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1. Solution synthesis of oligonucleotide conjugates with a TFA-protected basic peptide.

In order to be able to follow the approach where the peptide is deprotected in presence of a complementary sequence we developed an alternative solution phase conjugation procedure between a peptide-PATA derivative and 5'-azido-functionalized oligonucleotide. To benefit from our convenient TFA protection procedure the alternative approach requires a solution phase procedure, which means that the oligonucleotide has to be cleaved from support prior to conjugation. As the PATA linker does not survive the standard ammonolysis condition at 55-60 ºC, for removal of commercial oligonucleotides from support, we converted the oligonucleotide into the azido component (most conveniently done on solid support). The C-terminal of the peptide was extended with ethylenediamine to give 17 that was then turned into the activated alkyne derivative (TFA-SV40-PATA 18, Supplementary Scheme 1) which could be securely prepared due to the TFA-protection. This approach (Supplementary Scheme 1) then gives the conjugate of the deprotected LNA oligonucleotide with the TFA-protected peptide (entry 5, Table 1, Supplementary Figure 4).

Supplementary Scheme 1. Schematic presentation of the synthesis of oligonucleotide conjugates with a TFA-protected basic peptide by “click” reaction in solution. A: 1. Functionalisation of oligonucleotide with an azide linker (L2), pre-activation of L2-azide with HBTU, and NMM, DMF, 0.5h, r.t., then addition to solid supported oligonucleotide 2h; 2. Cleavage from support and removal of base protecting groups with NH₃, (aq.) sat. 55º C over night; B: TFA protection of amino groups: ethyl trifluoroacetate, methanol, 3 days, r.t.; C Extension with ethylenediamine: pre-activation of peptide 2 with HBTU, and NMM, DMF, 0.5h, r.t., then addition of ethylenediamine 2h; D: Functionalisation with the active alkyne linker PATA: pre-activation of PATA (11) with HBTU, and NMM, DMF, 0.5h, r.t., then addition to peptide 2h; E: CuSO₄/ascorbate catalyzed cycloaddition of oligonucleotide azide derivative with peptide-PATA: tBuOH/H₂O (1:1), CuSO₄ /Na ascorbate, overnight at r.t.
Lysine-rich peptide PATA (TFA-SV40-PATA), for C-terminal conjugation in solution.
The commercially available peptide PKKKRKVG-COOH (obtained deprotected but not purified, 25 mg, 0.0266 mmol) was dissolved in 1 mL of methanol in a glass vial which is then put on an ice-water bath. Triethylamine (100 μL) was added followed by ethyl trifluoroacetate (300 μL) whereupon the reaction vessel is sealed and allowed to slowly warm up to r.t. The reaction was complete after 3 days, whereafter it was evaporated to dryness and additionally dried under reduced pressure over night. The protected peptide (2) was then dissolved in 200 μL of dry DMF whereupon HBTU (1.1 eq., 11 mg) and NMM (2 eq., 6 μL) were added stepwise. The reaction was stirred at r.t. for 0.5 h after which time ethylenediamine (1.1 eq., 2 μL) was added and the reaction was left standing for an additional 1 h (solution A). The active linker PATA (2 eq., 11 mg) was dissolved in 200 μL of dry DMF. HBTU (2 eq., 20mg) and NMM (2 eq., 5.8 μL) were then added whereupon the reaction was agitated on a vortex for 0.5 h (solution B). After this time the solutions A and B were mixed together and allowed to react for 2 h at r.t. After evaporation to dryness the target peptide was dissolved in 1 M TEAA pH 6.5 containing 30% acetonitrile (buffer C) and purified by RP-HPLC using a linear gradient of buffer D (1 M TEAA pH 6.5 containing 90% of acetonitrile) in C from 60-100% in 30 min (UV-detection at 228 nm). t_R = 24.2 min, yield = 3.3 mg, 8% (after HPLC purification). ES-MS, calc m/z (M-H)^− 1645.7, found 1645.8.

5'-Azido-oligonucleotides (for solution synthesis of POCs).
The oligonucleotide on solid support (with a 5'-aminolinker, 0.2 μmol, 6 mg) was placed in a sealed Eppendorf tube. 2(2-Azidoethoxy)ethoxycetic acid (100 eq., 3.8 mg) was dissolved in DMF (280 μL) and first HBTU (100 eq., 7.6 mg) and then NMM (1230 eq., 27 μL) was added. The tube was sealed and agitated on a vortex at high speed for 2 h, centrifuged, and the solution was carefully removed with a syringe, whereupon 200 μL of DMF was added. The tube was sealed, centrifuged and the solution was removed from the solid support. This type of washing is performed 3 times. Deprotection solution (28% NH₃ (aq), 500 μL) is then added, and left at 55 ºC over night. The tube was centrifuged and the solution was collected with a syringe. Another portion of ammonia was added and the suspension was agitated and centrifuged, whereupon the solution was collected. The combined solutions were evaporated to dryness, dissolved in buffer A and purified by RP-HPLC using a linear gradient of buffer B in A from 0-50% in 20 min, detector at 260 nm, at 50 ºC, t_R = 15.0 min, ES-MS, calc (M) 2659, found 2659.

Synthesis of POCs by reaction of a peptide-PATA derivative with a 5'-azido-oligonucleotide, in solution. The oligonucleotide azide derivative from the procedure above (1 mM, 0.075 μmol) was freeze-dried in a 500 μL Eppendorf tube with a seal. The protected TFA-SV40-PATA (18, 2 eq., 0.15 μmol) was dissolved in 65 μL of tBuOH/H₂O (1:1) and added to the tube containing the oligonucleotide. Copper sulfate (5 μL, from a stock solution containing 3.6 mg/mL in H₂O, 1.2 eq., 0.09 μmol) was added and the reaction was gently shaken, whereupon a sodium ascorbate solution (5 μL, from a stock containing 8.9 mg/mL in tBuOH/H₂O (1:1), 3 eq., 0.225 μmol) was added. The tube was sealed and placed horizontally on a vortex and shaken overnight at r.t. The conjugate was purified by RP-HPLC using 1 M TEAA buffer pH 6.5 containing 30% acetonitrile (buffer C) and a linear gradient of buffer D (1 M TEAA containing 90 % acetonitrile) in C from 20-80% in 45 min, detector at 260 nm. TFA-SV40-LNA (entry 5, Table 1): t_R = 10.0 min (broad); ES-MS, calc m/z (M) 4307, found 4307.
2. **Spectroscopic data for AcMIF1-L1-azide** (see also 19 A). $^1$H-NMR (400 MHz, CDCl$_3$), $\delta$ (ppm): 0.85 (3H, d, CH$_3$ Leu, $J = 6.2$ Hz), 0.90 (3H, d, CH$_3$ Leu), 1.59 (2H, m, CH Leu + 1H from CH$_2$ Leu), 1.71 (1H, m, 1H from CH$_2$ Leu), 2.00 (3H, m, CH$_2$ Pro), 2.07 (3H, s, CH$_3$, Ac from Ac-Pro), 2.20 (1H, m, from CH$_2$ Pro), 3.39 (4H, bs, 2 x CH$_2$, ethylene from azido-amine), 3.48 (1H, m, from CH$_2$ Pro), 3.59 (1H, m, from CH$_2$ Pro), 3.88 (2H, ABq, CH$_2$ Gly, $J = 5.8$ Hz, $J = 11.0$ Hz), 4.17 (1H, m, $\alpha$CH Leu), 4.42 (1H, m, $\alpha$CH Pro), 7.21 (2H, bs, 2 x NH, Leu + Gly) $^{13}$C-NMR (100.62 MHz, CDCl$_3$), $\delta$ (ppm): 21.7 (CH$_3$, Ac from Ac-Pro), 22.7 (CH$_3$ Leu), 23.1 (CH$_3$ Leu), 25.2 (CH$_2$ Leu + CH$_2$ Pro), 28.5 (CH$_2$ Pro), 39.1 (CH$_2$, ethylene from azido-amine), 40.6 (CH$_2$ Leu), 43.4 (CH$_2$ Gly), 48.7 (CH$_2$ Pro), 50.5 (CH$_2$, ethylene from azido-amine), 53.3 (CH Leu), 60.7 (CH Pro), 169.9 (C=O), 171.1 (C=O), 172.7 (C=O), 172.9 (C=O). ES-MS, calc m/z (M-H)$^{-1}$ 394.2281, found 394.2279

3. **Spectroscopic data for PATA-linker** (11, see also 18 C) $^1$H-NMR (400 MHz, DMSO d$_6$), $\delta$ (ppm): 4.20 (1H, s, CH), 4.34 (2H, d, CH$_2$, $J = 6.1$ Hz), 7.32 (2H, d, 2 x CH Ph, $J = 8.1$ Hz), 7.90 (2H, d, 2 x CH Ph, $J = 8.1$ Hz), 9.32 (1H, t, NH, $J = 6.1$ Hz), $^{13}$C-NMR (100.62 MHz, CDCl$_3$), $\delta$ (ppm): 42.1 (CH$_2$), 76.3 (CH), 78.1 (CH), 127.1 (2 x CH Ph), 129.4 (2 x CH Ph), 142.9 (C=O), 151.8 (C=O) ES-MS, calc m/z (M-H)$^{-1}$ 202.0582, found 202.0570.

4. **Preparation of non-activated triple bond-derivatives of oligonucleotides.** Commercially available oligonucleotide (LNA-Am5) on solid support possessing at 5'-aminolinker with free amino function (0.2 $\mu$mol, 3 mg) was placed in sealed Eppendorf tube. Pentyonoic acid (100 eq., 1.9 mg) was dissolved in DMF (280 $\mu$L) and HBTU (100 eq., 7.6 mg) was added followed by NMM (1230 eq., 27 $\mu$L). The tube was sealed and agitated on a vortex for 0.5 h at r.t. The activated acid was then added to the oligonucleotide on solid support, the tube was sealed and the reaction was agitated on a vortex at high speed for 2 h. The tube was then centrifuged and the solution was carefully removed by a syringe whereupon 200 $\mu$L of DMF is added. The tube was vortexed, centrifuged and the supernatant was removed. This type of washing was performed 3 times. Ammonia (28% NH$_3$ (aq), 500 $\mu$L) was added, and deprotection was carried out in a sealed Eppendorf tube at 55 °C overnight. The Tube was centrifuged and the solution was carefully collected with a syringe. Another portion of ammonia solution was added and the suspension was agitated and centrifuged. The combined solutions were evaporated to dryness, dissolved in 50 mM triethylammonium acetate buffer pH 6.5 and purified by RP-HPLC using a linear gradient of buffer B in A from 0-35% in 40 min at 25 °C (UV-detection at 260 nm). $t_R = 28.2$ min, yield = 35%, 0.07 $\mu$mol (isolated). ES-MS, calc m/z (M) 2567.4, found 2568.

5. **Supplementary Scheme 2.** Conjugation of triple bond donor and oligonucleotide: Reagents and conditions: i = preactivation of linker with HBTU, and NMM, DMF, ii = conjugation with oligonucleotide, Abbreviations: HBTU = O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate, NMM = N'-methyl morpholine, DMF = dimethylformamide.
6. **General Method of the preparation of azido-peptides for C-terminus conjugation (TFA-SV40-L1-azide and SV40-L1-azide).**

(A) Commercially available peptide PKKKRKVG-COOH (1) obtained as deprotected but not purified (25 mg, 0.0266 mmol) is dissolved in 5mL of methanol and put to an ice-bath. Triethylamine is added (100 μL) followed by ethyl trifluoroacetate (300 μL), reaction is sealed and allowed to slowly warm up to r.t.. Reaction is completed after 3 days, evaporated to dryness and additionally dried on vacuum over night. After evaporation to dryness the target peptide TFA-SV40 is dissolved in 50 mM triethylammonium acetate buffer pH 6.5 containing 30% acetonitrile and purified on RP HPLC using linear gradient of buffer B (containing 50% of acetonitrile), detector at 228 nm. tR = 47.5 min, ESI-MS, calc m/z (M-H)+1 1420.55, found 1420.55.

(B) Purified peptide TFA-SV40 (3 mg, 0.002 mmol) was dissolved in 25 μL of DMF solution (made from the stock) containing HBTU (19 mg, 0.005 mmol) and NMM was added (43 mg, 0.42 mmol). This solution was left shaking on vortex for 30 min. After this preactivation, 25 μL of THF solution (made from the stock) containing 2-azidoethylamine (0.18 mg) was added and the solution was kept shaking for 2h. The solution was then lyophilized, dissolved in the HPLC starting buffer and purified by HPLC, tR = 47.4 min, ESI-MS, calc m/z (M-H)+1 1486.59, found 1486.68.

7. **Supplementary Scheme 3. Synthesis of protected peptide TFA-SV40-L1-azide.**

Reagents and conditions: i = ethyl trifluoroacetate, methanol, 3 days, r.t. ii = preactivation of peptide 1 with HBTU, and NMM, 0.5h, r.t., DMF, iii = conjugation with ethylenediamine 2h, r.t., Abbreviations: HBTU = O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate, NMM = N-methyl morpholine, DMF = dimethylformamide, TFA = trifluoroacetic

8. **Synthesis of water soluble C-terminal azide derivatized peptides.**

The peptides were synthesized like described above, but after the 2h coupling reaction, the mixture was transferred to a glass vial followed by 2 mL of conc. Ammonia (aq), the vial was sealed and kept at 55° C overnight. The solution was then lyophilized, dissolved in the HPLC starting buffer and purified by RP-HPLC, tR = 9.4 min, HPLC purification and analysis was done at 50° C, on a Vydac RP-18 column (218TP510, 10 x 250 mm, 5μmol) using a flow rate of 4 mL/min and first a linear gradient from 15% to 35% aqueous acetonitrile in 40 min followed by 35% to 70% in 10 min (with 0.1% trifluoroacetic acid in all solutions). ESI-MS, calc m/z (M-H)+1 1008.69, found 1008.69.

9. **Synthesis of POCs by conjugation of oligonucleotides on solid support with peptide-azides in the presence of [tris [(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine.** This conjugation was performed as described in the general method for conjugation without ligand except that a tBuOH/DMSO (2:1) solution of [tris [(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine was added to the support after the peptide solution was added.

tR = 15.1 min, conversion > 90% (HPLC). ES-MS, calc m/z (M) 4870, found 4870.
10. **Supplementary Figure 1.** Chromatograms of the crude products from synthesis of LNA-Am5-MIF1-Ac at different concentrations A) $c = 4 \text{ mM}$, B) $c = 2 \text{ mM}$, C) $c = 1 \text{ mM}$, with 2 eq. of peptide excess.

![Chromatograms A, B, C](image)

11. **Supplementary Figure 2.** Chromatograms of the crude products from synthesis of DNA-C6-C-myc performed with or without the ligand TBTA [tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine: A) without ligand, B) with 2.4 eq. of ligand.

![Chromatograms A, B](image)
12. Supplementary Figure 3. Chromatograms of the crude products from synthesis of LNA-Am5-C-myc performed with A) C-myc-L2-azide (7), or with B) TFA-C-myc-L2-azide (8)

13. Supplementary Figure 4. Chromatograms of the crude products from synthesis of TFA-SV40-LNA conjugate
14. Supplementary Figure 5. Chromatograms of crude reactions of conjugation of 18mer solid supported oligonucleotide with Ac-MIF1-L1-azide: A: using 1 eq. CuI/3eq sodium ascorbate; B: using 1 eq. CuI; C: using 1 eq. CuCl; D: using 1.2 eq. CuSO₄ and 3 eq. of sodium ascorbate.

Procedures:
CuSO₄/ascorbate: 4 eq. of peptide-azide (0.316 mg of Ac-MIF1-L2-azide) was dissolved in 80 μL of tBuOH/H₂O (1:1) and added into the vial. 1.2 eq. of CuSO₄ in 10 μL of H₂O (from a stock solution of 6 mg/mL) was added to the mixture followed by Na ascorbate solution (3 eq., 0.6 μmol, 10 μL from a stock solution of 12 mg/mL in tBuOH/H₂O (1:1)) and the solution was agitated on a vortex at low speed at r.t overnight.

CuCl: 4 eq. of peptide-azide (0.316 mg of Ac-MIF1-L2-azide) were dissolved in 80 μL of tBuOH/H₂O (1:1) and added into the vial. 1 eq. of CuCl in 10 μL of DMSO (from a stock solution of 2.2 mg/mL) was added to the mixture followed by DIPEA solution (8 eq., 1.6 μmol, 10 μL from a stock solution of 28 μL in 1 mL of tBuOH/H₂O (1:1)) and the solution was agitated on a vortex at low speed and at r.t overnight.

CuI: The protocol was the same as described above but using 1 eq. of Cul (from a stock solution of 4.3 mg/mL in DMSO) instead of CuCl.

CuI/ ascorbate: 4 eq. of peptide-azide (0.316 mg of Ac-MIF1-L2-azide or 1.1 mg of TFA-C-myc-L2-azide) were dissolved in 80 μL of tBuOH/H₂O (1:1) and added into the vial. The procedure was the same as described above but adding Na ascorbate (3 eq., 0.6 μmol, 10 μL from a stock solution of 12 mg/mL in tBuOH/H₂O (1:1))
15. Supplementary Figure 6. Chromatograms of purified conjugates and oligonucleotides and its intermediates. 

A: TFA-Cmyc-L2-azide, B: LNA-L2-azide, C: LNA-AcMIF1, D: LNA-C-myc, E: LNA-TFA-SV40, F: RNA-Am5-C-myc, G: RNA-Am5-AcMIF1
16. Estimation of copper content in crude POC preparations using the PAR reagent (4-(2'-Pyridylazo)resorcinol). Reagents: 1. 210 g citric acid/500ml H₂O; 2. 0,02g PAR (CAS: 1142-59-9)/500 ml H₂O; 3. 1:2 (1:1), freshly made; 4. 60% HNO₃/H₂O (1:2); 5. 3 M NaOH Procedure: To a 1 ml sample is added 0.03 ml reagent 4, and the sample is kept for 30 min in 100 °C water bath. Then 0.085 ml reagent 5 (NaOH) and 0.05 ml reagent 3 is added. After 3 to 5 min. the absorbance is recorded (400-700 nm).

Samples:
Blank = 100 μL milliQ-water that is treated the same way as sample.
Reference samples: A) 100 μL of drinking water diluted to 1 mL with milliQ water B) 45 μM aqueous (milliQ) CuSO₄.

Samples: Solid supported oligonucleotide-peptide conjugate was after reaction washed with water, acetonitrile, dichloromethane and then dried. C) 1 mg of that prepared support was put directly into a plastic vial, 0.5 mL of aqueous ammonia was added and reaction was kept at 55° C overnight. The vial was centrifuged, the solution collected by a syringe, whereupon the support was washed with water and the solutions were combined and evaporated to dryness.; D) 1mg of washed solid supported oligonucleotide-peptide conjugate was put into a disposable reaction vessel (a syringe with a filter) and washed with 0.05 M EDTA in water (20 mL) after which it was dried and put into plastic vial and treated with ammonia deprotection as for sample C. The respective solutions from C and D were evaporated to dryness and dissolved in 1mL milliQ water. 100 μL was taken out and diluted to 1 mL with milliQ water.

Supplementary Figure 7. UV absorption spectra of the copper analysis experiment using PAR reagent (4-(2'-Pyridylazo)resorcinol).
17. Mass spectra for peptide-oligonucleotide conjugates. The spectra represents authentic mass analysis results: spectrum signals used for calculation [m/z], combined spectrum raw data [m/z], and Maximum Entropy (MAXENT) recalculation of mass [M]

A. Supplementary Figure 8. Mass Spectroscopy data for LNA-AcMIF1, ES-MS, calc. m/z (M) 3067, found 3067.
B. Supplementary Figure 9. Mass Spectroscopy data for DNA-Am5-AcMIF1, ES-MS, calc. m/z (M) 4138, found 4138.

C. Supplementary Figure 7. Supplementary Figure 10. Mass Spectroscopy data for RNA-Am5-AcMIF1, ES-MS, calc. m/z (M) 6570, found 6570.
D. Supplementary Figure 11. Mass Spectroscopy data for LNA-Am5-C-myc, ES-MS, calc. m/z (M) 3802, found 3803.

E. Supplementary Figure 12. Mass Spectroscopy data for DNA-Am5-C-myc, calc. m/z (M) 4870, found 4870.
F. Supplementary Figure 13. Mass Spectroscopy data for DNA-C6-C-myc; ES-MS, calc. m/z (M) 4882, found 4882.

G. Supplementary Figure 14. Mass Spectroscopy data for RNA-Am5-C-myc
ES-MS, calc. m/z (M) 7306, found 7304.
18. A: Supplementary Figure 16. $^1$H-NMR spectrum of :AcMIF1-L1-azide (4).
B: Supplementary Figure 17. H-phosphonate-Am5-MMtr, triethylammonium salt (13)

C: Supplementary Figure 18. PATA reagent (11)
19. References:

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