Supporting information

Exploration of a Au(I)-mediated three-component reaction for the synthesis of DNA-tagged highly substituted spiroheterocycles

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1. Materials and instruments

Unless otherwise noted, chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany), Thermo Fisher Scientific (Karlsruhe, Germany), AppliChem (Darmstadt, Germany), and VWR (Langenfeld, Germany). Fmoc-NH-PEG(4)-COOH was purchased from Iris Biotech (Marktredwitz, Germany). 5'-Aminolinker-modified DNA oligonucleotides attached to controlled pore glass solid phase (CPG, 1000 Å porosity) were synthesized by IBA (Göttingen, Germany). Controlled pore glass solid phase was filtered on a synthesis column plugged onto a vacuum manifold (Vac-Man®, Promega). Oligonucleotide-small molecule conjugates were purified by ion pair reverse-phase high-pressure liquid chromatography (HPLC, Shimadzu Prominence) using a C_{18} stationary phase (Phenomenex, Gemini; 5 µm, C18, 110 Å; 100*10.0 mm or 50*10.0 mm) and a gradient of 100 mM aqueous triethylammonium acetate/MeOH. The triethylammonium acetate buffer was set to pH 8. Oligonucleotide-small molecule conjugates were analyzed by ion pair reverse phase 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/MeOH. The oligonucleotide-small molecule conjugates were detected with a UV detector at 254 nm. Oligonucleotide concentrations were determined by UV spectroscopy using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific). All oligonucleotide-conjugates were analyzed by MALDI MS (Bruker Daltonics) using THAP matrix (Dichrom). ^{1}H-NMR-spectra were measured at 400, 500, or 600 MHz on a Bruker DRX400, Bruker DRX500, Varian Inova 500, or Varian Inova 600 spectrometer. ^{13}C-NMR-spectra were measured at 101 MHz, 126 MHz or 150 MHz on a Bruker DRX400, Bruker DRX500, Varian Inova 500 or Varian Inova 600 spectrometer. For NMR measurements, the pure substance was dissolved in deuterated chloroform ($CDCl_3$, 99.8 %, VWR), dimethyl sulfoxide-d_6 (DMSO-d_6, 99.8 %, VWR), or deuterated benzene (C_6D_6, 99.6 %, Sigma-Aldrich) as indicated below. 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.

2. Synthesis of DNA-conjugates by amide coupling, and by reductive amination

2.1. Procedure for coupling of carboxylic acids to 5'-aminolinker-modified DNA

The MMt-protective group of any 5'-(C6)-aminolinker-modified DNA strand 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 CH_2Cl_2 (3 x 200 µL) for 3 x 1 min. A yellow to orange color indicated successful removal of the protective group. The CPG containing the amino-modified DNA was washed three times with each 200 µL of 1 % TEA in MeCN, DMF, MeOH, MeCN and CH_2Cl_2. The CPG, a carboxylic acid, and HATU were then 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 a carboxylic acid (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 µmol, 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 4 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 CH_2Cl_2. 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 CH_2Cl_2, 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 (hexT/C/TC) or for 4 hours (hexA/G/ACGT) 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 purified by RP-HPLC (Gemini, 5u, C18, 110A column, 50*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20 % – 80 % of methanol over 18 min), and analyzed by analytical RP-HPLC (Gemini, 5u, C18, 110A column; 100*4.6 mm) with a gradient of aqueous triethylammonium acetate buffer (10 mM, pH= 8) and methanol (30 % – 90 % of methanol over 15 min).

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

The MMt-protective group of the 5'-amino-linker-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 CH_2Cl_2 (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 CH_2Cl_2. 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 µmol, 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 4 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 CH_2Cl_2. 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 CH_2Cl_2, 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, and re-dissolved in 100 µL of distilled water. After purification product was analyzed by analytical RP-HPLC (Gemini, 5u, C18, 110A column; 100*4.6 mm) with a gradient of aqueous triethylammonium acetate buffer (10 mM, pH= 8) and methanol (30 % – 90 % of methanol over 15 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 3 x 200 µL of DMF, MeOH, MeCN and CH_2Cl_2.

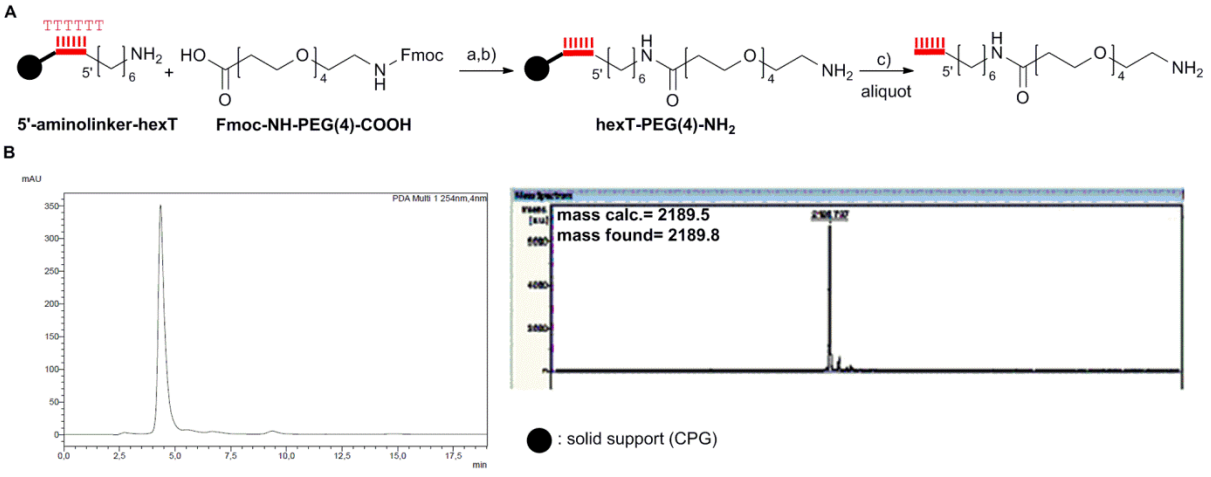

Fig S1. Synthesis of the 5'-amino-PEG(4)-linker modified **hexT**. A) Synthesis scheme. Reagents and conditions: a) HATU, DIPEA, dry DMF, room temperature, 4 hours; b) 20 % piperidine in dry DMF; c) cleavage of an aliquot of the hexT from the solid support: AMA (aqueous ammonia (30 %)/ aqueous methylamine (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 circle denotes solid support (CPG).

2.3 Synthesis of DNA 2-(4-formylphenoxy)acetic acid amide conjugates

DNA strands were coupled with 2-(4-formylphenoxy)acetic acid (18 mg, 100 µmol, 100 eq.) to furnish DNA-**1a** conjugates according to the procedure for coupling of carboxylic acids to amino-modified DNA. For analysis, an aliquot of ca. 10 nmol of each conjugate 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 (hexT/C/TC) or for 4 hours (hexA/G/ACGT) at room temperature. Then, 20 µL of 1 M Tris buffer (pH= 7.5) were added, the mixture was dried in a SpeedVac, and re-dissolved in 100 µL of distilled water. After purification product was analyzed by analytical RP-HPLC (Gemini, 5u, C18, 110A column; 100*4.6 mm) with a gradient of aqueous triethylammonium acetate buffer (10 mM, pH= 8) and methanol (30 % – 90 % of methanol over 15 min).

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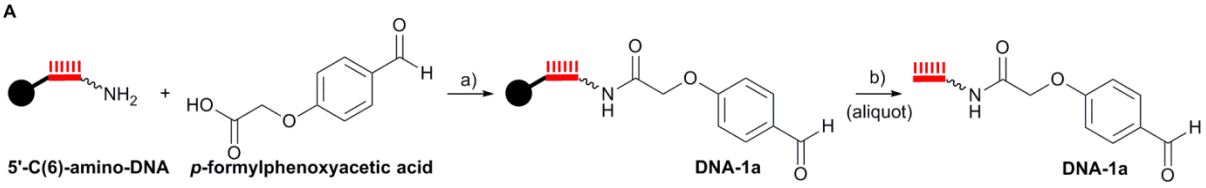

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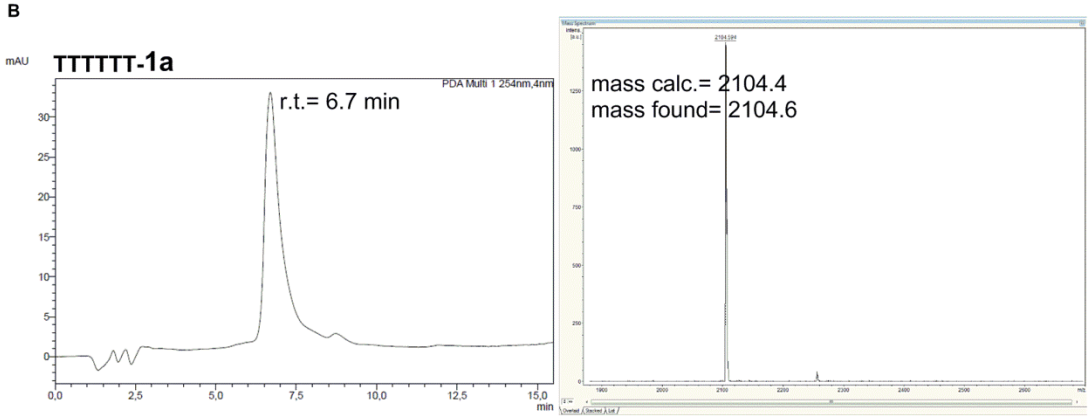

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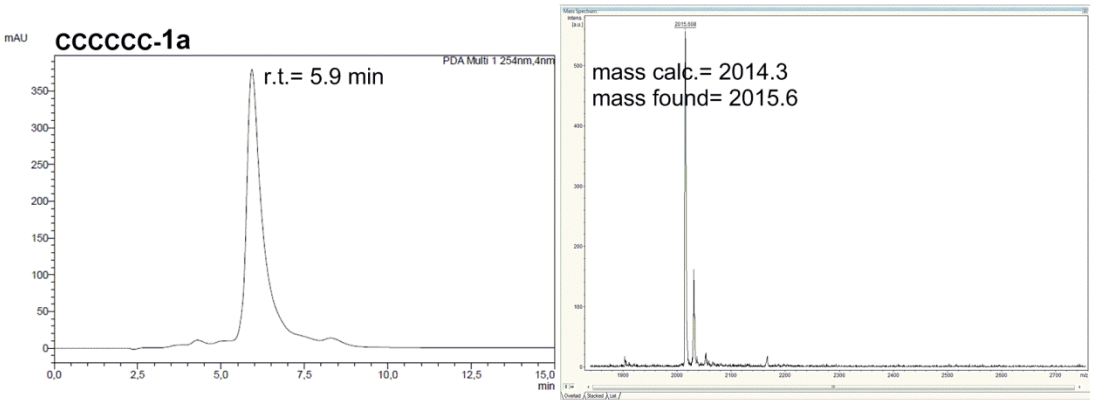

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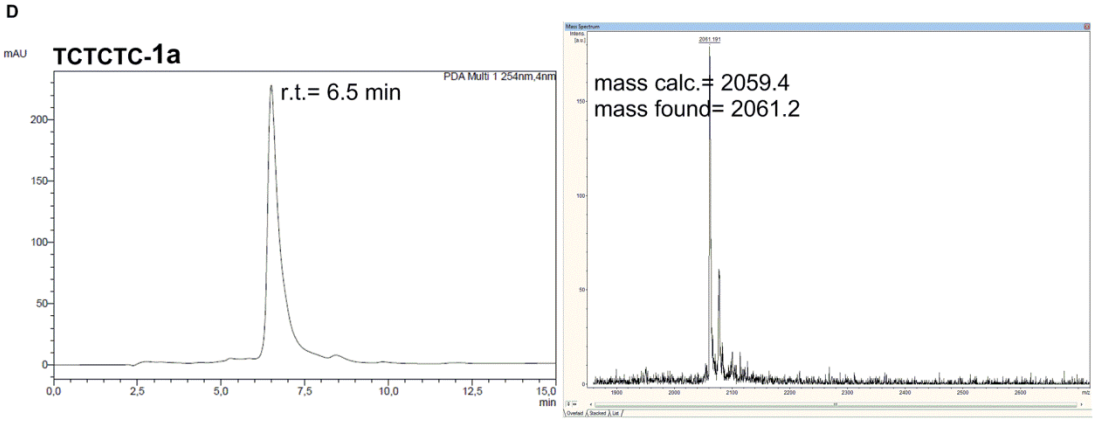
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min

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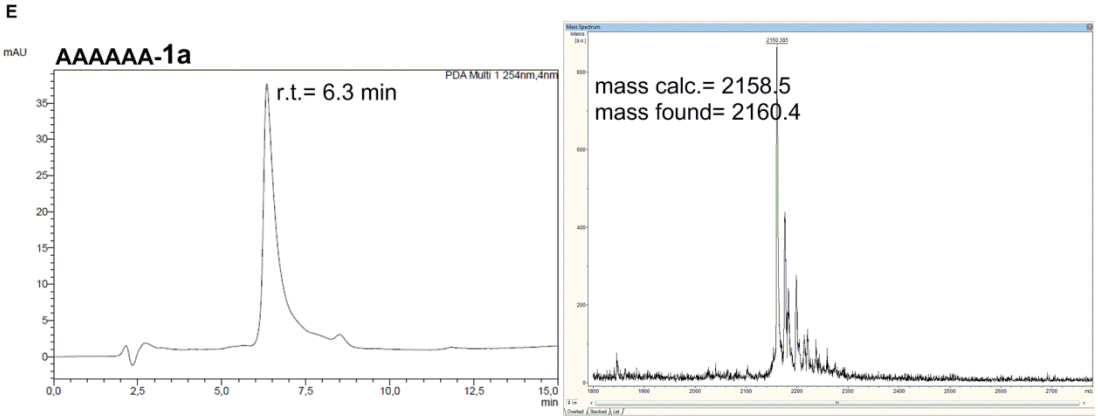
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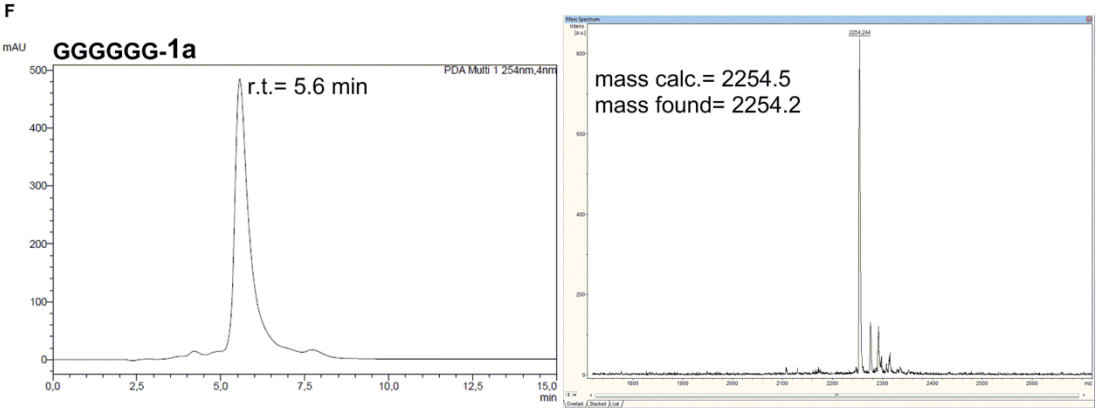
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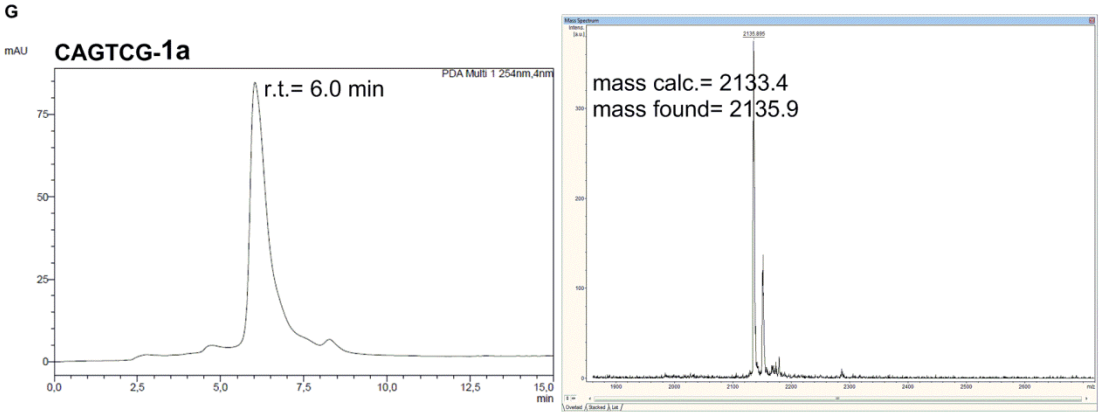
G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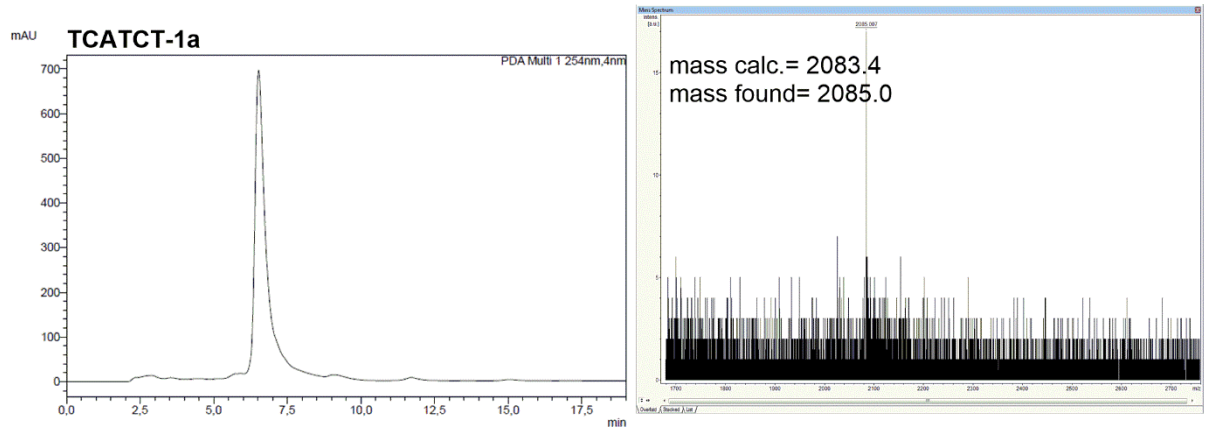


Fig S2. Synthesis of DNA-hexamer-aldehyde conjugates **DNA-1a**. A) Synthesis scheme. Reagents and conditions: a) HATU, DIPEA, dry DMF, room temperature, 4 hours; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min (hexT/C/TC) or for 4 hours (hexA/G/ACGT) at room temperature; B) HPLC trace and MALDI MS spectrum of the purified conjugate **hexT-1a**; C) HPLC trace and MALDI MS spectrum of the purified conjugate **hexC-1a**; D) HPLC trace and MALDI MS spectrum of the purified conjugate **hexTC-1a**; E) HPLC trace and MALDI MS spectrum of the purified conjugate **hexA-1a**; F) HPLC trace and MALDI MS spectrum of the purified conjugate **hexG-1a**; G) HPLC trace and MALDI MS spectrum of the purified conjugate **hexACGT-1a**; H) HPLC trace and MALDI MS spectrum of the purified conjugate **hexATC-1a**. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

2.4 Synthesis of DNA conjugate hexT-2d by reductive amination

A solution *tert*-butyl hydrazinecarboxylate **7** (12.9 mg, 97.5 µmol, 325 eq.) in 10 µL of DMF was added to sodium cyanoborohydride (6.1 mg, 97.5 µmol, 325 eq.), followed by addition of glacial acetic acid (5.58 µL, 97.5 µmol, 325 eq.). Then, 960 µL of 300 mM aqueous MOPS buffer was added. The whole mixture was added to the CPG-bound conjugate **hexT-1a**, and the reaction mixture was shaken at 37°C overnight. Then, the CPG containing the DNA-conjugate **hexT-2d** was filtered over a filter column and washed subsequently with each 3 x 200 µL of DMF, MeOH, MeCN and CH_2Cl_2. For analysis, an aliquot of ca. 10 nmol of the DNA-conjugate **hexT-2d** 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 purified/analyzed by RP-HPLC (Gemini, 5u, C18, 110A column; 50*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20 % – 80 % of methanol over 18 min).

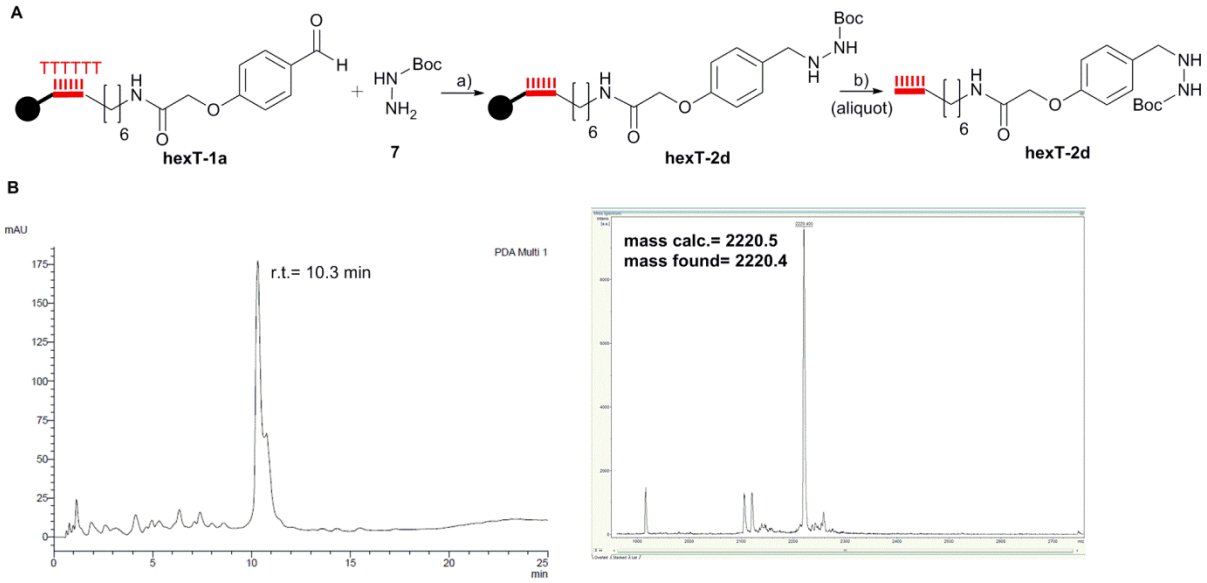


Fig S3. Synthesis of hexT-hydrazide conjugate **hexT-2d**. A) Synthesis scheme. Reagents and conditions: a) $NaBH_3CN$, glacial AcOH, 37°C, overnight; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature (aliquot); B) HPLC trace of the crude and MALDI MS spectrum of the purified conjugate **hexT-2d**. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

2.5 Synthesis of DNA conjugate hexT-3c

The hexT DNA was coupled with 4-(5-((*tert*-butyldimethylsilyl)oxy)pent-1-yn-1-yl)benzoic acid **19** (31.8 mg, 100 µmol, 100 eq.) to furnish **hexT-20** according to the procedure for coupling of carboxylic acids to amino-modified DNA. Then, the TBMS-group of CPG-bound conjugate **hexT-20** was removed with 200 µL of 1 M TBAF solution in THF at room temperature for 30 seconds yielding **hexT-3c**. The CPG was washed as described above. The procedure was repeated three times. For analysis, each an aliquot of ca. 10 nmol of conjugates **hexT-3c** and **hexT-20** 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, and re-dissolved in 100 µL of distilled water. After purification the product was analyzed by RP-HPLC (Gemini, 5u, C18, 110A column; 100*4.6 mm) with a gradient of aqueous triethylammonium acetate buffer (10 mM, pH= 8) and methanol (30 % – 90 % of methanol over 15 min).

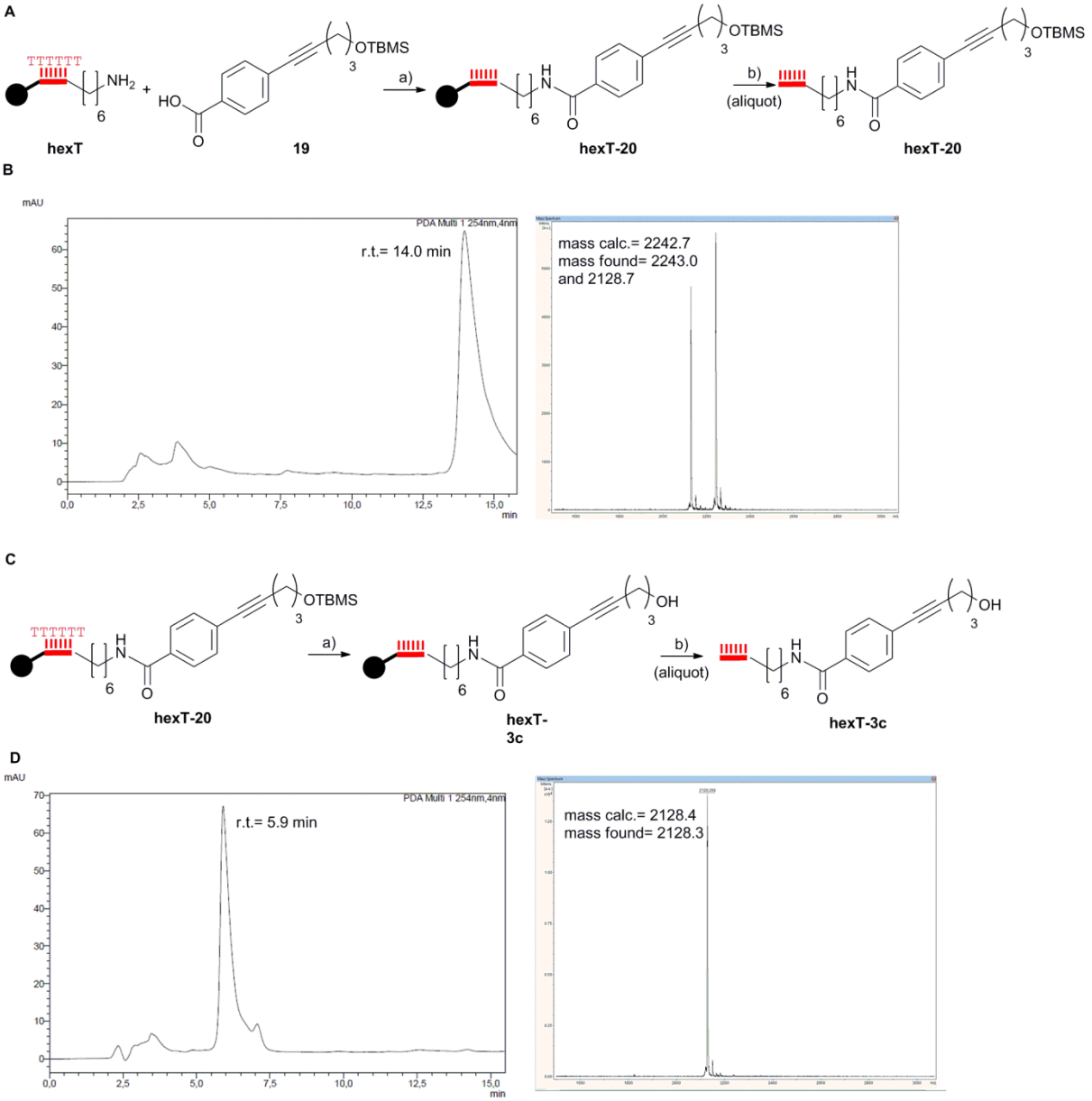


Fig S4. Synthesis of hexT-alkynol conjugates **hexT-20** and **hexT-3c**. A) Synthesis scheme for compound **hexT-20**. Reagents and conditions: a) HATU, DIPEA, dry DMF, room temperature, 4 hours; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature (aliquot); B) HPLC trace and MALDI MS spectrum of the purified conjugate **hexT-20**; partial loss of *tert*-butyldimethylsilyl protective group was observed upon irradiation in the mass spectrometer; C) synthesis scheme for compound **hexT-3c**. Reagents and conditions: a) 1 M TBAF solution in THF; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature (aliquot); D) HPLC trace and MALDI MS spectrum of the purified conjugate **hexT-3c**. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

3. Au(I)-mediated synthesis of hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugates from hexT-aldehyde conjugate 1a

3.1 Optimization of reaction conditions of the Au(I)-mediated three-component reaction

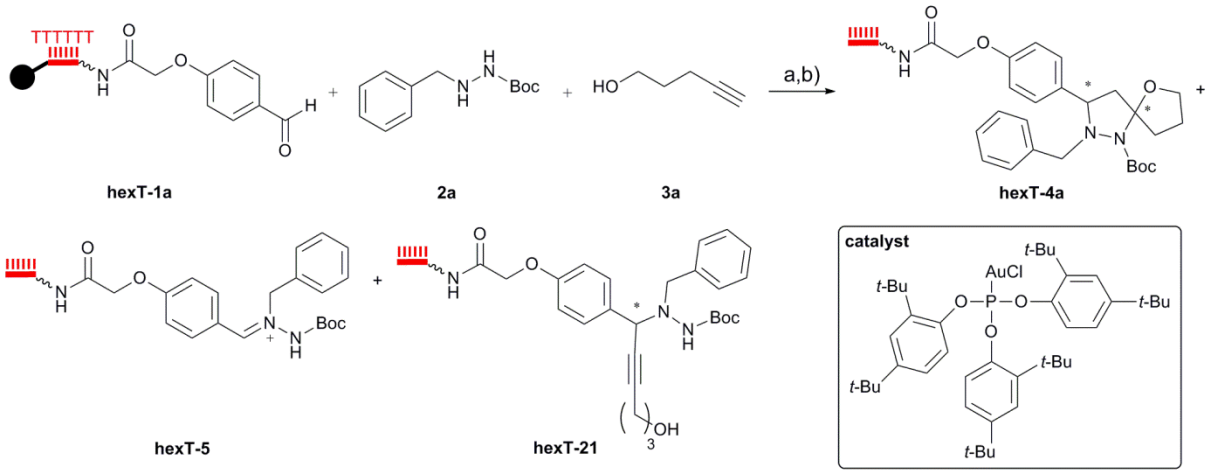


Fig S5. Au(I)-mediated synthesis of hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate **4a** from hexT-aldehyde conjugate **1a**. a) For conditions see Table S1; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature. Figs S6-S12 show HPLC traces of the crude reaction mixtures. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

The solid support containing the hexamer DNA-aldehyde conjugate **1a** (30 nmol, ca. 1.1 mg) was suspended in 15 µL of a solvent (see Table S1). Then, *tert*-butyl 2-benzylhydrazinecarboxylate **2a** and pent-4-yn-1-ol **3a**, at the amounts given in Table S1, dissolved in 15 µL of the same solvent (prepared as stock) were added to **1a**. This was followed by an equimolar mixture of Au(I)/$AgSbF_6$ at the amounts given in Table S1, which was suspended (Au(I)) or dissolved ($AgSbF_6$), respectively in 15 µL of the same solvent. The suspension of the catalyst was prepared as stock. Prior addition to **1a** it was vortexed and pipetted up and down. The reactions were shaken at room temperature overnight in Eppendorf tubes under normal atmosphere. Then, the CPG was filtered off, washed with each 3 x 200 µL of 0.1 M EDTA, DMF, MeOH, MeCN, and CH_2Cl_2, and dried *in vacuo* for 15 min. The hexamer-conjugates were deprotected and cleaved from the CPG by treatment with 500 µL of AMA for 30 minutes (hexT/C/TC) or for 4 hours (hexA/hexG/hexACGT) at room temperature. Then, 20 µL of 1 M Tris buffer (pH= 7.5) were added, the mixtures were dried, dissolved in 100 µL of distilled water, and purified by RP-HPLC (Gemini, 5u, C18, 110A column; 50*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20 % – 80 % of methanol over 18 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 RP-HPLC (Gemini, 5u, C18, 110A column; 100*4.6 mm) with a gradient of aqueous triethylammonium acetate buffer (10 mM, pH= 8) and methanol (30 % – 90 % of methanol over 15 min).

Table S1: Optimization of reaction conditions for the Au(I)-mediated MCR to furnish **hexT-4a**.

	No.	sequence	hydrazide [eq][a]	alkynol [eq][a]	catalyst [eq][a]	time [h]	solvent	T [°C]	catalyst[b]	**hexT-4a** [%][c]	**hexT-5** [%][c]
a) equivalents of reagents and catalyst[d]	**1**	hexT	100	200	10	20	THF	25	Au(I)/$AgSbF_6$	-	<5
	2	hexT	500	1000	10	20	THF	25	Au(I)/$AgSbF_6$	-	<5
	3	hexT	500	1000	50	20	THF	25	Au(I)/$AgSbF_6$	40	<5
	4	hexT	500	1000	100	20	THF	25	Au(I)/$AgSbF_6$	50	<5
	5	hexT	500	1000	250	20	THF	25	Au(I)/$AgSbF_6$	90	<5
	6	hexT	-	-	250	20	THF	25	Au(I)/$AgSbF_6$	-	-
	7	hexT	500	1000	250	20	THF	25	**-**	-	-
b) solvent	**8**	hexT	500	1000	250	14	MeOH	25	Au(I)/$AgSbF_6$	0	90
	9	hexT	500	1000	250	14	DMF	25	Au(I)/$AgSbF_6$	0	75
	10	hexT	500	1000	250	14	MeCN	25	Au(I)/$AgSbF_6$	45	45
	11	hexT	500	1000	250	14	THF	25	Au(I)/$AgSbF_6$	55	35
	12	hexT	500	1000	250	14	1,2-DCE	25	Au(I)/$AgSbF_6$	10	55
	13	hexT	500	1000	250	14	toluene	25	Au(I)/$AgSbF_6$	15	50
	14	hexT	500	1000	250	14	CH_2Cl_2	25	Au(I)/$AgSbF_6$	10	70
c) time	**15**	hexT	500	1000	250	14	THF	25	Au(I)/$AgSbF_6$	55	35
	16	hexT	500	1000	250	18	THF	25	Au(I)/$AgSbF_6$	75	20
	17	hexT	500	1000	250	20	THF	25	Au(I)/$AgSbF_6$	90	<5

[a] versus the solid support-bound hexT-aldehyde conjugate **hexT-1a**; [b] Au(I): tris(2,4-di-*tert*-butylphenyl)phosphite]gold chloride; [c] % conversion estimated based on the area under curve of the product peak versus the starting material peak in the HPLC-trace of the crude; [d] note that the conjugates contain PEG(4) linker.

a) Screening different equivalents of reactants, and catalyst Au(I)/$AgSbF_6$ versus the hexathymidine-aldehyde conjugate **hexT-1a** for the Au(I)-mediated MCR to DNA-spirocycle conjugate **hexT-4a**

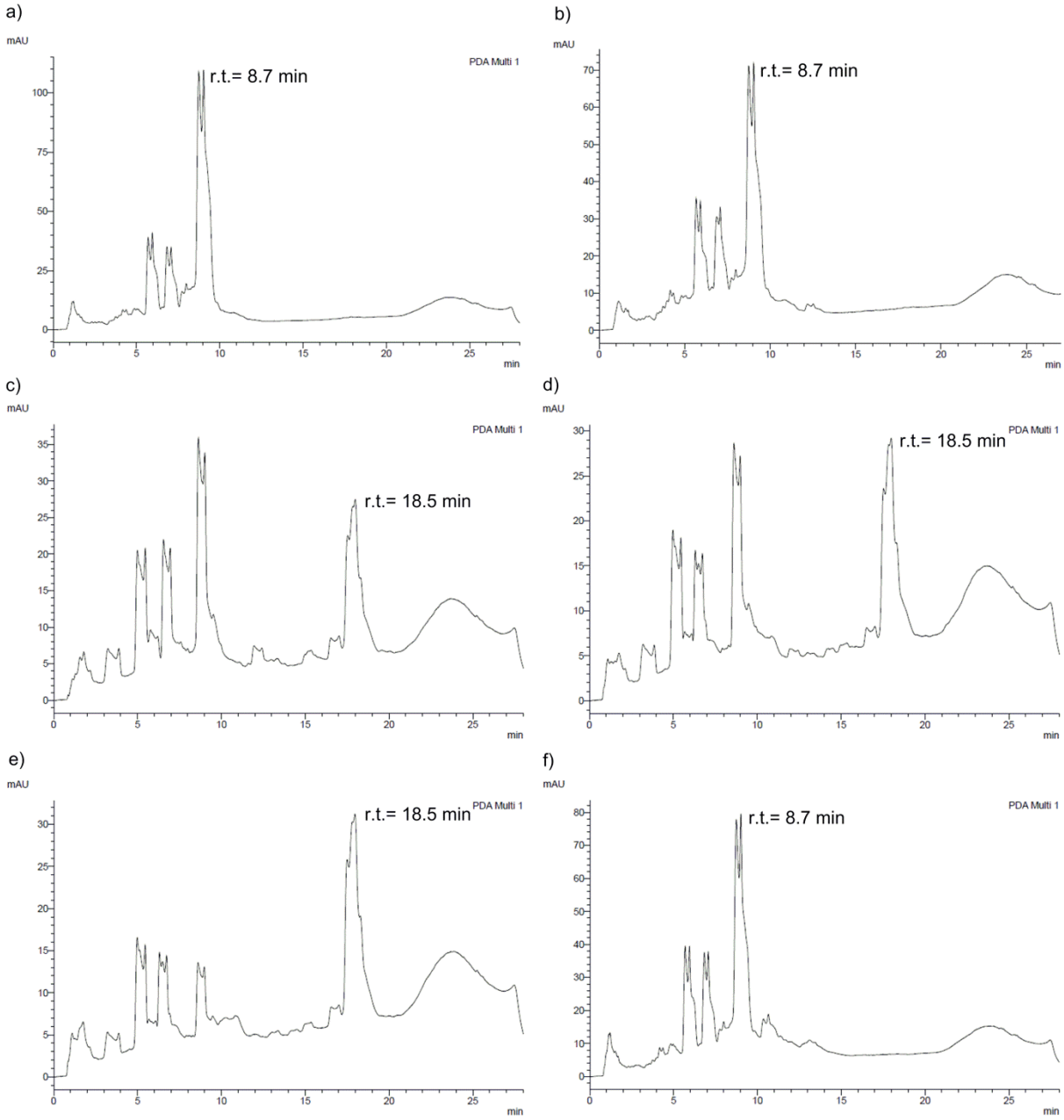


Fig S6. Effect of different equivalents of reactants, and catalyst on the Au(I)-mediated reaction of the hexathymidine-aldehyde conjugate **hexT-1a**, hydrazide **2a**, and alkynol **3a**, for reaction conditions see Table S1a. a) HPLC trace of the starting material (aldehyde conjugate **hexT-1a**); b) HPLC trace of experiment **No. 1**; formation of the target spirocycle **hexT-4a** was not observed (same result: experiment **No. 2**); c) HPLC trace of experiment **No. 3**; 40 % conversion of the starting material to the target spirocycle **hexT-4a**; d) HPLC trace of experiment **No. 4**; 50 % conversion of the starting material to the target spirocycle **hexT-4a**; e) HPLC trace of experiment **No. 5**; 90 % conversion of the starting material to the target spirocycle **hexT-4a**; f) HPLC trace of experiment **No. 6** and **No. 7**; formation of the target spirocycle **hexT-4a** was not observed. Note that the **hexT-1a** conjugates in Fig. **S6** contain PEG(4) linker, and were purified/analyzed on Gemini, 5u, C18, 110A; 100*10.0 mm column, with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20 % – 80 % of methanol over 19 min).

b) Screening different solvents for the Au(I)-mediated reaction to DNA-spirocycle conjugate **hexT-4a**

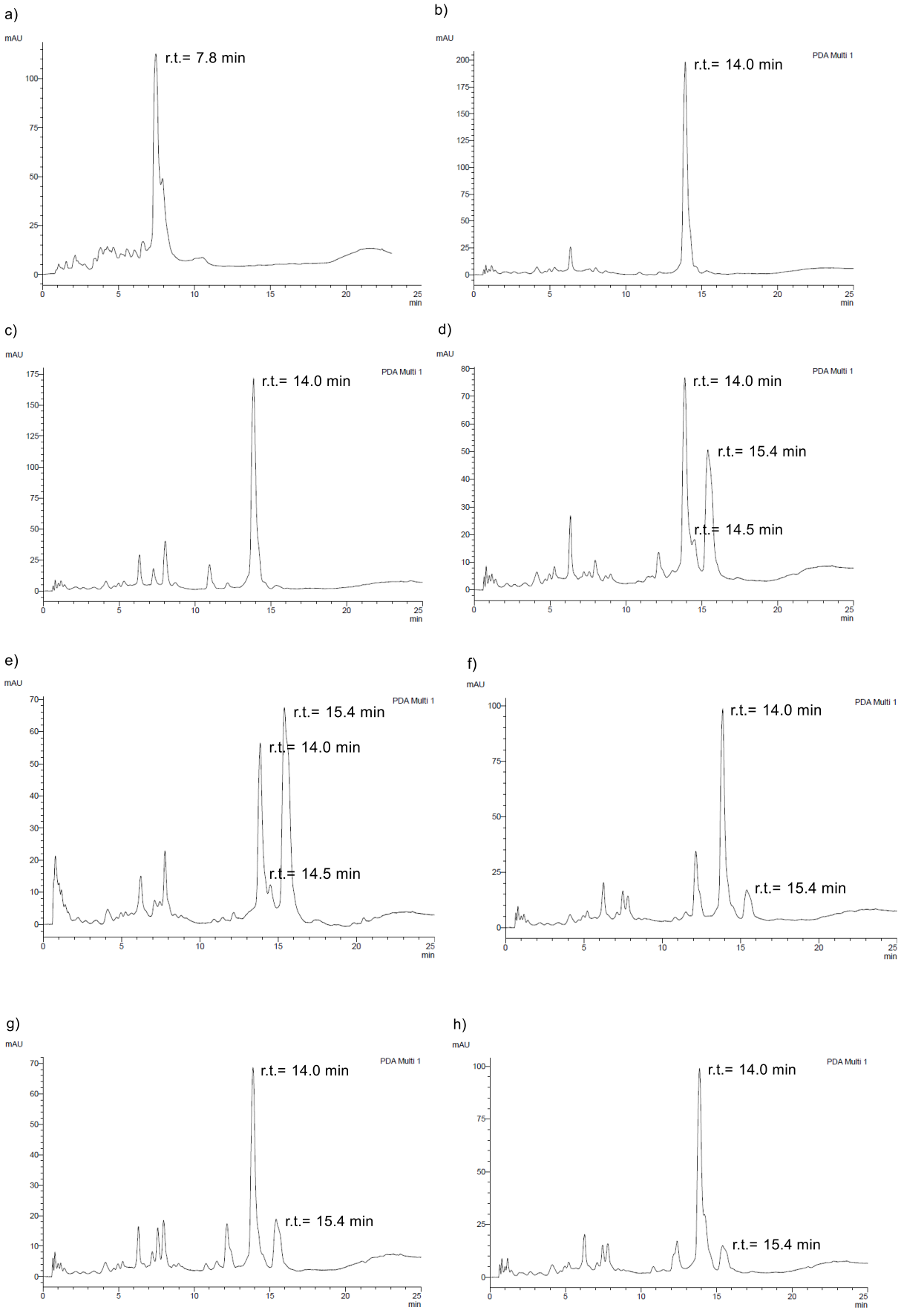


Fig S7. Effect of different solvents on the Au(I)-mediated MCR of the hexathymidine-aldehyde conjugate **hexT-1a**, hydrazide **2a**, and alkynol **3a**, for reaction conditions see Table S1b. a) HPLC trace of the starting material (aldehyde conjugate **hexT-1**); b) HPLC trace of experiment **No. 8**; formation of condensation product **hexT-5** (r.t.= 14.0 min); c) HPLC trace of experiment **No. 9**; formation of condensation product **hexT-5**; d) HPLC trace of experiment **No. 10**; formation of mixture of condensation product **hexT-5** and spirocycle **hexT-4a** (r.t.= 15.4 min); e) HPLC trace of experiment **No. 11**; formation of mixture of condensation product **hexT-5** and spirocycle **hexT-4a**; f) HPLC trace of experiment **No. 12**; formation of condensation product **hexT-5**, target spirocycle **hexT-4a** formed as minor product; g) HPLC trace of experiment **No. 13**; formation of condensation product **hexT-5**, target spirocycle **hexT-4a** formed as minor product; h) HPLC trace of experiment **No. 14**; formation of condensation product **hexT-5**, target spirocycle **hexT-4a** formed as minor product.

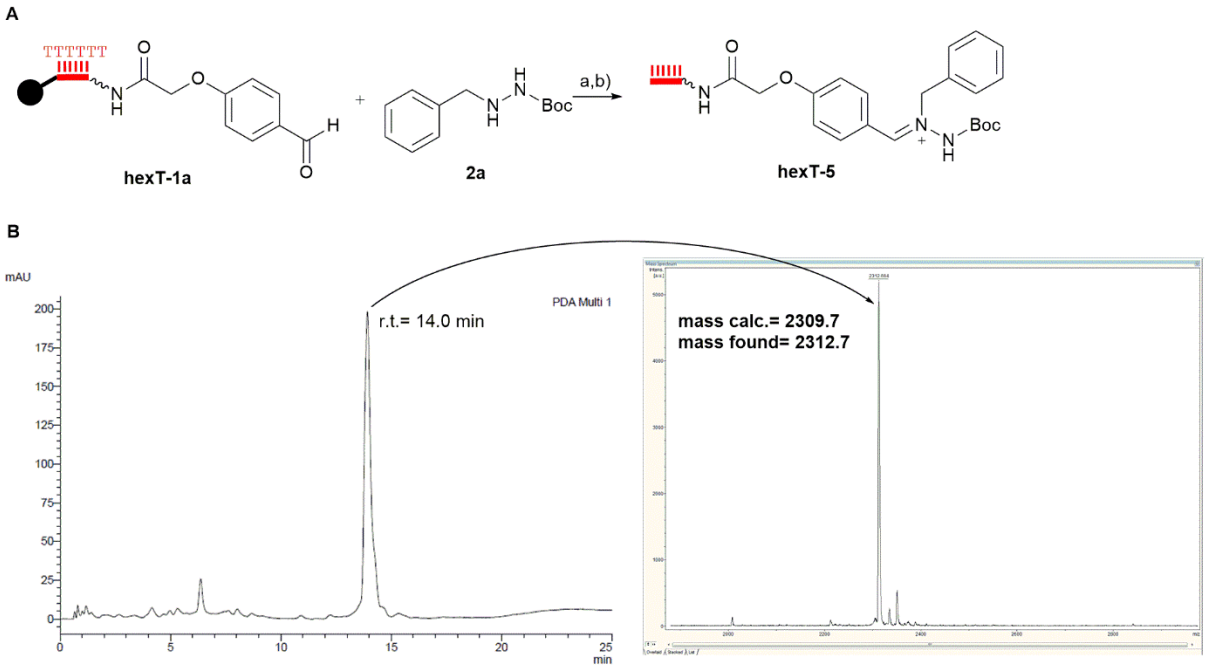


Fig S8. MALDI MS of the isolated hexathymidine conjugate **hexT-5** (r.t.= 14.0 min) from Fig S7b. A) Reaction scheme of the **hexT-5** formation; a) reaction condition **No. 8** (Table S1b); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; B) MALDI MS of the isolated hexathymidine conjugate **hexT-5**. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

c) Screening different reaction times for the Au(I)-mediated reaction to DNA-spirocycle conjugate **hexT-4a**

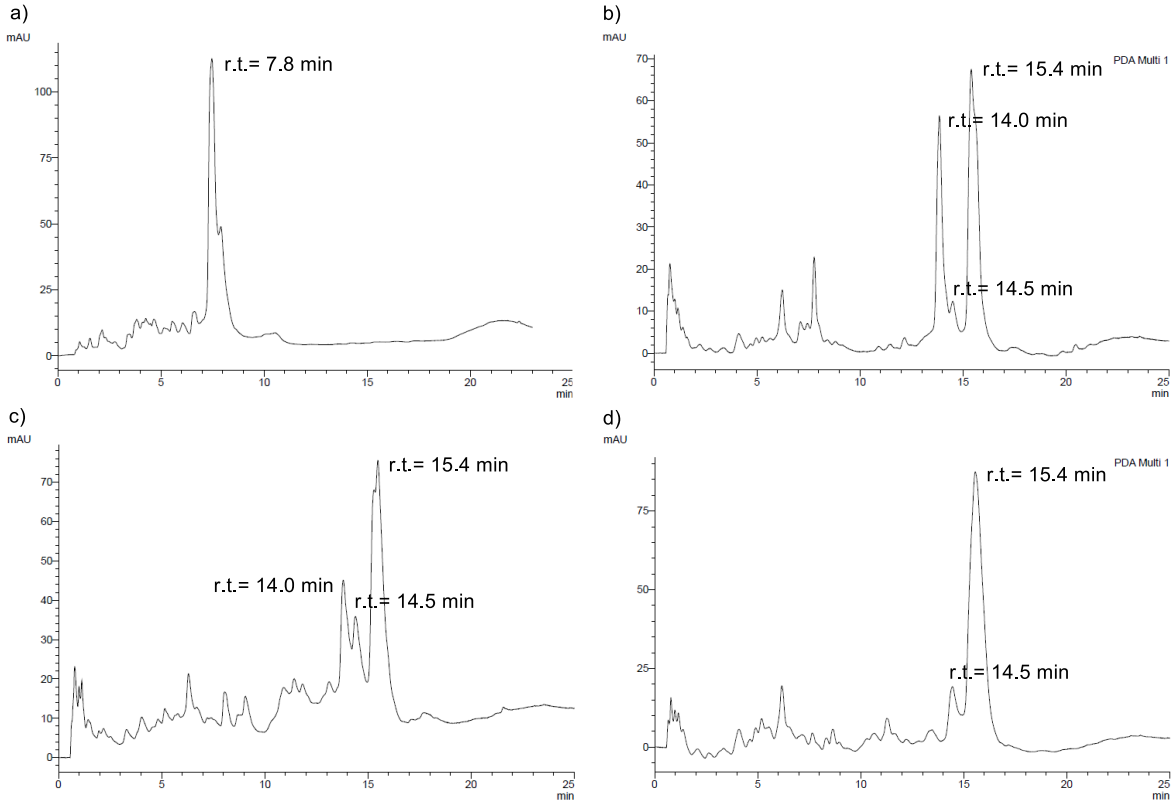


Fig S9. Effect of different reaction times on the Au(I)-mediated reaction to DNA-spirocycle conjugate **hexT-4a**, for reaction conditions see Table S1c. a) HPLC trace of the starting material **hexT-1a**; b) HPLC trace of experiment **No. 15**; formation of a mixture of products **hexT-4a** and **hexT-5**; c) HPLC trace of experiment **No. 16**; formation of a mixture of products **hexT-4a**, **hexT-21** and **hexT-5**; d) HPLC trace of experiment **No. 17**; target spirocycle **hexT-4a** formed as major product.

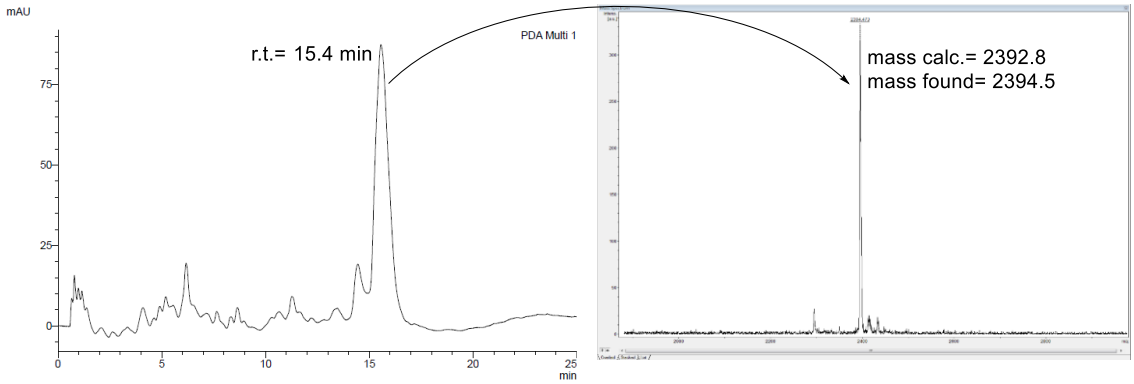


Fig S10. HPLC trace of the reaction mixture of experiment **No. 17** (Table S1c), and MALDI MS spectrum of the isolated hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate **hexT-4a**.

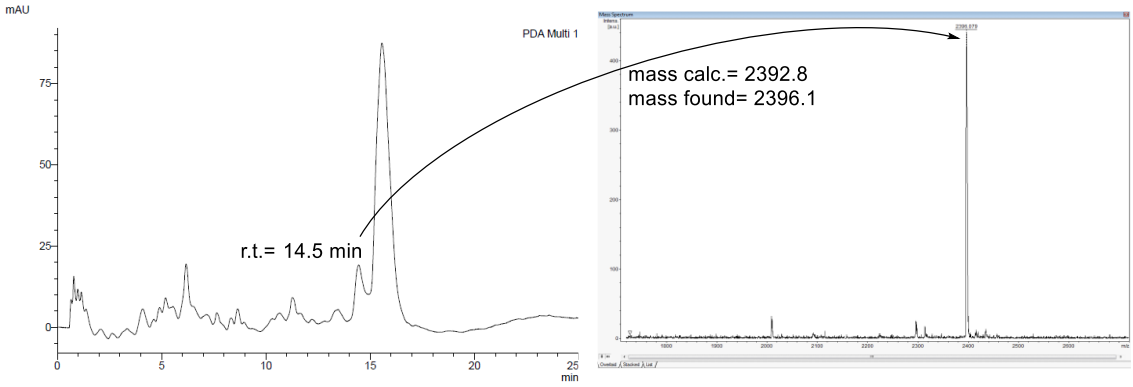


Fig S11. MALDI MS spectrum of the isolated side product **hexT-21** (Fig. S5) from Fig. S9d (r.t.= 14.5 min). *We tentatively assigned this product the structure of the Mannich-type product. It displays the same mass as the hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate* ***hexT-4a****. A product of this type was also found as side product in the Au(I)-mediated annelation reaction described in ref. 2.*

3.2 Comparison of the spirocycle conjugate hexT-4a with the reference molecule ref-hexT-4a

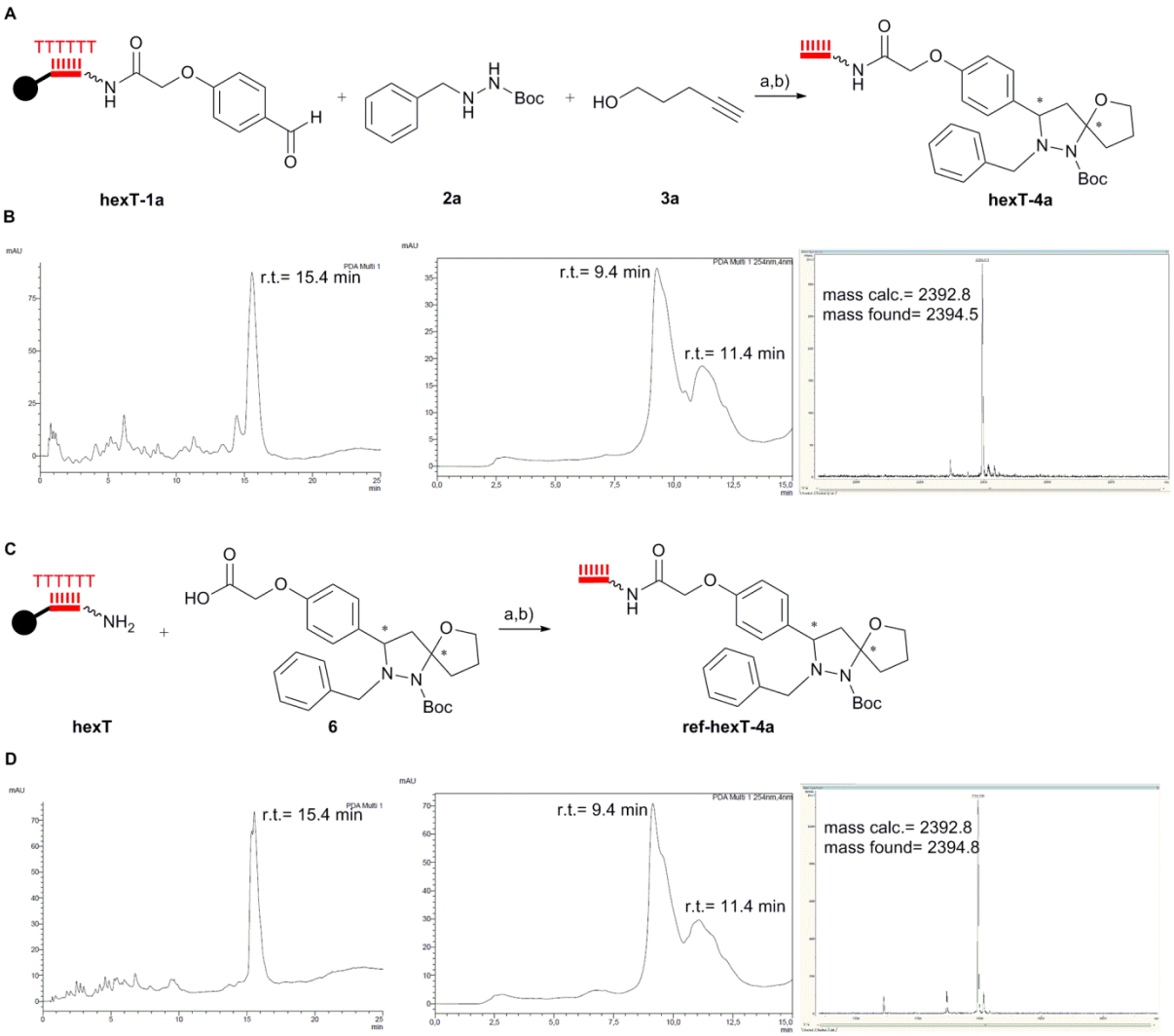
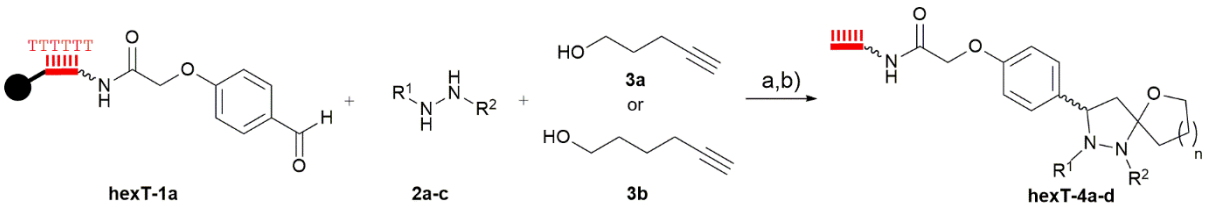
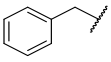
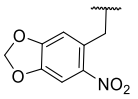
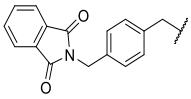
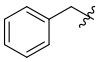


Fig S12. Comparison of the hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate **hexT-4a** synthesized from the hexT-aldehyde conjugate **hexT-1a** by the Au(I)-mediated reaction (Fig S5) with the reference hexT-spirocycle conjugate **ref-hexT-4a** synthesized from the 6-oxa-1,2-diazaspiro[4.4]nonane **6** (Fig. S37), and 5'-(C6)-aminolinker modified **hexT** by amide coupling. A) Scheme for the synthesis of the hexT-spirocycle conjugate **hexT-4a**; conditions: a) reaction condition **No. 17** (Table S1c); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature); B) preparative HPLC trace of the crude reaction mixture, analytical HPLC trace of the purified conjugate **hexT-4a**, and MALDI MS spectrum of the purified conjugate **hexT-4a**; C) scheme for the synthesis of the hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate **ref-hexT-4a** by amide coupling; conditions: c) HATU, DIPEA, room temperature, 4 hours; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; D) preparative HPLC of the crude reaction mixture, analytical HPLC of the purified conjugate **ref-hexT-4a**, and MALDI MS spectrum of the purified conjugate **ref-hexT-4a**. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

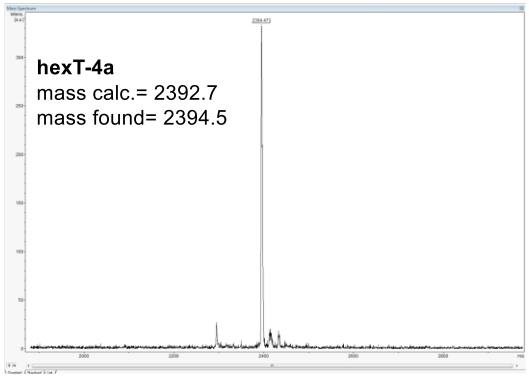
3.3 Reaction scope

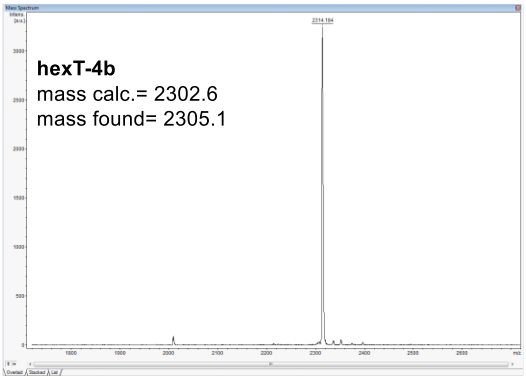
Table S2 Scope of the reaction to hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugates **hexT-4**. a) Reaction condition **No. 17** (Table S1c); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature). Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

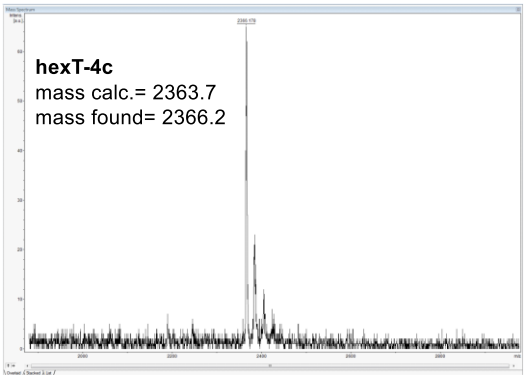


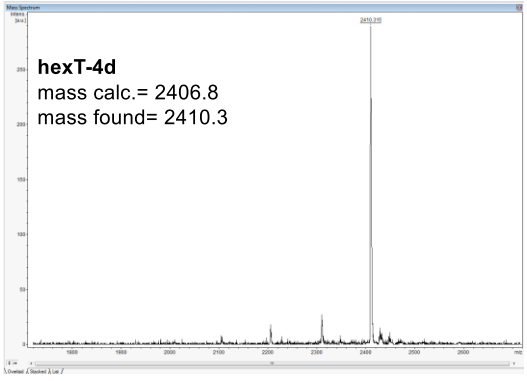
entry	hexT	hydrazide	alkynol	R^1	R^2	n	yield/ [nmol][a]	*mass calc.* mass found[b]
1	**hexT-4a**	**2a**	pentynol		*tert*-Boc	1	3.6	*2392.7* 2394.5
2	**hexT-4b**	**2b**	pentynol		*tert*-Boc	1	1.4	*2302.6*[c] 2305.1[c]
3	**hexT-4c**	**2c**	pentynol		acetyl	1	1.5	*2363.7* 2366.2[d]
4	**hexT-4d**	**2a**	hexynol		*tert*-Boc	2	1.8	*2406.8* 2410.3

[a] measured by Nanodrop; [b] measured by MALDI MS; [c] loss of the photolabile 3,4-methylendioxy-6-nitrobenzyl group upon irradiation in the mass spectrometer; [d] phthalimide group removed in the product.









4. Au(I)-mediated synthesis of hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugates from hexT-hydrazide conjugate 2d

4.1 Optimization of reaction conditions for the Au(I)-mediated reaction

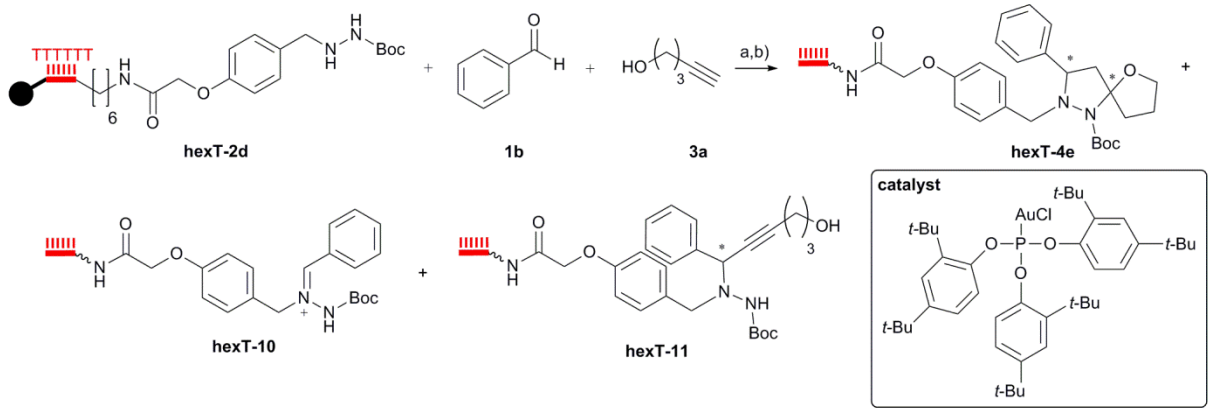


Fig S13. Au(I)-mediated synthesis of hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate **4e** from hexT-hydrazide conjugate **2d**. a) For conditions see Table S3; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature. Figs S14-S20 show HPLC traces of the crude reaction mixtures. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

The solid support (controlled pore glass, CPG) containing the hexathymidine-hydrazide conjugate **hexT-2d** (30 nmol, ca. 1.1 mg) was suspended in 15 µL of a solvent (see Table S3). Then, benzaldehyde **1b** and pent-4-yn-1-ol **3a**, at the amounts given in Table S3, dissolved in 15 µL of the same solvent (prepared as stock) were added to **hexT-2d**. This was followed by an equimolar mixture of the Au(I) catalyst/$AgSbF_6$ at the amounts given in Table S3 which was suspended (Au(I)) or dissolved ($AgSbF_6$), respectively in 15 µL of the same solvent. The suspension of the catalyst was prepared as stock. Prior addition to **hexT-2d** it was vortexed and pipetted up and down to add a homogeneous suspension. The reactions were shaken at room temperature overnight in Eppendorf tubes under normal atmosphere. Then, the CPG was filtered off, washed with each 3 x 200 µL of 0.1 M EDTA, DMF, MeOH, MeCN and CH_2Cl_2 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. 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 products were purified by RP-HPLC (Gemini, 5u, C18, 110A column; 50*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20 % – 80 % of methanol over 18 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 S3: Optimization of reaction conditions for the Au(I)-mediated reaction to 6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4e**.

	No.	aldehyde [eq][a]	alkynol [eq][a]	catalyst [eq][a]	time/h	solvent	T/[°C]	catalyst[b]	**hexT-4e** [%][c]	**hexT-10** [%][c]	**hexT-11** [%][c]
a) solvent	**1**	500	1000	250	20	MeOH	25	Au(I)/$AgSbF_6$	15	25	10
	2	500	1000	250	20	DMF	25	Au(I)/$AgSbF_6$	35	30	30
	3	500	1000	250	20	MeCN	25	Au(I)/$AgSbF_6$	25	30	10
	4	500	1000	250	20	THF	25	Au(I)/$AgSbF_6$	40	25	25
	5	500	1000	250	20	1,2-DCE	25	Au(I)/$AgSbF_6$	5	-	-
b) time	**6**	500	1000	250	14	THF	25	Au(I)/$AgSbF_6$	20	15	15
	7	500	1000	250	20	THF	25	Au(I)/$AgSbF_6$	40	25	25

[a] versus the solid support-bound hexT-hydrazide conjugate **hexT-2d**; [b] Au(I): tris(2,4-di-*tert*-butylphenyl)phosphite]gold chloride; [c] % conversion estimated based on the area under curve of the product peak versus the starting material peak in the HPLC-trace of the crude.

a) Screening different solvents for the Au(I)-mediated reaction to **hexT-4e**

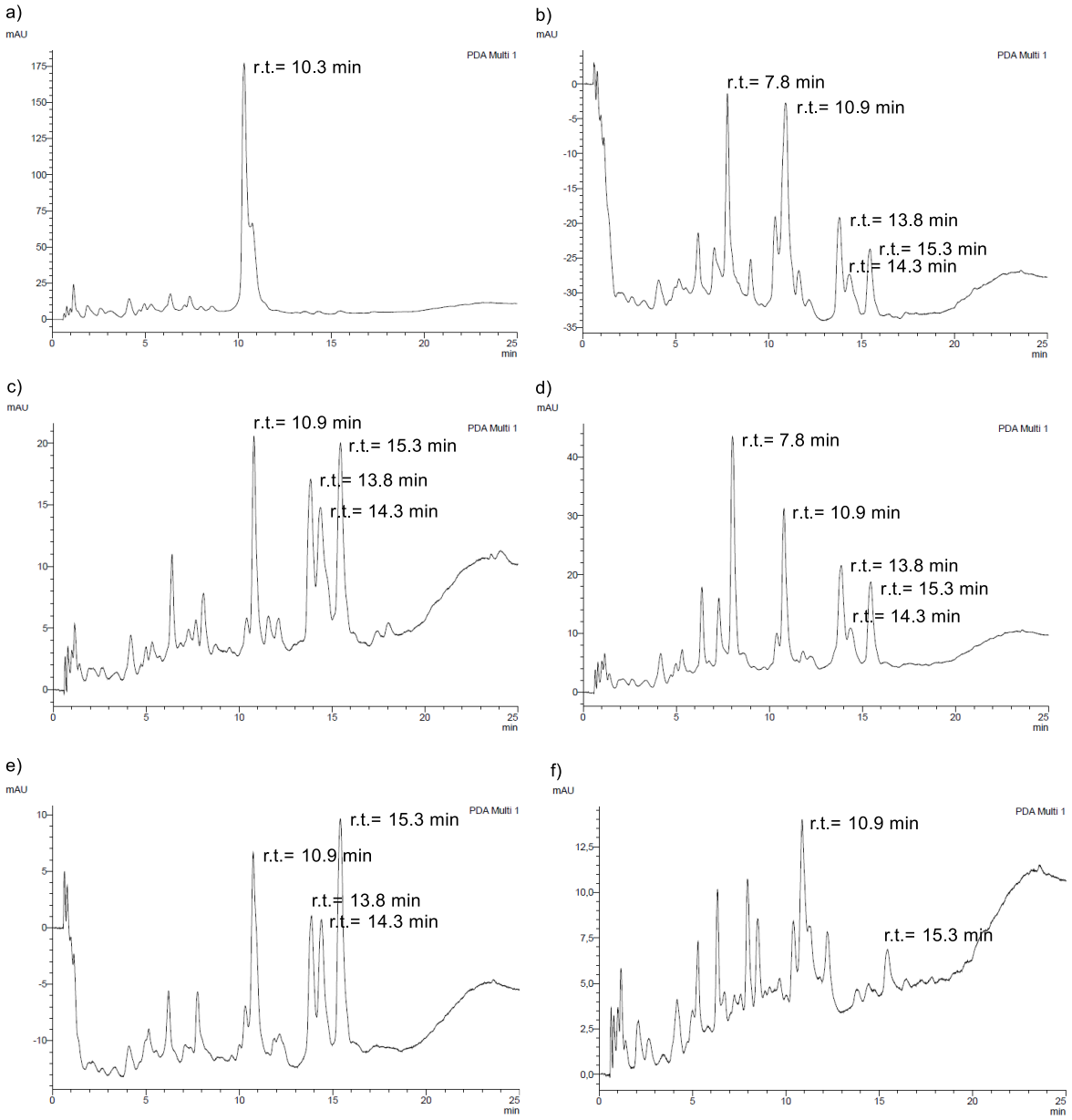


Fig S14. Effect of different solvents on the Au(I)-mediated reaction to **hexT-4e**, for reaction conditions see Table S3. a) HPLC trace of the starting material (hexathymidine-hydrazide conjugate **hexT-2d**); b) HPLC trace of experiment **No. 1**; formation of mixture of condensation product **hexT-10** (r.t.= 13.8 min), suggested *Mannich-type product* **hexT-11** (r.t.= 14.3 min), and spirocycle **hexT-4e** (r.t.= 15.3 min); c) HPLC trace of experiment **No. 2**; formation of mixture of condensation product **hexT-10**, suggested *Mannich-type product* **hexT-11**, and spirocycle **hexT-4e;** d) HPLC trace of experiment **No. 3**; formation of mixture of products **hexT-10**, **hexT-11**, and **hexT-4e**; e) HPLC trace of experiment **No. 4**; formation of mixture of products **hexT-10**, **hexT-11**, and **hexT-4e**; f) HPLC trace of experiment **No. 5**, target spirocycle **hexT-4e** formed as minor product.

b) Screening different reaction times for the Au(I)-mediated reaction to 6-oxa-1,2-diazaspiro[4.4]nonane conjugate **hexT-4e**

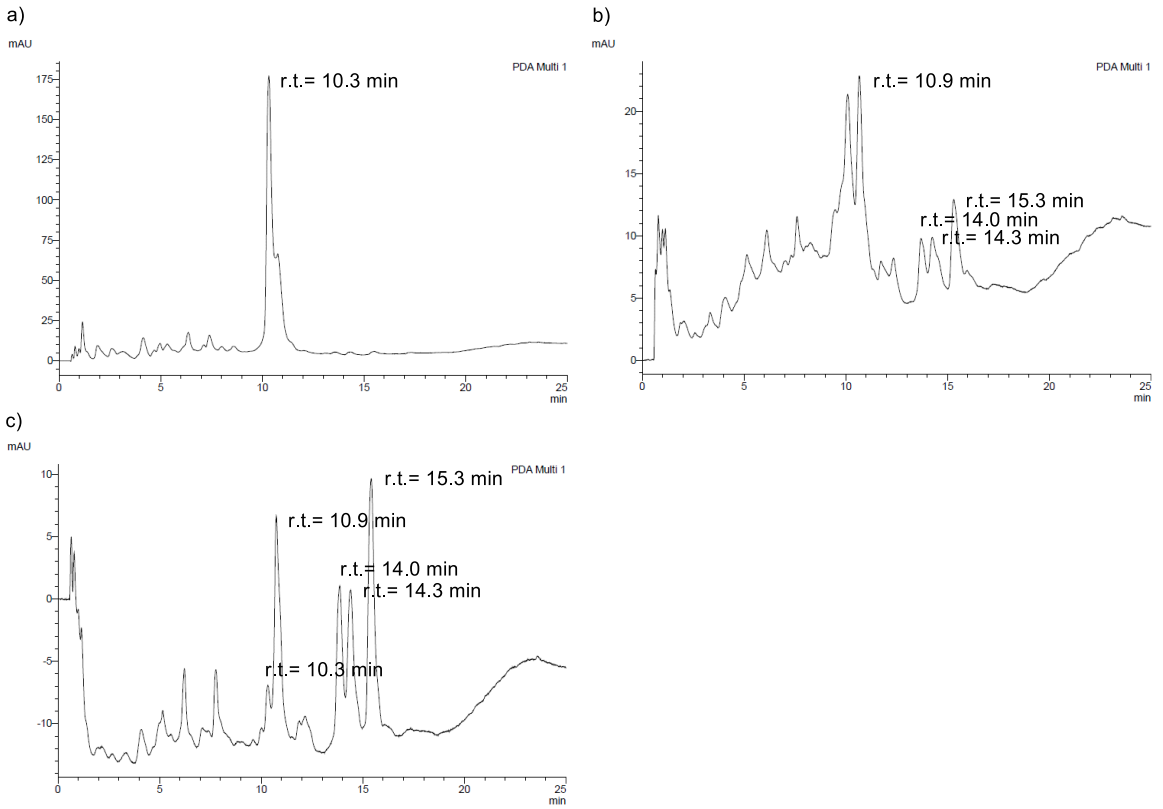


Fig S15. Effect of different reaction times on the Au(I)-mediated reaction to spirocycle conjugate **hexT-4e**, for reaction conditions see Table S3b. a) HPLC trace of the starting material (hexathymidine-hydrazide conjugate **hexT-2d**); b) HPLC trace of experiment **No. 6**, Table S3b; target spirocycle **hexT-4e** (r.t.= 15.3 min) formed as minor product in mixture of a products; c) HPLC trace of experiment **No. 7**, Table S3b; identical to Fig S14e.

4.2 MALDI MS analysis of different fractions isolated from the reaction to spirocycle conjugate hexT-4e

4.2.1 Detection of the starting material **hexT-1a** and Boc-deprotected **hexT-8/hexT-9**

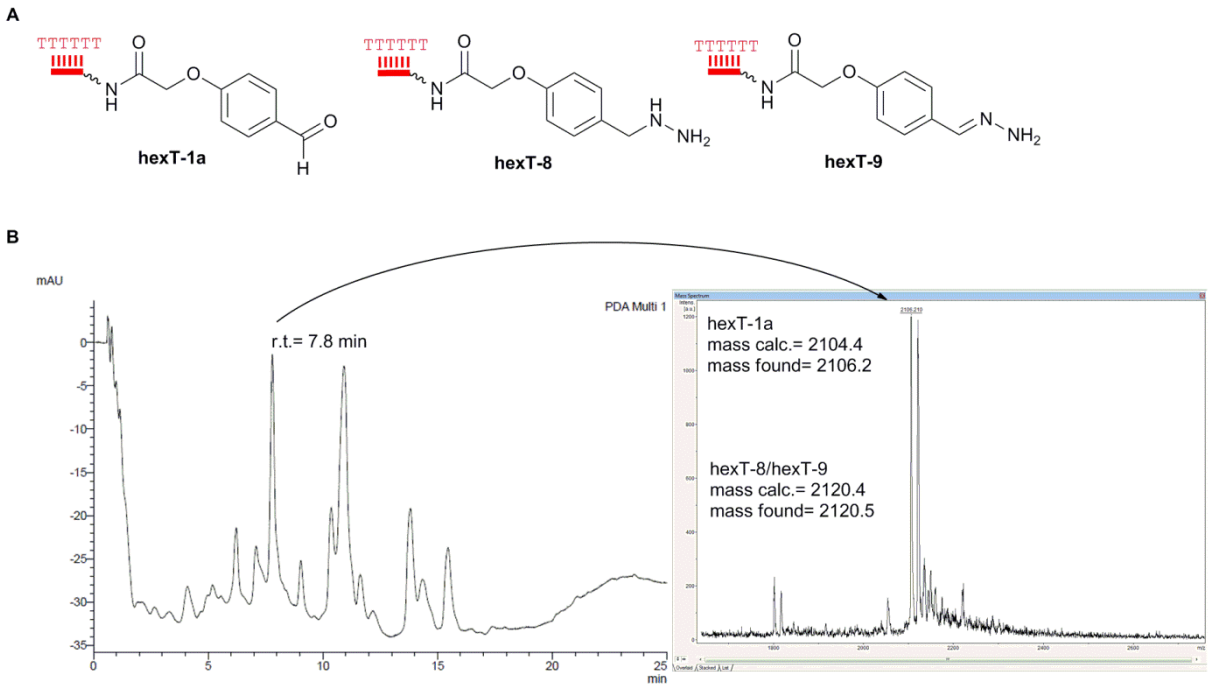


Fig S16. MALDI MS analysis of the isolated product **hexT-1** and **hexT-8/9** (r.t.= 7.8 min) from the experiment No.1 (Fig. S14b, Table S3a). A) Structures of starting material **hexT-1a**, **hexT-8** which forms upon removal of the Boc group, and **hexT-9** which forms upon removal of the Boc group from the unreduced hydrazone; B) MALDI MS spectrum of the isolated mixture of hexathymidine conjugates **hexT-1** and **hexT-8/9** from experiment **No.1**. Conjugates **hexT-1a** and **hexT-8/9** are eluting together at r.t.= 7.8 min, and based on MALDI MS spectrum they are present in a 1:1 mixture. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

4.2.2 Detection of the condensation product of the reaction of aldehyde **hexT-1a** with carbazate **7**

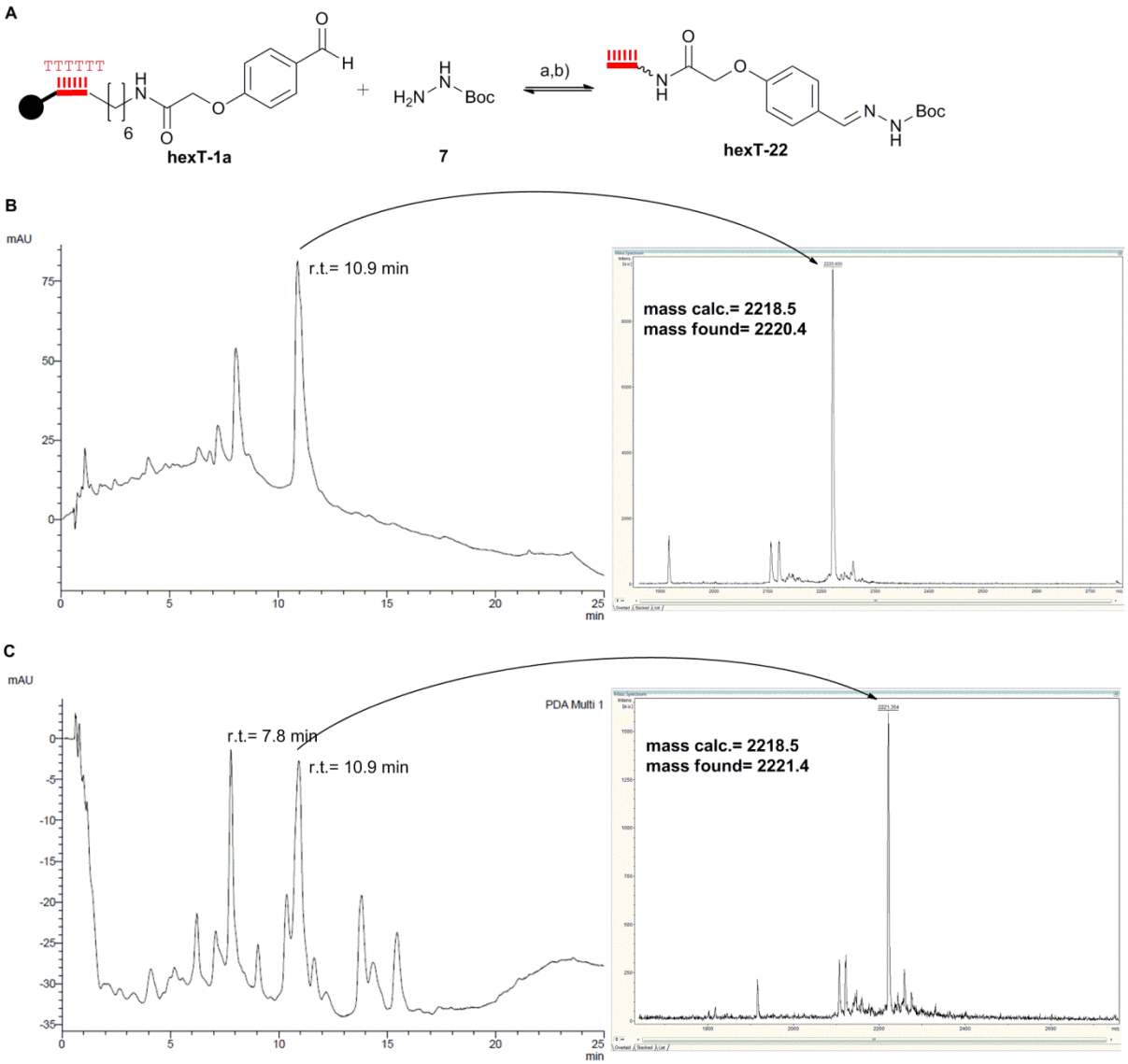


Fig S17. MALDI MS analysis of the isolated hydrazone conjugate **hexT-22** (r.t.= 10.9 min) from the condensation reaction between aldehyde **hexT-1a** and carbazate **7**. A) Reaction scheme of the formation of hydrazone **hexT-22** by condensation of **hexT-1a** with carbazate **7**; reaction conditions: a) 300 mM MOPS buffer, room temperature, overnight, *note that we did not add any reducing agent in this experiment*; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; B) MALDI MS spectrum of the isolated hexathymidine conjugate **hexT-22** from the condensation reaction; C) MALDI MS spectrum of the isolated hexathymidine conjugate **hexT-22** from the reaction mixture of experiment **No. 1** (Table S3a, Fig. S14b). Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

4.2.3 Detection of the condensation product **hexT-10**

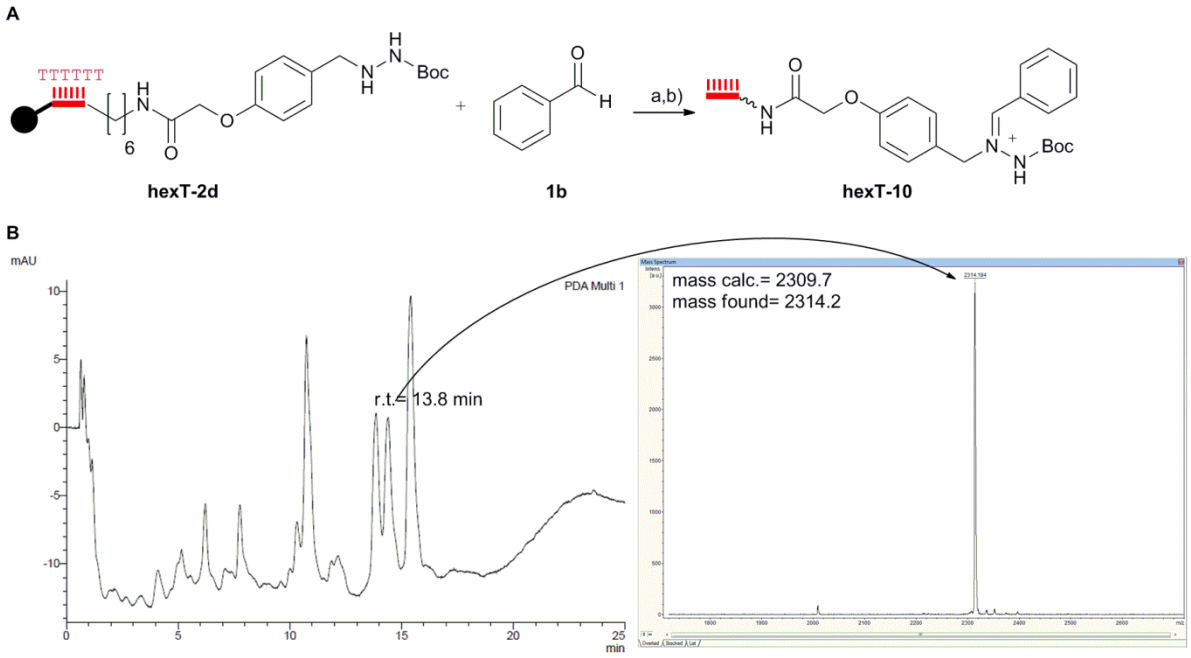


Fig S18. MALDI MS analysis of the isolated product **hexT-10** (r.t.= 13.8 min) from Fig. S14e. A) Reaction scheme of the **hexT-10** formation; a) reaction condition **No. 4** (Table S3a); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; B) MALDI MS spectrum of the isolated conjugate **hexT-10**. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

4.2.4 Detection of the putative Mannich-type adduct **hexT-11**

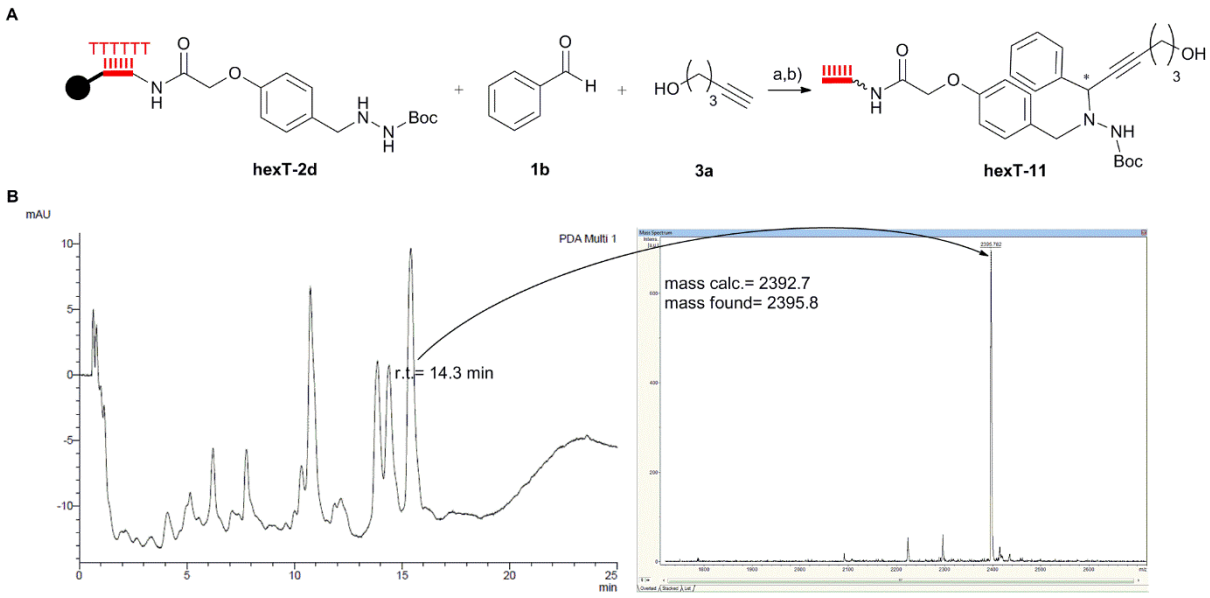


Fig S19. MALDI MS analysis of the isolated linear intermediate **hexT-11** (r.t.= 14.3 min) from Fig S14e. A) Reaction scheme of the linear intermediate **hexT-11** formation; a) reaction condition **No. 4** (Table S3a); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; B) MALDI MS spectrum of the isolated linear intermediate **hexT-11**. *We tentatively assigned the structure of the Mannich-type adduct to the product, which elutes at 14.3 min. It displays the same mass as the hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate* ***hexT-4e****. A product of this type was also found as side product in the Au(I)-*

mediated annelation reaction described in ref. 2. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

4.2.5 Detection of the hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate **4e**

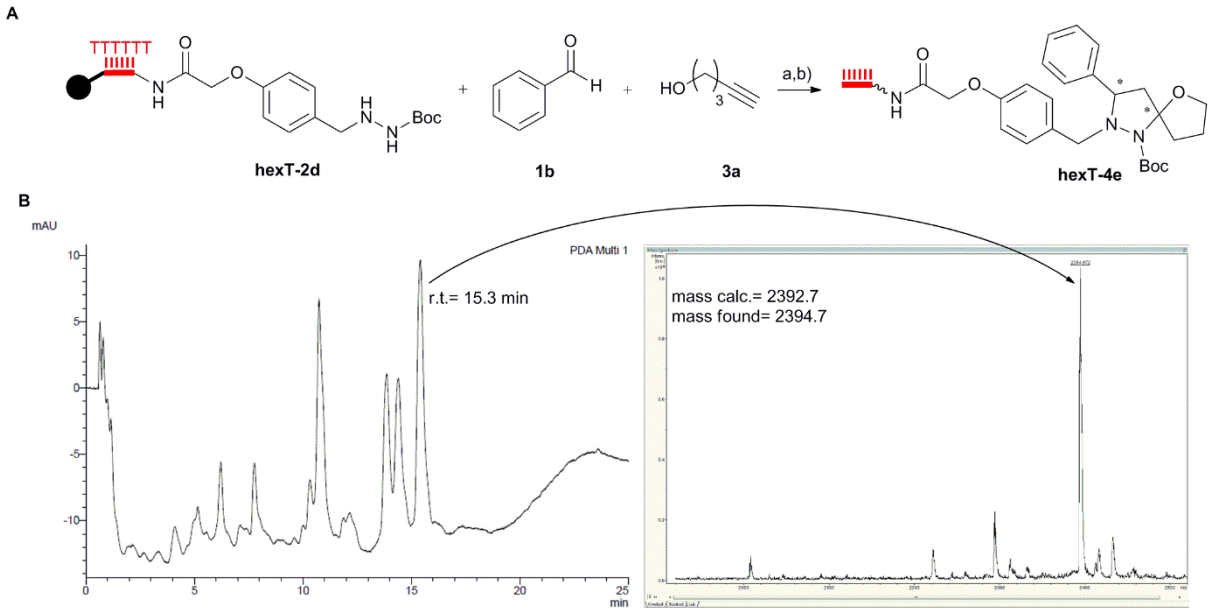
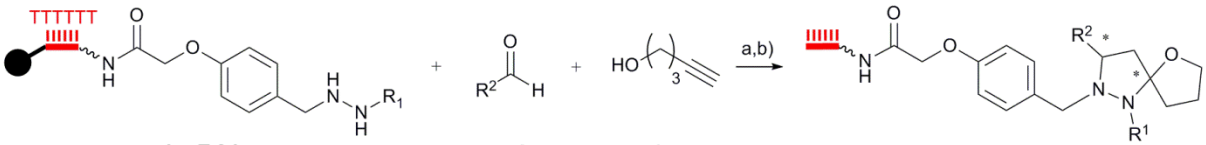


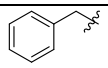
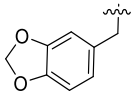
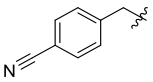
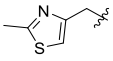
Fig S20. MALDI MS analysis of the isolated spirocycle conjugate **hexT-4e** (r.t.= 15.3 min) from Fig S14e. A) Reaction scheme of the hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate **4e** formation; a) reaction condition **No. 4** (Table S3a); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; B) the hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate **hexT-4e** elutes at the similar retention time as the hexT-spirocycle conjugate **4a** (Fig. S10); MALDI MS spectrum of the isolated spirocycle conjugate **hexT-4e**. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

4.3. Reaction scope

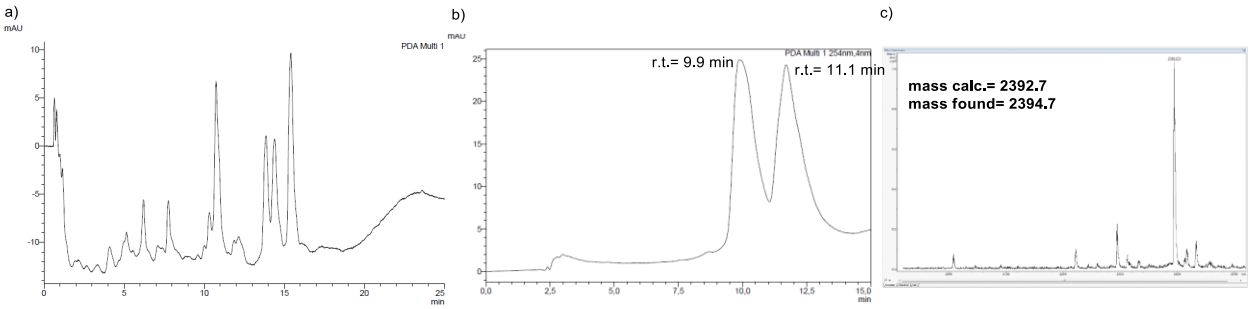
Table S4 Scope of the reaction to hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugates **hexT-4**. a) Reaction condition **No. 4** (Table S3a); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature). Filled circle denotes controlled pore glass, CPG; wavy bond to hexT: 5'-(C6)-amino-linker.



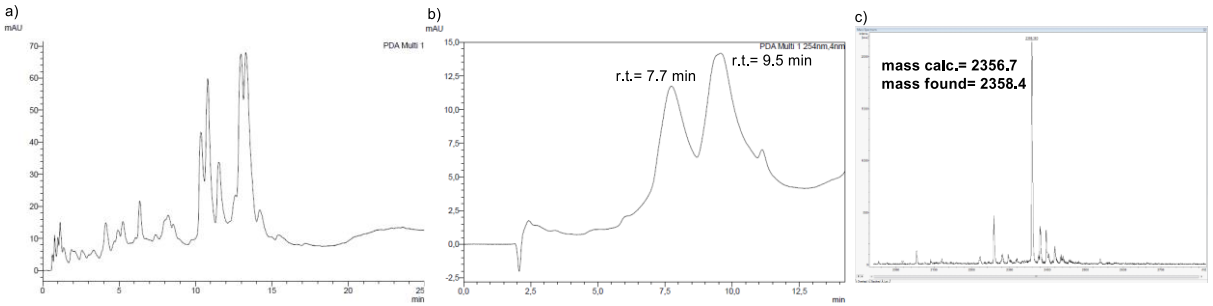
hexT-2d **1b-f** **3a** **hexT-4e-i**

entry	hexT	aldehyde	R^1	R^2	yield/ [nmol][a]	mass calc. mass found[b]
1	**hexT-4e**	**1b**	*tert*-Boc		1.1	*2392.7* 2394.7
2	**hexT-4f**	**1c**	*tert*-Boc		1.4	*2436.8* 2438.9
3	**hexT-4g**	**1d**	*tert*-Boc		3.8	*2417.8* 2419.7
3	**hexT-4h**	**1e**	*tert*-Boc		1.5	*2356.7* 2358.4
5	**hexT-4i**	**1f**	*tert*-Boc		2.7	*2413.8* 2415.6

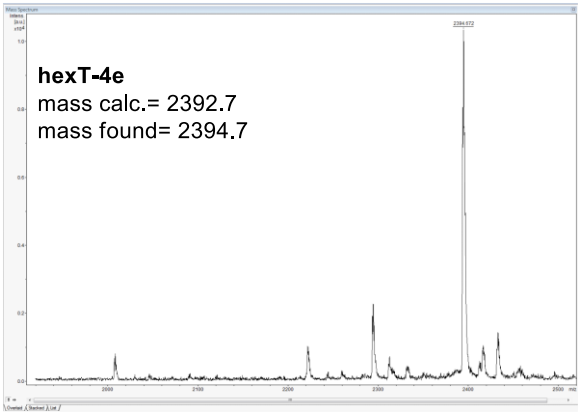
[a] measured by Nanodrop; [b] measured by MALDI MS.

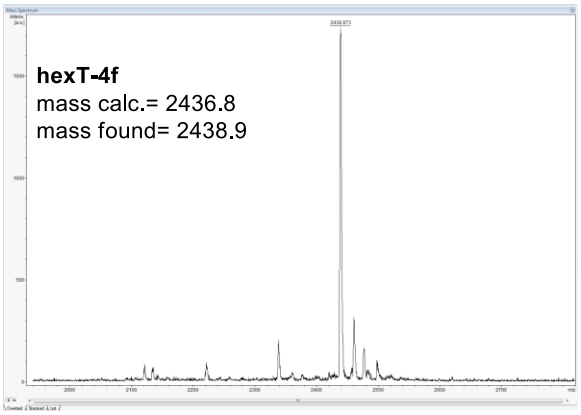


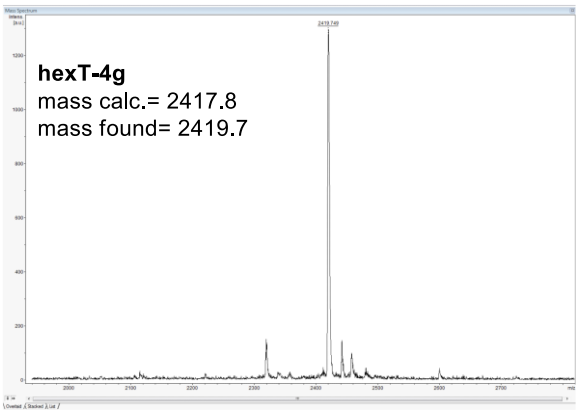
a) HPLC trace (preparative HPLC) of the crude hexT-spirocycle conjugate **hexT-4e**; b) HPLC trace (analytical HPLC) of the purified hexT-spirocycle conjugate **hexT-4e**; c) MALDI MS spectrum of the purified hexT-spirocycle conjugate **hexT-4e**.

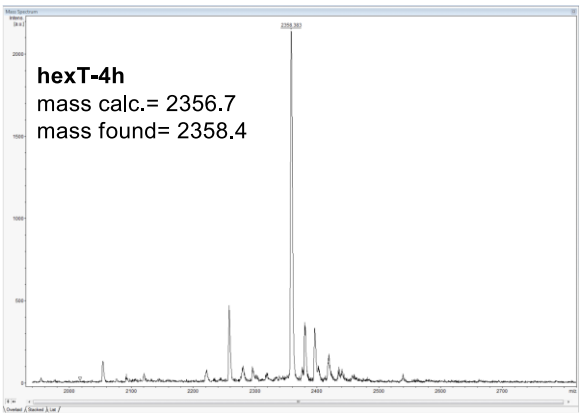


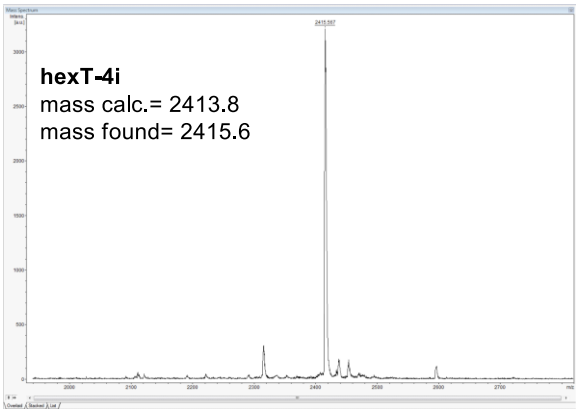
a) HPLC trace (preparative HPLC) of the crude hexT-spirocycle conjugate **hexT-4h**; b) HPLC trace (analytical HPLC) of the purified hexT-spirocycle conjugate **hexT-4h**; c) MALDI MS spectrum of the purified hexT-spirocycle conjugate **hexT-4h**.


hexT-4e
mass calc.= 2392.7
mass found= 2394.7


hexT-4f
mass calc.= 2436.8
mass found= 2438.9


hexT-4g
mass calc.= 2417.8
mass found= 2419.7


hexT-4h
mass calc.= 2356.7
mass found= 2358.4


hexT-4i
mass calc.= 2413.8
mass found= 2415.6

5. Synthesis of hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugates from hexT-alkynol conjugate 3c

5.1 Optimization of reaction conditions of the Au(I)-mediated spirocycle formation

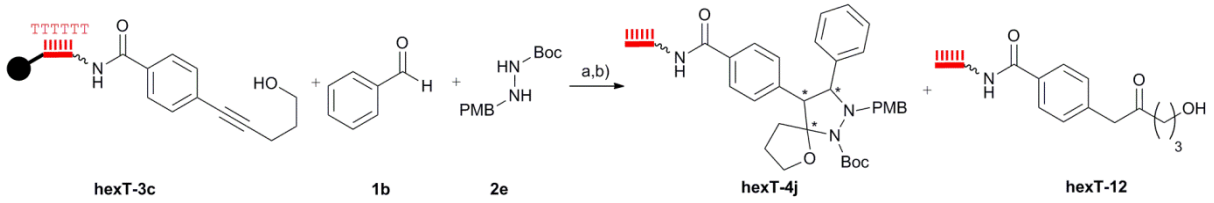


Fig S21. Au(I)-mediated synthesis of hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate **4j** from hexT-alkynol conjugate **3c**. a) For conditions see Table S5; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature. Figs S22-S28 show HPLC traces of the crude reaction mixtures (preparative HPLC). Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

The solid support (controlled pore glass, CPG) containing the hexathymidine-alkynol conjugate **hexT-3c** (30 nmol, ca. 1.1 mg) was suspended in 30 µL of a solvent (see Table S5). Then, benzaldehyde **1b**, and *tert*-butyl 2-(4-methoxybenzyl)hydrazinecarboxylate **2e**, each 30 µmol, dissolved in 30 µL of a same solvent (prepared as stock) were added to **hexT-3c**. This was followed by an equimolar mixture of catalyst Au(I)/$AgSbF_6$ (see Table S5) at the amounts given in Table S5 which were suspended (Au(I)) or dissolved ($AgSbF_6$), respectively in 30 µL of the same solvent. The suspension of the catalyst was prepared as stock. Prior addition to **hexT-3c** it was vortexed and pipetted up and down to add a homogeneous suspension. The reactions were shaken at the indicated temperatures overnight in Eppendorf tubes. The reactions were run in a presence of drying agents or without (see Table S5). Then, the CPG was filtered off, washed with each 3 x 200 µL of 0.1 M EDTA, DMF, MeOH, MeCN and CH_2Cl_2 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. 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 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 % – 80 % 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 S5: Optimization of reaction conditions for the Au(I)-mediated reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**.

	No.	hydrazide [eq][a]	aldehyde [eq][a]	catalyst [eq][a]	time [h]	solvent	drying agent	catalyst[b]	T [°C]	**hexT-4j** [%][c]
a) solvent	**1**	500	1000	250	17	THF	-	Au(I)/$AgSbF_6$	45	-
without	**2**	500	1000	250	17	$C_2H_4Cl_2$	-	Au(I)/$AgSbF_6$	45	-
drying agent	**3**	500	1000	250	17	toluene	-	Au(I)/$AgSbF_6$	45	-
	4	500	1000	250	17	MeCN	-	Au(I)/$AgSbF_6$	45	-
	5	500	1000	250	17	DMF	-	Au(I)/$AgSbF_6$	45	-
b) drying agent	**6**	500	1000	250	17	MeCN	MS 5Å	Au(I)/$AgSbF_6$	45	40
	7	500	1000	250	17	MeCN	$MgSO_4$	Au(I)/$AgSbF_6$	45	35
c) solvent	**8**	500	1000	250	17	THF	MS 5Å	Au(I)/$AgSbF_6$	45	30
	9	500	1000	250	17	$C_2H_4Cl_2$	MS 5Å	Au(I)/$AgSbF_6$	45	25
	10	500	1000	250	17	toluene	MS 5Å	Au(I)/$AgSbF_6$	45	trace
	11	500	1000	250	17	MeCN	MS 5Å	Au(I)/$AgSbF_6$	45	40
	12	500	1000	250	17	DMF	MS 5Å	Au(I)/$AgSbF_6$	45	-
d) time	**13**	500	1000	250	17	THF	MS 5Å	Au(I)/$AgSbF_6$	45	30
	14	500	1000	250	41	THF	MS 5Å	Au(I)/$AgSbF_6$	45	10
e) temperature	**15**	500	1000	250	17	MeCN	MS 5Å	Au(I)/$AgSbF_6$	25	-
	16	500	1000	250	17	MeCN	MS 5Å	Au(I)/$AgSbF_6$	45	40
	17	500	1000	250	17	MeCN	MS 5Å	Au(I)/$AgSbF_6$	50	30
	18	500	1000	250	17	MeCN	MS 5Å	Au(I)/$AgSbF_6$	55	25
f) catalyst	**19**	500	1000	250	17	MeCN	MS 5Å	Au(I)	45	-
	20	500	1000	250	17	MeCN	MS 5Å	$AgSbF_6$	45	-
	21	500	1000	250	17	MeCN	MS 5Å	Au(I)/$AgSbF_6$	45	40

[a] versus the solid support-bound hexT-alkynol conjugate **hexT-3c**; [b] Au(I): tris(2,4-di-*tert*-butylphenyl)phosphite]gold chloride [c] % conversion estimated based on the area under curve of the product peak versus the starting material peak in the HPLC-trace of the crude.

a) Screening different solvents without drying agent for the Au(I)-mediated reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**

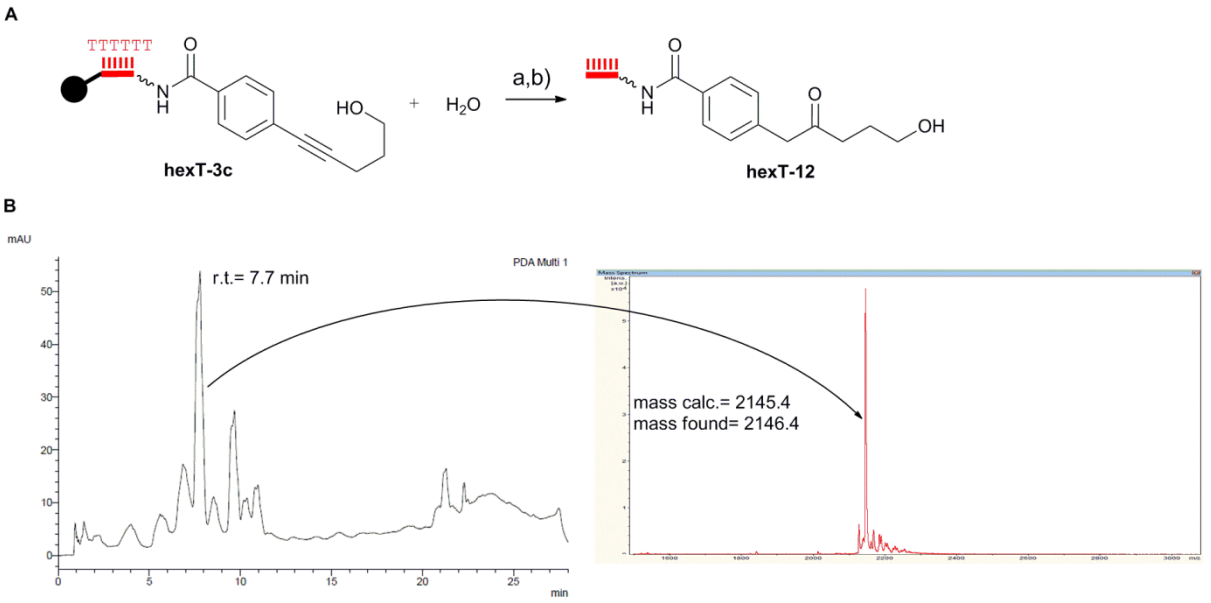


Fig S22. In all reactions, we obtained the product **hexT-12** (r.t.= 7.7 min) of the Au(I)-mediated water addition. A) Reaction scheme of the water addition reaction; a) reaction condition **No. 1-5** (Table S5a); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature; B) MALDI MS spectrum of the isolated hexathymidine conjugate **hexT-12**. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.

b) Screening different drying agents for the Au(I)-mediated reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**

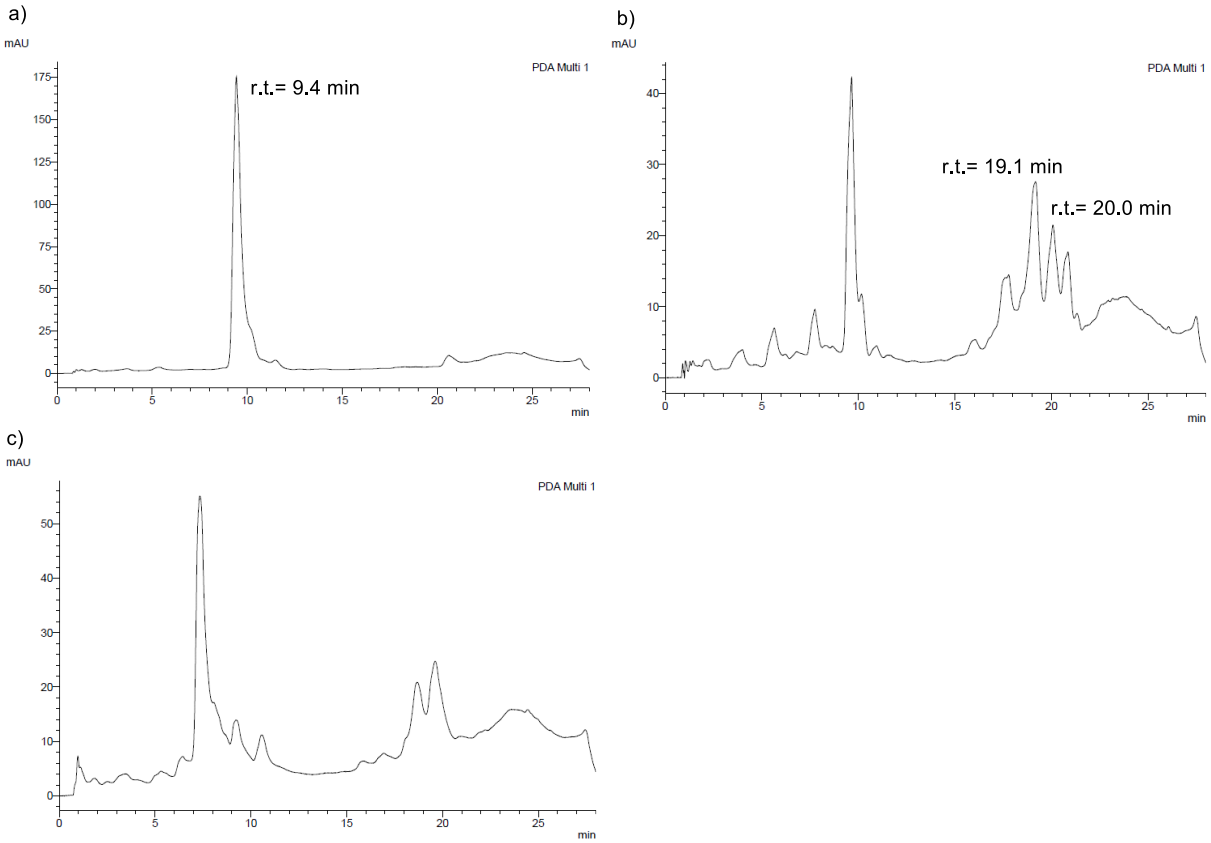


Fig S23. Effect of different drying agents on the Au(I)-mediated reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**, for reaction conditions see Table S5b. a) HPLC trace of the starting material (hexathymidine-alkynol conjugate **hexT-3c**); b) HPLC trace of experiment **No. 6**, formation of a product mixture with the target spirocycle **hexT-4j** (r.t.= 19.1 min and r.t.= 20.0 min), and lipophilic side products; c) HPLC trace of experiment **No. 7**, formation of the target spirocycle **hexT-4j**, and water addition product **hexT-12** (r.t.= 7.7 min) as main product. The spirocycle conjugate **hexT-4j** was formed as a complex mixture of closely eluting diastereoisomers (r.t.= 19.1 min and r.t.= 20.0 min), and lipophilic side products that were not identifiable by MALDI MS.

c) Screening different solvents for the Au(I)-mediated reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**

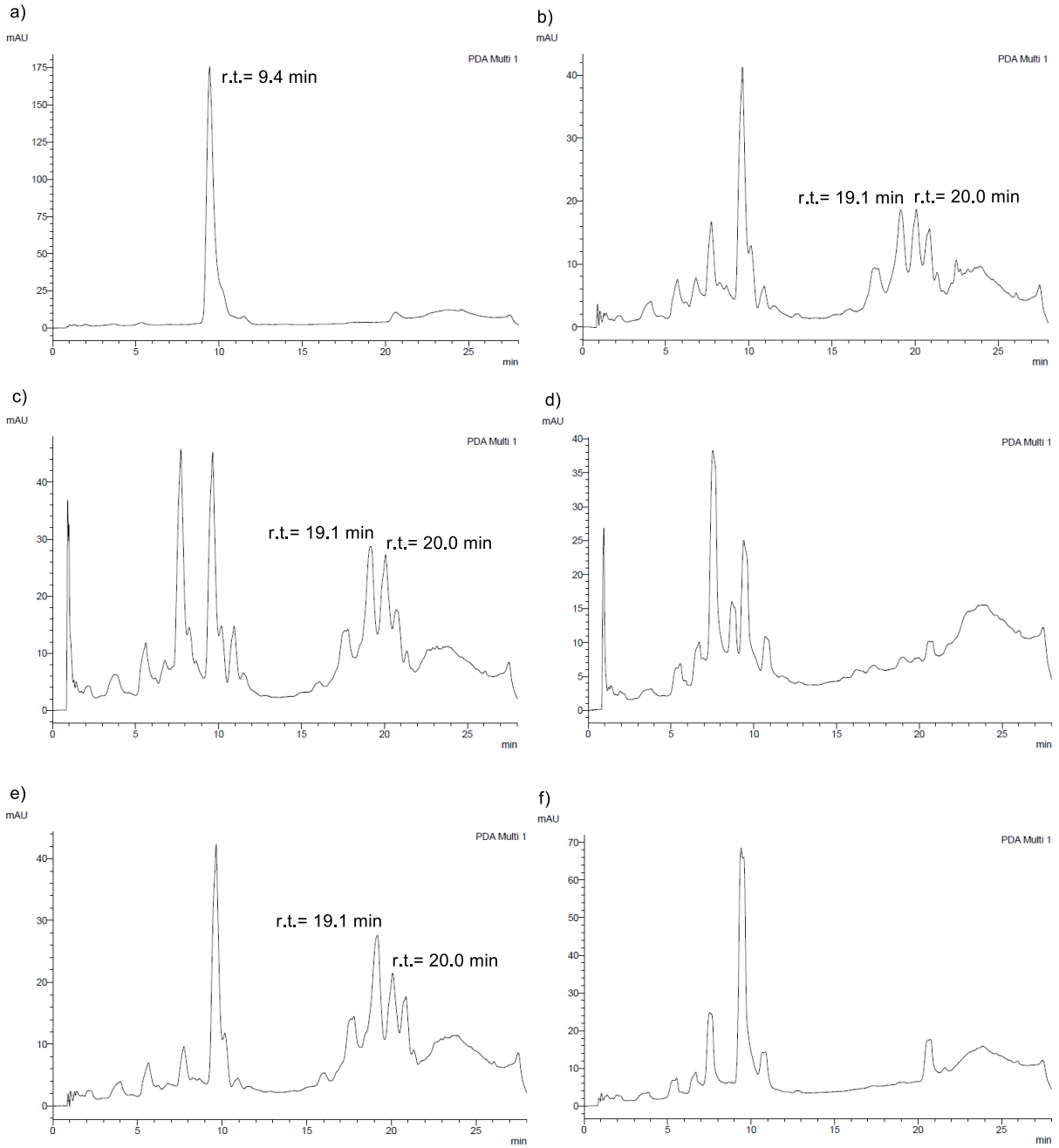


Fig S24. Effect of different solvents on the Au(I)-mediated reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**, for reaction conditions see Table S5c. a) HPLC trace of the starting material (hexathymidine-alkynol conjugate **hexT-3c**); b) HPLC trace of experiment **No. 8**; formation of a product mixture with the target spirocycle **hexT-4j** (r.t.= 19.1 min and r.t.= 20.0 min), lipophilic side products and the water addition product **hexT-12** (r.t.= 7.7 min); c) HPLC trace of experiment **No. 9**; formation of a product mixture with the spirocycle **hexT-4j**, lipophilic side products, and the water addition product **hexT-12** (r.t.= 7.7 min) as main product; d) HPLC trace of experiment **No. 10**; formation of the water addition product **hexT-12** (r.t.= 7.7 min) as main product; e) HPLC trace of experiment **No. 11** (experiment identical to Fig S23b); f) HPLC trace of experiment **No. 12**, formation of the water addition product **hexT-12** as minor product. The spirocycle conjugate **hexT-4j** was formed as a complex mixture of closely eluting diastereoisomers (r.t.= 19.1 min and r.t.= 20.0 min), and lipophilic side products that were not identifiable by MALDI MS.

d) Testing different reaction times for the Au(I)-mediated reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**

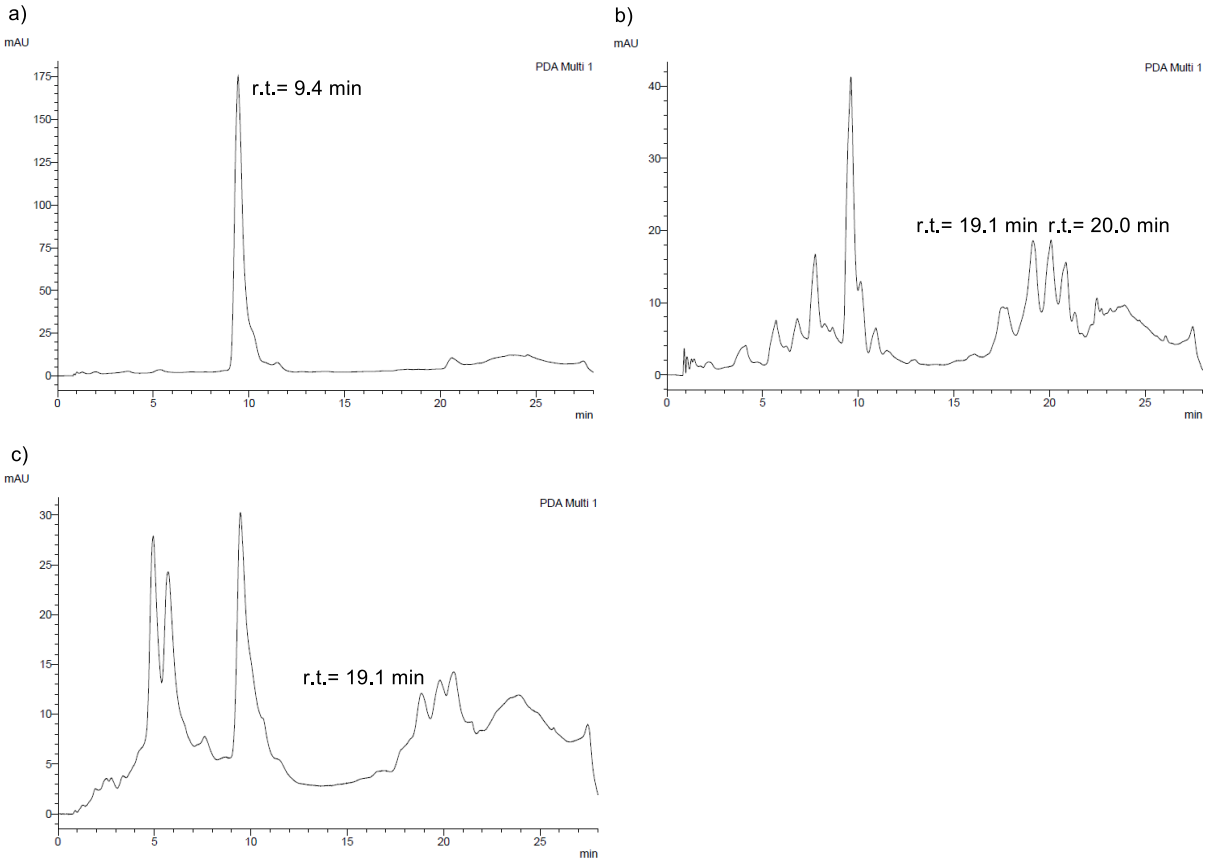


Fig S25. Effect of different reaction times on the Au(I)-mediated reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**, for reaction conditions see Table S5d. a) HPLC trace of the starting material (hexathymidine-alkynol conjugate **hexT-3c**); b) HPLC trace of experiment **No. 13** (same experiment as Fig S24b); c) HPLC trace of experiment **No. 14**; formation of a product mixture with the target spirocycle **hexT-4j**, lipophilic side products that were not identifiable by MALDI MS, and two polar side products as main products. Prolonged reaction time had no positive effect on reaction progress. The spirocycle conjugate **hexT-4j** was formed as a complex mixture of closely eluting diastereoisomers (r.t.= 19.1 min and r.t.= 20.0 min), and lipophilic side products that were not identifiable by MALDI MS.

e) Testing different reaction temperatures for the Au(I)-mediated reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**

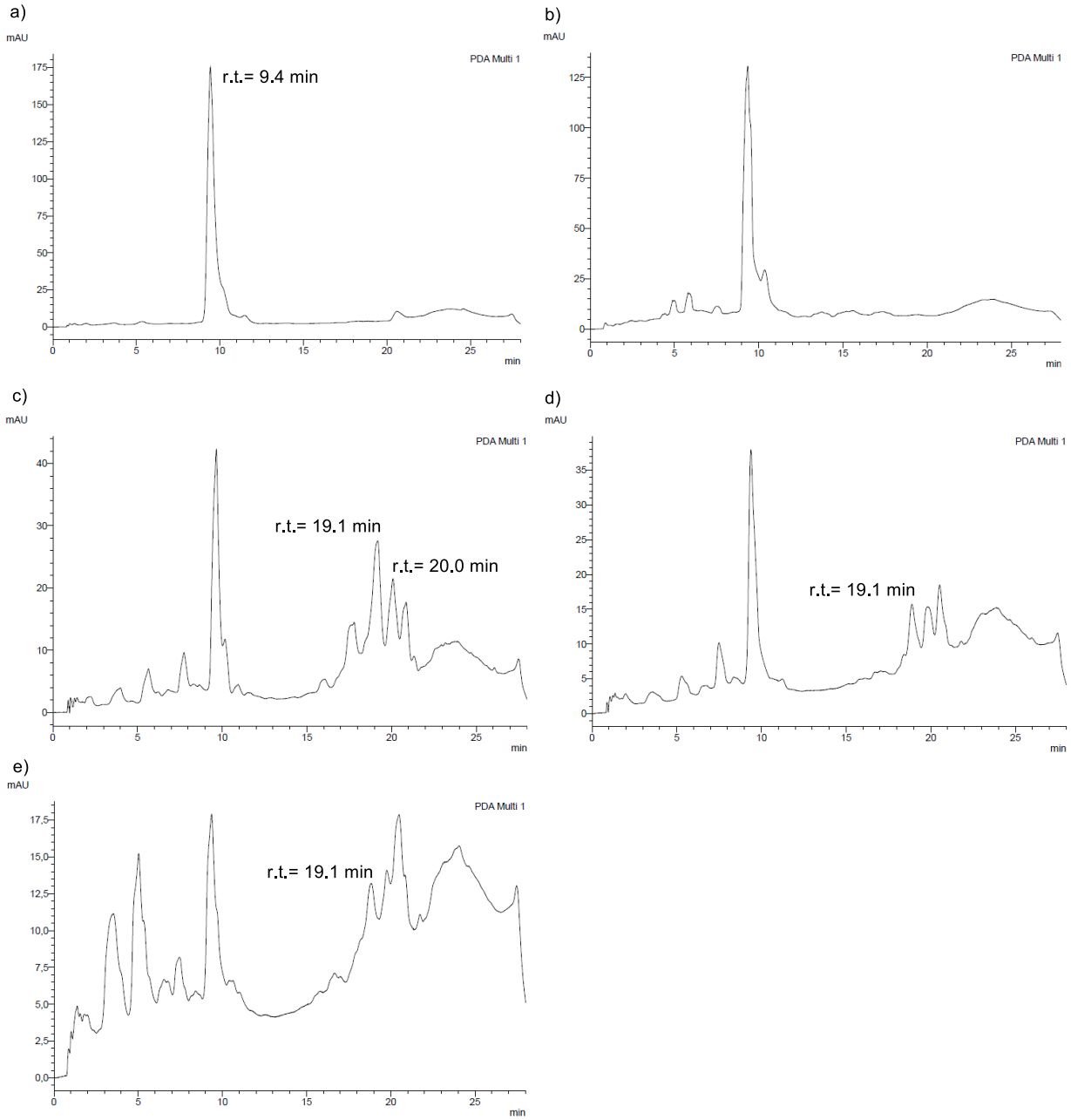


Fig S26. Effect of different temperatures on the Au(I)-mediated reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**, for reaction conditions see Table S5e. a) HPLC trace of the starting material (hexathymidine-alkynol conjugate **hexT-3c**); b) HPLC trace of experiment **No. 15**; no product formation; c) HPLC trace of experiment **No. 16** (experiment identical to Fig S23b); d) HPLC trace of experiment **No. 17**; formation of a product mixture with the target spirocycle **hexT-4j** (r.t.= 19.1 min and r.t.= 20.0 min), and lipophilic side product; e) HPLC trace of experiment **No. 18**; formation of a product mixture with the target spirocycle **hexT-4j**, lipophilic side products and several more polar side products. The spirocycle conjugate **hexT-4j** was formed as a complex mixture of closely eluting diastereoisomers (r.t.= 19.1 min and r.t.= 20.0 min), and side products that were not identifiable by MALDI MS.

f) Control experiment with different catalysts for the reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**

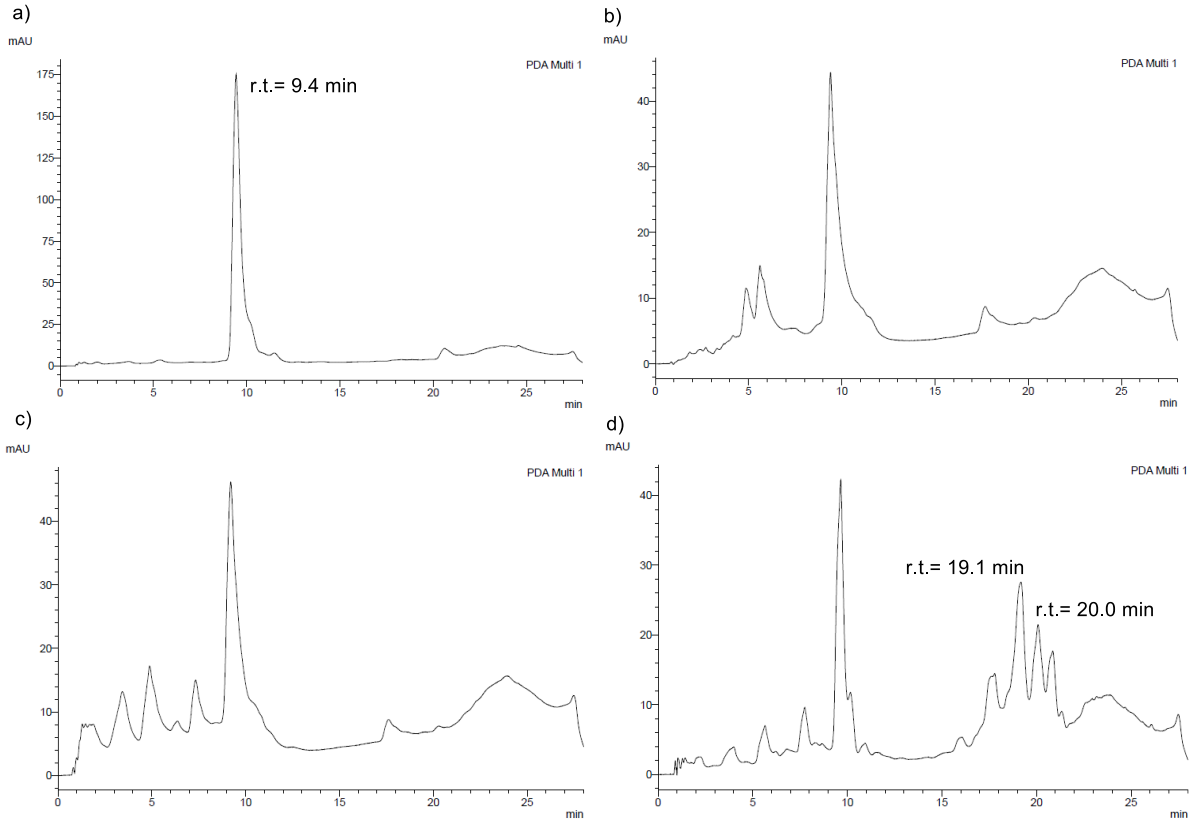


Fig S27. Control experiment with different catalysts for the reaction to hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j**, for reaction conditions see Table S5f. a) HPLC trace of the starting material (hexathymidine-alkynol conjugate **hexT-3c**); b) HPLC trace of experiment **No. 19**; no product formation; c) HPLC trace of experiment **No. 20**; no product formation; d) HPLC trace of experiment **No. 21** (experiment identical to Fig S23b). The spirocycle conjugate **hexT-4j** was formed as a complex mixture of closely eluting diastereoisomers (r.t.= 19.1 min and r.t.= 20.0 min), and lipophilic side products that were not identifiable by MALDI MS.

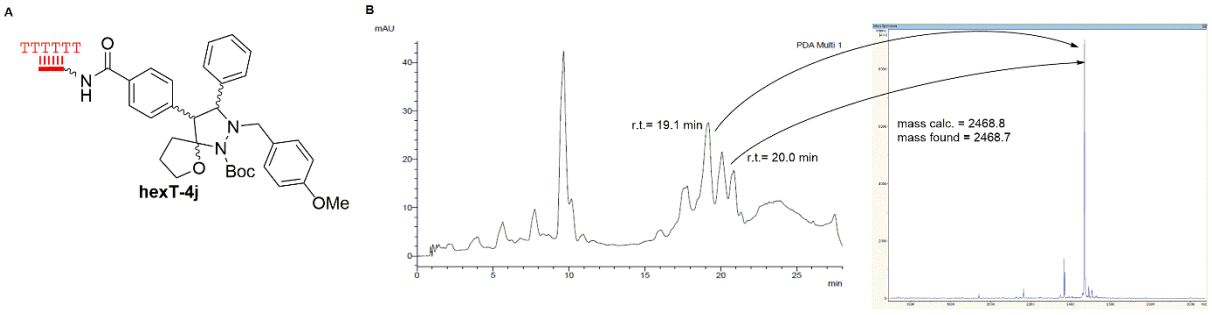
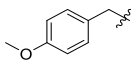
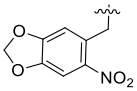
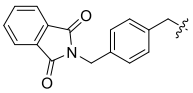
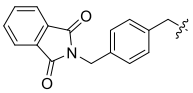
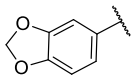
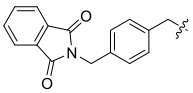
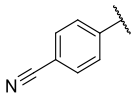
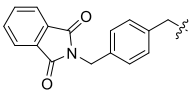


Fig S28. MALDI MS of the isolated hexT-6-oxa-1,2-diazaspiro [4,4]nonane conjugate **hexT-4j** (HPLC trace from Fig S23b, compound eluted at r.t.= 19.1 min and r.t.= 20.0 min). A) Structure of **hexT-4j**; B) MALDI MS of the isolated hexathymidine conjugate **hexT-4j**. Spirocycle **hexT-4j** is formed as a complex mixture of diastereoisomers. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker. *In contrast to the spirocycles **4a** and **4e**, which are expected to form two pairs of diastereoisomers, spirocycle **4j** has three stereocenters thus giving rise to a far more complex product mixture. This might explain the observation of two distinct product peaks in the HPLC trace that could be assigned to the*

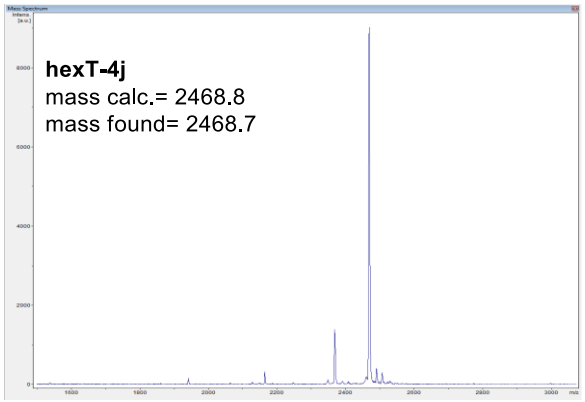
*target spirocycle (instead of one product peak which we observe in the HPLC traces (preparative HPLC) of the crude products **4a** and **4e**).*

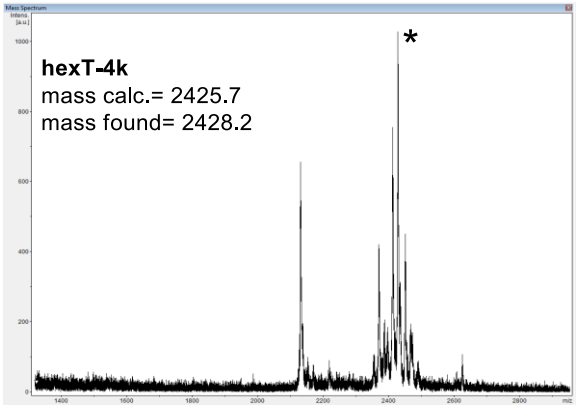
5.2. Reaction scope

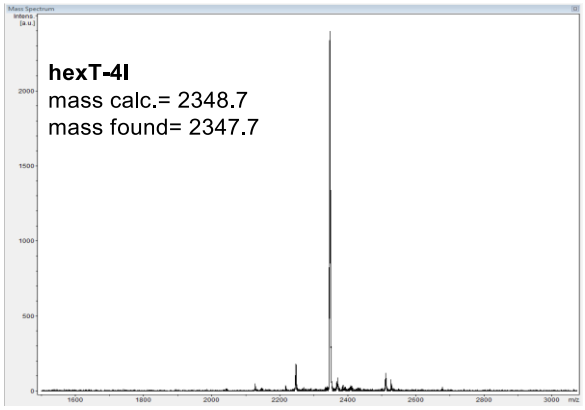
Table S6 Scope of the reaction to hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugates **hexT-4**. a) Reaction condition **No. 4** (Table S5c, **No. 4**); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature).

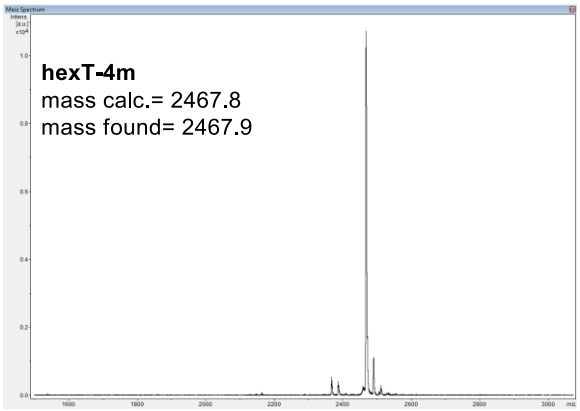
entry	hexT	hydrazide	aldehyde	R^1	R^2	R^3	yield/ [nmol] [a]	*mass calc.* mass found[b]
1	**hexT-4j**	**2e**	**1b**	*tert*-Boc			2.5	*2468.8* 2468.7
2	**hexT-4k**	**2f**	**1b**	Ac			0.9	*2425.7* 2428.2
3	**hexT-4l**	**2b**	**1b**	*tert*-Boc			2.0	*2348.7*[c] 2347.7[c]
4	**hexT-4m**	**2g**	**1b**	*tert*-Boc			2.9	*2467.8*[d] 2467.9
5	**hexT-4n**	**2g**	**1c**	*tert*-Boc			1.5	*2759.2*[d,e] 2759.2
6	**hexT-4o**	**2g**	**1d**	*tert*-Boc			1.8	*2740.2*[d,e] 2739.2
7	**hexT-4p**	**2g**	**1e**	*tert*-Boc			0.4	*2679.1*[d,e] 2681.0

[a] measured by Nanodrop; [b] measured by MALDI MS; [c] loss of the photolabile 3,4-methylendioxy-6-nitrobenzyl group upon irradiation in the mass spectrometer; [d] phthalimide group removed in the product; [e] conjugates contain PEG(4) linker.


hexT-4j
mass calc.= 2468.8
mass found= 2468.7


*
hexT-4k
mass calc.= 2425.7
mass found= 2428.2


hexT-4l
mass calc.= 2348.7
mass found= 2347.7


hexT-4m
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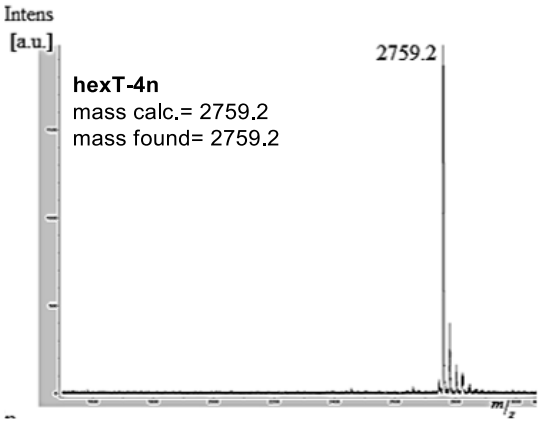
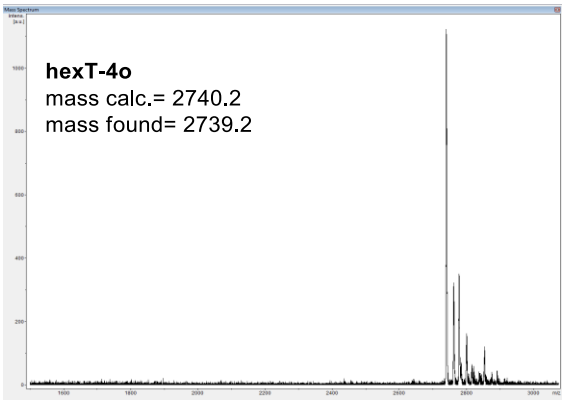
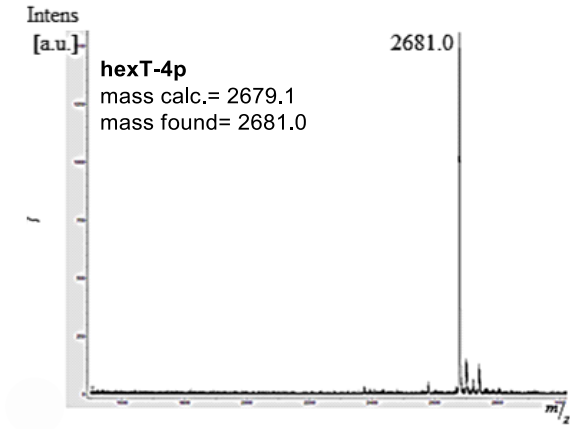
mass found= 2467.9


Intens
[a.u.]
2759.2
hexT-4n
mass calc.= 2759.2
mass found= 2759.2
m/z


hexT-4o
mass calc.= 2740.2
mass found= 2739.2


Intens
[a.u.]
2681.0
hexT-4p
mass calc.= 2679.1
mass found= 2681.0
m/z

6. Au(I)-mediated synthesis of DNA-6-oxa-1,2-diazaspiro[4.4]nonane conjugates 4 from DNA-aldehyde conjugates 1a

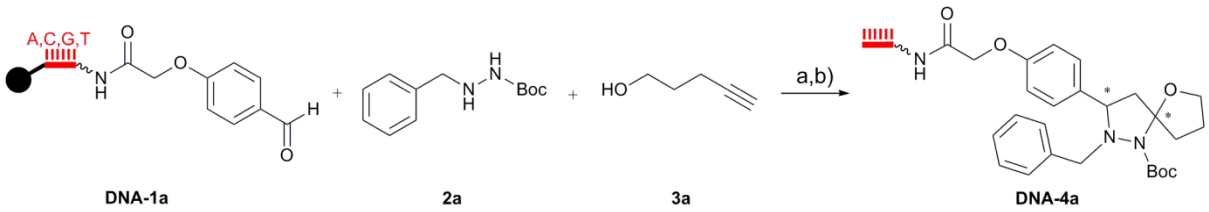


Fig S29. Au(I)-mediated synthesis of DNA-6-oxa-1,2-diazaspiro[4.4]nonane conjugates **4a** from DNA-aldehyde conjugates **1a**. a) Reaction condition **No. 17** (Table S1); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to DNA: 5'-(C6)-amino-linker.

The solid support containing a hexamer DNA-aldehyde conjugate **1a** (30 nmol, ca. 1.1 mg) was suspended in 15 µL of THF. Then, *tert*-butyl 2-benzylhydrazinecarboxylate **2a** (15 µmol, 500 eq.) and pent-4-yn-1-ol **3a** (30 µmol, 1000 eq.), dissolved in 15 µL of THF (prepared as stock) were added to **1a**. This was followed by an equimolar mixture of Au(I)/$AgSbF_6$ (7.5 µmol, 250 eq.), which was suspended (Au(I)) or dissolved ($AgSbF_6$), respectively in 15 µL of THF. The suspension of the catalyst was prepared as stock. Prior addition to **1a** it was vortexed and pipetted up and down. The reactions were shaken at room temperature overnight in Eppendorf tubes under normal atmosphere. Then, the CPG was filtered off, washed with each 3 x 200 µL of 0.1 M EDTA, DMF, MeOH, MeCN, and CH_2Cl_2, and dried *in vacuo* for 15 min. The DNA hexamer-conjugates were deprotected and cleaved from the CPG by treatment with 500 µL of AMA for 30 minutes (hexT/C/TC) or for 4 hours (hexA/hexG/hexACGT) at room temperature. Then, 20 µL of 1 M Tris buffer (pH= 7.5) were added, the mixtures were dried, dissolved in 100 µL of distilled water, and purified by RP-HPLC (Gemini, 5u, C18, 110A column; 50*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20 % – 80 % of methanol over 18 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 RP-HPLC (Gemini, 5u, C18, 110A column; 100*4.6 mm) with a gradient of aqueous triethylammonium acetate buffer (10 mM, pH= 8) and methanol (30 % – 90 % of methanol over 15 min).

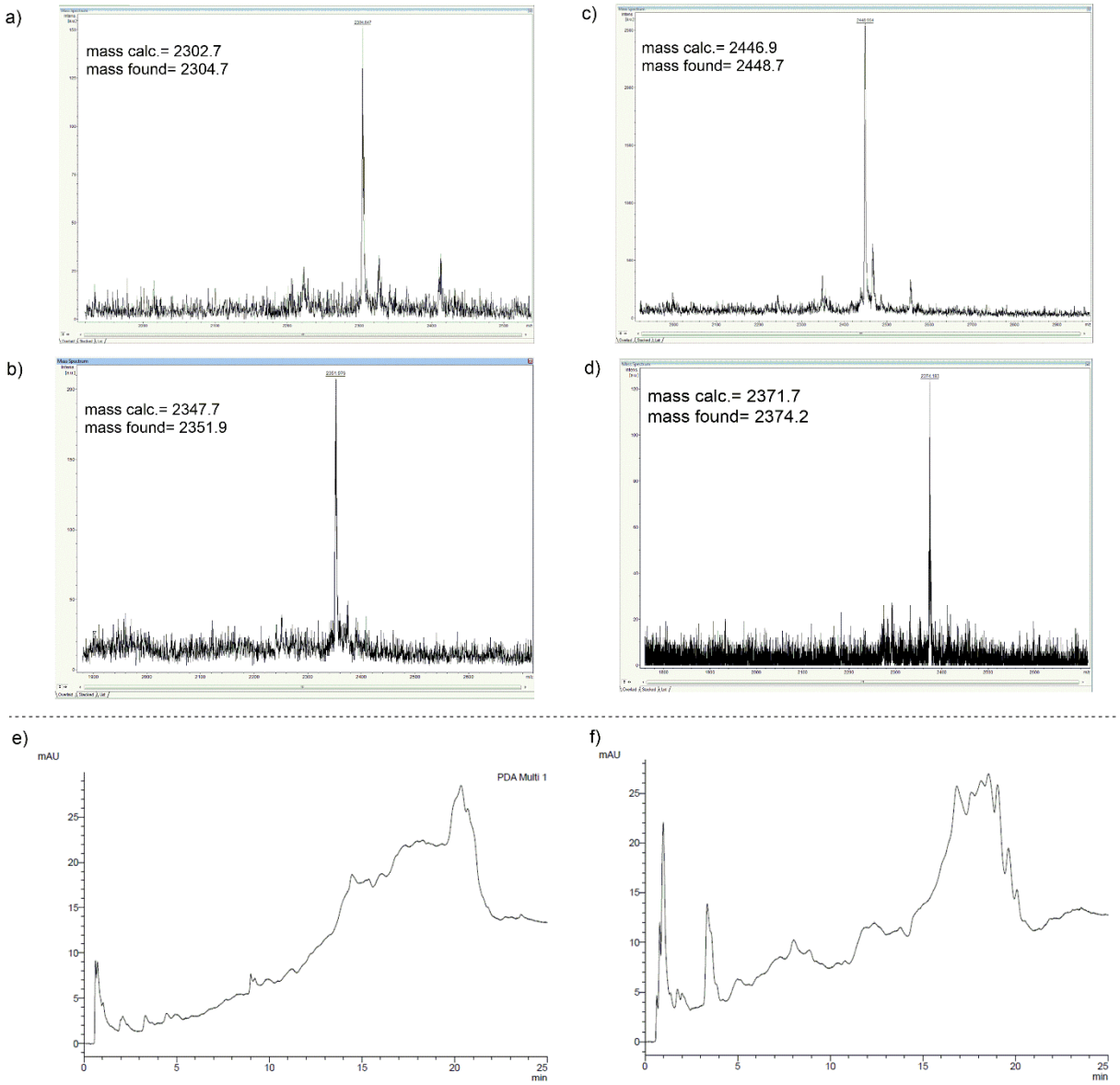


Fig S30. Synthesis of DNA-6-oxa-1,2-diazaspiro[4.4]nonane conjugates **4a** from DNA-aldehyde conjugates **1a**, using reaction condition **No. 17** (Table S1); a) MALDI MS spectrum of **hexC-4a**; b) MALDI MS spectrum of **hexTC-4a**; c) MALDI MS spectrum of **hexA-4a**; d) MALDI MS spectrum of **hexTCA-4a**; e) HPLC trace of **hexG-4a**, showing massive degradation of the DNA; f) HPLC trace of **hexACGT-4a**, showing massive degradation of the DNA.

7. Synthesis of hexapyrimidine-pyrazoline- and ß-carboline-conjugates

7.1 Synthesis of TC-DNA tryptophane conjugate hexTC-14 and TC-DNA alkyne conjugate hexTC-17

The hexTC oligonucleotide (250 nmol, ca. 9 mg) was coupled either with Fmoc-L-tryptophane **13** (10.7 mg, 25 µmol, 100 eq.) to furnish **hexTC-14**, or with *p*-ethynylbenzoic acid **16** (3.7 mg, 100 µmol, 100 eq.) to furnish **hexTC-17** according to the procedure for coupling of carboxylic acids to amino-modified DNA sequences. For analysis, an aliquot of ca. 10 nmol of each conjugate **hexTC-14** and **hexTC-17** 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 mixtures were dried in a SpeedVac, re-dissolved in 100 µL of distilled water, and the conjugates were purified by RP-HPLC (Gemini, 5u, C18, 110A column; 50*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20 % – 80 % of methanol over 18 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.

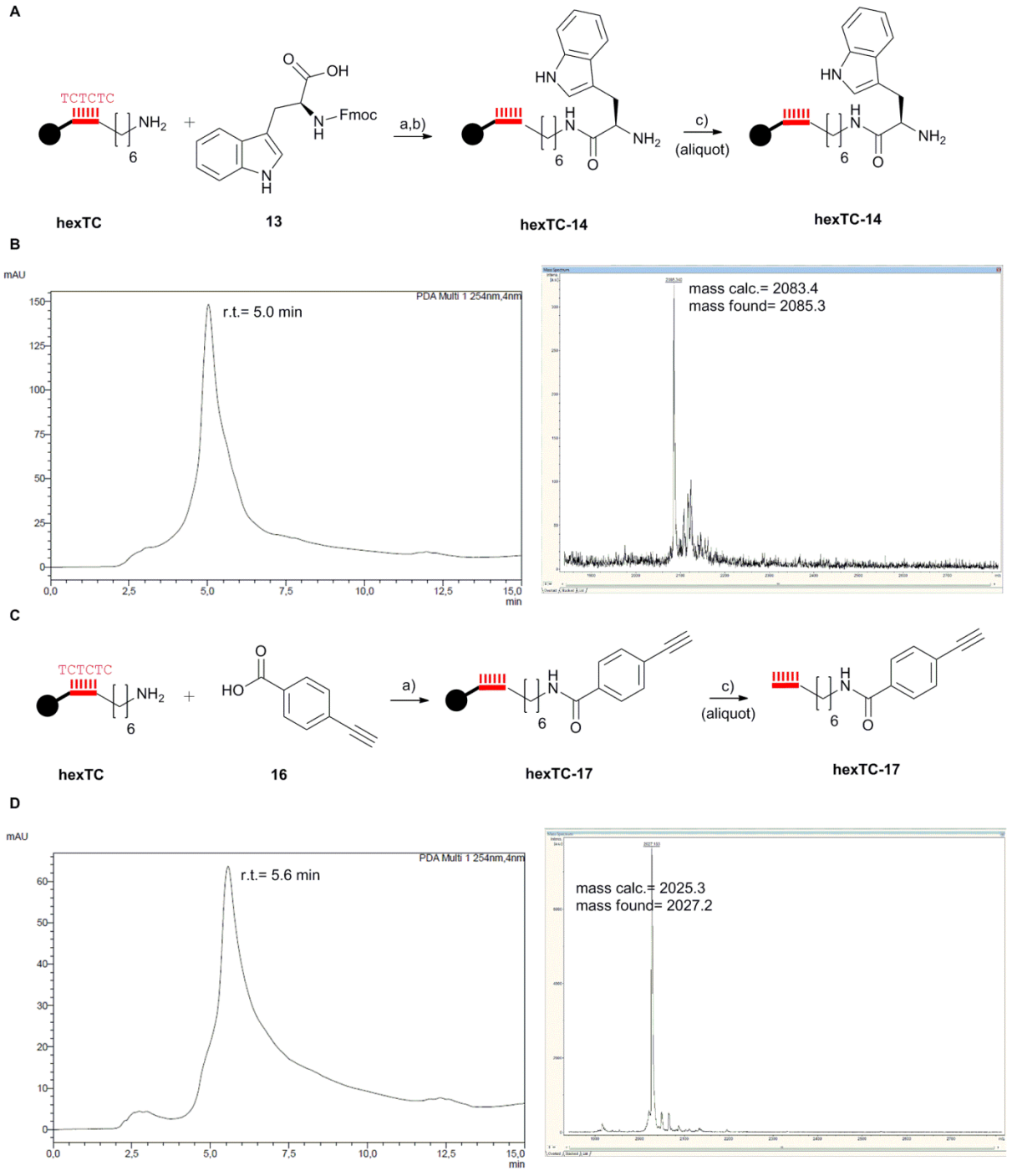


Fig S31. Synthesis of hexTC-tryptophane conjugate **hexTC-14** and hexTC-alkyne conjugate **hexTC-17**. A) Synthesis scheme for compound **hexTC-14**. Reagents and conditions: a) HATU, DIPEA, dry DMF, room temperature, 4 hours; b) 20 % piperidine in dry DMF; c) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature (aliquot); B) HPLC trace and MALDI MS spectrum of the purified conjugate **hexTC-14**; C) synthesis scheme for compound **hexTC-17**; D) HPLC trace and MALDI MS spectrum of the purified conjugate **hexTC-17**. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexTC: 5'-(C6)-amino-linker.

7.2 Synthesis of hexTC-β-carboline conjugate hexTC-15 by Pictet-Spengler reaction[2]

The solid support with the hexTC conjugate **hexTC-14** was dried *in vacuo* for 15 min. A solution of 2 % TFA in dry CH_2Cl_2 was prepared. Then, benzaldehyde **1b** (30 µmol, 1000 eq.) was dissolved in 45 µL of 2 % TFA/ CH_2Cl_2 solution and added to the CPG containing conjugate **hexTC-14** (1.1 mg, ca. 30 nmol). The reaction mixture was shaken at room temperature overnight. Then, the CPG was filtered off, washed with each 3 x 200 µL of DMF, MeOH, MeCN and CH_2Cl_2 and dried *in vacuo* for 15 min. The hexapyrimidine conjugate **hexTC-15** 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 minutes 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 purified by RP-HPLC (Gemini, 5u, C18, 110A column; 50*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20 % – 80 % of methanol over 18 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.

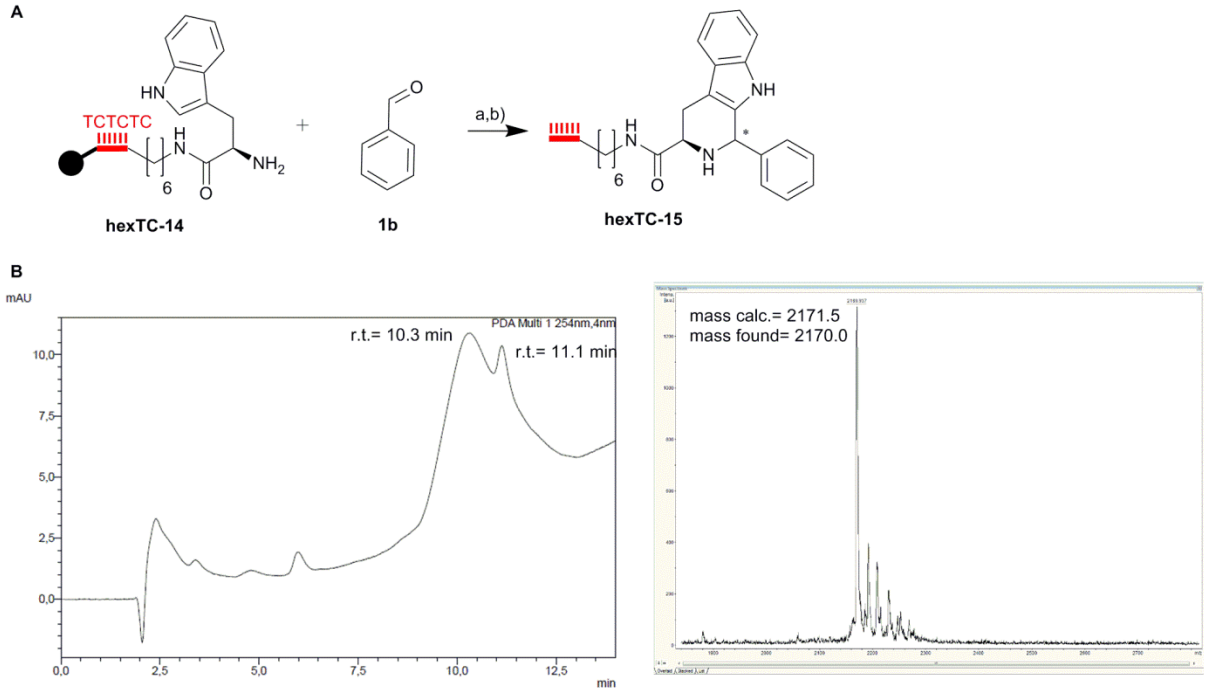


Fig S32. Synthesis of hexTC-ß-carboline conjugate **hexTC-15**. A) Synthesis scheme. Reagents and conditions: a) 2 % TFA, dry CH_2Cl_2, room temperature, overnight; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature (aliquot); B) HPLC trace and MALDI MS spectrum of the purified conjugate **hexTC-15**. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexTC: 5'-(C6)-amino-linker.

7.3 Synthesis of hexTC-pyrazoline conjugate hexTC-18 by Au(I)-mediated annelation reaction[2]

The solid support with the hexTC conjugate **hexTC-17**, hydrazide **2a**, Au(I) catalyst (see Fig S5) and $AgSbF_6$ were dried *in vacuo* for 15 min. Then, isobutyraldehyde **1g** (30 µmol, 1000 eq.), hydrazide **2a** (30 µmol, 1000 eq.), both dissolved in 30 µL of dry MeCN, and the catalyst Au(I)/$AgSbF_6$ (7.5 µmol, 250 eq. of both) suspended in 30 µL of dry MeCN were added to the solid support containing **hexTC-17** (1.1 mg, ca. 30 nmol) suspended in 30 µL of dry MeCN, giving a total reaction volume of ca. 120 µL. The reaction mixture was shaken at 50°C overnight. Then, the CPG was filtered off, washed with each 3 x 200 µL of 0.1 M EDTA, DMF, MeOH, MeCN and CH_2Cl_2 and dried *in vacuo* for 15 min. The hexapyrimidine conjugate **hexTC-18** 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 minutes 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 purified by RP-HPLC (Gemini, 5u, C18, 110A column; 50*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20 % – 80 % of methanol over 18 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.

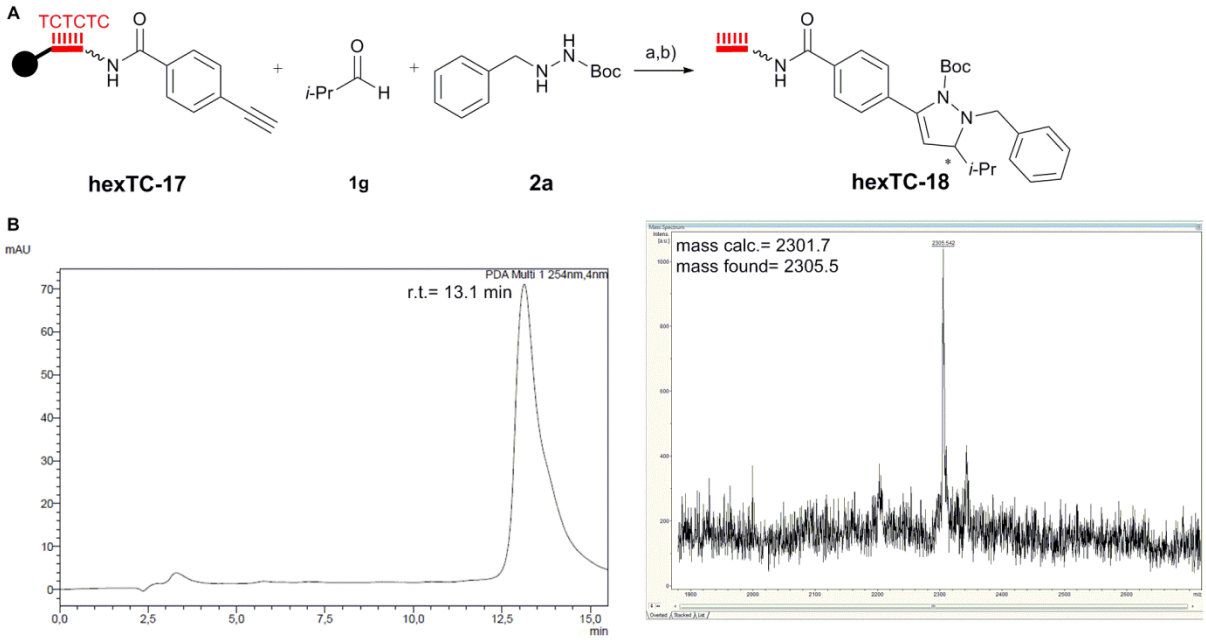


Fig S33. Synthesis of hexTC-pyrazoline conjugate **hexTC-18**. A) Synthesis scheme. Reagents and conditions: a) Au(I)/$AgSbF_6$, dry MeCN, 50°C, overnight; b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature (aliquot); B) HPLC trace and MALDI MS spectrum of the purified conjugate **hexTC-18**. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexTC: 5'-(C6)-amino-linker.

8. Synthesis of intermediates, and reference molecule 6

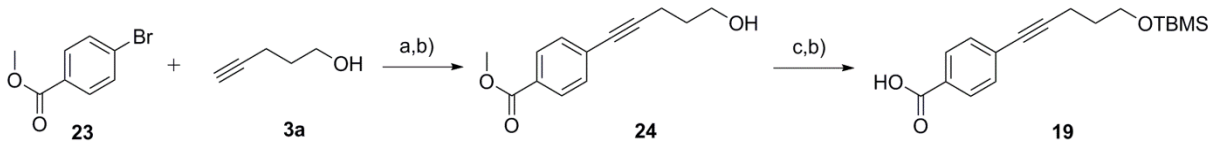
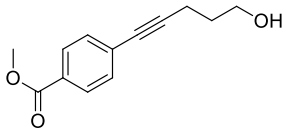


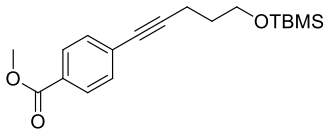
Fig S34. Synthesis of alkynol **19**. Reagents and conditions: a) $Pd(PPh_3)_2Cl_2$, CuI, TEA, 90°C, 18 hours; b) TBMS-Cl, imidazole, dry CH_2Cl_2, room temperature, 18 hours; c) dry THF/MeOH (1/1), room temperature, 6 hours.

Methyl 4-(5-hydroxypent-1-yn-1-yl)benzoate 24[3]



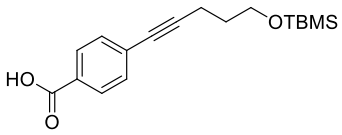
bis(Triphenylphosphine)palladium(II) dichloride (164.6 mg, 0.23 mmol, 0.05 eq.) and copper(I) iodide (59.8 mg, 0.35 mmol, 0.075 eq.) were added to a suspension of methyl 4-bromo-benzoate **23** (1 g, 4.65 mmol, 1.0 eq.) in triethyl amine (9.3 mL). The suspension was flushed with argon for 5 min before pent-4-yn-1-ol **3a** (432.7 µL, 4.65 mmol, 1 eq.) was added. The reaction mixture was stirred at 90°C for 18 hours. After that, the solvent was evaporated *in vacuo,* and the resulting crude product was filtered over Celite pad on top of a layer of silica, and eluted with petroleum ether. Then, the solvent was evaporated *in vacuo,* and the crude product was purified by column chromatography (solvent system: hexanes/ ethyl acetate 100:0 to 50:50) to provide **24** (540 mg, 53 % yield). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.91 (d, ^3J= 8.5 Hz, 2H), 7.40 (d, ^3J= 8.3 Hz, 2H), 3.87 (s, 3H), 3.77 (t, ^3J= 6.1 Hz, 2H), 2.52 (t, ^3J= 7.0 Hz, 2H), 2.36 (br s, 1H), 1.81–1.86 (quint, ^3J= 6.6 Hz, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 166.8, 131.5, 129.4, 128.7, 93.0, 80.5, 77.2, 61.5, 52.3, 31.3, 16.1. LC-MS (ESI) *m/z* Calcd. for $[C_{13}H_{15}O_3, M+H]^+$: 219.1, found 219.17. Purity (HPLC): 95 %.

Methyl 4-(5-((*tert*-butyldimethylsilyl)oxy)pent-1-yn-1-yl)benzoate 25[4]



Imidazole (156.0 mg, 2.29 mmol, 2.5 eq.), and subsequently *tert*-butyldimethilslyl chloride (151.9 mg, 1.01 mmol, 1.1 eq.) were added under argon atmosphere over 1 min at 0°C with stirring to a solution of methyl 4-(5-hydroxypent-1-yn-1-yl)benzoate **24** (200 mg, 0.92 mmol, 1.0 eq.) in dry CH_2Cl_2 (1.5 mL). The reaction mixture was allowed to warm to room temperature and stirred for 18 hours. Then, water was added to the reaction mixture, the crude product was extracted with CH_2Cl_2 (3x20 mL), and the combined organic layers were dried over $MgSO_4$. The solvent was evaporated *in vacuo,* and the crude product was purified by column chromatography (solvent system: hexanes/ ethyl acetate 100:0 to 50:50) to provide **25** (229 mg, 75 % yield). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.96 (d, ^3J= 8.1 Hz, 2H), 7.44 (d, ^3J= 8.1 Hz, 2H), 3.92 (s, 3H), 3.76 (t, ^3J= 6.1 Hz, 2H), 2.53 (t, ^3J= 7.1 Hz, 2H), 1.79–1.85 (quint, ^3J=6.5 Hz, 2H), 0.92 (s, 9H), 0.08 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 168.8, 131.6, 129.5, 128.9, 93.6, 80.3, 61.7, 60.6, 52.3, 31.7, 18.5, 16.0, 14.3, -5.2. LC-MS (ESI) *m/z* Calcd. for $[C_{19}H_{29}O_3Si, M+H]^+$: 332.19, found 333.51. Purity (HPLC): 97 %.

4-(5-((*tert*-Butyldimethylsilyl)oxy)pent-1-yn-1-yl)benzoic acid 19



Aq. NaOH (1.6M, 10 mL) was added to a solution of methyl 4-(5-((*tert*-butyldimethylsilyl)oxy)pent-1-yn-1-yl)benzoate **25** (219 mg, 658.6 mmol, 1.0 eq.) in 10 mL of a THF/MeOH mixture (1:1), and the reaction mixture was stirred at room temperature for 6 hours. Water (20 mL) was added and the reaction mixture was acidified with aq. 2M HCl solution to pH= 1. The crude mixture was extracted with ethyl acetate (3x20 mL), the combined organic layers were washed with brine (20 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo* and the crude product was purified by column chromatography (solvent system: hexanes/ ethyl acetate 100:0 to 50:50) to provide **19** (144 mg, 69 % yield). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.03 (d, ^3J= 8.3 Hz, 2H), 7.47 (d, ^3J= 8.2 Hz, 2H), 3.76 (t, ^3J= 6.1 Hz, 2H), 2.53 (t, ^3J= 7.2, 2H), 1.80–1.85 (quint, ^3J= 6.5 Hz, 2H), 0.91 (s, 9H), 0.08 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.8, 131.7, 130.2, 129.9, 128.1, 94.2, 80.3, 61.7, 31.6, 26.1, 18.5, 16.1, -5.2. LC-MS (ESI) *m/z* Calcd. for [$C_{19}H_{30}O_3Si$, M+H] $^+$: 319.17, found 319.20. Purity (HPLC): 97 %.

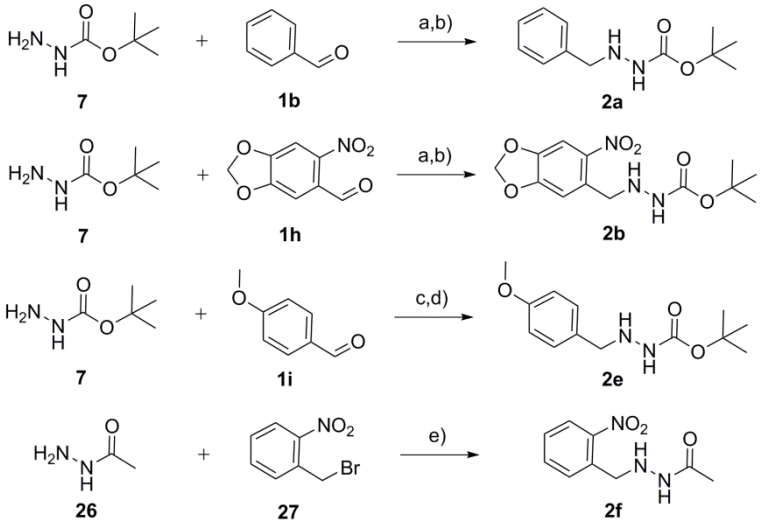
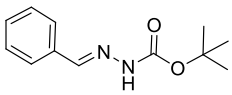


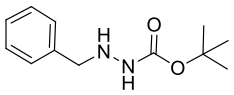
Fig S35. Synthesis of hydrazides **2a, 2b, 2e,** and **2f**. Reagents and conditions: a) dry THF, room temperature, 18 hours; b) dry THF, $NaBH_3CN$, room temperature, 18 hours; c) dry MeOH, 50°C, 18 hours; d) 10 % Pd/C, room temperature, 1.5 hours; e) K_2CO_3, dry DMF, room temperature, 30 min.

***tert*-Butyl-2-benzylidenehydrazine-1-carboxylate 28**[1]



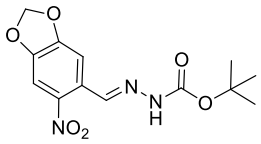
Benzaldehyde **1b** (772 µL, 7.57 mmol, 1.0 eq.) was added to a solution of *tert*-butyl carbazate **7** (1 g, 7.57 mmol, 1.0 eq.) in dry THF (10 mL). The reaction mixture was stirred at room temperature for overnight. The reaction was concentrated *in vacuo*, and the crude product was purified by column chromatography (solvent system: CH_2Cl_2/ ethyl acetate 100:0 to 90:10) to provide **28** (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 2a



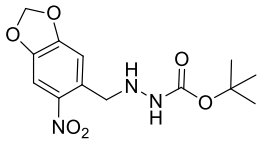
$NaBH_3CN$ (636.6 mg, 10.12 mmol, 2.5 eq.) was slowly added to a solution of *tert*-butyl-2-benzylidenehydrazine-1-carboxylate **28** (892 mg, 4.05 mmol, 1.0 eq.) in dry THF (9 mL) at 0°C. Then, acetic acid (6.07 mL, 106.1 mmol, 26.2 eq.) was added, and the reaction mixture was allowed to warm to room temperature, and stirred for 18 hours. An additional amount of $NaBH_3CN$ (636.6 mg, 10.12 mmol, 2.5 eq.) was added, and stirring was continued 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 a mixture of methanol (8 mL) and 1M aqueous NaOH (8 mL), and stirred at room temperature for 2 days. After that, the solution was concentrated under reduced pressure, and the product was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed 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 **2a** (567 mg, 62 % yield). mp 39-40°C ^{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 29



tert-Butyl 2-((6-nitrobenzo[d][1,3]dioxol-5-yl)methylene)hydrazine-1-carboxylate **29** was synthesized according to the procedure for synthesis of *tert*-butyl-2-benzylidenehydrazine-1-carboxylate **28** from *tert*-butyl carbazate **7** (1 g, 7.57 mmol, 1.0 eq.) and 6-nitro-benzo[d][1,3]dioxole-5-carbaldehyde **1h** (1.476 g, 7.57 mmol, 1.0 eq.). The product **29** 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 %.

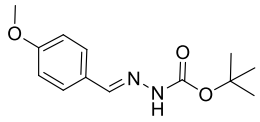
tert-Butyl 2-((6-nitrobenzo[d][1,3]dioxol-5-yl)methyl)hydrazinecarboxylate 2b



Compound **29** (1.03 g, 3.34 mmol, 1.0 eq.) was dissolved in methanol (21 mL), and $NaBH_3CN$ (1.05 g, 16.72 mmol, 5.0 eq.) was slowly added. Then one tip of a spoon of bromocresol green as indicator was added to the reaction mixture to monitor the pH value (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). The reaction mixture was stirred at room temperature for 18 hours. The crude product was extracted with ethyl acetate (3 x 50 mL). The 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: CH_2Cl_2/ethyl acetate 100:0 to 90:10) to provide **2b** (747 mg, 72 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51 (s, 1H), 7.01 (s, 1H),

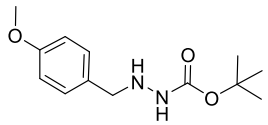
6.10 (s, 2H), 6.08 (br. s, 1H), 4.56 (br. s, 1H), 4.19 (s, 2H), 1.41 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.7, 151.8, 147.3, 143.3, 130.9, 110.5, 106.1, 103.0, 80.9, 53.8, 28.4. LC-MS (ESI) *m/z* Calcd. for $[C_{13}H_{17}N_3NaO_6$, M+Na]$^+$: 334.10, found 333.99. Purity (HPLC): 97 %.[5]

tert-Butyl (E)-2-(4-methoxybenzylidene)hydrazine-1-carboxylate 30[6]



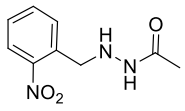
Anisaldehyde **1i** (2.06 g, 15.1 mmol, 1 eq.) was added to a solution of *tert*-butyl carbazate **7** (2 g, 15.1 mmol, 1 eq.) in dry methanol (75 mL). The reaction mixture was stirred at 50°C for overnight. After that, the reaction was filtered, the precipitate was washed with pentane, and dried *in vacuo* to provide **30** 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, ^3J= 8.8 Hz, 2H), 6.88 (d, ^3J= 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 are consistent with the literature values.[5]

tert-Butyl 2-(4-methoxybenzyl)hydrazine-1-carboxylate 2e[6]



To a solution of *tert*-butyl-2-benzylidenehydrazine-1-carboxylate **30** (2.0 g, 7.99 mmol, 1.0 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 hours. Then, the reaction mixture was filtered over Celite pad, and concentrated *in vacuo*. The crude product was dissolved in hot ethanol, and kept for crystallization at -2°C for overnight to provide **2e** (1.16 g, 58 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (d, ^3J= 8.4 Hz, 2H), 6.88 (d, ^3J= 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 are consistent with the literature values.[6]

***N*'-(2-Nitrobenzyl)acetohydrazide 2f**



Acetocarbazate **26** (781.9 mg, 10.55 mmol, 7.6 eq.), and potassium carbonate (211.3 mg, 1.53 mmol, 1.1 eq.) were added to a solution of 2-nitrobenzyl bromide **27** (300 mg, 1.39 mmol, 1.0 eq.) in dry DMF (2 mL). The reaction mixture was stirred at room temperature for 30 minutes. After that, the 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 **2f** (102 mg, 35 % yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.93 (dd, ^3J= 7.8 Hz, 1H), 7.52-7.58 (m, 2H), 7.42-7.46 (m, 1H), 7.11 (br. s, 1H), 4.53 (br. s, 1H), 4.03 (s, 2H), 1.88 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.02, 133.08, 131.69, 128.73, 124.97, 53.20, 21.12. LC-MS (ESI) *m/z* Calcd. for $[C_{12}H_{18}N_3O_4$, M+H]$^+$: 209.2, found 210.0. Purity (HPLC): 99 %.

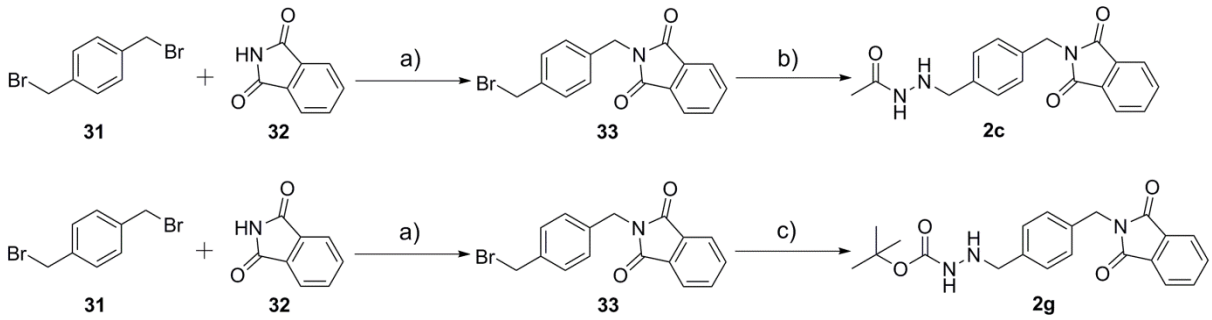
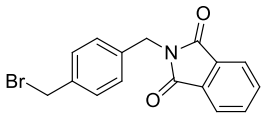


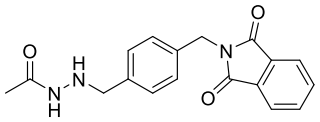
Fig S36. Synthesis of the hydrazides **2c** and **2g**. Reagents and conditions: a) K_2CO_3 in MeCN, room temperature, 18 hours; b) **26**, K_2CO_3, dry CH_2Cl_2, room temperature, 18 hours; c) **7**, K_2CO_3, dry CH_2Cl_2, room temperature, 18 hours.

2-(4-(Bromomethyl)benzyl)isoindoline-1,3-dione 33[7]



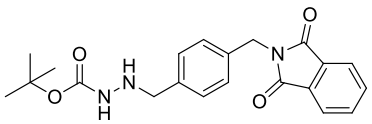
1,4-bis(Bromomethyl)benzene **31** (1794 mg, 6.8 mmol, 1.0 eq.), 18-crown-6 (26.9 mg, 1.0 mmol, 0.15 eq.), and potassium carbonate (3.8 g, 27 mmol, 4.0 eq.) were added to a solution of phthalimide **32** (1.0 g, 6.8 mmol, 1.0 eq.) in dry acetonitrile (60 mL). The reaction mixture was stirred under argon atmosphere at room temperature for 18 hours. After that, ethyl acetate (200 mL) was added and the reaction mixture was filtered. 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 **33** (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 %.

***N*'-(4-((1,3-Dioxoisoindolin-2-yl)methyl)benzyl)acetohydrazide 2c**



Acetohydrazide **26** (852.6 mg, 11.51 mmol, 7.6 eq.) and potassium carbonate (229.4 mg, 1.5 mmol, 1.1 eq.) were added to a solution of **33** (500 mg, 1.51 mmol, 1.0 eq.) in dry CH_2Cl_2 (12 mL). The reaction mixture was stirred under argon atmosphere at room temperature for 18 hours. After that, ethyl acetate (100 mL) was added, and the reaction mixture was filtered off. 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 **2c** (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, ^3J= 7.8 Hz, 2H), 7.29 (d, ^3J= 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 2-(4-((1,3-dioxoisoindolin-2-yl)methyl)benzyl)hydrazinecarboxylate 2g**



tert-Butyl carbazate **7** (1521 g, 11.51 mmol, 7.6 eq.) and potassium carbonate (229.4 mg, 1.66 mmol, 1.1 eq.) were added to a solution of **33** (500 mg, 1.51 mmol, 1.0 eq.) in dry CH_2Cl_2 (12 mL). The reaction mixture was stirred under argon atmosphere at room temperature for 18 hours. 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 **2g** (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; *m/z* Calcd. for [$C_{21}H_{24}N_3NaO_4$, M+Na] $^+$: 404.1586, found: 404.1579. Purity (HPLC): 95 %.

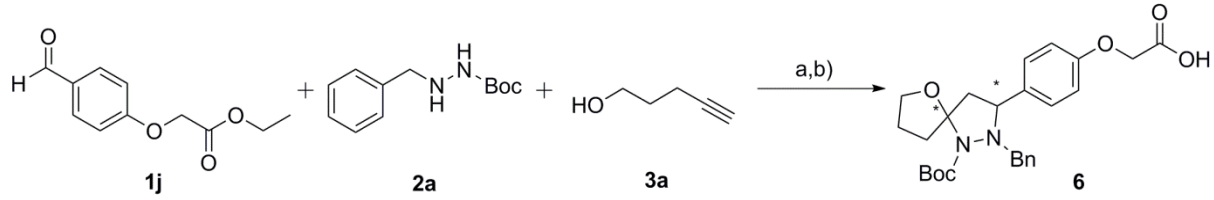
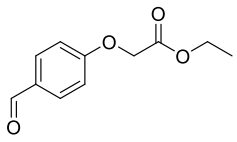


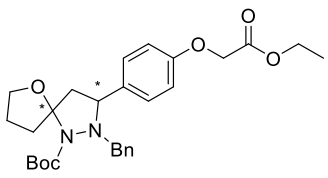
Fig S37. Synthesis of reference molecule **6**. Reagents and conditions: a) Au(I)/$AgSbF_6$, THF, 60°C, 3 hours; b) aq. 1 M NaOH, THF, room temperature, 2 hours.

Ethyl 2-(4-formylphenoxy)acetate 1j



Conc. H_2SO_4 (0.13 mL) was added dropwise to a solution of 2-(4-formylphenoxy)acetic acid (500 mg, 2.78 mmol, 1.0 eq.) in ethanol (13.0 mL). After stirring 3 hours at 80°C the mixture was cooled to room temperature, neutralized with sat. aq. $NaHCO_3$ solution and extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to afford compound **1j** (561 mg, 97 % yield) as a yellowish powder. ^{1}H-NMR (600 MHz, $CDCl_3$): *δ* 9.90 (s, 1H), 7.86-7.82 (m, 2H), 7.03-6.99 (m, 2H), 4.70 (s, 2H), 4.28 (q, ^3J = 7.1 Hz, 2H), 1.30 (t, ^3J= 7.1 Hz, 3H). ^{13}C-NMR (150 MHz, $CDCl_3$): *δ* 190.9, 168.2, 162.8, 132.1, 130.9, 115.0, 65.4, 61.8, 14.3. LC-MS (ESI) *m/z* Calcd. for [$C_{11}H_{13}O_4$, M+H] $^+$: 209.08, found 209.10. Purity (HPLC): 95 %.

***tert*-Butyl 2-benzyl-3-(4-(2-ethoxy-2-oxoethoxy)phenyl)-6-oxa-1,2-diazaspiro[4.4]nonane-1-carboxylate 34[1]**



Hydrazide **2a** (50.0 mg, 0.23 mmol, 1.0 eq.), aldehyde **1j** (58.3 mg, 0.28 mmol, 1.2 eq.), and pent-4-yn-1-ol **3a** (23.6 mg, 0.28 mmol, 1.2 eq.) were dissolved under argon atmosphere in THF (3.0 mL). Chloro[tris(2,4-di-*tert*-butylphenyl)phosphite]gold (10.6 mg, 12.0 µmol, 5 mol %), and $AgSbF_6$ (4.12 mg, 12.0 µmol, 5 mol %) were added to the solution with stirring, and the reaction mixture was stirred at 60°C for 3 hours. It was filtered over Celite, and concentrated *in vacuo*. The crude product was purified by column chromatography (solvent system: cyclohexane/EtOAc 98:2 to 95:5). Compound **34** was isolated as a mixture of

diastereomers (diasterometric ratio 3:1). The major diastereomer (11.2 mg, 10 % yield) was isolated as colorless oil. Major diastereomer: ^{1}H NMR (500 MHz, C_6D_6): δ 7.50 (d, ^{3}J= 7.4 Hz, 2H), 7.27 (d, ^{3}J= 8.5 Hz, 2H), 7.12 (t, ^{3}J= 7.5 Hz, 2H), 7.05-7.02 (m, 1H), 6.81 (d, ^{3}J= 8.7 Hz, 2H), 4.47 (d, ^{3}J= 12.7 Hz, 1H), 4.25 (m, 1H), 4.19 (s, 2H) 4.15-4.04 (m, 2H), 3.86 (q, ^{3}J= 7.1 Hz, 2H), 3.78 (td, ^{3}J= 7.7 Hz, ^{2}J= 3.0 Hz, 1H), 3.00 (dd, ^{3}J= 13.1, ^{2}J= 7.4 Hz, 1H), 2.76-2.70 (m, 1H), 2.11 (dd, ^{3}J= 13.1, ^{2}J= 2.1 Hz, 1H), 2.00-1.94 (m, 1H), 1.49 (m, 9H), 1.37-1.31 (m, 2H), 0.84 (t, ^{3}J= 7.1 Hz, 3H). ^{13}C NMR (125 MHz, C_6D_6) δ 168.5, 157.4, 152.6, 138.8, 129.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.6, 114.7, 102.3, 79.6, 69.0, 65.5, 62.2, 61.6, 60.8, 48.3, 35.3, 28.7, 26.7, 14.1.HRMS (ESI) *m/z* Calcd. for $[C_{28}H_{37}N_2O_6, M+H]^+$: 497.2646, found 497.2642. Purity (HPLC): 95 %.

2-(4-(2-Benzyl-1-(*tert*-butoxycarbonyl)-6-oxa-1,2-diazaspiro[4.4]nonan-3-yl)phenoxy)-acetic acid 6

To a solution of compound **34** (17.0 mg, 34.0 µmol, 1.0 eq.) in THF (2.0 mL) aq. NaOH solution (1 M, 0.34 mL) was added at room temperature. After 2 hours, the reaction mixture was neutralized with aq. 1 M HCl and distilled water (5.0 mL) was added. The solution was extracted with EtOAc and the combined organic layers were dried over anhydrous $MgSO_4$. Compound **6** (13.0 mg, 81 % yield) was isolated as a light orange oil (diastereometric ratio 3:1). ^{1}H NMR (600 MHz, C_6D_6) δ 7.50 (d, ^{3}J= 7.4 Hz, 2H), 7.47 (d, ^{3}J= 7.3 Hz, 0.6H), 7.36 (d, ^{3}J= 8.5 Hz, 0.6H), 7.24 (d, ^{3}J= 8.5 Hz, 2H), 7.14-7.12 (m, 2H), 7.09-7.03 (m, 1.4H), 6.74-6.72 (m, 2.5H), 4.46 (d, ^{3}J= 12.6 Hz, 1H), 4.23 (m, 1H), 4.13 (s, 2H), 4.11-4.07 (m, 2H), 3.78 (td, ^{3}J= 7.8, ^{2}J= 3.1 Hz, 1H), 3.00 (dd, ^{3}J= 12.9, ^{2}J= 7.4 Hz, 1H), 2.82-2.77 (m, 0.3H), 2.74-2.69 (m, 1H), 2.24-2.00 (m, 0.3H), 2.11 (dd, ^{3}J= 13.1, ^{2}J= 2.5 Hz, 1H), 1.98-1.94 (m, 1.4H), 1.51 (s, 3H), 1.48 (s, 9H), 1.37-1.31 (m, 2.3H). ^{13}C-NMR (150 MHz, $CDCl_3$): δ 172.5/172.3*, 157.1/157.0*, 152.8/152.6*, 138.7/138.4*, 135.6, 129.7/129.6*, 128.6/128.4*, 128.3, 128.1, 128.0, 127.7/127.6*, 114.7/114.7*, 102.3/101.7*, 79.9/79.8*, 69.8/68.9*, 65.0/64.9*, 62.3, 61.5, 48.3/46.4*, 37.26/35.3*, 28.7/28.6*, 26.6 ppm. *= diastereomeric carbon signal. HRMS (ESI) *m/z* Calcd. for $[C_{26}H_{33}N_2O_6, M+H]^+$: 469.2333, found 469.2321. Purity (HPLC): 95 %.

9. Literature

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