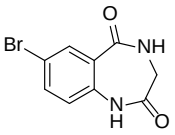
Compound **16b** (100 mg, 1 eq, 0.41 mmol) was dissolved in water (1.4 mL) and sodium bicarbonate (68.2 mg, 2 eq, 0.81 mmol) was added with stirring. The solution was cooled to 0 °C and *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (205 mg, 1.5 eq, 0.61 mmol), pre-dissolved in 1,4-dioxane (1.4 mL), was added slowly. The reaction mixture was stirred at 0 °C for 1 hour and at room temperature for 20 hours. Then, water (20 mL) was added and the product was extracted with EtOAc (3 x 20 mL). The organic layers were combined and back extracted with sat. aqueous $NaHCO_3$ solution (60 mL). The aqueous solution was acidified to pH 1 with 10 % aq. HCl, and extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by column chromatography (solvent system: DCM/MeOH 100:0 to 65:35) to provide **7** (135 mg, 71 % yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: δ ppm: 8.17 (s, 1H), 7.86 (d, ^3J= 7.6 Hz, 2H), 7.54 (2 x d, ^3J= 7.6 Hz, and ^3J= 7.5 Hz, 2H), 7.39 (m, 2H), 7.28 (2 x d, ^3J= 7.9 Hz, 2H), 4.75-4.70 (m, 1H), 4.64-4.59 (s, 1H), 4.56-4.51 (m, 1H), 4.35-4.31 (m, 1H), 4.20-4.18 (m, 2H), 4.16-4.13 (m, 1H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ ppm: 171.06, 156.6, 156.0, 155.2, 153.3, 143.8, 140.8, 127.8, 127.3, 125.9, 125.4, 125.3, 120.3, 101.0, 86.9, 74.9, 66.0, 53.4, 47.7, 46.6. MS (ESI): calc. 468.15, found 469.10 ($[M+H]^+$). Purity (HPLC): 99 %. In the NMR spectrum of the title compound we observed two sets of signals that indicate racemization of the amino acid. Racemization of amino acids is reported to occur under basic conditions that we applied for the synthesis of compound **16a**.

5-Bromoisatoic anhydride (**18**)



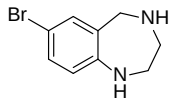
Bromine (10.8 g, 1.1 eq, 67.4 mmol) was added dropwise at 50 °C to a suspension of isatoic anhydride (10 g, 1 eq, 61.3 mmol) in water (300 mL). The mixture was stirred for 1 hour at 50 °C then it was allowed to cool to room temperature. The solid filtered off, washed with water, and with acetone giving 5-bromoisatoic anhydride as an off-white solid (6.9 g, 47 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 8.04 (br. s, 1H), 7.99 (d, ^{4}J= 2.4 Hz, 1H), 7.86 (dd, ^{3}J= 8.6 Hz and ^{4}J= 2.4 Hz, 1H), 7.11 (d, ^{3}J= 8.6 Hz, 1H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ ppm: 158.9, 146.9, 140.7, 139.4, 130.7, 117.8, 114.7, 112.4; MS (ESI): calc. 242.03, found 243.03 ([M+H]$^{+}$). Purity (HPLC): 97%.

7-Bromo-3,4-dihydro-1*H*-benzo[e][1,4]diazepine-2,5-dione (**19**)



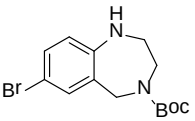
To a solution of 5-bromoisatoic anhydride (10 g, 1 eq, 41.3 mmol) in water (40 mL) was added glycine (4.4 g, 1.42 eq, 58.6 mmol) at room temperature. The reaction mixture was stirred at room temperature for 4 hours to give a cloudy solution. The reaction mixture was concentrated *in vacuo*. Glacial acetic acid was added and the reaction mixture was refluxed for 4.5 hours. The reaction mixture was cooled down slowly to room temperature. A precipitate formed. The reaction mixture was diluted with diethyl ether, then filtered through a sintered funnel to yield the title product (7.6 g, 73 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 8.02 (t, 1H), 8.04 (s, 1H), 7.84 (d, ^{4}J= 2.2 Hz, 1H), 7.70 (dd, ^{3}J= 8.6 Hz and ^{4}J= 2.2 Hz, 1H), 7.07 (d, ^{3}J= 8.7 Hz, 1H), 3.63 (d, ^{2}J= 13.3 Hz, 2H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ ppm: 168.9, 167.5, 137.4, 135.7, 130.2, 128.2, 124.1, 116.5, 45.1. MS (ESI): calc. 253.97, found 254.4 ([M+H]$^{+}$). Purity (HPLC): 98 %.

7-Bromo-2,3,4,5-tetrahydro-1*H*-benzo[e][1,4]diazepine (**20**)



A solution of borane in THF (1M, 15 eq, 28 mL) was slowly added to a stirred solution of **19** (5 g, 1 eq, 19.6 mmol) in dry THF. The reaction mixture was refluxed for 18 hours. After cooling to 0 °C, MeOH (9 mL) was added carefully, the solvent was evaporated and the residue was dissolved in MeOH (20 mL). To this solution, 7N aq. HCl (5 mL) was added and the mixture was heated to dryness. The resulting solid was suspended in $NaHCO_3$ (saturated aqueous solution, 100 mL) and the suspension was brought to pH 9 with 5N aq. NaOH. The product was extracted with 3 x 100 mL of CH_2Cl_2 and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (solvent system: DCM/MeOH 90:10) afforded **20** as a light yellow solid (2.55 g, 58 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 7.21 (dd, ^{3}J= 8.4 Hz and ^{4}J= 2.4 Hz, 1H), 7.18 (d, ^{4}J= 2.4 Hz, 1H), 6.46 (d, ^{3}J= 8.4 Hz, 1H), 5.61 (br. s, 1H), 3.65 (s, 2H), 2.89-3.00 (m, 2H), 2.75-2.82 (m, 1H), 2.12 (m, 1H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ ppm: 151.2, 135.5, 132.2, 130.1, 121.2, 110.7, 54.5, 52.3, 50.6. MS (ESI): calc. 226.01, found 227.95 ([M+H]$^{+}$). Purity (HPLC): 98 %.

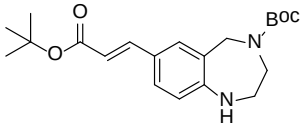
***tert*-Butyl 7-bromo-2,3-dihydro-1*H*-benzo[e][1,4]diazepine-4(5H)-carboxylate** (**21**)



To a stirred solution of compound **20** (2.73 g, 1 eq, 12.1 mmol) in dry methanol (10 mL) was added di-*tert*-butyl dicarbonate (2.64 g, 1 eq, 12.1 mmol) at 0° C under argon. The mixture was stirred at room temperature for 18 hours. The solvent was removed and the residue was purified by column chromatography (solvent system: hexanes/EtOAc 80:20), affording the pure compound **21** as a beige

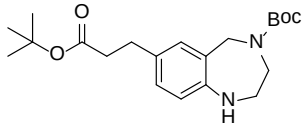
solid (2.29 g, 58 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 7.42 (dd, ^{3}J= 8.0 Hz and ^{4}J= 2.6 Hz, 1H), 7.39 (d, ^{4}J= 2.6 Hz, 1H), 6.42 (d, ^{3}J= 8.0 Hz, 1H), 4.25 (d, ^{2}J= 16.0 Hz, 2H), 4.11 (br. s, 1H), 3.33 (dd, ^{2}J= 13.6 Hz and ^{3}J= 4.8 Hz, 2H), 3.19 (dd, ^{2}J= 13.6 Hz and ^{3}J= 8.3 Hz, 2H), 1.39 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ ppm: 154.3, 147.3, 133.5, 130.7, 123.4, 113.8, 111.3, 79.8, 56.3, 55.5, 43.6, 28.4. MS (ESI): calc. 326.06, found 327.22 ([M+H]$^{+}$). Purity (HPLC): 96 %.

(E)-*tert*-Butyl 7-(3-(*tert*-butoxy)-3-oxoprop-1-en-1-yl)-2,3-dihydro-1*H*-benzo[e][1,4]diazepine-4(5*H*)-carboxylate (**22**)



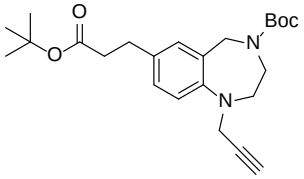
Palladium (II) acetate (200 mg, 0.2 eq, 0.92 mmol) was added to a degassed solution of **21** (1.5 g, 1 eq, 4.6 mmol), tri-o-tolylphosphine (700 mg, 0.5 eq, 2.3 mmol), triethylamine (2.6 mL, 4 eq, 18.4 mmol) and *tert*-butyl acrylate (4.1 mL, 6 eq, 27.9 mmol) in dry acetonitrile (8 mL). The mixture was refluxed for 18 hours. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (solvent system: DCM/MeOH 90:10) giving the title compound **22** (870 mg, 51 % yield). ^{1}H-NMR: (500 MHz, $CDCl_3$) δ ppm: 7.95 (dd, ^{3}J= 8.4 Hz and ^{4}J= 2.1 Hz, 1H), 7.44 (d, ^{2}J= 15.8 Hz, 1H), 7.38 (d, ^{4}J= 2.1 Hz, 1H), 7.06 (d, ^{3}J= 8.4 Hz, 1H), 6.29 (d, ^{2}J= 16.0 Hz, 1H), 4.25 (d, ^{2}J= 15.2 Hz, 2H), 4.05 (br. s, 1H), 3.34 (dd, ^{2}J= 13.6 Hz and ^{3}J= 4.8 Hz, 2H), 3.20 (dd, ^{2}J= 13.6 Hz and ^{3}J= 8.3 Hz, 2H), 1.48 (s, 9H), 1.39 (s, 9H). ^{13}C-NMR: (126 MHz, $CDCl_3$) δ ppm: 166.5, 154.3, 147.5, 145.1, 127.9, 126.3, 123.4, 121.1, 116.2, 107.8, 81.2, 79.8, 57.4, 55.5, 43.6, 28.8, 28.4. MS (ESI): calc. 374.22, found 375.47 ([M+H]$^{+}$). Purity (HPLC): 98 %.

***tert*-Butyl 7-(3-(*tert*-butoxy)-3-oxopropyl)-2,3-dihydro-1*H* benzo[e][1,4]diazepine-4(5*H*)-carboxylate** (**23**)



To a stirred solution of **22** (700 mg, 1 eq, 1.87 mmol) in dry methanol (5 mL) at room temperature under hydrogen was added palladium on carbon, Pd/C (10 %, 50 mg, 0.25 eq, 0.5 mmol). The reaction mixture was stirred under hydrogen for 18 hours, filtered and the residue was additionally washed with methanol. The filtrate was then concentrated and the product was purified by column chromatography (solvent system: DCM /MeOH 90:10), affording pure compound **23** (476 mg, 68 % yield). ^{1}H-NMR: (500 MHz, DMSO-d_6) δ ppm: 7.12 (d, ^{4}J= 2.1 Hz, 1H), 6.99 (dd, ^{3}J= 8.4 Hz and ^{4}J= 2.1 Hz, 1H), 6.56 (d, ^{3}J= 8.4 Hz, 1H), 4.25 (d, ^{2}J= 15.2 Hz, 2H), 4.05 (br. s, 1H), 3.34 (dd, ^{2}J= 13.6 Hz and ^{3}J= 4.8 Hz, 2H), 3.20 (dd, ^{2}J= 13.6 Hz and ^{3}J= 8.3 Hz, 2H), 2.92 (dd, ^{2}J= 12.6 Hz and ^{3}J= 4.5 Hz, 2H), 2.55 (dd, ^{2}J= 12.6 Hz and ^{3}J= 8.1 Hz, 2H), 1.48 (s, 9H), 1.39 (s, 9H). ^{13}C-NMR: (126 MHz, DMSO-d_6) δ ppm: 171.7, 154.3, 145.5, 129.3, 127.6, 126.8, 121.1, 113.2, 82.1, 79.8, 57.3, 55.5, 43.6, 34.7, 28.7, 28.4. MS (ESI): calc. 376.24, found 377.49 ([M+H]$^{+}$). Purity (HPLC): 97 %.

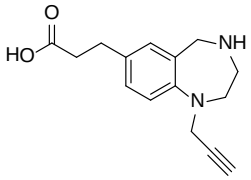
***tert*-Butyl 7-(3-(*tert*-butoxy)-3-oxopropyl)-1-(prop-2-yn-1-yl)-2,3-dihydro-1*H* benzo[e][1,4]diazepine-4(5*H*)-carboxylate** (**24**)



In a 2-neck round-bottomed flask fitted with argon gas and septum was placed compound **23** (535 mg, 1 eq, 1.42 mmol) dissolved in dry DMF (3 mL). To the suspension was added Cs_2CO_3 (930 mg, 2 eq, 2.84 mmol) of at room temperature. Stirring was continued for 20 minutes and propargyl bromide (189 µL, 2 eq, 2.5 mmol) was slowly added. Stirring was then continued at 60 °C overnight. Then, the solvent was evaporated, and the residue was purified by column chromatography (solvent system: DCM/MeOH 95:5 to 90:10, the solvent contained 0.5 % of

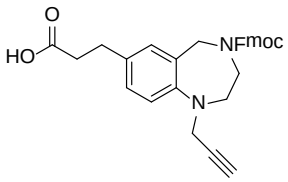
TEA) yielding pure compound **24** (278.2 mg, 47 % yield). ^{1}H-NMR: (400 MHz, DMSO-d_6) δ ppm: 7.16 (d, ^{4}J= 2.1 Hz, 1H), 7.03 (dd, ^{3}J= 8.4 Hz and ^{4}J= 2.1 Hz, 1H), 6.59 (d, ^{3}J= 8.4 Hz, 1H), 4.25 (d, ^{2}J= 15.2 Hz, 2H), 4.07 (d, ^{2}J= 15.8 Hz, 2H), 3.62 (dd, ^{2}J= 13.6 Hz and ^{3}J= 8.3 Hz, 2H), 3.18 (dd, ^{2}J= 13.6 Hz and ^{3}J= 4.8 Hz, 2H), 2.92 (dd, ^{2}J= 12.6 Hz and ^{3}J= 4.5 Hz, 2H), 2.63 (s, 1H), 2.55 (dd, ^{2}J= 12.6 Hz and ^{3}J= 8.1 Hz, 2H), 1.48 (s, 9H), 1.39 (s, 9H). ^{13}C-NMR: (101 MHz, DMSO-d_6) δ ppm: 171.7, 154.3, 145.5, 129.3, 127.6, 126.8, 121.1, 113.2, 82.1, 79.8, 78.1, 73.2, 57.3, 55.5, 46.7, 43.6, 34.7, 28.7, 28.4. MS (ESI): calc. 414.54, found 415.34 ([M+H]$^{+}$). Purity (HPLC): 95 %.

3-(1-(Prop-2-yn-1-yl)-2,3,4,5-tetrahydro-1*H*-benzo[e][1,4]diazepin-7-yl)propanoic acid (**24a**)



TFA (4.4 mL) was added to a solution of **24** (440 mg, 1.1 mmol) in dry dichloromethane (12 mL). The reaction was stirred overnight at room temperature for 19 hours. The solvent was removed *in vacuo* and the residue was triturated with diethyl ether to afford compound **24a** (276 mg, 97 % yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 10.79 (s, 1H), 7.16 (d, ^{4}J= 2.1 Hz, 1H), 7.03 (dd, ^{3}J= 8.4 Hz and ^{4}J= 2.1 Hz, 1H), 6.59 (d, ^{3}J= 8.4 Hz, 1H), 4.07 (d, ^{2}J= 15.8 Hz, 2H), 3.89 (d, ^{2}J= 15.2 Hz, 2H), 3.42 (dd, ^{2}J= 13.6 Hz and ^{3}J= 8.3 Hz, 2H), 2.75 (dd, ^{2}J= 13.6 Hz and ^{3}J= 4.8 Hz, 2H), 2.81 (dd, ^{2}J= 12.6 Hz and ^{3}J= 4.5 Hz, 2H), 2.63 (s, 1H), 2.49 (dd, ^{2}J= 12.6 Hz and ^{3}J= 8.1 Hz, 2H), 1.99 (m, 1H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ ppm: 174.4, 143.4, 129.4, 128.7, 126.9, 121.9, 120.2, 78.1, 73.2, 59.2, 53.3, 50.1, 46.7, 34.2, 30.5. MS (ESI): calc. 258.14, found 259.32 ([M+H]$^{+}$). Purity (HPLC): 98 %.

3-(4-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-1-(prop-2-yn-1-yl)-2,3,4,5-tetrahydro-1*H*-benzo[e][1,4]diazepin-7-yl)propanoic acid (**8**)



Compound **24a** (350 mg, 1eq, 1.35 mmol) was dissolved in water (2 mL) and sodium bicarbonate (220 mg, 2 eq, 2.6 mmol) was added with stirring. The resulting solution was cooled to 5 °C and Fmoc-OSu (685 mg, 1.5 eq, 2.03 mmol) was added slowly as a solution in *para*-dioxane (also cooled, 4 mL). The resulting mixture was stirred at 0 °C for 1 hour and allowed to warm to room temperature overnight. Water was then added and the aqueous layer was extracted 2 times with EtOAc. The organic layer was extracted back twice with saturated sodium bicarbonate solution. The combined aqueous layers were acidified to a pH of 1 with 10 % aq. HCl, and extracted 3 times with EtOAc. The combined organic layers were dried (sodium sulfate) and concentrated *in vacuo*. The residue was purified by column chromatography (solvent system: DCM/MeOH 90:10). (457.4 mg, 71 % yield). ^{1}H-NMR (500 MHz, DMSO-*d*6) δ ppm: 10.79 (s, 1H), 7.88 (d, ^{3}J= 6.8 Hz, 2H), 7.53 (d, ^{3}J= 7.4 Hz, 2H), 7.38 (dd, ^{3}J= 7.1 Hz and ^{3}J= 5.1 Hz, 2H), 7.27 (dd, ^{3}J= 7.9 Hz and ^{4}J= 2.1 Hz, 2H), 7.16 (d, ^{4}J= 2.1 Hz, 1H), 7.03 (dd, ^{3}J= 8.4 Hz and ^{4}J= 2.1 Hz, 1H), 6.59 (d, ^{3}J= 8.4 Hz, 1H), 4.73 (dd, ^{2}J= 15.4 Hz and ^{3}J= 4.3 Hz, 2H), 4.48 (d, ^{3}J= 5.1 Hz, 1H), 4.27 (d, ^{2}J= 15.2 Hz, 2H), 4.07 (d, ^{2}J= 15.8 Hz, 2H), 3.66 (dd, ^{2}J= 13.6 Hz and ^{3}J= 8.3 Hz, 2H), 3.22 (dd, ^{2}J= 13.6 Hz and ^{3}J= 4.8 Hz, 2H), 2.81 (dd, ^{2}J= 12.6 Hz and ^{3}J= 4.5 Hz, 2H), 2.63 (s, 1H), 2.49 (dd, ^{2}J= 12.6 Hz and ^{3}J= 8.1 Hz, 2H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ ppm: 174.4, 162.8, 143.6,143.4, 142.6, 129.4, 128.7, 126.9, 126.7, 126.2,125.2, 121.9, 120.5, 120.2, 78.1, 73.2, 67.6, 57.6, 56.4, 52.4, 47.0, 46.7, 34.2, 30.5. MS (ESI): calc. 480.55, found 481.57 ([M+H]$^{+}$), and 503.01 ([M+Na]$^{+}$). Purity (HPLC): 98 %.

3. Coupling of carboxylic acid building blocks to DNA scaffold conjugates

a) Synthesis of DNA-PEG-linker conjugates 25, 26

The MMt-protective group of the 5'-aminolinker-modified DNA bound to 1000 Å controlled pore glass (CPG) solid support (1 µmol, ca. 40 mg) was removed by addition of 3 % trichloroacetic acid in dry DCM (3 x 200 µL) for 3 x 1 min. A yellow color indicated successful removal of the protective group. The CPG containing the deprotected DNA was then washed three times with each 200 µL of 1 % TEA in MeCN, then DMF, MeOH, MeCN and DCM. The CPG, the MMt-NH-PEG(8)-COOH linker or Fmoc-NH-PEG(4)-COOH linker, and HATU as a coupling reagent were dried *in vacuo* for 15 min. Stock solutions of all reactants in dry DMF were prepared immediately before reaction was started. To 150 µL of a solution of the MMt-NH-PEG(8)-COOH linker (71 mg, 100 µM, 100 eq.) or the Fmoc-NH-PEG(4)-COOH linker (54 mg, 100 µmol, 100 eq.) in dry DMF were added HATU (19 mg, 50 µM, 100 eq.) dissolved in 150 µL of dry DMF and DIPEA (42 µL, 250 eq.). This reaction mixture was shaken for 5 min and added to the solid support-bound DNA suspended in dry DMF (150 µL). The amide coupling reaction was shaken at room temperature for 2 hours. Then, the CPG containing the DNA-PEG linker conjugate was filtered off using a filter column and washed subsequently with each 3 x 200 µL of DMF, MeOH, MeCN and DCM. Unreacted amines were capped with acetic acid anhydride (a 1:1 mixture of THF/methylimidazole, 9:1, vol/vol, and THF/pyridine/acetic acid anhydride, 8:1:1, vol/vol was used), and the CPG was again washed subsequently with each 3 x 200 µL of DMF, MeOH, MeCN and DCM, and dried *in vacuo* for 15 min. For analysis, an aliquot of ca. 10 nmol of the DNA-PEG conjugate was deprotected and cleaved from the CPG by treatment with 500 µL of AMA (AMA= aqueous ammonia (30 %)/ aqueous methylamine (40 %)= 1:1, vol/vol) for 4 hours at room temperature. Then, 20 µL of 1 M Tris buffer (pH= 7.5) were added, the mixture was dried in a SpeedVac, re-dissolved in 100 µL of distilled water, and the product was analyzed by RP-HPLC (Gemini, 5u, C18, 110A column; 100*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20% – 70% of methanol over 19 min).

b) Synthesis of DNA-scaffold conjugates 27-29

Prior coupling of the scaffold to the PEG-linker, the protective group of the linker was removed: The MMt-protective group of the DNA-PEG(8) linker conjugate bound to 1000 Å controlled pore glass (CPG) solid support (1 µmol, ca. 40 mg of the solid support) was removed by addition of 3 % trichloroacetic acid in dry DCM (3 x 200 µL) for 3 x 1 min. A yellow color indicated successful removal of the protective group. The Fmoc-group of the DNA-PEG(4) linker conjugate bound to 1000 Å controlled pore glass (CPG) solid support (1 µmol, ca. 40 mg of the solid support) was removed by addition of 20 % piperidine in dry DMF (1 mL). The reaction mixture was shaken for 5 min at room temperature. The CPG containing the deprotected DNA-PEG linker conjugate was washed three times with each 200 µL of 1 % TEA in MeCN, then DMF, MeOH, MeCN and DCM. The solid support-bound DNA-PEG linker conjugates, one of the compounds **6**-**8** and HATU as a coupling reagent were dried *in vacuo* for 5 min. To a 250 µL of a solution of the compound **6**-**8** (100 µmol, 100 eq.) in dry DMF were added HATU (38 mg, 100 µmol, 100 eq.) dissolved in 250 µL of dry DMF, and DIPEA (43 µL, 250 µmol, 250 eq.). Compounds **6**-**8** were activated for 5 min at room temperature in a shaker. Then, the activated carboxylic acids were added to the solid support-bound DNA-PEG linker conjugates **25, 26** suspended in dry DMF (250 µL). The amide coupling reactions were shaken at room temperature for 4 hours. The CPGs containing the amide coupling products **27**-**29** were filtered off using a filter column and washed subsequently with each 3 x 200 µL of DMF, MeOH, MeCN and DCM, they were then capped with acetic acid anhydride (a 1:1 mixture of THF/methylimidazole, 9:1, vol/vol, and

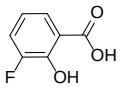
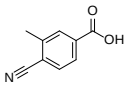
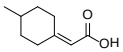
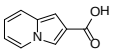
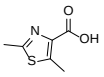
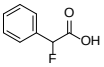
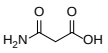
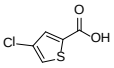
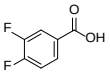
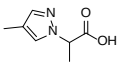
THF/pyridine/acetic acid anhydride, 8:1:1, vol/vol was used), washed again with each 3 x 200 µL of DMF, MeOH, MeCN and DCM, and dried *in vacuo* for 15 min. For analysis, an aliquot of ca. 10 nmol of the each DNA-small molecule conjugates **27**-**29** was deprotected and cleaved from the CPG by treatment with 500 µL of AMA (AMA= aqueous ammonia (30 %)/ aqueous methylamine (40 %)= 1:1, vol/vol) for 4 hours at room temperature. Then, 20 µL of 1 M Tris buffer (pH= 7.5) were added, the mixture was dried in a SpeedVac, re-dissolved in 100 µL of distilled water, and the product was analyzed by RP-HPLC (Gemini, 5u, C18, 110A column; 100*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20% – 70% of methanol over 19 min).

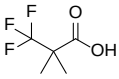
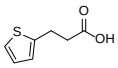
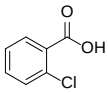
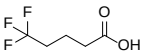
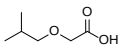
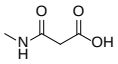
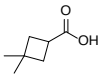
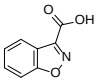
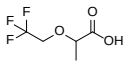
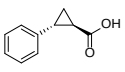
c) Coupling reactions of 114 carboxylic acids to DNA-scaffold conjugates 27-**29**

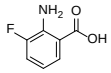
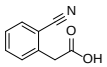
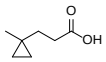
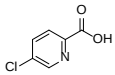
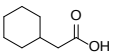
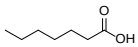
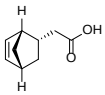
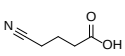
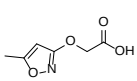
For library synthesis, sets of 20 carboxylic acids (see **Table S1**, building blocks **A**-**CV**) were coupled to the DNA-scaffold conjugates **27**-**29** in parallel (a total of 60 reactions was performed in parallel).

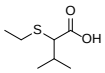
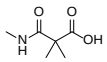
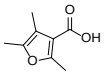
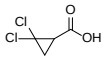
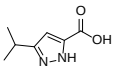
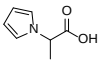
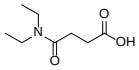
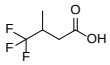
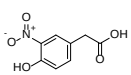
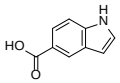
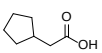
For Fmoc-deprotection of DNA-small molecule conjugates **27**-**29** 400 nmol (ca. 16 mg) of each DNA-small molecule conjugate bound to 1000 Å controlled pore glass (CPG) solid support was treated with 20 % piperidine in dry DMF (400 µL) for 5 min at room temperature. The solid support containing the Fmoc-deprotected conjugates **27**-**29** was filtered off and washed with each 3 x 200 µL of DMF, MeOH, MeCN and DCM. Then, the three batches of solid support with the DNA conjugates **27**-**29**, a set of 20 carboxylic acids (each 2 µmol per coupling reaction, a total of 6 µmol) , and HATU as a coupling reagent were dried *in vacuo* for 15 min. Then, each of the three batches of solid support containing one of the DNA conjugates **27**-**29** (each 16 mg) was split in 4 Eppendorf tubes (each 4 mg) on a balance, giving a total of 12 sub-batches (3 x 4). Each of these 12 sub-batches was suspended in 100 µL of dry DMF. These 12 suspensions were distributed to a total of 60 reaction vessels of a 96well plate, so that each reaction vessel contained ca. 20 nmol of a DNA-conjugate **27**-**29** suspended in 20 µL of dry DMF. Then, each of the 20 carboxylic acids (6 µmol;) was dissolved in 60 µL of dry DMF. To these solutions were added each HATU (6 µmol), dissolved in 60 µL of DMF, (taken from a stock solution: 120 µmol, 76 mg in 2 mL of dry DMF), and 2.6 µL (250 eq.) of DIPEA giving a total volume of ca. 120 µL. The 20 carboxylic acids were activated for 5 min with shaking. Then, from each solution of activated carboxylic acids 40 µL were added to each of the three DNA conjugates **27**-**29**, so that the coupling reaction was performed with 100 eq. of the carboxylic acid. The reaction mixtures were shaken at room temperature for 4 hours. The solid phase containing the amide coupling products were filtered off using a filter plate and washed subsequently with each 3 x 200 µL of DMF, MeOH, MeCN and DCM and dried *in vacuo* for 15 min. Then, the DNA-conjugates were deprotected and cleaved from the CPG by treatment with 500 µL of AMA (AMA= aqueous ammonia (30 %)/ aqueous methylamine (40 %)= 1:1, vol/vol) for 5 hours at room temperature. Samples were filtered directly from a filter plate into a 96-deep well plate. Then, 20 µL of 1 M Tris buffer (pH= 7.5) was added, the mixtures were dried in a SpeedVac, re-dissolved in 100 µL of distilled water, and all coupling products were purified by RP-HPLC (Gemini, 5u, C18, 110A column; 100*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20% – 70% of methanol over 19 min). Fractions containing the product were collected, evaporated in a SpeedVac, co-evaporated with 3 x 200 µL of ethanol/distilled water (1:1) and analyzed by MALDI-TOF-MS analysis and by analytical HPLC to assert purity and identity. This procedure was repeated until all 114 carboxylic acids were coupled to DNA- conjugates **27**-**29**.

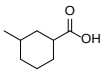
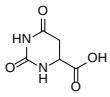
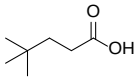
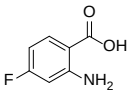
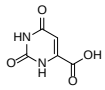
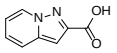
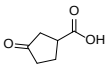
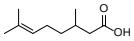
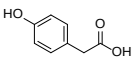
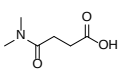
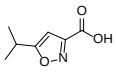
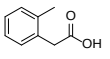
Table S1: Yield, conversion rates and MALDI MS data of compounds **27A-CV** – **29A-CV**.[1]

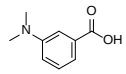
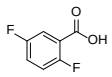
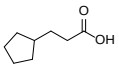
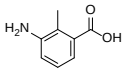
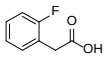
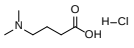
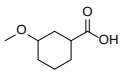
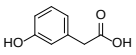
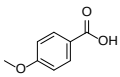
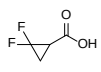
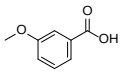
		27			28			29		
No.	**Structure**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**
A		1.17	50	7842.3 7849.0	1.14	50	7935.4 7942.9	0.10	50	7971.3 7974.0
B		3.65	60	7847.3 7850.0	0.10	60	7940.5 7949.8	0.91	65	7976.3 7977.3
C		3.39	55	7840.4 7846.0	1.74	55	7933.5 7940.1	0.41	60	7969.4 7971.0
D		1.36	75	7847.4 7845.0	1.43	70	7940.5 7949.4	0.10	80	7976.3 7977.5
E		2.54	100	7843.4 7848.9	1.67	90	7936.5 7942.6	4.66	95	7972.3 7976.6
F		0.19	25	7840.4 7843.0	0.10	10	7933.5 7934.5	0.10	30	7969.3 7967.8
G		4.04	100	7825.3 7825.5	1.27	100	7918.4 7917.0	1.95	100	7954.3 7954.6
H		3.73	95	5012.5 5010.8	0.10	85	5145.6 5148.0	1.33	95	5157.7 5154.6
I		0.10	95	7848.8 7845.2	0.10	85	7941.9 7949.0	3.16	95	7977.7 7979.8
J		0.10	90	7844.3 7842.7	0.10	85	7937.4 7941.0	2.85	90	7973.2 7974.5
K		5.26	20	7830.3 7829.0	1.41	15	7923.4 7918.9	0.58	30	7959.2 7957.1
L		2.83	60	7832.4 7832.0	0.10	20	7925.5 7925.0	0.10	60	7961.3 7960.4
M		0.10	90	7840.4 7856.0	2.79	90	7933.5 7931.8	0.19	95	7969.3 7974.6
N		0.08	95	7856.3 7858.0	0.10	90	7949.4 7943.6	1.19	95	7985.3 7988.1

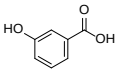
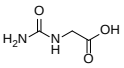
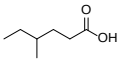
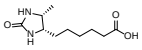
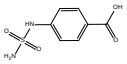
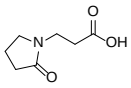
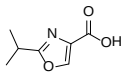
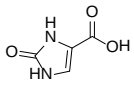
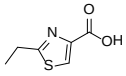
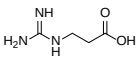
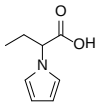
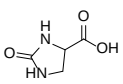
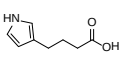
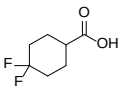
		27			28			29		
No.	**Structure**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**
O		0.10	40	7822.3 7822.6	0.67	10	7915.4 7922.0	0.74	15	7951.2 7949.8
P		2.29	90	7842.3 7841.5	0.68	85	7935.4 7945.1	5.73	90	7971.2 7969.2
Q		6.49	65	7842.4 7835.0	1.25	75	7935.5 7940.4	4.88	85	7971.3 7974.4
R		3.84	75	7847.4 7846.3	1.88	70	7940.5 7944.8	2.29	85	7976.3 7977.6
S		1.76	80	7842.8 7845.4	1.13	75	7935.9 7940.8	1.20	80	7971.3 7974.6
T		0.41	75	7816.4 7820.2	3.26	65	7909.5 7910.6	0.75	75	7945.3 7943.7
U		0.49	95	7842.3 7845.8	1.19	90	7935.4 7936.3	3.39	90	7971.2 7971.5
V		2.67	90	7804.3 7811.0	0.38	60	7911.5 7910.8	0.82	90	7947.3 7949.5
W		4.40	75	7817.3 7815.0	4.11	70	5159.6 5161.0	1.68	80	5171.7 5179.0
X		0.57	90	7814.4 7817.3	1.91	75	7907.5 7912.4	1.63	90	7943.3 7945.1
Y		0.10	85	7849.3 7854.3	0.10	80	7942.5 7942.5	1.49	85	7978.3 7981.2
Z		0.53	10	7843.8 7844.6	1.26	5	7936.9 7936.9	2.17	10	7972.7 7978.2
AA		4.03	85	7858.3 7860.2	1.59	10	7951.4 7952.1	0.59	80	7987.3 7988.2
AB		1.03	50	7848.4 7850.5	1.01	45	7941.5 7948.0	2.74	55	7977.3 7979.3
AC		0.10	75	5066.6 5067.0	/	/	/	0.60	80	7977.3 7978.3

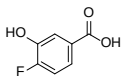
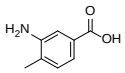
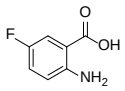
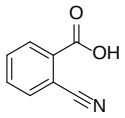
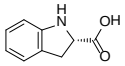
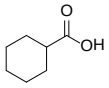
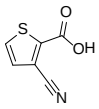
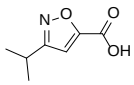
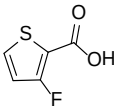
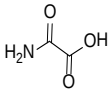
		27			28			29		
No.	**Structure**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**
AD		0.80	85	7825.3 7829.5	0.10	70	5199.7 5196.0	1.14	80	5209.7 5212.0
AE		3.10	85	5064.6 5064.5	0.88	70	7918.5 7919.5	3.69	80	7954.3 7954.7
AF		1.49	10	5070.6 5066.0	2.64	40	5203.7 5202.2	2.82	40	5215.8 5215.3
AG		3.49	60	5037.6 5045.0	0.68	10	4994.5 4987.5	1.41	100	5182.8 5181.2
AH		1.13	60	5067.0 5070.0	1.47	55	5200.1 5204.8	4.59	100	5212.2 5212.0
AI		2.42	80	5071.6 5073.0	1.92	20	5028.5 5030.0	3.70	70	5216.8 5217.0
AJ		0.10	90	4875.4 4878.4	1.09	10	5008.5 5005.6	2.75	85	5196.8 5195.0
AK		3.18	75	5049.6 5055.0	3.29	35	5182.7 5183.5	2.75	100	5194.8 5194.0
AL		2.59	65	5039.6 5047.0	0.78	40	5172.7 5171.5	4.42	100	5184.8 5184.0
AM		2.40	75	5011.5 5014.4	0.10	25	4968.4 4968.2	1.76	100	5156.7 5157.0
AN		1.84	100	5061.6 5069.0	0.60	30	5018.5 5015.9	1.39	100	5206.8 5206.0
AO		0.10	100	4875.4 4879.6	0.46	35	5008.5 5009.2	1.05	100	5196.8 5197.0
AP		0.86	90	5022.5 5024.6	0.97	50	4979.4 4979.3	1.74	90	4991.5 4992.0
AQ		1.89	90	5035.6 5034.0	0.67	30	4992.5 4997.7	2.65	100	5180.8 5179.0
AR		1.36	55	5066.5 5074.0	1.36	50	5199.6 5204.7	4.51	55	5211.7 5207.1

		27			28			29		
No.	**Structure**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**
AS		0.10	70	4895.4 4897.4	0.45	55	5204.8 5203.0	/	/	/
AT		0.10	25	4878.4 4879.2	1.45	30	5011.5 5015.1	3.89	100	5199.8 5198.0
AU		0.10	80	4887.4 4888.0	0.10	60	5196.7 5198.3	2.67	65	5208.8 5211.6
AV		3.10	90	5023.5 5024.0	1.02	35	4980.5 4977.0	0.10	90	4992.5 4992.0
AW		2.61	40	5064.4 5063.0	2.61	30	5197.5 5193.0	0.90	55	5209.6 5210.0
AX		0.10	90	4887.4 4890.4	0.76	60	5020.5 5017.5	1.60	100	5208.8 5208.0
AY		7.20	90	5048.6 5053.0	0.81	50	5181.7 5183.1	3.30	100	5193.8 5194.0
AZ		13.14	100	5082.6 5088.0	/	/	/	5.12	100	5227.8 5226.0
BA		8.55	95	5062.6 5067.0	0.20	45	5195.8 5195.6	7.18	95	5207.8 5208.0
BB		11.17	100	5065.5 5065.0	/	/	/	4.88	100	5210.7 5210.0
BC		1.23	100	5063.5 5071.0	0.16	40	5196.6 5197.3	5.46	100	5208.7 5208.7
BD		20.77	100	5071.5 5078.0	/	/	/	17.87	100	5216.7 5215.0
BE		16.68	80	5106.5 5106.0	4.80	20	5239.7 5238.7	13.46	100	5251.8 5251.8
BF		11.78	100	5070.6 5076.0	7.64	25	5203.7 5207.7	6.76	100	5215.8 5215.0
BG		3.67	100	5037.6 5046.0	0.10	15	5170.7 5170.0	5.23	100	5182.8 5184.5

		27			28			29		
No.	**Structure**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**
BH		8.84	90	5052.4 5051.0	6.47	80	5185.5 5185.1	4.36	100	5197.6 5197.0
BI		27.78	90	5045.6 5052.0	7.97	85	5178.7 5180.3	28.80	90	5190.8 5190.0
BJ		12.91	90	5051.6 5056.0	4.21	80	5184.7 5184.0	15.80	100	5196.8 5195.0
BK		7.04	90	5067.5 5070.0	2.79	80	5200.6 5203.4	4.13	100	5212.7 5212.0
BL		6.00	90	5039.6 5047.0	2.05	80	5172.7 5171.7	8.40	100	5184.8 5184.0
BM		1.48	80	5064.5 5069.0	5.12	60	5197.7 5205.0	3.71	75	5209.7 5210.0
BN		3.40	80	5065.5 5070.0	0.98	60	5198.6 5197.2	10.51	85	5210.7 5210.0
BO		8.55	100	5071.5 5077.0	0.22	85	5204.7 5204.7	11.50	95	5216.8 5215.0
BP		14.09	100	5037.5 5037.8	3.52	85	5170.7 5162.1	9.91	90	5182.7 5182.0
BQ		2.22	70	5079.7 5081.0	1.07	55	5212.8 5210.8	0.15	60	5224.9 5225.0
BR		7.71	100	5061.6 5063.5	0.17	90	5194.7 5190.1	13.16	95	5206.8 5209.7
BS		0.17	100	4833.3 4839.4	5.99	80	5142.6 5144.9	0.47	100	4978.5 4978.8
BT		1.54	100	5054.6 5061.0	0.26	70	5187.8 5184.3	3.36	95	5199.8 5198.0
BU		0.10	60	5064.6 5066.8	2.27	55	5197.7 5195.2	1.66	55	5209.8 5211.8
BV		5.11	100	5059.6 5068.0	2.44	65	5192.7 5188.5	0.10	100	5204.8 5207.5

		27			28			29		
No.	**Structure**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**
BW		5.13	95	5074.6 5082.6	/	/	/	0.10	100	5219.8 5221.0
BX		2.09	95	5067.5 5068.5	1.50	90	5200.6 5196.3	0.10	100	5212.7 5215.8
BY		2.20	100	5051.6 5057.0	1.67	100	5184.7 5188.8	2.44	100	5196.8 5197.0
BZ		0.10	80	5060.6 5061.6	0.10	85	5193.7 5192.3	1.49	90	5205.8 5205.0
CA		1.48	50	5063.6 5063.0	0.17	50	5196.7 5201.3	/	/	/
CB		1.34	20	5077.1 5078.0	1.24	15	5210.2 5208.8	1.11	35	5222.2 5222.0
CC		2.25	90	5051.6 5055.0	/	/	/	0.10	90	5196.8 5195.0
CD		0.98	90	5025.6 5031.0	/	/	/	/	/	/
CE		0.17	95	5025.6 5026.0	/	/	/	0.91	95	5170.8 5170.0
CF		2.93	95	5023.5 5033.3	/	/	/	1.92	95	5168.8 5168.0
CG		0.10	95	5067.6 5062.9	/	/	/	3.74	90	5212.8 5212.0
CH		2.64	80	5061.6 5071.0	/	/	/	5.68	85	5206.8 5205.0
CI		2.16	95	5061.6 5062.0	/	/	/	3.59	100	5206.8 5206.0
CJ		0.10	100	5031.5 5032.0	0.48	75	5164.6 5165.4	0.10	100	5176.7 5171.4
CK		4.52	90	5061.5 5062.3	/	/	/	/	/	/

		27			28			29		
No.	**Structure**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**	**Yield[2] [nmol]**	**Con. (%)[3]**	**Mass calc. Mass found[4]**
CL		3.81	100	5067.5 5065.0	/	/	/	/	/	/
CM		0.10	95	5047.5 5050.0	1.99	90	5180.6 5183.0	0.34	100	5192.7 5192.0
CN		1.53	40	5027.5 5037.0	/	/	/	/	/	/
CO		/	/	/	/	/	/	0.68	100	5184.8 5185.0
CP		1.57	90	5215.6 5219.0	0.57	60	5258.7 5251.5	2.09	95	5270.8 5270.0
CQ		1.70	80	5123.7 5122.0	0.59	50	5256.8 5263.2	3.23	90	5268.9 5269.0
CR		0.49	100	7843.4 7844.2	1.19	100	7947.5 7949.6	3.39	100	7983.6 7987.3
CS		2.67	100	7841.4 7845.6	0.38	100	7945.5 7945.7	0.82	100	7981.6 7982.6
CT		1.03	100	7814.3 7816.4	1.01	100	7907.4 7907.1	2.74	100	7943.5 7944.5
CU		0.10	100	7843.4 7846.6	0.20	100	7936.5 7936.7	0.60	100	7972.6 7974.0
CV		3.10	100	5040.6 5043.5	0.89	100	5173.7 5175.6	1.68	100	5185.8 5186.2
CW			/			/			/	
CX			/			/			/	
CY			/			/			/	
CZ			/			/			/	

No.	Structure	27	28	29
DA		/	/	/
DB		/	/	/
DC		/	/	/
DD		/	/	/
DE		/	/	/
DF		/	/	/
DG		/	/	/
DH		/	/	/
DI		/	/	/
DJ		/	/	/

[1] **27-29A-G**, **27-29I-AB** and **27-29CR-CU** were coupled to a 23mer DNA, all other compounds to a 14mer DNA

[2] measured by nanodrop

[3] % conversion estimated based on the area under the curve of the product versus the educt in the HPLC-chromatograms, 100 %: no starting material detectable.

[4] measured by MALDI MS

All carboxylic acids **A**-**CV** were coupled to DNA-scaffold conjugates containing a PEG(8) linker serving as spacer between DNA and scaffold, except for carboxylic acids **AJ**, **AO**, **AS, AT**, **AU**, **AX** and **BS** that were coupled to the DNA-scaffold **27** conjugate containing a PEG(4) linker, carboxylic acids **AG**, **AI**, **AJ**, **AM**, **AN**, **AO**, **AP**, **AQ**, **AT**, **AV** and **AX** that were coupled to the DNA-scaffold **28** conjugate containing a PEG(4) linker, and carboxylic acids **AP**, **AV** and **BS** that were coupled to the DNA-scaffold **29** conjugate containing a PEG(4) linker.

DNA conjugates 27	
Conversion	acids
0%	15
1-25 %	6
26-50 %	6
51-75 %	18
76-100 %	69
total	**99**

DNA conjugates 28	
Conversion	acids
0%	30
1-25 %	14
26-50 %	18
51-75 %	24
76-100 %	28
total	**84**

DNA conjugates 29	
Conversion	acids
0%	20
1-25 %	2
26-50 %	5
51-75 %	12
76-100 %	75
total	**94**

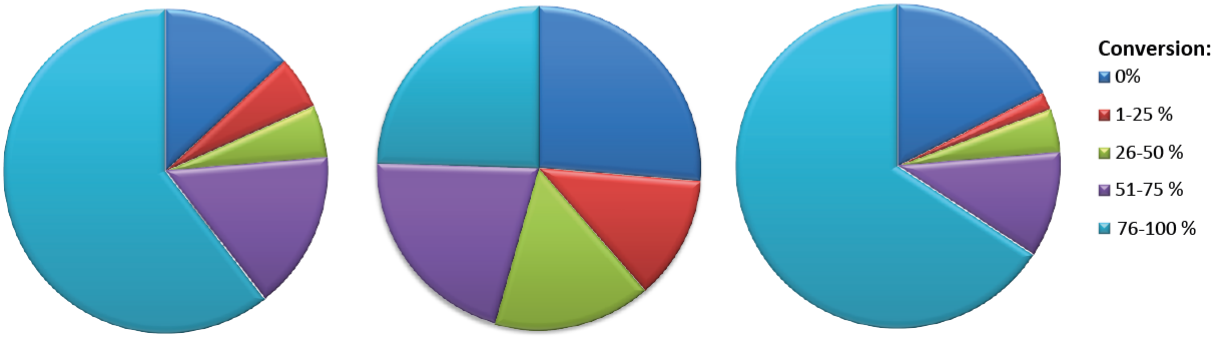


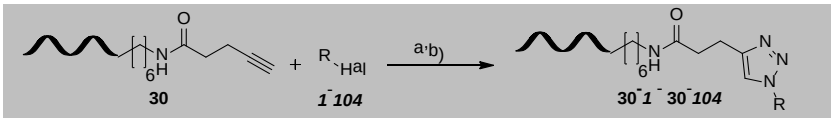
Figure S1: Statistical analysis of the coupling efficiency of carboxylic acids **A**-**DJ** to the DNA-scaffold conjugates **27**-**29**.

4. Evaluation of halides for library synthesis

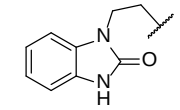
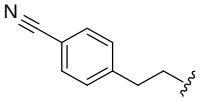
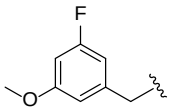
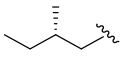
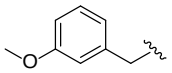
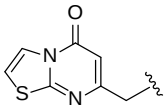
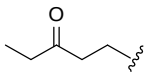
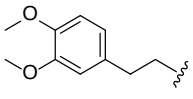
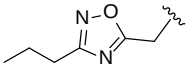
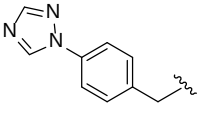
DEAE sepharose (100 µL) was washed with 10 mM aq. NaAc buffer (2 × 350 µL) and water (2 × 350 µL). Then, 400 pmol of the DNA alkyne conjugate **30** (**Table S2**; sequence of the DNA see **Table S4**,) was immobilized on DEAE anion exchange resin by incubation of an aqueous solution of the oligonucleotide conjugate (2 µL) with the resin for 15 min, followed by washing of the resin with 10 mM aq. NaAc buffer (2×350 µL), distilled water (2×350 µL) and MeOH/H_2O/DMF (2:2:1) mixture (2×350 µL). For the synthesis of the azide for *in situ*-CuAAC, 10 µmol of a halide 1-104 (**Table S2**) was dissolved in 800 µL of DMF in an Eppendorf tube, to this were added 100 µL of an aqueous Na-azide solution (123 µmol, 8 mg/100 µL) and 100 µL of TBAI in DMF (20 µmol, 7.5 mg/100 µL). Both Na-azide and TBAI solutions were prepared as stock solutions immediately before the substitution reaction. In order to generate the azide from halide, the reaction was shaken for 8 hour at rt. In case of aliphatic bromides and all chlorides, azide formation was performed for 4 h at 70 °C. For parallel CuAAC, the DEAE sepharose carrying the oligonucleotide conjugate was suspended in 100 µL of DMF. Subsequently, 380 µL of H_2O/MeOH (1:1), the azide (1 µmol in 100 µL of DMF/H_2O (9:1), 2500 eq.), TBTA (0.026 mg in 20 µL of DMF, 0.05 µmol, 125 eq.), Na-ascorbate (0.01 mg in 10 µL of H_2O, 0.05 µmol, 125 eq.) and $CuSO_4$*$5H_2O$ (0.00125 mg in 10 µL of H_2O, 0.005 µmol, 12.5 eq.) were added to the suspension in this order. All solutions had been prepared as stock solutions immediately before the CuAAC reaction was started (TBTA: 2.9 mg in 2.2 mL of dry DMF; Na-ascorbate: 1.1 mg in 1.1 mL

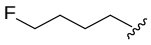
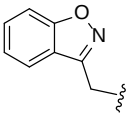
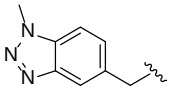
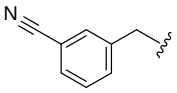
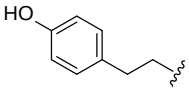
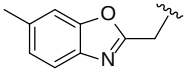
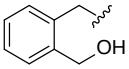
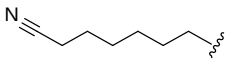
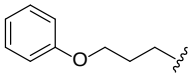
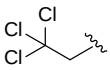
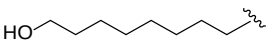
of distilled water; $CuSO_{4*}5H_2O$: 1.25 mg in 10 mL of distilled water). The reaction mixtures were shaken at 45°C for overnight. Then, DEAE sepharose was filtered over receiver plate (20 µm) and washed subsequently with each 3x200 µL of DMF, 0.1 N aqueous EDTA to remove the copper-ion contaminants, $MeOH/H_2O/DMF$ (2:2:1) mixture, water and 10 mM aq. NaAc buffer. After that, the oligonucleotide conjugates were eluted from DEAE sepharose by shaking with 60 µL of 3 M NaAc buffer (pH= 4.75) for 30 min. The products were analyzed by MALDI-TOF-MS analysis.

Table S2: Evaluation of halides ***1***-***104***.



a) NaN_3 TBAI, DMF/H_2O; b) $CuSO_4$, TBTA, Na-ascorbate, DMF, MeOH, H_2O, DEAE-sepharose

No.[1]	R	halide	Mass calc. Mass found[2]
1		Cl	4692.1 4689.3
2		Br	4661.1 4669.0
3		Br	4670.1 4669.0
4		Br	4602.1 4600.0
5		Br	4652.1 4653.0
6		Cl	4696.1 Not found
7		Cl	4616.0 4618.3
8		Br	4696.2 4693.0
9		Cl	4656.1 4661.0
10[3]		Br	4689.1 4698.0

11		Br	4606.0 4610.0
12		Br	4663.1 4664.0
13		Br	4677.1 4672.1
14		Br	4647.1 4653.0
15		Br	4652.1 4656.7
16[3]		Cl	4677.1 4668.5
17		Br	4652.1 4651.3
18		Br	4641.1 4650.0
19		Br	4680.2 4682.5
20		Cl	4663.4 4670.6
21		Br	4660.2 4663.0
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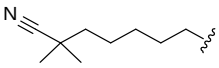
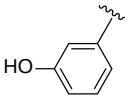
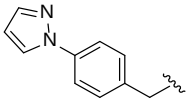
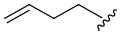
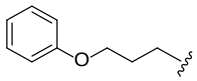
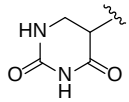
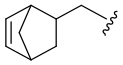
23		Br	4655.2 4648.0
24		Cl	4652.1 4653.9
25		Br	4688.1 4693.6
26		Br	4586.0 4583.1

27		Br	4666.1 4654.3
28		Br	4644.0 4647.6
29		Cl	4638.1 4639.4
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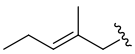
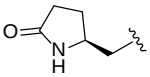
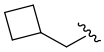
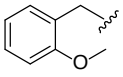
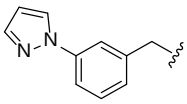
31		Br	4614.1 4619.3
32		Br	4629.1 4629.9
33		Br	4600.1 4581.6
34		Cl	4651.8 4657.8
35		Cl	4688.1 4686.9
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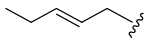
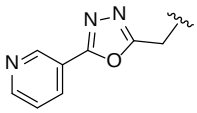
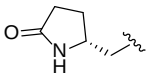
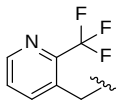
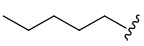
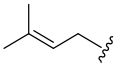
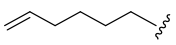
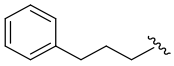
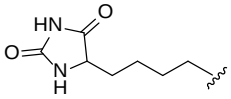
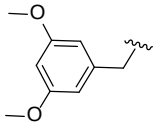
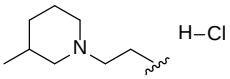
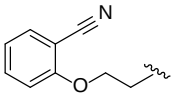
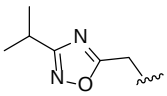
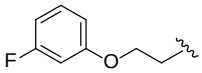
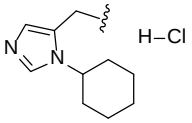
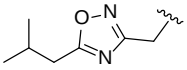
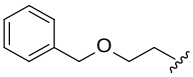
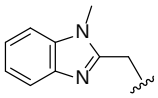
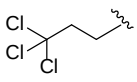
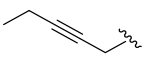
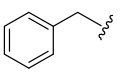
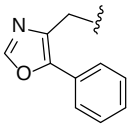
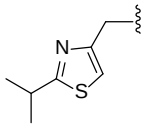
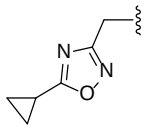
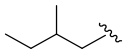
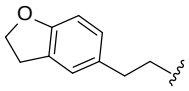
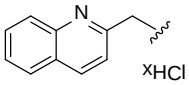
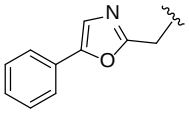
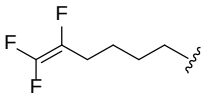
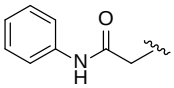
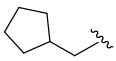
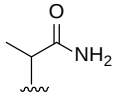
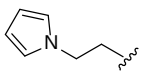
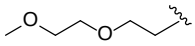
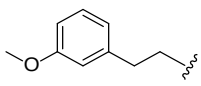
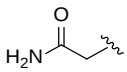
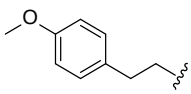
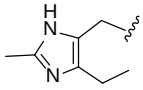
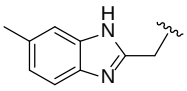
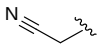
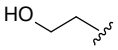
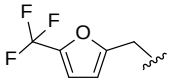
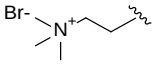
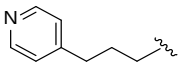
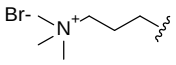
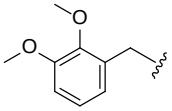
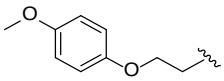
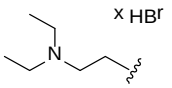
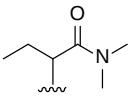
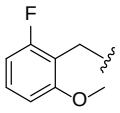
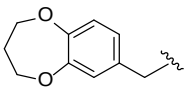
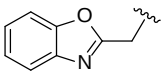
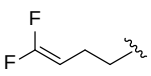
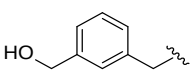
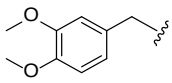
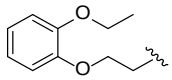
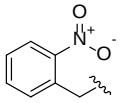
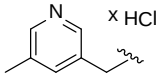
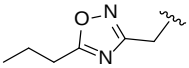
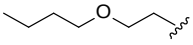
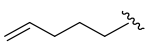
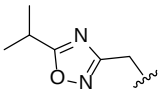
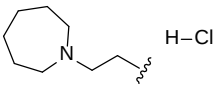
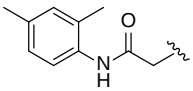
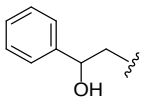
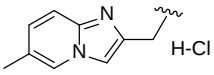
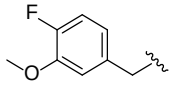
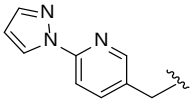
37		Br	4600.1 4600.2
38[3]		Cl	4691.1 4691.9
39		Br	4629.1 4637.4
40		Cl	4691.1 4691.5
41		Br	4602.1 4602.8
42		Br	4600.1 4599.8

43		Br	4599.1 4600.4
44[3]		Cl	4650.1 4649.3
45		Br	4686.1 4679.0
46		Br	4682.1 4683.1
47		Cl	4693.6 4691.5
48		Br	4677.1 4675.2
49		Cl	4656.1 4644.2
50		Br	4670.5 4670.5
51[3]		Cl	4731.0 4731.8
52		Cl	4670.1 4670.2
53[3]		Cl	4710.6 4710.9
54		Br	4676.1 4676.9
55		Br	4677.4 4677.1
56		Br	4598.1 4598.1
57		Br	4622.1 4622.4
58		Br	4689.1 4689.1

59		Cl	4671.2 4671.3
60		Cl	4654.1 4654.6
61		Br	4602.1 4602.4
62[3]		Br	4678.1 4672.0
63		Cl	4673.1 4673.1
64		Cl	4689.1 4694.5
65		Br	4668.0 4667.8
66		Br	4665.1 4666.2
67		Br	4614.1 4626.7
68[3]		Br	4603.0 4602.6
69		Br	4625.1 4625.1
70		Br	4634.1 4622.8
71		Br	4666.1 4666.5
72		Br	4589.0 4585.2
73		Cl	4666.1 4664.7
74		Cl	4654.1 4655.1

75[3]		Cl	4676.1 4665.1
76[3]		Br	4571.0 4571.1
77[3]		Br	4576.0 4574.1
78		Br	4680.0 4680.2
79		Br	4603.1 4592.1
80		Cl	4651.1 4650.2
81		Br	4632.1 4630.5
82		Cl	4682.1 4686.2
83		Br	4682.1 4683.3
84		Br	4631.1 4637.1
85		Br	4645.1 4646.1
86		Br	4670.1 4671.0
87		Cl	4694.1 not found
88		Cl	4663.0 4660.1
89		Br	4622.0 4613.2
90		Br	4652.1 4655.2

91		Cl	4682.1 4682.7
92		Br	4696.1 4698.2
93		Br	4667.1 4670.3
94		Cl	4637.1 4638.0
95		Cl	4656.1 4634.7
96		Cl	4632.1 4630.8
97		Br	4600.0 4609.3
98		Cl	4656.1 4655.1
99		Cl	4657.1 4655.3
100		Br	4693.1 4665.0
101[3]		Br	4652.1 4658.0
102[3]		Cl	4676.1 4675.0
103		Br	4670.1 4665.4
104[3]		Cl	4689.1 4690.0

[1] building blocks that did not yield products are marked in red

[2] measured by MALDI MS

[3] incomplete conversion, more than 50 % conversion as estimated by MALDI MS analysis

Table S3: Conversions of the DNA alkyne conjugate **30** to triazole.

DNA alkyne conjugate	
	azides
fully converted	88
incomplete conversion (>50 %)	14
no product observed	2
total	104

5. Optimization of DNA ligation with T4 ligase and optimization of primer sequences

5a. Optimization of DNA ligation with T4 ligase

Prior DNA ligation, the oligonucleotides were 5'-phosphorylated with polynucleotide kinase (PNK, see below). The ligation reactions were carried out with 100 pmol of dsDNA **I/I´** in a reaction volume of 20 µL (with the 10x buffer) or 30 µL (with the 2x buffer) testing different parameters described in **table S4**.

Table S4: Optimization of conditions for ligation with T4 ligase. The parameters were tested in combinatorial manner. The combination of parameters finally used for encoding of the DNA-encoded library are marked in bold.

Parameter	1	2	3	4
buffer	2 x buffer containing PEG6000[1]	**10 x buffer**[2]	2 x buffer containing PEG6000,[1] additional BSA[3]	10 x buffer;[2] additional BSA[3]
reaction time	2h	**4h**	6h	**16h**
equivalents of phosphorylated to non-phosphorylated oligonucleotides	**1**	1.4	2	2.5
temperature	16°C	20°C	**25°C**	37°C
enzyme concentration	10 units/µl	20 units/µl	**30 units/µl**	

[2] 132 mM Tris-HCl; 20 mM $MgCl_2$; 2 mM DTT; 2mM ATP; 15% PEG6000, pH 7.6 at 25 °C.
[3] 500 mM Tris-HCl; 100 mM $MgCl_2$; 50 mM DTT; 10 mM ATP; pH 7.6 at 25 °C.
[4] BSA: bovine serum albumin, 0.1 mg/mL.

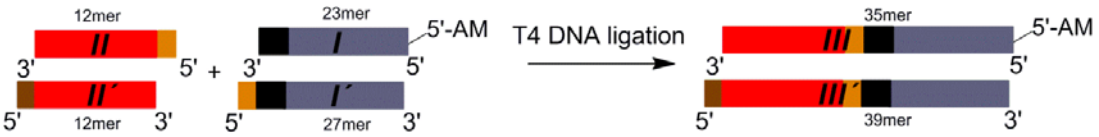


Figure S2: Ligation of two ds DNA sequences. 5'-AM: 5'-C6-Aminolinker.

Table S5: Sequences of oligonucleotides for the T4 ligation reaction as shown in **figures S2-6**.[1]

Oligonucleotide	Sequence (5'-3')
I	5'-AM-GTC TTG CCG AAT TCC GCT TAC CG
I´	ATAC CG GTA AGC GGA ATT CGG CAA GAC
II	GTAT GT ATG TAC
II´	TAGG GTA CAT AC
III	5'-AM-GTC TTG CCG AAT TCC GCT TAC CGG TAT GT ATG TAC
III´	TAGG GTA CAT AC ATAC CG GTA AGC GGA ATT CGG CAA GAC

[1] 5'-AM: 5'-C6-Aminolinker

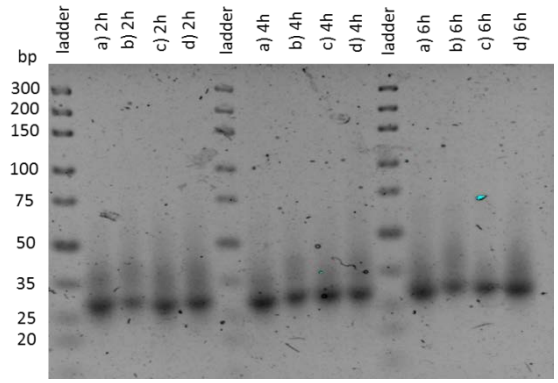


Figure S3: Gel analysis of ligation reactions of DNA duplexes ***I*/*I´*** with ***II*/*II´*** at **16°C** for different reaction times. a) 10 x ligation buffer, b) 2 x buffer with PEG6000, c) 10 x buffer with BSA, d) 2 x buffer with PEG6000 and BSA. bp, base pairs. The conversion to the expected ligation products (39/35mer) was low under these conditions. For buffer composition see **table S4**.

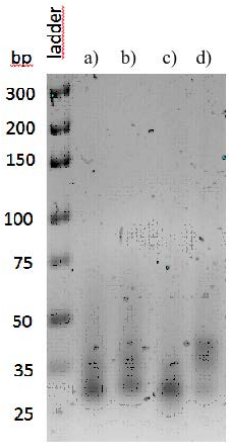


Figure S4: Gel analysis of ligation reactions of DNA duplexes ***I*/*I´*** with ***II*/*II´*** at **16°C overnight**. a) 10 x ligation buffer, b) 2 x buffer with PEG6000, c) 10 x buffer with BSA, d) 2 x buffer with PEG6000 and BSA. bp, base pairs. Higher yields of the expected ligation product (39/35mer) were observed only with the buffer containing PEG6000 and BSA. For buffer composition see **table S4**.

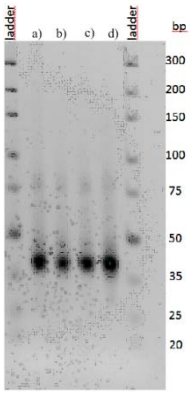


Figure S5: Gel analysis of ligation reactions of DNA duplexes ***I*/*I´*** with ***II*/*II´*** at **20°C overnight**. a) 10 x ligation buffer, b) 2 x buffer with PEG6000, c) 10 x buffer with BSA, d) 2 x buffer with PEG6000 and BSA. bp, base pairs. A high conversion to the expected ligation products (39/35mer) was observed. For buffer composition see **table S4**.

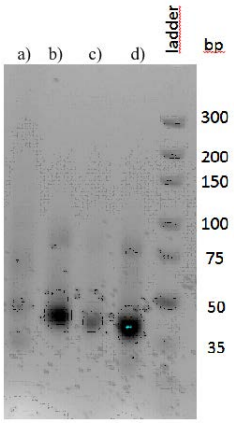


Figure S6: Gel analysis of ligation reactions of DNA duplexes ***I*/*I´*** with ***II*/*II´*** at **37°C overnight**. a) 10 x ligation buffer b) 2 x buffer with PEG6000 c) 10 x buffer with BSA d) 2 x buffer with PEG6000 and BSA. bp, base pairs. High conversions to the expected ligation products (39/35mer) were observed. For buffer composition see **table S4**.

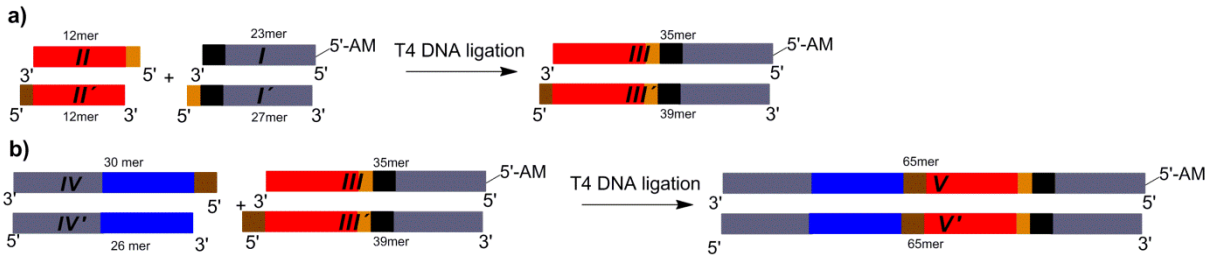


Figure S7: Ligation scheme for the ligation of double stranded DNA sequences shown in **figure S8**.

Table S6: Sequences of oligonucleotides for the T4 ligation reaction as shown in **figure S8**.[1]

Oligonucleotide	Sequence (5'-3')
I	**5'-AM**-GTC TTG CCG AAT TCC GCT TAC CG
I´	ATAC CG GTA AGC GGA ATT CGG CAA GAC
II	GTAT GT ATG TAC
II´	TAGG GTA CAT AC
III	**5'-AM**-GTC TTG CCG AAT TCC GCT TAC CG GTAT GT ATG TAC
III´	TAGG GTA CAT AC ATAC CG GTA AGC GGA ATT CGG CAA GAC
IV	CCTA TTC AGG AT CGA CTG CTG TGT GAC TTC
IV´	GAA GTC ACA CAG CAG TCG AT CCT GAA
V	**5'-AM**-GTC TTG CCG AAT TCC GCT TAC CG GTAT GT ATG TAC CCTA TTC AGG AT CGA CTG CTG TGT GAC TTC
V´	TAGG GTA CAT AC ATAC CG GTA AGC GGA ATT CGG CAA GAC GAA GTC ACA CAG CAG TCG AT CCT GAA

[1] **5'-AM**: 5'-C6-Aminolinker

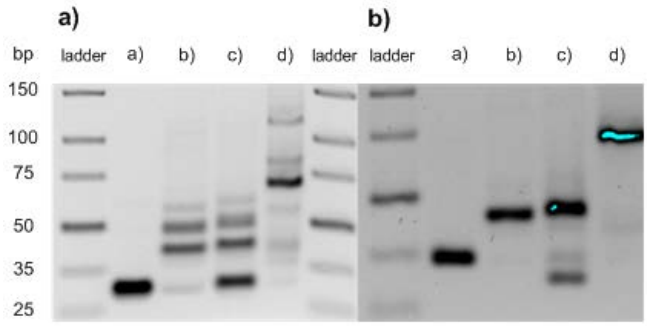


Figure S8: Gel analysis of ligation reactions of DNA duplexes ***I***/***I´*** with 1 eq. of ***II***/***II´*** and of ligation reactions of DNA duplexes ***III***/***III'*** with 1 eq. of ***IV***/***IV'*** (**table S6**) according to scheme **S7** at **25 °C overnight**. a) with 2 x buffer with PEG6000: lane a) first ligation w/o T4 DNA ligase (**figure 7a**), lane b) first ligation reaction (**figure 7a**), lane c) second ligation w/o T4 DNA ligase (**figure 7b**), lane d) second ligation reaction (**figure 7b**); b) with 10 x buffer: lane a) first ligation w/o T4 DNA ligase (**figure 7a**), lane b) first ligation reaction (**figure 7a**), lane c) second ligation w/o T4 DNA ligase (**figure 7b**), lane d) second ligation reaction (**figure 7b**). bp, base pairs. For buffer composition see **table S4**.

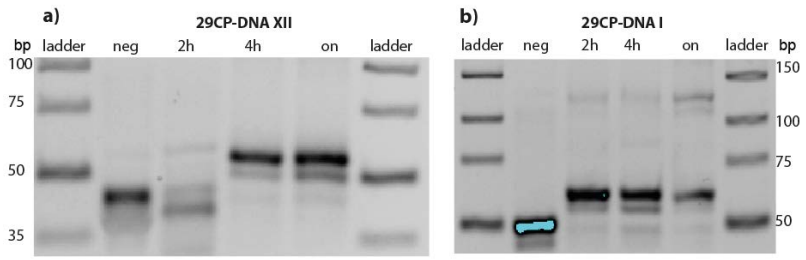


Figure S9: Gel analysis of the first ligation step for encoding of a representative library member **29CP** (**29**: benzodiazepine, building block **CP**: desthiobiotin, see **table S1**) employing the ligation conditions marked in bold in **table S4**. a) The small molecule **29CP** was coupled to the 14mer sequence **XII** (**table S10**) and the ligation was performed according to the scheme shown in **figure S14a** with the sequences given in **table S10**; b) for comparison **29CP** was coupled to the initially used 23mer sequence **I** and the encoding was performed according to the scheme shown in **figure S13a** with the sequences given in **table S10**. We tested the ligation efficiency in dependence of incubation time (2 h, 4 h and overnight) and found an incubation time of 4 h and overnight to furnish the encoded compound in both cases in good yield. Neg denotes the annealed sequences prior addition of T4 DNA ligase; on, overnight; bp, base pair.

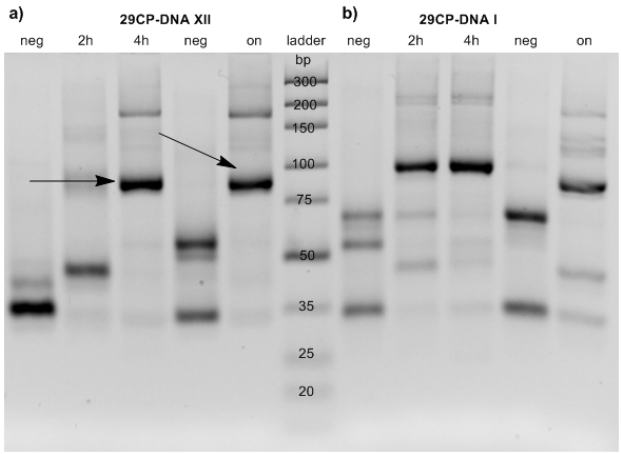


Figure S10: Gel analysis of the second ligation step for encoding of a representative library member **29CP** (**29**: benzodiazepine, building block **CP**: desthiobiotin, see **table S1**) employing the ligation conditions marked in bold in **table S4**. a) The small molecule **29CP** was coupled to the 14mer sequence **XII** (**table S10**) and the ligation was performed according to the scheme shown in **figure S14b** with the sequences given in **table S10**, the ligation product **29CP**-**XVI**/**XVII** (indicated) was used to establish the selection assay (**figure 5**); b) for comparison **29CP** was coupled to the initially used 23mer 5'-aminolinker modified sequence **I** (**table S10**) and the encoding was performed according to the scheme shown in **figure S13b** with the sequences given in **table S10**. We tested the ligation efficiency in dependence of incubation time (2 h, 4 h and overnight) and found an incubation time of 4 h and overnight to furnish the encoded compound in good yield. Neg denotes the annealed sequences prior addition of T4 DNA ligase; on, overnight; bp, base pair.

5b. Optimization of the primer sequences

Real-time PCR was carried out using the Light Cycler® 480 II (Roche) and the Light Cycler® 480 software (release 1.5.1.62 SP2). In a 96well plate (Sarstedt) were combined to a total volume of 20 µl a DNA template at 100 pM, 1 pM or 100 fM (2 fmol, 20 amol or 2 amol in 1 µl of water), 250 nM forward primer (5 pmol in 1 µL of water), 250 nM reverse primer (5 pmol in 1 µL of water), 10 µl SYBR Green Mastermix (2x FastStart Universal Probe Master (Rox); 04913949001 or Takyon™ No Rox Probe MasterMix dTTP from Eurogentec) and 7 µl purified water. The mastermix contained DNA polymerase (Takyon™ DNA polymerase or Taq polymerase by Roche), $MgCl_2$ (5.5 mM final concentration), dNTPs, SYBR Green I as fluorescent dye and stabilizers. Negative controls containing water only; water and mastermix; water, mastermix and primer pair; water, mastermix and template were prepared for every experiment. All qPCR experiments performed using SYBR Green were conducted at 42°C for 2 min, 95°C for 5 min, and then 40 cycles of 95°C for 10 s, 56°C for 10 s, 72°C for 12 s. The reactions were again incubated 95°C for 10 s, 60°C for 30 s, 95°C for 1 s and 40°C for 40 s. The specificity of the reactions was verified by melt curve analysis.

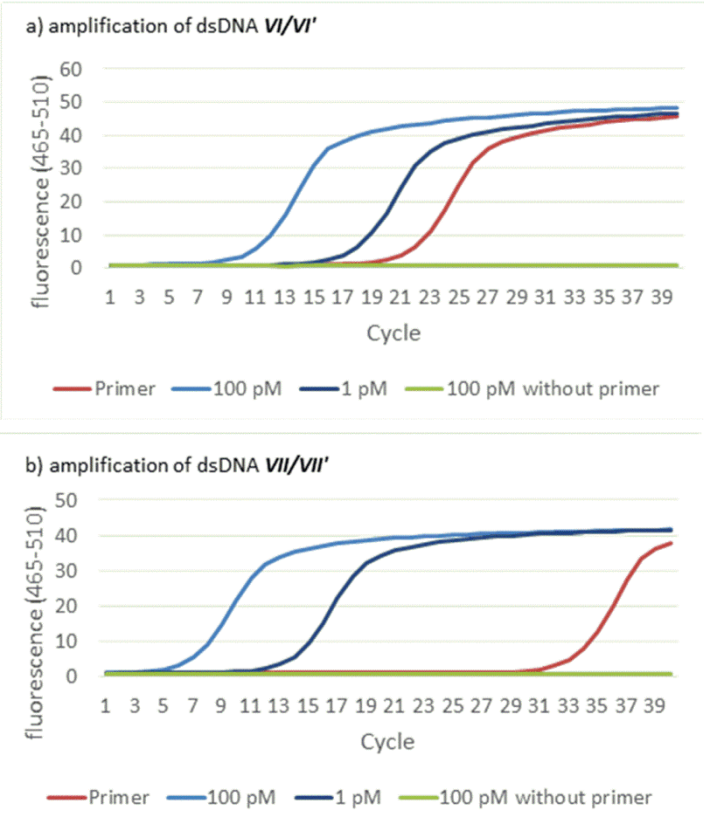


Figure S11: qPCR amplification of two template dsDNA sequences with different primer pairs (**table S8**). a) qPCR amplification of 100 pM and 1pM template dsDNA ***VI***/***VI´*** with primer sequences ***VIII*** and ***IX***; b) qPCR amplification of 100 pM and 1pM template dsDNA ***VII***/***VII´*** with primer sequences ***X*** and ***XI***. The latter primer pair was used for library synthesis.

Table S7: ct-values for different template dsDNAs ***VI***/***VI´*** and ***VII***/***VII´***.

sample	**template *VI*/*VI´***	**template *VII*/*VII´***
primer	21.3	32.8
100 pM template DNA	10.6	6.5
1 pM template DNA	17.4	13.4
100 pM template DNA w/o primer	> 40	> 40

Table S8: Sequences of oligonucleotides for primer optimization

Oligonucleotide	Sequence (5'-3')
VI	GTC TTG CCG AAT TCC GCT GCACT AGG TCG GTG TGA ACG GAT TTG GCA GTAT AAC ATAGG CCT A AC TGT TCA TGA CCT CAA CTA CAT GGT CTA CA
VI´	T AGG CC TAT GTT ATAC TGC CAA ATC CGT TCA CAC CGA CCT AGTGC AGC GGA ATT CGG CAA GAC TG TAG ACC ATG TAG TTG AGG TCA TGA ACA GT
VII	AGG TCG GTG TGA ACG GAT TTG GTC ATA ACA TAG GCC TAA CTG TTC ATG ACC TCA ACT ACA TGG TCT ACA
VII´	TGT AGA CCA TGT AGT TGA GGT CAT GAA CAG TTA GGC CTA TGT TAT GAC CAA ATC CGT TCA CAC CGA CCT
VIII (primer for ***VI***/***VI´***)	TGT AGA CCA TGT AGT TGA GGT CA
IX (reverse primer for ***VI***/***VI´***)	AGG TCG GTG TGA ACG GAT TTG
X (primer for ***VII***/***VII´***)	TGT AGA CCA TGT AGT TGA GGT CA
XI (reverse primer for ***VII***/***VII´***)	AGG TCG GTG TGA ACG GAT TTG

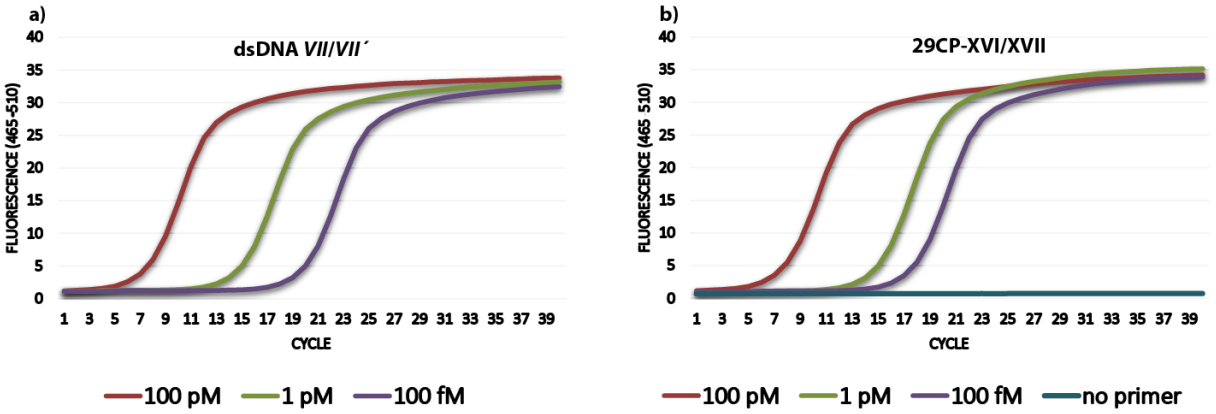


Figure S12: a) qPCR-amplification of 100 pM, 1pM, and 100 fM of the chemically synthesized reference dsDNA ***VII***/***VII´*** (provided by IDT, Leuven, Belgium, **table S8**); b) qPCR-amplification of 100 pM, 1pM, and 100 fM of the compound **29CP-XVI/XVII** (**29**: benzodiazepine, building block **CP**: desthiobiotin, see **table S1**) encoded by T4 ligation with dsDNA sequences (**table S10**) according to the ligation scheme shown in **figure S13**. The two ligation reactions yielding **29CP-XVI/XVII** are depicted in **figures S9** and **S10**. dsDNA ***VII***/***VII´*** has the same primer and insert sequence as the encoded compound **29CP-XVI/XVII**.

6. Synthesis of the DNA-encoded library

5'-phosphorylation of double stranded DNA

20 µL phosphorylation reactions contained 14 µM oligonucleotides, 10 units of PNK (T4 PNK, Thermo Fisher Scientific), 10 × PNK Buffer A (500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT, 1 mM spermidine, pH 7.6, 25°C, Thermo Fisher Scientific), 2 µL 10 µM ATP (100 mM aqueous solution of ATP, titrated to 7.3-7.5 with NaOH, Thermo Fisher Scientific). Reactions were carried out at 37°C for 20 minutes and stopped by heat inactivation at 75°C for 10 minutes.

Ligation

The ligation scheme for encoding of the library is shown in **figures S13** and **S14**. The sequences are stated in **tables S9** and **S10**. The oligonucleotides **I**-**V**, oligonucleotide **I** being the HPLC-purified 23mer sequence with the 5'-appended small molecules (see **table S1**), depicted in **figure S13a** or the oligonucleotides **XII**/**XIII**, and **II**, **III**, and **V**, oligonucleotide **XII** being the HPLC-purified 14mer sequence with the 5'-appended small molecules (see **table S1**), depicted in **S14a** were annealed at 85°C for 10 min and slowly cooled down to room temperature (25°C). The total reaction volume was 20 µL. This contained 2 µM oligonucleotides equaling a total amount of 40 pmol of each DNA oligonucleotide, 600 units of T4 DNA Ligase (T4 DNA ligase rapid, Biozym), 10 × T4 DNA Ligase Buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT, 10 mM ATP, pH 7.6 @ 25°C). Ligations were carried out at 25°C for 4 hours and stopped by heat inactivation at 75°C for 10 minutes. Five control reactions were performed without T4 DNA ligase. The encoded molecules were combined and precipitated as described below. The pellet was redissolved in IDTE buffer (pH 8) and again precipitated. The success of the ligation reaction was analyzed by agarose gel electrophoresis and the products of the first T4 DNA ligation reactions were splitted into 102 wells of 96well plates. Then, the 5'end of the duplex DNA strands **VI**/**VII** or **XIV**/**XV** and the oligonucleotides **VIII** that contained the code for the azide building block and the reverse primer sequence were phosphorylated as described above. The ligation of the codes for the second set of building blocks and the reverse primer was analogously performed using 20 pmol of each oligonucleotide **VI**/**VII** or **XIV**/**XV** and 40 pmol of each oligonucleotide **VIII**/**IX** (**figure S13b**) or **XIV**/**XV** (**figure S14b**). For ligation, the dsDNA sequences **VI**/**VII**, and **VIII**/**IX** (**figure S13b**), or **VIII**/**IX**, and **XIV**/**XV** (**figure S14b**) were annealed at 85°C for 10 minutes and slowly cooled down to room temperature. The total reaction volume was 30 µL. This contained the oligonucleotides, 900 units of T4 DNA Ligase (T4 DNA ligase rapid, Biozym), and 10 × T4 DNA Ligase Buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT, 10 mM ATP, pH 7.6 @ 25°C). The ligation reactions were carried out at 25°C for 16 hours and stopped by heat inactivation at 75°C for 10 minutes. Control reactions were performed without T4 DNA ligase.

Analysis of DNA ligation reactions

For analysis of ligation reactions, agarose gel electrophoresis (5.5 % agarose) was used. Electrophoresis was carried out in 1× TBE (0.1 M Tris, 0.1 M H_3BO_3, 0.2 mM EDTA, pH 8.3) at 150 V constant voltage for 75 minutes. For staining, Midori Green Direct (Biozym) and as reference the GeneRuler Ultra Low Range DNA Ladder (Thermo Fisher Scientific) was used. The gels were imaged by UV illumination with the Bio-Rad Gel Doc™ XR system.

Precipitation of DNA samples

After every ligation step the samples were precipitated. EtOH was added to 70 volume percent. The samples were stored at – 80°C for 1 hour and centrifuged for 30 minutes (15000 rpm, 4 °C, centrifuge 5424 (Eppendorf)). The supernatant was removed, 70 % EtOH was added and the samples were stored at – 80°C for 1 hour. After centrifugation for 30 minutes (15000 rpm, 4°C, centrifuge 5424 by Eppendorf) the supernatant was removed and the DNA was dissolved in IDTE storage buffer (10 mM Tris, pH 7.5, 0.1 mM EDTA, IDT).

Reaction of the set of azides *1-102* with the DNA-encoded library

DEAE sepharose (50 µL) was pipeted into 102 wells (102 x 50 µL) of a two 96well plates, washed with 10 mM aq. NaAc buffer (2 × 350 µL) and water (2 × 350 µL). Then, 20 pmol of pooled and encoded DNA small molecule conjugates **27A-CV** – **29A-CV** (**Table S1**) was immobilized on DEAE anion exchange resin by incubation of an aqueous solution of the oligonucleotide conjugate (2 µL) with the resin for 15 min, followed by washing of the resin with 10 mM aq. NaAc buffer (2×350 µL), distilled water (2×350 µL) and MeOH/H_2O/DMF (2:2:1) mixture (2×350 µL). For the synthesis of the azide for *in situ*-CuAAC, 500 nmol of a halide **1**-**102** (**Table S2**) was dissolved in 40 µL of dry DMF in an Eppendorf tube, to this were added 5 µL of an aqueous Na-azide solution (61.5 µmol, 4 mg/5 µL, taken from a stock solution: 6273 µmol, 408 mg in 510 µL of distilled water) and 5 µL of TBAI in DMF (1 µmol, 0.37 mg/5 µL, taken from a stock solution: 102 µmol, 37.7 mg in 510 µL of dry DMF). Both Na-azide and TBAI solutions were prepared as stock solutions immediately before the substitution reaction. In order to generate the azide from halide, the reaction was shaken for 8 hour at rt. In case of aliphatic bromides and all chlorides, azide formation was performed for 4 hours at 70 °C. For parallel CuAAC, the DEAE sepharose from each well carrying the DNA small molecule conjugates **27A-CV** – **29A-CV** was suspended in 50 µL of dry DMF and transported each well into each glass vial. Subsequently, 190 µL of H_2O/MeOH (1:1), the azide (50 nmol in 5 µL of DMF/H_2O (9:1), 2500 eq.), TBTA (0.0013 mg in 1 µL of DMF, 2.5 nmol, 125 eq.), Na-ascorbate (0.0005 mg in 0.5 µL of H_2O, 2.5 nmol, 125 eq.) and $CuSO_4$*$5H_2O$ (0.0000625 mg in 0.5 µL of distilled water, 250 pmol, 12.5 eq.) were added to the suspension of DNA-small molecule conjugates in this order. All solutions had been prepared as stock solutions immediately before the CuAAC reaction was started (TBTA: 1.3 mg in 1

mL of dry DMF; Na-ascorbate: 5 mg in 5 mL of distilled water; $CuSO_{4*}5H_2O$: 1.25 mg in 10 mL of distilled water). The reaction mixtures were shaken in a glass well shaker at 45°C for overnight. Then, DEAE sepharose was filtered over receiver plate (20 µm) and washed subsequently with each 3 x 200 µL of DMF, 0.1 N aqueous EDTA to remove the copper-ion contaminants, MeOH/H_2O/DMF (2:2:1) mixture, water and 10 mM aq. NaAc buffer. After that, the oligonucleotide conjugates were eluted from DEAE sepharose by shaking with 60 µL of 3 M NaAc buffer (pH= 4.75) for 30 min and filtered directly from a filter plate into a 96-deep well plate. The products of the CuAAC were pooled and precipitated twice as described above.

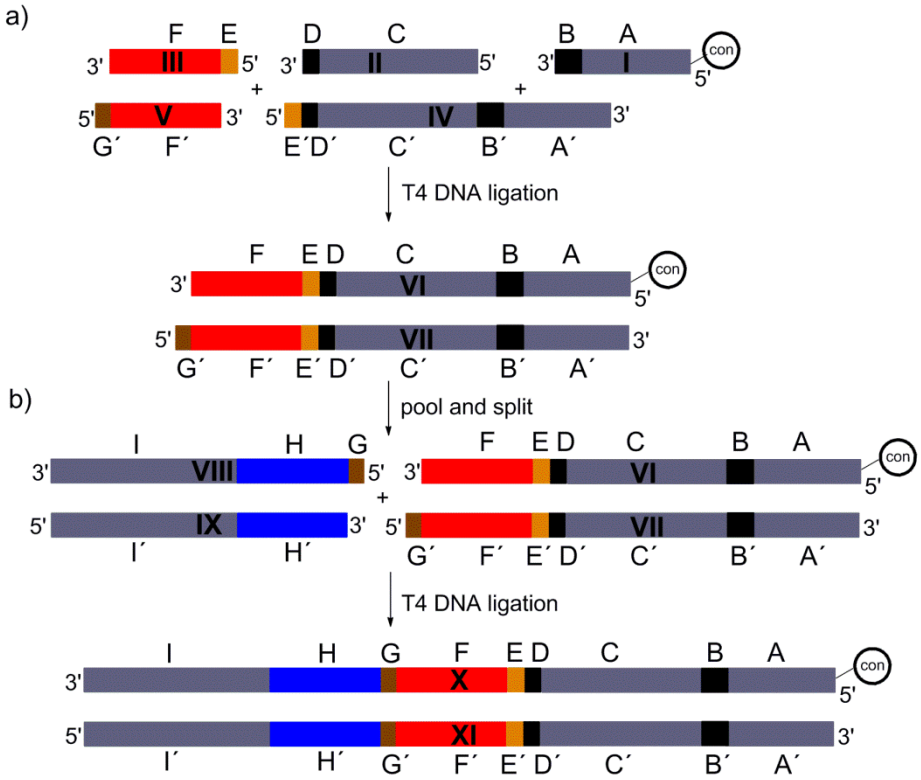


Figure S13: Encoding scheme for the 23mer-coupled compounds **27-29A-G**, **27-29I-AB** and **27-29CR-CV**. Latin numbering denotes the different oligonucleotides that were used for encoding (see **table S9**), while letters denote the functional partial sequences (see **table S10**); con: conjugated small molecule **27-29A-G**, **27-29I-AB** and **27-29CR-CV** (**table S1**).

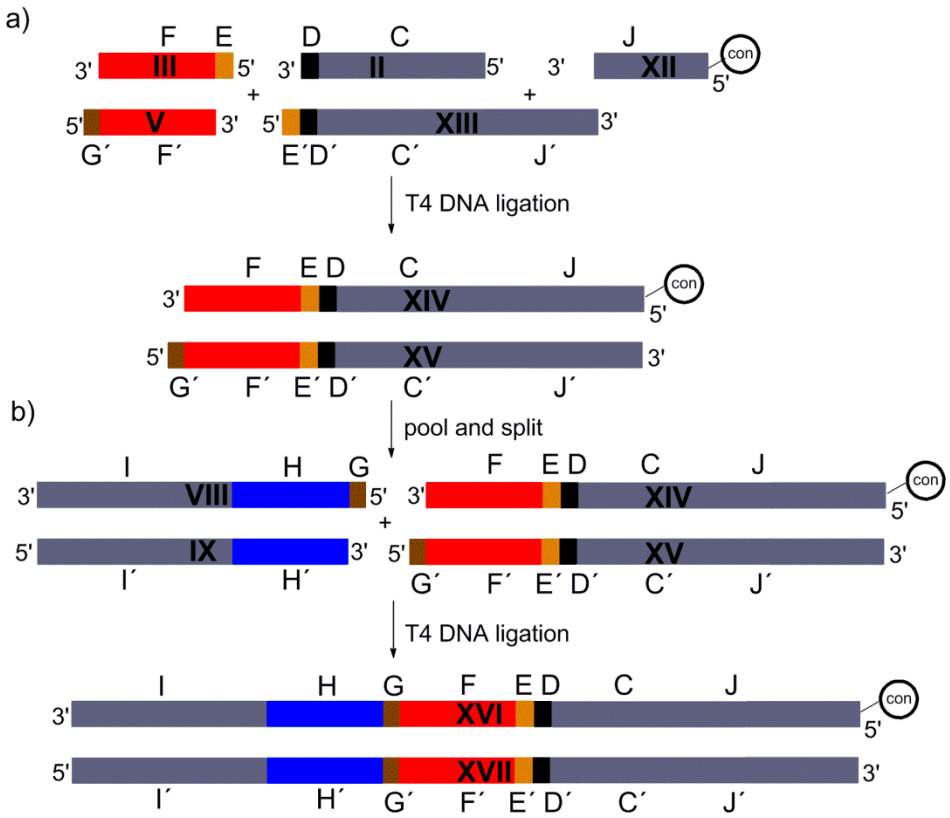


Figure S14: Encoding scheme for the 14mer-coupled compounds **27-29H**, **27-29AD-CQ**. Latin numbering denotes the different oligonucleotides that were used for encoding (see **table S9**), while letters denote the functional partial sequences (see **table S10**); con: conjugated small molecule **27-29H**, and **27-29AD-CQ** (**table S1**)**.**

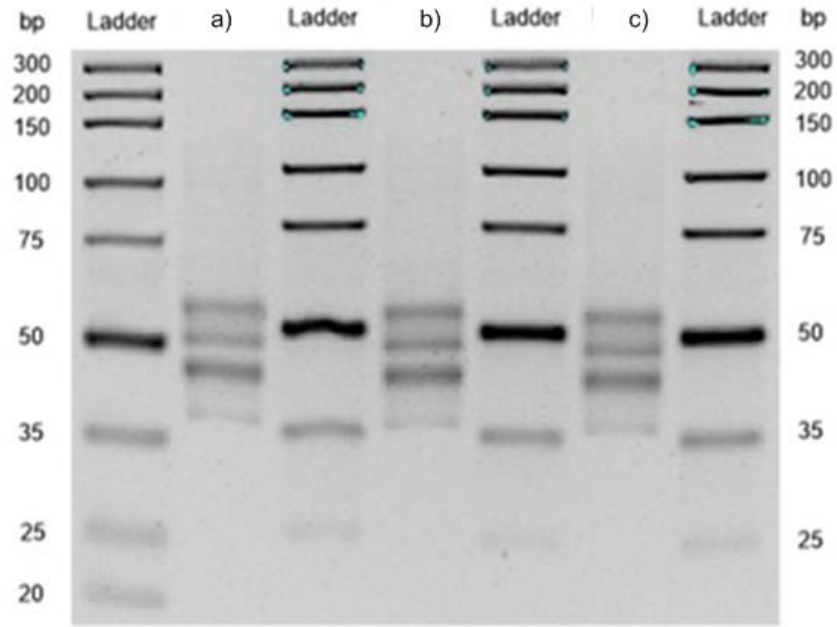


Figure S15: Gel analysis of the first ligation step after pooling and precipitation of the encoded compounds **27A-CV** (a), **28A-CV** (b), and **29A-CV** (c). The educts of the ligation reactions are hardly detectable in the pools. bp, base pair.

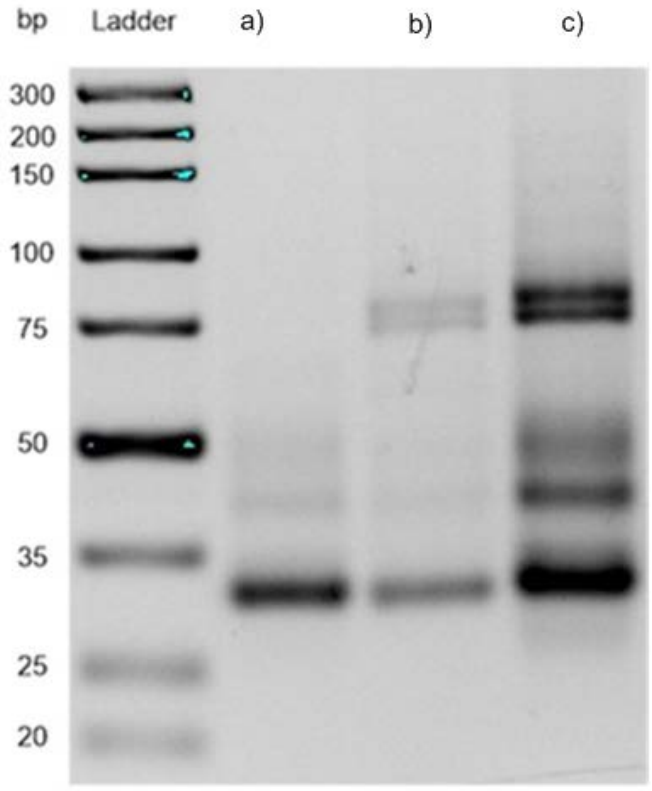


Figure S16: Gel analysis of the second ligation step (**figures S13b** and **S14b**) after CuAAC on DEAE sepharose and pooling of the libraries **9**-**11**; a) gel analysis of a pooled aliquot of the library prior ligation; b) gel analysis of a pooled aliquot (ca. 10 pmol) of the library after ligation (**figures S13b** and **S14b**) and CuAAC (**figure 4**, step f)); c) gel analysis of a pooled aliquot (ca. 50 pmol) of the library after ligation (**figures S13b** and **S14b**) and CuAAC (**figure 4**, step f)). Note that the coding sequences **VIII**/**IX** were added in twofold excess. The ligation yield was estimated to be ca. 70 % based on this gel. bp, base pair.

Table S9: qPCR-analysis of pooled libraries **9**-**11** after CuAAC and comparison to template dsDNA ***VII***/***VII´*** (data from **table S7**).

Sample	Ct-Value
100 pM of chemically synthesized dsDNA ***VII***/***VII´***	6.5
100 pM of libraries **9**-**11** after CuAAC (step f), **fig 4**)	7.5

Table S10: Sequences of the oligonucleotides **I – XVII**.

DNA	partial sequences[1]	length	sequence (5'-3')[2,3]
I	A,B	23mer	**5'-AM**-GTC TTG CCG AAT TCC GCT XXX XX
II	C,D	24mer	AGG TCG GTG TGA ACG GAT TTG XXX
III	E,F	12mer	GTA TXX XXX XXX
IV	A',B',C',D',E'	51mer	ATA CXX XCA AAT CCG TTC ACA CCG ACC TXX XXX AGC GGA ATT CGG CAA GAC
V	F',G'	12mer	TAG GXX XXX XXX
VI	A,B,C,D,E,F	59mer	**5'-AM**-GTC TTG CCG AAT TCC GCT XXX XXA GGT CGG TGT GAA CGG ATT TGX XXG TAT XXX XXX XX
VII	A'B',C',D',E',F',G'	63mer	TAG GXX XXX XXX ATA CXX XCA AAT CCG TTC ACA CCG ACC TXX XXX AGC GGA ATT CGG CAA GAC
VIII	G,H,I	35mer	CCT AXX XXX XXX TGA CCT CAA CTA CAT GGT CTA CA
IX	H',I'	31mer	TGT AGA CCA TGT AGT TGA GGT CAX XXX XXX X
X	A,B,C,D,E,F,G,H,I	94mer	**5'-AM**-GTC TTG CCG AAT TCC GCT XXX XXA GGT CGG TGT GAA CGG ATT TGX XXG TAT XXX XXX XXC CTA XXX XXX XXT GAC CTC AAC TAC ATG GTC TAC A
XI	A',B',C',D',E',F',G',H',I'	94mer	TGT AGA CCA TGT AGT TGA GGT CAX XXX XXX XTA GGX XXX XXX XAT ACX XXC AAA TCC GTT CAC ACC GAC CTX XXX XAG CGG AAT TCG GCA AGA C
XII	J	14mer	**5'-AM**-GTC TTG CCG AAT TC
XIII	C',D',E',J'	42mer	ATA CXX XCA AAT CCG TTC ACA CCG ACC TGA ATT CGG CAA GAC
XIV	C,D,E,F,J	50mer	**5'-AM**-GTC TTG CCG AAT TCA GGT CGG TGT GAA CGG ATT TGX XXG TAT XXX XXX XX
XV	C',D',E',F',G',J'	54mer	TAG GXX XXX XXX ATA CXX XCA AAT CCG TTC ACA CCG ACC TGA ATT CGG CAA GAC
XVI	C,D,E,F,G,H,I,J	85mer	**5'-AM**-GTC TTG CCG AAT TCA GGT CGG TGT GAA CGG ATT TGX XXG TAT XXX XXX XXC CTA XXX XXX XXT GAC CTC AAC TAC ATG GTC TAC A
XVII	C',D',E',F',G',H',I',J'	85mer	TGT AGA CCA TGT AGT TGA GGT CAX XXX XXX XTA GGX XXX XXX XAT ACX XXC AAA TCC GTT CAC ACC GAC CTG AAT TCG GCA AGA C

[1] see figures S13 and S14

[2] X denotes variable position used for encoding of building blocks

[3] **5'-AM** denotes 5'-C6-Aminolinker

Table S11: Functions of the partial sequences of oligonucleotides **I – XVII**.

partial sequence	function of partial sequence	length	sequence (5'-3')
A	adapter[1]	18mer	GTC TTG CCG AAT TCC GCT
A'	complementary sequence to A	18mer	AGC GGA ATT CGG CAA GAC
B	scaffold code	5mer	XXX XX
B'	complementary sequence to B	5mer	XXX XX
C	primer	21mer	AGG TCG GTG TGA ACG GAT TTG
C'	complementary sequence to C	21mer	CAA ATC CGT TCA CAC CGA CCT
D	scaffold code	3mer	XXX
D'	complementary sequence to D	3mer	XXX
E	overhang for T 4 ligation	4mer	GTA T
E'	complementary sequence to E	4mer	ATA C
F	code for building block I	8mer	XXX XXX XX
F'	complementary sequence to F	8mer	XXX XXX XX
G	overhang for T 4 ligation	4mer	CCT A
G'	complementary sequence to G	4mer	TAG G
H	code for building block II	8mer	XXX XXX XX
H'	complementary sequence to H	8mer	XXX XXX XX
I	complementary sequence to I‘	23mer	TGA CCT CAA CTA CAT GGT CTA CA
I'	primer	23mer	TGT AGA CCA TGT AGT TGA GGT CA
J	adapter[1]	14mer	GTC TTG CCG AAT TC
J'	complementary sequence to J	14mer	GAA TTC GGC AAG AC

[1] the (partial-)sequence was initially used as primer sequence, however we found it to be inefficient and thus use it only as adapter sequence.

7. Selection of the encoded desthiobiotin conjugate 29CP-XVI/XVII versus the non-modified dsDNA *VII*/*VII'*

2 µL (20 µL) Dynabeads® MyOne™ Streptavidin C1 (10 mg/mL) were transferred in an eppendorf tube and placed into a magnetic rack. The supernatant was discarded and the beads were resuspended in 200 µL washing buffer (50 mM Na-phosphate, pH 8.0, 150 mM NaCl, 0.01% Tween®-20). This procedure was repeated five times. The beads were resuspended in 500 µL selection buffer (3.25 mM Na-phosphate, pH 8.0, 70 mM NaCl, 0.01% Tween®-20, 0.2 mg/mL sheared herring sperm DNA) and incubated for 1 hour at 4°C mild shaking. Afterwards the beads were incubated with 190 µL selection buffer and 10 µL of the DNA small molecule conjugate for 1 ½ h at 4°C under mild shaking conditions. This was done for the reference conjugate **29CP-XVI/XVII** and for the negative control dsDNA ***VII***/***VII'*** in parallel. After incubation the beads were washed eight times with 500 µL selection buffer (without sheared herring sperm DNA). The DNA was eluted in 100 µL selection buffer by incubating the beads for 10 minutes at 85°C in order to denaturate the streptavidin. The eluate was selected a second time in analogous manner. Afterwards 10% (10 µL) of the eluate were analyzed by qPCR as described above.

8. Chemoinformatic analysis of the DNA-encoded libraries

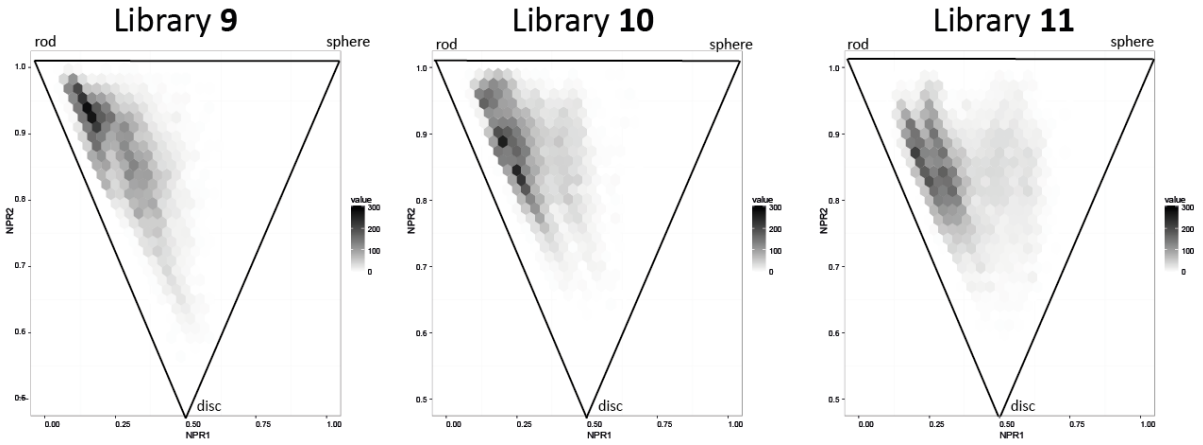


Figure S17: Hexagonally binned normalized principal moments of inertia (NPR)-plots for libraries **9**-**11**. The most frequently populated areas are different for each of the libraries, indicating different shape distribution for each of the libraries.

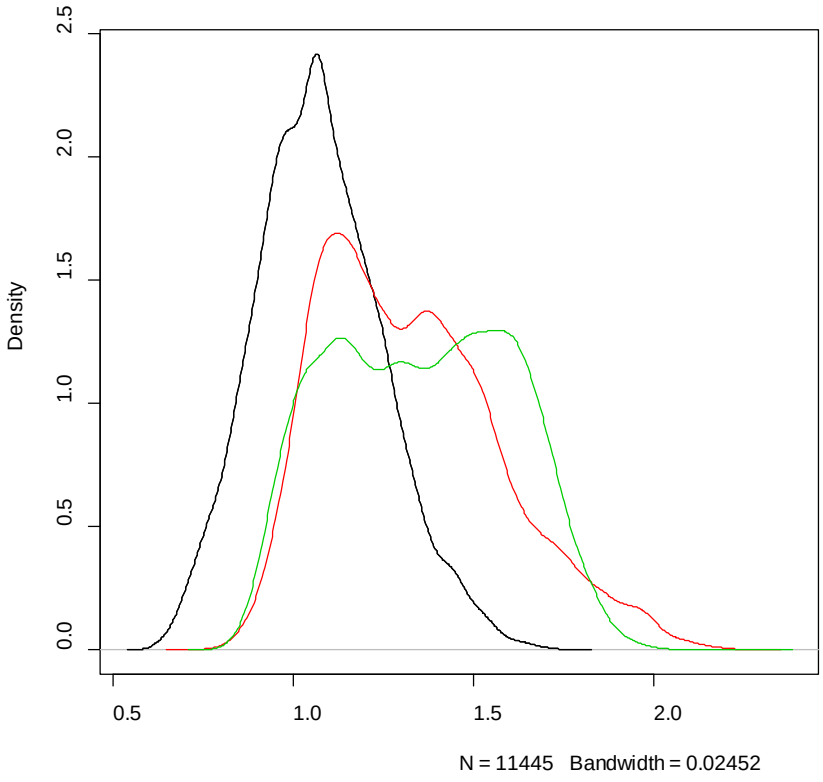


Figure S18: Kernel Density Plot of Plane of Best Fit score (PBF)**.** Scores for library **9** (black), Library **10** (red) and Library **11** (green). Most of the molecules have high 3D-character (PBF_Score > 1.0). The scores were calculated using the PBF-calculator implemented in RDKit.

9. HPLC traces and MALDI MS analysis of representative library members from table S1

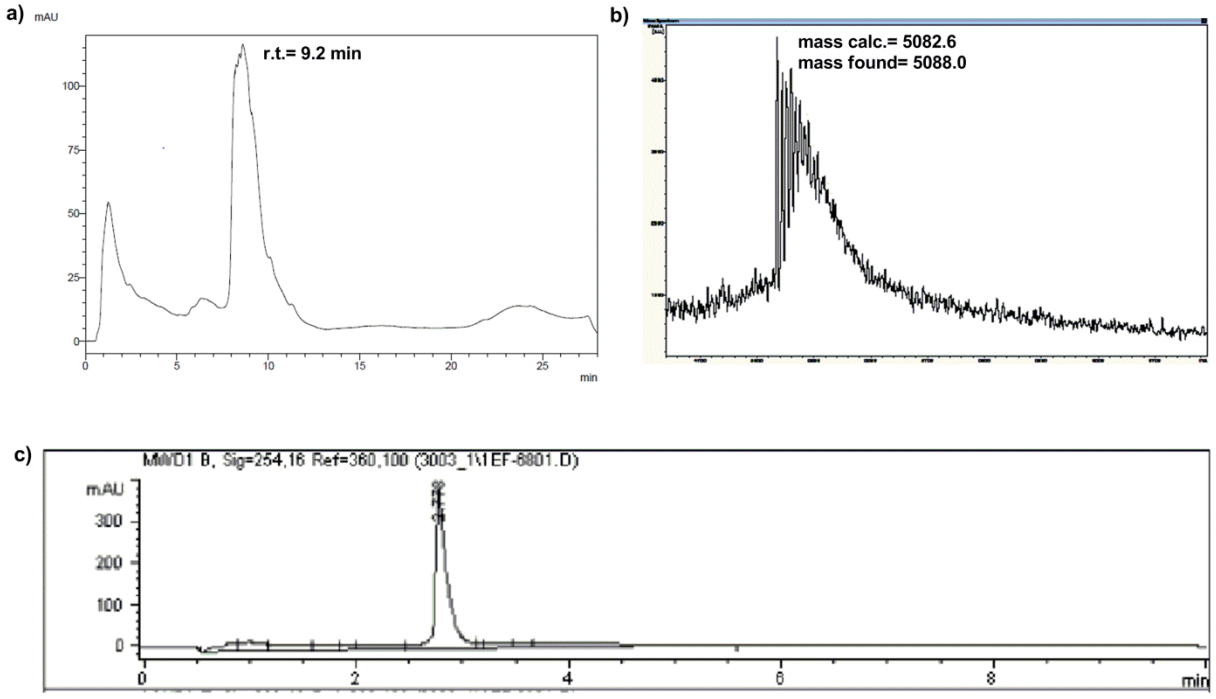


Figure S19: a) HPLC trace (preparative HPLC) of the crude conjugate **27AZ**; b) MALDI-TOF/TOF-MS analysis of purified conjugate **27AZ**; HPLC trace of purified conjugate **27AZ**.

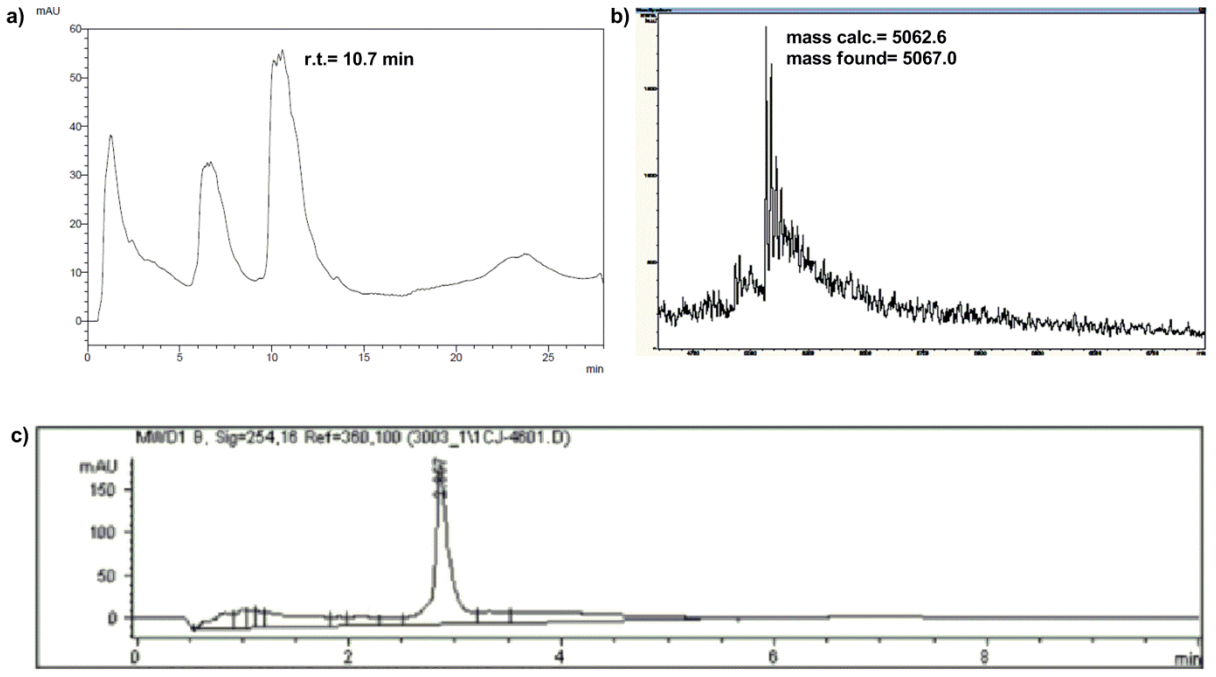


Figure S20: a) HPLC trace (preparative HPLC) of the crude conjugate **27BA**; b) MALDI-TOF/TOF-MS analysis of purified conjugate **27BA**; HPLC trace of purified conjugate **27BA**.

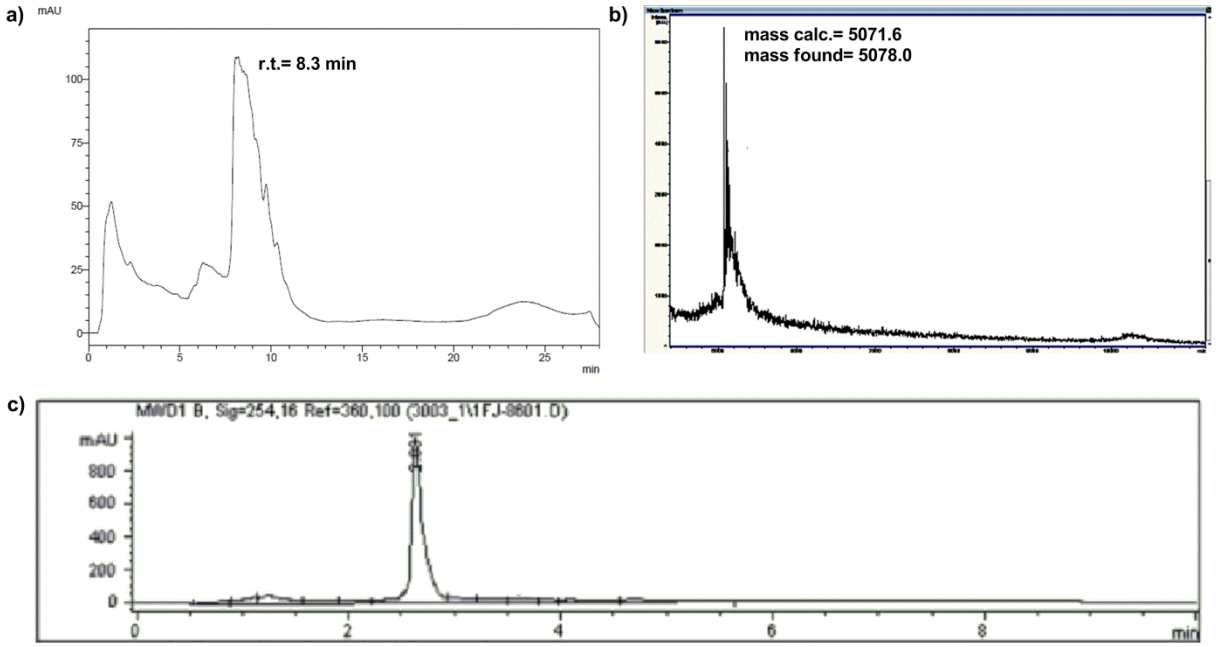


Figure S21: a) HPLC trace (preparative HPLC) of the crude conjugate **27BD**; b) MALDI-TOF/TOF-MS analysis of purified conjugate **27BD**; HPLC trace of purified conjugate **27BD**.

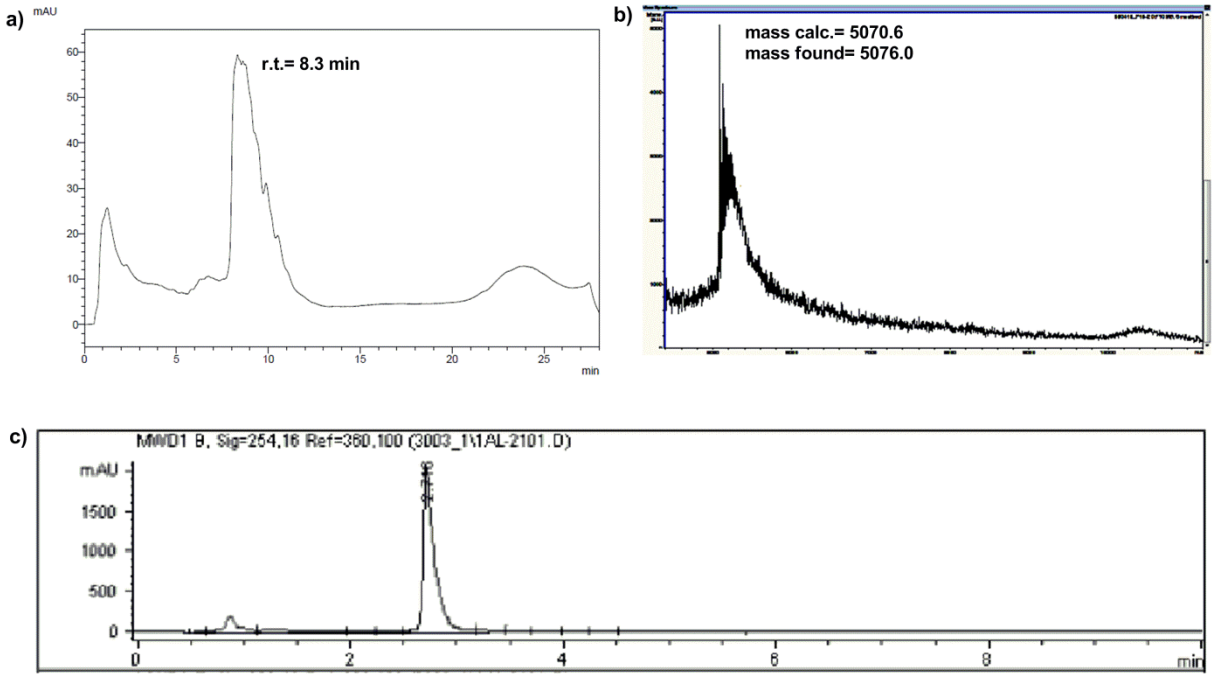


Figure S22: a) HPLC trace (preparative HPLC) of the crude conjugate **27BF**; b) MALDI-TOF/TOF-MS analysis of purified conjugate **27BF**; HPLC trace of purified conjugate **27BF**.

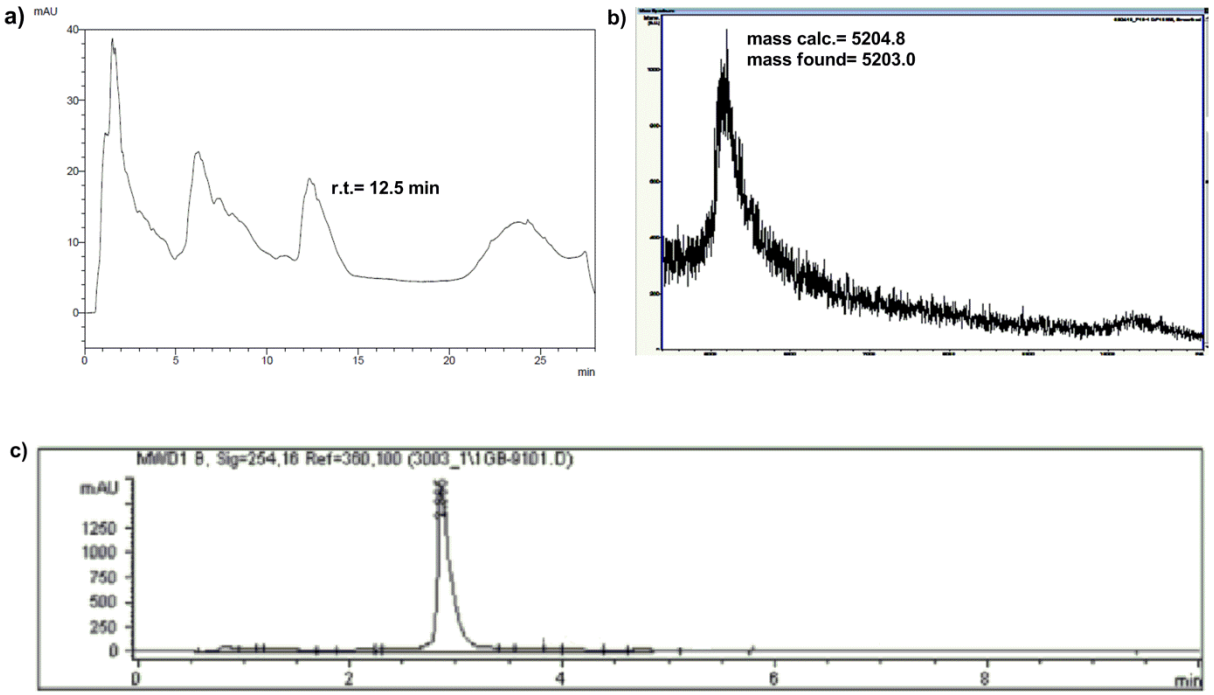


Figure S23: a) HPLC trace (preparative HPLC) of the crude conjugate **28AS**; b) MALDI-TOF/TOF-MS analysis of purified conjugate **28AS**; HPLC trace of purified conjugate **28AS**.

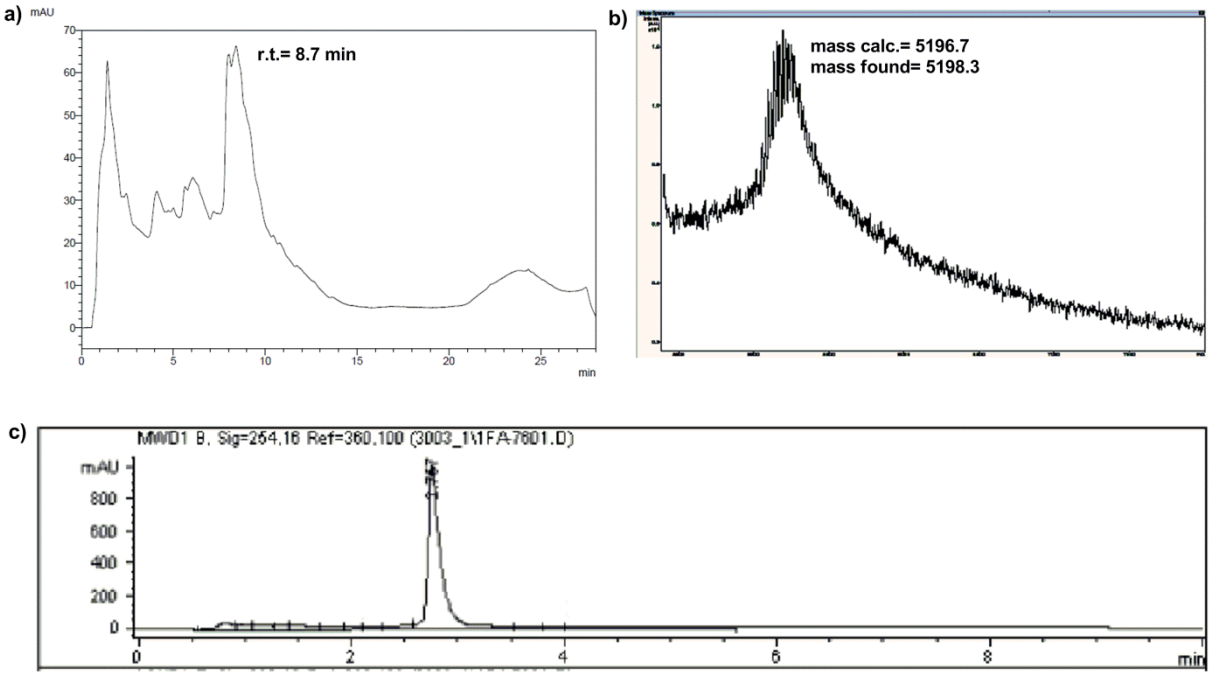


Figure S24: a) HPLC trace (preparative HPLC) of the crude conjugate **28AU**; b) MALDI-TOF/TOF-MS analysis of purified conjugate **28AU**; HPLC trace of purified conjugate **28AU**.

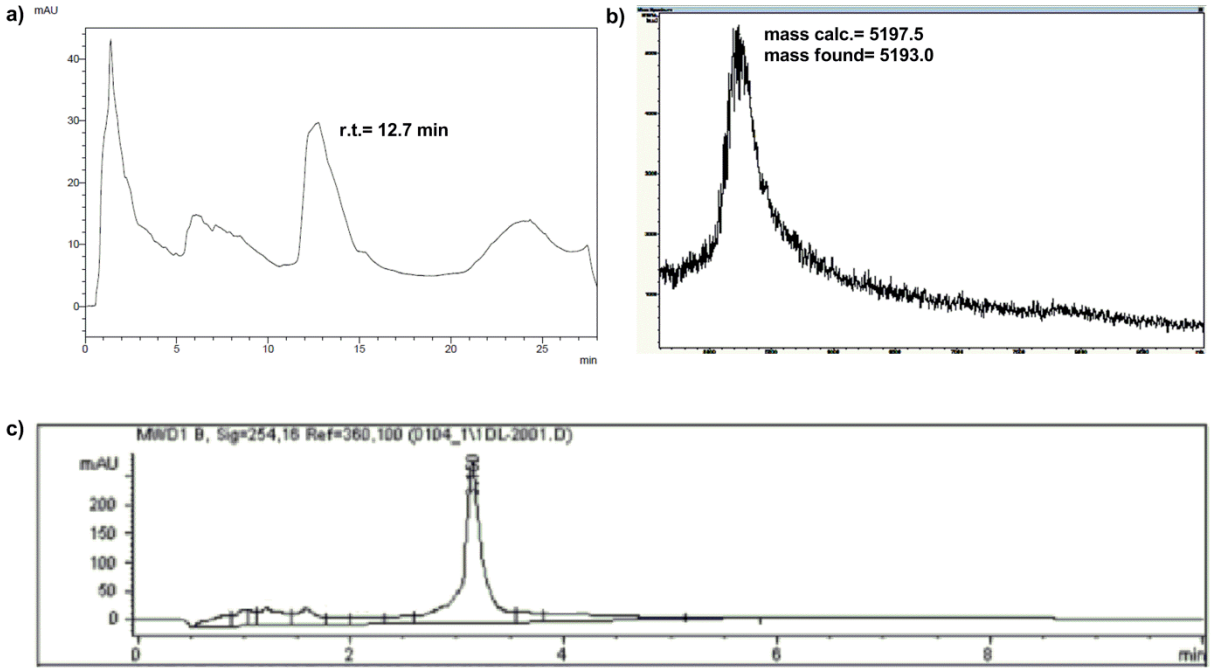


Figure S25: a) HPLC trace (preparative HPLC) of the crude conjugate **28AW**; b) MALDI-TOF/TOF-MS analysis of purified conjugate **28AW**; HPLC trace of purified conjugate **28AW**.

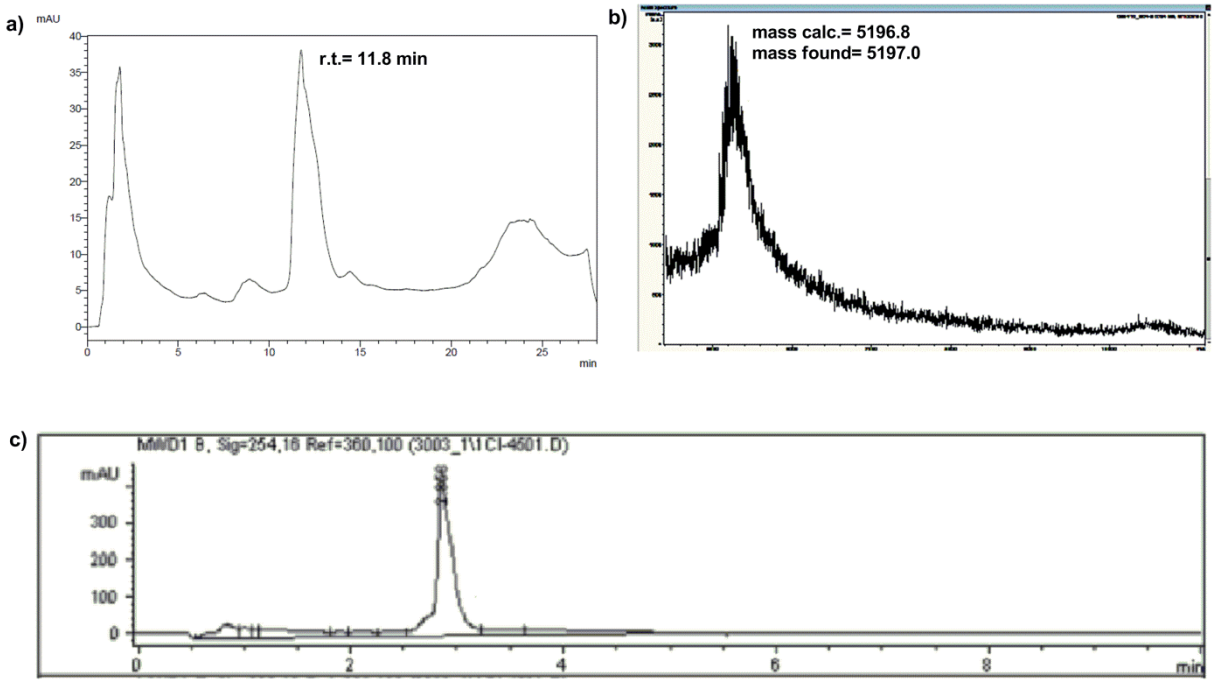


Figure S26: a) HPLC trace (preparative HPLC) of the crude conjugate **29AO**; b) MALDI-TOF/TOF-MS analysis of purified conjugate **29AO**; HPLC trace of purified conjugate **29AO**.

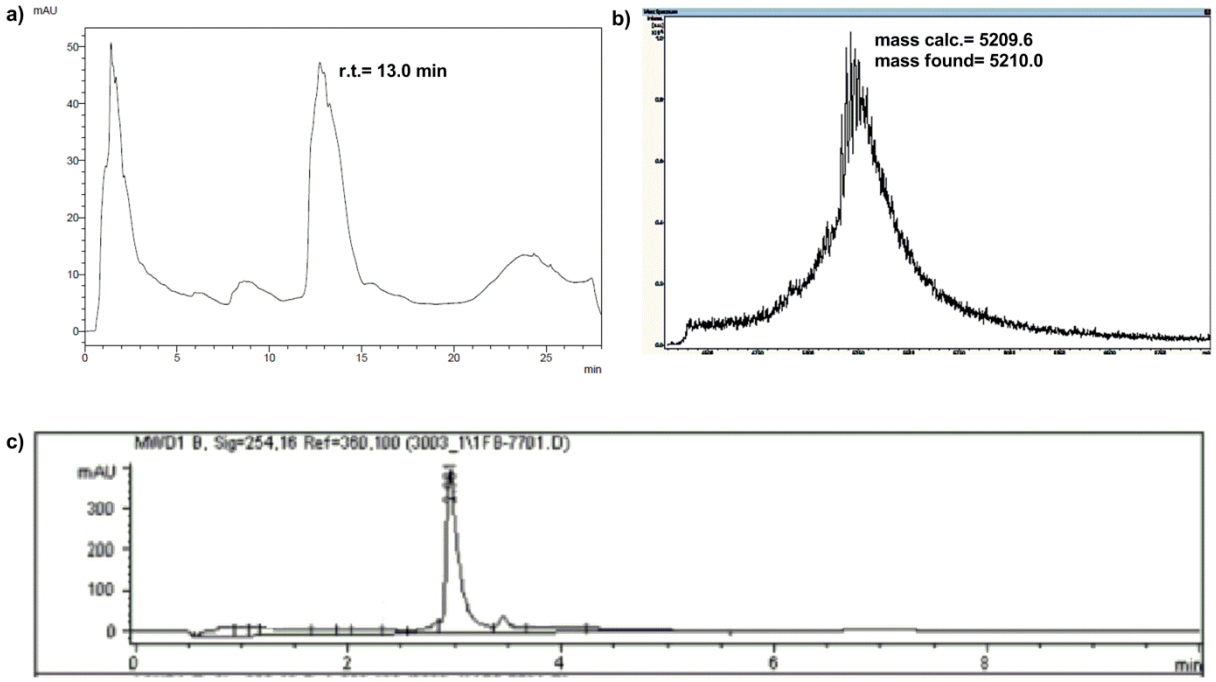


Figure S27: a) HPLC trace (preparative HPLC) of the crude conjugate **29AW**; b) MALDI-TOF/TOF-MS analysis of purified conjugate **29AW**; HPLC trace of purified conjugate **29AW**.

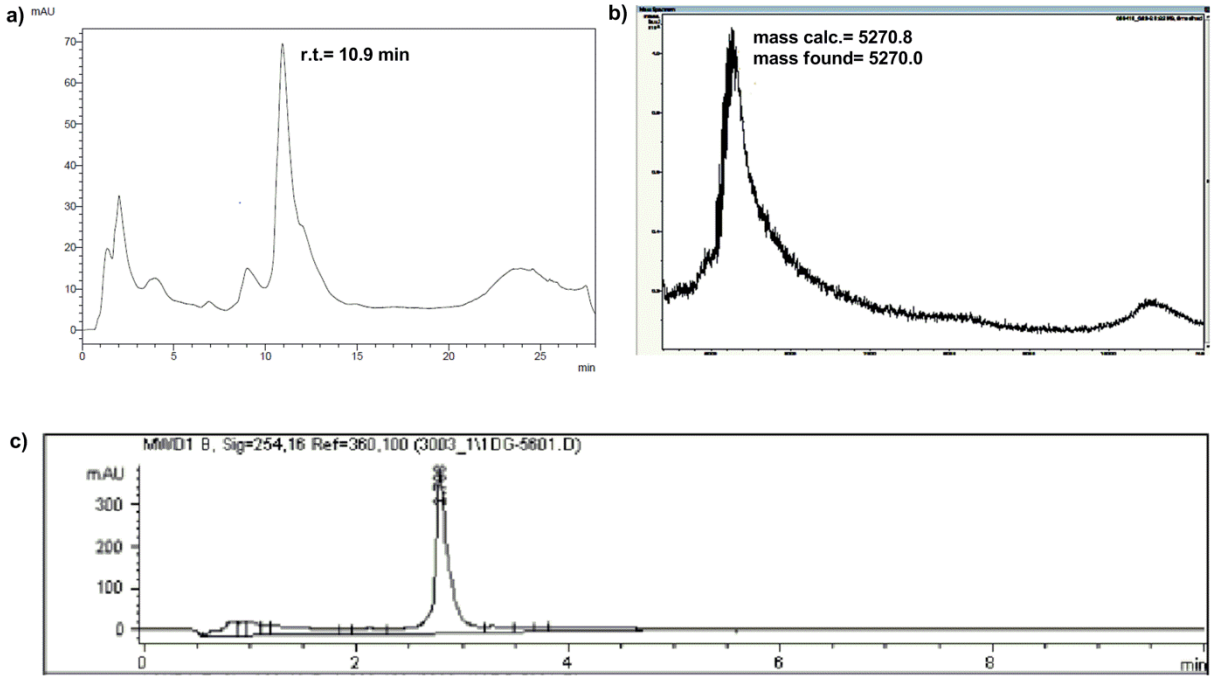


Figure S28: a) HPLC trace (preparative HPLC) of the crude conjugate **29CP**; b) MALDI-TOF/TOF-MS analysis of purified conjugate **29CP**; HPLC trace of purified conjugate **29CP**.

10. Validation of the CuAAC reaction with a DNA-conjugate of the amino acid 6

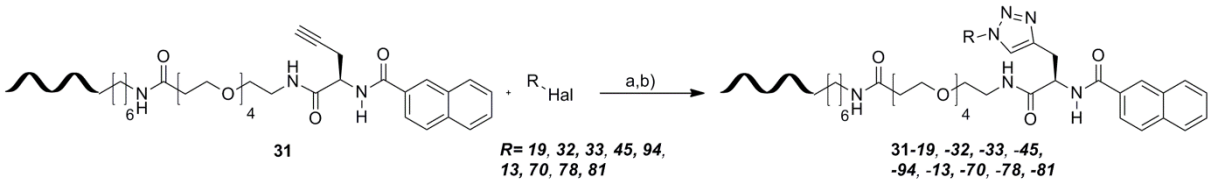
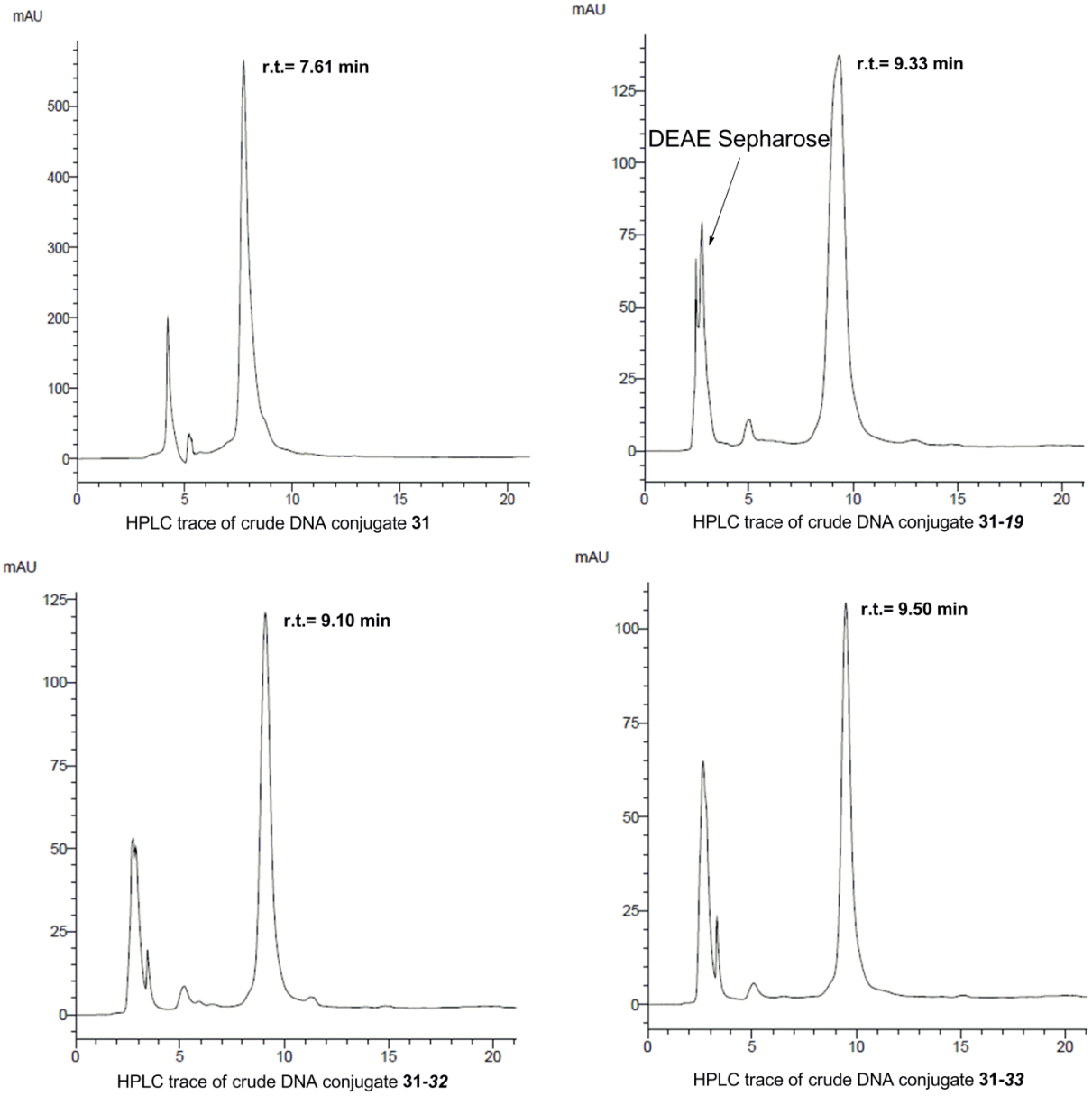
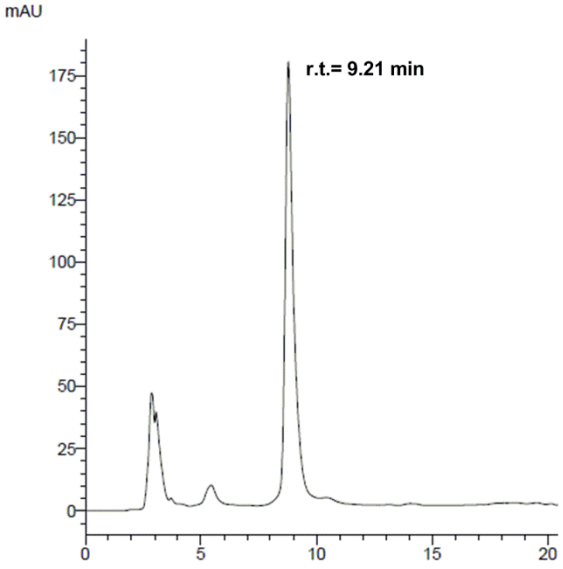
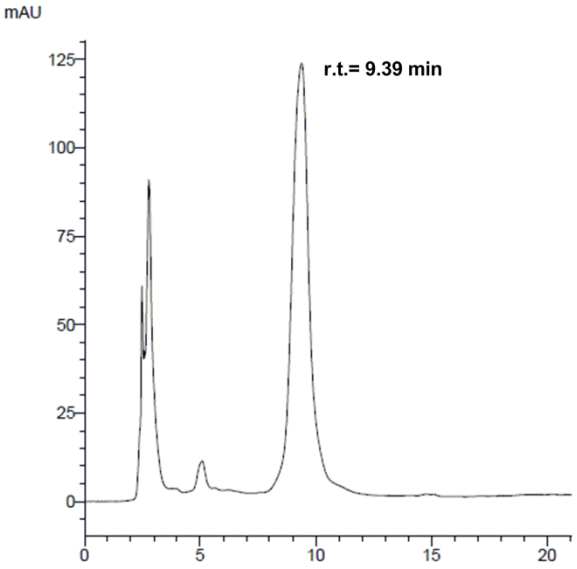


Figure S29: a) NaN_3 TBAI, DMF/H_2O; b) $CuSO_4$, TBTA, Na-ascorbate, DMF, MeOH, H_2O, DEAE-sepharose

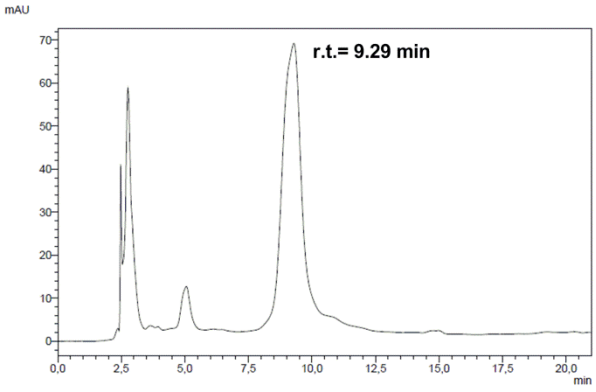




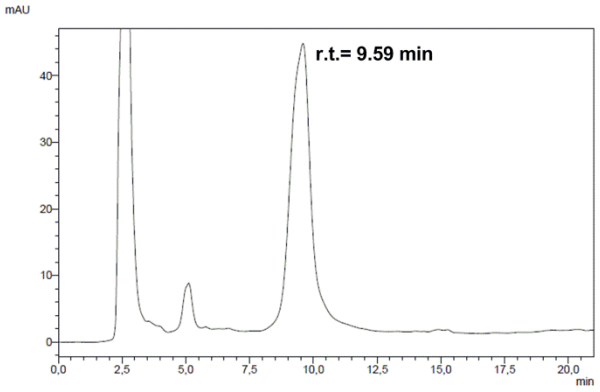
HPLC trace of crude DNA conjugate **31-*45***



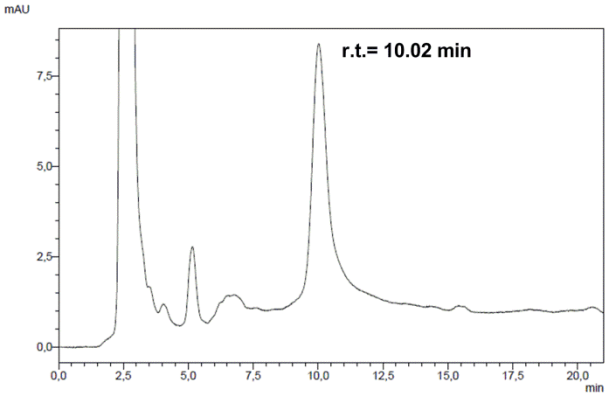
HPLC trace of crude DNA conjugate **31-*94***



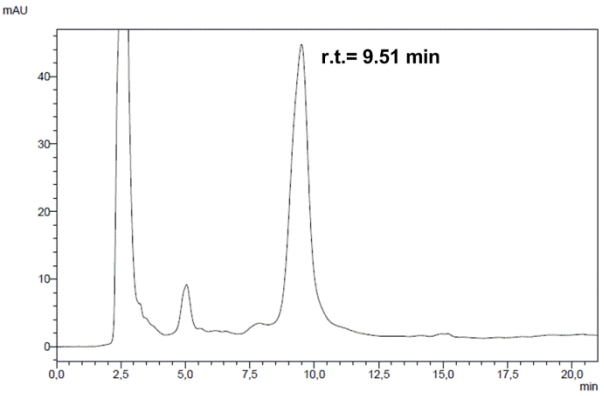
HPLC trace of crude DNA conjugate **31-*13***



HPLC trace of crude DNA conjugate **31-*70***



HPLC trace of crude DNA conjugate **31-*78***



HPLC trace of crude DNA conjugate **31-*81***