Supporting information

Acid- and Au(I)-mediated synthesis of hexathymidine-DNA-heterocycle chimeras, an efficient entry to DNA-encoded libraries inspired by drug structures

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1. Materials and instruments

Unless otherwise noted, chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany), Bachem (Bubendorf, Switzerland), Thermo Fisher Scientific (Karlsruhe, Germany), AppliChem (Darmstadt, Germany), and VWR (Langenfeld, Germany). Fmoc-NH-PEG(4)-COOH was purchased from Iris Biotech (Marktredwitz, Germany). PNK was purchased from Thermo Fisher Scientific, T4 DNA ligase (Rapid) from Biozym Scientific GmbH (Hessisch Oldendorf, Germany), Taq DNA Polymerase (5 U/µL), 10x PCR Rxn Buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), and $MgCl_2$ (50 mM aqueous solution) were purchased from Invitrogen (Carlsbad, CA). All primers were synthesized by Sigma-Aldrich Co. LLC (Munich, Germany). The dNTP Mix (25 mM each, 100 mM total) was obtained from Biozym Scientific GmbH. Agarose was purchased from Biozym Scientific GmbH. All PCR reactions were performed in an Eppendorf mastercycler personal (Eppendorf, Hamburg, Germany). GeneRuler Ultra Low Range DNA marker (Thermo Fisher Scientific) and PCR products were stained with Midori Green Direct (Nippon Genetics Europe, Düren, Germany). Gel documentation was done with Gel Doc XR+ from Bio-Rad (Hercules, CA). 5'-Aminolinker-modified DNA oligonucleotides attached to controlled pore glass solid phase (CPG, 1000 Å) were synthesized by IBA (Göttingen, Germany); DNA oligonucleotides used for encoding of the library were purchased from Integrated DNA Technologies (Leuven, Belgium). Controlled pore glass solid phase was filtered on a synthesis column plugged onto a vacuum manifold (Vac-Man®, Promega). Parallel reactions for library synthesis were performed in a DESYRE vortexer in 0.5 mL glass vials (Zinsser Analytic, Franfurt a. M., Germany). The oligonucleotide-small molecule conjugates were purified by ion pair reverse-phase high-pressure liquid chromatography (HPLC, Shimadzu Prominence) using a C_{18} 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. The oligonucleotide-small molecule conjugates were detected with a UV detector at 254 nm. The oligonucleotide-small molecule conjugates were analyzed by ion pair reverse phase high-pressure liquid chromatography (HPLC, Shimadzu Prominence) using a C_{18} stationary phase (Phenomenex, Gemini; 5 µm, C18, 110 Å, 100*4.6 mm) and a gradient of 10 mM aqueous triethylammonium acetate (pH 8)/MeOH. The oligonucleotide-small molecule conjugates were detected with a UV detector at 254 nm. Oligonucleotide concentrations were determined by a Nanodrop UV spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific). The oligonucleotides were analyzed by MALDI-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 = singulett, 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 (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 C_{18} 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. High resolution mass spectrometry (ESI) was performed on an Thermo LTQ Orbitrap coupled with a Accela HPLC system (Thermo Fisher Scientific).

1. Encoding hexT-conjugates, T4 ligation of duplex DNA sequences

5'-phosphorylation of DNA oligonucleotides

20 µL phosphorylation reactions contained 14 µM (280 pmol) oligonucleotides, 10 units of PNK (T4 PNK, Thermo Fisher Scientific), 2 µL of 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), 20 nmol of ATP (from a 10 mM aqueous solution of ATP, Thermo Fisher Scientific). The reactions were carried out at 37°C for 30 minutes, and stopped by heat inactivation at 75°C for 10 minutes.

Enzymatic ligation of double-stranded DNA

All DNA sequences are given in Tables S1 and S2. The indicated DNA oligonucleotides (Figure S1) were annealed in a total reaction volume of 20 µL at 85°C for 10 min and slowly cooled down to room temperature (25°C). The ligation reactions contained 2 µM oligonucleotides (40 pmol of each oligonucleotide), 600 units of T4 DNA ligase, and 2 µL of 10 × T4 DNA ligase buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT, 10 mM ATP, pH 7.6 at 25°C). The ligations were carried out at 25°C at the indicated times (overnight for library synthesis), followed by heat inactivation at 75°C for 10 minutes. Negative control reactions were performed in the same manner without addition of T4 DNA ligase.

Analysis of DNA ligations

Ligation reactions were analyzed by agarose gel electrophoresis (5.5 % agarose). Electrophoresis was carried out in TBE buffer (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. DNA was stained with Midori Green Direct, and the GeneRuler Ultra Low Range DNA Ladder was used as reference. The hexT-fluorescein conjugate **hexT-fluo** and its ligation products were detected by a laser scanner (Typhoon Trio+; GE Healthcare Life Sciences, Pittsburgh, PA).

Precipitation of DNA oligonucleotides

For precipitation of the DNA, EtOH was added to 70 percent v/v. The precipitates were stored at –80°C overnight, and centrifuged for 30 minutes (15000 rpm, 4°C, centrifuge 5424 (Eppendorf AG, Hamburg)). 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) the supernatant was removed and the DNA was dissolved in IDTE storage buffer (10 mM Tris, pH 7.5, 0.1 mM EDTA, IDT).

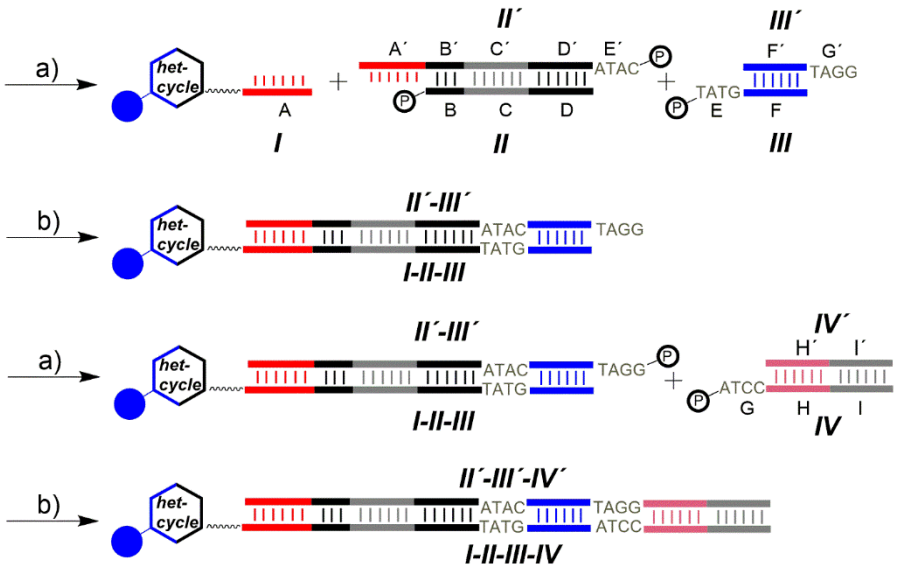


Figure S1. Scheme for enzymatic ligation of hexT conjugates (sequence ***I***); for the designation of the sequences see Tables S1 and S2; a) 5'-end phosphorylation and annealing; b) T4-ligation. **hetcycle**: β-carboline or pyrazol(in)e scaffold; blue filled circle denotes a substituent.

Table S1: Sequences of DNA oligonucleotides ***I*** – ***IV/IV'***

DNA	partial sequences (5'-3')	length	sequence (5'-3')[1]
I	A („hexT" sequence)	6mer	*5'AM*-TTT TTT
II	B-C-D	30mer	GAA TTC AGG TCG GTG TGA ACG GAT TTG XXX
II'	E'-D'-C'-B'-A'	40mer	ATA CXX XCA AAT CCG TTC ACA CCG ACC TGA ATT CAA AAA A
III	E-F	12mer	GTA TXX XXX XXX
III'	G'-F'	12mer	TAG GXX XXX XXX
I-II-III	A-B-C-D-E-F	48mer	*5'AM*-TTT TTT GAA TTC AGG TCG GTG TGA ACG GAT TTG XXX GTA TXX XXX XXX
II´-III´	G'-F'-E'-D'-C'-B'-A'	52mer	TAG GXX XXX XXX ATA CXX XCA AAT CCG TTC ACA CCG ACC TGA ATT CAA AAA A
IV	G-H-I	35mer	CCT AXX XXX XXX TGA CCT CAA CTA CAT GGT CTA CA
IV'	I´-H´	31mer	TGT AGA CCA TGT AGT TGA GGT CAX XXX XXX X
I-II-III-IV	A-B-C-D-E-F-G-H-I	83mer	*5'AM*-TTT TTT GAA TTC AGG TCG GTG TGA ACG GAT TTG XXX GTA TXX XXX XXX CCT AXX XXX XXX TGA CCT CAA CTA CAT GGT CTA CA
II´-III´-IV´	I´-H´-G'-F'-E'-D'-C'-B'-A'	83mer	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 TCA AAA AA

[1] *5'-AM* denotes C6-aminolinker; X denotes coding region

Table S2: Functions of the partial sequences of oligonucleotides ***I*** – ***IV/IV'***

partial sequence	function of partial sequence	length	sequence (5'-3')
A	hexT	6mer	*5'AM*-TTT TTT
A´	complementary sequence to A	6mer	AAA AAA
B	EcoRI restriction site	6mer	GAA TTC
B´	complementary sequence to B	6mer	GAA TTC
C	forward primer sequence	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 T4 ligation	4mer	GTA T
E´	complementary sequence to E	4mer	ATA C
F	codes for aldehyde synthons[1]	8mer	XXX XXX XX
F´	complementary sequence to F	8mer	XXX XXX XX
G	overhang for T4 ligation	4mer	CCT A
G´	complementary sequence to G	4mer	TAG G
H	codes for carboxylic acid synthons[1]	8mer	XXX XXX XX
H´	complementary sequence to H	8mer	XXX XXX XX
I	reverse primer sequence	23mer	TGA CCT CAA CTA CAT GGT CTA CA
I´	complementary sequence to I	23mer	TGT AGA CCA TGT AGT TGA GGT CA

[1] see Tables S3, S6, S7; [2] see Table S8

Validation of the encoding strategy

For validation of the encoding strategy a hexathymidine-fluorescine conjugate **hexT-fluo** was synthesized, and subsequently ligated with dsDNA sequences according to the encoding scheme depicted in figure S1.

Synthesis of the hexT-fluoresceine conjugate hexT-fluo

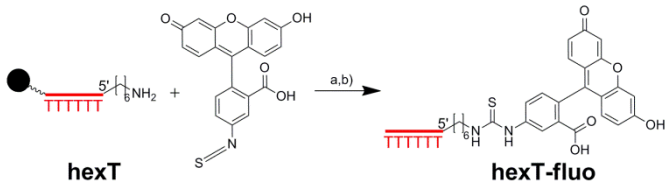


Figure S2. Synthesis of the hexT-fluorescein conjugate **hexT-fluo**; a) DMF, room temperature, 16h; b) K_2CO_3 in methanol, room temperature, 1h. Filled black denotes controlled pore glass (CPG) solid support.

The MMt-protective group of hexT bound to 1000 Å controlled pore glass (CPG) solid support (100 nmol, ca. 3.6 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 washed three times with each 200 µL of 1 % TEA in MeCN, then with DMF, MeOH, MeCN and DCM. Fluorescein isothiocyanate (10 µmol, 3.9 mg), dissolved in 50 µL of dry DMF was added, and the reaction was shaken overnight at room temperature. The CPG was washed three times with each 200 µL of DMF, MeOH, MeCN and DCM. The DNA was deprotected and removed from the CPG with 0.05 M K_2CO_3 in methanol for 60 minutes at room temperature (50 µL). The solution was neutralized with aq. acetic acid (10 %). Then, 20 µL of 1 M Tris buffer (pH= 7.5) was added, the mixture was concentrated to ca. 20 µL in a SpeedVac, filled up to 100 µL with distilled water, and the product **hexT-fluo** was purified by HPLC on a Gemini 5u C_{18} 110A column; 100*10.0 mm with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20% to 80% of methanol over 19 min). The purified sample was analyzed by MALDI-MS analysis. MS (calc.): 2333.3; MS (found): 2333.7.

The oligonucleotides **hexT-fluo**, 5'-phosphorylated ***II***, and ***II'*** (Table S1, Figure S3A) were annealed at 85°C for 10 min, slowly cooled down to room temperature (25°C), and ligated with T4 ligase for four hours. The ligation product was analyzed by agarose gel electrophoresis, and visualized by DNA stain (Figure S3B), or by fluorescence detection (Figure S3C).

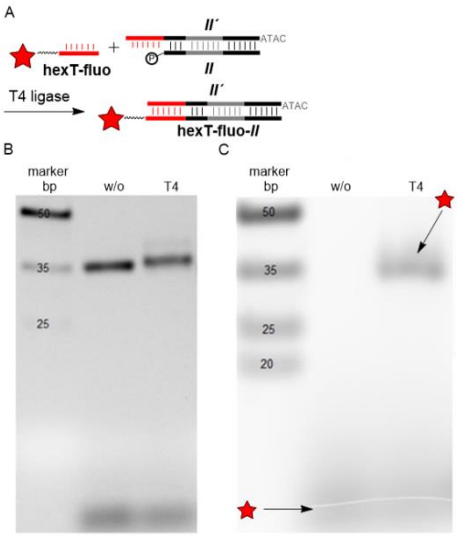


Figure S3. Ligation of the hexT-fluorescein conjugate **hexT-fluo** to the duplex ***II***/***II´*** (Table S1); A) ligation scheme; B) detection of the oligonucleotides with DNA stain. The product of the ligation reaction **hexT-fluo-*II*/*II'*** (right lane, T4) migrated slightly higher than the non-ligated duplex ***II***/***II´*** (left lane, w/o); C) fluorescence detection confirms formation of the fluorescently labeled duplex **hexT-fluo-*II*/*II´*** upon incubation with T4 ligase (right lane, T4); as control experiment the oligonucleotides were incubated without T4 ligase (left lane, w/o). The red star denotes fluorescein.

The oligonucleotides **hexT-fluo**, 5'-phosphorylated ***II***/***II´***, 5'-phosphorylated ***III***, and ***III´*** (Table S1, Figure S4A) were annealed at 85°C for 10 min, slowly cooled down to room temperature (25°C), and ligated with T4 ligase for four hours. The ligation product was analyzed by agarose gel electrophoresis, and visualized by DNA stain (Figure S4B), or by fluorescence detection (Figure S4C).

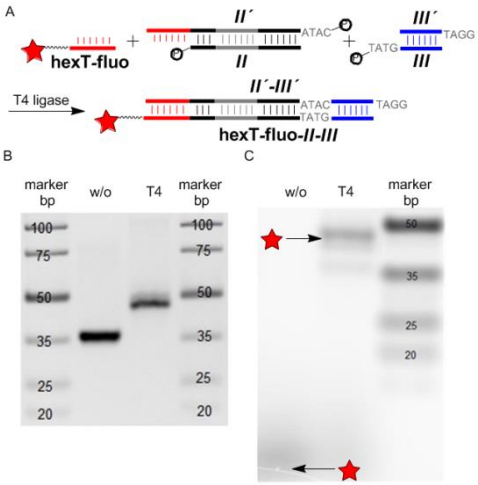


Figure S4. Ligation of the hexT-fluoresceine conjugate **hexT-fluo** to the 5'-phosphorylated duplex ***II***/***II´*** (Table S1), and the 5'-phosphorylated duplex ***III***/***III´*** (Table S1) *in one pot* yielding the labeled duplex **hexT-fluo-*II-III*/*II´-III´***; A) ligation scheme; B) detection of the oligonucleotides by staining with Midori Green, left lane (w/o): incubation of the oligonucleotides without T4 ligase; right lane (T4): incubation of the oligonucleotides with T4 ligase; C) fluorescence detection confirms formation of the fluorescently labeled duplex **hexT-fluo-*II-III*/*II´-III´*** upon incubation with T4 ligase (right lane, T4); as control experiment the oligonucleotides were incubated without T4 ligase (left lane, w/o). The red star denotes fluorescein.

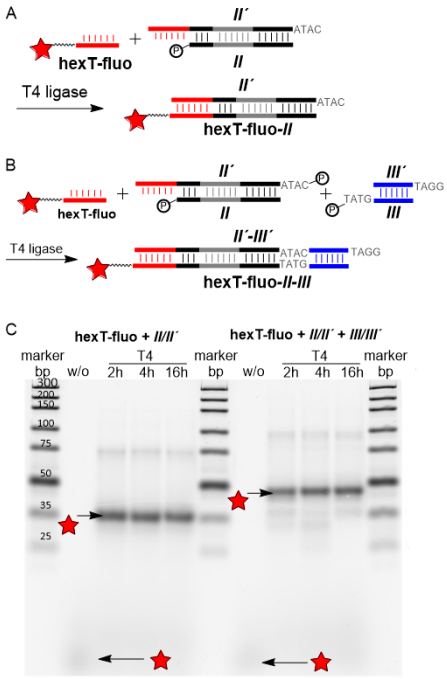


Figure S5. Time course of the ligation reactions depicted in Figures S3 and S4; A) scheme of the ligation of **hexT-fluo** to the duplex ***II***/***II´*** (Table S1); B) scheme of the ligation of **hexT-fluo** to the duplex ***II***/***II´***, and the duplex ***III***/***III´*** *in one pot*; C) fluorescence detection shows formation of the fluorescently labeled duplex DNA **hexT-fluo-*II*/*II´*** upon ligation for 2h, 4h, and 16h, w/o: incubation of the oligonucleotides without T4 ligase; fluorescence detection of the fluorescently labeled duplex duplex **hexT-fluo-*II*-*III*/*II´*-*III*'** upon ligation for 2h, 4h, and 16h, w/o: control incubation of the oligonucleotides without T4 ligase. The red star denotes fluorescein.

The duplex **hexT-fluo-*II-III*/*II´-III´*** (Figure S6) was isolated by precipitation (Figure S6B, left lane), 5'-phosphorylated, annealed at 85°C for 10 min with the 5'-phosphorylated ***IV*/*IV´***, slowly cooled to room temperature (25°C), and ligated for four hours with T4 ligase. The ligation product was analyzed by agarose gel electrophoresis, and visualized by DNA stain (Figure S6B), or by fluorescence detection (Figure S6C).

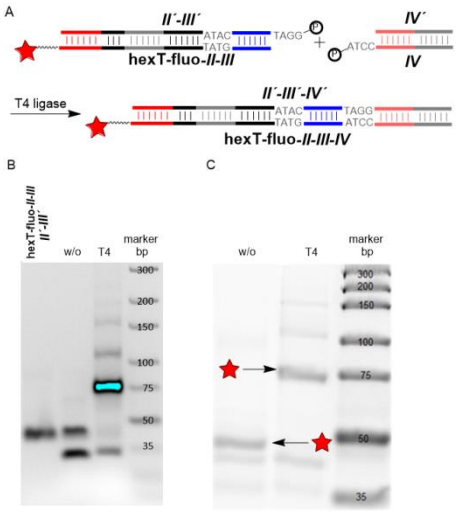


Figure S6. Gel analysis of the ligation reaction of the hexT-fluoresceine conjugate **hexT-fluo-*II-III*/*II´-III´*** with the duplex ***IV*/*IV´*** (Table S1); A) ligation scheme; B) detection of the oligonucleotides with DNA stain, **hexT-fluo-*II-III*/*II´-III´***: gel electrophoresis of the duplex **hexT-fluo-*II-III*/*II´-III´***, w/o: incubation of the two DNA duplexes **hexT-fluo-*II-III*/*II´-III´*** and ***IV*/*IV´*** without T4 ligase, T4: incubation of the two duplexes **hexT-fluo-*II-III*/*II´-III´*** and ***IV*/*IV´*** with T4 ligase for four hours; C) fluorescence detection of the fluorescently labeled duplex DNA **hexT-fluo-*II-III-IV*/*II´-III´-IV´*** formed upon ligation (right lane, T4), as control experiment the oligonucleotides were incubated without T4 ligase (left lane, w/o). The red star denotes fluorescein.

In order to validate the ligation reaction for the encoding of hexT-conjugates, seven hexT-β-carboline conjugates **hexT-11** (see Table S3 for numbering of aldehyde building blocks) were ligated for four hours to a duplex DNA ***II***/***II´*** in analogous manner to the ligation reaction of **hexT-fluo** shown in Figure S3. The ligation products **hexT-11-*II*/*II´*** were analyzed by agarose gel electrophoresis (Figure S7B), they migrated in similar manner as the labeled **hexT-fluo-*II*/*II´*** relative to negative control experiments (Figure S3B).

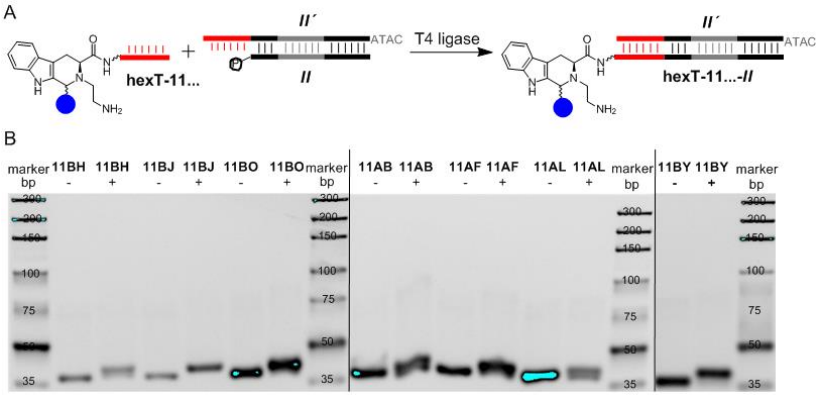


Figure S7. Gel analysis of the ligation reaction of seven hexT-β-carboline conjugates **hexT-11** (see Table S3 for numbering of aldehyde building blocks) with the duplex ***II***/***II'*** (Table S1); A) ligation scheme; B) analysis of the ligation reactions by gel electrophoresis. The products of the ligation reactions migrated slightly higher than the non-ligated duplex ***II***/***II´***; - denotes negative control experiment without T4 ligase; + denotes ligation reaction for four hours at 25°C. Note that Figure S7B was concatenated from three different gels.

Seven representative hexT-pyrazoline library members **hexT-20** (see Table S6 for numbering of aldehyde building blocks) were ligated for four hours to a duplex DNA ***II***/***II´*** in analogous manner to the ligation reaction of **hexT-fluo** shown in Figure S3. The ligated compounds were analyzed by agarose gel electrophoresis (Figure S8B), they migrated in similar manner as the labeled **hexT-fluo-*II*/*II´*** relative to negative control experiments (Figure S3B).

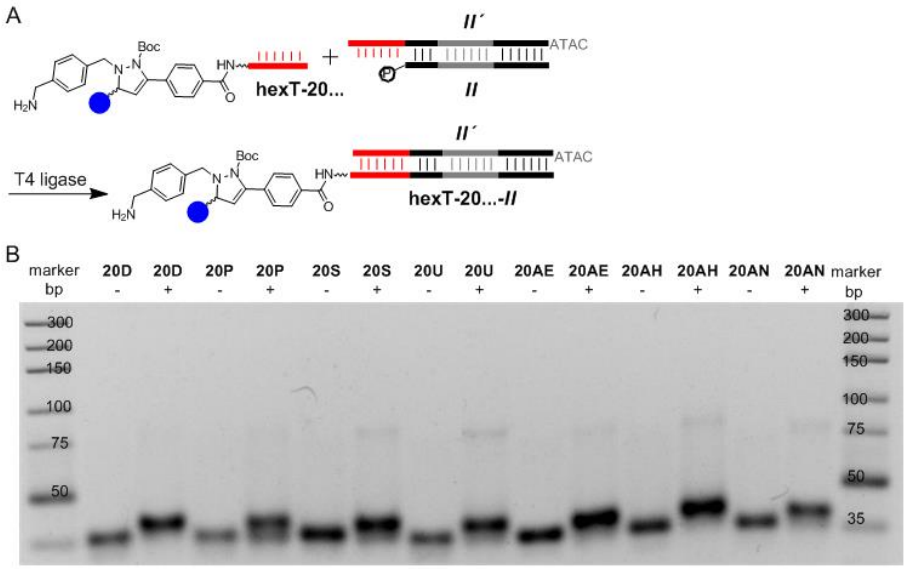


Figure S8. Gel analysis of the ligation reaction of seven hexT-pyrazoline conjugates **hexT-20** (see Table S6 for numbering of aldehyde building blocks) with the duplex ***II***/***II'*** (**Table S1**); A) ligation scheme; B) analysis of the ligation reactions by gel electrophoresis. The products of the ligation reactions migrated slightly higher than the non-ligated duplex ***II***/***II'***; - denotes negative control experiment without T4 ligase; + denotes ligation reaction for four hours at 25°C.

Seven representative hexT-β-carboline library members **hexT-11** (see Table S3 for numbering of aldehyde building blocks) were ligated for **four hours** to DNA duplexes ***II*/*II´***, *and* ***III*/*III´*** *in one pot* (Figure S9A) in analogous manner to the ligation of ***hexT-fluo*** shown in Figure S4. The ligation products were analyzed by agarose gel electrophoresis (Figure S9B). The ligation efficiency of the one-pot ligation was mostly higher than 50 % in this experiment.

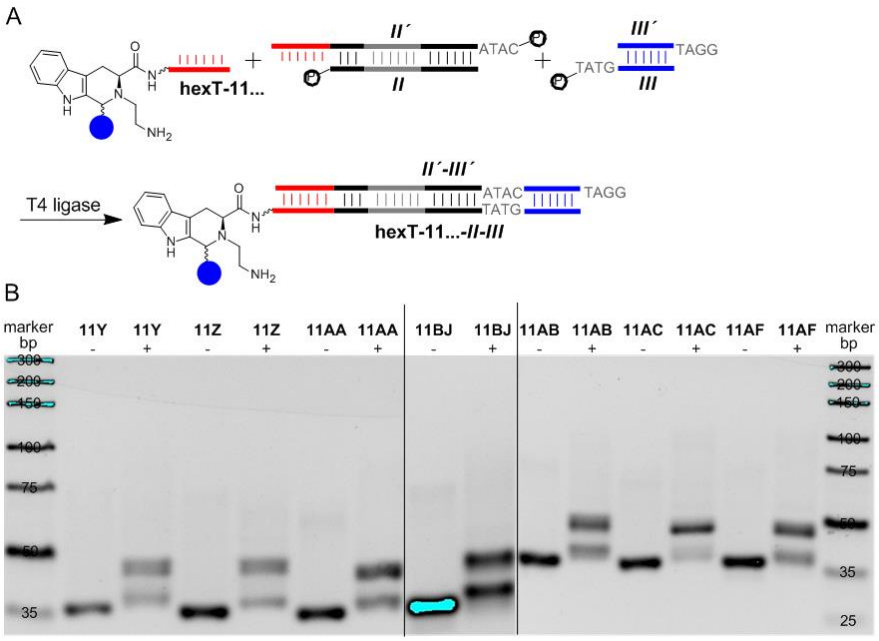


Figure S9. Gel analysis of the ligation reaction of hexT-β-carboline library members **hexT-11** (see Table S3 for numbering of aldehyde building blocks) with the duplex ***II*/*II´*** and ***III*/*III´*** in one pot (Table S1); A) ligation scheme; B) detection of the oligonucleotides by DNA staining; - denotes negative control experiment w/o ligase; + denotes ligation reaction for four hours at 25°C. Note that Figure S9B was concatenated from three different gels.

Seven representative hexT-pyrazoline library members **hexT-20** (see Table S6 for numbering of aldehyde building blocks) were ligated for **four hours** to DNA duplexes ***II*/*II´***, *and* ***III*/*III´*** *in one pot* (Figure S10A) in analogous manner to the ligation of **hexT-fluo** shown in Figure S4. The ligation products were analyzed by agarose gel electrophoresis (Figure S10B). The ligation efficiency of the one-pot ligation was mostly higher than 50 % in this experiment.

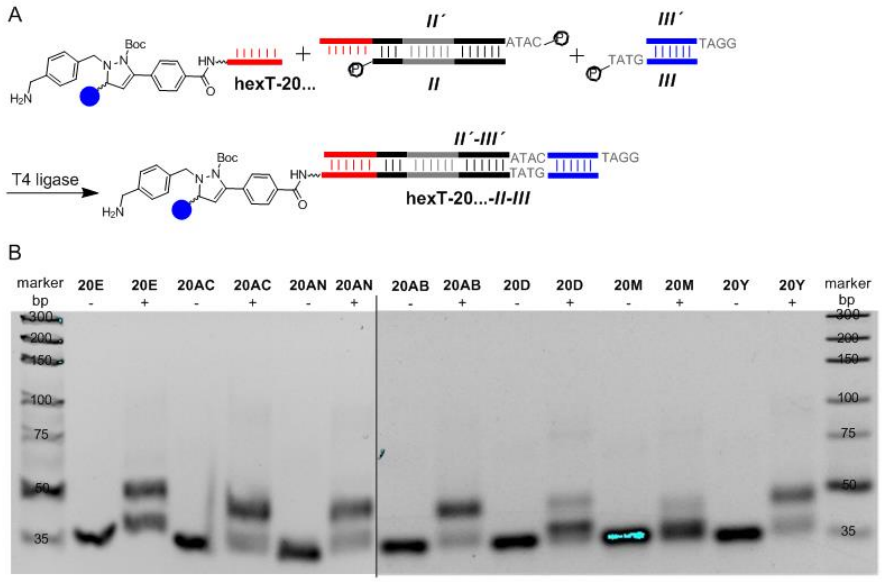


Figure S10. Gel analysis of the ligation reaction of seven hexT-pyrazol(in)e conjugates **hexT-20** (Table S6) with the duplexes ***II*/*II´*** and ***III*/*III´*** in one pot (Table S1); A) ligation scheme; B) detection of the oligonucleotides by staining with Midori Green; - denotes negative control experiment w/o ligase; + denotes ligation reaction for four hours at 25°C. Note that Figure S9 was concatenated from two different gels.

Four randomly selected hexT-pyrazolines **hexT-20** (see Table S6 for numbering of aldehyde building blocks), hexT-pyrazoles **hexT-21** (see Table S7 for numbering of aldehyde building blocks), and four hexT-β-carbolines **hexT-11** (see Table S3 for numbering of aldehyde building blocks) were ligated **overnight** to DNA duplexes ***II***/***II´***, and to duplexes ***II***/***II´***and ***III***/***III´*** *in one pot* (Figure S11) in analogous manner to the ligation of **hexT-fluo** shown in **Figure S3** and **S4**. The ligation products were analyzed by agarose gel electrophoresis (Figure S10B). The ligation efficiency of the one-pot ligation was higher than 90 % in this experiment.

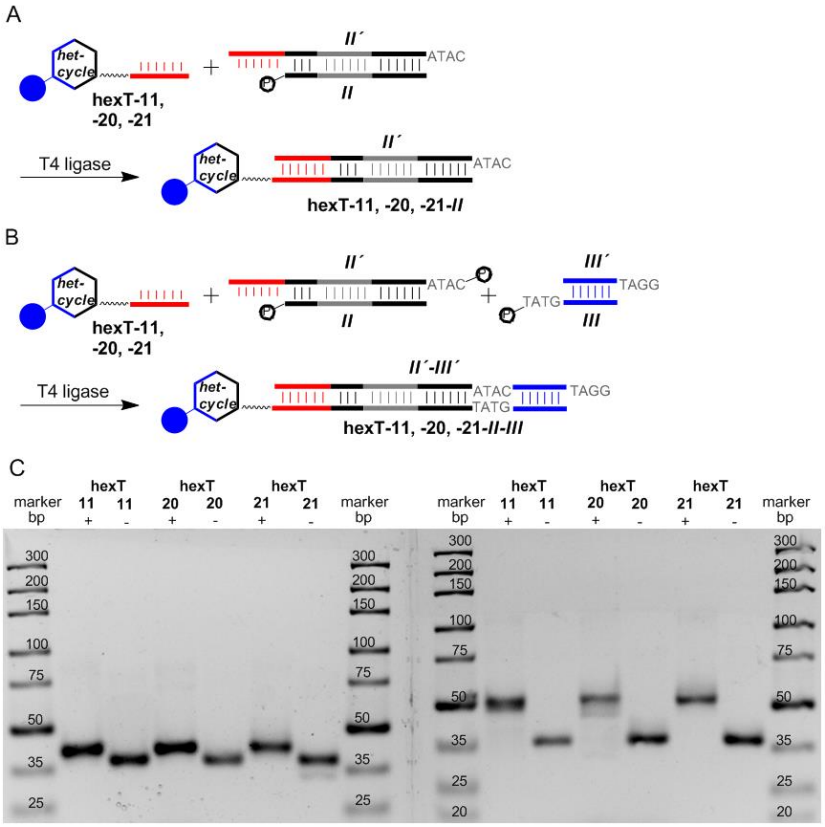


Figure S11. ***Overnight*** ligation of four hexT-pyrazoline conjugates **hexT-20** (**D**, **Y**, **AM**, **BC**), four hexT-pyrazole conjugates **hexT-21** (**D**, **E**, **AG**, **AK**), and four hexT-β-carboline conjugates **hexT-11** (**D**, **S**, **AT**, **BM**) to the duplex ***II***/***II´***, and to the duplexes ***II***/***II´*** and ***III***/***III´*** *in one pot* (Table S1); A) ligation scheme for the ligation to the duplex ***II***/***II´***; B) ligation scheme for the ligation to the duplexes ***II***/***II´*** and ***III***/***III´*** *in one pot*; C) for analysis of the ligation reactions each four reactions were pooled and analyzed by gel electrophoresis, left gel shows the ligation of each four conjugates **hexT-11**, **-20**, **-21** to the duplex ***II***/***II´***, right gel shows the ligation of each four conjugates **hexT-11**, **-20**, **-21** to the duplexes ***II***/***II´*** and ***III***/***III´*** *in one pot*; - denotes negative control experiment w/o ligase; + denotes ligation reaction for 16 h at 25°C. Note that Figure S11C was concatenated from two different gels.

3. Synthesis of hexT–conjugates by amide coupling

Procedure for coupling of carboxylic acids to 5'-aminolinker-hexT–conjugates on 1 µmol scale

The MMt-protective group of 5'-aminolinker-modified hexT bound to 1000 Å controlled pore glass (CPG) solid support (1 µmol, ca. 36 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 amine-deprotected DNA was washed three times with each 200 µL of 1 % TEA in MeCN, DMF, MeOH, MeCN and DCM. The CPG, a carboxylic acid, and HATU were then dried *in vacuo* for 2 h. Stock solutions of all reactants in dry DMF were prepared immediately before the reaction was started. To 150 µL of a solution of a carboxylic acid (100 µmol, 100 eq.) in dry DMF were added HATU (38 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-conjugate was filtered over 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 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 DNA-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 30 min at room temperature. To this solution 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).

a) Synthesis of the 5'-amino-PEG(4)-hexT conjugate

The MMt-protective group of the 5'-aminolinker-modified hexathymidine bound to 1000 Å controlled pore glass (CPG) solid support (1 µmol, ca. 36 mg) was removed by addition of 3 % trichloroacetic acid in dry DCM (3 x 200 µL) for 3 x 1 min. The CPG containing the deprotected DNA was then washed three times with each 200 µL of 1 % TEA in MeCN, DMF, MeOH, MeCN and DCM. The CPG, Fmoc-NH-PEG(4)-COOH, and HATU were dried *in vacuo* for 15 min. Stock solutions of all reactants in dry DMF were prepared immediately before the reaction was started. To 150 µL of a solution of the Fmoc-NH-PEG(4)-COOH linker (49 mg, 100 µmol, 100 eq.) in dry DMF were added HATU (38 mg, 100 µmol, 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 over 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 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 30 min at room temperature. To this solution 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). For further coupling of carboxylic acids to the linker, the Fmoc-group (1 µmol, ca. 36 mg of the solid support) was removed with 20 % piperidine in dry DMF (0.4 mL). The reaction mixture was shaken for 5 min at room temperature. The CPG containing the deprotected hexT was washed three times with each 200 µL of DMF, MeOH, MeCN and DCM.

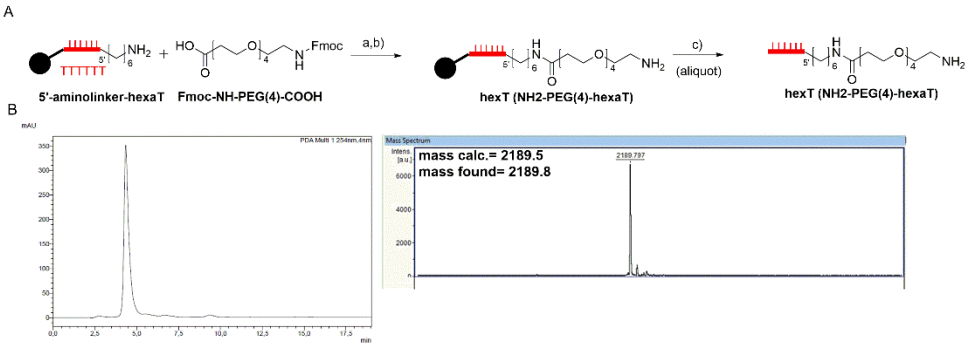


Figure S12. Synthesis of the 5'-amino-PEG(4)-linker modified **hexT**. A) Synthesis scheme. Reagents and conditions: a) HATU, DIPEA, dry DMF, room temperature, 2 hours; b) 20 % piperidine in dry DMF; c) cleavage of an aliquot of the hexT from the solid support: AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %)= 1:1, vol/vol), 30 min, room temperature. B) HPLC trace and MALDI-MS spectrum of the 5'-amino-PEG(4)-linker modified hexT. Filled black circle denotes solid support (CPG).

b) Synthesis of DNA conjugates hexT-8 and hexT-12

The pegylated hexT (1 µmol) was coupled either with Fmoc-L-tryptophane (100 µmol, 100 eq.) to furnish **hexT-8**, or with *p*-ethynylbenzoic acid (100 µmol, 100 eq.) to furnish **hexT-12** according to the procedure for coupling of carboxylic acids to amino-modified hexT. For analysis, an aliquot of ca. 10 nmol of each conjugate **hexT-8** and **hexT-12** was deprotected and cleaved from the CPG with 500 µL of AMA (AMA= aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol) for 30 min 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).

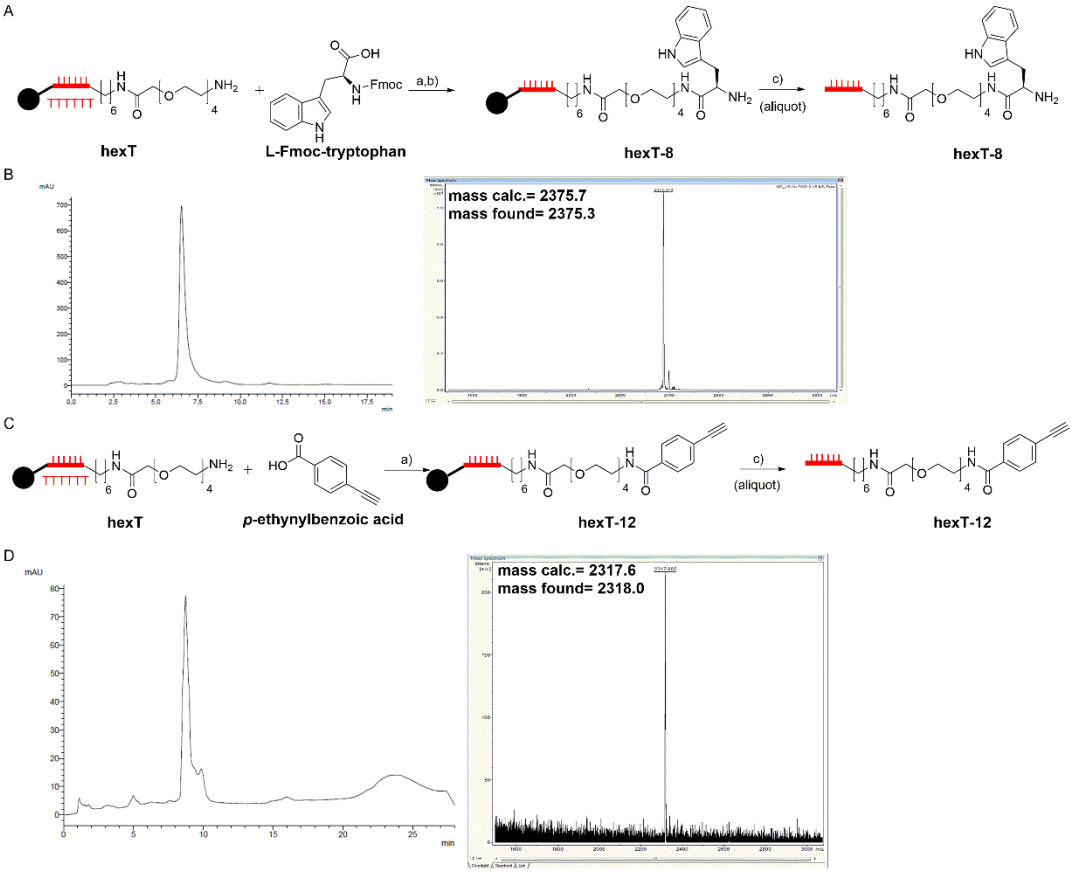


Figure S13. Synthesis of hexT-tryptophane conjugate **hexT-8** and hexT-alkyne **hexT-12**. A) Synthesis scheme for compound **hexT-8**. Reagents and conditions: a) HATU, DIPEA, dry DMF, room temperature, 2 hours; b) 20 % piperidine in dry DMF; c) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature (aliquot); B) HPLC trace and MALDI-MS spectrum of **hexT-8**; C) synthesis scheme for compound **hexT-12**; D) HPLC trace and MALDI-MS spectrum of **hexT-12**. Filled circle denotes solid support (controlled pore glass, CPG); bold bond denotes linkage of the hexT-DNA to the solid support.

4. Synthesis of hexT-β-carboline conjugates 11A – 11DJ by Pictet-Spengler reaction and reductive amination

4.1 Optimization of the reaction conditions for the synthesis of hexT-β-carboline conjugates

A solution of a catalyst in a solvent (Table 1) was freshly prepared as stock. Aldehyde **BO** (20 µmol, Table S3) was dissolved in this solution to a final concentration of 0.44 M. This solution (45 µL, 20 µmol of **BO**) was added to 20 nmol (ca. 0.7 mg) of CPG-bound **hexT-8**. The reaction was run for different times as given in Table 1. The CPG was filtered off, washed successively with DMF (3 x 200 µl), MeCN (3 x 200 µl), MeOH (3 x 200 µl) and DCM (3 x 200 µl), and dried *in vacuo* for 15 min. The DNA was removed from the CPG by treatment with 500 µL of AMA (AMA= aqueous ammonia (30 %)/ aqueous methylamine (40 %) 1:1 vol/vol) at room temperature for 30 min. Then, 20 µL of 1M Tris-buffer (pH=7.5) was added, the mixture was dried in a SpeedVac, re-dissolved in 100 µL of distilled water and the product was purified by HPLC on a Gemini 5u C_{18} 110A column; 100*10.0 mm with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20:80 – 70:30 of methanol over 19 min).

4.2 Synthesis of hexT-β-carboline conjugates hexT-11A – 11CB

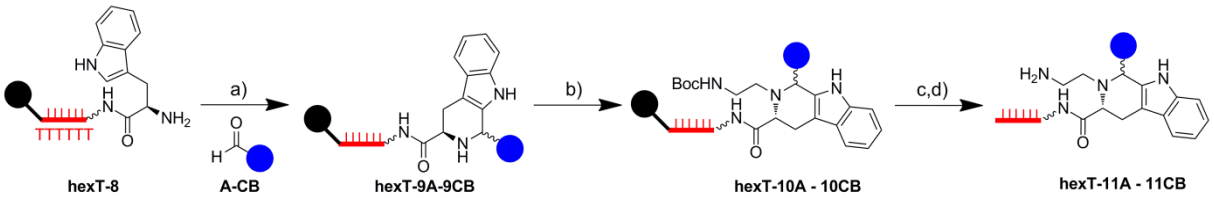


Figure S14. Synthesis of hexT-conjugates **11A**-**CB**. a) 2% TFA in CH_2Cl_2; b) $NaCNBH_4$, MOPS buffer; c) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %)= 1:1, vol/vol), 30 min, room temperature; d) 10 % TFA in dry DCM. Filled black circle: CPG solid support; wavy bond: 5'-PEG-linker; bold bond: linkage of the hexT oligonucleotide to the CPG.

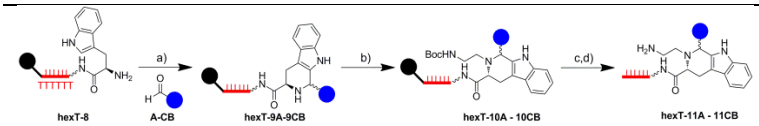
The CPG (600 nmol, ca. 22 mg) containing the deprotected **hexT**-**8** was suspended in dry CH_2Cl_2 and split into 20 aliquots of each ca. 30 nmol in glass vials. The supernatant was removed by pipette, and the CPG was dried in a desiccator. A solution of 2 % TFA in dry CH_2Cl_2 was freshly prepared as stock. Aldehydes **A** – **CB** were dissolved in this solution (final amount of 30 µmol of each aldehyde **A** – **CB** in 45 µL). These were added to the 20 nmol aliquots of CPG-bound **hexT**-**8**. Typically, 20 reactions were run in parallel at room temperature for 18 h. The CPG was filtered off, washed successively with DMF (3 x 200 µl), MeCN (3 x 200 µl), MeOH (3 x 200 µl) and CH_2Cl_2 (3 x 200 µl), and dried *in vacuo* for 15 min.

From each a 100fold stock solution, *N*-Boc-2-aminoacetaldehyde (5 µl, 20 µmol, 0.32 mg, dissolved in dry DMF), sodium cyanoborohydride (95 µl, 20 µmol, 0.13 mg, dissolved in MOPS-Puffer (300 mM, pH 7.4)), and 0.8 µl of acetic acid were combined, and added to the ca. 30 nmol of a CPG-bound **hexT-9A**-**CB**. The reaction was run at 40°C for 16 h, the CPG was filtered off, washed with DMF (3 x 200 µl), MeCN (3 x 200 µl), MeOH (3 x 200 µl) and CH_2Cl_2 (3 x 200 µl), and dried *in vacuo* for 15 min. The products **hexT-10A**-**CB** were removed from the CPG with 200 µL of AMA (AMA= aqueous ammonia (30 %)/ aqueous methylamine (40 %) 1:1 vol/vol) at room temperature for 30 min. The

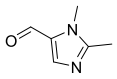
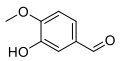
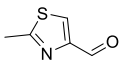
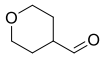
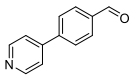
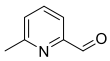
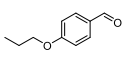
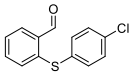
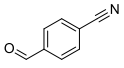
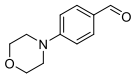
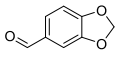
solution was filtered off, dried in a SpeedVac, re-dissolved in 100 µL of distilled water, and optionally purified by HPLC on a Gemini 5u C_{18} 110A column; 100*10.0 mm with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20:80 – 70:30 of methanol over 19 min). All products were quantified by Nanodrop measurement.

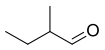
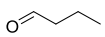
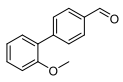
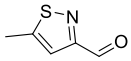
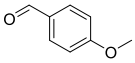
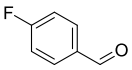
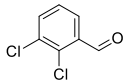
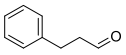
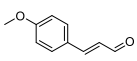
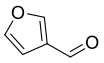
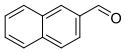
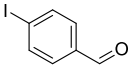
For the deprotection of the Boc-group, the conjugates **hexT-10** (each ca. 1 nmol) were dried in a SpeedVac, and three times co-evaporated with a mixture of ethanol/water (1:1, each 100 µl). A solution of 10 % of TFA in 20 µl of dry DCM was added to the dry conjugates **hexT-10**, and shaken at room temperature for 6 h to obtain the conjugates **hexT-11**. The solvent was removed in a SpeedVac, and the product was co-evaporated twice with ethanol/water (1:1, each 100 µl). Quantification of the conjugates by Nanodrop measurement showed quantitative recovery of the products, and MALDI-MS analysis proved the integrity of the products. A number of compounds were additionally analyzed by HPLC to corroborate the results of the mass analysis.

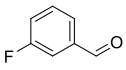
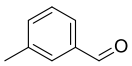
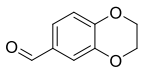
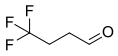
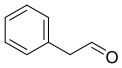
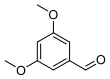
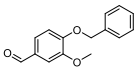
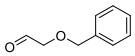
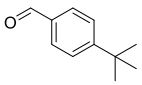
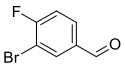
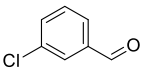
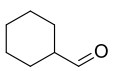
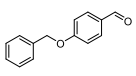
Table S3: MALDI-MS data of hexT-conjugates **9A-9CB**-**11A**-**11CB**.

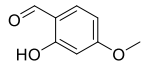
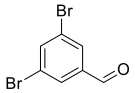
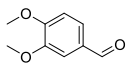
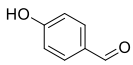
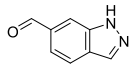
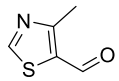
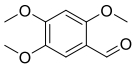
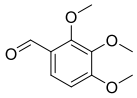
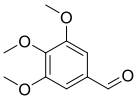
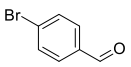
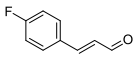
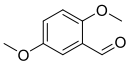
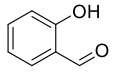
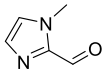


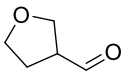
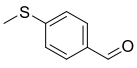
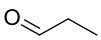
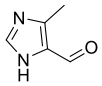
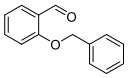
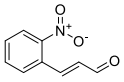
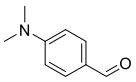
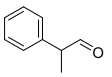
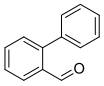
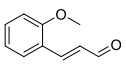
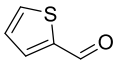
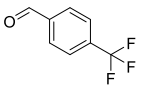
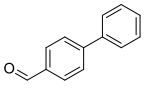
Reagents and conditions: see figure S14

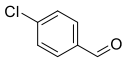
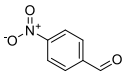
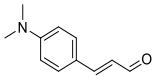
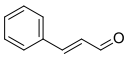
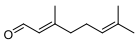
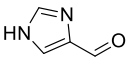
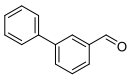
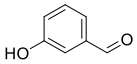
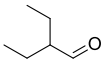
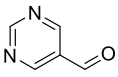
		hexT-9	hexT-10		hexT-11
No.	**structure**	**mass calc. mass found[a]**	**mass calc. mass found[a]**	**Yield[b] [nmol]**	**mass calc. mass found[a]**
A		2481.8 2483.9	2629.8 2630.7[c]	2.5	2530.8 2528.8
B		2509.9 2510.5	2655.9 2656.7[c]	1.5	2554.8 2556.1
C		2484.9 2482.2	2631.4 2630.4	3.5	2532.4 2549.2
D		2471.8 2473.5	2618.8 2619.6[c]	2.8	2519.8 2533.0
E		2540.9 2540.6	2685.9 2686.2	18.4	2587.9 2589.4
F		2478.8 2479.7	2625.8 2626.2	9.2	2526.8 2527.4
G		2521.9 2521.6	2668.9 2668.5	24.4	2569.9 2567.8
H		2606.4 2606.5	2751.4 2751.6	11.2	2653.4 2651.9
I		2504.8 2505.4	2650.8 2651.1[c]	5.9	2551.8 2552.7
J		2488.8 2487.5	2634.8 2634.7	24.6	2535.8 2536.6
K		2548.9 2548.7	2694.9 2695.3	22.8	2595.9 2596.7
L		2507.8 2507.5	2653.8 2653.9	16.7	2553.8 2554.1

No.	structure	hexT-9	hexT-10		hexT-11
		mass calc. mass found[a]	mass calc. mass found[a]	Yield[b] [nmol]	mass calc. mass found[a]
M		2444.8 2445.5	2588.8 2590.1	15.7	2489.8 2489.9
N		2429.8 2429.5	2575.8 2575.0	15.7	2476.8 2477.5
O		2569.9 2569.5	2715.9 2715.5	17.9	2616.9 2615.4
P		2484.9 2483.1	2630.9 2630.4	2.9	2531.9 2533.9
Q		2493.9 2494.5	2639.9 2638.9	16.5	2540.9 2539.2
R		2478.8 2477.7	2627.8 2627.3	22.7	2524.9 2526.8
S		2532.7 2533.3	2678.7 2678.1	2.4	2576.7 2577.2
T		2491.9 2492.4	2635.9 2636.9	6.1	2536.9 2537.4
U		2519.9 2519.5	2665.9 2665.1	5.3	2566.9 2567.4
V		2453.8 2455.0	2599.8 2600.1	24.4	2501.8 2503.3
W		2453.8 2453.5	2599.7 2599.8	19.6	2496.8 2497.3
X		2513.6 2513.9	2659.9 2659.5	22.1	2560.9 2560.3
Y		2429.8 2430.1	2574.8 2575.6	15.4	2476.8 2474.5
Z		2589.7 2590.2	2734.7 2735.9	18.8	2636.7 2636.4

No.	structure	hexT-9 mass calc. mass found[a]	hexT-10 mass calc. mass found[a]	hexT-10 Yield[b] [nmol]	hexT-11 mass calc. mass found[a]
AA		2443.8 2444.6	2589.8 2589.9	16.5	2490.8 2491.4
AB		2481.8 2479.7	2627.8 2627.0	18.7	2528.8 2529.7
AC		2477.9 2477.7	2623.9 2624.4	17.8	2524.9 2525.6
AD		2521.9 2521.8	2667.9 2667.8	11.3	2568.9 2568.7
AE		2483.8 2484.1	2629.8 2629.5	13.4	2530.8 2532.9
AF		2477.8 2478.5	2623.9 2623.9	13.7	2524.9 2524.4
AG		2523.9 2524.4	2669.9 2670.3	17.4	2570.9 2567.4
AH		2599.9 2600.4	2746.0 2745.2	13.6	2647.0 2648.1
AI		2507.9 2508.3	2653.9 2653.6	22.3	2554.9 2556.2
AJ		2519.9 2520.5	2665.9 2665.3	23.7	2566.9 2565.7
AK		2560.7 2557.2	2706.7 2706.5	21.5	2607.7 2608.2
AL		2498.3 2494.5	2644.3 2645.1	17.3	2545.3 2545.9
AM		2469.9 2469.1	2615.9 2615.7	15.3	2516.9 2518.0
AN		2569.9 2570.4	2715.9 2716.1	11.1	2616.9 2616.0

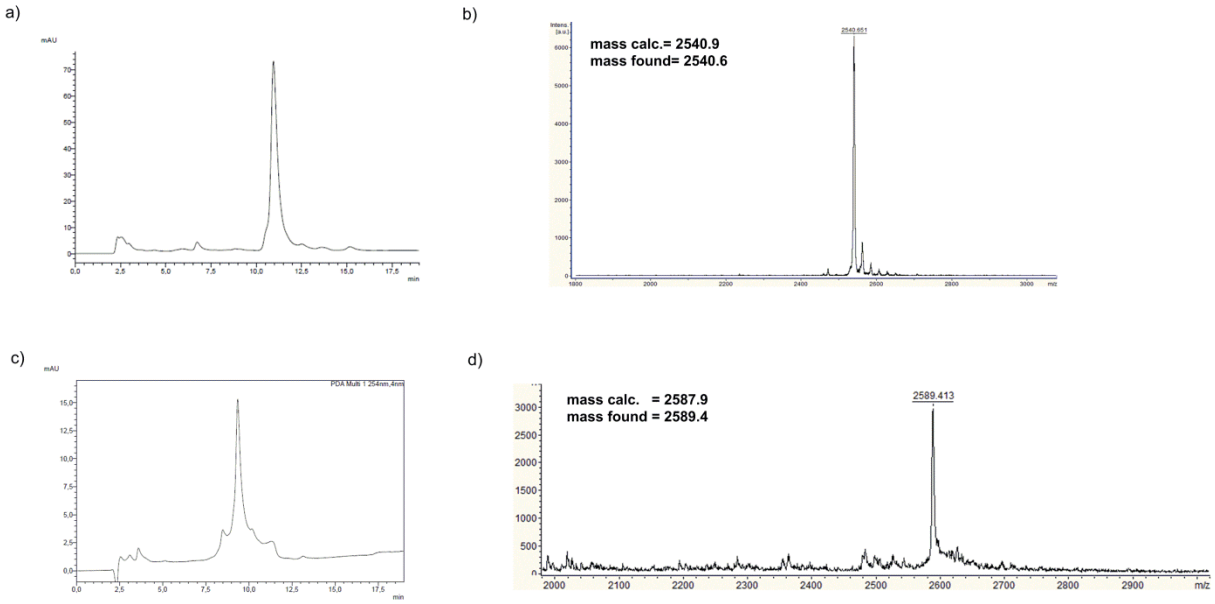
No.	structure	hexT-9 mass calc. mass found[a]	hexT-10 mass calc. mass found[a]	hexT-10 Yield[b] [nmol]	hexT-11 mass calc. mass found[a]
AO		2523.9 2524.4	2655.9 2665.4[c]	2.2	2556.9 2557.5
AP		2621.6 2622.3	2767.6 2767.9	15.6	2668.6 2669.8
AQ		2523.9 2524.2	2669.9 2669.3	15.4	2570.9 2572.2
AR		2479.8 2482.5	2625.8 2626.5[c]	5.4	2526.8 2526.1
AS		2503.8 2505.4	2649.9 2650.3	18.3	2550.9 2552.2
AT		2484.9 2485.5	2630.9 2630.8	14.0	2531.9 2534.9
AU		2553.9 2553.9	2699.9 2700.9[c]	4.2	2600.9 2600.7
AV		2553.9 2554.1	2699.9 2700.1	7.2	2600.9 2601.4
AW		2553.9 2553.9	2699.9 2699.1	9.9	2600.9 2597.4
AX		2542.7 2541.1	2688.7 2688.9	17.3	2589.7 2589.9
AY		2507.8 2508.2	2653.9 2655.1	19.7	2554.9 2555.8
AZ		2523.9 2524.4	2669.9 2669.9	11.5	2569.9 2567.7
BA		2481.8 2482.5	2625.8 2625.1	14.2	2525.8 2523.6
BB		2467.8 2468.6	2613.8 2615.8	16.2	2514.8 2516.3

No.	structure	hexT-9	hexT-10		hexT-11
		mass calc. mass found[a]	mass calc. mass found[a]	Yield[b] [nmol]	mass calc. mass found[a]
BC		2457.8 2458.4	2603.8 2603.5	15.7	2504.8 2504.2
BD		2509.9 2510.4	2655.9 2655.6	15.8	2556.9 2557.5
BE		2415.8 2415.1	2561.8 2562.1	11.7	2462.8 2462.6
BF		2467.8 2466.3	2613.8 2614.7[c]	3.4	2514.8 2515.8
BG		2569.9 2470.4	2715.9 2716.1	18.9	2614.1 2613.6
BH		2534.9 2536.1	2680.9 2684.0	16.9	2581.9 2583.9
BI		2506.9 2507.4	2652.9 2653.5[c]	1.3	2553.9 2554.7
BJ		2491.9 2492.1	2637.9 2641.2[c]	3.7	2538.9 2539.3
BK		2539.9 2541.6	2685.9 2685.1	12.2	2585.9 2586.3
BL		2519.9 2520.2	2665.9 2666.8	14.4	2566.9 2571.3
BM		2469.9 2469.1	2615.9 2615.6	17.4	2516.9 2516.4
BN		2528.8 2527.3	2677.8 2678.7[c]	4.4	2578.8 2579.7
BO		2539.9 2539.5	2685.9 2685.7	14.4	2586.9 2588.3
BP		2401.8 2402.5	2547.8 2548.4	14.3	2448.8 2449.8

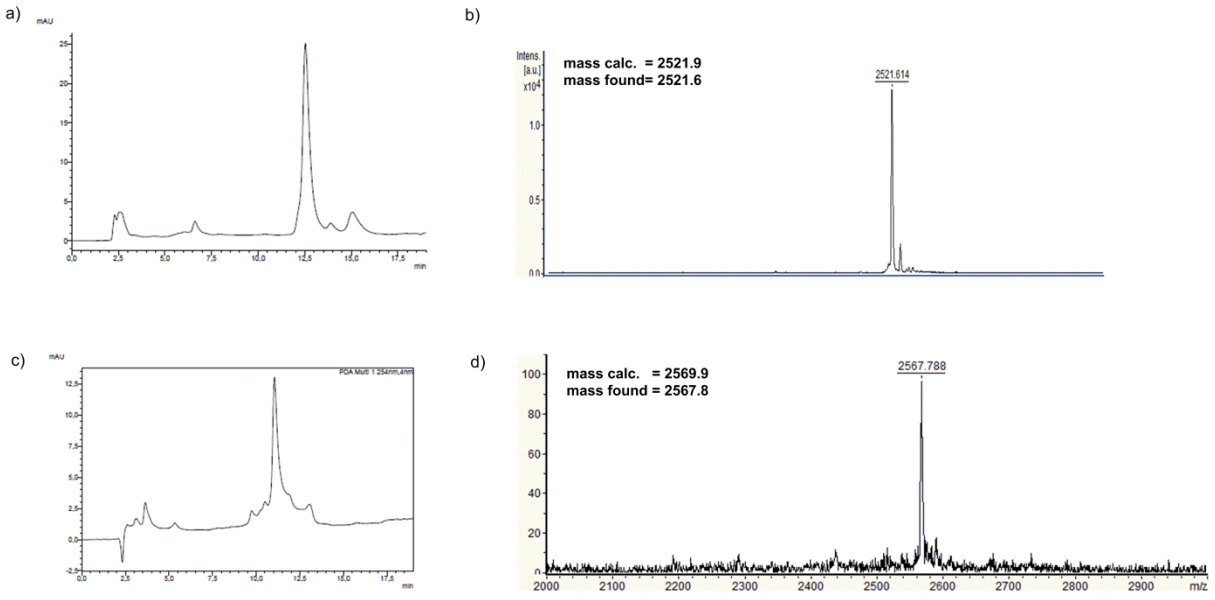
No.	structure	hexT-9	hexT-10		hexT-11
		mass calc. mass found[a]	mass calc. mass found[a]	Yield[b] [nmol]	mass calc. mass found[a]
BQ		2498.3 2493.9	2644.3 2645.9	4.0	2545.3 2546.8
BR		2508.8 2509.4	2654.8 2656.5	11.9	2555.8 2557.5
BS		2535.4 2536.7	2678.9 2679.3	15.6	2579.9 2580.7
BT		2489.9 2489.9	2635.9 2637.2	17.8	2536.9 2537.7
BU		2519.9 2520.2	2655.9 2657.5	6.5	2556.9 2557.2
BV		2463.8 2462.1	2609.8 2609.6	28.3	2510.8 2512.3
BW		2453.8 2451.1	2599.8 2599.6	17.0	2500.8 2501.9
BX		2539.9 2539.8	2685.9 2685.9	18.1	2586.9 2587.7
BY		2479.8 2482.1	2625.8 2627.6[c]	3.7	2526.8 2527.1
BZ		2457.9 2459.1	2603.9 2604.7	5.9	2504.9 2505.3
CA		2427.8 2430.1	2573.8 2575.7[c]	15.3	2474.8 2476.2
CB		2465.8 2465.8	2611.8 2615.7[c]	1.0	2512.8 2513.5

[a] measured by MALDI-MS; [b] measured by Nanodrop; [c] HPLC-purified.

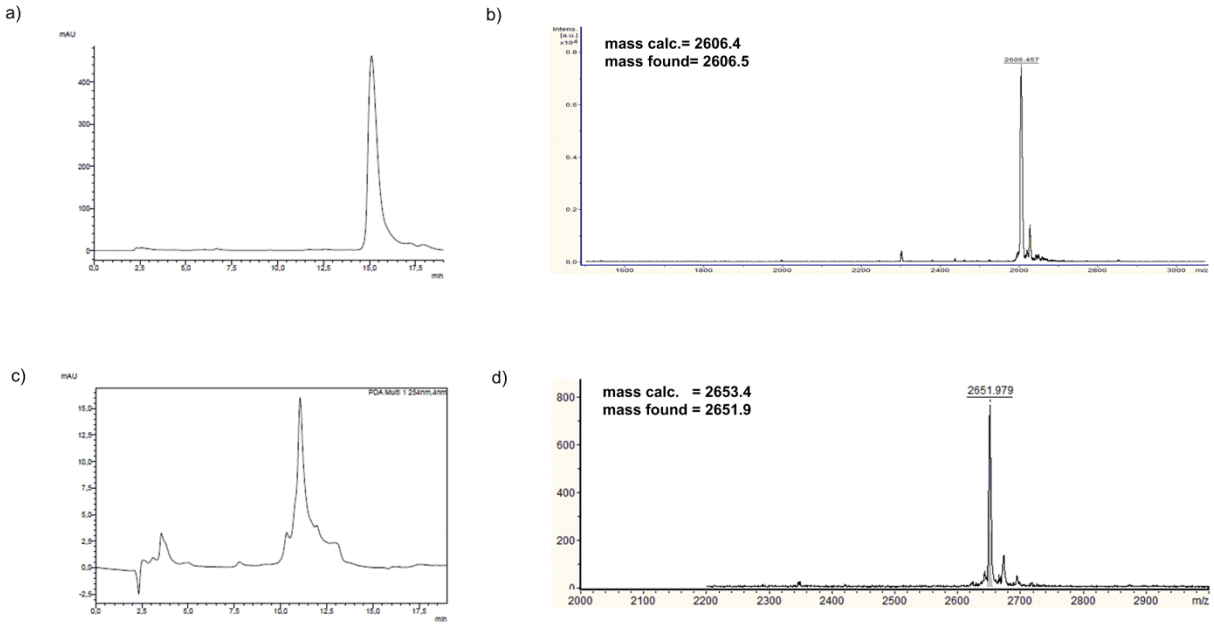
HPLC traces and MALDI MS spectra of hexT-β-carboline conjugates



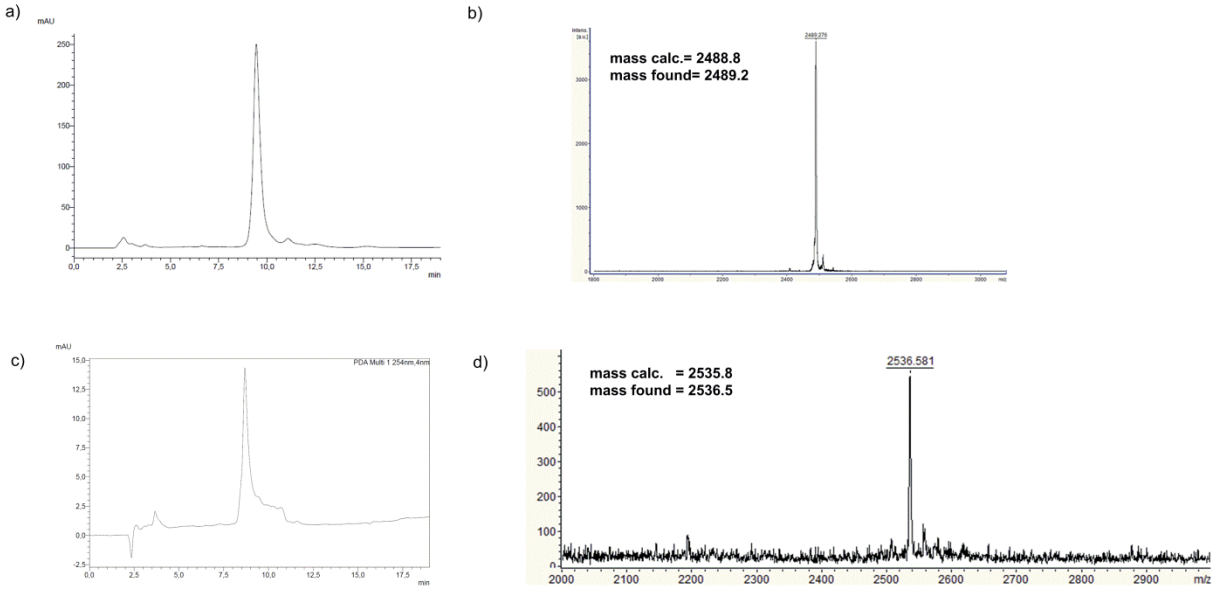
a) Analytical HPLC chromatogram of the conjugate **hexT-9E**; b) MALDI-MS analysis of the conjugate **hexT-9E**; c) Analytical HPLC chromatogram of the conjugate **hexT-11E**; d) MALDI-MS analysis of the conjugate **hexT-11E**.



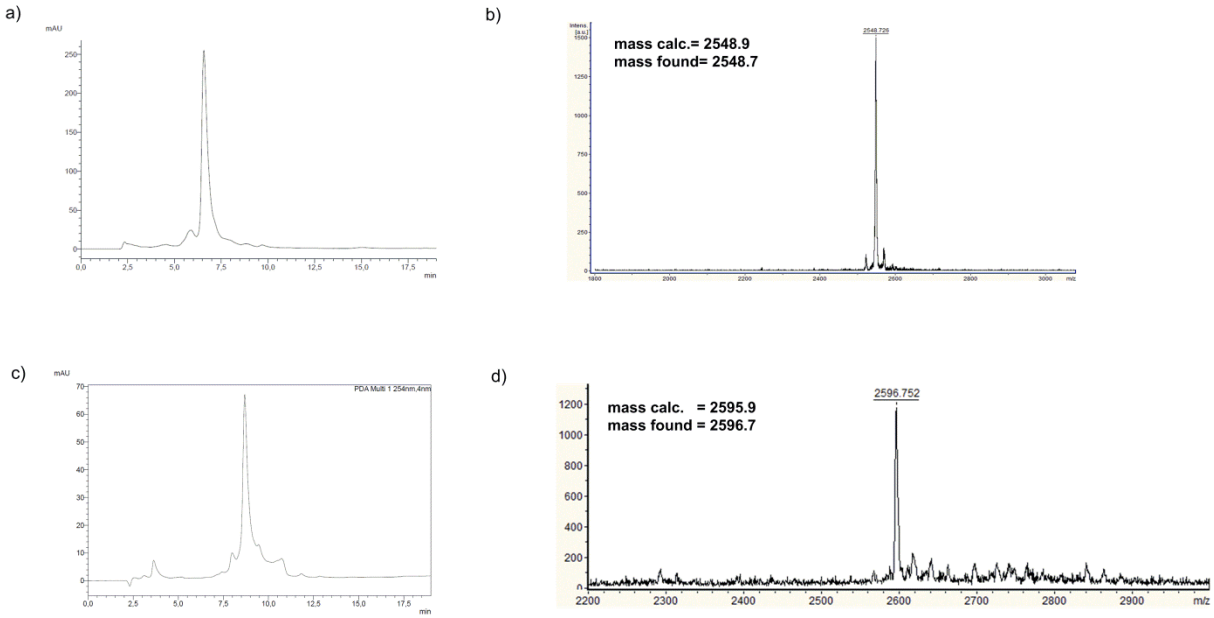
a) Analytical HPLC chromatogram of the conjugate **hexT-9G**; b) MALDI-MS analysis of the conjugate **hexT-9G**; c) Analytical HPLC chromatogram of the conjugate **hexT-11G**; d) MALDI-MS analysis of the conjugate **hexT-11G**.



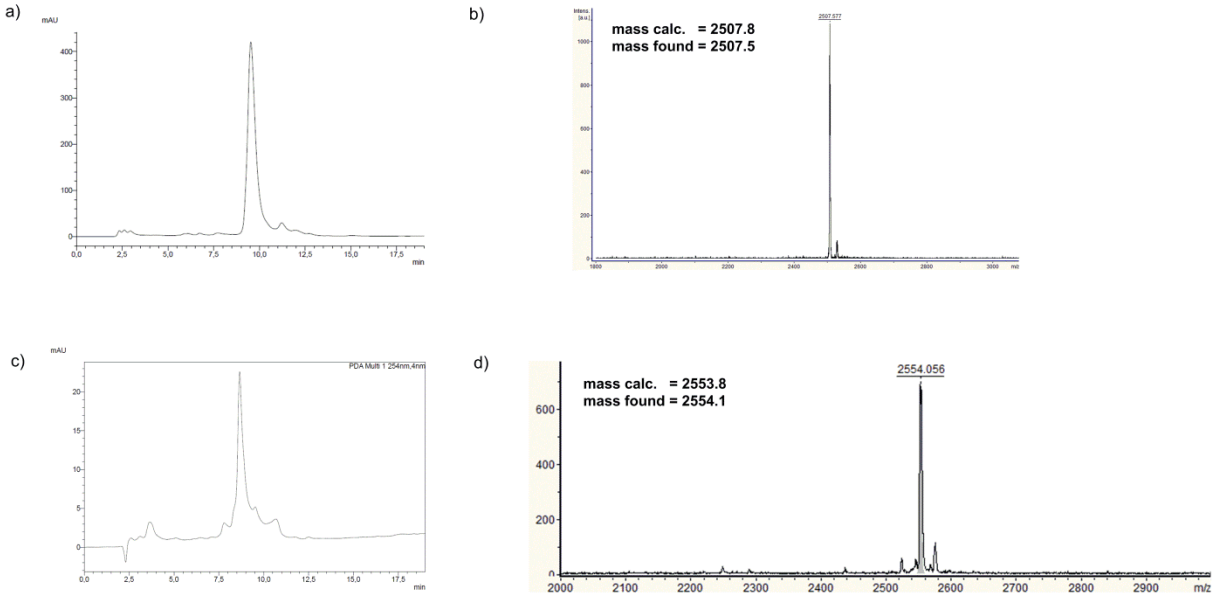
a) Analytical HPLC chromatogram of the conjugate **hexT-9H**; b) MALDI-MS analysis of the conjugate **hexT-9H**; c) Analytical HPLC chromatogram of the conjugate **hexT-11H**; d) MALDI-MS analysis of the conjugate **hexT-11H**.



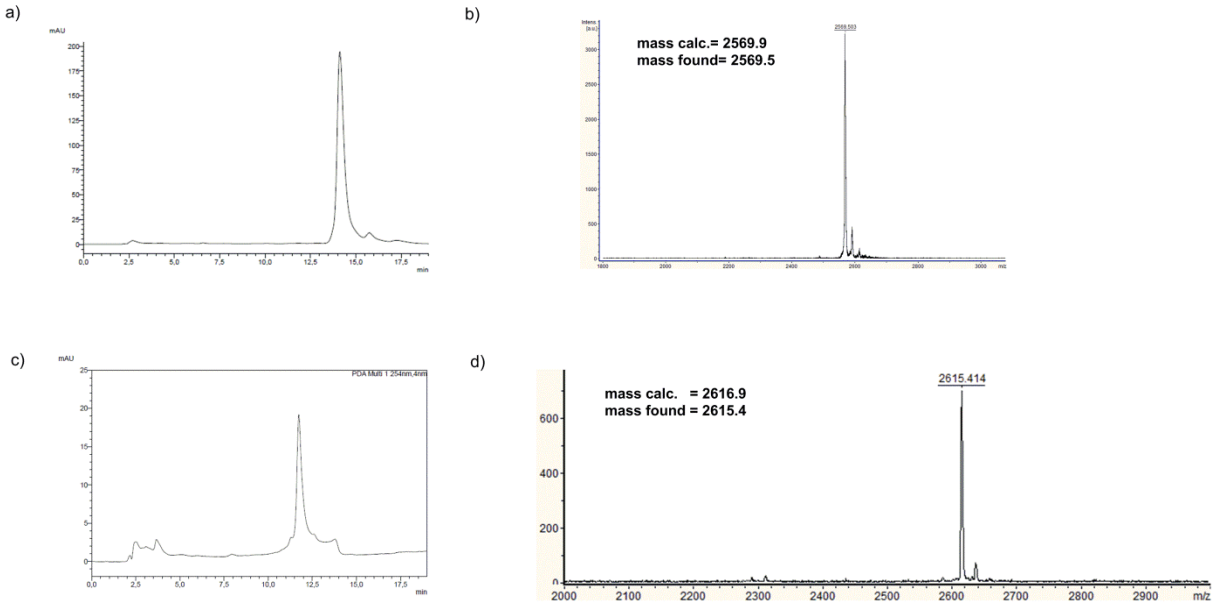
a) Analytical HPLC chromatogram of the conjugate **hexT-9J**; b) MALDI-MS analysis of the conjugate **hexT-9J**; c) Analytical HPLC chromatogram of the conjugate **hexT-11J**; d) MALDI-MS analysis of the conjugate **hexT-11J**.



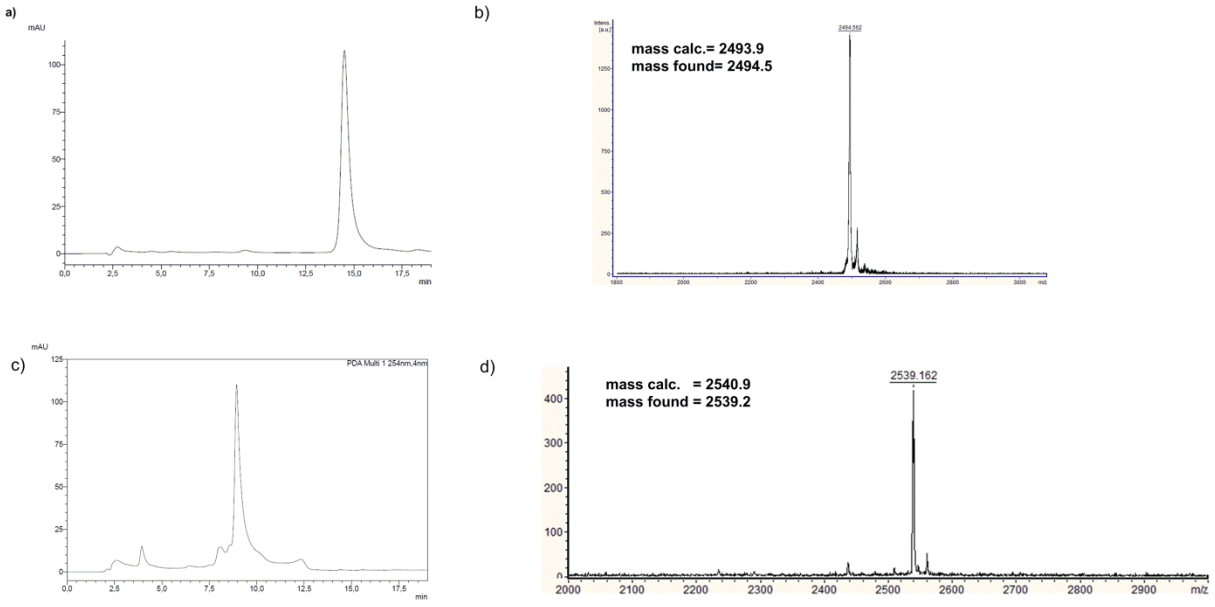
a) Analytical HPLC chromatogram of the conjugate **hexT-9K**; b) MALDI-MS analysis of the conjugate **hexT-9K**; c) Analytical HPLC chromatogram of the conjugate **hexT-11K**; d) MALDI-MS analysis of the conjugate **hexT-11K**.



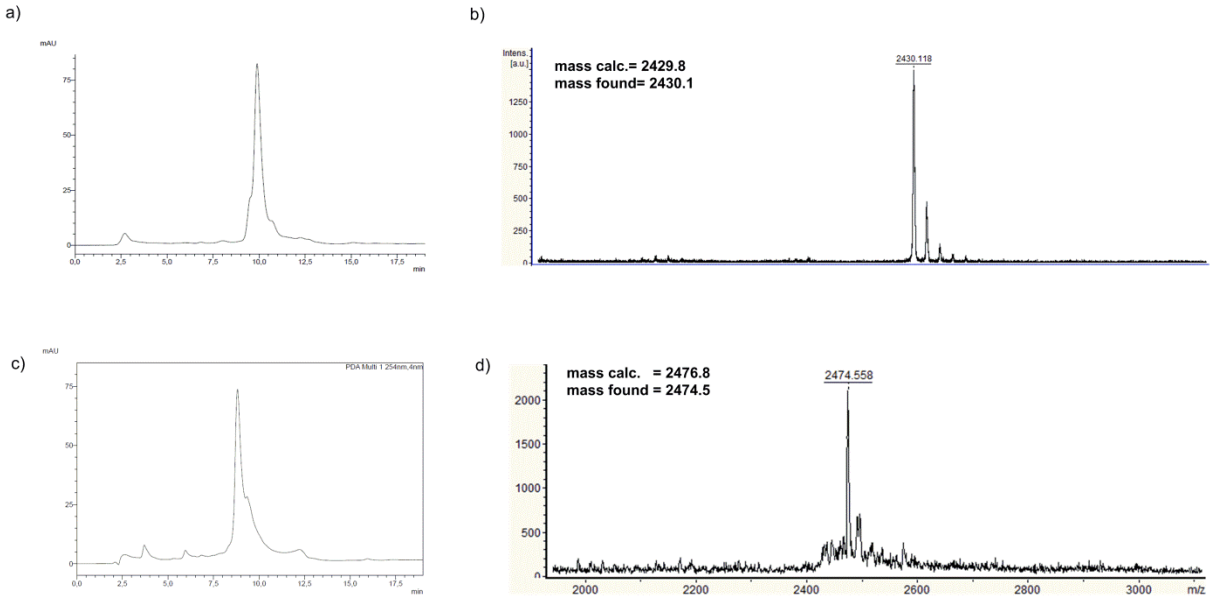
a) Analytical HPLC chromatogram of the conjugate **hexT-9L**; b) MALDI-MS analysis of the conjugate **hexT-9L**; c) Analytical HPLC chromatogram of the conjugate **hexT-11L**; d) MALDI-MS analysis of the conjugate **hexT-11L**.



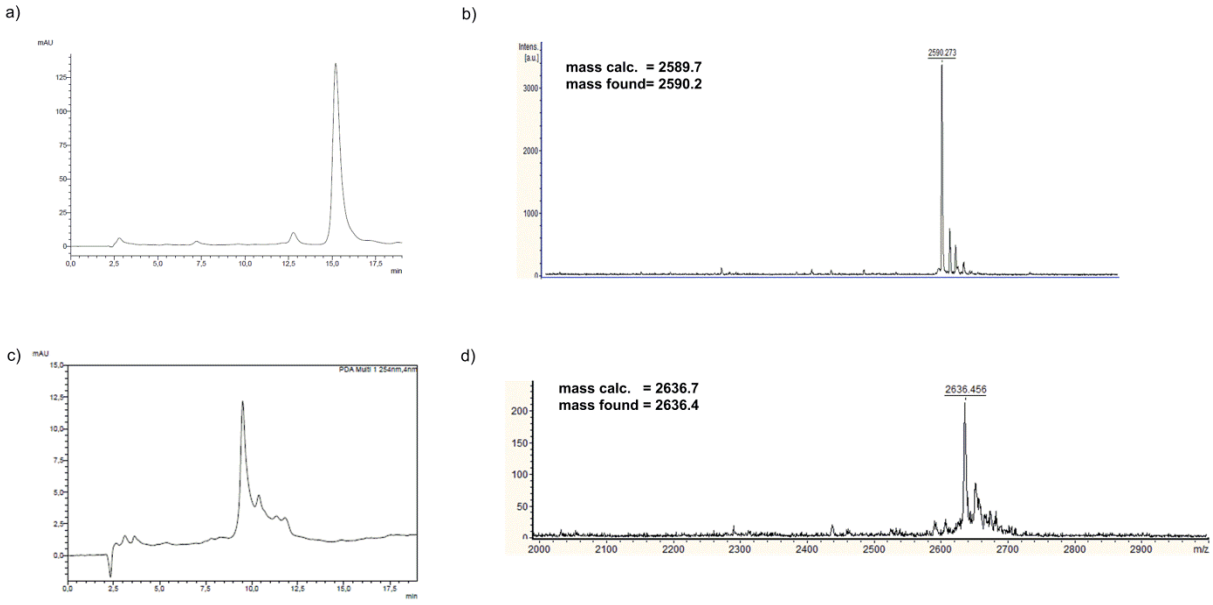
a) Analytical HPLC chromatogram of the conjugate **hexT-9O**; b) MALDI-MS analysis of the conjugate **hexT-9O**; c) Analytical HPLC chromatogram of the conjugate **hexT-11O**; d) MALDI-MS analysis of the conjugate **hexT-11O**.



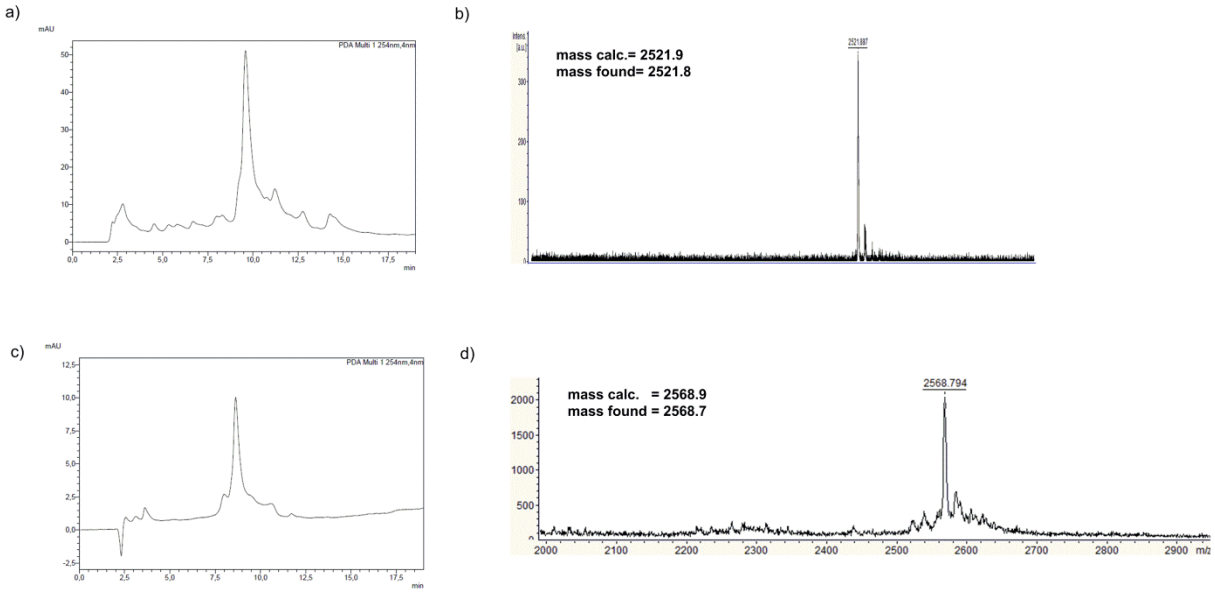
a) Analytical HPLC chromatogram of the conjugate **hexT-9Q**; b) MALDI-MS analysis of the conjugate **hexT-9Q**; c) Analytical HPLC chromatogram of the conjugate **hexT-11Q**; d) MALDI-MS analysis of the conjugate **hexT-11Q**.



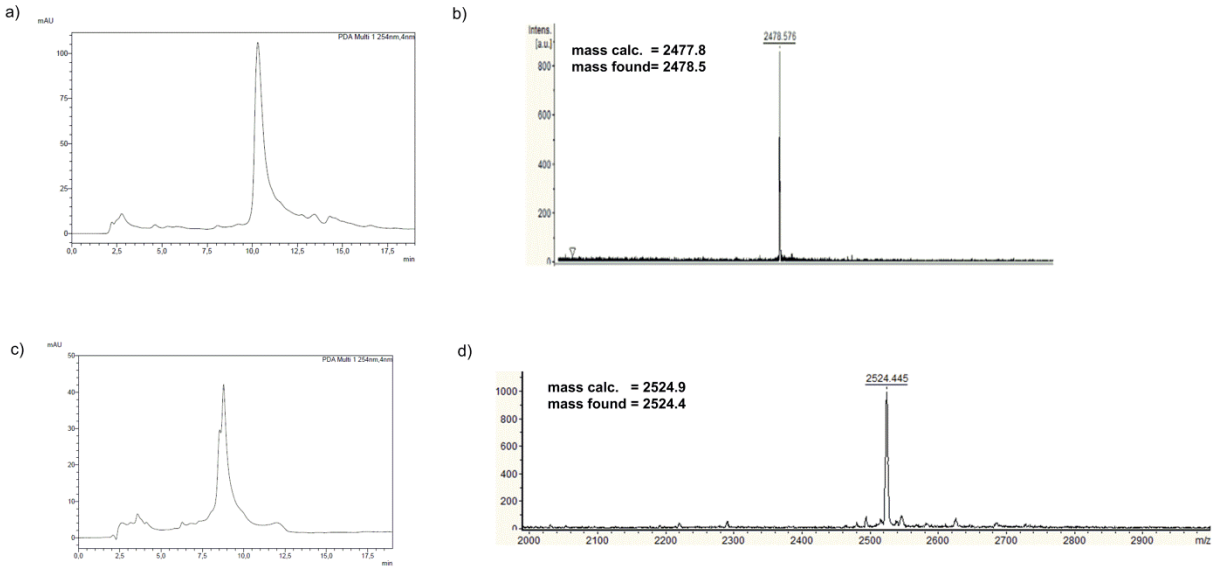
a) Analytical HPLC chromatogram of the conjugate **hexT-9Y**; b) MALDI-MS analysis of the conjugate **hexT-9Y**; c) Analytical HPLC chromatogram of the conjugate **hexT-11Y**; d) MALDI-MS analysis of the conjugate **hexT-11Y**.



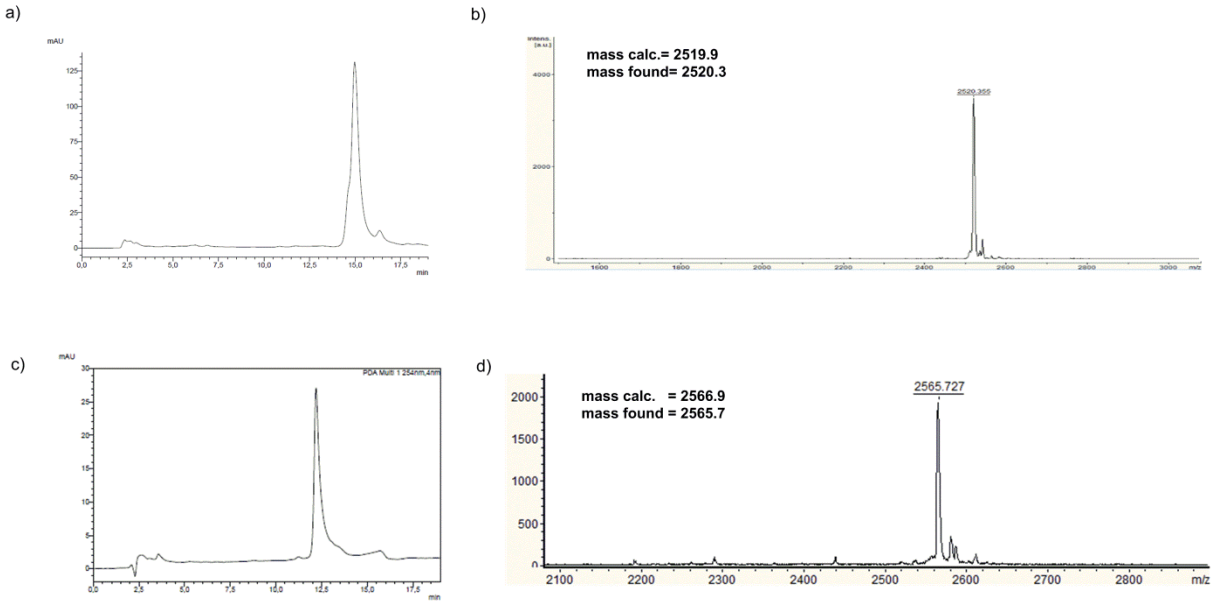
a) Analytical HPLC chromatogram of the conjugate **hexT-9Z**; b) MALDI-MS analysis of the conjugate **hexT-9Z**; c) Analytical HPLC chromatogram of the conjugate **hexT-11Z**; d) MALDI-MS analysis of the conjugate **hexT-11Z**.



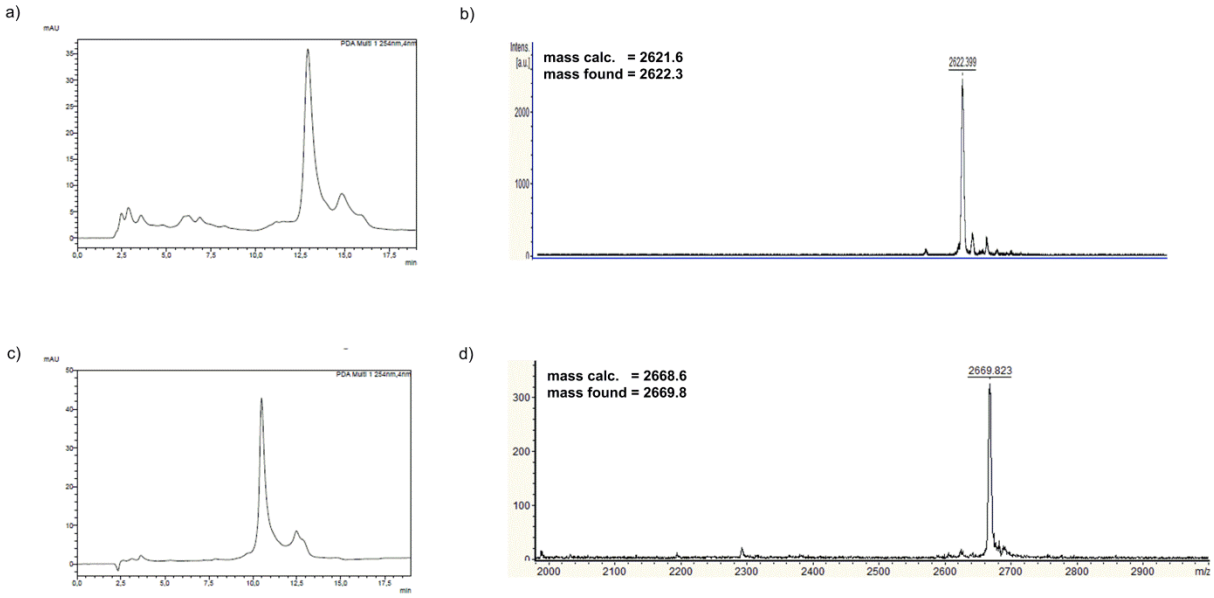
a) Analytical HPLC chromatogram of the conjugate **hexT-9AD**; b) MALDI-MS analysis of the conjugate **hexT-9AD**; c) Analytical HPLC chromatogram of the conjugate **hexT-11AD**; d) MALDI-MS analysis of the conjugate **hexT-11AD**.



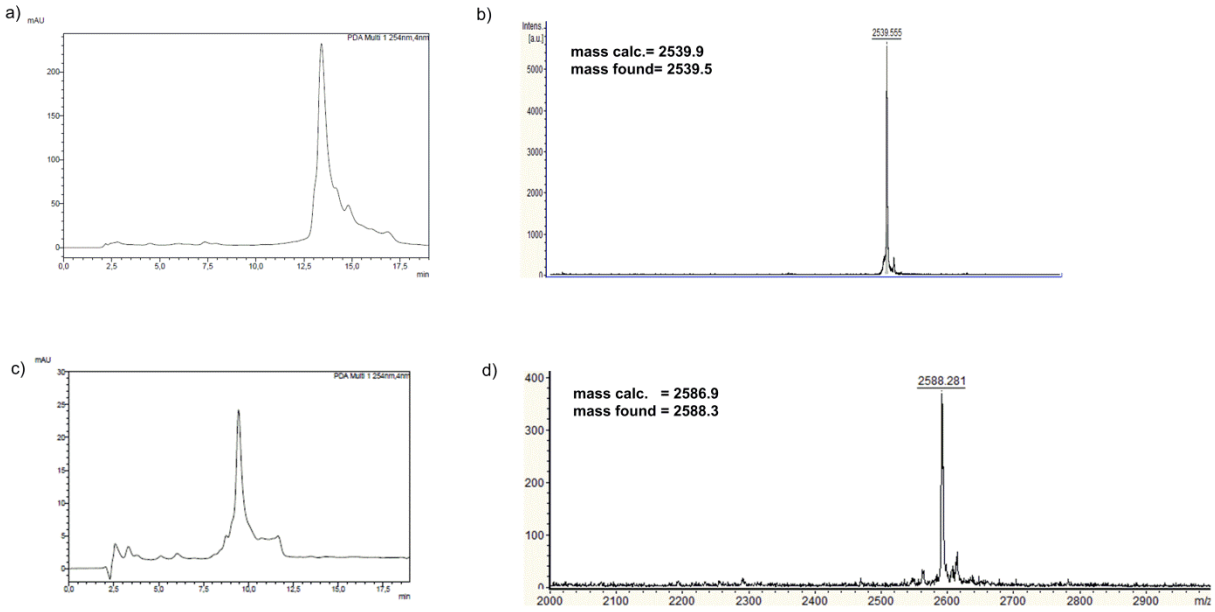
a) Analytical HPLC chromatogram of the conjugate **hexT-9AF**; b) MALDI-MS analysis of the conjugate **hexT-9AF**; c) Analytical HPLC chromatogram of the conjugate **hexT-11AF**; d) MALDI-MS analysis of the conjugate **hexT-11AF**.



a) Analytical HPLC chromatogram of the conjugate **hexT-9AJ**; b) MALDI-MS analysis of the conjugate **hexT-9AJ**; c) Analytical HPLC chromatogram of the conjugate **hexT-11AJ**; d) MALDI-MS analysis of the conjugate **hexT-11AJ**.

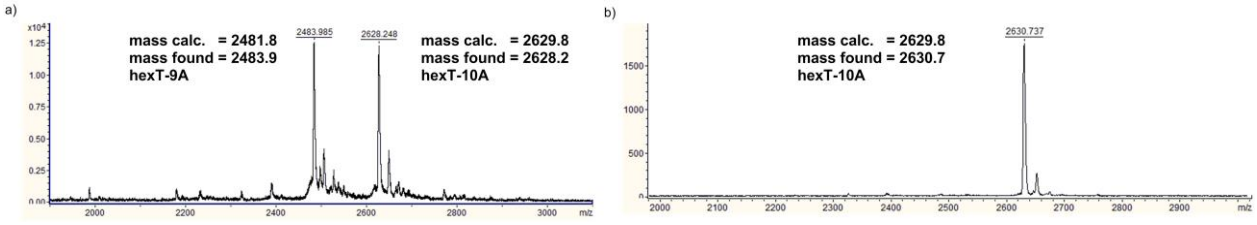


a) Analytical HPLC chromatogram of the conjugate **hexT-9AP**; b) MALDI-MS analysis of the conjugate **hexT-9AP**; c) Analytical HPLC chromatogram of the conjugate **hexT-11AP**; d) MALDI-MS analysis of the conjugate **hexT-11AP**.

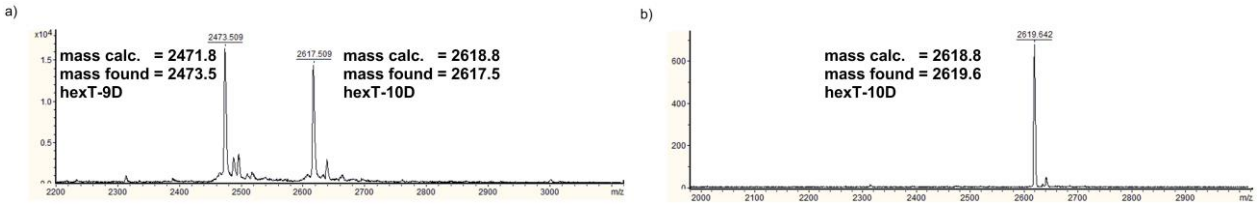


a) Analytical HPLC chromatogram of the conjugate **hexT-9BO**; b) MALDI-MS analysis of the conjugate **hexT-9BO**; c) Analytical HPLC chromatogram of the conjugate **hexT-11BO**; d) MALDI-MS analysis of the conjugate **hexT-11BO**.

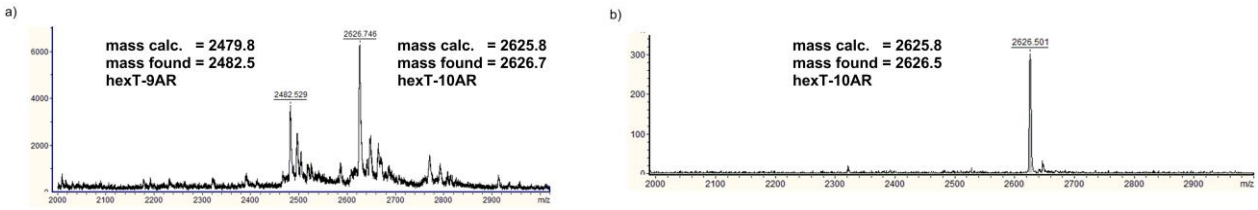
Exemplary MALDI-MS spectra of hexT-β-carboline conjugates before and after HPLC purification



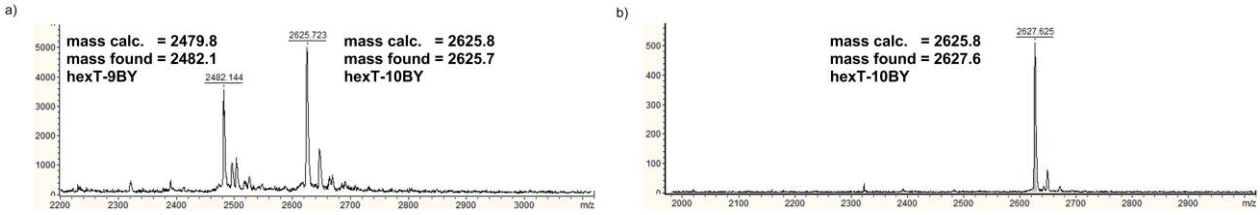
a) MALDI-MS analysis of the conjugate **hexT-10A** before HPLC-purification, showing a 1:1 mixture of the β-carboline **hexT-9A** and the amino-linker substituted **hexT-10A**; b) MALDI-MS analysis of the conjugate **hexT-10A** after HPLC purification.



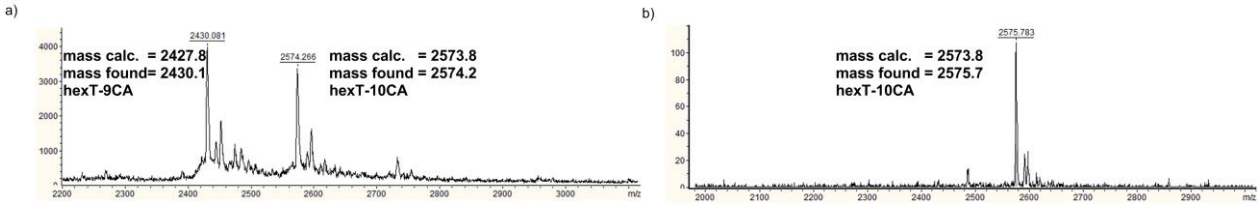
a) MALDI-MS analysis of the conjugate **hexT-10D** before HPLC-purification, showing a 5.5:4.5 mixture of the β-carboline **hexT-9D** and the amino-linker substituted **hexT-10D**; b) MALDI-MS analysis of the conjugate **hexT-10D** after HPLC purification.



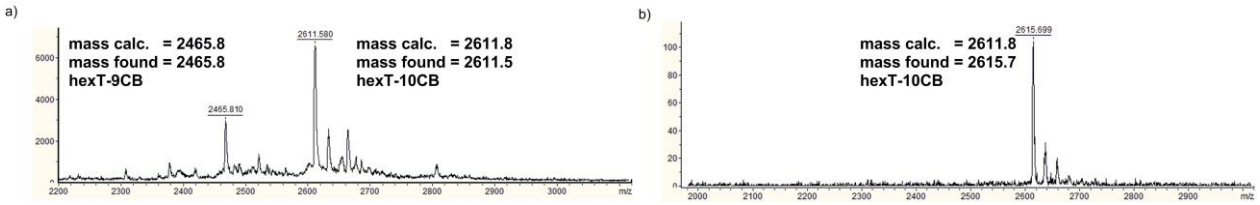
a) MALDI-MS analysis of the conjugate **hexT-10AR** before HPLC-purification, showing a 3:7 mixture of the β-carboline **hexT-9AR** and the amino-linker substituted **hexT-10AR**; b) MALDI-MS analysis of the conjugate **hexT-10AR** after HPLC purification.



a) MALDI-MS analysis of the conjugate **hexT-10BY** before HPLC-purification, showing a 4:6 mixture of the β-carboline **hexT-9BY** and the amino-linker substituted **hexT-10BY**; b) MALDI-MS analysis of the conjugate **hexT-10BY** after HPLC purification.



a) MALDI-MS analysis of the conjugate **hexT-10CA** before HPLC-purification, showing a 6:4 mixture of the β-carboline **hexT-9CA** and the amino-linker substituted **hexT-10CA**; b) MALDI-MS analysis of the conjugate **hexT-10CA** after HPLC purification.



a) MALDI-MS analysis of the conjugate **hexT-10CB** before HPLC-purification, showing a 2:8 mixture of the β-carboline **hexT-9CB** and the amino-linker substituted **hexT-10CB**; b) MALDI-MS analysis of the conjugate **hexT-10CB** after HPLC purification.

4.2 Comparison of the hexT-β-carboline conjugate hexT-9BO with the reference ref-hexT-9BO

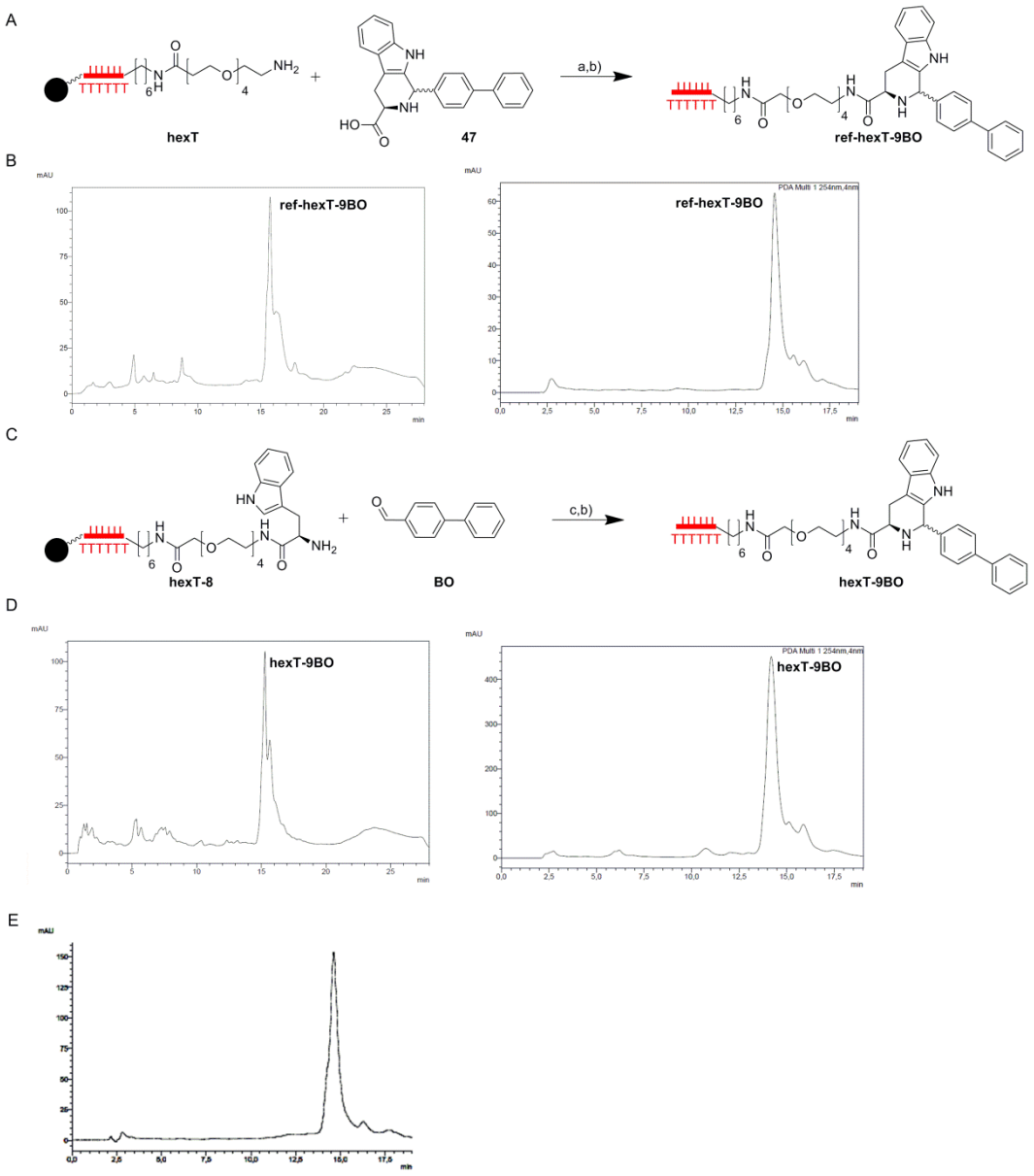


Figure S15. Comparison of the product of the Pictet-Spengler reaction **hexT-9BO** with a reference compound **ref-hexT-9BO** synthesized from the β-carboline **47** (Figure S32) and **hexT** by amide coupling. A) Synthesis scheme for the synthesis of the reference molecule, a) HATU, DIPEA, two hours, room temperature; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; B) HPLC trace (preparative HPLC) of the crude reference product **ref-hexT-9BO** (left hand), and HPLC trace (analytical HPLC) of the pure reference product **ref-hexT-9BO**; C) synthesis scheme for the Pictet-Spengler reaction of hexT conjugate **hexT-8** with the aldehyde **BO** furnishing **hexT-9BO**, c) 1 % TFA in CH_2Cl_2, room temperature, 16 hours; D) HPLC trace (preparative HPLC) of the crude product **hexT-9BO** (left hand), and HPLC trace (analytical HPLC) of the purified product **hexT-9BO**; E) HPLC trace (analytical HPLC) of the co-injected product **hexT-9BO**, and reference **ref-hexT-9BO**. Filled circle denotes controlled pore glass (CPG) solid support; wavy bond denoted lingage of the hexT-DNA to the CPG.

5. Synthesis of hexT-pyrazol(in)e conjugates

5.1 Optimization of reaction conditions of the Au(I)-catalyzed annulation reaction

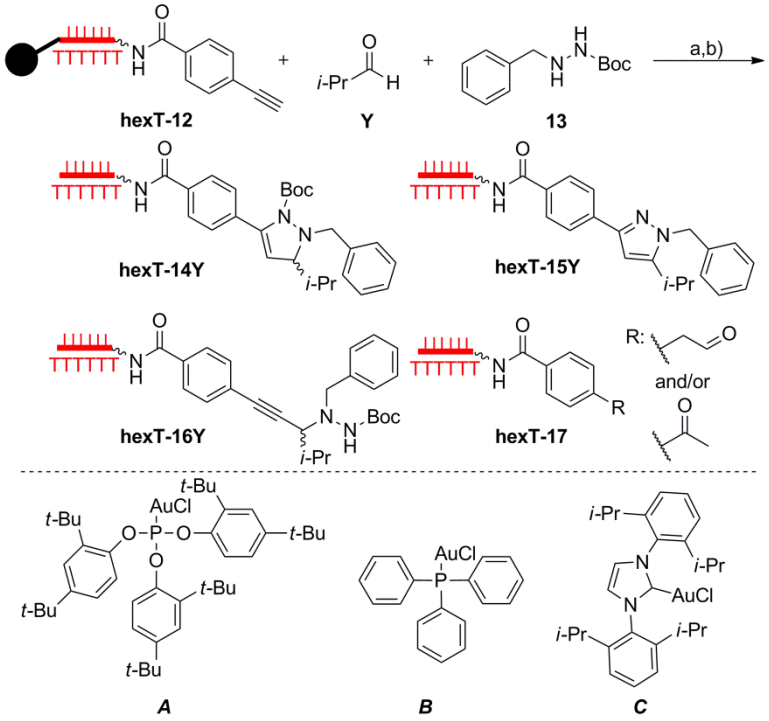


Figure S16. Adapting a Au(I)-catalyzed annulation reaction to TiDEC.[1,2] a) For conditions see Table S4; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature. Figures S17-S21 show HPLC traces of the crude reaction mixtures (preparative HPLC). Figure S22 shows HPLC traces of the crude reaction mixtures (analytical HPLC). Filled black circle: CPG solid support; wavy bond: C6-amino-linker; bold bond: connection of the hexT oligonucleotide to the CPG.

The solid support (controlled pore glass, CPG) containing the hexathymidine-alkyne conjugate **hexT-12** (30 nmol, ca. 1.1 mg, please note that the PEG-linker is missing in this compound) was suspended in 30 µL of a solvent (see Table S4). Then, hydrazide **13** and aldehyde **Y**, each 30 µmol, dissolved in 30 µL of the same solvent (prepared as stock) were added to **hexT-12**. This was followed by an equimolar mixture of either catalyst ***A*-*C***/AgOTf or ***A*-*C***/$AgSbF_6$ (see Table S4) at the amounts given in Table S4 which were suspended (***A*-*C***) or dissolved (AgOTf or $AgSbF_6$), respectively in 30 µL of the same solvent. The suspensions of the catalysts were prepared as stocks. Prior addition to **hexT-12** these were vortexed and pipetted up and down to add a homogeneous suspension. The reactions were shaken at the indicated temperatures overnight in Eppendorf tubes or in sealed 96well deep well plates. The reactions were neither run under protective gas nor were molecular sieves used. Then, the CPG was filtered off, washed with each 3 x 200 µL of 0.1 M EDTA, DMF, MeOH, MeCN and DCM and dried *in vacuo* for 15 min. The hexT-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 30 minutes 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) were 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-MS analysis and by analytical HPLC.

Table S4: Optimization of reaction conditions for the Au(I) catalyzed A^3 annulation reaction to furnish hexT-pyrazol(in)e conjugates.

	No.	hydrazide and aldehyde [eq][a]	catalyst [eq][a]	solvent	T [°C]	catalyst[1,2]	**hexT-14** [%][b]	**hexT-15** [%][b]	**hexT-16** [%][b]	**hexT-17** [%][b]
a) equivalents	**1**	1000	5	THF	50	***A***/$AgSbF_6$	-	-	-	-
of catalyst	**2**	1000	50	THF	50	***A***/$AgSbF_6$	15	-	55	-
A/$AgSbF_6$	**3**	1000	250	THF	50	***A***/$AgSbF_6$	50	-	25	10
b) solvent	**1**	1000	250	THF	50	***A***/$AgSbF_6$	50	-	25	10
	2	1000	250	$C_2H_4Cl_2$	50	***A***/$AgSbF_6$	40	-	10	50
	3	1000	250	CH_2Cl_2	50	***A***/$AgSbF_6$	20	-	10	50
	4	1000	250	toluene	50	***A***/$AgSbF_6$	50	15	15	15
	5 (A)	1000	250	MeCN	50	***A***/$AgSbF_6$	75	-	25	-
	6	1000	250	DMF	50	***A***/$AgSbF_6$	10	-	20	10
	7	1000	250	AcOH	50	***B***/AgOTf	10	55	10	-
c) temperature	**1**	1000	250	$C_2H_4Cl_2$	45	***A***/$AgSbF_6$	40	-	25	30
	2	1000	250	$C_2H_4Cl_2$	50	***A***/$AgSbF_6$	40	-	10	50
	3	1000	250	$C_2H_4Cl_2$	55	***A***/$AgSbF_6$	40	15	20	20
	4	1000	250	$C_2H_4Cl_2$	60	***A***/$AgSbF_6$	15	35	<5	35
	5	1000	250	toluene	50	***A***/$AgSbF_6$	50	15	15	15
	6	1000	250	toluene	55	***A***/$AgSbF_6$	30	25	10	30
	7	1000	250	MeCN	50	***A***/$AgSbF_6$	75	-	20	-
	8	1000	250	MeCN	55	***A***/$AgSbF_6$	75	-	25	-
	9	1000	250	MeCN	60	***A***/$AgSbF_6$	70	20	-	-
	10 (B)	1000	250	AcOH	50	***B***/AgOTf	10	55	10	-
	11 (C)	1000	250	AcOH	60	***B***/AgOTf	<5	90	-	-
	12	1000	250	MeCN	rt	***A***/$AgSbF_6$	-	-	-	-
d) catalyst	**1**	1000	250	toluene	50	***B***	-	-	5	-
	2	1000	250	toluene	50	AgOTf	40	-	60	-
	3	1000	250	$C_2H_4Cl_2$	50	***B***/AgOTf	85	-	-	-
	4	1000	250	$C_2H_4Cl_2$	50	***C***/AgOTf	85	-	-	-

[a] versus the solid support-bound hexathymidine-alkyne conjugate **hexT-12**; [b] HPLC analysis of the crude, missing percentage to 100 %: **hexT-12**.

a) Screening different equivalents of catalyst ***A***/$AgSbF_6$ versus the hexathymidine-alkyne conjugate **hexT-12** for the Au(I) catalyzed A^3 MCR of the hexathymidine-alkyne conjugate **hexT-12**, isobutyraldehyde **Y**, and hydrazide **13.**

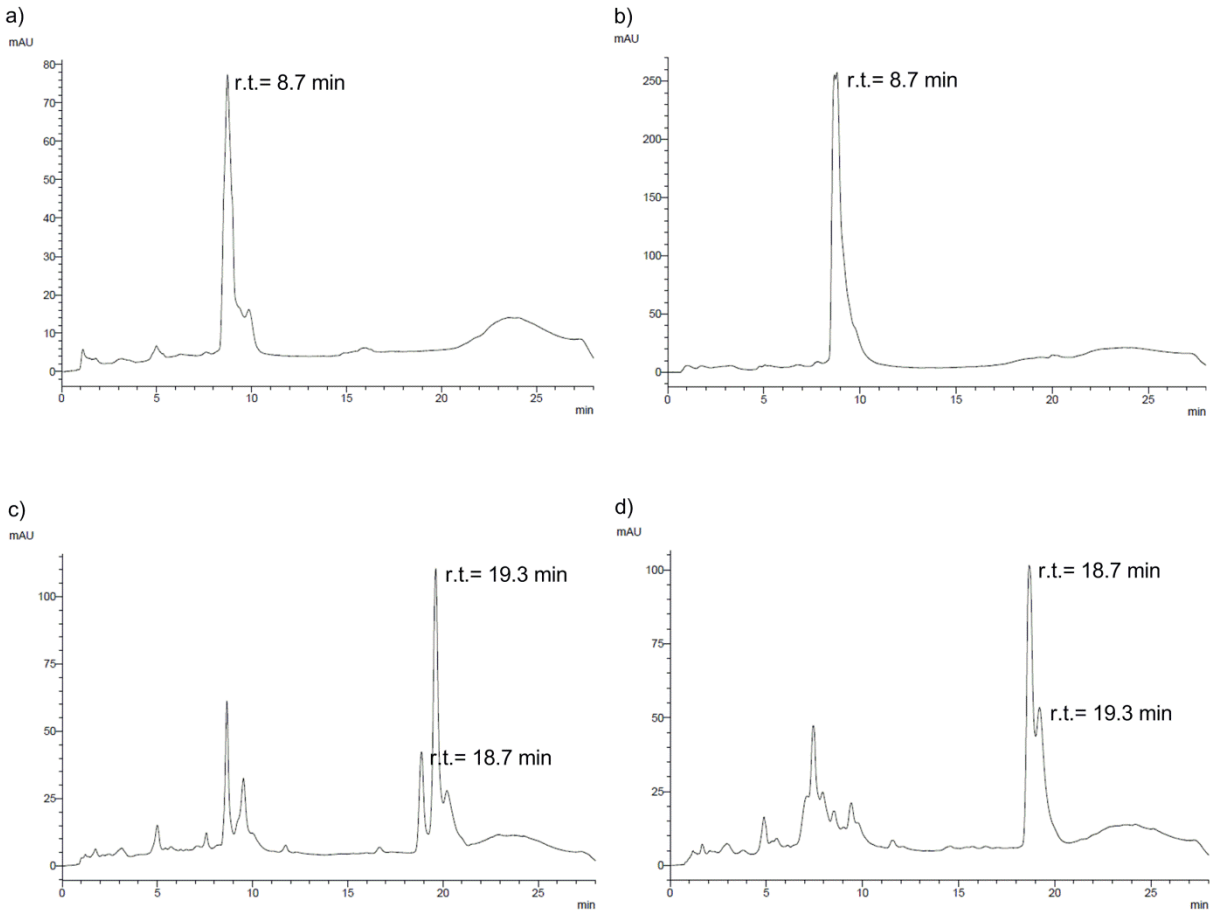


Figure S17. Effect of catalyst concentration on the Au(I) catalyzed A^3 MCR of the hexathymidine-alkyne conjugate **hexT-12**, isobutyraldehyde **Y**, and hydrazide **13**, for reaction conditions see Table S4a. a) HPLC trace of the starting material (hexathymidine-alkyne conjugate **hexT-12**); b) HPLC trace of experiment **No. 1**, no product formation visible; c) HPLC trace of experiment **No. 2**, formation of a product mixture with the later eluting component **hexT-16Y** as main product; d) HPLC trace of experiment **No. 3**, formation of a product mixture with the earlier eluting component **hexT-14Y** as main product. For a comparison of the retention times of the pyrazoline **hexT-14Y** with the propargyl amine **hexT-16Y** see Figure S23.

b) Screening different solvents for the Au(I) catalyzed A^3 MCR of the hexathymidine-alkyne conjugate **hexT-12**, isobutyraldehyde **Y**, and hydrazide **13.**

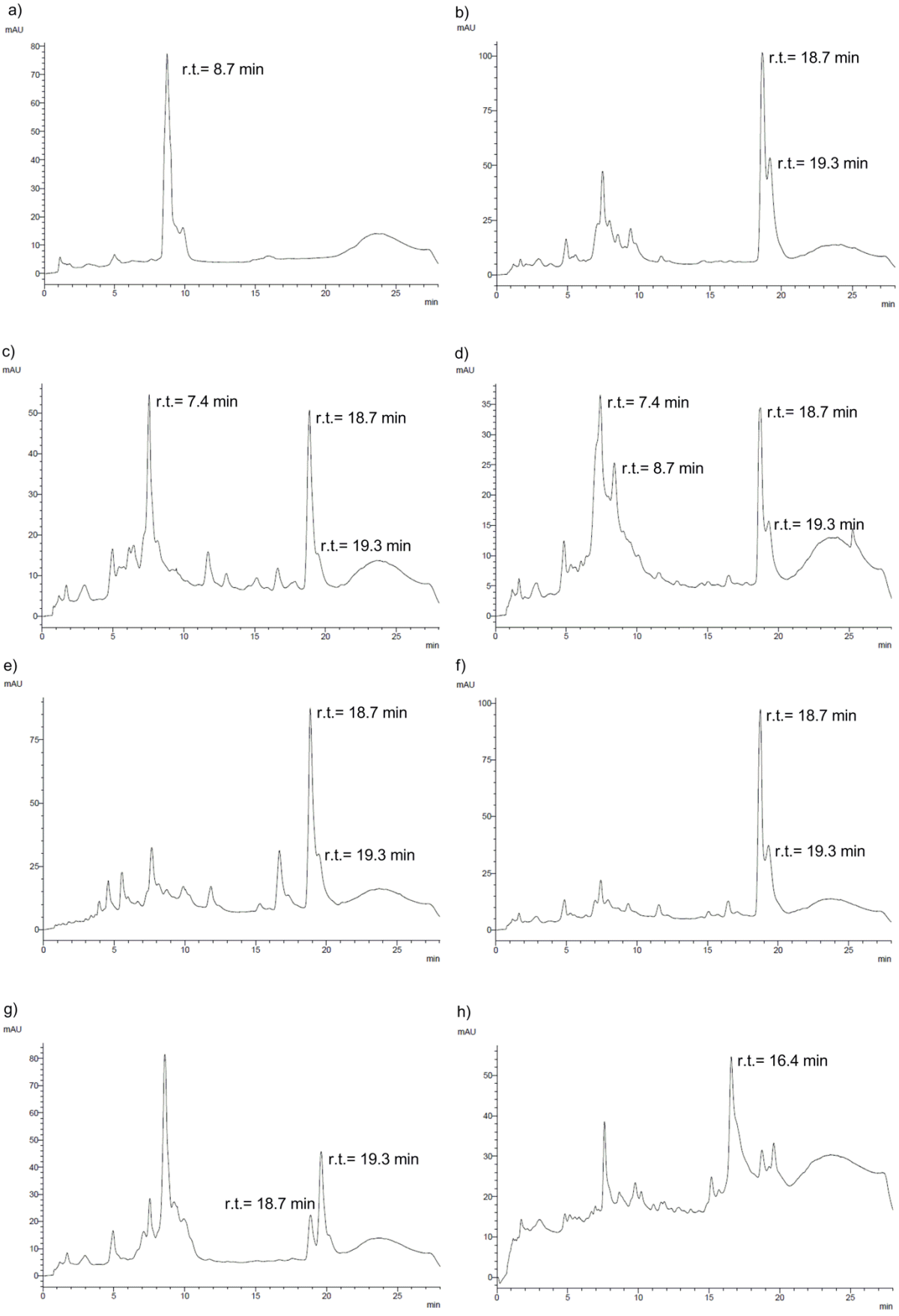


Figure S18. Effect of different solvents on the Au(I) catalyzed A^3 MCR of the hexathymidine-alkyne conjugate **hexT-12**, isobutyraldehyde **Y**, and hydrazide **13**, for reaction conditions see Table S4b. a) HPLC trace of the starting material (hexathymidine-alkyne conjugate **hexT-12**); b) HPLC trace of experiment **No. 1** (same experiment as Figure S17d); c) HPLC trace of experiment **No. 2**, formation of a product mixture with the pyrazoline **hexT-14Y** and the hydration product **hexT-17** (r.t.: 7.4 min) as main products; d) HPLC trace of experiment **No. 3**, similar result as experiment **No. 2**; e) HPLC trace of experiment **No. 4**, formation of the pyrazoline **hexT-14Y** as main product, formation of the oxidized pyrazole **hexT-15Y** as a side product (r.t.: 16.4 min, see also Figure S21); f) HPLC trace of experiment **No. 5(A)**, formation of the earlier eluting pyrazole **hexT-15Y** as main product; g) HPLC trace of experiment **No. 6**, low conversion to the target heterocycle; h) HPLC trace of experiment **No. 7**, formation of the pyrazole **hexT-15Y** as a main product (r.t.: 16.4 min). For a comparison of the retention times of the pyrazoline **hexT-14Y** with the propargyl amine **hexT-16Y** see Figure S23.

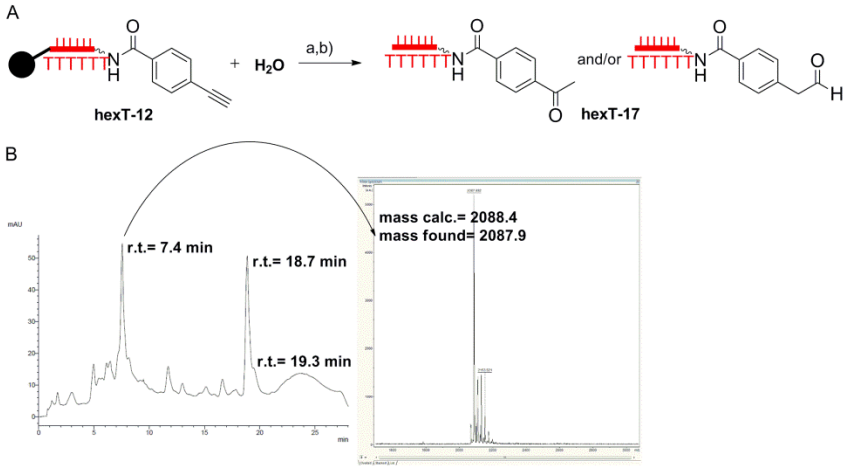
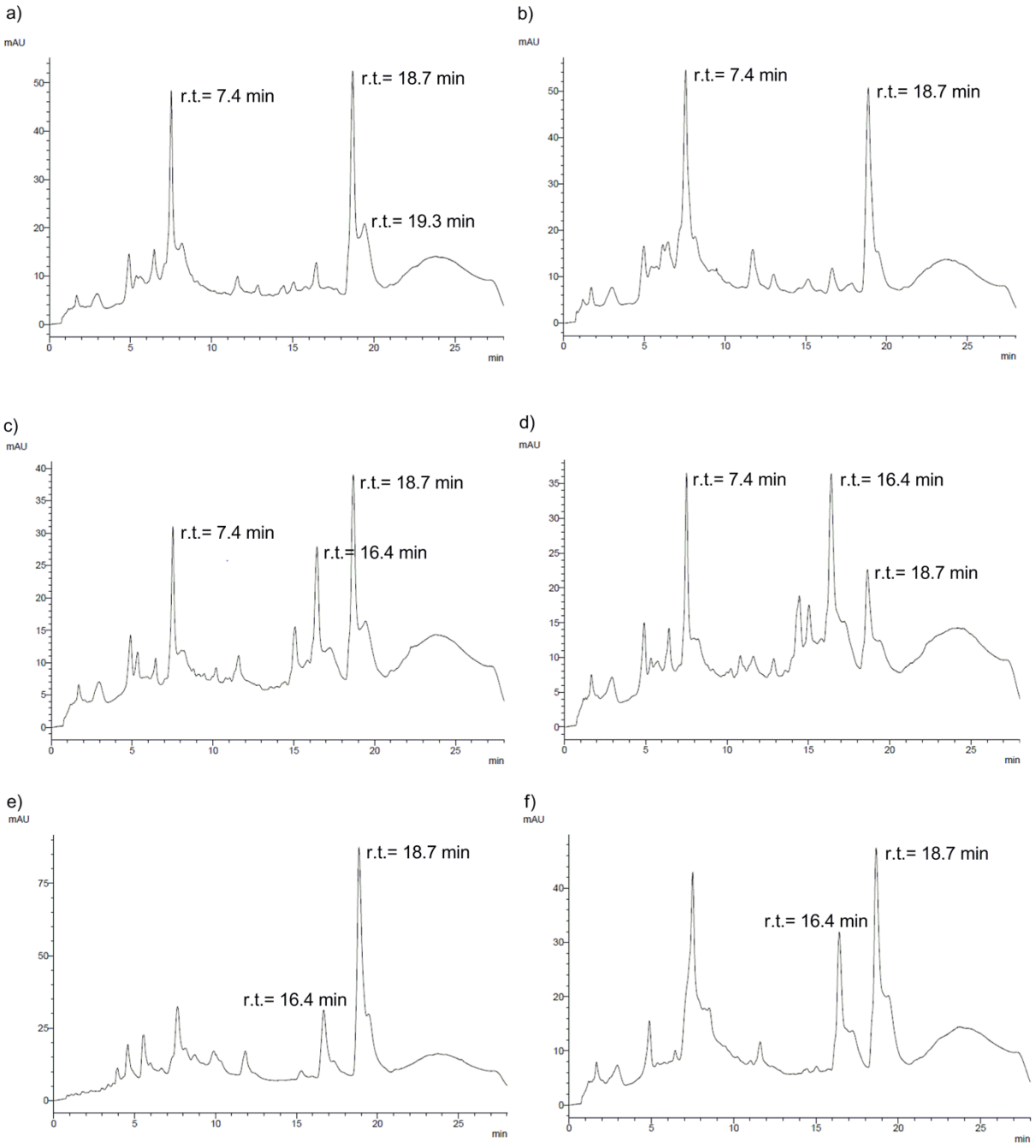


Figure S19. MALDI-MS analysis of the isolated hydration product **hexT-17** from **figure S18c**. A) Reaction scheme of the hydration reaction; a) reaction condition **No. 2** (Table S4b); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; B) MALDI-MS of the isolated hexathymidine conjugate **hexT-17**. Filled black circle: CPG solid support; wavy bond: C6-amino-linker; bold bond: connection from the hexT oligonucleotide to the CPG.

c) Screening different solvents and temperatures for the Au(I) catalyzed A^3 MCR of the hexathymidine-alkyne conjugate **hexT-12**, isobutyraldehyde **Y**, and hydrazide **13**.



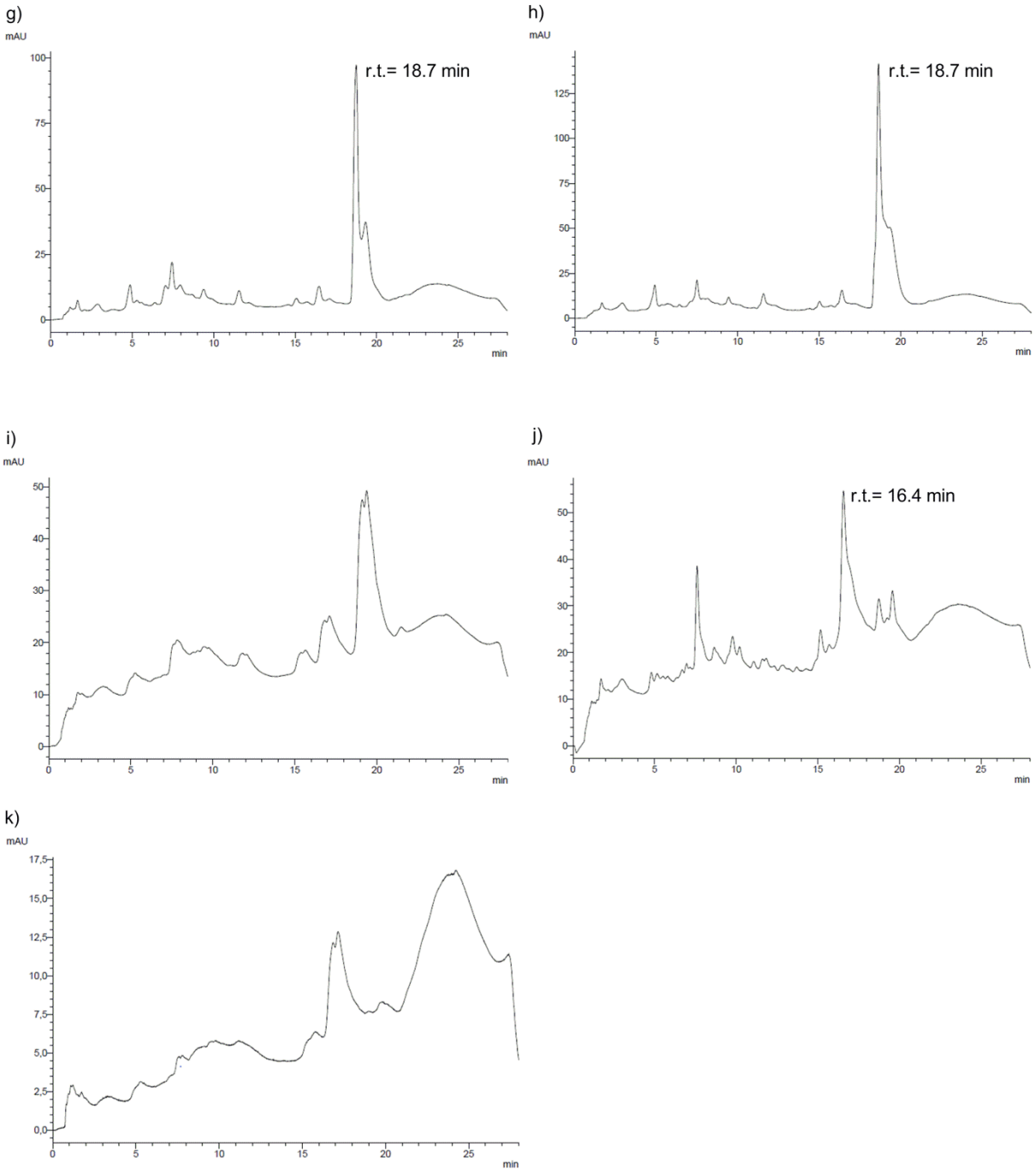


Figure S20. Effect of different solvents and temperatures on the Au(I) catalyzed A^3 MCR of the hexathymidine-alkyne conjugate **hexT-12**, isobutyraldehyde **Y**, and hydrazide **13**, for reaction conditions see Table S4c. a) HPLC trace of experiment **No. 1**, formation of a mixture of products **hexT-14Y**, **hexT-16Y** and **hexT-17**; b) HPLC trace of experiment **No. 2**, formation of a mixture of products **hexT-14Y** and **hexT-17**, less formation of **hexT-16Y** than in **No. 1**; c) HPLC trace of experiment **No. 3**, formation of a mixture of products **hexT-14**-**17**, at 55°C formation of the pyrazole **hexT-15Y** becomes notable; d) HPLC trace of experiment **No. 4**, the pyrazole **hexT-15Y** and the hydration product **hexT-17** are the main products formed under these conditions; e) HPLC trace of experiment **No. 5**, formation of compound **hexT-14Y** as main product, formation of the oxidized pyrazole **hexT-15Y** as a side product (r.t.: 16.4 min); f) HPLC trace of experiment **No. 6**, formation of a mixture of products **hexT-14**-**17**; g) HPLC trace of experiment **No. 7**, formation of compound **hexT-14Y** as main product; h) HPLC trace of experiment **No. 8**, formation of compound **hexT-14Y** as main product; i) HPLC trace of experiment **No. 9**, formation of a mixture of products **hexT-14**-**17**; j) HPLC trace of experiment **No. 10**, formation of pyrazole

hexT-15Y as main product; k) HPLC trace of experiment **No. 11**, formation of pyrazole **hexT-15Y** as main product**.**

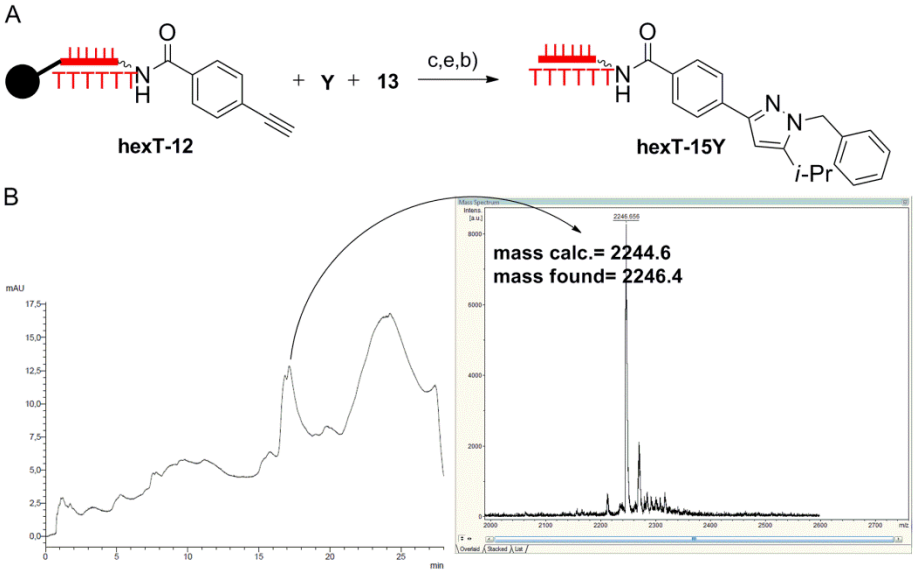


Figure S21. MALDI-MS of the isolated pyrazole **hexT-15Y** from Figure S20k. A) Reaction scheme of the pyrazole formation; a) reaction condition **No. 11** (Table S4c); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; B) MALDI-MS of the isolated hexathymidine conjugate **hexT-15Y**. Filled black circle: CPG solid support; wavy bond: C6-amino-linker; bold bond: connection of the hexT oligonucleotide to the CPG.

d) Screening of different catalysts for the Au(I) catalyzed A^3 MCR of the hexathymidine-alkyne conjugate **hexT-12**, isobutyraldehyde **Y**, and hydrazide **13**.

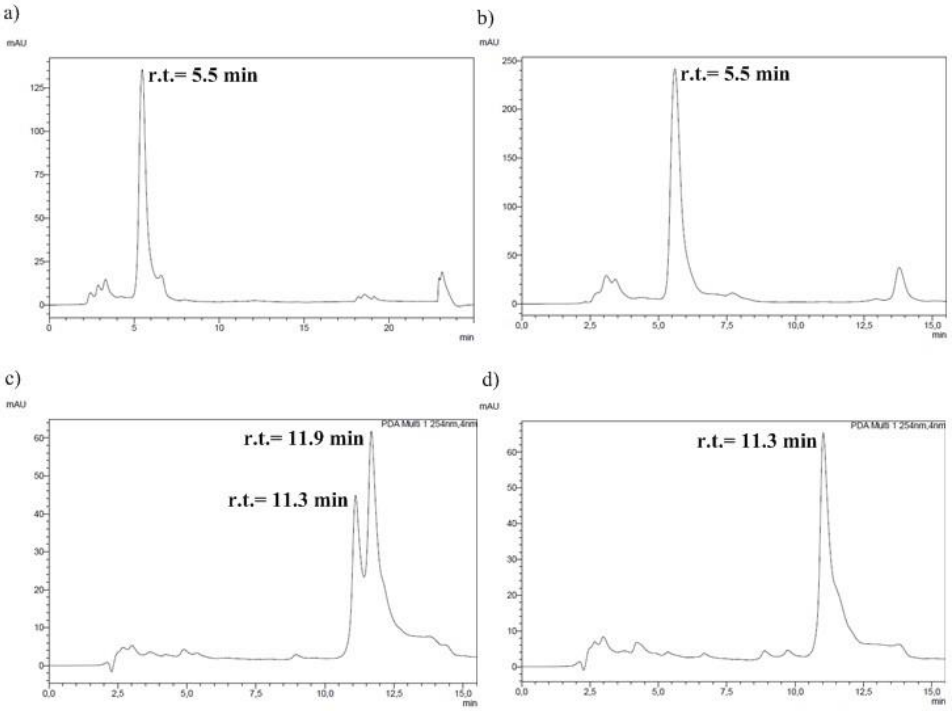


Figure S22. Effect of different catalysts on the Au(I) catalyzed A^3 MCR of the hexathymidine-alkyne conjugate **hexT-12**, isobutyraldehyde **Y**, and hydrazide **13**, for reaction conditions see Table S4d. a) HPLC trace of the starting material **hexT-12**; b) HPLC trace of experiment **No. 1**, only trace amounts of the product **hexT-16Y** were formed upon reaction with catalyst ***B*** alone; c) HPLC trace of experiment **No. 2**, catalysis with AgOTf gave rise to a mixture (40:60) of products **hexT-14Y** and **hexT-16Y**; d) HPLC trace of experiment **No. 3**, the pyrazoline **hexT-14Y** was formed as the sole product under these conditions; experiment **No. 4** gave the same result as experiment **No. 3**. Please note that a-d) were analyzed on a different HPLC system: 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 10 mM aqueous triethylammonium acetate/MeOH (30-90 % over 15.5 min). HPLC trace a) shows column washing, and equilibration, too.

5.2 Comparison of the hexT-pyrazoline conjugate hexT-14Y with the reference molecule ref-hexT-14Y, and with hexT-propargyl amine conjugates hexT-16Y and ref-hexT-16Y

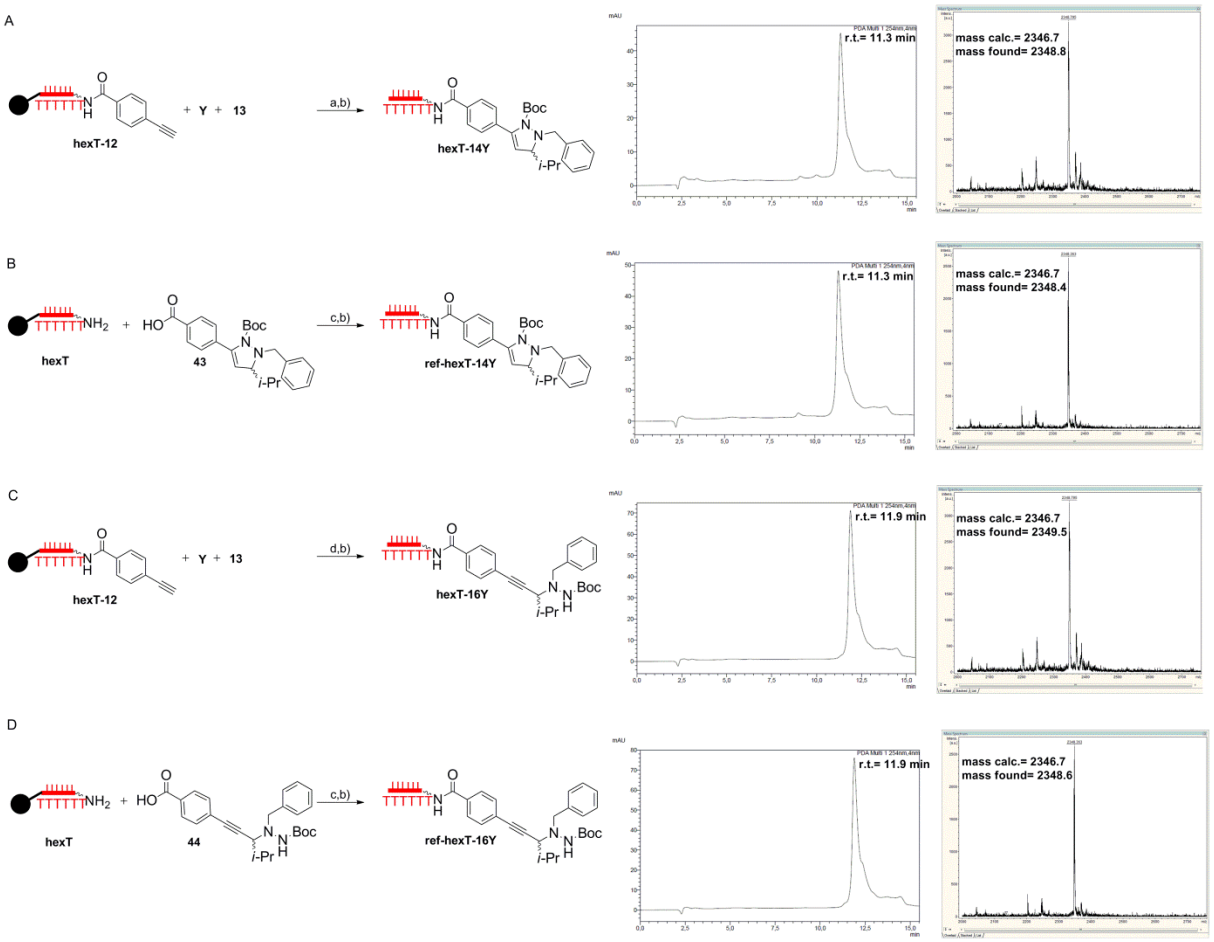


Figure S23. Comparison of the hexT-pyrazoline **hexT-14Y** synthesized from the hexT-alkyne conjugate **hexT-12** by the Au(I)-catalyzed annulation reaction (Figure S16) with the reference hexT-pyrazoline **ref-hexT-14Y** synthesized from the pyrazoline **43**, and 5'-(C6)-aminolinker modified **hexT** by amide coupling, and comparison of the propargyl amine **hexT-16Y** with the reference compound **ref-hexT-16Y** synthesized by amide coupling of the propargyl amine **44** with the 5'-(C6)-aminolinker modified **hexT**. A) scheme for the synthesis of the hexT-pyrazoline conjugate **hexT-14Y** and analysis of the product; conditions: a) method **A** (Table S4b, entry 5); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature); B) scheme for the synthesis of the hexT-pyrazoline conjugate **ref-hexT-14Y** by amide coupling and analysis of the product; conditions: c) HATU, DIPEA, room temperature, two hours; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; C) scheme for the synthesis of the hexT-propargylamine conjugate **hexT-16Y** and analysis of the product; conditions: d) AgOTf, toluene; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; D) scheme for the synthesis of the hexT-propargylamine conjugate **ref-hexT-16Y** by amide coupling and analysis of the product; conditions: c) HATU, DIPEA, room temperature, two hours; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature. Filled black circle: CPG solid support; wavy bond: C6-amino-linker; bold bond: connection of the hexT oligonucleotide to the CPG.

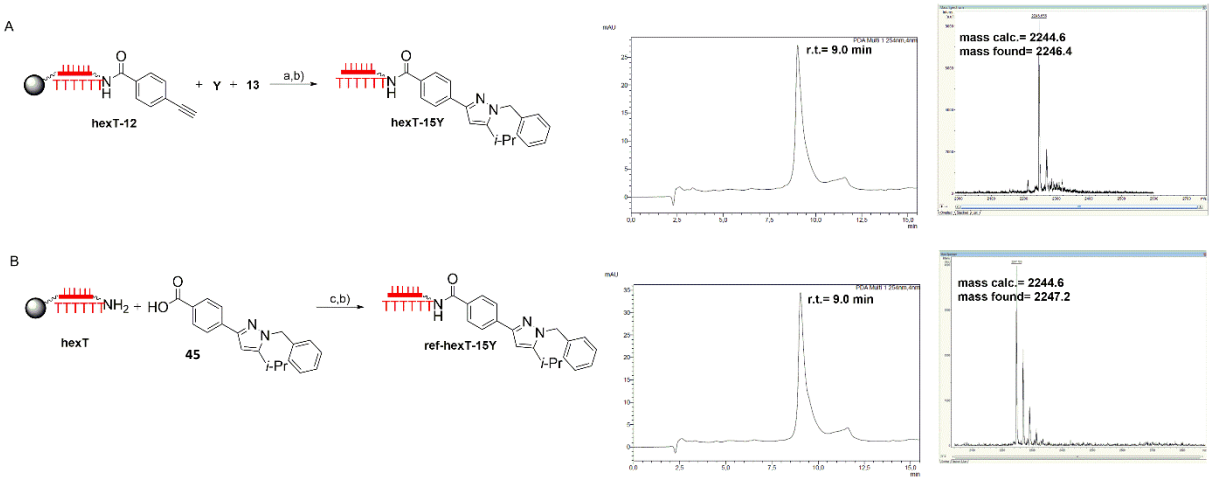


Figure S24. Comparison of the hexT-pyrazole **hexT-15Y** synthesized from the hexT-alkyne conjugate **hexT-12** by the Au(I)-catalyzed annulation reaction with the reference hexT-pyrazoline **ref-hexT-15Y** synthesized from the pyrazole **45**, and 5'-(C6)-aminolinker modified **hexT** by amide coupling. A) Scheme for the synthesis of the hexT-pyrazole conjugate **hexT-15Y** and analysis of the product; conditions: a) method **C** (Table S4c, entry 11); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature); B) scheme for the synthesis of the hexT-pyrazole conjugate **ref-hexT-15Y** by amide coupling and analysis of the product; conditions: c) HATU, DIPEA, room temperature, two hours; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature. Filled black circle: CPG solid support; wavy bond: C6-amino-linker; bold bond: connection of the hexT oligonucleotide to the CPG.

5.3 Synthesis of the hexT-pyrazole conjugate hexT-15Y from the hexT-alkyne conjugate hexT-12, and from hexT-pyrazolines hexT-14Y and hexT-18Y

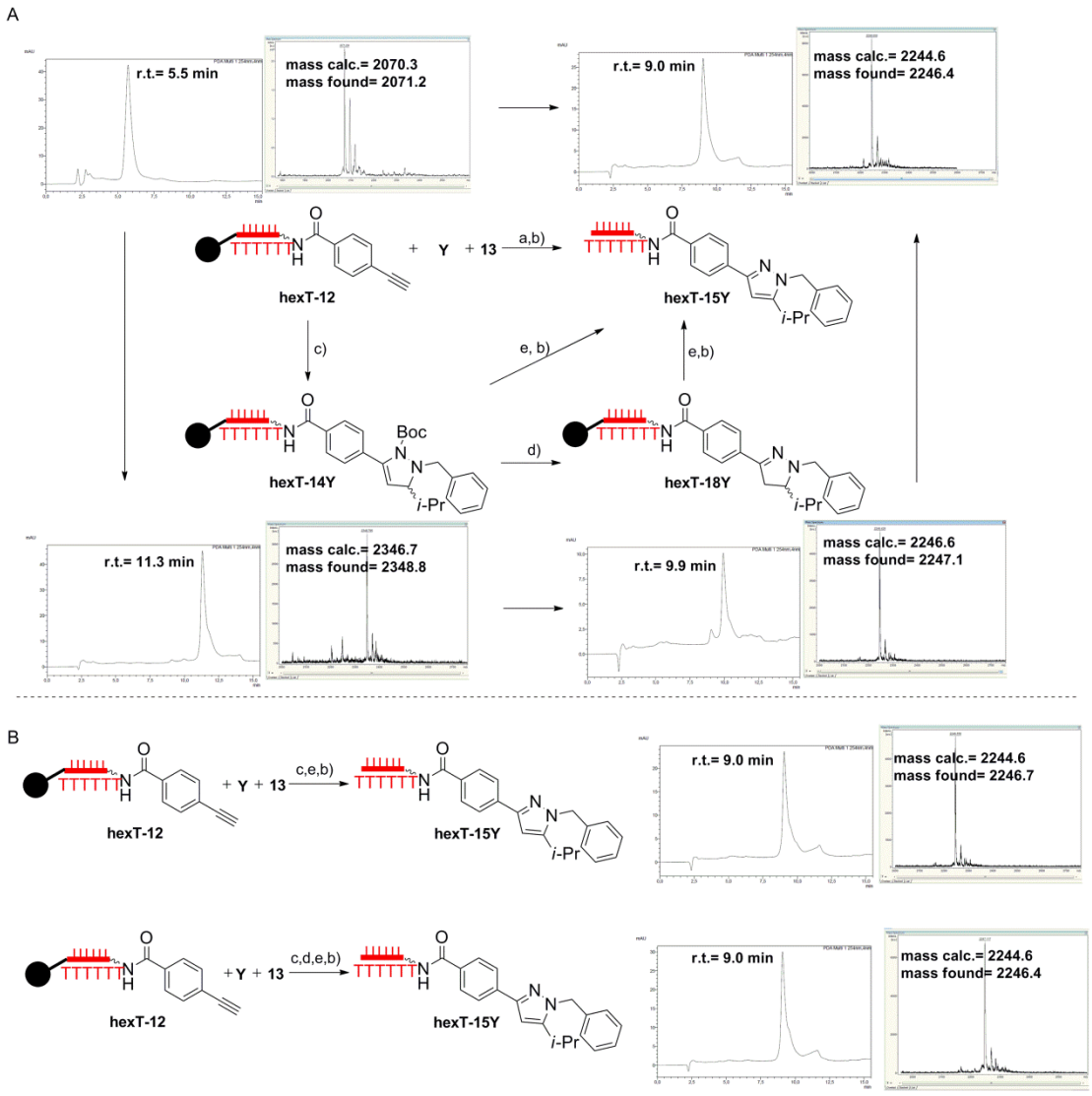
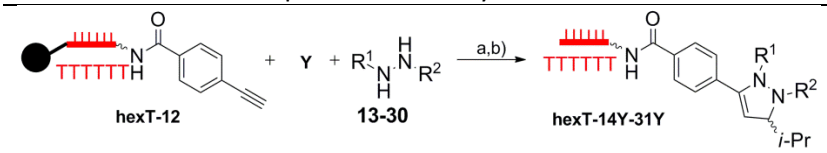


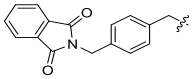
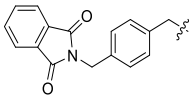
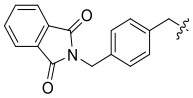
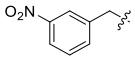
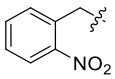
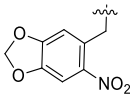
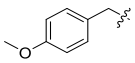
Figure S25. Synthesis of the hexT-pyrazole conjugate **hexT-15Y** from the hexT-alkyne conjugate **hexT-12** by the Au(I)-catalyzed annulation reaction in glacial acetic acid at 60°C (Figure S16), and synthesis of the same conjugate from the pyrazoline conjugates **hexT-14Y** and **hexT-18Y** by oxidative aromatization with DDQ. A) Synthesis scheme for the synthesis of the hexT-pyrazole conjugate **hexT-15Y** from **hexT-12**, **hexT-14Y** and **hexT-18Y**. See above or below each hexT-conjugate a corresponding HPLC trace and MALDI-MS spectrum of the compound; conditions: a) method **C** (Table S4c, entry 11); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; c) method **A** (Table S4b, entry 5); d) 10 % TFA, DCM; e) 100 eq. of DDQ, CH_2Cl_2, room temperature, 30 min; B) synthesis scheme and analytical data for hexT-pyrazole conjugate **hexT-15Y** synthesized from the pyrazoline conjugates **hexT-14Y** and **hexT-18Y** by oxidative aromatization. The conjugate **hexT-18Y** (pyrazoline) was stable upon storage in water at -20°C over several weeks, as well as upon storage at room temperature for several days. We did not observe oxidation to the pyrazole **hexT-15Y** upon storage as analyzed by HPLC. Black filled circle: controlled pore glass solid support; wavy bond: C6-amino-linker; bold bond: connection of the hexT oligonucleotide to the CPG.

5.4 Investigation of the scope of unsymmetrically substituted hydrazides for Au(I) mediated pyrazol(in)e synthesis

Table S5. Reaction scope of different hydrazides

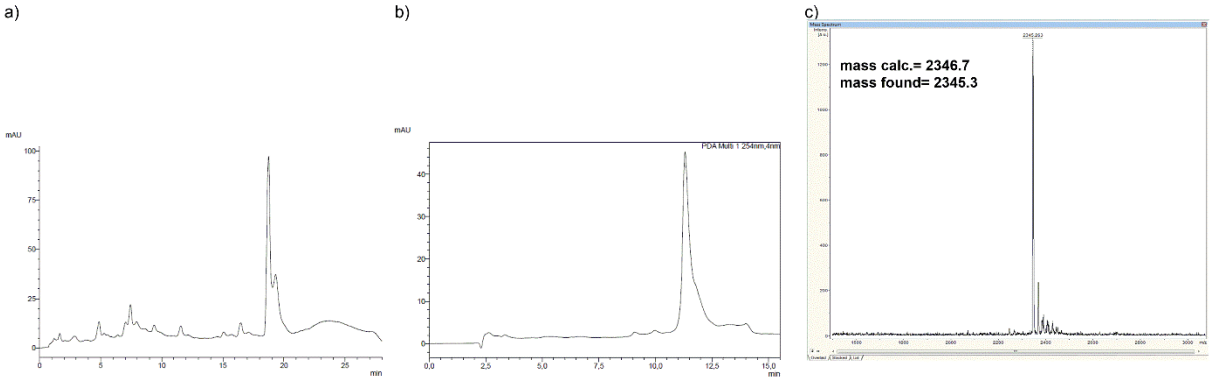


Conditions: a) method **A** (Table S4b, entry 5), b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature. All compounds with 5'-C6-aminolinker.

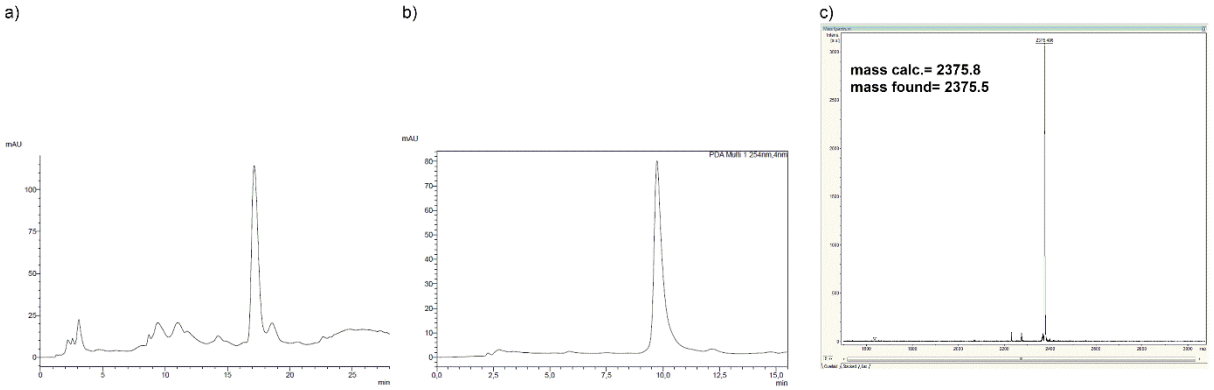
entry	hydrazide No.	hexT No.	R^1	R^2	yield [nmol][a]	conversion [%][b,c]	mass calc. mass found[d]
1	**13**	**hexT-14Y**	*tert*-Boc		4.4	75 (25)	2346.7 2345.3
2[e]	**19**	**hexT-20Y**	*tert*-Boc		4.9	80 (15)	2375.8 2375.5
3[e,f]	**19**	**hexT-21Y**	*tert*-Boc		5.0	90 (0)	2244.6 2246.4
4[e]	**22**	**hexT-23Y**	Ac		4.0	95 (0)	2317.7 2317.6
4	**24**	**hexT-25Y**	*tert*-Boc		4.0	80 (10)	2391.7 2392.3
5	**26**	**hexT-27Y**	*tert*-Boc		4.1	75 (0)	2391.7 2392.4
6	**28**	**hexT-29Y**	*tert*-Boc		0.9	25 (35)	2435.7 2437.2 2257.7[g]
7	**30**	**hexT-31Y**	*tert*-Boc		4.7	30 (15)	2376.7 2377.8

[a] measured by Nanodrop; [b] % conversion estimated based on the area under the curve of the product peak versus the educt peak in the HPLC-trace (preparative HPLC), missing percentage to 100 %: **hexT-12**; [c] conversions in parentheses show those of corresponding propargyl hydrazides; [d] measured by MALDI-MS; [e] phthalimide protective group was removed in the product; [f] product synthesized by method **C** (Table S4c, entry 11), thus leading to the oxidized compound **hexT-21Y**; [g] loss of the photolabile 3,4-methylendioxy-6-nitrobenzyl group upon irradiation in the mass spectrometer.

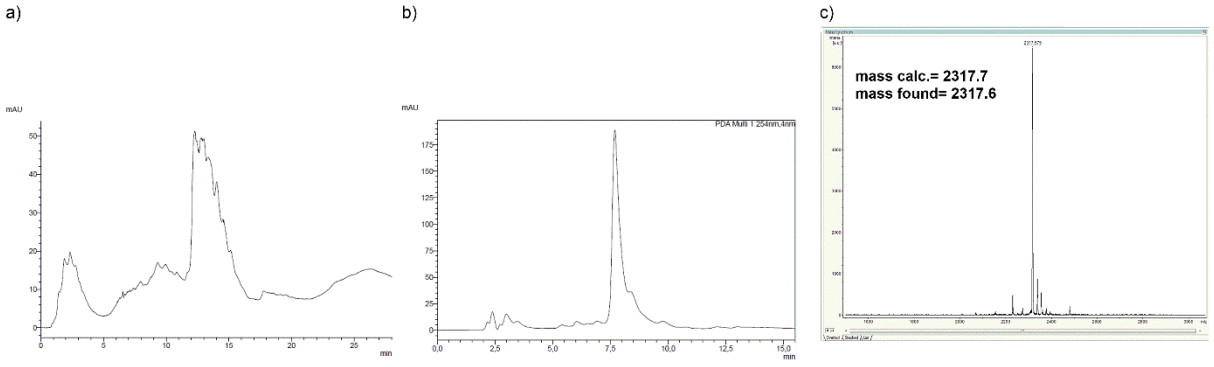
HPLC chromatograms and MALDI-MS spectra of hex-T-pyrazoline conjugates hexT-14Y, hexT-20Y, hexT-23Y, hexT-25Y, hexT-27Y, hexT-29Y, and **hexT-31Y**



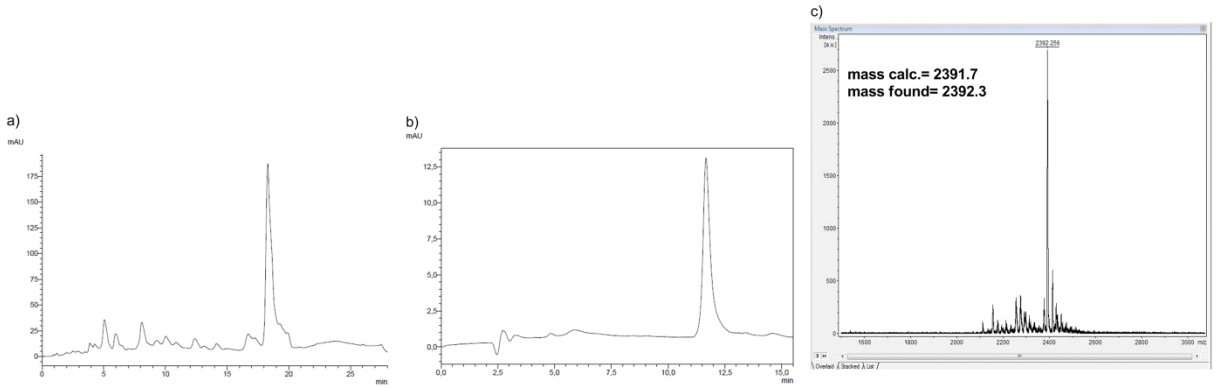
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-14Y**; b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-14Y**; c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-14Y**.



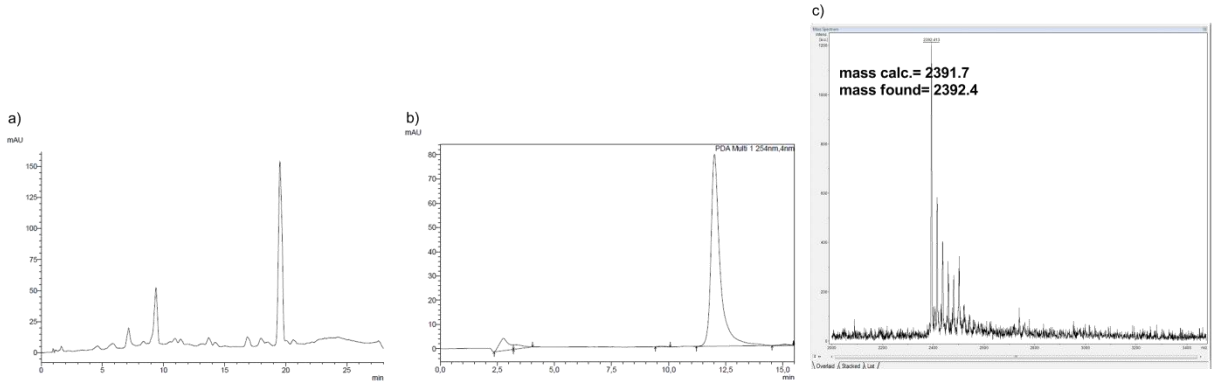
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-20Y**; b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-20Y**; c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-20Y**.



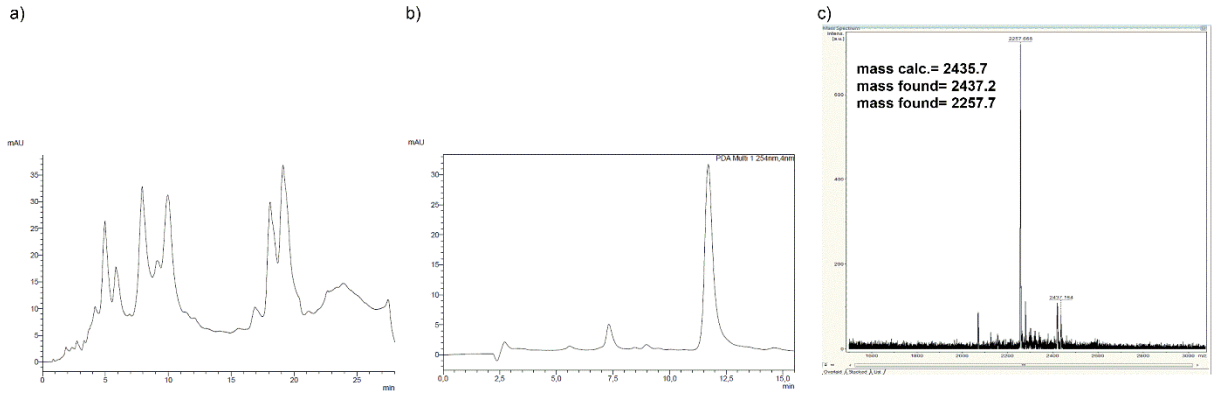
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-23Y**; b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-23Y**; c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-23Y**.



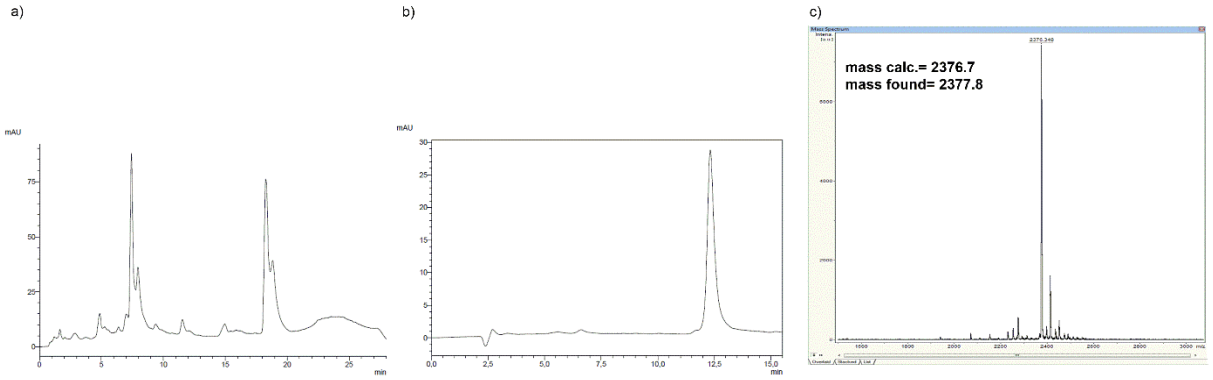
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-25Y**; b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-25Y**; c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-25Y**.



a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-27Y**; b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-27Y**; c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-27Y**.



a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-29Y**; b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-29Y**; c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-29Y**, we observed the expected mass and the product mass – 180 Da due to loss of the photolabile 3,4-methylendioxy-6-nitrobenzyl group upon irradiation.



a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-31Y**; b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-31Y**; c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-31Y**.

5.5 Au(I) catalyzed A^3 three-component reaction to hexT-pyrazol(in)e conjugates hexT-21 and hexT-22

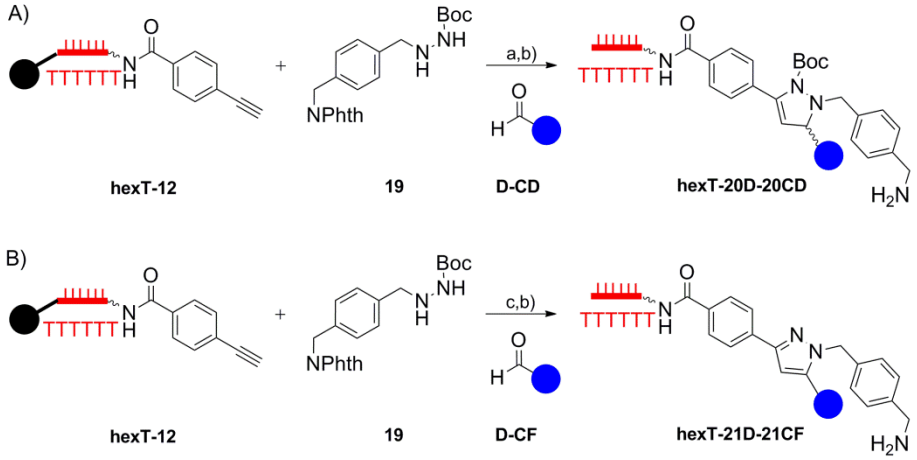


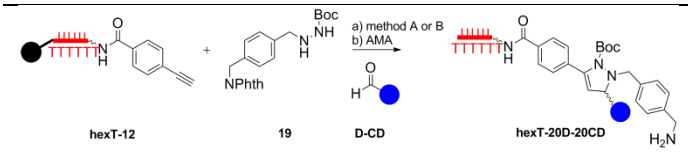
Figure S26: A) Synthesis of a pyrazoline library **hexT-20D-20CD**; a) method **A** (Table S4b, No. 5) for aliphatic aldehydes; method **B** (Table S4c, No. 10**)** for aromatic aldehydes; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; B) Synthesis of a pyrazole library **hexT-21D-21CF**; c) method **C** (Table S4c, No. 11); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature. Filled black circle: CPG solid support; wavy bond: PEG(4)-linker; bold bond: connection of the hexT oligonucleotide to the CPG.

Method A (Table S4b, No.5): The solid support with the hexT conjugate **hexT-12**, aldehydes **D-CD** (Table S6), and hydrazide **19**, catalyst ***A*** (Figure S16) and $AgSbF_6$ (each 12.5 µmol, 250 eq) were dried *in vacuo* for 15 min. Then, a batch of solid support containing hexT conjugate **hexT-12** (18 mg, ca. 500 nmol) was suspended in 330 µL of dry MeCN. This suspension was distributed to a total of 10 reaction vessels of a 96well plate, so that each reaction vessel contained ca. 50 nmol of **hexT-12** suspended in 30 µL of dry MeCN. Then, 10 aldehydes from **D-CD** (50 µmol, 1000 eq) each dissolved in 30 µL of dry MeCN were added to the splitted hexT conjugate **hexT-12**. To these suspensions were added hydrazide **19** (50 µmol), dissolved in 30 µL of dry MeCN (taken from a stock solution: 2750 µmol, 1048.9 mg in 1650 µL of dry MeCN), and the catalyst ***A***/$AgSbF_6$ (12.5 µmol) suspended in 30 µL of dry MeCN (taken from a stock solution: 687.5 µmol, 604.5 mg of catalyst ***A*** and 236.1 mg of $AgSbF_6$ mixed in 1650 µL of dry MeCN) giving a total reaction volume of ca. 120 µL. The reaction mixtures were shaken at 50°C overnight. The solid phase containing the pyrazolines **hexT-20D-20CD** was filtered off on a filter plate and washed subsequently with each 3 x 200 µL of 0.1 M EDTA, DMF, MeOH, MeCN and DCM and dried *in vacuo* for 15 min. Then, the hexT-pyrazoline conjugates **hexT-20D-20CD** were deprotected and cleaved from the CPG with 500 µL of AMA (AMA= aqueous ammonia (30 %)/ aqueous methylamine (40 %)= 1:1, vol/vol) for 30 minutes at room temperature. The samples were filtered directly on 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-MS analysis and by analytical HPLC to assert purity and identity.

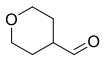
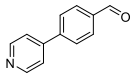
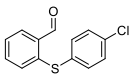
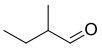
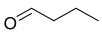
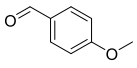
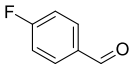
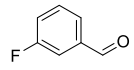
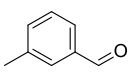
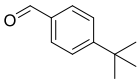
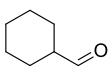
Method B (Table S4c, No.10)**:** The solid support with the hexT conjugate **hexT-12**, aldehydes (Table S6) (each 50 µmol, 1000 eq), hydrazide **19**, catalyst ***B*** (Figure S16) and AgOTf were dried *in vacuo* for 15 min. Then, a batch of solid support containing the **hexT-12** DNA conjugate (18 mg, ca. 500 nmol) was suspended in 330 µL of glacial acetic acid. This suspension was distributed to a total of 10 reaction vessels of a 96well plate, so that each reaction vessel contained ca. 50 nmol of a hexT conjugate **hexT-12** suspended in 30 µL of glacial acetic acid. Then, 10 aldehydes from **D**-**CD** (50 µmol, 1000 eq) each dissolved in 30 µL of glacial acetic acid were added to the splitted hexT conjugate **hexT-12**. To these suspensions were added hydrazide **19** (50 µmol, 1000 eq), dissolved in 30 µL of glacial acetic acid (taken from a stock solution: 2750 µmol, 1048.9 mg in 1650 µL of glacial acetic acid), and catalyst ***B***/AgOTf (12.5 µmol) suspended in 30 µL of glacial acetic acid (taken from a stock solution: 687.5 µmol, 340.1 mg of catalyst ***B*** and 176.6 mg of AgOTf mixed in 1650 µL of glacial acetic acid) giving a total reaction volume of ca. 120 µL. The reaction mixtures were shaken at 50°C overnight. The solid phase containing the pyrazolines **hexT-20D**-**20CD** was filtered off on a filter plate and washed subsequently with each 3 x 200 µL of 0.1 M EDTA, DMF, MeOH, MeCN and DCM and dried *in vacuo* for 15 min. Then, the hexT-pyrazolines **hexT-20D**-**20CD** 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 30 minutes 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-MS analysis and by analytical HPLC to assert purity and identity.

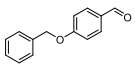
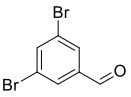
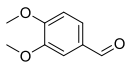
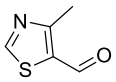
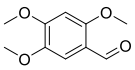
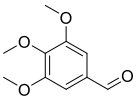
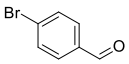
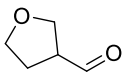
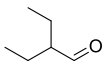
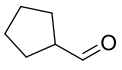
Method C (Table S4c, No.11): as method **B**, reactions run at 60°C.

Table S6. Yield, and MALDI-MS data of compounds **hexT-20D-20CD**.



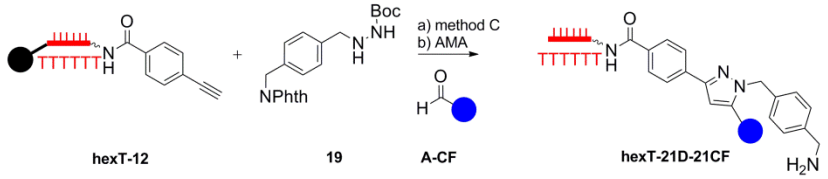
For reaction conditions see Fig. S26

No.	structure	yield [nmol][a]	mass calc. mass found[b]
D		3.8	2665.1[c] 2664.9
E		1.0	2734.2[d] 2733.7
H		1.7	2799.7[d] 2800.8
I		0.6	2698.1[d] 2699.1
M		2.7	2637.1[c] 2636.1
N		-	-
Q		0.7	2687.1[d] 2687.8
R		0.7	2675.1[d] 2675.9
Y		4.9	2623.1[c] 2623.7
AB		0.7	2675.1[d] 2675.2
AC		0.9	2671.1[d] 2671.8
AJ		0.6	2713.2[d] 2714.0
AM		2.7	2663.1[c] 2663.5

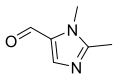
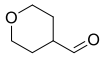
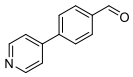
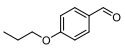
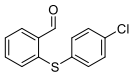
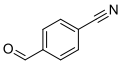
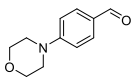
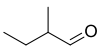
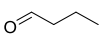
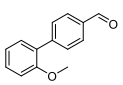
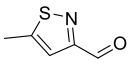
No.	structure	yield [nmol][a]	mass calc. mass found[b]
AN		0.9	2763.2[d] 2763.0
AP		0.4	2814.9[d] 2815.4
AQ		0.9	2717.1[d] 2718.1
AT		5.2	2678.1[d] 2678.4
AU		0.5	2747.2[d] 2747.9
AW		0.8	2747.2[d] 2748.5
AX		0.9	2736.0[d] 2736.3
BC		2.1	2651.1[c] 2651.6
BZ		3.5	2651.1[c] 2651.7
CA		0.8	2621.0[c] 2620.4
CD		4.5	2649.1[c] 2649.1

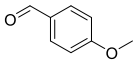
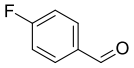
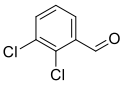
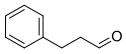
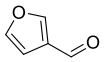
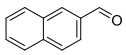
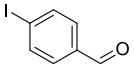
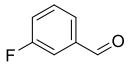
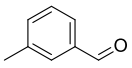
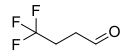
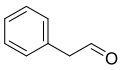
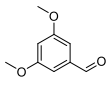
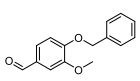
[a] Measured by Nanodrop; [b] measured by MALDI-MS; [c] synthesized according to the method **A** (Tables 2, S4); [d] synthesized according to method **B** (Tables 2, S4).

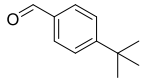
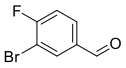
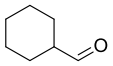
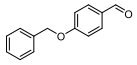
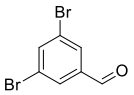
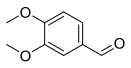
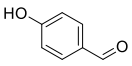
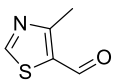
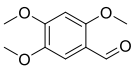
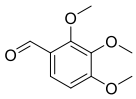
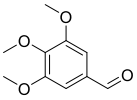
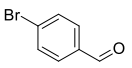
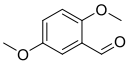
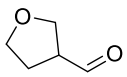
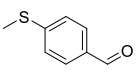
Table S7: Yield, and MALDI MS data of compounds **hexT-21A**-**21CF**.

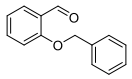
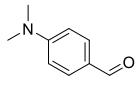
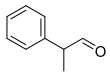
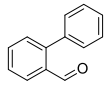
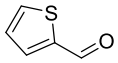
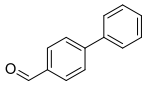
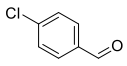
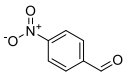
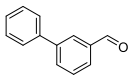
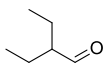
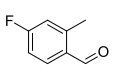
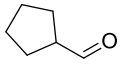


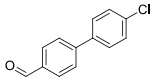
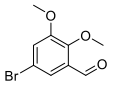
For reaction conditions see Fig. S26

No.	structure	yield [nmol][a]	mass calc. mass found[b]
A		-	-
D		4.3	2563.0 2564.1
E		1.7	2632.0 2633.6
G		1.5	2613.0 2617.4
H		2.4	2697.5 2699.4
I[c]		1.1	2598.0 2598.4
J		2.3	2580.0 2582.7
K		-	-
M		1.8	2535.0 2535.2
N		-	-
O		0.7	2661.1 2661.1
P		3.0	2576.0 2578.2

No.	structure	yield [nmol][a]	mass calc. mass found[b]
Q		1.1	2585.0 2586.8
R		1.9	2573.0 2574.5
S		0.5	2623.8 2628.1
T		-	-
V		-	-
AW		-	-
X		1.4	2605.0 2605.9
Y		5.0	2520.9 2520.9
Z		0.4	2680.8 2680.4
AB		1.7	2573.0 2572.1
AC		3.3	2569.0 2569.5
AE		-	-
AF		-	-
AG		2.9	2615.0 2616.4
AH		1.0	2691.1 2692.4

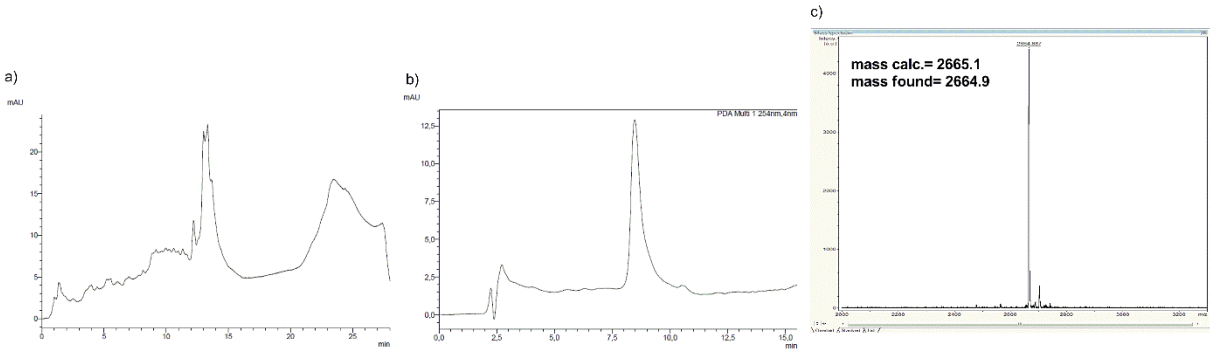
No.	structure	yield [nmol][a]	mass calc. mass found[b]
AJ		1.4	2611.0 2610.8
AK		3.6	2651.8 2655.6
AM		2.8	2561.0 2561.3
AN		1.3	2661.1 2661.8
AP		0.6	2712.7 2715.1
AQ		0.9	2615.0 2613.7
AR		-	-
AT		7.0	2576.0 2578.1
AU		0.9	2645.0 2645.4
AV		0.6	2645.0 2643.9
AW		2.8	2645.0 2646.9
AX		2.1	2633.8 2635.9
AZ		1.4	2615.0 2618.6
BC		2.1	2548.9 2552.7
BD		1.1	2601.0 2601.2

No.	structure	yield [nmol][a]	mass calc. mass found[b]
BG		1.9	2661.1 2661.1
BI		1.3	2598.0 2599.5
BJ		-	-
BK		2.0	2631.0 2634.9
BM		4.5	2561.0 2561.5
BO		3.0	2631.0 2632.5
BQ		2.6	2589.4 2590.6
BR		1.2	2600.0 2601.7
BV		1.3	2554.9 2554.2
BX		3.1	2631.0 2630.6
BZ		4.5	2549.0 2548.7
CA		1.1	2518.9 2518.7
CC		0.9	2587.0 2587.2
CD		3.3	2547.0 2549.0

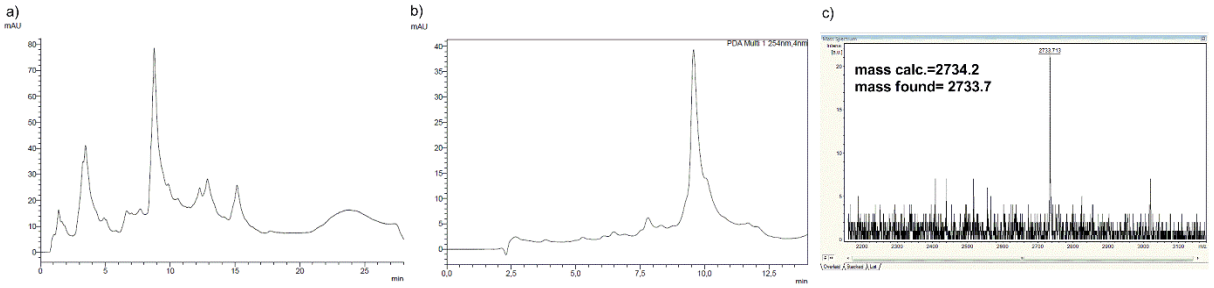
No.	structure	yield [nmol][a]	mass calc. mass found[b]
CE		1.1	2665.5 2666.5
CF		2.2	2693.9 2697.7

[a] Measured by Nanodrop; [b] measured by MALDI-MS; [c] Boc-deprotected pyrazoline was obtained.

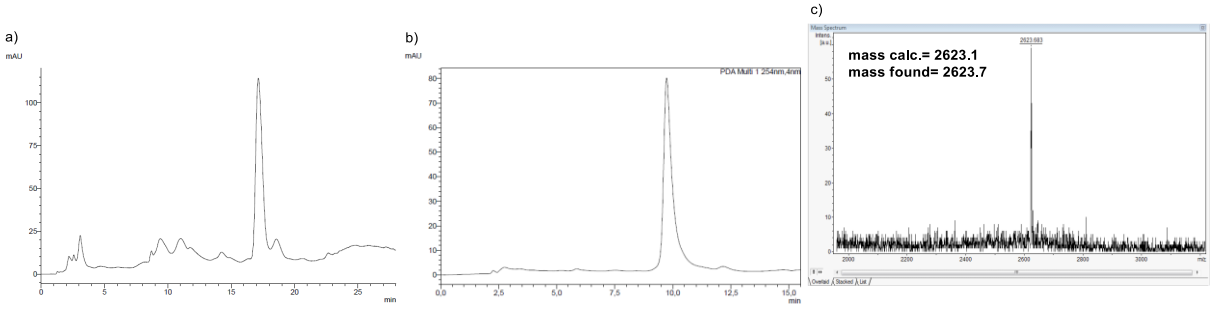
HPLC chromatograms and MALDI-MS spectra of 20 representative library members



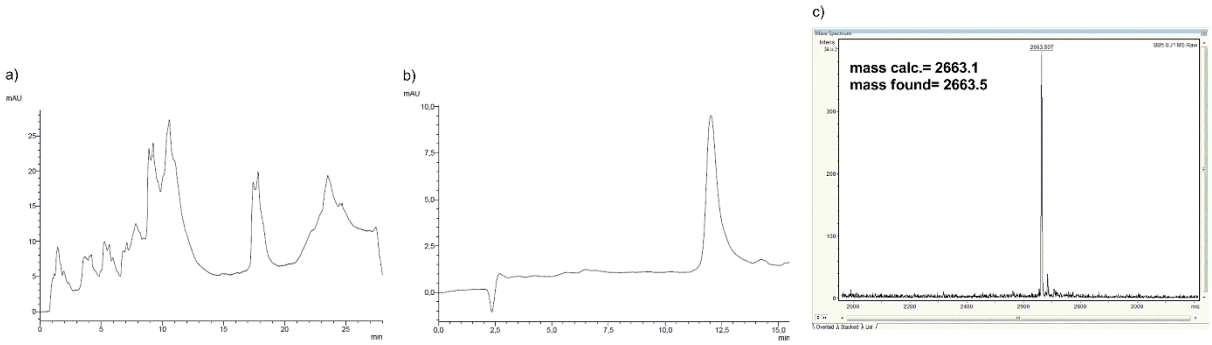
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-20D**. b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-20D**. c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-20D**.



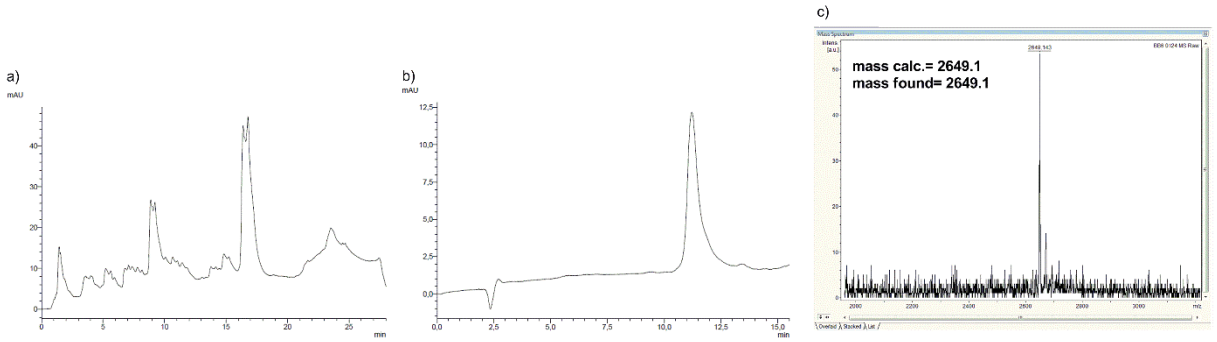
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-20E**. b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-20E**. c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-20E**.



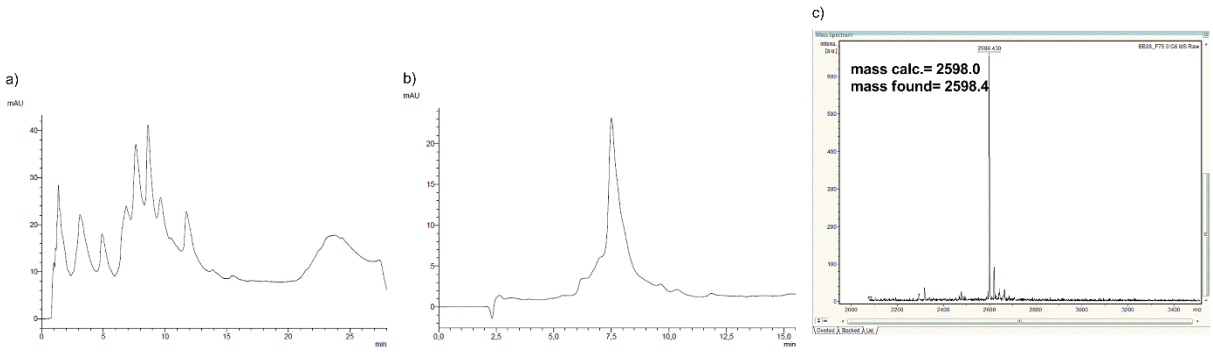
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-20Y**. b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-20Y**. c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-20Y**.



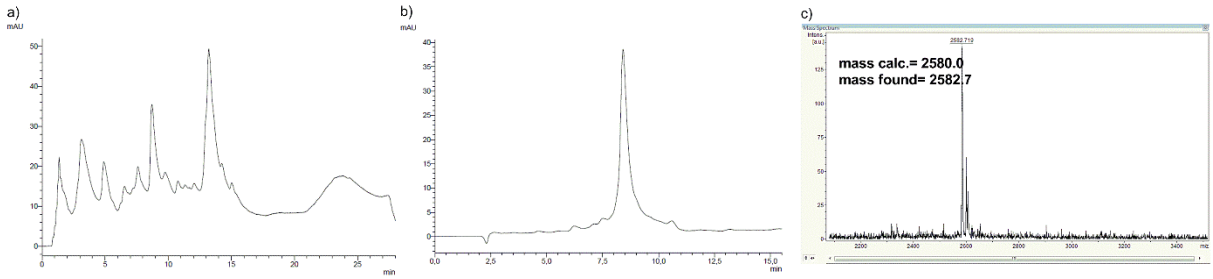
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-20AM**. b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-20AM**. c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-20AM**.



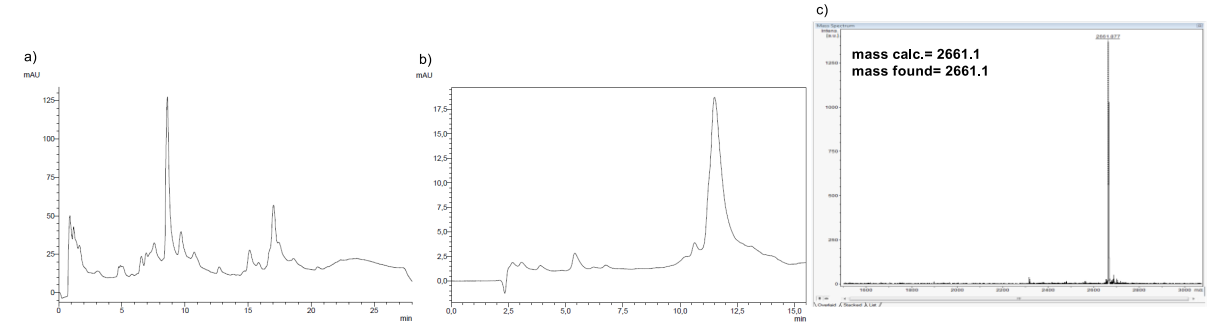
a) HPLC trace of the crude hexT-pyrazoline conjugate **hexT-20CD**. b) HPLC trace (preparative HPLC) of the purified hexT-pyrazoline conjugate **hexT-20CD**. c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-20CD**.



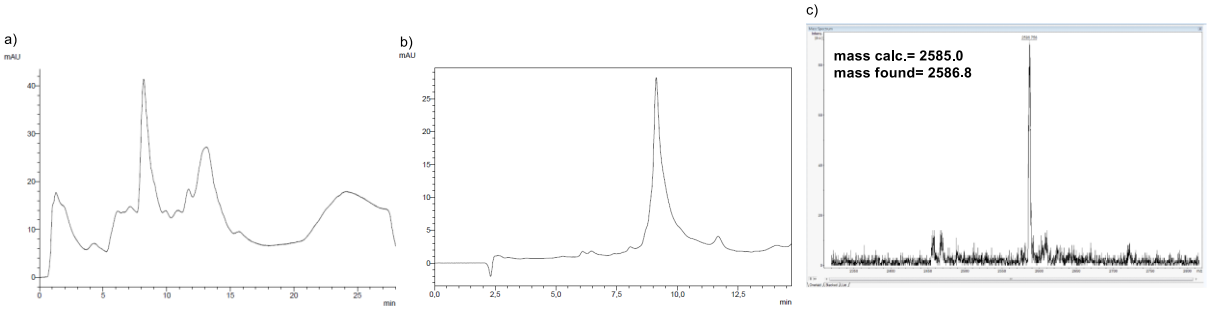
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazoline conjugate **hexT-21I**. b) HPLC trace of the purified hexT-pyrazoline conjugate **hexT-21I**. c) MALDI-MS analysis of the purified hexT-pyrazoline conjugate **hexT-21I**.



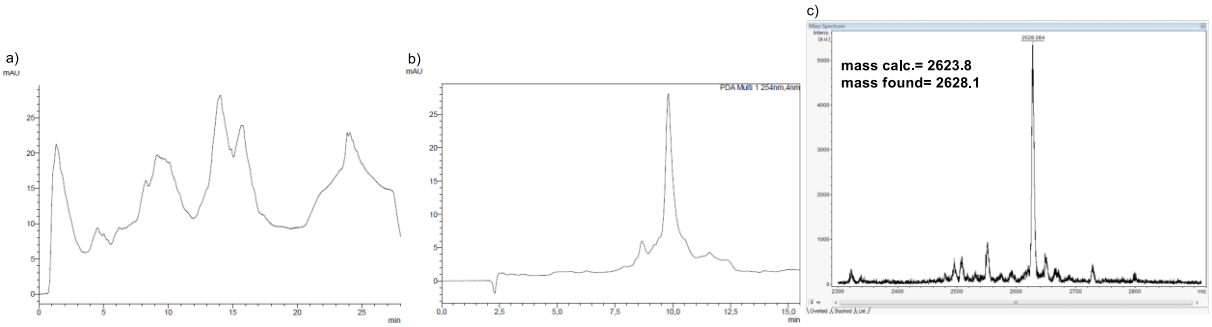
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21J**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21J**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21J**.



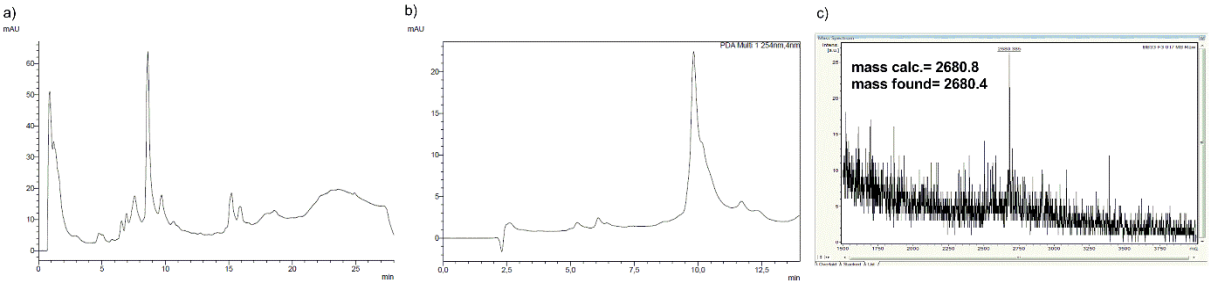
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21O**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21O**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21O**.



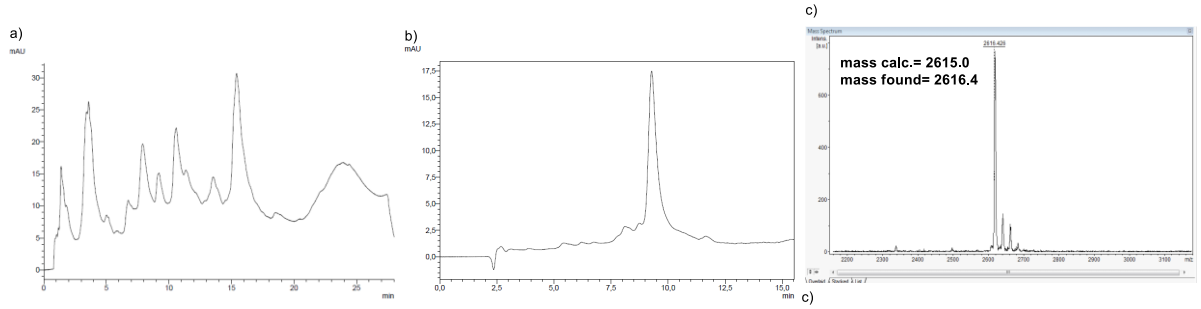
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21Q**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21Q**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21Q**.



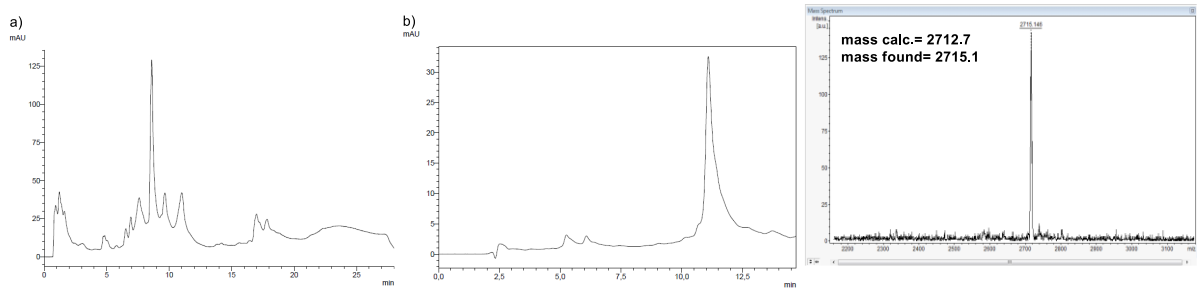
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21S**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21S**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21S**.



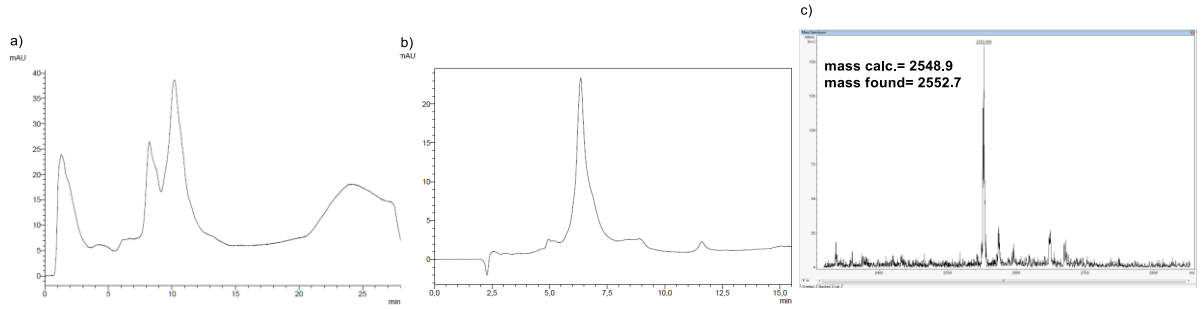
a) HPLC trace of (preparative HPLC) the crude hexT-pyrazole conjugate **hexT-21Z**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21Z**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21Z**.



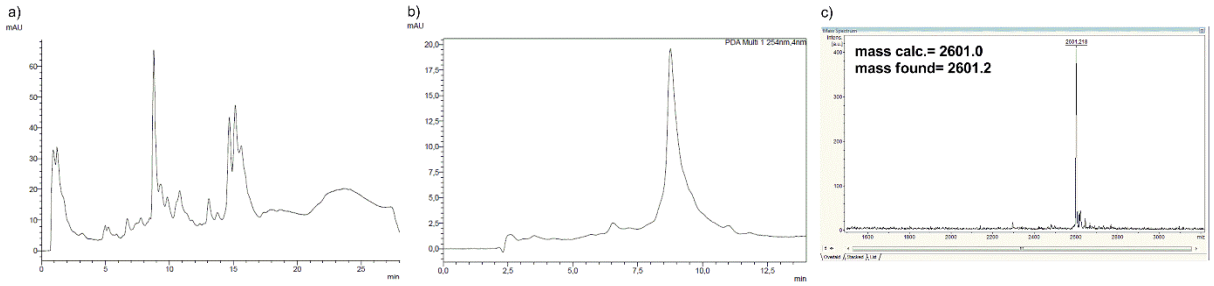
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21AG**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21AG**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21AG**.



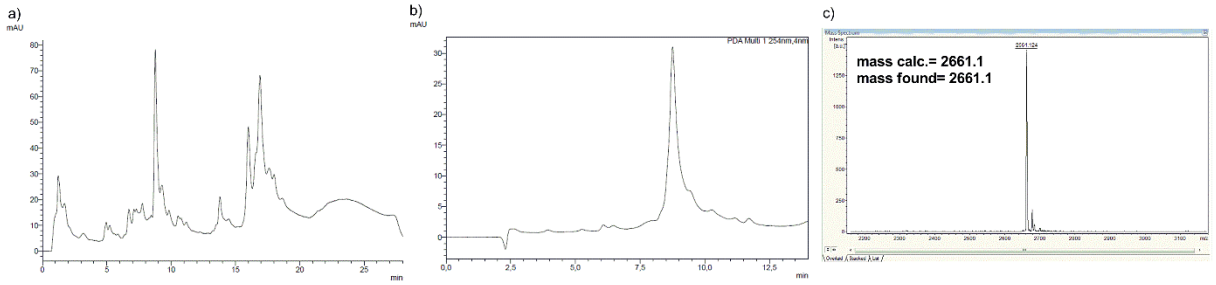
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21AP**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21AP**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21AP**.



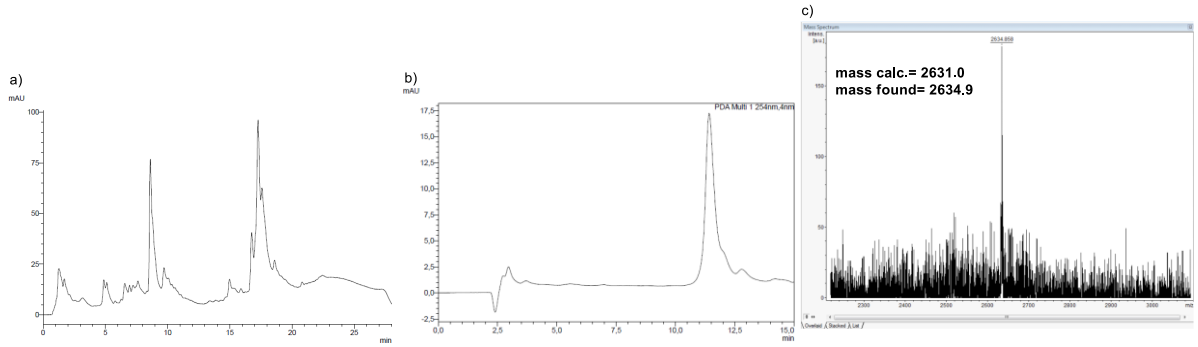
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21BC**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21BC**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21BC**.



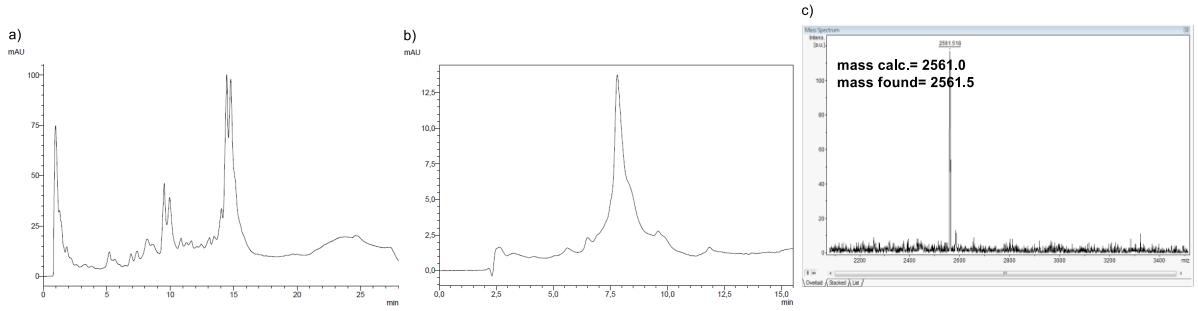
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21BD**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21BD**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21BD**.



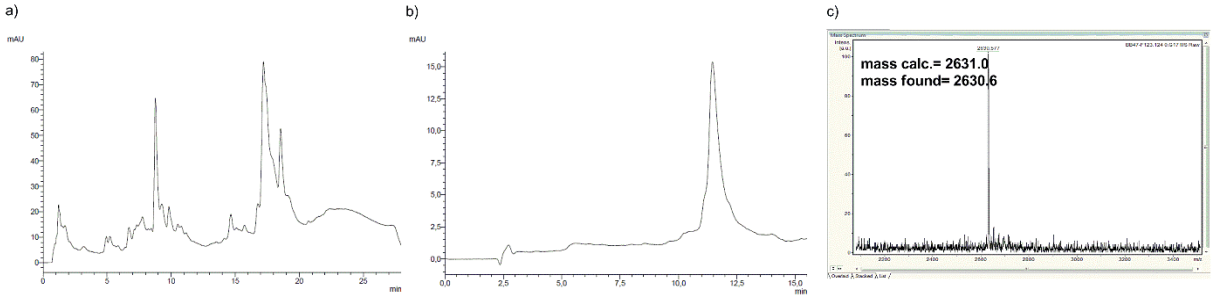
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21BG**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21BG**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21BG**.



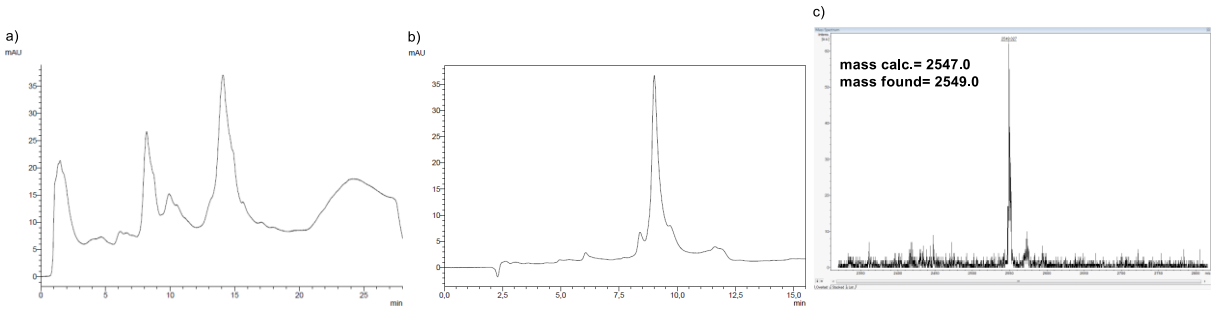
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21BK**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21BK**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21BK**.



a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21BM**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21BM**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21BM**.



a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21BX**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21BX**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21BX**.

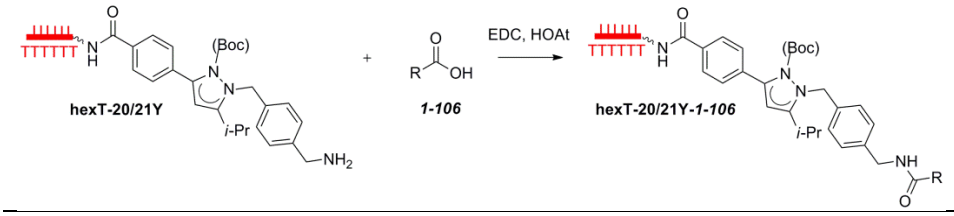


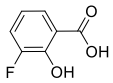
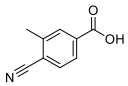
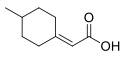
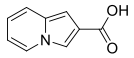
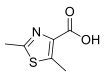
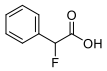
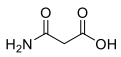
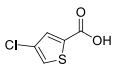
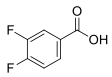
a) HPLC trace (preparative HPLC) of the crude hexT-pyrazole conjugate **hexT-21CD**. b) HPLC trace of the purified hexT-pyrazole conjugate **hexT-21CD**. c) MALDI-MS analysis of the purified hexT-pyrazole conjugate **hexT-21CD**.

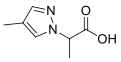
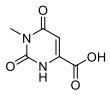
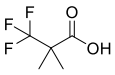
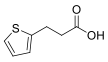
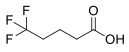
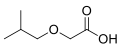
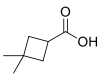
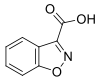
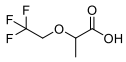
6. Evaluation of carboxylic acids for library synthesis[3]

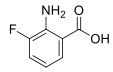
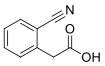
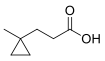
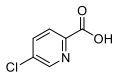
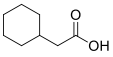
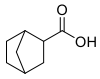
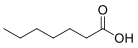
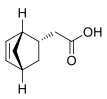
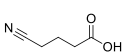
DEAE sepharose was pipetted on 96well filter plate (96 x 100 µL), washed with water (2 × 350 µL) and 10 mM aq. NaAc buffer (2 × 350 µL). Then, 500 pmol of the hexT-pyrazoline conjugate **hexT-20Y**, **hexT-20-PEG(4)-Y** or hexT-pyrazole conjugate **hexT-21Y** was immobilized on DEAE anion exchange resin placed in each well position, by incubation of an aqueous solution of the oligonucleotide conjugate (2-20 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 DMSO (2 × 350 µL). For activation of carboxylic acids, 25 µmol of each carboxylic acid (**Table S8**) was dissolved in 300 µL of dry DMSO in an Eppendorf tube, to this were added HOAt (2.5 µmol) dissolved in 50 µL of dry DMSO (taken from a stock solution: 275 µmol, 37.4 mg in 5.5 mL of dry DMSO) and EDC x HCl (25 µmol) dissolved in 50 µL of dry DMSO (taken from a stock solution: 2.75 mmol, 526.9 mg in 5.5 mL of dry DMSO). Activation mixtures were shaken at room temperature for 30 minutes. For parallel amide coupling, the DEAE sepharose carrying the hexT-pyrazoline conjugate **hexT-20Y**, **hexT-20-PEG(4)-Y** or hexT-pyrazole conjugate **hexT-21Y** was suspended in 100 µL of dry DMSO and transferred from the 96well filter plate into a 96well deep well plate. After that, the activated carboxylic acids were added, and the reaction mixtures were shaken at room temperature for 16h. Then, the DEAE sepharose was filtered over a 96well filter plate (20 µm), washed subsequently with each 3 x 200 µL of DMSO, distilled water and 10 mM aq. NaAc buffer. After that, the oligonucleotide conjugates were eluted from DEAE sepharose into a receiver plate by shaking with 60 µL of 3 M aq. NaAc buffer (pH= 4.75) for 1 hour. The oligonucleotide conjugates were analyzed by MALDI-MS.

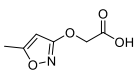
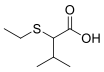
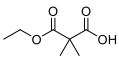
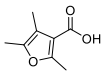
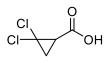
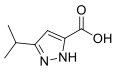
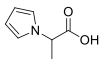
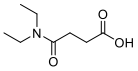
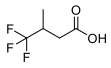
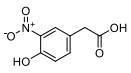
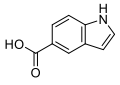
Table S8. MALDI-MS data of hexT-pyrazol(in)e conjugates after amide coupling with carboxylic acids ***1*-*106***.

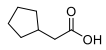
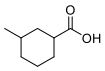
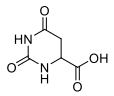
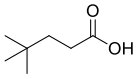
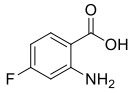
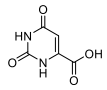
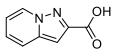
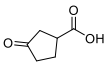
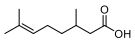
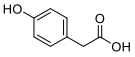
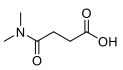
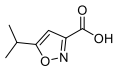


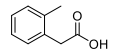
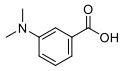
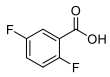
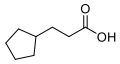
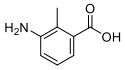
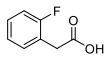
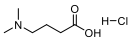
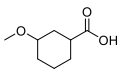
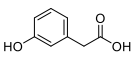
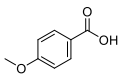
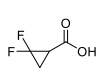
No.	structure	estimated conversion[a]	mass calc.	mass found[b]
1		-	2513.9	starting material
2		> 90 %	2518.9	2520.5
3		> 90 %	2512.0	2512.2
4		> 90 %	2416.8	2417.0[d]
5		> 90 %	2515.0	2516.2
6		> 90 %	2511.9	2514.6
7		> 90 %	2496.9	2497.0
8		-	2460.9	starting material
9		80 %	2520.3	2519.9
10		> 90 %	2515.3	2517.9
11		-	2501.3	starting material
12		> 90 %	2503.4	2505.6

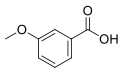
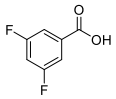
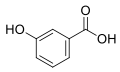
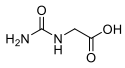
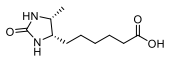
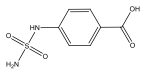
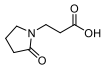
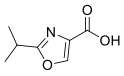
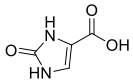
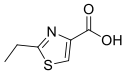
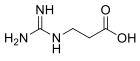
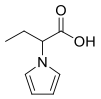
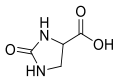
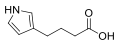
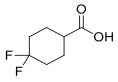
No.	structure	estimated conversion[a]	mass calc.	mass found[b]
13		> 90 %	2511.4	2512.8
14		-	2527.8	starting material
15		70 %	2493.3	2494.9
16		-	2513.9	starting material
17		> 90 %	2514.0	2515.4
18		70 %	2518.9	2520.5
19		> 90 %	2514.0	2515.8
20		> 90 %	2487.9	2489.0
21		> 90 %	2513.9	2515.0
22		> 90 %	2489.8	2490.6
23		> 90 %	2485.9	2487.6
24		80 %	2520.9	2523.2
25		> 90 %	2515.3	2516.5
26		-	2529.9	starting material
27		> 90 %	2519.9	2521.4

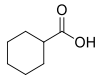
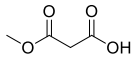
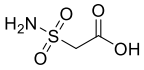
No.	structure	estimated conversion[a]	mass calc.	mass found[b]
28		> 90 %	2764.2	2765.4[c]
29		> 90 %	2757.2	2756.4[c]
30		> 90 %	2401.8	2403.0[d]
31		70 %	2416.8	2414.9[d]
32		80 %	2733.2	2734.9[c]
33		80 %	2413.2	2414.6[d]
34		> 90 %	2417.8	2419.9[d]
35		> 90 %	2500.0	2499.3
36		60 %	2497.9	2499.9
37		> 90 %	2735.2	2734.9[c]
38		> 90 %	2459.9	2461.6
39		> 90 %	2757.3	2762.1[c]
40		> 90 %	2500.0	2502.5
41		> 90 %	2718.2	2716.7[c]
42		> 90 %	2731.2	2732.8[c]

No.	structure	estimated conversion[a]	mass calc.	mass found[b]
43		> 90 %	2762.1	2763.2[c]
44		> 90 %	2417.9	2419.0[d]
45		> 90 %	2415.8	2417.2[d]
46		> 90 %	2759.2	2762.2[c]
47		> 90 %	2369.7	2371.2[d]
48		> 90 %	2410.6	2411.8[d]
49		> 90 %	2409.8	2411.1[d]
50		> 90 %	2496.9	2498.2
51		-	2428.8	starting material[d]
52		> 90 %	2510.9	2512.4
53		> 90 %	2513.9	2515.7
54		> 90 %	2511.8	2512.6
55		> 90 %	2519.9	2521.0
56		> 90 %	2802.2	2805.4[c]
57		60 %	2518.9	2520.5

No.	structure	estimated conversion[a]	mass calc.	mass found[b]
58		> 90 %	2485.9	2488.0
59		> 90 %	2500.7	2502.3
60		> 90 %	2493.9	2495.3
61		80 %	2499.9	2502.5
62		> 90 %	2515.9	2517.7
63		> 90 %	2487.9	2489.2
64		> 90 %	2512.9	2514.7
65		10 %	2513.9	2514.4
66		> 90 %	2519.9	2520.5
67		> 90 %	2485.9	2488.4
68		> 90 %	2528.0	2530.8
69		> 90 %	2509.9	2511.4
70		> 90 %	2457.9	2459.6
71		80 %	2502.9	2503.9
72		80 %	2512.9	2515.3

No.	structure	estimated conversion[a]	mass calc.	mass found[b]
73		> 90 %	2507.9	2510.1
74		> 90 %	2522.9	2524.1
75		30 %	2515.9	2518.8
76		> 90 %	2500.0	2501.7
77		> 90 %	2406.8	2407.7[d]
78		> 90 %	2511.9	2513.8
79		> 90 %	2488.9	2489.0
80		> 90 %	2500.0	2501.9
81		80 %	2473.9	2475.8
82		> 90 %	2371.8	2374.9[d]
83		> 90 %	2471.9	2471.5
84		> 90 %	2413.8	2411.3[d]
85		> 90 %	2407.8	2410.6[d]
86		> 90 %	2407.8	2409.2[d]
87		> 90 %	2377.7	2379.5[d]

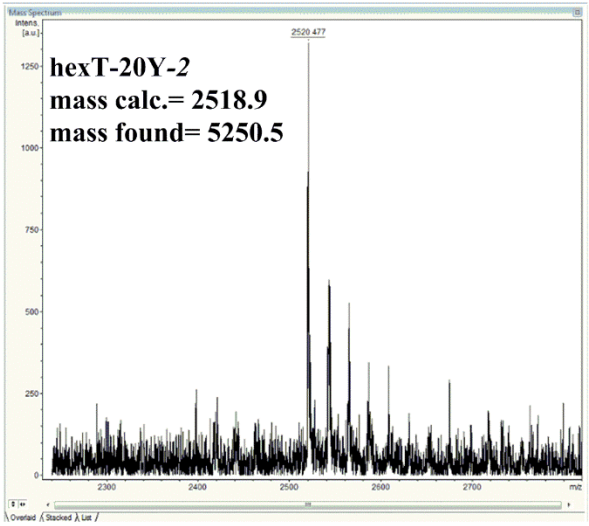
No.	structure	estimated conversion[a]	mass calc.	mass found[b]
88		> 90 %	2509.9	2511.8
89		-	2413.7	starting material[d]
90		> 90 %	2495.9	2497.6
91		> 90 %	2373.7	2375.8[d]
92		> 90 %	2487.9	2489.0
93		> 90 %	2821.3	2820.7[c]
94		> 90 %	2574.0	2575.5
95		> 90 %	2514.9	2518.7
96		> 90 %	2512.9	2512.9
97		> 90 %	2485.8	2485.2
98		> 90 %	2514.9	2515.1
99		> 90 %	2488.9	2488.1
100		> 90 %	2510.9	2511.9
101		> 90 %	2487.8	2490.5
102		> 90 %	2510.9	2512.6
103		> 90 %	2521.9	2522.9

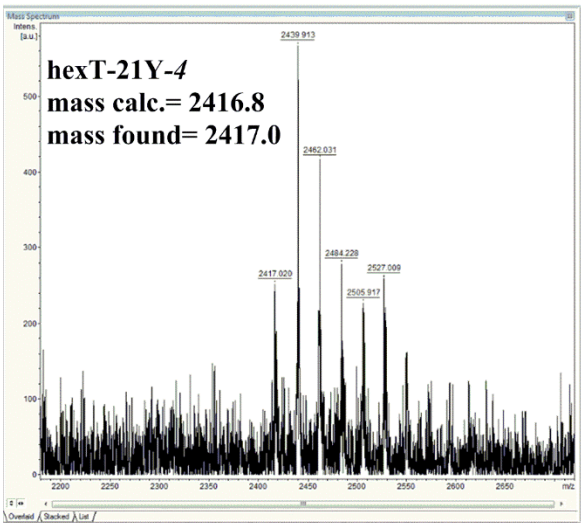
No.	structure	estimated conversion[a]	mass calc.	mass found[b]
104		> 90 %	2485.9	2488.2
105		> 90 %	2475.8	2477.6
106		-	2496.9	starting material

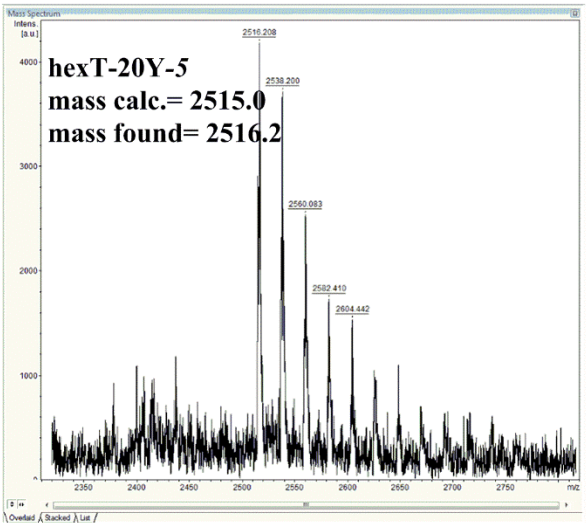
[a] % conversion estimated based on the MALDI-MS data; [b] measured by MALDI-MS;[c] carboxylic acids coupled to the hexT-pyrazoline conjugate **hexT-20-PEG(4)-Y** containing PEG(4) linker,[d] carboxylic acids coupled to the hexT-pyrazole conjugate **hexT-21Y**.

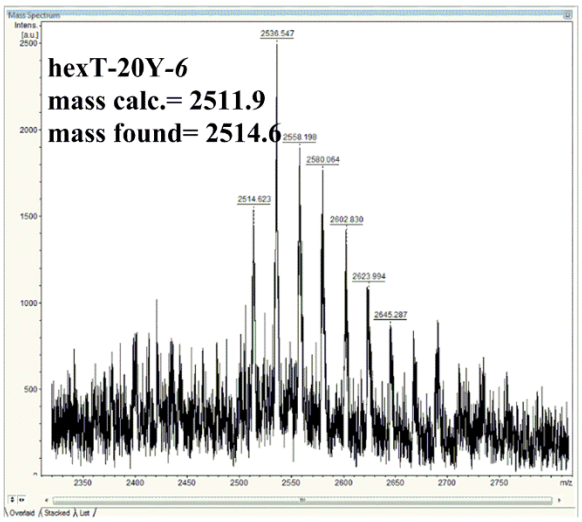
Carboxylic acids **28, 29, 32, 37, 39, 41, 42, 43, 46, 56** and **93** were coupled to the hexT-pyrazoline conjugate **hexT-20-PEG(4)-Y** containing a PEG(4) linker serving as spacer between hexT and pyrazoline scaffold, while carboxylic acids **4, 30, 31, 33, 34, 44, 45, 47, 48, 49, 51, 77, 82, 84, 85, 86, 87, 89** and **91** were coupled to the hexT-pyrazole conjugate **hexT-21Y**. All other carboxylic acids (76) were coupled to the hexT-pyrazoline conjugate **hexT-20Y**.

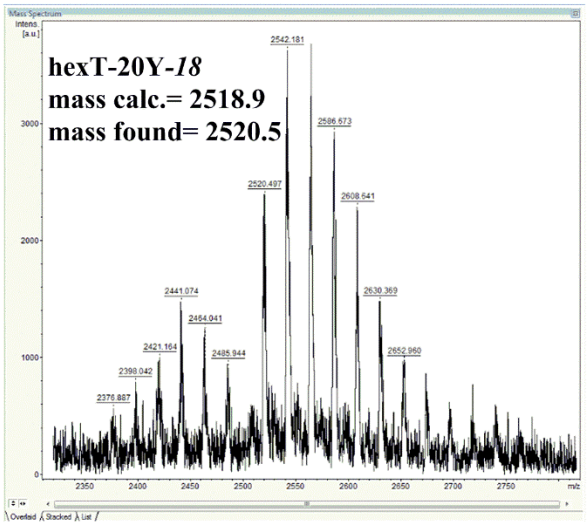
MALDI-MS spectra of 20 representative amide coupling products

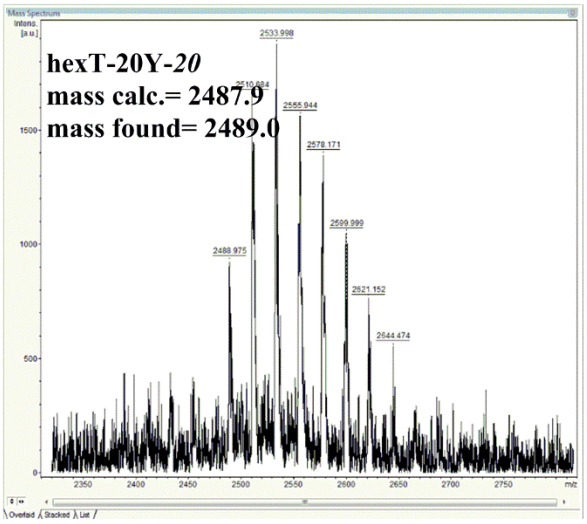


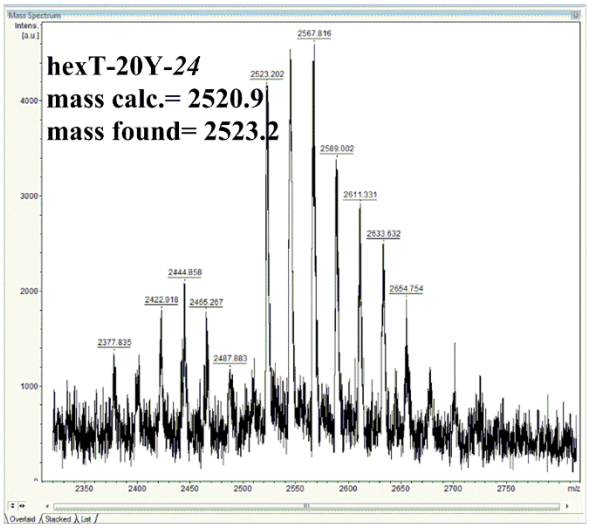


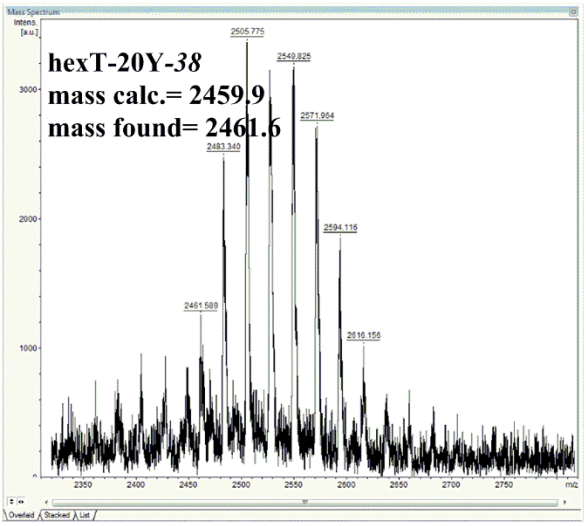


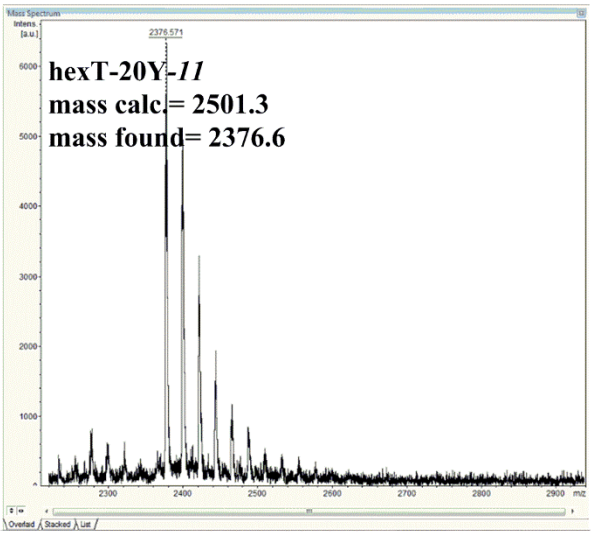


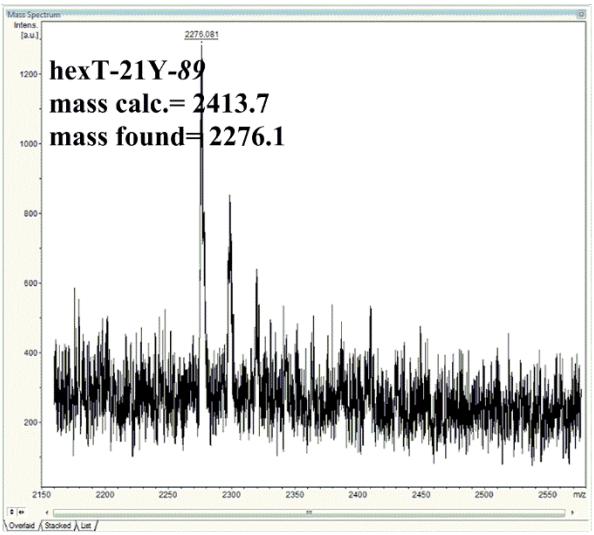

hexT-20Y-*18*
mass calc.= 2518.9
mass found= 2520.5

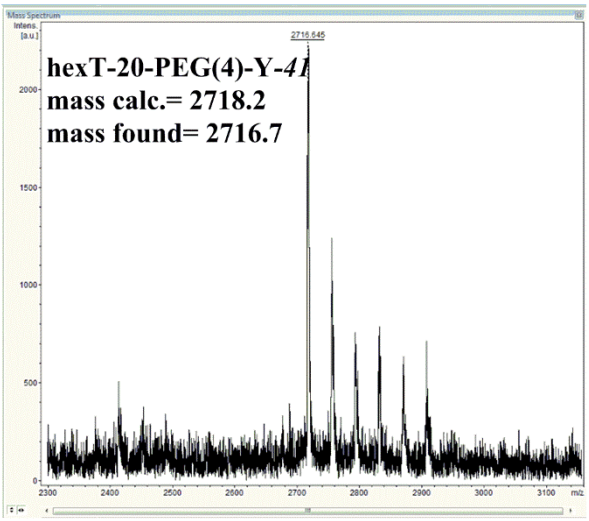

hexT-20Y-*20*
mass calc.= 2487.9
mass found= 2489.0

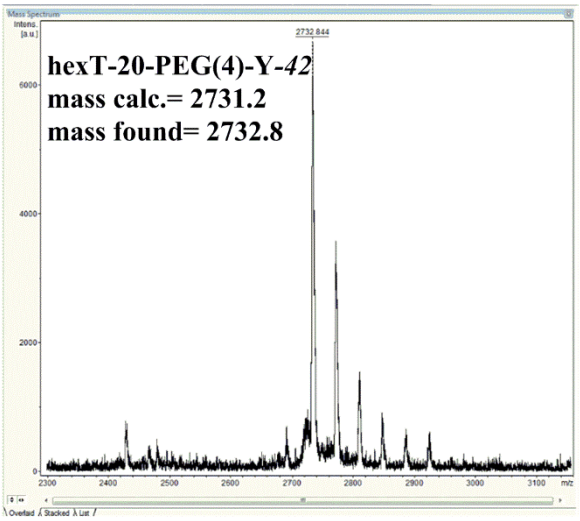

hexT-20Y-*24*
mass calc.= 2520.9
mass found= 2523.2

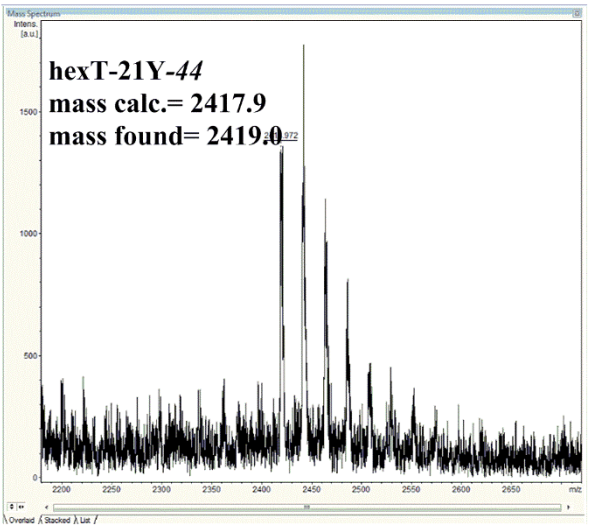

hexT-20Y-*38*
mass calc.= 2459.9
mass found= 2461.6

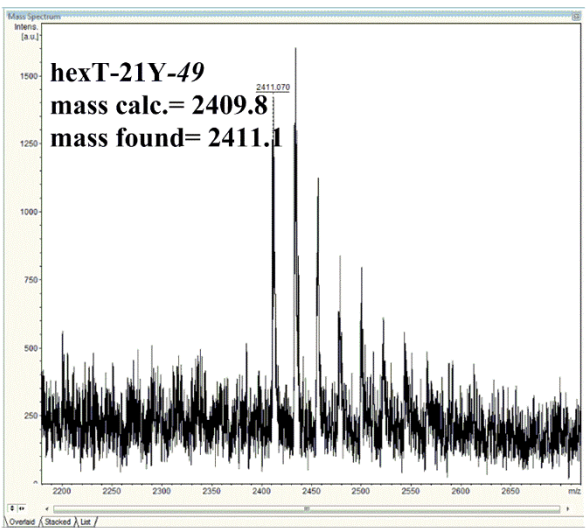

hexT-20Y-*11*
mass calc.= 2501.3
mass found= 2376.6

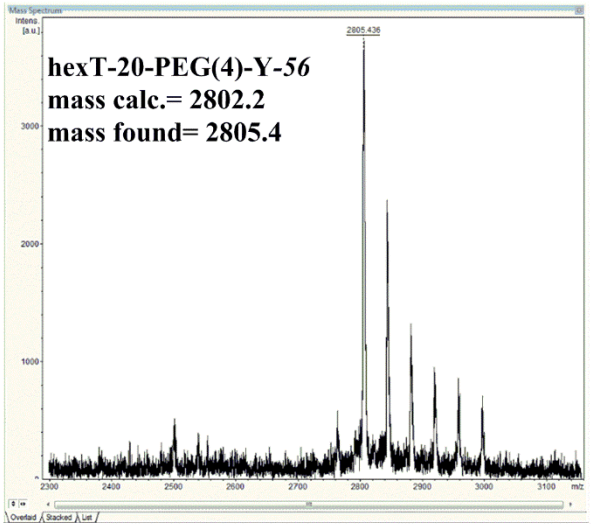

hexT-21Y-*89*
mass calc.= 2413.7
mass found= 2276.1

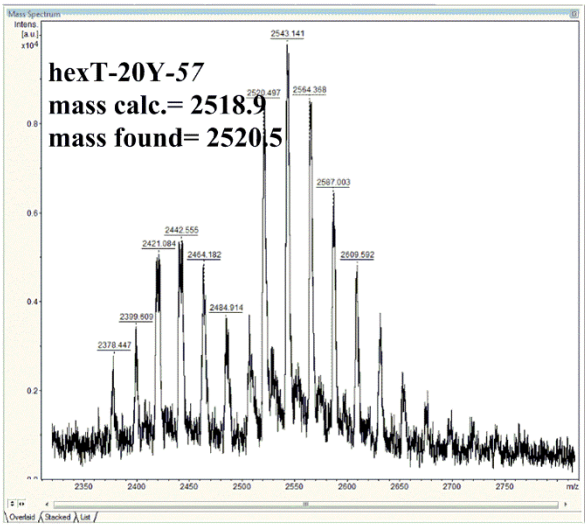

hexT-20-PEG(4)-Y-41
mass calc.= 2718.2
mass found= 2716.7

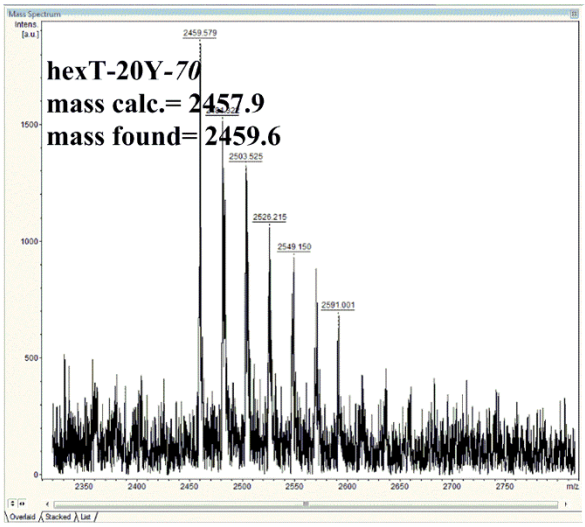

hexT-20-PEG(4)-Y-42
mass calc.= 2731.2
mass found= 2732.8

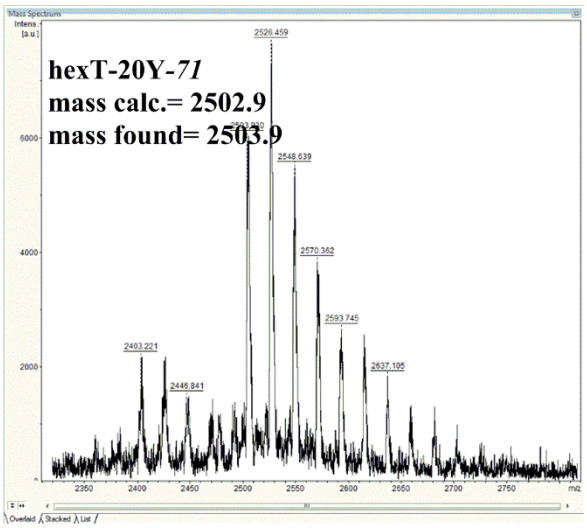

hexT-21Y-44
mass calc.= 2417.9
mass found= 2419.0

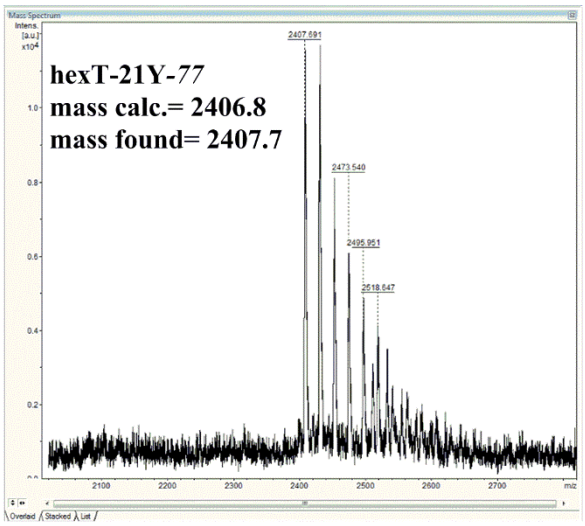

hexT-21Y-49
mass calc.= 2409.8
mass found= 2411.1

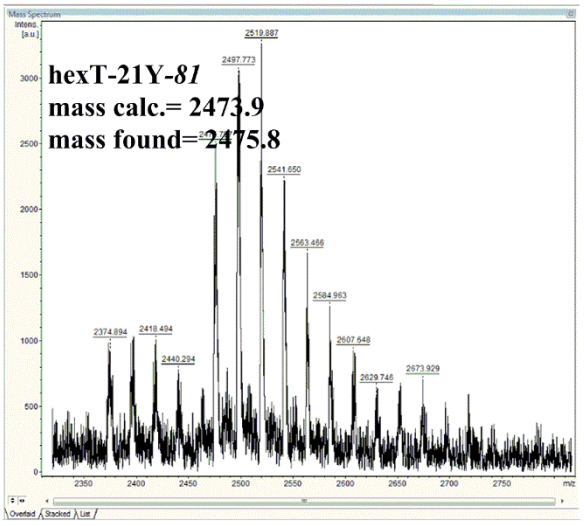

hexT-20-PEG(4)-Y-*56*
mass calc.= 2802.2
mass found= 2805.4
2805.436

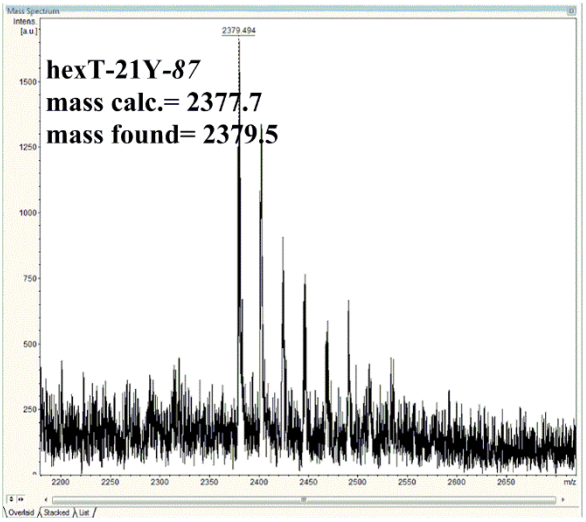

hexT-20Y-*57*
mass calc.= 2518.9
mass found= 2520.5
2543.141
2520.497
2564.368
2587.003
2442.555
2421.084
2464.182
2399.609
2484.914
2378.447
2699.592

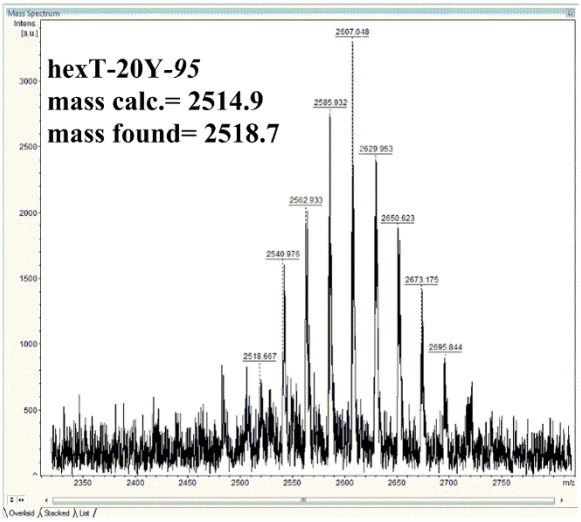

hexT-20Y-*70*
mass calc.= 2457.9
mass found= 2459.6
2459.579
2503.525
2526.215
2549.150
2591.001


hexT-20Y-*71*
mass calc.= 2502.9
mass found= 2503.9
2526.459
2548.639
2570.362
2593.745
2403.221
2446.841
2637.105


hexT-21Y-77
mass calc.= 2406.8
mass found= 2407.7
2407.691
2473.540
2495.951
2518.647


hexT-21Y-81
mass calc.= 2473.9
mass found= 2475.8
2519.887
2497.773
2541.650
2563.466
2584.963
2607.548
2629.746
2673.929
2374.894
2418.494
2440.294


hexT-21Y-87
mass calc.= 2377.7
mass found= 2379.5
2379.494


hexT-20Y-95
mass calc.= 2514.9
mass found= 2518.7
2607.048
2585.932
2629.953
2562.933
2650.623
2540.976
2673.175
2695.844
2518.667

7. tiDEL synthesis

7.1. Synthesis of the oligothymidine-initiated DNA-encoded library (tiDEL)

In total, 151 HPLC-purified hexT-conjugates (71 hexT-pyrazol(in)es **hexT-20/21** and 80 hexT-β-carbolines; **Table S3, S6** and **S7**) depicted as oligonucleotide **I** in **Figure S1** were placed in two 96well plates and annealed with enzymatically phosphorylated oligonucleotides ***II/II´*** and ***III/III´*** at 85°C for 10 min and slowly cooled down to room temperature (25°C) (ligation scheme: Figure S1, sequences: Tables S1 and S2). The total reaction volume was 20 µL. This contained 40 pmol of each DNA oligonucleotide. Ligations were carried out at 25°C for overnight and stopped by heat inactivation at 75°C for 10 minutes. Control reactions were performed without T4 DNA ligase. To control the success of the ligation reaction an aliquot of 0.5 µL was taken from each reaction, pooled, and analyzed by agarose gel electrophoresis (Figure S27). All duplex DNA strands ***I-II-III/II´-III´*** corresponding to encoded hexT-conjugates **11A-11CB, 20D-20CD** and **21D-21CF** were pooled and precipitated. The pellet was dissolved in 540 µL of IDTE buffer and half of the total volume was distributed in 96 wells of a 96well plate in a way that each well position contained 20 pmol of pooled and encoded hexT-conjugates **11A-11CB, 20D-20CD** and **21D-21CF** (96 × 2.8 µL, duplex ***I-II-III/II´-III´*** in Figure S1). Then, the free 5'-end of the duplex DNA ***I-II-III/II´-III´*** and 96 oligonucleotides ***IV/IV´*** that contained the code for the carboxylic acid building block and the reverse primer sequence were phosphorylated with PNK**.** For ligation, the duplex DNA sequences ***I-II-III/II´-III´***, and ***IV/IV'*** (Figure S1) were annealed at 85°C for 10 minutes and slowly cooled down to room temperature. Control reactions were performed without T4 DNA ligase. After the ligation, the duplex DNA ***I-II-III-IV/II´-III´-IV´*** (83mer) encoded hexT-conjugates **11A-11CB, 20D-20CD, 21D-21CF** were transferred to a 96well filter plate containing DEAE anion exchange resin (96 x 80 µL) that was prepared as described above. In parallel, 96 carboxylic acids (Table S8) were activated as described above. For parallel amide coupling, the DEAE sepharose from each well carrying the pooled and encoded DNA small molecule conjugates **hexT-11A-11CB, hexT-20D-20CD**, and **hexT-21D-21CF** was suspended in 100 µL of dry DMSO and transported from the 96well filter plate into 96 glass vials. Amide coupling reactions were performed at room temperature for overnight. Then, the DEAE sepharose was transferred to a 96well filter plate, and washed subsequently with each 3 x 200 µL of DMSO, distilled 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 aq. NaAc buffer (pH= 4.75) for 1 hour and filtered directly from the filter plate into a 96-deep well plate. The products of the amide coupling were pooled and precipitated twice as described above.

7.2. Analysis of the tiDEL

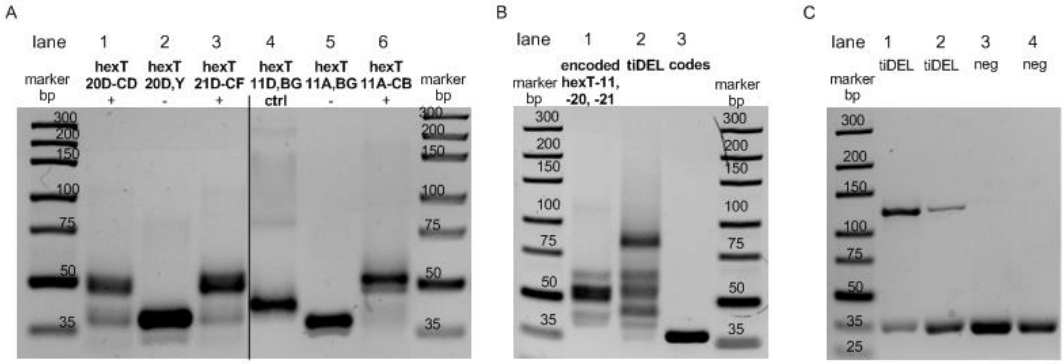


Figure S27. Analysis of the tiDEL, for the encoding scheme see Figure S1. A) Gel electrophoresis of the ligation of hexT-conjugates to DNA duplexes ***II***/***II´*** and ***III***/***III´*** *in one pot* yielding the duplex ***I-II-III/II´-III´***; lane 1: ligation of duplexes ***II***/***II´*** and ***III***/***III´*** to pyrazolines **hexT-20** (Table S6) yielding the duplex **hexT-20-*II-III/II´-III´***; lane 2: negative control for two compounds w/o T4 ligase; lane 3: ligation of duplexes ***II***/***II´*** and ***III***/***III´*** to pyrazoles **hexT-21** (Table S7) yielding the duplex **hexT-21-*II-III/II´-III´***; lane 4: ligation of two hexT-conjugates to duplex ***II***/***II´***; lane 5: negative control for two compounds w/o T4 ligase; lane 6: ligation of duplexes ***II***/***II´*** and ***III***/***III´*** to β-carbolines **hexT-11** (Table S3) yielding the duplex **hexT-11-*II-III/II´-III´***; B) ligation of DNA duplex sequences ***I-II-III/II´-III´*** to the DNA duplex ***IV***/***IV´*** to encode carboxylic acid building blocks (Table S8); lane 1: gel electrophoresis of the pool of all encoded hexT-conjugates (DNA duplex sequences ***I-II-III/II´-III´***); lane 2: gel electrophoresis of the ligation of DNA duplex sequences ***I-II-III/II´-III´*** to the DNA duplexes ***IV***/***IV´*** to encode carboxylic acid building blocks *1-96* (Table S8); lane 3: gel electrophoresis of a duplex sequence ***IV***/***IV´;*** C) PCR amplification of the pooled tiDEL with barcoded primers (forward primer: 5'- TAC ACG ACG CTC TTC CGA TCT CGATGT AGG TCG GTG TGA ACG GAT TTG; reverse primer: 5'- CAG ACG TGT GCT CTT CCG ATC CGATGT TGT AGA CCA TGT AGT TGA GGT CA);[4] lane 1: amplification of 10 pM of the tiDEL; lane 2: amplification of 100 fM of the tiDEL; lane 3: negative control, amplification of the primers without template; lane 4: negative control, gel electrophoresis of primers.

PCR protocol:

The final volume of each PCR reaction was 40 µL and contained 4 µL of 10 x PCR Rxn buffer, 1 µM of each primer, 3 mM of $MgCl_2$, 0.625 mM of each dNTP (dATP, dGTP, dTTP, and dCTP, corresponding to 2.5 mM of the mixture of dNTPs), and 10 µL of the template DNA. For amplification of the template DNA five units (1 µL) of Taq DNA polymerase were used. The PCR program started with predenaturation at 95°C for one minute, followed by denaturation for 15 s at 95°C, annealing for 15 seconds at 56°C, and elongation for 30 seconds at 72°C. After 25 cycles in total, the time for elongation was prolonged to seven minutes.

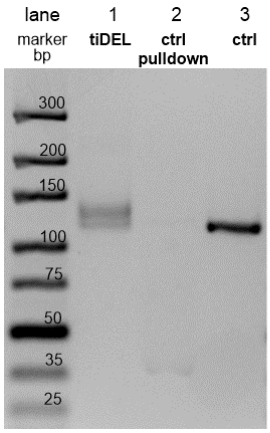


Figure S28. Streptavidin pull-down of the tiDEL. The streptavidin pulldown was performed with 0.1 pmol of the tiDEL, corresponding to ca. 1 fmol of streptavidin-binding desthiobiotin-conjugates (carboxylic acid building block *96*, Table S8) according to a previously published procedure.[5] The DNA was amplified by PCR as described above. Lane 1: gel electrophoresis of the streptavidin pulldown of the tiDEL; lane 2: gel electrophoresis of the streptavidin pulldown of 10 fmol of a 69mer duplex DNA corresponding to duplex ***I-II-III-IV/II´-III´-IV´*** (Table S1) serving as negative control. Only a faint trace of this DNA is visible on the gel after the streptavidin pull-down; lane 3: PCR amplification of the negative control 69mer DNA (10 fmol total amount).

8. Synthesis of reference molecules and intermediates

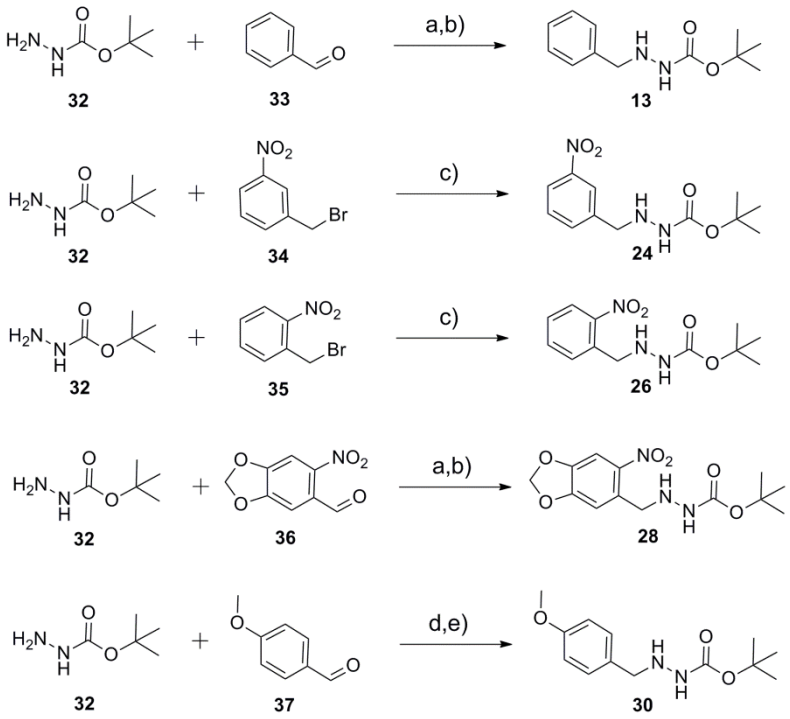
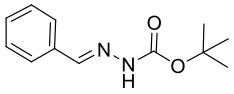


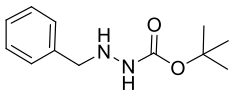
Figure S29. Synthesis of hydrazides **13-30**. Reagents and conditions: a) dry THF, room temperature, 18 h; b) dry THF (compound **13**) or dry MeOH (compound **28**), $NaBH_3CN$, room temperature, 18 h; c) K_2CO_3, dry DMF, 90°C, 30 min (compound **24**) or two h (compound **26**); d) dry MeOH, 50°C, 18 h; e) 10 % Pd/C, room temperature, 1.5 h.

tert-Butyl-2-benzylidenehydrazine-1-carboxylate 13a[2]



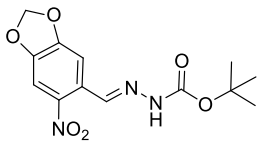
To a solution of *tert*-butyl carbazate **32** (1 g, 7.57 mmol, 1 eq.) in dry THF (10 mL) benzaldehyde **33** was added (772 µL, 7.57 mmol, 1 eq.). The reaction mixture was stirred at room temperature for overnight. The reaction was concentrated *in vacuo* to a residue and the crude product was purified by column chromatography (solvent system: CH_2Cl_2/ ethyl acetate 100:0 to 90:10) to provide **13a** (892 mg, 53 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (s, 1H), 7.85 (br. s, 1H), 7.65-7.68 (m, 2H), 7.33-7.36 (m, 3H), 1.53 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.0, 143.7, 134.0, 129.9, 128.7, 127.3, 81.6, 28.4. LC-MS (ESI) *m/z* Calcd. for $[C_{12}H_{17}N_2O_2, M+H]^+$: 221.13, found 221.20. Purity (HPLC): 90 %.

tert-Butyl 2-benzylhydrazine-1-carboxylate 13[2]



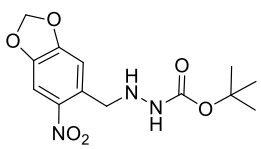
To a solution of *tert*-butyl-2-benzylidenehydrazine-1-carboxylate **13a** (892 mg, 4.05 mmol, 1 eq.) dissolved in dry THF (9 mL), $NaBH_3CN$ (636.6 mg, 10.12 mmol, 2.5 eq.) was slowly added at 0°C. Then acetic acid (6.07 mL, 106.1 mmol, 26.2 eq.) was added and the reaction mixture was allowed to warm to room temperature and stirred for overnight. Next day, an additional amount of $NaBH_3CN$ (636.6 mg, 10.12 mmol, 2.5 eq.) was added and the stirring was prolonged for one day. The crude product was extracted with ethyl acetate (3 x 30 mL). Organic layers were combined, washed with sat. aq. $NaHCO_3$ solution (100 mL) and brine (100 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product obtained as white solid was dissolved in methanol (8 mL) and 1M NaOH solution (8 mL) and stirred at room temperature for 2 days. After that, methanol was removed under reduced pressure and extraction with ethyl acetate (3 x 30 mL) was performed. The combined organic layers were washed extensively with brine (3 x 100 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (solvent system: CH_2Cl_2/ ethyl acetate 100:0 to 90:10) to provide **13** (567 mg, 62 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32-7.37 (m, 4H), 7.28-7.30 (m, 1H), 6.17 (br. s, 1H), 4.19 (br. s, 1H), 3.99 (s, 2H), 1.46 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.8, 137.8, 129.1, 128.6, 127.6, 80.6, 55.9, 28.5. LC-MS (ESI) *m/z* Calcd. for $[C_{12}H_{19}N_2O_2, M+H]^+$: 223.14, found 223.16. Purity (HPLC): 95 %.

(E)-_tert_-Butyl 2-((6-nitrobenzo[d][1,3]dioxol-5-yl)methylene)hydrazinecarboxylate 28a[6]



tert-Butyl 2-((6-nitrobenzo[d][1,3]dioxol-5-yl)methylene)hydrazine-1-carboxylate **28a** was synthesized according to the procedure for synthesis of *tert*-butyl-2-benzylidenehydrazine-1-carboxylate **13a** making use of *tert*-butyl carbazate **32** (1 g, 7.57 mmol, 1 eq) and 6-nitro-benzo[d][1,3]dioxole-5-carbaldehyde **36** (1.476 g, 7.57 mmol, 1 eq). Desired product was obtained as a yellow solid (1.635 g, 70 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.44 (s, 1H), 8.25 (s, 1H), 7.63 (s, 1H), 7.50 (s, 1H), 6.14 (s, 2H), 1.53 (s, 9H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.2, 152.3, 149.0, 144.4, 142.7, 126.5, 107.0, 105.3, 103.4, 82.1, 28.4 ppm. LC-MS (ESI) *m/z* calcd. for $[C_{13}H_{15}N_3NaO_6, M+Na]^+$: 332.09, found 331.92. Purity (HPLC): 96 %.

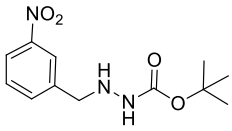
tert-Butyl 2-((6-nitrobenzo[d][1,3]dioxol-5-yl)methyl)hydrazinecarboxylate 28[6]



Compound **28a** (1.03 g, 3.34 mmol, 1 eq.) was dissolved in methanol (21 mL) and $NaBH_3CN$ (1.05 g, 16.72 mmol, 5 eq.) was slowly added. Then one tip of a spoon of bromocresol green as indicator was added to turn the reaction mixture blue (pH > 5.4). To further acidify the solution, 1M pTsOH · H_2O solution in MeOH was added until the solution turned yellow (pH < 3.8). Then the reaction mixture was stirred at room temperature for overnight. The crude product was extracted with ethyl acetate (3 x 50 mL). Organic layers were combined, washed with sat. aq. $NaHCO_3$ solution (100 mL) and brine (100 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (solvent system: DCM/ethyl acetate 100:0 to 90:10) to provide **28** (747 mg, 72 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51 (s, 1H), 7.01 (s, 1H), 6.10 (s, 2H), 6.08 (br. s,

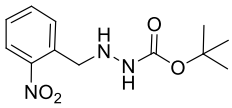
1H), 4.56 (br. s, 1H), 4.19 (s, 2H), 1.41 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.7, 151.8, 147.3, 143.3, 130.9, 110.5, 106.1, 103.0, 80.9, 53.8, 28.4. LC-MS (ESI) *m/z* calcd. for [$C_{13}H_{17}N_3NaO_6$, M+Na]$^+$: 334.10, found 333.99. Purity (HPLC): 97 %.

tert-Butyl 2-(3-nitrobenzyl)hydrazinecarboxylate 24



To a solution of 3-nitrobenzyl bromide **34** (100 mg, 0.46 mmol, 1.0 eq.) in dry DMF (2 mL), *tert*-butyl carbazate **32** (464.9 mg, 3.52 mmol, 7.6 eq.) and potassium carbonate (70.4 mg, 0.51 mmol, 1.1 eq.) were added. The reaction mixture was stirred at 90°C for 30 minutes. After that, solvent was evaporated *in vacuo.* The crude product was extracted with diethyl ether (3 x 30 mL), combined organic layers were washed with brine (90 mL) and dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography (solvent system: DCM/ ethyl acetate 100:0 to 75:25) to provide **24** (18 mg, 14 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.23 (s, 1H), 8.13 (dd, ^3J= 8.3 Hz and ^4J= 1.5 Hz, 1H), 7.68 (d, ^3J= 7.8 Hz, 1H), 7.5 (t, ^3J= 8.1 (x 2) Hz, 1H), 6.07 (br. s, 1H), 4.11 (s, 2H), 1.45 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 157.4, 149.8, 140.3, 135.1, 129.5, 123.8, 122.6, 81.0, 55.0, 28.5. HRMS (ESI) *m/z* calcd. for [$C_{12}H_{18}N_3O_4$, M+H]$^+$: 268.1297, found 268.1293; MS (ESI) *m/z* calcd. for [$C_{12}H_{18}N_3NaO_4$, M+Na]$^+$: 290.1117, found: 290.1112. Purity (HPLC): 98 %.

tert-Butyl 2-(2-nitrobenzyl)hydrazinecarboxylate 26



To a solution of 2-nitrobenzyl bromide **35** (300 mg, 1.39 mmol, 1.0 eq.) in dry DMF (2 mL), *tert*-butyl carbazate **32** (1394.8 mg, 10.55 mmol, 7.6 eq.) and potassium carbonate (211.3 mg, 1.53 mmol, 1.1 eq.) were added. The reaction mixture was stirred at 90°C for 2 hours. After that, solvent was evaporated *in vacuo.* The crude product was extracted with diethyl ether (3 x 50 mL), combined organic layers were washed with brine (150 mL) and dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography (solvent system: CH_2Cl_2/ ethyl acetate 100:0 to 75:25) to provide **26** (340 mg, 91 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95 (d, ^3J= 7.95 Hz, 1H), 7.54-7.58 (m, 2H), 7.42-7.45 (m, 1H), 6.05 (br. s, 1H), 4.53 (br. s, 1H), 4.28 (s, 2H), 1.39 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.8, 149.6, 133.3, 133.1, 131.9, 128.6, 125.0, 80.8, 53.4, 28.4. HRMS (ESI) *m/z* calcd. for [$C_{12}H_{18}N_3O_4$, M+H]$^+$: 268.1297, found 268.1293; MS (ESI) *m/z* calcd. for [$C_{12}H_{18}N_3NaO_4$, M+Na]$^+$: 290.1117, found: 290.1112. Purity (HPLC): 99 %.

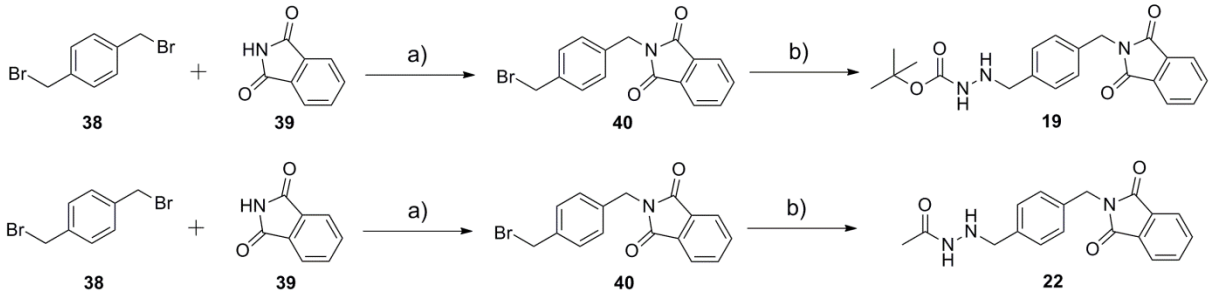
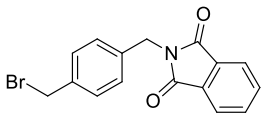


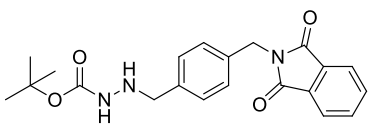
Figure S30. Synthesis of the hydrazides **19** and **22**. Reagents and conditions: a) K_2CO_3 in MeCN, room temperature, 18 hours; b) *tert*-butyl carbazate (**32**) or acetic acid hydrazide (**41**), K_2CO_3, dry CH_2Cl_2, room temperature, 18 hours.

2-(4-(Bromomethyl)benzyl)isoindoline-1,3-dione 40[7]



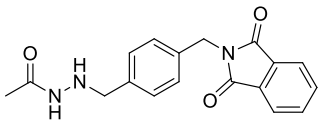
To a solution of phthalimide **39** (1.0 g, 6.8 mmol, 1.0 eq.) in dry acetonitrile (60 mL), 1,4-bis(bromomethyl)benzene **38** (1794 mg, 6.8 mmol, 1.0 eq.), 18-crown-6 (26.9 mg, 1.0 mmol, 0.15 eq.) and potassium carbonate (3.8 g, 27 mmol, 4.0 eq.) were added. The reaction mixture was stirred under argon atmosphere at room temperature for overnight. After that, ethyl acetate (200 mL) was added and the reaction mixture was filtered over filter paper. The filtrate was concentrated *in vacuo* and the crude product was purified by column chromatography (solvent system: hexanes/CH_2Cl_2 100:0 to 90:10) to provide **40** (1.4 g, 63 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83-7.85 (m, 2H), 7.70-7.72 (m, 2H), 7.41 (d, ^3J= 8.2 Hz, 2H), 7.34 (d, ^3J= 8.2 Hz, 2H), 4.85 (s, 2H), 4.44 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.1, 137.5, 136.7, 134.2, 132.1, 129.5, 129.2, 123.5, 41.3, 33.2. LC-MS (ESI) *m/z* calcd. for $[C_{16}H_{13}BrNO_2, M+H]^+$: 330.01, found 330.05. Purity (HPLC): 99 %.

***tert*-Butyl 2-(4-((1,3-dioxoisoindolin-2-yl)methyl)benzyl)hydrazinecarboxylate 19**



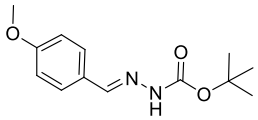
To a solution of **40** (500 mg, 1.51 mmol, 1.0 eq.) in dry DCM (12 mL), *tert*-butyl carbazate **32** (1521 g, 11.51 mmol, 7.6 eq.) and potassium carbonate (229.4 mg, 1.66 mmol, 1.1 eq.) were added. The reaction mixture was stirred under argon atmosphere at room temperature for overnight. After that, ethyl acetate (100 mL) was added and the reaction mixture was filtered over filter paper. The filtrate was concentrated *in vacuo* and the crude product was purified by column chromatography (solvent system: hexanes/ ethyl acetate 100:0 to 75:25) to provide **19** (481 mg, 84 % yield). mp 144-147°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83-7.85 (m, 2H), 7.68-7.72 (m, 2H), 7.40 (d, ^3J= 7.9 Hz, 2H), 7.29 (d, ^3J= 7.95 Hz, 2H), 6.01 (br. s, 1H), 4.83 (s, 2H), 4.12 (br. s, 1H), 3.94 (s, 2H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.18, 156.79, 135.67, 134.14, 132.20, 129.63, 129.43, 128.90, 123.49, 80.67, 55.45, 41.43, 28.46. HRMS (ESI) *m/z* calcd. for $[C_{21}H_{24}N_3O_4, M+H]^+$: 382.1767, found 382.1761; MS (ESI) *m/z* calcd. for $[C_{21}H_{24}N_3NaO_4, M+Na]^+$: 404.1586, found: 404.1579. Purity (HPLC): 95 %.

_N_'-(4-((1,3-Dioxoisoindolin-2-yl)methyl)benzyl)acetohydrazide 22



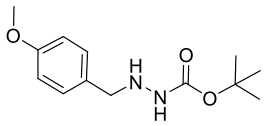
To a solution of **40** (500 mg, 1.51 mmol, 1.0 eq.) in dry DCM (12 mL), acetohydrazide **41** (852.6 mg, 11.51 mmol, 7.6 eq.) and potassium carbonate (229.4 mg, 1.5 mmol, 1.1 eq.) were added. The reaction mixture was stirred under argon atmosphere at room temperature for overnight. After that, ethyl acetate (100 mL) was added and the reaction mixture was filtered over filter paper. The filtrate was concentrated *in vacuo* and the crude product was purified by column chromatography (solvent system: ethyl acetate/ MeOH 100:0 to 95:05) to provide **22** (150 mg, 31 % yield). mp 136-138°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83-7.85 (m, 2H), 7.70-7.72 (m, 2H), 7.39 (d, ^{3}J= 7.8 Hz, 2H), 7.29 (d, ^{3}J= 7.95 Hz, 2H), 6.98 (br. s, 1H), 4.83 (s, 2H), 4.88 (br. s, 1H), 3.92 (s, 2H), 1.89 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.2, 137.5, 135.8, 134.2, 129.3, 128.8, 123.5, 123.2, 55.4, 41.4, 21.3. LC-MS (ESI) *m/z* calcd. for $[C_{18}H_{18}N_3O_3, M+H]^+$: 324.13, found 323.97. Purity (HPLC): 98 %.

***tert*-Butyl (E)-2-(4-methoxybenzylidene)hydrazine-1-carboxylate 30a**[8]



To a solution of *tert*-butyl carbazate **32** (2 g, 15.1 mmol, 1 eq.) in dry methanol (75 mL) anisaldehyde **37** was added (2.06 g, 15.1 mmol, 1 eq.). The reaction mixture was stirred at 50°C for overnight. After that, the reaction was filtered, the precipitate was washed with pentane and dried *in vacuo* to provide **30a** that was used in the next step without further purification (3.63 g, 96 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.80 (s, 1H), 7.61 (d, ^{3}J= 8.8 Hz, 2H), 6.88 (d, ^{3}J= 8.8 Hz, 2H), 3.81 (s, 3H), 1.53 (s, 9H). MS (ESI) *m/z* calcd. for $C_{13}H_{19}N_2O_2$: 251.14; found 251.21 $[M+H]^+$. Purity (HPLC): 97 %. The analytical data is consistent with the literature values.[8]

***tert*-Butyl 2-(4-methoxybenzyl)hydrazine-1-carboxylate 30**[8]



To a solution of *tert*-butyl-2-benzylidenehydrazine-1-carboxylate **30a** (2.0 g, 7.99 mmol, 1 eq.) dissolved in dry methanol (40 mL) was 10 % Pd/C (0.01 eq) added. The reaction mixture was stirred at room temperature under argon atmosphere for 1.5 hour. Then, the reaction mixture was filtered over Celite pad and concentrated *in vacuo*. The crude product was dissolved in hot ethanol and placed for crystallization at -2°C for overnight to provide **30** (1.16 g, 58 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (d, ^{3}J= 8.4 Hz, 2H), 6.88 (d, ^{3}J= 8.8 Hz, 2H), 6.25 (br. s, 1H), 3.96 (s, 2H), 3.81 (s, 3H), 3.33 (br. s, 1H), 1.47 (s, 9H). LC-MS (ESI) *m/z* calcd. for $[C_{13}H_{21}N_2O_3, M+H]^+$: 253.16, found 253.25. Purity (HPLC): 97 %. The analytical data is consistent with the literature values.[8]

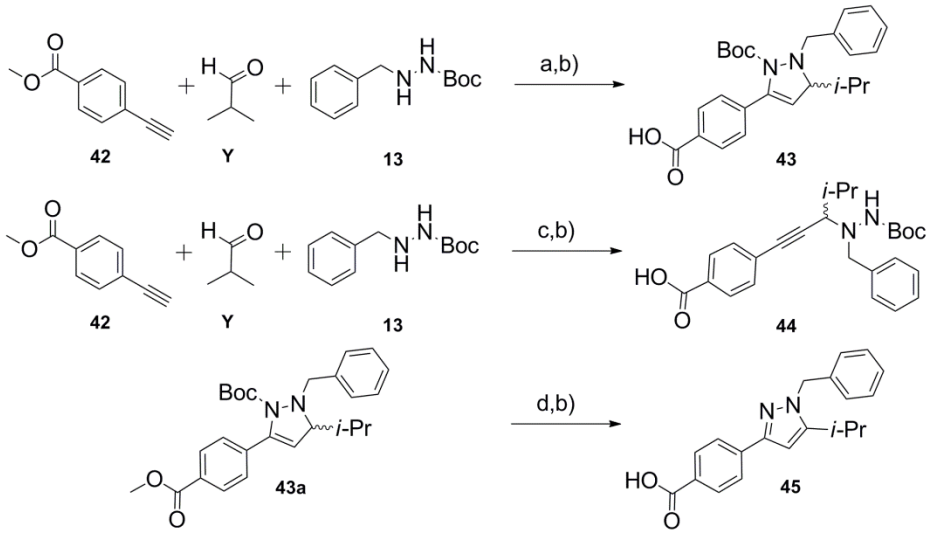
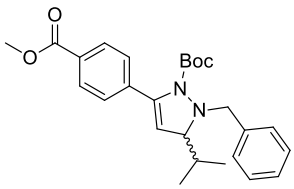


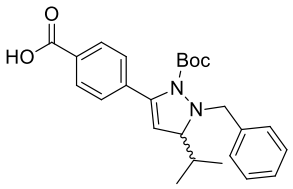
Figure S31. Synthesis of reference molecules **43**, **44** and **45**. Reagents and conditions: a) Au(I)/Ag(I), toluene, 50°C, four hours; b) NaOH in MeOH/H_2O, room temperature, 16 hours; c) AgOTf, dry toluene, 50°C, overnight; d) DDQ, DCM, room temperature, three hours.

***tert*-Butyl 2-benzyl-3-isopropyl-5-(4-(methoxycarbonyl)phenyl)-2,3-dihydro-1*H*-pyrazole-1-carboxylate 43a**[1]



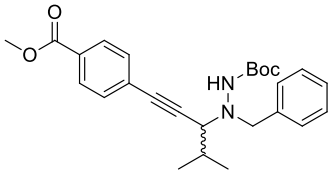
To a solution of methyl 4-ethynylbenzoate **42** (120.0 mg, 0.75 mmol, 1.2 eq.), *tert*-butyl 2-benzylhydrazine-1-carboxylate **13** (138.6 mg, 0.62 mmol, 1 eq.) and isobutyraldehyde **Y** (68.4 µL, 0.75 mmol, 1.2 eq.) in dry toluene (2.7 mL), chloro(triphenylphosphine)gold(I) (PPh_3AuCl, 15.4 mg, 0.03 mmol, 0.05 eq.) and silver trifluoromethanesulfonate (AgOTf, 8.0 mg, 0.03 mmol, 0.05 eq.) were added. The reaction mixture was stirred at 50°C for 4 hours under argon atmosphere. After that, it was filtered over celite pad and concentrated *in vacuo*. The crude product was purified by column chromatography (solvent system: hexanes/ ethyl acetate 100:0 to 90:10) to provide **43a** (262 mg, 96 % yield). mp 84-88°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, ^3J= 8.8 Hz, 2H), 7.45-7.50 (m, 4H), 7.27-7.35 (m, 3H), 5.58 (d, ^3J= 2.9 Hz, 1H), 4.08 (d, ^2J= -12.2 Hz, 1H), 3.92 (s, 3H), 3.75 (d, ^2J= -12.2 Hz, 1H), 3.30 (dd, ^3J= 6.1 Hz and ^3J= 3.2 Hz, 1H), 1.47-1.57 (m, 1H), 1.24 (s, 9H), 0.79 (d, ^3J= 6.8 Hz, 3H), 0.76 (d, ^3J= 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.0, 154.9, 141.9, 138.0, 137.0, 130.6, 129.5, 129.4, 128.1, 127.5, 126.9, 113.0, 81.0, 74.1, 63.0, 52.2, 32.9, 28.0, 18.5, 18.3. HRMS (ESI) *m/z* calcd. for $[C_{26}H_{33}N_2O_4, M+H]^+$: 437.2440, found 437.2433; MS (ESI) *m/z* calcd. for $[C_{26}H_{32}N_2NaO_4, M+Na]^+$: 459.2260, found: 459.2250. Purity (HPLC): 98 %.

4-(1-Benzyl-2-(*tert*-butoxycarbonyl)-5-isopropyl-2,5-dihydro-1*H*-pyrazol-3-yl)benzoic acid 43



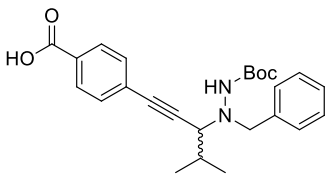
To a solution of **43a** (200 mg, 0.37 mmol, 1 eq.) in dry MeOH (3.8 mL), 2 M aqueous NaOH (3.8 mL) was added. The reaction mixture was stirred at room temperature for 16 h. After that, MeOH was evaporated *in vacuo* and reaction mixture was extracted with DCM (3 x 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, concentrated *in vacuo* and purified by column chromatography (solvent system: CH_2Cl_2/ MeOH 100:0 to 95:5) to provide the compound **43** (67.7 mg, 43 % yield). mp 107-109°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01-8.08 (m, 2H), 7.34-7.43 (m, 4H), 7.21-7.31 (m, 3H), 5.44 (br. s, 1H), 4.04 (d, ^2J= -11.7 Hz, 1H), 3.67 (d, ^2J= -12.2 Hz, 1H), 3.21 (br. s, 1H), 1.41-1.48 (m, 1H), 1.20 (s, 9H), 0.73 (d, ^3J= 6.8 Hz, 3H), 0.69 (d, ^3J= 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 154.8, 142.1, 139.5, 137.0, 130.6, 129.9, 129.9, 128.09, 127.5, 126.6, 104.6, 80.9, 73.8, 62.9, 32.8, 28.0, 18.4, 18.3. HRMS (ESI) *m/z* calcd. for $[C_{25}H_{31}N_2O_4, M+H]^+$: 423.2284, found: 423.2276. Purity (HPLC): 98 %.

***tert*-Butyl 2-benzyl-2-(1-(4-(methoxycarbonyl)phenyl)-4-methylpent-1-yn-3yl)hydrazinecarboxylate 44a**[1]



To a solution of methyl 4-ethynylbenzoate **42** (100.0 mg, 0.62 mmol, 1.2 eq.), *tert*-butyl 2-benzylhydrazine-1-carboxylate **13** (115.6 mg, 0.52 mmol, 1 eq.) and isobutyraldehyde **Y** (57.0 µL, 0.62 mmol, 1.2 eq.) in dry toluene (2.6 mL), silver trifluoromethanesulfonate (AgOTf, 2.7 mg, 0.01 mmol, 0.02 eq.) was added. The reaction mixture was stirred at 50°C for overnight under argon atmosphere. After that, it was filtered over celite pad and concentrated *in vacuo*. The crude product was purified by column chromatography (solvent system: hexanes/ ethyl acetate 100:0 to 90:10) to provide **44a** (62 mg, 27 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02 (d, ^3J= 8.2 Hz, 2H), 7.54 (d, ^3J= 7.9 Hz, 2H), 7.39-7.50 (m, 2H), 7.27-7.34 (m, 3H), 3.93 (s, 3H), 3.25 (br. s, 1H), 1.86-1.93 (m, 1H), 1.39 (s, 9H), 1.13 (d, ^3J= 6.4 Hz, 3H), 1.05 (d, ^3J= 6.7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.6, 166.4, 132.6, 131.9, 129.7, 129.7, 128.3, 127.7, 126.2, 100.0, 82.5**,** 82.0, 63.8, 52.5, 52.4, 31.2, 28.3, 20.3, 20.2. HRMS (ESI) *m/z* calcd. for $[C_{26}H_{33}N_2O_4, M+H]^+$: 437.2440, found 437.2434; MS (ESI) *m/z* calcd. for $[C_{26}H_{32}N_2NaO_4, M+Na]^+$: 459.2260, found: 459.2252. Purity (HPLC): 95 %.

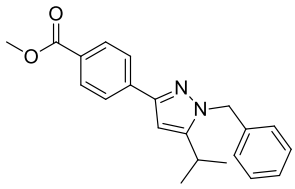
4-(3-(2-(*tert*-Butoxycarbonyl)-1-(2-nitrobenzyl)hydrazinyl)-4-methylpent-1-yn-1-yl)benzoic acid 44



To a solution of **44a** (37 mg, 0.08 mmol, 1 eq) in dry MeOH (0.8 mL), 2 M aqueous NaOH (0.8 mL) was added. The reaction mixture was stirred at room temperature for 16 h. After that, MeOH was evaporated *in vacuo* and reaction mixture was extracted with DCM (3 x 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, concentrated *in vacuo* and purified by column chromatography (solvent system: CH_2Cl_2/ MeOH 100:0 to 95:5) to provide the compound **44** (23.6 mg, 66 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65-7.74 (m, 2H), 7.47 (d, ^3J= 6.8 Hz, 2H), 7.28-7.43 (m, 5H), 4.00-4.04 (m, 1H), 3.91-3.98 (m, 1H), 3.16-3.25

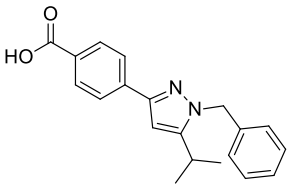
(m, 1H), 1.83-1.93 (m, 1H), 1.39 (s, 9H), 1.14 (d, ^{3}J= 6.4 Hz, 3H), 1.05 (d, ^{3}J= 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.0, 166.8, 132.4, 131.9, 131.8, 130.0, 129.8, 128.3, 127.6, 100.1, 82.4, 81.4, 65.0, 55.7, 31.5, 28.3, 20.3. HRMS (ESI) *m/z* calcd. for [$C_{25}H_{31}N_2O_4$, M+H]$^+$: 423.2284, found: 423.2276. Purity (HPLC): 99 %.

Methyl 4-(1-benzyl-5-isopropyl-1H-pyrazol-3-yl)benzoate 45a



To a solution of pyrazoline **43a** (150.0 mg, 0.34 mmol, 1.0 eq.) in dry dichloromethane (1.6 mL), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 115.8 mg, 0.51 mmol, 1.5 eq.) was added. The reaction mixture was stirred at room temperature for 3 hours. After that, reaction was quenched by addition of sat. aq. $NaHCO_3$ solution (0.7 mL). Then, the reaction mixture was extracted with DCM (3 x 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, concentrated *in vacuo* and purified by column chromatography (solvent system: hexanes/ ethyl acetate 100:0 to 80:20) to provide **45a** (109 mg, 95 % yield). mp 92-94°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.98 (d, ^{3}J= 8.2 Hz, 2H), 7.82 (d, ^{3}J= 8.2 Hz, 2H), 7.22-7.25 (m, 2H), 7.17-7.19 (m, 1H), 7.03 (d, ^{3}J= 7.6 Hz, 2H), 6.41 (s, 1H), 5.32 (s, 2H), 3.85 (s, 3H), 2.77-2.85 (m, 1H), 1.12 (d, ^{3}J= 6.7 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.3, 151.6, 149.4, 138.3, 137.4, 130.1, 128.8, 127.7, 126.6, 125.4, 100.5, 53.2, 51.2, 25.7, 23.0, 18.8, 18.5. HRMS (ESI) *m/z* calcd. for [$C_{21}H_{23}N_2O_2$, M+H]$^+$: 335.1760, found: 335.1753. Purity (HPLC): 99 %.

4-(1-Benzyl-5-isopropyl-1H-pyrazol-3-yl)benzoic acid 45



To a solution of pyrazole **45a** (80 mg, 0.24 mmol, 1 eq.) in dry MeOH (2.5 mL), 2 M aqueous NaOH (2.5 mL) was added. The reaction mixture was stirred at room temperature for 16 h. After that, MeOH was evaporated *in vacuo* and reaction mixture was extracted with DCM (3 x 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, concentrated *in vacuo* and purified by column chromatography (solvent system: CH_2Cl_2/ MeOH 100:0 to 95:5) to provide the compound **45** (54.5 mg, 71 % yield). mp 167-169°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07 (d, ^{3}J= 8.2 Hz, 2H), 7.86 (d, ^{3}J= 8.2 Hz, 2H), 7.24-7.17 (m, 3H), 7.05 (d, ^{3}J= 7.6 Hz, 2H), 6.43 (s, 1H), 5.35 (s, 2H), 2.78-2.86 (m, 1H), 1.13 (d, ^{3}J= 7.0 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.8, 155.3, 150.3, 141.9, 138.6, 133.5, 132.6, 132.5, 130.1, 128.7, 128.5, 126.8, 124.6, 114.4, 81.4, 76.6, 59.7, 33.0, 27.9, 18.8, 18.5. HRMS (ESI) *m/z* calcd. for [$C_{20}H_{21}N_2O_2$, M+H]$^+$: 321.1603, found: 321.1618. Purity (HPLC): 99 %.

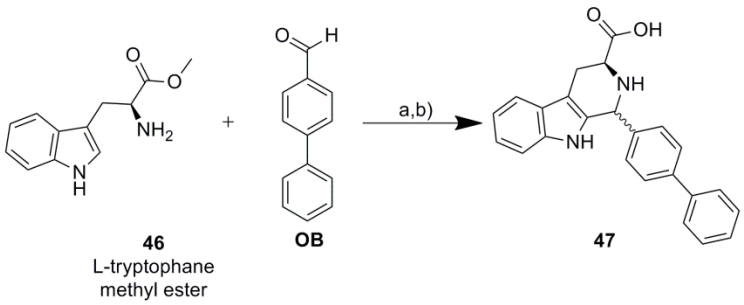
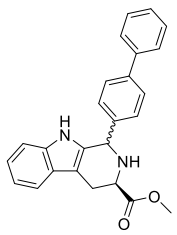


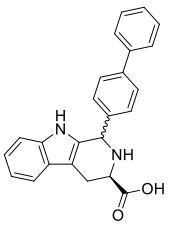
Figure S32. Synthesis of the β-carboline **47**. Reagents and conditions: a) 5 % TFA in CH_2Cl_2, room temperature, 18 hours; b) LiOH (H_2O/THF, 1:1), room temperature, 18 hours.

Methyl (R)-1-([1,1'-biphenyl]-4-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-3-carboxylate 47a



In a 50 mL two-necked round-bottomed flask equipped with a magnetic bar and an argon-filled balloon, L-tryptophan methyl ester hydrochloride **46** (637 mg, 2.5 mmol, 1 eq.) was suspended in dry CH_2Cl_2 (10 mL). The solution was cooled to 0°C in an ice bath, and biphenyl-4-carboxaldehyde **BO** (911 mg, 5 mmol, 2 eq.) was added. To this solution TFA (0.5 mL) was added dropwise and the reaction was stirred at room temperature for 18 hours under argon atmosphere. After completion of the reaction, the reaction mixture was basified with dilute NH_4OH solution and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was washed with water, brine, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using a gradient of CH_2Cl_2/MeOH (100:0 to 95:5) to yield the β-carboline **47a** as a mixture of two diastereomers in a ratio of 1:1 according to NMR (gum, 231 mg, 24 % yield). ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.34 (br. s, 1H), 7.62 (d, ^3J= 7.68 Hz, 1H), 7.61 (d, ^3J= 8.34 Hz, 2H), 7.57 (d, ^3J= 7.68 Hz, 1H) 7.55 (d, ^3J= 8.34 Hz, 2H), 7.44 (t, ^3J= 7.86 Hz, 2H), 7.35 (t, ^3J = 7.38 Hz, 2H), 7.31 (d, ^3J = 8.04 Hz, 2H), 7.20 (d, ^3J = 7.86 Hz, 2H), 7.14-7.07 (m, 4H) (overlap due to diastereomerism), 6.22 (s, 1H), 5.30 (br. s, 1H), 3.88 (dd, ^3J = 10.08 Hz, 1H), 3.84 (s, 3H), 3.79 (dd, ^3J = 10.08 Hz, 1H), 3.74 (s, 3H), 3.39 (dd, ^3J = 10.08 Hz, ^2J = 2.94 Hz, 1H), 3.08 (dd, ^3J = 14.52 Hz, ^2J = 2.4 Hz, 1H). ^{13}C NMR (600 MHz, $CDCl_3$): δ 175.3, 139.5, 137.7, 136.6, 135.2, 134.5, 127.8, 127.8, 127.6, 126.6, 126.3, 126.1, 126.0, 120.5, 117.9, 117.2, 109.7, 54.0, 51.4, 40.2, 28.4. MS (ESI) *m*/*z* calcd. for $C_{25}H_{22}N_2O_2$: 382.17, found 383.51 $[M+H]^+$. Purity (HPLC): 97 %.

1-(R,S)-([1,1'-Biphenyl]-4-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-3-(R)-carboxylic acid 47



In a 50 mL round-bottomed flask equipped with a magnetic bar, a solution of ester **47a** (200 mg, 0.52 mmol, 1 eq.) in tetrahydrofuran (0.5 mL) and water (0.5 mL) was cooled to 0°C. To this mixture was added lithium hydroxide monohydrate (87 mg, 2.08 mmol, 4 eq.), and it was allowed to slowly warm up to room temperature overnight. Then it was neutralized to pH 7 with hydrogen chloride (1 M in water, 30 mL) and cooled in an ice bath to obtain a yellow precipitate. The precipitate was filtered off and the residue was resolved in methanol and then concentrated. The filtrate was extracted with CH_2Cl_2 (2 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by C_{18}-reverse phase preparative HPLC (gradient of H_2O and MeOH) to give **47** as a yellow powder (135 mg, 85 % yield). mp 208°C (decomposition); ^{1}H

NMR (500 MHz, $CDCl_3$): δ 10.87 (s, 1H), 7.62 (d, ^3J= 7.5 Hz, 1H), 7.55 (d, ^3J= 6.8 Hz, 2H), 7.51 (t, ^3J= 7.4 Hz, 2H), 7.49 (d, ^3J = 7.7 Hz, 1H), 7.46 (t, ^3J = 6.8 Hz, 1H), 7.35 (d, ^3J = 7.4 Hz, 2H), 7.28 (t, ^3J = 7.6 Hz, 1H), 7.02 (dt, ^3J = 7.1 Hz and ^4J= 1.0 Hz, 1H), 7.06 (t, ^3J = 6.3 Hz, 1H), 6.31 (d, ^3J = 7.6 Hz, 1H), 5.58 (s, 1H), 3.96 (t, ^3J = 6.9 Hz, 1H), 3.03 (dd, ^2J = 10.5 Hz and ^3J= 2.8 Hz, 1H), 2.75 (dd, ^2J = 10.5 Hz and ^3J= 4.1 Hz, 1H), 1.89 (br. s, 1H). ^{13}C NMR (600 MHz, $CDCl_3$): δ 175.3, 139.5, 137.7, 136.6, 135.2, 134.5, 127.8, 127.8, 127.6, 126.6, 126.3, 126.1, 126.0, 120.5, 117.9, 117.2, 109.7, 59.2, 56.4, 28.4. MS (ESI) *m/z* calcd. for $C_{24}H_{20}N_2O_2$: 368.43, found: 369.20 $[M+H]^+$. Purity (HPLC): 99 %.

9. Literature

1 Y. Suzuki, S. Naoe, S. Oishi, N. Fujii and H. Ohno, *Org. Lett.*, 2012, **14**, 326–329.

2 B. Wagner, W. Hiller, H. Ohno and N. Krause, *Org. Biomol. Chem.*, 2016, **14**, 1579–1583.

3 D. R. Halpin, J. a Lee, S. J. Wrenn and P. B. Harbury, *PLoS Biol.*, 2004, **2**, E175.

4 W. Decurtins, M. Wichert, R. M. Franzini, F. Buller, M. A. Stravs, Y. Zhang, D. Neri and J. Scheuermann, *Nat Protoc.*, 2016, **11**, 764-780.

5 M. Klika Škopić, O. Bugain, K. Jung, S. Onstein, S. Brandherm, T. Kalliokoski and A. Brunschweiger, *Med. Chem. Commun.*, 2016, **7**, 1957–1965.

6 E. Wieczerzak, J. Kozlowska, L. Lankiewicz And Z. Grzonka, *Pol. J. Chem.*, **76**, 1693–1697.

7 M. S. Gibson and R. W. Bradshaw, *Angew. Chemie Int. Ed.*, 1968, **7**, 919–930.

8 T. P. Burkholder, J. R. Clayton, L. Ma*, US2010152181 (A1)*, **2010**.