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 C₁₈ 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 C₁₈ 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). ¹H-NMR-spectra were measured at 400, 500, or 600 MHz on a Bruker DRX400, Bruker DRX500, Varian Inova 500, or Varian Inova 600 spectrometer. ¹³C-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 (CDCl₃, 99.8 %, VWR), dimethyl sulfoxide-d₆ (DMSO-d₆, 99.8 %, VWR), or deuterated benzene (C₆D₆, 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 = singulet, 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 F₂₅₄ 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 C₁₈ column stationary phase (Phenomenex, Luna; 5 µm, C18, 100 Å, 250*4.6 mm) and MeOH/1 % aq. formic acid, 50:50 to 100:0 over 13 min. High resolution mass spectrometry (ESI) was performed on an Thermo LTQ Orbitrap coupled with an 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 CH₂Cl₂ (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 CH₂Cl₂. 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 CH₂Cl₂. 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 CH₂Cl₂, 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 CH₂Cl₂ (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 CH₂Cl₂. 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 CH₂Cl₂. 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 CH₂Cl₂, 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 CH₂Cl₂.

**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).

![Diagram of the synthesis process](image1)

**A**

5'-C(6)-amino-DNA + p-formylphenoxyacetic acid → a) DNA-1a

![Chromatograms](image2)

**B**

TTTTTTT-1a: r.t. = 6.7 min

mass calc.= 2104.4

mass found= 2104.6

**C**

CCCCCCC-1a: r.t. = 5.9 min

mass calc.= 2014.3

mass found= 2015.6
D

**TCTCTC-1a**
- r.t. = 6.5 min
- Mass calc.: 2059.4
- Mass found: 2061.2

E

**AAAAAA-1a**
- r.t. = 6.3 min
- Mass calc.: 2158.5
- Mass found: 2160.4

F

**GGGGGG-1a**
- r.t. = 5.6 min
- Mass calc.: 2254.5
- Mass found: 2254.2

G

**CAGTCG-1a**
- r.t. = 6.0 min
- Mass calc.: 2133.4
- Mass found: 2135.9
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 CH₂Cl₂. 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) NaBH₃CN, 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-((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

![Chemical structure diagram](image)

**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$_2$Cl$_2$, 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.

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[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, Su, 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 $\text{hexT-1a}$, hydrazide $2a$, and alkyol $3a$, for reaction conditions see Table S1b. a) HPLC trace of the starting material (aldehyde conjugate $\text{hexT-1}$); b) HPLC trace of experiment No. 8; formation of condensation product $\text{hexT-5}$ (r.t. = 14.0 min); c) HPLC trace of experiment No. 9; formation of condensation product $\text{hexT-5}$; d) HPLC trace of experiment No. 10; formation of mixture of condensation product $\text{hexT-5}$ and spirocycle $\text{hexT-4a}$ (r.t. = 15.4 min); e) HPLC trace of experiment No. 11; formation of mixture of condensation product $\text{hexT-5}$ and spirocycle $\text{hexT-4a}$; f) HPLC trace of experiment No. 12; formation of condensation product $\text{hexT-5}$, target spirocycle $\text{hexT-4a}$ formed as minor product; g) HPLC trace of experiment No. 13; formation of condensation product $\text{hexT-5}$, target spirocycle $\text{hexT-4a}$ formed as minor product; h) HPLC trace of experiment No. 14; formation of condensation product $\text{hexT-5}$, target spirocycle $\text{hexT-4a}$ formed as minor product.

**Fig S8.** MALDI MS of the isolated hexathymidine conjugate $\text{hexT-5}$ (r.t. = 14.0 min) from Fig S7b. A) Reaction scheme of the $\text{hexT-5}$ 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 $\text{hexT-5}$. 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. 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 methylvamine (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.

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<th>alkynol</th>
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<th>$R^2$</th>
<th>n</th>
<th>yield/[nmol]</th>
<th>mass calc.</th>
<th>mass found</th>
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<tr>
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<td>hexynol</td>
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<td>1.8</td>
<td>2406.8</td>
<td>2410.3</td>
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</table>

[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

![Chemical Structures](image)

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/AgSbF₆ at the amounts given in Table S3 which was suspended (Au(I)) or dissolved (AgSbF₆), 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 CH₂Cl₂ 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.

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<td>14</td>
<td>THF</td>
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<td>15</td>
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<td>25</td>
<td>Au(I)/AgSbF_6</td>
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<td>25</td>
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[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**

![HPLC traces](image)

**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

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.

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

![Reaction scheme of the hexT-10 formation](image)

**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

![Reaction scheme of the linear intermediate hexT-11 formation](image)

**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'-[(6)-amino-linker.

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[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

![Diagram](image)

**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)/AgSbF6 (see Table S5) at the amounts given in Table S5 which were suspended (Au(I)) or dissolved (AgSbF6), 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 CH2Cl2 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, Su, 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.

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[a] versus the solid support-bound hexT-alkynol conjugate hexT-3c; [b] Au(I): tris(2,4-di-tert-butyl)phenylphosphite 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-diaza
[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-diaza
[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-diazaaspiro[4,4]nonane conjugate hexT-4j

**Fig S27.** Control experiment with different catalysts for the reaction to hexT-6-oxa-1,2-diazaaspiro[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-diazaaspiro[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'-O-(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).

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[a] measured by Nanodrop; [b] measured by MALDI MS; [c] loss of the photolabile 3,4-methylenedioxy-6-nitrobenzyl group upon irradiation in the mass spectrometer; [d] phthalimide group removed in the product; [e] conjugates contain PEG(4) linker.
hexT-4j
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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
mass calc. = 2467.8
mass found = 2467.9

hexT-4n
mass calc. = 2759.2
mass found = 2759.2

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

hexT-4p
mass calc. = 2679.1
mass found = 2681.0
6. Au(I)-mediated synthesis of DNA-6-oxa-1,2-diazaspiro[4.4]nonane conjugates 4 from DNA-aldehyde conjugates 1a

![Diagram of the synthesis process]

**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$_2$Cl$_2$ 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 β-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-ethylbenzoic 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-trypophane 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\(_2\)Cl\(_2\) was prepared. Then, benzaldehyde 1b (30 µmol, 1000 eq.) was dissolved in 45 µL of 2 % TFA/ CH\(_2\)Cl\(_2\) 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\(_2\)Cl\(_2\) 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 methyamine (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.

![Synthesis scheme](image)

**Fig S32.** Synthesis of hexTC-β-carboline conjugate hexTC-15. A) Synthesis scheme. Reagents and conditions: a) 2 % TFA, dry CH\(_2\)Cl\(_2\), 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\textsuperscript{2}

The solid support with the hexTC conjugate hexTC-17, hydrazide 2a, Au(I) catalyst (see Fig S5) and AgSbF\(_6\) were dried \textit{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\(_2\)Cl\(_2\) and dried \textit{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.

![Synthesis scheme](image)

**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$_3$)$_2$Cl$_2$, Cul, TEA, 90°C, 18 hours; b) TBMS-Cl, imidazole, dry CH$_2$Cl$_2$, 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(II) 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, $^3$J= 8.5 Hz, 2H), 7.40 (d, $^3$J= 8.3 Hz, 2H), 3.87 (s, 3H), 3.77 (t, $^3$J= 6.1 Hz, 2H), 2.52 (t, $^3$J= 7.0 Hz, 2H), 2.36 (br s, 1H), 1.81–1.86 (quint, $^3$J= 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$_{11}$H$_{15}$O$_3$, M+H]$^+$: 219.1, found 219.17. Purity (HPLC): 95 %.

Methyl 4-{(tert-butylidimethylsilyloxy)pent-1-yn-1-yl}benzoate 25$^4$

Imidazole (156.0 mg, 2.29 mmol, 2.5 eq.), and subsequently tert-butylidimethylsilyl 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$_2$Cl$_2$ (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$_2$Cl$_2$ (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, $^3$J= 8.1 Hz, 2H), 7.44 (d, $^3$J= 8.1 Hz, 2H), 3.92 (s, 3H), 3.76 (t, $^3$J= 6.1 Hz, 2H), 2.53 (t, $^3$J= 7.1 Hz, 2H), 1.79–1.85 (quint, $^3$J=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$_{15}$H$_{26}$O$_3$Si, 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 MgSO₄. 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). ¹H-NMR (500 MHz, CDCl₃): 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); ¹³C-NMR (125 MHz, CDCl₃): δ 171.8, 131.7, 130.2, 129.9, 128.1, 94.2, 80.3, 61.7, 31.6, 18.5, 16.1, -5.2. LC-MS (ESI) m/z Calcd. for [C₁₉H₃₅O₃Si, 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, NaBH₃CN, room temperature, 18 hours; c) dry MeOH, 50°C, 18 hours; d) 10 % Pd/C, room temperature, 1.5 hours; e) K₂CO₃, dry DMF, room temperature, 30 min.

tert-Butyl-2-benzylidenehydrazine-1-carboxylate 2b

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: CH₂Cl₂/ethyl acetate 100:0 to 90:10) to provide 2b (892 mg, 53 % yield). ¹H NMR (400 MHz, CDCl₃): 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). ¹³C NMR (100 MHz, CDCl₃): δ 153.0, 143.7, 134.0, 129.9, 128.7, 127.3, 81.6, 28.4. LC-MS (ESI) m/z Calcd. for [C₁₂H₁₇N₂O₂, M+H]⁺: 221.13, found 221.20. Purity (HPLC): 90 %.
**tert-Butyl 2-benzylhydrazine-1-carboxylate 2a**

NaBH₃CN (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 NaBH₃CN (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. NaHCO₃ solution (100 mL) and brine (100 mL), dried over anhydrous MgSO₄ 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 MgSO₄ and concentrated to provide 2a (567 mg, 62 % yield). mp 39-40°C. ¹H NMR (500 MHz, CDCl₃): 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). ¹³C NMR (125 MHz, CDCl₃): 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 [C₂₂H₂₉N₂O₃, M+H⁺]: 332.09, found 331.92. 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). ¹H NMR (500 MHz, CDCl₃): 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. ¹³C NMR (125 MHz, CDCl₃): 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 [C₁₃H₁₅N₃NaO₆, 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 NaBH₃CN (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 · H₂O 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. NaHCO₃ solution (100 mL) and brine (100 mL), dried over anhydrous MgSO₄ and concentrated in vacuo. The crude product was purified by column chromatography (solvent system: CH₂Cl₂/ethyl acetate 100:0 to 90:10) to provide 2b (747 mg, 72 % yield). ¹H NMR (500 MHz, CDCl₃): δ 7.51 (s, 1H), 7.01 (s, 1H), 7.28 (m, 4H), 6.17 (s, 1H), 4.19 (br. s, 1H), 3.99 (s, 2H), 1.46 (s, 9H).
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$_3$NaO$_6$ 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, $^3$J = 8.8 Hz, 2H), 6.88 (d, $^3$J = 8.8 Hz, 2H), 3.81 (s, 3H), 1.53 (s, 9H). LC-MS (ESI) m/z Calcd. for C$_{13}$H$_{19}$N$_2$O$_2$: 251.14; found 251.21 [M+H$^+$]. Purity (HPLC): 97 %. The analytical data are consistent with the literature values.$^6$

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, $^3$J = 8.4 Hz, 2H), 6.88 (d, $^3$J = 8.8 Hz, 2H), 6.25 (br. s, 1H), 3.96 (s, 2H), 3.81 (s, 3H), 3.33 (br. s, 1H), 1.47 (s, 9H). LC-MS (ESI) m/z Calcd. for C$_{13}$H$_{21}$N$_2$O$_3$: 253.14; found 253.25 [M+H$^+$]. 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$_2$Cl$_2$/ 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, $^3$J = 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$_3$O$_4$, M+H$^+$]: 209.2, found 210.0. Purity (HPLC): 99 %.
Fig S36. Synthesis of the hydrazides 2c and 2g. Reagents and conditions: a) K$_2$CO$_3$ in MeCN, room temperature, 18 hours; b) 26, K$_2$CO$_3$, dry CH$_2$Cl$_2$, room temperature, 18 hours; c) 7, K$_2$CO$_3$, dry CH$_2$Cl$_2$, room temperature, 18 hours.

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

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$_2$Cl$_2$ 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, $^3$J = 8.2 Hz, 2H), 7.34 (d, $^3$J = 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$_2$Cl$_2$ (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, $^3$J = 7.8 Hz, 2H), 7.36 (d, $^3$J = 7.95 Hz, 2H), 6.98 (br. s, 1H), 4.83 (s, 2H), 4.88 (br. s, 1H), 3.92 (s, 2H), 1.89 (s, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 168.2, 137.5, 135.8, 134.2, 129.3, 128.8, 123.5, 123.2, 55.4, 41.4, 21.3. LC-MS (ESI) m/z Calcd. for [C$_{18}$H$_{22}$N$_2$O$_3$, M+H]$^+$: 324.13, found 323.97. Purity (HPLC): 98 %.
**tert-Butyl 2-(4-((1,3-dioioisindolin-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 CH2Cl2 (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; 1H NMR (500 MHz, CDCl3): δ 7.83-7.85 (m, 2H), 7.68-7.72 (m, 2H), 7.40 (d, J= 7.9 Hz, 2H), 7.29 (d, J= 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). 13C NMR (125 MHz, CDCl3): 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 [C21H24N3O4, M+H]⁺: 382.1767, found 382.1761; m/z Calcd. for [C21H24N3NaO4, M+Na]⁺: 404.1546, found: 404.1579. Purity (HPLC): 95 %.

![Diagram](image-url)

**Fig S37.** Synthesis of reference molecule 6. Reagents and conditions: a) Au(I)/AgSbF6, THF, 60°C, 3 hours; b) aq. 1 M NaOH, THF, room temperature, 2 hours.

**Ethyl 2-(4-formyloxy)acetate 1j**

Conc. H2SO4 (0.13 mL) was added dropwise to a solution of 2-(4-formyloxy)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. NaHCO3 solution and extracted with EtOAc. The combined organic layers were dried over anhydrous MgSO4, and concentrated *in vacuo* to afford compound 1j (561 mg, 97 % yield) as a yellowish powder. 1H-NMR (600 MHz, CDCl3): δ 9.90 (s, 1H), 7.86-7.82 (m, 2H), 7.03-6.99 (m, 2H), 4.70 (s, 2H), 4.28 (q, J= 7.1 Hz, 2H), 1.30 (t, J= 7.1 Hz, 3H). 13C-NMR (150 MHz, CDCl3): 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 [C11H13O4, 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**

Hydrazone 2a (50.0 mg, 0.23 mmol, 1.0 eq.), aldehyde 1j (58.3 mg, 0.28 mmol, 1.2 eq.), and pent-4-yne-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 AgSbF6 (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\(_6\)D\(_6\)): \(\delta\) 7.50 (d, \(^3J= 7.4\) Hz, 2H), 7.27 (d, \(^3J= 8.5\) Hz, 2H), 7.12 (t, \(^3J= 7.5\) Hz, 2H), 7.05-7.02 (m, 1H), 6.81 (d, \(^3J= 8.7\) Hz, 2H), 4.47 (d, \(^3J= 12.7\) Hz, 1H), 4.25 (m, 1H), 4.19 (s, 2H) 4.15-4.04 (m, 2H), 3.86 (q, \(^3J= 7.1\) Hz, 2H), 3.78 (td, \(^3J= 7.7\) Hz, \(^2J= 3.0\) Hz, 1H), 3.00 (dd, \(^3J= 12.9\) Hz, \(^2J= 3.1\) Hz, 1H), 3.00 (dd, \(^3J= 13.1\) Hz, \(^2J= 7.4\) Hz, 1H), 2.76-2.70 (m, 1H), 2.11 (dd, \(^3J= 13.1\) Hz, \(^2J= 2.1\) Hz, 1H), 2.00-1.94 (m, 1H), 1.49 (m, 9H), 1.37-1.31 (m, 2H), 0.84 (t, \(^3J= 7.1\) Hz, 3H). \(^{13}\)C NMR (125 MHz, C\(_6\)D\(_6\)) \(\delta\) 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, 65.0, 62.2, 61.6, 60.8, 48.3, 35.3, 28.7, 26.7, 14.1. HRMS (ESI) m/z Calcd. for \([\text{C}_{28}\text{H}_{37}\text{N}_{2}\text{O}_{6}]^+\): 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\(_6\)D\(_6\)) \(\delta\) 7.50 (d, \(^3J= 7.4\) Hz, 2H), 7.47 (d, \(^3J= 7.3\) Hz, 0.6H), 7.36 (d, \(^3J= 8.5\) Hz, 0.6H), 7.24 (d, \(^3J= 8.5\) 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, \(^3J= 12.6\) Hz, 1H), 4.23 (m, 1H), 4.13 (s, 2H), 4.11-4.07 (m, 2H), 3.78 (td, \(^3J= 7.8\) Hz, \(^2J= 3.1\) Hz, 1H), 3.00 (dd, \(^3J= 12.9\) Hz, \(^2J= 7.4\) 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, \(^3J= 13.1\) Hz, \(^2J= 2.5\) Hz, 1H), 1.98-1.94 (m, 1.4H), 1.51 (s, 3H), 1.48 (s, 3H), 1.37-1.31 (m, 2.3H). \(^{13}\)C-NMR (150 MHz, CDCl\(_3\)): \(\delta\) 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\(_2\)O\(_6\), M+H]^+: 469.2333, found 469.2321. Purity (HPLC): 95 %.
9. Literature